Original Article

Effect of Antagonist Start Day on Cycle Outcomes in Poor Responders

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ABSTRACT

Background: Despite the great advances in Assisted Reproductive Technologies (ART), management of poor responders has remained a great challenge. Gonadotropin releasing hormone antagonist (GnRH-ant) has been offered as a patient friendly protocol. In the literature, conflicting data exists about the effect of the GnRH-ant starting day on cycle outcomes. **Aim:** The aim of this study is to evaluate the effect of GnRH-ant starting day on cycle outcomes of patients with poor ovarian response defined by Bologna criteria. **Setting and Design:** This retrospective cohort study was conducted at an ART clinic of a tertiary hospital. **Materials and Methods:** A total of 361 cycles using flexible GnRH-ant, 195 in Group A (GnRH-ant administered before day 6 of stimulation) and 166 cycles in Group B (GnRH-ant started on or after day 6), were selected retrospectively for the study. **Statistical analysis:** Statistical analysis of data was carried out using using IBM SPSS Statistics Software (20.0, SPSS Inc., Chicago, IL, USA). Independent samples t-test and Mann–Whitney U test were used to analyze the variables. **Results:** Total antral follicle count was significantly higher in Group A compared to Group B ($P = 0.009$). Duration of stimulation was significantly shorter ($P < 0.01$) and total dose of gonadotropin used was lower in Group A when compared to Group B ($P < 0.01$). While higher number of oocytes was retrieved from Group A ($P = 0.037$), no between-group differences were observed in number of mature oocytes, fertilized oocytes, clinical pregnancy rate or ongoing pregnancy rate (OPR) per embryo transfer ($P > 0.05$). **Conclusion:** Early GnRH-ant start may point out a favourable response to ovarian stimulation in poor responders. However, clinical or OPRs were not different from the late GnRH-ant start group.

KEYWORDS: *Bologna criteria, controlled ovarian stimulation, decreased ovarian reserve, gonadotropin releasing hormone-antagonist*

INTRODUCTION

*D*espite the great advances in assisted reproductive technologies (ART), management of patients with decreased ovarian reserve has remained a great challenge, which comprises about 9%–24% of ART cycles.[1] Studies demonstrated that these patients have a poor ovarian response (POR) to controlled ovarian stimulation (COS) and associated with a decreased number of retrieved oocytes, decreased pregnancy rates, and increased cancellation rates when compared to normoresponders.[2,3] However, there has been no uniformity in the definition of POR until the Bologna criteria, which is the first concrete attempt to standardize the definition of POR in the literature. Briefly, two out of three criteria are required; (1) advanced maternal age (>40) or presence of any other risk factors, (2) previous poor response, (3) abnormal ovarian reserve tests.[4] Despite the various treatment protocols and interventions that have been investigated to improve ovarian response, including the use of high doses of gonadotropins, addition of growth hormone, androgens and androgen-modulating agents, there is still no consensus on the optimal stimulation method

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to increase success rates.[5,6] Gonadotropin-releasing hormone antagonist (GnRH-ant) has been offered as an alternative protocol for poor responders with several theoretical advantages over microdose flare and long luteal down-regulation protocols.[7,8] GnRH-ant's competetively bind to GnRH receptors which prevent binding of endogenous GnRH to this receptor. Therefore suppression of gonadotropin release occurs within a few hours without a flare-up effect and gonadal function resumes without a lag effect following the use of GnRH-ant.[9] A shorter duration of stimulation and fewer injections are other advantages. In flexible antagonist protocol, GnRH-ant is administered when the leading follicle has reached a diameter of 12–14 mm, while in fixed dose regimen GnRH-ant is started at day six of the COS.

In the literature, conflicting data exists about the effect of the antagonist starting day on cycle outcomes in ART cycles.[10-12] This study was designed to evaluate the effect of antagonist start day on *in vitro* fertilization (IVF)/ intracytoplasmic sperm injection (ICSI) cycle outcome of poor responders diagnosed by Bologna criteria that used flexible GnRH-ant protocol.

MATERIALS AND METHODS

Medical records of the patients treated at ART unit of a tertiary hospital between 2007 and 2016 were analyzed retrospectively. Flexible antagonist protocol cycles of patients with POR, defined by the Bologna criteria,[4] were selected for the study. Informed patient consent had been taken from all patients at the time of treatment for use of anonymised data for research or educational purpose. Anti-mullerian hormone <1,1 ng/mL and antral follicle count (AFC) <5 were taken as cut-off values to determine decreased ovarian reserve. Group A consisted of cycles that GnRH-ant was started before day 6 of COS according to flexible antagonist protocol (GnRH-ant started when leading follicle reached a diameter of 14 mm), whereas Group B consisted of cycles that GnRH-ant was started at or after day 6 of COS according to the flexible protocol. Freeze-thaw embryo transfer (ET) cycles, cycles that used protocols other than GnRH ant's, cycles that did not fullfill the Bologna criteria and cycles with male factor, tubal factor or unexplained infertility were excluded from the study.

