Imbalance of Steroid Hormones in Hamsters Infected with *Schistosoma mansoni*

Katia C. Oliveira[1], Rita Cardoso[2], Antonio C. Dos Santos[1], Ruben Fernandes[3,4] and Monica C. Botelho[2,4,*]

[1]Disciplina de Parasitologia, Departamento de Microbiologia, Imunologia e Parasitologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, Brazil; [2]INSA, National Institute of Health Dr. Ricardo Jorge, Department of Health Promotion and Chronic Diseases, Porto, Portugal; [3]ESS, Escola Superior de Saúde, Instituto Politécnico do Porto, Portugal; [4]I3S, Instituto de Investigação e Inovação da Universidade do Porto, Portugal

Abstract: ***Objective:*** Schistosomiasis is a debilitating disease that affects 200 million people worldwide. *Schistosoma haematobium* and *Schistosoma mansoni* are the major causative agents of this disease. Cancer-association and infertility-association in *Schistosoma haematobium* infection have already been described and it is known that the parasite produces a catechol-estrogen molecule that induces a hormonal imbalance in the host.

Methods: In order to better understand the relation of hormonal imbalance in experimental *Schistosoma mansoni* infection, we investigated a serum panel of steroid hormones in *Schistosoma mansoni* infected hamsters.

Results: We found a decrease in the serum levels of Estradiol (E2), Testosterone and Progesterone in infected females and an increase of Testosterone and a decrease in Progesterone in infected males in comparison with controls.

Conclusion: These results indicate that *S. mansoni* alters the levels of steroid hormones in infected males and females and it will increase the repertoire of data about the host-parasite molecular interplay and its relation with the endocrine system.

Keywords: Steroid hormones, *Schistosoma mansoni* infection, host-parasite interaction, estradiol, testosterone, progesterone, infertility.

1. INTRODUCTION

Human schistosomes currently infect more than 200 million people in 74 countries worldwide in the endemic areas of Africa, the Caribbean, Central America, South America, eastern Asia, and the Middle East [1]. Prevalence is thought to be rising mainly due to increasing travelers from the USA and Europe to these endemic regions for business or leisure [2].

Praziquantel is the main antihelminthic drug currently used to treat this infection. This drug is effective in eliminating adult worms, but is unsuccessful in the prevention of reinfection and does not treat severe liver damage nor bladder cancer [3].

Steroid hormones are generally synthesized from cholesterol in the adrenal glands and gonads [4]. These include corticosteroids (adrenal glands) and androgens, estrogens and progestogens (gonads) [5].

Schistosome eggs produce catechol-estrogens. These estrogenic molecules are metabolized by cytochrome P450 oxygenases to active quinones that cause alterations in DNA, known to promote breast and thyroid cancer [6-8]. Our group has shown that schistosome egg-associated catechol estrogens induce tumor-like phenotypes in urothelial cells, possibly due to the formation of parasite estrogen-host cell chromosomal DNA adducts [7]. These estrogen metabolites also contribute to schistosomiasis-associated infertility in *Schistosoma (S.) haematobium* infection [8].

The hormonal microenvironment also modulates gene expression of female and male *S. mansoni* adult parasites [9].

At present, there are very few reports associating infertility with *S. mansoni* infection, and in general, it is caused by the presence of egg and the formation of granuloma in reproductive organs [10-13]. Few works described a relation of *S. mansoni* infection and hormonal changes, such as it has been explored in *S. haematobium* [13].

The aim of this study was to study a panel of steroid hormones in hamsters infected with *S. mansoni*. We now hypothesize the induction of infertility in individuals infected with *S. mansoni* also through a hormonal imbalance.

2. MATERIAL AND METHODS

2.1. Animals and Infection

Twelve Nine-week-old Syrian hamsters (*Mesocricetus auratos*), six females and six males were used. Three male and three female hamsters were infected with 250 *S. mansoni* cercarie by subcutaneous injection. Other three male and female hamsters were kept without infection as negative controls.

After 7 weeks the hamsters were euthanized using Ketamine (50 mg/kg) and Xylazin (5mg/kg) followed by CO_2 administration according to CONCEA (Conselho Nacional de Controle de Experimentação Animal) recommendation.

During the experimental work, animal activity and welfare were evaluated. No animal died, and all animals exhibited healthy cage activity. The weight of the animals remained normal throughout the experiment (data not shown).

