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AGE-RELATED ULTRASTRUCTURAL CHANGES IN THE PRINCIPAL CELLS OF THE EPIDIDYMIS OF THE AFRICAN GREATER CANE RAT (*Thryonomys swinderianus*, Temminck 1827)

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ABSTRACT

This study was designed to investigate age-related changes in the epididymal ultrastructure of the African greater cane rats (AGCR). Electronmicroscope technique was utilized for the study. Twelve African greater cane rats were used for this study. The rats were randomly assigned into 4 groups of 3 rats each as: prepubertal (≤4 months), pubertal (>4≤12 months), adult (>12≤30 months) and aged (>30 months). Following intra-cardiac perfusion with Karnovsky glutaraldehyde fixative, epididymal (caput, corpus and cauda segements) tissues were excised and processed for Transmission Electron Microscopy. Ultrastructurally, the principal cell of the caput epididymis in the pre-pubertal rats had numerous mitochondria when compared to others. There was a progressive increased nuclear indentation in the principal cell of corpus epididymis from pre-pubertal to aged rats. In addition, copious amount of lysosomal granules were remarkably observed in the caudal epididymis of the aged rats. This study has highlighted notable changes in the ultrastructure of the epididymis of different age categories of the cane rat that could possibly be linked to the reproductive quiescence and activeness as the case may be in the groups evaluated.

Keywords: Age, ultrastructure**,** epididymis, cane rat

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INTRODUCTION

The epididymis remains an essential site for spermatozoa maturation and storage [1]. It is classically divided into three distinct regions or segments; the *caput* which is situated at the testicular cranial pole, the *corpus* occupies the side of the testes and the *cauda* segments located at the caudal pole of the testis [2]. Histologically, the epididymis has epithelium that houses several cell types (principal, basal, apical, clear and halo cells) with diverse functions [3]. Based on the epithelial cell population, two of these cell types (principal and basal cells) are regarded as main epididymal cells while others are accessory cells [4].

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Specific age-related ultrastructural changes in the epithelial cells of corpus and caudal epididymis have been reported to include increased accumulation of secondary lysosomes, residual bodies, lipofuscin pigments, mitochondrial damages and increased cytoplasmic filament bundles in aged hamster [5]. In addition, epididymis undergoing ageing has been found to demonstrate striking vacoulated principal cells with varying vacuolar sizes depending on species; being smaller in the principal cells of the distal epididymal region of aged rabbits and of giant dimensions in the proximal caudal epididymis of older rats [5, 6].

African greater cane rat (AGCR) is a wild hystricomorphic grass-eating rodent presently being domesticated as a prospective research animal model and an alternative animal protein source in sub-Saharan Africa [7,8,9]. Age-related changes in the reproductive organs, more importantly the epididymis of the male AGCR is highly essential in its breeding as only one male is used for mating several females [10]. Previous reports on cane rat epididymis focused only on the adult [11, 12, 13]. There is paucity of information on age-related changes in the epididymal ultrastructure of AGCR. Therefore, this study was conceptualized to examine age-related changes in the ultrastructure of the epididymis of AGCR.

MATERIALS AND METHODS

Experimental Animals

Twelve (12) apparently healthy male African greater cane rats used for this study were purchased from a commercial cane rat farm in Lagos State, Nigeria. Birth records of the rats were taken at the point of purchase. The rats were transported in wired cages to the Experimental Animal Unit, Department of Veterinary Anatomy, University of Ibadan, Ibadan, Oyo State, Nigeria. They were acclimatized for one week, fed daily on dry corn feed while water was provided *ad libitum*. The ethical clearance (***UI-ACUREC/18/*0120)** for the use of this animal was obtained from the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC)**.**

Experimental Design

The modified age-grouping of Soro *et al.* [10] was adopted. The male AGCR were randomly divided into four groups of three (n=3) animals each as shown below:
i. Prepubertal (Pre); ≤4 months, ii. Pubertal (Pub); >4≤12 months, iii. Adult; >12≤30 months and iv. Aged (AG); >30 months). On the 8th day of acclimatization, the animals were anaesthetized using a combination of xylazine and ketamine (20:80 mg/kg body weight correspondingly) injected intramuscularly. The thoracic cage was dissected open via a ventral midline incision to access the heart. Subsequent to this, 0.5 L of 0.9% sodium chloride (Aventra, Fidson, Nigeria) and 25, 000 IU of heparin (2IU\ML) (Heparinum; Polfa) were perfused into the heart. This was succeeded with the secondary perfusion of Karnovsky glutaraldehyde fluid for transmission electron microscopy (TEM).

