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Effect of *Toxoplasma gondii* Parasite Infection on Levels of AMH, IL-2 and IL-12 in Serum of Pregnant Women

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Abstract:

Toxoplasmosis is one of the most common parasitic infections that occur during pregnancy, particularly in early pregnancy. The parasite activates the immune system, often leading to miscarriage. After the immune response to the parasite is activated, changes occur in the secretion of inflammatory cytokines and the ovarian reserve hormone, anti-Mullerian hormone (AMH). Most studies have observed a link between AMH and the parasite Toxoplasma gondii. The study aimed to determine the serum levels of AMH, interleukin-2, and interleukin-12 in women pregnant and infected with Toxoplasma gondii and compare these levels with those of uninfected pregnant women. Samples were taken from 100 pregnant women in their first trimester. Fifty of these women were infected with toxoplasmosis, as their serological tests for both IgG and IgM antibodies were positive. The second group consisted of 50 pregnant women of similar ages who were healthy and served as the control group. Ovarian hormone (AMH), interleukin-2, and interleukin-12 levels were measured in the serum of these groups using ELISA. Data were analyzed using SPSS software, and a p-value < 0.05 was statistically significant. Pregnant women with toxoplasmosis had significantly higher serum AMH levels than those in the control group (5.22 ± 0.399 vs. 2.66 ± 0.552 ng/mL; $P = 0.040$). IL-2 was also significantly elevated among infected women (10.75 ± 0.68 vs. 3.38 ± 0.40 pg/mL; $P = 0.029$), as was IL-12 (16.24 ± 0.60 vs. 5.08 ± 0.50 pg/mL; $P = 0.027$). Together, these findings show a significant association between T. gondii infection, immune activation, and increased serum AMH levels during pregnancy. Toxoplasma gondii infection during pregnancy is associated with significant increases in serum Anti-Müllerian Hormone (AMH), Interleukin-2 (IL-2), and Interleukin-12 (IL-12). The positive relationship observed between AMH and these inflammatory cytokines suggests that the immune response triggered by toxoplasmosis may affect ovarian function during pregnancy.

Key words: Toxoplasma gondii, Anti-Mullerian Hormone (AMH), Interleukin-2, Interleukin-12, Pregnancy, Immunological markers.

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Introduction

Toxoplasma gondii is a parasite obligate intracellular protozoan parasite that infects many warm-blooded animals, including humans, and ranks among the most successful parasitic pathogens worldwide. Nearly one-third of the global population is estimated to have been exposed to it, though prevalence differs substantially with geography, environmental conditions, dietary habits, and socioeconomic status.

Toxoplasmosis infection is often asymptomatic in strong immunity individuals. However, in pregnant women and immunocompromised or weakened individuals, the parasite can invade various tissues and cause serious clinical symptoms [1;2]. The life cycle of the *Toxoplasma gondii* parasite consists of both a definitive (final host) and an intermediate host; cats are considered the definitive host for this parasite. The parasite undergoes sexual reproduction in them and produces environmentally resistant oocysts, which are shed in feces. Humans and other warm-blooded animals serve as intermediate hosts. Infection can follow ingestion of sporulated oocysts in contaminated food, water, or soil; eating undercooked meat or raw meat containing tissue cysts that transmit from an infected mother to fetus; organ transplantation; or blood transfusion [3;24]. After entering the human body, the parasite first develops into rapidly multiplying tachyzoites, which cause acute infection. It then changes into slowly replicating bradyzoites within tissue cysts that can remain in the body for life [4;5]; fetal damage is generally more severe when infection occurs early in pregnancy. [6]. Most of the symptoms that occur in the fetus due to congenital toxoplasmosis are miscarriage, hydrocephalus, stillbirth, intracranial calcifications, retinal and choroidal infections, and neurological disorders [7;8]. Appropriate monitoring of *T. gondii* infection during pregnancy remains essential for reducing maternal and fetal complications [9;10]

The host immune response helps restrict parasite dissemination and establish chronic infection. producing several pro-inflammatory cytokines that coordinate the response against it. Interleukin-12 is one of these important mediators. It promotes the differentiation of type 1 T lymphocytes and is crucial for the production of interferon-gamma (IFN-γ), thereby increasing the production of antimicrobial macrophages, which play a very clear role in inhibiting the intracellular replication of Toxoplasma gondii. Interleukin-2 also plays a significant role in the activation, proliferation, and differentiation of T lymphocytes. It thus controls the parasite through these enhancements [11, 12].

