DOI : 10.35124/bca.2019.19.2.4393

Biochem. Cell. Arch. Vol. 19, No. 2, pp. 4393-4398, 2019 | www.connectjournals.com/bca | *ISSN 0972-5075*

SOME PHYSIOLOGICAL AND HISTOLOGICAL CHANGES IN THE MALE REPRODUCTIVE SYSTEM OF RATS (*RATTUS RATTUS*) EXPERIMENTALLY INFECTED WITH *TOXOPLASMA GONDII*

Halla Abdul-Hadi Chabuk

Department of Biology, College of Science, University of Babylon, Iraq.
e-mail : halahadi30@gmail.com

(Received 22 February 2019, Revised 20 May 2019, Accepted 2 June 2019)

ABSTRACT : *Toxoplasmosis* is a zoonotic infection of animals caused by the protozoan parasite *Toxoplasma gondii*. It is one of the most important pathogen in humans and other warm-blooded animals that have effect on reproductive function. The aim of this study was detected the effect of *toxoplasmosis* on some Physiological parameters and Histological structure in the male Rats testes infected with parasite *Toxoplasma gondii*. In this study used 16 white Swiss Male Rats (*Rattus rattus*). They divided into three groups 1st group were control group that treated orally with distilled water, 2nd group were infected with suspension of parasite (5×10^3 tissue cysts) that isolated from sheep meat, these animals killed after six weeks from infection and 3rdgroup were infected with suspension ofparasite but they killed after Twelve weeks from infection. Results showed a significant decrease ($P \leq 0.05$) in the sperm parameters (including: sperm motility, viability, concentration and number of spermatozoa and testes weights) compared with the control groups; also it showed a significant decrease in the levels of testosterone and LH hormones in the serum of infected rats. Acute *Toxoplasma gondii* infection can cause impairment on the tissues of epididymis andtestes in the male rats were necrosis and erosion epithelial cells of the seminiferous tubules and epididymis in addition to, inflammation and congestion veins up to 12 weeks after infection with *Toxoplasma gondii*. These findings suggest that toxoplasmosis can cause damage on the reproductive parameters of human or animal male as well as decline of different hormones *T. gondii* infectionnot only effect on female reproduction, also cause male reproductive.

Key words : *Toxoplasma gondii*, testosterone and LH hormones, testes.

INTRODUCTION

Toxoplasma gondii is an intracellular protozoan that reproduces sexually in the intestine of cats act as definitive host and asexualreproductive occur in tissues of any warm-blooded animal (including human, birds, mice and other mammals) acts as an intermediate hosts (Montoya and Liesenfeld, 2004).Toxoplasmosis is widely prevalent in man and animals throughout the world, including Iraq. The infection in human occurs through consuming food or drink contaminated with feces of cats containing Oocysts or tissue cysts (Torda, 2001; Tenter *et al*, 2000). Infection in humans is asymptomatic, who enjoy healthy immune, either people who suffer from HIV or congenitally infected children, it causes serious damage may to death (Weiss and Dubey, 2009). The last studies have shown that the toxoplasmosis causes inflammation andnecrotic foci in the Spleen, liver, Lung and brain furthermore, congenital toxoplasmosis a result of transmission of parasites from an acutely infected mother to the fetus (Haziroglu *et al*, 2003).

Mention, Khaki *et al* (2011), Dalimi And Abdoli (2012) that the parasite effect on the male reproductive function may cause severe disease and it can occur pathological changes in the tissues of male reproductive such as testes, epididymis, prostate gland (Shen, 2001).

MATERIALS AND METHODS

Expermintal animals

In this study, used 16 white Swiss male rats ranged weights (250-300) were obtained from animal house of the Department of Biology, University of Babylon. These animals divided to three groups each group contain five animals,1st group animals served as controls were inoculated with 0.5 ml normal saline while 2nd group and 3d group animals were inoculated orally with suspension parasite (5×10^3 tissue cysts for each rats) that isolated from sheep meat (Eid, 2004). After the inoculation, 2nd group animals were sacrificed after 6 weeks while 3rd group animals were sacrificed after 12 week, then isolating the organs (Testes and Epididymis) and it keep in formalin

material for the study of histological sections (Humason, 1972).

