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POLIMORFISMO GÊNICO E HAPLÓTIPO DO *MICRORNA146a* HUMANO EM MULHERES IRAQUIANAS INFÉRTEIS INFECTADAS COM TOXOPLASMOSE LATENTE

GENE POLYMORPHISM AND HAPLOTYPE OF HUMAN *MICRORNA146a* IN INFERTILE IRAQI WOMEN INFECTED WITH LATENT TOXOPLASMOSIS

Amna Mohammed Hilal
University of Baghdad, College of Science for Women, Biology Department. Iraq.

Asmaa M. Salih Almohaidi
University of Baghdad, College of Science for Women, Biology Department. Iraq.

Naksheen Merza Ardalan
University of Baghdad, College of Science for Women, Biology Department. Iraq.

** Corresponding author*
e-mail: asmaams_bio@csw.uobaghdad.edu.iq.

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RESUMO

Introdução: A toxoplasmose é uma doença parasitária causada pelo *Toxoplasma gondii* que tem sido associada à infertilidade em mulheres. **Objetivos**: Este estudo investigou a relação entre polimorfismos do gene *miRNA146a* e infertilidade em mulheres iraquianas infectadas com toxoplasmose. **Métodos**: Um total de 80 mulheres com idades entre 18 e 45 anos foram inscritas, divididas em dois grupos: 40 mulheres inférteis com toxoplasmose latente e 40 controles férteis. A genotipagem do *miRNA146a* foi realizada usando reação em cadeia da polimerase (PCR) e sequenciamento. **Resultados e discussão:** Os resultados mostraram que o genótipo CC de rs2910164 foi associado a um maior risco de infertilidade e infecção por toxoplasmose. Além disso, o haplótipo CAAA foi considerado um fator de risco para infertilidade e toxoplasmose, enquanto os haplótipos CAAG e GTGA foram protetores contra a doença. o estudo sugere que os polimorfismos do gene miRNA146a podem desempenhar um papel crucial na suscetibilidade à infecção por toxoplasmose e infertilidade em mulheres iraquianas. Essas descobertas têm implicações importantes para o diagnóstico e prevenção da infertilidade relacionada à toxoplasmose. **Conclusão**: Este estudo fornece evidências para a associação entre variantes genéticas específicas e suscetibilidade à infertilidade relacionada à toxoplasmose, contribuindo para nossa compreensão dos fatores de risco genéticos nessa condição.

Palavras-chave: *Infertilidade, Toxoplasmose, gene miRNA146a, polimorfismo, Single Nucleotide Polymorphisms (SNPs), Haplótipo.*

ABSTRACT

Introduction: Toxoplasmosis is a parasitic disease caused by *Toxoplasma gondii* that has been associated with infertility in women. **Aims**: This study investigated the relationship between *miRNA146a* gene polymorphisms and infertility in Iraqi females infected with toxoplasmosis. **Methods**: A total of 80 females aged 18-45 years were enrolled, divided into two groups: 40 infertile women with latent toxoplasmosis and 40 fertile controls. Genotyping of *miRNA146a* was performed using polymerase chain reaction (PCR) and sequencing. **Results and Discussion**: The results showed that the CC genotype of rs2910164 was associated with a higher risk of infertility and toxoplasmosis infection. Additionally, the CAAA haplotype was found to be a risk factor for infertility and toxoplasmosis, while the CAAG and GTGA haplotypes were protective against the disease. The study suggests that *miRNA146a* gene polymorphisms may play a crucial role in the susceptibility to toxoplasmosis infection and infertility in Iraqi females. These findings have important implications for the diagnosis and prevention of toxoplasmosis-related infertility. **Conclusion**: This study provides evidence for the association between specific genetic variants and susceptibility to toxoplasmosis-related infertility, contributing to our understanding of genetic risk factors in this condition

Keywords: *Infertility, Toxoplasmosis, miRNA146a gene, polymorphism, SNPs, Haplotype*.

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1. INTRODUCTION:

Within the Apicomplexa phylum, *Toxoplasma gondii* is an intracellular protozoan parasite that can cause miscarriage in addition to stillbirths, infection, intrauterine growth retardation, fetal anomalies, or preterm deliveries (Kazemi *et al*., 2023; El-Sherbini *et al*., 2019). Infection occurs when humans consume food or water contaminated with oocysts from feline feces or live tissue cysts in undercooked or raw meat (Shapiro *et al*., 2019). Toxoplasmosis is typically asymptomatic or moderate, similar to influenza. However, immunocompromised patients may experience severe symptoms such as pneumonia and encephalitis, which can be fatal (Khairullah *et al*., 2024).

