

# Antiovarian Antibody may be Used as A Predictor for Poor *In Vitro* Fertilization Outcome

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## Abstract

**Objective:** The aim of this study is to demonstrate if there is any difference in the presence of antiovarian antibody (AOA) in normal fertile women and the ones having poor ovarian response and to examine if serum AOA levels could be used as a marker to detect patients at risk for poor ovarian reserve.

**Materials and Methods:** 51 premature ovarian insufficiency (POI) patients, 47 poor responder (POR) patients to *in vitro* fertilization (IVF) and 51 healthy controls were included in the study. The patients were examined for their basal hormone values and antral follicle counts. Venous blood sampling for the detection of AOA was applied and ELISA technique was used to detect AOA concentrations.

**Results:** The AOA levels were detected as  $7.91 \pm 1.11$  U/mL in POI group,  $5.72 \pm 1.00$  U/mL in the fertile group and  $8.30 \pm 0.75$  U/mL in the POR group. It was significantly elevated in the POI and POR groups when compared to the fertile group ( $p < 0.001$ ). There was not any difference between the AOA levels of POI and POR groups ( $p = 0.053$ ). The cut off levels of AOA for diagnosis of POI was detected to be 6.58 U/mL and for POR it was 7.78 U/mL. When these cut off levels were used, the sensitivity ratio was calculated as 90.2% for POI, and 94.74% for POR groups; whereas the specificity was 86.27% for POI and 83.05% for POR groups. In multivariate regression analysis of the POR group, AOA level was demonstrated to be the only significant independent variable in predicting the patients.

**Conclusion:** Antiovarian autoimmunity plays a role in the etiopathogenesis of POI and POR; but the exact mechanism still needs to be elucidated with more clinical trials. AOA can be used as serum marker to detect the POR patients and predict IVF results. Immunosuppressive treatment of these patients may enhance the pregnancy outcomes.

**Keywords:** Antiovarian antibody; Premature ovarian insufficiency; IVF; Poor responder

## Abbreviations

**AFC:** Antral Follicle Count; **AOA:** Antiovarian Antibody; **AUC:** Area Under the Curve; **BMI:** Body Mass Index; **E2:** Estradiol; **FSH:** Follicle Stimulating Hormone; **LH:** Luteinizing Hormone; **IVF:** *In Vitro* Fertilization; **OD:** Optic Density; **POI:** Premature Ovarian Insufficiency; **POR:** Poor Responder; **PRL:** Prolactin; **ROC:** Receiver Operating Characteristic; **SD:** Standard Deviation; **TSH:** Thyroid Stimulating Hormone

## Introduction

The role of autoimmunity in ovarian pathologies has been investigated in some studies [1,2]. The exact mechanism of autoimmunity playing role in the pathogenesis of idiopathic infertility, premature ovarian insufficiency (POI) and in poor responders (POR) to *in vitro* fertilization (IVF) has not been clearly elucidated, yet. Especially, antiovarian antibody (AOA), has been suspected to be a significant marker of the autoimmunity existing in the pathogenesis of POI and POR [3-5]. It was also shown to play role in the mechanism causing polycystic ovary syndrome, unexplained infertility and endometriosis, developing in the autoimmunity background [6].

AOA positivity was observed in 0-4% of normal population and, it was observed more in the patients of POI and POR to IVF [7]. The circulating AOA together with lymphocytic infiltrates in the ovaries were demonstrated in primary ovarian insufficiency patients and; an immune mechanism causing the situation was proposed [8]. Ovarian dysfunction is commonly related to autoimmune endocrine diseases like Hashimoto's disease, Graves' disease, and Addison's disease; and non-endocrine autoimmune diseases like, systemic lupus erythematosus [9,10]. Therefore, AOA may be a demonstrator of ovarian autoimmunity in the patients ovarian dysfunction;

beside of the other specific antibodies to other autoimmune diseases. So, it is possible for AOA to be used in the prediction of ovarian insufficiency in unexplained infertility patients [11,12].

Our aim in this study was to demonstrate if there was any difference in the presence of AOA in the normal fertile women and the ones having poor ovarian response and to examine if serum AOA levels could be used as a marker to detect patients at risk for poor ovarian reserve.

## Materials and Methods

51 POI and 47 POR patients, who admitted to infertility clinic of Dr. Zekai Tahir Burak Women Health Education and Research Hospital, were included in the study group. 51 healthy controls, who admitted to the gynecology clinic of the same hospital, were included as the control group. All of the patients were homogenized for their ages and body mass indices (BMI).

