http://ijmpes.com
doi:10.34172/ijmpes.6283
Open Access
Original Article
APMI Press
Aras Part Medical International Press
A scientific publishing imprint of PMC Pharmaceutique France
Int J Med Parasitol Epidemiol Sci 2026;7(3): x-x
eISSN 2766-6492
International Journal of Medical Parasitology & Epidemiology Sciences

Serum Interferon-Gamma, Interleukin-4, and Transforming Growth Factor-Beta Profiles in Infertile Women with Latent Toxoplasma gondii Infection: A Case-Control Study

Aws Maher Khaleel[1*], Amal Khudair Khalaf[1], Enaas Salih Jawad[2]

[1]Department of Microbiology, College of Medicine, University of Thi-Qar, Thi-Qar, Iraq
[2]Department of Gynecology & Obstetrics, College of Medicine, University of Thi-Qar, Thi-Qar, Iraq

Abstract

Introduction: Latent Toxoplasma gondii infection is highly prevalent worldwide and has been linked to adverse reproductive outcomes. However, the immunological mechanisms through which chronic toxoplasmosis may contribute to unexplained female infertility remain poorly understood, particularly regarding cytokine imbalance at the maternal-fetal interface. Aim of this study is determine whether latent T. gondii infection is associated with altered serum levels of pro-inflammatory (IFN-γ) and anti-inflammatory (IL-4, TGF-β) cytokines in females with unexplained infertility, and to evaluate whether this cytokine profile reflects a Th1-dominant immune imbalance.

Methods: A case-control study was conducted at Fatima Al-Zahraa Hospital, Baghdad, Iraq (February–May 2026). A total of 250 females were enrolled: 150 with unexplained infertility (cases) and 100 healthy fertile controls. All participants were tested for anti-T. gondii IgG antibodies by ELISA and subgrouped accordingly. Serum IFN-γ, IL-4, and TGF-β were quantified using sandwich ELISA. Data were analyzed using independent t-tests and two-way ANOVA.

Results: Toxoplasma seropositivity was significantly higher among infertile females (24.0% vs. 12.0%; OR = 2.32, $P = 0.018$). Seropositive infertile females exhibited significantly elevated IFN-γ (45.39 ± 9.76 vs. 33.54 ± 3.84 pg/mL; $P = 0.026$). Neither IL-4 ($P = 0.714$–0.901) nor TGF-β ($P = 0.397$–0.883) differed significantly across subgroups, indicating failure of compensatory anti-inflammatory regulation.

Conclusion: Latent T. gondii infection in females with unexplained infertility is associated with a Th1-dominant immune state that is not counterbalanced by regulatory cytokines, potentially disrupting endometrial receptivity and embryo implantation.

Keywords: *Toxoplasma gondii*, Infertility, Interferon-gamma, Interleukin-4, Transforming growth factor beta, Reproductive immunology

Received: April 12, 2026, **Revised:** June 5, 2026, **Accepted:** July 28, 2026, **ePublished:** August 10, 2026

Introduction

Infertility as a condition is defined as the inability to achieve a pregnancy after 12 months of regular unprotected intercourse (1). It affects a large number of people worldwide as some estimates put it at 10–15% of couples globally (2). In females, infertility can have a wide range of causes such as ovulatory disorders, tubal issues, uterine abnormalities and endometrium-related issues (3). However, there is a large portion of infertility cases that are labeled as "unexplained infertility" about 10–30% in some estimates, in these cases a clear cause of infertility cannot be established via the standard diagnostic route (4). Many studies link unexplained infertility to subtle immune dysregulation as a possible mechanism for the infertility in these cases especially cytokine imbalance at the maternal-fetal environment (5).

Toxoplasma gondii is a very common pathogen globally. It is an intracellular parasitic agent that infects a large percentage of the human population with some estimates putting the infection rate at one third of the population (6). *Toxoplasma gondii* life cycle is complex, having a sexual reproduction cycle in the definitive host which is exclusively felines and an asexual reproduction in intermediate hosts such as humans and other warm-blooded animals (7). Humans often get infected after ingestion of the tissue cyst in undercooked meat from intermediate hosts or from materials contaminated with cat feces that contain the sporulated oocyst (8). After the infection the parasite transforms from the rapid replicating form known as tachyzoite into the slowly growing form called the bradyzoite which is enclosed in a tissue cyst and is the latent phase form which resides in the brain, muscles and other organs (9). In people who are immunocompetent the latent Toxoplasmosis phase is asymptomatic but remains in a dormant state (10). The response to *Toxoplasma gondii* from the immune system is generally driven by Th1 cytokines. Interferon-gamma IFN-γ, which is produced by natural killer cells, CD4+ T helper cells and CD8+

