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ORIGINAL ARTICLE

Assessment of the long Pentraxin 3 level in Polycystic Ovarian Syndrome-related infertility

Short title: *PTX3 in PCOS-related infertility.*

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ABSTRACT

Objective. To evaluate the role of Pentraxin 3 level in PCOS-related infertility and its correlation with the disease's hormonal profile.

Patients and Methods. A case-control study involves a total of 90 women for one year, 30 women diagnosed with PCOS who are fertile, 30 women diagnosed with PCOS who are infertile, and 30 healthy controls, all women in the early follicular phase were sent for baseline investigations: FSH, LH, AMH, Fasting blood sugar, Insulin, Testosterone, TSH, and Pentraxin 3 that were measured by sandwich electrochemiluminescence immunoassay.

Results. There were statistically significant variations in LH, LH/FSH ratio, Testosterone levels, and AMH across the groups. The mean of Pentraxin for the fertile PCOS group (4.14 ± 1.97) ng/mL was significantly higher than for the infertile PCOS group (1.39 ± 1.10 ng/Ml) and control (1.99 ± 1.66 ng/mL). For the infertile PCOS group, the correlation of Pentraxin was significantly positive with age and negative with AMH. ROC Curve analysis showed a cutoff value of 1.05 ng/ml with a sensitivity of 46.67% and specificity of 83.33% for the infertile group.

Conclusions. Pentraxin 3 level is significantly higher in fertile PCOS patients and lower in the infertile PCOS group in comparison to the control group suggesting its possible role in PCOS-related infertility.

Key words

Infertility; Pentraxin 3; Polycystic Ovarian Syndrome.

Introduction

Polycystic ovary syndrome (PCOS) is among the common endocrinopathies seen in females and is manifested with hyperandrogenism, oligo-ovulation, and polycystic ovaries on ultrasound examination with established long-term metabolic disorders [1]. The main underlying pathology is not fully understood for which the researchers postulated many possible theories underlying its etiology as the microcystic ovarian inflammation theory, the ovarian congestion theory, and the ovarian dystrophy theory, with possible morphological changes expressed as thick ovarian struma that is reflected in its endocrine function [2].

The ovarian folliculogenesis is disrupted with consequent abnormal recruitment of the follicles resulting from inadequate Follicle stimulating hormone production and local action with excessive Antimullerian hormone and other factors mediating follicular growth a fact that differentiates PCOS from other pathologies leading to ovarian dysfunction [3].

A meta-analysis described four phenotypes of this syndrome depending on the presenting complaints as Phenotype A: the full PCOS picture including hyperandrogenism, oligo-ovulation, and polycystic ovarian ultrasound morphology, Phenotype B: hyperandrogenism and oligo-ovulation. Phenotype C: hyperandrogenism and polycystic ovarian ultrasound morphology, Phenotype D: oligo-anovulation and polycystic ovarian ultrasound morphology [4].

Investigating its pathogenesis is paramount, which may help in the treatment as the available treatment till now is directed towards the symptomatology rather than the disease process itself. Among the inflammatory markers studied in PCOS, the long Pentraxin 3 (PTX3) was chosen to be evaluated in the current study, as it is a multifunctional glycoprotein implicated in innate immunity response, regulation of inflammation, angiogenesis and formation and remodeling of the extracellular matrix [5].

The PTX3 is released by white blood cells and myeloid dendritic cells following stimulation with pro-inflammatory cytokines (Interleukin-1 and Tumour necrosis factor-α), agonists of Tool Like receptors, or microbial components [6]. The production is also stimulated in myeloid cells by the anti-inflammatory cytokine Interleukin-10, which is essential for suppressing inflammation and minimizing tissue damage. Human neutrophils store PTX3 in lactoferrin-positive granules and rapidly release them at the inflammatory site. Additionally, it is produced in many body cells, such as muscles, fibrous tissues, fatty tissues, cartilage, and ovarian tissue, due to its potential role in modulating the inflammatory reaction by binding elements of the complement cascade and regulating complement activation [7]. PTX3 is specifically expressed by the cumulus cells in the ovary, following the Luteinizing hormone or human chorionic gonadotropin stimulation of pre-ovulatory follicles [8].

The presence of PTX3 in the cumulus matrix and in the follicular fluids aspirated from In Vitro Fertilization cases suggest its role in human female fertility [9].

The study aims to evaluate the role of PTX3 level in PCOS-related infertility and to determine its correlation with the disease's hormonal profile.

