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A brief histological profile of the ovary of the hog badger (*Arctonyx collaris*)

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Abstract

This study presents the first microanatomical description of the ovary of the hog-badger (*Arctonyx* spp.) based on specimen obtained from post mortem. The ovary displayed a typical mammalian organization, closely with carnivores and wild ungulates, consisting of a follicle-rich cortical region and a medulla with loose connective tissue, blood vessels, nerves, and lymphatics reflecting the ovary's high metabolic and endocrine demands. The ovarian surface was lined by a tall cuboidal to columnar germinal epithelium with an average thickness of $4.86 \pm 0.17 \mu\text{m}$, beneath by a distinct tunica albuginea. The cortex was with follicles at various stages of development, like primordial follicles ($10 \pm 1 \mu\text{m}$) mostly concentrated within the sub-germinal zone, primary follicles ($50 \pm 3 \mu\text{m}$), surrounded by cuboidal granulosa cells, secondary follicles ($104 \pm 7.57 \mu\text{m}$) multilayered granulosa cell arrangements, and comparatively few tertiary follicles ($180 \pm 4.2 \mu\text{m}$). Tertiary follicles were with an antral cavity ($68 \pm 4.19 \mu\text{m}$), a distinct zona pellucida, and a prominent corona radiata. Oocytes measured an average of $51.2 \pm 2.09 \mu\text{m}$ and were positioned either eccentrically or centrally. Atretic follicles were characterised by pyknotic granulosa cells and degenerating oocytes. Both active and regressing corpora lutea were observed, indicating recent or ongoing ovulatory activity. Luteal tissue was made up of large luteinized granulosa cells measuring $4.8 \pm 0.29 \mu\text{m}$ in diameter. The thecal layer was distinct in tertiary to mature follicle with a cellular internal layer ($11 \pm 0.73 \mu\text{m}$) and a fibrous outer layer ($9.77 \pm 0.44 \mu\text{m}$). The average diameter of the thecal cells was noted $1.85 \pm 0.11 \mu\text{m}$. These findings establish essential baseline histological data for the hog-badger ovary and provide a valuable foundation for future investigations into reproductive function, seasonality, and conservation-oriented reproductive management.

Keywords: Histology, hog badger, ovary

Introduction

The Greater Hog badger (*Arctonyx collaris*) is a large, terrestrial carnivorous mammal belonging to Mustelidae with robust forelimbs, enlarged distinct white claws, a tail with long white hairs, and distinct, generally omnivorous in feeding. In 2008, three subspecies of hog badgers were recognized, namely the Greater, Indonesian, and Burmese hog badgers. The animal possesses medium length fur (white to brown), white throat, elongated pig like snout pink colored and compact body (Helgen *et al.*, 2008) [4]. This species is mostly found in the eastern Himalayan region, including northeastern India, Thailand, Assam, and Myanmar (Wozencraft, 2005) [8]. A major conservation concern is the rapid decline in hog badger populations, primarily due to poaching and anthropogenic pressures (Duckworth *et al.*, 2016; Baker, 2012) [2, 1].

Adult individuals typically measure 65-104 cm in body length, with a tail length of 19-29 cm and a body weight ranging from 7-14 kg (Helgen *et al.*, 2008) [4]. Females attain early sexual maturity (2-3 month), whereas males, around one year of age. Independence timing has been documented in American badgers (Edmunds, 2003) [3]. Hog badgers exhibit relatively low levels of physical activity and active during both day and night (Helgen *et al.*, 2008) [4].

Successful reproduction and offspring survival are critical for the long-term sustainability of the species; however, limited information is available regarding the structural organization of the reproductive system, particularly in hog badgers. Ovarian histomorphology is an important indicator of reproductive efficiency (Souza *et al.*, 2005; Silva *et al.*, 2006) [6, 5]. In

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order to establish baseline anatomical and histological data on the ovary of the hog badger (*Arctonyx collaris*), the present study was undertaken to examine the morphological profile of reproductive tissues obtained during post-mortem examinations conducted at Sepahijala Zoo, Tripura.