*Corresponding Author: Aws Maher Khaleel, Email: Aws.ms.24@utq.edu.iq

cytotoxic T cells, plays an important role in the control of the parasite replication (11). IFN-γ functions by activating macrophages and promotes antimicrobial pathways which restrict the intracellular bradyzoite replication and prevent tissue cysts from rupturing (12). While Th1 response is important in countering invading pathogens it must be balanced to prevent damage to the host; This is done by anti-inflammatory cytokine release. In healthy individuals the anti-inflammatory response is mediated by cytokines such as interleukin-4 (IL-4) and transforming growth factor-beta (TGF-β) which prevent excessive damage to host tissues and promote tolerance (13). When this immune balance is affected, many disorders can occur. The immune environment in the reproductive system of females especially the uterus is quite complex and in order to achieve a successful embryo implantation the maternal immune system has to shift towards a tolerogenic state that permits the semi-foreign embryo to be implanted into the endometrium (14). This tolerance is mediated partly by the cytokine TGF-β and other regulatory cytokines that suppress the hostile cytotoxic immune agents in the uterine environment (15). An unregulated Th1 immune state dominated by IFN-γ has been shown to be damaging to trophoblast invasion and implantation (16), Which proves that the immune balance needed is a critical factor in fertility. Previous studies in Iraq and across the Middle East have shown a high seroprevalence for the *Toxoplasma gondii* parasite. Studies from Baghdad, Erbil, Babylon, Duhok and other cities have reported high percentages of *Toxoplasma gondii* infection in females of reproductive age reaching 30–60% (17-19). Many studies in Iraq have also linked *Toxoplasma gondii* to miscarriages, hormonal disturbances and reproductive failures (20-23). But only a few have focused on the cytokine mechanisms by which the latent infection phase was likely to interfere with normal fertility in females suffering from unexplained infertility. This study was designed to bridge this gap in knowledge. We measured the serum concentration levels of IFN-γ, IL-4 and TGF-β in a group of infertile females and healthy fertile females used as controls and these groups were subdivided based on their *Toxoplasma gondii* IgG seropositivity status. The results were analyzed using a two-way ANOVA to determine the independent and interactive effects of Toxoplasma infection and infertility status on the cytokine levels. We hypothesized that latent Toxoplasmosis would be involved in a Th1 immune shift in infertile females and that the regulatory cytokine would not sufficiently compensate for the inflammatory state.

Materials and Methods

Study design

A case-control study was conducted at the fertility clinics in Fatima Al-Zahraa hospital in Baghdad province, Iraq between the months of February 2026 and May 2026. Ethical approval was obtained from the institutional ethics committee and all participants provided informed consent before the collection of samples. All procedures done in this study were performed in accordance with the principles of the Declaration of Helsinki.

Study population

A total of 250 females were included in this study and were divided into two main groups. The case group which included 150 females who were diagnosed with unexplained infertility. Whereas the control group included 100 healthy fertile females who had at least one prior full-term pregnancy and had no history of miscarriages or infertility.

Exclusion criteria

Acute viral or bacterial infections, confirmed autoimmune diseases, recent use of immunosuppressive drugs and male factor infertility.

Serological classification

All females were tested for anti-*Toxoplasma gondii* IgG antibodies using a commercial ELISA kit by DRG diagnostics (Cat. Number EIA-3519). According to the results of this test participants were subdivided into four subgroups: Subgroup A (Infertile/Toxo-Negative, n = 114) Subgroup B (Fertile/Toxo-Negative, n = 88), Subgroup C (Infertile/Toxo-Positive, n = 36), and Subgroup D (Fertile/Toxo-Positive, n = 12).

Sample collection

5 ml of venous blood was collected from each participant using a standard gel-tube and the blood was left for 20 minutes at room temperature to clot. Then it was centrifuged for 10 minutes at 3500 rpm and the serum was transferred into Eppendorf tubes and stored at -20 degrees Celsius until testing.

Cytokine measurement

Serum concentration levels of IFN-γ, TGF-β and IL-4 were performed using BT-Labs Sandwich ELISA kits (Cat. Numbers E0105Hu, E3051Hu and E0092Hu respectively), following the manufacturer guidelines precisely.

Statistical analysis

The data were analyzed using IBM SPSS statistics v.26. Continuous variables are shown as mean ± standard deviation (SD). The age difference between the case and control groups was tested using an independent t-test. Cytokine levels were analyzed by two-way ANOVA with anti-*Toxoplasma gondii* IgG status and fertility status as fixed factors.

Results

Anti-Toxoplasma gondii IgG

Results for this test showed higher prevalence of latent Toxoplasmosis in the infertile case group at 24% (36 out of 150) compared to 12% (12 out of 100) for the healthy controls as shown in Table 1.

Chi-square analysis showed that this finding was statistically significant ($X^2 = 5.56$, $P = 0.018$). Risk modelling showed that infertile females included in this study had more than two times the odds of being infected with latent

toxoplasmosis compared to the healthy fertile control group (OR = 2.32, 95% CI = 1.13 - 4.73).

Participants demographics

A total of 250 females were included in this study: 150 infertile females (case group) and 100 healthy fertile females (control group). The mean age of the case group was 27.53 ± 5.92 years and the mean age of the control group was 28.85 ± 3.25 years as shown in Table 2.

An independent samples t-test was performed and showed no statistically significant differences between the groups in regards to age (t = -1.303, df = 248, $P = 0.198$). This shows that the groups age-matched and age can be excluded as a factor in the cytokine comparisons.

