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Correlation Analysis of Hormonal and Immunological Biomarkers in Women with Primary Unexplained Infertility

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Abstract

Background

Unexplained infertility remains a clinical problem, with emerging evidence that subclinical immunological factors may contribute to diminished ovarian reserve even when standard clinical parameters appear normal.

Methodology

A 90 women had 60 patients with primary unexplained infertility and 30 fertile controls included in a case-control study. Anti-ovarian antibodies were investigated by measuring the level of anti-ovarian antibodies, TNF-α, anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and the quantity of E 2, TSH, and prolactin (PRL).

Results

For analysis, we used the Mann-Whitney U test, Spearman correlation, and the receiver operating characteristic (ROC) method. No clinical differences were observed in absolute levels of the measured marker for each of the patients and controls, but a correlation was found between TNF-α and AMH ($r = -0.37$, $p < 0.05$) and anti-ovarian antibodies and AMH ($r = -0.27$, $p < 0.01$), and correlations with age-dependent AMH in combination with hypothalamic-pituitary-ovarian feedback were confirmed. In addition, ROC analysis indicated poor diagnostics and diagnosis for AMH and TNF-α (AUC 0.515 and 0.498, respectively)

Conclusion

Consistent with normal absolute levels, anti-ovarian antibodies and TNF-α show significant associations with ovarian reserve in women with unexplained infertility. These analyses may also reflect a potential role of subclinical immunological disruption on ovarian reserve over a continuum in normal ranges. While such markers are not diagnostic tools, they may play a role in understanding the reason for unexplained infertility and may make it possible to predict reproductive outcomes. More should be explored for their therapeutic impact.

تحليل الارتباط بين المؤشرات الهرمونية والمناعية لدى النساء المصابات بالعقم الاولي الغير مفسر

ملاك رشيد يحيى ورؤى جمال عبد الخالق

الخلاصة

لا يزال العقم غير المفسر يمثل مشكلة سريرية، مع ظهور أدلة جديدة تشير إلى أن العوامل المناعية دون السريرية قد تساهم في انخفاض مخزون المبيض حتى عندما تبدو المعايير السريرية القياسية الطبيعية

العينات وطرق العمل

شملت الدراسة 90 امرأة ، 60 مريضة يعانين من العقم الأولي غير المفسر و30 امرأة خصبة كمجموعة ضابطة. تم فحص الأجسام المضادة للمبيض عن طريق قياس مستوى الأجسام المضادة للمبيض، وعامل نخر الورم ألفا (TNF-α)، والهرمون مخزون المبيض(AMH)، والهرمون المنبه للجريب (FSH)، والهرمون اللوتيني (LH)، وكمية الإستراديول (E2)، والهرمون المنبه للغدة الدرقية (TSH)، والبرولاكتين (PRL).

النتائج

استخدمنا اختبار مان-ويتني يو، ومعامل ارتباط سبيرمان، وطريقة منحنى خصائص التشغيل (ROC). لم تُلاحظ أي اختلافات سريرية في المستويات المطلقة للعلامة المقاسة لكل من المرضى والمجموعات الضابطة، ولكن وُجد ارتباط بين TNF-α و(r=-0.37 AMH، ($p<0.05$ والأجسام المضادة للمبيض و(r=-0.27 AMH، ($p<0.01$، وتم تأكيد الارتباطات مع AMH المرتبط بالعمر بالإضافة إلى التغذية الراجعة بين الغدة النخامية والمهاد والمبيض. علاوة على ذلك، أشار تحليل ROC إلى ضعف التشخيص لكل من AMH وTNF-α (AUC 0.515 و0.498 على التوالي).

