



The Comparison the Impact of Flare-up GnRH Agonist with Delayed-Start GnRH Antagonist Protocols on the IVF Outcome of Poor Responder Patients: A Randomized Controlled Trial

Shirzad Hosseinishenatal¹, Fardin Amidi², Mohammad Ebrahim Parsanezhad³, Sirous Rostami⁴, Mojtaba Eslami², Aligholi Sobhani^{5*}

Abstract

Objectives: The current research was established to make a comparison between the delayed-start GnRH antagonist and flare-up GnRH agonist protocols in poor response patients.

Methods: The present study is a randomized, prospective, controlled trial that was performed on 150 women who referred to two distinct in vitro fertilization (IVF) centers in Iran. Patients were randomly assigned to two experimental groups, as one group was treated with the delayed-start GnRH antagonist protocol (delayed-start group), while another group was treated with the flare-up protocol (flare-up group).

Results: The serum concentrations of estradiol and progesterone, along with the thickness of endometrial tissue and the number of follicles ≥ 13 mm was significantly increased in the delayed-start group compared with the flare-up group. Also, the number of total oocytes, retrieved mature oocytes, total embryos, fertilized oocytes, as well as the quality of embryos were markedly higher in the delayed-start group when compared with the flare-up group. No statistically significant difference was found in the rates of fertilization, implantation, and pregnancy between the two experimental groups.

Conclusions: According to the above evidence, it seems that the effect of delayed-start protocol on ovarian responsiveness was more pronounced during controlled ovarian stimulation in comparison with the flare-up protocol and the delayed start protocol probably lead to better implantation and pregnancy rates in comparison with the flare up agonist protocol cycle in poor responders.

Keywords: GnRH analogues, Flare-up GnRH agonist, Delayed-start GnRH antagonist, Poor responsiveness

Introduction

The epidemiological studies have indicated that one out of six couples is affected with infertility at reproductive ages (1). When it comes to males, 10%-15% of adult men are affected by infertility. Due to the multifactorial etiology of the infertility, the precise mechanism underlying the pathogenesis has not yet been fully understood, and that causality of the disease remains idiopathic in half of the patients. Several factors, such as varicocele, chronic diseases, ejaculatory disorders, lifestyle, malignancies, chromosomal defects, and infectious diseases, can affect male fertility (2). In females, in addition to the age, some factors, including lifestyle, environmental factors, dietary habits, obesity, gynecological diseases, and systemic disorders, have been shown to play a negative effect on female fertility (3). Recently, assisted reproductive technology (ART) has provided an excellent opportunity for couples afflicted with infertility to have children (4). Although much progress has been made in ART,

sometimes, a group of patients, usually 24%, do not adequately respond to ART, called "poor-responders"(5). However, the success rate of in vitro fertilization (IVF) is solely dependent on obtaining an adequate number of oocytes to fertilize high-quality embryos, without putting patients at risk of excessive ovarian stimulation (6). Folliculogenesis is a complicated and dynamic process, which is controlled by autocrine and paracrine factors (7). Numerous factors, including endocrine hormones, antioxidants, nutrition, culture media, vitamins, and local-acting factors, influence the development of Folliculogenesis (8). It is now known that different agents, including age, endometriosis, ovarian surgery, genetic predisposition, and iatrogenic factors could be associated with poor ovarian response (POR) (9). Although various trials have been conducted on infertile women who were clinically POR, the rate of successful pregnancy has not been satisfactory, and some investigations have reported less than 10% of women can deliver live birth (10). Thus,

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¹Department of Anatomy, School of Medicine, International Campus, Tehran University of Medical Sciences (IC-TUMS), Tehran, Iran.

²Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran. ³Department of Gynecology and Obstetrics, Division of Reproductive endocrinology and infertility, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran. ⁴Shiraz Fertility Center, Shiraz, Iran. ⁵Department of Anatomy, School of Medicine, International Campus, Tehran University of Medical Sciences (IC-TUMS), Tehran, Iran

*Corresponding Author: Aligholi Sobhani, Tel: +98 21 66597378, Email: sobhania@sina.tums.ac.ir



the stimulation of the ovary in POR patients to ovulate remains a challenge (11). The application of high-dose gonadotropins, GnRH-a flare-up protocol, GnRH antagonist protocol, recombinant follicle-stimulating hormone (FSH), and adjuvant therapy have been broadly employed for the stimulation of ovulation in POR patients (12). There is compelling evidence for the use of ovarian stimulation protocols to improve the IVF outcomes in poor responders (13,14). Recently, a new antagonist protocol has been introduced, named Delayed-Start GnRH Antagonist protocol that is applied for infertile female patients with POR. The hypothesis is the delay in the initiation of ovarian stimulation with GnRH antagonist pretreatment for one week could inhibit endogenous FSH during the early phase of folliculogenesis, leading to the increased number of FSH-responsive follicles and synchronous follicular growth. For this aim, we compared the IVF outcome of the delayed-start GnRH antagonist protocol with the flare-up GnRH agonist in female patients with POR.

Materials and Methods

The Procedures of the Study and Patient Enrollment

The present research is a randomized, prospective, and controlled trial that was carried out on 150 women who referred to two distinct IVF centers in Shiraz, Iran, between May 2017 and June 2018.

Inclusion and Exclusion Criteria

According to the Bologna criteria all participants must have met 2 of the following three criteria to be included in our study. Inclusion criteria were having a history of POR (≤ 3 oocytes) with standard stimulation protocols, having the maternal age equal to or above 40 years, and abnormalities in ovarian reserve parameters, including having 5-7 antral follicles and the serum concentration of anti-Mullerian hormone with a range between 0.5-1.1 ng/mL. The age range of subjects was between 18-44 years old with regular menstruation cycles and normal serum prolactin. The presence of uterine cavities in hysterosalpingography was also clinically considered normal. The body mass index (BMI) was in a range between 18-30. Patients with a history of the hypothalamus, pituitary tumor, endocrine disorders, and endometriosis were excluded from the study.

Randomization Procedures

Randomization was performed by the computer-assisted randomization list. Each patient that was eager to participate in the trial was free to be joined in the delayed-start GnRH antagonist protocol (group 1) or the flare-up GnRH agonist protocol (group 2), and the probability to be included in each of the two groups was equal for each patient. The assignment of experimental groups was conducted by a third person who was not involved in the clinical trial.

Key Messages

- ▶ The delayed-start protocol can efficiently improve ovarian responsiveness during stimulation, consequently it can improve the pregnancy rate in poor responders.

Intervention(s)

The baseline concentrations of FSH and LH were measured in all participants in a natural cycle on day 3 of the menstrual cycle. Prior to the invitation of therapeutic protocols, patients received a low dose of an oral contraceptive tablet (Yasmin; Bayer) that was started on the previous cycle on day 1 of the menstrual cycle and terminated on day 22 (15).

Flare-up Agonist Protocol

In the flare-up group, three days after taking the last tablet, Triptorelin was daily injected, at a dose of 0.025 mg/day, to female patients and continued until the day of hCG administration (9). For the stimulation of the ovary, hMG (Menopur; Ferring) and FSH (Follistim; Merck; or Gonal-F; EMD-Serono) were administered at doses of 150 IU and 300 IU, respectively. The last phase of oocyte maturation accomplished by the injection of Human Chorionic Gonadotropin (Pregnyl; Schering Plough) at a dose of 10,000 IU, when at least the largest two follicles with an average diameter of 18 mm were observed (15).

