Original article

Mosaic-cellular patterns of seminiferous tubules in rats gavaged *Momordica charantia* for four intervallic spermatogenic phases

Yama, OE.*, Noronha, CC. and Okanlawon, AO.

Department of Anatomy, College of medicine of the University of Lagos, P.M.B. 12003, Lagos, Nigeria
*E-mail: dro_yama@yahoo.com

Abstract

Introduction: The male contraceptive effect of the methanolic seed extract of *Momordica charantia* (MC) has been studied but the parallel microscopic testicular response is yet to be described. **Methodology:** A total of 100, 6-8 weeks' old male Sprague-Dawley (S-D) rats were used in this study. They were distributed into three groups A to C. Group A: treated daily with MC extract (50 mg.100 g^{-1}) based on their individual weights. Group B: rats were pre-treated with MC extract (50 mg.100 g^{-1}) between 8-40 weeks and later distilled water for 8 weeks. They were compared with rats in the control group C gavaged daily equal volumes of distilled water. The total experimental duration was 48 weeks. Rats were sacrificed after the last dose extract/distilled water was administered; the testes were harvested and processed for histology. **Results:** The testicular sections were compared to control. Group A showed a duration dependent distortions, with diminished tubular epithelium and luminal hypo-cellularity. The interstitial spaces appeared markedly reduced with few Leydig cells. The histological sections after cessation of extract administration showed a 'mosaic' pattern of increasing recovery towards baseline control. **Conclusion:** This study has demonstrated a time dependent reversible alterations in the morphologies seminiferous tubules of the testes treated with MC (50 mg.100 g^{-1}).

Keywords: *Momordica charantia*, Sprague-Dawley, testis, seminiferous tubule.

1 Introduction

Regardless of the momentous progress in contraceptive alternatives for women over past five decades, world population continues to flourish (MATZUK and LAMB, 2002; PAGE, AMORY and BREMNER, 2008). Scientists and activists' alike point to the devastating ecological impacts that population pressures have caused. These include global warming from the industrialized countries, starvation and disease in less developed nations (WANG and SWERDLOFF, 1999). Moreover half of all pregnancies are still unwanted or unplanned (HENSHAW, 1998). Clearly, there is a need for expanded, reversible, contraceptive options (PAGE, AMORY and BREMNER, 2008). Multicultural surveys demonstrate men's willingness to participate in contraception and enjoying full support from their spouses (PAGE, AMORY and BREMNER, 2008). An oral contraceptive from plant source seems to fit in perfectly because it allows the duos to control fertility without seeking advice from a health provider. This in turn increases the number of couples practicing family planning. Other advantages include the familiarity rural people have with herbal medicines, the fewer side effects associated with herbal preparations (CHAUDHURY 1993), their ready availability from local sources, and protection of privacy (CHAUDHURY 1993). There are many studies on herbal preparations in literature with male antifertility properties (RIAR, DEVAKUMAR, ILAVAZHAGAN et al., 1990; GU, MAO, WANG et al., 2000; MDHLULI and VANDER HORST 2002; AKPANTAH, OREMOSU, NORONHA et al., 2005).

In a brief review of previous work on *Momordica charantia* (MC) plant, a deliberate attempt was made to explore its contraceptive benefit. The effect of the seed extract on the rats' testes was understudied. It was discovered it decreased the testicular testosterone concentrations and testicular volume (YAMA, DURU, OREMOSU et al., 2011a) with an attendant decrease in sperm production (SP). In another study it was shown that the extract resulted in changes in the testicular oxidative status of the treated rats which played a role in testicular dysfunction that compromised the fertility of S-D rats (YAMA, DURU, OREMOSU et al., 2011b).

In this present study the investigative protocol was extended to centre on light microscopic studies on the seminiferous morphologies of S-D rats treated with MC extract for prolonged periods (8-40 weeks) and also possible reversibility.

2 Materials and methods

The *modus operandi* in this study matched the guiding principles for research relating animals as suggested by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (AMERICAN…, 2002). This was meticulously approved by the Departmental Ethical Committee responsible for the use of laboratory animals in conformity with international acceptable standards.

