Anim. Reprod., v.6, n.4, p.509-515, Oct./Dec. 2009

Aqueous stem-bark extract of *Ficus sycomorus* increases sperm production and pH of sperm microenvironment in growing albino rat

N.A. Igbokwe[1,*], U.K. Sandabe[1], S. Sanni[1], B. Wampana[1], I.M. Wiam[2], I.O. Igbokwe[3]

[1]Department of Veterinary Physiology, Pharmacology and Biochemistry, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria.
[2]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Maiduguri, Maiduguri, Nigeria.
[3]Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Maiduguri, Maiduguri, Nigeria.

Abstract

Sixty post-pubertal male albino rats, above 50 days of age and weighing 98.0 ± 22.9 g, were used to study the effects of prolonged oral administration of aqueous extract of *Ficus sycomorus* stem bark on sperm cell production and pH of sperm microenvironment. They were divided into four groups of 15 each and treated through an esophago-gastric tube at doses of 0, 200, 400 and 600 mg/kg, respectively, using extract concentration of 200 mg/ml for 30 days. Five rats from each group were euthanized on days 10, 20, and 30 of treatment and the testes were dissected out. Sperm head counts (SHCs) were estimated from testicular and epididymal homogenates by using hemocytometer and the pH of the homogenates were determined with a pH meter. The testicular and epididymal (head, body, tail, total) SHCs increased during the period of treatment with significant ($P < 0.01$) time-dependent linear trends which were apparently higher in the treated ($r^2 = 0.70$-0.91) than control ($r^2 = 0.50$-0.54) animals. Treatment effects were not observed between days 10 and 20 of treatment until day 30 when only testicular and total epididymal SHCs significantly ($P < 0.05$) increased in dose-dependent linear trends which were higher for testicular SHCs ($r^2 = 0.53$; $P < 0.01$) than total epididymal SHCs ($r^2 = 0.17$; $P < 0.01$). The treatment also significantly ($P < 0.05$) increased the pH of testicular and epididymal (head, body, tail) homogenates on days 20 and 30 of treatment with dose-dependent linear trends which were higher on day 30 ($r^2 = 0.52$-0.81; $P < 0.05$) than day 20 ($r^2 = 0.28$-0.59; $p < 0.05$) of treatment. At 400-600 mg/kg doses of treatment, the pH of the testicular and epididymal homogenates increased with time-dependent linear trends ($r^2 = 0.42$-0.85; $P < 0.05$), while no significant ($P > 0.05$) variations occurred in the same parameters in the controls. It was concluded that the extract increased sperm cell production and pH of homogenates of testes and epididymes of albino rats.

Keywords: albino rat, *Ficus sycomorus* extract, sperm, testicular and epididymal pH,

Introduction

Spermatogenesis in the rat is similar to that of other mammals (Hernandez *et al*., 2007). The time of onset of puberty is day 25 after birth and by day 53, the rat starts to produce mature spermatozoa (Bourguignon *et al.,* 1992; Gleen and Levine, 2003). The duration of spermatogenesis in rats ranges from 51.6-56 days (Rosiepen *et al.,* 1994*,* 1995; Penida, 2003). Spermatogenesis extends over 4 cycles of seminiferous epithelium (Penida, 2003) and each cycle of seminiferous epithelium ranges from 12-13 days (Aslam *et al.,* 1999; Penida, 2003). There are 14 stages of cells in spermatogenesis in rat as defined by the number of morphologically recognizable germ cell associations within the testes (Russel *et al.,* 1990; O'Donnel *et al.*, 2001).

Quantitative evaluation of spermatogenesis may be done by counting of sperm cells in the ejaculate, cauda epididymis or testicular homogenate (Berndston, 1977; Collins *et al*., 2006). Testicular sperm head count (SHC) has a direct relationship to fertility and can be applied to the quantitative assessment of reproductive risk (Meistrich, 1989). Epididymal sperm cell count is linearly related to fertility (Chapin *et al*., 1997). However, it must be noted that testicular and epididymal SHCs, as indicators of spermatogenesis, do not provide information on sperm cell motility, fertilizing potential or genetic integrity when a biologically active substance has been administered; and any substance being evaluated for its effect on spermatogenesis should be administered for a prolonged period to affect sperm cell production to the extent that the effect may be quantified by SHCs (Collins *et al*., 2006).

