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Variation in the Immunohistochemical expression of S-100 in the Testes and Epididymides of Different Age groups of Cane Rat (*Thryonomys swinderianus*)

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ABSTRACT

Introduction: the saturated (S-100) protein, a family of calcium binding proteins, has been found to be expressed in the different parts of the mammalian testis and epididymis with diverse functions including secretory, absorptive and blood-testis barrier formation. There is dearth of report on S-100 expression in the testis and epididymis of different age groups of cane rats (*Thryonomys swinderianus*). Therefore, this study investigated the variation in the S-100 immunohistochemical expression in the testes and epididymides of different age categories of domesticated cane rats.

Materials and Methods: twenty healthy male cane rats were used for this study. The cane rats were randomly sorted into four different groups (n=5); pre-pubertal, pubertal, adult and aged. The testicular and epididymal tissues were excised, trimmed and immunohistochemically processed using anti S-100 marker.

Results: testicular S-100 was majorly expressed in the Sertoli cell nuclei and cytoplasm of pubertal, adult and aged cane rats. While in the epididymal segments, S-100 was exclusively evident in the perimuscular coats and perivascular tissue of the interstitium of the corpus and cauda segments of the epididymal duct in the different cane rat groups. The testicular parenchyma S-100 intensity was significantly higher ($p<0.05$) in the aged cane rat relative to others and the intensity increases with age. Regarding epididymis, the S-100 intensity was significantly higher in the pubertal when compared to others.

Conclusion: the sets of data from our study have shown that the aged cane rat testes and epididymal segments (corpus and cauda) in pubertal rats had higher S-100 intensities which are suggestive of robust reproductive vigour.

Keywords: S-100 immunohistochemical expression; Testes;Epididymides; Cane rat.

Introduction

The saturated (S-100) protein, a family of calcium binding proteins, bears a low molecular weight ranging between 10 to 12kDa[1]. It was named for its dissolving ability in ammonium sulphate solution saturated at 100% and at pH of 7 (neutral)[2]. Based on plethora of works, this protein is known for diverse roles that includes; motility, secretion, transcription, apoptosis, neurite extension and chemotaxis [1,3,4,5] and a potential axonal marker[6].

S-100 proteins have been immuno-localized in the testis and epididymis of different mammalian species including rat and cat[4,7], farm animals[7,8,9], buffalo[10], rabbit and human[11,12]. In these diverse species, S-100 has been suggested to participate in the secretory and absorptive functions and may also play roles in blood-testis barrier formation[3,4,5,13].

Generally, S-100 immunostaining of the testicular parenchyma and epididymis have been reported to present varied staining intensity. Strong staining intensity occurred within the vasculature (arteries, veins and capillaries) of testis and epididymis especially in rat, pig, sheep, goat and European bison and man[3,7,8,14,15] as well as in the nuclear and cytoplasmic portions of the Leydig cells of rats, cats, and human[7]. Also, distinct immunostaining was reported in peritubular cells of the testis of tom, dog and rat[4,7].

However, weak S-100 immunostaining was demonstrated in the testicular Leydig cell of pig and stallion[3,7]. Similarly, the epididymal basal and principal cells nuclear and cytoplasmic parts have been reported to show intense S-100 immunostaing in bovine, donkey, buffalo and camel[9,10,16]. It is essential to state that in bovine the ciliated cells of the epididymis have been found to be strongly stained by S-100 marker[9].

Age-related changes in the expression of S 100 in the testis and epididymis in mammals is less reported. Czykier *et al.*[15] observed a weak expression of S-100 in the smooth muscle cells of epididymal arteries and vein of young European Bison relative to their adult counterpart and an equally strong positive expression in the endothelium of the vasculature (arteries, veins and lymphatics) of both young and adult epididymis.

However, there is dearth of information on the variation in S-100 localization in the reproductive organs (testes and epididymides) of different age

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category of cane rat otherwise known as African greater cane rat [AGCR] (*Thryonomys swinderianus*), a wild herbivorous rodent which is at present being massively bred as alternative to livestock protein source and a potential laboratory rodent of African origin[17,18]. Therefore, this study seeks to investigate post-natal variations in the expression of S-100 in the testes and epididymides of cane rat.

