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Evaluation of ovarian reserve in unexplained ınfertile cases: a case-controlled study

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Abstract

Background The aim of this study was to evaluate the effectiveness of current ovarian reserve tests in unexplained infertile cases.

Material and methods This case–control study was conducted on 70 unexplained infertile women who were included in a tertiary university hospital. Both groups of basal FSH, estradiol, antimullerian hormone (AMH), inhibin B, ovarian volume, total antral follicle count (AFC), ovarian volume, and ovarian stromal blood flow (peak systolic velocity (PSV), S/D (systole and diastole ratio), resistance index (RI), and pulsatility index (PI)) values were compared.

Results The mean AMH, inhibin B, PSV, and stromal blood flow values of the control group patients were higher than those in the unexplained infertility group. However, the values of the means of RI and PI of the cases in the control group were lower than those in the infertility group. When PI's value was ≥ 2.00, its sensitivity was 65.7%, and its specificity was 64.3%. In the case of the RI, its value was ≥ 0.745, its sensitivity was 65.7%, and its specificity was 62.9%. In order to show ovarian reserve in unexplained infertile cases, the sensitivity values can be sorted from high to low as follows: PI > RI > estradiol > FSH > ovarian volume > AFC > inhibin B > stromal blood flow > PSV > S/D.

Conclusion In the unexplained infertile patient group with normal ovarian reserve test results, basal estradiol, decreased PI, and RI values may be used as good ovarian reserve predictors.

Keywords Ovarian reserve, Ovary, Subfertility, Ultrasonography

Background

Infertility is defined as the absence of pregnancy despite regular sexual intercourse for at least 1 year without using any contraceptive method by a couple of reproductive age. Infertility affects 15% of couples of reproductive age. The female factor is determined in 40–50% of all infertile couples. An infertility cause cannot be identified as the result of all diagnostic examinations in 15% of couples. Unexplained infertility is defined as the absence of any pathology in the basic evaluation (sperm analysis, ovulation tests, hysterosalpingography) in couples who cannot achieve pregnancy after 1 year of unprotected coitus. Myoma uteri, endometriosis, and findings of poor ovarian reserve can be detected with the addition of ultrasonographic evaluation to the basic evaluation in some of the infertile couples [1, 2].

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addition of ultrasonographic evaluation to the basic evaluation in some of the infertile couples [2, 3].

Currently, there is no strict standard for determining ovarian reserves worldwide. There are still controversies and counter-studies about what is the most reliable parameter. Therefore, research continues. The main purpose of this study was to evaluate the effectiveness of current ovarian reserve tests in unexplained infertile cases.

Materıal and methods

Seventy participants who applied to infertility and gynecology outpatient clinics in the Department of Obstetrics and Gynecology at the medical faculty for 3 months (March–May 2014) were included in the current study (35 cases, unexplained infertility group, 35 cases, control group).

Those included in the patient group (unexplained infertile) should not have been able to achieve pregnancy despite unprotected sexual intercourse for at least 12 months. Unexplained infertility group selection and exclusion criteria are given below. The inclusion criteria of the cases in the study were (1) regular menstrual cycles, (2) being under the age of 35, (3) a marriage period of 1 year and/or more, (4) no pathology in hysterosalpingography (HSG), (5) no abnormality in the urological examination and semen analysis results of the spouse according to 2010 WHO evaluation reports, and (6) the presence of normal ovulation findings. Exclusion criteria are as follows: (1) there is a risk in the anamnesis and family history for premature ovarian failure, (2) having had pelvic surgery for any reason, (3) any endocrine pathology or systemic disease, (4) having gynecological diseases such as menorrhagia or dysfunctional uterine bleeding, (5) finding mental illness, and (6) long-term drug use, and (7) body mass index value is not within normal limits (normal BMI range: 18.5–25 kg/m^2).

In the control group, women under the age of 35 who had at least one child and had no history of infertility were admitted to the clinic for a normal gynecological examination.

Study protocol

For all cases, hormonal and transvaginal ultrasonographic evaluations on the 3rd day of menstruation were performed.

Antecubital venous blood samples were obtained for serum baseline FSH, estradiol, inhibin B, and anti-müllerian hormone levels. On the same day, total basal ovarian volume measurement, stromal blood flow, and antral follicle count were performed using transvaginal ultrasonography.

Five milliliters of antecubital venous blood from all cases included in the study group was collected on the 3rd day of menstruation, and centrifugation was performed for 10 min at 3000 p.m. The sera obtained were stored in 1.5-ml polypropylene tubes in a refrigerator at − 80 °C.