2.1 Extract pharmacognosy and LD_{50} determination

The plant materials (ripe fruits and seeds of MC) were procured from the local market in Lagos between June and July in the year. It was authenticated by Prof. J.D. Olowokudejo in the Botany/Microbiology Department of the University of Lagos. The plant (voucher no. FHI 108422) was deposited in the departmental forest herbarium. The

ripe fruits were plucked and desiccated between 30-38 °C in an oven. The dried seeds were then separated, sheaved and weighed ready for processing. Soxhlet extraction using absolute methanol as solvents was done at Pharmacognosy Department College of Medicine of the University of Lagos (CMUL). A percentage yield of 23.0% w/w was obtained, from which 50 mg.100 g^{-1} body weight of rat (suspended in distilled water) were prepared. Appropriate volumes based on the animal's individual weight were calculated by simple proportion and administered by gastric gavages. The dose used was arrived at, after prior determination of the LD_{50}. The LD_{50} for the oral route methanolic seed extract of MC was determined at 463.21 mg.100 g^{-1} body weight of rat from a Probit *vs.* Log dose curve.

2.2 Population, source and maintenance of animals

A total of 100 adult male S-D rats weighing 162 ± 52 g were used for the experiments. The rats, obtained from the Laboratory animal centre of CMUL were authenticated by a taxonomist in the department of Zoology of the University of Lagos (MALAKA, 2005, personal communication). They were kept in plastic cages in the animal room of the Department of Anatomy and allowed to acclimatize for two weeks under standard laboratory conditions of temperature 27-30 °C, with a photoperiodicity of twelve hours light alternating with twelve hours of darkness. They were fed with commercially available rat chow [Livestock feeds Plc. Ikeja, Lagos] and had access to water *ad libitum*.

2.3 Animal randomisation, experimental design and durations

The rats were arbitrarily allotted into three (3) groups A-C, made up of experimental (A and B) and control (C) groups. Groups A and B: comprised 5 sub-groups A_1-A_5 ($A_{8WK,}$ $A_{16WK,}$ $A_{24WK,}$ A_{32WK} and A_{40WK}) and B_1-B_5 ($B_{S8(R8)WK}$, $B_{S16(R8)WK}$, $B_{S24(R8)WK}$, $B_{S32(R8)WK}$ and $B_{S40(R8)WK}$). Group C (control): had 2 main groups C_S and C_R used to contrast events in A and B groups. They were divided further into 10 sub-groups ($C_{S8WK,}$ $C_{S16WK,}$ $C_{S24WK,}$ $C_{S32WK,}$ C_{S40WK} and C_{R16WK}, C_{R24WK}, C_{R32WK}, C_{R40WK} C_{R48WK}). It is important to note that in both experimental and control the sub-groups comprised 5 rats.

2.4 The experimental design was fragmented into 2 identical protocols

In protocol I, $suppression_{[S]}$ phase; the morphological effect of MC extract on the seminiferous tubules were studied. These involved animals in group A; treated daily with appropriate aliquots of MC based on their individual weights. They were then compared with rats in the control group C_S gavaged daily with distilled water. The total treatment duration was 8-40 weeks (as indicated in the respective sub-script).

In protocol II, $reversibility_{[R]}$ phase; testicular sections of animals fed MC extract were verified for possible recoveries at different points of reverse discontinuations estimated 8 weeks apart. They were initially pre-treated with MC extract from 8-40 weeks and later distilled water for 8 weeks. This means for each interval of extract treatment the rats were allowed 8 weeks period of recuperation. They were contrasted with rats in C_R (C_{R16WK}, C_{R24WK}, C_{R32WK}, C_{R40WK}, C_{R48WK}) gavaged distilled water daily. The total duration was 48 weeks.

The experimental durations were selected to correspond to intervals of the spermatogenic cycles of 8 weeks in rats (JEGOU, PINEAU and TOPPARI, 2002) and its multiples so as to study events in a complete cycle(s). Where required, morphologic association were adopted at similar intervals to address or eliminate the confounding effect of hormonal variations in the rats.

2.5 Orchidectomy and necropsy report

The sacrifices in both protocols were after the last dose was administered (i.e. at the end of durations 8, 16, 24, 32, 40, 48 weeks). The procedure was done under mild anaesthesia with intra-peritoneal injection of 7 mg.kg^{-1} body weight Ketamine HCl (SAALU, ADESANYA, OYEWOPO et al., 2007). The dose titrated against consciousness starting with 0.01 mL. The abdominal contents accessed via ventral incisions (laparotomy). The spermatic cord identified, testes delivered per abdomen aided by externally controlled scrotal traction. The harvested testes were weighed and prepared for histological processing.

