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The comparison of microdose flare-up and multiple dose antagonist protocols based on hCG day estradiol (E2), progesterone (P) and P/E2 ratio among poor responder patients in ICSI-ET cycles

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Abstract. – OBJECTIVE: Elevated progesterone levels surpassing exact treshold values impede endometrial receptivity and decrease clinical pregnancy rates in different responder patients during assisted reproductive techniques. A progesterone (P): estradiol (E2) ratio of > 1 on the day of hCG administration has also been suggested to be a manifestation of low ovarian reserve. The clinical significance of P/E2 ratio on the day of hCG administration was investigated among poor responder patients.

PATIENTS AND METHODS: Based on the ESHRE Bologna consensus criteria related to poor ovarian response diagnosis, 48 poor responder patients were treated with the microdose flare-up regimen and 34 patients were treated with the multiple-dose GnRH antagonist protocol. All patients were destined to perform a ICSI-ET procedure at the end of the stimulation protocols. Progesterone levels and P/E2 ratios have been detected during controlled ovarian hyperstimulation.

RESULTS: In the microdose flare-up group; the duration of stimulation, total gonadotropin dose used and hCG day E2 levels were significantly higher than the multiple dose antagonist group. However, the mean hCG day P/E2 rate in the microdose flare-up group was less than that in the multiple-dose antagonist group. The clinical pregnancy rates were non significantly higher in the multiple dose antagonist protocol group than in microdose flare-up group.

CONCLUSIONS: Impaired endometrial receptivity caused by elevated P levels results with lower pregnancy rates. Regardless of the selected stimulation protocol, poor responder patients are not prone to exhibit high P and E2 secretion. Increased P/E2 ratio of > 1 on hCG day has limited value to predict cycle outcomes in poor responder patients because of ovarian follicle depletion.

Key Words:
Poor responder, GnRH agonist, GnRH antagonist, Progesterone, Clinical pregnancy rate.

Introduction

Poor ovarian response (POR) caused by a reduction in follicular reserve in the ovaries, usually results with a reduced number of retrieved oocytes during *in vitro* fertilization-embryo transfer (IVF-ET) procedure. Based on the ESHRE consensus criteria on 2011 in Bologna, Italy; at least two of the three features regarding an advanced maternal age (≥ 40 years) or any other risk factor for POR, a previous POR (≤ 3 oocytes) to conventional ovarian stimulation and an abnormal ovarian reserve test like antral follicle count < 5-7 or antimullerian hormone levels of 0.5-1.1 ng/mL must be present to define a patient as "poor responder" or two episodes of POR after maximal ovarian stimulation also classifies a patient as poor responder in the absence of advanced maternal age or abnormal ovarian reserve test[1,2]. Increasing the dosage of gonadotropins, utilization of recombinant FSH, microdose GnRH-a flare-up, GnRH antagonist protocols, administration of DHEA or growth hormone, assisted hatching, preimplantation aneuploidy screning and *in vitro* maturation have been widely used as treatment strategies with various success rates to overcome POR.

During IVF-ET procedures, an adequate response to COH that was achieved in the expense of elevated progesterone (P) levels ($p > 0.9$

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ng/mL) secreted by the cohort of maturing follicles was not found to be associated with lower pregnancy rates (PR)[3]. Contrarily, when the response to COH (controlled ovarian hyperstimulation) was found to be weak, premature P elevation led to lower PRs[4]. Using a defined P/E2 ratio > 1 as a better indicator of progesterone levels (PL) could differentiate physiologic P secretion from multiple healthy mature follicles from that secreted from dysmature follicles[5]. So, it has been suggested that P/E2 ratio more accurately reflects PL than a single hormone level. In women with unexplained infertility undergoing COH with HMG (human menopausal gonadotropin), the physiologic increase in late follicular P levels in women undergoing COH defined as a P/E2 ratio of > 1 on the day of hCG (human chorianic gonadotropin) administration has been suggested to be a manifestation of low ovarian reserve[6].