Primary infection during pregnancy may cause spontaneous abortion or stillbirth. In-utero infection may cause congenital toxoplasmosis with ocular and neurological manifestations. Previous studies on laboratory animals reported that infection with *T. gondii* could be a cause of infertility in experimental animals (Taherimoghaddam *et al*., 2021). Recently, the relationship between female infertility and toxoplasmosis was reported, which raises concern about the importance of toxoplasmosis in female fertility problems and encourages further research. Several studies have shown that toxoplasmosis may invade the female reproductive and cause secondary infertility (Delgado *et al*., 2022). Moreover, it was reported that endometrium changes can lead to infertility among infected women (Al-Ani *et al*., 2021).

MicroRNAs, or small non-coding single-strand RNA molecules, range in length from (~21–23) nt. They bind to messenger RNAs in a specific sequence to adversely control the expression of target genes post-transcriptionally (Machowska *et al*., 2022). The miRNAs regulate the majority of human mRNAs, which have an impact on nearly all diseases and developmental processes. The regulatory process is disrupted when miRNA function is lost.

The miRNA gene has a variety of phenotypes, including immunological disorders, cancer, epilepsy, deafness, retinal degeneration, infertility, and problems that impact the development of the breast, ovaries, testes, and placenta, in addition to physiological, cellular, and behavioral issues (Bartel DP, 2018). Mutations or single-nucleotide polymorphisms (SNPs) can alter the maturation and/or expression of miRNAs, hence altering their function (Duan *et al*., 2007). The microRNAs are thought to be prime candidates for toxoplasmosis loci infection and infertility because small changes in them can impact thousands of mRNAs.

It is primarily unknown yet how genetic variations affect miRNAs. The miRNA has been convincingly shown to be involved in the regulation of embryo implantation, embryo-maternal communication, and embryo growth processes in a number of studies (Tesfaye *et al*., 2016; Liang *et al*., 2017). For a pregnancy to be successful, each event must be successful. A growing body of research indicates that *miR-146a, miR-149, miR-196a2*, and *miR-499* are essential for these processes in female reproduction (Alipour *et al*., 2019).

When it comes to parasitic infections, parasites have evolved tactics to control the expression of host miRNA-146 for their benefit (Alizadeh et al., 2020). Conversely, parasitic infections affect host miRNA expression [For example, research has demonstrated that parasites can cause host cells to up- or down-regulate *miRNA-146*, which can either suppress host immune responses or encourage parasite growth (Acuña *et al*., 2020) and can influence host immunological responses, elude immune surveillance, and increase their chances of survival by changing the expression of miRNA-146 (Mahami-Oskouei *et al*., 2021).

The apicomplexan parasite Toxoplasma gondii changes the innate and adaptive immune responses to stay alive inside the host. It does this by changing the amounts of microRNAs and mRNAs in infected host cells. Zhong et al., 2024 showed in their study that harnessing miR-142a may be a possible therapeutic approach for adverse pregnancy caused by immune imbalances, particularly those induced by T. gondii infection. Furthermore, Naguib and his colleagues discovered that the expression of *miRNA-146a* is significantly higher in women suffering from pregnancy complications compared to the control group, and their findings demonstrated the ability to use these microRNAs as biomarkers for diagnosis in both uncomplicated and complicated cases (Naguib et al., 2024).

Thus, this study aims to investigate *miRNA146a* gene polymorphism in relation to infertility in toxoplasmosis-infected individuals. Moreover, infertility is a real problem in the Iraqi population, and many previous local studies studied candidate gene variants that may be related to infertility (Saeed *et al*., 2021; Mirza *et al*., 2022a).

2. MATERIALS AND METHODS:

2.1. Materials

The study enrolled 80 Iraqi females aged 18-45 years from December 2021 to September 2022 at Al-Elweia Hospital in Baghdad. The participants were divided into two groups: 40 infertile women with latent toxoplasmosis and 40 fertile controls. The control group consisted of women with at least one child, no history of infertility, negative toxoplasma tests, normal CRP and ESR levels, and no history of gestational diabetes, hypertension, or other chronic diseases.