Serum IFN-γ concentration

The concentration levels of IFN-γ showed a clear stepwise elevation that is associated with infertility and *Toxoplasma gondii* seropositivity. The lowest mean IFN-γ was seen in the *Toxoplasma gondii* IgG negative healthy controls (Subgroup B: 33.54 ± 3.84 pg/ml), while the highest levels were seen in the *Toxoplasma gondii* IgG positive infertile group (Subgroup C: 45.39 ± 9.76 pg/ml). The IFN-γ levels in IgG positive fertile females (Subgroup D: 38.38 ± 4.71 pg/ml) and IgG negative infertile females (Subgroup A: 39.27 ± 9.47 pg/ml) as shown in Table 3.

Levene's test for equality of variance was non-significant (Levene statistic = 2.282, $P = 0.086$), indicating that the assumption of homogeneity of variance was met and standard two-way ANOVA was appropriate.

The two-way ANOVA test showed a statistically significant effect of the fertility status on the levels of IFN-γ (F = 5.182, $P = 0.026$). *Toxoplasma gondii* IgG serostatus showed a strong trend that was very close to the significance threshold (F = 3.830, $P = 0.054$). The interaction between *Toxoplasma gondii* infection and the fertility status was non-significant (F = 0.052, $P = 0.820$), which suggests that infertility and latent Toxoplasmosis act independently on IFN-γ levels rather than in unison. The overall ANOVA model was highly significant (F = 4.848, $P = 0.004$) and has accounted for approximately 16% of the variance in IFN-γ concentrations in total ($R^2 = 0.162$).

Serum IL-4 concentrations

Serum IL-4 levels showed similar results across all four subgroups of this study and no meaningful differences were observed. Mean values ranged from 137.83 ± 47.02 pg/ml for the infertile Toxoplasma IgG positive group (Subgroup C) to 144.04 ± 26.89 pg/ml for the fertile Toxoplasma IgG negative group (Subgroup B) while the infertile Toxoplasma IgG negative group (Subgroup A) had a mean concentration of 140.82 ± 36.20 pg/ml and lastly the fertile Toxoplasma IgG positive group (Subgroup D) had a mean of 137.99 ± 17.76 pg/mL as shown in Table 4.

Levene's test of variance showed unequal variance among the Subgroups in this study (Levene statistic = 3.085, $P = 0.032$) therefore robust post-hoc tests (Games-Howell) were applied for pairwise comparison. The two-way

Table 1. Seroprevalence and Odds Ratio of Anti-T. gondii IgG Between Study groups

Study groups	Total Examined (N)	IgG Positive (n, %)	IgG Negative (n, %)	χ^2 Value	*P*-value	Odds Ratio (95% CI)
Infertile Cases	150	36 (24.0%)	114 (76.0%)	5.56	0.018 (Significant)	2.32 (1.13 - 4.73) (95% Confidence)
Healthy Controls	100	12 (12.0%)	88 (88.0%)			

Table 2. Age distribution

Measured Parameter	Patient Group	Sample Size (N)	Average Age (Years)	Standard Deviation (±SD)
Participant Age	Infertile Cases	150	27.53	5.92

Table 3. Serum IFN-γ concentrations (pg/mL) across the four study subgroups with two-way ANOVA results

Subgroup	n	Mean (pg/mL)	SD	Fertility / Infection
Subgroup A – (Infertile / Toxo-Negative)	114	39.27	9.47	Infertile /Uninfected
Subgroup B – (Fertile / Toxo-Negative)	88	33.54	3.84	Fertile /Uninfected
Subgroup C – (Infertile / Toxo-Positive)	36	45.39	9.76	Infertile / Infected (highest)
Subgroup D – (Fertile / Toxo-Positive)	12	38.38	4.71	Fertile / Infected

Two-Way ANOVA: Overall model: F = 4.848, $P = 0.004$ ($R^2 = 0.162$) | Fertility status: F = 5.182, $P = 0.026$ | Infection status: F = 3.830, $P = 0.054$ | Interaction: F = 0.052, $P = 0.820$ | Levene's test: $P = 0.086$ (equal variances)

Table 4. Serum IL-4 concentrations (pg/mL) across the four study subgroups with two-way ANOVA results

Subgroup	n	Mean (pg/mL)	SD	Fertility / Infection
Subgroup A – (Infertile / Toxo-Negative)	114	140.82	36.20	Infertile / Uninfected
Subgroup B – (Fertile / Toxo-Negative)	88	144.04	26.89	Fertile / Uninfected
Subgroup C – (Infertile / Toxo-Positive)	36	137.83	47.02	Infertile / Infected
Subgroup D –(Fertile / Toxo-Positive)	12	137.99	17.76	Fertile / Infected

Two-Way ANOVA: Infection status: F = 0.136, $P = 0.714$ (NS) | Fertility status: F = 0.019, $P = 0.890$ (NS) | Interaction: F = 0.015, $P = 0.901$ (NS) | Levene's test: $P = 0.032$ (unequal variances; Games-Howell post-hoc applied)

ANOVA test showed no significant effect of Toxoplasmosis status (F = 0.136, $P = 0.714$) and no effect of the fertility status (F = 0.019, $P = 0.890$) as well as no significant interaction between the two factors mentioned (F = 0.015, $P = 0.901$). These findings show that IL-4 did not differ between the study groups in a significant manner.

