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# Visualization of Epididymides by Light and Scanning Electron Microscopy in Mali Pig of Tripura, India

## Visualización de Epidídimos Mediante Microscopía Óptica y Electrónica de Barrido en Cerdos Mali de Tripura, India

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**SUMMARY:** This study aimed to investigate the histological, histochemical and scanning electron microscopic characteristics of the developmental epididymides of Mali Pig of Tripura. The samples for this study were collected from the fifteen Mali pigs arranged in five different age groups. The collagen, reticular, elastic and nerve fibers were found in the epididymal capsule, basement membrane and the blood vessels for all three regions of the epididymides and it has been recorded on their developmental basis. Spermatozoa were recorded from some ducts of the corpus and caudal epididymides at three months of age. Histochemical studies were revealed for glycogen, acidic mucopolysaccharides, keratin and pre-keratin for all the age groups separately in their caput, corpus and caudal regions. The basement membrane of the tubules and stereocilia was recorded for glycogen and acidic mucopolysaccharides activity. The pre-keratin activity was recorded for the cytoplasm of the basal and principal cells in three months of aged animals. The scanning electron microscopic studies revealed the structural morphology of the epididymal ducts and provided detailed evidence of the development of spermatozoa in different regions of the epididymides.

**KEY WORDS: Epididymis; Histology; Histochemistry; Mali pig; Scanning electron microscopy.**

## INTRODUCTION

The pig population is dispersed throughout the Tripura state, located in India's Northeast region. According to the 20th Livestock Census, Tripura has 2.06 lakh pigs, of which about 50 percent are indigenous pigs (20th Livestock Census, 2019; Sarkar *et al*., 2024). Domestic pigs in this area are thought to share a common origin with the wild pig *Sus scrofa cristatus*. They are commonly referred to as "local pigs" or "desi pigs" and exhibit very little phenotypic variation among the many subgroups (Dandapat *et al*., 2010). Mali pigs are characteristically black with medium to small size, widely dispersed bristles, erected ears, a concave snout, a compact body with short legs, pot-bellied and just above the hoof, white markings can occasionally be observed. Epididymis is a cylindrical organ that is tightly connected to the testicles and divided into caput (head), corpus (body) and cauda (tail), respectively. Spermatogenesis originates from the seminiferous tubules of the testes, which discharge their contents through several straight tubes into the epididymis head, where the immature spermatozoa get their maturity. It also acts as a spermatozoa storage reservoir and discharge from the male reproductive system, controlling their maturation under androgenic regulation. It also helps in the re-absorption of testicular fluid and regulates the secretory activities, which is essential for the maturation of spermatozoa (Konig & Liebich, 2014). Postnatal anatomical investigations of the epididymides at various ages are necessary to understand the anatomical growth and development of the reproductive system. The epididymal postnatal development of Mali pigs is still unknown, and this study is the first to report age-related morphological changes in the epididymides of Mali pigs. The study aimed to illustrate the histological, histochemical and scanning electron microscopic features of the postnatal development of the epididymides. This study provides the morphological characteristics of the individual regions of the developing epididymides and also offers essential literature and baseline information for future research in developmental anatomy.

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## MATERIAL AND METHOD

**Animals.** For this study, 15 male Mali pigs were used. The animals were arranged in five different age groups viz. group-I (day-old piglets), group-II (3 months), group-III (4 months), group-IV (5 months) and group-V (6 months), with three animals in each group. The samples were collected from November 2023 to May 2024, after due approval from the Institutional Animal Ethical Committee (IAEC), College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram. Approval reference number. CVSC/CAU/IAEC/22-23/P-13 dated 31st October 2023.

**Samples.** The samples were collected immediately after surgical exposure of the scrotum (castration) with all aseptic precautions. After recording all the morphometrical characteristics, the epididymis was separated from the testis and cut into caput (head), corpus (body), and caudal (tail) regions. Then, biological samples were cleaned using normal clean water and carried to the department in an icebox for light and electron microscopic studies.

**Processing of the Samples for the Light Microscopy.** For the histological and histochemical studies, the tissue samples from all the regions of epididymides were cut into 3 to 5 mm thickness and fixed into the 10 % neutral buffer formalin solutions. The tissue processing was done by ascending grading of alcohol and sections were cut into 3 to 5 µm in size (Luna, 1968). The histological staining was done with Van Gieson's stain (VnGi) for collagen fibers (Luna,1968), Gomori's stain (Gomori) for reticular fibers (Bancroft & Gamble, 2008), Hartt's stain (Hartt) for elastic fibers (Luna, 1968), Berg's method (Berg) for spermatozoa (Luna, 1968), Bielschowsky's method (Bel) for nerve fibers (Luna, 1968) and the histochemical staining was carried out through periodic acid Schiff (PAS) for glycogen, PAS-Alcian blue (PAS-AB) for acidic mucopolysaccharides at pH 2.5 and Ayob-Shklar (Ayob) method for keratin and pre keratin (Luna, 1968).

**Observation and Photomicrography.** All sections were examined and imaged under a BX-51 Olympus Advance Trinocular Research Microscope equipped with DP software for computed image analysis.

**Processing of the Samples for the Scanning Electron Microscopy**. The tissue samples for scanning electron microscopy were cut into 1 to 2 mm sizes and fixed in 2.5 % glutaraldehyde solutions in phosphate buffer at pH 7.2 for 4 h at 4 ºC temperature. After fixation, the samples were placed in phosphate buffer solutions as per the standard procedure (Penchev, 2011; Choudhary & Priyanka, 2017). The samples were sent to the Sophisticated Analytical Instrumentation Facility (SAIF), North-Eastern Hill University (NEHU), Shillong, Meghalaya, for further processing and imaging under a scanning electron microscope, model no. SEM JEOL JSM 6360, manufactured by Japan Electron Optics Laboratory Company, Limited (Nihon Denshi Kogaku Kenkyujo), Japan.

## RESULTS

**Histological Observations.** The epididymal capsule was predominantly composed of collagen, reticular and elastic fibers for all age groups. In group-I, the thin capsule and trabeculae were mainly formed by procollagen fibers (Fig. 1a). The reticular fibers were observed in the basement membrane of the epididymal ducts. Very few elastic fibers were recorded in the trabeculae and the basement membrane, but there was no evidence of spermatozoa in the ducts of epididymides (Fig. 1c, d) in any regions. The nerve fibers located between the epididymal ducts were less noticeable (Fig. 1e).

In group-II, the epididymal capsule was recorded as thick and mainly formed by collagen fibers. The tunica albuginea of the 3-month epididymides exhibited some connective tissue septa, which formed a fine meshwork of collagen fibers (Fig. 1f). The blood vessels and the basement membrane of the epididymal ducts were noticed for reticular fibers (Fig. 2a). Some spermatozoa were observed in the epididymal duct of the corpus and caudal region and there was no spermatozoa were visualized for caput epididymides. The well-developed nerve fibers were noticed in between the ducts (Fig. 2d).

In group-III, the collagen fibers were located in the capsule as well as in the trabeculae of the epididymides (Fig. 2e). The well-formed elastic and reticular fibers were arranged around the basement membranes of all the regions of epididymal ducts and some reticular fibers also found in the connective tissue of the epididymides (Figs. 2f and 3a). It was observed that the number of blood vessels in the caput epididymis gradually decreased towards the caudal epididymis. Nerve fibers encircled the periphery, and a large number of spermatozoa were observed in the lumens of all three regions of the epididymal ducts (Fig. 3d). The concentration of spermatozoa was found to be higher in the corpus and caudal regions compared to the caput epididymides (Fig. 3b,c).

