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MORPHOLOGICAL AND HISTOLOGICAL ANALYSIS OF MALE DONKEY REPRODUCTIVE DUCTS: FROM DEFERENT DUCTS TO EJACULATORY PATHWAYS

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ABSTRACT:

The anatomical and histological characteristics of the terminal deferent ducts, excretory ducts of the seminal vesicles, and ejaculatory ducts were examined in detail. The terminal deferent duct, located ventromedially to the excretory duct of the seminal vesicle, exhibited a small, irregular lumen with cyst-like invaginations into the vascular, fibromuscular connective tissue. Its lumen was lined by bi-layered columnar epithelium, with principal cuboidal cells and occasional basal flat cells. The connective tissue of the cranial deferent duct contained collagen, elastic fibers, and smooth muscle, transitioning towards diminished smooth muscle at the duct's terminal region. The ducts from either side merged to form common ejaculatory ducts, which entered the urethra at the Colliculus seminalis. At this junction, the epithelium changed to either stratified columnar or cuboidal types. The ejaculatory ducts exhibited lumens characterized by branched folds, which were lined with stratified columnar epithelium and reinforced by a dense network of collagenous and elastic connective tissue. Scanning electron microscopy revealed that the luminal surface of the ejaculatory duct was folded, irregularly oval, had nearly hexagonal epithelial cells with distinct borders, and abundant microvilli on the luminal surfaces. These findings provide a comprehensive understanding of the structural organization of these ducts and their role in the male reproductive system.

KEYWORDS: Seminal vesicle ducts, Deferent ducts, Ejaculatory ducts, Mullerian ducts

1. INTRODUCTION

The complex anatomy and unique histological characteristics of the male reproductive system guarantee the effective transportation, maturation, and delivery of spermatozoa. Reproductive physiology depends heavily on the animal's accessory genital glands, such as the prostate gland (Abou-Elhamd *et al*., 2013), ampulla ductus deferens and seminal vesicles (Abou-Elmagd and Kelany, 1992; Noda and Ikawa, 2019; Abou-Elhamd *et al*., 2020), and bulbourethral glands (Abou-Elhamd *et al.,* 2019; Abou-Elhamd *et al.,* 2021), which they played a crucial role in reproductive physiology. Among the critical structures in these glands are the deferent ducts and ejaculatory ducts, which serve as conduits for sperm and seminal fluid. Clarifying these ducts' functions in male fertility and reproductive physiology requires an understanding of their histological and structural specializations

The deferent duct, also called the ductus deferens, plays an essential role in sperm transport. It begins in the epididymis and travels to the pelvic cavity via the spermatic cord before joining the seminal vesicle excretory ducts to form the ejaculatory ducts (Ross and Pawlina, 2006).

The final route for the movement of seminal fluid into the urethra is represented by the ejaculatory ducts, which are created by the union of the deferent ducts and seminal vesicle ducts. A stratified columnar epithelium and branched luminal folds are two distinctive histological characteristics of these ducts that are tailored to aid in the mixing and passage of seminal plasma and sperm (Standring *et al*., 2005; Standring, 2021).

This study aims to characterize the histological and structural features of the deferent ducts and ejaculatory ducts, with an emphasis on their connective tissue and epithelial components.

2. MATERIALS AND METHODS

The current study was performed on 10 sexually mature, apparently healthy male donkeys (jacks) aged between 5 and 7 years. The animals were anesthetized and humanely euthanized by exsanguination via the common carotid artery (Abou-Elhamd *et al*., 2012, 2013; Abou-Elhamd *et al*., 2019; Abou-Elhamd *et al*., 2020; Abou-Elhamd *et al*., 2021).

After dissection, Tissue specimens were collected from the prostatic and membranous regions of the pelvic urethra. They were subsequently fixed in the following fixatives: neutral buffered formaldehyde and Bouin's fluid for routine histological analysis and 5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) at 4°C for scanning electron microscopy (SEM). Paraffin sections, 5–7 µm thick, were prepared and stained with Harris hematoxylin and eosin (Harris, 1900), Crossmon's trichrome (Crossmon, 1937), Verhoeff's method (Verhoeff, 1908), and Gomori's reticulin stain (Gomori, 1937). Microscopic analysis and imaging were conducted using an Olympus microscope with a DP72 camera.