Serum TGF-β concentration

Serum TGF-β concentration was observed to have a descending pattern, with healthy Toxoplasma seronegative controls having the highest levels and seropositive infertile females having the lowest levels. Despite this, there was no statistically significant difference across all subgroups. The mean concentrations were as follows, Subgroup B (fertile/Toxo IgG negative) 177.45 ± 54.59 pg/ml, Subgroup A (infertile/Toxo IgG negative) 169.14 ± 60.53 pg/ml, Subgroup D (fertile/Toxo IgG positive) 165.29 ± 25.94 pg/ml and Subgroup C (infertile/Toxo IgG positive) 151.92 ± 52.53 pg/ml as shown in Table 5.

Levene's test was non-significant (Levene statistic = 1.395, $P = 0.251$), which confirms the homogeneity of variance. The two-way ANOVA test was performed and showed no significant effect of *Toxoplasma gondii* IgG status (F = 0.727, $P = 0.397$) and no significant effect of fertility status as well (F = 0.396, $P = 0.531$) as well as no significant interaction between the two factors (F = 0.022, $P = 0.883$).

Summary of All ANOVA Results

Table 6 shows a summary of all two-way ANOVA results for all the three cytokines and their respective findings.

Discussion

This study examined the serum cytokine levels of 250 Iraqi females divided by infertility status and whether they had latent Toxoplasmosis or not. The main finding was that females who were infertile and positive for latent Toxoplasmosis (anti-*Toxoplasma gondii* IgG) showed significantly higher concentrations of IFN-γ (F = 5.182, $P = 0.026$), while serum concentrations of IL-4 ($P = 0.714$–0.901) and TGF-β ($P = 0.397$–0.883) were not significantly different across all subgroups of this study. These results show a Th1-dominant pro-inflammatory immunological state in infected infertile females which is not being balanced by the anti-inflammatory or regulatory factors of the immune system, which can have direct effects on endometrial receptivity and embryo implantation as these processes need a tightly balanced immune environment. The IFN-γ level in Toxo-positive infertile females was (45.39 ± 9.76 pg/ml) and (33.54 ± 3.84 pg/ml) in Toxo-negative fertile healthy controls which is in line with the well-established role of IFN-γ being the main cytokine effector in the defense against *Toxoplasma gondii*. IFN-γ is produced by NK cells and T lymphocytes in response to *Toxoplasma gondii* antigens and promotes activation of macrophages and the intracellular killing of *Toxoplasma gondii* (24-26). In the latent phase of infection, the parasite exists as bradyzoites enclosed in tissue cysts and the immune system in this case continues IFN-γ production at a subclinical but biologically relevant levels in order to protect against reactivation (27, 28). This study demonstrates this continuous IFN-γ production and the effect of *Toxoplasma gondii* infection on IFN-γ levels showed a strong trend (F = 3.830, $P = 0.054$) suggesting a strong trend that chronic Toxoplasmosis contributes independently to higher levels of IFN-γ. Several Iraqi and regional studies support this finding. For example, Azab Hameed and Khalaf [2024] reported higher levels of IFN-γ in patients who were seropositive for *Toxoplasma gondii* in Baghdad which

Table 5. Serum TGF-β concentrations (pg/mL) across the four study subgroups with two-way ANOVA results

Subgroup	n	Mean (pg/mL)	SD	Fertility / Infection
Subgroup A – (Infertile / Toxo-Negative)	114	169.14	60.53	Infertile / Uninfected
Subgroup B – (Fertile / Toxo-Negative)	88	177.45	54.59	Fertile / Uninfected (highest)
Subgroup C – (Infertile / Toxo-Positive)	36	151.92	52.53	Infertile / Infected (lowest)
Subgroup D – (Fertile / Toxo-Positive)	12	165.29	25.94	Fertile / Infected

Two-Way ANOVA: Infection status: F = 0.727, $P = 0.397$ (NS) | Fertility status: F = 0.396, $P = 0.531$ (NS) | Interaction: F = 0.022, $P = 0.883$ (NS) | Levene's test: $P = 0.251$ (equal variances)

Table 6. Summary of two-way ANOVA results for serum IFN-γ, IL-4, and TGF-β across all study subgroups

Cytokine	Effect	F-value	*P*-value	Significance	Levene's P
IFN-γ	Fertility status	5.182	0.026	*	0.086
	Infection status	3.830	0.054	†	—
	Interaction	0.052	0.820	NS	—
IL-4	Fertility status	0.019	0.890	NS	0.032 [a]
	Infection status	0.136	0.714	NS	—
	Interaction	0.015	0.901	NS	—
TGF-β	Fertility status	0.396	0.531	NS	0.251
	Infection status	0.727	0.397	NS	—
	Interaction	0.022	0.883	NS	—

