



## Serum Testosterone-Estradiol Ratio in Toxoplasma-Seropositive Infertile Men: A Prospective, Single-Center Study

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### Abstract

**Background:** The purpose of the current study was to compare the testosterone-estradiol (T:E2) ratio in Toxoplasma gondii seropositive infertile men with seropositive and seronegative normozoospermic controls.

**Methods:** Totally, 200 men with normal virilization, 100 with idiopathic infertility and 100 normozoospermic men, were included. Participants underwent medical history assessment, physical examination, semen analysis, testing for T. gondii IgM/IgG, and estimation of serum T:E2 ratios. Statistical comparisons were done using t-test and Chi-square with  $p < 0.05$  significance level.

**Results:** Infertile cases were diagnosed with oligozoospermia (63%), oligoasthenozoospermia (34%), and oligoasthenoteratozoospermia (3%). Regarding anti-Toxoplasma IgG and IgM antibodies, among infertile men, 34 tested positive for IgG and 8 tested positive for IgM. Among cases tested positive for IgG antibodies, 13 (38.2%) had disturbed T:E2 ratios. Also, among the 12 IgG-positive controls, 5 (41.7%) had disturbed T:E2 ratios ( $p = 0.834$ ). However, only 2 out of the 83 seronegative controls (2.5%) had disturbed T:E2 ratios ( $p < 0.001$ ). Furthermore, 6 out of 8 IgM-positive cases had altered T:E2 ratios, compared to 3 out of 5 IgM-positive controls ( $p = 0.568$ ) and 2 out of 83 seronegative controls ( $p < 0.001$ ). The T:E2 ratio was significantly lower ( $8.68 \pm 1.95$ ) among IgM-positive and higher ( $13.04 \pm 3.78$ ) among IgG-positive cases when compared to seronegative controls ( $10.45 \pm 0.54$ ) ( $p < 0.001$ ). There were no significant differences in T:E2 ratios between infertile men with positive IgM or IgG serology and the control group with the same serology.

**Conclusion:** A substantial number of infertile men with toxoplasmosis showed disrupted T:E2 ratios, highlighting the significance of anti-T. gondii-IgG testing in individuals with abnormal ratios.

**Keywords:** Asthenozoospermia, Estradiol, Immunoglobulin G, Immunoglobulin M, Male Infertility, Oligospermia, Testosterone, Toxoplasma.

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### Introduction

Infertility is the failure of a couple to achieve a pregnancy after 12 months or more of regular, unprotected sexual intercourse (1). Approximately one-third of infertility cases are due to male factors (2). However, the cause of infertility remains unknown in approximately 50% of infertile men (3).

The local and systemic hormonal balance plays a role in spermatogenesis regulation. The hypothalamic-pituitary gonadal axis is a component of the systemic hormonal environment, whereas the balance of the testicular testosterone to estradiol ratio (T:E2 ratio) represents the local hormonal regulation (4).

The fine balance between testosterone (T) and estradiol (E2) is essential for normal spermatogenesis, and this is reinforced by the discovery of reduced T levels and increased E2 levels in both semen (5) and serum (6) in infertile men. Thus, calculating the T:E2 ratio appears to be a more reliable tool than measuring serum testosterone only for the assessment of male fertility (7-8). Serum rather than seminal T:E2 ratio better reflects fertility status because of the lack of reference values for the seminal hormonal profile (9). Toxoplasmosis, caused by the intracellular protozoan parasite *T. gondii*, is one of the most prevalent diseases globally, affecting a third of the population, with humans as intermediate hosts and domestic and wild cats as definitive hosts (10). Human toxoplasmosis generally occurs through the ingestion of infective stage oocysts or tissue cysts containing the bradyzoites. Also, transmissions by organ transplantation and through the placenta (from the infected mother to the foetus) were documented as routes of infection (11). Present hypotheses suggest *Toxoplasma* transmission during sexual intercourse (12) and oral sex (13) in humans, with a high prevalence observed in men who have sex with men, sex workers, and fathers of congenitally infected children (10).