FSH and estradiol were studied with the immunological method at the Beckman Coulter DXI 800 autoanalyzer with its original kit using the paramagnetic particle chemiluminescence immune analysis method. Estradiol has been studied at the Beckman Coulter DXI 800 autoanalyzer with its original kit using a paramagnetic particulate chemiluminescence immune analysis method. The blood samples taken into biochemistry tubes were centrifuged at 3000 rpm in the Medical Biochemistry Laboratory to separate the serum parts. Separated serum samples were aliquoted and kept at − 80℃. Commercially available Elisa kits for AMH and inhibin B were used in serum samples. The concentration of samples was calculated as ng/mL using the equation in this graph.

All ultrasonographic evaluations were done by one of the authors. Ovarian volume was measured using the prolate ellipsoid formula ($Vol = D1 \times D2 \times D3 \times \pi/6$) by measuring the widest longitudinal (D1), anteroposterior (D2), and transverse (D3) diameters of both overs. Total basal ovarian volume was obtained by summing both ovarian volume values. Round or oval anechoic structures observed in the ovaries were accepted as antral follicles. All follicles between 2 and 10 mm observed in both ovaries were counted and recorded as antral follicle counts (AFC). The average of the measurements of the diameters was taken in the two planes to calculate the follicle diameters.

The most centrally located vascular blood flows were measured in the stroma of the ovary, whose volume was measured for Doppler measurements. Vascular signal distribution properties were subjectively evaluated and divided into mild (1–2 spot blood), moderate (3–5 spot blood), and advanced (> 5 spot blood) grades. When more than one artery was viewed, the best-imaged arterial blood flow measurements were taken as the basis. Peak systolic velocity (PSV), S/D (systole and diastole ratio), resistance index (RI), and pulsatility index (PI) were calculated by taking the average of three waveforms in a row.

Statistical analysis

A Student's *t* test was used since the data were suitable for normal distribution in comparing the measurement data between the two groups. The Pearson correlation test was used to examine the relationship between the data and each other. The suitability of the data for normal distribution was evaluated with the one-sample Kolmogorov–Smirnov test. All statistical analyses were made using Statistical Package for the Social Sciences, version

13.0, SPSS Inc., Chicago, Illinois, USA (SPSS). A *P* value was considered statistically significant.

Results

The average age of cases in the unexplained infertility and control groups was 29.28 ± 3.99 and 29.74 ± 2.84, respectively. The comparison of clinical, laboratory, and ultrasonographic findings in unexplained infertility and control groups is given in Table 1.

The mean AMH (9.19 ± 2.86 vs. 7.86 ± 2.11, $p = 0.031$) and inhibin B (58.53 ± 9.99 vs. 53.46 ± 9.42, $p = 0.032$) values of the control group patients were higher than those in the unexplained infertility group.

The values of means of peak systolic flow (20.22 ± 7.06 vs. 24.70 ± 8.52, $p = 0.019$), S/D (4.01 ± 1.11 vs. 6.02 ± 2.59, $p < 0.001$), and stromal blood flow (3.28 ± 1.20 vs. 3.97 ± 1.38, $p = 0.030$) in the control group were higher than the mean value of the unexplained infertility group. The values of the means of RI (0.77 ± 0.10 vs. 0.70 ± 0.11, $p = 0.007$) and PI (2.51 ± 0.63 vs. 2.06 ± 0.53, $p = 0.002$) of the cases in the control group were lower than those in the unexplained infertility group.

Considering the unexplained infertility group, there was a negative correlation ($r = -0.345$, $p = 0.041$) between baseline FSH and estradiol levels, a positive correlation between AMH and PSV levels ($r = 0.359$, $p = 0.034$), a positive correlation between ovarian volume and AFC levels ($r = 0.460$, $p = 0.005$), and a positive correlation between RI and PI levels ($r = 0.563$, $p < 0.001$). No statistically significant correlation was found between other sonographic and laboratory parameters.