For SEM, specimens were fixed in 5% phosphate-buffered glutaraldehyde for 24 hours at 4°C, post-fixed in 1% buffered osmium tetroxide, and dehydrated in a graded ethanol series followed by amyl acetate. They were subjected to critical point drying with liquid CO_2, mounted on stubs, and sputter-coated with gold. They were examined and imaged using a JEOL 5400 LV scanning electron microscope.

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All procedures of the current study were conducted in accordance with the University guidelines for the care of experimental animals. Ethical approval (10936-24-2005-002) was obtained from the Committee of the Faculty of Veterinary Medicine, Assiut University, Egypt.

3. RESULTS

The terminal part of each deferent duct was located ventromedial to the excretory duct of the seminal vesicle (Fig. 1). Initially, the deferent duct featured a relatively small, irregular lumen with cyst-like invaginations extending into the underlying highly vascular, dense fibromuscular connective tissue. The lumen was lined with a bi-layered columnar epithelium, while the cysts were lined with principal and, occasionally, basal cells. The principal cells were cuboidal, with a faintly foamy, acidophilic cytoplasm and oval or rounded vesicular nuclei positioned basally. The basal cells were flat and small, with flattened nuclei (Figs. 2A & B).

In the cranial part of the deferent duct, the surrounding connective tissue layer contained abundant collagenous (Fig. 3A) and elastic fibers (Fig. 3B), along with circularly arranged smooth muscle fibers. Reticular fibers supported the lining epithelium and the endothelium of the cavernous spaces (Fig. 3C). While smooth muscle and elastic fibers were prominent in the outer regions of this layer (Fig. 3A), the smooth muscle fibers gradually decreased as the duct approached its terminal region. Near the terminal area, the deferent ducts and the excretory ducts of the seminal vesicles from both sides approached each other, and the smooth muscle fibers disappeared entirely (Figs. 4A & B). Eventually, the ducts merged before opening into the urethra (Figs. 4C & D), where the lining epithelium transitioned to stratified columnar or cuboidal, with occasional patches of bi-layered epithelium (Figs. 4C & D).

The fusion of the ducts on the right and left sides resulted in the formation of two common ejaculatory ducts, which opened into the urethra at the elevated Colliculus seminalis. Adjacent to the Colliculus seminalis, two deep invaginations of the urethral mucosa were observed (Fig. 5).

The lumens of the ejaculatory ducts displayed branched folds (Fig. 5), lined by stratified columnar epithelium (Figs. 6A & B). These ducts were supported by a dense connective tissue layer composed of collagenous (Fig. 5) and elastic fibers (Figs. 6C & D). Scanning electron microscopy of cross-sections of the ejaculatory ducts revealed an irregular oval shape with numerous folds (Figs. 7A & B). The luminal surfaces of these folds consisted of nearly hexagonal-shaped cells with well-defined boundaries. At higher magnification, the luminal surfaces covered numerous microvilli (Figs. 7C & D).

