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Gross Morphological, Histological and Histochemical Studies on the Epididymis of Local Pig (Zovawk) of Mizoram

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ABSTRACT

The aim of the research was to investigate the gross morphological, histological and histochemical structure of epididymis in local pig of Mizoram. The epididymis was collected from six apparently healthy adult Zovawk pigs. The epididymis appeared as a straight elongated tubular structure with reddish-white colour comprises of three regions; head, body and tail. The biometrical parameter including the weight, length and thickness (head, body and tail) were found to be higher in the left side of epididymis than the right side. Histologically, the duct of epididymis was surrounded by irregularly arranged tissue. The parenchyma of epididymis consists of tubules with distinct boundaries of connective tissues. The collagen and reticular fibers were abundant in tunica albuginea and connective tissue present between the tubules. However, few elastic fibers occurred in the capsule and only in the blood vessels in both the left and right epididymis of Zovawk pig. Histochemical studies revealed that basement membrane of tubules, blood vessels and stereocilia shows strong Periodic acid Schiff activity, whereas the basement membrane of tubules and stereocilia showed more concentration of acidic mucopolysaccharides. This is the first study to provide the detailed morphological, histological and histochemical structure of Zovawk epididymis.

Keywords: Epididymis, histological, histochemical, morphological, pig, Zovawk

The north-eastern region has the highest population and concentration of pig per household than any other state or region of the country. The total pig population of Mizoram is around 1,17,675; whereas the Zovawk population of Mizoram is about 43,000 (Livestock Census, 2007). Zovawk is newly identified breed of the pig of Mizoram approved by breed registration committee of Indian Council of Agricultural Research (ICAR), New Delhi (Choudhary *et al.,* 2018; 2019). The size of the Zovawk is small, attained puberty at the age of 2.5 months (Hmar *et al*., 2010). The population of this pig is decreasing significantly because of lack of scientific breeding practice (Kalita *et al*., 2014). Therefore, there is a need to increase the population and productivity of Zovawk in order to meet its demand in the market as well as a part of the conservation of the pig (Mayengbam *et al.,* 2014; Choudhary, 2018).

The knowledge of spermatogenesis, especially in the male pig is important for protection against extinction. It is also necessary for the improvement of species management as well as to boost male reproductive capacity in natural and artificial breeding programs particularly in those that are currently undergoing domestication and captive-rearing (Costa *et al*., 2010). The literature pertaining to the gross morphology, histology and histochemical studies on the epididymis in Zovawk is scanty. Therefore, the present investigation was designed to study the gross morphological, histological and histochemical components in the epididymis of Zovawk. The results of the present study will serve as the basis for further studies on the epididymis of this species.

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MATERIALS AND METHODS

The samples of the epididymis were collected from six apparently healthy adult Zovawk pigs (aged about 2-2.5 year) in the month of July, 2018 to June, 2019 after castration from the animal house of the Department of Livestock Production and Management, College of Veterinary Sciences and Animal Husbandry, Mizoram and from the local slaughterhouses near the Aizawl city, Mizoram. The gross morphological, histological and histochemical studies on the epididymis of the local pig of Mizoram were carried out in the Department of Veterinary Anatomy and Histology, College of Veterinary Sciences and Animal Husbandry, Aizawl, Mizoram. Immediately after collection of the samples, the gross morphological and biometrical details like weight, length, thickness (i.e. head, body and tail) were recorded afresh. For histological studies, the tissue from head, body and tail parts of the epididymis were collected and fixed in 10% neutral buffered formalin for 24-48 hours. Paraffin blocks were made by alcohol-xylene sequence using cedarwood oil (Luna, 1968). The paraffin sections of 3-5µm were cut and stained by routine Mayer's hematoxylin and eosin for general tissue reaction and cytoarchitectural studies (Mayer, 1891), Masson's trichrome for collagen fibers (Masson, 1929), Verhoeff's for elastic fibers (Verhoeff's 1908), Gomori's silver for reticular fibers (Gomori, 1937), Periodic acid Schiff for glycogen and various glycoproteins (Bancroft and Gamble, 2008) and Alcian blue for acidic mucopolysaccharides (Bancroft and Gamble, 2008). The data of the present investigation was analyzed by the standard statistical procedure (Snedecor and Cochran, 1994) with the help of SPSS 20.0 (2013).

RESULTS AND DISCUSSION

Gross observations

The epididymis appeared as adjacent and firmly attached along with the testis as an epididymal duct. The epididymal duct was located at the caudolateral border of the testicles, medial to the mesonephros. The epididymal duct was divided into head, body and tail regions. Head and tail developed at the cranial and caudal pole of the testis. The Body of the epididymis was connected to the testis along its cranial border (Fig. 1 & 2) as also reported in pig (Ziehmer *et al*., 2013) and (Konig and Liebich, 2014).

