ARTICLE IN PRESS

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
xx (xx): xx-xx, 2024
DOI: 10.9775/kvfd.2024.32597

Research Article

Assessment of Age-Related Morphological Changes in the Testes of Mali Pig of Tripura, India

Rupan SARKAR [1] Pranab Chandra KALITA [1] Arup KALITA [1] Probal Joyti DOLEY [1]
Om Prakash CHOUDHARY [2 (*)]

[1] Central Agricultural University, College of Veterinary Sciences and Animal Husbandry, Department of Veterinary Anatomy and Histology, Selesih, Aizawl-796015, Mizoram, INDIA
[2] Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), College of Veterinary Science, Department of Veterinary Anatomy, Rampura Phul, Bathinda-151103, Punjab, INDIA

(*) **Corresponding authors:**
Om Prakash CHOUDHARY
Phone: +91 9928099090
E-mail: om.choudhary@gadvasu.in

How to cite this article?
Sarkar R, Kalita PC, Kalita A, Doley PJ, Choudhary OP: Assessment of age-related morphological changes in the testes of Mali pig of Tripura, India. *Kafkas Univ Vet Fak Derg,* 2024 (Article in Press).
DOI: 10.9775/kvfd.2024.32597

Article ID: KVFD-2024-32597
Received: 03.07.2024
Accepted: 22.09.2024
Published Online: 03.10.2024

Abstract

This study aimed to investigate the histological, histochemical and electron microscopic features of the testes of Mali pigs of Tripura. The samples were collected from fifteen Mali pigs in five different age groups. Collagen, reticular, elastic, and nerve fibers were observed in the tunica albuginea, seminiferous tubules, germinal epithelium, mediastinum testis and blood vessels across all age groups. Spermatids were found in the seminiferous tubules at three months of age. Histochemical studies revealed glycogen, acidic mucopolysaccharides, keratin and pre-keratin activity in various testicular structures, with staining affinities differing among age groups. Scanning electron microscopy showed the structural morphology of the seminiferous tubules, interstitial tissues, and spermatozoa at different stages of development. The parenchyma of day-old piglets exhibited numerous small round seminiferous or sex cords. Well-defined seminiferous tubules were observed at three months of age and defined spermatogenic cells were present in the lumen at five to six months. The morphological characteristics of the testicular tissues in animals aged five to six months were observed to be almost similar. Sertoli cells, Leydig cells, and spermatozoa were also visualized in the seminiferous tubules under scanning electron microscopy.

Keywords: Histology, Histochemistry, Mali pig, Scanning electron microscopy, Testis

Introduction

The Mali pigs, also known as "local pigs" or "desi pigs," located in Tripura exhibit very little phenotypic variation among different subgroups. It is believed that domestic pigs in this area share a common origin with the wild pig *Sus scrofa cristatus* [1]. Male pigs weigh an average of 68 kg and reach puberty at around 138.3±6.4 days [2]. The testis is the primary male reproductive organ responsible for sperm production (spermatogenesis). Histological investigation of the testis provides critical insights into the cellular and structural organization that supports male reproductive activity and also helps in understanding the complex structure of the seminiferous tubules, where sperm production occurs and the interstitial cells, which secrete testosterone [3]. This understanding is crucial for comprehending how male fertility and hormone production are controlled. Male germ cell production is the exocrine function, while the production of male sex hormones is its primary endocrine function. Sperm production requires a lower temperature than the average body temperature provided by the scrotum and testicles [4]. Postnatal anatomical investigations of the testes at various ages are necessary to understand anatomical growth and development. The testicular postnatal development of Mali pigs is still unknown and this study is the first to report on the age-related morphological changes in the testes of Mali pigs. The purpose of the study to illustrate the histological, histochemical and scanning electron microscopic features of the postnatal development of the testes. These studies provided the morphological characteristics of the individual regions of the developing testes. While incorporating important literature, it also provides baseline information for future scientific research.

Material and Methods

Ethical Statement

The present study was conducted after approval from the Institutional Animal Ethical Committee (IAEC), Approval reference number. CVSC/CAU/IAEC/22-23/P-13 dated 31st October 2023.

Animals

For this study, 15 male Mali pigs from Tripura were used. The animals were designed for this experiment in five different age groups. Group-I (day-old piglets), group-II (3 months), group-III (4 months), group-IV (5 months) and group-V (6 months) and each of the group was carried three numbers of animals. The samples were collected in November 2023 to May 2024.

Samples

The samples for this study were collected immediately after surgical exposure of the scrotum (castration) with all aseptic precautions. After cleaning, the testicles were recorded for gross and morphometrical characteristics. Subsequently, the biological samples were carried to the department through the ice box for light and electron microscopic studies.

Processing of the Samples for The Light Microscopy

The tissue testicular samples from cranial, middle and caudal regions were cut into 3 to 5 mm thickness and fixed into 10% neutral buffer formalin solutions for the histological and histochemical studies. The tissue processing was carried out by ascending grading of alcohol and paraffined embedded sections were cut into 3 to 5 µm in size [5]. The histological staining was done with Van Gieson's stain (VnGi) for collagen fibers [5], Hartt's stain (Hartt) for elastic fibers [5], Berg's method (Berg) for spermatozoa [5], Bielschowsky's method (Bel) for nerve fibers [5], Gomori's stain (Gomori) for reticular fibers [6] 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 method for keratin and pre-keratin [5]. The prepared slides were observed 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°C temperature. Following fixation, the samples were placed in phosphate buffer solutions [7]. 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 histological study revealed the collagen, reticular, elastic and nerve fibers in all the age groups predominantly. In group-I, the tunica albuginea for day-old piglets were recorded procollagen fibers and the basement membrane of numerous seminiferous or sex cords exhibited elastic and reticular fibers *(Fig. 1-a, Fig. 2-a, Fig. 3-a)*. The lumen of the seminiferous cords was visualized with some gonocytes in the center and undifferentiated Sertoli cells-like structures in the periphery of the cords. No spermatids were present in the seminiferous cords of the day-old piglets *(Fig. 4-a)*. Fine nerve fibers were observed around the seminiferous cords and in the parenchyma of the testes *(Fig. 5-a,b)*.

