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Original Article

Assessment of Immunological Parameters among Women with Pregnancy Loss in Babylon City, Iraq: A Seroprevalence Study

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Abstract

Background: Pregnancy loss is attributed to several factors involved in human reproduction. Genetic and uterine abnormalities, endocrine and immunological dysfunctions, infectious agents, environmental pollutants, psychogenetic factors, and endometriosis are among the most significant causes of spontaneous abortion. **Objectives:** This study aimed to determine the potential role of anticardiolipin (ACL) and anti-phospholipid (APS) antibodies, alongside *Toxoplasma gondii* and Rubella virus infections, in the occurrence of pregnancy-related complications and recurrent miscarriages. **Methods:** A case-control study was conducted involving 300 participants aged 18-45 years in Babylon city from March 15 to May 25, 2023. The case group comprised 250 women with a history of one or more consecutive unexplained spontaneous abortions. The control group consisted of 50 healthy pregnant women with no history of miscarriage. Serum levels of ACL, APS, and antibodies (IgM and IgG) against *T. gondii* and Rubella virus were evaluated using the automated MiniVIDAS apparatus (Enzyme-Linked Fluorescent Assay - ELFA system) according to manufacturer protocols (bioMérieux, Marcy-l'Étoile, France; ref. 30400, 30410, 30420). **Results:** A highly significant association was observed between the gestational age (trimesters) and the seroprevalence of the studied etiological agents. The highest seropositivity rates for *T. gondii* IgM (81.6%), Rubella IgM (69.6%), ACL IgM (66.0%), and APS IgM (62.8%) were recorded among the cases. First-trimester miscarriages exhibited significantly higher seropositivity rates for all IgM markers compared to the second and third trimesters ($P < 0.001$). Notably, the control group showed 0% positivity for all IgM markers, highlighting a highly specific risk association ($P < 0.001$). Spearman's correlation analysis revealed strong negative correlations between IgM titers and gestational age: *T. gondii* ($\rho = -0.72$, $P < 0.001$), Rubella ($\rho = -0.58$, $P < 0.01$), ACL ($\rho = -0.61$, $P < 0.01$), and APS ($\rho = -0.65$, $P < 0.001$).**Conclusion:** This study demonstrates that acute infections and autoimmune markers are significantly associated with spontaneous abortion, particularly during the first trimester. The findings highlight the importance of early serological screening for TORCH infections and anti-phospholipid antibodies to improve maternal-fetal outcomes and reduce recurrent miscarriage rates in Iraq.

Keywords: *Anticardiolipin; Anti-Phospholipids; Gestational Ag; Miscarriage; Pregnancy Loss; Seroprevalence; Rubella; Toxoplasma Gondii*

Introduction

Abortion or miscarriage refers to the termination of a pregnancy by removing or expelling an embryo or fetus before it can survive outside the uterus. Spontaneous abortion occurs without any medical or

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surgical intervention, whereas induced abortion is deliberately carried out to end a pregnancy (Mohammed & Al-Janabi, 2019). Miscarriage, defined as the loss of pregnancy prior to 20–24 weeks of gestation, occurs in approximately 15% of all clinically recognized pregnancies (Quenby *et al*., 2021) and represents one of the most common adverse pregnancy outcomes worldwide (Dimitriadis *et al*., 2020). Beyond its physical consequences, miscarriage significantly impacts the psychological well-being of affected women, with evidence demonstrating elevated risks of anxiety, depression, and post-traumatic stress that may persist for months following the loss (Farren *et al*., 2020).

Spontaneous abortion is a complex, multifactorial outcome driven by a spectrum of anatomical, genetic, environmental, and immunologic dysfunctions, with chromosomal abnormalities remaining the primary etiology in early pregnancy loss (Hyde & Schust, 2015). In addition, infectious etiologies—most notably *Toxoplasma gondii* and Cytomegalovirus (CMV)—substantially contribute to adverse maternal-fetal outcomes and contribute to roughly 10–15% of total miscarriage cases worldwide (Megli & Coyne, 2022).

T. gondii is an intracellular parasite that infects humans primarily through the ingestion of contaminated food, water, soil, or undercooked meat containing tissue cysts (Kakayi *et al*., 2021). The global seroprevalence of toxoplasmosis in women of reproductive age ranges from 9% to 77%, with higher rates reported in the Middle East and North Africa (Pappas *et al*., 2009). Similarly, Rubella virus, which is often asymptomatic in adults, can lead to severe congenital defects or fetal death (congenital rubella syndrome) if infection occurs during the first trimester of pregnancy, with up to a 90% risk of transmission to the fetus during maternal viremia (Mahmood *et al*., 2022; Lambert *et al*., 2015).

