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AFC *vs.* AMH: prediction of ovarian response in women with endometrioma undergoing controlled ovarian stimulation

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Abstract. – OBJECTIVE: To assess the clinical value of antral follicle count (AFC) and anti-Mullerian hormone (AMH) for the prediction of ovarian response in women with endometrioma undergoing controlled ovarian stimulation for IVF using GnRH antagonist treatment.

PATIENTS AND METHODS: Fifty patients with endometrioma who underwent their first IVF/ICSI cycle with GnRH antagonist treatment were included in the study. The average AMH values were recorded as 1.5-2 ng/mL. Fifty infertile women are not suffering from endometrioma were selected from those with male factor infertility as control. They were matched according to both serum AMH levels and age. Serum samples have been collected before the IVF treatment for determining AMH levels in both groups of subjects. Likewise, each group of subject underwent ultrasound scan for AFC on day 3. Total number of oocytes retrieved during OPU, the number of transferred embryo, implantation and clinical pregnancy rates, live birth and abortion rates, total dose of rhFSH were noted in both groups of subjects.

RESULTS: Day 3 AFC was significantly higher in the control group compared to women with endometrioma. Both the number of retrieved oocytes during oocyte pick-up, MII oocytes and 2 PN embryo were significantly lower in the endometrioma. Likewise, the fertilization, implantation, clinical pregnancy and live birth rates of endometrioma group were significantly lower than those in the control group. The total rFSH dose was higher in the endometrioma group than those in control. The percentage of abortion in the endometrioma group was found to higher compared to those with controls.

CONCLUSIONS: AFC is more sensitive than the AMH in detecting ovarian response in women with ovarian endometrioma. The individualization of GnRH antagonist protocols in subjects having endometrioma might be improved by using an AFC-tailored approach instead of AMH.

Key Words:
Anti müllerian hormone, Endometrioma, Antral follicle count, Ovarian response.

Introduction

There is an ongoing debate in the field of reproduction biology which ovarian marker provides an accurate estimation of potential success for infertile women with endometrioma before IVF/ICSI treatment. The two markers that stand out in this discussion are antral follicle count (AFC) and anti-Mullerian hormone (AMH). Both markers have been proposed as predictors of ovarian reserve and clinical outcome in different types of infertility states[1]. Unfortunately, the determinative role of these markers has been studied in the same group of patients with different causes of infertility. Patients scheduled for IVF due to endometrioma were also included in this group. Similarly, predicting studies were usually performed on patients who underwent agonist protocol.

AMH prevents premature depletion of the ovarian follicle[2], and regulates steroidogenesis in granulosa cells[2]. It has been reported that predictor role of AMH in COS cycles is more prominent than in AFC for estimating of both high[3] and low ovarian response[4]. Similar to AMH, AFC has a good predictive value, exhibiting a linear association with the total number of retrieved oocytes[5]. The role of AFC and AMH in ovarian response prediction has been studied extensively[6] however, little information is avail-

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able regarding the clinical value of AFC and AMH in subjects with ovarian endometrioma undergoing IVF/ICSI with antagonist protocol. The aim of the study is to assess the clinical value of AFC and AMH for the prediction of ovarian response in women with endometrioma undergoing controlled ovarian stimulation for IVF using GnRH antagonist treatment.

Patients and Methods

To determine the minimum number of endometrioma subjects that needed to be enrolled in this study to ensure sufficient statistical power sample size calculation was carried out before the study. The probability of a type I error (α) (i.e., finding a difference although a difference does not exist) was calculated. We used an alpha cut-off of 5% (.05). Demographic and clinical variables were considered to be significant at a type I error cut-off of .05 with a power of 0.85. Together, 50 patients in the study and control groups were required to ensure adequate study power, so 50 patients with the diagnosis of endometrioma and 50 control cases diagnosed as male factor infertility were included in the study. Participants with endometrioma and control participants with male factor infertility were matched according to their serum AMH levels (1.5-2.0 ng/mL) and age. Ovarian endometrioma was diagnosed with a transvaginal ultrasonography. The endometrioma was suspected when a diffuse, regular margined cyst with a low level internal echo. The study protocol was approved by the local Academic Committee of Bahcesehir University IVF center. All the patients signed an informative written consent to participate in the study after full explanation. The exclusion criteria included the women with suspicious ovarian malignancy detected by ultrasound appearance. Due to their negative impact on ovarian reserve women with the diagnosis of premature ovarian aging and severe peritoneal endometriosis were not included. Women with a single ovary, a previous history of ovarian cystectomy, a history of chronic smoking, or previous chemotherapy and/or radiotherapy treatment were excluded.