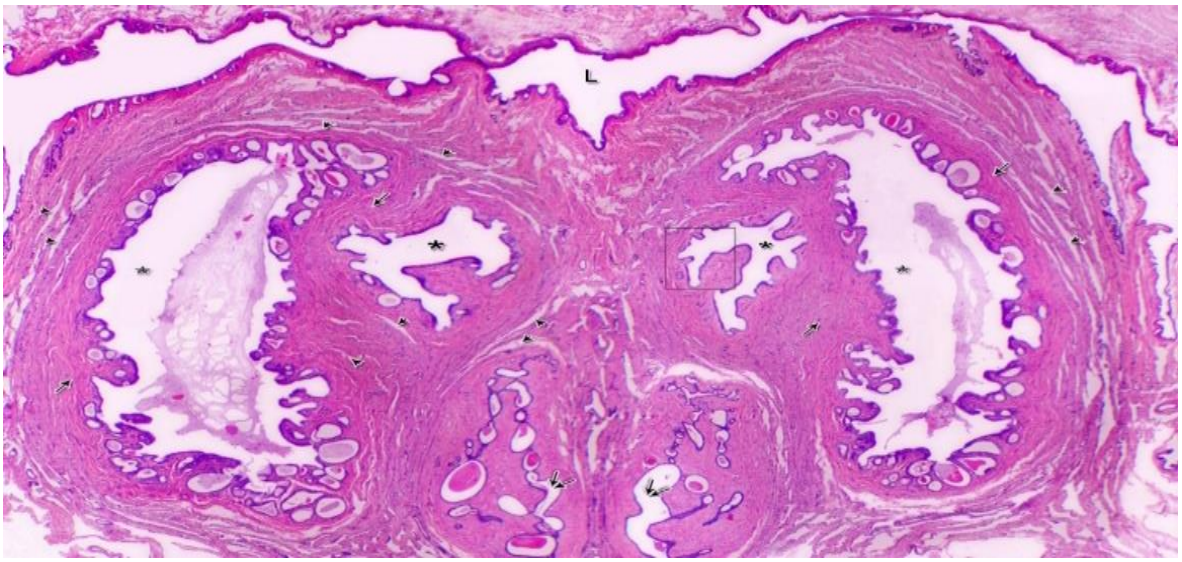

Figure. 1: Photomicrograph of cross-sectioned prostatic urethra at its dorsal aspect showing the deferent (asterisk) and seminal vesicle ducts (star) surrounded with a dense fibrous connective tissue (arrow), cavernous spaces (arrowhead), remnants of the Mullerian ducts (double arrow), urethral lumen (L). Haematoxylin & eosin stain. X 50.

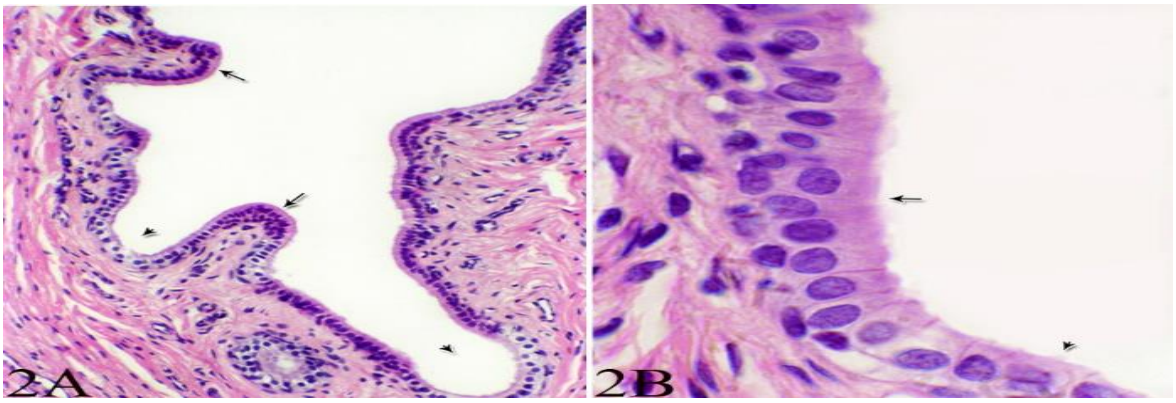

Figure. 2: (A) Photomicrograph of the marked area in Fig. 1 showing the lining epithelium of the deferent duct. Bi-layered columnar epithelium (arrow), cyst-like invaginations (arrowhead) with characteristics faintly acidophilic cytoplasm of their cuboidal cells. (B) High magnification photomicrograph showing the bi-layered epithelium (arrow) and the cyst-like invaginations (arrowhead). Haematoxylin & eosin stain. A (X400); B: (X1000).