* $P < 0.05$ (statistically significant) † $P = 0.054$ (borderline / strong trend) NS = not significant ($P > 0.05$)
[a] Levene's $P = 0.032$ for IL-4 (unequal variances); robust post-hoc tests applied. NS = not significant

supports the findings of this study (29). Additionally, Ibrahim et al. [2025] reported that Th1 response dominated in Egyptian females with both unexplained infertility and *Toxoplasma gondii* infection(30). Also a study in Baghdad reported significantly higher IFN-γ levels in infertile females infected with *Toxoplasma gondii* (31), a finding that is directly comparable to the significant main effect of fertility status observed in this study (F = 5.182, *P* = 0.026). Internationally, a [2023] systematic review by Dos Santos et al. revealed that the imbalance between pro-inflammatory and regulatory cytokines during gestational toxoplasmosis has harmful effects on the fetus, which suggests that chronic toxoplasmosis can promote consistent inflammatory cytokine production (16).

The main finding of this study is not just IFN-γ elevation but also that IL-4 and TGF-β levels remain the same and fail to counter the pro-inflammatory response. In normal conditions a strong Th1 response is countered by Th2 cytokines like IL-4 and regulatory factors like TGF-β to prevent excessive tissue damage and promote immune tolerance (32, 33). The data in this study show that the Th2 signals fail to increase in infertile females with latent Toxoplasmosis. IL-4 levels were very similar across all subgroups ranging from 137.83 to 144.04 pg/ml, *P* = 0.714–0.901, confirming Th2 response was not activated proportionally to the Th1 activation. This is explained by the known *Toxoplasma gondii* immune evasion strategy in which the parasite suppresses signals of IL-4 and Th2 differentiation as a survival mechanism, since a proper IL-4 and Th2 responses were shown to be of protective value against toxoplasmic encephalitis in murine models (34, 35). The lack of such response in this study can be expected to have significant consequences on the fertility of females with latent Toxoplasmosis.

The TGF-β cytokine is important in the immunology of the reproductive system as it promotes tolerance at the maternal-fetal interface, suppresses T cytotoxic cells and NK cells in the endometrium and also supports trophoblast invasion(14, 15, 36). In this study infertile females with latent Toxoplasmosis showed the lowest mean concentration of TGF-β (151.92 ± 52.53 pg/ml) which was lower than the uninfected fertile controls (177.45 ± 54.59 pg/ml) by almost 25 pg/ml. Although this was not statistically significant (F = 0.396, *P* = 0.531) it should not be dismissed. Especially when combined with higher IFN-γ level even a small decrease of TGF-β level can affect the tolerance of the uterine environment needed for successful implantation and shift it towards a hostile state. This is supported by Dos Santos [2023] findings who showed that cytokine imbalance in gestational Toxoplasmosis where pro-inflammatory cytokines dominate regulatory responses is associated with adverse pregnancy outcomes across the board (16). Haddad et al. [2025] from Iraq linked *Toxoplasma gondii* infection to altered TGF-β levels supporting the idea that the parasite affects the regulatory cytokines(37). Rico-Torres et al. [2025] showed that a single nucleotide polymorphism in the TGF-β1 regulatory gene region can influence the congenital Toxoplasmosis severity confirming the importance of TGF-β to *Toxoplasma gondii* related reproductive issues (13). The overall picture of this study shows an immune imbalance in the infertile females with chronic toxoplasmosis as the immune system is being consistently stimulated by the chronic infection to produce IFN-γ but at the same time the regulatory arms of the immune system fail to counter the inflammatory signals to balance this response. IL-4 and TGF-β levels relatively remain the same in all groups failing to achieve a tolerogenic state that is essential for a successful implantation (38). Furthermore, IFN-γ at the endometrium has been shown to alter adhesion molecules expression as well as impairment of trophoblast normal functions and promotion of a hostile NK phenotype (11, 39). And without TGF-β to suppress these effects and IL-4 to shift towards Th2 tolerance the uterine environment becomes hostile towards the embryo and is more likely to reject it than accept it.

These findings are in line with the research internationally that links *Toxoplasma gondii* to unexplained infertility and implantation failure. El Tantawy et al. [2014] in Egypt reported a significantly higher seroprevalence of *Toxoplasma gondii* in infertile females compared to healthy fertile controls and suggested immune mechanisms to be a possible link(40). Zamaniyan et al. [2023] in Iran reported some serological evidence that latent Toxoplasmosis could be associated with primary infertility in females(41). Kheirandish et al. from Iran also reported a significant link between *Toxoplasma gondii* infection and miscarriages(42). Fallahi et al. [2018] in a review of international literature on *Toxoplasma gondii* effects on reproductive health reported that both acute and chronic infections can cause reproductive issues through immune related pathways(43). Abdulhasan [2022] from Iraq reported higher seroprevalence of *Toxoplasma gondii* in females with miscarriages in Thi-Qar province(20). Also, Kadhim [2020] reported a significant association between spontaneous miscarriages in Iraqi females and *Toxoplasma gondii* infection(21). This shows the significance of the parasite effects on reproductive health of females in the region and all the worlds (44-47).

