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The comparison of microdose flare up and flexible antagonist protocols in poor responders undergoing IVF treatment: A prospective randomized controlled trial

İVF tedavisi alan zayıf ovaryan rezervli hastalarda mikrodoz flare-up ve fleksible antagonist protokollerinin karşılaştırılması: Prospektif randomize kontrollü çalışma

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Abstract

Aim: Ovarian reserve is one of the most important prognostic factors to predict probability of pregnancy in in vitro fertilization (IVF) cycles. Poor ovarian response is associated with high cycle cancellation rate and diminished pregnancy rates. Therefore, the management of women who demonstrate an inadequate response to controlled ovarian hyperstimulation (COH) are a challenge to treat with IVF.

Methods: A hundred consecutive infertile women, defined as poor responder, were recruited to this study. It was conducted at the assisted reproductive technology (ART) unit of the Ankara Etlik Zubeyde Hanim Women's Health Teaching and Research Hospital during the period September 2009 to September 2011. All patients in Group 1(n=50) were treated by using flexible gonadotropin releasing hormone (GNRH) antagonist protocol and in Group 2 (n=50) were treated by using GnRH microdose flare-up protocol. Exogenous gonadotropin (Gonal F, Serono, Istanbul, Turkey) was initiated on the second day of menstruation in all patients in Group 1(n=50) and GnRH antagonist (0.25 mg, Cetrotide; Serono, Geneva, Switzerland) was started when the leading follicle reached 12 mm in mean diameter and were continued until the day of hCG administration.

Results: Total dosage of gonadotropins was significantly higher in group 2 (2625 IU in group 1 vs 4050 IU in group 2; p<0.001). The pregnancy rate was higher in group 2 but not statistically significant (25.7% in group 1 vs 33.3% in group 2; p=0.501).

Conclusion: There is no consensus on the best standard treatment option for assisted reproductive technology (ART) cycles of poor responders. GnRH antagonist and microdose flare-up protocols seem to have similar outcomes in poor responder patients in intracytoplasmic sperm injection (ICSI) cycles except consumption of gonadotropins. Further prospective randomized trials with large sample size are needed to assess the efficacy of the two protocols in the poor responders.

Keywords: Poor responders, Microdose flare up, Antagonist, In vitro fertilization

Öz

Amaç: Ovaryan rezerv, in vitro fertilizasyon (İVF) sikluslarında gebelik olasılığını gösteren en önemli prognostik faktörlerden birisidir. Azalmış ovaryan rezerv, azalmış gebelik oranları ve artmış siklus iptalleriyle alakalıdır. Bu nedenle kontrollü ovaryan hiperstimülasyona (KOH) zayıf yanıt veren kadınlarda İVF ile tedavi bir zorunluluktur.

Yöntemler: Bu çalışmaya, eylül 2009-2011 tarihleri arasında Ankara Etlik Zübeyde Hanım Eğitim Araştırma Hastanesi Yardımcı Üreme Teknolojileri (ART) Ünitesi' nde tedavi alan zayıf ovaryan yanıtlı 100 infertil hasta katıldı. Grup 1'deki (n=50) hastalara fleksible antagonist protokolü ve grup 2' deki (n=50) hastalara gonadotropin serbestleştirici hormon (GnRH) mikrodoz flare-up protokolü uygulandı. Ekzojen gonadotropin (Gonal F, Serono, İstanbul, Turkey) grup 1' deki (n=50) tüm hastalara menstrüasyonun 2. gününde başlandı ve folikül büyüklüğü 12 mm olunca GnRH antagonist (0,25 mg, Cetrotide; Serono, Cenevre, İsviçre) başlanarak hCG' nin uygulandığı güne kadar devam edildi.

Bulgular: Kulanılan gonadotropin total dozu grup 2' de anlamlı derecede yüksek bulundu (grup 1; 2625 IU ve grup 2; 4050 IU ; p<0,001). Gebelik oranları grup 2' de yüksek ancak istatistiksel olarak anlamlı bulunmadı (grup 1; 25,7% ve grup 2; 33,3% p=0,501).

