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Original Article

Microdose flare-up Gonadotropin-Releasing Hormone (GNRH) agonist protocol and GNRH antagonist protocol: Effects on in-vitro fertilization in patients with poor-responder diagnosis according to Bologna Criteria

Gökşen Görgülü [a, †], Merve Çakır Köle [b], Oya Aldemir [c], Emre Köle [d], Serdar Dilbaz [c]

[a] *Department of Gynecologic Oncology, University of Health Sciences, Tepecik Training and Research Hospital, İzmir/Turkey*

[b] *Department of Gynecologic Oncology, Kocaeli University Faculty of Medicine, Kocaeli/Turkey*

[c] *Department of Obstetrics and Gynecology, University of Health Sciences Ankara Etlik Zübeyde Hanım Gynecology and Obstetrics Training and Research Hospital, Ankara/Turkey*

[c] *Department of Obstetrics and Gynecology, Kocaeli Darica Farabi Training and Research Hospital, Kocaeli/Turkey*

A B S T R A C T

Objective: The aim of the study was to evaluate microdose flare-up Gonadotropin-Releasing Hormone (GnRH) agonist protocol and GnRH antagonist protocol with respect to their effects on in-vitro fertilization (IVF) results in patients with poor ovarian response according to the Bologna Criteria.

Material and methods: This was a retrospective cohort study conducted in the Assisted Reproduction clinic of University of Health Sciences, Etlik Zübeyde Hanım Gynaecology Training and Research Hospital. A total of 645 patients who had been diagnosed as poor responders in our clinic, between 2007 and 2018, and received treatment with either microdose flare-up GnRH agonist protocol (n=250, 38.8%) or GnRH antagonist protocol (n=395, 61.2%), were included in the study.

Results: The mean age of the study group was 34.5±5.5 years. Comparisons showed that IVF cycle cancellation frequency (p<0.01), third day estradiol level (p=0.04) and third day follicle stimulating hormone level (p<0.01) were significantly greater in patients who underwent the microdose flare-up protocol. In the GnRH antagonist group, the number of surviving children (p=0.01), antral follicle count (p<0.01), follicle count on day of human chorionic gonadotropin (hCG) administration (p<0.01), endometrial thickness on hCG day (p<0.01), number of oocytes collected (p<0.01), mature oocyte count (p<0.01), embryo transfer number (p<0.01) were higher compared to the microdose flare-up protocol group. The two groups were similar in terms of clinical pregnancy rate.

Conclusion: In terms of clinical pregnancy rate, the IVF results of microdose flare-up and GnRH antagonist protocols are similar. Further studies are needed to reach more comprehensive results on the subject.

Keywords: infertility; in-vitro fertilization; gonadotropin-releasing hormone agonists; gonadotropin-releasing hormone antagonists

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Introduction

Infertility may occur in women due to ovulatory diseases, tubal diseases and uterine diseases [1]. Poor response (or poor ovarian response) related to decreased ovarian reserve, which refers to the decrease in oocyte production ability of the ovaries due to various reasons, is one of the increasing causes of infertility [2].

Within the scope of in vitro fertilization (IVF), the ovaries are stimulated with a combination of fertility drugs, then one or more oocytes are aspirated from the ovarian follicles and the fertilization process is performed in the laboratory environment (in vitro). One or more embryos are transferred to the uterine cavity 3-5 days after fertilization [3,4]. Different treatment options can be used to stimulate the ovaries for IVF in patients with poor response to stimulation, termed as poor-responders. The use of gonadotropin-releasing hormone (GnRH) agonists together with gonadotropins provides a decrease in IVF cycle cancellation rates, an increase in the number of follicles and oocytes, higher quality embryos for transfer and better pregnancy chance. The adverse effect of this protocol, namely ovarians suppression, in patients with poor response has led to the development of new treatment strategies [5]. These options include the use of GnRH antagonists in combination with gonadotropins to reduce the dose or diminish the need for GnRH agonists initiated in the luteal phase, to reduce suppression time (short, ultra-short, mini and microdose flare-up protocols) by shortening the duration of weak GnRH agonist use, and to prevent early Luteinizing Hormone (LH) increase in the mid-late period follicular phase [5].