The pH of the sperm microenvironment varies in the testis, excurrent duct, ejaculate and the female reproductive tract. The luminal pH is higher in the efferent ductules of the rat than in the epididymis (Newcombe *et al*., 2000). The pH of the fluid in the seminiferous tubules and rete testis of rats is about 7.4 (Tuck *et al*., 1970; Levine and Marsh, 1971), but decreases to about 6.5 in the epididymal head (Levine and Marsh, 1971). Acidification of fluid leaving the testis occurs primarily in the initial segment and to a lesser extent in the intermediate zone of the epididymis (Levine and Kelly, 1978). The acidic luminal pH of the epididymis keeps the spermatozoa in an immotile quiescent state during maturation and storage (Setchell *et al*., 1993; Shum *et al*., 2009) because of the activation of a pH-

*Corresponding author: nanaigb@yahoo.com
Received: May 5, 2009
Accepted: December 14, 2009

dependent inhibitory factor (Acott and Carr, 1984). The nominal pH of seminal plasma in man is 7.0 and its buffering agents are provided by the vesicular gland, but pH of 7.2-7.8 is required for sperm motility (WHO, 1999). The seminal plasma pH in most domestic animals is 6.7-7.4 (Roberts, 1986). The vaginal fluid pH in women is ≤5, but the seminal ejaculate has the potential to neutralize the vaginal acidity and prevent sperm immobilization after coitus (Suarez and Pacey, 2006). Thus, reduction of sperm acidity is necessary for the commencement and sustainability of sperm motility; and factors which promote a drift toward increasing pH may enhance sperm motility and fertility, since sperm hyperactivation is promoted by alkaline-stimulated calcium ion influx (Marquez and Suarez, 2007).

Folkloric information is available on the use of *Ficus sycomorus* extracts in the treatment of infertility and sterility in humans (Malgras, 1992; Pakia and Cooke, 2003; Kone and Atindehou, 2008). *Ficus capensis Thunb*. extract was used in the treatment of azoospermia (Gelfand *et al.,* 1985). *Ficus asperifolia* extract was reported to have an estrogenic effect in female rats which supported increases in the number of implantation sites and the litter size (Watcho *et al.,* 2009). The aqueous extract of *Ficus sycomorus* stem bark was reported to contain pharmacologically active substances such as gallic tannins, saponins, reducing sugars, alkaloids and flavone aglycones, and was relatively safe in rats with LD_{50} of 720 mg/kg, causing no haematological, hepatic and renal toxicities (Sandabe, 2002; Sandabe *et al.,* 2006), but the male reproductive effect of the extract has not been studied. Folkloric information from the local communities in Borno State, Nigeria, indicates that both men and women use the aqueous stem-bark extracts to treat infertility. It is not clear whether the plant extract has phytoandrogens and/or phytoestrogens. However, we speculate that the constituents of the extract may have gonadotrophic activities which affect both male and female reproductive functions.

The present study is the first experimental assessment of the effects of prolonged oral administration of the aqueous extract of *Ficus sycomorus* stem bark in rats on sperm cell production, evaluated by SHCs, and sperm microenvironment, estimated by the pH of homogenates of the tissue source of sperm.

Materials and Methods

Animals

Sixty post-pubertal male albino rats, above 50 days of age and weighing 98.0 ± 22.9 g, were selected from Ayuba Animal Farm, Maiduguri, 14 days prior to commencement of the experiment. They were housed in standard plastic cages containing 5 rats each with comparable mean live body weights and kept in a room with ambient temperature not exceeding 30°C. They were fed *ad libitum* with commercial pelletized feed (Vital Feed, Jos). Clean drinking water was provided freely in nipple drinkers.

Plant identification and extraction

The tree of *Ficus sycomorus* was identified by a taxonomist in the Department of Biological Sciences, Faculty of Science, University of Maiduguri, Maiduguri, Nigeria. The tree, which is perennial, is planted for shade in the sahel region of the country. It grows to 20 m tall and 6 m wide with a dense round crown of spreading branches, green-yellow to orange bark and heart-shaped leaves having round apex (Sandabe, 2002; Ficus, 2009).