Sonuç: Zayıf ovaryan yanıtlı hastalarda ART siklus tedavilerinde henüz standart protokol bulunamamıştır. GnRH antagonist ve mikrodoz flare-up protokolleri, zayıf ovaryan yanıtlı olup intrastoplazmik sperm enjeksiyonu (ICSI) tedavisi alan hastalarda, kullanılan gonadotropin toplam dozları haricinde benzer sonuçlar içermektedir. İleride, zayıf ovaryan yanıtlı hastalarda bu iki protokolün etkinliğini göstermek için geniş prospektif randomize çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Zayıf ovaryan rezerv, Mikrodoz flare up, Antagonist, İnvitro fertilizasyon

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Introduction

Some of variables including woman's age and ovarian reserve, embryo quality, endometrial receptivity and embryo transfer (ET) technique influences either positively or negatively pregnancy rates (PRs) in in vitro fertilization (IVF) [1-3]. Ovarian reserve is one of the most important prognostic factors to predict probability of pregnancy in IVF cycles. Poor ovarian response is associated with high cycle cancellation rate and diminishes pregnancy rates. Therefore, the management of women who demonstrates an inadequate response to controlled ovarian hyperstimulation (COH) is a challenge to treat with IVF.

There is still no consensus on the optimum COH protocol in poor responders. Several approaches have been used to manage patients who poorly respond to COH for increasing ovarian response, maximizing pregnancy rate and minimizing cancellation rate [4,5]. The microdose agonist gonadotropin releasing hormone (GnRH-a) flare-up and gonadotropin releasing hormone (GnRH) antagonist protocols are two of commonly used protocols for poor responders to improve ovarian response and clinical outcomes. The microdose agonist (GnRH-a) flare-up protocol is to stimulates follicular recruitment by the initial rise of endogenous gonadotropins in the early follicular phase and to enhances ovarian response to the subsequent administration of exogenous gonadotropins. GnRH antagonist protocols are also to reduce suppression in the early follicular phase and to potentially improve follicular recruitment and ovarian response.

Previous studies have shown that GnRH antagonist or agonist flare-up protocols might be better than the standard long protocol in these patients [6]. The aim of this prospective, randomized-controlled study was to compare the effects of gonadotropin-releasing hormone antagonist and agonist microdose flare-up protocols on cycle outcomes and pregnancy rates in poor responder patients.

Materials and methods

Participants

A hundred consecutive infertile women, defined as poor responder, were recruited to this study. It was conducted at the assisted reproductive technology (ART) unit of the Ankara Etlik Zubeyde Hanim Women's Health Teaching and Research Hospital during the period of September 2009 to September 2011. The study protocol was approved by the institutional local ethics committee and Institutional Education and Planning Committee. An informed consent was obtained from all patients. The definition of poor responders included at least one of the following: (1) a poor ovarian response in a previous stimulation protocol (<4 oocytes retrieved), (2) a prior cancelled stimulation cycle, (3) basal follicular stimulating hormone (FSH) level >10 IU/L, (4) age >38 years, (5) basal antral follicle count <6. Data were collected for cancellation rate, peak estradiol (E2) level, total dosage of FSH administered, the number of total and mature oocytes retrieved, no of pronucleus (2PN), duration of stimulation, cycle days, quality of oocytes, quality of embryos and pregnancy rate.

The sample size of the study was calculated with the G-power statistical packages. The required sample size for 95% power α=0.05 type 1 error, β=0.05 type 2 error and d=0.80, effect size was calculated as 84. To protect the study from potential loss to follow-ups, study was considered to be completed with a sample size 100.

Treatment protocols:

Patients were randomly divided into two groups. Random allocation was performed by using Random Allocation Software (Ver. 1.0.0 © Mahmood Saghaei, Isfahan University of Medical Sciences, Isfahan, Iran). All patients in Group 1 were treated by using flexible GNRH antagonist protocol and in Group 2 were treated by using GNRH microdose flare-up protocol. Exogenous gonadotropins (Gonal F, Serono, Istanbul, Turkey) was initiated on the second day of menstruation in all patients in Group 1(n=50) and GnRH antagonist (0.25 mg, Cetrotide; Serono, Geneva, Switzerland) was started when the leading follicle reached 12 mm in mean diameter and was continued until the day of human chorionic gonadotropin (hCG) administration. In all patients in group 2 (n=50) GnRH agonist (Lucrin; Abbott, Cedex, France) was started on the third day of menstruation twice daily 40 µg SC after a 21-day course of an oral contraceptive (Desolette; Organon, Istanbul, Turkey: 0.03 mg of ethinyl E2 and 0.15 mg of desogestrel). Exogenous gonadotropins (Gonal F, Serono, Istanbul, Turkey) was initiated on the fourth day of menstruation. Both of them were continued until the day of hCG administration. The starting doses of gonadotropin (range between 150 and 450 IU) was dependent on age of women, baseline serum FSH and E2 levels, body mass index and ovarian response to previous cycle (if present), with individual adjustments performed based on ovarian response via serial transvaginal scanning. Ovarian response was monitored with serum E2 measurements and transvaginal ultrasound. Cycle cancellation was recommended when not suitable endometrium for implantation, fertilization failure, no oocyte received and degenerated oosit. hCG was administered when the mean diameter of leading follicles reached ≥18 mm. Transvaginal ultrasound-guided oocyte retrieval was performed 36 hours after hCG administration. Clinical pregnancy was defined by the presence of a gestational sac or a fetus with cardiac activity on ultrasound examination.