Delayed-Start Antagonist Protocol

In the delayed-start group, on the second day of the menstrual cycle, Ganirelix acetate, a GnRH antagonist, was administered at a dose of 0.25 mg/d was administered until day 9 of the menstrual cycle. A week after the pretreatment with GnRH antagonist, the process of ovarian stimulation was initiated. For this aim, hMG (Menopur; Ferring) and FSH (Follistim; Merck; or Gonal-F; EMD-Serono) were administered at doses of 150 IU and 300 IU, respectively. For the prevention of the premature ovulation, Ganirelix acetate was administered at a dose of 0.25 mg/d, when the largest follicle attained a mean diameter above 12mm, and the injection of Ganirelix acetate was continued until the day of hCG administration. The final step of oocyte maturation was initiated by the administration of Human Chorionic Gonadotropin (Pregnyl; Schering Plough) at a dose of 10000 IU, when, at least, two largest follicles with an average diameter of 18 mm were apparent (15).

Intracytoplasmic Sperm Injection

After 36 hours of the administration of hCG, ovum pickup (OPU) was conducted, and the analysis was run on oocytes at metaphase II stage. The intracytoplasmic sperm injection method was utilized for the fertilization process in both groups. In this strategy, after washing and preparation, spermatozoa were injected into the mature oocytes by microinjection. The resulting embryos

were cultured and then transferred after the fertilization process on day 3 (15). Luteal support was daily provided to all female patients by the intravaginal administration of progesterone (Cyclogest, Actavis, United Kingdom) at a dose of 800 mg that was initiated on the day of OPU and continued to the day of beta hCG test. For the assessment of chemical pregnancy, the serum level of hCG was measured on day 14 following the embryo transfer. Transvaginal ultrasound was carried out on day 28 following the embryo transfer to approve the ongoing pregnancy by visualizing the intrauterine sac (16).

Embryo Grading

Morphological characteristics of embryos were scored in terms of the embryo's quality ranging from 1 to 3 based on a grading system introduced by the Istanbul consensus workshop on embryo assessment (17) (Table 1).

Outcome Measurements

The frequency of matured oocytes was regarded as the primary outcome, while the serum levels of estradiol and progesterone, the number of dominant follicles on the day of hCG administration, the total number of retrieved oocytes, Total Embryo number, as well as the rates of fertilization, implantation, and successful pregnancy were considered the secondary outcome.

Statistical Analysis

The Univariate statistical analysis was performed by the paired student *t* test. The non-parametric values were analyzed by the McNemar test with Yates correction for

Table 1. Embryo Grading System

Rank	Quality	Descriptions
1	Good	Stage-specific cell size Less than 10% fragmentation Lack of multi-nucleation
2	Fair	The stage-specific cell size for most of the cells A range of 10–25% fragmentation No evidence of multi-nucleation
3	Poor	Cell size not stage-specific Extensive fragmentation (above 25%) Partial Signs of multi-nucleation

continuity. The multivariable analysis was carried out by the mixed Poisson or negative binomial regression analyses where appropriate. Crude and adjusted incidence rate ratio (IRR), along with respective 95% confidence interval (CI), was employed. The obtained values were analyzed by the STATA software (version 11, StataCorp). The level of statistical significance was set at $P < 0.05$.

Results

At the first step of the clinical trial, 160 women were enrolled to participate in our study; however, 150 women met the required criteria, and 10 patients were excluded from the experiment. The procedures of patient selection are depicted in Figure 1.

The number of previous IVF cycles, as well as the concentration of estradiol and anti-Mullerian hormone (AMH) on day 3, was significantly higher in the flare-up group in comparison with the delayed-start group. However, the statistical analysis showed that the lack of