When the microdose flare-up and GnRH antagonist protocols were compared in previous studies, their possible superiorities over each other (cycle cancellation rates, number of oocytes per cycle and clinical pregnancy rates) could not be clearly proven [6,7]. In some studies, it was reported that the number of oocytes and implantation rates obtained with the microdose flare-up technique indicated higher success compared to the GnRH antagonist protocol, and therefore, the microdose flare-up protocol was suggested to increase success in IVF cycles [8-10]. Whereas, in other studies, GnRH antagonists are reported to be more advantageous [5].

† Corresponding author.
E-mail: goksengorgulu1923@gmhotmail.com

It is important to investigating these options further to increase the success of IVF.
Thus, the aim of the study was to evaluate the effects of microdose flare-up GnRH agonist protocol and GnRH antagonist protocol on IVF results in women who had been diagnosed as poor responders according to the Bologna Criteria.

Material and methods

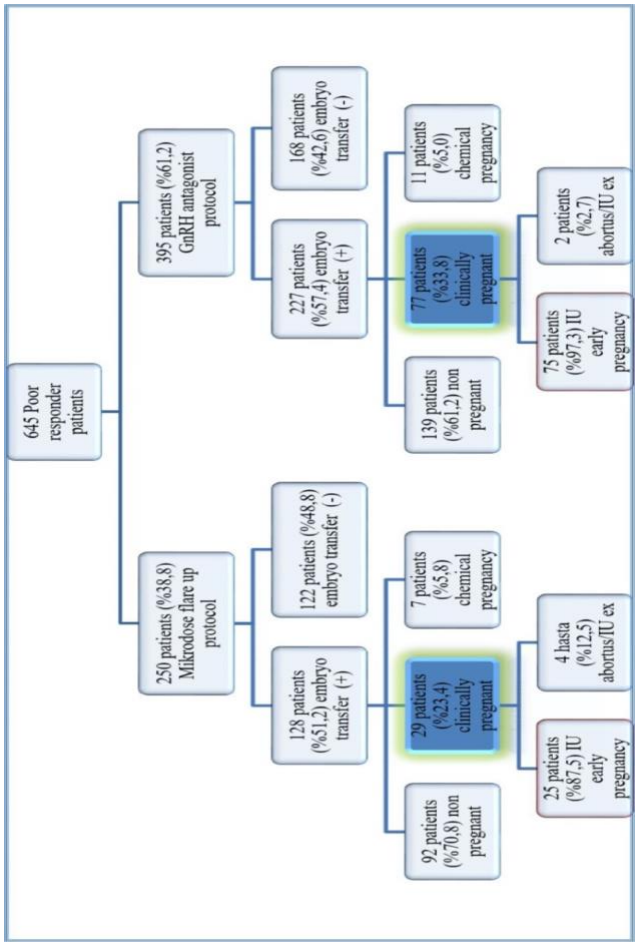
Study groups and data acquisition

This research is a retrospective cohort type study conducted between May 2007 and January 2018 at the Assisted Reproduction Clinic (UYTEM) of the University of Health Sciences, Ankara Etlik Zübeyde Hanım Gynaecology Training and Research Hospital.

Ethics committee approval was obtained from the Medical Specialty Education Board of Health Sciences University, Etlik Zübeyde Hanım Gynaecology Training and Research Hospital in affiliation with the Ankara Second District Public Hospitals Association, Republic of Turkey Ministry of Health.

Within the scope of the study, the medical records of patients admitted to the UYTEM with a poor responder diagnosis according to the Bologna Criteria, between May 2007 and January 2018, were reviewed, and those who had undergone microdose flare-up GnRH agonist and GnRH antagonist protocols were retrospectively evaluated. Cases which were identified to meet any one of the following criteria were excluded from the study: male factor, tubal factor, unexplained infertility, hormone ovulatory disorder, severe pelvic adhesion, endometriosis, patients who were applied aromatase inhibitor + GnRH antagonist protocol, and women who were applied natural cycle.

Figure 1. Treatment methods and results applied to the patients included in the study



Poor responder definition

The Bologna criteria was developed by the European Society of Human Reproduction and Embryology (ESHRE). According to the ESHRE consensus, those with two of the following criteria are defined as poor responders: (i) maternal advanced age (> 40 years) or other risk factors associated with poor response, (ii) history of previous poor response (cycle cancellation with traditional stimulation protocol or ≤3 oocyte count), and (iii) abnormal ovarian reserve test result (AFC <5–7 follicles with AMH level <0.5-1.1 ng/ml) [11].