Exclusion criteria included chronic conditions such as cardiovascular diseases, diabetes, and hypertension. Participants were categorized according to age, educational level, family history, workplace, presence of pets, and history of abortion.

2.2. Methods

A peripheral blood sample was divided into two parts: a gel tube for serum and an EDTA tube. The sera from all cases underwent testing for specific IgM/IgG using ELISA kits (Foresight kit/Germany), following the instructions provided by the manufacturer. DNA extraction from the EDTA tube, conventional PCR, and sequencing were performed to explore the microRNA 146 gene polymorphism. Patients with chronic diseases such as cardiovascular diseases, diabetes, and hypertension were excluded from the study based on the exclusion criteria.

2.2.1. DNA extraction and Genotyping:

Genomic DNA was extracted from blood samples using EasyPure® Blood Genomic DNA Kit (TransGen Biotech, EE101-01) according to the manufacturer instructions. The miRNA146a genotyping was performed using polymerase chain reaction (PCR) and sequencing. The PCR amplification targeted two segments. The first segment in Exon 2 was 452 bp, amplified using the forward primer 5'-GGTCTCCTCCAGATGTTTATA-3' and the reverse primer 5'-ATCATTCATTTAGCTACTTGG-3' (Luo *et al*., 2011). The second segment of 837 bp was amplified using the forward primer 5'-GACCAAGGAAAGGAAGCTAT-3' and the reverse primer 5'-TCTTGCAGCACGTGTCAG-3', which were modified by the third author using NCBI. The PCR protocol consisted of initial denaturation at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 54 °C for 45 seconds, and extension at 72 °C for 45 seconds, with a final extension at 72°C for 7 minutes. The primers for the 837 bp fragment were used as previously described by Luo *et al*. (2011). PCR products were analyzed on 1.5% agarose gel.

2.2.2. Sequencing of DNA

The purified PCR products from miRNA146a gene regions were sent to Macrogen in Korea for DNA sequencing. Subsequently, the acquired sequences were aligned using Mega-6 software (Tamura, 2013). These nucleotide sequences were compared to information in the National Center for Biotechnology Information (NCBI) GenBank website databases, employing the BLAST search tool to scrutinize for any polymorphic variations.

2.2.3. Statistical analysis

Statistical analysis of *miRNA146a* gene SNPs genotypes and alleles was performed using frequencies and percentages. The odds ratio (OR) and 95% confidence interval (CI) were calculated using logistic regression analysis through WINPEPI software for epidemiologists. Statistical significance was set at $P < 0.05$. Haplotype frequencies and linkage disequilibrium (LD) between SNPs were analyzed using SHEsis software version 4.2 (http://analysis.bio-x.cn), with LD coefficient (D') used to define linkage disequilibrium.

3. RESULTS AND DISCUSSION:

3.1. Results

Two fragments of *miRNA146a* were studied. The genomic segment is shown in Figure 1. Each fragment was sent for sequencing.

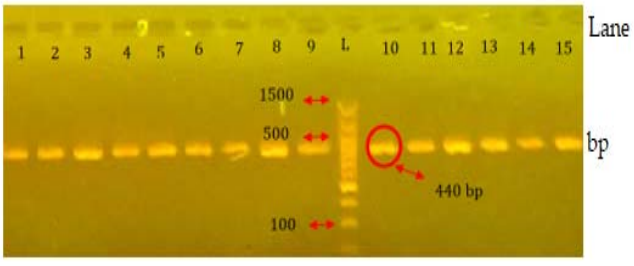

Figure 1. Electrophoresis of the miRNA146a gene PCR reaction outcomes in a 440 bp PCR product size; A DNA marker ladder (100–1500bp) and a 1% agarose gel 70 volts/cm were utilized. Lane 1–7 patients samples, lane 10–15 control samples

The nucleotide number from the gene of gene bank outcomes is the sequence. There was no expected nucleotide sequence for the miRNA146a gene, meaning the query and NCBI reference sequences (ID: NG_106437) were a 90% match. This high similarity, with only a 2% gap and a substantial score of 717 bits, underscores the remarkable alignment between the two sequences.