Oocyte quality assessments

Retrieved oocytes were denuded by 80 IU/ml hyaluronidase (Vitrolife, Sweden) enzyme, and the morphology of oocytes at the time of intracytoplasmic sperm injection (ICSI) was evaluated under an inverted microscope with Hoffman modulation at 4006 magnification (Olympus IX71, Olympus Co, Japan). Morphology assessment was performed based on the previously suggested morphological features [7-9]. Basically, abnormal features were grouped as extracytoplasmic, including fragmented first polar body, abnormal first polar body, large perivitelline space, abnormal zona pellucida and abnormal oocyte shape; and cytoplasmic, including vacuoles, granularity, refractile body and brown oocytes. Morphologically evaluated oocytes were scored from best to worst as Score 7; MII oocytes with no abnormal feature, Score 6; MII oocytes with one abnormal feature, Score 5; MII oocytes with more than one abnormality, Score 4; MI oocytes with no abnormal feature, Score 3; MI oocytes with one abnormal feature, Score 2; MI

oocytes with more than one abnormality and finally Score 1 for GV with any abnormality.

Embryo quality

Inseminated oocytes were cultured in an appropriate culture medium (G5 series, Vitrolife, Sweden), and the embryo development was evaluated every day. In this study, it was focused on the scores of cleaved and blastocyst stage embryos. The embryo evaluations for

cleavage stage were performed 40–45 (day 2) and 65–70 (day 3) hours later after ICSI and scored from best (5) to worst (1) based on the previously reported embryo evaluation criteria including, the number and equality of blastomeres, the percentage of fragmentation and the existence of multinucleus [9]. On day 5, blastocyst stage embryos were scored from best (5) to worst (1) subjecting the expansion of blastocyst, the structure of inner cell mass and trophoectoderm [10].

Implantation rate was defined as the ratio of number of implanted embryos to the number of embryos transferred. Clinical pregnancy was defined as a positive intrauterine gestational sac with fetal heart beat visible by ultrasound, and ongoing pregnancy was defined as pregnancy continuing beyond 28 weeks' gestation.

Statistical Analysis

All statistical analyses were performed using SPSS for Win. Ver. 15.0 (SPSS Inc. Chicago, IL, USA). Student's t test or Mann Whitney U test was used to compare the continuous variables and the chi-square test or Fisher's exact test was used to compare categorical variables. P<0.05 was considered statistically significant.

Results

Demographic characteristics of all patients were similar between the two groups (Table 1). The clinical and laboratory outcomes related to COH are as shown in Table 1. The median age of women participating in this study was 38. Cycle cancellation rate was higher in Group 1 (2 (4%) vs 1 (2%)) than group 2 due to an impaired ovarian response. Total dosage of gonadotropins was significantly higher in group 2 (2625 IU in group 1 vs 4050 IU in group 2; p<0.001). There were no significant differences peak E2 levels, the mean number of total oocytes and mature oocytes retrieved, no of 2PN, duration of stimulations, cycle days, quality of oocytes and embryos between groups (Table 1). Thirty-five in Group 1 (72.9%) and 30 (61.2%) patients in group 2 underwent embryo transfer procedure. 13 cycles in Group 1 (one patient owing to not suitable endometrium for implantation, 9 to total fertilization failure, 3 to no oocyte retrieved) and 19 cycles in Group 2 (fifteen patients owing to total fertilization failure, 3 to no oocyte retrieved, one to degenerated oocyte) did not reach ET (Table 2).

The pregnancy rate was higher in group 2 but it was not statistically significant (25.7% in group 1 vs 33.3% in group 2; p=0.501) (Figure 1). No significant differences were observed in pregnancy parameters including 'live birth'', 'ongoing pregnancy', ''abortion'' and ''biochemical pregnancy'' (p=0.497).