After euthanasia, all animals were submitted to total bleeding by the puncture of inferior vena cava, for the infected animals, the perfusion of the portal system with PBS (Phosphate Buffered Saline) was performed to confirm the presence of adult worms. Respective non-infected animals (negative controls), with the same age, were submitted to the same procedure.

2.2. Hormone Serum Levels

Estradiol, Testosterone and Progesterone were determined, using a solid phase competitive chemiluminescent enzyme immunoassay with the Cobas E411 (Roche) according to the manufacturer's instructions. Briefly, the solid phase (bead) is coated with rabbit anti-hormone polyclonal antibody. The reagent contains alkaline phosphatase (bovine calf intestine) conjugated to the hormone analyzed. The hormone-enzyme conjugate competes with the tested hormone in the patient sample for limited antibody binding sites on the bead. The excess sample and reagent are removed by a centrifugal wash. Finally, the chemiluminescent substrate is added to the bead and the signal is generated in proportion to the bound enzyme.

2.3. Statistical Analysis

For the groups' comparison, chi-square tests with Yate's correction were used or Fisher's exact test (two-sided) was used when expected values were below 5. For independent samples, Student's T-test was used for the comparison of means (Epi-Info software, version 3.03).

3. RESULTS

3.1. Animals' General Aspect

During the experimental work, no animal died, and all animals exhibited normal cage activity. The weight of the animals remained normal throughout the experiment (data not shown).

3.2. Hormonal Analysis

In a series of hamsters infected with *S. mansoni*, we attempted to identify and quantify the sex hormones estradiol (E2), testosterone and progesterone in their sera by ECLIA (electrochemoluminescence) (Fig. **1**; Table **1**). We found a decrease in the serum levels of E2, Testosterone and Progesterone in infected females and an increase of Testosterone and a decrease in Progesterone in infected males in comparison with controls (Fig. **1**; Table **1**).

4. DISCUSSION

The host endocrine system can not only impact the progression of parasitic infection by modulating the immune system but also can be exploited directly by parasites [14]. The capability of a parasite to affect a female or male host of the same species differentially (sexual dimorphism of an infection) can be facilitated by hormonal regulation of the immune reaction of the host or through hormonal effects on the parasite [14]. In the present work, we demonstrated that infection with *S. mansoni* alters significantly the levels of steroid hormones both in infected females and males hamsters.

Estrogen is the primary female sex hormone. It is responsible for the development and regulation of the female reproductive system and secondary sex characteristics [15]. Previous studies from our group already demonstrated an increase of hormonal levels of Estradiol in *S. haematobium* and *S. mansoni* infected male patients and showed that the total extract from both parasites has an estrogen-related molecule [2]. In the present work, we were not able to detect estradiol (the levels were below the detection limit) in infected male hamsters and therefore one could not compare the levels of Estradiol between infected and not-infected males. Surprisingly, in infected female hamsters, we detected a significant decrease in estradiol (Fig. **1**). To our knowledge, this is the first report describing a decrease in estradiol levels in infected females. Estrogens are responsible for maturation and maintenance of the vagina and uterus and are also involved in ovarian function, such as maturation of ovarian follicles. In addition, estrogens play an important role in the regulation of gonadotropin secretion. For these reasons, estrogens are required for female fertility [15]. This new data could very well explain infertility observed in women infected with schistosomiasis [8].

Progesterone is an endogenous steroid and progestogen sex hormone is involved in the menstrual cycle, pregnancy, and embryogenesis of humans and other species [16]. Regarding progesterone levels among infected and non-infected hamsters in the present study, both males and females showed the same profile: *S. mansoni* infection caused a decrease of progesterone levels. It is interesting to note that it was already described an increase in the expression level of estrogen and progesterone receptors in the uterus and endometrium of *S. mansoni* infected female mice. These authors also reported that infected females do not develop the secretory phase in acute and chronic schistosomiasis [16]. This phenomenon may happen as a mechanism of compensation due to the decreasing hormonal levels of estrogen and progesterone. In females, progesterone is sometimes called the "hormone of pregnancy", and it has an important role relating to the development of the fetus [17]. In males, progesterone regulates vital sperm functions, such as capacitation and motility. Progesterone is known to affect receptors on the sperm's acrosomal membrane. Sperms that lose their acrosomes before encountering the oocyte are unable to bind to the zona pellucida and thereby

