Iraqi Journal of Science, 2022, Vol. 63, No. 7, pp: 2850-2860
DOI: 10.24996/ijs.2022.63.7.9

ISSN: 0067-2904

The Role of Hormones and *Toxoplasma gondii* Infection to Change the Secondary Sex Ratio

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Received: 20/8/2021 Accepted: 11/11/2021 Published: 30/7/2022

Abstract

The secondary sex ratio (SSR) is affected by many factors, including the concentration of hormones and infection with some pathogens such as *Toxoplasma gondii*. This study aimed to evaluate the effect of hormone variability concentrations and toxoplasmosis infection on the secondary sex ratio. 150 pregnant women were selected, 60 infected with late toxoplasmosis (LT), 60 infected with early toxoplasmosis (ET) and 30 seronegative to toxoplasmosis. After tracking the birth outcomes of these women, we calculated SSR in each group. During the second and third trimesters of pregnancy, estrogen, testosterone, progesterone, TSH, T4 and T3 concentrations were measured. The results of the three groups were compared. The results showed that female births outpaced in LT group, with SSR equal to (0.9:1.45), while the number of male births was higher in ET group and seronegative groups, with SSR was (1.5:1; 1.3:1), respectively. The difference in hormones concentration was significant at $P \geq 0.05$. In LT group, progesterone and estrogen increased significantly in male pregnant women than in female pregnant women. Testosterone increased significantly in male pregnant women in all study groups. TSH level was the highest among female pregnant women in the LT and seronegative groups. The superiority of male pregnant women in the concentration of T3 hormone in all groups was significant. Male pregnant women were significantly superior with T4 concentration in LT and seronegative groups.
In conclusion, *T. gondii* has an indirect role in SSR. Important pregnancy hormones levels differ between male pregnant women and female pregnant women.

Key words :*Toxoplasma gondii*, Secondary sex ratio, Estrogen, Progesterone, Testosterone

دور الهرمونات والاصابة بالمقوسة الغوندية في تغيير نسبة الجنس الثانوية

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الخلاصة:

تتأثر نسبة الجنس الثانوية بالعديد من العوامل، بما في ذلك تركيز الهرمونات والعدوى ببعض مسببات الأمراض مثل *Toxoplasma gondii* ، تهدف هذه الدراسة إلى تقييم تأثير تغاير تركيزات الهرمونات والعدوى بداء المقوسات على نسبة الجنس الثانوية. أُختيرت 150 امرأة حامل، 60 امرأة مصابة بداء المقوسات

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المتأخر(بعد الحمل)، 60 امرأة مصابة بداء المقوسات المبكر (قبل الحمل) و 30 غير مصابة، بعد تتبع نتائج ولادة هؤلاء النساء، قمنا بحساب SSR في كل مجموعة. خلال أشهر الحمل قمنا بقياس تراكيز هرمونات الإستروجين،التستوستيرون، البروجستيرون، TSH، T4 و T3. قورنت نتائج المجموعات الثلاث. أظهرت النتائج أن الولادات الأنثوية تفوقت في مجموعة التوكسوبلازما المتاخرة حيث كانت النسبة (0.9:1.45)، في حين كان عدد المواليد الذكور أعلى في مجموعات مجموعة التوكسوبلازما المبكرة والسالبة (1.5:1) ؛ (1.3:1)على التوالي. كان الفرق في تركيز الهرمونات معنويًا عند $P \geq 0.05$ في مجموعة التوكسوبلازما المتاخرة، زاد البروجسترون والاستروجين بشكل أكبر في النساء الحوامل بالذكور مما كانت عليه في النساء الحوامل بالإناث، كذلك لوحظ زيادة في تركيز هرمون تستوستيرون بشكل ملحوظ في النساء الحمل الذكري وفي جميع مجموعات الدراسة، كان مستوى TSH الأعلى بين النساء الحوامل في مجموعات التوكسوبلازما المتاخرة و السالبة، تفوقت نساء الحمل الذكري في تركيز هرمون T3 وفي جميع المجموعات بشكل معنوي، بينما كانت نساء الحمل الذكري متفوقة بشكل ملحوظ مع تركيز T4 في مجموعتي التوكسوبلازما المتاخرة و السالبة. يمكن القول بأن للمقوسة الغوندية دور غير مباشر في تغيير نسبة الجنس الثانوية، هذا التأثير يكون في اختلاف مستويات هرمونات الحمل الهامة بين نساء الحمل الذكري ونساء الحمل الإنثوي.