In group-II, the outermost layer of the testes, the tunica albuginea, was formed by the thick connective tissue and mainly consisted of collagen fibers *(Fig. 1-b)*. The fine elastic fibers and the reticular fibers were observed in the basement membrane of seminiferous tubules along

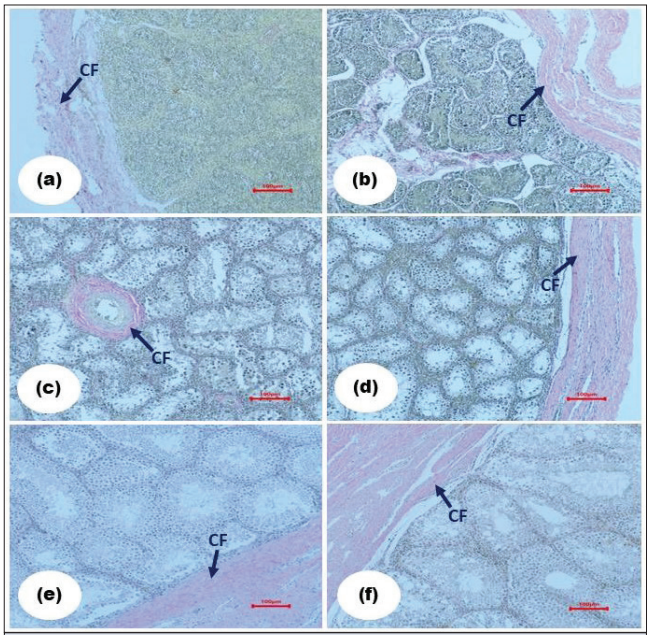

Fig 1. Photomicrographs from group-I showing (a) collagen fibers (CF) in tunica albuginea (VnGi, X100). From group-II showing (b) collagen fibers (CF) in tunica albuginea (VnGi, X100). From group-III showing (c) collagen fibers (CF) in blood vessels (VnGi, X100) and (d) collagen fibers (CF) in tunica albuginea (VnGi, X100). From group-IV showing (e) collagen fibers (CF) in tunica albuginea (VnGi, X100). From group-V showing (f) collagen fibers (CF) in tunica albuginea (VnGi, X100)

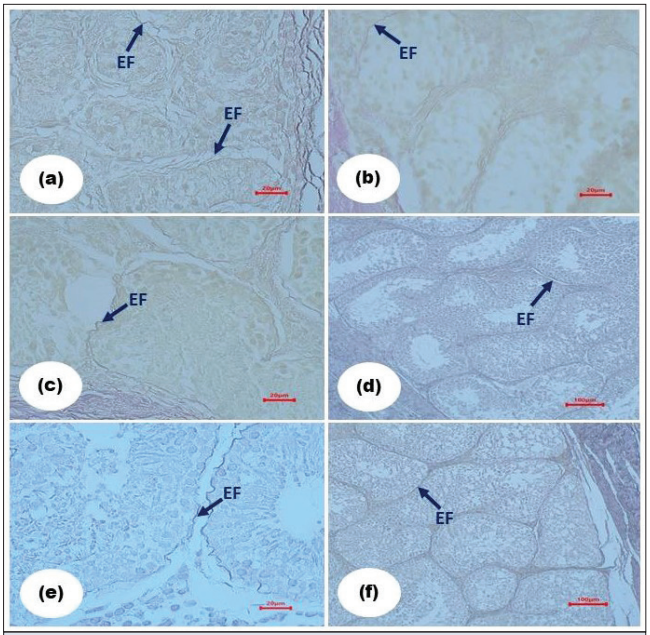

Fig 2. Photomicrographs from group-I showing (a) elastic fibers (EF) in basement membrane of seminiferous or sex cords (Hartt, X400). From group-II showing (b) elastic fibers (EF) in basement membrane of seminiferous tubules (Hartt, X400). From group-III showing (c) elastic fibers (EF) in basement membrane of seminiferous tubules (Hartt, X400). From group-IV showing (d) elastic fibers (EF) in basement membrane of seminiferous tubules (Hartt, X100) and (e) elastic fibers (EF) in basement membrane of seminiferous tubules (Hartt, X400). From group-V showing (f) elastic fibers (EF) in basement membrane of seminiferous tubules (Hartt, X100)

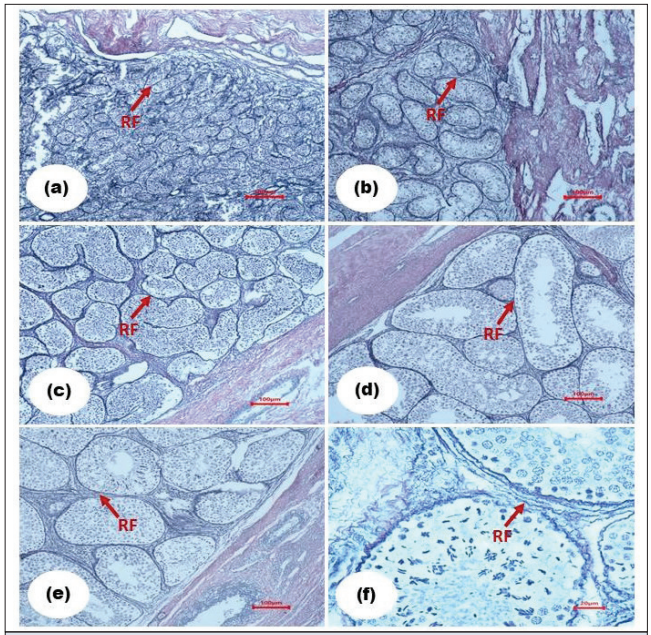

Fig 3. Photomicrographs from group-I showing (a) reticular fibers (RF) in basement membrane of seminiferous cords (Gomori, X100). From group-II showing (b) reticular fibers (RF) in basement membrane of seminiferous tubules and mediastinum testis (Gomori, X100). From group-III showing (c) reticular fibers (RF) in basement membrane of seminiferous tubules (Gomori, X100). From group-IV showing (d) reticular fibers (RF) in basement membrane of seminiferous tubules (Gomori, X100). From group-V showing (e) reticular fibers (RF) in basement membrane of seminiferous tubules (Gomori, X100) and (f) reticular fibers (RF) in basement membrane of seminiferous tubules (Gomori, X400)