*T. gondii* infections are primarily diagnosed by serological detection of IgM and IgG antibodies, and in some cases, IgA, targeting specific parasitic protein antigens (14). The detection of parasite-specific human IgG and IgM antibodies is sufficient to obtain estimates of the prevalence of acute and chronic infections (15).

Toxoplasmosis is one of the most significant infections that negatively impact reproductive function (12, 16). *T. gondii* infection may induce germ cell apoptosis in infertile men (17). One study has confirmed that a mutation in the ND1 gene, which is located in the mitochondria of sperm, occurs in infertile men as a result of *T. gondii* infection (18). According to a recent prospective study, latent toxoplasmosis affects certain aspects of male fertility, including sperm motility and count, but not sperm morphology and semen volume (10).

Emerging research has revealed the parasite's ability to trigger hormone and neurotransmitter secretion in the host, including dopamine and sex hormones. Notably, testosterone has been found to be particularly affected by the parasite (19). Shirbazou et al. (20) demonstrated a substantial relationship between anti-*Toxoplasma* IgG antibodies and plasma testosterone and cortisol levels

in both men and women. However, there are controversial issues about the effect of *T. gondii* infection on testosterone production; in some studies, it increased (20–22), and in other studies, it decreased the level of testosterone, with related hormones influencing testosterone production (23). According to Zhang et al. (24), estradiol can promote *T. gondii* infection in vitro and in vivo, and this may be related to its Tg-hydroxysteroid dehydrogenase (HSD) gene.

Due to the absence of uniform data addressing the effects of *T. gondii* on serum T:E2 ratio in infertile men, the aim of the current study was to estimate the difference in T:E2 ratio in *T. gondii* seropositive infertile men compared with seropositive and seronegative normozoospermic controls.

### Methods

This prospective study was conducted between May 2022 and January 2023. Participants were recruited from the Andrology Department of Beni-Suef University Hospital (Beni-Suef governorate, Egypt).

Eligible participants were asked to sign an informed consent before enrolment in this study, according to the regulations mandated by the research ethics committee (REC) of Beni-Suef Faculty of Medicine that conform to Declaration of Helsinki (2013) (25). Data and samples were assigned anonymous numbers to ensure confidentiality. Laboratory results were merely disclosed to the participants during a post-test counselling session. Ethical approval was registered under number: FMBSUREC/10042022.

**Study participants:** The present study recruited 200 participants who met the inclusion criteria outlined below. The study included 100 men with idiopathic infertility (Group I) and 100 normozoospermic controls (Group II). The inclusion criteria for patients were as follows: (i) men at least 20 years of age or older; (ii) experiencing primary or secondary infertility; (iii) having idiopathic oligozoospermia, oligoasthenozoospermia, or oligoasthenoteratozoospermia based on WHO (2010) guidelines (26); and (iv) demonstrating adequate development of masculine characteristics.

**The exclusion criteria were as follows:** (i) men with azoospermia or cryptozoospermia; (ii) individuals with known causes of infertility such as bilateral undescended testicles or anorchia, epididymo-orchitis, leukocytospermia, bilateral large varicocele or testicular torsion, testicular tumors or prior

radio (chemo) therapy, heavy smoking, morbid obesity, and end-stage liver or kidney diseases; (iii) individuals receiving drugs for chronic illnesses or hormonal formulations for hypogonadism or hyperprolactinemia; and (iv) those who were immunocompromised. The inclusion criteria for the control group were as follows: apparently healthy men exhibiting normal virilization and normal semen parameters based on WHO (2010) guidelines (26) who attended the andrology clinic for any concern other than fertility over the same time span. Cases and controls were matched for age and body mass index (BMI).

**Data collection:** A pre-designed structural questionnaire was utilized to precisely collect the subject's socio-demographic characteristics and their clinical and laboratory data. Basic demographic characteristics, medical history, fertility history, as well as general and genital examinations were documented for all participants.

