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Original research

Immunolocalization of Desmin, Vimentin and S-100 proteins in testis and epididymis of African striped ground squirrel (*Xerus erythropus*)

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ABSTRACT

The aim of the study was to immunolocalize desmin, vimentin and S-100 proteins in the testes and epididymis of African striped ground squirrel (*Xerus erythropus*) and establish their spatial distribution in the two organs. Formalin fixed-paraffin-embedded sections of the testis and epididymis obtained from ten apparently healthy adult male African striped ground squirrels were processed routinely for immunohistochemistry, using primary antibodies specific to desmin, vimentin and S-100. S-100The results showed that desmin reacted intensely in the myoid cells of the seminiferous tubules and smooth muscle cells of the epididymal ducts. It showed a moderate positive immunoreaction in the interstitial cells of Leydig, and the epididymal inter-ductal loose connective tissue. However, there was no immunoreaction of desmin in the spermatogonia or any other spermatogenic cell of the seminiferous tubule. Vimentin reacted intensely in the Leydig cells and spermatogonia. It showed moderate positive reaction in the myoid cells and the epididymal inter-ductal loose connective tissue. There was no immunoreaction of vimentin in the other spermatogenic cells (other than the spermatogonia). The S-100 proteins expressed a mild positive immunoreaction at the interstitial cells of Leydig, but negative immunoreaction in all other parts of testis and epididymis. Also, there was intense immunoreaction of desmin and vimentin and moderate immunoreaction of S-100 in the vascular endothelium in the testis and epididymis. In conclusion, the spatial distribution of desmin, vimentin and S-100 proteins in the testes and epididymis in the African striped ground squirrel showed some similarities and contrast with other mammals, giving insight into the functions of the proteins in these organs of the rodent.

KeyWords: Desmin, epididymis, immunohistochemistry, S-100, testes, vimentin.

INTRODUCTION

The African striped ground squirrel is a diurnal rodent which belongs to family Sciuridae, genus Xerus, species *Xerus erythropus* (Thorington & Hoffmann, 2005). They are moderately large, ranging from 22 to 29 centimeters in length, with a tail that is 19 to 26 centimeters, nearly as long as the body; their adults weigh between 0.5 to 1 kilogram. They have a sandy-brown to dark brown fur and whitish underparts which have a lateral stripe of pure white fur extending from the shoulders to the hip (Herron & Waterman, 2004). The squirrel is found across Africa, south of the Sahara and savannah countries, usually around cultivated lands (Thorington *et al*., 2012). It is well distributed in Nigeria particularly in North-eastern and South-eastern parts of the country (Ibe *et al*., 2020). Six sub-species are currently recognized, two of which are in Nigeria; *Xerus erythropus erythropus* and *Xerus erythropus chadensis* (Herron & Waterman, 2004). This rodent is an adaptable species and no particular threats have been identified. Thus, the International Union for Conservation of Nature has rated its conservation status as being of least concern (Grubb *et al*., 2008).

Desmin is the major muscle-specific intermediate filament (IF) protein. This 53-kDa type III IF protein is found mainly in the Z-disk of striated muscles and in the dense bodies of smooth muscle cells. It plays an essential role in maintaining muscle cyto-architecture by forming a 3-dimensional scaffold around the myofibrillar Z-disk, and by connecting the entire contractile apparatus to the sub-sarcolemmal cytoskeleton, the nuclei, and other organelles (Vogl *et al*. 2000; He *et al*. 2007).

The IF protein vimentin is expressed in the cells and tissues of many different organisms. The expression of vimentin variants showing high sequence homology and similar expression in major tissues in organisms down to shark, indicates that vimentin has an evolutionary role (Schaffeld *et al*., 2001). Although vimentin was first described in a limited number of physiological and pathophysiological contexts,

more recent findings have suggested that it has diverse roles across a broad range of cell and tissue functions and is coupled to a large variety of human diseases (Satelli & Li, 201). It has been expressed in different parts of the testis of rats, with evidence of decreased intensity as the rats advanced in age (Moustafa, 2012).

