

## The role of *Toxoplasma gondii* in concentration of some sex hormones in infertile individual

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**Abstract:** The main objective: Link the incidence of the *Toxoplasma gondii* present and manifestations of some sex hormones at infertile persons.

Follicle-stimulating hormone (FSH), 17- $\beta$  Estradiol (E2), and testosterone are key regulators for the development and progress of germline cells. Also affect many defensive functions to the immune system. *Toxoplasma gondii* attempts to manipulating these Hormones concentration to their survive.

The study methodology: since October 2018 till 31th March 2019, 100 samples of the blood of infertile patients have been collected from both sexes from outpatient clinics in different areas of Qadisiyah governorate, Iraq. All there examined with Elisa test to detect IgM and IgG antibodies against *T. gondii* and to estimate concentration of sex hormones.

Results: 39 samples (39 %) were positive to *T. gondii*. IgM positive samples were (6 and 10) with (12% and 20%) at male and female, Positive IgG samples for male and female were (10 and 13) with (20% and 26%). In male, Testosterone concentrations were increased in infected where were (5.4 and 5) ng/ ml with IgG and IgM test. Also E2 were (33.6 and 35) pg/ ml with IgG and IgM infected men . While FSH concentrations were decreased in (12.1 and 12.3) IU with IgG and IgM. In female, FSH and E2 concentrations were increased in infected female, where were (5.45 and 5.9)IU/ ml and (260 and 250)pg/ ml from both hormones with IgG and IgM test. Testosterone concentration was decreased (0.98)ng/ ml with IgG, but increased (1.35) ng/ ml with IgM in female.

The study has been showed that a significant difference in the concentrations of hormones studied between non-infected and infected samples in the acute case, and did not register a significant difference between chronic infection and non-infection, a significant increase in hormones Testosterone and E2 in male and female while FSH in female only, FSH concentration was lower in male after infection.

Conclusion: in light the study results the author see that: Infection with the parasite is associated with significant changes in the concentrations of sex hormones in infertile peoples, therefore it can be said that infection with *Toxoplasma gondii* is one of the main causes of infertility in both sexes.

Suggestions:

Conduct broader studies by examining more people and determine the relationship between infertility and sex hormone concentration with infection by *T. gondii* in girls and boys before or after puberty.

**Keywords:** *Toxoplasma gondii*, fertility, sex hormones, Elisa.

أثر الإصابة بطفيلي المقوسة القندية على تراكيز بعض الهرمونات الجنسية  
عند الأشخاص العقيمين

## مسافر هندي صفر العارضي

المديرية العامة لتربية القادسية || وزارة التربية || العراق

**الملخص:** الهدف الرئيسي: ربط نسبة حدوث الطفيل وبعض المظاهر الهرمونية لدى الأشخاص المصابين بالعمق. يعتبر الهرمون المنبه للجريب ((FSH، 17- $\beta$  استراديول (E2)، والتستوستيرون منظمات رئيسية لتطور خلايا الخط الجرثومي وتقدمها، وتؤثر أيضاً على العديد من الوظائف الدفاعية لجهاز المناعة، لذا تحاول طفيلي المقوسة التلاعب بتركيز هذه الهرمونات للبقاء على قيد الحياة.

منهجية الدراسة: من أكتوبر 2018 حتى مارس 2019، تم جمع 100 عينة من دم مرضى العمق من كلا الجنسين من العيادات الخارجية في مناطق مختلفة من محافظة القادسية، العراق. تم فحص جميع العينات باختبار الأليزا للكشف عن الأجسام المضادة IgM و IgG ضد *T. gondii* ولتقدير تركيز الهرمونات الجنسية.