الاستنتاج

بما يتوافق مع المستويات المطلقة الطبيعية، فإن الأجسام المضادة للمبيض تُظهر الأجسام المضادة وعامل نخر الورم ألفا ارتباطات كبيرة باحتياطي المبيض لدى النساء المصابات بالعقم غير المبرر. قد تعكس هذه التحليلات أيضًا دورًا محتملاً للاضطراب المناعي تحت السريري على احتياطي المبيض على امتداد نطاق طبيعي. في حين أن هذه المؤشرات ليست أدوات تشخيصية، إلا أنها قد تلعب دورًا في فهم سبب العقم غير المبرر وقد تجعل من الممكن التنبؤ بالنتائج الإنجابية. ينبغي استكشاف المزيد لتأثيرها العلاجي.

1. Introduction

Infertility is defined as the failure to attain a clinical pregnancy after twelve months or more consistent, unprotected sexual intercourse(Mburu et al., 2026). Approximately 10-30% of affected couples are categorized as having Unexplained Infertility (UI). In these instances, Standard diagnostic assessments reveal a normal ovulation pattern, tubal patency, and semen is present without evidence of fertility (Ehsani et al., 2019). Although conventional hormonal and anatomical examination of the fetus would be nothing unusual in their own life stage, in those cases for which the subtle endocrine and immune system problems have not been reported(Saito, 2024). The standard clinical evaluation of unexplained infertility is to assess the hypothalamic-pituitary-ovarian axis (HPO) level. FSH and LH are critical components in controlling follicle development (Valera et al., 2025). Prolactin (PRL) is the main protein that does not function in our natural lactation cycle, but increases levels in the HPO axis of hormone secretion are not as readily seen to show any hypothalamic gonadotropin-releasing hormone (GnRH) pulsatility, effect on hormone secretion to lead to low levels of follicle growth and ovulation(Szukiewicz, 2024). Fertility and production are affected by thyroid (TSH) and may be depressed if our fertility is lower (but not subclinical dysfunction)(Poppe et al., 2021). Anti-Müllerian hormone (AMH) makes it difficult to treat for infertility, but is excellent for ovarian reserve and good for assessing its effects through contraceptive treatment, and is used to determine a future course for infertility when pregnant(Karaviti et al., 2025). Autoimmune mechanisms are known as contributors to infertility. Autoimmune infertility is when the immune system is not well-regulated, leading to the production of autoantibodies that affect the reproductive process (S. Chen et al., 2023). As anti-ovarian antibodies are seen because they are found to directly affect the ovarian surface and the development of the ovarian follicles, potentially affecting ovarian function and reserve (J. L. Luborsky et al., 2011; Šemeklienė & Gradauskienė, 2025). As granulosa cells are the major sources of AMH, changes in the immune system caused by AOA can affect ovarian reserve markers even in otherwise normal endocrine systems of human women, while inflammatory cytokines are growing in interest in terms of ovarian physiology. Tumor necrosis factor-alpha (TNF-α) is a prominent pro-inflammatory marker mediating follicular development, oocyte maturation, and ovulation. However, decreased expression of TNF-α can adversely affect the ovarian microenvironment and could affect fertility(Gorman et al., 2023; Moolhuijsen & Visser, 2020). Despite the growing evidence of immune determinants in infertility, the role of anti-ovarian antibodies and TNF-α is still poorly understood, especially by those whose hormonal profiles are normally stable. This study attempted to understand the relationship between anti-ovarian antibodies and TNF-α in primary unexplained infertility and to identify them as diagnostic signs in patients living with illness.

2. Materials and Methods

2.1. Study Design and Location

This case-control study was directed from Rooh Al-Hayat Center for Infertility Treatment and In Vitro Fertilization at the AL-Razi IVF Center, Al-Ramadi, Iraq. The study is conducted for around four months from July 20 to December 1, 2025.