The stem bark was chopped off and air dried away from direct sunlight for eight days. The dried stem bark was pounded into powdered form using pestle and mortar. The powdered stem bark (550 g) was dissolved in distilled water (3000 ml) in a round bottom flask. The mixture was refluxed for 2 hours and filtered while hot with No. 1 Watman filter paper. The refluxing was done three times using fresh distilled water each time. After filtration, the filtrate was poured on a tray and placed in a hot air oven at 40-50°C for 2 hours for the water to evaporate. The dried sample left on the tray was weighed and used for the experiment.

Experimental design

The rats were divided into four groups of 15 rats each. The concentration of the extract used was 200 mg/ml (Sandabe, 2002). Rats in group A, B and C were administered orally through an esophago-gastric tube (Onu *et al.,* 2007) with 200 mg/kg, 400 mg/kg and 600 mg/kg, respectively, of the extract daily for 30 days. Rats in group D served as control and were sham-treated. Five rats from each group were euthanized on days 10, 20 and 30 of treatment by decapitation through cutting the cervical blood vessels with a sharp scalpel blade (Close *et al*., 1997). The testes and epididymes were dissected out.

Sperm head counts

The tunica albuginea was removed from each testis before its homogenization in 5 ml of normal saline (Dana, Ilorin). The head, body and tail of the epididymes were separately homogenized in 2 ml of normal saline. The sperm head count per milliliter of the homogenate was done using a hemocytometer (Almquist and Amann, 1961; Amann and Lambaise, 1969; Foote, 1969; Igboeli and Cardoso, 1984; Kwari, 1990; Seung *et al*., 2003; Wannang *et al.,* 2008; Parrish, 2009). The total sperm head count per homogenate was determined using the formula: (Volume of homogenate) x (Count in 5 squares) x (0.05×10^6).

pH of homogenates

A pH meter (ExStik™, Extech Instrument Corp., Waltham, MA, USA, http://www.extech.com) was used to determine the pH of homogenized samples of the testes, and head, body and tail of the epididymes.

Statistical analyses

The data obtained are presented as means ± standard deviations and variations in means were assessed by one-way analysis of variance (ANOVA) with Dunnet or Tukey post-test, and coefficient of determinations, r^2 , for linear trends in relation to time and dose (as independent variables) were determined using computer software (GraphPad Instat, 1993 version, http//:www.graphpadinstat.com).

Results

Sperm head output

The testicular and epididymal (head, body, tail, total) SHCs during the period of treatment are presented in Tables 1 and 2, respectively. Treatment effects were not observed between 10 and 20 days of treatment. At 30 days of treatment, testicular and total epididymal SHCs significantly ($P < 0.05$) increased in dose-dependent linear trend, which was higher for testicular SHCs ($r^2 = 0.53$; $P < 0.01$) than total epididymal SHCs ($r^2 = 0.17$; $P < 0.01$). All the SHCs at all doses increased with significant ($P < 0.01$) time-dependent linear trends which were higher in the treated ($r^2 = 0.70$-0.91) than control ($r^2 = 0.50$-0.54) animals.

Table 1. Effect of oral administration of aqueous extract of *Ficus sycomorus* stem bark on testicular sperm head counts ($x10^6$) in albino rats.

	Dose (mg/kg)			
Days of treatment	Control	200	400	600
10	14.4 ± 3.1[a]	17.3 ± 5.8[a]	17.7 ± 4.0[a]	14.4 ± 2.5[a]
20	15.8 ± 6.7[a]	29.8 ± 11.2[a]	27.0 ± 12.2[a]	27.7 ± 6.5[a]
30	36.0 ± 11.6[a]	44.0 ± 5.0[ab]	48.5 ± 5.6[b]	54.8 ± 3.1[b]

[a,b] Means ± standard deviations with different superscripts in rows are significantly different ($P < 0.05$).

Table 2. Effect of oral administration of aqueous extract of *Ficus sycomorus* stem bark on epididymal sperm counts (10^6) in albino rats.

