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MORPHOLOGICAL STUDY OF THE TESTIS AND EPIDIDYMIS IN SPOTTED DEER (*AXIS AXIS*)

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ABSTRACT

Spotted deer (*Axis axis*) is a medium sized deer classified under family *Cervidae*. Deer is reported as seasonal breeders in many temperate and tropical regions. The study was conducted on the testis and epididymis collected from three adult spotted deer. Tissue samples were preserved in 10 per cent neutral buffered formalin, processed for routine paraffin embedding procedures. 5 µm thick sections were stained with Haematoxylin and Eosin and examined to record morphological and morphometrical parameters. Testis was oval in shape and was covered by a thick, dense irregular connective tissue capsule. Mediastinum testis was centrally placed. Testicular parenchyma enclosed coiled seminiferous tubules and interstitial tissue containing isolated Leydig cells. Each seminiferous tubule had a stratified epithelium and an enveloping lamina propria. The epithelium enclosed Sertoli cells and various developmental stages spermatozoa, indicating active spermatogenesis. The basal zone of epithelium contained spermatogonia whereas primary spermatocytes formed the middle zone. More matured spermatids constituted a layer towards the lumen. Elongated spermatid had an oval sperm head. Simple cuboidal to simple squamous cells lined the rete testis. Efferent ductules lined by alternating groups of simple cuboidal and simple columnar cells were present in the caput epididymis in addition to the epididymal tubules. The entire corpus and cauda contained the highly coiled ductus epididymis. Pseudostratified epithelium with stereocilia lined the ductuli epididymis. The height of cells decreased significantly from the caput to cauda epididymis. Encircling smooth muscle cells of the epididymal tubules exhibited an increasing trend from caput to cauda. The study revealed morphological features of

testis and epididymis in spotted deer which will form a baseline data of the species.

Keywords: Spotted deer, testis, epididymis, morphology, spermatogenic cells

INTRODUCTION

Spotted deer (*Axis axis*) is a medium sized deer species native of India and Srilanka. It belongs to the family *Cervidae* under the suborder Ruminantia and order Artiodactyla. The animals are found in a wide range habitat and the International Union for Conservation of Nature had listed the species under animals of least concern. Seasonality in breeding had been described in spotted deer (*Axis axis*) by Umapathy *et al*. (2007) in India; by Schön and Blottner (2009) in roe deer (*Capreolus capreolus*) in Germany and by Sohn and Kimura (2012) in Korean water deer (*Hydropotes inermis*) in Korea. Studies on the skeletal system of spotted deer (Rajani *et al.* 2013a; 2013b and 2013c) and topography of viscera (Shil *et al*., 2014) are available. But only a few papers describe sparse anatomical peculiarities of the reproductive organs in deer including korean water deer (*Hydropotes inermis*) (Sohn and Kimura, 2012) and sika deer (*Cervus nippon*) (Hayakawa *et al*., 2009). A comprehensive data regarding morphological features of testis and epididymis in spotted deer appears to be scanty. Hence, the present work was conducted to elucidate the gross and histological features of testis and epididymis of spotted deer.

MATERIALS AND METHODS

Testis and epididymis were collected from three adult spotted deer that died due to natural causes at Central Kerala. Chief Wildlife Warden, Kerala had permitted the study as per order no KFDHQ-915/2019-CWW/WL10 dated 11/03/2019. Samples were obtained during the period May to July, 2020. The testis and epididymis along with its coverings was carefully detached from the scrotum during the post-mortem examination and gross morphological parameters of testis and epididymis were recorded. Representative tissue samples from the testis and different regions of epididymis were preserved in 10 per cent neutral buffered formalin. The samples were processed for routine paraffin embedding and 5 µm thick sections were taken and stained with Haematoxylin and Eosin (Singh and Sulochana, 1996). Morphological and morphometric parameters were recorded under Olympus binocular light microscope CX21i attached with Magvision software.

