SHORT COMMUNICATION

A Brief Insight into the Histological Profile of Testis of the Hog Badger *(Arctonyx collaris)*

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Abstract

The general histoarchitecture of the testis of the Hog Badger (*Arctonyx collaris*) in the present study was similar to that of other mammals, with a few peculiarities. The tunica albuginea of the testicular capsule was the thickest, with an abundance of collagen fibres with darkly stained thin outer serosal layer. The conspicious subcapsular layer was rich in connective tissue fibres, blood vessels, and interstitial cell masses arranged in clumps or cords. The Leydig cells were abundant between the seminiferous tubules, tubule recti, mediastinum, and subcapsular space with variable size and shape. Fibroblast- or mesenchyme-like cells with small elongated nuclei were found in between the interstitial cells. The large Leydig cells were more distinct with foamy cytoplasm, large, round nuclei compared to the smaller ones. Occasional eccentric nuclei of Leydig cells were also present. Primary spermatocytes were the largest. Small sized Leydig cells with small nuclei were noticed, particularly in the subcapsular area and around the mediastinum. Presence of secretory scalloped pseudostratified columnar epithelium of efferent ductules, and extensive rete with interstitial cell mass at the mediastinum can only be functionally correlated with a larger sample size in regards to their territorial behaviour and seasonal sexual activity.

Key words: Histology, Hog Badger, Leydig cell, Testis.

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Introduction

The greater Hog Badger (*Arctonyx collaris*) is a very large terrestrial carnivore mammal (mustelid), with larger claws on the front feet and a tail with long white hairs and white claws on the front feet. Three different subspecies have been identified in the year 2008 which are Greater, Indonesian, and Burmese. Hog Badgers are generally omnivorous (Helgen *et al.,* 2008). They are mostly prevalent in the eastern Himalayan region, in northeastern India, Thailand, and Assam to Myanmar with very few distinctive features with each other (Wozencraft, 2005). The point of great concern is the rapid decline in their population due to poaching (Duckworth *et al.,* 2016; Baker, 2012). The Hog Badger has medium-length white brownish to gray hair, a compact body, and a white throat, with two black stripes on an elongated face with a pink snout-like pig. The body length is approximately 65-104 cm, the tail 19-29 cm and the body weight is 7-14 kg (Helgen *et al.,* 2008). The age of sexual maturity in female is after 2 to 3 months, whereas the males do not reach sexual maturity until one year. Time of independence is noticed in American badgers (Edmunds, 2003). The physical activities of these animals either day or night are very less (Helgen *et al.,* 2008). Successful reproduction and survival of the offspring is the key to species sustainability, but very little information is available about structural profile of the male reproductive system, The testicular morphology can provide information on the reproductive efficiency compared with species concerning sperm production (Souza *et al.,* 2005; Silva *et al.,* 2006). To curate some basic anatomical clues about the testis of Hog Bedger (*Arctonyx collaris*), present work was undertaken to study the morphological profiles of testis obtained for the post-mortem at College from Sepahijala Zoo, Tripura.

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Materials and Methods

Necropsy of the sub-adult male Hog Badger was performed at College of Veterinary Sciences and Animal Husbandry, R.K. Nagar, Tripura vide order File No: F5C/SPZ/ZVH/ 2013/162-166 dated 14th August 2020 from the Sepahijala Zoo,

Tripura, India. On necropsy, the animal was found to have died due to acute gastroenteritis. During the necropsy, the male genital system was found to be normal. The whole testis was separated from the surrounding fascia and 2-3 mm testicular tissue was collected in 10% neutral buffered formalin for 72 h for fixation for histological studies followed by standard tissue processing procedures (Suvarna *et al.,* 2019). 5 µ thick tissue sections were obtained using a semi-automatic rotary microtome and stained with hematoxylin and eosin, Massons-Tri-chrome for collagen fibers (Suvarna *et al.,* 2019). Micro-metrical observations for seminiferous lumen diameter, epithelium profile, testicular lobular length, capsule thickness, interstitial space area, nucleus length, and breadth were recorded randomly at least in ten fields. Photography and measurements were performed using Lynx-Lawrence and Mayo Trinocular Microscope with an attached computer installed with Scope Image 10 software.