Isolation of Parasite

Muscles samples were collected from sheep's meat infected with *T. gondii* from Al-Hilla abattoir for detection of tissue cyst for parasite. These samples were collected in clean plastic and it brings immediately to the laboratory, they were cut in to small pieces after removing adipose tissues from them. After that, the minced meat was digested by acid-pepsin solution as described by Dubey and Thulliez (1993).

Estimate the level of testosterone and LH hormones

Blood samples (5 ml) were collected directly from the heart using medical syringes five ml. and it put in Jel tube, and stored under 4°C temperature overnight. Serums were isolated by centrifugation of blood samples at a rate of 2500 rpm for 10-15 minutes. Serum samples use for Measurement Testosterone, FSH and LH hormoneswere determined by ELISA method (Wistom, 1976).

Epididymal Sperm concentration and total number

The epididymis was excised immediately from animals after anesthesia and placed in petri dish containing 1 ml of normal saline (5%). This solution was stirred, one drop was placed on the slide and acoverslip was placed over the droplet. At least 10 microscopic fields were observed at 400x magnification to find concentration of sperm (N) in 10 fields according to the equation (Hinting, 1989)

$$\text{Sperm Concentrationin(1ml) of theepididymis} = \frac{N}{10} \times 10^6$$

Epididymis was cut by sharp scalpel added (10 ml of formalin (40%) and 90% normal saline thenthesperm countin 80 a small box to slice count (chamber Haemocytometer) (the number of sperm were extracted according to the following equation: Total number of sperms= (N/80) x4000 x1000 x 10. After dividing the total number of sperm on the totalweight of epididymis lead to sperm extracted in(mg /total epididymis weight) (Sakamoto and Hashimoto, 1986).

Sperms motility percent

Put one drop of the epididymis homogenate solution on clean slide to calculate the percentage of moving sperm in 10 random fields, according to the equation (Hinting, 1989).

$$\text{Sperms Motility Percentage} = \frac{\text{Number of sperm motile}}{\text{Number of total sperm}} \times 100$$

Sperm Viability and sperm abnormality percent

Put one drop of the epididymis homogenate solution on clean slide and the spermatozoa suspension was allowed to dry in air, then it was stained with (1% Eosin and 5% Nigrosin), then calculate the percentage of live sperm (which did not take the dye) and the morphological abnormalities.Normal sperm has an oval head, a piece of medium and long tail, there are form of abnormal of sperm includecircular head ,pin head, the head is too big or the presence of sperm with double head or tail lost (Wyrobek and Bruce, 1975; Linder *et al*, 1995; Perreault and Cancel, 2001).

Statistical analysis

Results were analyzed statistically using ANOVA tests, also used the method of Multiple comparisons, adoption of the method of Least significant difference (LSD) to find a moral difference younger and results proven form the arithmetic average and standard error.

RESULTS

The results showed significant decreased in the percentage of sperm motility in the infected animals groups compared to control group. Also infection in the experimental group caused a significant decreased in sperm concentration, total sperm number, percentage of normal and abnormal sperm compared with the control group. But the observed significantincrease in the pus cells or white blood cells s in the bloodrats infected with *Toxoplasmosis* compared with the control group (Table 1).

The results also show a significant decrease in the levels of testosterone hormone between infected animal groups compared with the control group and also between the groups themselves. The levels of LH and FSH hormones showed a significant decrease between the first and second groups compared with the control group, while the differences did not reach for significantly when comparing the first group with the second group. No statistically significant differences were found in the testes weight in the infected animals (2nd group) compared with the control group, but significant differences presence between the groups themselves (Table 2).