Materials and Methods

Twenty (20) disease free male cane rats used for this study were sourced from Pavemgo commercial farm, Ibereko, Badagry, Lagos state, Nigeria. The birth records of the cane rats were obtained at purchase. Thereafter, they were acclimatized for one week in the Experimental Animal Unit of Faculty of Veterinary Medicine, University of Ibadan. The rats were fed daily on dry corn and water *ad libitum*. The experimental protocol was approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UIACUREC) and was assigned UIACUREC/18/0120.

Experimental design

The experimental grouping earlier reported in our previous work Omirinde *et al.* [19] was adopted. Briefly, procured rats were randomly divided into four groups of five animals (n = 5); i. Prepubertal (Pre; ≤4 months), ii. Pubertal (Pub; >4 ≤12 months), iii. Adult (>12 ≤30 months), and iv. Aged (>30 months) based on birth records. The cane rats were sedated via intramuscular injection of xylazine (20 mg/kg) and ketamine (80 mg/kg). Thereafter, sodium chloride (0.9%), heparin (25,000 IU/ml) and buffered formalin (10%) were intracardially perfused. The testicular and epididymal (caput, corpus and cauda) tissue segments were excised for further processing.

Immunohistochemistry Protocols

The immunohistochemistry protocols reported by Alkafafy *et al.*[10] was used. Briefly, testicular and epididymal tissue sections were dewaxed at 60°C, deparaffiinized in double changes in xylene and rehydrated in ascending grades of alcohol. This was followed by antigen retrieval from the sections using 10mM citrate buffer (pH = 6.0) for 25 minutes. Three percent of H2O2 /methanol was used to remove both endogenous peroxidase and non-specific antibody from the sections for 15 minutes. Thereafter, the sections were washed in phosphate buffered saline (PBS) and encircled with PAP pen to establish a hydrophobic barrier. The sections were in turn incubated for an hour in a mixture of 2% PBS and 5% bovine serum albumin. This was followed by immunolabeling using primary antibody; polyclonal rabbit anti-S 100 (Dako 1:400). For rapid penetration of antibody, sections were diluted in 1% PBS milk and 0.1% Triton X detergent and incubated overnight at 4°C for a duration of 18hrs. To determine bound antibody in the incubated section, horseradish peroxidaseconjugated secondary antibodies were used by strictly adhering to the manufacturer protocol. The resultant product of the reaction in the sections was enhanced using 3, 3'- diamino-benzidine (DAB; Vectastain ABC kit) chromogen at a dilution ratio of 1:25 for 5 minutes. Sequel to the enhancement, sections were dehydrated in grades of alcohol concentrations, dealcoholized in xylene, mounted on slides with DPX mountant, cover-slipped and allowed to dry. The slides were then viewed and captured with light microscope (Olympus BX3-CBH, USA). The captured images were analyzed for staining intensity using Image J software (1.46r version) and results obtained were presented as bar charts.

Statistical analysis

The data obtained from the imageJ staining intensity quantification of the S-100 immunolabeling were analyzed using GraphPad Prism Version 4.00 for Window (GraphPad Software Inc., La Jolla California, USA) statistical package. The differences in the staining intensity of S-100 immunolabeling was compared between the different cane rat groups using oneway analysis of variance (ANOVA) and Tukey test was used for multiple comparisons *post hoc*. The level of significance was considered at $\alpha 0.05$.

Results

Immunohistochemical Expressions of Structural Proteins (S-100) in the Testis and Epididymis

With the exception of the negative reaction observed in the prepubertal testis as well as in the seminiferous interstitium of all age group of cane rats, Sertoli cell nuclei and cytoplasm of pubertal, adult and aged cane rats were positive to S-100 staining (Fig. 1). In addition, conspicuous S-100 positive areas were exclusively evident in the perimuscular coats and perivascular tissue of the interstitium of the corpus and cauda segments of the epididymal duct in the different age groups of cane rats (Figs. 3 and 4).

On the intensity of S-100 in the testicular parenchyma of different cane rats, significantly higher ($p<0.05$) intensity was seen in the aged cane rats relative to others and the intensity increases with age (Fig. 1). The caput epididymal S-100 intensity was not possible due to the observed negative immunoreactivity (Fig. 2). The S-100 intensity was markedly increased in the corpus epididymis of the pubertal cane rat compared to the prepubertal rat (Fig. 3). However, the S-100 intensity in the cauda epididymal segments showed significantly increased values in pubertal rat when compared to others (Fig. 4).