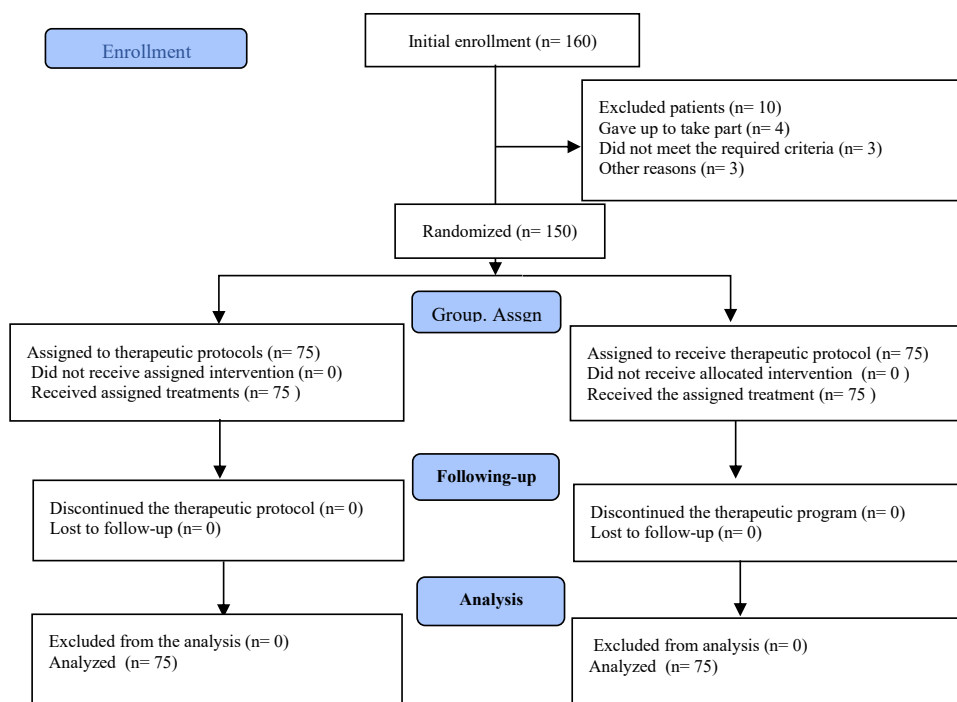


Figure 1. The Flowchart of the Study.

significant differences between the two experimental groups in terms of BMI, age, type of infertility, duration of infertility, along with the serum levels of LH, FSH, progesterone, and antral follicle count (AFC) on day 3 (Table 2).

According to univariate analysis, the serum estradiol levels, progesterone, endometrial thickness, and the number of follicles ≥ 13 mm were elevated by delaying ovarian stimulation with GnRH antagonist on the day of hCG trigger compared with the flare-up group. In delayed-start antagonist protocol, a higher number of total oocyte and mature oocyte, total embryos and good embryos, and a higher number of fertilized oocytes were observed compared with flare-up agonist cycles (Table 3).

The multivariable analysis showed that the number of

total and mature oocytes, as well as the frequency of whole embryos with good and fair qualities, was increased in the delayed-start group. Moreover, the increased number of fertilized oocytes was observed in the delayed-start group, as compared with the flare-up group (Table 3).

According to univariate analysis, despite the significant difference was found in the number of immature and post-mature oocytes between the two experimental groups, however, when it comes to the multivariable analysis, the frequency of immature and post-mature oocytes was not statistically significant (Table 3). The delayed-start GnRH antagonist protocol slightly (but not significantly) improved the rates of fertilization (58.68 ± 21 vs. 55.9 ± 30.94), implantation (16 ± 27.83 vs. 8.33 ± 24.07), and clinical pregnancy (28 ± 0.45 vs. 12 ± 0.33) (Figure 2).

Table 2. Demographic Characteristics of Patients

Data	Delayed-start	Flare-up	P Value*
Age (y)	33.28 \pm 4.25	34.56 \pm 5.97	0.38
BMI (kg/m ²)	29.98 \pm 1.52	22.98 \pm 1.83	0.98
Duration of infertility (month)	25.32 \pm 4.56	25.40 \pm 5.11	0.95
Type of infertility			
Primary (n)	18.00 (72%)	19.00 (78%)	0.73
Secondary (n)	7.00 (28%)	6.00 (23%)	
No. Of Previous IVF cycle	1.44 \pm 0.50	1.88 \pm 0.66	0.01
FSH (IU/L) on day 3	9.59 \pm 2.15	8.84 \pm 4.61	0.46
LH (IU/L) on day 3	6.66 \pm 1.5	7.42 \pm 6.57	0.57
Estradiol (pg/mL) on day 3	121.08 \pm 21.50	143.60 \pm 19.50	0.00
Progesterone (ng/mL) on day 3	0.79 \pm 0.18	0.70 \pm 0.19	0.08
AMH (ng/mL)	1.68 \pm 0.63	2.43 \pm 1.12	0.00
AFC	3.60 \pm 0.64	3.20 \pm 0.86	0.07