Treatment protocols

Patients in microdose flare-up group were pre-treated with combined oral contraceptives at the previous menstrual cycle. A total of 2 x 40 mcg leuprolide acetate was started 3 days after oral contraceptive use. High-dose gonadotropins were added from the third day of this treatment. The change in the ovaries was monitored by transvaginal ultrasonography from the fifth day of stimulation and the gonadotropin dose was adjusted according to the response [12].

Within the scope of the antagonist protocol, ovulation stimulation with recombinant follicle stimulating hormone (FSH) was initiated from the second day of the menstrual cycle. The change in the ovaries was monitored by transvaginal ultrasonography from the fifth day of stimulation, and the dose of gonadotropins was changed according to the response. When the size of a follicle was >14 mm, the GnRH-antagonist Cetrorelix (Cetrotide; Merck Serono, Mumbai, India) was started subcutaneously at a dose of 0.25 mg / day.

In both groups, recombinant human chorionic gonadotropin (hCG) was administered subcutaneously 250 IU to induce ovulation when at least two follicles reached an average diameter of 18 mm. Oocyte retrieval was performed under intravenous sedation 35-36 hours after hCG triggering. Intracytoplasmic sperm injection (ICSI) was performed 2–4 hours after oocyte retrieval, and fertilization was checked 16–18 hours later. Embryos were graded as Grade 1–4 according to zonal thickness, fragmentation and blastomere size. Embryo transfer was performed with ultrasonography on the second or third day with a maximum of two embryos. For luteal support, administrations were in the form of micronized P4, 100 mg intramuscularly or 400 mg perivaginally, from the day of oocyte retrieval. Chemical pregnancy was confirmed by serum β-hCG measurement 12–14 days after embryo transfer. Clinical pregnancy was defined as the presence of fetal cardiac activity 4–5 weeks after embryo transfer. Cycle cancellation was defined as the development of less than three follicles 14 mm in size after 12 days of stimulation with maximum stimulation doses (450 IU gonadotropin).

Statistical analysis

The data obtained from the medical records of the patients were evaluated with the SPSS v15 (SPSS Inc., Chicago, IL, USA) statistical package program. Number, percentage, mean, standard deviation, median, minimum and maximum values were used in the evaluation of descriptive data. For the data, compliance with normal distribution was tested with Shapiro-Wilk test, and the Independent Sample t Test (Student t Test) was used to determine the relationship between the independent variables comply with the normal distribution and the Mann Whitney U test to determine the relationship between the independent variables that do not comply with the normal distribution. Chi-square test was used to determine the relationships between categorical variables. Statistical significance level was accepted as $p < 0.05$.

Results

In the study group, there were a total of 645 patients diagnosed as poor responders according to Bologna Criteria; 250 (38.8%) were treated with microdose flare-up GnRH agonist protocol and 395 (61.2%) were treated with GnRH antagonist protocol. The ages of the patients ranged from 21 to 48, with a mean of 34.5 ± 5.5 years. 128 of the patients (19.8%) were older than 40 years, 345 (53.4%) had previous cycle cancellation or less than 3 oocyte retrieval, 365 (56.5%) had an AMH level < 1.1 ng/ml, AFC < 7 in 354 (54.8%).

Embryo transfer was performed in 51.2% of the patients who underwent microdose flare-up protocol, and clinical pregnancy was observed in 23.4% of them. 87.5% of these patients with clinical pregnancy were identified as intrauterine early pregnancy. Embryo transfer could be performed on 57.4% of GnRH antagonist recipients, and clinical pregnancy was observed in 33.8% of these. Intrauterine early pregnancy was identified in 97.3% of these patients with clinical pregnancy (Figure 1).

The frequency of patients younger than 40 years of age was significantly higher in the GnRH antagonist protocol group (p <0.01). The frequency of IVF cycle cancellation was found to be significantly higher in patients who were applied microdose flare-up protocol compared to those who received the GnRH antagonist protocol (p <0.01). The groups that were applied microdose flare-up protocol and GnRH protocol were found to be similar in terms of BMI, embryo transfer status, embryo transfer result and clinical pregnancy outcome (Table 1).