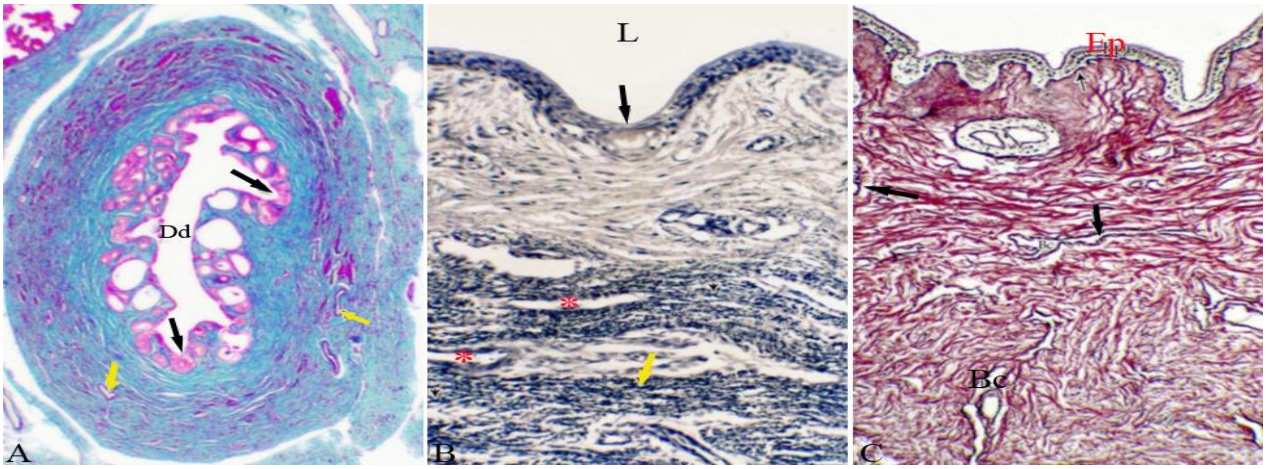

Figure. 3: Photomicrograph of the deferent duct (Dd) at the cranial part of the prostatic urethra: (A) collagenous fibers (green colour) and smooth muscle fibers (red colour). Cyst-like invaginations of the epithelium (arrow), and blood vessels (yellow arrowhead). (B) Elastic fibers (Yellowwhead) in the outer portion of the wall of the deferent duct. Lumen (L), cyst-like invaginations (black arrow); blood vessels (red asterisk). (C): The reticular fibers network (black arrow) supporting the lining epithelium (Ep) as well as the endothelium of the blood cavernae (Bc). Collagen fibers (brown colour). A: Crossmon´s trichrome X 25, B: Verhoff´s stain. X200, C: Gomori´s stain. X200.

