

# Age-Related Changes in the Testicular Morphophysiology of the Cane Rat (*Thryonomys Swinderianus*)

Jamiu Oyewole Omirinde, Samuel Gbadebo Olukole<sup>1</sup>, Bankole Olusiji Oke<sup>1</sup>

Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Jos, Jos, Plateau State, <sup>1</sup>Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria

## Abstract

This study evaluated age-related changes in the testicular morphophysiology of the cane rat (*Thryonomys swinderianus*) using histological, histochemical, and sex hormonal profile approaches. Twenty (20) pathogen-free male cane rats were used for the investigation. Cane rats were divided into four groups: prepubertal ( $\leq 4$  months), pubertal ( $>4 \leq 12$  months), adult ( $>12 \leq 30$  months), and aged ( $>30$  months) of 5 rats each. Blood was collected from the different cane rat groups and processed for sex serum hormonal levels. Testes were also excised and processed routinely for variations in histology, histochemistry (using Masson's trichrome [MT] and Periodic acid-Schiff [PAS]), and histomorphometric evaluations using GIMP2 software. Testosterone concentrations were significantly elevated ( $P < 0.05$ ) in the prepubertal to adult, while there were no significant differences ( $P > 0.05$ ) in this hormone between adult and aged. The concentrations of follicle-stimulating hormone (FSH) decreased significantly ( $P < 0.05$ ) for prepubertal, pubertal, and adult, respectively. There were no significant differences ( $P > 0.05$ ) between adult and aged for FSH and luteinizing hormone. Histologically, there were scanty interstitial cells, lack of patent lumen, and incomplete spermatogenic cell series in prepubertal compared to other age groups. Testicular capsular (MT and PAS) staining intensity increased with age advancement, while in the parenchyma, remarkably high intensity was displayed by the pubertal compared to others. Seminiferous tubular and luminal diameters (LD) significantly ( $P < 0.05$ ) increased with advancing age whereas epithelial height (EH) was markedly increased in pubertal relative to other groups. In conclusion, these sets of data have shown that reproductive activity is directly related to age and is at maximum in adult cane rat.

**Keywords:** Age-related, cane rat, morphophysiology, testes

## INTRODUCTION

The male reproductive system in mammals is composed of the paired testicles and its appendages (rete testes and ductuli efferentes), paired duct system (epididymis and ductus deferens), accessory glands (vesicular, prostate, ampullary, and bulbourethral), urethra, and penis.<sup>[1,2]</sup> The reproductive system is crucial for continuation of life in all species of animal.<sup>[3]</sup> Reproduction wise, the testis has the prime function of producing viable spermatozoa required for fertilization within the female genitalia and also reputed for secreting gonadal steroids, androgens, and estrogens which in conjunction with the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) have been shown to be very important in the male reproductive function.<sup>[4-7]</sup>

Age-related alterations in the anterior pituitary gonadotropes' physiology regulating LH and FSH secretions have been associated with reproductive senescence.<sup>[8]</sup> A report on the profile of pituitary gonadotropins (LH and FSH) of mammals, especially man, showed that their levels in the serum increase with age advancement.<sup>[9]</sup> Elevated gonadotropin secretion with aging has been found to coincide with the consistent decrease in level of testosterone, provided that the feedback pathway is normal to permit suboptimal testosterone level to exert influence on hypothalamic-pituitary axis for FSH and LH secretions.<sup>[10]</sup> Although, Woerdeman *et al.*<sup>[11]</sup> reported that

**Address for correspondence:** Dr. Jamiu Oyewole Omirinde, Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Jos, Jos, Plateau State, Nigeria. E-mail: omirindejamiu@gmail.com

Received: 20-08-2020    Revised: 26-12-2020  
Accepted: 20-01-2021    Published: 09-07-2021

### Access this article online

Quick Response Code:



Website:  
<http://www.jmau.org/>

DOI:  
10.4103/jmau.jmau\_84\_20

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

**For reprints contact:** WKHLRPMedknow\_reprints@wolterskluwer.com

**How to cite this article:** Omirinde JO, Olukole SG, Oke BO. Age-related changes in the testicular morphophysiology of the cane rat (*Thryonomys swinderianus*). J Microsc Ultrastruct 2022;10:118-26.

a reasonable decrease in the synthesis of testosterone may not be entirely accompanied by elevated LH production even in young male subject which is suggestive of level of moderation within the system to variation in the levels of gonadal steroids.

Concerning age-related changes in testosterone level in mammals, Woerdeman *et al.*<sup>[11]</sup> reported a decline in testosterone level with age increment in man and such finding has been found not to be conclusive as testosterone level may be elevated or unchanged with advancement in age in a number of men and rodents such as hamster rats.<sup>[12,13]</sup> However, serum estrogen level unlike testosterone has been found to increase with increasing age mainly because of the tendency of increase in aromatase activity, an enzyme modulating the formation of estrogen precursors from other gonadal steroids,<sup>[14]</sup> during postnatal development. The elevated levels of the aromatase most times do result in decline in testosterone level. Although, the influence of age variation on the pattern of estrogen levels has variously been reported to either decline or remain steady.<sup>[15,16]</sup>

The mammalian testis is enveloped by a capsule which is made up of two main tunics: tunica albuginea (TA) and vaginalis. The former constitutes the bulk of the testicular capsule, and is composed of collagen, elastic fibers, and abundant fibroblasts; while, the latter forms the innermost layer of the capsule and is poorly differentiated.<sup>[17,18]</sup> The testicular parenchyma is divided into tubular and intertubular portions. The tubular part houses the seminiferous tubules, the centers of spermatogenesis, while the intertubular or interstitium contains Leydig cells, mastocytes, macrophages, lymphatic, vascular, and neural elements.<sup>[19]</sup> Remarkable age-related changes in the tubular and intertubular compartments of the testis have been documented in several animals: ram,<sup>[20]</sup> pig,<sup>[21]</sup> bull,<sup>[22]</sup> Japanese quails,<sup>[23]</sup> and mice.<sup>[24]</sup>

Cane rat (*Thryonomys swinderianus* Temminck, 1827), otherwise known as the African Greater Cane rat (AGCR) is a wild herbivorous rodent presently undergoing massive domestication in the South of Sahara to improve the ongoing animal protein shortages and constitutes a prospective laboratory research animal model of African origin.<sup>[25,26]</sup> With exception of the report of Olukole and Obayemi<sup>[27]</sup> on the histomorphometric parameter of the adult cane rat testis, there is a dearth of literature on the age-related changes in the testicular morphophysiology. Therefore, this study seeks to investigate such changes from prepubertal to aged cane rats using the profiles of histology, histochemistry, histomorphometry, and sex serum hormonal levels. It is hoped that results generated from this study will constitute beneficial baseline data to rodent researchers, wildlife veterinary practitioners, and cane rat breeders.