The protein S-100 is so named because it is soluble in a 100% saturated solution of ammonium sulphate. It belongs to a multifunctional subfamily of Ca^{2+} binding proteins (including calmodulin, troponin C, parvalbumin, light chain of myosin and intestinal calcium-binding protein) that have many functions including motility, chemotaxis, and secretion (Heizmann *et al*., 2002). It regulates cell division and cell morphology in a calcium-dependent manner (Abd-Elmaksoud *et al*., 2014). It has been suggested to be involved in the processes of spermatogenesis and steroidogenesis in the African four-toed hedgehog (Olukole *et al*., 2020). It has also been demonstrated in the testis of other rodents like the pine vole and mouse (Czykier *et al*. (2000).

The present global demographic upheaval is exerting pressure on the supply of animal protein, so that several rodent species have become source of food in Nigeria, especially among the rural populace (Ibe *et al*., 2023). The African striped ground squirrel is not an exception, and may become endangered in the nearest future. Already, in a study to examine the consumption of rodents as a food source in the south of Benin, Edo state, Nigeria, by Assogbadjo *et al*. (2005), the African squirrel was 1 of the 10 most consumed rodent species in the region. The African striped ground squirrel has also been indicated for cultural ceremonies by farmers and herbal medicinal purposes as components of anti-venom, anti-convulsant and aphrodisiac (Ajayi, 1979; Adeola 1992). Despite these economic values of the African striped ground squirrel, literature on their reproductive biology is dearth; available in extant literature are histological study of their epididymis (Korzerzer *et al*., 2018) and testis (Korzerzer *et al*., 2019a), and gross anatomical description of the entire male reproductive tract (Korzerzer *et al*., 2019b). Elsewhere, Bahmani *et al*. (2015) described the gross anatomy of male reproductive tract in the Persian squirrel, while Akbari & Kianifard (2017) described the anatomy of the accessory sex glands in the male Persian squirrel (*Sciurus anomalus*). However, this is the first study to demonstrate the spatial distribution of desmin, vimentin and S-100 proteins in the testis and epididymis in the African striped ground squirrel. This study will boost existing literature and improve the understanding of the testicular and epididymal architecture and function in the African striped ground squirrel. It will also serve as a lead for future studies on the reproductive biology and medicine in the African striped ground squirrel geared towards successful breeding and domestication of the species.

MATERIALS AND METHODS

The study was carried out in the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. The University is located in the Northern Guinea Savannah zone of Nigeria between latitude 11° 03/ 60// N and longitude 07° 41/ 59.99// E, at an altitude of 550-700 meters with an average annual temperature of 18.0 + 3.7°C to 31.8 + 3.2°C. The monthly average rainfall during the rainy season (May-October) is 148.1++ 68.4 mm (69.2-231.9 mm), while mean monthly relative humidity is 71.1 ± 9.7%.

EXPERIMENTAL ANIMALS

Ten (10) apparently healthy adult male ASGS (*Xerus erythropus*) were caught from the wild, alive and unharmed, by the use of traps, between the months of January and May. The animals were conveyed in constructed iron cages to the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, for the research study.

EXPERIMENTAL PROCEDURE

Ethical approval was sought and obtained from the Care on Animal Use Committee (CAUC), Ahmadu Bello University, Zaria, with approval number ABUCAUC/2018/025. The parameters used for determining if the rodents had attained sexual maturity were anogenital distance (Sacramento *et al*., 2013), body weight and radiographs of the hind-limbs to observe their epiphyseal growth plates. The research animals were acclimatized for a period of twenty-one days, during which they were fed with groundnut, water melon, sweet potatoes, tomatoes and water ad libitum. Blood samples were analysed at the Parasitology Department, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria for haemoparasites. The body length (distance from the nose to the tip of the tail) of each animal was measured using a measuring thread and rule. The radiographs were taken at the imaging unit of Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria using the conventional mobile X-ray machine. The procedure involved placing the research animals on the X-ray table on ventral recumbency with their limbs stretched out, and the X-ray beams passed on the limbs to obtain the caudo-cranial view of the limbs. The X-ray photographs were processed manually, almost immediately

