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EXPERIMENTAL TOXOPLASMOSIS IMPACT ON MALE GENERATIVE SYSTEM: HISTOPATHOLOGY AND HORMONAL ASSAYS

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Abstract

The protozoan *T. gondii* infects human and animals. The parasite can alter the generative system affecting fertility. This work studied the impact of toxoplasmosis on male generative system in rats. 49 laboratory breed Wistar rats were divided into group I containing 35 rats and group II 14 control. About 0.5ml of the withdrawn peritoneal exudates contained 2 x 10^6 tachyzoites RH strain type I was inoculated intraperitoneally into each rat of GI. On days 10, 20, 30, 40, 50, 60 & 70 post-inoculation (PI). Seven rats were anesthetized 5 from GI and 2 from GII each time. Both testes were removed, weighed, body and testis weight ratio (BTR) was calculated. Left testis was kept for histopathology and right testis was processed for tissue homogenate preparation and estimation of intratesticular testosterone (ITT) and lactate dehydrogenase (ITLDH). For BTR, slightly or no difference was detected. Mean level of ITT was significantly decreased in GI throughout the 70 days, ITLDH was higher in GI especially on days 40, 50, 60 & 70 ($P < 0.05$). Detection of tachyzoites within seminiferous tubules on day 10 PI, with aggregation on day 30. Engorged blood vessels, degenerated, oedematous vessels, empty seminal vesicle with increasing number of tachyzoite and bradyzoite in cyst were detected. In GII, germinal cells were available with non detectable pathology. Toxoplasmosis altered hormonal ITT and ITLDH which was linked to testicular damage in male rats.

Key words: Toxoplasmosis, Rats, Experimental, Hormonal, Histopathology

Introduction

Toxoplasma gondii infects human and all animals as well as birds (Długońska, 2008). The sequels caused by toxoplasmosis varied from asymptomatic to congenital affection passing with abortion, still birth, neonatal death and fertility alteration (Dubey, 2010).

Besides, *Toxoplasma* adverse outcomes on female genital tract (Jones *et al*, 2003), the parasite has the ability to affect male generative system (Garcia *et al*, 1996).

Chronic infection of *T. gondii* in animals alter the reproductive parameters in males (Arantes *et al*, 2009; Kaňková *et al*, 2011). Terpsidis *et al*. (2009) reported *Toxoplasma* isolation from semen in male rats and infection was a fertility predictive factor.

Experimentaly, secondary hypogonadism was reported among the mice infected with toxoplasmosis, by virtue of the hypothalamic dysfunction (Antonios *et al*, 2000). Hypogonads in man in behalf of toxoplasmosis was mentioned, but without satisfactory determinations in the human beings (Oktenli *et al*, 2004).

Sufficient data were needed to clarify the effect of *T. gondii* on male reproductive system.

This study aimed to analyse the impact of toxoplasmosis on male reproductive system in clean laboratory bred Wistar rats by using the histopathology and hormonal assay.

Material and Methods

Ethical considerations: All experiment procedures were carried out in accordance to the guiding principles of the European Council Directive (86/609/EU) in what concern utilizing and breeding of laboratory animals. The experiment protocol was approved by The National Research Center, Egypt.

Animals and infection procedures: A total number of 49 laboratory breeding Wistar rats were obtained and breed at the animal house laboratory in The National Research Center, Egypt. Rats were housed in optimal

conditions of temperature and humidity, kept in clean cages and fed on pelleted food and water. They were about 30days old, with body weight varies from 200 to 250gm. The experiment was started at day 40 of age of rat, 10 days was taken for adjustment of experiment conditions. All rats were confirmed to be toxoplasmosis-free using modifyed agglutinatin Test (MAT) after Dubey and Desmonts (1987) at a dilution of 1:25.

Male rats were divided into GI included 35 rats (cases) that later were infected by *T. gondii* and GII included 14 non infected rats (control).

T. gondii strain: RH-Virulent type I *T. gondii* local strain was used for experimental infection obtained by Shaapan *et al.* (2008), using bioassay trails on the suspected infected sheep tissues and in order to isolate the *T. gondii* infective stages, cats and mice were used. The recovered tachyzoites of *T. gondii* local strain was maintained in the Laboratory of Zoonotic Diseases Department, National Research Center, Egypt.