Recent studies have highlighted the importance of inflammatory cytokines and their active role not only in supporting the body's defenses but also in influencing endocrine functions through complex interactions between the glandular and immune systems. When an infection activates immune cells, these cells release cytokines capable of altering hormone secretion, positively affecting tissue activity. This demonstrates a two-way communication between the immune and glandular systems, and therefore, scientists have focused on this important aspect, as it may explain many of the glandular changes observed in infectious and inflammatory diseases [13;14]. One of the hormones that has recently gained considerable interest in

reproductive medicine is Anti-Mullerian Hormone (AMH). AMH is primarily secreted by granulosa cells in pre-follicular and microfollicular ovarian follicles and belongs to the transforming growth factor-beta (TGF-β) family [15;16]. AMH is considered a biomarker of ovarian reserve in women, as the number of developing follicles remaining within the ovary reflects its concentration in the bloodstream, unlike many other reproductive hormones [17;18]. Anti-Mullerian Hormone exhibits relatively stable serum concentrations throughout the menstrual cycle, making it an important laboratory marker for evaluating ovarian function, predicting ovarian response during assisted reproductive techniques, and assessing reproductive potential. [19;20].

The current study aimed to determine the serum levels of anti-Mullerian hormone, interleukin-2, and interleukin-12 in women pregnant and infected with *Toxoplasma gondii* and to compare these levels with those of uninfected pregnant women.

Materials and Methods

Study Design

This study was conducted to find out if there is a relationship between toxoplasmosis infection and serum levels of anti-Mullerian hormone, interleukin-2, and interleukin-12 among pregnant women in the first trimester of pregnancy. This study was carried out from November 2025 to February 2026; blood samples were collected from pregnant women attending gynecological and obstetric clinics for routine antenatal care. Following sample collection, laboratory investigations were performed at the Governorate, Baghdad, Iraq, where serological and immunological analyses were conducted.

The study included 100 samples taken from pregnant women (during the first trimester of pregnancy). Participants were selected according to specific inclusion and exclusion criteria to minimize the effect of factors that may influence hormonal or immunological parameters. The participants were divided into two groups:

A\\ (Group I): This group consisted of 50 apparently healthy pregnant women with negative serological results for *Toxoplasma gondii* antibodies (IgG and IgM). The control group was selected to be comparable with the infected group regarding pregnancy stage and general characteristics. Participants were excluded if they had:

* Chronic systemic diseases.

* Autoimmune disorders.

* Endocrine disorders affecting reproductive hormones.

B\\ (Group II): This group consisted of 50 pregnant women with serological evidence of toxoplasmosis. The parasite was detected by specific IgG and IgM antibodies to toxoplasmosis, using enzyme-linked immunosorbent assay (ELISA).

Blood Sample Collection and Serum Preparation

Under sterile conditions, venous blood (5 milliliters) was drawn from the patients into gelatin tubes. It was allowed to clot at room temperature for 10 minutes, followed by centrifugation at 3000 rpm for ten minutes to separate the serum from the cellular components. The resulting serum was then

transferred carefully into sterile Eppendorf tubes and stored at -20°C for further analysis.

Serological Detection of *Toxoplasma gondii* Infection

Toxoplasma gondii infection was detected using the Latex Agglutination Test (LAT) and an enzyme-linked immunosorbent assay (ELISA) for the detection of anti-Toxoplasma gondii IgG and IgM antibodies using ELISA kits according to the manufacturer's instructions. The presence of IgG antibodies was considered an indicator of previous or chronic exposure to the parasite, while the presence of IgM antibodies suggested recent or active infection. Participants showing positive results for IgG and IgM were classified as infected cases.

Measurement of Serum Anti-Mullerian Hormone (AMH(

Serum levels of Anti-Mullerian Hormone (AMH) were determined using a quantitative ELISA method [21]. The ELISA technique depends on the specific interaction between AMH molecules present in serum samples and antibodies immobilized on the microplate. After addition of enzyme-linked detection antibodies and substrate solution, the intensity of the developed color reaction was measured spectrophotometrically.

Measurement of Immunological Markers (IL-2 and IL-12(

The ELISA procedure was performed according to the manufacturer's protocol; samples from patients and healthy women's serum were added to antibody-coated wells, then incubation and washing steps to remove unbound substances. Enzyme conjugates and substrate solutions were then added, resulting in a measurable color reaction proportional to cytokine concentration; the absorbance was then measured using an ELISA microplate reader, and cytokine concentrations were calculated based on standard calibration curves. [21].

Table 1 Specifications of ELISA kits used in this study.