		Dose (mg/kg)			
Epididymal tissue	Days of treatment	Control	200	400	600
Head	10	3.1 ± 2.3[a]	3.0 ± 3.0[a]	2.2 ± 0.4[a]	2.8 ± 2.4[a]
	20	3.8 ± 2.1[a]	7.8 ± 3.2[a]	8.1 ± 4.8[a]	7.5 ± 3.0[a]
	30	15.2 ± 7.0[a]	21.6 ± 0.8[a]	19.5 ± 2.0[a]	21.0 ± 3.1[a]
Body	10	1.2 ± 0.4[a]	1.4 ± 1.1[a]	1.3 ± 0.2[a]	1.0 ± 0.2[a]
	20	1.9 ± 1.8[a]	6.2 ± 2.8[a]	5.5 ± 3.3[a]	8.5 ± 5.7[a]
	30	8.9 ± 5.0[a]	12.8 ± 2.3[a]	16.6 ± 6.2[a]	16.1 ± 3.8[a]
Tail	10	7.0 ± 2.7[a]	4.2 ± 3.8[a]	6.0 ± 1.5[a]	4.9 ± 3.1[a]
	20	9.3 ± 1.8[a]	12.8 ± 6.7[a]	13.7 ± 9.3[a]	8.7 ± 4.1[a]
	30	19.4 ± 7.6[a]	24.0 ± 0.9[a]	24.9 ± 1.8[a]	25.0 ± 1.2[a]
Total	10	11.2 ± 4.9[a]	8.3 ± 7.5[a]	9.5 ± 1.7[a]	8.8 ± 5.5[a]
	20	14.2 ± 7.4[a]	26.8 ± 12.3[a]	23.1 ± 12.7[a]	24.7 ± 10.1[a]
	30	43.5 ± 19.0[a]	58.4 ± 2.6[ab]	61.6 ± 8.1[b]	62.1 ± 3.5[b]

[a,b] Means ± standard deviations with different superscripts in rows are significantly different ($P < 0.05$).

pH of testicular and epididymal homogenates

The effects of treatment on pH of testicular and epididymal (head, body, tail) homogenates are presented in Tables 3 and 4, respectively. In the untreated control rats, the pH of testicular and epididymal homogenates did not vary significantly ($P > 0.05$). The administration of the extract significantly ($P < 0.05$) increased the pH of testicular and epididymal (head, body, tail) homogenates on days 20 and 30 of treatment with significant ($P < 0.05$) dose-dependent linear trends which were higher on day 30 ($r^2 = 0.52$-0.81) than day 20 ($r^2 = 0.28$-0.59). Also, the pH of the testicular and epididymal homogenates of treated rats significantly ($P < 0.05$) increased with time-dependent linear trends ($r^2 = 0.42$-0.85; $P < 0.001$) at 400-600 mg/kg doses of treatment.

Table 3. Effect of oral administration of aqueous extract of *Ficus sycomorus* stem bark on the pH of testicular homogenates of albino rats.

Days of treatment	Dose (mg/kg)			
	Control	200	400	600
10	6.6 ± 0.0[a]	6.6 ± 0.1[a]	6.6 ± 0.1[a]	6.9 ± 0.1[a]
20	6.6 ± 0.0[a]	6.8 ± 0.2[a]	7.0 ± 0.2[b]	7.1 ± 0.1[b]
30	6.7 ± 0.1[a]	6.9 ± 0.1[b]	7.1 ± 0.1[bc]	7.2 ± 0.1[bc]

[a,b] Means ± standard deviations with different superscripts in rows are significantly different (P < 0.05).

Table 4. Effect of oral administration of aqueous extract of *Ficus sycomorus* stem bark on pH of epididymal homogenates of albino rats.

	Days of treatment	Dose (mg/kg)			
		Control	200	400	600
Head	10	6.5 ± 0.1[a]	6.4 ± 0.0[a]	6.5 ± 0.1[a]	6.8 ± 0.1[a]
	20	6.7 ± 0.1[a]	7.0 ± 0.2[b]	6.9 ± 0.1[a]	7.1 ± 0.2[b]
	30	6.7 ± 0.1[a]	6.8 ± 0.0[a]	7.1 ± 0.1[b]	7.1 ± 0.1[b]
Body	10	6.5 ± 0.0[a]	6.5 ± 0.1[a]	6.4 ± 0.1[a]	6.7 ± 0.1[a]
	20	6.7 ± 0.1[a]	7.0 ± 0.2[b]	6.9 ± 0.1[a]	7.0 ± 0.1[b]
	30	6.7 ± 0.2[a]	6.8 ± 0.1[ab]	7.0 ± 0.1[b]	7.0 ± 0.0[b]
Tail	10	6.5 ± 0.1[a]	6.4 ± 0.0[a]	6.4 ± 0.1[a]	6.7 ± 0.1[a]
	20	6.6 ± 0.1[a]	7.0 ± 0.2[b]	7.0 ± 0.1[b]	7.0 ± 0.1[b]
	30	6.6 ± 0.1[a]	6.8 ± 0.1[b]	7.0 ± 0.1[bc]	7.0 ± 0.0[bc]

[a,b,c] Means ± standard deviations with different superscripts in rows are significantly different(P < 0.05).

