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Histomorphological study on the ductus epididymis of camel (*Camelus dromedarius*)

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Abstract
The epididymal tubules were made up of the ductus epididymis, which was very wavy. The epididymis's head, body, and tail body, and tail of the epididymis were each a separate area. Circular smooth muscle fibers, a thin layer of loose epididymal connective tissue, and implanted blood and lymphatic vessels encircled the ductus epididymis. There were four main cell types in the ductus epididymis epithelium: primary cells, basal cells, apical cells, and dark cells. This epithelium was a pseudostratified ciliated columnar epithelium.

Keywords: Histomorphology, ductus epididymis, camel

Introduction
A camel is an even-toed ungulate from the genus Camelus that has distinctive fatty deposits known as "humps" on its back. The three-remaining species of camel are the dromedary, or one-humped camel (*Camelus dromedarius*), which lives in the Middle East and the Horn of Africa, the Bactrian, or two-humped camel (*Camelus bactrianus*), which lives in Central Asia, and the critically endangered wild Bactrian camel (*Camelus ferus*), which has small populations in remote regions of northwest China and Mongolia. Both dromedaries and Bactrian camels have been domesticated; they supply milk, meat, and hair for textiles and other products like felted bags. They are working animals that perform a variety of jobs, including carrying things and moving people.

The testes are the main and most important organ of the male genital system, followed by the epididymis, the ductus deferens, the ducts for ejaculation, the penis, and several accessory sex glands. The location of sperm collection, storage, and subsequent maturing is the highly convoluted ductus epididymis (Singh and Bharadwaj 1980).

Materials and Methods
Six pairs of left and right epididymis were used to collect small pieces of tissue (4-5mm) for histological analysis. To investigate regional differences, tissues from three identical anatomical locations of each epididymis were taken (Fig. 1). The tissues were prepared for light microscopy after being preserved for 48 hours, 18 hours, or 15 hours in 10% Formal saline, Zenker's fluid, or Bouin's fluid, respectively. The melting point of the paraffin wax utilized to make the blocks was 58-60 °C. Using a microtome and sections cut from albumenized slides, the paraffin blocks were divided into 5-6 m thick slices, which were subsequently heat fixed. To show the various parts of the epididymis, the fixed slides were stained with a series of histological stains.

1. Eharlich's Haematoxylin and Eosin method for routine observation (Singh and Sulochana 1997) [21].
1. Azan trichrome stain demonstrates the collagen, reticular and muscle fibres (Singh and Sulochana, 1997) [21].
2. Crossman's modification of mallory's triple stains methods for elastic, collagen and muscle fibres (Singh and Sulochana, 1997) [21].
3. Weigert's method used for elastic fibres (Singh and Sulochana, 1997) [21].
4. Masson's trichrome method used to find collagen and muscle fibres (Singh and Sulochana, 1997) [21].
5. Verhoeff's elastin stain for evaluate connective tissue fibers (Singh and Sulochana, 1997) [21].

6. Mallory's phosphotungastic acid haematoxylin PTAH stain used for collagen and muscle fibers (Singh and Sulochana, 1997) [21].
7. Selective demonstration of reticulum, elastic, and collagen by silver orcein and aniline blue stain (Singh and Sulochana, 1997) [21].
8. Gomori's silver impregnation method attempt for reticular, elastic and collagen fibres (Luna, 1968).

Ductus epididymis

The epididymal tubules, which were contained in the highly convoluted ductus epididymis, were encircled by circular smooth muscle fibers and thin, loose connective tissue (Figs. 2, 3, and 4). The loose connective tissue had collagen, elastic and reticular fibers in which blood vessels, lymph vessels were distributed (Fig 5, 6, 7, 8, 9, 10, 11 and 12), These findings appear to be consistent with those made by Abdou *et al.* (1985) [2] in buffalo, Victor (2008) [24] in human, Zayed (2012) [25] in one-humped camels, and Deshmukh *et al.* (2014) [7] in adult birds of Aseel and Vanaraja. While Jacks (2001) [11] revealed that the duct was circumscribed externally by two to three layers of myoepithelial cells in the mouse epididymis, Egger and Witter (2009) [8] explained that in the mammalian testis, a basal lamina encased, at least partially, the whole tubular system of the canine testis and epididymis, which was surrounded by contractile cells expressing smooth muscle actin, smooth muscle myosin, and desmin, Sarma *et al.* (2011) [19] reported that the substantial fibrous capsule encased the corpus epididymis. There were only a few reticular and elastic fibers in it; collagen fibers made up the majority. Procollagen, which is an immature form of collagen (procollagen), made up the majority of the capsule in day-old children. There were no reticular fibers and only a few elastic fibers in the capsule, which were detected in the blood vessels of the capsule. in assam goat and Alkafafy *et al.* (2011) [5] mentioned that several layers of circularly and obliquely oriented SMCs made up the peritubular muscle coat (PMC), which encircled the epididymal epithelium (smooth muscle cells). The epididymal blood, lymph, and nerve fibers were scattered throughout the interstitium, which was made up of loose connective tissue. The interstitium had a wide variety of cell types.