ELISA test	Manufacturer	Country of origin	Catalog No.	Detection range	Sensitivity	Intra-assay CV%	Inter-assay CV%
Toxoplasma IgG	Novus Biologicals / Bio-Techne	USA	NBP2-77055	Not stated	Not stated	<15%	<15%
Toxoplasma IgM	Novus Biologicals / Bio-Techne	USA	NBP2-60174	Not stated	Not stated	<15%	<15%
AMH	Abcam	UK	ab267629	0.164–40 ng/mL	0.18 ng/mL	<10%	<12%
IL-2	Invitrogen / Thermo Fisher Scientific	Austria	BMS221-2	18.8–1,200 pg/mL	9.1 pg/mL	7%	5%
IL-12 p70	Invitrogen / Thermo Fisher Scientific	Austria	BMS238	3.1–200 pg/mL	2.1 pg/mL	3%	4.8%

ELISA: Enzyme-Linked Immunosorbent Assay; AMH: Anti-Müllerian Hormone; IL-2: Interleukin-2; IL-12 p70: Interleukin-12 (p70); CV: Coefficient of Variation.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS), version 26.0. continuous variables were expressed as mean ± standard error (Mean ± SE). Differences between infected and control groups.

P-value ≤ 0.05 was statistically significant; the significance of differences in serum AMH, IL-2, and IL-12 levels between groups was evaluated.

Results

A total of 100 samples were enrolled in this study and equally allocated into the pregnant women with toxoplasmosis group (n1 = 50) and the pregnant women without Toxoplasmosis control group (n2 = 50), as shown in Table 2. No statistically significant differences were observed (P > 0.05).

Table 2. Baseline Characteristics of the Study Participants

Variables	Group I (50 pregnant women without Toxoplasmosis)		Group II (50 pregnant women with Toxoplasmosis))		P- value*
	mean	±SE	mean	±SE	
Maternal age	27.86	1.622	27.65	1.830	0.411
Gravidity	2.78	1.255	2.88	1.340	0.580

*Statistical significance: P < 0.05.

3.1: Comparison of Serum AMH Levels Between Pregnant Women With and Without Toxoplasmosis

Table (2) presents a comparison of serum Anti-Mullerian Hormone (AMH), IL-2 Interleukin-2 and IL-12 Interleukin-12 levels between pregnant women with and without *Toxoplasma gondii* infection. Pregnant women with toxoplasmosis (Group II) showed significantly higher serum Anti-Mullerian Hormone (AMH) levels (5.22 ± 0.399 ng/mL) than pregnant women without toxoplasmosis (Group I) (2.66 ± 0.552 ng/mL; P = 0.040). Likewise.

Serum Interleukin-2 (IL-2) levels were significantly higher in the second group (10.75 ± 0.68 pg/mL) than in the first Group (3.38 ± 0.40 pg/mL; P = 0.029). Similarly, serum In second group nterleukin-12 (IL-12) levels were significantly elevated (16.24 ± 0.60 pg/mL) compared with the first group (5.08 ± 0.50 pg/mL; P = 0.027). These findings indicate that *Toxoplasma gondii* infection during pregnancy was associated with significantly increased serum levels of Anti-Mullerian Hormone, Interleukin-2 and Interleukin-12, as shown in Table (3).

Table 3. Comparison of serum AMH, IL-2 and IL-12 in the study groups.

Variables	Group I (50 pregnant women without Toxoplasmosis)		Group II (50 pregnant women with Toxoplasmosis)		P value*
	mean	±SE	mean	±SE	
AMH	2.66	0.552	5.22	0.399	0.040
IL-2	3.38	0.40	10.75	0.68	0.029
IL-12	5.08	0.50	16.24	0.60	0.027