Infection procedures: At the start of the procedure the rat's age was 40 days. GI were infected (Dube, 2010) by peritoneal inoculation of about 0.5ml of taspirated peritoneal exudates containing ($2x10^6$) tachyzoites (RH strain type 1). Then, GI rats were confirmed to be infected with *T. gondii* on 6^{th} day post infection according to Baumgarth *et al.* (2000) by serological examination using Latex Agglutination Test (LAT), detected IgM antibodies. Rats were left for 4 days and then on days 10^{th}, 20^{th}, 30^{th}, 40^{th}, 50^{th}, 60^{th} & 70^{th} days post-ino-culation (PI) 5 rats from GI & 2 rats from GII were anesthetized.

Calculation of body and testis weight ratio (BTR): The body weight of each anesthetized rat was measured then both testes were immediately removed and weighed. The mean weight of both testes were recorded for each animal. Then ratio of body weight to testes weight (BTR) was estimated by measuring the mean weight of both testes in relation to the terminal body weight just before necrosis. The left testis was kept in saline at room temperature for testicular histopathological examination while the right testis was stored at -20^oC in saline solution for later preparation of tissue homogenate and hormonal assay.

Hormonal assays: The tissue homogenate from right testis was processed for measurement of ITT using ELISA assay (ElA Testosterone; Dia plus Inc., San Francisco, CA, USA) according the manufacturer's instructions. In addition to measuring ITLDH using spectrophotometer using commercal kits (EIA LDH Dia plus. Inc. CA, USA)

Histopathological examination: The left testis was taken to make 5-µm paraffin sections after being suspended in the Bouin's fluid for two days. The sections were stained with hematoxylin and eosin (H & E). The stained tissue sections were examined under the microscope at power magnification of X100 for identification of toxoplasma (Culling, 1974).

Statistical analysis: Data was analyzed by SPSS software version 16. The quantitative data were conferred as mean ± standard deviation. Comparison of quantitative variants between two groups was assessed by student t-test. Differences with P values less than 0.05 were considered significant.

Results

All the rats in GI inoculated intraperitoneal proved to be infected as shown by the LAT. But, GII rats showed negative reaction.

Impact of toxoplasmosis on BTR: There was slightly or no differences between GI and GII on the BTR ($P > 0.05$).

Impact of toxoplasmosis on ITT: The mean level of tissue testosterone was lower in GI throughout the seventy days. Diminished ITT mean level in GI was significantly during 10^{th}, 60^{th}, 70^{th} days PI ($P < 0.05$). The lowest mean level was on 70^{th} day PI.

Impact of toxoplasmosis on ITLDH: The mean level of tissue ITLDH was higher in GI compared to GII throughout the 70 days especially on days 40^{th}, 50^{th}, 60^{th} and 70^{th} ($P < 0.05$).

Histopathology results: Among GI, abnor-

malities were recorded by tachyzoites within the seminiferous tubules on the10th day PI, that increased from the 30th till 60th day PI with aggregation (Fig. 1). The engorged blood vessels appeared on the 20th day PI reached a peak on the 40th day PI, degenerated and oedematous vessels within the seminiferous tubules appeared on 40th day PI (Fig. 2). On the 50th day, some of the seminal vesicle appeared empty with increasing number of tachyzoite and bradyzoite in cyst on the 70th day PI (Fig. 3). Aggregated tachyzoites was detec- ted within the blood vessel wall on the 70th day PI (Fig. 4).

Regaring GII, the germinal cells within the seminiferous tubules were available on the 10th day and the 30th day with normal sperm and seminiferous tubules filled with seminal fluid.

Details were given in tables (1, 2 & 3) and figures (1, 2, 3 & 4).

