The Levels of Testosterone, FSH and LH in Pregnant Women with Chronic Toxoplasmosis in Najaf Province

Raad A. AL-Asady, College of Medicine, University of Kufa.

الخلاصة:

تعتبر الاصابة بطفيلي داء المقوسات واسعة الانتشار بين البشر والفقريات. ان مدى كبير من تاثيرات داء المقوسات قد درست سابقا, لكن تبقى جوانب اخرى يجب ان تكتشف. الدراسة الحالية تبحث التغيرات الحاصلة في الهرمونات الجنسية testosterone و FSH وLH عند النساء الحوامل المصابات بداء المقوسات المزمن باستخدام تقنية mini-Vidas. شملت الدراسة 59 امراة حامل ذات فحص مصلي موجب للاجسام المضاد للمقوسات نوع IgG بالاضافة ل28 امراة حامل ذات فحص مصلي سالب لنفس الاجسام الضادة. النتائج بينت ان النساء الحوامل المصابات بداء المقوسات المزمن اظهرن مستويات اعلى لهرموني testosterone و 0.44 ,1.74 mIU/ml) LH (ng/mlو مستويات اوطأ لهرمون FSH (1.78 mIU/ml) مقارنة بالنساء الحوامل غير المصابات, لكن بدون فروق احصائية مهمة لكل الهرمونات الثلاث (0.42 ng/ml و 1.68 mIU/ml وmIU/ml 2.25) . هذه النتائج تقترح بان الاصابة المزمنة بطفيلي داء المقوسات لا يرتبط بتغيرات مهمة لمستويات هذه الهرمونات عند النساء الحوامل.

Abstract

Infection with the intracellular protozoan parasite *Toxoplasma gondii* is widely prevalent in human and vertebrate animals. A wide range effects of toxoplasmosis has been studied, but there are still unknown aspects which must be explored The present study investigates the changes of testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels in pregnant women with chronic toxoplasmosis using mini-VIDAS technique. A total number of 59 toxoplasma-IgG positive pregnant women and 28 healthy toxoplasm-IgG seronagative pregnant women were involved. The results showed that pregnant women with *Toxoplasma* chronic infection revealed insignificant higher levels of testosterone and LH (0.44 ng/ml, 1.74 mIU/ml respectively), and insignificant lower levels of FSH (1.78 mIU/ml) in compared to non-toxoplasmic pregnant women (0.42 ng/ml, 1.68 mIU/ml, 2.25mIU/ml). These findings are to suggest that chronic infection with *T. gondii* has no association with significant changes of these hormones in pregnant women.

Keyword: Toxoplasmosis, *Toxoplasma gondii*, testosterone, FSH and LH.

Introduction

Toxoplasmosis caused by *Toxoplasma gondii*, is one of the most common zoonotic diseases that has infected approximately one-third of the world's human population (1). *T. gondii* is most common in warm, moist areas, and has been reported from man, pigs, sheep cattle, horses, dogs, cats and other domestic animals, as well as rodents, wild carnivores, and birds (2). Infection with *T.gondii* is generally initiated by ingesting either the tissue cyst stage, found in the meat of infected animals, or the oocyst stage, released in the feces of infected cats (3). Adult acquired toxoplasmosis is normally mild to asymptomatic, but disease can be severing in the immunosuppressed (4).

Numerous epidemiological and clinical studies have noted differences in the incidence and severity of parasitic diseases between males and females. Although in some instances this may be due to gender-associated differences in behavior, there is overwhelming evidence

that sex-associated hormones can also modulate immune responses and consequently directly influence the outcome of parasitic infection (5).

There is considerable evidence that steroid hormones affect the course of toxoplasmosis in humans and mice. In 1976, Henry and Beverley were the first to demonstrate differences in the immune and inflammatory responses of male and female mice following infection with *T. gondii*. In these studies, female mice developed more severe brain inflammation than male mice following infection (6). Moreover, a direct role for sex hormones was demonstrated in experiments which found that gonadectomy increased resistance, whereas oestrogen administration exacerbated disease in mice. Similarly, simultaneous gonadectomy and oestrogen treatment predisposed guinea pigs to increased parasite burdens compared with non treated control animals (7).