## MATERIALS AND METHODS

### Experimental animals

Twenty pathogen-free male cane rats with a known record of birth were purchased from a commercial cane rat farm in Lagos

State, Nigeria, and used for this study. The purchased rats were acclimatized in the Experimental Animal Unit of the Faculty of Veterinary Medicine for 1 week during which they were daily fed on dry corn and water provided *ad libitum*. The use of cane rat was approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC), and an ethical clearance (UI-ACUREC/18/0120) was subsequently issued.

### Experimental design

The age-grouping earlier reported in our previous work Omirinde *et al.*<sup>[28]</sup> was adopted. Briefly, the twenty male cane rats were randomly divided into four groups of five ( $n = 5$ ) animals each as presented below:

i. Prepubertal (Prepub/Pre):  $\leq 4$  months, ii. Pubertal (Pub):  $>4 \leq 12$  months, iii. Adult:  $>12 \leq 30$  months, and iv. Aged:  $>30$  months. Sequel to acclimatization, cane rats were anesthetized precisely on day 8 using the combination of xylazine and ketamine (20:80 mg/kg body weight correspondingly) injected intramuscularly. Blood was taken intracardially for hormonal analysis and was succeeded by initial intracardiac perfusion of primary perfusion agents (500 ml of 0.9% sodium chloride [Aventra, Fidson, Nigeria] and 25,000 IU/ml of heparin [Heparinum; Polfa]) and then followed by secondary perfusion of 10% buffered formalin.

### Blood sample collection for hormonal assay

Two (2) milliliters of blood was drawn from each of the cane rat's heart into a 5 ml gauge syringe (Agary-ject, China) and released into a plain test tube (Micropoint Bioscience, China). The blood samples were centrifuged at 3500 revolutions per minute (rpm) for 5 min using a centrifuge (Gallenkamp, England). Subsequent to the centrifugation, blood was separated into two layers (serum and aggregated blood cell portions). The tube was gently tilted and a 1 ml insulin syringe was inclined into it to draw the serum into Eppendorf tubes (Micropoint Diagonistica, China). The tubes were stored at  $-20^{\circ}\text{C}$  before subsequent hormonal (testosterone, estrogen, progesterone, LH, and FSH) assays which were conducted by strict adherence to manufacturers protocols within 48 h.

### Hormonal assay determination

The serum level of the sex hormones (FSH, LH, progesterone, testosterone and estrogen) was measured using ELISA method as previously described by Brahm.<sup>[29]</sup> The commercial kits of Dialab, Germany was procured and used for FSH, LH, progesterone, and testosterone determinations while Rapid Labs Ltd., UK kit was used to determine estrogen level.

### Processing of testicular tissue for histology

Concerning histology, formalin-fixed testes (about 5 mm thick) were consistently excised from the testicular equator and processed using the protocol of Salami *et al.*<sup>[30]</sup> Briefly, the excised formalin-fixed testicular tissues were subjected to dehydration in grades of ascending alcohol concentrations (70%–100%), cleared in xylene, embedded in paraffin, and sectioned at 5  $\mu\text{m}$ . Sections of the testicular tissues

were stained with hematoxylin-eosin and viewed with light microscope (Olympus BX3-CBH, USA) for histoarchitectural differences with variation in age.

### Testicular histomorphometry

Testicular histomorphometric measurement was carried out using the modified method of Kalwar *et al.*<sup>[31]</sup> Briefly, from a photographed ( $\times 10$  objective lens) section of the testis in the different age groups of cane rats ( $n = 5$ ), twenty most circular seminiferous tubules from the peripheral and central areas of the testicular section were selected for the determination of seminiferous tubular diameter (TD), EH, and luminal diameter (LD) using GIMP2 software. The seminiferous tubular and LDs in a tubule were determined from four orthogonal lines with a common intersection, while the seminiferous EH remained a linear measurement between the seminiferous epithelial basement and its apex.

### Testicular histochemical demonstration

#### Masson trichrome

The Masson trichrome (MT) technique was carried using the procedure of Drury and Wallington.<sup>[32]</sup> Dewaxed testicular sections were rinsed in descending ethanol grades (100%–70%), stained in filtered celestine blue solution, counterstained in Lillie Mayer's haematoxylin, and blued under tap water. The blued tissues were further stained with Biebrich Scarlet Fuchsin solution, mordanted in phosphotungstic-phosphomolybdic acid, and counter-stained in aniline blue. The counter-stained tissues were then differentiated in 1% glacial acetic acid, dehydrated in alcohol (96 and 100%), cleared in xylene, and mounted on Entellan slide. Collagen fiber-positive tissues stained bluish while the nuclei appeared black and cytoplasm, keratin, muscle fiber, and intercellular fibers stained reddish.

#### Periodic acid–Schiff

Testicular glycogen content was demonstrated with periodic acid–Schiff (PAS) stain using the method of Mazaud-Guittot *et al.*<sup>[33]</sup> Briefly, dewaxed testicular sections were rinsed in descending alcohol grades (100%–70%), treated with periodic acid, and exposed to Schiff solution. Schiff exposed tissues were then stained in Lillie Mayer's haematoxylin, blued under tap water, dehydrated in ascending grades of ethanol (96 and 100%), cleared in xylene, and mounted in Entellan. Glycogen-positive part of the tissues appeared magenta with bluish-stained nuclei. Furthermore, the respective photomicrographs of MT- and PAS-stained slides were quantified using Image J software (NIH, Bethesda, MD, USA).

### Statistical analysis

Data obtained were expressed as mean  $\pm$  standard error. One way analysis of variance was used to evaluate the significant difference between groups and the value of  $P < 0.05$  was considered statistically significant. Tukey's *post hoc* test was used to evaluate the significant difference between groups using GraphPad Prism 4.0 (GraphPad Software Inc., La Jolla California, USA) statistical package.

## RESULTS

### Histological changes in the testes of different age groups of the cane rat

#### Testicular capsule

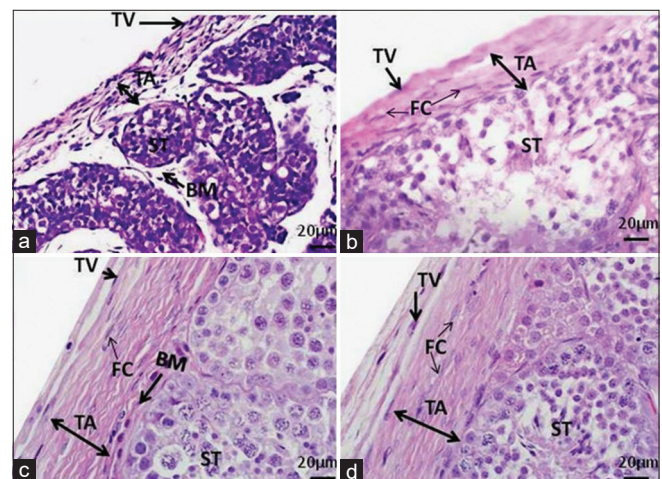
The testis in all the groups is enveloped by a capsular covering which is made up of two distinct divisions: TA and tunica vaginalis (TV) [Figure 1]. The TA is the closest division to the testicular parenchyma and is composed of dense connective tissue rich in blood vessels, fibrocytes, and collagens. TV is the outermost layer of thin mesothelium. The thickness of the testicular coverings seen in this study increases with age.