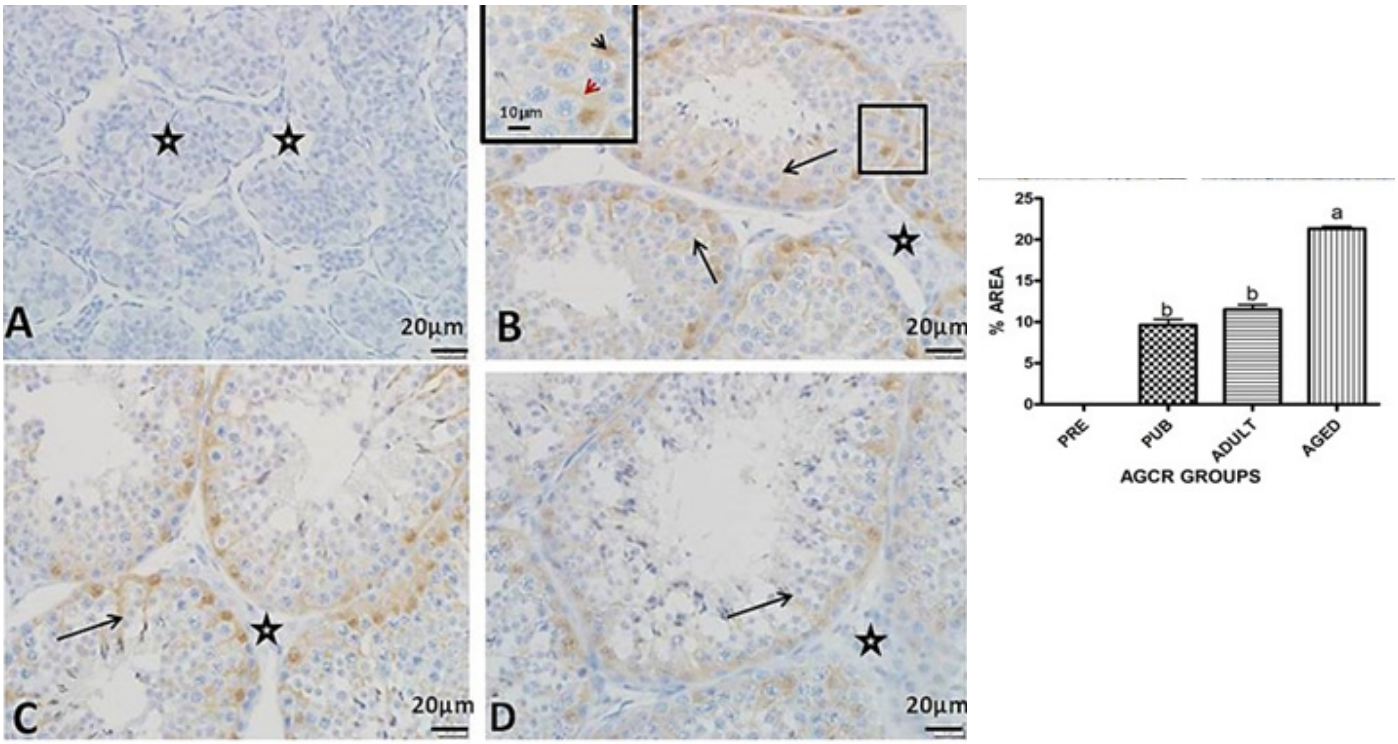

Figure 1. Photomicrographs of S-100 expression in the testis of different age groups of AGCR. A. Prepubertal: B. Pubertal: C. Adult: D. Aged: Note the positive S-100 staining in the Sertoli cells (inset; nuclei [black arrow head] and cytoplasm [red arrow head] (arrows), negative staining in the interstitial tissue (star) and slide A. Scale bar: 20µm (main) and 10µm (inset). Bars with different superscripts are significantly different.