In addition to macrophages, lymphocytes, and plasma cells, the majority of these cells in the camel were fibroblasts. Each of the three epididymal zones was lined by pseudostratified columnar ciliated epithelium, which was made up of four different cell types: principal cells, basal cells, apical cells, and dark cells. Along the whole length of the duct, principal cells (PC) and basal cells (BC) were visible, and PC had stereocilia on the apical border. Additionally, the dark cells (DC) occurred alongside the major cells as thin, tall, and deeply stained cells, extending from the basement membrane to the lumen (Figs. 13 and 14). Apical cells (AC) were also identified at various locations throughout the duct. The whole epididymal duct included black cells with dark, elongated, and fusiform nuclei; nevertheless, their frequency increased near the cauda epididymis (Fig 15). This interpretation was comparable to those made by Abdou *et al.* (1985) [2] on buffalo, Alkafafy *et al.* (2011) [5] in camels, Pasha *et al.* (2013) [16] in camels, Danmaigoro *et al.* (2014) [6] for bats, and Sakhawy *et al.* (2016) [9] in camels in Egypt. Nevertheless, Goyal and Dhingra (1975) [10] noted that at 3-30 weeks, the epithelium was simply high columnar in region I and simply low columnar in region II. regions III, IV, and V were pseudostratified low columnar, and region VI was pseudostratified low columnar. In buffalo, the epithelium grows taller with advancing age and tends toward advanced pseudo-stratification, Tingari (1989) [22] noted that the camel's epididymis contained principal, basal, halo, apical, and dark cells, as well as four other cell types. In camels, the principal cells made up the majority of the epithelial lining, Jacks (2001) [11] argues that the duct was internally lined with a pseudostratified epithelium. All mammals' epididymis contains a main cell, which was the predominant cell type there. Although it was present across the entire duct, there are structural variations in each area. Only the regions of the caput, corpus, and cauda have clear cells, whereas the beginning segment and intermediate zone were the only locations where narrow cells may be detected. The entire length of the duct was occupied by basal and halo cells, Moonjit and Suwanpugdee (2007) [14] stated about a very tall pseudostratified columnar epithelium bordered the surface of the epididymis. Long stereocilia protruded from the surface of the majority of epithelial cells, commonly known as principal cells, Victor (2008) [24] found that the tall columnar principal cells with long, immobile stereocilia and smaller basal cells that make up the pseudo-stratified columnar epithelium in humans, Uppal *et al.* (2009) [23] resemble that Principal and basal cells were the predominant cell types. Segments I to IV had more noticeable stereocilia. Specifically, segments IV and V contained apical cells. Segment V showed cytoplasmic blebbing, an indication of apocrine secretion. Segment VII had the thickest peritubular smooth muscle cuff in guinea pig, Razi *et al.* (2010) [17] concluded that the pseudostratified columnar epithelium of the epididymis was immersed in a loose connective tissue. Its diameter was similarly less than that of the efferent ductules, Alkafafy *et al.* (2012) [4] found that In both species, a pseudostratified columnar epithelium bordered the epididymal duct. Along the duct's whole length, there were mainly two sorts of cells to be spotted. A basal membrane supports the basal cells (BCs) and the main cells (PCs). Stereocilia (SC) lined the apical margins of the PCs. In some areas of the duct, apical cells were also commonly observed in addition to both cell types. Dark cells (DCs) had dark, elongated, fusiform nuclei and were visible as narrow, tall, and darkly colored cells. They were noticed in all epididymal segments, although their frequency rises near the cauda in donkeys and dromedary camels, Kishore *et al.* (2012) [12] explaned that at birth, the simple epithelium developed gradual pseudo stratification. At various stages of postnatal development, stereocilia, several epithelial cell types, and the smooth muscle layers surrounding the epididymal tubules all appeared in ram's, Schimming *et al.* (2013) [20] pointed out the cellular lining of the epithelium comprised the population of principal cells, basal cells, apical cells, narrow cells, and hallo cells in paca epididymis, Deshmukh *et al.* (2014) [7] mentioned that most of the ductus epididymis lacked mucosal folds in grower, and in both the right and left epididymis in adults, Aseel had much less mucosal folds than Vanaraja did, Akbarsha *et al.* (2015) [3] elucidate that the ductus epididymis's histological differentiation and shifting luminal microenvironment were prompted by the different epithelial cell types lining the duct. The principal cell (PC), narrow cell (NC), apical cell (AC), clear cell (CC), basal cell (BC), and intraepithelial leukocyte cell populations, also known as halo cells, intraepithelial lymphocytes (IELs), or intraepithelial macrophages (IEMs), made up the pseudo-stratified epithelium of the ductus epididymis. Pale vacuolated epithelial cells in the mammalian epididymis were recently highlighted as yet another cell type, El-Sakhawy *et al.* (2016) [9] reported that the pseudo-stratified columnar epithelium that bordered the epididymal duct was present. There were five different cell types that made up the epididymal epithelium in Egyptian camels: primary, basal, apical, dark, and halo cells and Nurliani *et al.* (2020) [15] explained that the principal cells, which had stereocilia and apical blebs, were the predominant cell type in the epididymis in Sunda porcupine.