Although the incidence of *T. gondii* infection was similar in males and females, disease manifestations varied according to gender and age (6). In those under 15 years of age, lymphadenopathy was more frequently observed in males than in females. However, in sexually mature adults (over 25 years of age), lymphadenopathy was more frequently observed in females (6, 8). The prevailing hypothesis for immunological differences between the sexes is that sex hormones, in particular, testosterone, influence the immune system (9). The localization of sex hormone receptors in immune cells, including lymphocytes, macrophages, granulocytes, and mast cells, illustrates that there are direct connections between the endocrine and immune systems and that endocrine factors can directly modulate the expression of target genes in immune cells (9).

Latent toxoplasmosis is known to influence the morphology of infected persons and also increases the probability of the birth of male offspring in both humans and mice. All these traits can be related to the observed differences in the concentration of testosterone between *Toxoplasma*-infected and *Toxoplasma*-free subjects. However, it is not possible to decide, using the *Toxoplasma*-human model, whether toxoplasmosis influences the level of testosterone in the infected host or whether individuals with different levels of testosterone vary in the probability of toxoplasma infection (8, 10).

During pregnancy, maternal hormones alter the immune responses of the mother in the presence of fetal antigens. The increases in the susceptibility to infection and a diminished pro-inflammatory response have critical anti-parasitic properties that cause an unfavorable development of toxoplasmosis (11, 5, 12, 13). In addition, the ability of sex and pregnancy-associated hormones to influence the severity of *T .gondii* infection is of particular public health interest due to the ability of the parasite to cause congenital disease if infection occurs during pregnancy, moreover, female is more vulnerable than male to infection by *T. gondii* and the susceptibility to pathogens also varies according to the stage of the menstrual cycle in non-pregnant women and varies according to stage of gestation in pregnant women (14, 5). Two hormones secreted by the anterior pituitary gland called gonadotropins (follicle stimulating hormone “FSH” and lutienizing hormone “LH”) are pregnancy-associated hormones that control the cyclic changes in ovaries (15).

In present study we have attempted to find if there was a correlation between chronic infection by *T. gondii* and levels of testosterone, FSH and LH hormones in the study groups.

Materials and methods

-Samples:

In this cross sectional study, sera of 59 (21 first trimester, 29 second trimester and 9 third trimester) seropositive IgG anti-Toxoplasma antibodies pregnant women with history of one or more previous abortions as a patients group (previously identified by mini-VIDAS method) and 28 (9 first trimester, 8 second trimester and 11 third trimester) seronegative IgG anti-Toxoplasma antibodies pregnant women as a controls group were included for the estimation of testosterone, FSH and LH concentrations. These samples were obtained from the AL-Zahraa Maternity and Pediatrics Hospital in Najaf city. Their ages were 27.52 ± 6.54 with a range of 15-43 years for patients group and 24.79 ± 5.70 with a range of 15-42 years for controls group.

-Collection of blood:

Disposable syringes and needles were used for blood collection. Venous blood samples, about 4-5 ml were collected from pregnant women in plane tubes. After allowing the blood to clot at room temperature for 15 min, blood samples were centrifuged at 3000 xg for 15 min. Sera were separated, and store in -40 °C to determine testosterone, FSH and LH levels.

-Determination of testosterone, FSH and LH concentrations in serum:

For the quantitative determination of total testosterone, FSH and LH concentrations in serum of pregnant women , mini-VIDAS Testosterone EnzymeTestKit, FSH TestKit and LH TestKit manufactured by Biomerieux (England) were used. .

-Statistical analysis: .

