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Delayed Start Versus Conventional GnRH Antagonist Protocol in Poor Responders Pretreated With Estradiol in Luteal Phase: A Randomized Controlled Trial

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Abstract

Objective: To compare the new delayed start protocol against the conventional gonadotropin (Gn)-releasing hormone antagonist protocol in poor responders (PORs). **Study Design:** A total of 160 women with poor response to previous in vitro fertilization (IVF) cycle were randomized either to start Gn then Cetrotide 0.25 subcutaneously (sc) added when leading follicle (DF) reach >12 mm or Cetrotide 0.25 mg sc started first from day 2 to day 8 then Gn therapy was added and Cetrotide restarted when DF reach >12 mm. **Results:** There was a statistically significant difference between conventional and delayed start protocols regarding the needed dose of Gn for stimulation (4368 $\pm$ 643 and 3798 $\pm$ 515), level of estradiol (E2; 778 $\pm$ 371 and 1076 $\pm$ 453), and endometrial thickness at human chorionic gonadotropin triggering (8.6 $\pm$ 1.8 and 9.8 $\pm$ 1.9), the number of DF (3.4 $\pm$ 1.5 and 4.9 $\pm$ 2.1), the number of retrieved follicles (2.4 $\pm$ 2.1 and 4.3 $\pm$ 2.5), and successful embryo transfer (13 vs 16), respectively ($P < .05$). There was a highly statistically significant difference between the 2 study groups regarding the number of oocytes fertilized (1.2 $\pm$ 2.0 vs 3.3 $\pm$ 1.4), metaphase II oocytes (0.9 $\pm$ 1.0 vs 2.7 + 1.6), and grade I embryos (0.7 $\pm$ 0.9 vs 2.1 + 1.1; $P < .001$). The chemical pregnancy, clinical pregnancy, and abortion rate showed a statistically significant difference between the 2 study groups (P value .003 and .006, respectively). **Conclusion:** Delayed start protocol significantly improved clinical pregnancy rate and IVF cycle parameters in PORs.

Keywords

GnRH antagonist, conventional protocol, delayed start protocol, poor responders

Introduction

A poor response to controlled ovarian hyperstimulation is disappointing yet not uncommon in the practice of assisted reproduction technologies.[1] A diagnosis of poor responders (PORs) is confirmed by the failure of a standard (long protocol) ovarian stimulation or by the cancellation of at least 1 in vitro fertilization (IVF) cycle.[2] The PORs represent 5% to 35% of infertile patients undergoing controlled ovarian stimulation according to definition of poor response.[3,4]

Recently, the concept of treatment of PORs is to decrease follicle-stimulating hormone (FSH) in late luteal phase—early follicular period to prevent early follicular selection and allow recruitment of more follicles for induction and the use of estradiol (E2) pretreatment may help to suppress early rise in FSH in late luteal phase and allow better environment for higher number of follicle growth.[5]

Many protocols were suggested to improve cancellation and clinical pregnancy rate (CPR) such as increasing dose of gonadotropins (Gn) up to 450 U,[6] using flare agonist protocol (short protocol),[7,8] luetinizing hormone (LH) supplementation from day 6 onward in patients receiving recombinant FSH (rFSH),[9,10] Gn-releasing hormone (GnRH) agonist stop protocol,[11,12] mini dose luteal phase agonist,[13] decreasing agonist dose from day 2 onward in patient starting long protocol,[14] lutael phase FSH intake,[15] micro-dose flare protocol,[16,17] GnRH antagonist,[18] estrogen priming to antagonist,[5] and clomiphene citrate and aromatase inhibitor with antagonist.[19,20]

Cakmak et al introduced use of pretreatment E2 and start of antagonist alone from day 2 to day 8 without Gn therapy—the so-called delayed start protocol with aim to reduce early

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follicular FSH and synchronize high number of follicular development without impairing the quality of oocytes.[21] The aim of this study is to compare the new delayed start protocol against the conventional GnRH antagonist protocol in PORs.