The epithelium also contained intra-epithelial glands, the lumen of which was encircled by straightforward columnar or cuboidal cells. Although they were infrequently identified in other areas, the intra-epithelial glands were usually seen in the distal region of the corpus epididymis (Fig 16). Similar to that revealed by Alkafafy *et al.* (2011) [5] in the camel. The result was partially harmony with that of Abdel-Maksoud *et al.* (2019) [1] stated that, there were various stages to gland creation; it starts as tiny invaginations inside the epithelium of the epididymal duct; later, these invaginations develop into the gland, which has a lumen enclosed by surrounding epididymal epithelium. Although basal cells were rarely seen, the intraepithelial gland's epithelium was made up of columnar cells. The main (P) and dark cells (D), were two types of columnar cells that might be examined predicated on their nuclear and cytoplasmic contents in camel.

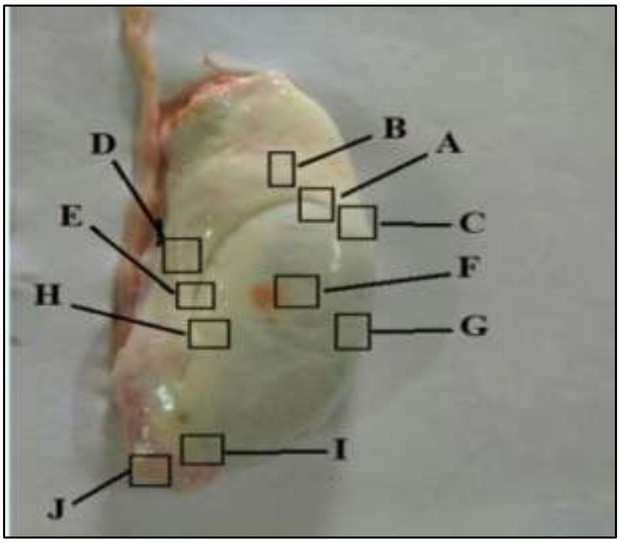

Fig 1: Photograph of testis showing, B-Head of epididymis, D- Body of epididymis and J- Tail of epididymis

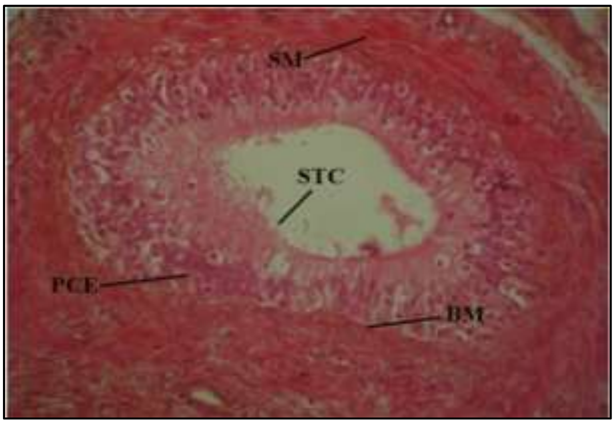

Fig 2: Photomicrograph of ductus epdidymis (head) showing smooth muscle (SM), basement membrane (BM), and pseudostratified columnar ciliated epithelium (PCE). H&E Stain 400x