Table 1. The distribution of characteristics according to treatment protocol

	Microdose flare-up (n=250) n (%)	GnRH antagonist (n=395) n (%)	Overall n (%)	χ^2;p
Age (years)				
<40	176 (70.4)	341 (86.3)	517 (80.2)	24.4;<0.01
≥40	74 (29.6)	54 (13.7)	128 (19.8)	
BMI (kg/m^2)				
Underweight (<18.5)	5 (2.2)	4 (1.0)	9 (1.4)	1.5;0.70
Normoweight (18.5-24.9)	80 (33.9)	133 (33.3)	213 (33.0)	
Overweight (25.0-29.9)	93 (39.1)	165 (39.1)	258 (40.0)	
Obese (>30.0)	59 (24.8)	106 (26.6)	165 (25.6)	
Antral Follicle count				
≥7	56 (22.5)	235 (59.4)	291(45.1)	83.8;<0.01
<7	194 (77.5)	160 (40.6)	354 (54.9)	
Cycle cancellation				
Yes	45 (18.1)	35 (8.7)	80 (12.4)	11.8;<0.01
No	205 (81.9)	360 (91.3)	565 (87.6)	
Embryo transfer				
Yes	128 (51.2)	227 (57.4)	355 (55.0)	2.3;0.12
No	122 (48.8)	168 (42.6)	290 (45.0)	
Embryo transfer outcome				
No pregnancy	92 (70.8)	139 (61.2)	231 (65.0)	4.0;0.13
Chemical pregnancy	7 (5.8)	11 (5.0)	18 (5.0)	
Clinical pregnancy	29 (23.4)	77 (33.8)	106 (30.0)	
Clinical pregnancy outcome				
Intrauterine early pregnancy	25 (87.5)	75 (97.3)	100 (94.3)	3.0; 0.10
Abortus / Intrauterine death	4 (12.5)	2 (2.7)	6 (5.7)	

BMI: Body mass index

The mean age (p <0.01), estradiol (E2) level on the third day (p = 0.04), FSH level on the third day (p <0.01), total human menopausal gonadotropin (hMG) dose (p <0.01) of the microdose flare-up protocol group in the study was found to be significantly higher than the GnRH antagonist group.

In the microdose flare-up group, the mean the number of living children (p = 0.01), antral follicle count (p <0.01), follicle count on hCG day (p <0.01), endometrium thickness on hCG day (p <0.01), number of oocytes collected (p < 0.01), mature oocyte count (p <0.01) and embryo transfer count (p <0.01) were significantly lower compared to the GnRH antagonist group (Table 2, Figure 2).

Table 2. Comparison of study groups with respect to demographic, treatment and laboratory characteristics

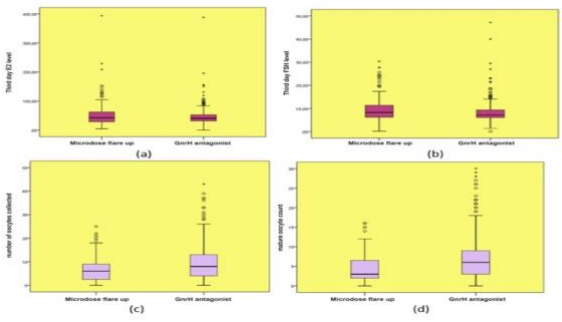
	Microdose flare-up Mean ± SD	GnRH antagonist Mean ± SD	p*
Age (years)	36.0±5.4	33.6±5.4	<0.01
BMI (kg/m^2)	26,9±5.2	27.3±4.7	0.34
Infertility duration (months)	97.9±71,4	97.6±58.0	0.95
Gravidity	0.5±0.9	0.6±1.1	0.08
Abortus	0.4±0.9	0.3±0.7	0.72
Alive children	0.04±0.2	0.1±0.5	0.01
Antral follicle count	5.0±3.3	11.3±8.5	<0.01
Third day E_2 (pg/mL)	55.3±43.9	48.6±36.3	0.04
Third day FSH (mIU/ml)	9.9±5.5	8.5±4.8	<0.01
Third day LH (mIU/ml)	5.4±3.4	5.4±2.9	0.78
Initiation hMG dose (IU)	105.9±65.5	106.2±72.2	0.96
Termination hMG dose (IU)	113.7±67.5	109.6±74.2	0.53
hMG total dose (IU)	3685.4±1275.8	2508.9±1113.9	<0.01
On hCG day:			
Number of ≥17 mm follicles	1.9±1.4	2.6±2.0	<0.01
Number of 15-17 mm follicles	2.2±2.1	3.3±2.8	<0.01
Number of 10-14 mm follicles	2.9±2.9	5.3±5.4	<0.01
Endometrial thickness (mm)	9.0±2.4	9.8±1.9	<0.01
Collected oocyte count	6,4±4,6	9,9±7,7	<0.01
Mature oocyte count	4,6±3,7	7,2±5,9	<0.01
Embryo transfer count	1.2±0.9	1.4±0.8	<0.01