The body weights of the animals were determined using a weighing balance (Fuji Keiryo weighing scale K-1, 210 × 210, 200g - 8kg), thereafter, they were euthanized with 30mg/ kg body weight of thiopental sodium, i/m. The euthanized animals were placed on dorsal recumbency and a thoracolumbar incision made up to the point of the pubic region, to expose the reproductive organs. The skin and superficial tissues were reflected to obtain the testes, epididymis, ductus deferens, penis and the accessory sex

glands. Photographs of the organs were taken using a digital camera (Samsung SH100, 14.2 megapixels), and the organs were exteriorized and weighed using an electronic balance (GG 1000 USA, with sensitivity 0.01).

IMMUNOHISTOCHEMISTRY TECHNIQUE

Sections of 4 µm thick were made from the embedded testes and epididymis using a microtome (Leica, Model RM2125RTS, Biosystems, made in China), and then mounted on positively charged glass slides. The specimens were then incubated at 65º C for 1 hour, and then placed in water. Sections were covered with 3% H2O2 for 10 minutes to quench endogenous peroxidase activity. Then washed in PBS and antigen retrieved at 95º C in 20mM Tris-Hcl buffer (pH 8) for 20 minutes, and then washed again in PBS.

The specimen were then blocked in PBS containing 5% bovine serum albumin for 1 hour, and then incubated with the primary antibody (Rabbit monoclonal anti-S-100, DB 055, Immunogen: Peptide derived from C-terminal sequence of human S-100-A1 protein), at a dilution of 1:100 for 30 minutes, DB BIOTECH, Slovakia, Anti-Desmin, Rabbit clonal antibody DB 148), at a dilution of 1:100 for 30 minutes and (anti-Vimentin monoclonal antibody (VIM-572-L-CE), at a dilution of 1:400 for 30 minutes in the closed humidified chamber for 60 minutes, and washed in PBS (3×). Staining was completed by a 5-minute incubation with 3,3' diaminobenzidine tetrahydochloride (DAB) in 3% H_2O_2 which results in a brown precipitate at antigen sites.

were obtained by omitting the primary antibody. Positive controls as suggested by the manufacturer were also employed. Photomicrographs of the tissues were obtained using an Amscope MT digital camera for microscope MD 500, connected to a Compact Presario computer.

RESULTS

IMMUNOREACTION OF DESMIN, VIMENTIN AND S-100 IN THE TESTIS OF THE ADULT AFRICAN STRIPED GROUND SQUIRREL

The scoring of desmin, vimentin and S-100 immunoreactive cells in the different parts of the epididymis of the African striped ground squirrel was represented in Table 2. Desmin reacted strongly in the vascular endothelium and smooth muscle cells of the peri-ductal spaces (Fig. 4: a and b), moderately in the inter-ductal loose connective tissue stroma (Fig. 4: c) and mildly in the epithelial apical cells and stereocilia of the epididymis (Fig. 4: e and f). It was unreactive in the basal cells of the epithelium (Fig. 4: d). Vimentin reacted strongly in the vascular endothelium (Fig. 5: a), moderately in the inter-ductal loose connective tissue stroma (Fig. 5: c) and non-reactive in the smooth muscle, epithelial basal, apical and stereocilia cells of the epididymis (Fig. 5: b, d-f). Lastly, S-100 was moderately reactive in the vascular endothelium (Fig. 6: a), and non-reactive in other parts of the epididymis (Fig. 6: b-f).

Table 1: Semi-quantitative analysis of desmin, vimentin and S-100 immunoreactive cells in the testis in the African striped ground squirrel.