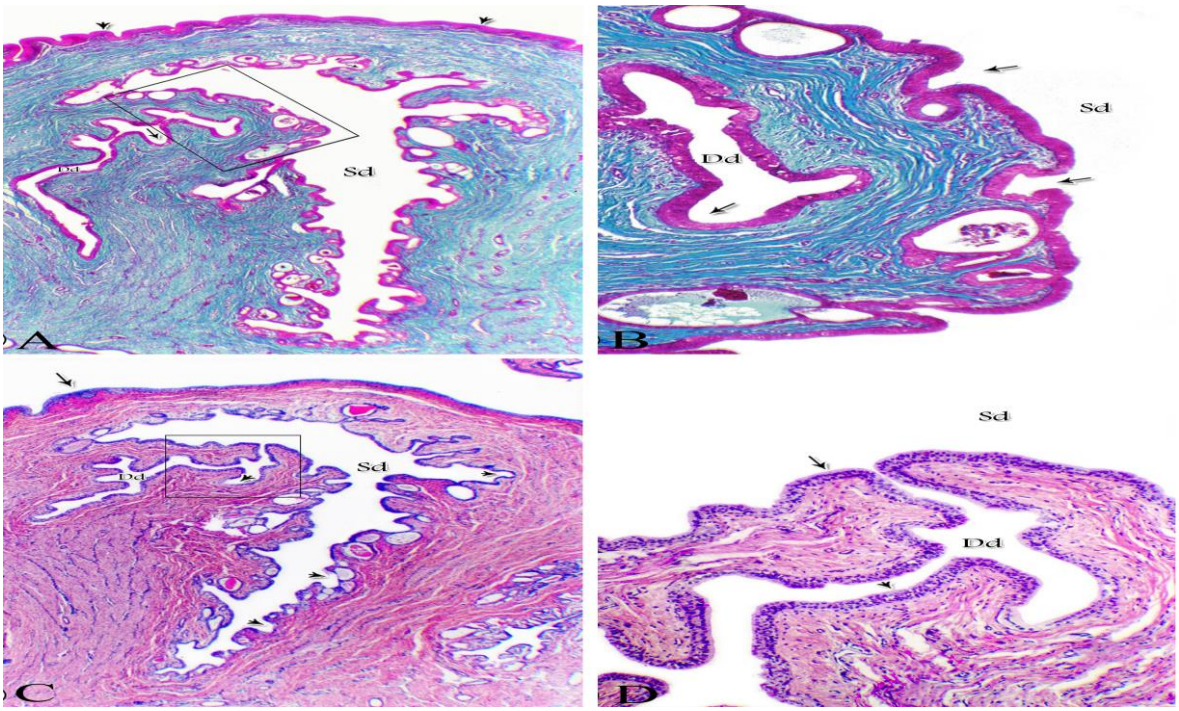

Figure. 4: (A): Photomicrographs of the deferent duct (Dd) just before joining the seminal vesicle duct (Sd), (B): Higher magnification of the marked area in (A) showing collagenous fibers (green colour) in the wall of the deferent duct. Cyst-like invaginations in the wall of both ducts (arrow), urethral epithelium (arrowhead). (C): Photomicrograph showing the joining of the deferent (Dd) to the seminal vesicle ducts (Sd). Cyst-like invaginations in both ducts (arrowhead), urethral epithelium (arrow). (D): High magnification of the marked area in (C) showing the stratified columnar (arrow) and cuboidal (arrowhead) epithelium lining the deferent (Dd) and seminal vesicle ducts (Sd). A& B Crossmon´s trichrome stain, C& D Haematoxylin & eosin stain. A&C: X50, B:X400, D:X200