FSH: follicle stimulating hormone; AFC: antral follicle count; LH: luteinizing hormone; AMH: anti-Mullerian hormone.

*P values of all variables, except for "Type of infertility," were analyzed according to Mann-Whitney test, and the P values of "Type of infertility" was obtained by the chi-square test.

Table 3. Comparison of Features and Results of Ovarian Stimulation in Delayed-Start and Flare-up Protocols

Outcome	Univariate Analysis			Multivariable Analysis		
	Delayed-Start	Flare-Up	P Value	Crude IRR (95% CI)	Adjusted IRR (95% CI)	
Stimulation day	Estradiol (pg/mL)	1262 \pm 172.44	943.84 \pm 89.2	0.00	300(155.93-444.06)	168(64.86-271.80)
	Progesterone (ng/mL)	1.52 \pm 0.48	1.22 \pm 0.33	0.01	0.05(-0.007-0.10)	0.08(0.19-0.02)
	Endometrial thickness (mm)	10.38 \pm 0.74	8.84 \pm 0.52	0.00	1.3(0.60-1.9)	1.44(1.01-1.87)
	No. of follicles ≥ 13	11.32 \pm 4.46	6.2 \pm 2.4	0.00	1.82(1.50-2.22)	1.61(1.2-2.00)
No. of retrieved Oocytes	Total	11.32 \pm 4.46	6.2 \pm 2.4	0.00	1.83(1.50-2.22)	1.61(1.30-2.00)
	Immature	1.44 \pm 0.86	0.88 \pm 0.78	0.02	1.64(0.96-2.78)	0.86(1.23-3.17)
	Mature	4.4 \pm 2.48	1.84 \pm 1.02	0.00	1.28(0.59-2.73)	2.39(1.69-3.37)
	Post	4.56 \pm 3.05	2.92 \pm 2.19	0.03	1.56(1.05-2.3)	0.23(0.64-2.38)
	Atretic	0.88 \pm 1.2	0.6 \pm 0.91	0.36	1.46(0.76-2.82)	3.35(5.23-49.73)
No. of Embryos	Total	6.28 \pm 3.11	3.24 \pm 2.06	0.00	1(0.54-1.84)	1.93(1.42-2.64)
	Good	1.84 \pm 1.72	0.56 \pm 0.82	0.00	2.99(1.49-6.01)	3.29(1.8-5.97)
	Faire	3.04 \pm 1.39	1.72 \pm 1.27	0.00	1.77(1.21-2.56)	1.77(1.21-2.56)
	Poor	1.4 \pm 1.70	1 \pm 1.22	0.34	1.40(0.84-2.34)	0.20(0.4-0.94)
No. of fertilized oocyte	6.48 \pm 3.04	3.24 \pm 2.18	0.00	1.55(1.09-2.20)	2.00(1.53-2.61)	
No. of Embryos transferred	1.88 \pm 0.33	1.72 \pm 0.54	0.21	1.09(0.72-1.65)	0.91(1.36-2.31)	

CI, confidence interval; IRR, incidence ratio rate.