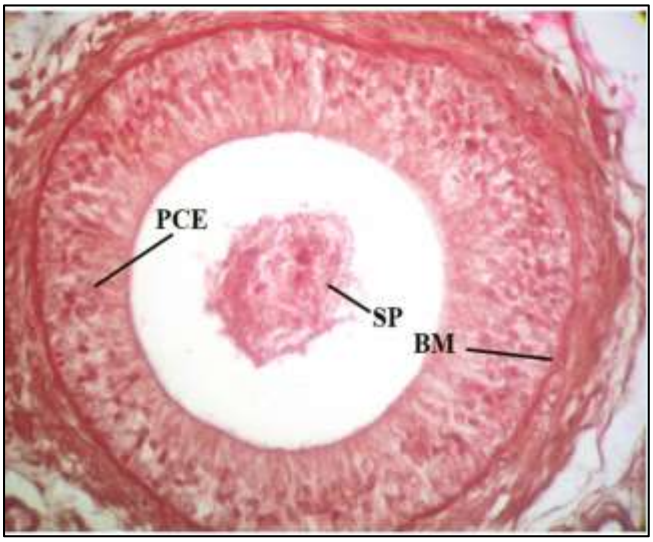

Fig 3: Photomicrograph of epididymis (body) showing, smooth muscle (SM), pseudostratified columnar ciliated epithelium (PCE), and spermatozoa (SP). H&E Stain 400x.

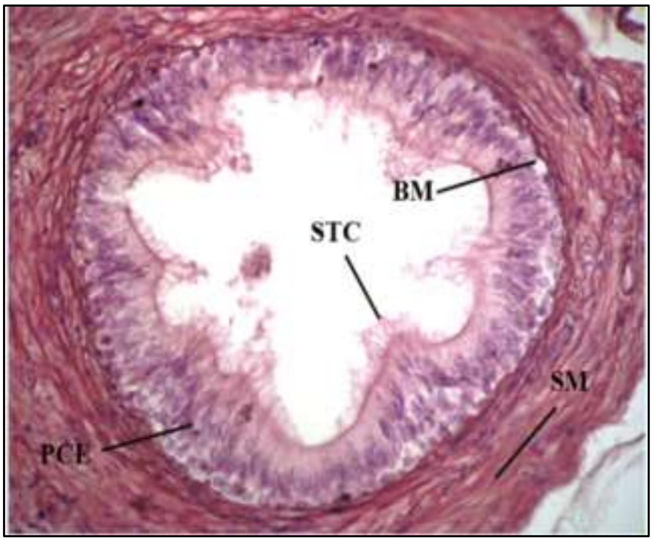

Fig 4: Photomicrograph of epididymis (tail) showing, smooth muscle (SM), pseudostratified columnar cliated epithelium (PCE), stereocilia (SC). H&E Stain 400x.

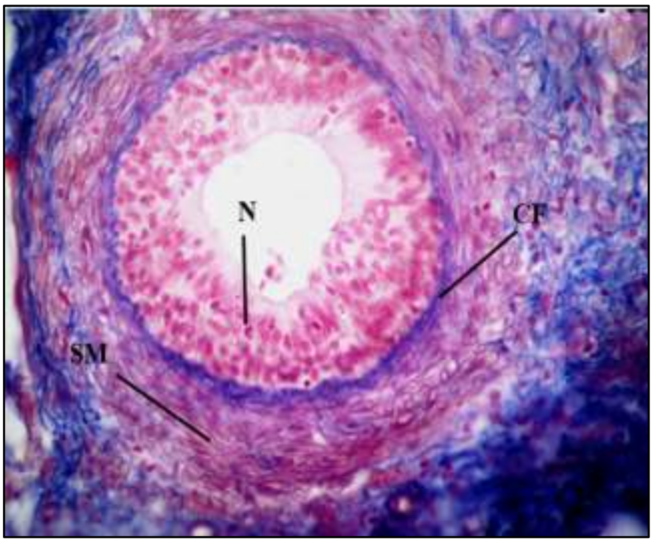

Fig 5: Photomicrograph of ductus epididymis (head) showing, collagen fibers blue (CF), muscle fibres (M), and nucleus red (N). Azan trichrome Stain 400x