The patients having oligo menorrhoea and/or amenorrhoea at least for four months and having follicle stimulating hormone (FSH) levels above 25 IU/L twice in a time period longer than four weeks, were accepted as POI [1]. POR criteria were as follows:

Advanced maternal age (above 40 years) or another risk factor like developing ovarian insufficiency after twice of maximum stimulation,

Previous experience of POR (3 or less oocytes with the conventional stimulation protocols),

Abnormal ovarian reserve (i.e. less than 5-7 antral follicle count (AFC), or antimullerian hormone level less than 0.5-1.1 ng/mL). The patients having 2 of these 3 criteria were accepted as POR [13].

The patients having menstrual periods longer than 45 days, or having 8 or less cycles in a year were defined as oligomenorrhoeic and the ones which did not have menstruation at least for 3 months were defined as amenorrhoeic [14]. The patients, who had proved their fertility with at least one delivery, and admitted to the family planning unit to obtain a birth control method during their menstrual days, were included in the control group.

The patients having any chromosomal abnormality, pregnancy, any chronic medical condition (i.e. kidney disease, diabetes, hypertension, metabolic syndrome, Cushing's syndrome, androgen secreting tumors, late onset 21-hydroxylase deficiency, hyperprolactinemia, polycystic ovary syndrome, endometrioma), and using pills for these diseases, smoking, older than 40 years of age and having previous ovarian surgery were excluded.

The committee of ethics of Dr. Zekai Tahir Burak Women Health Education and Research Hospital had approved the study protocol, and all of the patients included in the research signed their informed consents.

All of the patients of the study were studied in their early follicular phase, at the third day of their spontaneous menstrual cycles. The patients did not have any ovulation induction treatment with clomiphene citrate or gonadotropins, before venous sampling. FSH, luteinizing hormone (LH), estradiol (E2), prolactin (PRL), and thyroid stimulating hormone (TSH) levels were measured using UniCel DxI 800 Immunoassay System (Beckman Coulter, Fullerton, CA, USA). Ovaries were examined with 7 MHz transvaginal ultrasound convex transducer (SSD 1000; Aloka, Tokyo, Japan). BMI were calculated with the formula, weight (kg)/square of height (m<sup>2</sup>).

The venous blood samples of all of the patients were centrifuged at 5000 revolution per minute (2236 x g) within one hour and stored at -80°C, to be studied all together.

The presence of ovarian autoantibodies was evaluated by the enzyme-linked immunosorbent assay (ELISA) technique. The AOA ELISA Ig-classifying test used is a solid-phase sandwich enzyme immunoassay for the quantitative determination of AOA in human serum by optical density results, from Bioserv Diagnostics (Rostock, Germany).

The ELISA plate was coated with a mixture of ovarian proteins used as capture antigens for AOA. The samples and controls were pipetted into the wells and then incubated. During incubation, AOA were bound to the antigen and immobilized on the plate. A conjugate consisting of antibodies against different regions of different classes of human immunoglobulin (IgA, IgG, IgM) was bound to the antigen-antibody complex during incubation. After removal of the unbound conjugate by washing with horseradish peroxidase, the substrate 3, 30, 5, 50-tetramethylbenzidine was added, yielding a color reaction that was stopped with 0.25 M of sulfuric acid. The extinction was measured at a wavelength of 450 nm with a microplate reader.

We preferred to use the AOA concentration levels rather than OD levels, because of the linear relationship between concentration and OD levels.

## Statistical analysis

Data analysis was performed by using SPSS for Windows, version 17 (SPSS Inc., Chicago, IL, United States). The Kolmogorov Smirnov test was used to test whether continuous variables were distributed normally or not. Homogeneity of variances was evaluated by the Levene test. Continuous variables were shown as mean ± standard deviation (SD). Mean differences between groups were compared by ANOVA test. The optimal cut-off points of the clinical measurements, which discriminated groups from each other, were evaluated by receiver operating characteristic (ROC) analysis calculating area under the curve (AUC) as giving the maximum sum of sensitivity and specificity for the significant test. Sensitivity, specificity, positive and negative predictive values were also calculated at the best cut-off point for each clinical measurement. The best predictors which discriminated groups from each other were determined by multiple logistic regression analysis, where applicable. Any variable, whose univariate test had a p-value < 0.05 was accepted as a candidate

for the multivariable model, along with all variables of known clinical importance. Adjusted odds ratios, 95% confidence intervals and Wald statistics were calculated for each variable. Any p-value less than 0.05 were considered statistically significant.

