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# EFFECT OF TOXOPLASMOSIS AND/ OR ITS TREATMENTS (SULPHADIAZINE AND PYRIMETHAMINE) ON FEMALE RAT REPRODUCTIVE PERFORMANCE

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**ABSTRACT**

*Toxoplasma gondii* infection significantly decreased serum FSH, LH, estrogen and progesterone level in female rats. Infection also decreased ovary and uterus weight, decrease pregnancy rate, litter size, fetal weight, placental weight and increased fetal resorption and early fetal death in comparison with non infected females. Sulphadiazine and pyrimethamine didn't induced further changes in the serum level of serum FSH, LH, estrogen and progesterone, either used in non infected or infected females. Both drugs decreased pregnancy rate, litter size, fetal weight, placental weight and increased fetal resorption ratio and early fetal death in the non infected females. However, in the infected group, sulphadiazine decreased pregnancy rate, resorption rate and early fetal death and didn't changed litter size, fetal and placental weights. Pyrimethamine was also decreased pregnancy rate, fetal weight, placental weight and increased fetal resorption ratio and early fetal death in infected females. Both sulphadiazine and pyrimethamine improved the histopathological changes in the infected group.

**Keywords:** Females, Rats, Fertility, Toxoplasmosis, Sulphadiazine, Pyrimethamine.

## INTRODUCTION

Toxoplasmosis, probably the most widespread human parasitic infectious disease in developed countries, is caused by a coccidian protist, *Toxoplasma gondii.* It is generally initiated by ingestion either the tissue cyst stage, found in the meat of infected animals, or the oocyst stage, released in the feces of infected cats [1]. Several studies have investigated the association between infection with *Toxoplasma gondii* and fertility in females. Experimentally, it was found that toxoplasmosis induced hypogonadotropic hypogonadism which occurred secondary to hypothalamic dysfunction in mice, and associated with histopathological changes with estrus cycling impairment, impaired folliculogenesis and few corpora lutea [2-4]. *Toxoplasma gondii* altered ovarian follicular growth in mice and different phases of follicles and corpus luteum in the ovaries [5]. Histological examination of infected female mice showed accentuated hypertrophy of the endometrium and myometriumand a reduction in folliculogenesis and formation of corpora lutea in the ovaries on the infected mice [6]. Tachyzoite of Toxoplasma and DNA of this parasite were observed in sub mucosa and muscles of the uterus and in the villi of placenta [7].

*Toxoplasma gondii*-infected women reported to take a longer time to conceive than *Toxoplasma gondii*-free women (P=0.015). They also claimed to have more fertility problems than *Toxoplasma gondii*-free women (P<0.0001) [8]. However, *Toxoplasma gondii* infection in pregnant women may cause poor obstetric outcomes such as spontaneous abortion, still-born and sterility. A survey of *Toxoplasma gondii* infection in 68 cases of oviducal sterility revealed a prevalence of 44.1%, which was significantly different from that in normal pregnant women, indicating that *Toxoplasma* infection could result in oviducal sterility [9].The levels of follicles stimulating hormone and luteinizing hormone were measured in

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pregnant women suffering from toxoplasmosis. The concentration of LH hormone in infected pregnant were lower than normal. These result suggested that infection with *Toxoplasma gondii* interfered with hypothalamic-pituitary level [10].This study was designed to investigate the effect of toxoplasmosis on female reproductive performance.

## MATERIAL AND METHODS

The study was carried out on 84 female rats (*Rattus_norvegicus*) ranging in weight from 250 to 300g, all females were housed in an air-conditioned animal room at an ambient temperature of 23 ±2 °C and in a 12h light 12h dark cycle. Half of the females were infected with 100 tissue cysts of *T. gondii* intraperitoneally [11].

Infected females were examined for documentation of the infection with the using of real-time PCR. Each, infected group (42 females) and non infected group (42 females) were divided into 3 subgroups (14 females each) and treated with DMSO, sulphadiazine 200 mg/kg or pyrimethamine 12.5 mg/kg. Sulphadiazine and pyrimethamine were given in DMSO as a single oral daily dose for 2 menstrual cycles. At the end of the treatment period, blood samples were collected for hormonal analysis (ELISA) by cardiac puncture from half of females in each subgroup, and then they were killed by neck dislocation after light anesthesia. Ovary and uterus were weighed, then fixed in formalin for histological examination [12-14].