2.6 Tissue processing for histological studies

The testes were carefully dissected of all fat and splodge dry to eliminate any blood. The testicular tissues were processed by the method described below with slight modification (GRETCHEN, 2009). The testes were then fixed in 10% formal saline and transferred to graded successions of ethanol. On day 1, they were placed in 70% alcohol for 7 hours, then relocated to 90% alcohol and left in the latter overnight. On day 2, the tissues were passed through three variations of absolute alcohol for an hour each then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58 °C. Three changes of molten paraffin wax at one-hour intervals were made, after which the tissues were embedded in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicular to the long axis of the testes. The sections were designated "vertical sections". Serial sections of 5 µm thick were obtained from a solid block of tissue, fixed on clean slides to which Mayer's egg albumin had been coated to cement the sections to the slides properly and later stained with haematoxylin and eosin stains, after which they were passed through a mixture of equal concentration of xylene and alcohol. Following clearance in xylene the sections were oven-dried between 35-40 °C.

3 Results and discussion

The anatomic-physiological relevance of the male gonad is verified in its capability to generate sperm cells which is crux on the integrity of the seminiferous tubules. In this present qualitative study, several testicular sections were at intervals viewed from rats based on its temporal adaptation to MC extract. The demonstrated morphologies of seminiferous tubules were correspondingly compared to those of their control and withdrawal counterparts (Figures 1 and 2). The sections from the experimental and control rats were taken at particular intermissions (and its multiples) which tallied