Conclusion

This study demonstrates that latent Toxoplasma gondii infection in females with unexplained infertility is associated with a pronounced Th1-dominant immunological imbalance characterized by elevated serum IFN-γ in the absence of compensatory increases in IL-4 or TGF-β. Rather than merely reflecting infection-driven cytokine production, this pattern suggests a failure of immune regulatory mechanisms that are essential for maintaining the tolerogenic environment at the maternal-fetal interface. The resulting pro-inflammatory milieu likely impairs endometrial receptivity and trophoblast invasion, contributing to implantation failure. These findings position chronic toxoplasmosis as a modifiable immunological contributor to unexplained infertility and support the integration of T. gondii serological screening into routine infertility workups, particularly in endemic

regions such as Iraq. Clinically, immune-modulating interventions alongside anti-parasitic therapy may represent a promising adjunctive strategy for improving reproductive outcomes in seropositive infertile women. Larger prospective studies with endometrial cytokine profiling are warranted to confirm these systemic findings at the tissue level.

Acknowledgments
The authors would like to thank the staff and patients at Fatima Al-Zahraa Hospital for their cooperation. The authors would also like to thank the Public Health Laboratory in Baqubah for its technical assistance.

Authors' Contribution
Conceptualization: Aws Maher Khaleel, Amal Khudair Khalaf, Enaas Salih Jawad
Data curation: Aws Maher Khaleel, Amal Khudair Khalaf, Enaas Salih Jawad
Formal analysis: Aws Maher Khaleel, Amal Khudair Khalaf, Enaas Salih Jawad
Funding acquisition: Aws Maher Khaleel, Amal Khudair Khalaf, Enaas Salih Jawad
Investigation: Aws Maher Khaleel, Amal Khudair Khalaf, Enaas Salih Jawad
Methodology: Aws Maher Khaleel, Amal Khudair Khalaf, Enaas Salih Jawad
Project administration: Aws Maher Khaleel, Amal Khudair Khalaf, Enaas Salih Jawad Resources: Aws Maher Khaleel, Amal Khudair Khalaf, Enaas Salih Jawad
Software: Aws Maher Khaleel, Amal Khudair Khalaf, Enaas Salih Jawad
Supervision: Aws Maher Khaleel, Amal Khudair Khalaf, Enaas Salih Jawad
Validation: Aws Maher Khaleel, Amal Khudair Khalaf, Enaas Salih Jawad
Visualization: Aws Maher Khaleel, Amal Khudair Khalaf, Enaas Salih Jawad Writing – original draft: Aws Maher Khaleel, Amal Khudair Khalaf, Enaas Salih Jawad
Writing – review & editing: Aws Maher Khaleel, Amal Khudair Khalaf, Enaas Salih Jawad.

Competing Interests
The authors declare no conflicts of interest.

Data Availability Statement
The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval
This study was approved by the Institutional Ethics Committee of the College of Medicine, University of Thi-Qar. Informed consent was obtained from all individual participants included in the study.

Funding
This study was self-funded by the authors and received no external financial support from any funding organization.