Other half of females in each subgroup was mated with healthy males (1 male/1 females), during proestrus and for 24 hrs. Recovery of sperms in the vaginal smears was considered as day one of pregnancy. Pregnant females were killed at day 15 of gestation by cervical dislocation after light anesthesia. Number of corpus lutea were estimated in both ovaries of each female, fetuses were counted, weighted and examined for identification of resorption rate [15-16].

The significant differences among subgroups were determined by single sided student t-test and Chi square test.

## RESULTS

### Hormonal analysis

As shown in table 1, the level of serum FSH, LH, estrogen and progesterone in non infected females treated by DMSO were 52.906±2.120 ug/ml, 3.663±0.042 ug/ml, and 64.637±3.213 pmol/l and 52.063±1.624 ng/ml respectively. The levels of these hormones were significantly declined in *Toxoplasma gondi iinfected* females treated by DMSO to 47.777±1.116ug/ml ($P<0.05$), 3.103±0.034ug/ml ($P<0.05$), 56.667±2.527 ($P<0.01$) pmol/l and 39.526±1.783 ng/ml ($P<0.01$) respectively in comparison with non infected females treated by DMSO. However, sulphadiazine and pyrimethamine didn't induced further changes in the serum levels of these hormone either used in non infected or infected females, in comparison to non infected and infected females treated by DMSO respectively.

### Ovary and uterus weights

The relative weight of the ovary in non infected females treated by DMSO was 0.066±0.010 g/ 100 g body weight; it was significantly decreased in infected females treated by DMSO to 0.014±0.005g/100 g body weight ($P< 0.001$). In comparison with non infected females treated by DMSO, using of sulphadiazine or pyrimethamine treatments in non infected females was significantly decreased relative ovary weights ($P<0.001$), and in comparison with infected females treated by DMSO, using of sulphadiazine or pyrimethamine treatments in non infected females was also significantly decreased relative ovary weights ($P<0.001$). On the other hand, the relative weight of the uterus in non infected females treated by DMSO was 0.251±0.062g/100 g body weight, it was significantly decreased in infected females treated by DMSO to 0.115±0.014 g/ 100 g body weight ($P<0.01$). In comparison with non infected females treated by DMSO, using of sulphadiazine or pyrimethamine treatments in non infected females was significantly decreased relative uterus weights ($P<0.05$), while, in comparison with infected females treated by DMSO, using of sulphadiazine or pyrimethamine treatments in non infected females was significantly increased relative uterus weights ($P<0.05$) (table 2).

### Fertility parameters

As shown in the table 3, the pregnancy rates in the non infected females treated by DMSO, was 100%, it was significantly more that that recorded in the non infected females treated by sulphadiazine 86% ($P<0.01$) or pyrimethamine 43% ($P<0.0001$). The pregnancy rates in the infected females treated by DMSO was the same as non infected females treated by DMSO (100%), but when the infected females treated by sulphadiazine or pyrimethamine, the pregnancy rate was declined to 86% ($P<0.01$) and 26% ($P<0.0001$) respectively in comparison with DMSO treated infected females. The litter size in non infected females treated by DMSO was 11.29±1.08 fetus/dam, it was decreased to 6.14±0.063 fetus/dam in non infected females treated by sulphadiazine ($P<0.01$), and to 2.00±0.012 fetus/dam ($P<0.001$) in pyrimethamine treated non infected females. However, the litter size in infected females treated by DMSO was declined to 7.14±0.032 fetus/dam, it was significantly less ($P<0.01$) than that recorded in non infected females treated by DMSO. Treatment of infected females with sulphadiazine didn't exert further decreased in litter size, while it decreased to 2.00±0.012 ($P<0.001$) in pyrimethamine treated infected females in comparison with DMSO treated infected females.

The mean fetal weight in non infected female group treated by DMSO was 0.573±0.098g. It was

significantly declined in non infected females treated by sulphadiazine 0.548±0.092g (P<0.05) and pyrimethamine 0.449±0.086g (P<0.01). The mean fetal weight was also significantly decreased in the infected female group treated with DMSO to 0.486±0.082g compared with non infected group treated by DMSO (P<0.05). The mean fetal weight was not changed when the infected female treated by sulphadiazine 0.439±0.088g, but it significantly decreased in pyrimethamine treated group 0.344±0.062g (P<0.01) compared with infected females treated by DMSO.