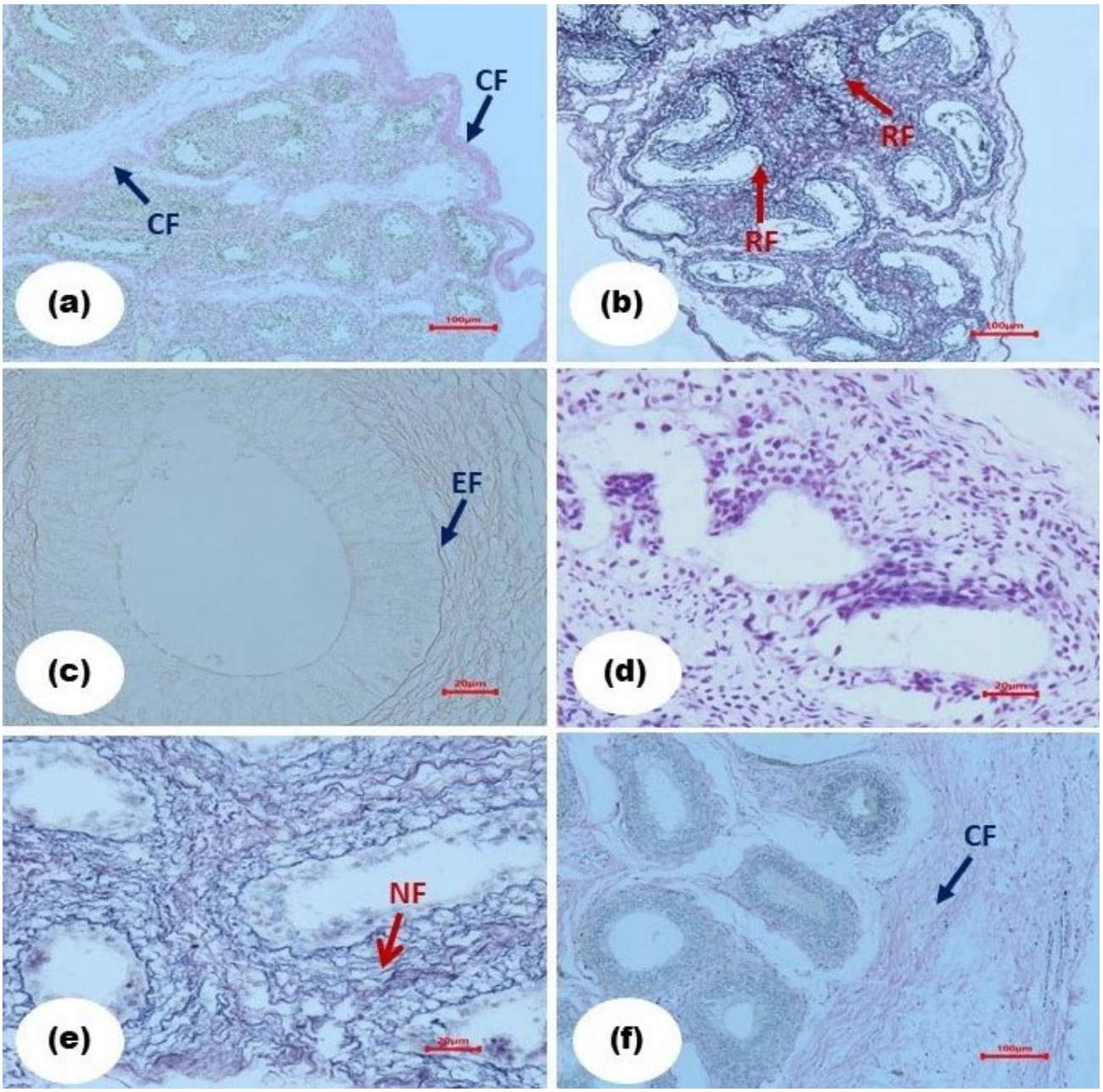


Fig. 1. Photomicrographs from group-I showing (a) collagen fibers (CF) in caput epididymis (VnGi, X100). (b) reticular fibers (RF) in corpus epididymis (Gomori, X100). (c) elastic fibers (EF) in cauda epididymis (Hartt, X400). (d) Berg's for spermatozoa in cauda epididymis (Berg, X400). (e) nerve fibers (NF) in caput epididymis (Bel, X400). From group-II showing (f) collagen fibers (CF) in caput epididymis (VnGi, X100).

In group-IV, the tunica submucosal connective tissue in between the epididymal ducts consisted of loose collagen fibers, few reticular and elastic fibers (Figs. 3e,f and 4a). The basement membranes showed fine reticular fibers, but the fibers were observed less in the capsule. Few elastic and nerve fibers were observed around the epididymal ducts. The bulk of spermatozoa were found in all regions of the epididymides from the 5 months of age (Figs. 4b and 5a).

In group V animals, collagen fibers were found in the thick epididymal capsule, trabeculae, and tubular stroma (Fig. 4d). The reticular and elastic fibers were noticed around the ducts of the epididymides (Fig. 4e,f).

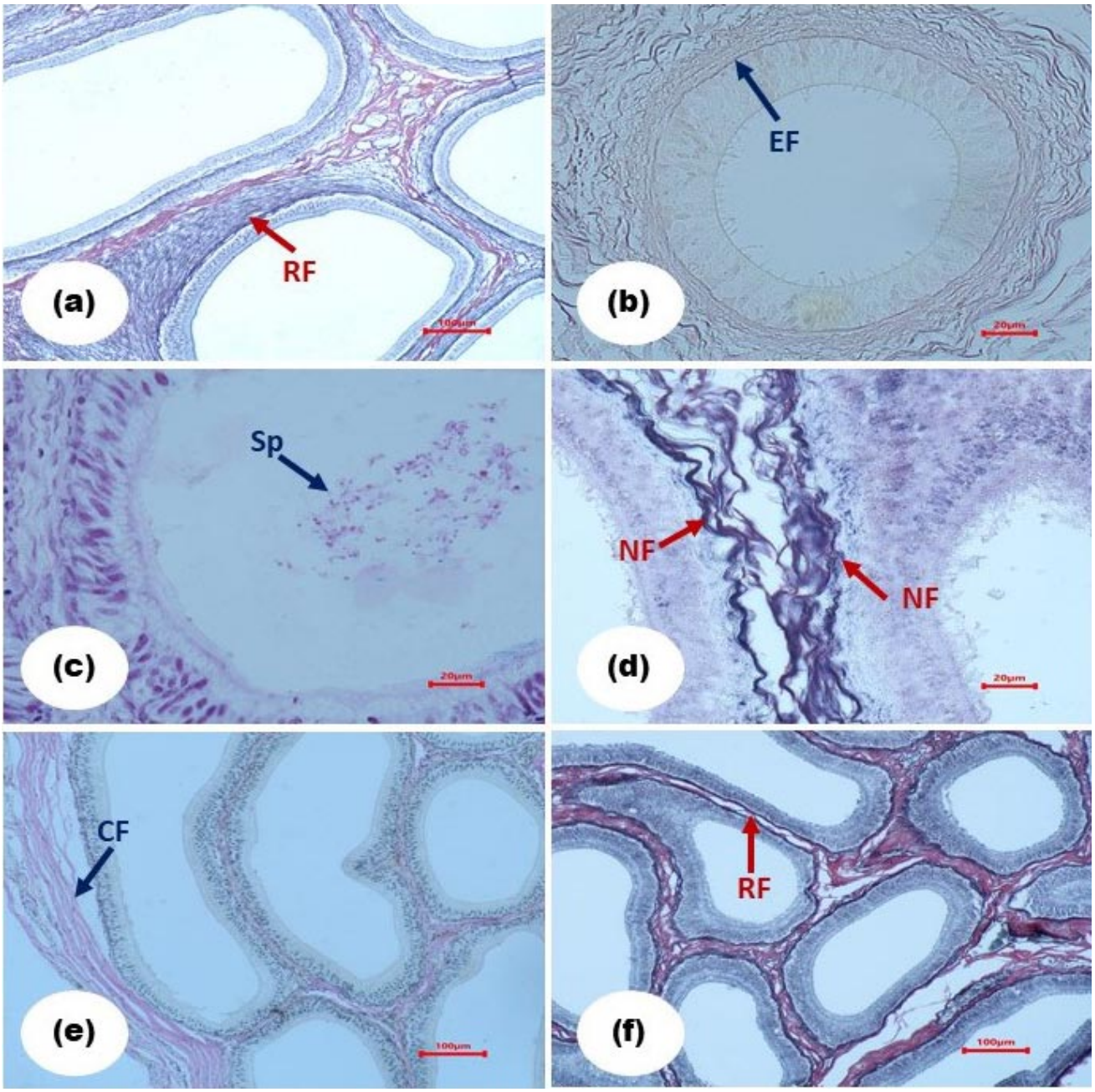


Fig. 2. Photomicrographs from group-II showing (a) reticular fibers (RF) in cauda epididymis (Gomori, X100). (b) elastic fibers (EF) in corpus epididymis (Hartt, X400). (c) Berg's for spermatozoa (Sp) in corpus epididymis (Berg, X400). (d) nerve fibers (NF) in corpus epididymis (Bel, X400). From group-III showing (e) collagen fibers (CF) in cauda epididymis (VnGi, X100). (f) reticular fibers (RF) in caput epididymis (Gomori, X100).

The fine nerve fibers were well arranged in the corpus and caudal regions compared to the caput of the epididymides (Fig. 5b,c).

**Histochemical Studies.** The distribution of glycogen particles, acidic mucopolysaccharides, and pre-keratin activity was recorded in the caput, corpus, and cauda epididymides during this study and the observations were tabulated in Table I.