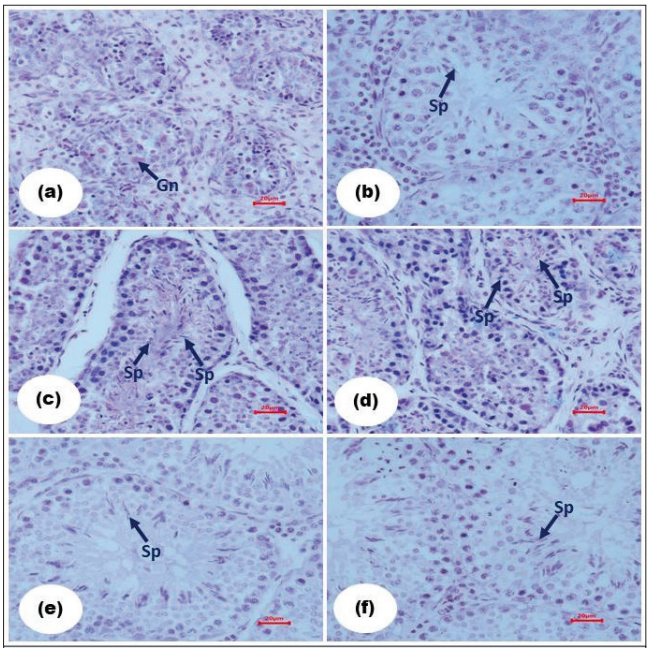

Fig 4. Photomicrographs from group-I showing (a) gonocytes (Gn) in seminiferous cords (Berg, X400). From group-II showing (b) elongated spermatids (Sp) in seminiferous tubules (Berg, X400). From group-III showing (c-d) round to elongated spermatids (Sp) in seminiferous tubules (Berg, X400). From group-IV showing (e) elongated spermatids (Sp) in the seminiferous tubules (Berg, X400). From group-V showing (f) elongated spermatids (Sp) in seminiferous tubules (Berg, X400)

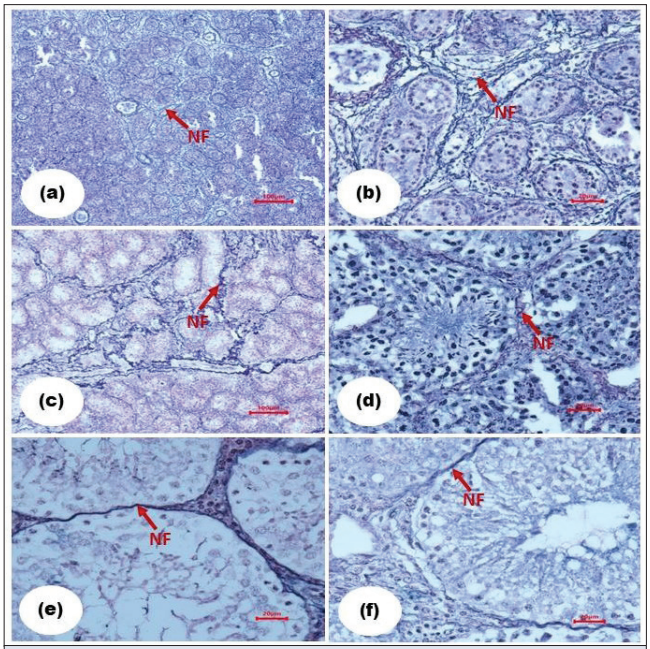

Fig 5. Photomicrographs from group-I showing (a) nerve fibers (NF) around the seminiferous cords (Bel, X100) and (b) nerve fibers (NF) around the seminiferous cords (Bel, X400). From group-II showing (c) nerve fibers (NF) in trabeculae of the testes (Bel, X100). From group-III showing (d) nerve fibers (NF) around the seminiferous tubules (Bel, X400). From group-IV showing (e) nerve fibers (NF) around the seminiferous tubules (Bel, X400). From group-V showing (f) nerve fibers (NF) in between the seminiferous tubules (Bel, X400)

with the lining epithelium of the rete testis *(Fig. 2-b, Fig. 3-b)*. The luminal place of the seminiferous tubules was observed from the 3 months of the age and some of the tubules have recorded few numbers of the elongated spermatids *(Fig. 4-b)*. Nerve fibers were observed in the basement membrane of seminiferous tubules and trabeculae, as well as in the interstitial tissues of the testes *(Fig. 5-c)*.

In group III, the luminal place and the size of seminiferous tubules were observed more in 4 months than in the 3 months of age. The collagen fibers were mainly observed in the tunica fibrosa and trabeculae and around the blood vessels of the testes *(Fig. 1-c,d)*. The elastic and reticular fibers were noticed in the basement membrane of seminiferous tubules and rete testis of the mediastinum *(Fig. 2-c, Fig. 3-c)*. The lumen also contained numerous rounds to elongated spermatids *(Fig. 4-c,d)*. Nerve fibers were found around the seminiferous tubules *(Fig. 5-d)*.

In group-IV, the tunica fibrosa and the tunica vasculosa of the capsule were observed for the collagen fibers *(Fig. 1-e)*. The diameter of the seminiferous tubules was found to be more in 5 months than in 4 months of age. Also, elastic, reticular and nerve fibers were observed in the basement membrane of seminiferous tubules, blood vessels and in

Table 1. *Distribution of histochemical characteristics in the testes of Mali pig of Tripura*

Group	**Testicular Components**	**Histochemical Stains**		
		PAS	PAS-AB	Ayob- Shklar method
Group - I	Tunica albuginea	+	+	-
	Spermatogonia	+	+	-
	Basement membrane of the seminiferous cords	++	+	-
	Interstitial tissue	+	+	+++
	Mediastinum	+	++	-
Group - II	Tunica albuginea	+++	++	-
	Spermatogonia	++	++	-
	Basement membrane of the seminiferous tubules	+++	++	-
	Interstitial tissue	++	+	++
	Mediastinum	++	+	-
Group - III	Tunica albuginea	+++	+	-
	Spermatogonia	++	+	-
	Basement membrane of the seminiferous tubules	+++	++	-
	Interstitial tissue	+++	+	+
	Mediastinum	+++	+	-
Group - IV	Tunica albuginea	+++	++	-
	Spermatogonia	++	+	-
	Basement membrane of the seminiferous tubules	+++	+	-
	Interstitial tissue	+++	+	-
	Mediastinum	+++	+	-
Group - V	Tunica albuginea	+++	+	-
	Spermatogonia	++	+	-
	Basement membrane of the seminiferous tubules	++	+	-
	Interstitial tissue	++	+	-
	Mediastinum	++	+	-

- Absent; + Weak; ++ Moderate; +++ Intense/Strong, (PAS)- Periodic Acid Schiff for glycogen; (PAS-AB)-PAS- Alcian blue for acidic mucopolysaccharides; Ayob- Shklar method for keratin and pre-keratin activity

the rete testis, respectively *(Fig. 2-d,e, Fig. 3-d, Fig. 5-e)*. The widest lumen of the seminiferous were viewed from the 5 months of age and contained numerous spermatids *(Fig. 4-e)*.