Fifty women with endometrioma and fifty control subjects fulfilled the eligibility criteria were invited to participate in this study. Participants in the endometrioma group were selected among infertile women who were referred to our IVF clinic, in Bahcesehir University Hospital, from January 2015 until June 2016 due to diagnosis of endometrioma. Forty out of 50 subjects had unilateral endometrioma, and 10 out of them had bilateral endometrioma. Both groups of infertile women underwent a routine laboratory and radiological examination to diagnose the underlying factors of infertility. At the initial visit, they underwent ultrasound scan for AFC as well as an AMH blood test. Transvaginal ultrasound is used to determine AFC in both groups of participants. All small antral follicles those between 2 and 10 mm in diameter were manually measured and counted. Subjects with endometrioma had normal early follicular hormone profiles, normal mid-luteal progesterone levels, normal semen analysis and normal hysterosalpingography with bilateral tubal patency and absence of intra-uterine mass forming pathology and the only detectable cause of infertility was endometrioma. The infertile women enrolled as the control group had no history of endometrioma or other benign ovarian cysts. Patient demographic and laboratory data were documented, including BMI (Table I). In addition to AFC and AMH, total oocyte number retrieved during OPU, the number of transferred embryo, implantation and clinical pregnancy rates, live birth and abortion rates, total dose of rhFSH were noted in both groups of subjects.

Antagonist Protocol

Both groups of subjects underwent controlled ovarian stimulation for IVF using GnRH antagonist protocols. The steps of the antagonist protocol can be found elsewhere[7]. COS was performed with recFSH (Gonal-f; Merck Serono, Modugno, Italy). While 375 IU rFSH was started on day 2 or 3 of cycle in endometrioma group starting dose of rFSH in the control group was 225 IU. A GnRH antagonist Cetrotide (Merck-Serono, Halle, Germany) was used daily subsequent to the leading follicle reached a diameter of 14 mm and carried on until and including the day of hCG injection. hCG (rhCG: Ovitrelle; Merck Serono, 250 mg, Modugno, Italy) was administered to induce final oocyte maturation when at lead two 17 mm or lead one 18 mm in diameter were visualized by ultrasound. Oocyte retrieval was performed 36 h after hCG administration. One embryo was transferred 3 or 4 days after oocyte retrieval. The luteal phase was supported progesterone vaginally initiated on the day of oocyte pick-up and continued until the 12th week of gestation in cases where a pregnancy was achieved.

Table I. Comparsion of clinical caharacteristics of each group.

	Endometrioma (n:50) AMH:1.5-2 (ng/mL)	Control (n:50) AMH:1.5-2 (ng/mL)	*p-value
Age (years)	33.5 ± 4.3	31.6 ± 5.8	NS
Body mass index (kg/m^2)	25.5 ± 3.8	24.5 ± 4.1	NS
Infertility duration (yr)	4.9 ± 3.3	4.1 ± 2.7	NS
Day 3 FSH (IU/L)	6.5 ± 2.4	6.3 ± 3.2	NS
Day 3 LH (IU/L)	3.7 ± 2.1	3.2 ± 1.8	NS
Day3 E2 (pg/ml)	41.2 ± 21.2	38 ± 21	NS
Total AFC	5.5 ± 3.1	12.6 ± 6.9	< 0.001
Total dose of rFSH (IU)	3300 (2500-5100)	1800 (1425-2400)	< 0.001
Duration of stimulation (day)	13 (9-14)	10 (9-11)	< 0.04
Total number ofoocytes	4.16 (1-6)	7.7 (4-13)	< 0.001
Number of MII oocytes	3.18 (1-4)	6.06 (3-13)	< 0.001
Numberof 2PN oocytes	2.94 (1-4)	5.52 (2-10)	< 0.001
Number of embryos transferred	0.7 ± 0.7	1.2 ± 0.6	< 0.01
Implantation rates (%)	27%	42%	< 0.001
Clinical pregnancy rate (%)	42%	64%	< 0.001
Abortion rates (%)	28.5%	12.5%	< 0.001
Live birth rates (%)	24%	52%	< 0.001

*$p < 0.05$ was accepted significiant, data are presented mean ± SD.

AMH Assay

After venous blood collection, serum for assay of AMH was separated and frozen. All samples were analyzed using an ultra-sensitive AMH Gen II ELISA kit (Beckman-Coulter, Inc., Webster, NY, USA).The lower limit of AMH detection was 0.16 µg/l. Inter-assay variation was 10% at 0.27 µg/l. All values are expressed in ng/mL.