Effect of *Toxoplasmo gondii* on the Testes and Epididymis tissues

Histological sections of the testes infected with toxoplasmosis after the6 weeks was observed Vascular Congestion, Degeneration in Seminiferous tubules cells in addition to necrosis in spermatogonia and spermatocyte, absence of sperm in the some tubules cavity with the occurrence of necrosis in Leydig cells and the occurrence oedema in the tissue between seminiferous tubule compared with the control group. But the pathological

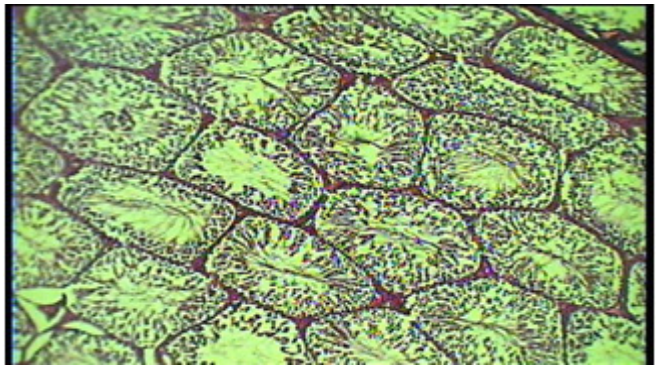

Fig. 1 : Transverse -section of the testicular tissue for Male Rats (control groups) showed the natural shape of the Seminiferous tubules and successive stages of the formation of sperm (Spermatogenesis) with a high level of sperm concentration Spermatozoa in the tubules cavity H.& E. (10 X).

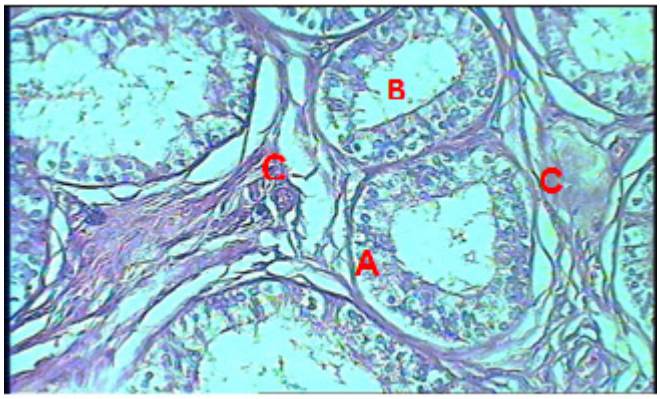

Fig. 2 : Transverse - section of the testicular tissue for Male Rats infected with Toxoplasmosis (after 6 weeks) showing vascular congestion (B) and sperm lost in the lumen of tubules (A) H & E. (4 X).

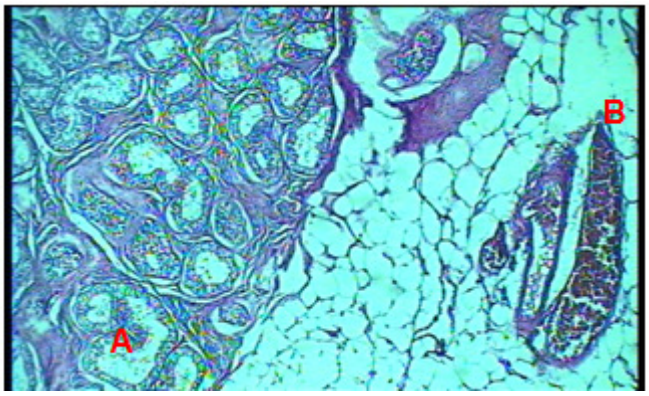

Fig. 3 : Transverse - section of the testicular tissue for Male Rats infected with Toxoplasmosis (after 6 weeks) observed Degeneration in the Seminiferous tubule cells and necrosis in spermatogonia and spermatocyte (A), absence of sperm in the some tubules cavity (B) with the occurrence of necrosis in Leydig cells and the occurrence oedema in the tissue between ýseminiferous tubules (C), H & E. (40 X).