النتائج: 39 عينة (39%) كانت إيجابية لطفيلي *T. gondii*، كانت العينات الموجبة لـ 6 (IgM و 10) بنسبة (12% و 20%) عند الذكور والإناث على التوالي، وكانت عينات IgG الموجبة عند الذكور والإناث (10 و 13) بنسبة (20% و 26%) على التوالي. في الذكور زادت تراكيز التستوستيرون عند المصابين حيث كانت (5.4 و 5) نانوغرام/ مل باختبار IgG و IgM على التوالي. وزادت E2 أيضاً فكانت (33.6 و 35) بيكوغرام/ مل مع IgG و IgM للرجال المصابين على التوالي. بينما انخفضت تراكيز FSH في (12.1 و 12.3) وحدة دولية مع IgG و IgM على التوالي. في الإناث، زادت تراكيز FSH و E2 في الإناث المصابة حيث كانت (5.45 و 5.9) وحدة دولية/ مل و (260 و 250) بيكوغرام/ مل من كلا الهرمونين مع اختبار IgG و IgM على التوالي. انخفض تركيز التستوستيرون (0.98) نانوغرام/ مل مع IgG، لكنه زاد (1.35) نانوغرام/ مل مع IgM في الإناث. أظهرت الدراسة فرقاً معنوياً في تراكيز الهرمونات المدروسة بين العينات غير المصابة والمصابة في الحالة الحادة، ولم تسجل فرقاً معنوياً بين العدوى المزمنة وعدم الإصابة بزيادة معنوية في هرموني التستوستيرون والاستراديول (E2) في الذكور والإناث بينما لوحظت الزيادة في هرمون المحفز للحويصلات في الإناث فقط، والذي كان تركيزه أقل عند الذكور بعد الإصابة.

الاستنتاج: في ضوء نتائج الدراسة يرى الباحث عن الإصابة بالطفيلي ترتبط مع تغيرات معنوية في تراكيز الهرمونات الجنسية عند الأشخاص العقيمين، وبالتالي يمكن القول إن الإصابة بالمقوسة أحد الأسباب الرئيسية للعمق عند الجنسين. إجراء دراسات أوسع من خلال فحص المزيد من الأشخاص وتحديد العلاقة بين العمق وتركيز الهرمونات الجنسية مع الإصابة بالتوكسوبلازما في الفتيات والفتيان قبل سن البلوغ أو بعده.

**الكلمات المفتاحية:** المقوسة القندية، الخصوبة، الهرمونات الجنسية، إلiza.

## Introduction.

*Toxoplasma gondii* obligate intracellular parasite, which causes toxoplasmosis (Dardé *et al.*, 2011), affects many hot-blooded animals (Dubey, 2010). Felines such as domestic cats are the definitive host of the parasite, in which sexually phases present and they also are an intermediate host (Al-Hadithi and Awad, 2010). The parasite affects many tissues and organs such as kidney, brain, eye and other tissues (Mahmud, 2017). The parasitic infection ranges from mild symptoms including lymph node enlargement and fever to blindness, cirrhosis, schizophrenia, infertility and recurrent abortion (Atifi, 2011).

In the Arab countries, Soliman *et al.*, 2001 recorded (81.4%) as the incidence of toxoplasmosis in Egypt, in Jordan, Jummain (2005) recorded (47.1%), Al-Harhi *et al.*, 2006, recorded (29.4%) in Saudi Arabia, in Kuwait, Iqbal and Khalid (2007) recorded (53.1%), and in Qatar (28. %) (Abu-Madi *et al.*, 2008).

In Iraq, Al-Khafaf (2001) in Nineveh Governorate, indicated an infection rate (69.2%) among pregnant women, and in the same governorate the infection rate was (48.7%) according to a study conducted by Al-Dulaimi (2002), Khudair *et al.*, 2011 recorded a case of 16.9%, Al-Nasiri and Daoud (2012) recorded a rate of (24%), Al-Mousawi *et al.*, 2015 indicated that the parasite prevalence increased

by 21.94% using the Enzyme linked Immunosorbant Assay (ELISA) technology, and Al Douri et al.2018 recorded an infection rate of 22.3% using the same technique, among pregnant women.

Among women with miscarriages, the incidence rates were (52.1%) in Basra (Yacoub et al., 2006), (61.6%) in Dhi Qar (Al Adlan, 2007), (60.3%) in Wasit (Al Khanq, 2009).