Processing of cane rat epididymal tissues for electron microscopy

Karnovsky glutaraldehyde-fixed epididymal tissues were processed for TEM as previously reported by Adebayo et al. [13] at the Electron-Microscopy Unit, Department of Veterinary Anatomy and Physiology, University of Pretoria, South Africa. Semi-thin sections were stained with toluidine blue and viewed under the light microscope (Olympus BX63 with a DP72 camera). Thereafter, ultra-thin sections were prepared, stained with uranyl acetate and lead citrate and examined using a transmission electron microscope (Philips CM 10 TEM, USA) functioning at 80 kv. Transmission electron micrographs of the caput, corpus and cauda segments of the epididymis were captured using a Gatan 785 Erlangshen digital camera (Gatan Inc., Warrendale PA).

RESULTS

Age-related ultrastructural changes in the epididymal architecture of the African greater cane rat

The principal cell of the caput epididymis in the prepubertal rat was characterized by the presence of numerous mitochondria in the basal and perinuclear portions of its cytoplasm when compared to other age groups (Fig. 1A). Rough endoplasmic reticulum were abundant in the cytoplasm of principal cells of the adult and aged (Fig. 1C and D)

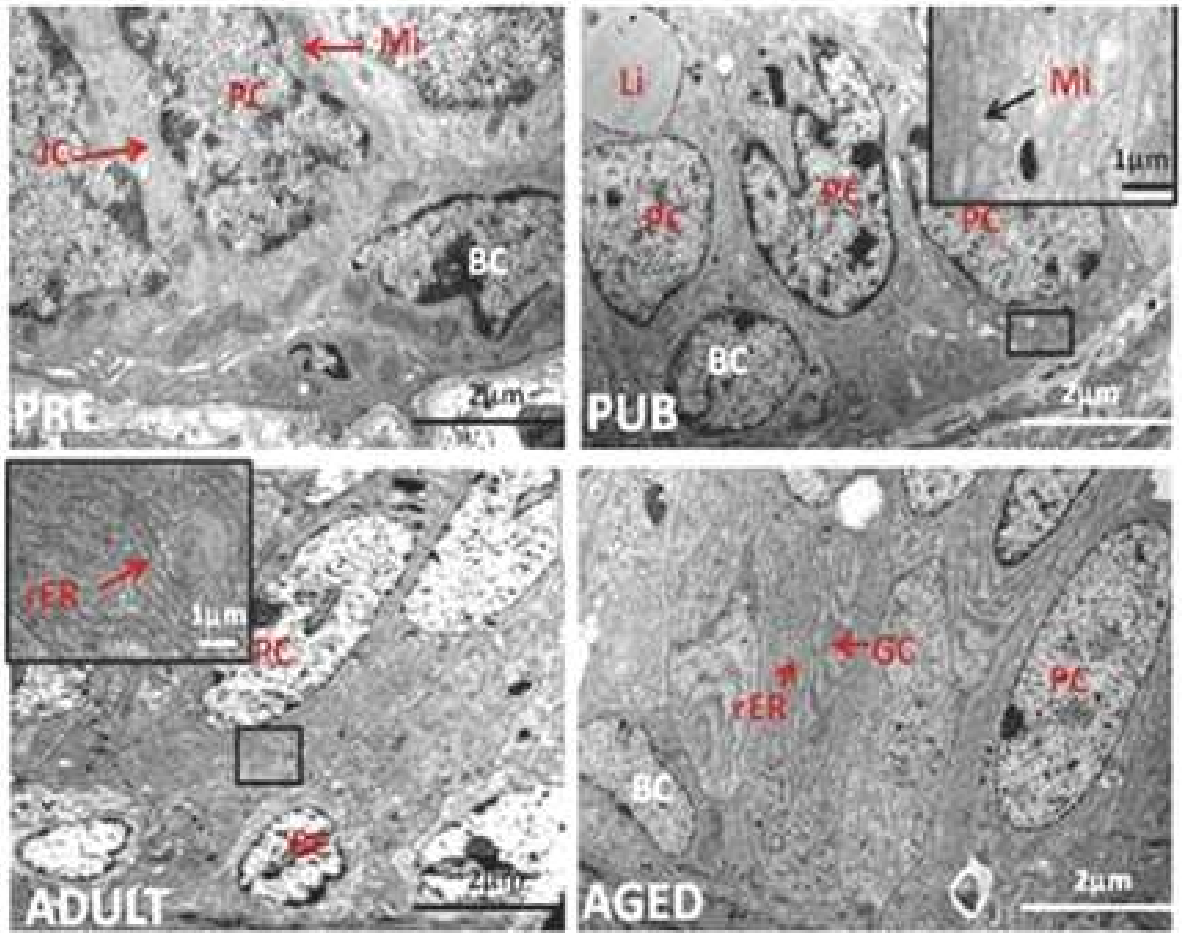