Discussion

Although much progress has been made in the therapeutic strategies used for the treatment of infertility, no consensus has yet approved on the optimal protocol for infertile women who are clinically considered poor responders (5). In the present research, we compared the clinical outcomes of the delayed-start GnRH antagonist protocol with the flare-up agonist protocol in female patients with POR. Our findings demonstrated that 7-day pre-treatment of female patients with a GnRH antagonist, prior to the initiation of ovarian stimulation, it can markedly boost the development of follicles. Correspondingly, the yield of mature oocytes was substantially increased, and higher numbers of fertilized oocytes and high-quality embryos were evident when compared with the flare-up GnRH agonist protocol. Our results also indicated that rates of implantation and successful pregnancy were slightly (although not significantly) higher in the delayed-start protocol, as compared with the flare-up protocol. The onset of puberty in the female gender is controlled by GnRH with a sudden change in the release of pituitary hormones, such as LH and FSH. As a result, such an alteration leads to the initiation of the folliculogenesis process and promotes oocyte maturation (18). In the process of ovarian stimulation, exogenous gonadotropins are required for the synchronous follicular growth and response. The presence of various follicular sizes is potentially capable of reducing the feasibility of the fertilization rate and oocyte maturation, leading to the restricted number of embryos (19). The difference in follicular sizes might be more pronounced in infertile women with reduced ovarian response. Studies have shown that inhibiting the production of endogenous FSH at the initial phase of folliculogenesis could result in the enhancement of follicular growth and synchronization of follicles (20). To date, few studies evaluated the impact of the delayed-start GnRH antagonist protocol on ovarian stimulation to improve the outcome of the controlled ovarian hyperstimulation (COH). Consistent with our results, Aflatoonian et al. compared the results of the delayed-start GnRH antagonist protocol with the conventional antagonist protocol in a group of patients with POR. They showed that although this protocol improved the rates of successful pregnancy and implantation, no significant difference was detected between the two types of protocols (21). Ashrafi et al also demonstrated that the delayed-start antagonist protocol can enhance the quality of embryos, fertility rate, and prevent the cycle cessation; however, it has no significant impact on the rate of a successful pregnancy (22). In another study by Frankfurter et al, they reported that, in patients with POR, a delay in ovarian stimulation using the delayed-start GnRH antagonist protocol is able to increase the frequency of transferred embryos and retrieved oocytes (23). They began the first injection of a GnRH antagonist on cycle day 5–8 for a period of eight days, and the second dose of a GnRH antagonist at a dose

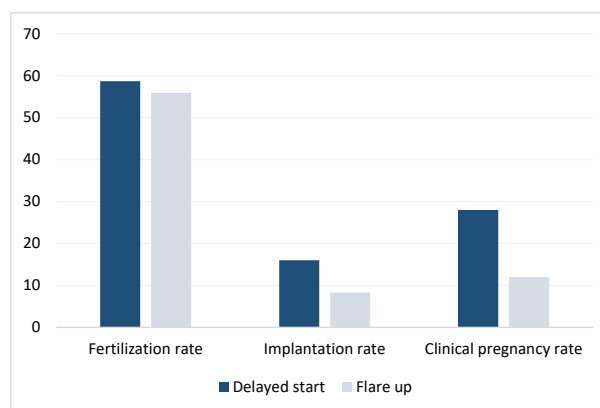


Figure 2. The Comparison of the Rates of Fertilization, Implantation, and Pregnancy rate Between the Two Experimental Groups.