Sensitivity and specificity calculations for baseline FSH, Estradiol, AMH, Inhibin B, ovarian volume, total AFC, PSV, S/D, RI, PI, and stromal blood flow were done using ROC (receiver operating characteristic curve) graphics (Fig. 1). When PI's value was ≥ 2.00, its sensitivity was 65.7%, and its specificity was 64.3%. In the case of the RI, its value was ≥ 0.745, its sensitivity was 65.7%, and its specificity was 62.9%. When estradiol's value was ≥ 040.0, sensitivity was 54.3%, and specificity was 54.3%. The RI value was found to have the highest sensitivity and specificity. It is seen that PI is at the top of the ROC curve, followed by RI and estradiol. The area under the curve was determined to be 0.702 for PI (95% CI 0.581–0.823, $p = 0.04$), 0.676 for RI (95% CI 0.149–0.387, $p = 0.01$), and 0.608 (95% CI 0.474–0.742, $p = 0.121$) for estradiol. In order to show ovarian reserve in unexplained infertile cases, the sensitivity values can be sorted from high to low as follows: PI > RI > estradiol > FSH > ovarian volume > AFC > inhibin B > stromal blood flow > PSV > S/D. (Graph 1).

Discussion

Unexplained infertility: basic infertility evaluation tests are for cases that are found to be normal and those who have difficulty getting pregnant. In other words, they are cases whose problems in the process of having a child cannot be revealed by current infertility assessment tests. In this context, it was planned in this study to determine whether unexplained infertile cases considered to have normal ovarian reserve have a peculiar ovarian reserve disorder, and particularly promising results in terms of ultrasonographic ovarian reserve tests such as PI and RI have been obtained.

The ovarian reserve shows the reproductive potential of the woman, the number of primordial follicles, and oocyte quality. A large number of studies on the tests used for ovarian reserve determination and extensive

Table 1 Comparison of clinical, laboratory, and ultrasonographic findings in unexplained infertility and control groups

	Unexplained infertility group ($n = 35$)	**Control group ($n = 35$)**	***p***
Mean age (year)	29.28 ± 3.99	29.74 ± 2.84	> 0.05
Mean body mass index (kg/m^2)	22.60 ± 1.54	22.70 ± 1.65	> 0.05
Basal FSH *(mIU/ml)*	6.00 ± 1.71	6.4 ± 2.41	> 0.05
Estradiol *(pg/ml)*	43.48 ± 16.43	38.85 ± 17.35	> 0.05
AMH *(ng/ml)*	7.86 ± 2.11	9.19 ± 2.86	**0.031**
Inhibin B *(pg/ml)*	53.46 ± 9.42	58.53 ± 9.99	**0.032**
Ovarian volume (cm^3)	14.21 ± 6.51	15.41 ± 6.95	> 0.05
Antral follicle count (no.)	11.62 ± 2.37	12.31 ± 2.23	> 0.05
PSV *(cm/s)*	20.22 ± 7.06	24.70 ± 8.52	**0.019**
S/D	4.01 ± 1.11	6.02 ± 2.59	**< 0.001**
RI	0.77 ± 0.10	0.70 ± 0.11	**0.007**
PI	2.51 ± 0.63	2.06 ± 0.53	**0.002**
Stromal blood flow	3.28 ± 1.20	3.97 ± 1.38	**0.030**

PSV Peak systolic velocity, *S/D* Systol/diastole, *RI* Resistivity index, *PI* Pulsatility index. Abbreviations are given. Student's *t* test was used for comparison

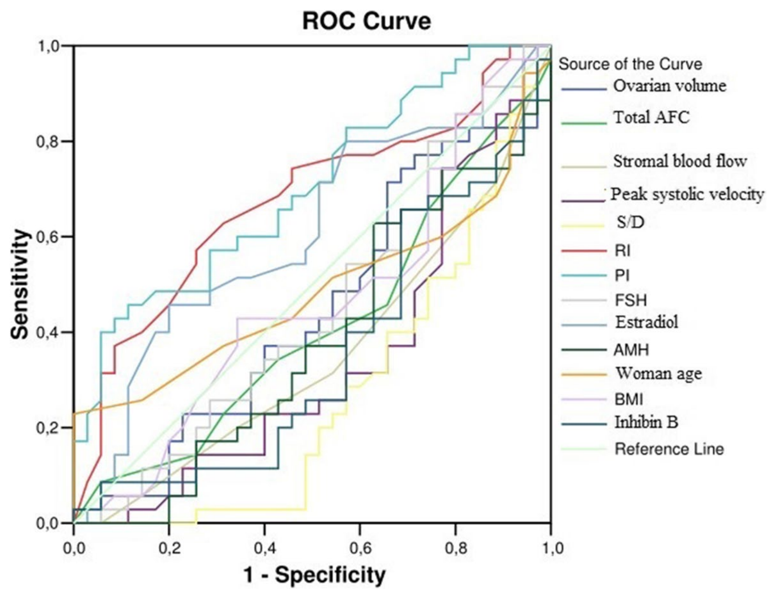

Fig. 1 ROC curves for basal FSH, Estradiol, AMH, Inhibin B, ovarian volume, total AFC (antral follicle count), PSV (peak systolic velocity), S/D (systolic/diastolic), RI (resistivity index), PI (pulsatility index), stromal blood flow, age, and BMI (body mass index) values

reviews have been published [4]. However, there is not enough data about which ovarian reserve test is ideal for unexplained infertile cases. The ideal marker to be used in evaluating the ovarian reserve should be cheap, easy to measure, minimally invasive, and have good predictive value.