Materials and Methods

Necropsy of the sub-adult female Hog Badger was performed at College of Veterinary Sciences and Animal Husbandry, R.K. Nagar, Tripura from the Sepahijala Zoo, Tripura, India. On necropsy, the animal was found to have died due to acute gastroenteritis. During the necropsy, the female genital system was found to be normal. The whole ovary was separated from the surrounding fascia and 3-4 mm ovarian tissue was collected and kept in 10% neutral buffered formalin for 72 hr for fixation for histological studies followed by standard tissue processing procedures (Suvarna *et al.*, 2019) [7]. 5 μ thick tissue sections were obtained using a semi-automatic rotary microtome and stained with haematoxylin and eosin, Massons-Tri-chrome for collagen fibers (Suvarna *et al.*, 2019) [7]. Micro-metrical observations for size of different follicles (primordial, primary, secondary and mature), germinal epithelium profile, thickness of thecal layer, germinal epithelium, zona pellucida, antral diameter, size of luteal granulosa cell, thickness of corona radiata, nucleus diameter etc breadth were recorded randomly at least in ten fields. Photography and measurements were performed using Zeiss Trinocular Microscope with an attached computer installed with Zen.3.11 micrometry software.

Results and Discussion

Microscopic Architecture

Microscopically, the ovary was composed outer cortex and inner medulla. The cortex was characterized by ovarian follicles of different generations, and medulla contained blood vessels, connective tissue, lymphatics, and nerves. Ovary was covered by a tall cuboidal to columnar single layer germinal epithelium, with a mean height of 4.86 ± 0.17

μ m. Beneath this surface epithelium, a thin distinct tunica albuginea clearly separated the epithelium from the underlying cortex. The ovarian cortex appeared darkly stained and consisted of ovarian follicles at various stages of development within a stromal connective tissue matrix. In contrast, the medulla was lightly stained and with loose connective tissue. Irregularly branched trabecular connective tissue extended from the medulla toward the cortex.

Histological sections revealed follicles ranging from primordial to tertiary stages. Primordial follicles measured 10 ± 1 μ m in diameter, primary follicles averaged 50 ± 3 μ m, secondary follicles measured 104 ± 7.57 μ m, and only a few tertiary follicles were observed, with an average diameter of 180 ± 4.2 μ m.

Primordial follicles, were surrounded by an incomplete single layer of flattened squamous cells with whorled arrangement of connective tissue fibers and cells. Primordial and primary follicles were predominantly distributed in the peripheral subgerminal region beneath the germinal epithelium. Primary follicles were larger and consisted of a single layer of cuboidal follicular (granulosa) cells surrounding an oocyte with a large, round nucleus.

Secondary follicles were single layered to multilayered granulosa cells, with indistinct thecal layer. Tertiary follicles were characterized by antrum, and granulosa cells. The granulosa cells were characterized by large, round, euchromatic nuclei, indistinct cellular boundaries, and cloudy cytoplasm. Secretory activity and the irregular outlines of these cells produced a flare-like appearance extending toward the zona pellucida. Large lipid droplets were observed within the antral cavity.

The primary oocytes within tertiary follicles were predominantly eccentrically positioned, although centrally located oocytes were observed. A thin layer of granulosa cells forming the cumulus oophorus surrounding the oocytes.

Key Microscopic observations noted

Table 1: Histological characteristics and morphometric measurements of ovarian structures

