Ovarian reserve markers in unexplained infertility patients treated with clomiphene citrate during intrauterine insemination

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Abstract

Introduction: The aim of this retrospective case control study was to identify predictors of ovarian response and pregnancy outcomes in intrauterine insemination (IUI).

Material and methods: One hundred women undergoing IUI cycles with clomiphene citrate were enrolled. The number of antral follicles and the total ovarian volume by ultrasound, and the basal levels of follicle-stimulating hormone (FSH), estradiol, and inhibin B on cycle day 3 were measured in groups that were divided according to ovarian response. The tests were also evaluated according to ovarian response and pregnancy outcomes. All analyses were performed using the Statistical Package for the Social Sciences, version 15.0 (SPSS, Chicago, IL, USA).

Results: The antral follicle count (AFC) was the best single predictor for ovarian response and pregnancy outcomes. The sensitivity and specificity for prediction of ovarian response were 81% and 78% for AFC at an optimum cutoff value of ≤ 13.1. Age was negatively correlated with ovarian volume (r = –0.280, p = 0.021) and AFC (r = –0.358, p = 0.003). Increasing FSH was associated with a reduction in AFC (r = –0.273, p = 0.025). The AFC was significantly correlated with ovarian volume (r = 0.660, p < 0.0001) and FSH (r = –0.273, p = 0.03).

Conclusions: Our data demonstrate that the AFC provides better prognostic information on the occurrence of ovarian response during clomiphene citrate stimulation for IUI.

Key words: antral follicle count, ovarian reserve, intrauterine insemination, clomiphene citrate, unexplained infertility.

Introduction

Infertility affects approximately 15–20% of reproductive aged couples [1]. Primary infertility is a term used to describe a couple that has never been able to conceive a pregnancy after a minimum of 1 year of attempting to do so through unprotected intercourse [2]. Causes of infertility include a wide range of physical as well as emotional factors [3]. Intrauterine insemination (IUI) with ovarian stimulation has been widely used as the primary therapeutic modality for infertility, especially that which is unexplained or caused by nonsevere male factor, anovulation or cervical mucus hostility. Moreover, IUI with controlled ovarian hyperstimulation is

commonly used as first-line treatment for couples with unexplained subfertility [4].

Ovarian reserve is the major factor in human fertility potential. Age is considered to be the single most important factor in determining quality and quantity of ovarian reserve. The most common tests are basal tests for follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2) and inhibin B, or dynamic endocrine tests such as the clomiphene citrate challenge test and gonadotrophin analogue stimulating test [5]. In recent years, great attention has been devoted to direct tests such as the antral follicle count (AFC), ovarian volume and ovarian biopsy results. The basal FSH concentration is the most common test utilized for ovarian screening. More recently, anti-Mullerian hormone (AMH) has been used by various groups to assess the ovarian reserve [6]. However, the availability of multiple ovarian reserve markers suggests that none is ideal [7].

Consequently, ovarian reserve can be screened using various tests. The aim of the present study is to compare the value of different tests for measuring ovarian reserve in unexplained infertility. In this study, we also evaluated the commonly used tests predicting ovarian reserve and IUI outcomes in couples with unexplained infertility.

Material and methods

In this study, a total of 127 IUI cycles in 70 couples (group 1) with unexplained infertility were evaluated. Data were collected from the records of 100 couples with unexplained infertility that underwent IUI in the Gynecology and Obstetric Medicine Unit of Erzincan University in 2013. Thirty women (group 2) had no ovarian response with ovarian stimulation.

Unexplained infertility was defined as infertility for couples with no definite reason. Patients with at least one patent tube in hysterosalpingography or laparoscopy and husbands with normal sperm parameters according to the World Health Organization classification were included. Women with myomas, endometriosis and uterine anomalies were excluded from the study. Patients with a basal FSH level > 12 mIU/ml, a maternal age > 40 years, polycystic ovarian syndrome or a coexisting chronic disease such as hyper- or hypothyroidism, diabetes mellitus or any history of previous reproductive surgery were also excluded from the study. The inclusion criterion was unexplained infertility couples without any systemic or anatomic disease.