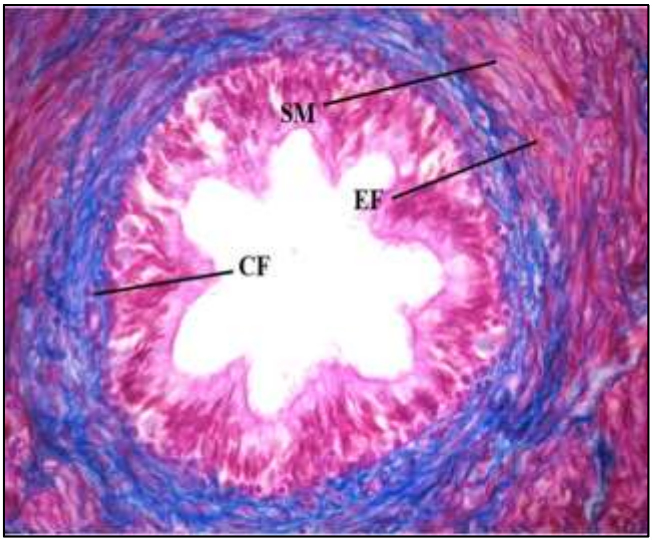

Fig 6: Photomicrograph of ductus epididymis (tail) showing, collagen fibers blue (CF) elastic fibers pink (EF) and muscle fibers pink (SM). Crossman's Triple Stain 400x.

Fig 9: Photomicrograph of ductus epididymis showing, collagen fibers pink (CF), elastic fibers brown (EF), and nucleus brown (N). Verhoeff's Stain 400x.

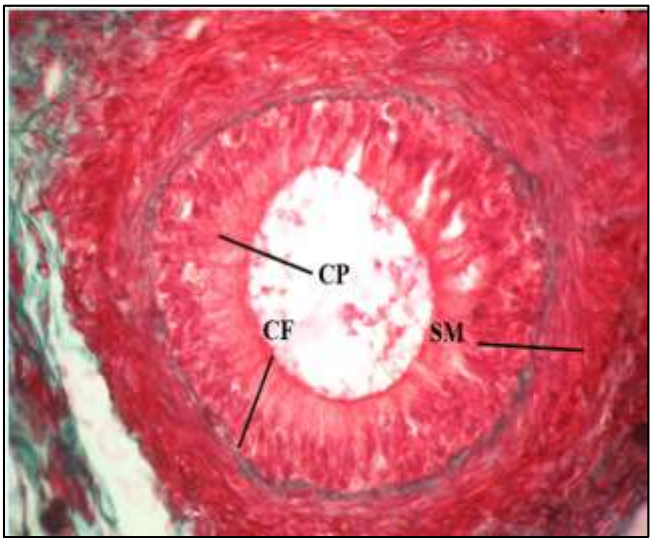

Fig 7: Photomicrograph of ductus epididymis showing, collagen fibers green (CF), smooth muscle red (SM) and cytoplasm red (CP). Masson's Trichrome Stain 400x.

Fig 10: Photomicrograph of ductus epididymis showing, collagen fibers pink (CF), elastic fibers black (EF) and smooth muscle yellowish pink (SM). Weigert's Stain 400x.

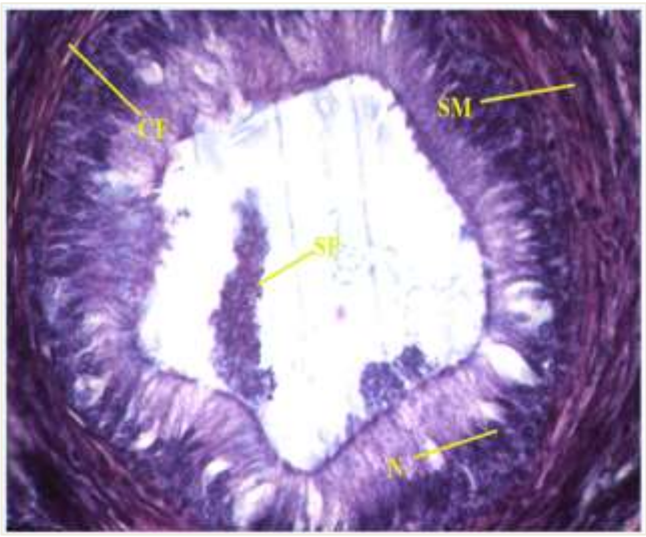

Fig 8: Photomicrograph of ductus epididymis showing, collagen fibers red (CF), smooth muscle blue (SM), and nucleus blue (N). PTAH Stain 400x.