In group-I, moderate reactivity for PAS was observed in the capsule, tubular stroma, and blood vessels. Weak reactivity for PAS-AB was recorded in the capsule and stroma. The cytoplasm of the undifferentiated cells

Table I. Distribution of histochemical characteristics in different regions of the epididymides of Mali pig of Tripura, India.

| Group | Histochemical | Caput | | | | Corpus | | | | Cauda | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CP | BM | TS | SC | CP | BM | TS | SC | CP | BM | TS | SC |
| Group I | Glycogen | + | + | + | - | + | + | + | - | + | + | + | - |
| | Acid mucopolysaccharides | + | + | + | - | + | + | + | - | + | + | + | - |
| | keratin and pre-keratin | - | - | +++ | - | - | + | +++ | - | - | - | ++ | - |
| Group II | Glycogen | + | +++ | + | +++ | + | +++ | + | +++ | + | +++ | + | ++ |
| | Acid mucopolysaccharides | + | +++ | + | +++ | + | +++ | + | +++ | + | +++ | + | +++ |
| | Keratin and pre-keratin | - | - | + | - | + | - | + | - | - | - | + | - |
| Group III | Glycogen | + | +++ | + | +++ | + | +++ | + | +++ | + | +++ | + | ++ |
| | Acid mucopolysaccharides | + | ++ | + | + | + | ++ | + | ++ | + | + | + | +++ |
| | keratin and pre-keratin | - | - | - | - | - | - | - | - | - | - | - | - |
| Group IV | Glycogen | + | +++ | + | +++ | + | +++ | + | +++ | + | ++ | + | ++ |
| | Acid mucopolysaccharides | + | ++ | + | + | + | + | + | ++ | + | ++ | + | ++ |
| | Keratin and pre-keratin | - | - | - | - | - | - | - | - | - | - | - | - |
| Group V | Glycogen | + | +++ | + | +++ | + | +++ | + | +++ | + | ++ | + | ++ |
| | Acid mucopolysaccharides | + | ++ | + | ++ | + | ++ | + | ++ | + | ++ | + | ++ |
| | Keratin and pre-keratin | - | - | - | - | - | - | - | - | - | - | - | - |

- Absent; + Weak; ++ Moderate; +++ Strong/Instance, CP- Capsule; BM- Basement membrane; TS-Intertubular stroma; SC- Stereocilia.

in the ducts of three regions of the epididymides showed early pre-keratin activity (Figs. 5d,e,f and 6a).

In group II, the epididymal basement membrane of the tubules showed long stereocilia starting from the 3 months of aged, and the blood vessels exhibited strong PAS reactivity (Fig. 6b). Strong PAS-AB activity was observed in the stereocilia and basement membrane of the ducts. Pre-keratin activity was detected in the basal and principal cells of the ducts (Fig. 6c,d).

In group III, the basement membrane, stereocilia, and blood vessels also exhibited strong PAS reactivity for glycogen (Fig. 6e). However, weak PAS-AB activity was observed in the basement membrane and stereocilia of the cauda epididymis. All the cells in the epididymal ducts tested negative for keratin and pre-keratin activity (Fig. 7a).

In group IV, strong PAS reactivity was observed in the basement membrane and moderate reactivity was recorded in the stereocilia. Moderate PAS-AB reactivity for acidic mucopolysaccharides was found in the basement membrane and stereocilia of the cauda (Fig. 7c).

In group V, the PAS reactivity was moderate for the basement membrane, stereocilia, and some intraepithelial glands (Fig. 7d). Strong PAS-AB reactivity was found in the basement membranes of the corpus epididymis, whereas the reactivity was recorded as moderate in the cauda epididymis (Fig. 7e). No keratin or pre-keratin activity was detected in any regions of the epididymides.

**Scanning electron microscopic studies**. In group I, only the epididymal capsule for the day-old piglets was viewed under scanning electron microscopy. No distinct epididymal ducts were observed in the epididymides. The epididymal stroma was recorded with numerous unarranged fibers running throughout the parenchyma (Fig. 8a,b,c).

In group II, the epididymal ducts were lined by pseudostratified columnar epithelium. The epithelium showed small vacuolated areas at the base and the terminal segments. At the site of the luminal surface in the ducts of caput epididymis, some circular bleb-like structures were observed placed at the terminal ends of the stereocilia. The ducts were no evidence for the spermatozoa in different segments of the epididymides under the scanning electron microscope (Figs. 8d and 9a,b).

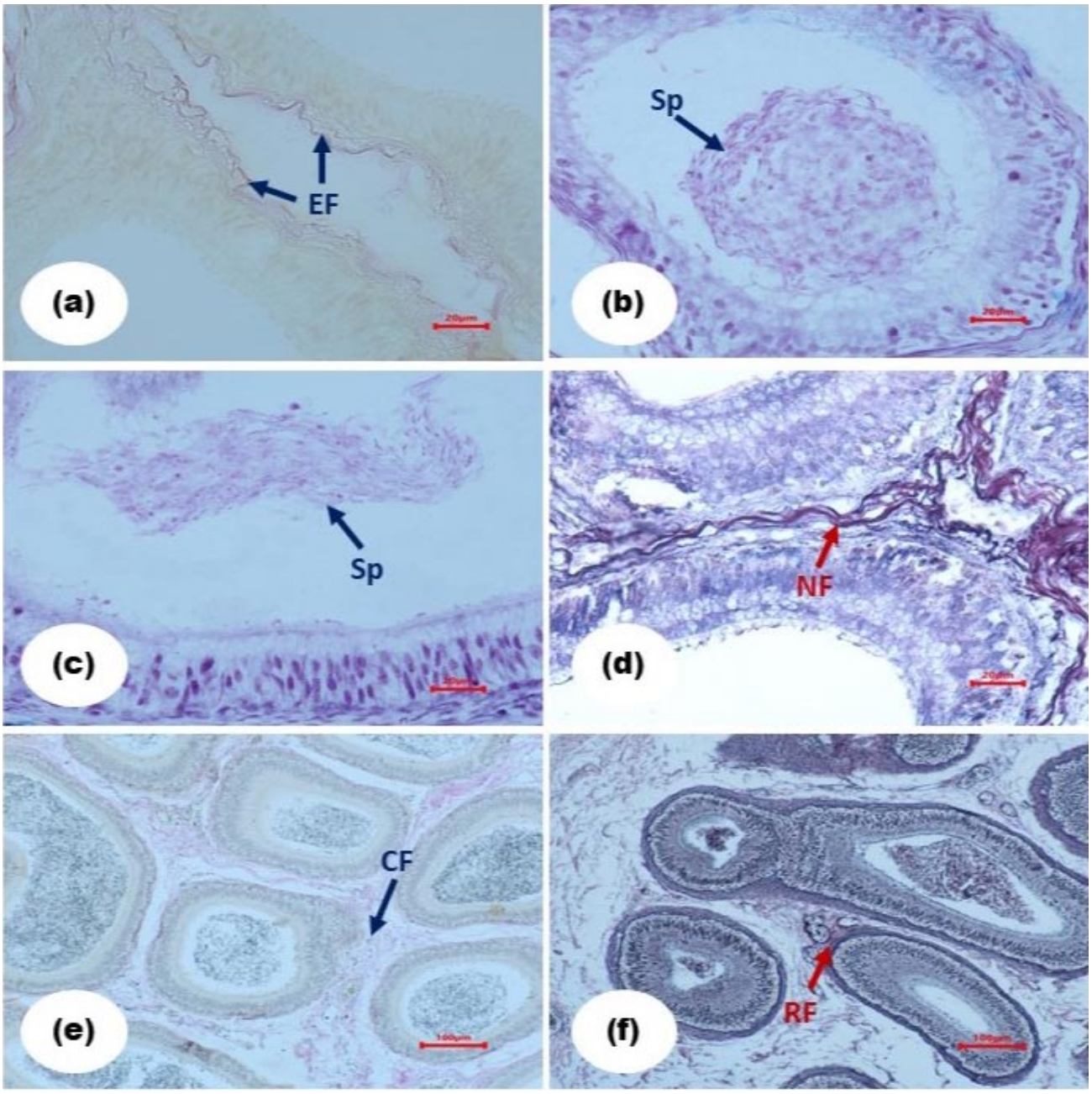


Fig. 3. Photomicrographs from group-III showing (a) elastic fibers (EF) in caput epididymis (Hartt, X400). (b) Berg's for spermatozoa (Sp) in caput epididymis (Berg, X400). (c) Berg's for spermatozoa (Sp) in corpus epididymis (Berg, X400). (d) nerve fibers (NF) in cauda epididymis (Bel, X400). From group-IV showing (e) collagen fibers (CF) in corpus epididymis (VnGi, X100). (f) reticular fibers (RF) in corpus epididymis (Gomori, X100).