#### **Laboratory evaluation**

**Seminal fluid collection and evaluation:** Semen samples were obtained by masturbation after 2–7 days of sexual abstinence. Following liquefaction at 37°C, the seminal fluid was investigated macroscopically for appearance, volume, pH, and viscosity and microscopically for sperm concentration, motility, and morphology. The lower reference levels specified in WHO (2010) manual (26) were employed to assess alterations in semen parameters.

**Serological diagnosis of *T. gondii* infection:** Sample collection involved obtaining 5 ml of venous blood from each participant using a sterile syringe. The samples were then transferred to sterile tubes and left to clot at room temperature. Subsequently, they were centrifuged at 3000 rpm for 5 min, and the isolated sera were transferred to separate tubes and stored at -20°C until serological analysis (27).

For detection of IgM and IgG antibodies, enzyme-linked immunosorbent assay (ELISA) kits for *Toxoplasma gondii*-specific human IgM and IgG antibodies (Catalog Number E 920Hu; Pre-check Bio, Inc., USA) were used for distinguishing an acute infection from a chronic one. The assay was conducted according to the manufacturer's instructions.

**Hormonal assay:** A fasting serum sample was collected in the morning for assessment of total

testosterone levels (normal value: 2.23–10.12 ng/ml) and estradiol (normal value: 0.0–56 pg/ml) levels using ELISA kits (MyBiosource, Inc., USA) according to the manufacturer's instructions.

The T:E2 ratio was calculated using the following formula: testosterone/(10\*estradiol) (28). A T:E2 ratio of 10 was established as the lower limit for a normal T:E2 ratio cutoff value (29).

**Statistical analysis:** Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., USA). Quantitative data was summarized using mean, standard deviation, median, minimum, and maximum and categorical data were presented as frequency and percentage. Comparisons between groups were done using the unpaired t-test for normally distributed quantitative variables, while the non-parametric Mann-Whitney U test was used for non-normally distributed quantitative variables. For comparing categorical data, the Chi square ( $\chi^2$ ) test was performed. Fisher's exact test was used instead when the expected frequency was less than 5. The p-values less than or equal to 0.05 were considered statistically significant.

**Sample size calculation:** The sample size calculation was based on the primary outcome, which was T:E2 ratio. Using G. power, an independent t-test was used to detect differences in T:E2 ratio between infertile men and normozoospermic controls. Assuming an effect size of 0.6,  $\alpha$  error of 0.05, power of 90%, and a distribution ratio of 1:1, the total sample size required was determined to be 200 patients, with 100 in the infertile group and 100 in the normozoospermic group. These participants were scheduled to undergo hormonal assay and serology for toxoplasmosis.

## **Results**

The age and BMI of the infertile men and normozoospermic controls were not significantly different ( $p=0.072$  and  $p=0.255$ , respectively). Primary and secondary infertility were identified in 21% and 79% of cases, respectively. Meanwhile, 63%, 34%, and 3% of cases were consecutively diagnosed with oligozoospermia, oligoasthenozoospermia, and oligoasthenoteratozoospermia (OAT). Most participants were employed as workers, farmers, and food handlers and were living in rural areas (Table 1).