A threshold level for premature increase in serum progesterone (P) levels on the day of hCG administration was arbitrarily defined within various studies[7-12]. Different cutoff levels for P levels on the day of hCG have been suggested among different populations with different ovarian responses[4,6,13]. Despite increasing evidence related to this controversial topic, whether the presence of these increased serum P levels or P/E2 ratios on the day of hCG administration have any detrimental effects on IVF-ET R results reflected as embryo implantation and clinical pregnancy rates (CPR) remains as a subject of debate[11,13]. Although conducted by the different GnRH analogue protocols, a meta-analysis review of the clinical studies that investigated the possible relationship between P levels and CPRs suggested that the increase in P levels does not correlate with cycle outcomes[14]. Whether the unfavorable pregnancy outcome is caused by low ovarian reserve or high P levels on the day of hCG has not been be studied extensively before among poor responder women. To our knowledge, investigations regarding the effect of elevation of both P and P/E2 on the day of hCG on pregnancy outcomes in women with poor ovarian reserve based on ESHRE consensus criteria determined in Bologna on 2011 are lacking. Supraphysiologic levels of steroid hormones during IVF-ET procedures altering P/E2 ratios can detrimentally affect subsequent endometrial receptivity[15-18]. As the higher serum oestradiol and progesterone concentrations may affect endometrial receptivity, for patients with an extremely high progesterone concentration on the day of HCG, transfer of frozen embryos in a natural cycle can be a treatment choice[19]. A serum P level of 1.5 ng/mL as the threshold for poor responders, 1.75 ng/mL for intermediate responders, and 2.25 ng/mL for high responders have been proposed by a study proceeded in 11,055 ICSI-ET cycles among different responders20. A combination of the elevated progesterone and estradiol concentrations rather than an elevated estradiol levels alone has also been found to confer a potential negative effect on CPRs[21]. The objective of the present study was to identify the possible detrimental effects of P and or the P/E2 ratio on the day of hCG to CPRs in women with poor ovarian reserve undergoing ICSI-ET by using either microdose flare-up or multiple dose antagonist protocols.

Patients and Methods

Participants

For selecting a more homogeneous population to be tested, in this current study we used ESHRE criteria established in Bologna, Italy, on 2011 to define poor responder patients. A total of 82 poor-responder women diagnosed and treated in our Infertility and IVF Unit were enrolled in this study as two groups. The poor responder patients, according to Bologna criteria, who have been treated with microdose flare-up or GnRH antagonist protocols were retrospectively recruited from the medical records of our IVF Unit from 2009 to 2012. Fortyeight patients were treated with the microdose flare-up regimen and 34 patients were treated with the multiple-dose GnRH antagonist protocol. All patients were destined to perform an ICSI-ET procedure at the end of the stimulation protocols. Clinical pregnancy was defined as the presence of a gestational sac with accompanying fetal heartbeat by ultrasound 4 weeks following the ET procedure. The implantation rate was defined as the ratio of gestational sac determination on transvaginal ultrasonography to the number of ETs.

Stimulation Protocols

Group 1 consisted of 48 patients in which lowdose OC (Desolett; Organon, Oss, The Netherlands) was started on day 1 of the previous cycle for 21 days. On the third day of menstruation, 40 mg SC twice daily of leuprolide acetate (Lucrin; Abbott, Cedex, France) fol-

lowed by 225 IU/day hMG (Menogon; Ferring, Istanbul, Turkey) and 225 IU/day recombinant FSH (Gonal-F; Serono, Istanbul, Turkey) stimulation was started. Group 2 consisted of 34 patients who have received 225 IU/day hMG and 225 IU/day recombinant FSH starting on day 3 and 0.25 mg cetrorelix (Cetrotide; Asta Medica, Frankfurt, Germany) administered daily when two or more follicles reached 13-14 mm in diameter. The doses of hMG and recombinant FSH have been adjusted according to the ovarian response for both groups. Recombinant hCG (250 micrograms sc., Ovitrelle, Serono, Istanbul, Turkey) was administered when at least two leading follicles reached a mean diameter of 18 mm and the serum E2 concentration was > 500 pg/mL. Transvaginal oocyte retrieval was scheduled 36 hours after hCG injection. Following oocyte retrieval, metaphase II oocytes were reviewed and good-quality embryos were transferred under ultrasonographic guidance on day 3 for all patients. Following the transfer, all patients received vaginal progesterone (Crinone 8% gel, Serono) supplementation twice a day until menstruation or for 8 weeks after ET procedure in case of a clinical pregnancy establishment. A clinical pregnancy was defined as the presence of a gestational sac with accompanying fetal heartbeat by ultrasound at least 4 weeks after ET.

Statistical Analysis

Statistical analysis was performed by using IBM SPSS Statistics Software (19.0, SPSS Inc., Chicago, IL, USA). Whether the distributions of continous variables were normal or not, was determined by the Kolmogorov-Smirnov test. The parametric results were presented as mean± standard deviation values and compared by using the Independent Samples t test when distributed normal. Mann-Whitney *U* test was used when the results were not found to be distributed normal or for comparison of nonparametric data. Categorical variables were compared with Fisher's exact or Pearson chi-square tests when available. *p* values < 0.05 were considered statistically significant.