Figure 1. Transmission electron micrographs of the basal part of caput epididymis of the African greater cane rat. Note the presence of numerous mitochondria (Mi) in the basal part of prepubertal (PRE) and pubertal (PUB) as well as abundant long rough endoplasmic reticulum (rER) in adult and aged. GC-Golgi complex, Mvb –Multi-vesicular bodies, V-Vacoules Ly- Lysosome, Li- Lipid, V- Vacoules, Mi- Mitochondria. BC- Basal cell, PC- Principal cell, JC –Junctional complex.

Regarding the age-related differences in corpus epididymal ultrastructure, the nucleus of the principal cell in the corpus epididymis of all AGCR was irregular in shape and bears some degree of indentations that appeared to increase with advancing age of the AGCR (Fig. 2). In addition, numerous lysosomal granules were more evident in the perinuclear aspect of the principal cells of aged corpus epididymis (Fig. 2D). Prominent apical vacuolations were observed in the principal cell of pubertal rats (Fig. 3B).

In the caudal epididymis, the principal cells of the aged AGCR were observed to bear numerous lysosomal granules as well as degenerating mitochondria in both perinuclear and supranuclear regions (Fig. 4D and 5D). In addition, prominent principal cell nuclear indentation was noticed in pubertal and aged rats (Fig 4B-D).