Statistical Analysis

For the statistical analysis of all demographic and laboratory findings from both groups of participants, SPSS version 21.0 (SPSS Inc., Chicago, IL, USA) was used. The normality of individual group parameters was assessed with the 1-sample Kolmogorov-Smirnov Z test. Variables with normal distributions were compared between groups using independent samples tests. Because they were found to be abnormally distributed in both groups the Mann-Whitney U and Wilcoxon tests were used for comparisons of endometrioma and control participants. Data were given as mean ± standard deviation. *p*-values < 0.05 were considered to indicate statistical significance.

Results

The baseline characteristics of the endometrioma and control groups are shown in Table I. There was no significant difference in age, bodyweight, BMI and AMH levels in each group. Table I also demonstrates the stimulation characteristics and cIVF outcome per group. There was a significant difference between endometrioma and control group for the number of oocytes retrieved, number of 2PN oocytes, number of transferred or cryopreserved embryos and the percentage of single embryo transfer. The day 3 AFC was significantly higher in the control group compared to women with endometrioma. The number of oocytes retrieved, MII oocytes and 2 PN embryo were obtained from the endometrioma group was significantly lower than those in control group ($p < 0.01$). The fertilization, implantation, and clinical pregnancy rates of endometrioma group were significantly lower than those in the control group ($p < 0.01$). Subjects with endometrioma were treated with the rFSH at a higher prime dose when compared to control subjects. Likewise, the duration of rFSH treatment in endometrioma group was found longer compared to controls. Total amounts of rhFSH were given to the subjects with endometrioma were significantly higher than in the control group (Table I).

Discussion

Both AFC and AMH measurement are good predictors of ovarian response during assisted reproduction techniques. However, their predictor role in women with endometrioma undergoing IVF/ICSI are not clear.The value of AMH

and AFC has primarily been studied in patients undergoing endometrioma surgery. However, it remains to be confirmed whether AMH or AFC has a comparable ability to predict the ovarian response in women with endometrioma undergoing IVF/ICSI with antagonist protocol. This prospective controlled study demonstrates that AFC as a single predictor test has substantial accuracy in the prediction of ovarian response in women having ovarian endometrioma using GnRH antagonist protocol. Furthermore, the number of retrieved oocytes and clinical pregnancy rates indicate that AFC is a good predictor of ovarian response in women with endometrioma.

AFC is an ultrasound measure of pre-treatment small antral follicles in both ovaries. Ultrasound measures AFC is recommended at the beginning of a cycle[8]. Interestingly, Deb et al[9] reported that AFC can be measured at any point in the cycle without compromising accuracy. The findings from the present work are in line with previous studies on the predictive value of AFC for ovarian response using GnRH antagonist treatment. In good agreement with, recent study has emerged to support AFC as preferred methods for predicting ovarian reserve[10]. Depending on the underlying causes of infertility AFC has more predictive value than the other markers of ovarian reserve[5]. In the current study, clinical pregnancy and 2 PN oocytes rates were found to decreased in endometrioma group suggesting predictor role of AFC in embryo quality and pregnancy rates. On the other hand, Chang and Jayaprakasan[11] demonstrated that AFC is not a good predictor of oocyte or embryo quality.

Despite similar AMH levels, there may be several possible causes of the low number of follicles in endometrioma patients undergoing oocyte pick-up. The structural anomalies in the microenvironment and vessel support of the follicle are of great importance. In good agreement with this, Maneschi et al[12] reported that subjects having endometrioma showed disturbed vascular patterns in their follicles. Other reasons for the low number of follicles may be the toxic and inflammatory properties of cystic content. Indeed, both superficial endometriosis and the presence of endometrioma negatively affect both follicle development and receptivity[13]. Correspondingly, it has been reported that presence of superficial endometriosis disturbs ovarian cortex and this is linked to toxic and inflammatory properties of implants[12]. The impaired apoptotic process within the follicle of endometrioma subjects may be another culprit. Concordantly, Fauvet et al[14] observed an increased expression apoptoti proteins in women with endometriomas.

Conclusions

When reviewing the literature, both AFC and AMH measurements have shown similar predictive value for ovarian response and reproductive outcome. On the other hand, in this study, we have demonstrated the ability of AFC to predict the number of retrieved oocytes and ovarian response following ovarian stimulation with GnRH antagonist protocol. Our findings showed that the women with endometrioma had a significantly lower oocytes retrieval rates than those in the controls, despite their AMH levels were the same. These results strongly suggest that AMH does not predict the ovarian response in women with endometrioma undergoing IVF/ICSI as strong as AFC. Early measurement of AFC was found to be better predictor ovarian response in women with endometrioma. AFC can be an ideal marker for the individualization of COS strategies in women with endometrioma undergoing COS with antagonist protocol.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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