Materials and Methods

This is a case-control study conducted at the Department of Obstetrics and Gynaecology of Al-Yarmouk Teaching Hospital; the study was conducted for one year, for January 2022 to December 2022.

Inclusion criteria: Of the total women involved in the study, ninety were assigned as:

Fertile PCOS group: Thirty women diagnosed with polycystic ovary syndrome according to Rotterdam's criteria [4] who are fertile corresponding to phenotype C [10].

Infertile PCOS group: Thirty women diagnosed with polycystic ovary syndrome according to Rotterdam's criteria [4] who are Infertile corresponding to phenotype B [10].

Control group: Thirty healthy women who are ovulating with normal ovarian morphology, matched for the age (mean age 27-28 years) and Body mass index (BMI) with PCOS groups.

After obtaining informed consent from all participants, a physical examination was performed, including general weight and height estimation, to calculate BMI and features of androgen excess.

All women in the early follicular phase of their menstrual cycle were subjected to blood sampling of three mills of venous blood in the morning between 8:00 and 9:00 am after overnight fasting state and had been sent for baseline investigations, including:

Luteinizing hormone (LH), Follicle stimulating hormone (FSH), Insulin, Fasting blood sugar (FBS), Antimullerian hormone (AMH), Testosterone, Prolactin, Thyroid stimulating hormone (TSH), and The Long Pentraxin 3. These parameters were analyzed using sandwich electrochemiluminescence immunoassay “ECLIA.”

Exclusion Criteria: Pregnancy, endocrine disorders, chronic diseases, drugs, smoking, alcohol intake, and other causes of infertility: tubal and male factors

Study registration, ethical and methodological standards

The protocol was approved by the Mustansiriyah Scientific Council of Obstetrics and Gynaecology /Iraq MOG-107 in DEC 2021; written informed consent was taken from all participants following the Declaration of Helsinki and local guidelines.

Statistical analysis

Analysis of data was explained using descriptive statistics; mean, standard deviation, standard error of the mean, median, minimum, and maximum. ANOVA Test was used to determine the difference in means among the groups. T-test was used to compare paired groups.

To assess the quality of the correlation using Pearson's method; small effect sizes were defined as correlation coefficients (r) between 0.10 and 0.29, moderate effect sizes as coefficients between 0.30 and 0.49, and large effect sizes as coefficients above 0.50.

ROC curve comparison of Pentraxin, AMH, and LH/FSH ratio as they differentiate between the study groups, the P value was significant if less than 0.05.

Patient and public involvement

Iraqi women at AL Yarmouk teaching hospital / Baghdad.

Results

The demographic, biochemical, and hormonal characteristics of the study groups are clarified in Table (1).

Age and BMI showed no statistically significant variations as the groups were matched for these two variables to minimize their influence on the study results.

Concerning LH level, compared to the infertile group (3.48±1.61) mIU/L, the control (7.22±2.93) mIU/L and the fertile group (9.31±5.07) mIU/L had significantly higher mean LH levels P < 0.001. While the mean FSH for infertile PCOS (4.79±0.95) mIU/L was lower than that of the control group (7.49±1.71) mIU/L, the mean FSH for fertile PCOS (6.98±1.95) mIU/L was higher than that of the non-fertile PCOS group P<0.01.

LH/FSH ratio for the infertile PCOS group had an average of (0.74±0.32) which is lower than The control group (1.05±0.58) and for the fertile PCOS group (1.37±0.74).

Insulin and FBS were significantly lower in the control group than in both PCOS groups.

The mean of AMH for the control (5.90±1.36) ng/mL was significantly lower than for the fertile PCOS group (8.21±1.61) ng/mL and for the infertile PCOS group (8.97±1.83) ng/mL, as P<.001. Likewise, for Testosterone, Control subjects had a significantly lower mean (0.39±0.25) ng/mL than those in the fertile PCOS group (0.62±0.20; p.001) ng/mL and then those infertile PCOS group 0.69±0.16) ng/mL P 0.001 for both comparisons.

Prolactin and TSH showed significant variations among the groups as P value<0.05; however, none exceeds the normal reference values.

Pentraxin levels were different among the groups (ANOVA P.001). Using the t-test, a pairwise comparison between groups was done. The mean of Pentraxin for the fertile PCOS group (4.14±1.97) ng/mL was significantly higher than both control (1.99±1.66) ng/mL, P.001, and infertile PCOS group (1.39±1.10) ng/mL, P.001. While infertile PCOS showed a non-significant lower value than the control group. The Pentraxin levels for PCOS patients and controls are shown in Figure (1).