Results

Forty-eight ICSI-ET cycles were performed with the microdose flare-up protocol, and 34 were performed with the multiple-dose antagonist protocol. The mean age and body mass index (BMI) values of the patients; duration of infertility in years; type of infertility either primary or secondary; basal FSH, LH and E2 levels (day 3); and number of previous IVF cycles were similar in both groups. Cycle cancellation rates were also statistically similar between groups. The clinical and laboratory characteristics of two stimulation protocols including duration of stimulation, total gonadotropin dose used, mean number of follicles, mean number of oocytes retrieved, mean number of embryos transferred, hCG day E2 levels (pg/mL), hCG day P levels (ng/mL), hCG day P/E2 rates and hCG day endometrium thicknesses (mm) have been demonstrated in Table I. In the microdose flare-up group, a total of ten cycles were cancelled (five to poor folliculogenesis, two cycles owing to premature LH surge, and three to fertilization failure), while in the multiple-dose antagonist group a total of eight cycles were cancelled (four to poor folliculogenesis, two cycles owing to premature LH surge, and two to fertilization failure). In the microdose flare-up group; the duration of stimulation, total gonadotropin dose used and hCG day E2 levels were significantly higher than the multiple dose antagonist group (*p* values = 0.001; 0.001 and 0.007 respectively).

However, the mean hCG day P/E2 rate in the microdose flare-up group was less than that in the multiple-dose antagonist group (*p* = 0.048; 0.78±0,41 versus 2.64±3.94). The mean number of high quality oocytes (grade 1) characterized with blastomeres of equivalent size, a single nucleus for blastomere, and no fragmentation was non significantly higher in the microdose flare-up group than in the multiple dose antagonist group (*p* = 0.152; 2.12±1.90 versus 1.41±1.25). The implantation rates were also similar betwen the two study groups (*p* = 0.984). The CPRs were non significantly higher in the multiple dose antagonist protocol group than in microdose flare-up group (*p* = 0.649; 6/26 (23.1%) versus 7/38 (18.4%) respectively). Regardless of the stimulation protocol, when we compared the patients who had achieved clinical pregnancy with the patients who did not, the mean hCG day E2, the mean hCG day P and hCG day P/E2 ratio were similar. The ROC curve analysis revealed that hCG day P level was not significantly associated with CPRs in poor responder patients and no relevant cutoff value for hCG day P level has been observed.

Table I. The comparison of microdose flare-up and multiple dose antagonist groups based on cycle characteristics and ART outcomes.

Parameter	Microdose flare-up (n = 48)	Multiple dose antagonist (n = 34)	*p* value
Age (years)	36.13 ± 4.39	37.97 ± 3.59	0.059‡
BMI (kg/height^2)	25.83 ± 3.50	26.31 ± 3.42	0.542*
Day 3 FSH level (mIU/mL)	10.01 ± 2.20	9.42 ± 3.58	0.361*
Day 3 LH level (mIU/mL)	5.68 ± 2.53	6.46 ± 3.28	0.231*
Day 3 E2 level (pg/mL)	58.29 ± 37.83	58.39 ± 33.80	0.990*
Stimulation (days)	10.88 ± 1.46	9.71 ± 1.54	0.001*
Total gonadotropin dose (IU)	5018 ± 1659	3911 ± 879	0.001*
Mean number of follicles	4.23 ± 2.58	3.74 ± 2.67	0.351‡
Cancellation rate (n, %)	10/48 (20.8%)	8/34 (23.5%)	0.771†
hCG day E2 level (pg/mL)	1414 ± 811	957 ± 614	0.007*
hCG day P level (ng/mL)	0.96 ± 0.52	1.19 ± 0.97	0.672‡
hCG day P/E2 rate	0.78 ± 0,41	2,64 ± 3,94	0.048‡
hCG day endometrium thickness (mm)	9.88 ± 1.68	8.94 ± 2.50	0.046*
Mean number of oocytes (metaphase II) retrieved	3.46 ± 2.61	2.65 ± 1.98	0.132*
Mean number of oocytes retrieved	4.42 ± 2.79	3.35 ± 2.54	0.082*
Mean number of high quality oocytes	2.12 ± 1.90	1.41 ± 1.25	0.152‡
Fertilization rate (%)	79.16%	76.47%	0.128†
Mean number of embryos transferred	2.02 ± 1.92	1.32 ± 1.22	0.213‡
Implantation rate, %	26.31%	26.92%	0.984†
Clinical pregnancy rate (n, %)	7/38 (18.4%)	6/26 (23.1%)	0.649†

*Independent Samples Test *p* value of mean ± SD values; ‡Mann Whitney U test p value of mean ± SD values; †Pearson chi-square *p* value.