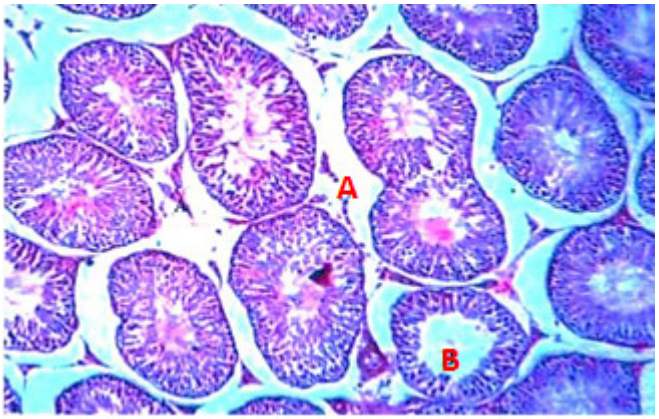

Fig. 4 : Transverse - section of the testicular tissue for Male Rats infected with Toxoplasmosis (after 12 week) showing the reduction in the size of Seminiferous tubule with the widening of distances between them (A), also notes the increased necrosis and damage in Leydig cells, with degeneration in the sperm formation and absence of sperm ýin the tubule lumen (B) H. & E. (10 X).

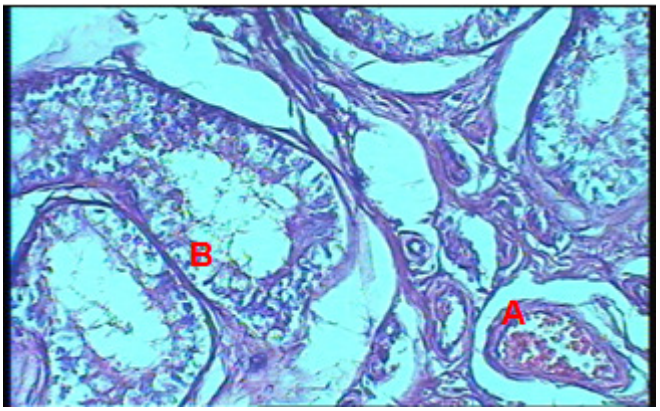

Fig. 5 : Transverse - section of the testicular tissue for Male Rats infected with Toxoplasmosis (after 12 week) showing Vascular congestion between tubules (A) and a decrease in their size with an increase in necrosis of Leydig cells and spermatogonia (B) H & E. (40 X).

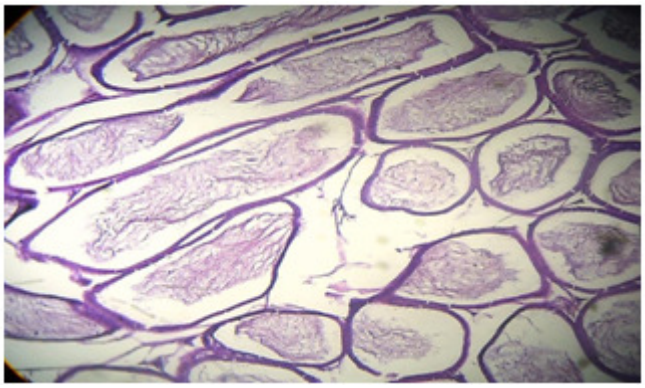

Fig. 6 : Transverse - section of the Epididymis tissue from the Rat control group showing epididymis cavities are full with sperm H & E, (10 X).

changes after 12 weeks were the more effect such as Congestion, severe Hemorrhage and Bleeding in blood vessels between the tubules with a reduction in the size of these tubules. Furthermore, cavity of the epididymis was empty from sperm as shown the reduction in the size of epididymis tubules in addition to necrosis and disappearance of most epithelial lining of epididymis after 6weeks from infection.