RESULTS AND DISCUSSION

Testes were located within the scrotum, a little cranial to the inguinal region. Epididymis was attached along the caudal border of the testis. Testis of spotted

deer was oval in shape with compressed surfaces (Fig. 1). The medial surface was more flattened than the lateral surface due to its apposition with the scrotal septum. The mean weight of formalin fixed testis along with epididymis of the left and right sides were 44.6 ± 0.8 g and 41.4 ± 0.5 g respectively. The average transverse circumference of the testis and epididymis of left and right sides were 12.2 ± 0.4 cm and 11.8 ± 0.6 cm respectively. The corresponding values of cranio-caudal circumference for the left and right sides were 13.6 ± 0.4 cm and 13.3 ± 0.5 cm respectively. The left testis was slightly larger than the right. There was no marked difference in the morphometric parameters among the three pairs of testes used in the study. A thick capsule, tunica albuginea, enclosed the testis which in turn is covered by the glistening visceral layer of tunica vaginalis. Microscopically, the capsule was made up of dense irregular connective tissue and composed of numerous collagen fibres. A distinct zona vasculosa was present in its inner zone. Microscopic observations of the capsule in the present study resembled the reports made by Shukla *et al*. (2013) in Chamurthi horse.

Longitudinal section of the testis revealed centrally positioned mediastinum testis (Fig. 2). The septulae testis arose from the capsule and converged towards mediastinum testis. The septae divided the testicular parenchyma into lobules which enclosed convoluted seminiferous tubules and a little interstitial tissue. The mediastinum testis contained irregular interconnecting channels, rete tubules, lined by simple cuboidal epithelium. The finding of mediastinum and rete testis is similar to the reports in ruminants (Wrobel and Bergman, 2006). However, in donkey, mule and stallion, the mediastinum was marginal in position (Pathak *et al*., 2013). The interstitial connective tissue was of loose areolar type and contained mainly collagen and reticular fibres. In addition, it enclosed blood vessels, fibrocytes and Leydig cells. Leydig cells were present mainly as solitary cells in the vicinity of blood capillaries. The large, polygonal Leydig cells contained spherical nuclei and prominent nucleoli.

Histologically, the testicular parenchyma revealed longitudinal, cross and tangential sections of seminiferous tubules. The tubules had a mean diameter of 346.49 ± 12.58 µm. Each seminiferous tubule rested on a lamina propria and enclosed stratified epithelium (Fig. 3). Collagen and elastic fibres and myofibroblasts were observed in the lamina. The presence of myofibroblasts encircling the seminiferous tubules indicated that their contractile activity may assist spermatozoa transport within the tubule (Wrobel and Bergmann, 2006). Myofibroblast cells

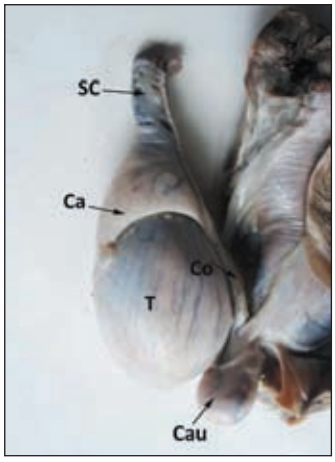

Fig. 1. Lateral view of left testis of spotted deer T- Testis, Ca- Caput epididymis, Co- Corpus epididymis, Cau- Cauda epididymis, Sp- Spermatic cord

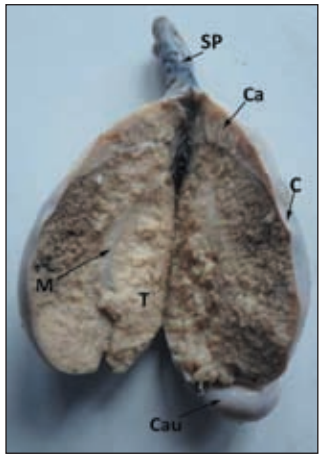

Fig. 2. Midsagittal section of left testis of spotted deer T- Testicular parenchyma, Ca- Caput epididymis, M- Mediastinum testis, C- Tunica albuginea capsule, Cau- Cauda epididymis, Sp- Spermatic cord

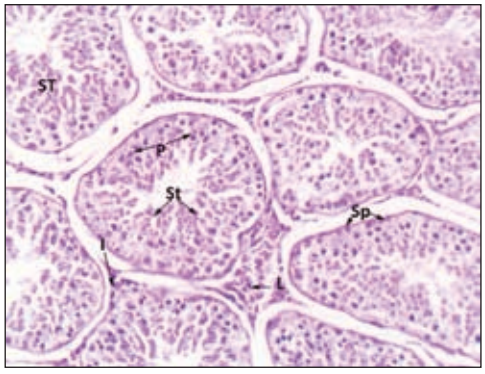

Fig. 3. Photomicrograph of testis of spotted deer ST- Seminiferous tubule, P- Primary spermatocyte, St- Spermatid, I- Interstitial tissue, L- Leydig cell (Haematoxylin and Eosin X 150)