Discussion

This study demonstrated that despite the serum P levels on the day of hCG was found to be higher in patients stimulated with multiple dose antagonist protocol than patients with microdose flare-up protocol administration during ICSI-ET procedures among poor responder women, this difference did not effect CPRs significantly. Increased P levels on the day of hCG is associated with a reduced implantation rates and ongoing PRs regardless of different ovarian responses have been proposed. The association between serum P levels and PRs was thought not to be identified by arbitrarily choosing a low threshold value of 0.9 ng/mL. A serum P level of 1.5 ng/mL on the day of hCG administration can serve as the threshold for poor ovarian response, a serum P level of > 1.75 ng/mL is associated with lower ongoing PRs for intermediate responders, and a P threshold of 2.25 ng/mL can be applied to high responders. Implantation rates and ongoing PRs decline with P level elevation on the day of hCG in all ovarian responses to COH by impeding the endometrial receptivity. Paradoxically, P supplementation for luteal phase support that is started on the day of hCG administration is a common clinical practice does not effect the PRs adversely[22]. As a general rule, clinicians observe the better the ovarian response the higher the P threshold concentration during IVF-ET procedures. Therefore, the negative association between P elevation and ongoing PRs should be interpreted with different P threshold concentrations according to the quality of ovarian response. Some investigators advocate to freeze all the embryos and transfer in a subsequent freezed embryo transfer (FET) cycle for patients with high ovarian response, for intermediate responders and for poor responders when the P concentration on the day of hCG administration is > 2.25 ng/mL, > 1 .75 ng/mL and > 1.50 ng/mL respectively to increase the PRs when compared with the fresh embryo transfer cycles. It has been suggested that a defined P/E2 ratio > 1 could be a better indicator of progesterone levels (PL) for differentiating physiologic P secretion by multiple healthy mature follicles from that secreted by dysmature follicles. However, in our study an increased P/E2 ratio of > 1 did not have an deleterious effect on PRs among poor responder patients either stimulated with microdose flare-up or multiple dose antagonist protocol during ICSI-ET (Table I). Even so, poor

responder patients who were stimulated with multiple dose antagonist protocol exhibited a significantly higher P/E2 ratio than microdose flare-up protocol without changing clinical pregnancy outcome. This result reflects the enhancing effect of gonadotropin releasing hormone agonists on ovarian folliculogenesis and ovarian E2 production during the microdose flare-up stimulation protocol. However, in this study, hCG day mean P levels of the patients have not surpassed the previously proposed treshold P level of > 1.50 ng/mL that is assumed to negatively effect endometrial receptivity. Besides, the implantation rates and CPRs of the patients stimulated with microdose flare-up and multiple dose antagonist protocols were found to be similar because of the low mean P levels achieved during both stimulation protocols. Poor ovarian reserve itself exhibits a limited activity to secrete P from granulosa cells of the growing follicles resultant with decreased E2 levels secreted by using P as a steroid hormone precursor. For poor responder patients, different stimulation protocols seem to have similar cycle outcomes achieved exclusively CPRs. Our study demonstrated that clinical utility of P/E2 ratio to predict cycle outcomes is limited for poor responder patients. Various P treshhold concentrations on the day of hCG administration for different ovarian response patients that can adversely effect endometrial receptivity should be used during decision making process of fresh embryo transfer or freezed-thawed embryo transfer procedures.

Higher progesterone concentration on day of HCG is an undesirable and common event in IVF treatment that is associated with poor pregnancy outcome. Higher number of retrieved oocytes during ovarian stimulation is responsible for this unwanted P elevation. Although administration of higher gonadotropin doses and regardless of the choice of stimulation protocol, poor responder patients seem to have low risk for P elevation because of the impaired ovarian follicle reserve. Even so, increased P levels on hCG day also exert deleterious effect on endometrial receptivity among poor responder patients like normoresponders and highresponders. Impaired endometrial receptivity caused by elevated P levels results with lower pregnancy rates. Increased P/E2 ratio of > 1 has been thought to be related to lower CPRs taking into account that dysmature follicles secrete lower E2 and higher P during COH.

Conclusions

Increased P/E2 ratio of > 1 on hCG day has limited value to predict cycle outcomes because of ovarian follicle depletion in poor responder patients. Selection of a suitable gonadotropin dose during ovarian stimulation for IVF-ET that limits uncontrolled P elevation is mandatory to increase pregnancy success. Despite paying great attention to control unwanted P level elevation during IVF-ET, freezing the embryos and transfering them via frozen-thawed cycle is a promising method since CPRs are higher in the FET cycles than the fresh embryo transfer cycles.

Conflict of Interest

We, as authors of this original research study, disclose that we do not have any financial and personal relationships with other people or organizations that could inappropriately influence our work.

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