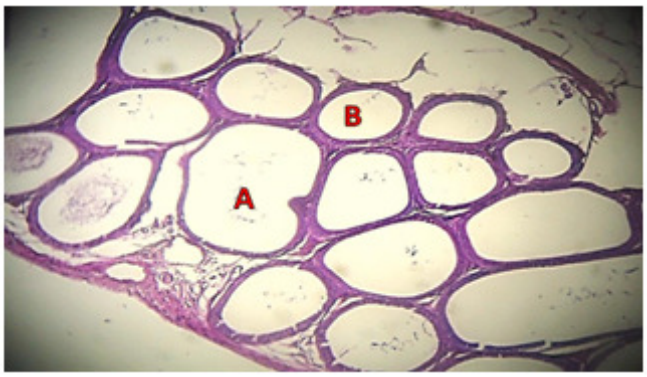

Fig. 7 : Transverse - section of the epididymis tissue (after 6 weeks) showing reduction in the size of the some epididymis tubules (B), in addition to cavity of the Epididymis was empty from sperm (A) H & E, (10 X).

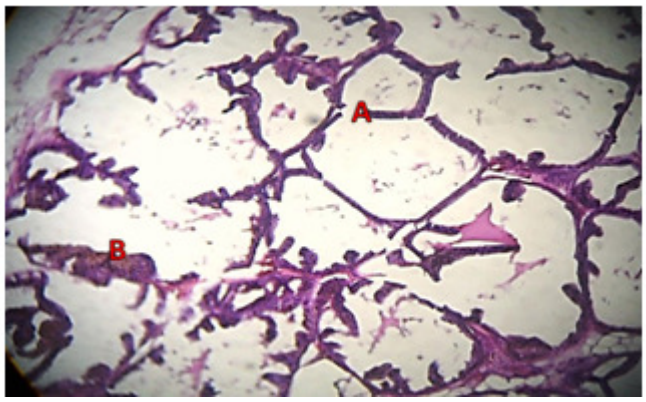

Fig. 8 : Transverse - section of the Epididymis tissue (after 12 week) showing thickening of the basement membrane because of the tissue cysts of parasite (B) in addition to necrosis and disappearance of most epithelial lining (A) H & E, (10 X).

Table 1 : Some physiological parameters of male's rat testes infected with *Toxoplasmosis* after 6 weeks and 12 week, respectively.

Parameters / Groups	Group 1 (control) n= 6	Group 2 (Animals infected after 6 weeks) n= 6	Group 3 (Animals infected after 12 week) n= 6	L.S.D.
Total sperm number x 10^6 M ± SE	93.57± 4.35	77.09 ± 2.92	57.95 ± 2.79	10.33
Sperm concentration x10^6/ml M ± SE	53. 61± 2.67	33.51± 1.87	15.66± 1.62	6.34
Spermatozoa motility(%) M ± SE	76.85 ± 3.73	65.41 ± 3.02	48.65 ± 2.76	9.63
Spermatozoa abnormalities (%) M ± SE	20.56 ± 1.56	31.25 ± 2.54	50.77 ± 2.9	7.21
Spermatozoa normality(%) M ± SE	80.56 ± 1.34	62.84 ± 2.12	48.51 ± 2.31	5.94
Testes weights (gm) M ± SE	1.26 ± 0.3	0.95 ±0.28	0.42 ± 0.09	0.76
Pus cells or white blood cells X 10^6/ml M ± SE	1.35 ± 0,54	5.40 ± 0.79	8.89 ± 0.93	2.32

Table 2 : The concentration of hormones (Testosterone, FSH and LH) in the serum of blood rats infected with *Toxoplasmosis* after 6 weeks and 12 week, respectively.