All data were analyzed using the Statistical Package for Social Sciences (SPSS) version 12 for Windows. Results are expressed as mean ± standard deviation (SD). Statistical significance and difference from control and test values were evaluated by Student s t-test. A probability value of $P<0.05$ indicated a statistically significant difference.

Results

The present study showed overall variations in levels of hormons (testosterone, FSH and LH) in pregnant women with seropositive IgG Toxoplasma antibodies and in controls are presented in (figure 1).

The result showed very slightly higher serum levels of testosterone (0.44 ng/ml) was detected in patients with chronic toxoplasmosis compared to controls (0.42 ng/ml) but with no statically significant difference (p= 0.86).

FSH serum level was lower (1.78 mIU/ml) in chronic toxoplasmosis patients when compared with controls (2.25 mIU/ml), but did not show significant variation (p= 0.46).

While the LH level was slightly higher (1.74 mIU/ml) in IgG positive pregnant women in comparison to IgG negative pregnant women (1.68 mIU/ml), but also without significant difference (p= 0.88).

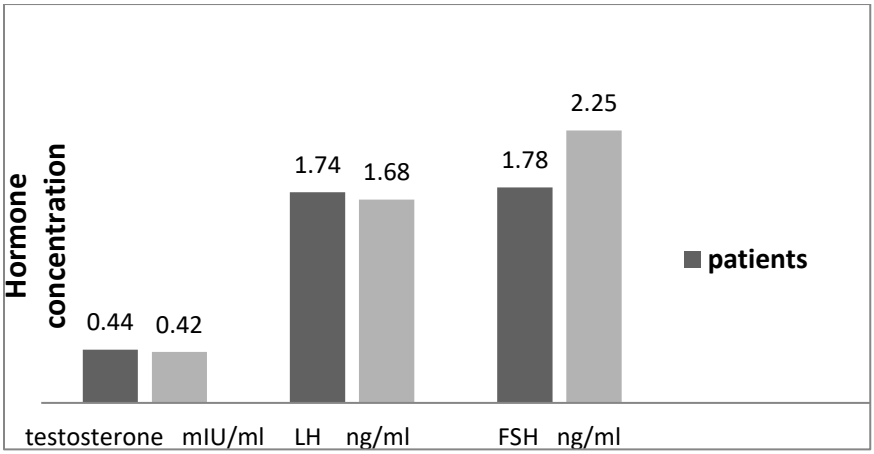

Figure (1): Testosterone, LH and FSH levels in patients and controls.

Regarding the age of patients and controls, comparison of testosterone levels in the two groups (patients and controls) showed slightly elevation in the 1st [15-24 year] and the 2nd [25-34 year] age groups (0.5 ng/ml and 0.34 ng/ml respectively) and slightly decrease in the 3rd (35-43 year) age group (0.62 ng/ml) in chronic toxoplasmosis patients as compared to controls (0.44 ng/ml, 0.39 ng/ml, and 0.70 ng/ml respectively), but without any statically significant differences between patients and controls of the three age groups (p= 0.66, p= 0.69 and p= 0.65 respectively) (figure 2).

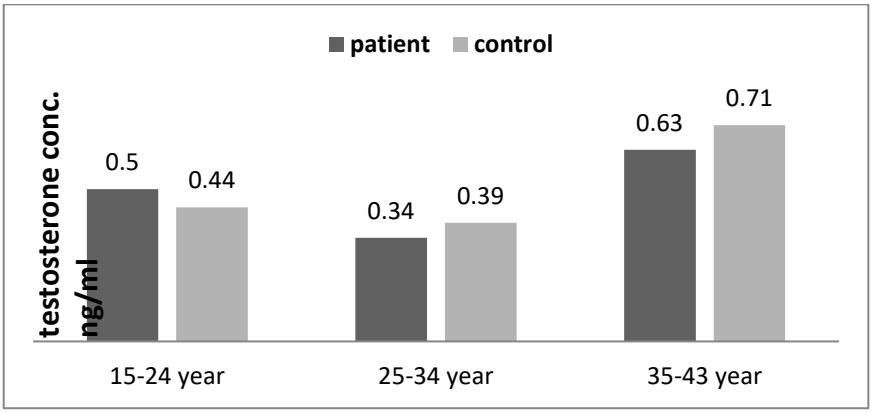

Figure (2): Testosterone levels in patients and controls according to age group.