Table 1: Mean of body weight in GI and GII

Test Day	GI (n=35)		GII (n=14)		P value
	Mean	Std. Deviation	Mean	Std. Deviation	
10 days	0.245	0.44	0.248	---	0.137
20 days	0.243	0.13	0.245	---	0.098
30 days	0.214	0.14	0.215	---	0.147
40 days	0.160	0.40	0.164	---	0.110
50 days	0.196	0.16	0.148	---	0.089
60 days	0.135	0.20	0.137	---	0.182
70 days	0.133	0.35	0.134	---	0.073

Table 2: Mean of ITT in GI and GII

Testosterone Test Day	GI		GII		P value
	Mean	Std. Deviation	Mean	Std. Deviation	
10 days	236.3	4.8	250	---	0.019*
20 days	241.3	10.3	250	---	0.321
30 days	240	14.1	260	---	0.132
40 days	238.8	11.8	250	---	0.273
50 days	238.8	8.5	255	---	0.064
60 days	233.8	4.8	260	---	0.002*
70 days	210	8.2	270	---	0.001*

**P* value significant

Table 3: Mean of ITLDH in GI and GII

LDH Test Day	GI		GII		P value
	Mean	Std. Deviation	Mean	Std. Deviation	
10 days	101	31.2	40	---	0.079
20 days	108.8	8.5	85	---	0.055
30 days	176.3	46.4	90	---	0.068
40 days	160	45.5	40	---	0.024*
50 days	157.5	42.7	30	---	0.016*
60 days	240	45.5	50	---	0.005*
70 days	325	117.3	30	---	0.029*

**P* value significant

Discussion

For years rats were used as an animal model for *Toxoplasma* infection (Dubey and Frenkel, 1998). In this experimental study, rats were used to encounter the impact of *T. gondii* on the male rat's reproductive system

Acute toxoplasmosis infection proved to be an agent for unproductiveness among experimentally tested male rats (Lu *et al,* 2005; Sun *et al* 2008; Terpsidis *et al* 2009; Abdoli *et al,* 2012). It induced harm to testicular tissue (Shen, 2001). Spermatogenesis was the crucial process occurred in testis that acquired healthy germinal cells (Patton and Battaglia, 2005).

Zhou *et al*. (2002) reported that the sterile men showed the high level of toxoplasmosis antibodies than fertile ones. Toxoplasmosis affection of human fertility was reported (Qi *et al*, 2005). Congenital *T. gondii* hypogonadotropic hypogonadism (Suresh Babu *et al,* 2007) and testicular toxoplasmosis

(Barreto *et al*, 2008) were reported. Besides, Eslamirad *et al*. (2013) reported that chronic toxoplasmosis influenced human reproductive parameters. Santana *et al*. (2010) suggested possibility of venereal transmission of *T. gondii* among goats which should be further assessed.

In the present study, there was limited or no differences between GI and GII on the BTR ($P > 0.05$). In the same way, Abdoli *et al*. (2012) reported no significant difference on BTR between infected and control. Also, Dvorakova-Hortova *et al*. (2014) did not find significant differences between infected and control mice.

Testosterone hormone is chiefly created in testis, the present results revealed that, the mean level of ITT was lower among group I throughout the 70 days, with significant difference during 10, 60 & 70 days PI ($P < 0.05$), reaching minimum level on 70^{th} day PI. This result agreed with Rui *et al*. (2009) attributing to induction of spermatogenic apoptosis by diminished local testosterone range and subsequent testicular damage (Zhou *et al*, 2004). Also, Abdoli *et al*. (2012) reported a decrease of ITT. Flegr *et al*. (2005) and Hodková *et al*. (2007) could n't drive either the diminished in testosterone levels or no changes occur in toxopla-smosis and they attributed hormonal changes to be subjectively.

LDH is an indicator of cell stress during tissue damage, it was released during anaerobic glycolysis (Adiga and Jagetia, 1999). The present results showed that, the mean level of tissue LDH was higher in GI compared to GII throughout the 70 days especially on days 40, 50, 60 & 70 ($P < 0.05$). Suzuki *et al*. (1971) reported LDH declined during acute toxoplasmosis There is a link between levels of ITLDH and degree of tissue damage, some authors showed minimal changes in tissue LDH due to minimal or non-detected pathology in testes by toxoplasmosis (Abdoli *et al*, 2012).

