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Morphohistological changes in the male genital duct system of experimentally blind rabbits (*Oryctolagus Cuniculus*)

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ABSTRACT

Background: The male genital duct system plays a critical role in the reproductive physiology of mammals, encompassing structures that transport, mature, and store spermatozoa.

Methods: This study investigates the effects of induced blindness on the morphology and histology of genital duct system of experimentally blind male rabbits. The experiment was conducted in the Department of Veterinary Anatomy and the Central Laboratory Animal House, College of Veterinary Science and Animal Husbandry, Jabalpur. Total sixteen male rabbits aged 1 to 1.5 years were housed under controlled environmental conditions with 12 hours of artificial light daily. Out of these, 12 rabbits were experimentally blinded through tarsorrhaphy and divided into three groups II, III and IV based on the duration of blindness: 30, 60, and 90 days, respectively. Group I comprised of four visually normal rabbits as control. At the end of the experimental periods, rabbits were euthanized using a Ketamine hydrochloride overdose, and their reproductive tracts were examined. After gross morphometric measurements tissue samples from the epididymis, vas deferens and penis were collected and processed for histological examination.

Results: Gross measurement of epididymis was not feasible. *Vas deferens* was looping around the ureter and connecting to the uterus musculinus. The lengths of the vas deferens, both left and right, showed no statistically significant differences ($P > 0.05$) among the four groups. For the left vas deferens, the mean lengths were 107.75 ± 1.03 mm in group I, 110.75 ± 0.75 mm in group II, 113.75 ± 0.75 mm in group III, and 112.50 ± 1.50 mm in group IV. Similarly, the mean lengths of the right vas deferens were 104.00 ± 1.77 mm in group I, 107.25 ± 0.85 mm in group II, 107.00 ± 0.40 mm in group III, and 106.75 ± 2.13 mm in group IV. The length of penis in group I (8.87 ± 0.42 mm), group II (9.00 ± 0.57 mm), group III (9.62 ± 0.85 mm), and group IV (8.25 ± 0.85 mm). Overall, no significant morphological changes were attributed to photoperiod or blindness. The morphometry of organs showed no significant difference ($P > 0.05$) across the experimental groups. Histological examination revealed significant alterations in the epididymal epithelium of group II. The epididymal diameter was 160.40 ± 7.15 μ m in group I which get reduced significantly to 85.41 ± 2.99 μ m in group II exhibiting the effect of blindness in luminal diameter. *Vas deferens* and penis showed no significant histological changes across groups, with stable duct diameters, epithelial thickness, and tissue organization.

Keywords: Blindness, Genital duct system, Photoperiod, Rabbit, Reproductive system

Introduction

The male genital duct system plays an essential role in sperm maturation, storage, and transport in mammals. In rabbits (*Oryctolagus cuniculus*), the epididymis, vas deferens, and other reproductive structures are critical for the maturation of sperm and its eventual passage through the reproductive tract. These organs are regulated by various factors, including hormonal signaling and environmental cues such as photoperiod, which can significantly influence reproductive cycles in seasonal breeders like rabbits (Arslan *et al.*, 2015) [1]. The impact of sensory input, particularly light, on the male reproductive system has been well-documented, but there is limited research on how induced blindness might alter the morphohistology of the male genital duct system. Rabbits are highly responsive to changes in environmental light, and their reproductive cycles are tightly regulated by photoperiodic cues (Goldman, 2001) [2]. The pineal gland, which secretes melatonin in response to light, plays a key role in regulating reproductive hormones in mammals (Bittman *et al.*, 1980) [3]. In seasonal breeders, melatonin secretion influences gonadotropins, which regulate testicular function and the maturation of sperm. Thus, blindness, which disrupts light-based environmental cues, may impact these hormonal pathways, potentially leading to changes in the structure and function of the male genital ducts. Despite the importance of photoperiod in regulating reproduction in rabbits (Dalton *et al.*, 2005) [4], the specific effects of blindness on the male genital duct system, including the epididymis and vas deferens,