3.1.1. Genotype and Allele Analysis of miRNA146a

The number of females in the toxoplasmosis group with the wild TT genotype (rs1766309047 T>C) did not differ significantly from those who could not have children. At the same time, infertile females infected with toxoplasmosis reported a lower significant frequency of heterozygous genotype TC (25% vs 45%) in infertile females with toxo than in fertile. Besides this, the CC genotype revealed an OR 2.64 and CI 95% =1.04 to 6.69, which indicates a potential risk factor associated with infertility and toxoplasmosis. Allele C recorded an odds ratio of 2.15 while allele T recorded an odds ratio of 0.47; therefore, allele C has a positive association with infertility and toxoplasmosis and a tendency to be a susceptible allele to infertile, but allele T has a negative association with infertility and toxoplasmosis. Therefore, allele C tends to be a protective factor against infection. The homozygous genotype CC is the common genotype in the present study because it recorded the high frequency in both studied groups from the Iraqi population; this finding is compatible with NCBI, which considers it a wild genotype Table 1.

According to the SNP rs2910164 result, the most common toxoplasmosis types in both study groups were CC-infected females who were unable to have children (75% of them) and 45% of them. In contrast, GG recorded the lowest frequency in both groups, 5% and 20%, respectively. Both homozygous genotypes show significant differences between the studied groups. The female with the CC genotype may act as an etiological factor associated with infertility and toxoplasmosis. The current research found that the frequency of the C allele was significantly higher among patients (compared with the control) and that the frequency of the G allele was lower in the infertile group infected with toxoplasmosis compared to the fertile group. In addition, the C allele was positively associated with infertility and toxoplasmosis susceptibility. At the same time, the G allele recorded a negative association with infertility and toxoplasmosis. Those carrying the C allele, or the CC genotypes of miR-146a rs29101164, were more likely to have infertility with infection, but those with the GG genotype were more likely to be free of both medical problems.

The distribution of SNP rs1226933433 A>G genotypes in the current research groups shows AA 28 (70%), AG 10 (25%), and GG 2 (5%). Significant differences were observed in the AA genotype distribution, which indicates that it may be related to infertility in Iraqi females with toxoplasmosis. The AG heterozygous genotype also showed no significant difference, with an OR of 3.86. On the contrary, the GG homozygous genotype showed a significant difference in protective tendencies against infertility with toxoplasmosis. The A allele shows a high percentage in the infertile group, with toxoplasmosis opposite to the fertile group. An allele may make Iraqi females more susceptible to being infected with toxoplasmosis. Moreover, the G allele shows significant differences and could make Iraqi females resistant to toxoplasmosis infection and infertility.

The SNP rs780488034 A>T results in Table 1 show significant differences in the AA homozygous. This suggests that it may be a factor that helps prevent infertility in Iraqi women who have toxoplasmosis. The TT genotype did not significantly differ between the groups, but the TT homo-genotype did, with a 1.59 OR. This suggests that it may make women more likely to have problems getting pregnant when they have toxoplasmosis. The A allele shows a high percentage in the fertile group, unlike the infertile group with toxoplasmosis. The A allele is considered a protective allele that makes females who carry it resist the disease (OR = 0.28). Moreover, the T allele recorded a high OR of 3.62. The T allele may be etiologically risky.

The frequency at which the second most frequent allele appears in a specific population is known as the minor allele frequency, or MAF. Single nucleotide polymorphisms (SNPs) with minor allele frequencies of 0.05 (5%) or above are the focus of the HapMap project. The primary allele of the SNP may be preserved and essentially fixed if the MAF is low, less than 0.05 (5%). This indicator provides insight into the genotype variation of a specific SNP within a population; given differently, it provides insight into the SNP's level of commonality (The International HapMap Consortium, 2005). Hence,

SNPs in the current study may be considered a source of variants within the Hap Map project. Haplotype analysis was performed for polymorphisms in *miRNA146a* that increase the risk of Toxoplasmosis parasite infection with infertility. Table 2 shows that the haplotype CAAA acts as a pathogenic associated with infertility and infection with toxoplasmosis. However, the CAAG with GTGA haplotypes in the *miRNA146a* gene was significantly linked to a lower incidence of toxoplasmosis with infertility. This means that women who have these haplotypes may be better protected against infertility caused by toxoplasmosis.

The blocks in Figure 2 indicate haplotype blocks, and the SNP names are the text above the horizontal numbers. Pairwise analysis of linkage disequilibrium (LD) between the four SNPs studied in this work revealed different values of D' linkage disequilibrium (LD). Some SNPs were in a strong LD (0.99), while others showed weak (0.42) or no LD (0.00). Such a profile was different in infertile women infected with toxoplasmosis (Figure 2). Therefore, these results indicate that those polymorphisms SNPs formed a single haplotype group as one block and may be co-inherited but separate in some samples.