## Results

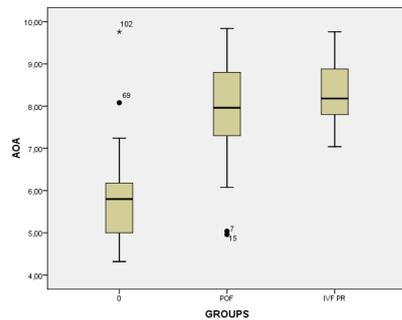
149 patients, including 51 POI, 47 POR patients having IVF treatment because of poor ovarian capacity, and 51 healthy controls, were studied. The groups were homogenized for their

ages and BMI. The clinical, ultrasonographic and andropometric parameters of the groups were demonstrated in Table 1. 27 patients (52.9%) of the POI group and only 1 patient (2.2%) of the POR group were amenorrheic, whereas fertile group did not possess any amenorrheic patients. This difference was significant when the POI and POR groups were compared with the fertile group individually ( $p < 0.001$  and  $p < 0.001$ , respectively). Having AFC less than 8 was present in 47 patients (92.2%) of POI group, 24 patients (52.2%) of POR group and 5 patients (9.8%) of the fertile group; which was significantly different ( $p < 0.001$  and  $p < 0.001$ ) (Table 1).

**Table1:** Comparison of clinical, ultrasonographic and andropometric parameters of the groups (\*Independent simple t test, \*\*Pearson's chi-square test. p value; within groups, \*\* p value between groups. **POF:** Premature Ovarian Failure; **POR:** Poor Responder; **AFC:** Antral Follicle Count; **BMI:** Body Mass Index).

	GROUP 1	GROUP 2 (CONTROL)	GROUP 3	p* value	p** value
	(POF)	N=51	(POR)		
	N=51		N=47		
Age*	31.82 ± 5.57	31.39 ± 5.36	32.39 ± 4.76	P(1-2)=0.621 P(2-3)=0.756 P(1-3)=0.596	0.523
BMI *	29.89 ± 3.82	29.03 ± 3.66	28.87 ± 4.12	P(1-2)=0.769 P(2-3)=0.069 P(1-3)=0.082	0.07
Amenorrhea **	27(52.9%)	0(0%)	1(22%)	P(1-2)<0.001 P(2-3)=0.474 P(1-3)<0.001	<0.001
POF in the mother **	5(9.8%)	6(11.8%)	3(6.5%)	P(1-2)=0.500 P(2-3)=0.298 P(1-3)=0.417	0.675
POF in the sister **	6(11.8%)	1(2.0%)	1(2.2%)	P(1-2)= 0.032 P(2-3)=0.726 P(1-3)=0.044	0.046
AFC<8**	47(92.2%)	5(9.8%)	24(52.2%)	P(1-2)<0.001 P(2-3)=0.001 P(1-3)<0.001	<0.001

The mean level of AOA was detected as  $7.91 \pm 1.11$  U/mL in the POI group;  $8.30 \pm 0.75$  in the POR group and  $5.72 \pm 1.00$  in the fertile group. AOA was found significantly elevated in the POI and POR groups than the control group ( $p < 0.001$  and  $p < 0.001$ , respectively), but did not differ when the two were compared ( $p = 0.053$ ) (Table 2, Figure 1).



**Figure 1:** Distribution of AOA in the POR, POI and control groups.

FSH and LH levels in the patients were significantly higher in POI group than the control and POR group ( $p < 0.001$  and  $p < 0.001$ , respectively). E2 levels in the POR group were higher when compared to the POI group ( $p < 0.001$ ); but did not differ from the E2 levels of the control group ( $p = 0.089$ ). PRL was significantly higher in the POR group than the other two groups ( $p = 0.030$  and  $p = 0.033$ , respectively) (Table 2).

**Table 2:** Comparison of hormonal parameters and AOA among groups (**FSH:** Follicle Stimulating Hormone; **LH:** Luteinizing Hormone; **E2:** Estradiol; **PRL:** Prolactin; **TSH:** Thyroid Stimulating Hormone; **AOA:** Anti Ovarian Antibody).