In addition to infectious agents, autoimmune factors play a critical role. Anti-cardiolipin (ACL) antibodies and Anti-Phosphatidylserine (APS) antibodies are classical markers of anti-phospholipid syndrome, an immune disorder that induces hypercoagulability affecting approximately 1-2% of the general population and 15-20% of patients with recurrent miscarriage (Ruiz-Irastorza *et al*., 2010). This condition increases the risk of thrombosis in placental vessels, leading to recurrent miscarriages and pregnancy complications, often occurring after 12 weeks of gestation (Aldabbagh *et al*., 2022; Miyakis *et al*., 2006).

This study aimed to investigate the seroprevalence of T. gondii and Rubella virus infections, evaluate the levels of ACL and APS antibodies, and assess their quantitative association with the risk of spontaneous abortion among women in Babylon city.

Material and Methods

Study Design

A case-control study was conducted in various private clinics in Babylon City, Iraq, between March 15 and May 25, 2023. A total of 300 women aged between 18 and 45 years were enrolled. The sample size was calculated using the formula for case-control studies with an expected odds ratio of 2.5, 80% power, and 95% confidence level, based on regional TORCH prevalence estimates from previous Iraqi studies (Mohammed & Al-Janabi, 2019; Naame *et al*., 2021). A case-to-control ratio of 5:1 was employed to maximize statistical power for detecting exposure-disease associations while optimizing recruitment feasibility. This ratio was deemed appropriate given the rarity of healthy pregnant controls meeting all inclusion criteria in the local setting. Participants were divided into two groups:

1. *Cases (n=250):* Pregnant women who experienced one or more consecutive, unexplained spontaneous abortions.

- *Inclusion Criteria:* Women aged 18-45 with a confirmed clinical history of unexplained recurrent or spontaneous miscarriage.

- *Exclusion Criteria:* Women with known anatomical (e.g., uterine malformations), genetic (e.g., chromosomal abnormalities), or hormonal (e.g., thyroid dysfunction, polycystic ovary syndrome) causes of miscarriage were excluded to isolate immunological and infectious factors.

2. Controls (n=50): Apparently healthy pregnant women matched by age, with a successful obstetric history and no prior miscarriages.

Blood Sample Collection

Venous blood samples (5 mL) were collected from all participants using standard sterile techniques by qualified phlebotomists. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at −20°C until further analysis, following standardized pre-analytical protocols (Ramamonjiarisoa *et al*., 2026).

Detection of Anticardiolipin and Anti-Phospholipid Antibodies

The serum levels of anticardiolipin (ACL) and antiphospholipid (APS) antibodies (IgM and IgG), as well as specific IgM and IgG antibodies for *Toxoplasma gondii* and Rubella virus, were measured using the automated MiniVIDAS® analyzer (bioMérieux, Marcy-l'Étoile, France) based on the Enzyme-Linked Fluorescent Assay (ELFA) principle. Commercially available diagnostic kits (VIDAS® TOXO IgM [Ref. 30202], VIDAS® TOXO IgG II [Ref. 30210], VIDAS® RUB IgM [Ref. 30214], and VIDAS® RUB IgG II [Ref. 30221]) were utilized strictly according to the manufacturer's instructions. Test results were automatically calculated and interpreted based on the manufacturer's cut-off values: for *T. gondii* IgM, an index of 0.60 was considered positive, while Rubella IgG levels of 10 IU/mL were interpreted as immune/positive.

Statistical Analysis

Data analysis was performed using SPSS version 21 (IBM Corp., Chicago, USA). Categorical variables were presented as frequencies and percentages. Pearson's Chi-square test was employed to compare proportions between groups. Quantitative IgM seropositivity levels (U/mL) were expressed as mean ± standard deviation (SD). To measure the strength of association, Odds Ratios (OR) and 95% Confidence Intervals (CI) were calculated. To resolve the zero-cell count issue in the control group (0% seropositivity) and prevent division by zero, the Haldane-Anscombe continuity correction—adding 0.5 to all cells of the 2×2 contingency table—was applied, and Fisher's exact test was used to determine statistical significance (Jewell, 2003).