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have not been extensively studied. While blindness has been shown to cause changes in general health and behavior in mammals, little is known about its impact on the structure of reproductive organs. This lack of understanding is significant because the male genital ducts are integral to the maturation, storage, and transport of sperm, and any alteration in their structure could have implications for fertility. The epididymis in rabbits is a critical site for sperm maturation and storage, while the vas deferens is responsible for transporting sperm from the epididymis to the urethra during ejaculation. These organs are lined by specialized epithelial cells, and their function is dependent on both hormonal regulation and environmental factors. Research on the effects of light deprivation on reproductive function has indicated that photoperiodic signals may play a role in maintaining the structural integrity of these organs (Wilson and Hsu, 1991 and Santos *et al.*, 2019) [5]. Changes in the morphology of the reproductive organs, such as the epididymis and vas deferens, have been observed in response to alterations in environmental cues such as light exposure (Stouffer *et al.*, 1995) [6] but the potential effects of blindness on the histology of these structures remain unclear. Thus the present study was planned to examine the morphohistological changes in the male genital duct system on experimentally induced blind rabbits. The findings of this study could have broader implications for understanding how sensory impairments affect reproductive physiology and may contribute to the development of interventions for managing reproductive disorders related to sensory deprivation.

Materials and Methods

The study was conducted at the Department of Veterinary Anatomy and Central Laboratory Animal House, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur (M.P.). The experimental work was performed under controlled environmental conditions, which were facilitated by

a centrally managed air handling unit ensuring the provision of 100% fresh air. Humidity levels were maintained within the range of 30% to 70%, and the temperature was controlled between 18 to 23 °C. The rabbits were housed in a dark room devoid of natural light, with only artificial lighting provided for a 12-hour light/dark cycle. The rabbits were provided *ad-lib* access to standard feed, fodder, and clean water throughout the study, ensuring proper nutrition as per standard protocol. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Nanaji Deshmukh Veterinary Science University, Jabalpur (No. 04/IAEC/Vety./2023, Dated 06/09/2023) and comply with CPCSEA guidelines (Registration No. 2071/GO/Re/S/19/CPCSEA, Dated: 08/07/2019). The experimental subjects comprised 16 male rabbits, aged between 1-1.5 years, reared in cages at the Central Laboratory Animal House. Of these, 12 rabbits were experimentally induced blind via tarsorrhaphy, while 4 male rabbits with normal visual function served as controls.

Procedure of tarsorrhaphy

Male rabbits (1-1.5 years old) were anesthetized with ketamine (40 mg/kg) and xylazine (5 mg/kg). The surgical site was cleaned and surrounding skin was sterilized. Under sterile conditions, the eyelids were retracted, and local anesthesia (lidocaine) was introduced. A continuous suture pattern with 3-0 silk sutures was used to close the eyelids, starting at the medial canthus. Post-surgery, rabbits were monitored and provided analgesics. Sutures were removed after 7-10 days.

Euthanasia and organ collection

At the end of the experimental period for each group, the rabbits were humanely euthanized using an overdose of ketamine hydrochloride (> 50 mg/kg BW). Rabbits were carefully dissected for the collection of organs.

Table 1. Experimental design

Groups	Day of Sample Collection	No. of animals
I (Control)	30 days	04
II	30 days after tarsorrhaphy	04
III	60 days after tarsorrhaphy	04
IV	90 days after tarsorrhaphy	04

Gross examination

Morphometric measurements of reproductive organs were conducted using a measuring scale, thread, and vernier caliper for the following parameters:

- **Vas deferens:** Length (mm)
- **Penis:** Length (mm)

Histological examination

Tissue samples from the reproductive organs were collected for histological examination. The samples were initially fixed in 10% neutral buffered formalin for 24-48 hours. Following fixation, the tissues were dehydrated using a graded series of alcohol and cleared in benzene. After dehydration, the tissues were embedded in paraffin wax. Paraffin blocks were sectioned into 5 µm thick sections using a rotary microtome. The sections were mounted on albuminized glass slides and stained with a standardized histological staining technique to evaluate general histomorphological features (Drury *et al.*, 1980) [7].