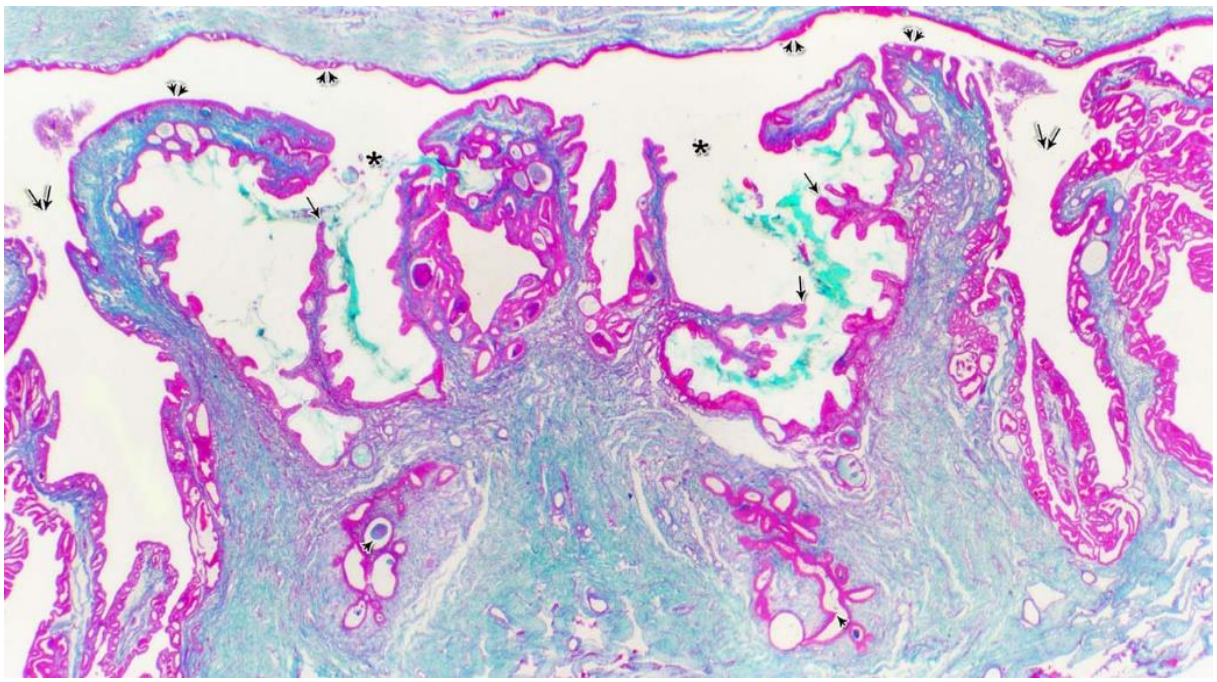

Figure. 5: Photomicrograph of a cross-sectioned prostatic urethra at the level of the Colliculus seminalis showing the right and left ejaculatory duct openings (asterisk), folded mucosa (arrow) and collagen fibers (green colour). Remnants of the two Mullerian ducts (arrowhead), lateral invaginations of the urethral mucous membrane (double arrow), urethral epithelium (double arrowhead). Crossmon´s trichrome stain X 50.