1. Introduction

Toxoplasmosis is a zoonotic parasitic disease. Reports indicate that about two-thirds of the world's population is infected. The danger of the disease lies in its ability to transmit from the mother to the fetus [1], which may lead to stillborn or malformations in the fetus. The parasite causes high pathological effects that may reach blindness, neurological imbalances or schizophrenia and others [2]. There are many molecular and serological methods were used to determent the parasite presence. Serological methods depend on the presence of IgM antibody as an evidence of early infection and IgG antibody as an indicator of late infection [3,4].

The ratio between male births to female births is known as the secondary sex ratio. It refers to the composition of society and which sexes are predominant. The normal is about 1.05:1 [5]. This ratio varies, increase or decrease under the influence of many internal factors (the human body) or external (the environment). In addition to various bacterial, viral and parasitic infections [6], parents age, stress and the concentration of hormones in the parents are factors that affect SSR [7].

Which of the parents determines SSR? Given that the man's semen contains two types of sperm carrying an X or Y chromosome, some studies have concluded that the father is responsible for determining this ratio or bias to one of the sexes[8]. It is known that the number of the two types of life is equal, which mean the health status and reproductive capacity of the father is the determinant of this ratio, man who has high reproductive will has more male births, but female births will increases when his ability decreases[9]. The other opinion concludes that it is the woman who determines the SSR, given that the mother is the incubator in which the fetus grows, and therefore any changes that affect the reproductive system or sex hormones and other factors such as the age of the mother and stress, which affect the health status of the mother lead to stillborn[10].

In different animals, many assumptions is affect the SSR variation, including the exposure of a woman to stress during pregnancy, may increase the chances of stillbirth of male fetuses and female birth alive at a greater rate[11], the mechanism that lead this affects don't clear, its maybe return to the fact which assumes that male fetuses are weaker than female fetuses, due to increase of testosterone concentration in male pregnant, or selectivity against Y chromosome[12]. Exposure to the Bruce effect, this effect assumes that a pregnant female tends to lose her newly formed fetus when exposed to multiple males, especially if she encounters a strong and dominant male in her region[13]. Follow the hypothesis of Trivers and Willard, this hypothesis is based on two basic principles: the first is that a healthy female tends to push the secondary sex ratio towards males. The second is that a healthy female gives

birth to more boys than girls[11]. The other view is due to the reproductive capacity of parents, where the chances of having males increase as parents are younger[14], but Ein-Mor *et al*. [15] confirmed that there is no relationship between age and SSR. Sex hormones and their concentrations in pregnant women play a clear important role in determining SSR[16]. James conducted that many physiological and pathological reasons or the genetic makeup of the parents make the variations in hormone concentrations[17].

Some studies indicated the responsibility of *T. gondii* in changing the SSR in infected women[4]. What effect does the mother have on certain parasites (especially *T. gondii*) in sex hormone concentrations? How much does the variation in hormone concentrations affect SSR? Is there a difference in the level of these hormones between women of male and female pregnancy? Answers of these questions may be keys that solve some of the SSR variation mystery. From this, we performed this work.

2.Materials and methods

2.1. Samples collection

From January 2020 to May 2021, (1226) pregnant women (beginning of the eighth week of pregnancy) aged (20-40 years) who visit private clinics were followed. With the help of a gynecologist and by ultrasound, the pregnancy and gender of the fetus were confirmed, then recorded data such as age, the number of previous births, live births and aborted children. From each patient, (5 ml) of intravenous blood using a sterile syringe was withdrawn, and put it in the test tube free of EDTA. The blood left at room temperature for 20 minutes, then the serum separated using a centrifuge at a speed of 3500 rpm/min for 15 minutes, after which the serum withdrawn by micropipette absorbent, placed in special containers and kept under freezing at (20-16°C) for laboratory testing[18].