**Table 1.** Sociodemographic and laboratory data of infertile men and normozoospermic controls

Variables	Infertile men (n:100)	Normozoospermic men (n:100)	p-value
Age (years)	33.85±7.50 (20-50)	35.83±7.98 (23-53)	0.072
Body mass index ( $kg/m^2$ )	23.90±3.49 (19.12-26.80)	24.39±2.50 (20-26.15)	0.255
Residence			
Urban	36 (%)	28 (%)	0.225
Rural	64 (%)	72 (%)	
Occupation			
Worker	19 (%)	25 (%)	0.464
Farmer	39 (%)	27 (%)	
Food handlers	17 (%)	12 (%)	
Others: [vet, doctor, chemist, engineer, teacher, accountant, police officer]	25 (%)	36 (%)	
Occupation risky for toxoplasmosis			
Yes	51 (%)	47 (%)	0.572
No	49 (%)	53 (%)	
Type of infertility			
Primary	21 (%)	--	--
Secondary	79 (%)	--	
Semen			
Normozoospermia	--	100 (%)	--
Oligozoospermia	63 (%)	--	--
Oligoasthenozoospermia	34 (%)	--	--
Oligoasthenoteratozoospermia	3 (%)	--	--
Anti-Toxoplasma IgG			
Positive	34 (%)	12 (%)	0.0001**
Negative	66 (%)	88 (%)	
Anti-Toxoplasma IgM			
Positive	8 (%)	5 (%)	0.390
Negative	92 (%)	95 (%)	
Seum estradiol ( $pg/ml$ )	47.98±25.68 (20-130.8)	36.29±11.94 (19-75)	0.0001*
Seum total testosterone ( $ng/ml$ )	5.40±3.60 (2.4-15.8)	3.8±1.28 (2.5-7.9)	0.0001*
T:E ratio in serum			
Value	11.03±3.67 (3-27.4)	10.46±0.81 (8.1-13.7)	0.131
Disturbed [ $<10$ ]	37 (%)	10 (%)	0.0001**
Normal [ $\geq 10$ ]	63 (%)	90 (%)	

Values are presented as mean±S.D. [min-max], or frequency (%)

\* The t-test was employed for quantitative variables

\*\* The  $\chi^2$  test was employed for categorical variables

The infertile cases, compared to the controls, demonstrated significantly higher levels of serum estradiol ( $47.98 \pm 25.68$   $pg/ml$  vs.  $36.29 \pm 11.94$   $pg/ml$ ) with  $p < 0.001$ . Similarly, 37% of cases reported a disturbed T:E ratio compared to 10% of controls ( $p < 0.001$ ) (Table 1).

Based on serological assay, normozoospermic controls were further classified as IgG seropositive (n=12), IgM seropositive (n=5), and seroneg-

ative controls (n=83). As illustrated in table 1, no statistically significant difference was observed between the percentage of cases and controls reporting positive anti-Toxoplasma IgM ( $p = 0.390$ ). In contrast, positive anti-Toxoplasma IgG was found in 34 out of 100 cases compared to 12 out of 100 controls, and the difference was statistically significant ( $p < 0.001$ ).

Of the 34 infertile men with positive IgG serolo-

gy, 18 (52.9%) were farmers, and 27 (79.4%) were from rural areas. On the other hand, among the 12 normozoospermic men with positive IgG, 5 (41.7%) were farmers and 11 (91.7%) were from rural areas. As demonstrated in table 2, seronegative controls (n=83) had significantly lower (p<0.001) levels of serum estradiol (pg/ml), total testosterone (ng/ml), and T:E2 ratio compared to infertile men with positive IgG serology (n=34). Similarly, serum estradiol and total testosterone, but not the T:E2 ratio, were significantly lower in normozoospermic men with positive IgG serology (n=12) compared to infertile men, seropositive for IgG (n=34).

A disturbed T:E2 ratio was detected in 13 out of 34 (38.2%) infertile men with positive IgG, compared to 5 out of 12 (41.7%) normozoospermic

controls with positive IgG. However, it was evident in only 2 out of 83 (2.5%) seronegative controls (Table 2).

According to table 3, a statistically significant difference was not noticed between IgM-positive normozoospermic men (n=5) and IgM-positive infertile men (n=8) regarding serum estradiol, total testosterone, and the T:E2 ratio. On the other hand, serum estradiol and total testosterone were significantly lower (p<0.001) and the T:E2 ratio was significantly higher (p<0.001) in seronegative controls (n=83) compared to IgM-positive infertile men (n=8). A disturbed T:E2 ratio was detected in 6 out of 8 (75%) IgM-positive infertile men and in 3 out of 5 (60%) IgM-positive normozoospermic men (Table 3).