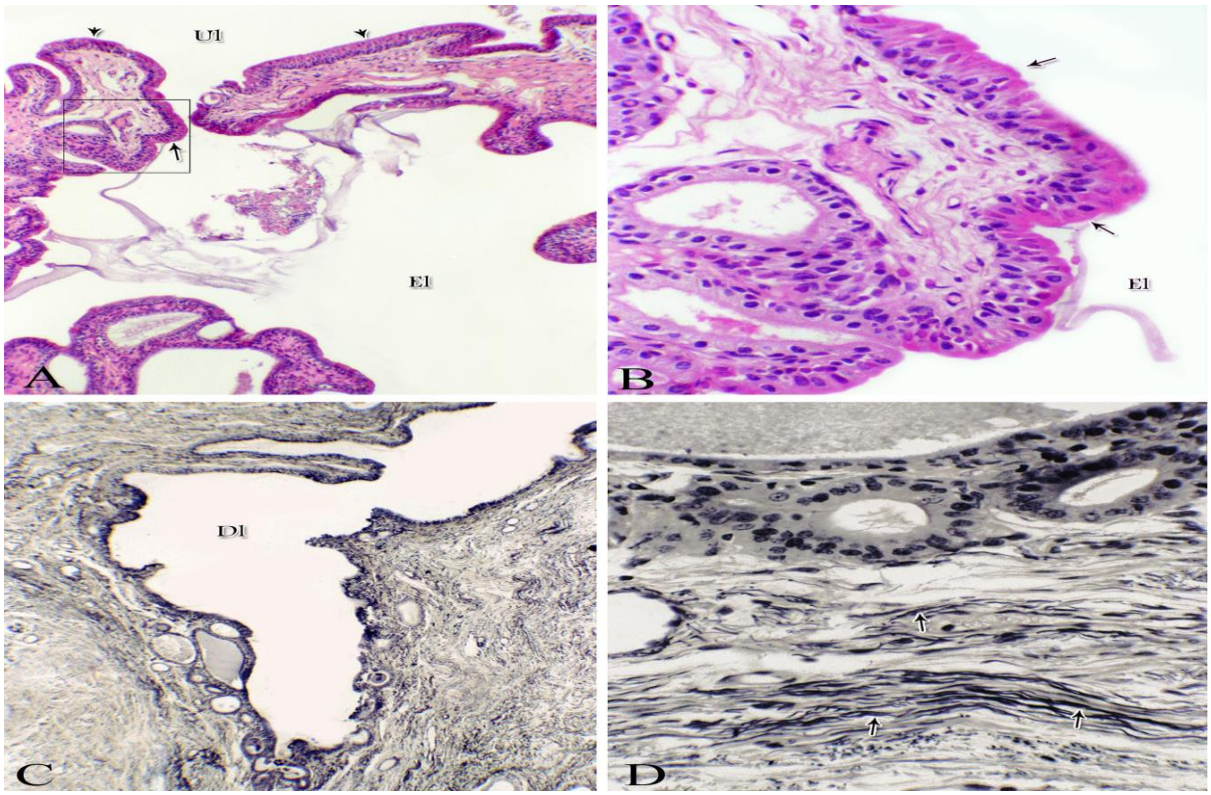

Figure 6: (A & B): Photomicrographs of the lining epithelium of the ejaculatory ducts (A) and its high magnification (B) showing the stratified columnar epithelium of the ejaculatory duct (arrow) as that of the urethra (arrowhead). C&D: Photomicrographs showing the distribution of the elastic fibers (arrow) in the wall of the ejaculatory duct and the high magnification (D- arrow) during winter. Duct lumen (Dl). Ejaculatory duct lumen (El), urethral lumen (Ul). Haematoxylin & eosin stain. (A): X100. (B): X400. Verhoff´s stain. (C): X50, (D): X400.

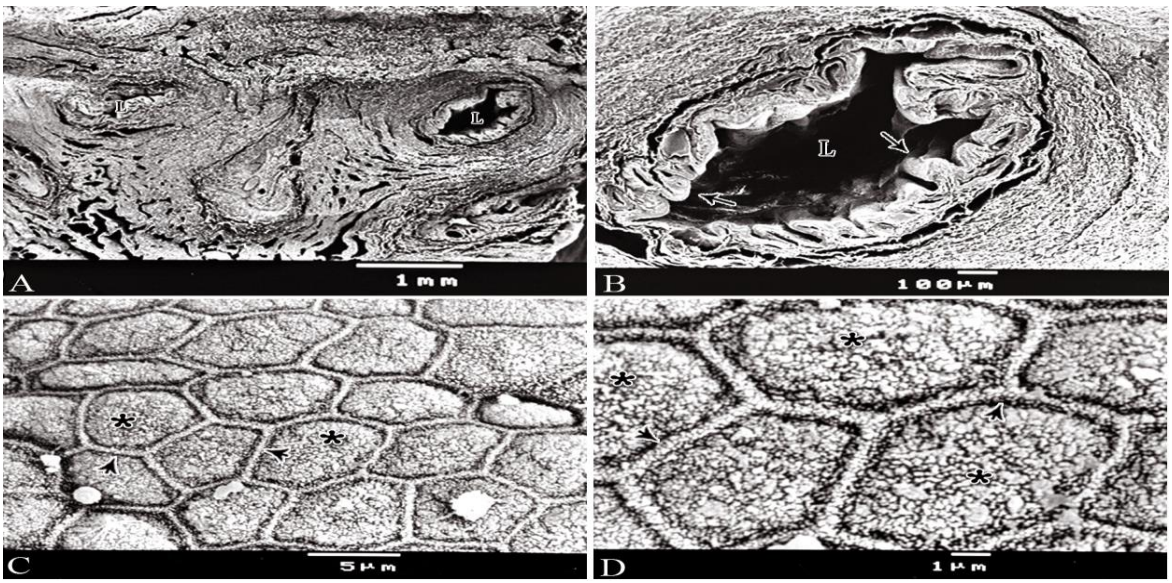

Figure.7: (A& B) Scanning electron micrographs of cross-sectioned ejaculatory ducts (A) and high magnification of the right one (B) showing the duct lumen (L) with numerous mucosal folds (arrows). (C& D)**:** The lining epithelium of the ejaculatory duct (C) and its high magnification (D) showing nearly hexagonal-shaped luminal cell surface with numerous microvilli (asterisk) and well-demarcated cell boundaries (arrowheads).

4. DISCUSSION

These histological and anatomical features of the deferent duct and ejaculatory ducts can provide useful information for understanding their functional specializations adapted for sperm transport and secretion of seminal fluids in donkey species. The findings underscore a complex organization of epithelial lining, connective tissue, and smooth muscle constituents in the functional unit for efficient sperm transport and preparation for ejaculation. These tissues support the ducts' physiological function.