The present histopathologic results detected tachyzoites within the seminiferous tubules on 10^{th} day PI and increased throughout the experiment to reach parasitic aggregation on 60^{th} day PI. The testicular architecture showed abnormalities in form of engorged blood vessels, degenerated and oedematous vessels within the seminiferous tubules, empty seminal vesicle and increased the number of tachyzoite and bradyzoite in cyst.
This result agreed with Arantes *et al*. (2009) who isolated *T. gondii* in seminal fluid, testicular and epididymal tissues from dogs Also, Koch *et al*. (2016) mentioned isolation of the parasite from dogs seminal fluid.In contrast, Terpsidis *et al*. (2009) did not detect abnormalities in histopathology of the testes of infected rates. Damage caused by toxoplasmosis pointed to Th1 dominated effect and the released cytokines especially nitric oxide, reactive oxygen species (Miller *et al*, 2009). However, they countered protective effect that caused cell damage. Oktenli *et al*. (2004) reported that acute *T, gondii* infection might cause temporary hypo gonadotrophic gonadal insufficiency regardless the course of toxoplasmsis. Another explanation was added by Dvorakova-Hortova *et al*. (2014) that toxoplasmisi has the ability to adjust epi genome of host testes. Through linking between unusual DNA methylation & comprom ised spermatogenesis. But, mechanism was quite obscure. It was vital to determine whether toxoplasmosis affect male reproductive system that would reflect on understanding its pathogensis and infection outcome.

Conclusion

Undoubtedly, since the discover of *T. gondii* over two hundred years ago was immediately recognized as a pathogen disease responsible for congenital complications.

The outcome data proved that *Toxoplasma* could affect male genital tract in rats, it can significantly alter the tissue hormones ITT & ITLDH which are allied with testicular damage in male rats. More studies are ongoing and will be published in due time elsewhere.

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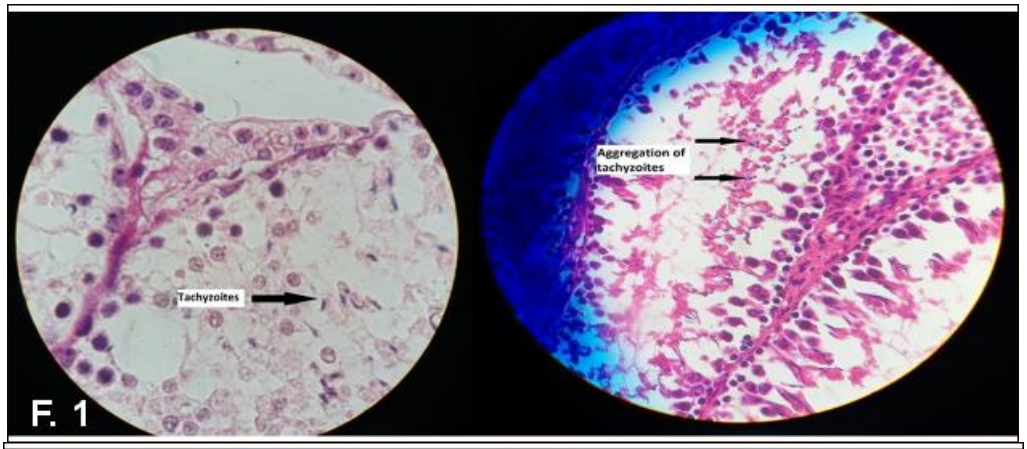
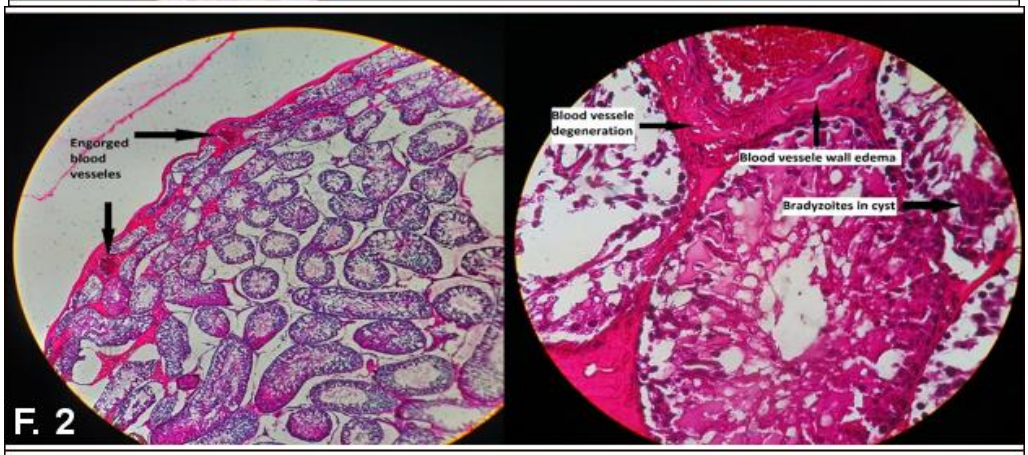
Explanation of figures