A comparison of the pooled means of the pH of testicular and epididymal homogenates during treatment is presented in Table 5. At the treatment doses of 200-400 mg/kg and in the controls, no significant differences occurred between the pooled mean of the pH of any part of the epididymal homogenate when compared with that of the testicular homogenate. However, the pH of the epididymal homogenates was lower (P < 0.05) than that of the testicular homogenate when the treatment was at 600 mg/kg, suggesting the acidification of the sperm microenvironment toward the epididymal tract, even though the treatment had increased the pH of both testicular and epididymal homogenates as shown in Tables 3 and 4.

Table 5. Comparison of the pooled means of the pH of testicular and epididymal homogenates of albino rats during oral administration of aqueous extract of *Ficus sycomorus* stem bark.

Dose (mg/kg)	pH of homogenate (n = 15, pooled from 10, 20 and 30 days of treatment)			
		Epididymis		
	Testis	Head	Body	Tail
Control	6.64 ± 0.06[a]	6.64 ± 0.12[a]	6.62 ± 0.15[a]	6.58 ± 0.10[a]
200	6.72 ± 0.24[a]	6.76 ± 0.29[a]	6.77 ± 0.28[a]	6.71 ± 0.25[a]
400	6.87 ± 0.28[a]	6.78 ± 0.38[a]	6.79 ± 0.29[a]	6.74 ± 0.29[a]
600	7.05 ± 0.19[a]	6.88 ± 0.15[bc]	6.88 ± 0.15[bc]	6.91 ± 0.15[ac]

[a,b,c] Means ± standard deviations with different superscripts in rows are significantly different (P < 0.05).

Discussion

Aqueous stem-bark extract of *Ficus sycomorus* might have increased sperm cell production in the rats by stimulating the hypothalamus-pituitary-testicular axis and this could only be ascertained through a subsequent endocrine study. A significant effect of the extract was observed at day 30 of treatment, because increased spermatogenesis was possible after at least 2 cycles of the seminiferous epithelium; one cycle being 12-13 days (Aslam *et al.,* 1999; Penida, 2003). Since the duration of spermatogenesis in rats ranges from 51.6-56.0 days (Rosiepen *et al.,* 1994*,* 1995; Penida, 2003), it was most probable that the extract did not act only on the dormant spermatogonia at the beginning of each cycle, but acted at intermediate stages of spermatogenesis to boost sperm production.

The increased sperm cell production of control rats suggested that the capacity of the seminiferous tubules to produce sperm was enhanced, probably by increase in tubular length and diameter. Treatment with the extract exaggerated the time-dependent increase in

sperm cell production as shown by higher coefficients of determination, an observation that suggested that the extract stimulated faster testicular growth through expansion of seminiferous tubular function, perhaps because of increased spermatogenic stimulus. Also, a dose-dependent effect of the extract indicated a graded potency of the active components responsible for the increased sperm cell production.

Normal saline (0.9% sodium chloride) was used to homogenize the testicular and epididymal tissues during sperm extrusion. The hydrogen ion concentrations, pH, of the homogenates estimated to assess the microenviromental effect of the extract on sperm was contributed to by the normal saline, which has a nominal pH of 5.5. Intravenous normal saline fluid was reported to have pH of 5.0 (Steele and Story, 2000), 5.9 (Anshu *et al*., 1985) or 5.5-6.5 (Breborowicz and Oreopoulos, 2005), and has an acidifying potential after intravenous infusion even as an isotonic solution (Ho *et al*., 2001; Criss, 2007). Thus, the homogenate fluid was acidified by the normal saline since it contained no buffer and might not reflect the true pH of the testicular and epididymal tissues.