	GROUP 1	GROUP 2	GROUP 3	P* VALUE WITHIN GROUPS	P** VALUE BETWEEN GROUPS
	(POI) N=51	(CONTROL) N=51	(POR) N=47		
FSH (mIU/ml)	46.43 ± 28.28	6.99 ± 2.00	7.06 ± 2.36	P(1-2)<0.001 P(2-3)=0.983 P(1-3)<0.001	<0.001
LH (mIU/ml)	27.73 ± 20.73	4.80 ± 2.74	4.45 ± 2.15	P(1-2)<0.001 P(2-3)=0.888 P(1-3)<0.001	<0.001
E2 (pmol/l)	41.41 ± 30.03	50.62 ± 27.62	59.92 ± 21.08	P(1-2)=0.083 P(2-3)=0.089 P(1-3)=0.001	0.004
PRL (ng/ml)	11.35 ± 8.48	11.42 ± 9.22	15.57 ± 8.29	P(1-2)=0.971 P(2-3)=0.033 P(1-3)=0.030	0.048
TSH (uIU/ml)	2.15 ± 1.44	1.88 ± 1.24	2.66 ± 1.80	P(1-2)=0.354 P(2-3)=0.011 P(1-3)=0.096	0.036
AOA (U/ml)	7.91 ± 1.11	5.72 ± 1.00	8.30 ± 0.75	P(1-2)<0.001 P(2-3)<0.001 P(1-3)=0.053	<0.001

ROC curve analysis was applied for the POI and POR groups. 6.58 U/mL and 7.78 U/mL were defined as cut off levels for serum AOA for the POI and POR groups, respectively [area under curve (95% CI); 0.926(0.867-0.985) and 0.782 (0.711-0.853)]. These cut off levels led to sensitivity ratios of 90.20% and 94.74% for the POI and POR groups, respectively;

whereas the specificity ratios were calculated as 86.27%, and 83.05%, respectively (Table 3).

**Table 3:** AOA cut off levels, sensitivity and specificity ratios and predictive values for the POF and POR groups (**POF:** Premature Ovarian Failure; **POR:** Poor Responder; **AOA:** Anti Ovarian Antibody).

AOA	Cut off (U/ml)	Sen(%)	Spe (%)	Positive predictivity (%)	Negative predictivity (%)	Area under the curve (95% CI)	P value
					(%)		
POF	6.58	90.20%	86.27%	86.79%	89.80%	0.926 (0.867-0.985)	<0.001
POR	7.78	94.74%	83.05%	78.26%	96.08%	0.782 (0.711-0.853)	<0.001

Multiple regression analysis was used to calculate the predictive independent variables in detecting increased POR risk. Above the AOA cut off level ( $\geq 7.780$  U/ml) with odds ratio: 13.978, 95% confidence interval CI: 4.383-44.573 was detected and AOA was calculated to be the only significant

independent variable in predicting POR patients ( $p < 0.001$ ) (Table 4). Age and AFC less than 8 was found to be related to POR, in univariate analysis, whereas in multivariate analysis, they were not related to POR risk prediction ( $p = 0.121$  and  $p = 0.261$ , respectively) (Table 4).

**Table 4:** Relations of the variables with POR (**FSH:** Follicle Stimulating Hormone; **LH:** Luteinizing Hormone; **AFC:** Antral Follicle Count; **AOA:** Anti Ovarian Antibody).

	Univariate		Multivariate	
	AUC (95% CI)	p value	AUC (95% CI)	p value
Age	1.123 (1.033-1.220)	0.006	1.174 (0.959-1.437)	0.121
Irregular menses	1.093 (0.310-3.864)	0.89		
POF experienced mother	0.523 (0.123-2.225)	0.381		
POF experienced sister	1.111 (0.068-18.289)	0.941		
Presence of autoimmune disease	0.516 (0.156-1.709)	0.279		
FSH (mIU/ml)	1.015 (0.844-1.221)	0.872		
LH (mIU/ml)	0.841 (0.793-1.116)	0.484		
AFC < 8	0.122 (0.044-0.342)	<0.001	0.295 (0.035-2.478)	0.261
AOA (U/ml)	16.489 (5.309-51.212)	<0.001	13.978 (4.383-44.573)	<0.001

Multiple regression analysis was applied to define independent variables in predicting increased POI risk. Only the FSH, with OR: 1.643, 95%CI: 1.063-2.539, was demonstrated to have positive predictive value in observing

POI ( $p = 0.025$ ) (Table 5). Although AOA, AFC < 8, LH, age and irregular menses were found related to POI in univariate analysis; they were not calculated so in multivariate analysis (Table 5).

**Table 5:** Relations of the variables with POF (**FSH:** Follicle Stimulating Hormone; **LH:** Luteinizing Hormone; **AFC:** Antral Follicle Count; **AOA:** Anti Ovarian Antibody).