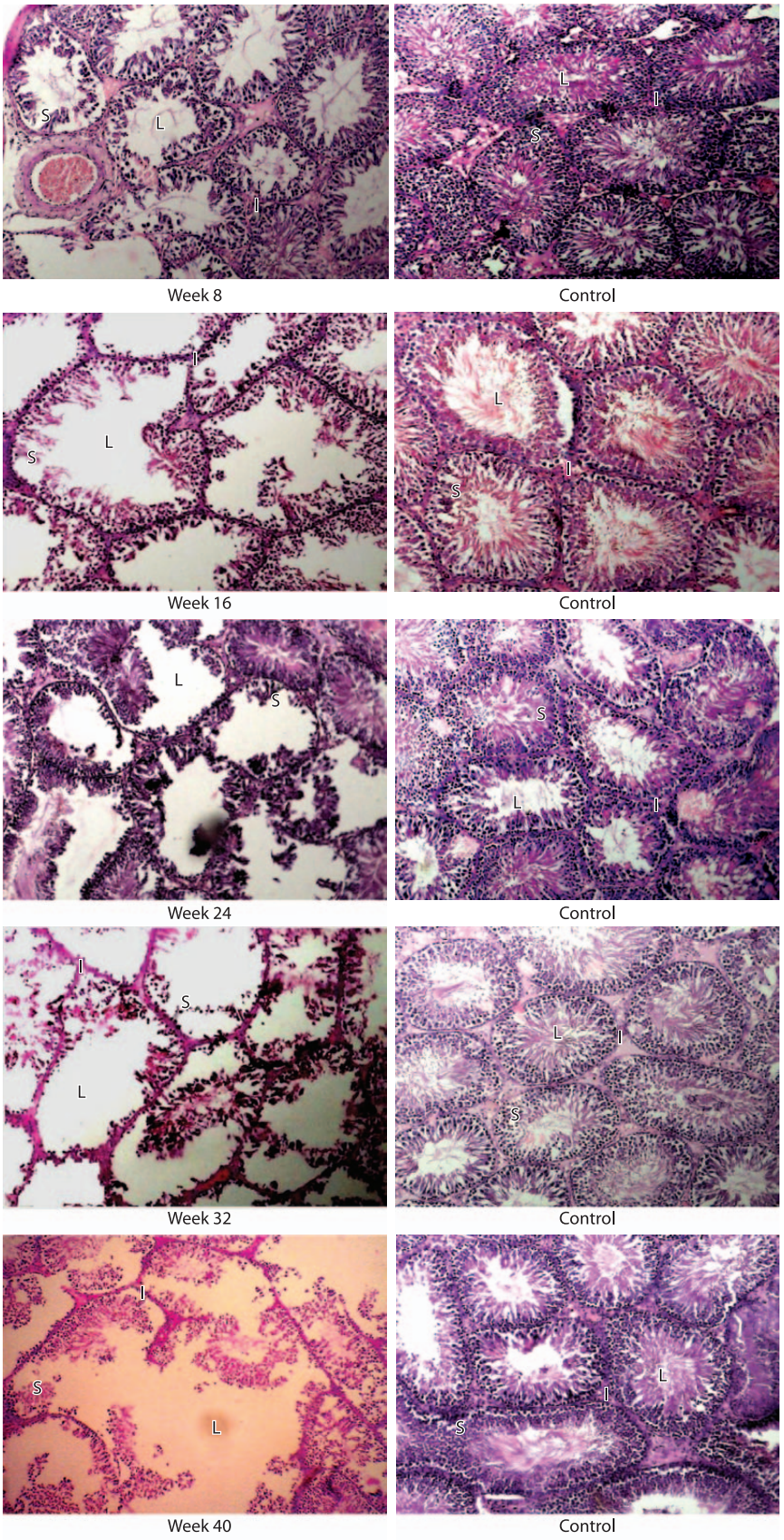

Figure 1. Seminiferous tubules taken from rats treated daily with 50 mg.100 g^{-1} body weight of *Momordica charantia* seed extract for 8, 16, 24, 32 and 40 weeks. Showing relative degrees of alterations compared to control gavaged daily with distilled water for similar durations respectively. Stains: Haematoxylin & Eosin; Mag. × 100; S: Spermatogenic series; L: Lumen of seminiferous tubule; I: Interstitium.