#### Testicular parenchyma

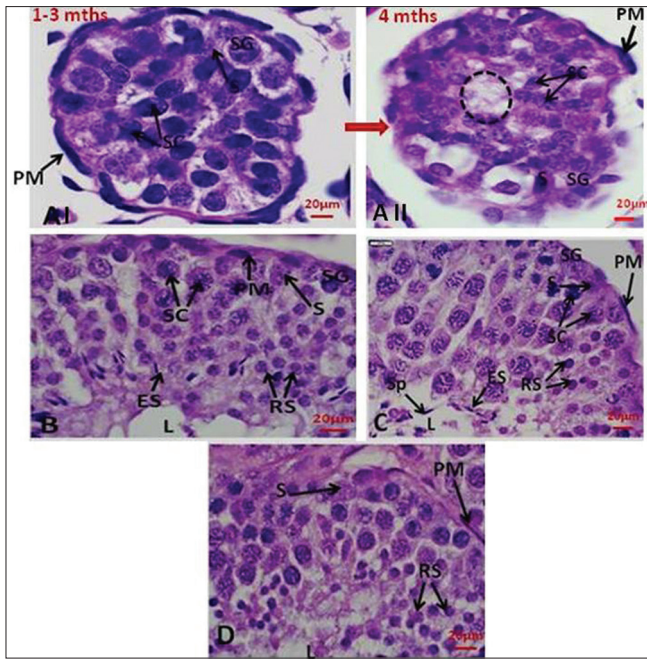
On the difference in the seminiferous tubular architecture [Figure 2], prepubertal cane rat (1–3 months) lacks a patent lumen within its tubule, and instead, the tubule is filled with cords of immature cells (spermatogonia, Sertoli cells, and spermatocytes) [Figure 2AI]. Evidence of patent seminiferous tubular lumen specifically begins in prepubertal 4-month-old rat [Figure 2AII]. Complete spermatogenic and sustentacular cells (spermatogonia, spermatocytes, spermatids [round and elongated], spermatozoa, and Sertoli cell) are visible only in the seminiferous tubules of pubertal to aged AGCR [Figure 2B–D]. Besides, the intertubular space (interstitium) in the prepubertal rat contains wide noncellular areas, i.e., scanty interstitial components [Figure 3a] as opposed to the high Leydig cell presence in the interstitium of pubertal to aged cane rats [Figure 3b–d]. The cellularity of the interstitium in this study seems to increase with age.

### Masson trichrome and periodic acid–Schiff stainings of the testes of different cane rat groups

With the use of MT, portions of the testes positive for collagen fibers were demonstrated in the capsule, peritubular tissue, and the interstices of seminiferous tubules in all the cane rat



**Figure 1:** Photomicrographs of the testes of different age groups of AGCR with their capsular coverings. (a) Prepubertal: (b) Pubertal: (c) Adult: (d) Aged: Note the tunica vaginalis with conspicuous elongated nuclei. TA: Tunica albuginea, FC: Fibrocytes, BM: Basement membrane, ST: Seminiferous tubule. Stain: H and E; Scale bar: 20  $\mu$ m



**Figure 2:** Photomicrographs of the testis of different age groups of AGCR. (A) Prepubertal: (B) Pubertal: (C) Adult: (D) Aged: Note the absence of patent lumen (l) and the different types of spermatids in A. PM: Peritubular myoid cell, SG: Spermatogonia, S: Sertoli, SC: Spermatocyte, ES: Elongating spermatids, RS: Round spermatids, S: Spermatocytes, oval outline – lumen. Stain: H and E

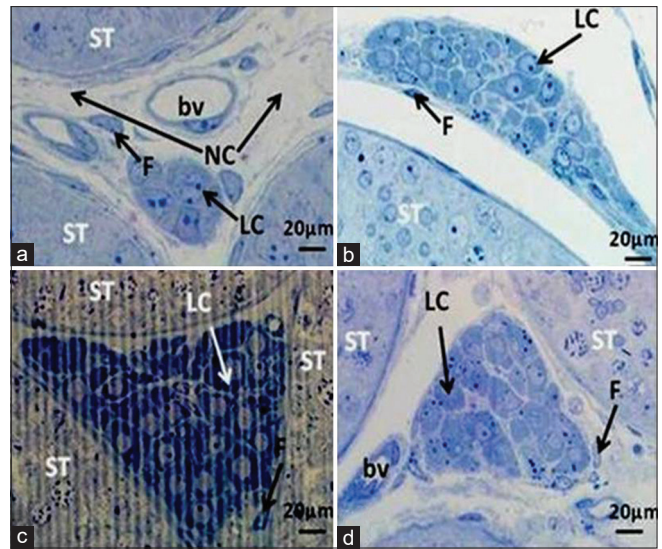
groups [Figures 4 and 5]. The quantification of the intensity of expression of the stains shows that the trend of both MT and PAS intensities was similar in the testicular capsule and they increase significantly ( $P < 0.05$ ) with the advancement in age of cane rats [Figure 4]. For the testicular parenchyma, the intensity of the PAS stain was significantly higher ( $P < 0.05$ ) in the pubertal cane rat compared to others [Figure 5]. However, there was no significant difference ( $P > 0.05$ ) in the MT intensity of all cane rat groups [Figure 5] though insignificantly increased MT intensity values were observed in both the adult and aged rats.

### Age-related changes in testicular histomorphometry

#### Testicular capsule thickness and percentage capsular tunic thickness

Testicular capsule thickness as shown in Figure 6A was significantly reduced in the prepubertal cane rat relative to others. Furthermore, there was no significant difference between the testicular capsule thickness of the adult and aged cane rats though the values are insignificantly higher in the aged. The pattern of testicular capsule thickness displayed in this study seemed to increase with age advancement.

On the percentage capsular tunic thickness [Figure 6B], the contribution of the TA to capsular thickness was significantly higher relative to the TV counterpart. Except for the significant increase ( $P < 0.05$ ) in TA thickness in prepubertal, TA thickness was not significantly different from pubertal onward. Similarly, there was no significant difference in the TV thickness from the pubertal to aged cane rats.