Lower levels of FSH and LH were detected in the 1st age group of patients (1.36 mIU/ml and 1.24 mIU/ml respectively) in comparison to controls (4.43 mIU/ml and 3.05 mIU/ml) with high significant differences of both hormones (p= 0.012 and p=0.005) (figure 3 & 4), while higher levels of FSH and LH were reported for the 2nd and 3rd age groupds of patients (1.94 mIU/ml, 2.19 mIU/ml for FSH and 1.98 mIU/ml, 1.97 mIU/ml for LH) compared to controls (0.66 mIU/ml, 1.66 mIU/ml for FSH and 0.73 mIU/ml, 0.1 mIU/ml for LH) with significant differences of LH levels in the 2nd and 3rd age groups and FSH levels in the 2nd age group (p= 0.002, p= 0.002 and p= 0.03 respectively), but no significant difference in the level of FSH for the 3rd age group

between patients and controls (p= 0.27). (fiigure 3 & 4).

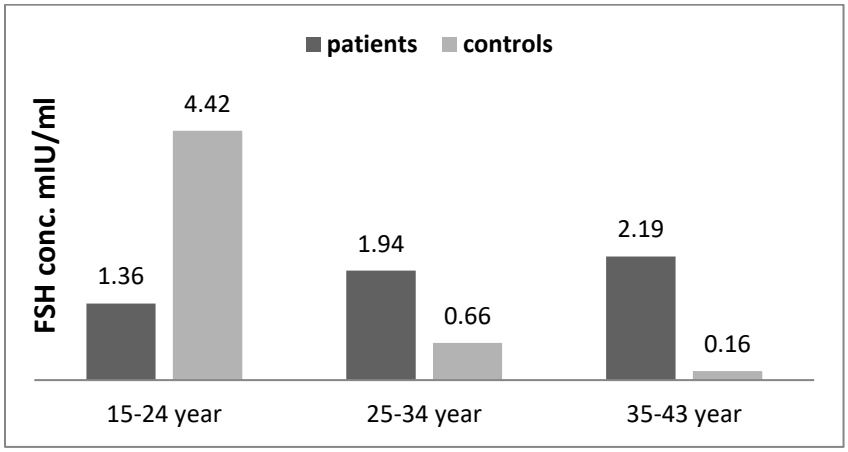

Figure (3): FSH levels in patients and controls according to age group.

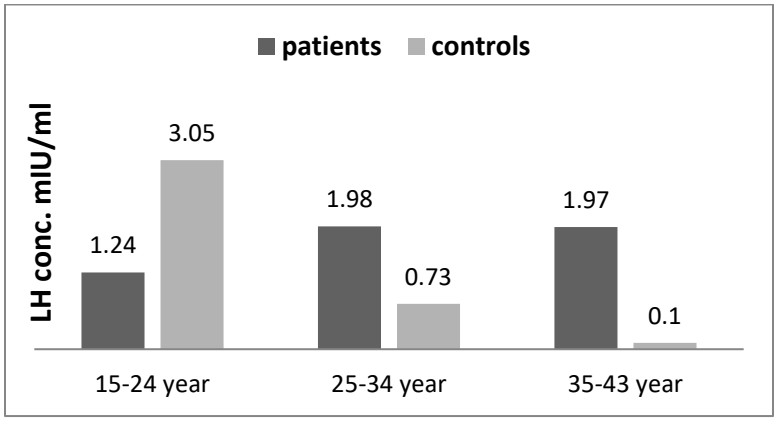

Figure (4): LH levels in patients and controls according to age group.