2.2. Detection T. gondii infection

ELISA kits produced by Demeditec Diagnostics GmbH, Germany were used according to the manufacturer's instructions.

2.3. Study groups

After excluding women with other diseases and missing data, 150 pregnant women were selected randomly and tracked, divided into three groups, the first included 60 women with late toxoplasmosis, the second included 60 women with early toxoplasmosis and the third group included 30 seronegative women .

2.4. Hormone level assessment

During the third trimester (7-9 months) of pregnancy, 5 ml of intravenous blood (from 150 women in the study groups) withdrawn, after blood clotting, serum used in ELISA test. An ElISA kit produced by Monobind (USA) was used, as instructed by the manufacturer.

2.5. Statistical analysis

Statistical analysis was carried out using the SPSS program, the mean of concentrations, standard deviation, and comparisons of means were calculated using Dunkin' method and T-test or ANOVA.

3. Results

3.1. Incidence of T. gondii

From tracking the case of (1226)(20 – 40 years) pregnant women, the study recorded a significant infection (p. value > 0.0001) in 203 pregnant women were infected with toxoplasmosis with an infection rate (16.55%). (114) infected with late toxoplasmosis (infected before pregnancy) (9.29%), and (89) infected with early toxoplasmosis (infected after pregnancy)(7.25%), this result was insignificant (P. value = 0.1520), after confirming the presence of IgG and IgM, respectively (Tab. 1).

Table 1-Overall seroprevalence of *T. gondii* in pregnant women

Group	number(%)	Statistical result
Latent Toxoplasmosis	114(9.29)	Odds ratio 0.1984 95 % CI: 0.1674 - 0.2352 z statistic 18.631 P. value < 0.0001
Early Toxoplasmosis	89(7.25)	
Negative	1023(83.44)	
Total	1226(100)	

One hundred fifty women were selected randomly as mentioned in 2.3, then distributed into three groups, the first with late toxoplasmosis, the second with early toxoplasmosis and the third was seronegative. There was no significant difference in age (P = 0.455) across the study groups, while the difference in the number of previous births across the three groups was significant (P = 0.002) (Tab. 2).

Table 2-study groups as age and seroprevalence .

Group	number	Age	mean	Standard deviation	P. value
Latent Toxoplasmosis	60	20-40	32.2	5.64	P = 0.455
Early Toxoplasmosis	60	20-40	31	7.02	
Negative	30	20-40	30.56	7.42	
Total	150	20-40	31.39	6.58	

The number of female births in LT group was higher than the number of male births in the same group, the ratio of males to females (0.9 : 1.45) was not significant (p = 0.67). The number of male births was higher in ET and seronegative groups, where SSR in both groups was (1.5:1 ; 1.3:1), respectively. This difference was not significant (p = 0.65; p = 0.48). The difference in SSR across the three groups was significant (P= 0.047), as well as within late and early toxoplasmosis (P = 0.03) but the difference within the late and early toxoplasmosis versus seronegative (p= 0.1; P=0.09), respectively was not significant (Tab. 3).

Table 3- Distribution of male and female live neonates among the study groups.

Groups	Male(%)	Female(%)	Total	Male: female	P. value
Latent Toxoplasmosis	18(30)	29(48.33)	47(78.33)	0.9:1.45a	p = 0.67
Early Toxoplasmosis	30(50)	20(33.33)	50(83.33)	1.5:1a	p = 0.65
Negative	16(53.33)	13(43.33)	29(96.66)	1.3:1b	p = 0.48
Total	64(42.66)	62(41.33)	126(84)	1.06:1	P= 0.047

Similar letters have significant difference
Different letters have no significant difference

The number of aborted males in LT was higher than aborted females number in the same group, as was the case in the seronegative group, the number of aborted females was higher in ET group. Despite the non-significance of the difference in the stillbirth rates across the three groups (P= 0.614). LT had the highest stillbirth rate (21.16%), followed by ET (16.66%). The stillbirth rate within the two infection groups was (P= 0.33), within LT and seronegative was (P= 0.62), and (P= 0.68) within ET and seronegative (Table-4).

