Vimentin expression profiles in the testis and epididymis of prepubertal to aged African greater cane rat (Thryonomys swinderianus)

**Introduction:** Vimentin is a cytoskeletal protein that is expressed in different regions of the testicular and epididymal parenchyma with principal function of structural support. There is dearth of literature on testicular and epididymal vimentin expression in different ages of African greater cane rat (AGCR). Hence, this study investigated vimentin expression profiles in the testis and epididymis of prepubertal to aged AGCR.

**Methods:** Twenty healthy AGCR procured from a commercial cane rat farm were used for this study. The rats were randomly divided into 4 groups (n=5) as follows; Group 1: prepubertal (1-4 months), Group 2: pubertal (5-11 months), Group 3: adult (12-30 months) and Group 4: aged (>30 months). Testicular and epididymal samples were harvested and processed for immunohistochemistry using anti-vimentin marker.

**Results:** Testicular vimentin was expressed in the interstitium, perinuclear region and basal aspect of Sertoli cells and along the tip of elongated spermatids. In the various segments of the epididymis, vimentin expression was confined to the peritubular coat and interstitium (stroma and perivascular components). Vimentin staining intensity in the testis was significantly higher (p < 0.05) in the adult group relative to others and appeared to increase with age. For the epididymis, significantly higher (p < 0.05) vimentin intensity was observed prepubertally and decreased with age advancement.

**Significance:** The result from this study has demonstrated that the testes of the adult AGCR as well as the epididymal segments of the prepubertal rat had increased vimentin intensities which are indicative of marked reproductive activity and robust content of undifferentiated cells respectively.

**Vimentin expression in the peritubular myoid cells of mammalian testis has been linked to its crucial role in the contractions of the seminiferous tubules in transporting spermatozoa and testicular fluid (Miyake *et al.*, 1986; Maekawa *et al.*, 1996; Sasaki *et al.*, 2010). Available data on age-related changes in vimentin expression intensity in testicular cells most especially Sertoli cell was documented by Sasaki *et al.* (2010) in the mouse deer. It was observed that despite the existence of variation in Sertoli cell sizes between immature and adult mouse deer, there was no remarkable difference in the localization and intensity of vimentin-positive reaction. Similarly, the sub-epithelial myoid cells and the stroma between the epididymal duct interstices positively expressed vimentin in both immature and adult deer (Sasaki *et al.*, 2010). However, there is scarcity of research documentation on age-related changes in vimentin expressions in the reproductive organs of African greater cane rat (AGCR), a wild grass-eating rodent currently being reared as a substitute to livestock protein source in sub-Saharan Africa (Fayenuwo *et al.*, 2003; Olude *et al.*, 2014). This study was therefore designed to investigate age-related changes in vimentin expressions in the testes and epididymis of the African greater cane rat.
Materials and methods

Experimental animals and design

A total of twenty (20) AGCR of known ages used for this study were procured from a commercial cane rat farm. They were acclimatized for 7 days in the Experimental Animal Unit of the Department of Veterinary Anatomy, University of Ibadan and provided with dry corn feed daily and water ad libitum. The protocols for the study were approved and assigned UI-ACUREC/18/0120 by the University of Ibadan Animal Care and Use Research Ethics Committee. The rats were randomly divided into 4 groups (n=5) as follows; Group 1: prepubertal (1-4 months), Group 2: pubertal (5-11 months), Group 3: adult (12-30 months) and Group 4: aged (>30 months). On day 8 of acclimatization, they were sedated with xylazine and ketamine combination (20:80 mg/kg body weight respectively) injected intramuscularly. The abdomen was dissected open via the ventral midline incision and extended cranially to the thoracic cage to expose the heart. This was then followed by the perfusion of 0.5 L of pre-perfusion (primary) solution (0.9% sodium chloride (Aventra, Fidson, Nigeria) and 25, 000 IU/ml of heparin (Heparinum, Polfa). This was succeeded by secondary perfusion of 10% buffered formalin. The perfusion was monitored till satisfactory change i.e. pale appearance devoid of vascular congestion was observed in the testes colouration. Both the testes and epididymides segments (caput, corpus and cauda) were excised for further processing.

Immunohistochemical localization of vimentin in the testes and epididymides of cane rat

The procedure described by Moustafa (2012) was used. Briefly, sections of the testes and epididymides were dewaxed at 60°C in an oven, deparaffinized in 2 changes of xylene and passed through ascending grades of alcohol for rehydration. Subsequent to this, antigens were removed from the sections using 10mM citrate buffer at pH of 6.0 for 25 minutes. The endogenous peroxidase activities as well as non-specific antibody binding were inhibited by subjecting sections of the tissues to 3% H₂O₂ /methanol for 15 minutes. The sections were then washed in phosphate buffered saline (PBS) and later encircled with PAP pen to create a hydrophobic barrier. This was succeeded by incubating in 2% PBS containing 5% bovine serum albumin for an hour. Each section was then immunolabeled using primary antibody; anti-vimentin (Dako 1:200). Thereafter, each section was diluted in 1% PBS milk and 0.1% Triton X detergent (for quick penetration of antibody) and then incubated overnight for 18hrs at 4°C. At this stage, HRP-conjugated secondary antibodies were consequently used by strictly adhering to the manufacturer protocol to detect the bound antibody. The 3, 3’-diaminobenzidine (DAB; Vectastain ABC kit) chromogen at 1:25 dilution ratio was used to enhance the end-product of the reaction within a duration of 5 minutes. Shortly after the enhancement, the sections were dehydrated in graded alcohol concentrations, dealcoholized in xylene, mounted on slides with DPX mountant, cover-slipped and allowed to dry. The slides were then viewed and photographed with light microscope (Olympus BX3-CBH, USA). The captured images were analysed for staining intensity with the use of Image J software (1.46r version) and results obtained were presented as bar charts.