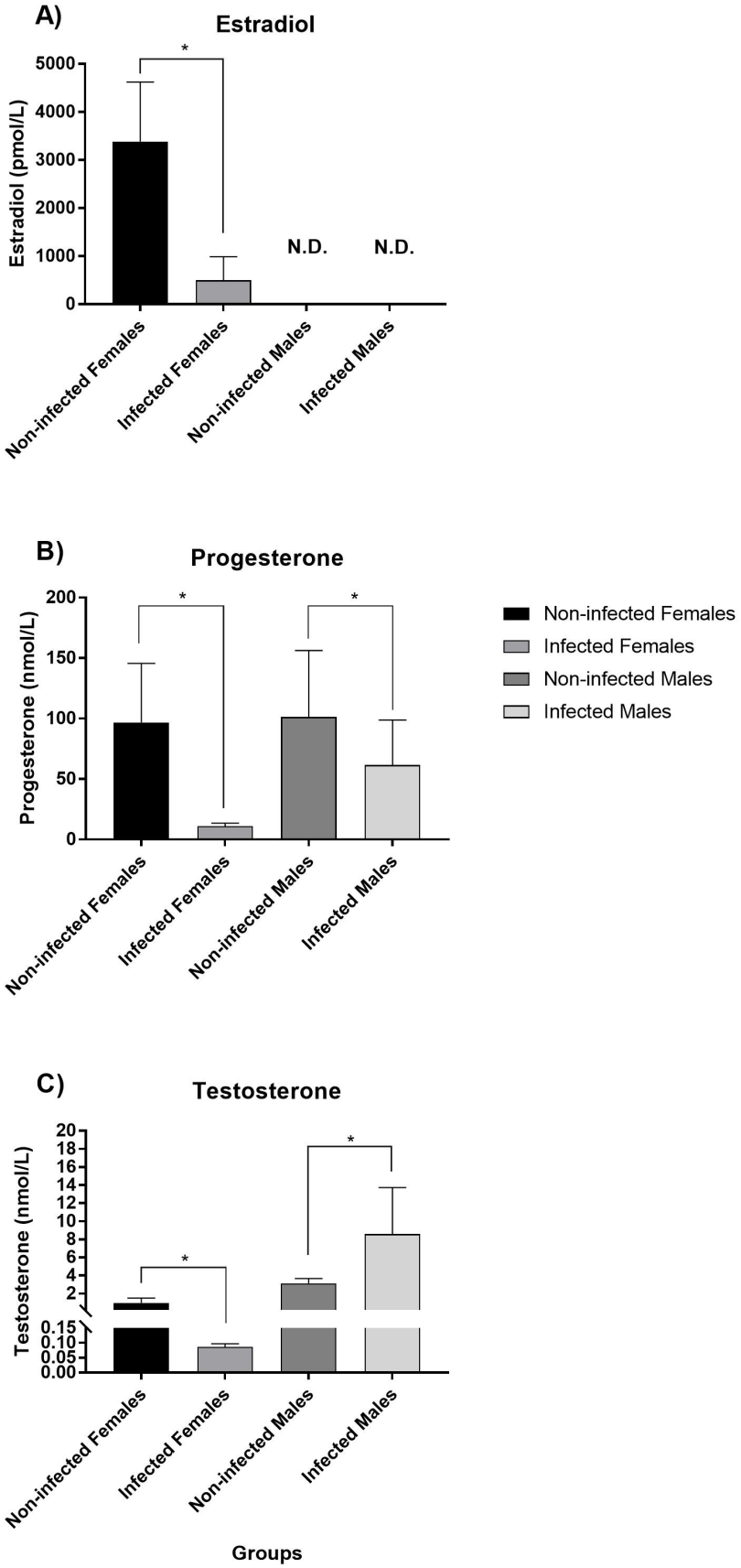

Fig. (1). Mean (with SD) of hormonal levels of Estradiol (**A**), Progesterona (**B**) and Testosterone (**C**) in females and males infected with *S. mansoni* and non-infected.

Table 1. Hormone serum levels among females and males that were Non-infected or infected with *S. mansoni*.