Table 4-Distribution of aborted male and female among the study groups

Groups	Total(%)	Male(%)	Female(%)	P. value
Latent Toxoplasmosis	13(21.66)a	8(13.33)	5(8.33)	0.614
Early Toxoplasmosis	10(16.66)b	4(6.66)	6(10)	
Negative	1(3.33)c	0(0)	1(3.33)	
Total	24(16)	12(8)	12(8)	

Similar letters have significant difference
Different letters have no significant difference

3.2. Hormone levels

At a significant level ($P \geq 0.05$), tab. 5 shows a significant superiority for LT group over the ET and seronegative groups in their effect on estrogen concentration. The results in this study showed that the progesterone concentration was higher in ET than in the other two groups. This difference was significant. The level of testosterone hormone showed a significant decrease in LT while its level was higher in the other two groups. The difference across the three groups was significant. Tab. 5 shows a significant decrease of the TSH level in seronegative and ET groups with a significant increase in the hormone concentration in LT. The same table shows a significant increase in T3 hormone in LT compared to the other two groups. The T4 hormone showed a significant difference in its concentration across the three groups. It was in the highest concentration in ET.

Table 5-Hormones level between study groups

Hormones	Pre-pregnancy		Post-pregnancy		Sero-negative	
	Mean	SD	Mean	SD	Mean	SD
Estrogen (pg/ml)	504.87^{a}	5.54	483.63^{b}	69.19	448.27^{c}	17.5
Progesterone(ng/ml)	18.73^{a}	0.79	23.56^{b}	24.55	18.93^{a}	0.6
Testosterone(ng/ml)	0.79^{a}	0.03	0.81^{b}	0.05	0.92^{c}	0.06
TSH (ng/ml)	4.68^{a}	6. 89	4.02^{b}	0.33	4.39^{c}	0.2
T3 (ng/ml)	1.38^{a}	0.11	1.31^{b}	0.19	1.09^{c}	0.12
T4 (ng/ml)	9.35^{a}	1.3	9.78^{b}	0.55	9.45^{a}	0.89

Similar letters have significant difference
Different letters have no significant difference

3.3. Hormones and gender

At a significant level ($P \geq 0.05$), it is noticeable from the tab. 6 that there is insignificant superiority of estrogen in female pregnancy compared to male pregnancy in LT. The superiority estrogen in male pregnancy was significant in ET and seronegative groups. The same table shows a high level of progesterone hormone in female pregnancy in LT and ET groups. In the seronegative group, the hormone level in male pregnancy was the highest. This superiority was insignificant. Tab. 6 shows the superiority of testosterone level in women of male pregnancy compared to women of female pregnancy in the three study groups. The TSH level showed a significant superiority in the female pregnant women in ET and seronegative groups. The hormone level in the male pregnant women in LT group was higher compared to the female pregnant women, although the difference was not significant. In all study groups, T3 hormone levels were higher in male pregnant women than female pregnant women. with a similarity of the hormone level in LT and seronegative groups. The study recorded a significant difference in the concentration of T4 hormone between male pregnant women and female pregnant women across all study groups, as well as within members of the same group.

Table 6-Effect of gender in hormones concentration

Parameters			Estrogen	Progesterone	Testosterone	TSH	T3	T4
Pre- pregnancy	Male	mean	501.07a	19.04a	0.96a	4.14a	1.18a	10.93a
		SD	4.18	0.6	0.02	0.53	0.11	0.19
	Female	mean	507.59a	18.12a	0.91b	4.95b	1.01b	8.38b
		SD	4.02	0.65	0.03	0.27	0.05	0.42
Post- pregnancy	Male	mean	511.79a	18.15a	0.82c	4.26b	1.45b	9.37c
		SD	8.62	1.99	0.012	0.04	0.06	0.15
	Female	mean	438.39b	22.73a	0.74d	3.63b	1.08b	10.47^{d}
		SD	93.88	0.38	0.04	0.16	0.03	0.12
Negative	Male	mean	461.38c	18.36a	0.85e	4.28b	1.19a	9.55^{e}
		SD	6.86	0.41	0.014	0.15	0.08	0.94
	Female	mean	429.33d	19.32a	0.74f	4.56a	1.07b	9.30^{f}
		SD	7.1	0.28	0.02	0.14	0.06	0.85