Discussion

Not only hormones of the host can change response to infection, but parasites can change hormones within the host.

The proximal mechanism of association between testosterone and toxoplasmosis is not clear. As stressed in the article of flgler *et al.* (16), a case control study cannot decide whether Toxoplasma infection induced changes in testosterone concentration or whether low-testosterone and high-testosterone subjects differ in the probability of acquiring *Toxoplasma* infection or both.

The purpose of this study is to evaluate three important sex hormones concentrations (testosterone, LH and FSH) in pregnant females with chromic toxoplasmosis and history of abortion (patients group). The results were compared with non-toxoplasmosis healthy pregnant women (controls group).

The results of the current study showed slightly higher concentration of testosterone in pregnant women with positive IgG toxoplasm antibody (patients) in compared to pregnant women with negative IgG toxoplasm antibody (controls) but with non-significant difference (p=0.86). This finding is

compatible with the findings of Flger (17), who detected insignificant increase in the testosterone concentration between toxoplasm-positive and toxoplasm-negative patients, but partially corroborated by the studies done by Shirbazou *et al.* (18), Khadim and Al-awaid (19) and Al-sherres (20) which find a significant increase in the plasma testosterone level in pregnant women with positive IgG toxoplasm antibody.

Increased testosterone concentration have immuo-suppressive effects characterized by decreased cellular immunity (5, 21), which represents the best explanation of the observed toxoplasmosis-testosterone association and an increased risk of toxoplasmosis in persons with higher concentrations of testosterone, and may explain the changed behavior induced by *T. gondii* as a side effect to suppressed host immunity and thus enhance the chances of organisms surviving in the host (22). In addition to suppressed immunity (as above), it is possible that persons with high concentrations of testosterone had more chances of being infected by *T. gondii* due to behavioral changes and personality profile, because their tendency to disregard rules of their community may lead to lower standards of hygiene and corresponding higher risk of contact with an infection source (10).

The result of this study partially support the idea of some researcher who believed that there are some direct and indirect evidences of an increased testosterone concentration in human infected with toxoplasmosis as infected males are taller, have lower hand 2D:4D ratio (23, 16), and are perceived as more dominant and mescaline (24); and infected females have a non significantly lower left hand 2D:4D ratio and more likely to give birth to boy than girls (25), the later is true for laboratory mice (26).

Flegr *et al*. (16) reported that men with toxoplasmosis had a higher concentration of testosterone while infected women had a lower concentration compared to controls. Whom attributed such opposite direction of the testosterone shift in men compared to women to the gender specificity of behavioral shifts in patients with toxoplasmosis (27).

In mice with induced infection, the observations of Kankova *et al.* (10) are in contrast with this study finding since they observed that there was significant decrease in the serum testosterone values for the male and female in their study, they suggested that the decrease of testosterone concentration could be an adaptive response of infected mice to Toxoplasma-induced immunosuppresion by decreasing the concentration of testosterone, the infected mice could partly compensate the latent toxoplasmosis associated down-regulated cellular immunity, namely the observed suppressed reactivity of macrophages and lymphocytes to the antigens in in vitro assays (28). Such compensation might increase the probability of the survival of infected mice after contact with various pathogens in their natural environment. It is also possible that the physiological reaction to Toxoplasma infection differs qualitatively between mice and humans because mice have short life comparable with the length of life in human.

Results of the study revealed lower FSH concentration in pregnant women with positive IgG toxoplasma antibodies compared to seronegative IgG pregnant women but with statically insignificant difference. This result is disagreed with the findings of Al-warid *et al*. (29) and Al-sherees (20), where significant increase in

the level of FSH in pregnant women in compared to healthy pregnant women was reported. In the current study significant increase in FSH concentration of patients group compared to controls in the second age group (25-34 year) which is compatible with the result of Al-warid *et al.* (29), this can be explained by the relative matching in the ages of patients and controls between the two studies.