Exposure to parasites shows a clear difference between the sexes, as most studies suggest that males are more likely than females to develop the disease, and this is explained by the behavior of males that tend to aggregation and be in groups (Roberts *et al.*, 2001). In addition to the immune capacity of females, which prevents and reduces the incidence of parasites (Moore and Wilson, 20002). However, the situation may be fabricated when infected with *Toxoplasma*, where females are more susceptible to infection (Liesenfeld,2001), some studies attribute it to the ability of males to produce anti-inflammatories (INF $\alpha$ , INF $\gamma$ , IL12) with higher concentrations than females (Morales-Montor, 2002). The inflammatory response is also higher in females, generating complications such as erythematosus lupus (Diodato, 2001).

Infertility is defined as the inability to have sexual relations with a partner for one year (Rashid et al., 2013). It results from the abnormal congenital or acquired embryogenesis of the reproductive organs, various infections, and hereditary or hormonal causes (Heinonen et al., 1983). All these factors work to vary the sex hormones concentrations, thus the incomplete formation of the reproductive organs during fetal development (Hussien et al., 2018).

*Toxoplasma* infection increases the concentration of the sex hormones FSH and testosterone (Obaid et al., 2016), there are two theories that explain this increase, the first assumes that the increase in the concentration of the hormone is a protective reaction to stimulating the immune system against the parasite (Kankova´ et al. 2011), the second assumes that the parasite itself stimulates the production of this increase to stay on Life (James, 2010).

The study of effect of sex hormones associated with the pregnancy on the parasite, if these effects are to prevent or increase the manifestation of infection, is a public health priority because the parasite causes many congenital problems of fetuses and newborns since infection occur during pregnancy (Roberts et al., 2001). In male, infection of reproduction system may be caused a weakness in the testis (Eslamirad et al., 1996), therefore lead to sterile (Martinez-Garcia et al., 1996). There are many shreds of evidence for the effect of steroid hormones on toxoplasmosis, study of Kittas and Henry (1978) showed a difference in rats immune and inflammatory response after *T. gondii* infection. The study showed that females showed higher inflammatory effects than males, while resistance to infection increased after gonadectomy and after estrogen therapy.

The effect of testosterone, 17- $\beta$ -oestradiol and progesterone on the immune response due to the existence of surface receptors on lymphocytes, macrophages, granulocytes and mast cells, that indicating an interaction between the immune system and the endocrine system (Klein et al., 2010).

Many infertile with toxoplasmosis people suffer from an increase in some sex hormones concentration, which may cause an overlap and an excessive increase in the concentration of these hormones when treating the infertility problem by hormones administration.

This study aimed to estimate some sex Hormones among infertile patients, and determent correlation between infection and concentration increase.

## Materials and Methods:

### Population of the study:

Infertile patients from both sexes from outpatient clinics in different areas of Qadisiyah governorate, Iraq.

### Study period:

since October 2018 tell 31th March 2019. :

### collecting of blood samples:

5 ml of venous blood was withdrawn with a sterile syringe. The blood was conveyed to sterile tubes and left until thrombus formed. Then the tubes were placed in the centrifuge to separate the serum. The residues were kept in other tubes at -20 ° C until they would be used in other tests (Rafiei et al., 2005).

**Sampling size determination:** Selected 20 from 100 patients.

**Sampling Method :** Samples were randomly selected from the patients in the private medical clinics and according to the review record in each.

**Inclusion and exclusion criteria :** The study included people with infertility more than a year ago, and was excluded who least of this period.

**Data collection technique and tool:** all patients data as age, sex, partner status recoded. Used Elisa test apparatus from pioneer company and kit test from follow companies.

### Enzymatic tests:

A. tests of IgG and IgM against T. gondii

The Kit produced from Bio Check, Inc. USA was used according to the manufacturer's instructions.

B. sex hormones test

The Kit was produced from ELISA Monobind kit, USA, and has been used to detect FSH, E2 and testosterone Hormones according to the manufacturer's instructions.

### Statistical Analysis

Results were tested by SPSS version 11software, extracting SD, mean, and chi square ( $\chi^2$ ) at P value  $\geq 0.05$ .

Ethical consideration: All patients have been informed of the test method, they were signed a paper included volunteering to conduct the experiment.

## Results:

### • Incident of *T. gondii*

Since 10/ 2018 till 31/ 3/ 2019, 100 samples of the blood of infertile patients have been collected from both sexes and outpatient clinics in different areas of Qadisiyah governorate, Iraq. 50 samples of male and female were between 20 and 45 years of age.