They underwent venipuncture at approximately 08:00 hours for baseline pretreatment antral follicle count assessment in the early follicular phase (days 2–4) of the menstrual cycle. Serum samples were stored at –20°C and assayed for LH, FSH, E2, and inhibin B. In the same morning of the blood tests, the total numbers of antral follicles measuring 2–10 mm in diameter were counted by the same operator. A 7.5 MHz transvaginal probe was used in all examinations. Ovarian volume was calculated with the equation of an ellipsoid (0.526 × length × height × width). The inclusion criteria were age between 20–39 years, both ovaries present on transvaginal ultrasound scan, no previous ovarian operation, adequate visualization of ovaries on transvaginal ultrasound, no exposure to cytotoxic drugs or pelvic radiation therapy, and no current hormone therapy.

For controlled ovarian hyperstimulation, 50 mg of clomiphene citrate (CC) was given orally from day 5 to day 10. Patients were monitored by transvaginal folliculometry. When one follicle reached at least ≥ 16 mm in diameter, a 250 µg of recombinant human chorionic gonadotropin (hCG; Ovitrelle, Serono) was subcutaneously administered. IUI with the husband's semen was performed 36 h after the administration of hCG. After semen preparation, a total of 1 ml of sperm preparation was slowly injected into the uterine cavity. Clinical pregnancy was defined as the ultrasound observation of fetal heart movements at 7–8 weeks of gestation. Ovarian response positivity was determined when at least one follicle reached ≥ 16 mm in diameter after ovarian stimulation by CC. If there was no follicle ≥ 16 mm in diameter, it was determined as ovarian response negativity.

Statistical analysis

Numerical variables were presented as means ± SD. After the confirmation of normal distribution, the *t* test or Mann-Whitney *U* test was used to compare the values of the groups. Spearman correlation coefficients were used to assess the relationships between the parameters. Logistic regression analysis was used to evaluate the value for the prediction of oocytes ≥ 16 mm in diameter. Value of $p \leq 0.05$ was considered statistically significant. All analyses were performed using the Statistical Package for the Social Sciences, version 15.0 (SPSS, Chicago, IL, USA).

Results

One hundred women were divided into two groups according to their response to ovarian stimulation. Seventy women had oocytes ≥ 16 mm in diameter (70%), while 30 women had no ovarian response (30%). Additionally, group 1 was analyzed for the pregnancy outcomes.

Nine (12.8%) of 70 patients were pregnant. The chance of pregnancy increased below the age of 35 (93% sensitivity, 33.3% specificity), below 7 of FSH level (73% sensitivity, 55.6% specificity), above 4.23 of ovarian volume (91.5% sensitivity, 55.6% specificity), and above 12 of AFC (85%

sensitivity, 77.8% specificity) according to receiver operating characteristic (ROC) analysis. Moreover, there was no statistically significant difference in age, FSH, AFC or ovarian volume between the groups ($p < 0.05$).

Table I summarizes patients' demographics, basal FSH, AMH, and AFC in the two study groups. There was no difference between the ages of the groups. Body mass index (BMI) was similar in both groups. Group 2 had a lower total AFC ($p < 0.001$) and smaller ovaries ($p < 0.05$), but there was no statistically significant difference between the serum FSH levels of groups. The other endocrine (basal LH, E2, and inhibin-B) markers were similar in both groups. Multivariate logistic regression analysis of the variables was performed for the prediction of oocytes ≥ 16 mm in diameter. The AFC was the only significant predictor on multivariate analysis (1.121, 95% CI: 1.014–1.372; $p = 0.035$). In group 1, similar results were obtained for prediction of pregnancy, with several of the measures being predictive on univariate logistic regression analyses, which again revealed AFC as a significant predictor (Table II). Additionally, ovarian volume was the other significant predictor of pregnancy (Table II). The ROC curve analysis (Figure 1) showed that AFC predicted the ovarian response, as demonstrated by a similar area under the curve (AUC) ($p < 0.001$). The optimum cutoff level of AFC was 13.1 with 81.4% sensitivity (95% CI: 69.1–90.3), 77.8% specificity (95% CI: 40.1–96.5), 3.66 positive likelihood ratio, and 0.24 negative likelihood ratio.