Correlation analysis of the infertile PCOS group: an examination of the relationship between Pentraxin and the other factors in the infertile PCOS group was performed. Pentraxin was shown to have a moderately positive correlation with age (r=.44, P=.014, 95% CI= [.10,.69]). The correlation between Pentraxin and advancing age is strong enough here to support the conclusion that Pentraxin tends to decrease with advancing age. The inverse relationship between Pentraxin and AMH was statistically significant (r=-.39, P=.031, 95.00% CI= [-.66, -.04]), with a moderate effect size.

Accordingly, as Pentraxin levels rise, AMH levels fall; this data is shown in Table (2).

ROC curve comparison of Pentraxin, AMH, and LH/FSH ratio as they differentiate the infertile PCOS group from the control group showed a good specificity of PTX3 with a high sensitivity of both AMH and LH/FSH ratio as clarified in Figure (2) and Table (3).

Discussion

Main findings

The hormonal investigations showed that there were significant variations in LH, FSH, and their ratio in infertile PCOS patients compared with control groups and the fertile PCOS.

The core result of this study is the significantly lower PTX3 in non-fertile versus fertile PCOS suggests its potential role in ovarian dysfunction and PCOS-related infertility.

The correlation of biochemical and hormonal variables of the infertile study group with Pentraxin showed an inverse relationship between Pentraxin and AMH that was statistically significant with a moderate effect size.

ROC curve analysis revealed a cutoff value of PTX3 (≤1.05) that shows low sensitivity but good specificity, suggesting its possible diagnostic role in PCOS infertility. The application of the ROC curve for AMH and LH/FSH ratio shows higher sensitivity than PTX3 but lower specificity as coincides with another study [34], and this suggests the beneficial application of combined parameters in the diagnosis of PCOS-related infertility.

Strengths and Limitations

The strength of the study is the evaluation of patients with PCOS according to phenotypes and fertility issues to determine the role of markers in different disease categories.

The limitations: enrolment of phenotypes B and C only to achieve a statistically significant sample study size.

Interpretation and comparison with other literature

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in young age women [11]. It presents with hyperinsulinemia, ovarian dysfunction, and metabolic syndrome. Pentraxin is released in response to inflammation, and many studies suggest its usefulness as an inflammatory marker released from peripheral blood leukocytes and myeloid dendritic cells following stimulation with pro-inflammatory cytokines. PTX3 had been evaluated in cases with PCOS with conflicting results regarding obesity, insulin resistance, and infertility, suggesting its role in ovarian function disturbances [12].

Age and BMI variation are common presenting features in PCOS. The current study design considered matching of these variables to minimize their biased effect on the results as accumulating evidence suggests that one of the most important mechanisms of PCOS pathogenesis is the insulin-resistance. For this reason, the use of insulin sensitizers, such as inositol isoforms, gained increasing attention due to their safety profile and effectiveness [13], and other studies showed that there are positive genetic correlations between PCOS and adult BMI [14], but the inverse correlation between PTX 3 and obesity in other studies [15].

The hormonal investigations showed that there were significant variations in LH, FSH, and their ratio in infertile PCOS patients compared with control groups and the fertile PCOS. LH was significantly lower in the infertile group than in controls, but it is higher in the fertile PCOS compared to the control, while other comparative studies revealed higher values in the infertile group versus the fertile group [16]. On the other aspect, the FSH values were significantly lower in the non-fertile PCOS group than in the control and fertile PCOS groups, and this is against another study which showed an insignificant difference between the PCOS group and the control [17], and this related to the study sample size, population, and study design.

LH/FSH ratio in literature presents controversial conclusions. Banaszewska et al. did not find significant differences between LH/FSH ratio means between women with and without PCOS [18]. In our study, we found it to be significantly lower in infertile PCOS than fertile PCOS group and control; a comparative study disagrees with our results regarding the non-fertile group only and states that LH and LH/FSH ratio showed insignificant results in the PCOS group [19, 20], this discrepancy in LH and the ratio possibly can be justified by the BMI of the participants as the means of our sample were in the overweight range for all groups and this tends to lower the LH value in the current study [21].