Age, body mass index (BMI), basal serum follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E2) values, AFC, duration of infertility and characteristics of COS were recorded from the charts of the patients. The study was approved by the local ethics committee of the institution (Date: 21.12.2016/Number: 219).

COS was performed using flexible GnRH antagonist protocol. Either pure recombinant FSH (Gonal-F, Merck Serono, Germany; Puregon, Organon, Netherlands) and/ or human menopausal gonadotropin (hMG) (Menogon, Ferring Pharmaceuticals, Germany; Merional, IBSA, Switzerland) was started on day 2 or 3 of the menstrual cycle in accordance with BMI, patient's age and the number of AFC. According to the follicular growth, monitored by serial transvaginal ultrasonography and serum E2 measurements, gonadotropin dose was adjusted for each patient. GnRH-ant (Cetrotide, 0.25 mg/day, Serono, Germany) was started when the dominant follicle reached at a diameter of 14 mm for inhibition of premature LH surge. Recombinant human chorionic gonadotropin (hCG) (Ovitrelle; Merck Serono, Germany) was administered when at least three follicles reached a mean diameter of ≥17 mm. Oocyte pick-up was performed by transvaginal ultrasound-guided aspiration 35.5–36 h after the hCG injection. ICSI was performed for all metaphase II oocytes.

ET was performed on day 3 or 5 under ultrasound guidance. Luteal phase support was provided by vaginal progesterone (Crinone 8% gel, Merck, Germany) twice daily or a combination of intramuscular (Progestan amp, Koçak Farma, Turkey) and vaginal progesterone starting from the day of oocyte retrieval. Pregnancy was determined by the β-hCG level in blood tests performed 12 days after ET and clinical pregnancy was defined as the presence of a gestational sac with accompanying fetal heartbeat by ultrasound 4 weeks following the ET procedure. Luteal support was continued up to 10–12 weeks of gestation in cases of pregnancy.

Statistical analysis

Statistical analysis of data was carried out using using IBM SPSS Statistics Software (20.0, SPSS Inc., Chicago, IL, USA). Variables with normal distributions were compared using independent samples *t*-tests. The Mann–Whitney *U*-test was applied to the variables that were not distributed normally. The results are presented as mean ± standard deviation. For the categorical variables, Pearson's Chi-square analysis and Fisher's exact tests were used. Statistical significance was assumed with a probability error of $P < 0.05$.

RESULTS

A total of 361 cycles that fulfilled the inclusion criteria were selected for the study. Of the 361 cycles, 195 were included in Group A and 166 in Group B. Twelve cycles in Group A (6.2%) and 15 cycles in Group B (9%) were cancelled due to inadequate response to stimulation. ET was performed in 122 cycles in Group A and 87 cycles in Group B. Flow chart of the cycles is presented in Figure 1.

No significant difference was detected between two groups regarding the age of the patients, BMI,

duration of infertility, baseline serum FSH, LH and E2 levels ($P > 0.05$) [Table 1]. Total AFC of Group A was found to be significantly higher than Group B (4.9 ± 1.7 vs. 4.4 ± 1.8, $P = 0.009$). Regarding COS parameters, serum E2 levels at GnRH-ant administration day were significantly higher (314.1 ± 12.8 vs. 246.4 ± 11.8 pg/mL, $P < 0.01$) and the duration of stimulation was significantly shorter in Group A when compared to Group B (8.8 ± 1.6 vs. 10.6 ± 1.6 days, $P < 0.01$). Also, total gonadotropin dose used was lower in Group A when compared to Group B (2758.2 ± 822.5 vs. 3755.6 ± 965.9 IU, $P < 0.01$). There was no difference between groups regarding the number of follicles >17 mm, 15–17 mm and 10–14 mm, but the E2 level on hCG day was significantly higher in Group A than Group B (1458.9 ± 900.8 vs. 1192.1 ± 876.9 pg/mL, $P = 0.013$). While a higher number of oocytes was retrieved from Group A (6.4 ± 4.1 vs. 5.5 ± 3.5, $P = 0.037$), no between-group differences were observed in the number of mature oocytes and fertilized oocytes ($P > 0.05$). Clinical pregnancy rate (CPR) (25.4% vs. 26.4%) or ongoing pregnancy rates (OPR) (21.3% vs 21.8%) per ET were similar in both groups ($P > 0.05$) [Table 2].