Epididymis	Vascular endothelium	Peri-ductal smooth muscle cells	Inter-ductal loose connective tissue stroma	Epithelial basal cells	Epithelial apical cells	Stereocilia
Desmin	+++	+++	++	-ve	+	+
Vimentin	+++	-ve	++	-ve	-ve	-ve
S-100	++	-ve	-ve	-ve	-ve	-ve

Table II: Semi-quantitative analysis of desmin, vimentin and S-100 immunoreactive cells in the epididymis in the African striped ground squirrel

Epididymis	Vascular endothelium	Peri-ductal smooth muscle cells	Inter-ductal loose connective tissue stroma	Epithelial basal cells	Epithelial apical cells	Stereocilia
Desmin	+++	+++	++	-ve	+	+
Vimentin	+++	-ve	++	-ve	-ve	-ve
S-100	++	-ve	-ve	-ve	-ve	-ve

A semi-quantitative subjective scoring was conducted by three independent observers to assess the immunoreaction. Immunohistochemical negative controls for each staining

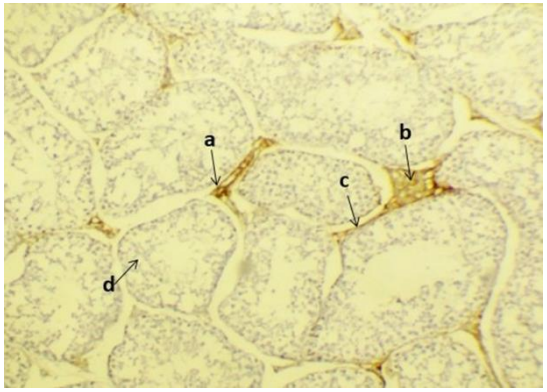

Fig. 1: Immunoreaction of Desmin in the Testis of the African Striped Ground Squirrel. a: strong immunoreaction of vascular endothelium; b: moderate immunoreaction of Leydig cells; c: strong immunoreaction of myoid cells; d: negative immunoreaction of spermatogenic cells. Mag. ×40.

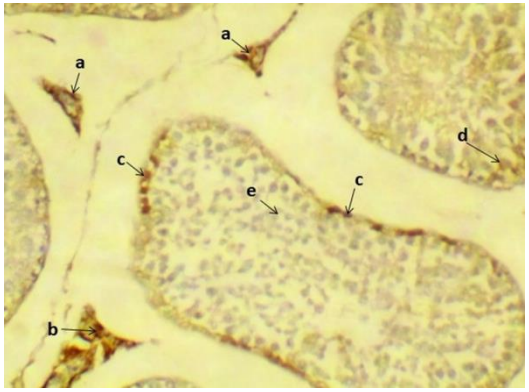

Fig. II: Immunoreaction of Vimentin in the Testis of the African Striped Ground Squirrel. a: strong immunoreaction of vascular endothelium; b: strong immunoreaction of Leydig cells; c: strong immunoreaction of spermatogonia; d: mild immunoreaction of Sertoli cells; e: negative immunoreaction of spermatocytes. Mag. ×100.

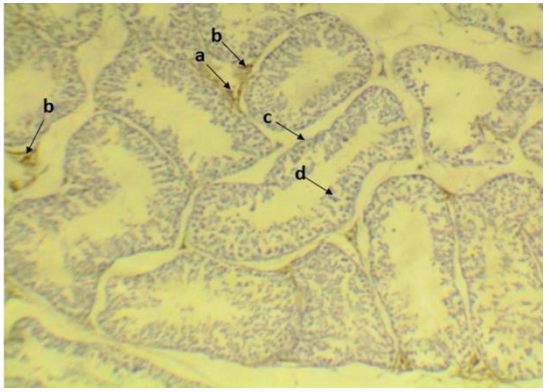

Fig. III: Immunoreaction of S-100 in the Testis of the African Striped Ground Squirrel. a: moderate immunoreaction of vascular endothelium; b: mild immunoreaction of Leydig cells; c: negative immunoreaction of myoid cells: d: negative immunoreaction of spermatogenic cells. Mag. ×40.