Materials and Methods

This study is a randomized controlled study conducted on 160 women attending 4 IVF centers in 2 countries Egypt and Kingdom of Saudi Arabia during the period from January 2014 to April 2015. The study was approved by local ethics committee, and informed consents about the study and expected value and outcome were obtained. All participants were PORs for previous IVF cycle defined as Bologna criteria; at least 2 of the following 3 features must be present: (1) advanced maternal age ($\geq$40 years) or any other risk factor for POR; (2) a previous POR ($\leq$3 oocytes with a conventional stimulation protocol); and (3) an abnormal ovarian reserve test (ie antral follicular count [AFC], 5-7 follicles or anti-Mullerian hormone [AMH], 0.5-1.1 ng/mL).[22]

All candidates were more than 35 years old, with normal menstrual cycle with a range of 24 to 35 days and normal seumprolactin. All women had normal uterine cavity determined by previous hysterosalpingography or hysteroscopy. Exclusion criteria included women older than 44 years and those with AMH <0.3 ng/mL. Women with endometriosis; abnormal endocrine functions such as diabetes mellitus, thyroid disorders, and adrenal abnormalities; general diseases, those with ovarian cysts, or those who have azospermia partners were also excluded.

The history of patients were collected including age, duration, type and cause of infertility, and medical history. Full examination including general, abdominal, and vaginal examination was done followed by ultrasound evaluation for the detection of any of the exclusion criteria. Basal day 3 hormonal evaluation for FSH, LH, and E2 was done in a natural cycle. Estradiol, FSH, and LH levels were determined using Immulite system (Siemens Healthcare Diagnostics, United Kingdom).

All patients received combined oral contaraceptive pills for 1 cycle (gynera; Bayer, Schering, Germany) from day 5 to day 25, then received E2 tablets of 2 mg daily for 1 week in the same time with oral pills from day 21 to day 28 (white tablet of climen; Bayer Scherring), and then transvaginal ultrasound (TVUS) was done to exclude any ovarian cyst or follicle >10 mm.

Participants were equally randomized using automated Web-based randomization system ensuring allocation concealment into 2 groups (randomization was done for each center individually): group I received 300 U recombinant FSH (Gonal-f; Merck Serono, Germany) + 150 U urinary Gn (Menogon; Ferring, Switzerland) from day 2 till the day of human chorionic gonadotropin (HCG), dose adjusted according to ovarian response monitored by serum E2 and ultrasound evaluation. All patients were followed up by TVUS scan daily or on alternate days according to the ovarian response to treatment starting on treatment cycle day 6 for folliculometry and endometrial thickness and pattern. Cetrotide (MerckSerono, Germany) 0.25 subcutaneously was added when the leading follicle (DF) reached >12 mm and HCG 10 000 IU intramuscularly (Pregnyl, Merck Sharp, United Kingdom)was given only if we have at least 3 mature follicles >14 mm and the leading one >17 mm; then, ovum pickup (OPU) was done after 36 hours of HCG and metaphase II ocytes were analyzed. The intracytoplasmic sperm injection (ICSI) procedure was performed in all cases to avoid low fertilization rate by conventional IVF. Fertilization was assessed 16 to 18 hours after ICSI, and embryo quality was evaluated 2 and 3 days after ICSI according to the number of blastomeres and the degree of fragmentation and multinucleation.[23] Oocytes were collected and embryos were cultured in ISM1 culture medium (Origiomedicult media, Denmark).

Transfer of cleaving embryos was done on day 3 after oocyte retrieval (using Labotect semirigid catheter; labotect GmbH, Germany), when we have at least 1 embryo group I (GI) otherwise canceled embryo transfer (ET). The ET was done by 1 person in each center. Both of the transferring consultants have more than 5 years experience in IVF unit. Group II received Cetrotide 0.25 mg subcutaneously only from day 2 to day 8, and then we initiated Gn therapy using the same dose (300 FSH + 150 U urinary GN). Same adjustment of dose was done and the antagonist restarted when DF >12 mm, till day of HCG. The HCG, OPU, and ET were done using the same method. All patients received luteal support in the form of daily progesterone cyclogest (Actavis, United Kingdom) 800 mg daily starting from day of ovum retrieval till day of hCG testing. Serum β hCG level was assessed on day 14 after ET and considered positive if >5 mIU/ml. The TVUS was performed 28 days after ET to confirm ongoing pregnancy by visualization of intrauterine sac.

The primary outcome parameters evaluated were clinical pregnancy (defined as the presence of gestational sac with accompanying fetal cardiacactivity) using ultrasonography.[24] Other parameters included occurrence of abortion, the needed dose of Gn, duration of stimulation, E2 level at day of HCG triggering, endometrial thickness at day of HCG triggering, number of follicles >16 mm, number of retrieved follicles, number of oocytes fertilized, number of good ET, and cancellation rate.