Parameters / Groups	Group 1 (control) n= 6	Group 2 (Animals infected after 6 weeks) n= 6	Group 3 (Animals infected after 12 week) n= 6	L.S.D.
Levels of Testosterone hormone (ng/ml) M ± SE	6.16 ± 0.42	3.76 ± 0,40	1.34 ±0.37	1.31
Levels ofLH hormone(ng/ml) M ± SE	7.20 ± 0.29	5.36 ± 0.32	4.89± 0.5	1.13
Levels ofFSH hormone(ng/ml) M ± SE	8.65 ± 0.61	3.89 ± 0.78	2.74 ± 0.81	2.23

- Represents values average ± standard error.
- Representsn = 6 the number of animals treated.
- LSD is least significant difference at the level of significance ($P \leq 0.05$).
- FSH: follicle stimulating hormone.
- LH:Luteinizing hormone.

DISCUSSION

Recent studies have shown that the infected with *Toxoplasma gondii* affects not only the female reproductive system, but also cause weakness in the male reproductive system, which will probably cause to infertility. The clinical studies showing the high prevalence of toxoplasmosis in men, who suffer from infertility (Dalimi and Abdoli, 2013). In another study in China showed 100 cases infertility in men, 39% of them were serologically *Toxoplasma* positive (Gao *et al*, 2005).

Rats considered as the best model for human Toxoplasmosis infection, whereas the chronic case of Toxoplasmosis in human is similar to rat (Dubey and Frenkel, 1998).

The results showed a significant decrease in the sperm concentration, sperm morphology,motility and the testosterone, LH and FSH levels hormones compared to the control group. This result was a identical to Sun *et al* (2008) and Khaki *et al* (2011), Terpsidis *et al* (2009), they found that the male albino rats infected with toxoplasmosis cause a significant decrease in the Sperm concentration, motility and morphology parameters, they showed also increase in abnormal sperm with occurrence of a significant decrease in testicular weight. May be reason to presence tissue cysts of bradyzoites, which

itcause necrosis, degeneration and disappearance in most of epithelium germinal cells (spermatogonia and spermatocyte) that lining the seminiferous tubules responsible for the synthesis of spermatogenesis leading to stop this process. Furthermore, the parasite cause damage and necrosis in the Leydig cells between the seminiferous tubule, it responsible for the secretion of the testosterone level, so it decreased level in the serum (Garedaghi and Bahavarnia, 2014).

Moreover, Toxoplasmosis is one of the most causes of focal lesions in brain, so it may cause disorder in hypothalamic dysfunction (Antonios *et al*, 2000). They concluded that changes in thalamus and hypothalamus may cause malfunctioning of release of GnRH. Two case reports of pituitary adenoma associated with *T. gondii* infection have been published by Zhang *et el* (2002) that effect on activity of the hypothalamus gland due to reduce the secretion of gonads resulting hormones (GnRH), which affects the pituitary gland activity that secrete the FSH and LH resulting hormones it lower levels in the serum, which in turn effects on secretion of testosterone hormone from Leydig cells so led to a decline in serum levels and stop the synthesis of sperm and reduce the total number of the sperm in the testes and epididymis (Dvorakova-Hortova *et al*, 2014) because the brain is one of the most important members of which contain on tissue cysts of parasite (Barakat, 2007). Minárovits (2009) he said that bacterial, viral and parasitic infection working on programming change of germ cells male genetic. Dvorakova-Hortova *et al* (2014) he found that toxoplasmosis can effect on epigenome of testes therefore, it result abnormal DNA, which leads to abnormalities in sperm. On the other hand, hypothalamic and pituitary gland disorder as a result to present of parasite effect on thyroid and sex steroid hormones which impact on male reproduction system (Choksi *et al*, 2003). Thyroid disorder (hypothyroxinaemia) cause decrease levels in both thyrotropin-releasing hormones (TRH), thyroid-stimulating hormone (TSH) in blood (Wagner *et al*, 2008). Stahl and Kaneda (1998), they said that decrease levels of thyroxine (T_4) in miceblood infected with *T. gondii.*

Choksi *et al* (2003) mention to natural thyroid hormone levels play very an important role in sperm development and its morphology, therefore any change in thyroid hormones (hypothyroidism) affects negative on gonadotropin secretion (such as testosterone) and semen quality.