**Student's t-test, SD: Standard deviation, BMI: Body Mass Index, E2: Estradiol, FSH: Follicle Stimulating Hormone, GnRH: Gonadotropin Releasing Hormone, hMG: Human Menopausal Gonadotropins, LH: Luteinizing Hormone, hCG: Human Chorionic Gonadotropin*

Discussion

The reproductive treatment of patients diagnosed as poor responders continues to be an important problem in all age groups. The optimal treatment protocol for patients with a diagnosis of poor response is evidently one that facilitates the highest chances of clinical pregnancy and healthy birth, preferably with maximum number of mature and good quality oocytes; however, it should also have acceptable cycle cancellation frequency, be applicable in an appropriate duration and must be affordable, while also resulting in the least number of undesirable outcomes.

Figure 2. Distribution of E2 on day 3, FSH levels on day 3 and total oocyte and mature oocyte counts of the patients included in the study



The use of GnRH antagonists in these patients has brought a new perspective to clinicians, and the studies on this subject have continued to increase. In some studies comparing GnRH agonist and antagonist applications, it was reported that more successful results were obtained in antagonist protocols [6,13]. Many different strategies are recommended for poor responders, but an ideal protocol has not yet been defined due to incompatible results that often demonstrate significant variations from study to study. The microdose flare-up and antagonist protocols are two of the most commonly used treatment approaches, and have been compared in several studies [14].

A BMI of less than 18.5 or greater than 25 in the reproductive age negatively affects fertility in women. BMI at these values decreases fertility by causing suppression of ovulation, and consequently increases the need for infertility treatment, and may result in difficult pregnancies and delivery complications [15]. It has been reported by Savadkouhi et al. that having a BMI value greater than 27 is a risk factor for infertility [16]. In the study by Anderson et al., it was reported that the frequency of infertility was higher in obese and overweight women and that fertility treatment success rates were also lower compared to other groups [17]. We found that 65% of infertile women were either obese or overweight in the present study. This percentage is quite high when compared to that of the general population of women living in Turkey (The Turkey Health Interview Survey Study, overall incidence: 54%). Our result is compatible with the literature. It appears that, prior to undertaking planned treatment, it may be crucial to suggest regular physical activity and balanced nutrition for infertile couples.

It has been reported that the microdose flare-up protocol reduces cycle cancellations in poor responders [18], and Surrey et al. found that the microdose flare up protocol provided more successful cycle results compared to other protocols applied to patients with poor responder diagnosis [19]. Çakıroğlu et al., it reported that cycle cancellation was more common in the GnRH antagonist / Letrozole group [20], which was a finding supported by the study of Nabati et al., who found 2.87-fold greater frequency of cycle cancellation in the GnRH antagonist / Letrozole-applied group compared to the microdose flare-up group [21]. However, in-line with the aforementioned inconsistency in results, various other publications have indicated that the microdose flare-up and GnRH antagonist / Letrozole are similar in terms of cycle cancellation [7,14,22-24]. Leonodris et al. investigated the results of the long GnRH agonist and microdose flare-up protocol in their study, and similar to our research results, they reported that there was a significantly higher frequency of cycle cancellation in the microdose flare-up group [8]. In this study, excessive cycle cancellation is identified as one of the disadvantages of microdose flare-up protocol when compared to the GnRH antagonist protocol.

Nabati et al. found that the levels of E2, endometrial thickness and luteinizing hormone (LH) measured on hCG day, the number of follicles with a diameter of >14 mm, and oocyte counts were significantly higher in the microdose flare-up protocol group compared to the GnRH antagonist group [21]. In two other studies, LH level, E2 level, the number of follicles with a diameter of >14 mm, and hCG-day endometrial thickness were reported to be higher in the microdose flare-up group [14,23]. In the study of Çakıroğlu et al., it was reported that stimulation time, total gonadotropin dose, peak E2 and hCG-day endometrial thickness were higher in the microdose flare-up group [20]. In this study, FSH and E2 levels were found to be significantly higher in the microdose flare-up group on the third day. Additionally, on hCG day, endometrial thickness was significantly higher in the GnRH antagonist group.