Results and Discussion

Histologically testicular parenchyma of Hog Badger (*Arctonyx collaris*) showed that the seminiferous tubules, interstitial tissue, capsule, inter-lobular, and subcapsular connective tissue area constitute approximately 47.5%, 35.2%, 11.8%, and 5.5% area of the testis, respectively. The testicular capsule was almost uniformly thick and average thickness was 181.27±9.87 µ (Table 1), except at the exit area of the efferent ductules. The capsule was composed of three layers and the middle layer was thickest (tunica albuginea). The outer serosal and inner vasculosa were very thin and lightly stained. Trabeculae arise from capsule, dividing the parenchyma in testicular lobules. The presence of dense collagen fibers, fibroblast cells and smooth muscle fibers with elongated and irregular nuclei were observed (Fig. 1c). Tortuous seminiferous tubule within testicular lobules and interstitial cell mass, connective tissue stroma, blood vessels and rete testis was noticed in the testicular parenchyma. Elongated, elliptical, small rounded testicular lobes covered by connective tissue fibers converging to mediastinum at rete testis (Fig. 1b,d). The average longitudinal length of the lobes was 2125±302.7 µ with a maximum length of 3000 µ and minimum of 1125 µ. Seminiferous tubules were lined by spermatogenic stratified cuboidal epithelium with distinct basement membranes. The mean diameter of the seminiferous tubules was 95.95±6.31 µ with an average cross-sectional area of 5395.49 ±324.15 μm^2. The mean height of the seminiferous epithelium was recorded 27.97±2.02 µ (Table 1).

The thick basement membrane of the tubules was composed of abundant collagen fibers, smooth muscle with thin elongated darkly stained fibroblast and myoid cells. Darkly stained small spermatogonium cells with distinct nuclei were located close to the basal lamina of seminiferous tubule. Large irregular Sertoli cells were lightly stained with indistinct cell boundary with large basally located irregular darkly stained nuclei. Sartolis cells were connected with the germ cells with distinct basement (Fig. 2a,b).

Presence of abundant interstitial tissue masses, Leydig cells, was prominent in the peritubular, subcapsular spaces. Mesenchyme or fibroblast-like cells were noticed in the interstatial space. Due to the presence of well-organized fine connective tissue fibers and fibroblast-like cells in the interstitial spaces, the interstitial cells were well demarcated particularly in between the seminiferous tubular space. Both dark stained and lightly stained interstitial cells were found. The arrangement of the interstitial cells (Leydig cells) was mostly in clump, cluster, and cord fashion-like nest of darkly stained cells with eosinophilic, electron-dense cytoplasm. The Leydig cells were characterized by a large round nucleus, darkly stained foamy cytoplasm, and a clear circumscribed nucleus with a well-defined cell membrane. The clusters of Leydig cells were held together with inter-digitating membrane (Fig. 1d, 2b). The mean diameter of Leydig cells and nucleus were 12.72±0.48 µm and 5.38±0.17 µm, respectively (Table 1). The average nucleus:cytoplasm ratio was 0.43. The Leydig cells in the subcapsular space and mediastinum were with a small round nucleus with pale cytoplasm. The mean interlobular connective tissue thickness was 36.4±3.89 µm (Table 1).

Table 1: The micro metrical parameters of testis of Hog Badger (*Arctonyx collaris*)

Parameters	Mean ± SE (µ)	Max value	Min Value
Seminiferous epithelium height	27.97± 2.02	42.3	17.49
Seminiferous tubular diameter	95.95±6.31	134.17	65.58
Leydig cell diameter	12.72 ±0.48	17.2	9.2
Leydig cell nucleus diameter	5.38 ±0.17	7.74	3.9
Nucleus : Cytoplasm of Ledyig cell	0.43	0.57	0.29
Efferent duct epithelium diameter	19.66±1.25	25.37	17.25
Cross sectional area of seminiferous tubule (round spherical tubule, µm2)	5395.49 ±324.15	7381.24	4209.05
Cross sectional area of seminiferous tubule (elongated tubule, µm2)	11677.68±118	17344.07	9516.7
Capsular thickness	181.27±9.87	227.5	155.5
Capsular thickness at hilus	733.3±79.49	875.5	450
Testicular lobes longitudinal length	2125±302.7	3000	1125
Thickness of subcapsular space	185.5±24.2	300	110
Interlobular connective tissue thickness	36.4±3.89	50	20