The mean placental weight in non infected female group treated by DMSO was 0.322±0.073g. It was significantly declined in non infected females treated by sulphadiazine to 0.273±0.052g and pyrimethamine to 0.229±0.042 g (P<0.05). The mean placental weight was also significantly decreased in the infected female group treated with DMSO 0.274±0.045 g compared with non infected group treated by DMSO (P<0.05). There was no further significant change (0.266±0.048) in the infected female treated by sulphadiazine, but pyrimethamine caused further decrease in the placental weight 0.181±0.021g (P<0.01) compared with infected females treated by DMSO.

The fetal resorption was not recorded in non infected DMSO-treated female rats. It increased to 3.84% (P<0.05) in infected DMSO-treated females. In comparison with non infected DMSO-treated females, the non infected females treated by sulphadiazine showed 4.00% resorption ratio (P<0.05), and 12.50% (P<0.01) in pyrimethamine-treated non infected females. On the other hand, treatment of infected females with sulphadiazine significantly decreased the resorption rate to 1.82% (P<0.05), while pyrimethamine was significantly increased the resorption rate to 28.57% (P<0.0001) in comparison with that recorded in infected females treated by DMSO.

The early fetal death ratio was not recorded in non infected DMSO-treated female rats. It increased to 12.870% (P<0.01) in infected DMSO-treated females. In comparison with non infected DMSO-treated females, the non infectedsulphadiazine treated females showed 12.82% (P<0.01) early fetal death ratio, while pyrimethamine-treated non infected females showed 24.073% (P<0.001) early fetal death rate. On the other hand, treatment of infected females with sulphadiazine decreased the resorption rate to 2.772 %( P<0.01), while pyrimethamine increased the resorption rate to 39.286% (P<0.0001).

**Histology**

Ovary sections in non infected groups treated with DMO, sulphadiazine, or pyrimethamine, showed normal histological pictures, the primary and secondary follicles and corpus luteum showed normal appearance. Ovary sections of infected female rats treated by DMSO revealed morphological differences compared to the non infected group treated with DMO, they showed decreased primary and secondary follicle with increased follicle atresia, the atretic follicles were characterized by degenerating oocytes, disorganized granulosa cell layers, folded zonapellucida, partially or completely separated from corona radiata and from granulosa cells of the oocyte. However, some of the primary follicles showed normal appearance, although with high congestion compared to the control group. In the infected groups treated with sulphadiazine or pyrimethamine, the numbers of healthy primary and secondary follicles were increased with decreasing of the number of atretic follicles.

Uterine sections in non infected groups treated with DMO, sulphadiazine, or pyrimethamine, showed normal histological pictures. The luminal and glandular epithelium were normal in appearance, normal thickness of endometrium and myometrium with low numbers of polymorphonuclear cells appeared within the lamina propria. Uterine section of infected female rats treated by DMSO showed hypertrophy of the endometrium and myometrium, polymorphic inflammatory infiltration, fewer glands, increased endometrial thickness with congestion of vessels in endometrium and myometrium. Uterine sections in non infected groups treated with sulphadiazine, or pyrimethamine, showed almost normal histological structure but with an increased polymorphic inflammatory infiltration and congestion of vessels and slide increased in the endometrium and myometrium.

**Table 1. Serum level of FSH (ug/ml), LH (ug/ml) and estrogen (pmol/l) and progesterone (ng/ml) of non infected and *Toxoplasma gondii* infected female rats treated with DMSO, sulphadiazine 200 mg/kg and pyrimethamine 12.5 mg/kg for 60 days.**

| Groups | FSH (ug/ml) | LH (ug/ml) | Estrogen (pmol/l) | Progesterone(ng/ml) |
|---|---|---|---|---|
| Non infected treated with DMSO | 52.906±2.120[a] | 3.663±0.042[a] | 64.637±3.213[a] | 52.063±1.624[a] |
| Non infected treated with sulphadiazine | 50.114±1.434[a] | 3.346±0.022[a] | 63.694±1.232[a] | 49.306±1.222[a] |
| Non infected treated with pyrimethamine | 51.548±0.982[a] | 3.531±0.036[a] | 62.148±1.823[a] | 50.008±1.729[a] |
| Infected treated with DMSO | 47.777±1.116[b] | 3.103±0.034[b] | 56.667±2.527[b] | 39.526±1.783[b] |
| Infected treated with sulphadiazine | 44.911±0.868[b] | 3.121±0.018[b] | 54.163±1.624[b] | 41.437±1.234[b] |
| Infected treated with pyrimethamine | 45.602±0.982[b] | 3.101±0.032[b] | 54.496±1.828[b] | 42.500±1.825[b] |

Vertically, similar letter means not significant.