Table 4 shows that there was no insignificant

**Table 2.** Serum estradiol, total testosterone, and T/E2 ratio in IgG positive infertile men vs. Toxoplasma free normozoospermic men and IgG positive normozoospermic men

Variables	IgG positive infertile men (n: 34)	Toxoplasma free normozoospermic men (n: 83)	IgG positive normozoospermic men (n: 12)	P1	P2
Estradiol (pg/ml)	76.30±21.46 (29-130.8)	32.23±7.6 (19-46)	55.75±9.26 (44-75)	0.0001*	0.0026*
Total testosterone (ng/ml)	9.64±2.75 (2.7-15.8)	3.372±0.799 (2.5-5.7)	6.03±1.20 (4-7.9)	0.001*	0.0001*
T/E2 ratio					
Value	13.04±3.78 (8.12-21.2)	10.45±0.54 (9.3-12.6)	10.85±1.67 (8.1-13.7)	0.0001*	0.060*
Disturbed	13 (38.2%)	2(2.5%)	5(41.7%)	0.0001**	0.834**
Normal	21 (61.8%)	81 (97.5%)	7(58.3%)		

Values are presented as mean± S.D. [min-max], or frequency (%)

\*The t-test was employed for quantitative variables.

\*\*Categorical variables were tested using the x<sup>2</sup> test or Fisher's exact test.

P1= P between IgG positive infertile men and Toxoplasma free normozoospermic men.

P2= P between IgG positive infertile cases and IgG positive normozoospermic men

**Table 3.** Serum estradiol, total testosterone, and T/E2 ratio in IgM positive infertile men vs. Toxoplasma free normozoospermic men and IgM positive normozoospermic men

Variables	IgM positive infertile men (n: 8)	Toxoplasma free normozoospermic men (n: 83)	IgM positive normozoospermic men (n: 5)	P1	P2
Estradiol (pg/ml)	50.0±19.99 (23-78)	32.23±7.6 (19-46)	57.0±9.75 (45-68)	0.0001*	0.485
Total Testosterone (ng/ml)	4.438±2.532 (2.51-9.8)	3.372±0.799 (2.5-5.7)	5.64±0.953 (4.2-6.6)	0.0072*	0.337
T/E2 ratio					
Value	8.68±1.95 (6-12.5)	10.45±0.54 (9.3-12.6)	9.94±1.42 (8.48-12.2)	0.0001*	0.240
Disturbed	6 (75%)	2 (2.5%)	3 (60%)	0.0001**	0.568
Normal	2 (25%)	81 (97.5%)	2 (40%)		

Values are presented as mean± S.D. [min-max], or frequency (%)

\*The t-test was employed for quantitative variables.

\*\*Categorical variables were tested using the x<sup>2</sup> test or Fisher's exact test.

P1= P between IgM positive infertile men and Toxoplasma free normozoospermic men

P2= P between IgM positive infertile cases and IgM positive normozoospermic men

**Table 4.** Serum estradiol, total testosterone, and T/E2 ratio in Toxoplasma free infertile men and Toxoplasma free normozoospermic men

Variables	Toxoplasma free infertile men (n:58)	Toxoplasma free normozoospermic men (n:83)	p-value
Estradiol (pg/ml)	31.11±7.83 (20-65.6)	32.23±7.6 (19-46)	0.396
Total testosterone (ng/ml)	3.12±1.11 (2.4-9.3)	3.372±0.799 (2.5-5.7)	0.122
T/E2 ratio			
Value	10.18±3.27 (3-27.4)	10.45±0.54 (9.3-12.6)	0.461
Disturbed	14 (24.1%)	2 (2.5%)	0.0006*
Normal	44 (75.9%)	81 (97.5%)	

Values are presented as mean± S.D. [min-max], or frequency (%)

\* The  $\chi^2$  test was employed for categorical variables

difference between seronegative infertile men (n=58) and seronegative normozoospermic men (n=83) concerning serum estradiol, total testosterone, as well as the T:E2 ratio. A disturbed T:E2 ratio was detected in 14 out of 58 (24.1%) seronegative infertile men and in 2 out of 83 (2.5%) seronegative normozoospermic men.