The rete testis was extensive with an anastomosing network of delicate tubules located in the hilum with abundant collagen fibers. Rete testis was lined by the simple lining of darkly stained cuboidal epithelium with an abundant interstitial tissue mass at the mediastinum. The straight tubules were smaller in dimension lined with simple tall cuboidal epithelium with basal nuclei (Fig. 2c). The efferent duct was lined by pseudostratified columnar epithelium with stereocilia like scalloped appearance. The epithelium was found actively involved in a secretory role. Two to three smooth muscle cell layers with distinct nuclei and collagen fibers were present around it (Fig. 3). The average diameter of the duct recorded was 106 ±19.65 µ with epithelial height of 19.66±1.25 µ (Table 1).

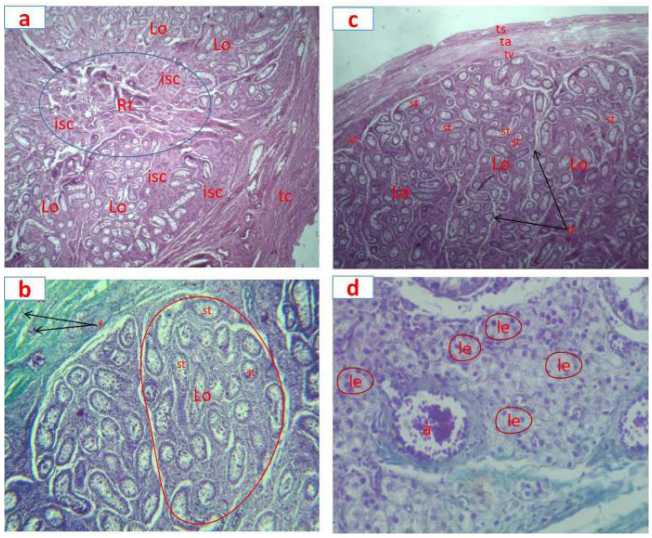

Fig. 1: a.Microphotograph showing general histological architecture of testis of Hog Badger with testicular capsule (tc) and parenchyma with testicular lobules (lo) filled with numerous seminiferous tubules (st) and rete testis (rt) at 40X magnification (H&E). b. testicular lobules (lo) with seminious tubules (st), testicular capsule (tc) fibroblast, smooth muscle fibers and collagen fibers (arrow) 40X magnification (Massons Tri-chrome). c. Three layers of testicular capsule viz outer tunica serosa (ts), middle tunica albugenia (ta) and inner tunica vasculosa (tv) 40X magnification (H&E). d. Microphotograph showing the subcapsular layer (sc) and peritubular space filled with interstitial cell mass (isc) in, and presence of thick collagen fibers in the testicular capsule and trabeculae and small interstatial leg cells (le) and artery (a) at 40 X magnification

Though the general histomorphological features of testis in the present study were alike other mammalian species, a few notable observations like, abundance of interstitial tissue mass with, presence of both large polymorphic Leydig and small sized Leydig cells, difference in staining intensity light and dark, foamy and pale cytoplsam were unique in the present study. Large Leydig cells were mostly observed in between the seminiferous tubule and smaller ones at mediastinum. Interstitial cells with eccentric nuclei were not uncommon. Variations in the Leydig cell population, location, staining intensity, nucleus size of the Leydig cell, in the present observation may not be conclusive for stating the seasonal reproductive changes, photoperiodicity, or state of independence, as reported in *Macropod marsupials* by Poole and Catling (1974), in tammar wallaby and Bennett's wallaby by Xie *et al.* (1998), in adult male swamp wallabies by Paplinska *et al.* (2007), and in giant otter by Gabriel *et al.* (2011). Kangawa *et al.* (2019) reported increase in the Leydig cells dimension of micromini pigs with age and noticed large active Leydig cells in the peritubular space, and smaller one in subcapsular and interlobar space as reported in tammar wallabies (Butler *et al.,* 2008). Two types of Leydig cells (fetal and adult) were reported in dasyurid and brown marsupial mice (Taggart *et al.*, 1993; Chen *et al.,* 2009). However, only one population was reported by Xie *et al.* (1998) in opossums (*Monodelphis domestica).*