At loci 2, 3, and 4 in Figure 2, the rs78048834, rs1226933433, and rs1766309047, respectively, SNPs act together synergistically because of their coexistence as a haplotype, females will inherit these three SNPs as one block.

Figure2. Linkage disequilibrium estimated between SNPs in *miRNA146a* gene

3.2. DISCUSSION

According to relative risk, several SNPs were significantly associated with diseases like rs2910164, rs1226933433, rs78048834, and rs1766309047. People with genotype CC in SNPs rs2910164 and rs1766309047 and genotype AA in SNPs rs1226933433 and rs78048834 are more likely to have trouble getting pregnant after getting toxoplasmosis. G allele in both rs2910164 and rs1226933433, in addition, allele A in rs78048834 and allele T in rs1766309047 were significantly not associated with infertility and toxoplasmosis infection, so that may be a protective factor from disease.

A study of SNPs in *miRNA146a* for infertility and toxoplasmosis infection showed that haplotypes CAAG and GTGA were not significantly linked to infertility, and toxoplasmosis infection tended to act as a protective factor. On the other hand, CAAA tends to act as a risk factor for infertility and toxoplasmosis infection. The current study goes with previous studies that emphasize the role of haplotype variants of several genes' role in facilitating infectious disease (Liu *et al*., 2005).

This study found that rs2910164 has alleles that are linked to the disease. The findings of this study agree with those of another one that found functional polymorphisms at the miRNA-146a SNP rs2910164 locus. These have been linked to the risk of different diseases in different inheritance models. The current study was in agreement with the study by Li and his colleagues, who hypothesized that alterations in the prevalence of miRNA single nucleotide polymorphism (SNP) variants *miR-146a* rs2910164 in women with PCOS in comparison to healthy controls demonstrated the significant correlation of both SNPs with an increased risk of PCOS (Li *et al.,* 2022). A study of the Chinese population showed more proof of the critical role of *miR-146b* and its changes in endometriosis. The study found that miR-146b is crucial in controlling the progression of endometriosis and the pain that comes with it (Zhang *et al*., 2019). An Iraqi study recently emphasized that polymorphisms in adhesion genes affect the endometrium's readiness for embryo attachment and the failure of implantation (Rashid *et al*., 2023).

A new study found that the miRNA-146a SNP rs2910164 C>G polymorphism is linked to a higher risk of infection by changing the way Notch-1/IL-6 signals work in the immune system (Keewan *et al*., 2020). In addition, SNP rs2910164 C>G may be a risky factor for the job

of miRNA and prevent the release of a transform from pre-miRNA to mature (Jodeiryzaer *et al.,* 2020; Liu *et al.,* 2020), like rs2910164, SNP C > G allele C may modify mature *miR-146a* synthesis, which may alter the inflammatory process, according to some experimental studies (Dai *et al*., 2007; Zhang *et al*., 2015). In the same way, a positive link between the disease and the C allele of *miRNA-146a* SNP rs2910164 is similar to what was found before as a risk factor for metabolic syndrome (Mehanna *et al*., 2015).

Moreover, current results agree with a study that revealed the association of microRNA polymorphisms with recurrent spontaneous abortion (RSA), which was frequently reported and showed a significant correlation of miR-146a rs2910164 C>G with idiopathic recurrent spontaneous abortion (Wang *et al*., 2020; Jeon *et al*., 2011).

Additionally, a study by (Liu *et al.,* 2021) revealed the association between miRNA polymorphisms and the risk of RA or SLE., it showed individuals who carried the CC genotype in *miR-146a* rs2910164 were found to confer protection against RA in Caucasians. In comparison with G allele carriers, the level of *miR-146a* was higher in C allele carriers, and they speculated that the C-allele *miR-146a* could decrease the expression of *miR-146a* and may serve as a specific biomarker to monitor the activity of patients with RA in Caucasians (Liu *et al.,* 2021).