In group-V, the histological findings were almost similar for the 5 and 6 months aged animals. The testicular capsule and basement membrane of seminiferous tubules predominantly contained collagen, fine elastic and reticular fibers. In contrast, the nerve fibers were recorded in the periphery of the seminiferous tubules *(Fig. 1-f, Fig. 2-f, Fig. 3-e,f, Fig. 5-f)*. The rounds to elongated shaped spermatids were well observed in the lumen of seminiferous tubules *(Fig. 4-f)*.

Histochemical Studies

In this present investigation, the histochemical distributions for glycogen, acidic mucopolysaccharides and pre-keratin activity were recorded in the cranial, middle and caudal regions of the testes on their developmental basis and observation was recorded in the *Table 1*. The magenta colour was found for the intense reactivity of PAS, which was shown by various locations in the testes. The PAS alcian blue appeared as blue for acidic mucopolysaccharides, whereas the Ayob-Shklar method showed an orange colour for the demonstration of pre-keratin activity in the testicular tissues. No significant differences in histochemical observations were found between the right and left testes.

In group I, weak to moderate activity for the PAS was recorded in the tunica albuginea, basement membrane of the seminiferous cords, mediastinum and the trabeculae of the testes *(Fig. 6-a)*. The mediastinum, tunica albuginea and the interstitial tissue recorded weak reactivity for the acidic mucopolysaccharides and the cytoplasm of some undifferentiated cells in the seminiferous cords showed positive for the pre-keratin activity *(Fig. 7-a, Fig. 8-a)*.

In group II, PAS activity was intense in the tunica albuginea, basement membrane of the seminiferous tubules, trabeculae and moderate PAS affinity was found in the interstitial tissues of the testes *(Fig. 6-b)*. Moderate PAS-AB reactivity was recorded in the tunica albuginea, seminiferous germinal epithelium and the basement membrane of the seminiferous tubules. Weak reactivity for the acidic mucopolysaccharides was found in the interstitial tissues of the testes *(Fig. 7-b)*. Some peritubular and interstitial cells' cytoplasm showed positive pre-keratin activity *(Fig. 8-b,c)*.

In group-III, intense PAS reactivity was recorded in the basement membrane of the seminiferous tubules, trabeculae, blood vessels in the capsules and the interstitial tissues. Spermatids in the seminiferous tubules showed PAS-positive activity from 4 months of age *(Fig. 6-c)*. Weak to moderate acidic mucopolysaccharides were observed in

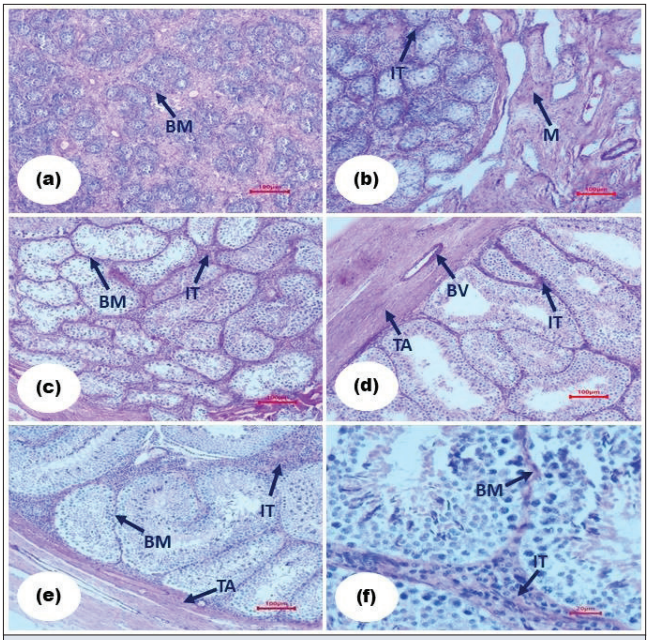

Fig 6. Photomicrographs from group-I showing (a) basement membrane (BM) of seminiferous cords (PAS, X100). From group-II showing (b) interstitial tissue (IT) and mediastinum (M) testis (PAS, X100). From group-III showing (c) basement membrane (BM) of seminiferous tubules and interstitial tissue (IT) in testis (PAS, X100). From group-IV showing (d) tunica albuginea (TA), blood vessels (BV) and interstitial tissue (IT) in testis (PAS, X100). From group-V showing (e) tunica albuginea (TA), blood vessels (BV) and interstitial tissue (IT) in testis (PAS, X100) and (f) basement membrane (BM) of seminiferous tubules and interstitial tissue (IT) in testis (PAS, X400)

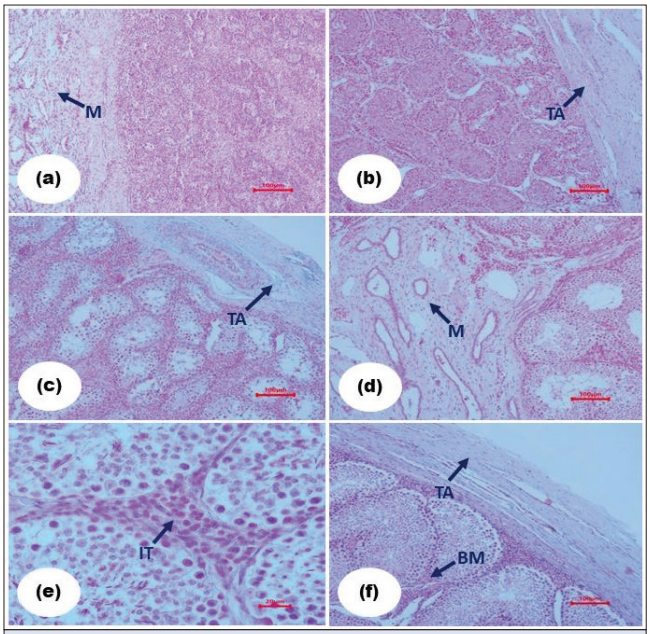

Fig 7. Photomicrographs from group-I showing (a) mediastinum (M) testis (PAS-AB, X100). From group-II showing (b) tunica albuginea (TA) in testis (PAS-AB, X100). From group-III showing (c) tunica albuginea (TA) in testis (PAS-AB, X100). From group-IV showing (d) mediastinum (M) testis (PAS-AB, X100) and (e) interstitial tissue (IT) in testis (PAS-AB, X400). From group-V showing (f) tunica albuginea (TA) and basement membrane (BM) of seminiferous tubules (PAS-AB, X100)