The current study showed changes in testicular tissue and epididymis tissue in animals infected with toxoplasmosis, may be reason to contact Tachyzoit with host cells and it penetrate these cells, leading to formation cysts, as a result, it divided and multiply within cysts and it continue in multiplication until the arrival of this number to a critical mass, Exploding the cell, which leads to apoptosis, necrosis this cells and severe inflammation in tissue, as well as the parasite secreted proteolytic enzyme, which analyzes cells and the presence of tissue cyst for years in various tissues of the body cause inflammation in the infected position (Topy *et al*, 1996). Also, we cannot seen Tachyzoiteby dyes routine tissue, but immune glow dye could show the parasite (Aghwan *et al*, 2010).

CONCLUSION

Toxoplasmosis can cause damage on the reproductive parameters of human or animal male as well as decline of different hormones *T. gondii* infection not only effect on female reproduction, also cause male reproductive.

REFERENCES

Aghwan S S, Al-Taee A F and Suliman E G (2010) Detection of *Toxoplasma gondii* infection in domestic rabbits by using multiple techniques. *Iraqi Journal of Veterinary Sciences* **24**(2), 65-69.

Antonios S N, Ismail H I and Essa T (2000) Hypothalamic origin of reproductive failure in chronic experimental toxoplasmosis. *J. Egypt Soc. Parasitol.* **30**(2), 593-599.

Barakat A M A (2007) Some Diagnostic Studies on Male New Zealand Rabbit Experimentally Infected with *Toxoplasma gondii* strain. *Global Veterinaria* **1**(1), 17-23.

Barakat A M A (2007) Some Diagnostic Studies on Male New Zealand Rabbit Experimentally Infected with *Toxoplasma gondii* Strain. *Global Veterimria* **1**(1), 17-23.

Choksi N Y, Jahnke G D, Hilaire C and Shelby M (2003) Role of thyroid hormones in human and laboratory animal reproductive health. *Birth Defects Res. B. Dev. Reprod Toxicol.* **68**(6), 479-491.

Dalimi A and Abdoli A (2013) *Toxoplasma gondii* and Male Reproduction Impairment: A new Aspect of Toxoplasmosis Research. *Jundishapur J. Microbiol.* **6**(8), e7184.

Dalimi A and Abdoli A (2013) *Toxoplasma gondii* and Male Reproduction Impairment: A new Aspect of Toxoplasmosis Research. *Jundishapur J. Microbiol.* **6**(8), e7184.

Dubey J P and Thulliez M D (1993) Persistance of tissue cysts in edible tissue of cattle fed *Toxoplasma gondii* oocysts. *Am J Vet Res*. **54**(2), 270-273.

Dubey J P and Frenkel J K (1998) Toxoplasmosis of rats: a review, with considerations of their value as an animal model and their possible role in epidemiology. *Vet Parasitol.* **77**(1), 1-32.

Dvorakova-Hortova K, Sidlova A, Ded L, Hladovcova D, Vieweg M, Weidner W, Steger K, Stopka P and Paradowska-Dogan A (2014) *Toxoplasma gondii* Decreases the Reproductive Fitness in Mice. *Journal.pone* 0096770.s002.

Gao X L, Qi R, Su X P and Liang X L (2005) Toxoplasma infection in males with sterility in Shenyang, China. *Zhonghua Nan KeXue* **11**(7), 503–514.

Garedaghi Y and Bahavarnia S R (2014) Repairing Effect of *Allium cepa* on Testis Degeneration Caused by *Toxoplasma gondii* in

The Rat. *Int. J. Women's Health Reproduction Sci*. **2**(2), 2330 – 4456.

Haziroglu R, Altintas K, Atasever A, Gulbahar M Y and Tunca O R (2003) Pathological and immunohistochemical studies in rabbits experimentally infected with *Toxoplasma gondii. Turk J Vet Anim Sci*. **27**, 285-293.