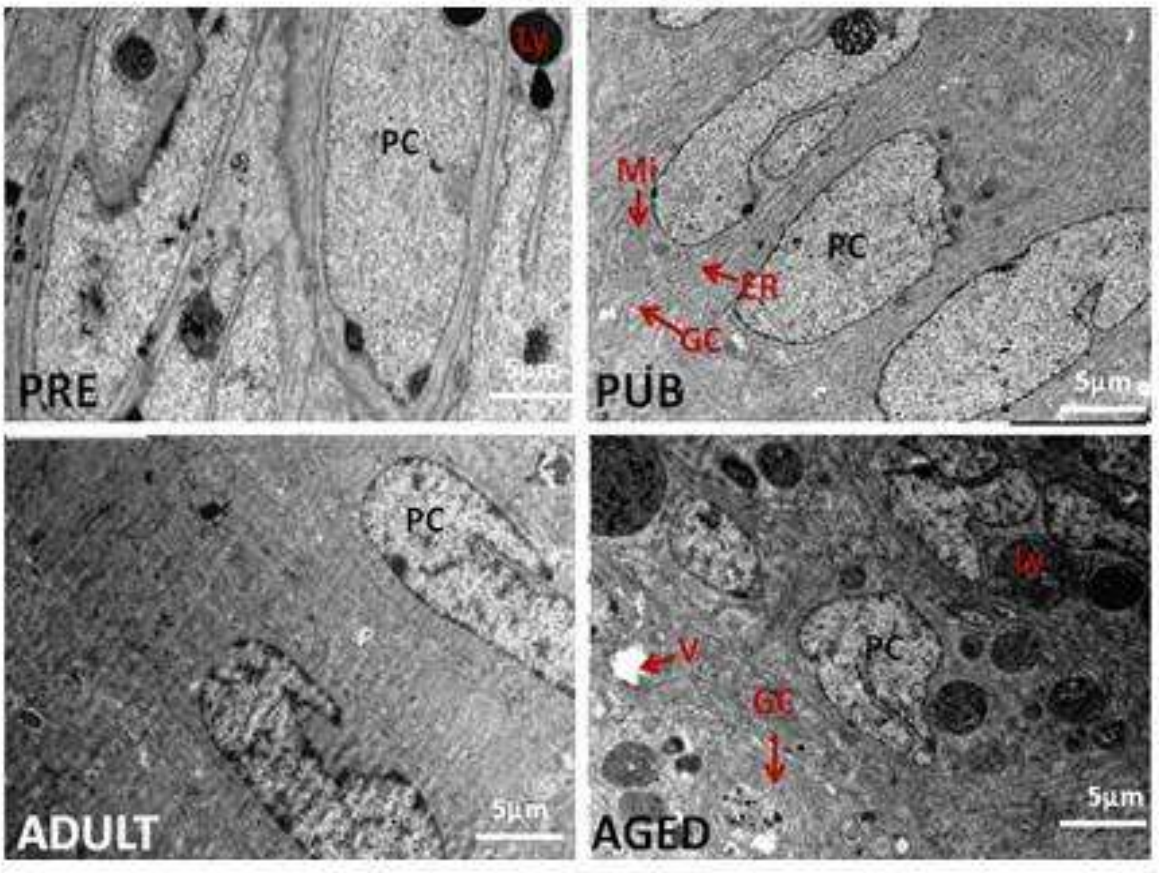

Figure 2. Transmission electron micrographs of the perinuclear aspect of the corpus epididymis principal cell of the African greater cane rat. Note the irregularly shaped nuclei and their indentations that appear to increase with age as well as the prominent increase in lysosomal granules in the principal cell cytoplasm of the aged rat. GC-Golgi complex, V-Vacoules Ly-Lysosome, V- Vacoules, Mi- Mitochondria. PC- Principal cell.

DISCUSSION

The numerous mitochondria displayed in the basal and the perinuclear parts of the principal cells of caput epididymis in pre-pubertal rat is suggestive of increased metabolic activities within the caput of this age group. Increased mitochondria have been suggested to be part of cellular provision needed for marked absorptive function that is peculiar to caput epididymis [13]. In addition, the abundant long rough endoplasmic reticulum (RER) and Golgi apparatus seen in the adult and aged rats are morphological indicators of protein synthesis. This finding concurs with report of Adebayo *et al*. [13] on the adult AGCR.

The progressive accumulation of lysosomal and lipofuschin granules as well as mitochondria degeneration in the principal cell of both corpus and cauda epididymis of aged rat could be attributed to aging process [5, 14, 15]. Lysosomes are known to house hydrolytic enzymes which are important in phagocytosis of both damaged cellular organelles and extracellular products as well as storage of lipofuschin, the major undigested material [16]. Therefore, the progressive accumulation of lipofuschin in the principal cell of aged rat could functionally impair the intracellular trafficking through combined oxidative damage and decline of the degradative pathways which are reputed causative factors in aging [17, 18].