2.2. Study Group and How to Choose Participants

Study Population and Participant Selection: These 90 women aged 20-44 years participated in this study. Participants were divided into two groups. An initial patient group of 60 with a diagnosis of primary idiopathic infertility, where the men were healthy, fertile, and their children had three or more successful pregnancies with no history of miscarriage or infertility. For the control group of 30 women who had no known history of infertility, the venous blood samples were administered at least three days before the date of onset of menstruation. The study is being run by strict criteria under

which such an association could appear. Women were excluded if they were diagnosed with identifiable infertility and were known as tubal obstruction, endometriosis, polycystic ovary syndrome, or ovulatory dysfunction. Other exclusion criteria may be a history of thyroid disorders, systemic autoimmune diseases, chronic inflammatory conditions, or hormone therapy.

2.3. Laboratory Measurements

Average tumor necrosis factor-alpha (TNF-α) SunLong Biotech Co., Ltd. (China) and anti -ovarian antibodies Elabscience (USA) were measured both in blood through commercially available enzyme-linked immunosorbent assay (ELISA) kits. Each assay was conducted according to the manufacturer's protocol. Serum hormonal levels were determined by electrochemiluminescence immunoassay (ECLIA) using the Cobas e411 automated analyzer (Roche Diagnostics, Germany).

2.4. Statistical Analysis

Statistical models were drawn using R software 4.4.1. The Shapiro-Wilk test was used to verify the normality of data distribution. Because most variables in our study were not normal, the Mann-Whitney U test was used to compare continuous variables between the patients and the control. Immunological and hormonal markers are correlated with Spearman's rank correlation. A binary logistic model was drawn in order to generate independent predictors of primary idiopathic infertility and to incorporate different hormonal and immune factors in the model as well. We verified the discriminative ability of our predictive model by calculating the average surface area (AUC) under the receiver operating characteristic curve in the Fig.1. It is acceptable to consider p -values > 0.05 significant.

3. Results

Table1 depicts the hormonal and immunological profiles of both groups. In this analysis, statistical analysis revealed no significant difference in the absolute levels of any particular parameter between patients and controls. All values were within clinically significant reference ranges. The levels of AMH showed no statistically significant difference between the patients (2.61 ± 1.63) and controls (2.59 ± 1.99), p-value is 0.586. Anti-ovarian antibodies remained comparable between patients (0.27 ± 0.33) and controls (0.23 ± 0.27), with a p-value of 0.976. No significant variation was found in TNF-α, which averaged 53.02± 13.80 in patients compared to 51.20 ± 12.36 in controls (p = 0.817). To the best of our knowledge, conventional hormonal and immunologic profiles when treated alone are indifferent to patients of unexplained infertility and fertile women.

Table1: Hormonal and Immunological Profiles of Study Participants

Marker	Patients (n=60), Mean ± SD	Controls (n=30), Mean ± SD	P-value
AMH (ng/mL)	2.61 ± 1.63	2.59 ± 1.99	0.5868
PRL (ng/mL)	19.00 ± 7.68	17.15 ± 7.39	0.2242
TSH (µIU/mL)	2.04 ± 0.98	1.83 ± 1.19	0.1516
E2 (pg/mL)	43.97 ± 27.12	42.09 ± 16.79	0.8139
LH (mIU/mL)	6.50 ± 3.02	7.06 ± 2.96	0.4591
FSH (mIU/mL)	8.52 ± 5.29	7.17 ± 2.52	0.4436
Anti-ovarian Ab (U/mL)	0.27 ± 0.33	0.23 ± 0.27	0.9761
TNF-alpha (pg/mL)	53.02 ± 13.80	51.20 ± 12.36	0.8172

Fig.1. has boxplots showing the distribution of hormonal and immunological markers as compared to the group with control samples, as shown in Table1. For each marker, the boxplots are very well overlapped. On most parameters, the median values are very similar, and interquartile ranges are almost all equally distributed. For anti-Müllerian hormone, the boxplots are mostly the same, except the group with control samples has a very large interquartile range. The boxes in anti-ovarian

antibodies have very similar distributions, and median values are even. TNF-α boxplots suggest a slightly higher variability in the patient sample, while the overall trend is still the same. These visual plots show no extreme difference between groups and support the findings that immunological disruption of unexplained infertility is subclinical and lacks clinical contribution of these antibodies.