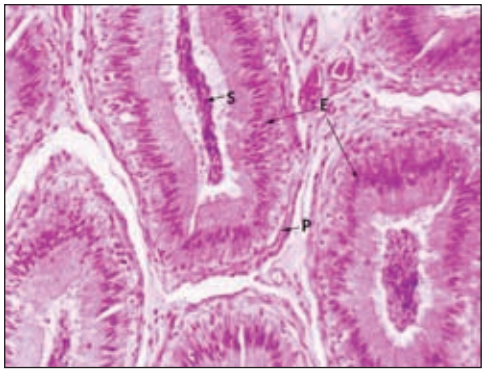

Fig. 4. Photomicrograph of caput epidiymis of spotted deer S- Clumps of sperm in the lumen, E- Pseudostratified epithelium with apical stereocilia, P- Peritubular connective tissue (Haematoxylin and Eosin X 150)

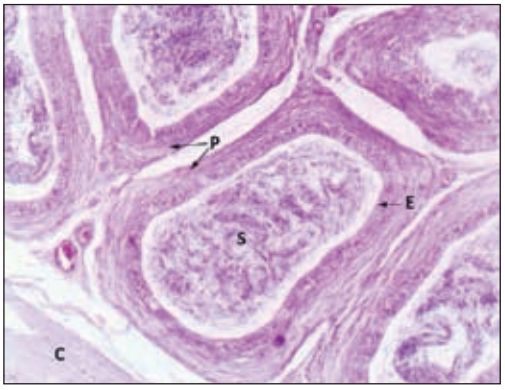

Fig. 5. Photomicrograph of corpus epidiymis of spotted deer S- Clumps of sperm in the lumen, E- Pseudostratified epithelium with apical stereocilia, P- Peritubular connective tissue, C- Capsule (Haematoxylin and Eosin X 150)

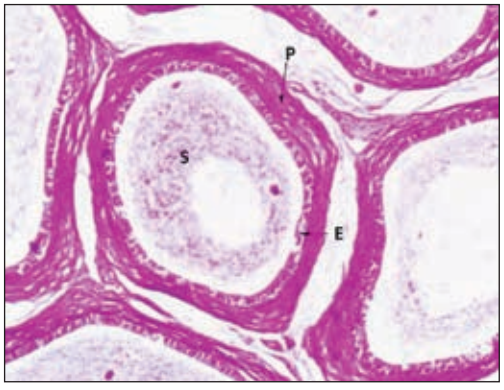

Fig. 6. Photomicrograph of cauda epidiymis of spotted deer S- Clumps of sperm in the lumen, E- Pseudostratified epithelium with apical stereocilia, P- Peritubular connective tissue (Haematoxylin and Eosin X 150)

were also described in the testis of Rusa deer (Moonjit and Suwanpugdee, 2007). The seminiferous epithelium presented two categories of cells: Sertoli or sustentacular cells and developmental stages of spermatogenic cells. The testis from all the animals contained various spermatogenic cells indicating active spermatogenesis. Sohn and Kimura (2012) in Korea recorded spermatids within the seminiferous tubules only in the specimens collected during October to November in Korean water deer (*Hydropotes inermis*). However, histological study by Umapathy *et al*. (2007) in spotted deer (*Axis axis*) in Hyderabad, India recorded spermatogenic stages in the testis specimens collected from deer with both hard and velvet antler. Moreover, the cyclic spermatogenic stages were more evident in the hard antler deer. It was also observed that epididymis contained spermatozoa only in the specimens collected during March to May. However, Umapathy *et al*. (2007) could not correlate fully the seasonal breeding activity to the histology of testis because spermatogonia and round spermatids were present in the testis of deer with velvet antler. The earlier study done in India suggested that spermatogenesis occurs throughout year, though at a diminished rate in spotted deer with velvet antler. The findings of the present study indicated that the animals were in reproductively active stage. However, a more extensive study incorporating collection of specimens from animals throughout the year needs to be carried out to ascertain the existence of breeding season in Kerala. In deer, in the temperate regions, photoperiod had an influence on antler cycle and breeding season, whereas in tropical regions, the annual rain fall was observed to be the important factor for seasonal breeding (Umapathy *et al*., 2007).

Sertoli cells had a uniform distribution and were the only cells that extended entire layer of epithelium. The cells had an irregular profile and enclosed large, irregular nucleus. The basally placed, lightly stained nucleus presented a prominent nucleolus. Different developmental stages of spermatogenic cells were observed along the basal lamina at the basal part of Sertoli cells or between adjacent Sertoli cells or in the apical recesses of the Sertoli cells. The characteristics of Sertoli cells observed in the study were similar to findings of Moonjit and Suwanpugdee (2007) in Rusa deer and Bashir *et al*. (2012) in goat.