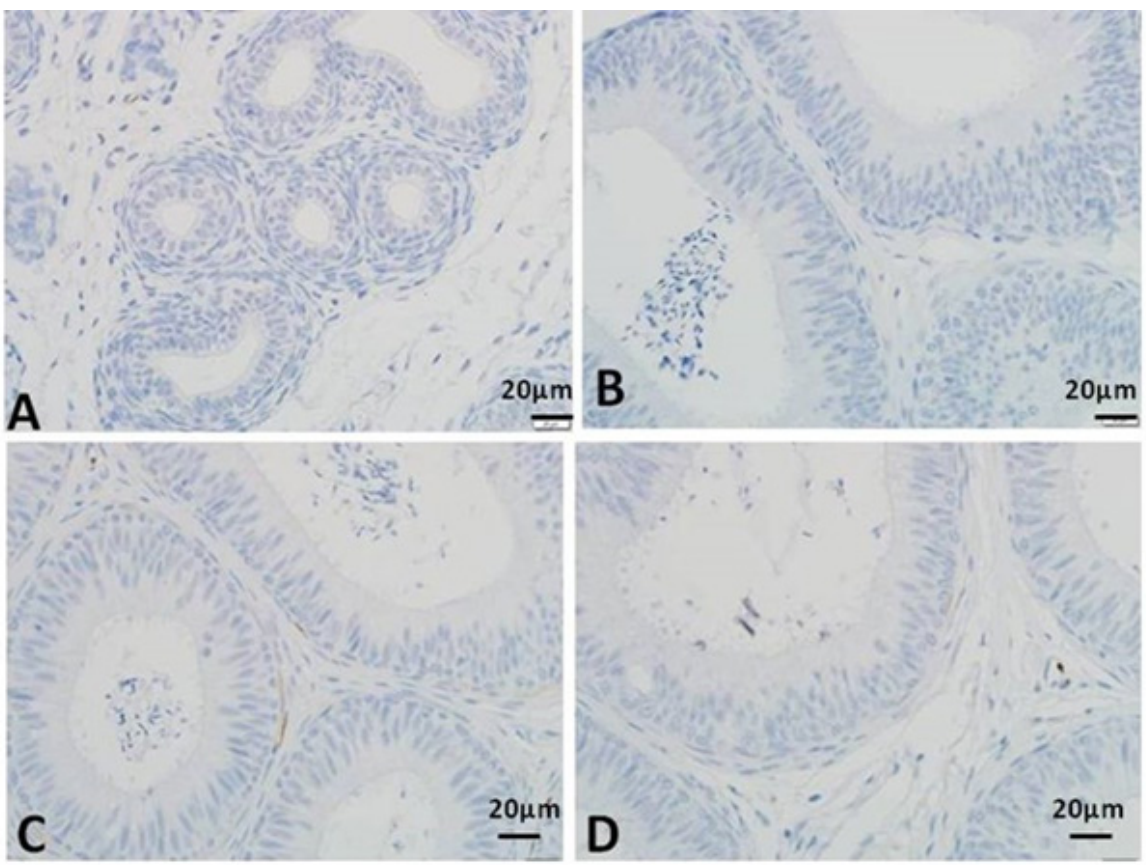

Figure 2. Photomicrographs of S-100 expression in the CAPUT segment of epididymis in AGCR. A. Prepubertal B. Pubertal C. Adult D. Aged Note: There was no positive S-100 staining in the caput epididymal parenchyma in A - D. Scale bar: 20µm.