Homones	Non-Infected Females (mean±SD)	Infected Females (mean±SD)	*P-Value*	Non-Infected Males (mean±SD)	Infected Males (mean±SD)	*P-Value*
Estradiol (pmol/L)	3385±1238.85	499.07±489.93	0.03	N.D.	N.D.	N.D.
Progesterone (nmol/L)	96,67±48.84	11.01±2.61	0.04	101.2±55.08	61.66±37.03	0.01
Testosterone (nmol/L)	0,974±0.52	0.09±0.01	0.04	3.14±0.52	8.57±5.16	0.04

Legend: SD - Standard deviation; ND - not determined.

unable to fertilize [18]. The reduction of progesterone levels shown by the present study could be a cause of infertility associated *S. mansoni* infection, both in females and males.

Testosterone is the primary male sex hormone. In males, testosterone plays a key role in the development of male reproductive tissues such as testes and prostate, as well as promoting secondary sexual characteristics. Testosterone is secreted primarily by the testicles of males and, to a lesser extent, the ovaries of females [19]. When we compare testosterone levels between males and females we observed that *S. mansoni* infection causes a decrease in female and an increase in male testosterone levels. The literature is somewhat discordant about the effect of *S. mansoni* on testosterone levels among different experimental models and humans. Kasilima *et al* [20] reported that in rabbits (42 and 70 days after infection with *S. mansoni*) testosterone levels were decreased, both in males and females. Lansoud-Soukate *et al.* [21] observed the decrease of testosterone in infected male rats and hamsters; however, Abdallah *et al.* [22] detected an increase in both infected males and females of testosterone, progesterone and estradiol.

In humans, Saad *et al.* [10] observed an elevation of estradiol and a decrease in testosterone levels in an infected man; on the other hand, Skelly *et al.* [23] did not observe any changes in testosterone in serum of an infected man, as well as our group [2]. The reason for this controversy might be based on the different methods used for the detection of serum levels of hormones. The techniques are increasingly more sensitive and this might lead to the differences of results encountered in the literature along the years. It is more likely that the levels of estradiol accompany the levels of testosterone since estradiol is also synthesized from testosterone [24], as our studies indicate. In human males, increased levels of testosterone cause infertility and low sperm count [25]. The increased levels of testosterone in infected males shown in our studies could be a cause of infertility associated *S. mansoni* infection, in males.

CONCLUSION

The infection with *S. mansoni* disrupts the host hormonal milieu, and triggers immune responses that result in disturbing the endocrine system. Furthermore, sex steroids may also directly modify parasite reproduction and molting. Existing information in the literature point out that parasites synthesize steroid hormones and the occurrence and action of related enzymes have been demonstrated [26]. Here, we contribute to this discussion with our data. Further studies are necessary to understand the physiological and molecular mechanisms involved in the complex relationship of host-parasite particularly host hormones.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the animal ethics committee of Universidade Federal de São Paulo, São Paulo, Brazil (protocol number 8250260618).

HUMAN AND ANIMAL RIGHTS

No humans were involved in the study. All animal experiments were performed in accordance with the National (DL 129/92; DL 197/96; P 1131/97) and European Convention for the Protection of Animals used for Experimental and Other Scientific Purposes and related European Legislation (OJ L 222, 24.8.1999).

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article is available in the [ResearchGate] at [https://www.researchgate.net/profile/Monica_Botelho5/research].

FUNDING

This work was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo – Grant 2012/50115-6) and FEDER - Fundo Europeu de Desenvolvimento Regional funds through the COMPETE 2020 - Operacional Programme for Competitiveness and Internationalisation (POCI), Portugal 2020, and by Portuguese funds through FCT - Fundação para a Ciência e a Tecnologia/ Ministério da Ciência, Tecnologia e Inovação in the framework of the project "Institute for Research and Innovation in Health Sciences" (POCI-01-0145-FEDER- 007274), and by UID/BIM/04293/2013.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

We thank Núcleo de Enteroparasitas/Centro de Parasitologia e Micologia at Instituto Adolfo Lutz for supplying S. mansoni cercariae for this study.