In group III, the well-formed epididymal capsule and the ducts were observed under the scanning electron microscope. Some small vacuoles were also observed on the terminal side of the epithelium. The ducts were surrounded by smooth muscle fibers and the luminal space appeared wider compared to 3 months of age. The corpus epididymides showed few spermatozoa (Fig. 9c,d).

In group IV, the most comprehensive luminal space in the ducts was observed. The epididymal capsule was well-defined, and smooth muscle fibers were also observed in the caput and corpus regions of the epididymides (Fig. 10a,d). The ducts in the caput and corpus epididymides contained a large number of spermatozoa, which underwent a regressive loss of cytoplasmic droplets into the tails of the spermatozoa (Figs. 10b,c and 11a).

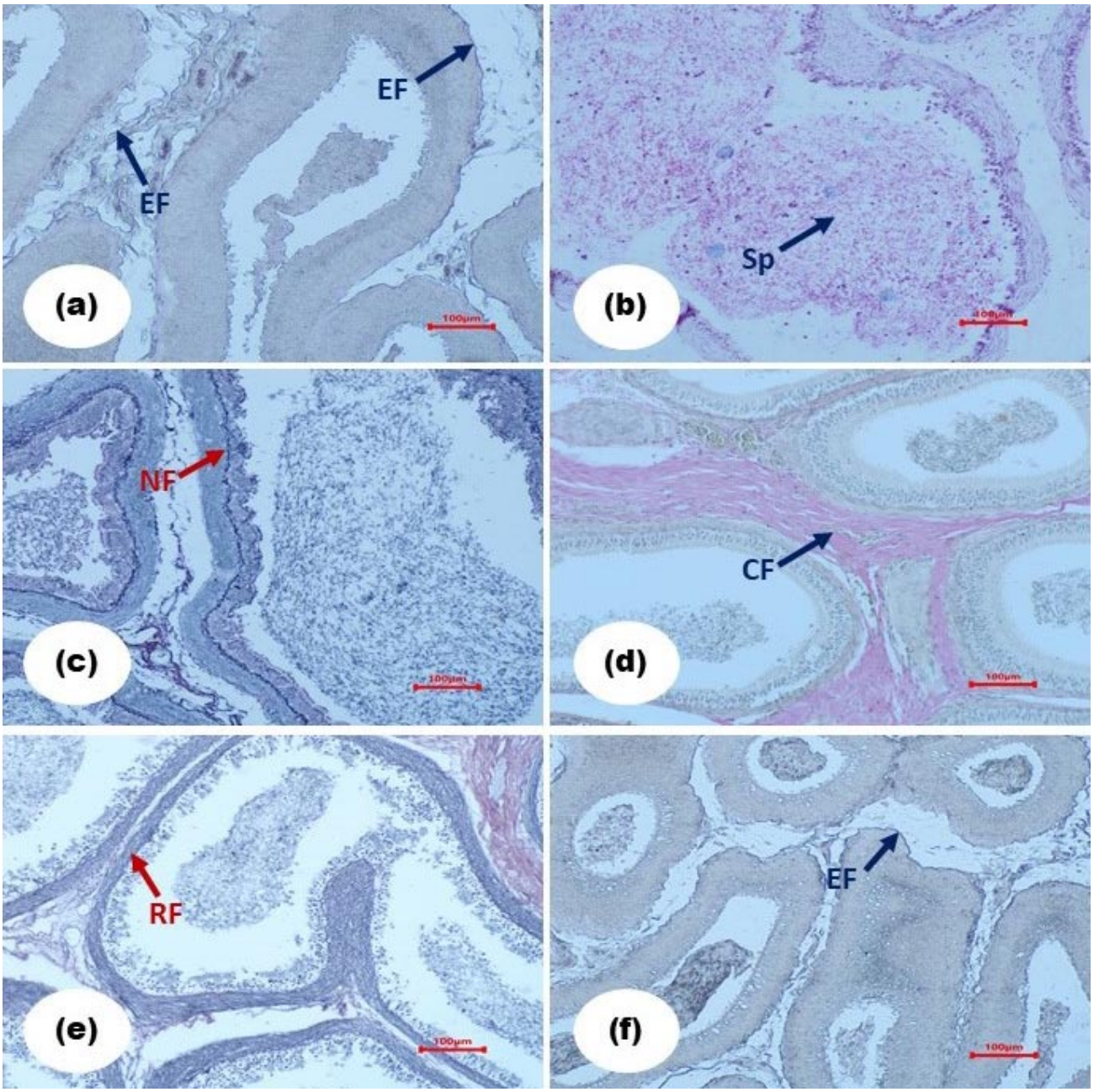


Fig. 4. Photomicrographs from group-IV showing (b) elastic fibers (EF) in caput epididymis (Hartt, X100). (b) Berg's for spermatozoa (Sp) in corpus epididymis (Berg, X100). (c) nerve fibers (NF) in cauda epididymis (Bel, X100).From group-V showing (d) collagen fibers (CF) in cauda epididymis (VnGi, X100). (e) reticular fibers (RF) in cauda epididymis (Gomori, X100). (f) elastic fibers (EF) in corpus epididymis (Hartt, X100).

In the group-V, under scanning electron microscopy, the epididymides of 6 months of age almost showed the same characteristics found for the 5 months of age. The luminal space of the ducts contained numerous spermatozoa in the caput and corpus regions (Figs. 11c and 12c). The cytoplasmic droplets containing spermatozoa have been recorded less in corpus epididymides as compared to the caput epididymides (Figs. 11d and 12a,b,d).

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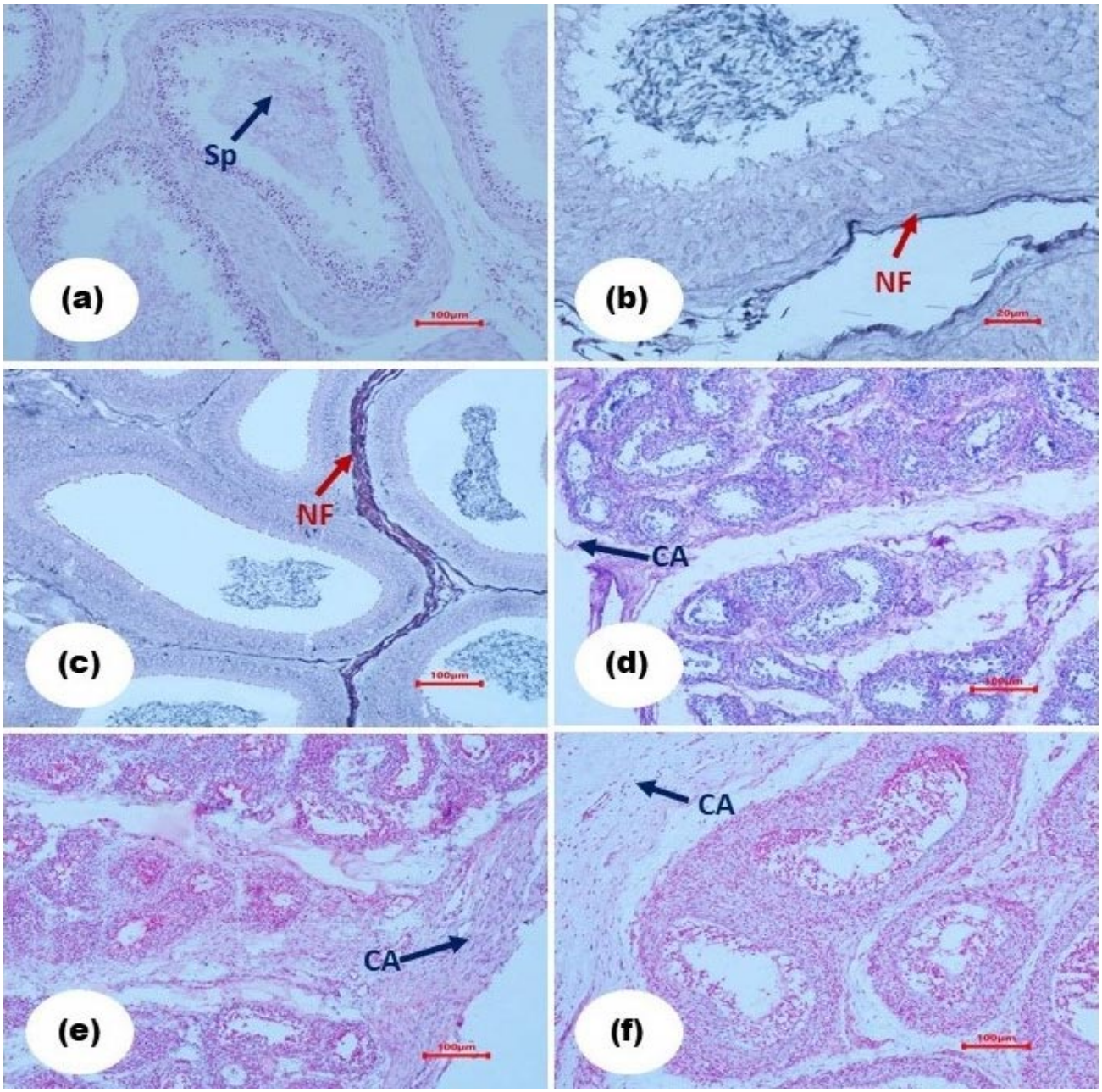


Fig. 5. Photomicrographs from group-V showing (a) Berg's for spermatozoa (Sp) in cauda epididymis (Berg, X100). (b) nerve fibers (NF) in corpus epididymis (Bel, X400) (c) nerve fibers (NF) in cauda epididymis (Bel, X100). From group-I showing (d) capsule (Cp) in caput epididymis (PAS, X100). (e) capsule (Cp) in caput epididymis (PAS-AB, X100).(f) capsule (Cp) in cauda epididymis (PAS-AB, X100).