***Statistical significance: P < 0.05.**

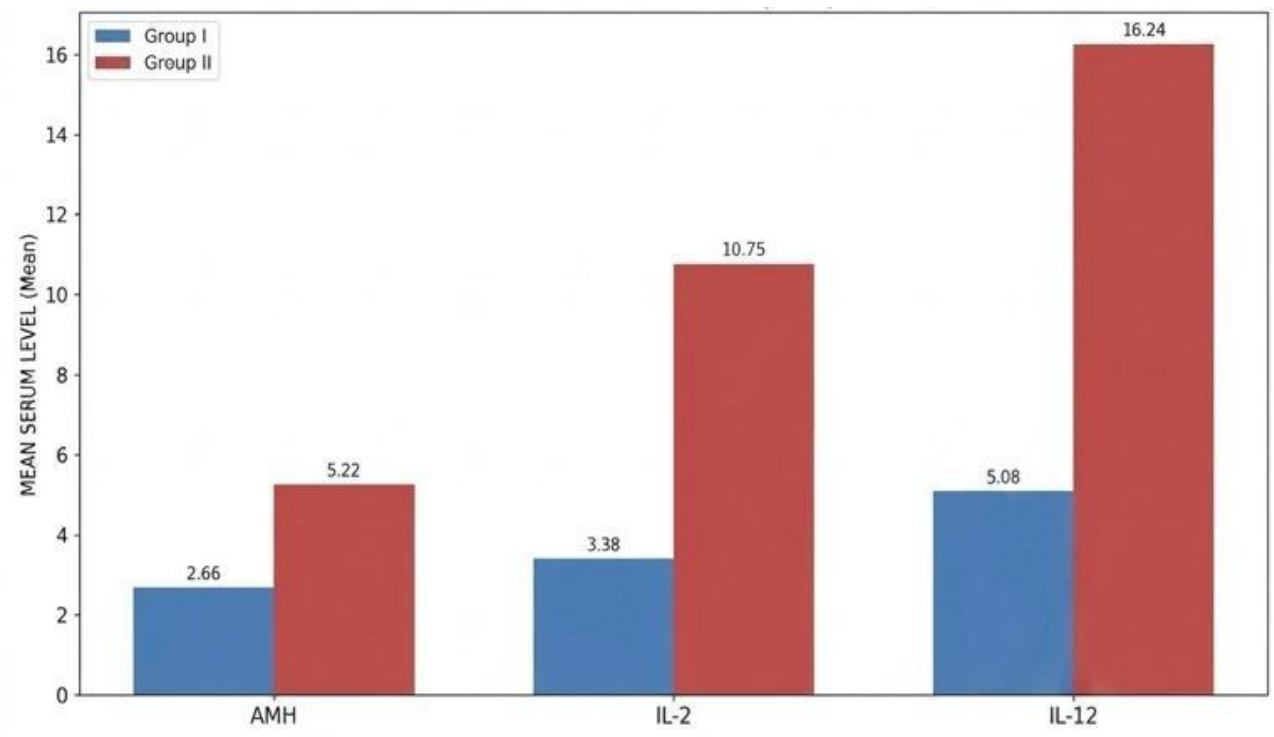

Figure (1): Mean Serum Levels of AMH, IL-2, and IL-12 in Groups I and II

Discussion

The present study found that serum Anti-Mullerian Hormone (AMH) levels were significantly higher in pregnant women infected with *Toxoplasma gondii* than in healthy pregnant women (P = 0.040).

Anti-Mullerian hormone (AMH) is secreted by granulosa cells in follicles and is the primary indicator of ovarian reserve. This increase may reflect the effects of the host immune response and inflammatory mediators induced by *T. gondii* infection on ovarian function. Such immunological changes could influence granulosa cell activity and, in turn, alter AMH secretion.

The present study's findings agree with those of Mushattat *et al*. (2024) [23], who reported significantly elevated serum AMH levels in women infected with *Toxoplasma gondii*. They proposed that activation of the immune response, along with increased production of inflammatory mediators, may alter ovarian endocrine function. In a similar report, Mazael and Shakir (2024) [24].found changes in AMH levels among women infected with *Toxoplasma gondii* who had experienced abortion. Their findings suggest that parasitic infection may influence reproductive hormonal regulation through immunological and inflammatory mechanisms. Taken together, these results support the hypothesis that immune activation associated with toxoplasmosis can affect ovarian physiology and hormone secretion.

In contrast, the present findings differ from those reported by Yahya Jirjees Salman (2014) [25], who observed lower serum AMH levels in women infected with *Toxoplasma gondii*. The discrepancy could reflect differences between the studies in population characteristics, sample size, reproductive status, laboratory methods, inclusion criteria, and the proportions of acute (IgM-positive) and chronic (IgG-positive) infections.

In this study, serum Interleukin-2 and Interleukin-12 levels were significantly higher in pregnant women infected with *Toxoplasma gondii* than in those in the control group (P < 0.05)

The present findings align with Mushattat *et al*. (2025) [26], who reported higher serum IL-2 and IL-12 levels among women infected with *Toxoplasma gondii*. They interpreted the increased cytokine levels as evidence of cellular immune activation and as a crucial immune response to the parasite. Similarly, our results show elevated IL-2 and IL-12, suggesting that pro-inflammatory cytokines help limit parasite replication and strengthen host resistance during toxoplasmosis.