**Table 2. Relative weights (g/ 100 g body weight) of ovary and uterus of non infected and *Toxoplasma gondii* infected female rats treated with DMSO, sulphadiazine 200 mg/kg and pyrimethamine 12.5 mg/kg for 60 days.**

| Groups | Relative weights g/ 100 g body weight | |
|---|---|---|
| | Ovary | Uterus |
| Non infected treated with DMSO | 0.066±0.010[a] | 0.251±0.062[a] |
| Non infected treated with sulphadiazine | 0.016±0.006[b] | 0.224±0.056[b] |
| Non infected treated with pyrimethamine | 0.020±0.008[b] | 0.213±0.048[b] |
| Infected treated with DMSO | 0.014±0.005[c] | 0.115±0.014[c] |
| Infected treated with sulphadiazine | 0.022±0.009[b] | 0.155±0.018[d] |
| Infected treated with pyrimethamine | 0.023±0.007[b] | 0.147±0.020[d] |

Vertically, similar letter means not significant.

**Table 3.Pregnancy%, litter size, fetal weight/ g, placenta weight/ g, fetal resorption ratio and early fetal lost ratio of non infected and *Toxoplasma gondii* infected female rats fertilized by healthy males and treated with DMSO, sulphadiazine 200 mg/kg and pyrimethamine 12.5 mg/kg for 60 days.**

| Groups | Pregnancy% | Litter size Fetuses/dam | Fetal weight/ g | Placenta weight/ g | Fetal resorption ratio | Early fetal lost ratio |
|---|---|---|---|---|---|---|
| Non infected treated with DMSO | 100%[a] | 11.29±1.08[a] | 0.573±0.098[a] | 0.322±0.073[a] | 0.00%[a] | 0.00%[a] |
| Non infected treated with sulphadiazine | 86%[b] | 6.14±0.063[b] | 0.548±0.092[b] | 0.273±0.052[b] | 4.00%[b] | 12.82%[b] |
| Non infected treated with pyrimethamine | 43%[c] | 2.00±0.012[c] | 0.449±0.086[bc] | 0.229±0.042[b] | 12.50%[c] | 24.073%[c] |
| Infected treated with DMSO | 100%[a] | 7.14±0.032[b] | 0.486±0.082[c] | 0.274±0.045[b] | 3.84%[b] | 12.870%[b] |
| Infected treated with sulphadiazine | 86%[b] | 6.43±0.030[b] | 0.439±0.088[c] | 0.266±0.048[b] | 1.82%[d] | 2.772%a |
| Infected treated with pyrimethamine | 26%[d] | 0.71±0.006[d] | 0.344±0.062[d] | 0.181±0.021[c] | 28.57%[e] | 39.286%[d] |

Vertically, similar letter means not significant.

## DISCUSSION

The present study showed that *Toxoplasma gondii* infection significantly decreased serum FSH, LH, estrogen and progesterone in female rats. Many authors found that toxoplasmosis induced hypogonadotropic hypogonadism which occurred secondary to hypothalamic dysfunction in animals [2-3, 4], and in women [10]. Antonios *et al*., mentioned that the endocrine effects of toxoplasmosis were documented by histopathological examination of the hypothalamus of toxoplasma infected mice which revealed that supraoptic and paraventricular hypothalamic nuclei were deformed and showed pyknotic neurons [3].

The biochemical studies also gave further evidence; Interleukin-1b (IL-1b) levels were increased in toxoplasmosis. The levels of IL-1b correlated significantly in a negative manner with FSH, LH in toxplasmosis [17]. Interleukin-1b was known to suppress the hypothalamic-pituitary- gonadal (HPG) axis, directly or indirectly through increased corticotrophin-releasing hormone (CRH) and/or cortisol. It was also found that cytokines released peripherally in response to the parasite reached the hypothalamus and initiated a sequence of events that inhibited the pulsatile release of gonadotropin-releasing hormone (GnRH), leading to the subsequent impairment of the pituitary-ovarian axis[18]. By these mechanisms, toxoplasmosis could interfere with pituitary and gonadal hormones secretion at hypothalamic level. The declined levels of estrogen and progesterone in *Toxoplasma gondii* infected females in this study, were in agreement with the results of Al-Warid and Al-Qadhi who recorded a low level of progesterone in *Toxoplasma gondii infected* women (11.19 ± 9.76 ng/ml) compared with non infected women (18.30 ±9.84 ng/ml), and a low level of estrogen in infected women (53.61 ± 76.24 pg/ml) *compared* with non infected women (88.19 ±101.10 ng/ml) [10]. However, the decreased estrogen and progesterone levels could be occurred as a result of declined pituitary FSH and LH.