## DISCUSSION

In group-I, the epididymal capsule and trabeculae were predominantly observed as procollagen fibers. Reticular fibers formed the basement membrane of the developing epididymal ducts. These current findings were also closely similar to the findings of Sarma & Devi (2015), in Assam goats and Kumari (2016), in black Bengal kids. In the present study from group-II the epididymal capsule was observed to be thick and the blood vessels were found more often, as also reported by Sarma *et al*. (2012b), in goat. The capsule and basement membrane of the ducts were observed with collagen and reticular fibers. Elastic fibers were also present in the blood vessels along with the basement membrane of

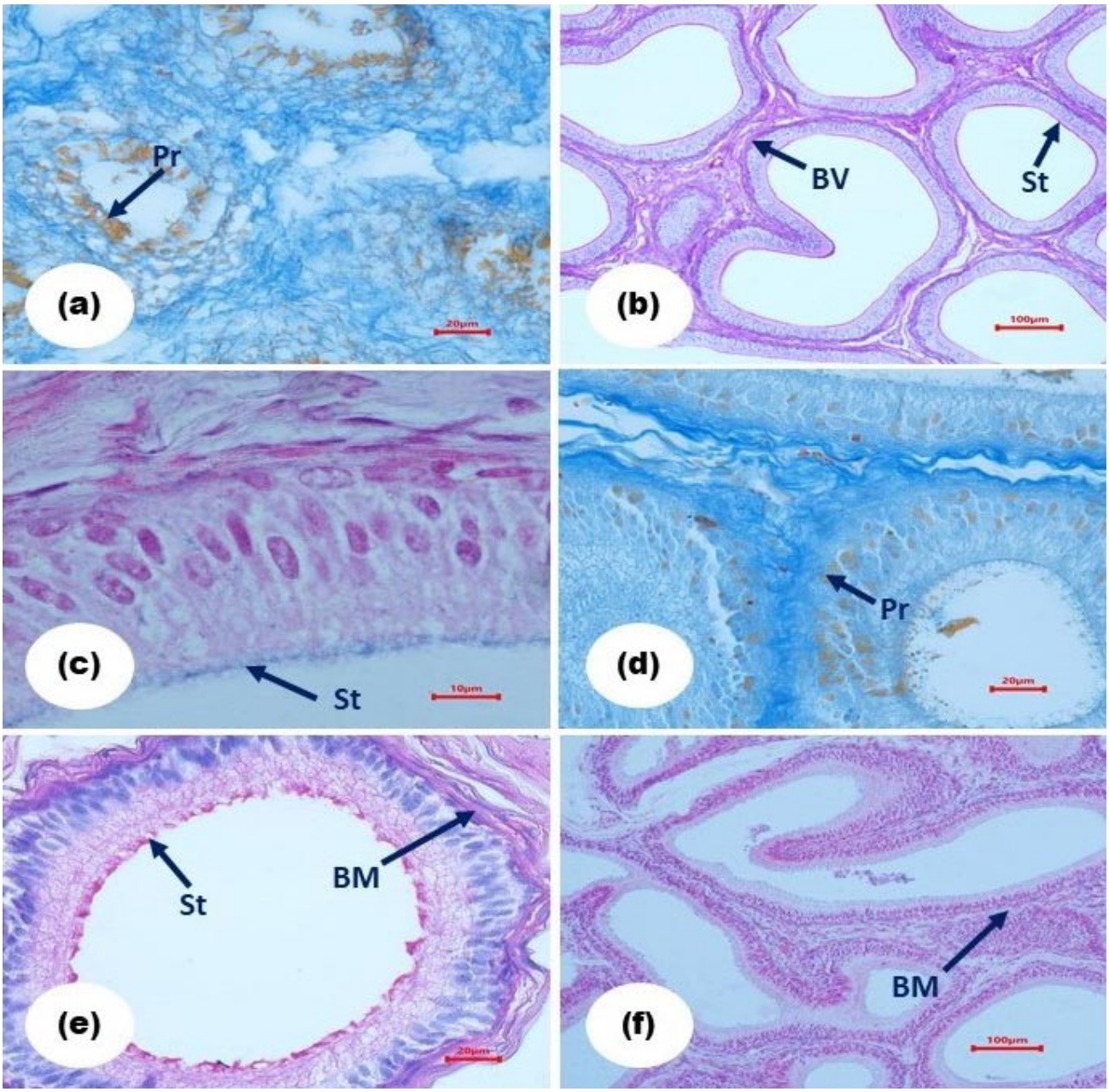


Fig. 6. Photomicrographs from group-I showing (a) pre-keratin (Pr) activity in caput epididymis (Ayob, X400). From group-II showing (b) basement membrane (BM) and stereocilia (St) in cauda epididymis (PAS, X100). (c) stereocilia (St) in cauda epididymis (PAS-AB, X1000).(d) pre-keratin (Pr) activity in corpus epididymis (Ayob, X400). From group-III showing (e) basement membrane (BM) and stereocilia (St) in corpus epididymis (PAS, X400). (c) basement membrane (BM) in cauda epididymis (PAS-AB, X100).

the ducts, whereas nerve fibers were noticed between the ducts. The present findings were closely agreed with the findings of Sarma *et al*. (2012b), in goat and Kaur (2012), in buffalo epididymis. The well-formed nerve fibers, also mentioned by Mohamed (2005), in bovine epididymis, help constrict the epididymal tubules. In this study, a few spermatozoa were observed in some of the caudal epididymal ducts of 3 months of aged, while Sarma (2009) reported observing spermatozoa in the tubular lumen from 6-month-old goats.

In this study, collagen fibers were noticed in the capsule and trabeculae of group III. The well-defined elastic and reticular fibers were placed around the basement membrane and connective tissue in all regions of epididymides. These findings were closely agreed with the