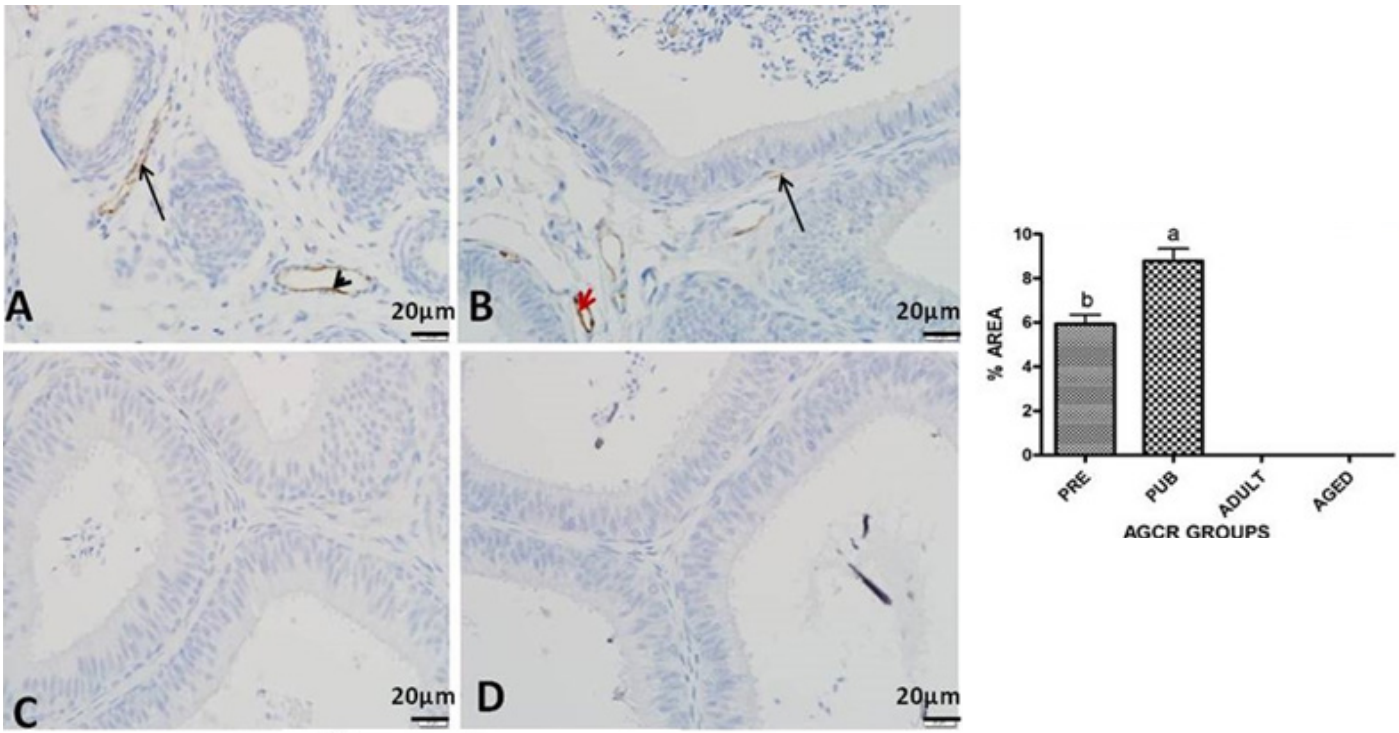

Figure 3. Photomicrographs of S-100 expression in the CORPUS epididymal segment in AGCR. A. Prepubertal: B. Pubertal: C. Adult: D. Aged: Note the positive S-100 staining in the perimuscular coats (arrows) and interstitial vessels (arrow heads) as well as negative staining in C and D. Scale bar: 20µm.