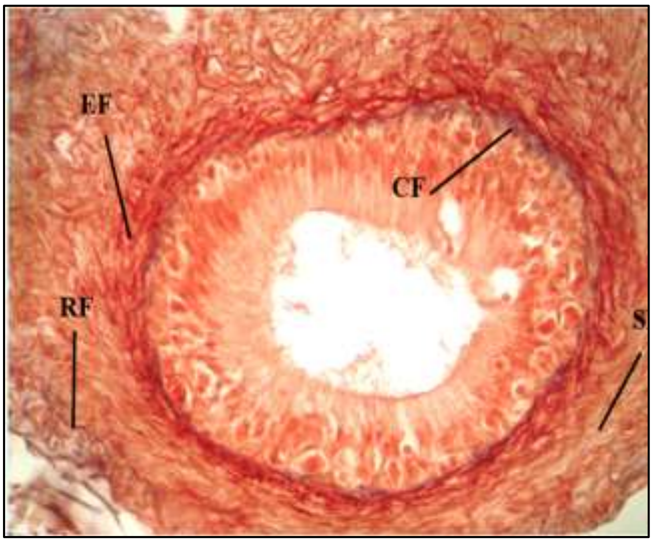

Fig 11: Photomicrograph of ductus epididymis showing, collagen fibers blue (CF), elastic fibers red (EF), reticular fibers black (RF), smooth muscle beige (SM). Selective demonstration of elastic, reticulum and collagen by silver orcein and aniline blue Stain 400x.

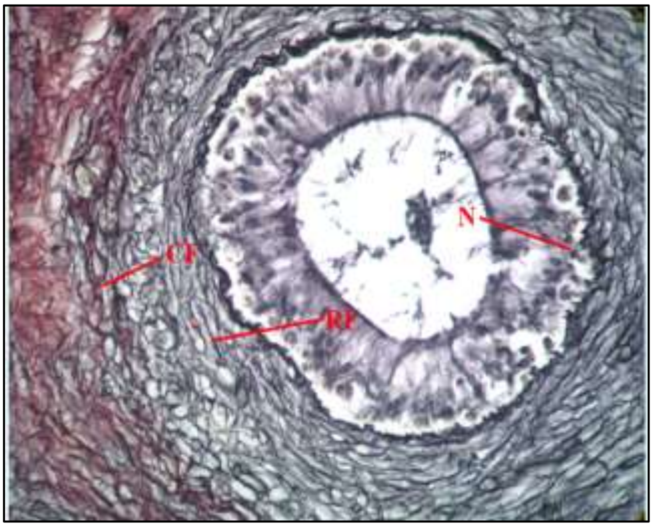

Fig 12: Photomicrograph of ductus epididymis showing, collagen fibers red (CF), nucleus gray (N), and reticular fibers black (RF). Gomori's Silver Impregnation Stain 400x.

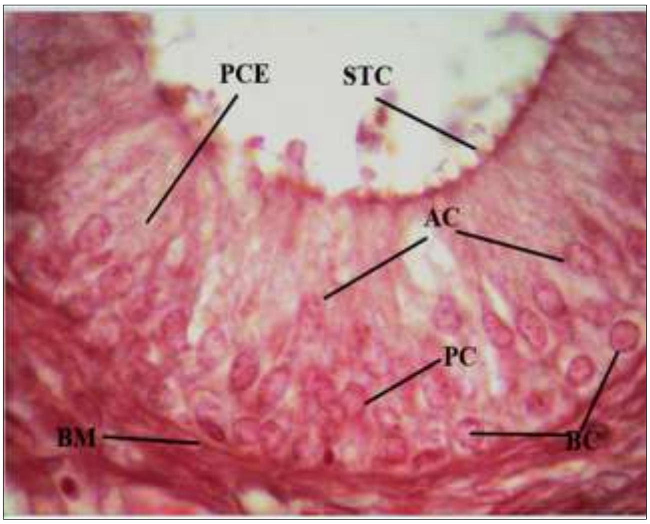

Fig 13: Photomicrograph of ductus epididymis (head) region showing, smooth muscle (SM), basement membrane (BM), pseudostratified columnar ciliated epithelium (PCE), with principal cell (PC), apical cell (AC) and stereocilia (SC). H&E Stain 1000X