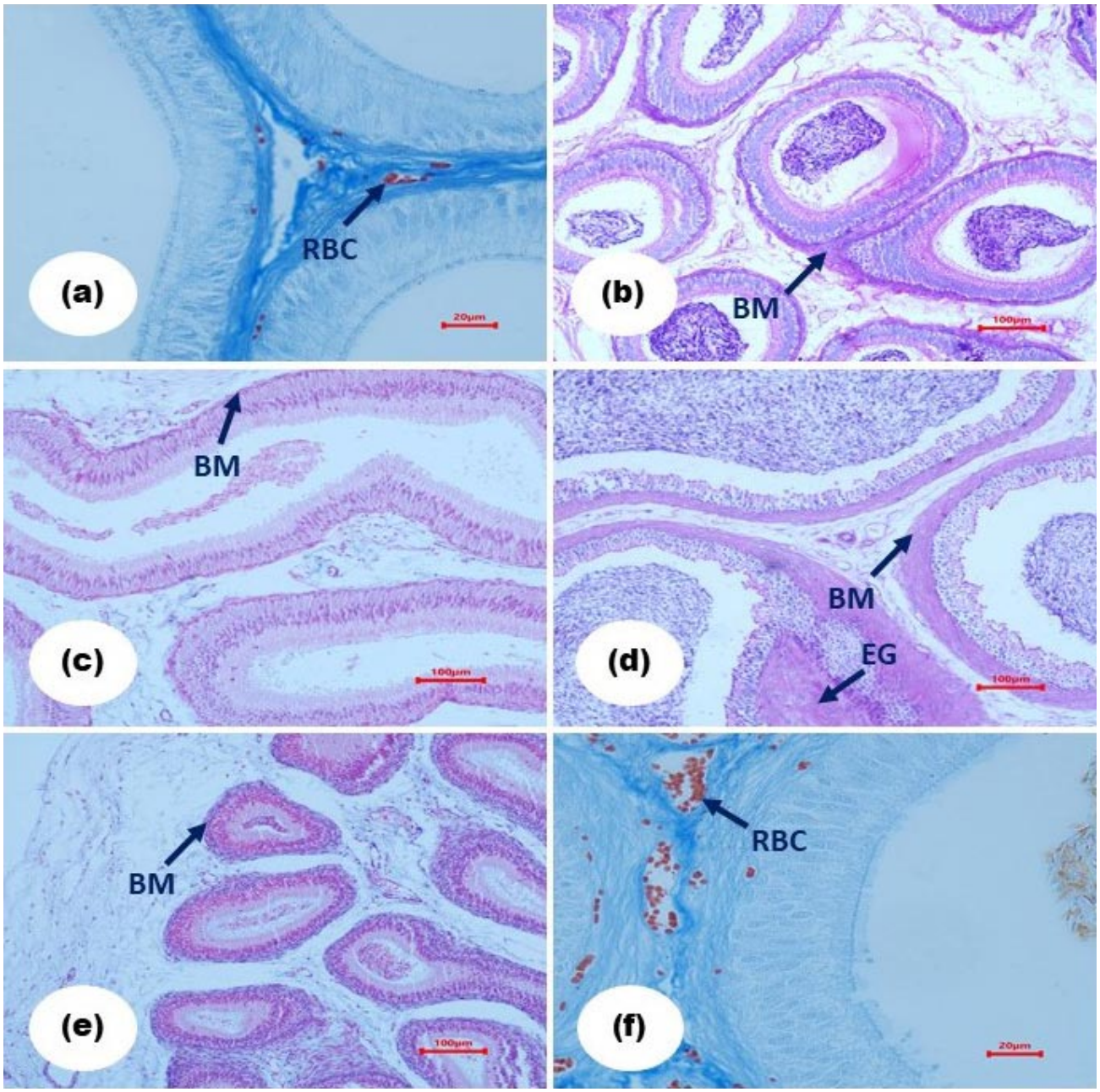


Fig. 7. Photomicrographs from group-III showing (a) erythrocytes (RBC) and negative for pre-keratin (Pr) activity in cauda epididymis (Ayob, X400). From group-IV showing (b) basement membrane (BM) in corpus epididymis (PAS, X100). (c) basement membrane (BM) cauda epididymis (PAS-AB, X100). From group-V showing (d) basement membrane (BM) and intraepithelial gland (EG) in cauda epididymis (PAS, X100). (e) basement membrane (BM) corpus epididymis (PAS-AB, X100). (f) erythrocytes (RBC) and negative for pre-keratin (Pr) activity in cauda epididymis (Ayob, X400).

reports of Zayed *et al*. (2012), in camel epididymis. The bulk of spermatozoa were observed in the luminal space of the corpus and caput epididymides. The concentration of the spermatozoa in corpus and caudal epididymis was also observed by Kaur (2012) in buffalo, Mohamed (2005), in bovine and Alkafafy *et al*. (2011), in camel epididymis. In the current study, the stromal connective tissue of group-IV animals was viewed for loose collagen fibers, few reticular and elastic fibers. This present finding closely agreed with the findings of Sikarwar (2018) in pig, Singh *et al*. (2019), in pig and Schimming *et al*. (2001), in dog epididymis. Numerous spermatozoa were found in the lumen of all three

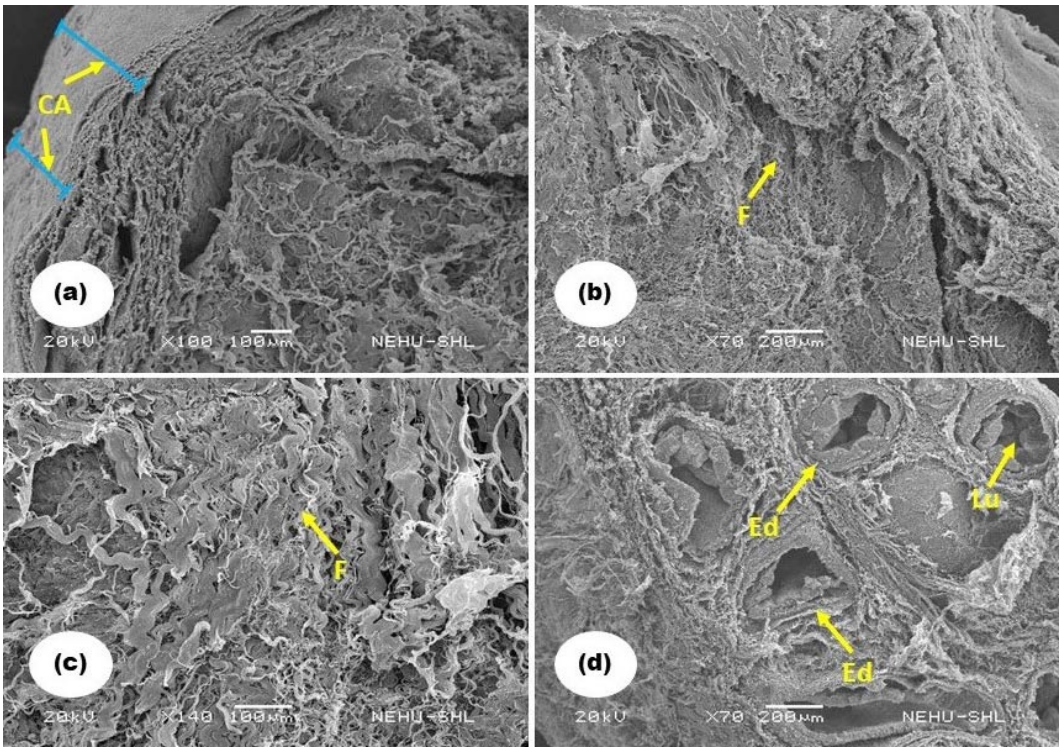


Fig. 8. Scanning electron micrographs from group-I showing (a) capsule (CA) in caput epididymis. (b) fibrous contains (F) in caput epididymis. (c) fibrous contains (F) in caput epididymis. From group-II shows (d) epididymal ducts (Ed) and lumen (Lu) in caput epididymis.

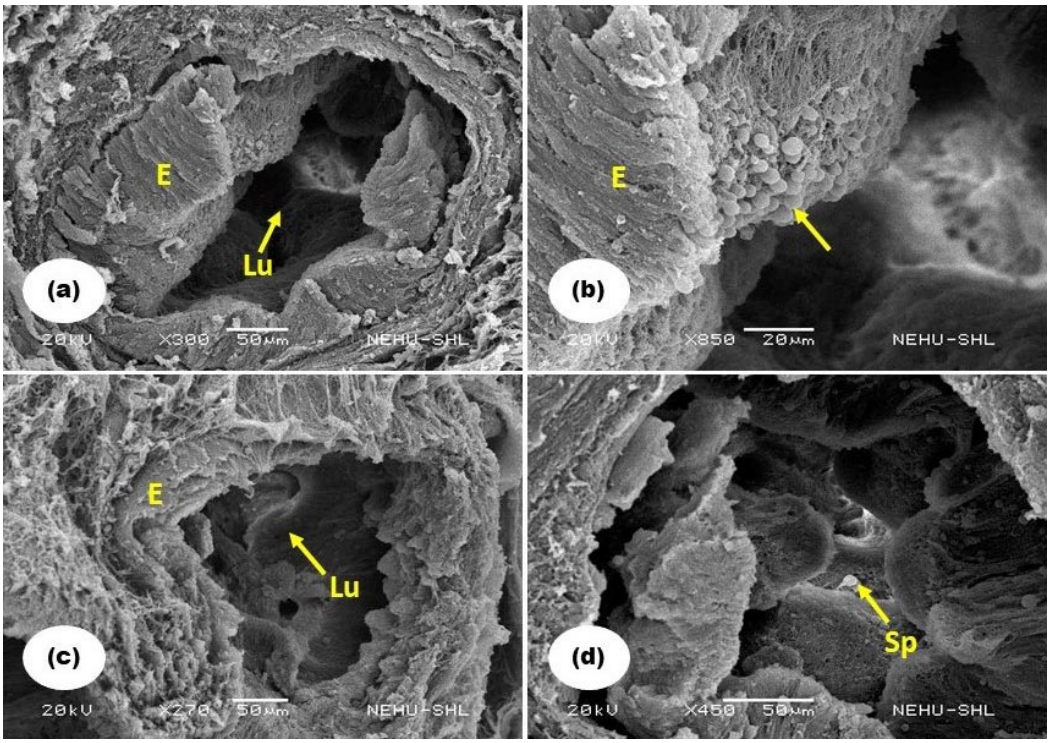


Fig. 9. Scanning electron micrographs from group-II showing (a) epithelium (E) and lumen (L) in the duct of caput epididymis. (b) epithelium (E) and round bleb (arrow) in the duct of caput epididymis. From group-III shows (c) epithelium (E) and lumen (Lu) in the duct of caput epididymis.(d) spermatozoa (Sp) in the duct of caput epididymis.