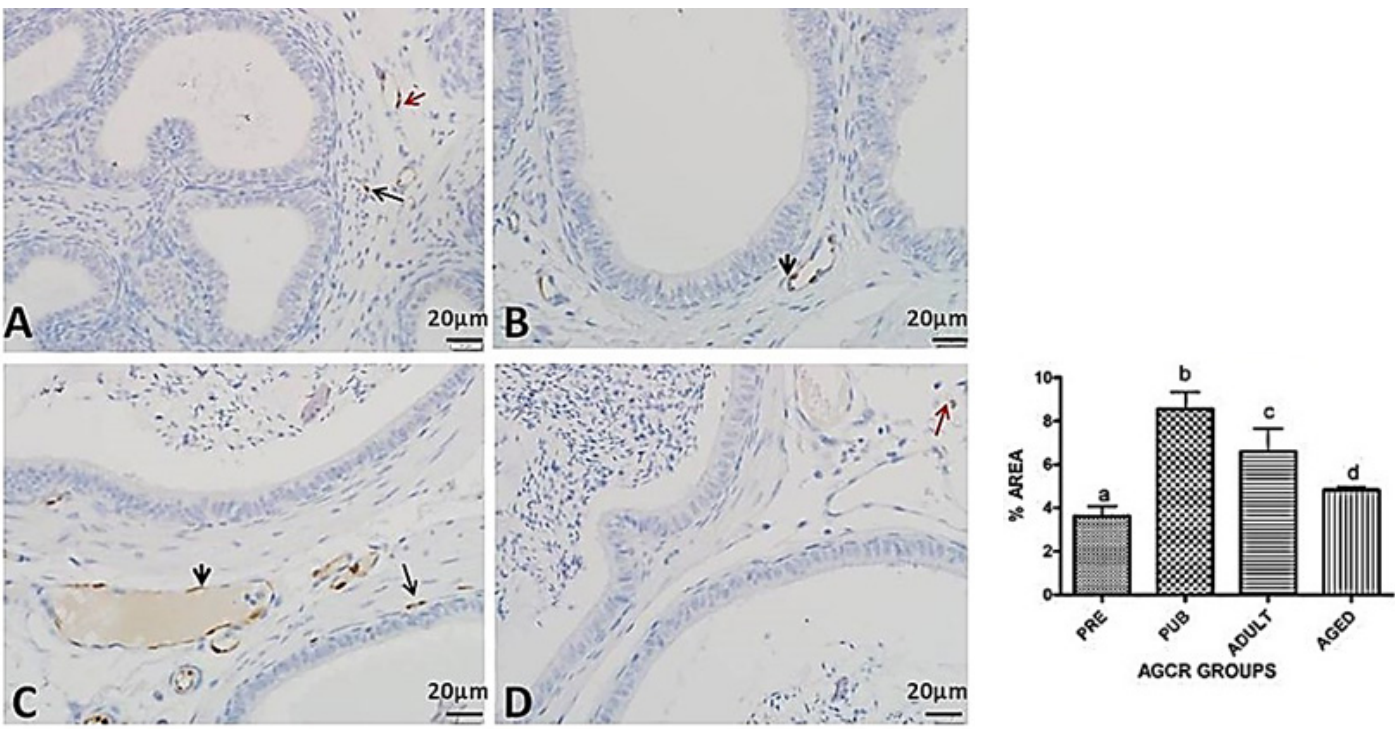

Figure 4. Photomicrographs of S-100 expression in the CAUDA segment of epididymis in AGCR. A. Prepubertal: B. Pubertal: C. Adult: D. Aged: Note the positive S-100 staining in the perimuscular coats (arrows), interstitium proper (red arrow) and interstitial vessels (arrow heads). Scale bar: 20µm

Discussion

Saturated (S)-100 is a structural protein with unclear biological function though available reports have shown that it may be involved in establishing the blood-testis barrier[3,4,5,13]. Therefore, the S-100 immunopositivity observed in the nuclear and cytoplasmic part of the Sertoli cells of the pubertal, adult and aged cane rat used in this study further validate the assertion stated above. Morphologically, the blood-testis barrier (BTB) is majorly formed by the Sertoli cell cytoplasmic processes extension around the testicular germ cells[20]. Going by the S-100 profile seen in this study, one can assumed that the testicular BTB seemed to be well established in the testes of pubertal to aged rats.

However, the negative immunolocalization of S-100 in prepubertal testis is difficult to explain for now because there are previous reports of completion of testicular BTB assemblage in rats by postnatal day[18,21,22,23]. Going forward, we hope to clear this doubt in our subsequent study that will incorporate prenatal testis along with the present prepubertal data so that the missing gap in S-100 profile in immature testes of African greater cane rat can be properly unraveled.

Considering the age-related increase in the intensity of S-100 expression in the testes of cane rat from pubertal to aged, it could be suggested that the intensity seems to correlate with maturity. Also, it could be deduced that the intensity is indicative of striking secretory and absorptive processes as well as blood- testis barrier strengthening which are peculiar to these age groups.

The observed positive immunoreactivity to S-100 proteins in the periductal muscle coat, interstitial stroma and perivascular part of the corpus and cauda epididymal segments in nearly all the AGCR is similar to the reports of the distribution of S-100 in European Bison, rat and mouse epididymis[3,14]. On the other hand, the negative S-100 immunoreactivity in the caput epididymis of all age category and in the corpus epididymis of the adult and aged cane rats could reflects that less or no secretory and absorptive activities occur in the highlighted regions. Although, this defy the well-established functions of those segments[24]. The strong intensity found in the corpus and cauda epididymal segments of pubertal AGCR especially in the interstitial vascular endothelium can be linked to S-100 involvement in transcytotic movement of materials within the interstitium[3,14].

In conclusion, this study has shown that the S-100 was expressed in both nuclear and cytoplasmic regions of the testicular Sertoli cell in all the age category and assumed a trend of increase intensity with advancement in age. Going by the results on the profile of S-100 in the cane rat testes and its contribution in blood-testes barrier (BTB) formation. It could be concluded that the strength of testicular BTB increases in relation to age increment. Also, our data consistently revealed that the epididymal segments in pubertal cane rat had increased S-100 intensity which can be linked to better reproductive vigour.

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Mini Curriculum and Author's Contribution

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