Four in group 1 (44.4%) and four pregnant patients in group 2 (40%) had biochemical pregnancies and one of all pregnant patients developed abortion (in group 1, 11.1%). Furthermore two in group 1 (22.2%) and four (40%) pregnant patients in group 2 gave healthy birth and four of all pregnant patients (two patients in group 1 (22.2%), two patients in group 2 (20%)) had ongoing pregnancy when this study stopped (Figure 2). The successful pregnancies were named ''healthy birth'' and "ongoing pregnancy". Likewise the unsuccessful pregnancies were named "abortion" and "biochemical pregnancy". As a result, the successful pregnancies in group 2 were higher than group 1 but it was not statistically significant (χ^2 = 0.461; p=0.497).

Table 1: Cycle characteristics

Characteristic	Group 1	Group 2	P value
Cancellation rate	2 (4%)	1 (2%)	NS
Peak E2 level (pg/ml)	1092.0 (IQR:956.8)	983.0 (IQR:1776.5)	0.513
Total dosage of gonadotropins	2625 (IQR: 1200)	4050 (IQR: 1200)	<0.001*
No. of oocytes retrieved	6.5 (IQR:6.0)	5.0 (IQR:7.0)	0.093
No. of mature oocytes	4.5 (IQR:5.8)	3.0 (IQR:4.3)	0.194
No. of 2PN	2.0 (IQR:3.8)	1.0 (IQR:3.0)	0.079
Duration of stimulations	8(IQR: 3.0)	10 (IQR: 3.0)	0.354
Cycle days	9.0 (IQR:2.8)	11.0 (IQR:3.0)	0.816
Oocyte quality index	5.7 (IQR:0.8)	5.2 (IQR:1.2)	0.097
Day 2 embryo quality	4.6 (IQR:1.0)	4.5 (IQR:1.0)	0.984
Day 3 embryo quality	3.9 (IQR:1.3)	4.2 (IQR:1.5)	0.247
Day 5 embryo quality	2.3 (IQR:1.5)	2.1 (IQR:1.1)	0.637

*P<0.05 is considered statistically significant difference, comparison of all groups, NS: not specified

Table 2: Number of embryo transfer and causes of unachievable embryo transfer *

Transfer and causes unachievable ET	Grup I		Grup II		Total	
	n	%	n	%	n	%
ET, successful	35	72.9	30	61.2	65	67.0
Unachievable ET, endometrium is thin on the day of transfer	1	2.1	0	0.0	1	1.0
Unachievable ET, Total Fertilization Failure (TFF)	9	18.8	15	30.6	24	24.7
Unachievable ET, no oocyte	3	6.3	3	6.1	6	6.2
Unachievable ET, degenerate oocyte	0	0.0	1	2.0	1	1.0
Total	48	100.0	49	100.0	97	100.0

ET: embryo transfer, *: The numbers indicate the women who were successful or unachievable on ET

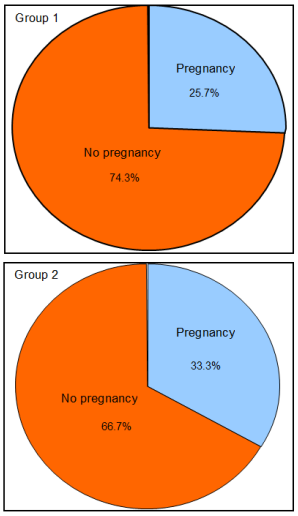

Figure 1: Group 1 and group 2 pregnancy rates after transfer

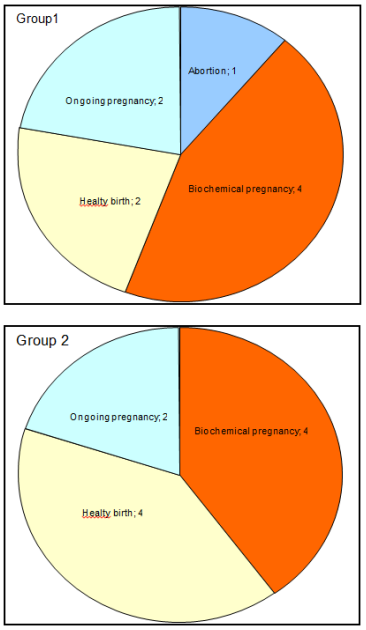

Figure 2: Group 1 and group 2 results of pregnancy

Discussion

There is no consensus on the best standard treatment option for ART cycles of poor responders. But the best stimulation protocol for the poor responder patients must be associated with the acceptable rate of minimum cancellation rate, maximum number of good quality oocyte retrieved and maximum chance of pregnancy [11]. As many stimulation protocols have been suggested for the poor responder patients,

microdose flare-up and antagonist protocols are the most popular regimes [12].