It has been reported that the use of GnRH agonists prevents premature luteinization and increases the number of oocytes collected, consequently increasing the pregnancy rate per cycle and per embryo transfer [25]. In the study by Malhotra et al., it was reported that the number of mature oocytes was significantly higher in those who were applied the microdose flare-up protocol compared to those who received GnRH antagonists [26]. Similarly, Demirol and Gurgan found that the number of mature oocytes was significantly higher in the microdose flare-up group [10]. Boza et al. also found an increased count of mature oocytes in the microdose flare-up group and their results also showed a significant increase in the frequency of implantation among recipients of the microdose flare-up protocol [27]. In a meta-analysis aggregating the outcomes of various studies, the number of oocytes collected in GnRH agonist protocols was documented to be higher than that of antagonist-receiving groups [7]. Akman et al. reported that the microdose flare-up and GnRH antagonist groups were similar in terms of total and mature oocyte numbers [6]. However, in the randomized controlled study by Davar et al., it was reported that the number of oocytes collected, the number of mature oocytes and the frequency of implantation were significantly higher in the late-onset GnRH antagonist group compared to the microdose flare-up group [28]. On the other hand, Uludağ et al. reported that higher oocyte count was obtained with a multidose GnRH antagonist protocol compared to the microdose flare-up GnRH agonist protocol in patients diagnosed as poor responders [29]. Similarly, in our study, the collected oocyte count and mature oocyte count were significantly higher in the GnRH antagonist group.

In the study by Nabati et al., it was reported that clinical pregnancy rate was significantly higher in the microdose flare-up group compared to the GnRH antagonist / Letrozole group [21]. In various previous studies, similar clinical pregnancy rates have been reported for the microdose flare-up GnRH agonist and the GnRH antagonist protocols, as is the case in our study [6,10,24,26-29]. A meta-analysis also reported similar frequencies of clinical pregnancy with the use of GnRH antagonists and agonists [7]. Mohamed et al. reported that, in order to obtain a significant difference in terms of pregnancy between agonist flare-up and antagonist protocols, at least 701 patients should be studied at 90% power and 5% significance level [30]. According to the Bologna Criteria, it is very difficult to carry out such a comprehensive study because it requires a multi-centred approach, long-term commitment, large amount of manpower, and the presence of reliable, standardized and regular records. In our study, this target number was all but reached with a retrospective analysis at a single centre (facilitating consistency in patient management, data records and outcome analyses); however, our results demonstrate a lack of difference between the two groups in terms of pregnancy rates, the ultimate goal of such treatments. Positive results were obtained for the GnRH antagonist group in terms of oocyte count, mature oocyte count, embryo transfer number and cycle cancellation rate; however, it appears that these advantages were not sufficiently influential on clinical pregnancy rates.

Limitations

The most important limitation of the study is its retrospective design, we could have obtained stronger results with a prospective type of research. Another limitation of the study is that it was conducted in a single-centred manner, and therefore, does not provide population-based data for such patients. The results may have limited generalizability to the

population at question in other centres or regions. Another limitation of the study is that the mean age of the microdose flare-up GnRH protocol group was higher than that of the GnRH antagonist group. This situation may have affected the results of the two groups; but due to the retrospective nature of the study, no intervention could be made on this issue. Despite these limitations, our research is valuable in terms of the relatively large sample size and the evaluation of many parameters from patients in a single centre –which increases consistency and reliability of data.

Conclusion

As a result of the analysis, the third day E2 level, third day FSH level and cycle cancellation frequency were found to be higher in the microdose flare-up group compared to the GnRH antagonist group. Although the antral follicle count, collected oocyte count, mature oocyte count, embryo transfer numbers, hCG day follicle count and hCG day endometrial thickness were significantly higher in the group treated with GnRH antagonist, the clinical pregnancy rate was similar in both groups. It can be feasible to suggest that the GnRH antagonist protocol, which has a lower cycle cancellation frequency, may be preferred in poor responders (according to Bologna Criteria), despite the fact that clinical pregnancy rates were similar with both treatments. It was concluded that prospective and population-based studies must be conducted in poor responders, which would enable better comparison of treatment protocols.

Disclosure

Authors have no potential conflicts of interest to disclose.

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