Different spermatogenic cells were distinguished based on their position within the seminiferous epithelium and other morphological features *viz*., shape, staining character of nucleus and chromatin. The least differentiated cells, spermatogonia, were located along the

basal lamina. These were oval or round cells and contained an oval vesicular nucleus and one or two prominent nucleoli (Fig. 3). The large, round to oval primary spermatocytes occupied a thick middle zone of seminiferous epithelium, above the spermatogonia. Primary spermatocytes were numerous in number and were in different stages of prolonged meiosis I. These findings agree with the reports of Moonjit and Suwanpugdee (2007) in Rusa deer during the rutting period. The spermatids formed the apical zone. Two varieties of spermatids *viz*., round spermatids and elongated spermatids were present. The small round spermatids represented Golgi and cap phase of spermiogenesis which were the smallest cells of spermatogenesis. Elongated spermatids contained deeply stained oval nucleus with dense chromatin and the tails protruded into the lumen of the seminiferous tubule. Elongated variety designated acrosome and maturation phases. The shape of sperm is determined by the shape of its nucleus. Elongated spermatid had an oval sperm head, broader in the cranial half than the caudal half and is in accordance with the reports of Ros-Santaella *et al*. (2019) in fallow deer.

The seminiferous tubules interconnected to form the rete testis. The lining cells of the straight tubules and rete testis were simple cuboidal to simple squamous type. Rete testis continued as efferent ductules and ductus epididymis. Grossly, three regions *viz*., caput, corpus and cauda were distinguished in the epididymis (Figs. 1 and 2). Efferent ductules and ductuli epididymis constituted the caput region. The entire corpus and cauda contained the highly coiled ductus epididymis. Alternating groups of simple cuboidal cells and groups of simple columnar cells lined the efferent ductules giving a wavy appearance to its lumen. Columnar cells presented distinct cilia at its apex. Pseudostratified epithelium containing basal and tall columnar principal cells constituted the lining cells of the ductuli epididymis (Fig. 4). The small polygonal basal cells enclosed small round nucleus while the principal cells contained an oval nucleus. The apical part principal cells exhibited branching microvilli, stereocilia. The villi were very long in the caput region. The height decreased in the corpus region and drastically reduced in the cauda epididymis (Figs. 5 and 6). These findings of stereocilia and pseudostratified epithelium within the epididymis are in accordance with the reports of Moonjit and Suwanpugdee (2007) in Rusa deer and Schön and Blottner (2009) in roe deer.

The lumen of the ductus epididymis was more even and enclosed sperm clumps. Sperm morphology revealed head and tail regions. The principal cells were the tallest in the caput region of the epididymis (Fig.

4). The lining epithelium was 66.67 ± 5.68 µm tall in the caput epididymis. However, the height declined to 35.93 ± 4.35 µm in the corpus and in the cauda the cells were 21.26 ± 2.52 µm tall. So, the height of the lining epithelium revealed a decreasing trend. Similarly, the length of stereocilia gradually decreased from the caput to cauda region of the epididymis (Figs. 5 and 6). The peritubular connective tissue enclosed smooth muscle cells. The number of smooth muscle cells significantly increased from the head to the tail of the epididymis (Fig. 6). The diameter of the lumen of the epididymal tubules in the caput, corpus and cauda region were 186.27 ± 13.56 µm, 424.67 ± 10.23 µm and 537.19 ± 16.68 µm respectively. The data suggested a radical increase in the lumen size from the caput to cauda region of the epididymis. The decreasing trend in the epithelial height from the cranial to caudal parts of epididymis observed in the present study agrees with the reports of Schön and Blottner (2009) in roe deer during the rutting period.

SUMMARY

Testis had an oval shape and centrally positioned mediastinum testis. Parenchyma of testis enclosed seminiferous tubules and interstitial tissue. Seminiferous epithelium revealed Sertoli cells and developmental stages of spermatocytes. Caput, corpus and cauda epididymis were lined by pseudostratified epithelium with stereocilia. The height of lining cells as well as of stereocilia exhibited a decreasing trend from caput to cauda region. The study revealed the gross and histological features of testis and epididymis in spotted deer. The data will form a basis for understanding pathological conditions affecting the organ.

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