An Iraqi report has demonstrated that polymorphisms in the miRNA gene impact its expression, biogenesis, and maturation, which may serve as an essential risk factor in the susceptibility to diseases, including infection and carcinogenesis. The functional variant of the SNP rs2910164, which influences miR-146a transcription and expression levels, has been identified. As a result, it aids in the pathogenesis of many inflammatory and autoimmune illnesses, such as toxoplasmosis infection (Abdulridha *et al.*, 2023). However, genetic polymorphism of different genes in Iraqi women has recorded a significant effect on fertility in many previous studies (Mirza *et al*., 2022b; Saeed *et al*., 2021) and may cause the prevention of embryo implantation on the endometrium (Rashid *et al*., 2024; Yousif *et al*., 2023).

The involvement of microRNAs in the development, progression, and control of toxoplasmosis disease, as well as the prospect of employing these microRNAs as biomarkers for diagnosis in both simple and complex cases. In support of these assumptions, Antil *et al.,* 2022 found novel autocrine/paracrine signaling mechanisms that could be related to host response altered by *T. gondii* via 74 differently regulated miRNAs and their 319 high-confidence mRNA targets. Furthermore, (Xie *et al*., 2022) discovered dysregulated non-coding RNAs, 88 common differentially expressed miRNAs, and 120 new differentially expressed PIWI-interacting RNAs in blood and urine samples of rabbits infected with *T. gondii* oocysts. Regarding the studied microRNAs, Cannella *et al*. (2014) detected expression of *miR-146a* and *miR-155* in the brains of mice challenged with toxoplasma and found *miR-146a* has modulatory action on the infection through the rhoptry kinase; additionally, *miR-146a* deficiency induced better control of parasite burden in the gut and its early brain dissemination and concluded that *miR-146a* and *miR-155* are immunomodulatory for toxoplasma infected cells. Thereafter, (da Cruz *et al*., 2020) detected overexpression of *miR-146a*, *miR-155, miR-21, miR-29c,* and *miR-125b* in serum-derived extravascular vesicles (EVs) from patients who had cerebral and gestational toxoplasmosis.

Furthermore, (Meira-Strejevitch *et al*., 2020) discovered that patients with ocular toxoplasmosis had considerably greater expression levels of *miR-155* and an insignificantly higher level of miR-146a than patients with asymptomatic toxoplasmosis. (Zou et al., 2022) found 177 and 77 differentially expressed miRNAs in livers during acute and chronic *T. gondii* infection stages, respectively. The *miR-146a* and *miR-150* were linked to liver immunity and toxoplasmosis pathogenesis. (Wang et al., 2022) found upregulation of *miR-146a* in a mouse model of *T. gondii* infection.

Yi-Hong *et al*. (2019) found that *T. gondii*-infected macrophages showed a greater than 4-fold increase in miR-155 expression. This elevation was correlated with increased mRNA synthesis of both inducible nitric oxide synthase and interleukin-12 (IL-12), as measured by PCR. Additionally, ELISA analysis revealed elevated levels of NO and IL-12 proteins.

In a mouse model infected with *T. gondii*, (Xu *et al*., 2021) found that a lack of *miR-155* led to increased parasite burden, decreased animal survival, reduced innate and adaptive immune responses with decreased pro-inflammatory mediators, and worsened CD8 + T cell exhaustion. They concluded that *miR-155* is a critical immune regulator for the control of *T. gondii* infection and could be used as a molecular

target for boosting immunity against *T. gondii*. Another study used liver tissue from mice infected with *T. gondii*.

The present linkage represents a good bio-indicator about the disease, which makes the females more susceptible to getting an infection and may lead to infertility; this result, in agreement with the study, revealed that *miR-146a* could be associated with the susceptibility to pulmonary TB in China and may be closely related to individual differences caused by genetic factors in a Chinese population (Zhang *et al.*, 2015). At the same time, all those women with the CAAA haplotype may produce a new generation of daughters who carry the CAAA haplotype and become more susceptible to infection and infertility. We need to do more research with a larger sample size to look into the role of miRNA SNPs in infertility linked to toxoplasmosis and its complications. This will help us understand how genes and the environment interact better and develop the best ways to diagnose, treat, and prevent this disease.

4. CONCLUSIONS:

The homozygous-genotype CC of SNP rs1766309047 may be a frequent genotype in the research group, as is SNP rs2910164. In contrast, SNPs rs1226933433 and rs780488034, which result in the homozygous genotype AA, were the most common in the Iraqi study sample. Females that possess the CAAA haplotype may be sterile. Females with the CAAG and GTGA haplotypes, on the other hand, are more fertile because GAAG and GTGA diminish susceptibility to infection and sterility, respectively. The current study contributes to establishing a link between genetic variants, risk factors such as miRNA146a polymorphisms, and susceptibility to toxoplasmosis infection and infertility.