References
1. Adebisi OY, Singh M, Tobler KJ. Female infertility. In: StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing; 2025.
2. Liu J, Qin Y, Liu H, Liu Y, Yang Y, Ning Y, et al. Global, regional, and national burden of female infertility and trends from 1990 to 2021 with projections to 2050 based on the GBD 2021 analysis. Sci Rep 2025;15(1):17559. doi:10.1038/s41598-025-01498-x
3. Carson SA, Kallen AN. Diagnosis and management of infertility: a review. JAMA 2021;326(1):65-76. doi:10.1001/jama.2021.4788
4. Raperport C, Desai J, Qureshi D, Rustin E, Balaji A, Chronopoulou E, et al. The definition of unexplained infertility: a systematic review. BJOG 2024;131(7):880-97. doi:10.1111/1471-0528.17697
5. Gómez-Chávez F, Cañedo-Solares I, Ortiz-Alegría LB, Flores-García Y, Luna-Pastén H, Figueroa-Damián R, et al. Maternal immune response during pregnancy and vertical transmission in human toxoplasmosis. Front Immunol 2019;10:285. doi:10.3389/fimmu.2019.00285
6. Smith NC, Goulart C, Hayward JA, Kupz A, Miller CM, van Dooren GG. Control of human toxoplasmosis. Int J Parasitol 2021;51(2-3):95-121. doi:10.1016/j.ijpara.2020.11.001
7. Sharma J, Rodriguez P, Roy P, Guiton PS. Transcriptional ups and downs: patterns of gene expression in the life cycle of *Toxoplasma gondii*. Microbes Infect 2020;22(10):525-33. doi:10.1016/j.micinf.2020.09.001
8. Robert-Gangneux F, Dardé ML. Epidemiology of and diagnostic strategies for toxoplasmosis. Clin Microbiol Rev 2012;25(2):264-96. doi:10.1128/cmr.05013-11
9. Matta SK, Rinkenberger N, Dunay IR, Sibley LD. *Toxoplasma gondii* infection and its implications within the central nervous system. Nat Rev Microbiol 2021;19(7):467-80. doi:10.1038/s41579-021-00518-7
10. Daher D, Shaghlil A, Sobh E, Hamie M, Hassan ME, Moumneh MB, et al. Comprehensive overview of *Toxoplasma gondii*-induced and associated diseases. Pathogens 2021;10(11):1351. doi:10.3390/pathogens10111351
11. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-γ: an overview of signals, mechanisms and functions. J Leukoc Biol 2004;75(2):163-89. doi:10.1189/jlb.0603252
12. Schneider WM, Chevillotte MD, Rice CM. Interferon-stimulated genes: a complex web of host defenses. Annu Rev Immunol 2014;32:513-45. doi:10.1146/annurev-immunol-032713-120231
13. Rico-Torres CP, Reyes-León A, Figueroa-Damián R, Vela-Amieva M, Macias-Parra M, Ruiz-García M, et al. SNPs at the IL-2, IL-12, TNF-α and TGF-β1 regulatory gene regions are probably important for *Toxoplasma gondii* congenital transmission and disease severity in humans. Microbes Infect 2025;27(8):105565. doi:10.1016/j.micinf.2025.105565
14. Mor G, Cardenas I. The immune system in pregnancy: a unique complexity. Am J Reprod Immunol 2010;63(6):425-33. doi:10.1111/j.1600-0897.2010.00836.x
15. Deng Z, Fan T, Xiao C, Tian H, Zheng Y, Li C, et al. TGF-β signaling in health, disease, and therapeutics. Signal Transduct Target Ther 2024;9(1):61. doi:10.1038/s41392-024-01764-w
16. Dos Santos PV, de Toledo DN, de Souza DM, Menezes TP, Perucci LO, Silva ZM, et al. The imbalance in the relationship between inflammatory and regulatory cytokines during gestational toxoplasmosis can be harmful to fetuses: a systematic review. Front Immunol 2023;14:1074760. doi:10.3389/fimmu.2023.1074760
17. Muwafaq SM, Ali AA, NoorEldin MY. The molecular detection of *Toxoplasma gondii* infection and its impact on leptin and ferritin levels in diabetic females. Rev Diabet Stud 2025;21(1):14-20. doi:10.1900/nz4h3041
18. Mala SF, Bakre HM. Association of seropositivity of toxoplasmosis and type 2 diabetes among adult females in Erbil city. Zanco J Med Sci 2023;27(3):234-43. doi:10.15218/zjms.2023.026
19. Mustafa KM, Mohammed AB, Mero WM. Seroprevalence of *Toxoplasma gondii* antibodies and associated risk factors among women in Zakho city, Iraq. Cureus 2024;16(3): e56328. doi:10.7759/cureus.56328
20. Abdulhasan SA. Prevalence of toxoplasmosis among miscarried women and the effect on embryo in Thi-Qar province, southern Iraq. Univ Thi-Qar J 2022;9(1):26-9. doi:10.32792/utq/utjsci.v9i1.868