Although there is a trend toward higher pregnancy rates and lower cancellation rates in microdose GnRH-a flare-up protocol, we found that microdose GnRH-a flare-up protocol has similar IVF outcomes with flexible GnRH antagonist protocol. Moreover, maximum E2 level, total and mature oocyte number, no of 2PN were less but total dosage of gonadotropins, duration of stimulations, cycle days were higher in microdose GnRH-a microdose flare-up protocol despite that there was no significant statistical difference between two groups in terms of these parameters. In our study a clear difference was not found in both groups in terms of embryo oocyte quality on 2^{nd}, 3^{rd} and 5^{th} days. Our single center randomized prospective trial confirmed no significant differences with regards to any outcome parameters except consumption of gonadotropins. Increasing total dosage of gonadotropins, it would be considered as a disadvantage in terms of cost. Furthermore, some advantages of the present study need to be pointed currently; (1) comparable demographic features of both groups to prevent potential bias (2) adequate sample size for the power of the study.

In 1994, Scoot and Navod [13], firstly defined microdose flare-up protocol (20 μg of leuprolide acetate twice daily) for poor responders, indicated that microdose flare-up protocol improved IVF outcomes when compared with GnRH agonist protocol. They reported that it decreased cycle cancellation rate, increased peak E2 level, total number of oocyte retrieved, implantation and clinical pregnancy rates. Surrey et al. [14] assessed the effects of the microdose agonist protocol (40 μg of leuprolide acetate twice daily) in poor responders and also showed that microdose GnRH-a improves ovarian response and clinical outcome in poor responders due to enhanced release of early follicular phase endogenous FSH. Consequently, some of studies demonstrated that the microdose agonist protocol was proven to increase total mature oocyst number and maximum E2 level and decreased cancellation rates and increased both clinical and ongoing pregnancy rates in poor responders. Therefore, it was well-demonstrated that microdose flare-up protocols an important approach to improved IVF outcomes for poor responders.

GnRH antagonists for the management of poor ovarian responders have recently been an encouraging protocol and gradually gained favor [15]. A recent review evaluated role of GnRH antagonists in the treatment of poor-responder patients indicates that GnRH antagonists may offer several advantages, including a shorter duration of stimulation, a decrease in the total amount of gonadotropins, lower cost, and a shorter interval between successive treatment cycles [16]. As a meta-analysis reported that there was no difference in clinical pregnancy rates between antagonist protocol and agonist protocol in the poor responder patients [17].

In a prospective, randomized, clinical study, included 42 poor responder patients, Kahraman et al. [12] compared the efficacy of microdose GnRH agonist (GnRH-a) flare-up and multiple dose GnRH antagonist protocols and they concluded that microdose GnRH-a flare-up protocol and multiple dose GnRH antagonist protocol seem to have similar efficacy in improving treatment outcomes of poor responder patients although E2 levels of microdose GnRH agonist (GnRH-a) flare-up were higher. As well, a prospective, randomized, clinical study compared microdose GnRH agonist (GnRH-a) flare-up and multiple dose GnRH antagonist protocols demonstrated that the impact of these two regimens in ovarian stimulation of poor responders seem to be similar despite that maximum serum E2 level and number of total oocyte retrieved were higher in microdose GnRH agonist (GnRH-a) flare-up protocol [18]. In a study which compared GnRH antagonist protocol with GnRH agonist flare-up protocol in poor responders, Berin at al. [19] found excellent and comparable pregnancy and live birth rates in poor responders of advanced reproductive age with the use of either GnRH antagonist or flare protocol.

Poor ovarian response is also associated with very nominal and low quality oocyst and embryo. Some studies reported that there are similar results in terms of 2PN between micro dose flare up and antagonist protocols in poor responders [20-22]. With the effort of bettering the choice of embryos which bring about successful pregnancies and their usage, the researchers benefited from the advantages of extending the in vitro culture span before the embryo transfer. In vıtro culture span brings about usage of embryos and chooses them even after 5 days after embryo formation. While some researchers recommend the extension of culture span until 5 days, the others recommend doing the transfer in 3^{rd} day if embryos including more than 3 or 8 cells in vitro culture were determined [3,23,24].

In conclusion, GnRH antagonist and microdose flare-up protocols seem to have similar outcomes in poor responder patients in ICSI cycles except consumption of gonadotropins. Further prospective randomized trials with large sample size are needed to assess the efficacy of the two protocols in the poor responders.

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