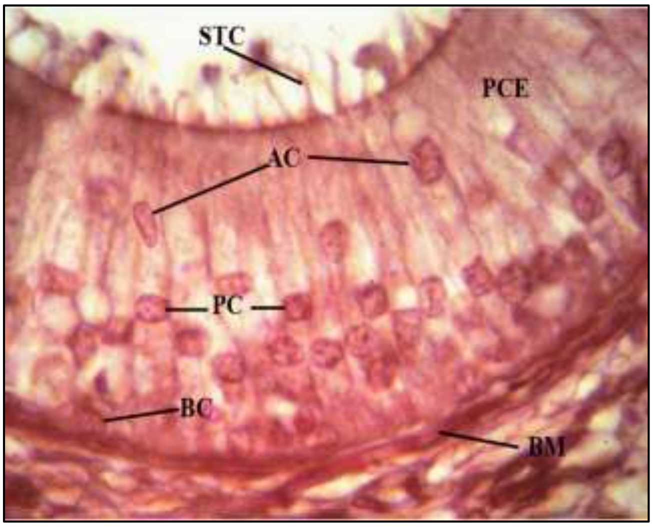

Fig 14: Photomicrograph of ductus epididymis (body) showing, basement membrane (BM), pseudostratified columnar ciliated epithelium (PE), with principal cell (PC), basal cells (BC), apical cell (AC), and stereocilia (SC). H&E Stain. 1000x.

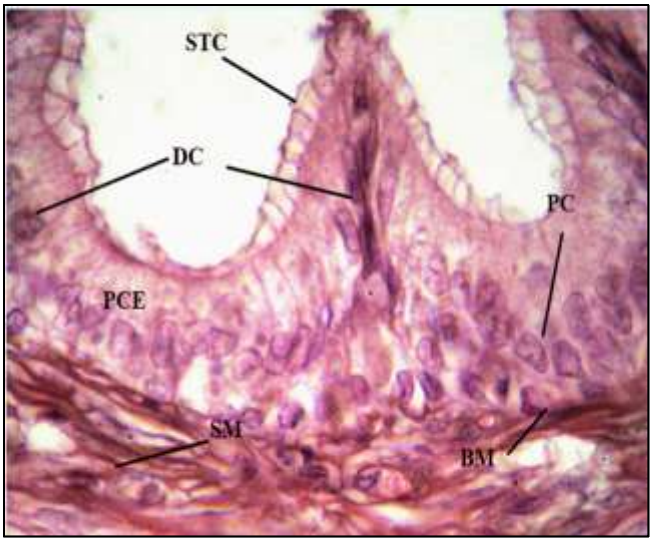

Fig 15: Photomicrograph of ductus epididymis (tail) showing, basement membrane (BM), smooth muscle (SM), dark cells (DC), pseudostratified columnar ciliated epithelium (PCE), principal cells (PC), and stereocilia (SC). H&E Stain 1000x.

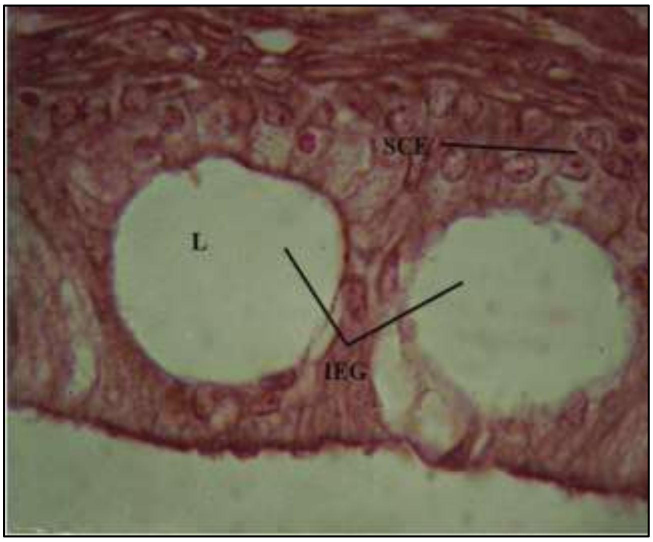

Fig 16: Photomicrograph of intra epithelial glands (IEG) in ductus epididymis showing, simple columnar epithelium (SCE) and lumen (L). H&E Stain 1000x.

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