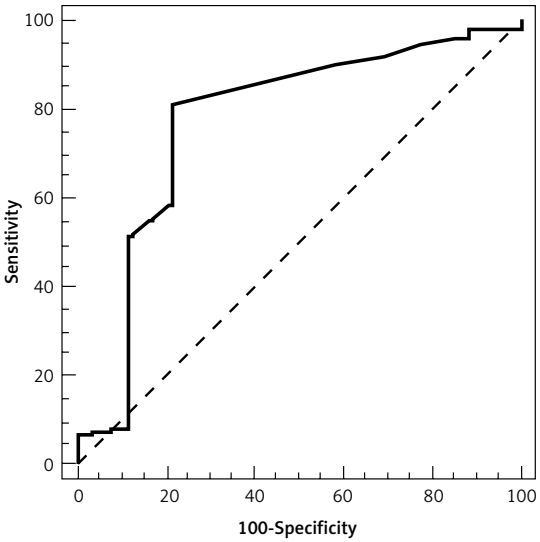

Figure 1. ROC curve analysis of antral follicle count

Table III summarizes the correlation of the clinical and hormonal parameters of the 70 patients. Age was negatively correlated with ovarian volume ($r = –0.280$, $p = 0.021$), and AFC ($r = –0.358$, $p = 0.003$). Increasing FSH was associated with a reduction in AFC ($r = –0.273$, $p = 0.025$). The AFC was significantly correlated with ovarian volume ($r = 0.660$, $p < 0.0001$), and FSH ($r = –0.273$, $p = 0.03$).

Discussion

The proportion of couples suffering from unexplained infertility is popularly quoted as 16%, but

Table I. Comparison of baseline clinical, endocrine, and ultrasound characteristics between groups

Parameters	Group 1 ($n = 70$)	Group 2 ($n = 30$)	Value of p
Age [years]	30.4 ±5.2	31.5 ±4.6	0.27
Body mass index [kg/m^2]	24.6 ±4.2	24.3 ±4.2	0.79
Basal FSH level [IU/l]	6.9 ±2.2	7.4 ±3.2	0.28
Basal LH level [IU/l]	6.3 ±3.1	6.9 ±3.6	0.16
Basal E_2 level [pmol/l]	66.6 ±358.0	67.9 ±117.7	0.31
Inhibin-B [pg/ml]	52.9 ±38.1	68.5 ±107.6	0.94
Total antral follicle count	18.7 ±6.4	12.2 ±8.7	0.009
Mean ovarian volume [cm^3]	10.1 ±4.2	7.9 ±3.5	0.04

Values are presented as mean ± SD.

Table II. Univariate analysis of baseline markers of ovarian reserve for prediction of pregnancy outcomes in group 1

Parameters	Odds ratio	95% CI	Value of p
Age	0.91	0.872–1.075	0.33
Basal FSH	0.707	0.627–1.076	0.13
Inhibin-B	0.997	0.981–1.004	0.19
Mean ovarian volume	1.316	1.015–1.707	0.03
Antral follicle count	1.325	1.055–1.536	0.01

Table III. Spearman correlation coefficient of clinical and hormonal parameters

Variable	Age	FSH	OV	AFC
Age	–	0.202	–0.280	–0.358
FSH	0.102	–	–0.189	–0.273
OV	0.021	0.125	–	0.660
AFC	0.003	0.025	< 0.0001	–

FSH – Follicle-stimulating hormone, OV – ovarian volume, AFC – antral follicle count.

the range varies from 0% to 37% [8]. Clomiphene citrate is the first-line treatment for unexplained infertility [9]. Although the effectiveness of the treatment was evaluated, a recently published review showed that clomiphene citrate may improve fertility as much as 2-fold [8]. In our study the pregnancy rate was 12.8%. The pregnancy rate in IUI cycles has varied between 11.4% and 12.6% [10]. In this study, we examined the relation between the prediction of ovarian reserve tests and the pregnancy outcomes and mature follicle in unexplained infertility patients treated by CC with IUI.