This study showed that pregnant women with chronic toxoplasmosis have statically insignificant higher LH concentration in comparison to non-toxoplasmic pregnant women, this result is agreed with the findings of Rui *et al*. (30), where no obvious changes in LH hormone in mice infected with toxoplasm was reported, but incompatible with the results of Al-warid *et al*. (29) where a significant lower LH levels in pregnant women with toxoplasmosis as compared to healthy pregnant women was detected. In this study, the result of the first age group (15-24 years) is agreed with the findings of Al-warid *et al.* (29) and Al-sherees (20), and this similarity may be due to close age matching of the current study with these studies.

The significant lower level of FSH and LH in the age group of 15-24 year may explained by the hypothesis of Stahil *et al*. (31) which stated that cytokines released peripherally in response to parasite reached the hypothalamus and initiate a sequence of events that inhibit the pulsatile release of gonadotrophic releasing hormone (GnRH), leading to subsequent impairment of the pituitary-ovarian axis.

In general, variations in the results among different studies may be due to differences in sample size, age range, environmental factors, parasite strain and technical procedure of hormone detection.

Conclusion

The result of current study showed no significant changes of testosterone, FSH and LH levels in pregnant women with chronic toxoplasmosis.

References

1-Montoya, J. G. and Liesenfeld, O. (2004). Toxoplasmosis. Lancet. 363: 1965-1976.

2-Nobel, E. R. and Nobel, G. A. (1982). Parasitology: The Biology of animal parasites. Lea & Febiger; Philadelphia. pp: 522.

3-Zeibig, E. A. (1997). Clinical Parasitology.W. B Saunders Company; Philadelphia. pp: 320.

4-Araujo, F. G. and Remington, J. S. (1987). Toxoplasmosis in immunocompromised patients. Eur. J. Clin. Microbiol. 6: 1-12.

5-Roberts, C.W.; Walker, W. and Alexander, J. (2001). Sex-associated hormones and immunity to protozoan parasites. Clin. Microbiol. Rev. 14: 476-488.

6- Henry, L. and Beverley**,** J. K. A. (1976). Age and sex differences in the response of lymph node post-capillary venules in mice infected with *Toxoplasma gondii*. J. Exp. Pathol. 57: 274-281. (Cited by Roberts, 2001).

7-Kittas, C. and Henry**,** L. (1980). Effect of sex hormones on the response of mice to infection with *Toxoplasma gondii*. Br. J. Exp. Pathol. 61(6): 590-600.

8-Oktay, K.; Eva-Maria, F.; Gijsbert, M.; Heikyoung, S.; Rudolf, J. and Hidde, L. (2010).New model tracks the immune response to a *T. gondii* Whithead Inst. Bio. Res. 12: 41-43.

9-Oktenli, C.; Dogansi, L. and Ozgurtas, T. (2004). Transient hypogon- adotrophic hypogonadism in males with acute toxoplasmosis: suppressive effect of interleukin-1β on the secretion of GnRH. Human Repro. 19(4): 859-866.

10-Kankova, S.; Kodym, P. and Flegr, J. (2011). Direct evidence of Toxoplasma-induced changes in serum testosterone in mice. Exp. Parasitol. 128: 181–183.

11-Craig, W. R.; Willian, W. and James A. (2001). Sex-associated hormones and immunity to protozoan parasites. Clin. Microbiol. Rev. 14: 476–488.