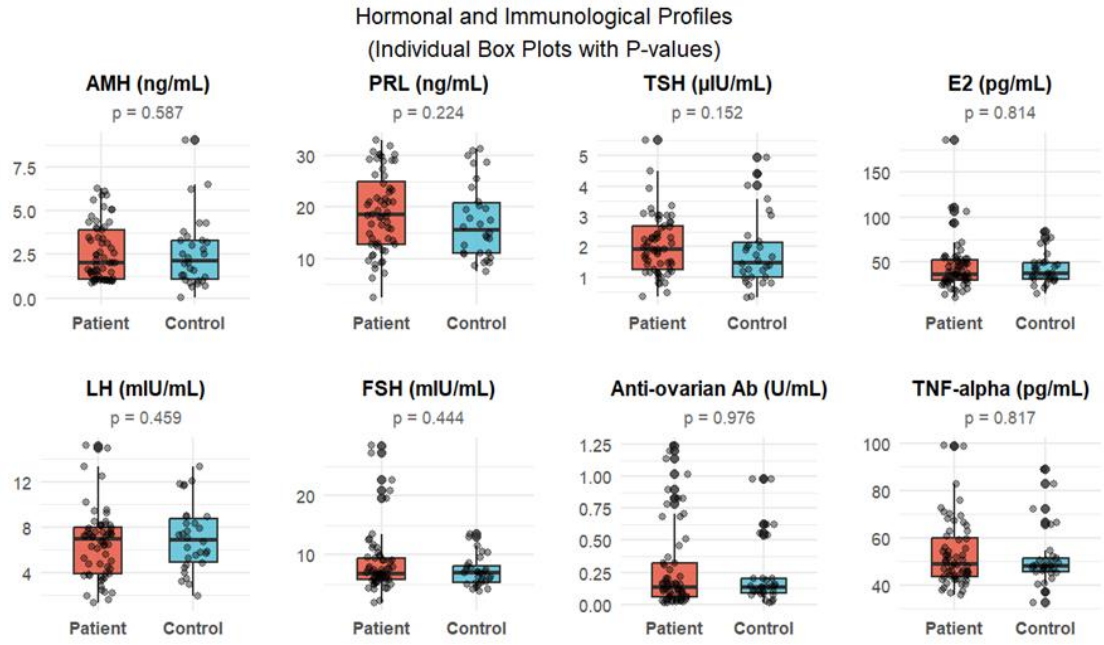

Figure1: Boxplot Representation of Hormonal and Immunological Markers

Table2 shows Spearman correlations between the various studied markers. A more significant correlation between TNF-α and AMH ($r = -0.37$ and $p < 0.05$) shows that high TNF-α is associated with lower ovarian reserve level. More negative correlations among anti-ovarian antibodies and AMH ($r = -0.27$, $p < 0.01$) are found, which indicates the greatest effect of this analysis. This is directly in line with our own intention within the research, as anti-ovarian antibodies show a significant association with AMH, even in subclinical ones with very low levels. TNF-α and anti-ovarian antibodies also exhibit very weak correlations with each other ($r = 0.059$ and $p > 0.05$), which indicates that the two markers interact via different mechanisms. And the matrix also confirmed established physiological relationships, which confirm the dataset and which are expected and are in relation to age with AMH ($r = -0.476$, $p < 0.001$) and with FSH ($r = -0.44$, $p < 0.001$), which also indicate a positive correlation for FSH with LH ($r = 0.26$, $p < 0.01$).