Data were statistically described in terms of mean $\pm$ standard deviation ($\pm$SD), median and range or frequencies (number of cases), and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student *t* test for independent samples. For comparing categorical data, chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. All statistical calculations were done using computer programs SPSS (SPSS Inc, Chicago, Illinois) version 14 for Microsoft Windows.

Results

There was no significant difference between the 2 study groups regarding age, body mass index (BMI), duration of infertility,

Table 1. Demographic Data of the Study Population.[a]

		Conventional Protocol	Delayed Start Protocol	*P* Value
Age, years		38.0 ± 1.9	38.5 ± 2.3	.5
BMI, kg/m^2		29.2 ± 4.1	29.4 ± 4.7	.85
Duration of infertility, years		6.0 ± 2.7	6.7 ± 1.7	.34
Type of infertility[b]	1ry	56 (70%)	64 (80%)	.27
	2ry	24 (30%)	16 (20%)	.25
Cause of infertility[b]	Male	28 (35%)	24 (30%)	.67
	Tubal	24 (30%)	24 (30%)	.87
	Ovarian dysfunction	20 (25%)	24 (30%)	.65
	Unexplained	8 (10%)	8 (10%)	.89
Failed previous IVF cycles		2.3 ± 1.1	1.9 ± 1.1	.57

Abbreviation: BMI, body mass index; IVF, in vitro fertilization; SD, standard deviation.
[a]All results are presented as means ± SD.
[b]Number (%).

Table 2. Cycle Characteristics.[a]

	Conventional Protocol	Delayed Start Protocol	*P* Value
Day 3 FSH, IU/L	9.4 ± 1.9	10.5 ± 2.5	.12
Day 3 LH, IU/L	6.7 ± 1.6	6.5 ±1.6	.7
AMH, ng/mL	1.3 ± 1.1	1.2 ± 0.7	.71
D3 estradiol, pg/mL	110 ± 54	123 ± 60	.45
Antral follicular count	2.6 ± 1.1	2.2 ± 0.9	.23
Gonadotropin dose, IU	4368 ± 643	3798 ± 515	.004[b]
Duration of stimulation, days	10.8 ± 1.2	10.8 ± 1.4	.9
E2 at HCG triggering	778 ± 371	1076 ± 453	.03[b]
Endometrial thickness at hCG injection	8.6 ± 1.8	9.8 ± 1.9	.044[b]
Mature follicle count	3.4 ± 1.5	4.9 ± 2.1	.01[b]
Ovum pickup	52/80	64/80	.002[b]
Number of retrieved oocytes	2.4 ± 2.1	4.3 ± 2.5	.02[b]
Number of oocytes fertilized	1.2 ± 2.0	3.3 ± 1.4	.001[b]
Embryo transfer	32/80	64/80	.02[b]
Metaphase II oocytes	0.9 ± 1.0	2.7 + 1.6	.001[b]
Grade I embryos	0.7 ± 0.9	2.1 + 1.1	.001[b]
Cancellation of OPU[b]	28/80	16/80	.002[b]

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone; AMH, anti-Mullerian hormone; HCG, human chorionic gonadotropin; SD, standard deviation.
[a]All results are presented as means ± SD.
[b]Number (%).

Table 3. Outcome Parameters.

	Conventional Protocol	Delayed Start Protocol	*P* Value
Positive β hCG	12/80 (15%)	28/80 (35%)	.006[a]
CPR (sac seen on 6th wk)	8/80 (10%)	24/80 (30%)	.003[a]
Abortions/pregnancies	4/80	4/80	NS

Abbreviation: CPR, clinical pregnancy rate; NS, nonsignificant.
[a]All results are presented as number (percentage).

type (primary infertility is more common), cause of infertility, and number of previous failed IVF cycles (Table 1).

There was no significant difference between the 2 study groups regarding day 3 FSH, LH, E2, anti-Mullerian hormone, or antral follicular count (Table 2). There was no significant difference between the 2 study groups regarding duration of stimulation, ovum pickup, and cycle cancellation (Table 2).

There was a statistically significant difference between the 2 study groups regarding the needed dose of Gns for stimulation, level of E2 and endometrial thickness at HCG triggering, the number of mature follicular count, the number of retrieved follicles, and successful ET (Table 2).