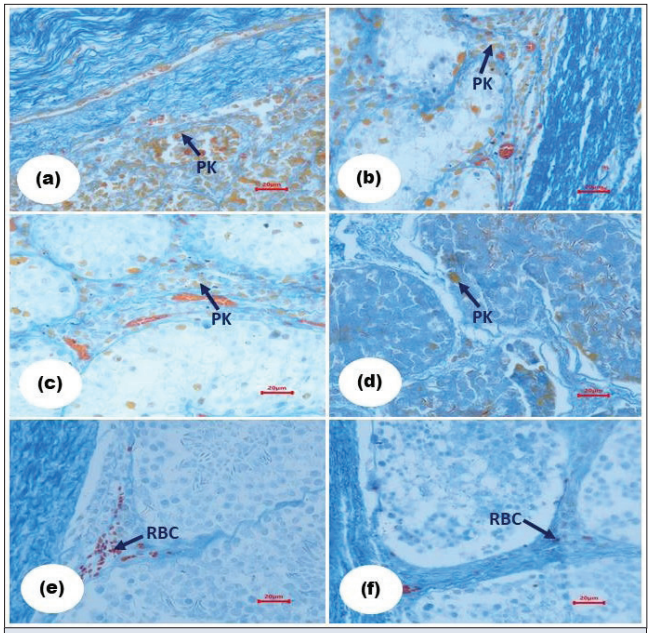

Fig 8. Photomicrographs from group-I showing (a) pre-keratin (Pr) activity in the seminiferous cords (Ayob, X400). From group-II showing (b-c) pre-keratin (Pr) activity in the interstitial cells (Ayob, X400). From group-III showing (d) pre-keratin (Pr) activity in the interstitial cells (Ayob, X400). From group-IV showing (e) erythrocytes (RBC) and negative for pre-keratin (Pr) activity in testis (Ayob, X400). From group-V showing (f) erythrocytes (RBC) and negative for pre-keratin (Pr) activity in testis (Ayob, X400)

the tunica albuginea, blood vessels and interstitial tissues *(Fig. 7-c)*. Few pre-keratin affinities also observed in the interstitial cells of the testes *(Fig. 8-d)*.

In group-IV, intense PAS activity was detected in the tunica albuginea, blood vessels, basement membrane of the seminiferous tubules, mediastinum and interstitial tissues of the testes *(Fig. 6-d)*. Weak PAS-AB reactivity was noticed in the tunica albuginea, seminiferous germinal epithelium, mediastinum and the basement membranes of the seminiferous tubules *(Fig. 7-d)*. No reactivity was noticed for keratin and pre-keratin in 5 months of age *(Fig. 8-e)*.

In group-V, the histochemical studies showed almost the same for the 6 and 5 months of age. A strong affinity for glycogen was observed in the tunica albuginea but was moderate in the basement membrane of the seminiferous tubules and the interstitial tissues of the testes *(Fig. 6-e,f)*. The acidic mucopolysaccharides were found to be weak in the tunica albuginea, blood vessels, interstitial tissues and in the mediastinum of the testes *(Fig. 7-f)*. No reactivity had been shown for the keratin and pre-keratin in the testes *(Fig. 8-f)*.

Scanning Electron Microscopic Studies

In this study, the scanning electron microscopic features were observed in the various locations of the testes during their postnatal development. In group-I, under

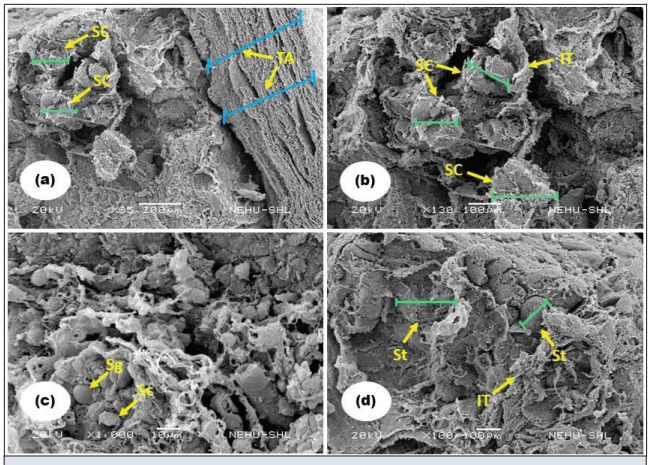

Fig 9. Scanning electron micrographs from group-I showing (a) seminiferous or sex cords (SC) and tunica albuginea (TA) in testis. (b) seminiferous cords (SC) and interstitial tissue (IT) in testis. (c) spermatogenic cell (Sg) and Sertoli cell (Sc) in seminiferous cords. From group-II showing (d) seminiferous tubules (St) and interstitial tissue (IT) in testis

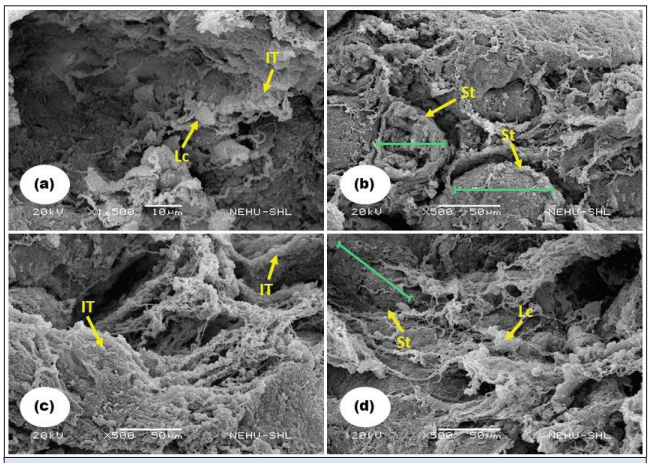

Fig 10. Scanning electron micrographs from group-II showing (a) interstitial tissue (IT) and Leydig cell (Lc) in testis. From group-III showing (b) seminiferous tubules (St) in testes. (c) interstitial tissues (IT) in testes.(d) seminiferous tubules (St) and Leydig cell (Lc) in testis

the scanning electron microscope, the tunica albuginea was viewed as the outermost covering of the testes. The parenchyma of the testes was recorded in numerous small-sized seminiferous or sex cords *(Fig. 9-a,b)*. The seminiferous cords contained round to oval-shaped spermatogonia and spherical-shaped Sertoli cells *(Fig. 9-c)*. The Leydig cells appeared irregularly polygonal in shape for the day-old piglets.