This discovery could help scientists better understand the infection. This study's linkage and haplotype indicate either protection or susceptibility to toxoplasmosis-related infertility. Females with haplotype CAAA are more sensitive to infection and infertility in subsequent generations. As a result, haplotypes may prove useful in future follow-up research.

Even though available data have provided much information on the association of *miR-146a* with this disease, more studies with large samples are required to elucidate the processes of pathogenesis and provide more hints for investigating the efficient influence on infertility and toxoplasmosis.

5. DECLARATIONS

5.1. Study Limitations

The sample is limited because most patients visit private medical clinics. Therefore, there are limited numbers of patients who visit hospitals, and most of them refuse to participate in the study.

5.2 Acknowledgments

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5.3. Funding source

The authors funded this research.

5.4 Competing Interests

The authors declare that they have no conflicts of interest.

6. HUMAN AND ANIMAL-RELATED STUDIES

6.1. Ethical Approval

The study protocol was approved by the Ethics Committee of the Department of Biology, University of Baghdad, College of Science for Women, and the Ministry of Health and Environment of Iraq (approval No. 55679, dated May 11, 2022). The protocol authorized the collection of blood samples from fertile and infertile women. Before participation, all individuals were informed about the study's purpose, potential risks, and benefits. Each participant provided written informed consent after receiving this information.

6.2. Informed Consent

The authors had provided informed consent for all patients and collected approval to publish this research work.

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Table 1. *Genotypes and alleles analysis of miRNA146a gene SNPs in infertile women infected with toxoplasmosis and fertile*

***miRNA 146a* SNP**	**Genotypes and Alleles**	**Infertile with toxoplasmosis NO.=40**	**Fertile NO.=40**	**Maf miRN146A**	**OR**	**p. value**
rs1766309047 T>C	TT	1 (2.5%)	2 (5%)	-	0.49	0.56 NS
	TC	10 (25%)	18 (45%)	-	0.41	0.006**
	CC	29 (72.5%)	20 (50%)	-	2.64	0.04*
	T	12 (15%)	22 (27.5%)	0.212(21.25)	0.47	0.0563
	C	68 (85%)	58 (72.5%)	-	2.15	0.056
rs2910164 C>G	CC	30 (75%)	18 (45%)	-	3.67	0.007**
	CG	8 (20%)	14 (35%)	-	0.46	0.14NS
	GG	2 (5%)	8 (20%)	-	0.21	0.05 *
	C	68 (85%)	50 (62.5%)	-	3.40	0.002**
	G	12 (15%)	30 (37.5%)	0.2625 (26.25)	0.30	0.002**
rs1226933433 A>G	AA	28 (70%)	16 (40%)	-	3.50	0.008**
	AG	10 (25%)	16 (40%)	-	0.50	0.15NS
	GG	2 (5%)	8 (20%)	-	0.16	0.02*
	A	66 (82.5%)	48 (60%)	-	3.14	0.002**
	G	14 (17.5%)	32 (40%)	0.288(28.8)	0.32	0.002**
rs780488034 A>T	AA	8 (20%)	28 (70 %)	-	0.11	<0.0001**
	AT	26 (65%)	8 (20 %)	-	7.43	0.001**
	TT	6 (15%)	4 (10%)	-	1.59	0.50 NS
	A	42 (52.5 %)	64 (80%)	-	0.28	0.003**
	T	38 (47%)	16 (20%)	0.3375(33.75)	3.62	0.003**

Significant * (p≤0.05), highly Significant ** (p ≤0.01), NS: Non-Significant, confidence interval 95 % CI, Odds ratio OR

Table 2. *Analysis of haplotypes in miRNA146a gene*

haplotypes	**Infertility with toxo (frequency)**	**Fertile (frequency)**	**Odds Ratio**	**95%CI**	**p-value**
CAAA*	29.99(0.375)	27.99(0.350)	1.116	0.583-2.137	0.74
CAAG*	12.00(0.150)	20.01(0.250)	0.527	0.237- 1.17	0.11
GTGA*	12.00(0.150)	16.00(0.200)	0.705	0.309~1.607	0.40

(frequency<0.03) in both fertile and infertile females has been dropped