The epididymis appeared as a straight elongated tubular structure. The epididymal head and tail were firmly attached to the testicular capsule (Fig. 1) as also reported in domestic animals (Konig and Liebich, 2014) and Gaddi sheep (Shukla, 2015). The epididymis was reddish-white in colour in both Zovawk and Large White Yorkshire pig (Fig. 1) as also reported in boar (Sisson and Grossman, 1953) and goat (Yaseen, 2009).

The present study revealed that the average weight of the right and left epididymis of adult Zovawk were 27.55±0.85 gm and 29.48±0.44, respectively. However, the average weight of the right and left epididymis of large white boars were 40.90±6.81 gm and 43.7± 8.55 gm, respectively (Samuel and Bankole, 2016).

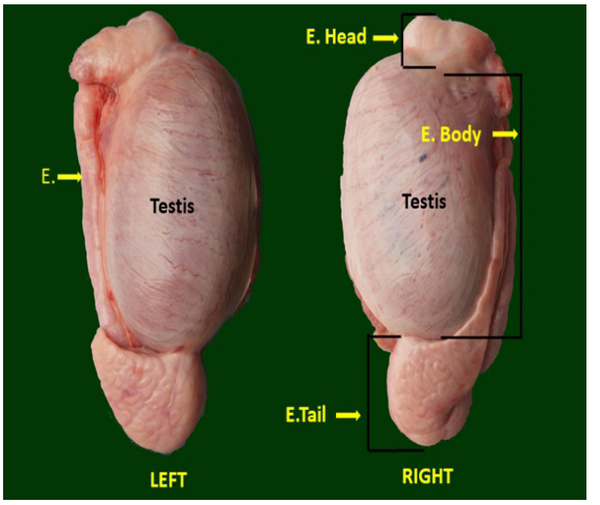

Fig. 1: Photograph showing Testis and Epididymis (E) of Zovawk showing the different parts of the epididymis (i.e. E. Head, E. Body and E. Tail) on Lateral aspect

The average length of the right and left epididymis of adult Zovawk was 9.32±0.35 cm and 9.47±0.33 cm, respectively. However, the average length of the right and left epididymis of large white boars were 18.0±1.98 cm and 17.6±1.76 cm, respectively (Samuel and Bankole, 2016). The average epididymal head thickness of the right and left epididymis of adult Zovawk was 2.71±0.12 cm and 2.75±0.08 cm, respectively. Shukla (2015) reported in Gaddi sheep that the right side epididymal head thickness at 1.0-2.0, 2.0-3.0 and 3.0-4.0 months of age was 0.54±0.06, 0.87±0.14 and 2.40±0.24 mm and that of the left side was 0.52±0.13, 0.85±0.14 and 2.37±0.15 mm, respectively.

The average epididymal body thickness of the right and left epididymis of adult Zovawk was 0.47±0.02 cm and 0.49±0.01 cm, respectively. Whereas, Shukla (2015) reported in Gaddi sheep that the right side epididymal body thickness at 1.0-2.0, 2.0-3.0 and 3.0-4.0 months of age was 0.54±0.05, 0.68±0.08 and 1.78±0.12 mm and that of the left was 0.51±0.05, 0.69±0.09 and 1.77±0.16 mm, respectively. The average epididymal tail thickness of the right and left epididymis of adult Zovawk was 2.61±0.09 cm and 2.74±0.08 cm, respectively. However, Shukla (2015) reported in Gaddi sheep that the right side epididymal tail thickness at 1.0-2.0, 2.0-3.0 and 3.0-4.0 months of age was 0.78±0.08, 1.09±0.25 and 3.22±0.18 mm and that of the left was 0.75±0.09, 1.08±0.24 and 3.25±0.22 mm, respectively.