Fig. 1: Tachyzoites within seminiferous tubules with aggregations (x100, H&E stain).
Fig. 2: Engorged blood vessels, degenerated and oedematous vessels within seminiferous tubules (x40, H&E stain)
Fig. 3: Empty seminal vesicle appeared empty with Increasing number of tachyzoite and bradyzoite in cyst (x100, H&E stain)..
Fig. 4: Aggregated tachyzoites detection within the blood vessel wall (x100, H&E stain)


Tachyzoites
Aggregation of tachyzoites
F. 1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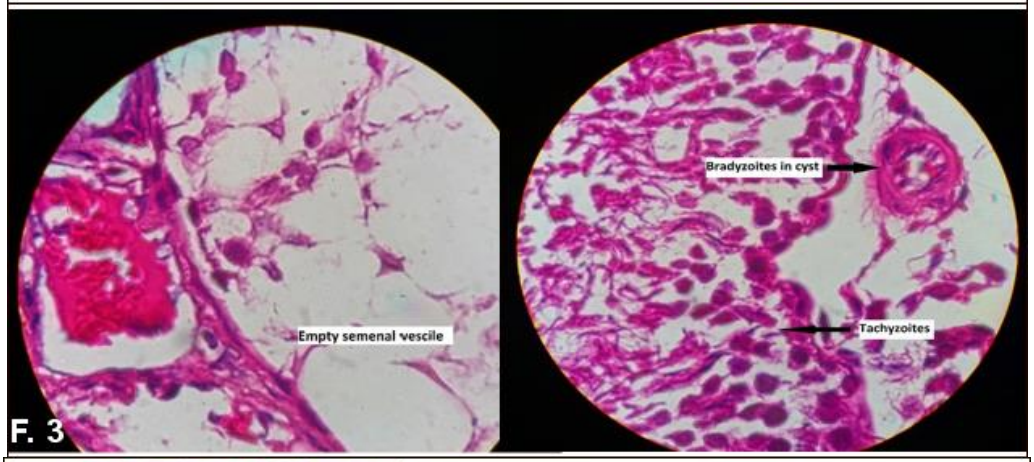
Blood vessele degeneration
Blood vessele wall edema
Bradyzoites in cyst
F. 2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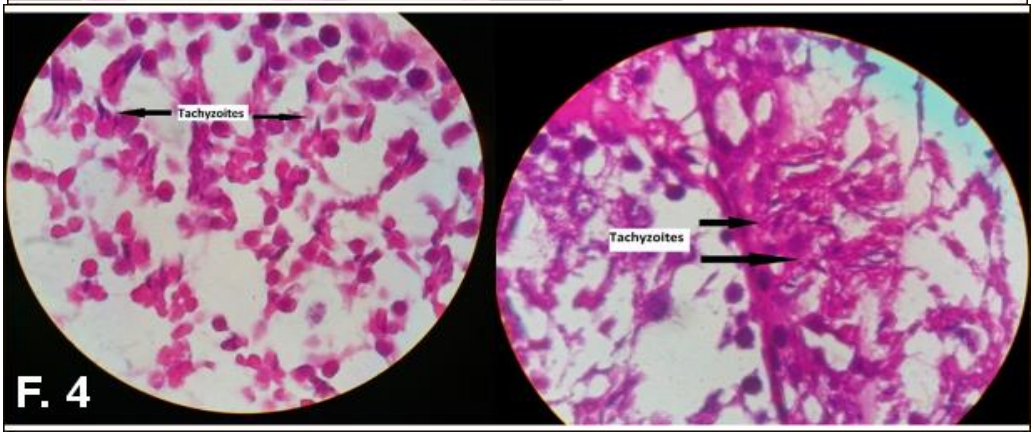
Bradyzoites in cyst
Tachyzoites
F. 3


Tachyzoites
Tachyzoites
F. 4