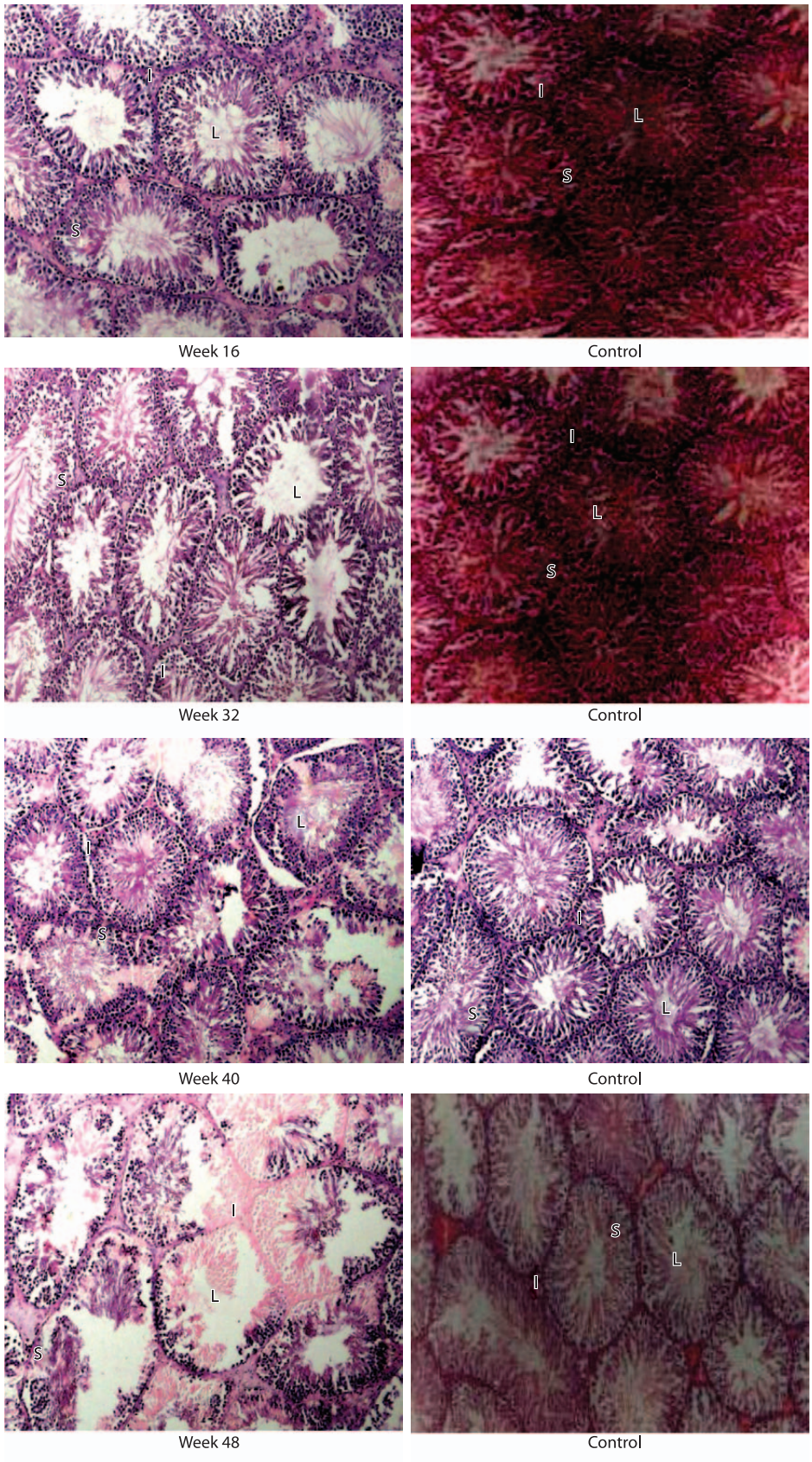

Figure 2. Seminiferous tubules taken from rats treated daily with 50 mg.100 g^{-1} body weight of *Momordica charantia* seed extract for 8, 16, 24, 32 and 40 weeks. They were later treated with distilled water daily for 8 weeks and sacrificed at 16, 24, 32, 40 and 48 weeks. Showing relative degrees of recovery compared to control. Stains: Haematoxylin & Eosin; Mag. × 100; S: Spermatogenic series; L: Lumen of seminiferous tubule; I: Interstitium.

with the period of time to complete a spermatogenic cycle (JEGOU, PINEAU and TOPPARI, 2002).

The cross-section of the seminiferous tubules (ST) of control rats showed profiles that were relatively oval or circular with normal epithelium. The germ cells well organised and stratified with all cells of the spermatogenic series represented. The tubular lumen enclosed a quantum of viable germ cells with numerous spermatozoa seen within (Figures 1 and 2). The cellular interstitium and cytoarchitectural skeleton were undistorted. This finding compared to previous experimental studies on the normal testes (OSINUBI, 2006) attesting to the fact that the testicular processing were unbiased.

In the suppression phase groups treated daily with MC extract, the histological sections of seminiferous tubules revealed very interesting findings. Sections from the rats sacrificed at the end of week 8, showed an oval outline, decreased tubular cellularity, as well as diminution in the interstitium (Figure 1). There were areas of focal necrosis, few to absent luminal spermatozoa and destruction of the spermatid layer. The sections from rats treated with the extract for longer durations (16, 24, 32 and 40 weeks), revealed tubular adaptations that depended on duration. These alterations were most marked in the testes as the weeks of extract administration increased (Figures 1). The ST epithelium for the 16th week show evidence of more destruction compared to the 8th week, also the tubular outline and alignment showed more relative irregularity (Figure 1). Further, extensive focal areas of degeneration in the ST with visibly absent viable germ cells in many areas (indicating marked hypospermiation and coagulative necrosis) were observed in sections from rats sacrificed at 24, 32 and 40 weeks. In the 32 and 40 weeks sections the nuclei of the cells were almost not visible; there were very scanty Leydig cells present compared to those in the earlier durations of the suppression phase. In *précis*, the histological testicular sections (compared to control) in the suppression phase animals showed a time dependent continuum of distortions, with diminished ST epithelium, loss of cellularity in the adluminal compartment leading to relative widening of the tubular lumen. The interstitial spaces appeared markedly reduced with few Leydig cells. The histological sections (together with sections from suppression phase) of animals taken at 16th, 24th, 32nd and 40th weeks, after cessation of administration of the extract (reversibility group) (Figure 2), viewed collectively at the different stages, show a 'mosaic' pattern of increasing resolution/recovery towards baseline control after 8 weeks (Figures 1 and 2). These findings compliment previous investigation where SP and testicular testosterone concentrations of rats fed MC 50 mg.100 g^{-1} significantly decreased (YAMA, DURU, OREMOSU et al., 2011a). A perceptive into the male gonadal functioning show the germinal epithelium produces sperm cells whereas the interstitial Leydig cells conscientious for testosterone required by the ST for cellular maturation and function (JOHN STEPHANIE and WILLIAM, 2006). It therefore means that this present study sheds further light to our previous study in which both SP and testicular testosterone were similarly compromised.

In conclusion from the compared micrographs, this study has demonstrated various mosaic-patterns produced as a consequence of MC (50 mg.100 g^{-1}) treatment. These responses were actual relative reversible distortions in the seminiferous tubular morphology that varied with interval. The reversibility clearly satisfies a main contraceptive requirement amongst others.

Acknowledgements: We wish to acknowledge Mr. ADELEKE of the Pharmacognosy Department Faculty of Pharmacy University of Lagos Nigeria for his assistance with the preparation of the herbal decoction and also support of this work. Also special thanks goes to the meticulous Dr. (Mrs.) DARAMOLA; consultant pathologist of the University of Lagos Teaching Hospital who made relevant comments on all the histological slides.