- **Epididymis:** Diameter and epithelial thickness (µm)
- **Vas deferens:** Diameter and epithelial thickness (µm)
- **Penis:** Epithelial thickness (µm)

Results and Discussion

The study examined the morphometry of the epididymis, vas deferens and penis in experimentally blind rabbits to determine the effects of photoperiod and blindness on these reproductive structures.

Epididymis: The epididymis of the rabbit (*Oryctolagus cuniculus*) was observed as a well-differentiated and elongated structure located dorsally along the testis. It was distinctly divided into three regions: caput (head), corpus (body), and cauda (tail), each demonstrating unique anatomical characteristics (Fig 01). The caput epididymis, positioned at the anterior pole of the testis, appeared broader and prominently defined. This region housed efferent ducts that converge into the initial portion of the epididymal duct, establishing the primary connection between the testis and the epididymis. The corpus epididymis extended along the dorsomedial margin of the testis and was comparatively narrower than the caput. It demonstrated a highly coiled ductal structure, maximizing its surface area within a confined space. This segment was firmly associated with the testis, running parallel to its longitudinal axis. The cauda epididymis, located at the posterior pole, was the largest and most bulbous segment, characterized by its thicker walls and dense connective tissue anchoring it to the testis (Donnelly *et al.*, 2020) [8]. It marked the transition of the epididymal duct into the vas deferens, facilitating sperm transport during ejaculation. The epididymal duct in all regions was surrounded by connective tissue and smooth muscle layers, which increased in thickness toward the cauda region. These layers likely support sperm

transport through peristaltic contractions. The gross measurement of the epididymis was not feasible due to its close anatomical attachment to the testes, which hindered precise separation and direct measurement. The histological examination of the epididymis revealed distinct structural changes across the different experimental groups. The epididymal duct was consistently lined by pseudostratified columnar epithelium, supported by a thin lamina propria, a layer of connective tissue beneath the epithelium. The histological observations of the present study were in the accordance to [Rakhmiyati et al. \(2019\)](#) [9]. In group I, the epididymal duct was filled with clumps of spermatozoa, and the lining epithelium appeared normal, exhibiting no significant histological abnormalities. The overall structure of the duct was intact, with healthy epithelial cells and minimal surrounding connective tissue (Fig 2A). In group II, significant histopathological changes were observed. The epididymal duct lumen lacked spermatozoa, and the epithelial layer showed severe derangement. The duct wall became thickened, and the lumen was filled with sloughed-off epithelial cells. Additionally, the epithelium appeared distorted and surrounded by an increased amount of connective tissue, indicating tissue damage and inflammation (Fig 2B). In groups III and IV, the lumen of the epididymal duct became enlarged and remained empty, similar to group II. However, the epithelial lining showed reduced degeneration compared to group II, with the epithelium almost returning to a near-normal condition. In both groups, there was an increase in the connective tissue present between the cross-sectional profiles of the epididymis,

suggesting ongoing repair or remodeling of the tissue (Fig 2C and 2D). The diameter of the epididymal duct varied significantly across the experimental groups. In group I, the mean diameter was $160.40 \pm 7.15 \mu\text{m}$, which was significantly larger than in group II, where the diameter was reduced to $85.41 \pm 2.99 \mu\text{m}$. In group III, the duct diameter increased significantly to $133.97 \pm 7.47 \mu\text{m}$, and further increased in group IV to $146.19 \pm 3.69 \mu\text{m}$, although this change was not statistically significant. The epithelial thickness also showed significant variation between groups. In group I, the mean thickness of the epithelium was $35.05 \pm 3.50 \mu\text{m}$, reflecting a healthy, normal epithelial structure. In group II, the epithelium became significantly thinner, with a mean thickness of $25.98 \pm 3.26 \mu\text{m}$, indicating epithelial damage and thinning. However, in groups III and IV, the epithelial thickness increased, with group III showing a significant recovery to $35.52 \pm 2.20 \mu\text{m}$, and group IV demonstrating a slight decrease to $32.19 \pm 2.20 \mu\text{m}$, which was still within a relatively normal range compared to group II (Table 03). Overall, these histological findings suggest that the epididymal duct in group II experienced significant degeneration as observed by [Kus et al. \(2003\)](#) [10], while groups III and IV showed varying degrees of recovery. The diameter of the duct and epithelial thickness closely correlated with the observed histopathological changes, providing insight into the structural integrity and functional recovery of the epididymis across the experimental groups.