**Figure 3:** Photomicrographs of the testicular interstitium of different age groups of AGCR. (a) Prepubertal: (b) Pubertal: (c) Adult: (d) Aged. Note the presence of wide non cellular (NC) area within the interstitium of A. LC: Leydig cell, F: Fibroblast, BV: blood vessel. Stain: Toluidine blue; Scale bar: 20µm

### Seminiferous epithelial height, luminal diameter, and tubular diameter

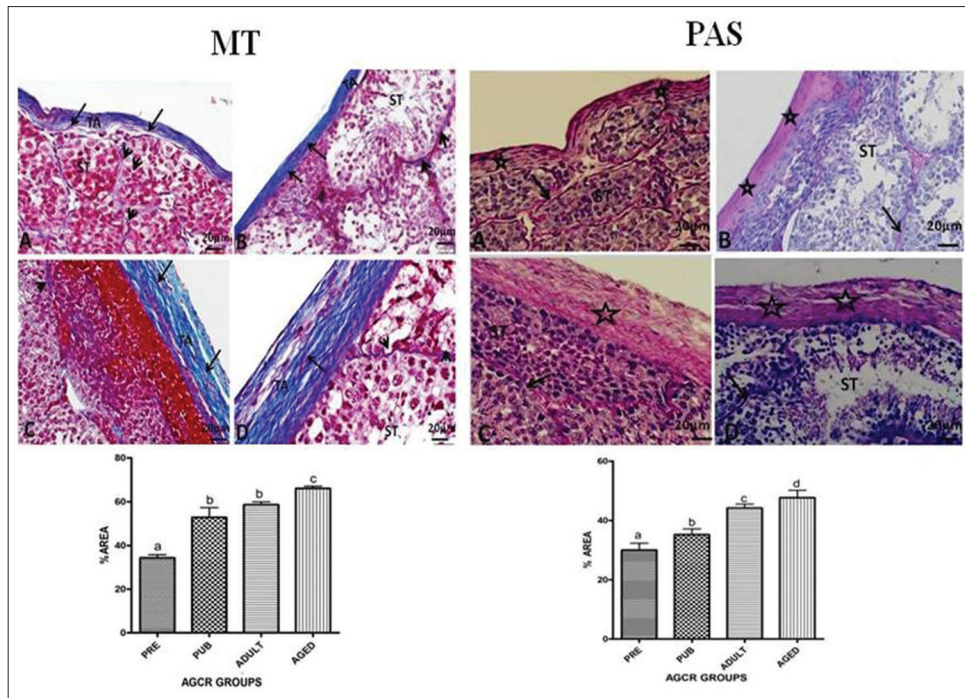
Due to the absence of patent lumen whose boundary is essential in determining the extent of seminiferous EH, it was difficult to correctly determine the EH as well as LD in prepubertal AGCR [Figure 7A and B]. In other cane rat groups [Figure 7A], the seminiferous EH trend displayed a decrease in value with increasing age of the animal, with the pubertal showing significantly higher level ( $P < 0.05$ ) while the adult and aged demonstrated no significant ( $P > 0.05$ ) difference.

For the seminiferous LD [Figure 7B], a significant increase ( $P < 0.05$ ) was noticed in both adult and aged relative to the pubertal cane rat, though insignificantly higher ( $P > 0.05$ ) values occur in the adult when compared to the aged rat [Figure 7B]. In this study, LD trend seemed to increase with age increment.

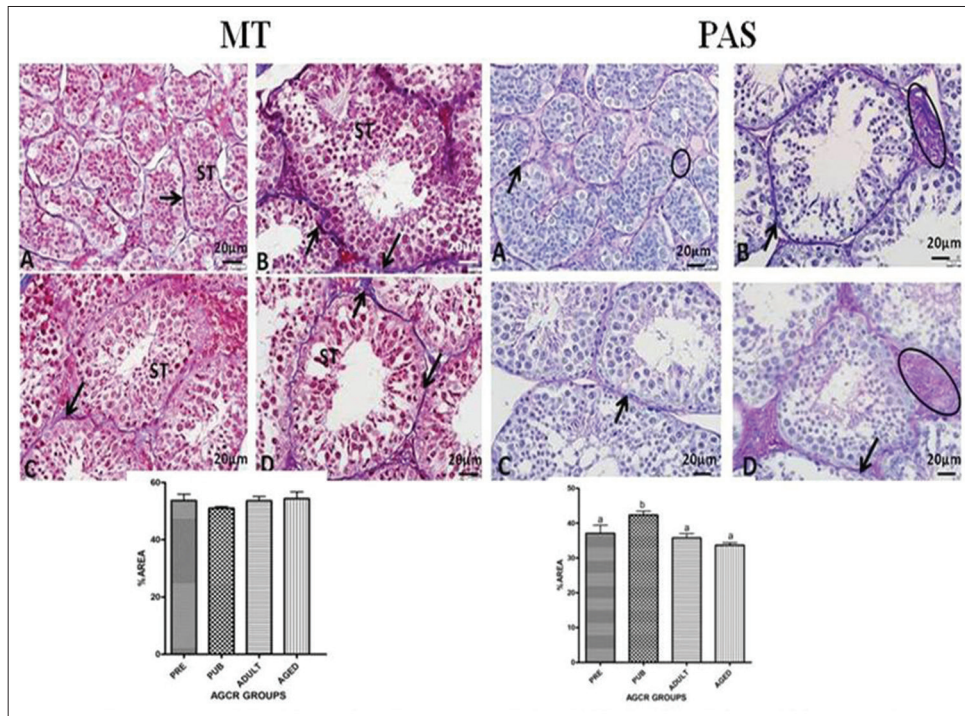
On the profile of seminiferous TD [Figure 7C], a significant increase ( $P < 0.05$ ) value was seen from pubertal to aged groups relative to prepubertal AGCR [Figure 7C]. Although increase in TD value was noticed in pubertal rats, this was not significant ( $P > 0.05$ ) enough to establish a difference between the three groups [Figure 7C]. Thus, with exception of the prepubertal, the trend of TD with age advancement observed in this study appears to be constant.