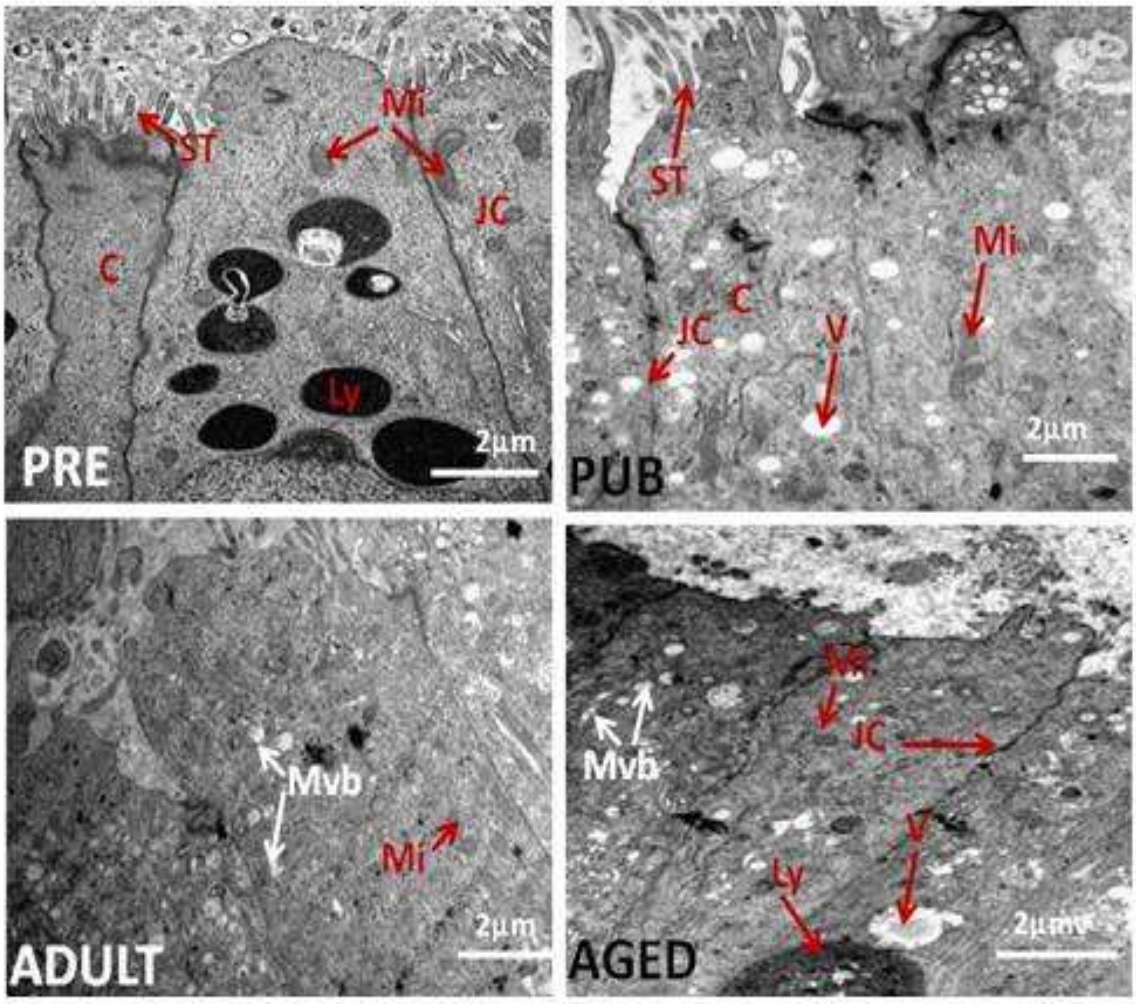

Figure 3. Transmission electron micrographs of the supranuclear aspects of the corpus epididymis of the African greater cane rat. Note the conspicuous apical vacoulations (V) especially in the pubertal rats. Also, observe the numerous lysosomal granules (Ly) in the apical region of prepubertal rat. In the cauda segment, note the degenerating mitochondria (Mi) within the apical PC of aged AGCR. JC - Junctional complex, Mvb – Multi-vesicular bodies, ST-Stereocilia, L-Lumen, C-Ciliated cell.

The age-related increase in nuclear shape indentation observed in the principal cell of corpus and cauda epididymis from pubertal to aged cane rats is consistent with the previously reported nuclear shape indentation in the brown rat [5], adult AGCR [13], Macaque monkey [19] and in ram [20]. These prominent principal cell nuclear shape indentations have been suggested to be associated with increased metabolic and synthetic activities with advancement in age [19].

In conclusion, this study has highlighted age-related changes in the epididymal ultrastructure of cane rat that could possibly be linked to the quiescence and physiological activeness as the case may be in the groups evaluated. Although authors have described the epididymis of the adult AGCR, this work is perhaps the first report of age-related changes in the ultrastructure of the epididymis in the cane rat.