Table 2. Length of vas deferens and penis (mm) in different groups

Parameters		Groups			
		I	II	III	IV
<i>Vas deferens</i>	Left	$107.75^a \pm 1.03$	$110.75^a \pm 0.75$	$113.75^a \pm 0.75$	$112.50^a \pm 1.50$
	Right	$104.00^a \pm 1.77$	$107.25^a \pm 0.85$	$107.00^a \pm 0.40$	$106.75^a \pm 2.13$
Penis		$8.87^a \pm 0.42$	$9.00^a \pm 0.57$	$9.62^a \pm 0.85$	$8.25^a \pm 0.85$

Mean value between the groups does not change significantly ($p < 0.05$)

Vas Deferens: The vas deferens originates from the cauda (tail) of the epididymis, which is the final part of the epididymal duct where sperm are stored and matured (Fig 01). and travels within the spermatic cord, along with blood vessels and nerves. The vas deferens passes through the inguinal canal and enters the pelvic cavity. It entered entering the pelvic cavity through inguinal canal and runs along the lateral wall of the bladder and then joining the duct of seminal vesicle to form ejaculatory duct ([Bozbiyik et al., 2022](#)) [11]. The length of the vas deferens varies, but in rabbits, it is typically about 10-12 cm long. It is well-formed and exhibits a thick, muscular wall, which is essential for the peristaltic movement of sperm during ejaculation. In group I, the left vas deferens had a length of 107.75 ± 1.03 mm, while the right measured 104.00 ± 1.77 mm, with the left being slightly longer than the right. In group II, the left and right vas deferens showed a slight increase in length i.e 110.75 ± 0.75 mm and 107.25 ± 0.85 mm respectively. Group III showed a further increase in both side vas deferens. In group IV, the length of both vas deferens showed decrease in length. However, it was non-significant (Table 02). Overall, no significant differences were found between the groups ($p > 0.05$), indicating that photoperiod or induced blindness had no notable effect on the length of the vas deferens in rabbits. Histologically the vas deferens was found to have a well-defined structure, with the mucosa being lined by pseudostratified columnar epithelium. The mucosal layer is supported by a loose connective tissue

sue known as the propria-submucosa, which is highly vascularized and rich in fibroblasts, providing the tissue with the necessary nutrients and support. The tunica muscularis, or muscle layer, consists of two distinct arrangements of smooth muscle: an inner concentric circular layer and an outer longitudinal layer. These muscle layers are responsible for the peristaltic movements that propel sperm through the vas deferens during ejaculation. The outermost layer, the tunica serosa, is composed of loosely arranged connective tissue fibers that help anchor the vas deferens to surrounding structures. The findings were in accordance with the study done by [Flickinger \(1975\)](#) [12]. The mean diameter of the vas deferens remained constant across all four groups. Group I and IV had a mean diameter of $270.10 \pm 8.39 \mu\text{m}$ and $273.69 \pm 9.04 \mu\text{m}$ (Fig 3A and 3D), while in group II and III slight increase in diameter was observed i.e. $278.33 \pm 4.05 \mu\text{m}$ and $280.34 \pm 6.62 \mu\text{m}$ respectively (Fig 3B and 3C). The mean thickness of the epithelium in the vas deferens showed minimal variation between the groups. In group I and III, the epithelial thickness was almost same (Table 03), while in group II and IV, it was slightly thinner i.e. $30.67 \pm 3.15 \mu\text{m}$ and $28.39 \pm 1.11 \mu\text{m}$ respectively. These results indicate that induced blindness did not significantly affect the gross and histological structure of the vas deferens in rabbits. The findings imply that other factors, apart from vision, might be responsible for maintaining the structural integrity of the vas deferens, further supporting the idea that blindness does not alter the function or histology of this reproductive organ in rabbits.