An ideal ovarian reserve parameter should be easily measurable, minimally invasive, and inexpensive and should have good predictive values [11]. In infertility treatment with ovulation induction and IUI, the fertility expert has limited parameters to predict the likelihood of success. Our study confirmed the efficacy of AFC as a biomarker of ovarian function. We observed an inverse correlation between AFC and serum FSH levels measured at the early follicular phase. We also noted that high levels of AFC were positively correlated with pregnancy after IUI in unexplained infertility. To our knowledge, this is the first study to evaluate the prediction of mature follicle and pregnancy with ovarian reserve markers in intrauterine insemination for treatment of unexplained infertility with CC.

Age is considered to be the single most important factor in determining ovarian reserve [12]. In our study, increased age (≥ 35 years) negatively affected the pregnancy outcomes (93% sensitivity, 33.3% specificity); however, there was no statistically significant effect to predict the pregnancy.

The FSH is the most studied and used endocrine test in determining ovarian reserve [5]. Previous reports have shown that elevated 3 FSH levels on the basal day were related to lower pregnancy rates [13]. However, this criterion cannot indicate a poor ovarian response with normal basal FSH levels. Van der Steeg *et al.* studied the predictive value of basal FSH for spontaneous pregnancy occurrence in ovulatory subfertile women younger than 40 years and observed reduced chances when the levels exceeded 8 IU/l, whereas no association could be determined for lower levels [14]. In a prospective study, Haadsma *et al.* found that basal FSH and AFC were significant predictors of spontaneous pregnancy [15]. Our study showed that FSH may not predict ovarian response or pregnancy in unexplained infertility couples treated with IUI, but pregnancy rates decreased when FSH levels exceeded 7 IU/l. Increasing FSH was also associated with a reduction in AFC.

Inhibin B has been postulated as an alternative endocrine marker of ovarian reserve. It has been suggested that measuring inhibin B concentrations would provide a more direct assessment of ovarian reserve [16]. However, other studies did not recommend the use of inhibin B alone as a reliable predictor of ovarian reserve [17, 18]. Our data showed that inhibin B did not predict the response to ovulation induction and pregnancy in IUI cycles of unexplained infertility stimulated with CC.

Ovarian volume and AFC values can be useful markers to determine ovarian reserve [19]. Erdem *et al.* suggest that transvaginal ultrasonography rather than hormonal parameters is the preferred method for ovarian reserve determination, as ultrasound assessment of ovarian volume and the AFC confer a stronger correlation with chronological aging than day 3 FSH level indices and aging [20]. Additionally, several investigators have reported the effectiveness of AFC and ovarian volume in predicting ovarian response to hormonal stimulation in *in vitro* fertilization (IVF) [21, 22]. In another study, AFC demonstrated the best correlation with women's age [23]. Women undergoing their first IVF cycle were evaluated with a battery of tests to compare several basal ovarian reserve markers [24]. Measurements were performed to determine the number of antral follicles, total ovarian volume, basal FSH, E2, and inhibin B on cycle day 3. The AFC was the best single predictor for a poor ovarian response [24]. However, the performance of the AFC for predicting failure to achieve pregnancy is poor [23–25]. Our results revealed that AFC displayed a significant positive correlation between ovarian volume and a negative correlation between age and FSH. Additionally, AFC was the most predictive test for the ovarian response and pregnancy outcomes. This study suggests that the AFC provides an optimum sensitivity and specificity of 0.81 and 0.78, respectively, at a cutoff value of 13.1 or less for the prediction of ovarian response. Ovarian volume was the other significant predictor of pregnancy but not ovarian response.

The IUI is the first step and is important for infertile women before IVF cycles [26]. The prediction of the treatment outcomes is pivotal for the treatment procedures in infertile therapy. Although there have been many trials for the prediction of IVF outcomes and the relation between ovarian reserve and ovarian response, there is no study to predict ovarian response in IUI cycles. Our study evaluated the tests of ovarian reserve markers in IUI treatment with CC and illustrated that AFC was more powerful to show an ovarian response. This study might help to estimate the tests of ovarian reserve before IUI cycles.

In conclusion, pretreatment AFC was the most significant predictor of ovarian response and pregnancy outcomes for ovarian stimulation with CC during IUI. Additionally, ovarian volume might predict the pregnancy outcomes. Our study is the first to demonstrate this in unexplained infertility couples. More prospective studies are needed in order to confirm the predictive value of the markers.

Conflict of interest

The authors declare no conflict of interest.

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