In group-II, well-defined seminiferous tubules were recorded from the 3 months of age *(Fig. 9-d)*. Interstitial tissue was observed between the tubules, which contained spherical to polygonal shaped Leydig cells. Under the scanning electron microscope, it is challenging to differentiate the spermatogonium and spermatogenic cells. Spermatogonium was noticed to be the largest cells in the seminiferous tubules *(Fig. 10-a)*.

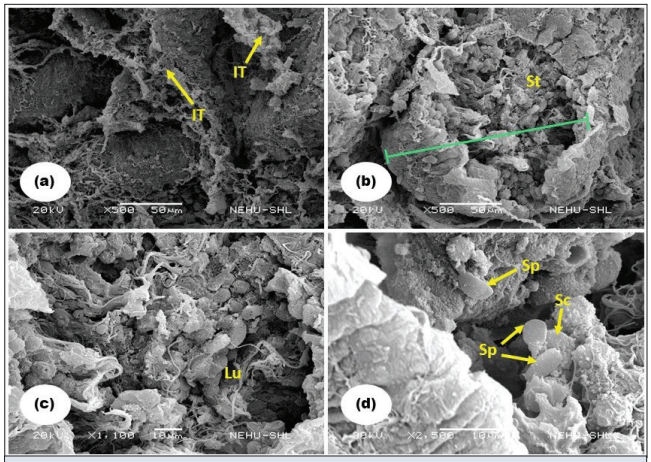

Fig 11. Scanning electron micrographs from group-III showing (a) interstitial tissues (IT) in testes. From group-IV showing (b) seminiferous tubule (St) contained spermatogenic cells in testes. (c) seminiferous lumen (Lu) contained spermatogenic cells in the testes. (d) seminiferous lumen contained spermatozoa (Sp) and Sertoli cells (Sc)

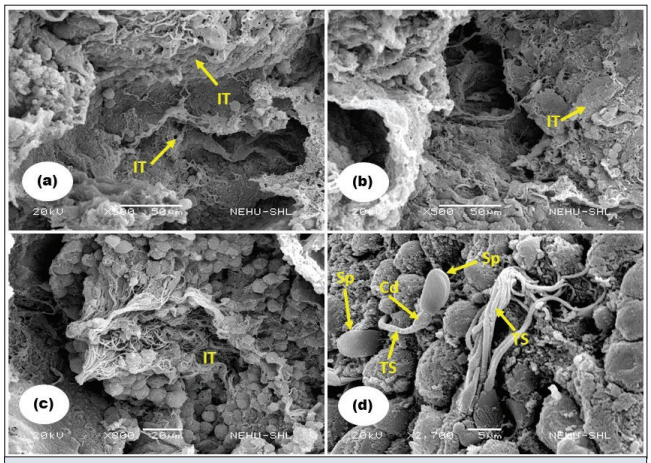

Fig 12. Scanning electron micrographs from group-V showing (a-c) interstitial tissues (IT) contained interstitial cells in testes. (d) the peripheral surface of the seminiferous lumen showing spermatozoa (Sp) and tail (TS) of the spermatozoa contained cytoplasmic droplets (cd) in the testis

In group-III, the scanning electron microscopy revealed numerous spermatogonia and spermatogenic cells in the seminiferous lumen *(Fig. 10-b)*. The round to elongated spermatids and the roughly triangular to elongated-shaped Sertoli cells were noticed in the seminiferous tubules. Additionally, polygonal-shaped Leydig cells were observed in the interstitial tissues *(Fig. 10-c,d, Fig. 11-a)*.

In group-IV, the widest lumen of the seminiferous tubules was viewed from the 5 months of age *(Fig. 11-b)*. The elongated to polygonal-shaped Leydig cells were observed in the in interstitial tissues. Sertoli cell, spermatogonium and spermatogenic cells were found in the luminal place of the seminiferous tubules *(Fig. 11-c)*. The heads of the spermatozoa were observed adjacent to the Sertoli cells in the tubules *(Fig. 11-d)*.

In group-V, the scanning electron microscopic observations were almost similar to the features of 5 and 6 months of age. The seminiferous luminal place exhibited Sertoli cells, spermatogonium and spermatogenic cells *(Fig. 12-a,b,c)*. Leydig cells were also observed in the interstitial tissues. Spermatozoa contained cytoplasmic droplets were seen on the luminal surface of the seminiferous tubules *(Fig. 12-d)*.

Discussion

In this study, the testicular capsule of the day-old piglets (group-I) contained predominantly collagen fibers [8]. The lumen of the seminiferous cords was visualized for some gonocytes and Sertoli cell-like structure in the periphery of the cords [9,10]. The fine elastic and reticular fibers were observed in the basement membrane of numerous seminiferous cords and the nerve fibers were noticed between the seminiferous cords and in the parenchyma of the teste [11]. Furthermore, the tunica albuginea of the testes was recorded for the collagen, elastic and reticular fibers at 3 months (group-II) of age [12,13]. The basement membrane of the seminiferous tubules, rete testis and the mediastinum testis were viewed for fine elastic and reticular fibers [14]. The lumen of the seminiferous tubules contained peripherally located Sertoli cells and a few elongated spermatids [8,15].

In the current investigation, the lumen of the seminiferous tubules observed rounds to elongated shaped spermatids in 4 months (group-III) of age [16]. The basement membrane of the seminiferous tubules and rete testis of the mediastinum testis contained collagen, reticular, and elastic fibers [17,18]. Elastic fibers gradually increased in the periphery of the seminiferous tubules in this study; this might be due to increased tubule elasticity to accommodate more luminal content. The study also observed an increase in the diameter of the seminiferous tubules from age 5 months (group-IV) onward. The basement membrane of seminiferous tubules, blood vessels and the rete testis were noticed for the elastic, nerve and reticular fibers [19]. In the present investigation, the histological findings in 6 months (group-V) were found to be almost similar to those of 5 months of age.