The histological changes in the ovary and uterus in *Toxoplasma gondii* infected female rats were also recorded previously. Eslamirad *et al*., mentioned that the ovaries of *Toxoplasma gondii* infected pregnant mice showed gross morphological differences compared to the control groups. The histological comparison of experimentally infected and control groups revealed that the primary follicles, secondary follicle and corpus luteum were significantly decreased in the infected females [5].

However, Fux et al., recorded accentuated hypertrophy of the endometrium and myometrium and a reduction in folliculogenesis and formation of corpora lutea in the ovaries of *Toxoplasma gondii* infected mice [6]. These changes could be attributed to the declined pituitary secretion and decreasing of its influence on ovary and uterus in the infected animals.

The decreasing of all fertility parameters in *Toxoplasma gondii* infected females was in agreement with previous results recorded by Fux et al [6], which could be resulted from the deterioration of endocrine function, and the pathological changes recorded in ovary and uterus of the infected animals.

Sulphadiazine and pyrimethamine didn't induced further changes in the serum level of endocrine and gonads hormones, either used in non infected or infected females. Both drugs decreased pregnancy rate, litter size, fetal weight, placental weight and increased fetal resorption ratio and early fetal death in the non infected females. However, in the infected group, sulphadiazine decreased pregnancy rate, resorption rate and early fetal death and didn't changed litter size, fetal and placental weights. Pyrimethamine was also decreased pregnancy rate, fetal weight, placental weight and increased fetal resorption ratio and early fetal death in infected females. However, sulphadiazine and pyrimethamine improved the histopathological changes induced by toxoplasmosis in the infected group. The absence of the effect of sulphadiazine and pyrimethamine on hypothalamic pituitary-gonads secretion was also recorded previously. Both pyrimethamine and sulfadoxine administered in therapeutic doses early in gestation resulted in decreased fertility and in complete embryo resorption in Wistar rats [20]. The antifertility effects of sulphadiazine and pyrimethamine could be attributable to its antifolate action [21-22]. Pyrimethamine is a drug used for the treatment of protozoal infections. It is commonly used as an antimalarial and to treat *Toxoplasma gondii* infections, particularly when combined with sulphadiazine. Both drugs inhibited the dihydrofolatereductase of protozoa and thereby blocked the folic acid synthesis [23]. Folate affected ovarian function, implantation, embryogenesis and the entire process of pregnancy. Many studies showed that folic acid deficiency decreased fertility, while supplementation enhanced fertility in males and females [24-25]. Therefore, the incidence of dead and abnormal fetuses in rats treated with 5 mg pyrimethamine was markedly reduced, from about 70 to 10%, by single intraperitoneal injections of 6 mg folinic acid; this result suggested that folinic acid might be potentially of value in lessening the antifertility and embryotoxicity of pyrimethamine when employed in toxoplasmosis therapy during early pregnancy [26].

However, the decreasing of fertility parameters and increasing of fetal resorption and early fetal death of non infected and toxoplasma infected female rats treated by either sulphadiazine or pyrimethamine could be attributed to the mutagenic characteristics of these drugs [27-30]. Pyrimethamine was found to produce a significant increase in structural chromosomal aberrations after acute treatment in bone marrow cells of mice. It also induced chromosome abnormalities in spermatogonial cells at the highest dose [26, 32]. Many sulphonamides were also mutagenic and cause fetal growth retardation [32]. According to the results of antifertility pyrimethamine, some authors recommended using of this drug as a contraceptive [33-34].

## CONCLUSION

According to these results, we can conclude that toxoplasmosis deteriorated the female reproductive performance. Sulphadiazine and pyrimethamine which usually used in treatment of toxoplasmosis also exerted adverse effects on the fertility of non infected and *Toxoplasma gondii* infected female rats.

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Nil

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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