Similar letters have significant difference
Different letters have no significant difference

4. Discussion

Toxoplasmosis is globally disease, with reports indicating that one-third of the world's population is infected [19]. Among pregnant women, a study carried out by (Colak and Asgin) recorded infection (0.7% and 15.7%) of women with acute and chronic toxoplasmosis, respectively [20]. The presence of anti-parasitic antibodies (IgG, IgM) among Iranian women were (32.95%; 1.13%), respectively[21]. In Angola, the incidence rate for chronic and acute infections was (39.4%; 0.2%), respectively [22]. In Mexico, the incidence was (5.7%)[23], France (31.3%) [24]. Rural areas of Brazil had an incidence rate of (68.3%) [25].

Several studies reported the effect of toxoplasmosis in determining the secondary sex ratio but differed in the direction of this effect. Bahreini *et al*. study indicated a significant superiority of female births to male births among women infected with late toxoplasmosis compared with the seronegative group[26]. Shojaee and his colleague found that women infected with *T. gondii* give more birth male than uninfected. Also, they confirmed that the rate of stillbirth among female increases with an increase in the titer of anti-Toxoplasma antibodies. *Toxoplasma* acts as a secondary factor that alters some factors (Hormones) affecting the formation of the egg implantation and embryo survival, and helps in activating other factors such as cytokines[27]. Another study confirmed that the infection with *T. gondii* elevated SSR ratio to 2.6:1, They reasoned that toxoplasmosis promotes the production of immunosuppressive components such as IL-10 and TGF-β that increase the chances of survival of sperm containing the Y chromosome because it reduces the high sensitivity against this sperm, and it improves implantation in the uterus [28].

The conclusion of a study conducted on female mice by kanˇ kova *et al*. indicated that after infection with *T. gondii*, the secondary sex ratio is low in females with chronic toxoplasmosis, while the opposite occurs in females infected with early toxoplasmosis[29]. In a systematic review of several countries, Madhukar *et al*. confirmed that the relationship between

toxoplasmosis infection and male birth is negative. They found that low concentrations of parasite antibodies stimulate more male birth[30]. These results are consistent with the findings of our current study, which can explain this result to the effect of sex hormones, including testosterone, as females with low testosterone concentration have an increased chance of giving birth to females[31]. The low hormone concentration may be due to infection with toxoplasmosis[32]. Toxoplasmosis decreases the concentration of testosterone in infected women and a rise in infected men [33]. The immune changes that occur after infection may have a role in determining the fetus gender or stimulating and protecting specific gender, thus increasing SSR. Immunosuppressive is one of the phenomenons in toxoplasmosis infection, Where the infection decreases NK cells, white cells and monocytes [34, 35], which gives the greatest opportunity for male fetuses to develop, especially since they have the Y production on their surface. It was found that the male fetus is 1.62 times more than the female fetus is developing in the womb[31].

Zhang *et al*. pointed out the important role of estrogen in parasite invasion of cells and production of MIC2 - Microneme protein in addition to stimulating sliding movement and parasite exit from cells. Therefore, the concentration of estrogen increases after *T. gondii* infection[36]. During the first stage of pregnancy, Estrogen also contributes to negative feedback and secretion of GnRH hormones from the hypothalamus and FSH from the pituitary to prevent ovulation, to prepare the uterine environment in cooperation with the hormone progesterone[37]. On the other hand, progesterone negatively affects the presence and activity of the parasite[36]. In normal pregnancies, the concentration of progesterone increases gradually with the progression of the pregnancy until it reaches its peak during the third stage of pregnancy, then begins to decline before birth, and the delay in its decline before birth leads to a case of delayed delivery from its natural time. Progesterone decreasing during the three stages of pregnancy result in stillborn[38].

During pregnancy, androgenic hormones turn into estrogen in the presence of the aromatase enzyme. Therefore, testosterone concentration decrease[39]. After infection, *Toxoplasma* works to excite the enzymes and factors that stimulate the conversion of testosterone to estrogen[40].