Spearman's rank correlation coefficient (ρ) was calculated to assess the relationship between quantitative IgM seropositivity levels (U/mL) and gestational age at miscarriage (coded as 1=first trimester, 2=second trimester, 3=third trimester), as suggested for non-parametric correlation analysis in reproductive health studies (Indryani*et al*., 2023). A *P*-value of < 0.05 was considered statistically significant and < 0.001 as highly significant.

Ethical Approval

Ethical approval was obtained from the Institutional Review Board (IRB) / Research Ethics Committee of Al Furat Al-Awsat Technical University, Kufa, Al-Najaf, Iraq, reference number 628 dated 22nd June 2026.

Results

The study included 250 cases (women with miscarriage) and 50 healthy controls. The cases were subdivided based on the number of miscarriages. Figure 1 illustrates that the frequency of having a first miscarriage was the highest (37%), followed by those experiencing a second (32%) and third (31%) miscarriage.

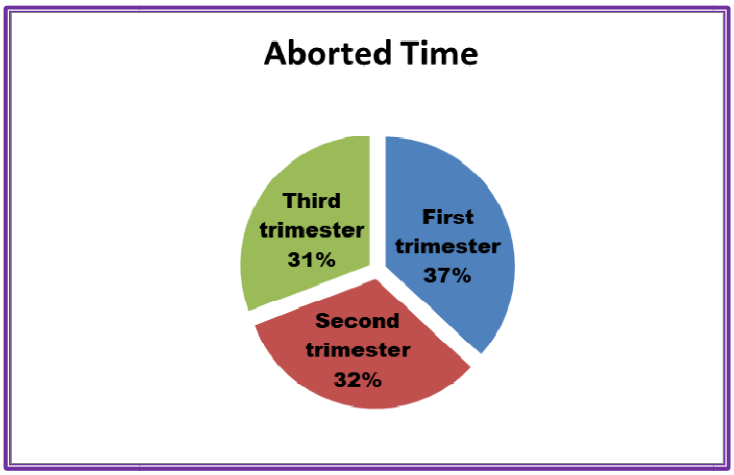

Figure 1: *Distribution of 250 Women with Pregnancy Loss with Seropositivity of ACL, APS, Rubella Virus and Toxoplasma Gondii According to Number of Miscarriages*

The highest incidence of miscarriages occurred in the 30-39 age group (40.4%), closely followed by the 20-29 age group (36.4%). Serological analysis (Table 1) revealed an extremely high prevalence of acute infections and autoimmune markers among the cases compared to controls. Out of 250 cases, 204 (81.6%) tested positive for T. gondii IgM, making it the most prevalent marker. This was followed by Rubella IgM (69.6%), ACL IgM (66.0%), and APS IgM (62.8%). Notably, the control group showed 0% positivity for all IgM markers, highlighting a highly specific risk association ($P < 0.001$).

Table 1: *Results of MiniVIDAS System in 250 Cases and 50 Controls*

Serological Analysis	Ig Type	Cases (N=250)	Cases (%)	Controls (N=50)	Controls (%)
Rubella	IgM	174	69.6%	0	0.0%
Rubella	IgG	76	30.4%	7	14.0%
Toxoplasma gondii	IgM	204	81.6%	0	0.0%
Toxoplasma gondii	IgG	46	18.4%	10	20.0%
Anticardiolipin (ACL)	IgM	165	66.0%	0	0.0%
Anticardiolipin (ACL)	IgG	85	34.0%	0	0.0%
Antiphosphatidylserine (APS)	IgM	157	62.8%	0	0.0%
Antiphosphatidylserine (APS)	IgG	93	37.2%	0	0.0%

(Note: Percentages are calculated accurately based on the total N = 250 for cases)

Furthermore, the study found a highly significant correlation between the gestational age (trimester) at the time of miscarriage and the seropositivity of these etiological agents. Table 2 demonstrates that the first trimester accounted for the vast majority of positive IgM cases across all markers ($P< 0.001$ for *T. gondii* and $P< 0.05$ for others).