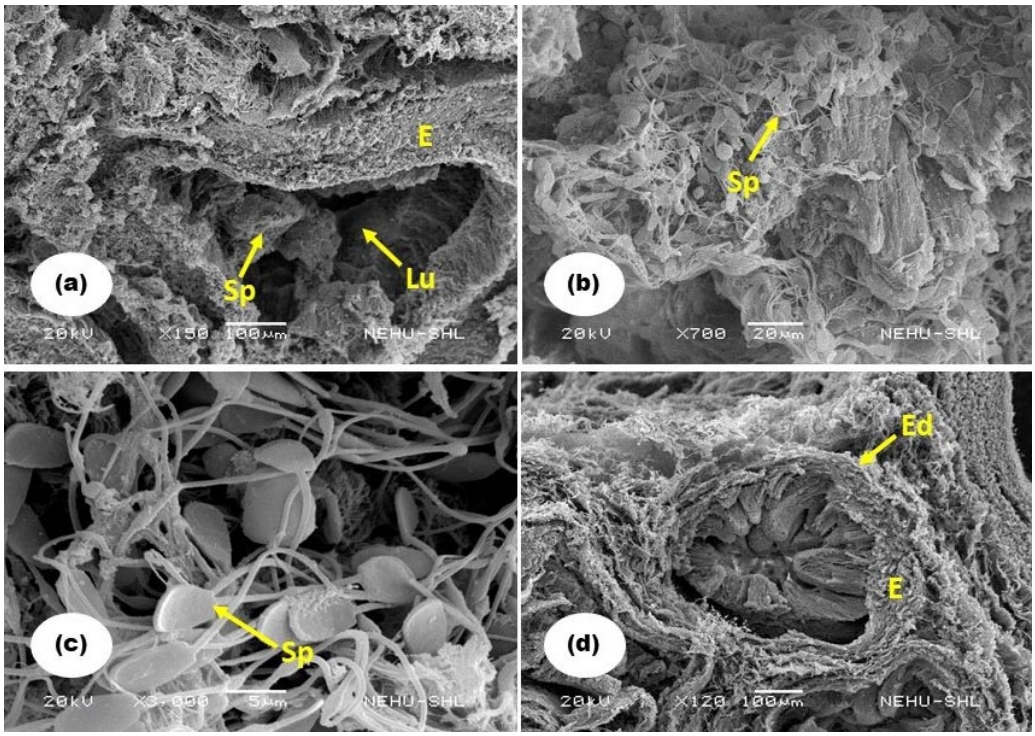


Fig. 10. Scanning electron micrographs from group-IV showing (a) epithelium (E) and lumen (Lu) contained spermatozoa (Sp) in the duct of caput epididymis. (b) lumen contained spermatozoa (Sp) in the duct of caput epididymis. (c) Spermatozoa have cytoplasmic droplets (Cd) in the duct of the caput epididymis. (d) epididymal duct (Ed) and epithelium (E) contained spermatozoa in the duct of corpus epididymis.

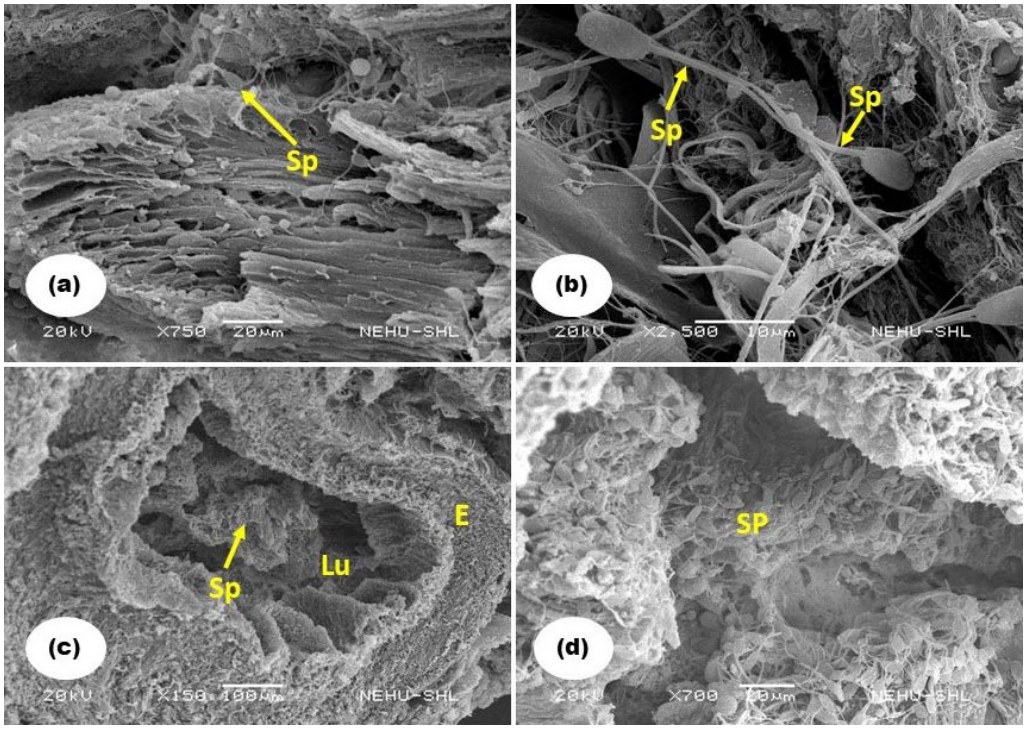


Fig. 11. Scanning electron micrographs from group-IV showing (a) luminal epithelium contained spermatozoa (Sp) in the duct of corpus epididymis. (b) lumen contained spermatozoa (Sp) in the duct of corpus epididymis. From group-V showing (c) epithelium (E) and lumen (Lu) contained spermatozoa (Sp) in the duct of caput epididymis. (d) lumen (Lu) contained bulk of spermatozoa (Sp) in the duct of caput epididymis.