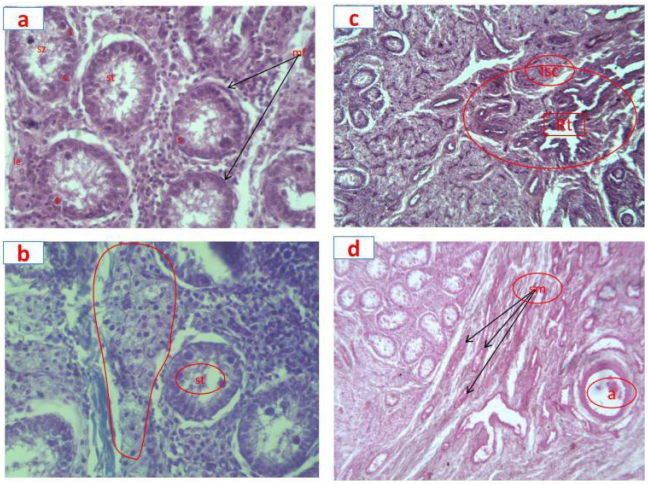

Fig. 2: Microphotographs showing the seminiferous tubules (st) with different generations of spermatogenic cells, sartolis cells(s), myofibroblast cells (arrow) in the basal membrane (bm) a, with myo-fibroblast cell (mf) of tubules and Leydig cells (le) in clump with cloudy cytoplasm and large round nucleus (b) at 400X magnification H&E. At 400X magnification c. Rete testis (rt) a clump or network space surrounded by interstatial tissue (isc) and d. presence of smooth muscle fibers with small elongated nuclei in the testicular capsule (tc)

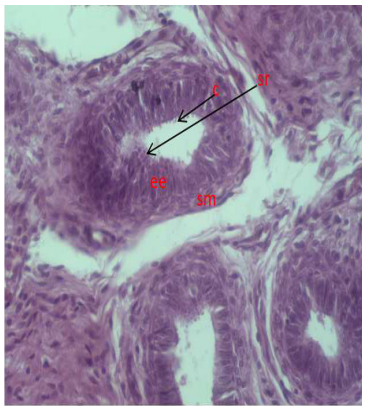

Fig. 3: Microphotograph showing the pseudostratified ciliated columnar (e) epithelium of efferent duct at the caput in secretory role (sr) surrounded by 2-3 layers of smooth muscle (sm), 400X magnification

Hog Badger being a seasonal and territorial animal, may show variations in Leydig cell activity during different seasons. In this aspect, very little information

is available regarding the period of independence, photoperiods, and temperature role which may influence the number and activity of Leydig cells, seminiferous tubule (https://animaldiversity.org). Surmacki *et al*. (2011) in Chinchilla have reported variations in Leydig cell's nuclear shape between low and high fertility periods. The nuclei of Leydig cells were smaller during the season of low fertility and close to the cell membrane. Environmental temperature, photoperiod, food, and health have a direct effect on the sexual characteristics and capabilities of animals (Paplinska *et al.,* 2008). The higher proportion of the Leydig cell in Hog Badger *(Arctonyx collaris)* in the present study could be explained due to increasing LH levels (Akingbemi *et al*., 2004). More specific information could be generated if serum testosterone and LH are measured. Secretory blebs of the efferent duct at the caput epididymis may explain the secretory role of efferent ductules. Further detailed investigations with more samples are required to interpret physiology, behaviour, and structural correlations. Adebayo and Olurode (2010) reported variations in the epididymal dimension (diameter and epithelial height) of the greater cane cat. Seminiferous tubular diameter and epithelium height in the present study were 95.95±6.3µ and 27.97±2.02 µ, respectively. Active spermatogenesis was not noticed. Oliveira *et al*. (2011) reported the mean diameter of the seminiferous tubule in Giant Otters as 126 ± 13.37 µ, with the territorial behavior and seasonal variation in breeding and sexual activity.

In short, in the present study the testis of Hog badger was like other mammals with abundance of interstitial tissue mass with more Leydig cells. The peritubular Leydig cells were large with distinct cell margin and foamy cytoplasm, while in subcapsular, mediastinum, Leydig cells were smaller in dimension with pale cytoplasm. Large Leydig cells may be associated with development of male sexual activities and duct system needs a detailed study with more sample number and seasonal consideration. The basic structural understanding about the anatomy of these animals is essential to study their behaviour.

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