Hinting A (1989) Methods of semen analysis. In: *Assessment of human sperm fertilizing ability*. *Ph.D. thesis* by Hinting A. University of Michigan state.

Humason G L (1972) *Animal tissue techniques.* 3rd Ed. Hiffreeman W and H. company, SanFrancisco, USA. P.641.

Khaki A, Farzadi L, Ahmadi S, Ghadamkheir E, Khaki A A, Shojaee S and Sahizadeh R (2011) Recovery of spermatogenesis by *Allium cepain Toxoplasma gondii* infected rats. *Africa Journal of Pharmacy and Pharmacology* **5**(7), 903-907.

Khaki A, Farzadi L, Ahmadi S, Ghadamkheir E, Ashin Khaki A, Shojaee S and Sahizadeh R (2011) Recvery of spermatogenesis by Allium Cepa in *Toxoplasma gondii* infected rats. *African Journal of Pharmacy and Pharmacology* **5**(7), 903-907.

Linder R E, Klinefetter G R and Strader L F (1995) Dibromoacetic acid affects reproductive competence and sperm quality in the male rats. *Fundamental and Applied Toxicology* **28**, 9–17.

Minárovits J (2009) Microbe-induced epigenetic alterations in host cells: the coming era of patho - epigenetics of microbial infections. A review. *Acta Microbiol Immunol Hung* **56**(1), 1–19.

Montoya J G and Liesenfeld O (2004) Toxoplasmosis. *Lancet J.* **363**(9425), 1965–1976.

Perreault S D and Cancel A M (2001) Significance of incorporating measures of sperm production and function into rat toxicology studies. *Reproduction* **121**, 207– 216.

Sakmoto J and Hashimoto K (1986) Reproductive toxicity of acrylaminde and related compounds in mice effects on fertility and sperm morphology. *Arch. Toxical.* **59**, 201- 205.

Shen L (2001) Pathology and pathogenetic study of the Toxoplasma gondii acute infection mice testis. *Chin J. Zoonos.* **17**, 75–77.

Stahl W and Kaneda Y (1998) Aetiology of thyroidal dysfunction in murine toxoplasmosis. *Parasitology* **117** (3), 223 -237.

Sun L H, Fan F, Wang J J and Gong J (2008) Acute *Toxoplasma gondii* infection affects the reproductive function of male mice. *Zhonghua Nan KeXue*. **14**(1), 55-57.

Terpsidis K I, Papazahariadou M G, Taitzoglou I A, Papaioannou N G, Georgiadis M P and Theodoridis I T (2009) *Toxoplasma gondii* : reproductive parameters in experimentally infected male rats. *Experimental Parasitology* **121**(3), 238-241.

Topy S E, Zimmeberg G E and Ward J (1996) *Toxoplasma* invasion. The parassitophorous vacuole is formed from host cell plasma membrane and pinches off via fusion pore. *Proc. Natl. Acad. Sci. USA* **93**, 8413-8418.

Torda A (2001) Toxoplasmosis : Are cats really the source?. *Aust. Fam. Physician* **30**(8), 743-750.

Wagner M S, Wajner S M and Maia A L (2008) The role of thyroid hormone in testicular development and function. *J Endocrinol.* **199** (3), 351-365.

Weiss L M and Dubey J P (2009) *Toxoplasmosis*: A history of clinical observations. *Int J. Parasitology* **39**(8), 895–901.

Wistom G B (1976) Enzyme- Immunoassy. *Clin. Chem*. **22**, 30 .

Wyrobek A and Bruce W (1975) Chemical induction of sperm abnormalities in mice, proc. *Nat. Acad. Sci*. **72**, 4425 – 4429.

Zhang X, Li Q H P, Cheng H and Huang G (2002) Two case reports of pituitary adenoma associated with *Toxoplasma gondii* infection. *J Clin Pathol.* **55**(12), 965-966.