21. Kadhim DK, Rahi AA. The burden of *Toxoplasma gondii* in spontaneous miscarriage and its association with rhoptry protein 5 gene and *Toxoplasma gondii* specific primers GRA 6 gene expression in Iraq women. Indian J Forensic Med Toxicol 2020;14(2):1496-500. doi:10.37506/ijfmt.v14i2.3136
22. Hussein RD, Mohammed AS. Relationship between *Toxoplasma gondii* and sex hormone. Egypt Acad J Biol Sci C Physiol Mol Biol 2022;14(2):229-36.
23. Al-Warid HS, Ali HZ, Muhamad SN. Detection of LTH, FSH and LH hormone level in pregnant women infected with *Toxoplasma gondii*. Int J Sci Res 2012;3(10):809-11.
24. Ihara F, Yamamoto M. The role of IFN-γ-mediated host immune responses in monitoring and the elimination of *Toxoplasma gondii* infection. Int Immunol 2024;36(5):199-210. doi:10.1093/intimm/dxae001
25. Dupont CD, Christian DA, Hunter CA. Immune response and immunopathology during toxoplasmosis. Semin Immunopathol 2012;34(6):793-813. doi:10.1007/s00281-012-0339-3
26. Sasai M, Pradipta A, Yamamoto M. Host immune responses to *Toxoplasma gondii*. Int Immunol 2018;30(3):113-9. doi:10.1093/intimm/dxy004
27. Eberhard JN, Shallberg LA, Winn A, Chandrasekaran S, Giuliano CJ, Merritt EF, et al. Immune targeting and host-protective effects of the latent stage of *Toxoplasma gondii*. Nat Microbiol 2025;10(4):992-1005. doi:10.1038/s41564-025-01967-z
28. Sullivan WJ Jr, Jeffers V. Mechanisms of *Toxoplasma gondii* persistence and latency. FEMS Microbiol Rev 2012;36(3):717-33. doi:10.1111/j.1574-6976.2011.00305.x
29. Azab Hameed F, Khalaf AK. The effect of infection with *Toxoplasma gondii* in inducing interferon-gamma in breast cancer patients. Arch Razi Inst 2024;79(1):138-43. doi:10.32592/ari.2024.79.1.138
30. Ibrahim A, Mostafa T, Abdelaziz AA, Ali Y. The imbalance of TNF-α level and gene polymorphisms involved in *Toxoplasma gondii* infection contributes to unexplained female infertility. Afr J Biol Sci 2025.
31. Ismael AK, Salih TA. Studying the role of IFN-γ, vitamin C, SOD, LH, FSH, APA and ACA in *Toxoplasma gondii* infected miscarriage women. Baghdad Sci J 2019;16(3):22. doi:10.21123/bsj.2019.16.3(Suppl.).0697
32. Sana M, Rashid M, Rashid I, Akbar H, Gomez-Marin JE, Dimier-Poisson I. Immune response against toxoplasmosis-some recent updates RH: *Toxoplasma gondii* immune response. Int J Immunopathol Pharmacol 2022;36:3946320221078436. doi:10.1177/03946320221078436
33. Heeb LE, Egholm C, Boyman O. Evolution and function of interleukin-4 receptor signaling in adaptive immunity and neutrophils. Genes Immun 2020;21(3):143-9. doi:10.1038/s41435-020-0095-7
34. Suzuki Y, Yang Q, Yang S, Nguyen N, Lim S, Liesenfeld O, et al. IL-4 is protective against development of toxoplasmic encephalitis. J Immunol 1996;157(6):2564-9.
35. Chaves AC, Cerávolo IP, Gomes JA, Zani CL, Romanha AJ, Gazzinelli RT. IL-4 and IL-13 regulate the induction of indoleamine 2,3-dioxygenase activity and the control of *Toxoplasma gondii* replication in human fibroblasts activated with IFN-γ. Eur J Immunol 2001;31(2):333-44. doi:10.1002/1521-4141(200102)31:2<333::aid-immu333>3.0.co;2-x
36. Jing H, Gao Y, Jing L, Yang H, Liu S. Recent advances in therapeutic use of transforming growth factor-beta inhibitors in cancer and fibrosis. Front Oncol 2025;15:1489701. doi:10.3389/fonc.2025.1489701
37. Haddad AH, Khalaf AK, Al-Waeli DK. Detection of *Toxoplasma gondii* among autoimmune thyroid disease (AITD) and estimation its association with transforming growth factor-β (TGF-β). Egypt J Med Microbiol 2025;34(1):65-70.
38. Vannuccini S, Clifton VL, Fraser IS, Taylor HS, Critchley H, Giudice LC, et al. Infertility and reproductive disorders: impact of hormonal and inflammatory mechanisms on pregnancy outcome. Hum Reprod Update 2016;22(1):104-15. doi:10.1093/humupd/dmv044
39. Ng CT, Fong LY, Abdullah MNH. Interferon-gamma (IFN-γ): reviewing its mechanisms and signaling pathways on the regulation of endothelial barrier function. Cytokine 2023;166:156208. doi:10.1016/j.cyto.2023.156208
40. El-Tantawy N, Taman A, Shalaby H. Toxoplasmosis and female infertility: is there a co-relation. Am J Epidemiol Infect Dis 2014;2(1):29-32. doi:10.12691/ajeid-2-1-6
41. Zamaniyan M, Fakhar M, Tabaripour R, Peivandi S, Keighobadi M, Ghasemi S, et al. Credible serological evidence of latent *Toxoplasma* infection among women with primary infertility: a ten-year registry-based study. Acta Parasitol 2023;68(2):400-5. doi:10.1007/s11686-023-00677-y
42. Kheirandish F, Ezatpour B, Fallahi S, Tarahi MJ, Hosseini P, Karimi Rouzbahani A, et al. *Toxoplasma* serology status and risk of miscarriage, a case-control study among women with a history of spontaneous abortion. Int J Fertil Steril 2019;13(3):184-9. doi:10.22074/ijfs.2019.5740
43. Fallahi S, Rostami A, Nourollahpour Shiadeh M, Behniafar H, Paktinat S. An updated literature review on maternal-fetal and reproductive disorders of *Toxoplasma gondii* infection. J Gynecol Obstet Hum Reprod 2018;47(3):133-40. doi:10.1016/j.jogoh.2017.12.003
44. Garedaghi Y, Safarmashaei S. Survey of *Toxoplasma* contamination in kidney recipient patients by ELISA method and comparison it with control group in Tabriz (East-Azerbaijan), Iran. Adv Environ Biol 2011;5(4):769-72.
45. Garedaghi Y, Bahavarnia SR. Repairing effect of *Allium cepa* on testis degeneration caused by *Toxoplasma gondii* in the rat. Int J Womens Health Reprod Sci 2014;2(2):80-9. doi:10.15296/ijwhr.2014.12
46. Garedaghi Y, Firozivand Y. Assessment of pregnant women toxoplasmosis by ELISA method in Miandoab city, Iran. Int J Womens Health Reprod Sci 2017;5(1):72-5. doi:10.15296/ijwhr.2017.13
47. Jannati R, Tavakoli Pasand, Garedaghi Y. Evaluation of different techniques in laboratory diagnosis of intestinal amoebiasis. Int J Med Parasitol Epidemiol Sci 2024;5(1):16-23. doi: https://doi.org/10.34172/ijmpes.3129