The increase in TSH and T3 concentrations in LT and ET compared to seropositive due to the high concentration of estrogen, which increases the concentrations of the proteins transporting T3 and T4 hormones, which results in an increase in the concentrations of these hormones during this stage due to its great and sensitive importance in the development of the fetus and the success of pregnancy[41]. On the other hand, the decrease in these hormones concentration plays a role in causing hyperthyroidism during the first trimester of pregnancy, causes a decrease in the pregnant weight by about 5% of body weight, thus a decrease in the level of the nervous system growth and development and the cerebral cortex in particular[42].

The reason for estrogen concentration superiority in female pregnancy women compared to male pregnancy women in LT and the opposite in ET and seronegative groups is due to the role of this hormone in indirectly raising the concentration of T4 hormone, which has a chief role in the nervous system development and other male fetuses body organs compared to female fetuses[43]. The increase in these hormones concentrations occurs after the beginning of the decrease in progesterone level, as it has a negative role on the estrogen hormone[44].

The concentrations of the progesterone hormone in the three groups are consistent with the rate of stillborns in them. It is known that the survival of the fetus and increase the stillborn rate effect by hormone decrease[38]. This concentration difference between the three groups is due to the positive effect of high estrogen in female pregnant women compared to male pregnant women[45]. Several studies indicated the positive effect of leptin on estrogen in female pregnant women when it recorded a high level of this hormone compared to male pregnant women[46].

The positive relationship between cortisone and Testosterone was the reason for the superiority of testosterone in male pregnant women in all groups. In the fetal stage, testosterone secreted from the adrenal cortex of the fetus under the influence of the cortisone, which secreted from the same gland under the effect of adrenocorticotropic hormone (ACTH) coming from the pituitary gland of the pregnant mother[47]. The placenta also releases Placental Corticotrophin Releasing Hormone (CRH), which stimulates the fetus adrenal gland to produce the enzyme Dehydroepiandrosterone – sulfate (DHEA-S), which is the precursor in the production of testosterone during female and male pregnancy[48]. On the other hand, during a female pregnancy, a large part of the testosterone converted into estrogen due to the presence of the aromatase enzyme in female pregnant women compared to male pregnant women, which explains why testosterone is higher in women of male pregnancy compared to female pregnancy[49]. The high level of the testosterone hormone necessary for the male pregnancy for its role in increasing brain weight and the area and thickness of the grey area of the central nervous system (cerebral cortex) compared to the female pregnancy. It is the reason why males outperform females in increasing brain weight and size after birth[50].

Low TSH concentrations in male pregnant women may be due to high hCG concentrations in female pregnant women, which has a negative relationship with TSH. The decline of hCG secretion in the third trimester is the main cause for elevating TSH concentrations in women in male pregnant women compared to female pregnant women[51]. The significant difference between T3 and T4 concentrations between male and female pregnant women is due to the effect of the hCG hormone. Because TSH and hCG are similar in their chemical and functional structure hCG competes with TSH for its receptors in the fetus and mother thyroid tissues, consequently, leads to an increase in the production of T3 and T4 hormones[52]. The hCG hormone also helps in causing sexual differentiation. It found that the higher its concentration during pregnancy, the greater the sexual differentiation of the fetus towards the male fetus. An increase in the hormone level leads to the development and increase the chance of sperm carrying the Y chromosome to fertilize the egg[53].

5. Conclusions

LT women tend to give birth to more females than males, with a higher rate of male fetus stillborn, consequently a lower rate of SSR. In women with early toxoplasmosis, the SSR rate is close to the normal range, with more males than females. *T. gondii* has indirectly affect the SSR through its effect on the levels of certain hormones. The tendency of women to give birth to females increases with the increase in their estrogen concentration, on the other hand, a decrease in progesterone has a role in stimulating the growth of female fetuses and increasing the chances of stillborn when its concentration drops to a minimum. The increase in testosterone concentration accelerates the growth and development of male fetuses. There is a clear significant difference between concentrations of different hormones in male pregnant women and female pregnant women.

Ethics approval: All volunteers were informed of the trial approach and steps, and their prior approval was taken before the proceedings began.

Funding: No funding

Conflict of interests: No conflict of interests is declared

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