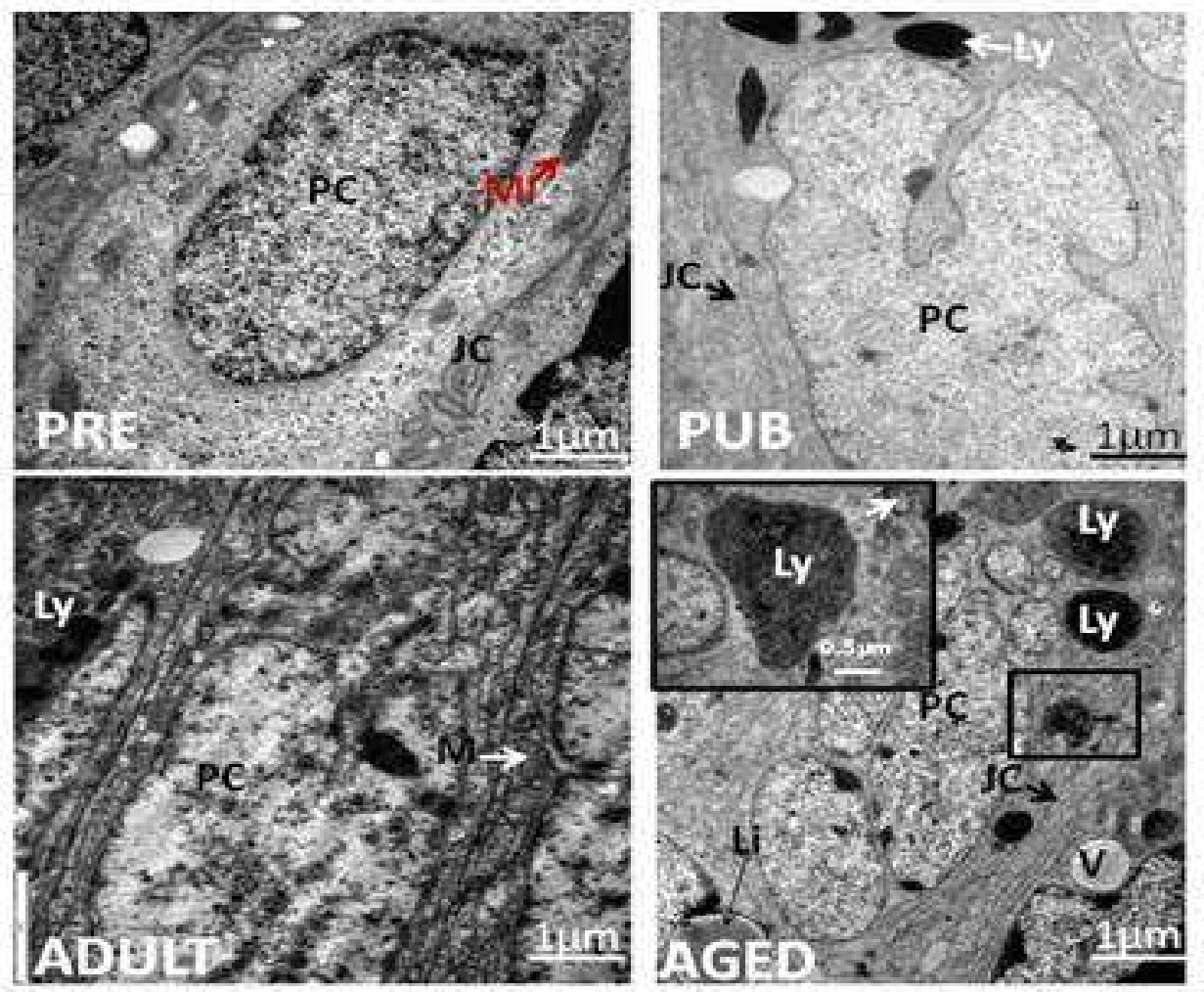

Figure 4. Transmission electron micrographs of the perinuclear aspect of the cauda epididymis principal cells of the African greater cane rat. Note the numerous lysosomal granules (Ly) and degenerating mitochondria (Mi: inset arrow) especially in the aged AGCR and prominent nuclear lobulations of PC in pubertal to aged rats. V-Vacoules, Li- Lipid, PC- Principal cell.

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REFERENCES

1. Cornwall, G. A. (2009). New insights into epididymal biology and function. *Human Reproduction Update,* 15 (2): 213 – 227.
2. Hermo, L. (1995). Structural features and functions of principal cells of the intermediate zone of the epididymis of adult rats. *Anatomical Record,* 242: 515 – 30.
3. Robaire, R., Hinton, B. T. and Orgebin-Crist, M. C. (2006). The epididymis. In: *Physiology of Reproduction*, 3rd Edn., Knobil, E. and Neill, J. D., Eds. Elsevier, New York. Pp. 1071 – 147.