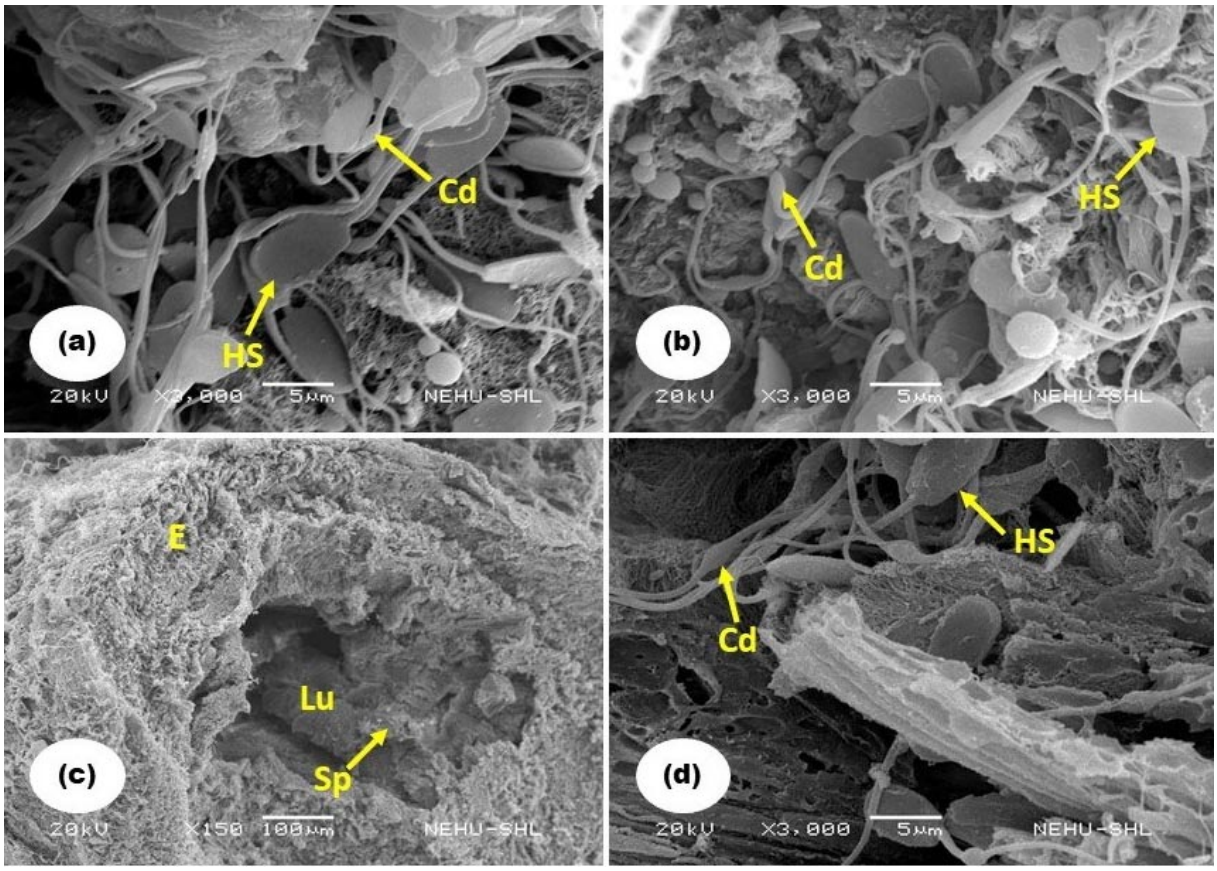


Fig. 12. Scanning electron micrographs from group-V showing (a) spermatozoa contained head (HS) and cytoplasmic droplets (Cd) in the duct of caput epididymis. (b) Spermatozoa contained head (HS) and cytoplasmic droplets (Cd) in the duct of caput epididymis. (c) epithelium (E) and lumen (Lu) contained spermatozoa (Sp) in the duct of corpus epididymis. (d) luminal space showing spermatozoa contained head (HS) and cytoplasmic droplets (Cd) in the duct of corpus epididymis.

regions of the epididymides. In the present study, the histological findings of group-V animals were also most similar to those of group-IV animals; the collagen fibers were recorded in the capsule, trabeculae, and tubular stroma of the epididymides. These findings closely agreed with the findings of Sikarwar (2018), in pig and Singh *et al*. (2019), in pig epididymis. The spermatozoa observed in all three regions of the epididymides are also closely related to the findings of Singh *et al*. (2019), in pigs and Khan *et al*. (2019), in the epididymis of Teddy goats

In day-old (group-I) animals the blood vessels, tubular stroma and capsule of caput epididymides showed moderate reactivity for glycogen. These current findings were also closely agreed with the findings of Sarma *et al*. (2012a), in Assam goats, Kumari (2016), in black Bengal kids and Mohamed (2005), in bovine foetus and Sharma (2010) in the epididymis of buffalo foetus. In this study, the cytoplasm showed instances of pre-keratin activity for the undifferentiated cells, and the PAS-AB was recorded for weak in the capsule and stroma in caput and corpus epididymides. However, Kumari (2016), found that black Bengal kids exhibit mild reactivity for PAS Alcian blue in prepubertal animals. In this study, at 3 months (group-II) of aged the basement membrane of the tubules, long stereocilia and blood vessels showed instance reactivity for PAS as also reported by Uppal *et al*. (2009), in guinea pig and Parul & Rajesh (2019), in horse epididymis. However, Sikarwar (2018) observed weak PAS positive reactivity in pig epididymis. The basement membrane and stereocilia were also recorded for strong PAS-AB activity. The stereocilia may provide an acidic environment for the spermatozoa, helping to maintain their motility during storage. The long stereocilia reabsorbs approximately 90 % of the testicular fluid as the spermatozoa starts to motile. The cytoplasm of the basal and principal cells showed orange pre-keratin activity in corpus epididymides.

In this study, the basement membranes, stereocilia and blood vessels in caput and corpus epididymides were also observed, for instance, PAS reactivity in 4 months (group-III) of aged. PAS-AB activity was noticed in the

basement membrane and long stereocilia. These current findings were also closely similar to the findings of Zayed *et al*. (2012) in camel and Singh *et al*. (2019) in pig epididymis. From the 4 months of age, no epididymal ducts were recorded for keratin and pre-keratin activity as Sikarwar (2018) reported in pig epididymis. In this present investigation, the histochemical studies for 5 months (group-IV) and the 6 months (group-V) of aged were recorded almost same. The moderate to strong reactivity for PAS was recorded to the basement membrane, stereocilia and intraepithelial glands as also reported by Zayed *et al*. (2012), in camel and Sarma *et al*. (2012a), in Assam goats. In the present study, the PAS-AB reactivity was recorded intense to the corpus epididymides for both the age groups, whereas the activity was moderate to the cauda epididymides. These present findings were also closely agreed to the findings of Archana *et al*. (2009), in Gaddi goats.

In the present study, only the epididymal capsule was viewed as group-I animals under scanning electron microscopy. No distinct parenchyma and epididymal tubules were for the day-old piglets, as reported by Sarma & Devi (2015) in their light microscopic studies for Assam goats. In the current study, the epididymal ducts were observed from the 3 months (group-II) of aged and it was lined by pseudostratified columnar epithelium. These present findings were closely similar to the findings of Sikarwar (2018) in pig epididymis. Some bleb-like round structures were also observed in the terminal ends of the stereocilia as also reported by Zayed *et al*. (2012) in camel. Under the scanning electron microscopy there was no evidence for the spermatozoa in the epididymides for 3 months of aged. In the present investigation, well-formed epididymal ducts and capsule were viewed from the 4 months (group-III) of aged. Some small vacuolated place was observed in the terminal parts of the epithelium. These present findings were closely agreed to the findings of Karmore *et al*. (2015) in goat epididymis. The lumen of the corpus epididymides also recorded few spermatozoa. In the current study, the widest luminal space of the ducts was recorded from the 5 months (group-IV) of age. The caput and corpus epididymides ducts showed numerous spermatozoa having cytoplasmic droplets. These present investigations closely agree with the findings of Sikarwar (2018), on pig epididymis. In the present study, the characteristics features under the scanning electron microscope were almost the same for the age of 5 months (group-IV) and 6 months (group-V). The spermatozoa contained cytoplasmic droplets have been recorded less in corpus epididymides at 6 months of age as also closely similar to the findings of Singh (2018) in pig epididymis. The data presented in this study can be the foundation for future research on the reproductive systems of domestic and wild animals in the era of artificial intelligence (Choudhary *et al*., 2023, 2024).

## CONCLUSION

The present study revealed the histological, histochemical and scanning electron microscopic features of the epididymides during postnatal development in Mali pigs of Tripura. The histological findings were recorded age-wise in the various segments of the epididymides, and spermatozoa were seen in the ducts of the corpus and caudal regions at 3 months of age. The epididymal capsule, ducts and stereocilia were examined for histochemical reactivity at varying intensities. The epithelium and the luminal space of the ducts provided detailed evidence of developing spermatozoa with cytoplasmic droplets in different regions of the epididymides under scanning electron microscopic studies. These studies provided the morphological characteristics of the individual regions of the developing epididymides. It also incorporated important literature and provided essential baseline information for future scientific research.

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**SARKAR, R.; KALITA, P.C.; KALITA, A.; DOLEY, P.J. & CHOUDHARY, O.P.** Visualización de epidídimos mediante microscopía óptica y electrónica de barrido en cerdos Mali de Tripura, India. *Int. J. Morphol., 43(2)*:702-716, 2025.

**RESUMEN:** Este estudio tuvo como objetivo investigar las características histológicas, histoquímicas y de microscopía electrónica de barrido de los epidídimos en desarrollo de cerdos Mali de Tripura. Las muestras para el estudio se recolectaron de quince cerdos Mali organizados en cinco grupos de edades diferentes. Las fibras de colágeno, reticulares, elásticas y nerviosas se encontraron en la cápsula epididimaria, la membrana basal y los vasos sanguíneos de las tres regiones de los epidídimos y se registraron en función de su desarrollo. Se observaron espermatozoides de algunos conductos del cuerpo y de los epidídimos caudales a los tres meses de edad. Los estudios histoquímicos revelaron la presencia de glucógeno, mucopolisacáridos ácidos, queratina y prequeratina para todos los grupos de edad por separado en sus regiones de cabeza, cuerpo y cauda. Se registró la actividad de glucógeno y mucopolisacáridos ácidos en la membrana basal de los túbulos y estereocilios. Se observó actividad de prequeratina para el citoplasma de las células basales y principales en animales de tres meses de edad. Los estudios de microscopía electrónica de barrido revelaron la morfología estructural de los conductos epididimarios y proporcionaron evidencia detallada del desarrollo de los espermatozoides en diferentes regiones de los epidídimos.

**PALABRAS CLAVE: Epidídimo; Histología; Histoquímica; Cerdo Mali; Microscopía electrónica de barrido.**

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