The anatomical morphology of the terminal deferent ducts, which include tortuous lumens and cyst-shaped invaginations, reinforces their functional complexity (Robaire, 1988; Eroschenko and Di Fiore, 2013). The mechanical propulsion of sperm and seminal fluids during ejaculation is probably supported by the extensive, highly vascular fibromuscular connective tissue surrounding these channels (Gurung *et al*., 2023). The columnar epithelium that appears to be organized in layers and cyst-lining cells seen here would further argue for active roles in secretion and fluid regulation (Junqueira, 2005). While the flat basal cells provide structural support and regeneration potential, the cuboidal main cells with acidophilic cytoplasm and basally located vesicular nuclei imply active secretory and absorptive capabilities. These results are consistent with the ductal epithelial specialization descriptions in Ross and Pawlina, 2006, which highlight the functional diversity of reproductive tissue epithelial cells, which emphasize the functional diversity of epithelial cells in reproductive tissues.

In the cranial part of the deferent duct, the surrounding connective tissue rich in collagen and elastic fibers, along with smooth muscle fibers, indicates a dual role of mechanical support and motility. The progressive reduction of smooth muscle fibers towards the duct's termination suggests a shift from active propulsion to passive transport as the ducts approach the urethra. Similar structural transitions have been recorded in (Junqueira *et al*., 1998; Junqueira, 2005), demonstrating how crucial tissue composition is for accomplishing functional objectives.

The close anatomical association and fusion of the deferent ducts with the excretory ducts of the seminal vesicles allows for the integration of sperm with seminal secretions. The disappearance of smooth muscle fibers close to the end part may represent a dependency on urethral peristalsis for ejaculation. Changing the epithelial lining from simple cuboidal to stratified columnar or cuboidal, sometimes with bi-layered regions, indicates the capacity of the ducts to respond to distinct functional requirements, such as fluid movement and protection. Other species have shown similar epithelial adaptations, highlighting the necessity of an effective lining that can endure biochemical and mechanical pressures (Cross, 1995).

The ejaculatory ducts are formed via the junction of the seminal and ampullary vesicle ducts, followed by their entry into the urethra at the colliculus seminalis, which emphasizes the anatomical integration necessary for effective ejaculation (Steger and Weidner, 2011). Deep mucosal invaginations close to the colliculus seminalis may serve as a reservoir to preserve seminal fluid volume during ejaculation or as a regulator of fluid dynamics (Cossu *et al*., 1983; Junqueira, 2005).

Scanning electron microscopy unveiled further specializations, particularly the irregular oval shape of the ejaculatory ducts, which are lined with hexagonal-shaped cells that possess numerous microvilli. These structures probably optimize fluid dynamics and aid in mixing sperm with seminal vesicle secretions (Cossu *et al*., 1983; Abou-Elmagd and Wrobel, 1990).

(Bozzola & Russell, 1998). These microanatomical characteristics support findings from electron microscopy research conducted by Cossu *et al.* (1983), which emphasizes the functional significance of modifications to the luminal surface. The presence of these microvilli likely enhances the absorptive and secretory functions of the epithelium, helping to maintain an environment conducive to sperm viability and motility (Mathangasinghe *et al*., 2020). These findings are consistent with functional studies of male reproductive ducts, as outlined by Hafez and Hafez (2013).

The deep mucosal invaginations of the urethra and the raised Colliculus seminalis seem to minimise retrograde flow and enable accurate ejaculate delivery into the urethral lumen. This structural arrangement is essential for successful reproduction and efficient ejaculation, Similar arrangements have been observed in other mammalian species by comparative anatomical studies, such as those conducted by (Eurell and Frappier, 2006), highlighting their evolutionary significance.

In Conclusion, the described findings provide a comprehensive understanding of the structural-functional relationship within the deferent, excretory, and ejaculatory ducts.

These adaptations ensure the efficient transport and integration of sperm and seminal fluid, highlighting their critical roles in male fertility. The observations contribute valuable insights into normal male reproductive anatomy, with potential implications for diagnosing and treating conditions associated with ductal obstruction or dysfunction. Future research could focus on the molecular mechanisms regulating epithelial and connective tissue interactions, as well as their implications for fertility treatments and reproductive health.

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Competing interests:

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