Table 3. Mean diameter and epithelium thickness (μm) of epididymal duct, vas deferens and penis in different groups

Parameters		Groups			
		I	II	III	IV
Epididymal duct	Diameter	160.40 ^a $\pm$ 7.15	85.41 ^b $\pm$ 2.99	133.97 ^a $\pm$ 7.47	146.19 ^a $\pm$ 3.69
	Epithelium	35.05 ^a $\pm$ 3.50	25.98 ^b $\pm$ 3.26	35.52 ^a $\pm$ 2.20	32.19 ^a $\pm$ 2.20
<i>Vas deferens</i>	Diameter	270.10 ^a $\pm$ 8.39	278.33 ^a $\pm$ 4.05	280.34 ^a $\pm$ 6.62	273.69 ^a $\pm$ 9.04
	Epithelium	32.02 ^a $\pm$ 3.00	30.67 ^a $\pm$ 3.15	32.29 ^a $\pm$ 2.73	28.39 ^a $\pm$ 1.11
Penis	Epithelium	36.59 ^a $\pm$ 4.37	34.90 ^a $\pm$ 4.30	36.56 ^a $\pm$ 2.85	35.59 ^a $\pm$ 2.88

Mean value between the groups (a and b) with different superscripts varied significantly ($p < 0.05$)

Penis: grossly there was an absence of glans in penis, the body of the penis was cylindrical and the diameter decreased at its end. It was comprised of three parts: the tip of the penis covered by the prepuce, the body and the base. The urethra, which extends from the urinary bladder to the glans penis, serves as a conduit for both urine and semen. It is divided into the prostatic and penile portions. The prostatic urethra, lined by transitional epithelium, transports both urine and semen, while the penile urethra, lined by stratified squamous epithelium, delivers semen during ejaculation. The mean length of the penis presented in Table 02. In group IV, the mean penis length was 8.25 ± 0.85 mm, which was lowest than all other groups. The slight variations in penis length observed in this study could be attributed to individual biological variation among rabbits, rather than any significant effects due to experimentally induced blindness. The penile urethra in rabbits demonstrated a well-organized histological structure, with consistent features observed across all experimental groups. It was surrounded by a thick layer of dense irregular connective tissue, which contained varying numbers of elastic fibers and smooth muscle cells. This connective tissue layer provided structural support and elasticity, crucial for the functional role of the urethra in both excretory and reproductive processes. The lining of the penile urethra consisted of pseudostratified columnar epithelium which was supported by the propria-submucosa (Fig 4). The propria-submucosa was composed of loose connective tissue enriched with numerous elastic fibers and smooth muscle cells, further enhancing the structural flexibility and functional integrity of the urethra. The findings of histology of penis were in accordance with Skonieczna *et al.* (2021) [13]. The thickness of the urethral epithelium showed minimal variation across the experimental groups. In group I, the mean epithelial thickness was recorded as 36.59 ± 4.37 μm . A slight decrease was noted in group II, where the thickness measured 34.90 ± 4.30 μm . In group III, the thickness returned to 36.56 ± 2.85 μm , and in group IV, it measured

35.59 ± 2.88 μm (Table 03). This study suggests that external factors like induced blindness has no observable impact on structure of penis.

Conclusion

The present study demonstrates that induced blindness and altered photoperiod exert differential effects on the male genital duct system of rabbits. Epididymis is sensitive to photoperiod-dependent neuroendocrine regulation, which may influence its structural and functional roles in sperm maturation, transport, and storage. The subsequent partial restoration of epididymal morphology indicates a degree of structural plasticity and adaptive response to prolonged alterations in photic input. In contrast, the vas deferens and penile structures are relatively less sensitive to photoperiodic disruption.