Structure / Follicle Type	Histological Characteristics	Morphometric Measurements (Mean $\pm$ SEM)
Primordial follicles	Small oocytes surrounded irregularly by flattened pre-granulosa cells; beneath germinal epithelium	Oocyte diameter: 10 ± 1 μ m
Primary follicles	Enlarged oocyte with a single layer of cuboidal granulosa cells; granulosa cells with large round nucleus	Follicle diameter: 50 ± 3 μ m
Secondary follicles	Multiple layers of granulosa cells; no distinct antrum	Follicle diameter: 104 ± 7.57 μ m
Tertiary (antral) follicles	Distinct antral cavity; theca interna and externa; presence of corona radiata	Follicle diameter: 180 ± 4.2 μ m Antrum diameter: 68 ± 4.19 μ m Corona radiata thickness: 14.2 ± 0.76 μ m Oocyte diameter: 51.2 ± 2.09 μ m
Germinal epithelium	Darkly stained tall cuboidal simple epithelium with large round nuclei; distinct basement membrane composed of connective tissue fibre's	Thickness: 4.86 ± 0.17 μ m
Zona pellucida	Distinct and thick surrounding growing oocytes	Thickness: 2 ± 0.05 μ m
Atretic follicles	Pyknotic granulosa cells, disrupted oocyte morphology, collapse of antral cavity	—
Granulosa cells (follicular)	Closely packed cells surrounding oocyte	Cell diameter: 4.06 ± 0.24 μ m Nucleus diameter: 1.86 ± 0.05 μ m
Luteal structures (corpora lutea)	Active corpora lutea with large pale luteal cells; regressing corpora lutea with eosinophilic fibrovascular infiltration; luteal cells large, irregular, granular cytoplasm with round nucleus	Luteinized granulosa cell diameter: 4.8 ± 0.29 μ m Nucleus diameter: 1.97 ± 0.15 μ m N:C ratio lower than follicular granulosa cells
Theca layer	Distinct in tertiary and mature follicles; cellular theca interna and fibrous theca externa	Theca interna thickness: 11 ± 0.73 μ m Theca externa thickness: 9.77 ± 0.44 μ m Thecal cell diameter: 1.85 ± 0.11 μ m
Stroma and vasculature	Medullary stroma with dense vascular networks; distinct basement membranes; Masson's trichrome highlights collagenous tunica albuginea and interstitial connective tissue	



Fig 1: Photomicrograph showing the presence of primary and primordial follicle in the cortex of ovary H&E (40X)



Fig 2: Photomicrograph showing the cortex (C) with follicles and medulla (M) with dense connective tissue of ovary H&E (10X)

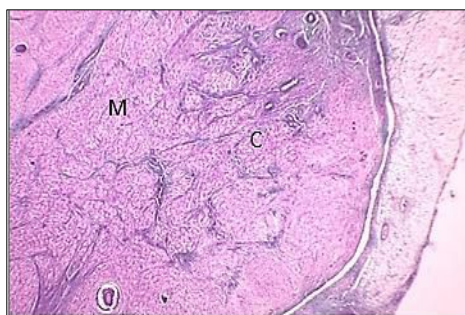


Fig3: Photomicrograph showing the trabecular branched orientation of connective tissue in the medulla and cortex of ovary (Massons Tri Crome 10X)

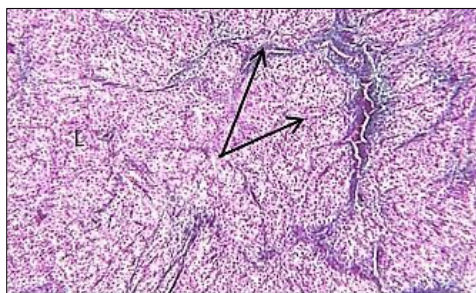


Fig 4: Photomicrograph showing connective tissue in the medulla and cortex with luteal cells (L) of ovary (Massons Tri Crome 40X)



Fig 5: Photomicrograph showing mature follicle of ovary (H&E 10X)

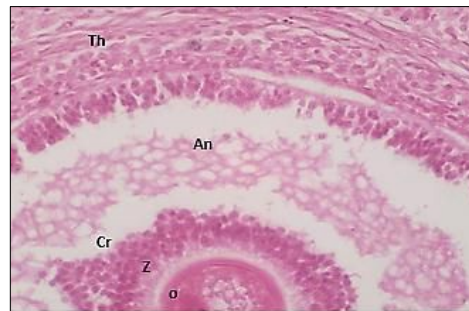


Fig 6: Photomicrograph showing mature follicle with zona pellucida (Z), corona radiata (Cr), oocytes(o), antrum(An) and thecal layers(Th) of ovary (H&E 40X)

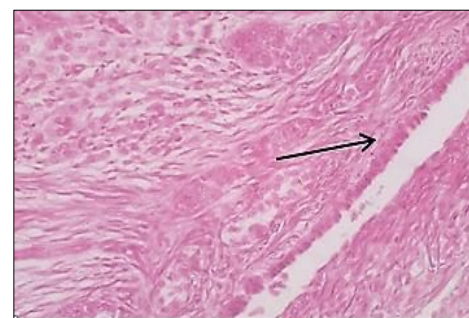


Fig 7: Photomicrograph showing tall cuboidal germinal epithelium of ovary (H&E 40X)