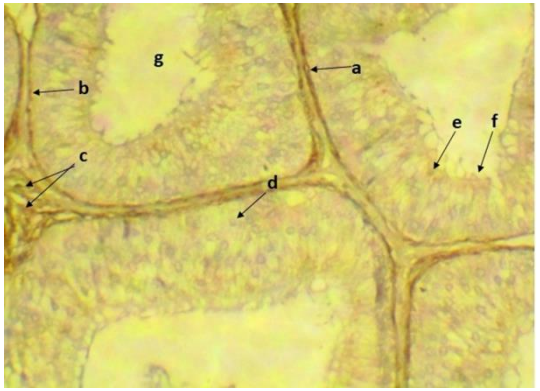

Fig. IV: Immunoreaction of Desmin in the Epididymis of the African Striped Ground Squirrel. a: strong immunoreaction of vascular endothelium; b: strong immunoreaction of peri-ductal smooth muscle cells; c: moderate immunoreaction of inter-ductal loose connective tissue stroma: d: negative immunoreaction of epithelial basal cells; e: mild immunoreaction of epithelial apical cells f: mild immunoreaction of stereocilia; g: lumen of duct of epididymis. Mag. ×100.

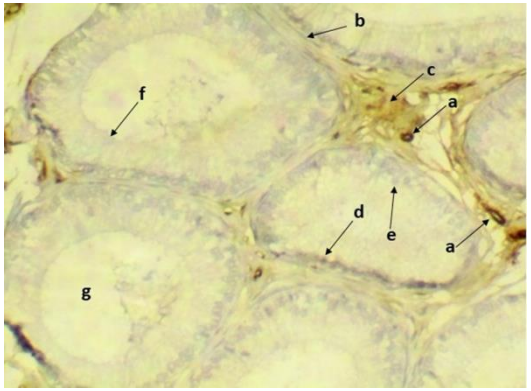

Fig. V: Immunoreaction of Vimentin in the Epididymis of the African Striped Ground Squirrel. a: strong immunoreaction of vascular endothelium; b: negative immunoreaction of peri-ductal smooth muscle cells; c: moderate immunoreaction of inter-ductal loose connective tissue stroma: d: negative immunoreaction of epithelial basal cells; e: negative immunoreaction of epithelial apical cells; f: negative immunoreaction of stereocilia; g: lumen of duct of epididymis. Mag. ×100.

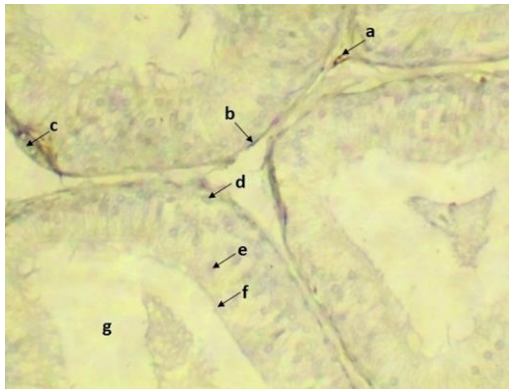

Fig. VI: Immunoreaction of S-100 in the Epididymis of the African Striped Ground Squirrel. a: moderate immunoreaction of vascular endothelium; b: negative immunoreaction of peri-ductal smooth muscle cells; c: negative immunoreaction of inter-ductal loose connective tissue stroma: d: negative immunoreaction of epithelial basal cells; e: negative immunoreaction of epithelial apical cells; f: negative immunoreaction of stereocilia; g: lumen of duct of epididymis. Mag. ×100.

IMMUNOREACTION OF DESMIN, VIMENTIN AND S-100 IN THE EPIDIDYMIS IN THE ADULT AFRICAN STRIPED GROUND SQUIRREL

The scoring of desmin, vimentin and S-100 immunoreactive cells in the different parts of the epididymis of the African striped ground squirrel was represented in Table II. Desmin reacted strongly in the vascular endothelium and smooth muscle cells of the peri-ductal spaces, moderately in the inter-ductal loose connective tissue stroma and mildly in the epithelial apical cells and stereocilia of the epididymis. It was unreactive in the basal cells of the epithelium. Vimentin reacted strongly in the vascular endothelium, moderately in the inter-ductal loose connective tissue stroma and non-reactive in the smooth muscle, epithelial basal, apical and stereocilia cells of the epididymis. Lastly, S-100 was moderately reactive in the vascular endothelium and non-reactive in other parts of the epididymis studied.