Statistical analysis

Data obtained from the image J quantification of staining intensities of the vimentin immunolabelling were analysed using GraphPad Prism Version 4.00 for Window (GraphPad software Inc., California, USA.) statistical package. The data were subjected to one-way analysis of variance (ANOVA) and Tukey was used for multiple comparisons post hoc. The results were expressed as group mean ± standard error of mean and the level of significance was considered as p < 0.05.

Results and Discussion

Vimentin-positive areas in the testes of all AGCR groups were observed as shown in Fig. 1. These areas included the Sertoli cell (from the perinuclear region to the cytoplasm), spermatids as well as the testicular interstitium. With respect to testicular vimentin staining intensity across the different groups of AGCR, significantly higher (p < 0.05) intensity was displayed by the adult group relative to others and the intensity appeared to increase with age (Fig. 1).

Vimentin positive areas comprised the peritubular coat and interstitium (stroma and perivascular components) in the various segments of the epididymal duct (Figs. 2-7). Vimentin expression intensities along the epididymal duct (initial segments, caput, corpus and cauda segments) in the different age-groups of the AGCR decreased with age with the prepubertal AGCR consistently presenting with strongest staining intensity when compared to other groups (Figs. 2-7).

The expression of vimentin in the Sertoli cell and interstitium of the testis as well as in the peritubular coat and interstitium (stroma and perivascular) in all the epididymal segments of the different cane rat groups further substantiates the earlier documentations on vimentin localisation in the mammalian testis and epididymis (Bilinska, 1989; Sasaki et al., 2010; Moustafa, 2012). The structural support and functions of vimentin in the testis are believed to include; the anchorage and translocation of spermatids in preparation for spermiation (He et al., 2007; Lie et al., 2010; Sasaki et al., 2010). Therefore, the increased testicular vimentin intensity with increase in age in the adult rat could reflect its marked reproductive activity.

Vimentin has also been found to be widely distributed in cells of mesenchymal origin (Kameda, 1995). Thus, it suffices to attribute the increased intensity observed in all the epididymal segments of the prepubertal rats to the presence of higher population of relatively undifferentiated cells when compared to other groups of the cane rat. To our knowledge, this is the first report on the profiles of vimentin expression in testes and epididymides of different age-groups of AGCR.

In conclusion, this study has demonstrated that with increase in age, the testes of the adult African greater cane rats as well as the epididymal segments of the prepubertal rat had increased vimentin intensities indicative of increased reproductive function in relation to age. In addition, the study has demonstrated decrease vimentin expression in both testes and epididymides of aged cane rat which can be associated with reduced reproductive vigour or vitality.
Figure 1. Photomicrographs of vimentin expression in the testis of different age groups of the AGCR. A. Prepubertal B. Pubertal C. Adult D. Aged. Note that the vimentin staining (arrows) is expressed in the Sertoli cell with intense staining located in perinuclear areas of Sertoli cell and along the base with strands of vimentin reaching out to the tips of elongated spermatids in B, C and D. Scale bar: 20µm.

Figure 2. Photomicrographs of vimentin expression in the proximal initial segment (PIS) of the epididymis in the AGCR. A. Prepubertal B. Pubertal C. Adult D. Aged: Note that the Vimentin staining (arrows) is expressed in the epididymal perimuscular coat (black arrow), peribasal cell region (arrow head) and interstitial vasculature (star). Intensity appears more in the pre-pubertal group.
Figure 3. Photomicrographs of vimentin expression in the middle initial segment (MIS) of the epididymis in AGCR. A. Prepubertal; B. Pubertal; C. Adult; D. Aged. Note that the Vimentin staining is expressed in the epididymal perimuscular coat (black arrow), peribasal cell region (arrow head) and interstitial vasculature (star). Intensity appears more in the pre-pubertal group.

Figure 4. Photomicrographs of vimentin expression in the distal initial segment (DIS) of the epididymis in AGCR. A. Prepubertal; B. Pubertal; C. Adult; D. Aged. Note that the Vimentin staining is expressed in the epididymal perimuscular coat (black arrow), peribasal cell region (arrow head) and interstitial vasculature (star). Intensity appears more in the pre-pubertal group.
Figure 5. Photomicrographs of vimentin expression in the CAPUT segment of the epididymis in AGCR. A. Prepubertal; B. Pubertal; C. Adult; D. Aged. Note that the staining is expressed in the epididymal perimuscular coat (black arrow), peribasal cell region (arrow head) and interstitial vasculature (star). Intensity appears more in the pre-pubertal group.

Figure 6. Photomicrographs of vimentin expression in the CORPUS segment of epididymis in AGCR. A. Prepubertal; B. Pubertal; C. Adult; D. Aged. Note that the Vimentin staining is expressed in the epididymal perimuscular coat (black arrow), peribasal cell region (arrow head) and interstitial vasculature (star). Higher intensity occurs in the pre-pubertal group.
Figure 7. Photomicrographs of vimentin expression in the CAUDA segment of the epididymis in AGCR. A. Prepubertal B. Pubertal C. Adult D. Aged. Note that the Vimentin staining is expressed in the epididymal perimuscular coat (black arrow), peribasal cell region (arrow head) and interstitial vasculature (star). Highest intensity occurs in the pre-pubertal group.

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Conflicts of interest

Authors declare that there is no conflict of interest regarding this manuscript.

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