Table 2: *Distribution of Seropositive Cases According to Gestational Age*

Marker	Gestational Trimester	(N)Positive Cases	(250of Total) %	value-*P*
	st1	158	%63.2	
	nd2	10	%4.0	
Rubella IgM	rd3	6	%2.4	(Significant) 0.05
	st1	184	%73.6	
	nd2	20	%8.0	
gondii IgM .T	rd3	0	%0	(.Highly Sig) 0.001
	st1	147	%58.8	
	nd2	15	%6.0	
ACL IgM	rd3	3	%1.2	(Significant) 0.05
	st1	154	%61.6	
	nd2	3	%1.2	
APS IgM	rd3	0	%0	(Significant) 0.05

Correlation Analysis between IgM Seropositivity and Gestational A

To further investigate the quantitative relationship between acute immunological markers and the timing of pregnancy loss, Spearman's rank correlation coefficient (ρ) was calculated between IgM seropositivity levels (continuous U/mL values) and gestational age at miscarriage (coded as 1=first trimester, 2=second trimester, 3=third trimester). A strong negative correlation was observed between *T. gondii* IgM levels and gestational age (ρ = -0.72, *P*< 0.001), indicating that higher acute antibody titers were predominantly associated with earlier pregnancy losses. Similarly, significant negative correlations were found for Rubella IgM (ρ = -0.58, *P*< 0.01), ACL IgM (*ρ* = -0.61, *P*< 0.01), and APS IgM (ρ = -0.65, *P*< 0.001). These findings quantitatively confirm that the pathological impact of acute infections and autoimmune activation is most pronounced during the first trimester, with diminishing effect in later gestational stages, consistent with established critical windows of trophoblast vulnerability (Sheridan *et al*., 2017; Lin *et al*., 2023).

Discussion

The present study investigated the seroprevalence of infectious and autoimmune markers in 250 women with spontaneous abortion compared to 50 healthy pregnant controls in Babylon City, Iraq. Several key findings emerged that warrant critical discussion. First, the absence of IgM positivity in all healthy controls (0%) contrasts sharply with the high seropositivity rates observed in cases, suggesting a strong association between acute infections and pregnancy loss. However, this finding must be interpreted cautiously given the cross-sectional design, which precludes the establishment of definitive temporal causality.

A remarkably high 81.6% of cases tested positive for *Toxoplasma gondii* IgM, which is substantially higher than previously reported seroprevalence rates in regional populations, which typically range between 35% and 65% (Rostami *et al*., 2020; Bigna *et al*., 2020). This discrepancy may reflect differences in sample selection, diagnostic assay sensitivities, or geographic variations in exposure risk. Such elevated seropositivity could be attributed to local dietary habits, agricultural practices involving contact with contaminated soil, or ingestion of undercooked meat. However, it is critical to acknowledge that IgM antibodies can persist for several months to over a year post-infection; thus, their isolated presence does not definitively confirm an acute primary infection during the critical window of pregnancy loss (Vargas-Villavicencio *et al*., 2022). Incorporating IgG avidity testing remains indispensable to delineate recent from remote infections, representing a methodological limitation of the present study. Furthermore, Rubella IgM was detected in 69.6% of cases. While this prevalence is clinically notable, rubella vaccination coverage in low- and middle-income regions has historically experienced regional suboptimality (Grant *et al*., 2019), and IgM reactivity may reflect persistent post-vaccination immune response, subclinical viral re-exposure, or non-specific immunological cross-reactivity rather than active congenital viremia at the time of miscarriage (Lambert *et al*., 2015).

Beyond infectious agents, autoimmune markers demonstrated a notable association with pregnancy loss. Anti-cardiolipin (ACL) IgM and broader antiphospholipid (APS) IgM reactivity were positive in 66.0% and 62.8% of cases, respectively, compared to 0% in controls. While these antibodies are established mediators of antiphospholipid syndrome and pregnancy complications (Miyakis *et al*., 2006; Ruiz-Irastorza *et al*., 2010), several methodological caveats must be considered. First, the clinical classification of antiphospholipid syndrome strictly requires the persistent positivity of these antibodies on two or more occasions at least 12 weeks apart (Miyakis *et al*., 2006), which was not evaluated in this single-point cross-sectional study. Second, the exclusion of women with known autoimmune diseases may have inadvertently selected for cases with isolated antibody positivity rather than clinically definitive antiphospholipid syndrome. Third, low-positive antibody titers may represent transient immunologic reactivity following infection or tissue injury rather than pathognomonic autoimmunity. Therefore, while the observed association is statistically robust, clinical interpretation should accommodate these methodological constraints.