FSH levels are regulated by the granulosa cell product and are indirectly related to the number of antral follicles. The direct evaluation of the number of primordial follicles in women is difficult, and the growing follicle, that is, the number of antral follicles, is correlated with the number of primordial follicles [5]. In a recent study, low 3rd day E2 levels combined with normal FSH levels were found to be associated with increased stimulation response, a high pregnancy rate, and decreased cycle cancellation rates [6]. Increased follicular estradiol levels in the early period are associated with decreased ovarian response [7]. However, we could not obtain statistically significant results in terms of classical ovarian reserve tests such as FSH, E2, and also AFC, female age, and ovarian volume.

AMH can be evaluated at any stage of the menstrual period, regardless of the cycle [8]. In recent years, the importance of AMH in determining the ovarian reserve has been increasingly understood. AMH had 83–84% sensitivity and 67–79% specificity in determining ovarian reserve and predicting oocyte count [9]. It has also been known that inhibin-B released from the ovaries inhibits the release of FSH. IVF cycles with poor outcomes were detected in patients with a low inhibin-B value on day 3 and whose FSH was not elevated. Some researchers have reported that inhibin-B is useful in predicting ovarian response [10, 11], while other studies have reported no contribution [12, 13]. Compared with the control group, the low AMH and inhibit B values in unexplained infertile cases suggested that there was an ovarian reserve misfunction (which may be at the molecular level) in unexplained infertile cases, which cannot be demonstrated by today's technology.

Ultrasonographically, ovarian reserve assessment is a fast, accurate, and convenient method in terms of cost and effectiveness. Decreased ovarian arterial blood flow in the early follicular phase was detected in women with low ovarian reserve. Ovarian stromal peak systolic velocity (PSV) is the single most important independent factor in showing ovarian reserve according to serum FSH levels. High PSV (≥ 10) found in patients was associated with high clinical pregnancy rates [14]. The mean PSV, stromal blood flow, and S/D values of the cases in the control group were higher than the mean values of the patient group. Although there was a statistically significant increase in these parameters compared to the patient group in the control group, the sensitivity of these tests was found to be low in this patient group. These findings support the conclusion that ovarian reserve function may be affected in unexplained infertile cases.

The mean PI and RI values of the cases in the control group were lower than the mean value of the patient group. In a previous study, it was observed that follicles that would be ovulated in the ovaries had a decrease in PI and RI [15]. Considering high sensitivity values for predicting unexplained infertility, RI stands out as the second most sensitive parameter after PI. The low arterial pulsatile index (PI) in the ovarian stroma was associated with high pregnancy rates and decreased gonadotropin use during ovarian stimulation [16]. In the current study, PI has the largest area under the ROC curve compared to other tests. Just as with the RI value, it can be used as a marker to evaluate the ovarian reserve in infertile cases whose PI value cannot be explained.

Strengths and limitations

The most important limitation of the study was the small number of cases and the infertility cases' inability to reveal the relationship between these ovarian reserve values and infertility treatment outcomes. A new large-series study including cases with infertility treatment may correct these limitations.

Conclusıon

Decreased PI and RI ratios in the Doppler ultrasound were found to have the highest sensitivity and specificity for predicting an unexplained infertile woman. All these results revealed the argument that in cases of unexplained infertility, even if the ovarian reserve tests were within normal limits, in these cases, different results compared to the control actually have a border ovarian reserve in etiology. Large-scale research will further strengthen this result.

Abbreviations

AMH	Antimullerian hormone
AFC	Total antral follicle count
PSV	Peak systolic velocity
S/D	Systole and diastole ratio
PI	Pulsatile index
RI	Resistivity index

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Authors' contributions

All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. MS, SG, HS, and ESGG conceived, designed, and performed the experiment; MS and SG analyzed the data.

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Availability of data and materials

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Declarations

Ethics approval and consent to participate

All procedures performed in the study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was conducted after the approval of our research ethics committee. Informed consent was obtained from all participants before recruitment.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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