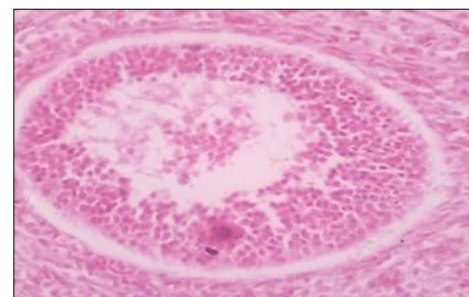


Fig 8: Photomicrograph showing an atretic follicle of ovary (H&E 40X)

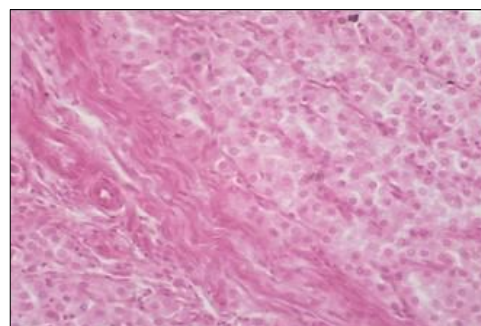
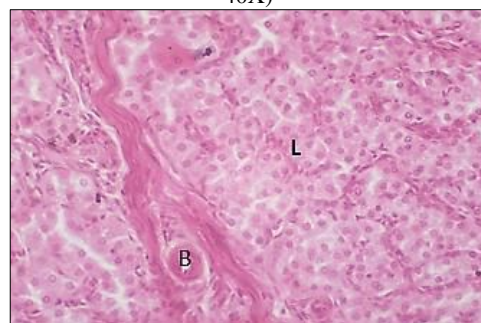


Fig 9: Photomicrograph showing the clusters of large luteal granulosa cells (L) with connective tissue and blood vessels (B) (H&E 40X)

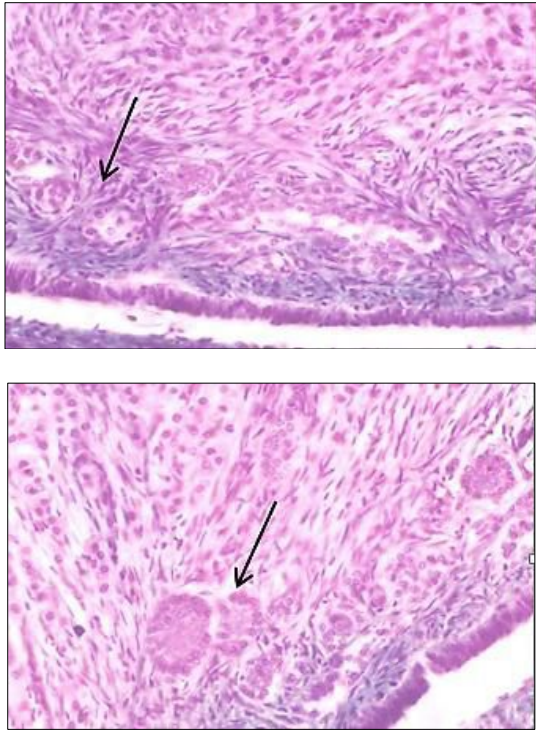


Fig 10: Photomicrograph showing the clusters primary and primordial follicle with abundance of connective tissue and elongated fibroblast cells vessels (H&E 40X)

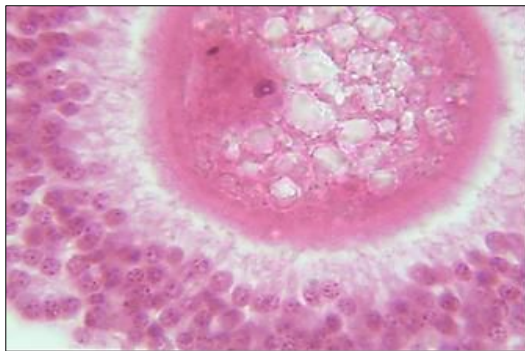


Fig 11: Photomicrograph showing the granular euchromatic cells of corona radiata, eccentric nucleus of oocytes with thick zona pelucida (H&E 100X)