### Age-related changes in the serum hormonal profiles of the cane rat

There was a significant elevation in the serum testosterone level of the pubertal cane rat when compared to others [Figure 8A]. Conversely, the serum testosterone level was markedly lower in the prepubertal rat relative to other groups [Figure 8A]. In this study, serum testosterone profile across the different age groups of



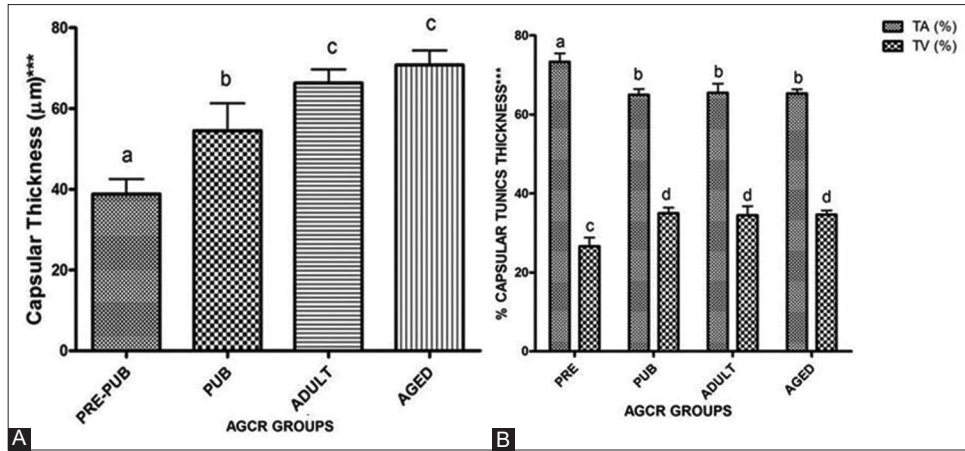
**Figure 4:** Photomicrographs of the testis of different age groups of AGCR. (A) Prepubertal: (B) Pubertal: (C) Adult: (d) Aged. In Masson trichrome, note the blue staining collagen fibers surrounding the seminiferous tubules. Also, note the demonstration of periodic acid–Schiff positive areas in the interstitium (oval) as well as in the seminiferous tubular surrounding (arrow). Scale bar: 20µm



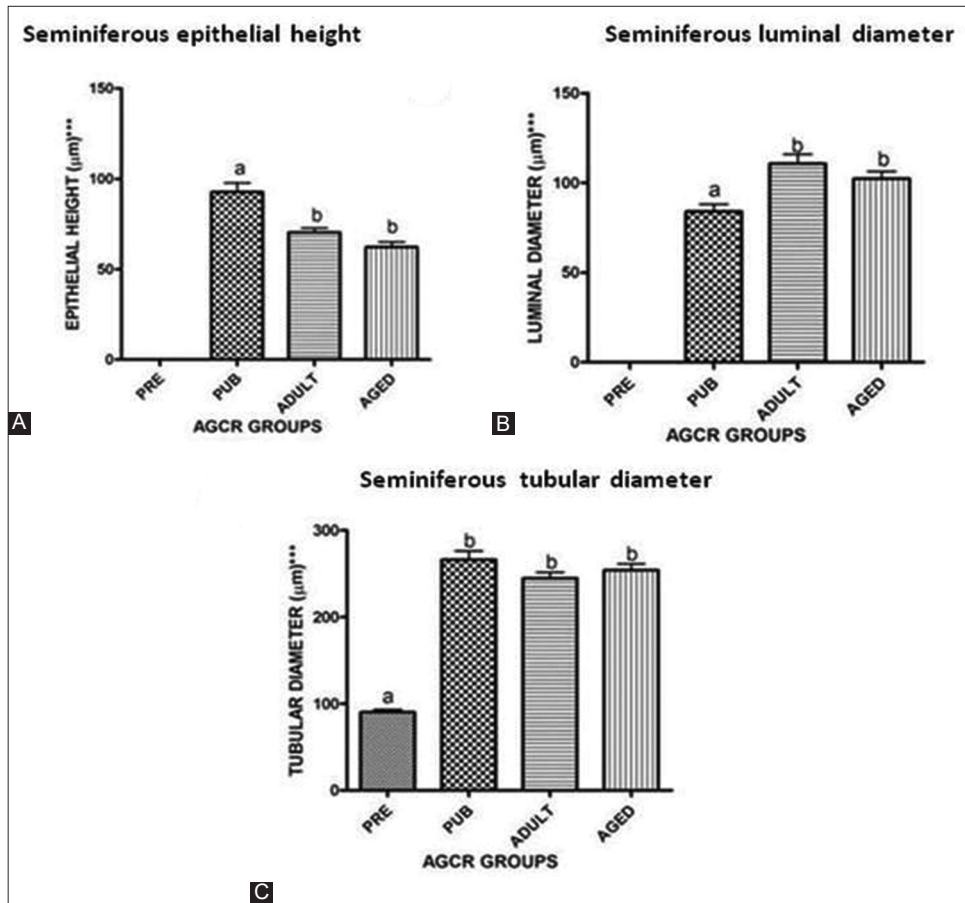
**Figure 5:** Photomicrographs of the testis of different age groups of AGCR. (A) Prepubertal: (B) Pubertal: (C) Adult: (D) Aged. In Masson trichrome, note the blue staining collagen fibers surrounding the seminiferous tubules. Also, note the demonstration of periodic acid–Schiff positive areas in the interstitium (oval) as well as in the seminiferous tubular surrounding (arrow). Scale bar: 20µm

cane rat appeared to progressively increase with age advancement with peak level observed in the pubertal rat and later followed by an insignificant ( $P > 0.05$ ) decline level in both adult and aged rats.

Serum LH level was significantly higher ( $P < 0.05$ ) in the adult cane rat relative to other groups [Figure 8B]. However, there was no significant difference ( $P > 0.05$ ) in the LH levels in both



**Figure 6:** Charts illustrating age related changes in the testicular capsule thickness (A) and percentage capsular tunics thickness (B) of different age groups of AGCR. Bars with different alphabet superscripts (a-d) are significantly different