Table2: Spearman Correlation Matrix Among Study Variables

Variable	AMH	FSH	LH	Age	TNF-alpha	Anti-ovarian Ab
AMH	1.000	-0.44**	0.14	-0.476***	-0.37	-0.27**
FSH	-0.44**	1.000	0.26*	0.085	0.08	-0.004
LH	0.14	0.26*	1.000	-0.188	0.15	-0.138
Age	-0.476***	0.085	-0.188	1.000	-0.001	0.131
TNF-alpha	-0.37	0.08	0.15	-0.001	1.000	0.059
Anti-ovarian Ab	-0.27**	-0.004	-0.138	0.131	0.059	1.000

Fig.2. (the receiver operating characteristic curve for TNF-α and anti-ovarian antibodies). The curve for TNF-α is in red, and that of anti-ovarian antibodies is in blue. Both curves are close to the diagonal line (i.e., random classification AUC = 0.5), and the TNF-α line shows a slight deviation above the diagonal line at higher values of specificity (52.200 pg/mL.

sensitivity (40.0%) and specificity (80.0%). The curve for anti-ovarian antibodies is mostly on the diagonal line, and its AUC is, of course, 0.498. The proximity of both curves to the diagonal line strongly suggests that they perform poorly as discriminative tests. This is an interesting finding and, at the same time, this can never be interpreted as a diagnostic guide to the women suffering from unexplained infertility and the fertile control women: although these immunologic markers had high correlations to AMH (that we know of), it is important to understand the function that immune status plays for ovarian reserve.