In this present study, PAS reactivity was recorded as weak to moderate in the tunica albuginea, basement membrane of the seminiferous cords, mediastinum and the trabeculae of the testes for group-I animals, which indicates less glycogen contained at their initial stage of life [8]. However, PAS reactivity was strong in the basement membrane of the seminiferous cords for the day-old kids [20,21]. The PAS-AB activity was found to be weak in the mediastinum, tunica albuginea and the interstitial tissues. The cytoplasm of some undifferentiated cells in the seminiferous cords was found positive for the

pre-keratin activity [17]. In the present investigation for group-II, the basement membrane of the seminiferous tubules, trabeculae and tunica albuginea were recorded instances PAS activity. The PAS-AB were moderate in the tunica albuginea, seminiferous germinal epithelium and the basemen membrane but the activity was weak in the interstitial tissues [8]. The pre-keratin activity was observed in the peritubular cells and the interstitial cells for 3 months of age, as reported earlier in pig testis [17].

In this study, the basement membrane of the seminiferous tubules, spermatids trabeculae, blood vessels in the capsules and the interstitial tissues were recorded strong PAS activity for group-III [19]. The peritubular cells and some interstitial cells also showed for pre-keratin affinity. The tunica albuginea, blood vessels, and interstitial tissues were observed to have weak to moderate PAS-AB activity [22]. In the current study, the PAS activity was intense in the tunica albuginea, basement membrane of the seminiferous tubules, mediastinum, interstitial tissues and the blood vessels for group-IV. However, the intense PAS reactivity in the tunica albuginea and basement membrane of the seminiferous tubules of pigs was also recorded earlier [23]. In this study, Sertoli cells were viewed as weak for glycogen activity [17]. The tunica albuginea, seminiferous germinal epithelium, mediastinum and the basement membrane of the seminiferous tubules recorded weak PAS-AB activity, this might provide the proper acidic environment for the spermatogenic cells. No major histochemical differences were observed between the 6 months (group-V) and 5 months aged testes in the present study. Intense PAS activity was noticed in the tunica albuginea, and moderate reactivity was observed in the basement membrane of the seminiferous tubules and interstitial tissues of the testes, which might be the reason for increased spermatozoa production at this stage. However, weak PAS-positive reactivity in the basement membrane of the seminiferous tubules also reported in pig and goat testes [17,21]. The PAS-AB activity was viewed as weak in the tunica albuginea, basemen membranes of the seminiferous tubules, blood vessels, interstitial tissues and the mediastinum of the testes [21]. However, the affinity for the PAS-AB activity was recorded as intense in the basement membranes of the seminiferous tubules and tunica albuginea of pig testes [8]. No reactivity was recorded for the keratin and pre-keratin in the testes of 6 months of age.

In the present investigation, the tunica albuginea was viewed under the scanning electron microscope as the most covering of the testes for group-I animals. Numerous small sized seminiferous or sex cords were viewed in the parenchyma of the testes. The interstitial tissues were viewed for irregular polygonal-shaped Leydig cells in the day-old piglets. However, Leydig cells were also viewed in the electron and light microscopic studies for piglets and microminipigs, respectively [8,24]. Under scanning electron microscopy, well-formed seminiferous tubules were observed from the testes of 3 months (group-II) of aged. The spherical to polygonal-shaped Leydig cells were noticed in the interstitial tissue and spermatogonium was noticed the largest cells in the seminiferous tubules [25]. In the current study, the seminiferous lumen was recorded in numerous spermatogonium and spermatogenic cells for the testes of group-III animals. The interstitial tissues were recorded and elongated to round-shaped Leydig cells [26]. In this study, the widest lumen of the seminiferous tubules was recorded from the 5 months (group-IV) of the aged. The polygonal-shaped Leydig cells were observed in the interstitial tissues [27]. The seminiferous luminal place was noticed for the Sertoli cells and the spermatogonium and spermatogenic cells [11]. In this study, the scanning electron microscopic features were observed to be almost similar for the 6 (group-V) and 5 months of the aged animals. The Sertoli cells and the spermatogonium and spermatogenic cells were viewed in the seminiferous tubules, whereas the Leydig cells were recorded in the interstitial tissues [7]. 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 [28]. The present study revealed the histological, histochemical, and scanning electron microscopic characteristics of the testes based on the post-natal development of Mali pigs in Tripura. The research provides morphological insights into the testes and contributes valuable baseline information for future scientific studies on the male genital system of domestic animals.

Declarations

Availability of Data and Materials: The data that support the findings of this study are available from the corresponding author (O.P. Choudhary) upon reasonable request

Acknowledgements: The authors are thankful to their respective institutions/ universities for the completion of this work. We would like to express our gratitude to staff of North Eastern Hill University (NEHU), Shillong for their technical support in electron microscopy.

Funding Support: This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Congress-Symposium Information: Not applicable.

Competing Interests: The authors declared that they have no competing interests.

Declaration of Generating Artificial Intelligence: This article and/or tables and figures were not written/created by Artificial Intelligence (AI) and AI-assisted technologies.

Ethical Statement: The present study was conducted after approval from the Institutional Animal Ethical Committee. (Approval reference number. CVSC/CAU/IAEC/22-23/P-13 dated 31st October 2023).

Author's Contributions: Writing the original draft and design, R.S and P.C.K; methodology, R.S and A.K; planned and investigation, R.S, P.C.K and A.K; software, R.S, A.K, and P.J.D; formal analysis, R.S, A.K, P.J.D and O.P.C; writing-review, editing and visualization, R.S, P.J.D and O.P.C. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. Dandapat A, Choudhury KD, Debbarma C, Das MK: Phenotypic characterization of Mali pig in Tripura, India. *Chest Livest Res Rural Dev,* 22 (4): 0-7, 2010.

2. Debbarma A, Koloi S, Sarkar D, Tripura S, Debbarma A, Debbarma K: Livestock and fodder production scenario of Tripura. *J Pharma Innov,* 10 (5): 18-20, 2021.

3. Rajathi S, Ramesh G, Kannan TA, Raja K: Postnatal morphological development of male reproductive organs in guinea pigs. *Haryana Vet,* 60 (2): 285-288. 2021.