39 positive samples was (39%). 6 and 10 (12%; 20%) samples for male and female jointly were positive to IgM. IgG samples positive were (10; 13) for male and female jointly (table 1).

The results showed significance infection at  $p \leq 0.05$ . The existence of IgM as an indicator of acute infection and the existence of IgG from the chronic parasitic infection. Positive and negative samples As control samples has been isolated to use in estimating hormone concentrations.

The results of this study has been agreed with the findings of Al-Jebouri et al. (2013) when they recorded a 42% prevalence among female in Salah al-Din, Iraq. But less than Muhsen et al. (2018) when recorded a total of 51% when they examined patients who have been referred to Al-Rusafa hospital in Baghdad. And Al-Asadi and Hussein (2015) recording among the infertile female in Al-Basra, Iraq. Greater than Abdulla et al. (27.7%) among infertile males in Baghdad and (Al-Masoudi) among female in Babil Governorate (23.3%) as prevalence rate.

**Table (1): Prevalence of *T. gondii* by enzymatic tests.**

Antibody	positive		Negative		Statically tests
	Male	female	male	female	
IgM	6 (12%)	10 (20%)	44 (88%)	40 (80%)	P.value = 0.232
IgG	10 (20%)	13 (26%)	40 (80%)	37 (64%)	
Total	16 (32%)	23 (46%)	34 (68%)	27 (54%)	

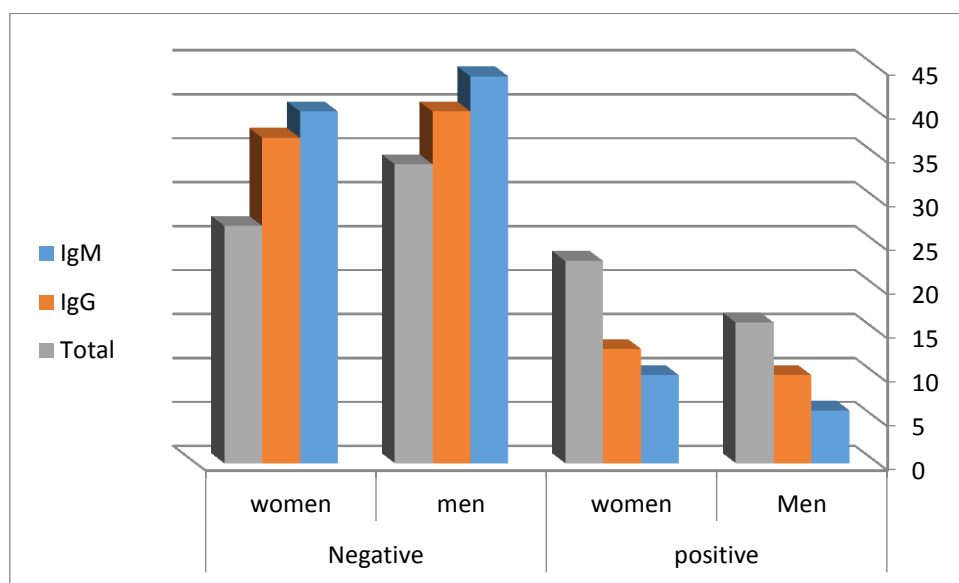


Fig (1) prevalence of *T. gondii*.

Results of this study showed an increase in the concentration of testosterone hormone in the infected samples compared to non-infected samples with *T.gondii* in male, where recorded (4.78; 5 and 5.4) in non-infected, acute and chronic infected samples, respectively, Table (2). (1.0; 1.35 and 0.98) were testosterone concentration in non-infected, acute and chronic infection samples, respectively, (Table 3) in female. Significant differences have been recorded between acute parasitic infection with both chronic infection and non-infection at a significant level of  $P \leq 0.05$ .

Some last studies have shown an increase in testosterone levels (Al-Saadii (2013), Mohammad (2018)), while other reported that the infection caused low levels of the hormone (Flegr *et al.* 2008), because testosterone appeared to play important roles in The different processes as reproduction, afraid and behavior. that difference factors - primarily parasite strains and changes in the host - have a variety of effects on the severity of *T. gondii* infection, which therefore have a variety effects on testosterone production and behavioral changes (Abdol, 2014).