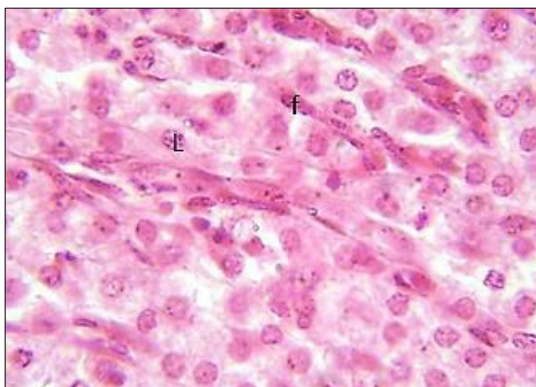


Fig 12: Photomicrograph showing the large irregularly shaped luteal granulosa cells with large round nucleus and long fibroblasts (f) in between (H&E 100X)

Discussion

This histomorphological study propose the first description of ovarian structure in the hog-badger (*Arctonyx* spp.) and provides morphological evidence of actively cycling normal

ovary. The basic organization of the ovary, with follicle-rich cortex and a highly vascularized medulla, is consistent with the ovarian anatomy described in other mammals and closely related carnivores such as mustelids, canids, and felids, as well as in several wild ungulate species.

Ovarian follicles representing different stages of development, primordial ($10 \pm 1 \mu\text{m}$) to tertiary follicles ($180 \pm 4.2 \mu\text{m}$), were identified, evident of ongoing folliculogenesis. The high proportion of primordial and primary follicles located in the sub germinal zone and it may be acting as reserve follicular pattern observed across mammalian species (Souza *et al.*, 2005; Silva *et al.*, 2006) [6, 5]. In contrast, relatively low number of tertiary follicles were observed. Which may be suggesting that the ovary was examined during an inter-ovulatory period. Well-defined distinct zona pellucida ($2 \pm 0.05 \mu\text{m}$) and corona radiata ($14.2 \pm 0.76 \mu\text{m}$) structures observed in mature tertiary follicles indicate normal oocyte development, while the eccentric positioning of oocytes within tertiary follicles is indicative of the approaching ovulation.

Both active and regressing corpora lutea were observed. Which provides the evidence of recent ovulation and progression of luteal phase. Luteinized granulosa cells were larger ($4.8 \pm 0.29 \mu\text{m}$) and displayed reduced nuclear-to-cytoplasmic ratios, reflecting the morphological changes due to luteinisation. These observations are suggestive of reproductive cyclicity in the species; however, conclusions regarding seasonal breeding cannot be drawn from a single specimen. Reports indicating that female hog-badgers reach sexual maturity at approximately 2-3 months of age (Edmunds, 2003; Helgen *et al.*, 2008) [3, 4] suggest comparatively early reproductive competence relative to other mustelids.

Few atretic follicles were observed in the present study, degenerating pyknotic granulosa cells and oocytes, suggestive of normal follicular atresia. The well-established connective tissue network within the medulla (Suvarna *et al.*, 2019) [7] highlights the ovarian considerable metabolic demands for steroidogenesis and follicular growth.

For *Arctonyx* species, these baseline histological findings collectively establish fundamental reproductive parameters (Duckworth *et al.*, 2016; Baker, 2012) [2, 1]. As per Wozencraft (2005) [8] and Helgen *et al.* (2008) [4], the species' wide range in the eastern Himalayan region, northeastern India, Thailand, Assam, and Myanmar coincides with regions that are experiencing severe habitat fragmentation. For exclusive understanding of breeding cyclicity, reproductive senescence, and morphophysiological relationships, future research is required, with larger sample sizes considering the seasons.

Conclusion

This study aimed to draw baseline information on ovarian microanatomical studies for this vulnerable species through. Typical mammalian ovarian organisation and active reproductive cyclicity are confirmed by the presence of follicles at all developmental stages, active and regressing corpora lutea, and a well-vascularized medullary stroma. For upcoming comparative and conservation-focused studies, quantitative morphometric measurements provide useful benchmarks.

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