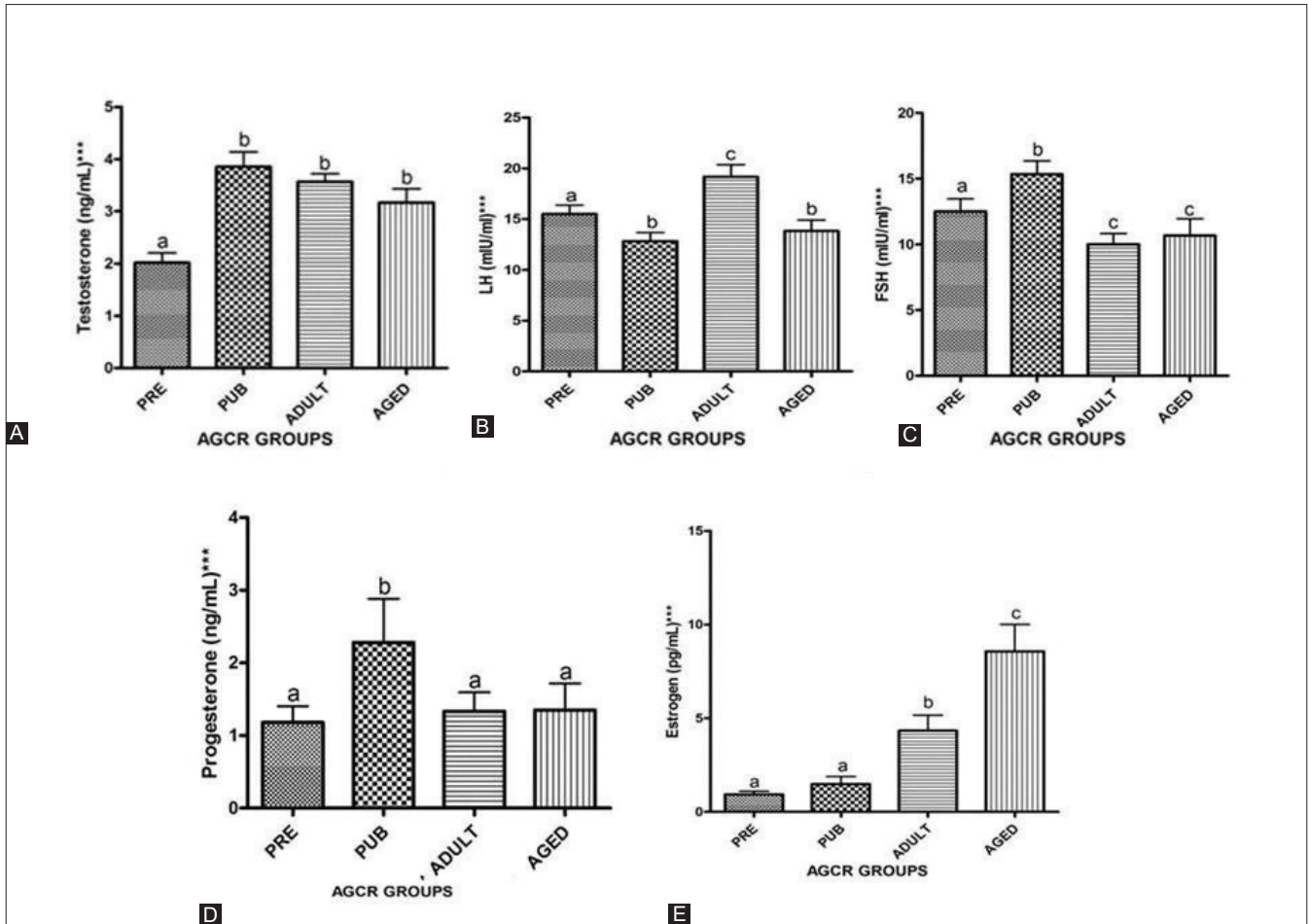
**Figure 7:** Charts illustrating age-related changes in the testicular parameters of AGCR. (A) Epithelial height (B) Luminal diameter (C) Tubular diameter. Bars with different alphabet superscripts (a and b) are significantly different

pubertal and aged cane rats [Figure 8B]. Except for the peak level of LH seen in the adult rat, the profile of LH produced across all cane groups seems to decrease with advancing age.

Serum FSH level was significantly elevated ( $P < 0.05$ ) in the pubertal rat when compared to other groups [Figure 8C]. On the contrary, there was no significant difference ( $P > 0.05$ ) in the FSH levels of adult and aged rats [Figure 8C]. The trend

of FSH levels across the different cane rat groups appeared to decrease with age variation except for the peak observed in the pubertal rat.

Serum progesterone level was significantly elevated in the pubertal cane rat compared to other groups [Figure 8D]. Except for the remarkable climax seen in the level of progesterone of pubertal, there was no significant difference



**Figure 8:** Age-related changes in the serum hormonal levels in AGCR. A. Testosterone B. Luteinizing Hormone (LH) C. Follicle Stimulating Hormone (FSH) D. Progesterone E. Estrogen. Bars bearing dissimilar superscripts (a-c) are significantly different

in estrogen level of others, though an insignificant increase exists in the values of adult and aged over prepubertal rat [Figure 8D]. Thus, a trend of a fairly stable level was seen with age advancement.

There was a significant increase in the serum estrogen level of the aged cane rat relative to other groups [Figure 8E]. Furthermore, there was no significant difference in the estrogen levels of prepubertal and pubertal rats, though a slight insignificant increase was seen in the estrogen value of the pubertal cane rat [Figure 8E]. Interestingly, the estrogen hormone level observed in this study progressively increased with age increment.

## DISCUSSION

This study demonstrated that testicular structure and function in cane rats were remarkably altered with age increment as evidenced by the age-dependent decrease in gonadotropins and gonadal hormones and progressive canalization of seminiferous tubular lumen. There was also an age-related increase in seminiferous tubular and LDs as well as an increase testicular capsule and parenchyma glycogen and collagen expression intensities. The observed age-related changes in

the testicular morphophysiology of cane rats were similar to previously reported works.<sup>[34-37]</sup>

The absence of patent testicular parenchymal lumen coupled with the reduced interstitial cell components in the testis of the prepubertal rat appeared to be suggestive of structural proof of the quiescent reproductive status of this age group. On the contrary, the morphological presence of canalized lumen in the seminiferous tubules of pubertal to aged cane rats could be linked to the possible spermiogenetic activities occurring in their seminiferous epithelium. The testicular histology observed in the prepubertal rats in this study is similar to those reported for immature rats<sup>[34]</sup> and some young avian species.<sup>[23]</sup>

The PAS-positive staining demonstrated in the testicular capsule, basement membrane, interstitium, and seminiferous tubular lumen agrees with previously recognized glycogen-rich regions in the testes.<sup>[35,38,39]</sup> Furthermore, the marked parenchymal PAS staining intensity in the pubertal cane rat relative to others could be attributed to the increase in demands for glycogen to meet up with the energy requirement for the reproductive climax and the initiation of spermatogenic activities. This finding corroborates the report of age-related changes in the testicular parenchymal staining in ram by Kishore *et al.*<sup>[36]</sup>

The Masson trichrome-positive staining observed in the testicular capsules and the interstices of seminiferous tubules suggest that collagen fibers are abundantly present in the highlighted parts of the testes. This finding attests to the earlier reports of collagen expression in the testes of mammals.<sup>[39,40]</sup> In the same vein, the age-related increase in capsular Masson trichrome staining intensity could be presumed to be due to collagen fiber amplification with the advancement in age, while the nonsignificant difference in the MT staining of seminiferous tubular parenchyma could be ascribed to the uniform distribution of collagen fibers across age groups.