Insulin is highly elevated in PCOS patients, and hyperinsulinemia is among the main pathophysiological mechanisms of this syndrome [22] and this increased secretion of insulin is a

consequence of insulin resistance that is commonly apparent in women with PCOS and could be due to defects in the expression and/or activity of insulin receptor even after matching the BMI [23].

The value of AMH in PCOS patients was higher than in control, and this is compatible with a study that found a significant relationship between AMH levels and inflammatory markers in PCOS due to a greater release of inflammatory factors into the systemic circulation, thus affecting AMH production in the ovaries [24]. The raised number of ovarian follicles and follicular arrest underlies the elevated AMH in PCOS with adverse effects on fertility outcomes [25]. These results are in concordance with a study that showed higher intra-follicular AMH levels in women with PCOS compared to controls [26]. In addition, the study demonstrated a Strong correlation between circulating AMH levels and antral follicle count on ultrasound in PCOS [27].

Testosterone serum levels were elevated in fertile and non-fertile PCOS compared with the control group; this finding resembles the findings of previous studies [27, 28]; the pathogenesis of the disease state could explain this.

Hyperandrogenism is an important clinical feature in patients with PCOS and it is overt in non-fertile PCOS versus both fertile and control in concordance with another previous study [20].

The prolactin and TSH levels showed a significant decrement in the infertile PCOS group compared with the control and the fertile group but still within normal range values, and this is against the study [29] that revealed a higher prolactin level in the PCOS group, possibly due to the sample size and population studied.

Accumulating evidence suggests that several vitamin disbalances, as well as nutraceutical supplementation to counteract them, may play a significant role in women's health and modulate molecular and endocrine as well as metabolic pathways [13,30].

The infertile PCOS group had significantly lower PTX3 values than the fertile PCOS group, while its value was higher in fertile PCOS versus control.

Variable findings were found in PCOS in different studies; for example, PTX3 was found to be low in Tosi et al. 2014 [31] in which the sample was entirely constituted of Caucasian women; additionally, the study design was a cohort of PCOS patients mostly consisted of women with the ‘classic’ phenotype. While in our study, we evaluated different ethnic groups with sub-classification according to fertility issues. While in another study showed high PTX3 levels in PCOS [32], which we agree is considered fertile PCOS to be compared with healthy control.

Another study showed the circulating PTX3 level was elevated in PCOS women and significantly associated with the presence of hyperandrogenism [29], which our study agrees definitely.

The core result of this study is the significantly lower PTX3 in non-fertile versus fertile PCOS, suggesting its potential role in ovarian dysfunction and PCOS-related infertility.

The correlation of biochemical and hormonal variables of the infertile study group with Pentraxin showed an inverse relationship between Pentraxin and AMH that was statistically significant with a moderate effect size. In contrast, other studies showed that other parameters (Body mass index, blood sugar, lipids, and total testosterone) had also significant correlations [33], as the study was a cohort study involving all cases with PCOS regardless of phenotypes.

ROC curve analysis revealed a cutoff value of PTX3 (≤1.05) that shows low sensitivity but good specificity, suggesting its possible diagnostic role in PCOS infertility, and a few studies discuss the possible fertility effect of PTX3; these studies demonstrate that PTX3 plays important roles in cumulus cell-oocyte interaction in the peri-ovulatory period as a downstream protein in the dominant follicle signal transduction cascade [34].

The application of the ROC curve for AMH and LH/FSH ratio shows higher sensitivity than PTX3 but lower specificity, as coincides with another study [35], and this suggests the beneficial application of combined parameters in the diagnosis of PCOS-related infertility.

Conclusions

Pentraxin 3 level is significantly higher in fertile PCOS patients and lower in the infertile PCOS group in comparison to the control group suggesting its possible role in PCOS-related infertility.

Compliance with Ethical Standards

Authors contribution:

NE: data collection, formal analysis, investigations, methodology, validation, visualization, writing the original draft.

BH: conceptualization, formal analysis, methodology, project administration, software, supervision, validation, visualization, writing review, and editing.

Funding

None.

Study registration

N/A.

Disclosure of Interests

The authors declare that they have no conflict of interests.

Ethical approval

The protocol was approved by the Mustansiriyah Scientific Council of Obstetrics and Gynaecology/Iraq MOG-107 in DEC 2021.

Informed consent

Written informed consent was taken from all participants following the Declaration of Helsinki and local guidelines.

Data sharing

Data are available under reasonable request to the corresponding author.

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Table 1. Summary Statistics Table for demographic, biochemical, and hormonal Variables by groups.