The temporal distribution of seropositive cases revealed a concentration during the first trimester, which is biologically plausible given the vulnerability of early placental development and trophoblast invasion. However, this observation must be interpreted with caution due to the retrospective study design. The timing of serological testing relative to the miscarriage event was not standardized, and IgM antibodies may persist for weeks to months post-infection, potentially overestimating the proportion of first-trimester associations. Additionally, the absence of IgG avidity data limits the ability to distinguish between recent and remote infections. The first trimester is indeed a critical period for embryonic development, and acute infections or thrombotic events during this window can disrupt organogenesis and early placental perfusion (Ahmed & Khudhair, 2023). Nevertheless, prospective longitudinal studies with serial serological sampling are needed to confirm these temporal relationships.

The correlation analysis provides additional quantitative depth to these observations. The strong negative correlations between IgM titers and gestational age (ρ ranging from -0.58 to -0.72) demonstrate a dose-response relationship where higher acute antibody levels are disproportionately concentrated in first-trimester losses. This is biologically plausible: the first trimester represents the period of maximal trophoblast invasion and placental vascularization, making it most susceptible to thrombotic and infectious insults. The diminishing correlations in later trimesters suggest either spontaneous resolution of acute infections or alternative compensatory placental mechanisms.

Regarding maternal age, our data exhibited a J-shaped distribution, with the majority of overall miscarriage cases clustered in the 20–39 age demographic (accumulating 76.8% of cases). While younger women may face distinct risks possibly linked to immunological factors or initial pathogen exposure, women over 30 experience a sharp escalation in age-specific miscarriage risk due to declining oocyte quality and accumulating metabolic or autoimmune predispositions (Magnus *et al*., 2019; Andersen *et al*., 2000). These findings align with large-scale prospective register-based studies demonstrating that maternal age and pregnancy history function as critical independent predictors of miscarriage risk (Magnus *et al*., 2019).

Limitations

The main limitations of this study include its cross-sectional design, which precludes establishing definitive causal relationships between seropositivity and miscarriage. Additionally, the relatively small control group (n=50) compared to the case group (n=250) may have affected the precision of comparative estimates. Furthermore, the study did not assess IgG avidity indices to differentiate recent from remote infections, nor did it evaluate other TORCH pathogens such as CMV and HSV.

Future Scope

Future research should employ prospective longitudinal designs with serial serological sampling during pregnancy to establish temporal causality. Future studies should also incorporate larger, matched control groups and evaluate comprehensive TORCH panels including CMV and HSV. Additionally, intervention studies to assess the efficacy of preconception screening and prophylactic treatment protocols in reducing miscarriage rates among high-risk populations in Iraq are warranted.

Conclusion

This case-control study demonstrates that acute markers of *T. gondii* infection (IgM: 81.6%), Rubella infection (IgM: 69.6%), ACL antibodies (IgM: 66.0%), and APS antibodies (IgM: 62.8%) are significantly associated with spontaneous abortion in Babylon City, Iraq. The complete absence of these IgM markers in healthy pregnant controls (0% vs. 62.8-81.6% in cases; $P < 0.001$) suggests a strong risk association. Spearman correlation analysis further confirmed negative correlations between IgM titers and gestational age (ρ = -0.58 to -0.72), indicating that the risk is highest during the first trimester.

The concentration of positive cases in the first trimester (73.6% for *T. gondii* IgM, 63.2% for Rubella IgM, 58.8% for ACL IgM, and 61.6% for APS IgM) aligns with the established critical window of

trophoblast invasion and early placental development. These findings suggest that early pregnancy represents the period of maximal vulnerability to both infectious and autoimmune insults. However, the cross-sectional design limits the ability to establish definitive temporal causality, and prospective studies with serial serological monitoring during pregnancy are recommended to confirm these observations.

Based on these findings, early-pregnancy or pre-marital screening for TORCH infections (*T. gondii*, Rubella, CMV, HSV) and anti-phospholipid antibody profiles (ACL, APS, lupus anticoagulant) may be beneficial for identifying at-risk women in the Babylon region. However, the implementation of such screening programs should be accompanied by confirmatory testing (e.g., IgG avidity, repeat antibody testing at 12-week intervals for autoimmune markers) to avoid over-interpreting transient serological findings. Additionally, cost-effectiveness analyses and feasibility studies should be conducted to ensure sustainable integration into existing maternal healthcare services in Iraq.

Conflict of Interest

The authors declare that they have no competing interests.

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