	Univariate		Multivariate	
	AUC (95% CI)	p value	AUC (95% CI)	p value
Age	1.085 (1.008-1.167)	0.029	1.466 (0.816-2.631)	0.2
Irregular menses	0.067 (0.020-0.222)	<0.001	6.318 (0.000-30.278)	0.737
POF experienced mother	0.815 (0.232-2.862)	0.75		
POF experienced sister	6.667 (0.773-57.518)	0.084		
Presence of autoimmune disease	0.584 (0.177-1.925)	0.377		

FSH (mIU/ml)	1.649 (1.265-2.150)	<0.001	1.643 (1.063-2.539)	0.025
LH (mIU/ml)	1.605 (1.283-2.010)	<0.001	1.825 (0.887-3.754)	0.102
AFC <8	0.011 (0.003-0.043)	<0.001	6.813 (0.025-18.499)	0.502
AOA (U/ml)	5.844 (3.050-11.196)	<0.001	0.947 (0.065-13.851)	0.947

## Discussion

In this study, we aimed to find out if AOA is predictive or not in the diagnosis of patients having poor ovarian capacity. When we compared the groups, we observed that POI and POR groups had significantly higher levels of AOA than the fertile control group; whereas they were not different from each other. We also observed decreased AFC in the POI and POR groups than the control group, as expected [15]. When the basal hormone levels were compared, FSH and LH levels were obviously higher; E2 levels were lower in the POI group than the POR and control groups. There was not a difference in hypothyroidism frequency between the groups; but the POR group had higher TSH and PRL levels than the other two; which made us think that subclinic hypothyroiditis was more frequent in the POR group.

The mechanisms of some specific antigens targeting ovaries and taking place in the development of POI, were described. Steroid cell antibodies [16], highly positive in especially Addison's disease, 3 $\beta$ -hydroxysteroid dehydrogenase autoantibodies [17], and gonadotropin receptor blocking antibodies [18] are examples to these. Although the case number is limited, Damewood et al. [19] showed AOA presence in 9 of 27 POI patients, immunohistochemically; and Luborsky et al. [1] demonstrated AOA in 33 of 45 POI patients having IVF.

The researches studying antiovarian autoimmunity and POI show that genetic factors may be effective on disease pathogenesis [20]. Familial prevalence of POI varies between 4%-31%. The wide range may be caused by the changes in the definition of POI, election of the study population, and the presentation of the data [21]. In our study, having a POI experienced mother or sister prevalence in the POI group was 17.6%, whereas it was 9.8% in the control group. The incidence was suitable with the literature, but the difference between the groups was not important. Besides, the AOA concentrations of the patients who had a family member experiencing POI and who had not, did not differ in our study.

We observed that only the elevation of the levels of FSH in the POI group, and elevation in the levels of the AOA in POR group, had predictive value in the diagnosis of the diseases. Although AOA level was increased in both of the POI and POR groups, it was only predictive in the POR group.

Antiovarian autoimmunity was shown to be relevant in the ethiopathogenesis of the POR group [22,23]. Similarly, our study demonstrated the significantly higher incidence of AOA in the POR group than the control group. In rat studies, anti-oocyte cytoplasm antibodies in the follicle aspirates of the ovarian insufficiency group were found in elevated levels than

the control group's [24]. A negative relationship between ovarian autoimmunity and outcome of IVF attempts has been demonstrated in a number of studies, and it has been speculated that repeated IVF attempts might induce AOA [3,25-27]. Measuring AOA before the treatment period; may help to inform patients about the success of the IVF attempt [28,29]. There are also studies, showing the success of immunosuppressive treatment in obtaining pregnancy, in POR and POI patients [25,30-33].

## Conclusion

The antiovarian autoimmunity plays a role in the ethiopathogenesis of POI and POR to IVF treatments; but the exact mechanism still needs to be elucidated with more clinical trials. De novo genetic anomalies (like mutation, deletion, duplication) which may cause ovulatory dysfunction, protein expression abnormalities (real-time PCR), the histone methylation mechanisms of the DNA, immune mechanisms and the biochemical changes in molecular levels must be investigated.

Our study has limitations because of small sample size; but we observed a definite difference in AOA concentrations of POR patients and the healthy, fertile controls; and detected a serum cut off level for AOA; which can be used as serum marker to detect the POR patients and predict IVF results. Besides, using some kind of steroid treatments may suppress the autoimmunity in these patients, and may help to enhance the pregnancy outcomes.

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