DISCUSSION

The present study has demonstrated the spatial distribution of some cytoskeletal proteins in the testis and epididymis in the African striped ground squirrel, as the first of its kind in this rodent. Research findings have demonstrated that cytoskeletal proteins such as desmin and vimentin compose the cytoskeleton of the mammalian testes, and are related to their structural support, among other functions (He *et al.*, 2007; Sasaki *et al*., 2010; Moustafa, 2012). Peritubular myoid cells, surrounding the seminiferous tubules in the testis, have been found in all mammalian species, as a cytoskeletal layer of smooth muscle cells in rodents and stratified layer of smooth muscle in primates (Maekawa *et al*., 1996). It was also observed as a single layer of smooth muscles in the African striped ground squirrel from this study. Apart from providing structural integrity to the seminiferous tubules, myoid cells have been linked to play regulatory roles in spermatogenesis by influencing Sertoli cell, androgens and retinol activities (He *et al.*, 2007). Cytoskeletal proteins such as desmin and vimentin have been identified in the myoid cells of some rodents and non-rodent mammals. In the present study, there was strong immunolocalisation of desmin in the peritubular myoid cells in the African striped ground squirrel. Frojdman *et al.* (1992) and Sohn *et al.* (2013) made similar observation of desmin in the testicular myoid cells in adult Wistar rats and water deer, respectively. However, Sasaki *et al.* (2010) did not observe desmin in the testicular myoid cells of the lesser mouse deer, but in their epididymal smooth muscles instead. The strong immunoreaction of desmin in myoid cells in the African ground stripped squirrel confirms the role of the protein in signaling between the extra-cellular matrix and sarcomere which regulates muscle contraction and movement. Desmin also plays a crucial role in maintaining the structure of sarcomeres, inter-connecting the Z-disks and linking them to the nucleus and mitochondria, thus providing strength for the muscle fibre during activity.

Desmin was not reactive in the Sertoli and spermatogenic cells in the present study. In fetal and neonatal testes of the Wistar rat, cytoskeletal proteins were expressed in the Sertoli cells, but completely became unreactive by adulthood (Paranko *et al.,* 1986). Furthermore, neither Sasaki *et al.* (2010) nor Sohn *et al.* (2013) observed desmin in the Sertoli and spermatogenic cells of adult lesser mouse deer and water deer, respectively, similar to the result of the present research. These findings imply that the immunoreaction of desmin in Sertoli and spermatogenic cells in mammals may be age-dependent. Further studies need to be undertaken in neonatal and foetal African striped ground squirrel to confirm or refute the present negative findings of desmin in the Sertoli and spermatogenic cells in the adult. The observation of desmin in vascular endothelium in the epididymis of African striped ground squirrel in this study was consistent with the finding in the lesser mouse deer by Sasaki *et al.* (2010).

In the present study, there was immunoreaction of vimentin in the vascular endothelial, Leydig, peritubular myoid and Sertoli cells, and spermatogonia, as well as in the vascular endothelium and inter-ductal loose connective tissue stroma of the epididymis in the African striped ground squirrel. Sasaki *et al.* (2010), and Sohn *et al.* (2013) and Omirinde *et al.* (2020) made similar observation of vimentin in the vascular endothelium, Leydig, peritubular myoid, Sertoli and spermatogenic cells in the testis of adult lesser mouse deer, and water deer cane rat, respectively. Similarly, while Wang *et al.* (2002) observed immunoreaction of vimentin in the Sertoli, Leydig and peritubular myoid cells of the Wistar rat testis. The immunolocalisation of vimentin in the vascular endothelium, Leydig and Sertoli cells in the African striped ground squirrel in the present study is also consistent with the findings in the testis of albino rats examined for vimentin by Moustafa (2012). However, Moustafa (2012) observed that the intensity of expression of vimentin in these testicular cells decreased with aging in the rat, and linked this to age-related changes, such as reduction in testicular size, and decrease in Leydig cell steroidogenesis. In another study, Omirinde *et al.* (2020) observed that the intensity of expression of vimentin in the epididymal cells decreased with age in the cane rat, and linked this to the presence of higher population of relatively undifferentiated cells in prepubertal cane rat relative to adult. This is so as vimentin is widely distributed in mesenchymal cells (Kameda, 1995).