of 3 mg was administered four days later. Meanwhile, progesterin was daily applied until ovarian suppression was confirmed (23). The administration intervals of the GnRH antagonist were comparable in our study (a seven-day course with a daily injection of ganirelix at a dose of 250 mg) with other similar investigations. The administration of the GnRH antagonist was begun on cycle day 2, before choosing the leading follicle, to suppress the premature folliculogenesis as a result of FSH increase. In line with our findings, Cakmak et al reported nearly similar results on poor response when used a similar protocol (15). They indicated that the application of the delayed-start protocol led to a higher rate of concurrent follicular development. Additionally, the yield of mature and fertilized oocytes, as well as the embryo transfer, was markedly increased in the delayed-start protocol in comparison with the conventional estrogen priming used by a GnRH antagonist (15). Davar et al showed that this protocol was able to enhance ovarian response in patients with POR, thereby synchronizing the follicular growth (24). In another study by Blockeel et al, it has been demonstrated that the administration of a GnRH antagonist for three consecutive days, at the early follicular phase, produced higher numbers of retrieved oocytes in normal responders; however, the rate of successful pregnancy was not considerably increased (25). Another clinical trial was conducted on women having a normal ovarian reserve. The results of the clinical trial showed that three days pretreatment with a GnRH antagonist, before COH, enhanced the rates of oocyte development and fertilization, whereas failed to increase the probability of successful pregnancy (26). In a recent study, 160 patients who were poor responders to the previous IVF cycle were assigned to two groups as follows; the first group received gonadotropin, then Cetrotide, at a dose of 0.25 mg, was added when the largest follicle reaches at a diameter of 12 mm. The second group received Cetrotide, at the same dose used for the first group, that was started from the second day to the 8th day of the cycle; then, gonadotropin was administered, and Cetrotide was resumed when the largest follicle attained a diameter of 12 mm. It was

shown that, at the initial stage of folliculogenesis, a seven-day pretreatment course with a GnRH antagonist led to a considerable number of fertilized oocytes, mature oocytes, and high-quality embryos. It was indicated that, in poor responders, the delayed-start protocol is able to significantly increase the rate of clinical pregnancy and IVF outcomes (16). In the current research, significant amounts of factors involved in ovarian stimulation are remarkably increased as decreased levels of FSH at the early follicular phase would be capable of reducing the exposure of follicles to increased concentrations of FSH, and it could result in better synchronization of follicular growth and suppression of the initial domination of a single follicle (13). In opposed to our findings, Zarei and colleagues indicated that this protocol has no marked impact on the rates of pregnancy and IVF outcomes in poor responders (27). Our obtained results could be employed for patients with POR to increase ovarian stimulation. The delayed-start GnRH antagonist protocol was shown to increase the frequency of high-quality embryos; however, the rate of successful pregnancy was not significantly increased in this group of patients. Similar to many other trials conducted on stimulation protocols for poor responders, our study suffers from the small sample size. It is suggested that the delayed-start GnRH antagonist protocol should be analyzed at larger randomized prospective studies by means of other ovarian stimulation methods for poor responders. The application of mini flare-up, stop GnRH agonist, and Shanghai or DuoStim protocols are exemplary strategies for this goal. The analysis of such studies could be performed at the level of gene expression for identifying genes involved in oocyte maturation.

Conclusions

It is now apparent that applying the delayed-start protocol can efficiently improve ovarian responsiveness during COH. The delayed-start protocol caused a higher rate of follicle growth and increased the number of mature oocytes, fertilized oocytes, and high-quality embryos to transfer. It also improved the rates of implantation and pregnancy in comparison with the flare-up GnRH agonist protocol. Despite the higher cost and duration of the delayed-start protocol in comparison with the flare-up protocol, the satisfactory results of the protocol can still compensate for the pitfalls early mentioned. However, further clinical trials with a larger sample size are warranted to confirm the superiority of the delayed-start protocol over other therapeutic approaches.

Authors' Contribution

Conceptualization: AS.
Methodology: MEP, SH.
Validation: FA.
Formal Analysis: ME.
Investigation: SH.
Resources: SR.
Data Curation: ME.

Writing—Original Draft Preparation: SH
Writing—Review and Editing: SH, AS.
Visualization: SH.
Supervision: AS, MEP.
Project Administration: AS.
Funding Acquisition: AS.

Conflict of Interests

Authors declare that they have no conflict of interests.

Ethical Issues

The research protocols of the study were corroborated by the Ethics Committee of Tehran University of Medical Sciences with an ethics code IR.TUMS.VCR.REC.1395.481. The written informed consent was obtained from all female patients, and the study was registered in IRCT with a trial code number IRCT-2016091722903N2.

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