Conclusion

The study showed that pregnant women infected with Toxoplasma gondii had significantly higher serum levels of AMH, IL-2, and IL-12 than healthy pregnant women. The increased levels of IL-2 and IL-12 indicate an enhanced immune response to the infection, while the higher AMH levels reflect changes in an ovarian-related marker. Overall, these findings indicate that T. gondii infection during pregnancy is associated with changes in both immune and ovarian-related biomarkers.

Conflict of Interest: Author declare there is no conflict of interest.

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تأثير الاصابة بطفيلي التوكسوبلازما جوندي على مستويات هرمون AMH، الإنترلوكين-2 والإنترلوكين-12 في مصل دم النساء الحوامل

منال ضياء محمد

الكلية التربوية المفتوحه - القسم الدراسي في الدجيل - العراق

الخلاصة:

تُعد الإصابة بطفيلي المقوسة الغوندية (*Toxoplasma gondii*) من أكثر الإصابات الطفيلية شيوعًا بين النساء في سن الإنجاب، وقد ارتبطت بحدوث مضاعفات الحمل نتيجة تنشيط الاستجابة المناعية والتغيرات في إفراز السيتوكينات الالتهابية. كما تشير الأدلة العلمية إلى أن هذه الاستجابة المناعية قد تؤثر في بعض المؤشرات الحيوية المرتبطة بوظيفة المبيض، ومن أبرزها الهرمون المضاد لمولر (Anti-Mullerian Hormone; AMH), هدفت هذه الدراسة إلى تقييم مستويات الهرمون المضاد لمولر (AMH)، والإنترلوكين-2 (IL-2)، والإنترلوكين-12 (IL-12) في مصل النساء الحوامل المصابات بعدوى *Toxoplasma gondii*، ومقارنتها بمستوياتها لدى النساء الحوامل السليمات, أُجريت دراسة من نوع الحالات المرضية والضابطة (Case-Control) إذ شملت 100 امرأة حامل في الثلث الأول من الحمل, وقُسمت المشاركات إلى مجموعتين متساويتين: ضمت المجموعة الأولى 50 امرأة حامل مصابة بعدوى *T. gondii* ومؤكدة مصليًا (إيجابية للأجسام المضادة IgG وIgM)، بينما ضمت المجموعة الثانية 50 امرأة حامل سليمة كمجموعة سيطرة. وتم قياس مستويات الهرمون المضاد لمولر (AMH)، والإنترلوكين-2 (IL-2)، والإنترلوكين-12 (IL-12) في مصل الدم باستخدام تقنية المقايسة المناعية المرتبطة بالإنزيم (ELISA). وأُجري التحليل الإحصائي باستخدام برنامج SPSS، واعتمدت القيمة $P < 0.05$ كمستوى للدلالة الإحصائية. أظهرت النساء الحوامل المصابات بداء المقوسات ارتفاعًا معنويًا في مستويات الهرمون المضاد لمولر مقارنة بمجموعة السيطرة (5.22 ± 0.399 مقابل 2.66 ± 0.552 نانوغرام/مل؛ P = 0.040). كما ارتفعت مستويات الإنترلوكين-2 بصورة معنوية لدى النساء المصابات (10.75 ± 0.68 مقابل 3.38 ± 0.40 بيكوغرام/مل؛ P = 0.029)، وكذلك ارتفعت مستويات الإنترلوكين-12 (16.24 ± 0.60 مقابل 5.08 ± 0.50 بيكوغرام/مل؛ P = 0.027). وتشير هذه النتائج إلى وجود ارتباط معنوي بين الإصابة بعدوى *T. gondii*، وتنشيط الاستجابة المناعية، وارتفاع مستويات الهرمون المضاد لمولر أثناء الحمل, نستنتج انه ترتبط الإصابة بطفيلي المقوسة الغوندية (*Toxoplasma gondii*) أثناء الحمل بارتفاع معنوي في مستويات الهرمون المضاد لمولر (AMH)، والإنترلوكين-2 (IL-2)، والإنترلوكين-12 (IL-12), كما تشير العلاقة الإيجابية بين الهرمون المضاد لمولر والسيتوكينات الالتهابية إلى أن الاستجابة المناعية الناجمة عن داء المقوسات قد تؤثر في وظيفة المبيض خلال الحمل.

الكلمات المفتاحية: المقوسة الغوندية (*Toxoplasma gondii*)، الهرمون المضاد لمولر (AMH)، الإنترلوكين-2 (IL-2)، الإنترلوكين-12 (IL-12)، المؤشرات المناعية.