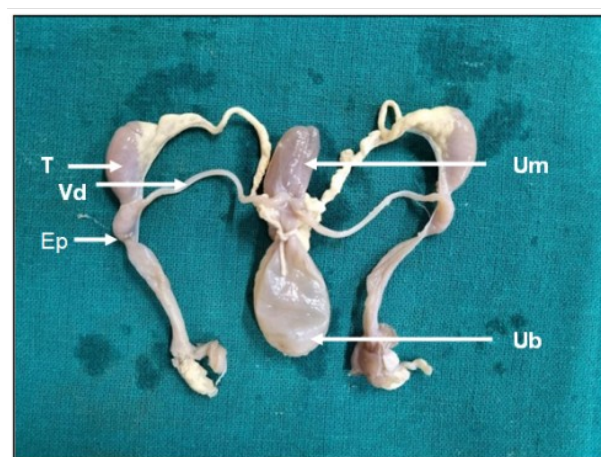
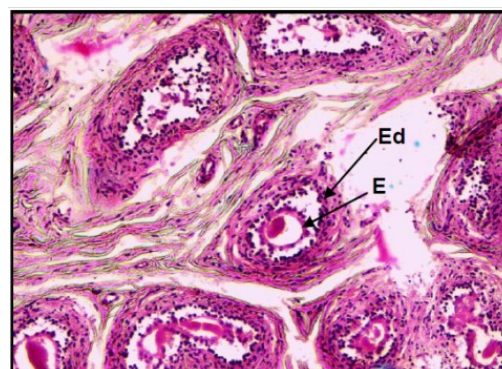


Figure 1. Male reproductive system of rabbit (group I) showing testis (T), epididymis (Ep), vas deferens (Vd), uterus musculus (Um), and urinary bladder (Ub)



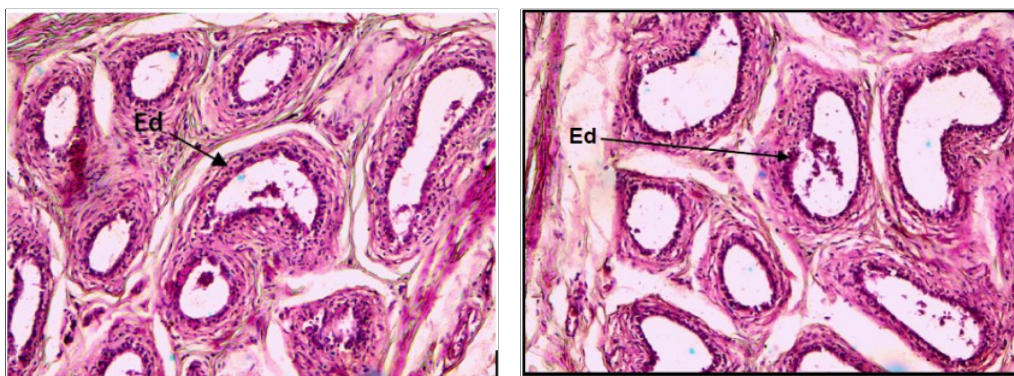


Figure 2. Photomicrograph of the epididymis (A) Group I, showing large epididymal ducts (Ed) filled with clumps of spermatozoa (S) (B) Group II, showing thickened wall and sloughed-off epithelium (E) of epididymal duct (Ed) (C) Group III, showing empty lumen of epididymal duct (D) Group IV, showing empty lumen of epididymal duct H&E X100.