There is no way to determine the mechanism by which the secretion of the hormone testosterone as response to *T.gondii* infection, there are two hypotheses the first is supposed to stimulate the secretion of the hormone in response to enter the parasite, where the high concentrations of the hormone act as stimulator and regulator to the work of the immune system, the second assumption that the infection of this parasite reduces hormone concentration, thus the parasite gets rid of the immune system's pressure against it (Larralde *et al.*, 1995).

The concentration of follicle-stimulating hormone (FSH) in the infected samples is lower than in non-infected samples among male table (2). The concentrations were (12.6; 12.3 and 12.1) for negative, acute and chronic infection, respectively. While FSH concentration was increase among infected female (4.7; 5.9 and 5.45) table (3). The differences were significant. The study will be showed significant

difference between acute and chronic infection, and between acute and non-infection at a significant level  $P. \leq 0.05$ .

Ologi (2003) has attributed the increase or decrease in the concentration of FSH to the ability of gonads receptors to respond to it, and its concentration is inversely proportional to the concentration of the hormone testosterone, whose high concentration inhibits the production of FSH. The high concentration of FSH is a sign of the destruction of germinal epithelial tissue, associated with many Distortions, and heterogeneity in sperm count (Zhang et al., 2003). Differences in the DNA methylation of selected genes have been detected between the positive toxoplasma group and the control group indicating a direct relationship Between the toxoplasmosis and reduced male reproductive fitness in mice, which may be contributed to increased infertility in humans (Dvorakova, et al., 2014).

The study showed a significant difference between the infected and non-infected samples, with significant difference between acute infection and both chronic or non-infection at a significant level  $P. \leq 0.05$ , for the concentration of estradiol (E2) in male, when recorded (32.3; 35 and 33.6) In non-infected samples, chronic and acute infection respectively, Table (2), in female recorded (249; 250 and 260) respectively Table (3).

The concentration of estrogen interferes with the activity of many immune cells and lymphoid organs, because of the existence of hormone receptors on these cells (Bini et al., 2011), Craig et al. (2001) Explained that High concentrations of estrogen facilitates infection of parasites, while Kittas et al. (1979) suggests that prolonged administration of hexo-estrol leads to atrophy of the thymus gland and the strong integration of the thymus-dependent areas of the lymph nodes and spleen, thus drop of immunity and severe disease in animals hose infected with parasites.

**Table (2) Hormones concentration in positive and negative samples, male.**

Hormones	Positive		Negative	P.value
	IgG	IgM		
Testosterone ng\ml	5.4 ± 0.06a	5 ± 0.173b	4.78 ± 0.047a	0.003
FSH (IU\ml)	12.1 ± 0.12a	12.3 ± 0.09b	12.6 ± 0.19a	0.00
E2 (pg\ml)	33.6 ± 1.6a	35 ± 1.78b	32.3 ± 1.75a	0.043

\* similar letter mean no significant difference.

**Table (3) Hormones concentration in positive and negative samples, female.**

Hormones	Positive		Negative	P.value
	IgG	IgM		
Testosterone ng\ml	0.98 ± 0.131b	1.35 ± 0.171b	1.0 ± 0.1414a	0.00
FSH (IU\ml)	5.45 ± 0.158b	5.9 ± 0.128b	4.7 ± 0.21a	0.00
E2 (pg\ml)	260 ± 6.04b	250 ± 4.92b	249 ± 4.886a	0.03

\* similar letter mean no significant difference.

## Conclusions:

Infection with *Toxoplasma* is one of the most important causes of infertility. The existence of the parasite affects the concentration of some sex hormones, which contribute to the maturation and differentiation of sex cells, in this study we recorded significant increase in hormones Testosterone and E2 in male and female while FSH in female only, FSH concentration was lower in male after infection.

## Recommendations:

Conduct broader studies by examining more people and determine the relationship between infertility and sex hormone concentration with infection by *T. gondii* in girls and boys before or after puberty.

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