### Discussion

Although the impact of *Toxoplasma gondii* infection on testosterone production has been demonstrated in some studies, there is still obscurity and incomprehensibility on this topic (30). Diversity in reports on testosterone levels in *Toxoplasma*-infected individuals has been attributed to the variability in duration of infection and type of measured testosterone (10).

This study was conducted to reveal the difference in the T:E2 ratio in *T. gondii* seropositive infertile men. To authenticate our results, seropositive infertile men against seropositive and seronegative normozoospermic controls were tested.

The present study showed that infertile men with positive anti-*Toxoplasma* IgG or IgM had higher rates of disturbed T:E2 ratios compared to seronegative controls. However, there were no significant differences in the T:E2 ratio between infertile men and controls with the same serology. Specifically, it was observed that 5 out of 12 IgG-positive normozoospermic men and 13 out of 34 IgG-positive infertile men had altered T:E2 ratios. Furthermore, in 3 out of the 5 IgM-positive normozoospermic men and 6 out of the 8 IgM-positive infertile men, a disrupted T:E2 ratio was noticed. To the best of our knowledge, this study is the first to suggest a potential impact of acute and chronic toxoplasmosis on serum hormone

balance in men regardless of their semen characteristics. Given the lack of consistent data on the impact of *T. gondii* on serum T:E2 ratio in infertile men, our results cannot be compared to previous studies as there is no published literature available on this specific topic. This study adds to the growing body of evidence linking infectious diseases to reproductive health issues and emphasizes the need for a comprehensive approach to addressing male infertility.

The current study showed seropositivity for *Toxoplasma*-IgM and IgG in a small percentage of healthy individuals with normal semen. This finding contrasts with previous studies (31-32) that reported higher seroprevalence rates among apparently healthy men.

Regarding the present findings, the results showed that 8% of infertile men tested positive for *Toxoplasma*-IgM, while 34% tested positive for *Toxoplasma*-IgG. Comparably, one study (33) found that 36% of infertile men tested positive for both *Toxoplasma*-IgG and IgM. In China, a higher prevalence (69.30%) of chronic toxoplasmosis was found in infertile patients, which was attributed to the presence of antisperm antibodies (34). Moreover, another study (35) showed that fertile men infected with chronic toxoplasmosis had a low percentage of anti-*Toxoplasma* IgG antibodies, while infertile men showed a significantly higher percentage.

According to the current study, 52.9% of IgG seropositive infertile men and 41.7% of IgG seropositive normozoospermic controls were engaged in agricultural occupations. Similar findings were reported by Rostami et al. (36), who identified a correlation between the high *T. gondii* seropositivity among farmers and their limited knowledge

about the disease, as well as greater exposure to contaminated soil.

The present work revealed that 79.4% of infertile men and 91.7% of normozoospermic controls with positive Toxoplasma IgG results were from rural areas. Comparable findings were reported by Kawashima et al. (37) and Rostami et al. (36). Conversely, previous researchers (38) demonstrated no significant differences in Toxoplasma infection according to residence, and this may be related to the socioeconomic level of selected subjects or the increased awareness of infection risks among rural populations (38).

Previous studies indicated that toxoplasmosis in men could affect serum testosterone concentrations (39–40). Abbasian (41) showed a significant correlation between Toxoplasma infection and testosterone increase in human serum. Two theories could explain this increase: protective immune response (42) or parasite-induced production of sex hormones (43).