The increase in pH of all the homogenates over time could be attributed primarily to the process of testicular maturation and invigoration of production of motile and fertile spermatozoa and secondarily, to the extract administration. The pH changes in the testicular tissues may arise from alterations in the pH of the extracellular fluid, but the pH of the epididymal fluid is adjusted by the clear cells of the epithelium that secrete high levels of protons contributing to the luminal acidification and the principal cells secreting bicarbonate (Shum *et al*., 2009). This study showed strong evidence that the extract increased the pH of the homogenates, an effect on the sperm microenvironment that might be considered pro-spermatic since decrease in pH in seminiferous tubules and epididymal lumen was deemed anti-spermatic (Caflisch, 1992).

Non-motile sperm cells freshly extruded from the rat caudal epididymis could be initiated to full motility immediately after diluting with normal saline, an event that was dependent on the pH, viscosity and osmolality (Morita and Chang, 1971; Chulavatnatol, 2008). The motility of epididymal sperm cell was initiated in diluents at pH of 4-8 (Chulavatnatol, 2008). The pH of bovine caudal epididymal sperm was approximately 5.8 and the motility of the sperm cells increased with elevation of the pH of the fluid (Acott and Carr, 1984). The sperm cell requires the alkaline medium to remain motile in the course of migration through the acidic vaginal fluid to reach the oocyte location, and to engage in the process of hyperactivation required for eventual fertilization to occur (Suarez and Pacey, 2006; Marquez and Suarez, 2007). However, the quiescent state of the sperm cell engendered by epididymal fluid acidification may be undermined if alkalinizing activity of the extract is not moderated, causing the early initiation of motility which may reduce the life span of the sperm cell during storage in the caudal epididymis. The pH of the testicular homogenates was higher than that of epididymal homogenates when the treatment was at 600 mg/kg, indicating that the acidifying potential of the epididymis was activated to moderate the alkalinizing role of the extract to favour the quiescent state.

Although the data provided in the present work appears to justify the folkloric use of the extract in treating male infertility, the validity of its usefulness needs to be further ascertained by assessing the effect of the extract on the endocrine profiles relating to the hypothalamus-pituitary-testicular axis, sperm motility and fertilizing ability, genetic preservation, libido, fecundity of co-habiting females and viability of offspring. Meanwhile, it could be concluded that oral administration of aqueous stem-bark extract of *Ficus sycomorus* to albino rats for 30 days led to a dose-dependent increase in sperm cell production and pH of testicular and epididymal homogenates, suggesting that the extract might have a pharmacologically active substance capable of affecting male reproduction.

Acknowledgments

Our appreciations go to Mr. Fine Akawo for the extraction of the stem bark in the Department of Chemistry, University of Maiduguri, Maiduguri. The project was privately funded by one of the authors under the Strategic Animal Research Initiative, Nigeria.

References

Acott TS, Carr DW. 1984. Inhibition of bovine spermatozoa by caudal epididymal fluid: II. Interaction of pH and a quiescence factor. *Biol Reprod*, 30:926-935.

Alamquist JO, Amann RP. 1961. Reproductive capacity of dairy bulls. II. Gonadal and extragonadal sperm reserves as determined by direct counts and depletion trials, dimensions and weight of genitalia. *J Dairy Sci*, 44:1668-1678.

Amann RP, Lambiase JT. 1969. The male rabbit. III. Determination of daily sperm production by means of testicular homogenates. *J Anim Sci*, 28:369-374.

Anshu JS, Endahl GL, Mottley JL. 1985. Microwave heating of intravenous fluids. *Am J Emerg Med*, 3:316-319.

Aslam H, Rosiepen G, Krishnamarthy H, Arslan H, Clemen G, Nieschlag E, Weinbauer GF. 1999. The cycle duration of the seminiferous epithelium remains unaltered during GnRH antagonist-induced testicular involution in rats and monkeys. *J Endocrinol*, 161:282-288.

Berndston WE. 1977. Methods of quantifying mammalian spermatogenesis: a review. *J Anim Sci*, 44:818-823.

Bourguignon JP, Gerard A, Acvarez Gonzales ML,

Franchimont D. 1992. Neuroendocrine mechanism of onset of puberty. Sequential reduction in activity of inhibitory and facilitatory N-methy-D-aspartate receptors. *J Clin Invest*, 90:1736-1744.

Breborowichz A, Oreopoulos DG. 2005. Is normal saline harmful to the peritoneum? *Perit Dial Int*, 25(suppl. 4):367-370.

Caflisch CR. 1992. Acidification of testicular and epididymal fluids in the rat after surgically-induced varicocele. *Int J Androl*, 15:238-245.