In our present study, we evaluated histomorphometric changes in the testes of different age groups of cane rats from sections of testicular tissue samples that were consistently excised from the testicular equator of each rat. Twenty most circular seminiferous tubules from both the peripheral and central areas of the testicular section were carefully selected for the determination of these histomorphometric changes. Seminiferous EH, an important testicular morphometric parameter, was observed to progressively decrease with accompanied concomitant increase in LD as age advances. This inverse association between seminiferous EH and LD can be assumed to coincide with the decrease in functional activity with aging. These findings partly agree with the report of Sarma *et al.*<sup>[35]</sup> on the goat. The seemingly uniform TD seen in the pubertal to aged cane rats in this study corroborates the earlier reports on TD profile in mammals.<sup>[41]</sup> However, it is at variance with the age-related increase in TD reported by Sarma *et al.*<sup>[35]</sup> and Kumari<sup>[40]</sup> in goats.

The significantly elevated serum testosterone level observed in pubertal rats with the subsequent decline with age advancement could be assumed to be indicative of the commencement of the active reproductive activity. In addition, it has been speculated that testosterone peak may be triggered by complementary Leydig cell hypertrophy and proliferative germ cell activity.<sup>[42]</sup> The trend of serum testosterone in this study concurs with the reports of Wang *et al.*<sup>[37]</sup> and Vom Saal *et al.*<sup>[43]</sup> It, however, contradicts the decrease and stable levels reported by Calvo *et al.*,<sup>[12]</sup> Trivison *et al.*,<sup>[13]</sup> and Horn *et al.*<sup>[44]</sup>

The significant increase in LH level observed in the adult rat relative to others provides remarkable suggestive evidence of a functional pituitary–gonadal relationship. Based on this relationship, the rise in the LH level is presumed to be capable of initiating a consequential triggering of testosterone release which is on the decline in this group. Interestingly, studies have shown that moderate or extreme testosterone decline in a male subject with an intact hypothalamic–pituitary relationship may or may not be compensated with LH climax.<sup>[9-11]</sup>

FSH is important in the regulation of spermatogenesis.<sup>[45-47]</sup> Thus, the significantly increased FSH level in pubertal rats could be attributed to the completion of maturation of spermatids in this group while the decline in the FSH of the adult and aged cane rats suggests an already intact, matured

seminiferous epithelium ready to exert a feedback influence on FSH secretion.

The observed increase in estrogen level with the advancement in age could be associated with the increase in aromatase activity, an enzyme modulating the formation of estrogen precursors from other gonadal steroids,<sup>[14]</sup> during postnatal development. Going by this assumption, the elevated level of the aromatase activity could in-turn affect the feedback for testosterone synthesis which might result in a consequential decline in its level. Contrary to the age-related elevation in estrogen observed in this study, trends of estrogen levels with advancing age have been variously reported to either decline or remain steady.<sup>[15,16,47]</sup>

Progesterone is believed to play a role in activating sperm in the female reproductive tract and as a modulator of the male sexual response and behavior.<sup>[48]</sup> Hence, the progesterone peak observed in pubertal rats suggests that maximum sexual response and behavior is attainable at this age.

## CONCLUSION

The sets of data on histology, histochemistry, histomorphometry, and hormonal profiles have demonstrated that reproductive activity is directly related to age advancement and is at maximum in adult cane rats.

## Acknowledgments

The authors are grateful to Mrs. R. Phaswane of the Pathology Laboratory Unit, University of Pretoria, for her excellent technical assistance on the histology and histochemistry protocols.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

## REFERENCES

1. Dyce KM, Sack WO, Wensing CJ. Textbook of Veterinary Anatomy. 3<sup>rd</sup> ed. Pennsylvania: Saunders; 2002. p. 183-92.
2. König HE, Liebich HG. Veterinary Anatomy of Domestic Mammals: Textbook and Color Atlas. In: Endocrine Gland (Glandulae endocrinae). König HE, Liebich HG, editors. 3<sup>rd</sup> ed. Stuttgart New York: Schattauer Press; 2014. p. 561-9.
3. Mehanna M, Ferreira AL, Ferreira A, Paz RC. Morphology of the testes and epididymal ducts in the Pampas cat *Leopardus colocolo* (Molina, 1782). *Pesqui Vet Bras* 2016;36:1014-20.
4. Nilsson S, Gustafsson JA. Estrogen receptor action. *Crit Rev Eukaryot Gene* 2002; 12: 237-258.
5. Hess RA. Estrogen in the adult male reproductive tract: A review. *Reprod Biol Endocrinol* 2003;1:52.
6. Welsh M, Saunders PT, Atanassova N, Sharpe RM, Smith LB. Androgen action via testicular peritubular myoid cells is essential for male fertility. *FASEB J* 2009;23:4218-30.
7. Wei L, Peng KM, Liu H, Song H, Wang Y, Tang L. Histological examination of testicular cell development and apoptosis in the ostrich chick. *Turk J Vet Anim Sci* 2011;35:7-14.
8. Hall, J.E and Gill, S. Neuroendocrine aspects of aging in women. *Endocrinol Metab Clin North Am* 2001;30:631-46.