Post hoc power analysis of this retrospective study was calculated by G-power 3.1.9.2 software and revealed the power of 99,9% with an effect size of 0.8, and sample size of Group 1, $n = 195$ and Group 2, $n = 166$ with the alpha probability of 0.05.

DISCUSSION

The study demonstrated that the early start of GnRH-ant resulted in a shorter duration of stimulation and decreased amount of gonadotropin use in poor responder patients. Although more oocytes were retrieved in the early start group, no difference was found between CPR or OPRs.

GnRH-ant is an attractive and patient-friendly management option for poor responders in ART. Clinical use of GnRH-ant provides a shorter duration of stimulation and a lower dose of gonadotropin consumption. Moreover, hormonal withdrawal symptoms that are commonly experienced with GnRH agonists are not seen with antagonist use.[13,14] A competitive block of pituitary GnRH receptors induces a rapid and reversible suppression of gonadotropin secretion.[15] GnRH-ant is started on day 5 or 6 of stimulation in fixed dosing independent of follicle size, whereas in the flexible dosing, the antagonist is started as the follicles reach 12–14 mm in diameter.[16] Although higher oocyte yield and lower total gonadotropin dose were reported in the
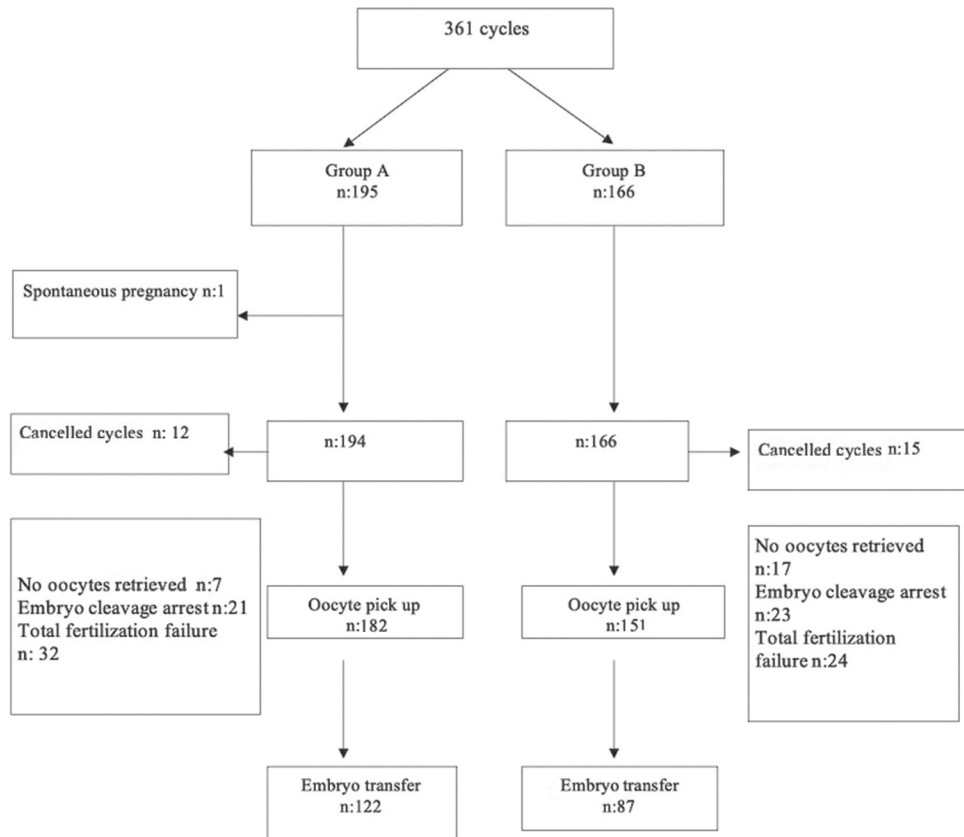

Figure 1: Flow chart of the study and control groups

Table 1: Demographic features of the patients and controlled ovarian stimulation parameters of the cycles