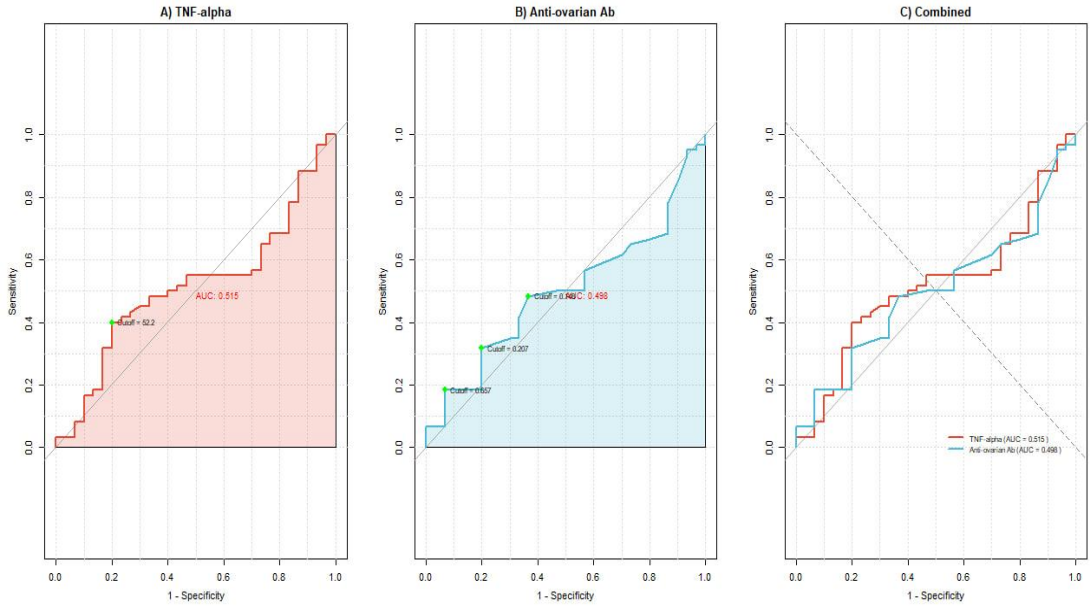

Figure2: ROC Curves For TNF-A and Anti-Ovarian Antibodies

4. Discussion

Our study was focused primarily on the hormonal and immunological issues in women with unexplained infertility, and especially anti-ovarian antibodies, TNF-α, and ovarian reserve. Despite not distinguishing between any of the indicators directly measured and controls, the correlation results demonstrated strong immunological associations. In addition, an inverse relationship between TNF-α vs AMH was reported ($r = -0.37$, $p<0.05$). These results indicate that TNF-α levels greater and TNF-α levels lower are associated with ovarian reserve. It is consistent with Arwan and Hendri's (2021) study, which demonstrated a similar inverse relationship of inflammatory cytokines with AMH in patients with endometriosis (Hendri, 2021). All three conditions seem to have the same cause and effect, as inflammation results in oxidative stress as well as granulosa cell apoptosis, which leads to TNF-α decline in AMH secretion that results in infertility. Ojo et al. show inflammation, oxidative stress, and apoptosis all contribute to infertility, and Mushattat et al(Mushattat et al., 2025; Ojo et al., 2023). We found a positive correlation between TNF-α and AMH in acute toxoplasmosis. Such a divergence points to the fact that the effect of TNF-α has a negative effect on AMH through chronic inflammatory illness and not acute immune responses to the incidental infection; on the other hand, it was reported to be a positive effect. We found a significant inverse correlation between anti-ovarian antibodies and AMH ($r = -0.27$; $p < 0.01$). Indeed, both variables stayed in normal ranges, suggesting that even subclinical anti-ovarian antibodies suppress ovarian follicles. Their significance is supported by Chan et al., who showed ovarian antibodies were major contributors to ovarian dysfunction in women with unexplained infertility and disrupted hormonal signals leading to follicular dysfunction even in less severe ovarian disease. Furthermore, Chen et al. demonstrated that detecting these autoantibodies and revealing their profiles would prove useful approaches for

diagnosing such diseases related to autoimmune infertility and poor reproductive status as the cause of infertility (X. Chen, 2022). The results demonstrated a significant negative correlation between age and ovarian reserve markers, as they are known to decline with age (Aslan et al., 2025)(Ramezani Tehrani et al., 2025). It was shown that the inverse of AMH ($r = -0.44$), with respect to FSH ($p < .001$), shows positive feedback going on between both hypothalamic-pituitary and ovarian axis (Demır et al., 2025; Robertson et al., 2021). The positive relationship between FSH and LH ($r = 0.26$, $p < 0.01$) also indicates that, of course, the two factors are being co-produced by these two components of the hypothalamic-pituitary axis from the pituitary. The modest ROC curve performance does not necessarily indicate a weak relationship between the index and AMH. Instead, it may reflect the subtle, graded clinical progression of unexplained infertility, where various immune factors may slowly affect the ovarian reserve without serving as a clear marker for disease onset. This aligns with Luborsky et al(J. Luborsky & Pong, 2000). who noted that anti- ovarian antibodies might be linked to lower IVF pregnancy rates, even when standard hormonal markers show no significant difference. Similarly, Huang et al(Huang et al., 2025) found comparable results concerning TNF-α levels. Therefore, these markers' importance may lie more in understanding the underlying mechanisms and predicting reproductive outcomes rather than providing definitive diagnostic criteria.

Conclusion

Based on these observations, it is plausible that the main cause of infertility in women with unexplained infertility may not be solely related to the endocrine system. Instead, it could involve subtle changes in the ovarian reserve that occur before visible hormonal shifts. The inverse relationship between AMH and both TNF-α and anti-ovarian antibodies suggests a possible underlying subclinical immune mechanism affecting ovarian reserve. However, given the preliminary nature of these findings, further studies with larger samples are needed to confirm these links and to clarify whether this immune activity is inflammatory or autoimmune in this specific group.

Conflicts of Interest

The authors declare that there are no competing financial interests or personal relationships that could have influenced the work presented in this manuscript.

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Ethical Approval

The study protocol was reviewed and approved by the Ethics Committee of the College of Applied Sciences, University of Fallujah (approval number AS-EC/0005). All individuals were communicated about the purpose of the study, and verbal informed consent of all participants was obtained according to the Declaration of Helsinki.

Authors' contributions

The study was designed and supervised by Assistant Professor Roua Jamal Abdulkhaliq, while Malak Rasheed Yahya collected the samples, performed the important diagnostic tests, and conducted the statistical analyses.

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