Chapin RE, Sloane RA, Haseman JK. 1997. The relationships among reproductive endpoints in Swiss mice using reproductive assessment by continuous breeding database. *Fundam Appl Toxicol*, 38:129-142.

Chulavatnatol M. 2008. Motility initiation of quiescent spermatozoa from rat caudal epididymis: effects of pH, viscosity, osmolality and inhibitors. *Int J Androl*, 5:425-436.

Close B, Banister K, Bauman V, Bernoth E-M, Bromage N, Bunyan J, Erhardt W, Flecknell P, Gregory N, Hackbarth H, Morton D, Warwick C. 1997. Recommendations for euthanasia of experimental animals: Part 2. *Lab Anim*, 31:1-32.

Collins TFX, Sprando RL, Shackleford MT, Gruber MF, Morse DE. 2006. Principles of risk assessment-FDA perspective. *In*: Hood RD (Ed.). *Developmental and reproductive toxicology:* A practical approach. 2nd ed. Boca Raton, FL: CRC Press. pp. 877-910.

Criss EA. 2007. *A comparison of normal saline and lactated Ringers's solution in the treatment of shock*. Tucson, AZ: University of Arizona. 57pp. MSc Project.

Ficus sycomorus. 1999. *In*: Wikipedia. Available from: http://en.wikipedia.org/wiki/Sycamore-fig. Accessed on: Sept. 5, 2009.

Foote RH. 1969. Research techniques to study reproductive physiology in the male. *In*: Techniques and procedures in animal science research. Champaign, IL: American Society of Animal Science. pp. 80-110.

Gelfand M, Mavi S, Drummond RB, Ndemera B. 1985. *The traditional medicinal practitioner in Zimbabwe*. Gweru, Zimbabwe: Mambo Press. 411pp.

Glenn CH, Levine JE. 2003. Pubertal acceleration of pulsatile gonadotropin-releasing hormone release in male rats as revealed by microdialysis. *Endocrinology*, 144:163-171.

Hernandez ME, Abraham S, Gonzalo EA, Rosaura D, Fausto R, Luis IG, Toledo R, Manzo J. 2007. A study of the prostate, androgens and sexual activity of male rats. *Reprod Biol Endocrinol*, 5:11. (abstract).

Ho AMH, Karmaker MK, Contardi LH, Ng SSW, Hewson JR. 2001. Excessive use of normal saline in managing traumatized patients in shock: a preventable contribution to acidosis. *J Trauma*, 51:173-177.

Igboeli G, Cardoso FM. 1984. Gonadal and extragonadal sperm reserves of indigenous Central African bulls. *J Reprod Fertil*, 25:107-109.

Kone WM, Atindehou KK. 2008. Ethnobotanical inventory of medicine in northern Cote d'Ivore (West Africa). *S Afr J Bot*, 74:76-84.

Kwari HD. 1990. *Some reproductive parameters in male M'Borro, Kuri. Borno White and Balami breeds of indigenous Nigerian livestock*. Ibadan: University of Ibadan. Dissertation.

Levine N, Marsh DJ. 1971. Micropuncture studies of the electrochemical aspects of fluid and electrolyte transport in individual seminiferous tubules, the epididymis and the vas deferens in rats. *J Physiol*, 213:557-576.

Levine N, Kelly H. 1978. Measurement of pH in the rat epididymis in vivo. *J Reprod Fertil*, 52:333-335.

Malgras D. 1992. *Arbres et arbustes guérisseurs des savanes maliennes*. Paris: Ed. Karthala, 480pp. Available from: http://www.metafro.be/prelude/view_plant?pi=05960. Accessed on: Oct. 28, 2008.

Marquez B, Suarez S. 2007. Bovine sperm hyperactivation is promoted by alkaline stimulated Ca^{2+} influx. *Biol Reprod*, 76:660-665.

Meistrich ML. 1989. Evaluation of reproductive toxicity by testicular sperm head counts. *Int J Toxicol*, 8:551-567.

Morita Z, Chang MC. 1971. Maintenance of the motility of rat epididymal spermatozoa in the presence of male accessory secretions. *J Reprod Fertil*, 24:247-254.

Newcombe N, Clulow J, Man SY, Jones RC. 2000. pH and bicarbonate in the ductuli efferences testis of the rat. *Int J Androl*, 23:46-50.