12-Prigione, I.; Chiesa, S.; Taverna, P.; Ceccarelli, R.; Frulio, R. and Morandi, F.; *et al*.(2006). T cell mediated immune responses to *Toxoplasma gondii* in pregnant women with primary toxoplasmosis. Microbes Infect. 8: 552–560.
13-Dionne, P.; Robinson, S. and Klein, L. (2012). Pregnancy and pregnancy-associated hormones alter immune responses and disease pathogenesis. Horm. Behav. 62: 263–271.
14-Styrt, B. and Sugarman, B. (1990). Estrogens and infection. Rev. Infect. Dise. 13: 1139-1184.
15-Antony, C. P. and Thibodeau, G. A. (1983).Textbook of Anatomy and Physiology. The C.V. Mosby Company; London. pp: 887.
16-Flegr, J.; Lindova, J. and Kodym, P. (2008). Sex-dependent toxoplasmosis-associated differences in testosterone concentration in humans. National Reference Laboratory for Toxoplasmosis. 18: 427- 431.
17-Flegr, J. (2011).Potential immunomodulatory effects of latent toxoplasmosis in humans. J. Parasitology. 135: 427- 431.
18-Shirbazou, S.; Abasian, L. and Meymand, F. T. (2011). Effect of *Toxoplasma gondii* infection on plasma testosterone and cortisol level and stress index on patients referred to Sina hospital,Tehran. Jundishapur J. Microbiol. 4(3): 167-173
19-Kadhima, R. A.; AL-awadib, H. M. (2014).Changes in testosterone, progesterone and prolactin levels in pregnant women with chronic toxoplasmosis. Medical Journal of Babylon. 10(3):
20-AL-sherres, H. A. (2014).Immunological and molecular study of toxoplasmosis on some levels of cytokines and hormones in women in Najaf province. M.Sc. Thesis. College of Medicine. University of Kufa. pp: 72-73.
21-Schuster, J. P.and Schaub, G. A. (2001). Experimental Chagas disease: the influence of sex and psychoneuro immunological factors. Parasitol. Res. 87: 994-1000.
22- Flegr, J. (2007). Effects of *Toxoplasma* on human behavior. Schizophrenia Bulletin. 33: 757-760.
23-Flegr, J.; Hruskova, M.; Hodny, Z.; Novotna, M. and Hanusova, J. (2005). Body height, body mass index, waist-hip ratio, fluctuating asymmetry and second to fourth digit ratio in subject with latent toxoplasmosis. J. Parasitology. 130(6): 621-628.
24- Hodkova, H.; Kolbekova, P.; Shallora, A.; Lindova, J. and Flegr, J. (2007). Higher perceived dominance in *Toxoplasma* infected men the new evidence for role of increased level of testosterone in toxoplasmosis –associated changes in human behavior. J. Neuro. Endocnol.Lett. 28(2): 110-114.
25-Kaňková, Š.; Kodym, P. and Fleg, J. (2011).Direct evidence of *Toxoplasma*-induced changes in serum testosterone in mice. Exp. Parasitol. 12(3):181-183.
26-Kankova, S.; Sulc, J.; Nouzora, K.; Fajfrlik, K.; Fynta, D. and Flegr, J. (2007) Women infected with parasite *Toxoplasma* have more sons . Natur Wissen Schaften. 94: 122-127.
27- Flegr J. (2010). Influence of latent toxoplasmosis on the phenotype of intermediate hosts. Folia Parasitol. 57(2): 81-87.
28-Kankova, S.; Hola, V.; Zajicova, A.; Kodym, P. and Flegr, J. (2010). Modulation of immunity in mice with latent toxoplasmosis–the experimental support for the immunosuppression hypothesis of Toxoplasma-induced changes in reproduction of mice and humans. Parasitol. Research. 107: 1421–1427.
29- Al-Warid, H. S.; Ali, H. Z. and Muhamad, S. N. (2012). Detection of LTH, FSH and LH hormone level in pregnant women infected with *Toxoplasma gondii*. International Journal of Recent Scientific Res. 10(3): 809 -811.
30-Rui, Y.; Qiang , X. and MingZhe, J. (2009). Effect of abnormal hormone secretion caused by *Toxoplasma gondii* in male fertility descent. Mod. Prev. Med. 36(21): 4130-4135.
31-Stahl, W.; Dias, J. A.; Turek, G. and Kaneda, Y. (1995). Etiology of ovarian dysfunction in chronic murine toxoplasmosis. Parasitol. Res. 81(2): 114-20.