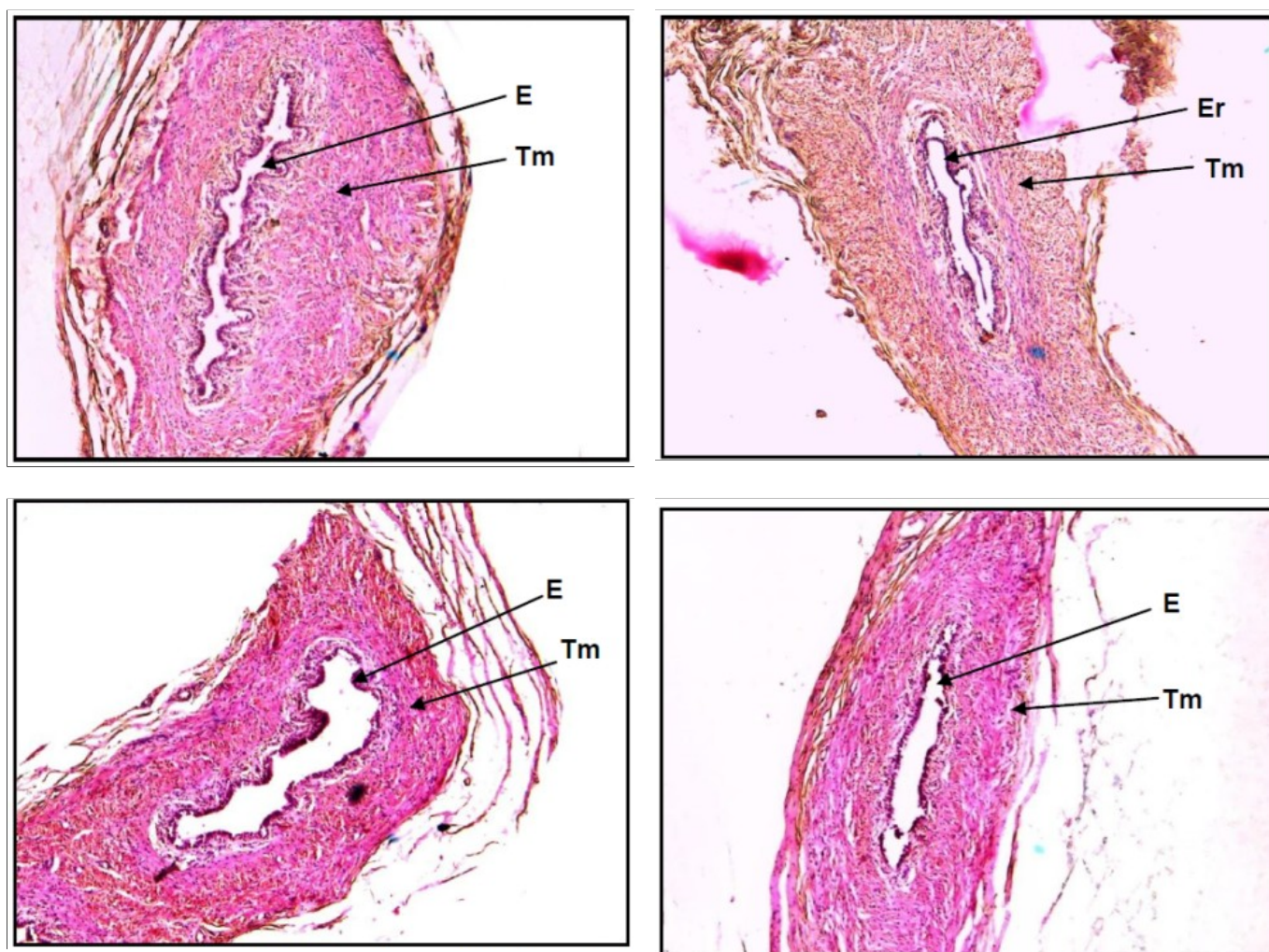


Figure 3. Photomicrograph of *Vas deferens* (A) Group I, showing epithelium (E) and tunica muscularis (Tm). (B) Group II, showing eroded epithelium (Er) and tunica muscularis (Tm). (C) Group III, showing intact epithelium (E) and tunica muscularis (Tm). (D) Group IV, showing intact epithelium (E) and tunica muscularis (Tm) H&E X50.



Figure 4. Photomicrograph of penis showing lumen (L), pseudostratified epithelium and tunica muscularis (Tm), H&E X100.

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