REFERENCES

[1] Botelho, M.C.; Ribeiro, R.; Vale, N. (...), Machado, J.C., Cor-reia Da Costa, J.M. Inactivation of estrogen receptor by Schis-tosoma haematobium total antigen in bladder urothelial cells. *Oncol. Rep.,* **2012**, *27*(2), 356-362.
[PMID: 22089035]

[2] Botelho, M.C.; Crespo, M.; Almeida, A.; Vieira, P.; Delgado, M.L.; Araujo, L.; Machado, J.C.; Correia da Costa, J.M. Schisto-soma haematobium and Schistosomiasis mansoni: production of an estradiol-related compound detected by ELISA. *Exp. Parasitol.,* **2009**, *122*(3), 250-253.
[http://dx.doi.org/10.1016/j.exppara.2009.04.001] [PMID: 19361503]

[3] Botelho, M.C.; Machado, J.C.; Brindley, P.J.; Correia da Costa, J.M. Targeting molecular signaling pathways of Schistosoma haemotobium infection in bladder cancer. *Virulence,* **2011**, *2*(4), 267-279.
[http://dx.doi.org/10.4161/viru.2.4.16734] [PMID: 21788729]

[4] Linda, J. *Heffner; Danny J. Schust. The Reproductive System at a Glance*; John Wiley and Sons, **2010**, p. 16.

[5] Frye, C.A. Steroids, reproductive endocrine function, and affect. A review. *Minerva Ginecol.,* **2009**, *61*(6), 541-562.
[PMID: 19942840]

[6] Botelho, M.C.; Machado, J.C.; da Costa, J.M. Schistosoma haema-tobium and bladder cancer: what lies beneath? *Virulence,* **2010**, *1*(2), 84-87.
[http://dx.doi.org/10.4161/viru.1.2.10487] [PMID: 21178421]

[7] Botelho, M.C.; Vale, N.; Gouveia, M.J.; Rinaldi, G.; Santos, J.; Santos, L.L.; Gomes, P.; Brindley, P.J.; Correia da Costa, J.M. Tumour-like phenotypes in urothelial cells after exposure to anti-gens from eggs of Schistosoma haematobium: an oestrogen-DNA adducts mediated pathway? *Int. J. Parasitol.,* **2013**, *43*(1), 17-26.
[http://dx.doi.org/10.1016/j.ijpara.2012.10.023] [PMID: 23260770]

[8] Santos, J.; Gouveia, M.J.; Vale, N.; Delgado, Mde.L.; Gonçalves, A.; da Silva, J.M.; Oliveira, C.; Xavier, P.; Gomes, P.; Santos, L.L.; Lopes, C.; Barros, A.; Rinaldi, G.; Brindley, P.J.; da Costa, J.M.; Sousa, M.; Botelho, M.C. Urinary estrogen metabolites and self-reported infertility in women infected with Schistosoma haematobium. *PLoS One,* **2014**, *9*(5)e96774
[http://dx.doi.org/10.1371/journal.pone.0096774] [PMID: 24848950]

[9] Hernández-Cervantes, R.; Quintanar-Stephano, A.; Moreno-Méndoza, N.; López-Griego, L.; López-Salazar, V.; Hernández-Bello, R.; Carrero, J.C.; Morales-Montor, J. Regulation of intestinal immune response by selective removal of the anterior, posterior, or entire pituitary gland in Trichinella spiralis infected golden ham-sters. *PLoS One,* **2013**, *8*(3)e59486
[http://dx.doi.org/10.1371/journal.pone.0059486] [PMID: 23555042]

[10] Saad, A.H.; Abdelbaky, A.; Osman, A.M.; Abdallah, K.F.; Salem, D. Possible role of Schistosoma mansoni infection in male hypogo-nadism. *J. Egypt. Soc. Parasitol.,* **1999**, *29*(2), 307-323.
[PMID: 10605485]

[11] Delmondes, L.M.; Cruz, M.A.; Guimarães, M.K.; Santana, L.G.; Gonçalves, V.P.; Brito, H.L. [Endocervical schistosomiasis: case report]. *Rev. Bras. Ginecol. Obstet.,* **2014**, *36*(6), 276-280.
[http://dx.doi.org/10.1590/S0100-720320140004827] [PMID: 25099468]

[12] Badiale, G.B.; Brandão, D.F.; Ribeiro-Silva, A. [Endocervical schistosomiasis]. *Rev. Soc. Bras. Med. Trop.,* **2005**, *38*(4), 351-352.
[http://dx.doi.org/10.1590/S0037-86822005000400015] [PMID: 16082486]

[13] Habib, F.A.; Zaki, M.M.; El-Aal, A.A.; Safwat, M.D.; Domiaty, M.A. Immunohistochemical expression of oestrogen and proges-terone receptors during experimental acute and chronic murine Schistosomiasis mansoni. *Rev. Soc. Bras. Med. Trop.,* **2010**, *43*(5), 531-535.
[http://dx.doi.org/10.1590/S0037-86822010000500012] [PMID: 21085864]