Variable	Groups (number)	Mean	SD	SE_M	Median	Min	Max	P value
Age (years)	Control (30)	27.20	5.05	0.37	23.45	18.70	27.40	0.14
	Fertile PCOS(30)	28.21	4.83	0.91	28.00	19.00	38.00	
	Infertile PCOS(30)	27.97	5.06	0.92	27.00	18.00	42.00	
BMI (Kg/m^2)	Control (30)	27.30	5.42	0.99	27.00	18.00	41.00	0.65
	Fertile PCOS(30)	28.61	6.09	1.15	27.50	18.00	41.00	
	Infertile PCOS(30)	27.50	5.6	1.02	26.50	20.00	39.00	
LH (mIU/L)	Control (30)	7.22	2.92	0.54	6.50	2.50	12.00	<.001
	Fertile PCOS(30)	9.31	5.08	0.96	7.96	0.62	20.80	
	Infertile PCOS(30)	3.48	1.63	0.29	2.85	2.12	8.10	
FSH (mIU/L)	Control (30)	7.49	1.72	0.31	7.55	4.90	10.80	<.001
	Fertile PCOS(30)	6.98	1.93	0.37	6.94	4.00	12.90	
	Infertile PCOS(30)	4.79	0.95	0.17	4.87	2.80	6.22	
LH/FSH ratio	Control (30)	1.05	0.56	0.11	0.86	0.32	2.24	<.001
	Fertile PCOS(30)	1.37	0.72	0.14	1.30	0.11	2.76	
	Infertile PCOS(30)	0.74	0.31	0.06	0.62	0.38	1.48	
Insulin(mIU/L)	Control (30)	8.05	2.92	0.53	8.08	1.50	13.40	.001
	Fertile PCOS(30)	25.49	24.78	4.68	18.00	6.16	104.80	

	Infertile PCOS(30)	22.78	22.35	4.08	15.71	3.71	121.10	
FBS (mg/dl)	Control (30)	101.40	12.82	2.34	98.00	82.00	120.00	<.001
	Fertile PCOS(30)	113.43	12.36	2.33	115.50	90.00	142.00	
	Infertile PCOS(30)	114.23	6.13	1.12	114.50	102.00	129.00	
AMH (ng/ml)	Control (30)	5.90	1.35	0.25	5.70	4.10	9.20	.008
	Fertile PCOS(30)	8.21	1.62	0.30	8.28	5.55	11.90	
	Infertile PCOS(30)	8.97	1.82	0.33	9.08	5.77	12.07	
Testosterone (mIU/L)	Control (30)	0.39	0.27	0.05	0.39	0.07	0.82	<.001
	Fertile PCOS(30)	0.62	0.23	0.04	0.56	0.33	0.98	
	Infertile PCOS(30)	0.69	0.18	0.03	0.67	0.40	0.99	
Prolactin (ng/ml)	Control (30)	16.98	6.28	1.15	17.45	6.88	27.70	<.001
	Fertile PCOS(30)	17.95	6.22	1.18	17.75	6.88	30.00	
	Infertile PCOS(30)	9.34	3.28	0.60	7.80	6.00	19.00	
TSH (mIU/L)	Control (30)	2.91	1.13	0.20	2.75	0.89	5.00	.008
	Fertile PCOS(30)	2.94	1.02	0.19	2.95	1.60	5.00	
	Infertile PCOS(30)	2.23	0.69	0.13	1.92	1.20	4.00	

Results for Testing the Mean Differences by groups using F-Tests; SD= Standard deviation; SEM=Standard error of the mean; Min and Max= Minimum and maximum respectively

Table 2. Pearson Correlation Results of Pentraxin 3 with Age, BMI, LH, FSH, LH/FSH ratio, TSH, PRL, Testosterone, AMH, FBS, and Insulin in the infertile group.