	Group A (*n*=195)	Grup B (*n*=166)	*P*
Age (years)	34.4±4.9	34.5±4.7	0.212
BMI (kg/m^2)	25.9±4.5	26.6±4.9	0.518
Basal FSH (IU/mL)	10.2±5.9	11.1±5.6	0.153
Basal LH (IU/mL)	5.5±2.7	5.5±2.8	0.311
Basal E2 (pg/mL)	53.7±40.4	45.1±29.8	0.056
Total AFC	4.9±1.7	4.4±1.8	0.009
Duration of GnRH antagonist use (days)	4.9±1.4	5.1±1.5	0.058
E2 level at antagonist administration day (pg/mL)	314,1±12,8	246,4±11,8	<0.01
Duration of stimulation (days)	8.8±1.6	10.6±1.6	<0.01
Total gonadotropin dose (IU)	2758.2±822.5	3755.6±965.9	<0.01
Number of follicles>17 mm on hCG day	2.4±1.6	2.0±1.5	0.088
Number of follicles between 15 and 17 mm on hCG day	1.9±1.61	2.1±1.8	0.597
Number of follicles between 10 and 14 mm on hCG day	3.2±3.6	2.1±1.8	0.057
E2 level on hCG day (pg/mL)	1458.9±900.8	1192.1±876.9	0.013
Endometrial thickness on hCG day (mm)	9.8±2.1	9.5±1.9	0.671
Number of total oocytes retrieved	6.4±4.1	5.5±3.5	0.037
Number of mature oocytes	4.5±3.3	3.9±3.1	0.121
Number of fertilized oocytes	2.4±2.2	2.2±2.1	0.129

BMI: Body mass index, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, AFC: Antral follicle count, GnRH: Gonadotropin releasing hormone, hCG: Human chorionic gonadotropin

Table 2: Results of the embryo transfer cycles

	Group A (*n*=122)	Group B (*n*=87)	*P*
Clinical pregnancy/ET, *n* (%)	31 (25.4)	23 (26.4)	0.588
Ongoing pregnancy/ET, *n* (%)	26 (21.3)	19 (21.8)	0.588

ET: Embryo transfer

flexible protocol than in the fixed protocol, no difference regarding the CPRs was observed.[17,18]

Retrieval of decreased number of oocytes is the major contributor of poor ART outcome in patients with decreased ovarian reserve. Fewer oocytes result in fewer embryos to select and transfer, which will eventually end up with decreased pregnancy rates compared with normoresponders.[19,20] AFC is a sensitive predictor of ovarian response which reflects the recruitable follicular cohort.[21,22] It was demonstrated in the literature that the number of antral follicles correlated closely with the number of retrieved oocytes.[23-25] Chang *et al.*, allocated patients into 3 groups according to their AFC (<3, 4–10, >10) and demonstrated a significant difference between groups regarding their number of retrieved oocytes.[23] In line with this study, a higher number of oocytes were retrieved from the group (Group A) which had a higher basal AFC in our study.

Previous research demonstrated that early start of GnRH-ant's resulted in a shorter duration of stimulation in antagonist cycles. Tannus *et al.*, reviewed 442 IVF cycles of patients from the general IVF population that used flexible antagonist protocol. They demonstrated that

the number of retrieved oocytes was decreased, duration of stimulation and total gonadotropin dose used was increased when the antagonist was started after day 6 of stimulation.[12] Although our study was performed on poor responders, it revealed similar results. Early start group had shorter duration of stimulation and consumed lower total dose of gonadotropins. Poor responder patients are characterised by early follicular recruitment, accelerated follicular growth and a relatively shorter follicular phase during COS.[26] So the poor responder group which has a relatively higher basal AFC, as in Group A, will produce more E2 which ends up with early antagonist start than the group that has lower AFC (Group B). Also this subgroup of patients will also reach the treshold follicle diameter required for ovulation trigger earlier than the other group, resulting in even shorter duration of stimulation.

There are conflicting data regarding the effect of GnRH-ant administration day on pregnancy rates.[10,11] Kolibianakis *et al.*, compared the cycle outcome of 111 normoresponder patients treated with either fixed or flexible antagonist protocols. They demonstrated a significantly lower implantation rate when the antagonist was delayed beyond the 6th day of stimulation, which could be attributed to altered endometrial receptivity.[10] In another study, 208 cases of normoresponders were categorized into three groups according to their antagonist starting day as group D4 for day 4, group D5 and D6 for day 5 and 6 respectively. The biochemical, clinical, OPRs per started cycle were higher for D4 and D5 than D6, which was interpreted by the researchers that

rapid response was a positive predictor of a favourable IVF outcome.[11] However, others reported no effect on pregnancy outcomes either GnRH antagonist was started before or after stimulation day 6.[12,18] In line with the later reports, our study revealed no difference in CPR or OPRs.

The weakness of this study is its retrospective design. However, participants were selected with strict inclusion criteria and according to our knowledge this is the first study investigating the effect of antagonist administration day on cycle outcomes in this specific subgroup of ART patients, namely poor responders diagnosed by Bologna criteria.

Conclusion

The results of this study showed that the early start of GnRH-ant may point out a subgroup of poor responders which has a higher total AFC and a favourable response. However, no difference in clinical or OPRs was detected.

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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