There was a highly statistically significant difference between the 2 study groups regarding number of oocytes fertilized, metaphase II oocytes, and grade I embryos (Table 2). The chemical pregnancy rate and CPR showed a statistically significant difference between the 2 study groups, while the number of patients who experienced abortion showed a nonstatistically significant difference between the 2 study groups (Table 3).

Discussion

Poor response usually leads to many detrimental effect on IVF cycles due to low oocyte count and quality that result in low pregnancy rate, implantation rate, and live birth rate.[6] In our study, we found that the delayed start protocol significantly decreased the needed dose of Gns needed for stimulation, improved level of E2 and endometrial thickness at HCG triggering, increased the number of mature follicular count, and the number of retrieved follicles.

Our novel protocol also significantly increased number of oocytes fertilized, number of metaphase II oocytes, and grade I embryos, with successful embryo transfer. In our study, most of the ovarian induction parameters improved significantly, and this can be explained by the fact that decreasing early exposure of high FSH in early follicular phase may allow more synchronization of multiple follicular growth and prevent early domination of single follicle.[10]

It is noticed that there is significant increase in chemical and CPR. This is the first randomized controlled trial (RCT) that demonstrated significant high CPR with delayed start protocol versus conventional antagonist one. To the best of our knowledge, our study is the first randomized study done to evaluate the delayed start GnRH antagonist protocol in PORs. The RCTs were done for normal responders, and their results revealed improved ovarian induction results without significant effect on CPR.[8,9]

Tannus and colleagues in their retrospective study tried to determine the proportion of patients stimulated on a flexible GnRH antagonist regimen who meet the criteria for antagonist administration after stimulation day 6 (S6) and to compare their clinical characteristics and cycle outcome with those patients who start the antagonist on S6 or earlier.

Ovarian stimulation was performed using Gns and GnRH antagonists. Group A (n = 323) patients met the criteria for antagonist administration (follicle size >12 mm and

E2 >300 pg/mL) on S6 or earlier. Group B patients (n = 119) started the antagonist later. A comparable implantation (30.4% vs 33.7%), CPR (47.4% vs 52.9%), and ongoing pregnancy rate (41.2% vs 47.9%) were observed in groups A and B, respectively. Group B patients had a significantly higher BMI, longer stimulation, increased Gns dosage, fewer oocytes and 2 pronuclei oocytes, fewer frozen embryos, and fewer cycles with embryo freezing. Patients with polycystic ovary syndrome were more likely to be in group B. They concluded that a considerable proportion of patients on a flexible regimen begin administration of GnRH antagonist later than S6 had different stimulation and laboratory characteristics; however, their reproductive outcome is not compromised when compared to patients with an earlier antagonist start.[25]

The only study done on our novel protocol is the one done by Cakmak and colleagues in 2014. They studied 30 PORs who failed to get pregnant with conventional estrogen-priming antagonist IVF protocol.

They found that the number of patients who met the criteria to proceed to oocyte retrieval was significantly higher in the delayed start protocol (21 vs 11/30). The number of dominant follicles was significantly higher in the delayed start (4.2 ± 2.7) compared to conventional (2.4 ± 1.3) protocol. In patients who had oocyte retrieval after both protocols (n = 9), the delayed start resulted in shorter ovarian stimulation (9.4 ± 1.4 days vs 11.1 ± 2.0 days), higher number of mature oocytes retrieved (4.9 ± 2.0 vs 2.2 ± 1.1), and a trend toward increased fertilization rates with ICSI (86 ± 17% vs 69 ± 21%) compared to conventional protocol. After delayed start, the average number of embryos transferred was 2.8 ± 1.4 with implantation rate of 9.8% and CPR of 23.8%. They concluded that the delayed start protocol improves ovarian response in PORs by promoting and synchronizing follicle development without impairing oocyte developmental competence. However, this study was a retrospective one.[21]

Younis et al performed a prospective, controlled, randomized study to investigate early and short follicular administration of GnRH antagonist using the flexible protocol. They found that early and short follicular GnRH antagonist supplementation using flexible GnRH antagonist treatment improves the meiotic status and competence of retrieved oocytes. It seems that early and short pituitary shutdown has the potential to improve clinical results in IVF ET GnRH antagonist cycles.[26]

Conclusion

Delayed start protocol improved IVF cycle parameters in PORs.

Declaration of Conflicting Interests

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