[14] Escobedo, G.; Roberts, C.W.; Carrero, J.C.; Morales-Montor, J. Parasite regulation by host hormones: an old mechanism of host exploitation? *Trends Parasitol.,* **2005**, *21*(12), 588-593.
[http://dx.doi.org/10.1016/j.pt.2005.09.013] [PMID: 16236553]

[15] Ryan, K.J. Biochemistry of aromatase: significance to female re-productive physiology. *Cancer Res.,* **1982**, *42*(8)(Suppl.), 3342s-3344s.
[PMID: 7083198]

[16] Patel, B.; Elguero, S.; Thakore, S.; Dahoud, W.; Bedaiwy, M.; Mesiano, S. Role of nuclear progesterone receptor isoforms in uter-ine pathophysiology. *Hum. Reprod. Update,* **2015**, *21*(2), 155-173.
[http://dx.doi.org/10.1093/humupd/dmu056] [PMID: 25406186]

[17] Gailly-Fabre, E.; Kerlan, V.; Christin-Maitre, S. [Pregnancy-associated hormones and fetal-maternal relations]. *Ann. Endocri-nol. (Paris),* **2015**, *76*(6)(Suppl. 1), S39-S50.
[http://dx.doi.org/10.1016/S0003-4266(16)30006-3] [PMID: 26776288]

[18] Shah, C.; Modi, D.; Gadkar, S.; Sachdeva, G.; Puri, C. Progester-one receptors on human spermatozoa. *Indian J. Exp. Biol.,* **2003**, *41*(7), 773-780.
[PMID: 15255380]

[19] Mooradian, A.D.; Morley, J.E.; Korenman, S.G. Biological actions of androgens. *Endocr. Rev.,* **1987**, *8*(1), 1-28.
[http://dx.doi.org/10.1210/edrv-8-1-1] [PMID: 3549275]

[20] Kasilima, Y.S.; Wango, E.O.; Kigondu, C.S.; Mutayoba, B.M.; Nyindo, M. Plasma bioactive LH and testosterone profiles in male New Zealand rabbits experimentally infected with Schistosoma mansoni. *Acta Trop.,* **2004**, *92*(3), 165-172.
[http://dx.doi.org/10.1016/j.actatropica.2004.06.004] [PMID: 15533284]

[21] Lansoud-Soukate, J.; Leonardelli, J.; Torpier, G.; Croix, D.; Ca-pron, A. [Role of Schistosoma mansoni bilharziasis in male hy-pogonadism]. *Pathol. Biol. (Paris),* **1991**, *39*(7), 681-685.
[PMID: 1758719]

[22] Abdalla, K.F.; Abdel-Aziz, S.M.; el Fakahany, A.F.; el-Hamshary, A.S.; Afifi, L.M. Effect of praziquantel on sex hormone levels in murine schistosomiasis mansoni. *J. Egypt. Soc. Parasitol.,* **1994**, *24*(3), 627-632.
[PMID: 7844428]

[23] Skelly, P.J.; Secor, W.E.; Reis, M.G.; Ramos, E.A.; Carmo, T.M.; Peixoto, E.E.; Harn, D.A. Failure of schistosomiasis to significantly decrease testosterone levels in Brazilian men. *Am. J. Trop. Med. Hyg.,* **1994**, *51*(1), 40-44.
[http://dx.doi.org/10.4269/ajtmh.1994.51.40] [PMID: 8059914]

[24] Shlomo Melmed. **2016***Williams Textbook of Endocrinology. El-sevier Health Sciences.,* , 710.

[25] Handelsman, D.J.; Conway, A.J.; Boylan, L.M. Suppression of human spermatogenesis by testosterone implants. *J. Clin. Endocri-nol. Metab.,* **1992**, *75*(5), 1326-1332.
[PMID: 1430094]

[26] Romano, M.C.; Jiménez, P.; Miranda-Brito, C.; Valdez, R.A. Para-sites and steroid hormones: corticosteroid and sex steroid synthesis, their role in the parasite physiology and development. *Front. Neu-rosci.,* **2015**, *9*, 224.
[http://dx.doi.org/10.3389/fnins.2015.00224] [PMID: 26175665]