References

AKPANTAH, AO., OREMOSU, AA., NORONHA, CC., EKANEM, TB. and OKANLAWON, AO. Effects of Garcinia Kola seed extract on ovulation, oestrous cycle and foetal development in cyclic female Sprague-Dawley rats. *Nigerian Journal of Physiological Sciences*, 2005, vol. 20, n. 1-2, p. 58-62. PMid:17220929.

American Physiological Society. Guiding principles for research involving animals and human beings. *American Journal of Physiology – Regulatory Integrative and Comparative Physiology*, 2002, vol. 283, p. 281-283.

CHAUDHURY, RR. The quest for a herbal contraceptive. *National Medical Journal of India,* 1993, vol. 6, n. 5, p. 199-201. PMid:8241931.

GRETCHEN, L. *Animal Tissue Techniques.* Humason Los Alamos Scientific Laboratories, 2009. chap. 1-3, p. 3-39.

GU, ZP., MAO, BY., WANG, YX., ZHANG, RA., TAN, YZ., CHEN, ZX., CAO, L., YOU, GD. and SEGAL, SJ. Low dose gossypol for male contraception. *Asian Journal of Andrology*, 2000, vol. 2, n. 4, p. 283-287. PMid:11202417.

HENSHAW, SK. Unintended Pregnancy in the United States. *Family Planning Perspectives*, 1998, vol. 30, n. 1.

JEGOU, B., PINEAU, C. and TOPPARI, J. *Spermatogenesis in vitro in mammals.* In JONGE CJ. and BARRATT, CLR. *Assisted Reproductive Technology*: Accomplishments and New Horizons. Cambridge: Cambridge University Press, 2002. vol. 1, p. 3-60.

JOHN, KA., STEPHANIE, TP. and WILLIAM, JB. Drug Insight: recent advances in male hormonal contraception. *Nature Clinical Practice Endocrinology and Metabolism,* 2006, vol. 2, p. 32-4. PMid:17082810. http://dx.doi.org/10.1038/ncpendmet0069

MATZUK, MM and LAMB DJ. Genetic dissection of mammalian fertility pathways. *Nature Cell Biology*, 2002, vol. 4, p. 41-49.

MDHLULI, MC. and VANDER HORST, G. The effect of oleanolic acid on sperm motion characteristics and fertility of male Wistar rats. *Laboratory Animals*, 2002, vol. 36, n. 4, p. 432-437. PMid:12396287. http://dx.doi.org/10.1258/002367702320389107

OSINUBI, AA. *Quinine-induced cytoarchitectural and morphometric alterations in the testes of Sprague-Dawley rats.* Lagos: University of Lagos, 2006. [Tese de Doutorado].

PAGE, ST., AMORY, JK. and BREMNER, WJ. Advances in Male Contraception. *Endocrine reviews*, 2008; 29, n. 4, p. 465. http://dx.doi.org/10.1210/er.2007-0041

RIAR, S., DEVAKUMAR, C., ILAVAZHAGAN, G., BARDHAN, J., KAIN, AK., THOMAS, P., SINGH, R. and SINGH, B. Volatile fraction of neem oil as a spermicide. *Contraception*, 1990, vol. 42, n. 4, p. 479-487. http://dx.doi.org/10.1016/0010-7824(90)90054-Y

SAALU, LC., ADESANYA, AO., OYEWOPO, AO. and RAJI, Y. An evaluation of the deleterious effect of unilateral cryptorchidism on the contralateral normally descended testis. *Scientific Research and Essay*, 2007, vol. 2, n. 3, p. 74-78.

WANG, C. and SWERDLOFF, RS. Male contraception in the 21ST Century. In WANG, C. Male Reproductive Function. 1999. chapt. 16. Available from: <www.scribd.com/doc/51249039/Male-Reproductive-Function>.

YAMA, OE., DURU, FI., OREMOSU, AA., OSINUBI, AA., NOROHNA, CC. and OKANLAWON, AO. Sperm quotient in Sprague–Dawley rats fed graded doses of seed extract of *Momordica charantia*. *Middle East Fertility Society Journal*, 2011a, vol. 16, n. 2, p. 154-158.

YAMA, OE., DURU, FI., OREMOSU, AA., NORONHA, CC. Testicular oxidative stress in Sprague-Dawley rats treated with bitter melon (*Momordica charantia*): the effect of antioxidant supplementation. *Bangladesh Journal of Medical Science*, 2011b, vol. 10, n. 2.

Received August 23, 2011
Accepted November 11, 2011