9. Feldman HA, Longcope C, Derby CA, Johannes CB, Araujo AB, Coviello AD, *et al.* Age trends in the level of serum testosterone and other hormones in middle-aged men: Longitudinal results from the Massachusetts male aging study. *J Clin Endocrinol Metab* 2002;87:589-98.
10. Bagatell CJ, Bremner WJ. Androgens in men--uses and abuses. *N Engl J Med* 1996;334:707-14.
11. Woerdeman J, Kaufman JM, de Ronde W. In young men, a moderate inhibition of testosterone synthesis capacity is only partly compensated by increased activity of the pituitary and the hypothalamus. *Clin Endocrinol (Oxf)* 2010;72:76-80.
12. Calvo A, Pastor LM, Martínez E, Vázquez JM, Roca J. Age-related changes in the hamster epididymis. *Anat Rec* 1999;256:335-46.
13. Travison TG, Araujo AB, Kupelian V, O'Donnell AB, McKinlay JB. The relative contributions of aging, health, and lifestyle factors to serum testosterone decline in men. *J Clin Endocrinol Metab* 2007;92:549-55.
14. Leder BZ, Rohrer JL, Rubin SD, Gallo J, Longcope C. Effects of aromatase inhibition in elderly men with low or borderline-low serum testosterone levels. *J Clin Endocrinol Metab* 2004;89:1174-80.
15. Orwoll E, Lambert LC, Marshall LM, Phillips K, Blank J, Barrett-Connor E. Testosterone and estradiol among older men. *J Clin Endocrinol Metab* 2006;91:1336-44.
16. Araujo AB, Travison TG, Leder BZ, McKinlay JB. Correlations between serum testosterone, estradiol, and sex hormone-binding globulin and bone mineral density in a diverse sample of men. *J Clin Endocrinol Metab* 2008;93:2135-41.
17. Banks JW. *Applied Veterinary Histology*. Mosby Year Book. 3<sup>rd</sup> ed. Baltimore, Boston, Chicago, London, Philadelphia, Sydney, Toronto: Inc. St. Louis; 1993. p. 429-45.
18. Singh I. *Textbook of Human Histology with Colour Atlas and Practical Guide*. 6<sup>th</sup> ed. New Delhi: Jaypee Brothers Medical Publishers Ltd; 2011. p. 290-303.
19. Maekawa M, Kamimura K, Nagano T. Peritubular myoid cells in the testis: Their structure and function. *Arch Histol Cytol* 1996;59:1-3.
20. Carmon JL, Green WW. Histological study of the development of the testis of the ram. *J Anim Sci* 1952;11:674-87.
21. Orsi AM, Dias SM, Moreira JE, Camilli JA. Morphological development of the seminiferous epithelium of the pig at different ages (*Sus scrofa* of the Landrace strain). *Anat Histol Embryol* 1987;16:97-102.
22. Wrobel KH. Prespermatogenesis and spermatogoniogenesis in the bovine testis. *Anat Embryol (Berl)* 2000;202:209-22.
23. Kannan TA, Ramesh G, Sivakumar M. Age-related changes in the gross and histoarchitecture of testis in Japanese quails (*Coturnix coturnix japonica*). *Int J Livest Res* 2015;5:26-33.
24. Kaavya R, Kannan TA, Basha SH, Vairamuthu S, Ramesh G, William BJ. Age-related changes in the histoarchitecture of seminiferous epithelium in mice. *Int J Curr Microbiol App Sci* 2017;6:1-7.
25. Fayenuwo JO, Akande M, Taiwo AA, Adebayo AO, Saka JO, Lawal BO, *et al.* Guidelines for Grasscutter Rearing. Ibadan: Technical Bulletin, IAR & T; 2003. p. 38.
26. Opara MN. The Grasscutter I: The livestock of tomorrow. *Res J Forestry* 2010;4:119-35.
27. Olukole SG, Obayemi TE. Histomorphometry of the testes and epididymis in the domesticated adult African great cane rat (*Thryonomys swinderianus*). *Int J Morphol* 2010;28:1251-4.
28. Omirinde JO, Olukole SG, Oke BO. Nerve and glial cell expressions in the testes and epididymides of different age groups of cane rat (*Thryonomys swinderianus*). *J Microsc Ultrastruct.* Advance online publication 2021. DOI: 10.4103/JMAU.JMAU\_6\_20.
29. Braham G. *Handbook of Radioimmunoassay*. New York: Marcel Dekker, Inc; 1977. p. 591-656.
30. Salami SA, Omirinde JO, Balogun ME, Raji Y. Testicular and epididymal histomorphometric assessment in F1 male Wistar rats after gestational Ricinus communis oil exposure. *J Morphol Sci* 2015;32:187-91.
31. Kalwar Q, Chu M, Ahmad AL, Ding X, Wu X, Bao P, *et al.* Morphometric evaluation of spermatogenic cells and seminiferous tubules and exploration of luteinizing hormone beta polypeptide in testis of Datong Yak. *Animals* 2020;10:1-16.
32. Drury RA, Wallington EA. *Carleton's Histological Technique*. 5<sup>th</sup> ed. Oxford New York: Oxford University Press; 1980. p. 140-2.
33. Mazaud-Guittot S, Gow A, Le Magueresse-Battistoni B. Phenotyping the claudin 11 deficiency in testis: From histology to immunohistochemistry. *Methods Mol Biol* 2011;763:223-36.
34. Lasheen SS, Refaat SH, El-Nefawy NE, Abd-Elgawad RA, Othman AI. Developmental characteristics of rat testicular tissue and the impact of chronic noise stress exposure in the prenatal and postnatal periods. *Anat Physiol* 2015;4:1-15.
35. Sarma K, Kalita SN, Devi J. Age-related changes in the cytomorphology of the seminiferous epithelium in Assam goat (*Capra hircus*) from birth to 10 month. *Folia Vet* 2011;55:45-9.
36. Kishore PV, Geetha R, Sabiha H. Postnatal differentiation and regional histological variations in the ductus epididymis of rams. *Tamil Nadu J Vet Ani Sci* 2012;8:145-51.
37. Wang C, Leung A, Sinha-Hikim AP. Reproductive aging in the male brown-Norway rat: A model for the human. *Endocrinology* 1993;133:2773-81.
38. Rajani C, Ramesh G, Vijayaragavan C. Histochemical studies of the epididymis in rat (*Rattus norvegicus*). *Indian J Ani Res* 2008;42:291-3.
39. Shagufta B, Sharma K, Suri S, Devi J. Histochemical studies on the testis of adult Bakerwali goat (*Capra hircus*). *Indian J Vet Anat* 2012;24:50-1.
40. Kumari P. Gross, Histomorphological and Histochemical Changes in Testis, Epididymis, vas, and seminal Vesicles of Developing (post-natal) Kids of Black Bengal Goats. (*Capra hircus*). A Thesis Submitted to West Bengal University of Animal and Fishery Sciences in Partial Fulfilment of the Requirements for the Degree of Doctorate of Veterinary Science, Belgachia, Kolkata, India; 2013. p. 32-82.
41. Nishimura S, Okano K, Yasukouchi K, Gotoh T, Tabata S, Iwamoto H. Testis developments and puberty in the male Tokara (Japanese native) goat. *Anim Reprod Sci* 2000;64:127-31.
42. Choi J, Smitz J. Luteinizing hormone and human chorionic gonadotropin: Distinguishing unique physiologic roles. *Gynecol Endocrinol* 2014;30:174-81.
43. Vom Saal FS, Finch CE, Nelson JF. The natural history of reproductive aging in humans, laboratory rodents, and selected other vertebrates. In: Knobil E, editor, *Physiology of Reproduction*. 2<sup>nd</sup> ed. Ch. 61, Vol. 2. New York: Raven Press; 1994. p. 1213-314.
44. Horn R, Pastor LM, Moreno E, Calvo A, Canteras M, Pallares J. Morphological and morphometric study of early changes in the ageing golden hamster testis. *J Anat* 1996;188(Pt 1):109-17.
45. Kumar TR. FSH beta knockout mouse model: A decade ago and into the future. *Endocrine* 2009;36:1-5.
46. Peltoketo H, Rivero-Muller A, Ahtiainen P, Poutanen M, Huhtaniemi I. Consequences of genetic manipulations of gonadotropins and gonadotropin receptors in mice. *Ann Endocrinol* 2010;71:170-6.
47. Araujo AB, Witter GA. Endocrinology of the aging male. *Best Prac Res Clin Endocrinol Metabol* 2011;25:303-19.
48. Oettel M, Mukhopadhyay AK. Progesterone: The forgotten hormone in men? *Aging Male* 2004;7:236-57.