Considering the present findings, infertile men serologically positive for Toxoplasma-IgG or IgM demonstrated significantly higher levels of serum total testosterone in comparison to seronegative controls. Similar findings were reported by prior studies assessing Toxoplasma-positive and negative men regarding their testosterone concentrations in serum (20–21, 44) as well as saliva (22). Likewise, Zghair et al. (31) demonstrated that both acute and chronic toxoplasmosis in men were associated with significantly higher mean concentrations of total and free testosterone hormone, respectively. Furthermore, Flegr et al. (45) revealed that Toxoplasma-infected men had a higher concentration of testosterone, and Toxoplasma-infected women had a lower concentration of testosterone than Toxoplasma-free controls. Alternatively, a study conducted on apparently healthy men revealed that testosterone concentrations in Toxoplasma-IgG-positive men were insignificantly higher than seronegative men, but significant differences were observed in men aged 15–29 (32).

In contrast to our results, Khan et al. (46) demonstrated a decrease in the levels of free and total testosterone in the Toxoplasma-infected infertile men. Likewise, a case-control study has shown that the serum concentration of testosterone in 365 men with latent toxoplasmosis was significantly lower than in testosterone-negative men (39). Additionally, Babu et al. (47) reported low testosterone concentrations in Toxoplasma-

infected patients with elevated levels of FSH and LH hormones, and they attributed these findings to the presence of primary hypogonadism in those patients. Instead, Oktenli et al. (23) demonstrated that total and free testosterone, FSH, and LH levels decreased in men during acute toxoplasmosis, and they related their findings to the suppressive effect of interleukin-1 beta on the secretion of GnRH.

There is considerable evidence that steroid hormones influence the course of toxoplasmosis in humans and mice (48). Estradiol could promote the invasion of PRU and VEG strains into various host cells and enhance the pathogenicity of *T. gondii* in mice (49). Zhang et al. (24) revealed that estradiol can enter the cytoplasm of parasites, rapidly induce intracellular calcium flux, promote the secretion of the key protein mediating the motility of the parasite (Tg-MIC2), and accelerate the gliding and egress ability of parasites, thus promoting parasite activity and pathogenicity in mice. Considering the present results, infertile men serologically positive for Toxoplasma-IgG or IgM exhibited significantly higher levels of serum estradiol in comparison to seronegative controls. Similarly, Alardi (44) showed a significant difference between the *T. gondii* infected and non-infected samples, with a significant difference between acute infection, chronic infection, and non-infection for the concentration of estradiol in infertile men.

Nevertheless, the present study has some shortcomings. The two initial main constraints are the smaller positive control sample in comparison to the negative control sample and the absence of randomization. However, obtaining a larger sample size was hampered by the existence of strict exclusion criteria. The fact that the controls and infertile men were chosen from a single center is another shortcoming of this study. Furthermore, the absence of hormone level monitoring following treatment for infected subjects is another limitation of this study. Additionally, it was not feasible to assess the length of the infection or how it affected the concentration of hormones. Lastly, because anti-*T. gondii*-specific IgM can persist for extended periods of time after infection, the absence of anti-*T. gondii* IgM antibodies in people with anti-*T. gondii* IgG antibodies suggests a chronic infection, while the presence of IgM antibodies does not always indicate an acute infection (50).

### Conclusion

Based on our findings, altered testosterone-estradiol (T:E2) ratios have been detected in a significant proportion of infertile cases exhibiting positive serology for Toxoplasma IgM-IgG antibodies. Nevertheless, conclusive findings about T:E2 ratios in IgM-positive infertile cases cannot be verified because of the small sample size. Therefore, the presence of an abnormal T:E2 ratio in infertile men may indicate the need for anti-Toxoplasma gondii-specific IgG testing. To confirm the current findings, further randomized multicenter studies are required. Moreover, additional research utilizing molecular techniques such as PCR and sequencing, as well as serological assays, is necessary to confirm recent infections and identify various Toxoplasma species and how they relate to the T:E2 ratio in infertile men.

### Conflict of Interest

The authors declare that they have no conflict of interest.

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