4. Agnes, V. F. and Akbarsha, M. A. (2001). Pale vacuolated epithelial cells in the epididymis of aflatoxin-treated mice. *Reproduction* (formerly *Journal of Reproduction and Fertility*), 122: 629 – 41.
5. Serre, V. and Robaire, B. (1998). Segment-Specific Morphological Changes in Aging Brown Norway Rat Epididymis. *Biology of Reproduction*, 58: 497 - 513.
6. Cran, D. G. and Jones, R. (1980). Aging of male reproductive system: changes in the epididymis. *Experimental Gerontology,* 15: 93 - 101.
7. Fayenuwo, J. O., Akande, M., Taiwo, A. A., Adebayo, A. O., Saka, J. O., Lawal, B. O., Tiamiyu, A. K. and Oyekan, P. O. **(**2003). *Guidelines for grasscutter rearing*. Technical Bulletin, IAR & T., Ibadan. p. 38.
8. Olude, M. A., Mustapha, O. A., Sonubi, A. C., Falade, T. E., Ogunbunmi, T. K., Adebayo, A. O. and Akinloye, A. K. (2014). Morphometric study of the skull of the greater cane rat (*Thryonomys swinderianus*, Temmnick). *Nigeria Veterinary Journal*, 35 (3): 1026 – 1037.
9. Monadjem, A., Taylor, P. J., Denys, C. and Cotterill, F. P. (2015). *Rodents of sub-Saharan Africa: a biogeographic and taxonomic synthesis*. Walter de Gruyter GmbH, Berlin, Germany.
10. Soro D., Karamoko Y., Kimse M. and Fantodji, A. (2014). Study of basic haematological parameters: indicators of the general state and immune competence in the male grasscutter (*Thryonomys swinderianus*, Temminck, 1827) bred in captivity in Côte d'Ivoire. *Journal of Animal and Plant Sciences*, 22 (1): 3379 - 3387.
11. Adebayo, A. O. and Olurode, S. A. (2010). The morphology and morphometry of the epididymis in the greater cane rat *(Thryonomys swinderianusTemmincks). Folia Morphologia,* 69 (4): 246 – 252.
12. Olukole, S. G., Oyeyemi, M. O. and Oke, B. O. (2009). Biometrical observations on the testes and epididdymis of the domesticated adult African greater cane rat (*Thryonomys swinderianus*). *European Journal of Anatomy,* 13 (2): 71 - 75.
13. Adebayo, A.O., Akinloye, A.K., Ihunwo, A.O., Taiwo, V.O. and Oke, B.O. (2016). Zonal Changes in the Ultrastructure of the Epididymal Principal cell of the Greater Cane Rat (*Thryonomys swinderianus*). *Alexandria Journal of Veterinary Sciences*, 48 (1): 99-106
14. Calvo, A., Pastor, L. M., Martinez, E., Vazquez, J. M. and Roca, J. (1999). Age-related changes in the hamster epididymis. *Anatomical Record,* 256: 335 – 346.
15. Hart, L. and Schoning, P. (1984). Age-related changes in the cat testis and epididymis. *American Journal of Veterinary Research,* 45: 2380 – 2384.
16. Ivy, G. O., Roopsingh, R., Kanai, S., Ohta, M., Sato, Y. and Kitani, K. (1996). Leptin causes an accumulation of lipofuscin substances and other signs of aging in kidneys of young rats: further evidences for the protease inhibitor model of aging. *Annals of the New York Academic of Sciences,* 786: 12 – 23.
17. Sohal, R. S. and Brunk, U. T. (1990). Lipofuscin as an indicator of oxidative stress and aging. In: *Lipofuscin and Ceroid Pigments.*Porta, E. A. Ed., Plenum Press, New York. Pp. 17 - 26.
18. Tabatabaie, T. and Floyd, R. A. (1996). Protein damage and oxidative stress. In: *Cellular Aging and Cell Death*. Holbrook, N., Martin, G. R . and Lockshin, R.A Eds., Wiley-Liss, New York Pp35 - 49.
19. Ramos, A. S. and Dym, M. (1977a). Fine structure of the monkey epididymis. *American Journal of Anatomy*, 149 (4): 501.- 532.
20. Elzoghby, I. M. A., Sosa, G. A., Mona, N. A. H. and Manshawy, A. A. (2014). Postnatal development of the epididymis in the sheep. *Benha Veterinary Medical Journal,* 26 (1): 67 - 74.