O'Donnel L, Robertson KM, Jones ME, Simpson ER. 2001. Estrogen and spermatogenesis. *Endocr Rev*, 22:289-318.

Onu E, Oke BO, Ozegbe PC, Oyewale JO. 2007. Effects of alcohol on testicular weight and testosterone levels in Wistar rats exposed during gestation and lactation. *In*: Proceedings of the 44th Annual Congress of the Nigerian Veterinary Medical Association, 2007, Effurun, Delta State: NVMA. pp. 116-121.

Pakia M, Cooke JA. 2003. The ethnobotany of Midzichenda tribes of the coastal forest areas in Kenya: 2. Medicinal plant uses. *S Afr J Bot*, 69:328-395.

Parrish J. 2009. Determining the concentration of sperm using a hemocytometer. Available from: http://www.ansci.wisc.edu/jjp1/ansci_repro/lab/procedures/hemacytometer/Hemocytometer%20use.html. Accessed in: Feb. 6, 2009.

Penida MH. 2003. *McDonald's Veterinary Endocrinology*. 5th ed. Ames, IA: Iowa State Press. pp. 220-253.

Roberts SJ. 1986. *Veterinary Obstetrics and Genital Diseases*. 3rd ed. Woodstock, VT: Stephen Roberts.

Rosiepen G, Weinbauer GF, Schlatts, Behre HM , Nieschlag E. 1994. Duration of the cycle of the seminiferous epithelium estimated by the 5-bromodeoxyuridine technique in laboratory and feral rats. *J Reprod Fertil*, 100:299-306.

Rosiepen G, Chapin RE, Weinbauer GF. 1995. The duration of the cycle of seminiferous epithelium altered

by administration of 2, 5-hexanedione in the adult Sprague-Dawley rat. *J Androl*, 16:127-135.

Russel LD, Ettlin RA, Sinhahikim AP, Clegg ED. 1990. *Histological and Histopathologic Evaluation of the Testis*. Clear Water, FL: Cache River Press.

Sandabe UK. 2002. *Pharmacological and toxicological studies of aqueous extract of Ficus sycomorus in laboratory animals*. Maiduguri: University of Maiduguri. Thesis.

Sandabe UK, Onyeyili PA, Chibuzo GA. 2006. Phytochemical screening and effect of aqueous extract of Ficus sycomorus L (moraceae) stem bark on muscular activity in laboratory animals. *J Ethnopharmacol*, 103:481-483.

Setchell BP, Sanduz-Partida LG, Chairussyuhr A. 1993. Epididymal constituents and related substances in the storage of spermatozoa: a review. *Reprod Fertil Dev*, 5:601-612.

Seung H, Wolfe G, Rocca M. 2003. Performing a testicular spermatid head count. *Curr Protoc Toxicol,* Unit 16.7. DOI: 10.1002/0471140856.tx1607s16.

Shum WWC, Da Silva N, Brown D, Brenton S. 2009. Regulation of human acidification in male reproductive tract via cell-cell crosstalk. *J Exp Biol*, 212:1753-1761.

Steele RG, Story RA. 2000. The effect of microwave heating on the acidity of 0.9% saline in 1litre and 100ml polyvinyl chloride packaging. *Crit Care Resusc*, 2:19-21.

Suarez SS, Pacey AA. 2006. Sperm transport in the female reproductive tract. *Hum Reprod Update*, 12:23-27.

Tuck RR, Setchell BP, Waites GMH, Young TA. 1970. The composition of fluid collected by micropuncture and catheterization from the seminiferous tubules and rete testis of rats. *Pflügers Arch ges Physiol*, 318:225-243.

Wanang NO, Jiman NS, Gyang SS, Bukar BB, Goton S. 2008. Effects of *Cucumis metuliferus* E. mey. Ex Naud (*cucurbitaceae)* fruit extract on some male reproductive parameters in adult rats. *Afr J Pharm Pharmacol*, 2:48-51.

Watcho P, Ngadjui E, Alango NP, Benoit NT, Kamanyi A. 2009. Reproductive effects of Ficus asperifolia (Moraceae) in female rats. *Afr Health Sci*, 9:49-53.

WHO Laboratory manual for the examination of semen and semen-cervical mucus interaction. 1999. 4th ed. Geneva: World Health Organization.