Variables	Pentraxin 3 n = 30		
	r	95.00% CI	p
Age	**0.44**	**[.10, .69]**	**.014**
BMI	-0.15	[-.49, .22]	.420
LH	-0.08	[-.42, .29]	.689
FSH	-0.02	[-.38, .34]	.917
LH/FSH ratio	-0.08	[-.43, .29]	.676
TSH	0.30	[-.07, .60]	.107
PRL	-0.13	[-.47, .24]	.485
Testosterone	-0.01	[-.37, .35]	.949
AMH	**-0.39**	**[-.66, -.04]**	**.031**
FBS	-0.01	[-.37, .35]	.953
Insulin	-0.25	[-.56, .12]	.183

r: correlation confidence, CI: confidence interval, P: P-value

Table 3. ROC curve comparison between different markers showing ROC criteria in the classification of infertility from control

Variable	AUC	SE	95% CI	Cutoff	Sen	Spe	+PV	-PV	P value
Pentraxin 3	0.68	0.07	0.55 to 0.79	≤1.05	46.67	83.33	73.7	61.0	<.001
AMH	0.91	0.03	0.81 to 0.97	>6.5	93.33	76.67	80.0	92.0	<.001
LH/FSH ratio	0.65	0.07	0.52 to 0.77	≤1.33	96.67	36.67	60.4	91.7	<.037

AUC: area under the curve, SE: standard error, CI: confident interval, Sen: sensitivity, Spe: specificity, PV: Predictive value

Figure 1. Means & Confidence interval of Pentraxin for PCOS patients and control.

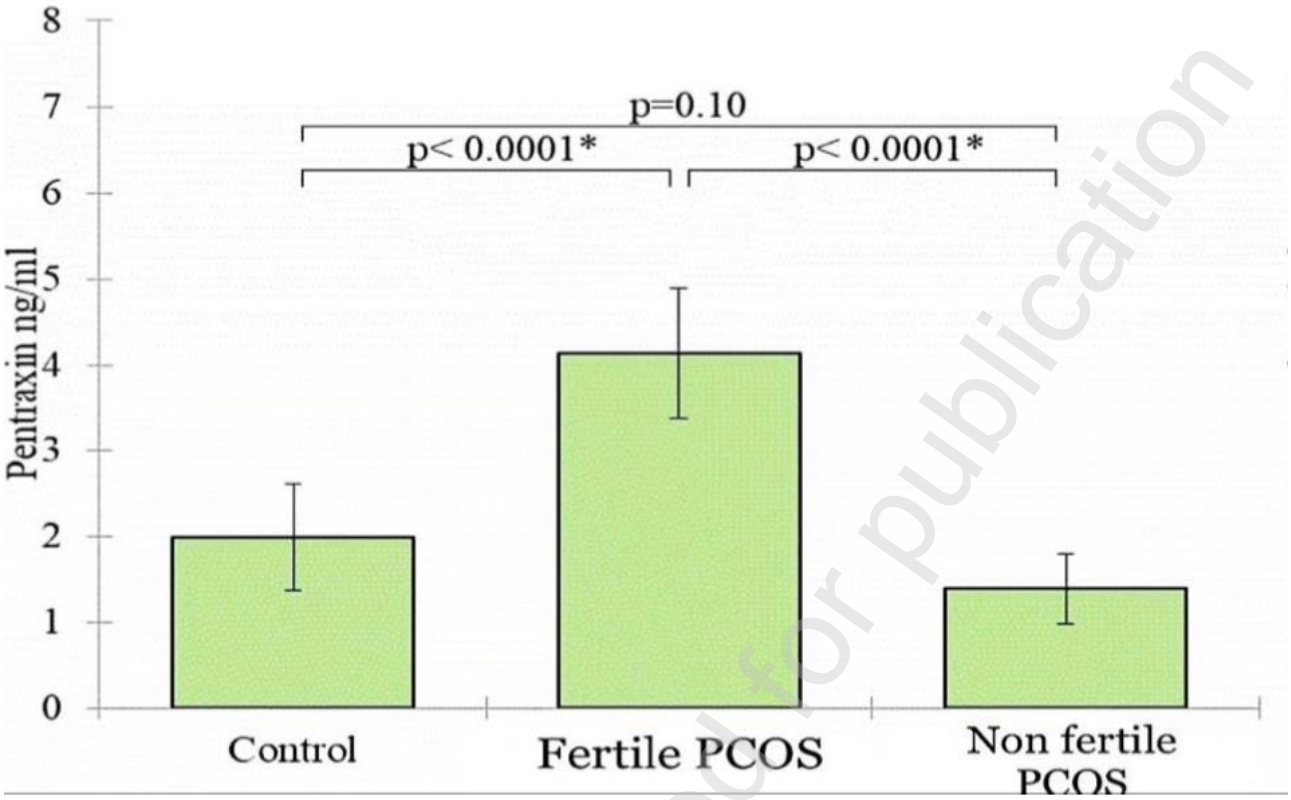

Figure 2. ROC curve comparison of Pentraxin 3, AMH, and LH/FSH ratio.