The immunoreaction of vimentin in the Sertoli and spermatogenic cells may be linked to the need of vimentin filaments to anchor spermatogenic cells to the Sertoli cells as demonstrated by He *et al.* (2007).

The spatial distribution of S-100 protein in the testis in the African striped ground squirrel from the present study is comparable to that of other mammals. The immunoreaction in the vascular endothelium is same as in the pine vole, rabbit and African four-toed hedgehog by Czykier *et al.* (2000), Abd-Elmaksoud *et al.* (2014) and Olukole *et al.* (2020), respectively. However, while the immunoreaction of S-100 in the Leydig cells in the African striped ground squirrel in the present study was also reported in other rodents such as the Wistar rat, bank vole (and mouse) and African four-toed hedgehog by Amselgruber *et al.* (1994), Czykier *et al.* (2000) and Olukole *et al.* (2020), respectively, it was unreactive in the Leydig cells of lagomorphs such as the rabbit (Abd-Elmaksoud *et al.* (2014). This presence of S-100 in vasculature of blood vessels have been attributed to the protective role of the protein against mitochondrial dysfunction and cell death due to intracellular calcium overload (Orrenius *et al*., 2003). Also, the immunoreaction of S-100 in the Leydig cell indicates the role of the protein in the regulation of androgen activities in rodents. The negative immunoreaction of S-100 in the spermatogenic cells in the present study was also reported in the Wistar rat and rabbit by Amselgruber *et al.* (1994) and Abd-Elmaksoud *et al.* (2014), respectively, but not in the African four-toed hedgehog as Olukole *et al.* (2020) observed moderate immunoreaction. There was no immunoreaction of S-100 in the Sertoli cells of the African striped ground squirrel in the present study. Amselgruber *et al.* (1994) also reported negative immunoreaction of S-100 in Sertoli cells of Wistar rats and proffered that immunoreactivity in the Sertoli cells may be dependent upon cyclic variations of the seminiferous epithelium. Conversely, Olukole *et al.* (2020) observed moderate immunoreaction of S-100 in Sertoli cells of African four-toed hedgehogs, while Omirinde *et al.* (2023) reported major expression in the Sertoli cell of cane rats (African giant pouched rats).

Result of the present study indicated negative immunoreaction of S-100 in the epididymal smooth muscle cells, epithelial basal and apical cells and inter-ductal connective tissue. This is similar to the report obtained in the dromedary camel by Alkafafy *et al.* (2011); they recorded varied immunoreaction of S-100 in different parts of the epididymis in the dromedary camel with the nuclei of epithelial basal cells, stereocilia of epithelial principal and apical cells being mostly negative. Conversely, the report of this study differs from the findings of Abd-Elmaksoud *et al.* (2014) who reported immunoreaction of S-100 in the epithelial basal cells and inter-ductal loose connective tissue stroma of rabbits. It also differed from the report of Czykier *et al.* (2000) who observed weak immunoreaction of S-100 in epididymal smooth muscle cells in pine vole and mouse. Furthermore, the immunoreaction of S-100 in the epididymal perimuscular coats and perivascular tissue of the cane rat as observed by Omirinde *et al.* (2023) differs from the observations in the present study. Although S-100 has been reported to be multifunctional in cells, its specific role in the mammalian epididymis is still largely unknown (Cruzana *et al*., 2003). Czykier *et al.* (2000) suggested that it may play role in blood-testis barrier, transcytosis and contractility in testis and epididymis.

CONCLUSION

The present study described the spatial distribution of desmin, vimentin and S-100 proteins in the testis and epididymis in the adult African striped ground squirrel. The findings were compared with similar findings from studies conducted on rodents and non-rodent mammals. Possible inferences were made from the findings of this study. It is expected that this study will narrow the literature gap in the anatomy and immunohistochemistry of the adult testis in the African striped ground squirrel (*Xerus erythropus*).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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