4. Konig HE, Liebich HG: Veterinary anatomy of domestic animals. 6th ed., 413-419, Manson Publishing Ltd, London, UK, 2014.

5. Luna LG: Manual of histologic staining methods, Armed Forces Institute of Pathology. 3rd ed., 76-163, McGraw Hill Book Co London, New York, 1968.

6. Bancroft JD, Gamble M: Theory and Practice of Histological Techniques. 6th ed., Churchill Livingstone, Elsevier, China, 2008.

7. Penchev G: Postnatal development of interstitial endocrine (Leydig) cells in pig's testes-electron microscopy study. *Bulg J Vet Med,* 14 (3): 131-136, 2011.

8. Babu LS: Gross anatomical, histomorphological and ultrastructural studies of testis during postnatal development in pig *(Sus domesticus). PhD Thesis*. Maharashtra Animal and Fishery Sciences University, Bombay Veterinary College, Nagpur, India, 2012.

9. Kim S, Shin T: Immunohistochemical study of osteopontin in boar testis. *J Vet Sci,* 8 (2): 107-110, 2007. DOI: 10.4142/jvs.2007.8.2.107

10. Contreras-Ortiz AJ, Vigueras RM, Mendoza-Elvira SE, Martínez-Castañeda FE, Gutiérrez-Pérez O, Trujillo-Ortega ME: Postnatal testicular development in Vietnamese pot-bellied pigs. *Acta Histochem,* 123 (5): 151741, 2021. DOI: 10.1016/j.acthis.2021.151741

11. Sarma K, Devi J: Changes in the seminiferous epithelium of the testes during postnatal development in Assam goat. *Anat Res Int,* 2012 (1):620924, 2012. DOI: 10.1155/2012/620924

12. Bhagyalakshmi J, Balasundaram K, Selvaraj P, Kathirvel S, Balachandran P, Chandana GSS: Gross, histology and histochemical analysis of the testis in dog *(Canis lupus familiaris). J Entomol Zool Stud,* 8 (4): 69-73, 2020.

13. Abhin-Raj KP, Sreeranjini AR, Maya S, Vasudevan VN, Sunilkumar NS: Histomorphological studies on the testicular capsule of crossbred Large White Yorkshire boars *(Sus scrofa domesticus). J Vet Anim Sci,* 53 (2): 154-157, 2022. DOI: 10.51966/jvas.2022.53.2.154-157

14. Singh TS, Kalita PC, Choudhary OP, Doley PJ, Kalita A: Gross morphological, histological and histochemical studies on the epididymis of local pig (Zovawk) of Mizoram. *J Anim Res,* 9 (6): 855-861, 2019. DOI: 10.30954/2277-940X.06.2019.9

15. Khan SA, Kalhoro IB, Gandahi JA, Vistro WA, Kalwer Q, Ali I, Khokhar TA, Mangi MH: Puberty associated micro anatomical modification in testes and epididymis of Teddy goat *(Capra hircus). Pure Appl Biol,* 8 (2): 1281-1286, 2019. DOI: 10.19045/bspab.2019.80070

16. Neves ES, Chiarini-Garcia H, França LR: Ultrastructural observation of the mule testis indicates normal function of somatic cells. *Anim Reprod,* 2 (4): 263-271, 2018.

17. Sikarwar S: Gross and histological studies on the testes of large white Yorkshire pig (*Sus scrofa*). *PhD Thesis*. Rajasthan University of Veterinary and Animal Sciences, College of Veterinary and Animal Science, Bikaner, Rajasthan, India, 2018.

18. Sikarwar S, Mathur R, Dangi A: Histological studies of seminiferous tubules in the testes of large white Yorkshire pig *(Sus scrofa). J Pharm Innov,* 11 (7): 505-508, 2022.

19. Gofur MR, Khan MZI, Karim MR, Islam MN: Histomorphology and histochemistry of testis of indigenous bull *(Bos indicus)* of Bangladesh. *Bangladesh J Vet Med*, 6 (1): 67-74, 2008. DOI: 10.3329/bjvm.v6i1.1341

20. Sarma K, Kalita SN, Devi J: Postnatal age related histochemical studies on the testes in Assam goat *(Capra hircus). Vet Pract,* 18 (1): 74-76, 2017.

21. Sarma K, Kalita SN, Devi J: Histochemical studies on the testicular stroma, seminiferous tubules and the Leydig cells in Assam goats. *Explor Anim Med Res,* 8 (2): 168-172, 2018.

22. Singh A: Gross and histological studies on the testes of Marwari sheep *(Ovis aries). MVSc Thesis*. Rajasthan University of Veterinary and Animal Sciences, College of Veterinary and Animal Science, Bikaner, Rajasthan, India, 2013.

23. Singh TS, Kalita PC, Choudhary OP, Kalita A, Doley PJ: Histological, micrometrical and histochemical studies on the testes of large white Yorkshire pig *(Sus scrofa domesticus). Indian J Anim Res,* 54 (12): 1595-1598, 2020. DOI: 10.18805/ijar.B-3914

24. Kangawa A, Otake M, Enya S, Yoshida T, Shibata M: Histological changes of the testicular interstitium during postnatal development in microminipigs. *Toxicol Pathol*, 47 (4): 469-482, 2019. DOI: 10.1177/0192623319827477

25. Sarma K: Studies on post natal development of testis and epididymis in relation to thyroid activity in Assam goats *(Capra hircus). PhD Thesis*. Assam Agricultural University, College of Veterinary Science, Assam, India, 2009.

26. Singh TS: Comparative gross morphological, light and electron microscopic studies on the testes and epididymis of Zovawk and Large White Yorkshire pig. *MVSc Thesis*. Central Agricultural University, College of Veterinary Sciences and Animal Husbandry, Imphal, Manipur, India, 2018.

27. Yazama F, Nishida T, Kurohmaru M, Hayashi, Y: A scanning electron microscopy of the interstitial tissue of the boar testis. *Exp Anim,* 39 (1): 117-120, 1990. DOI: 10.1538/expanim1978.39.1_117

28. Choudhary OP, Saini J, Challana A: ChatGPT for veterinary anatomy education: An overview of the prospects and drawbacks. *Int J Morphol,* 41(4):1198-1202, 2023. DOI: 10.4067/S0717-95022023000401198