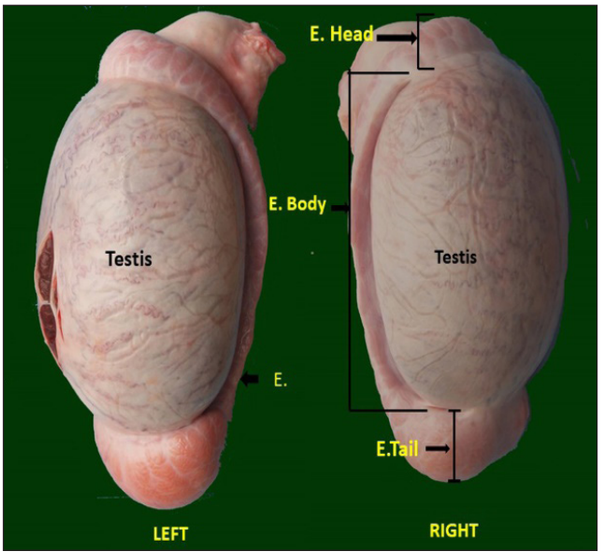

Fig. 2: Photograph showing Testis and Epididymis (E) of Zovawk showing the different parts of the epididymis (i.e. E. Head, E. Body and E. Tail) on Medial aspect

Histological observations

The epididymal duct was surrounded by irregularly arranged tissue i.e. capsule. The surrounding mesenchymal connective tissue got arranged in a regular manner and forms tunica albuginea and was further differentiated into tunica fibrosa and tunica vasculosa (Fig. 3). Similar in Gaddi sheep the mesenchymal connective tissue arranged and form the future tunica albuginea on the 65th day of gestation (Shukla, 2015).

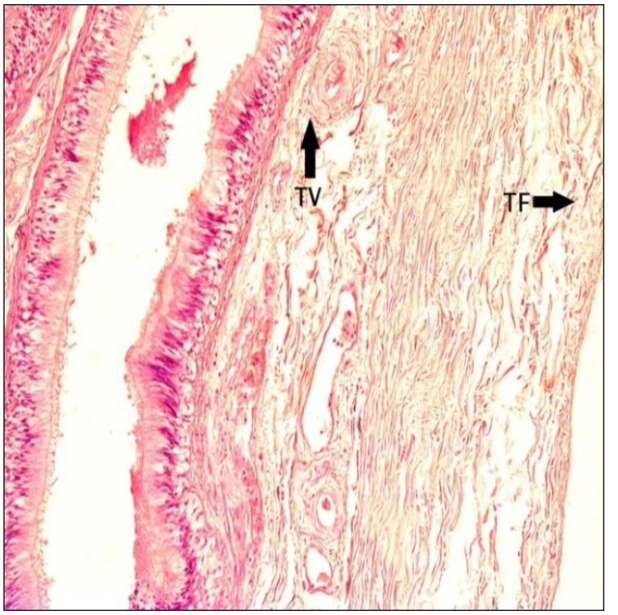

Fig. 3: Photomicrograph of the epididymis of Zovawk, showing differentiation of capsule into outer tunica fibrosa (TF) and inner tunica vasculosa (TV) surrounding the epididymal tubule. (Mayer's hematoxylin and eosin, 100X).

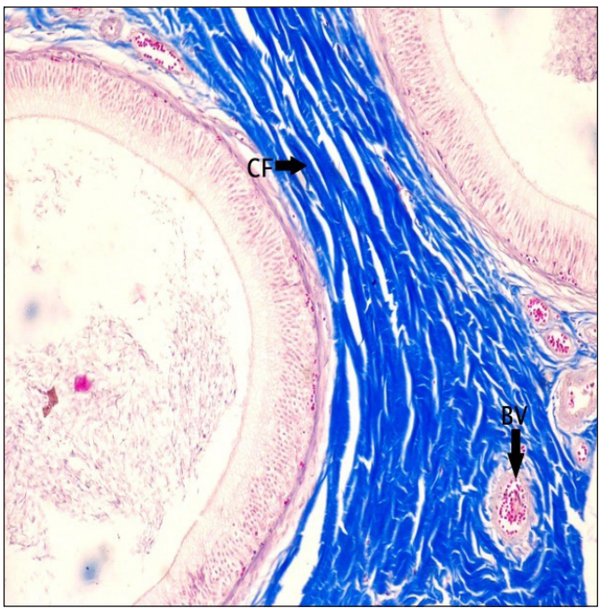

Fig. 4: Photomicrograph of the epididymis of Zovawk, arrow showing collagen fibers (CF) and blood vessel (BV). (Masson's trichrome, 100X).

The tunica albuginea consisted mainly of collagen and reticular fibers (Fig. 4 & 5) as also reported in Gaddi sheep (Shukla, 2015). A thin layer of smooth muscle cells surrounded the epithelium of entire epididymal tubules and the blood vessels (Fig. 6) as also reported in rat (Hoffer *et al*., 1973) and bull (Eurell and Brian, 2006). However, a thin layer of smooth muscle cells surrounded the epithelium of the entire epididymal duct in the bovine fetus and the thickness of these layers increased distally (Moustafa and Hafez, 1971).

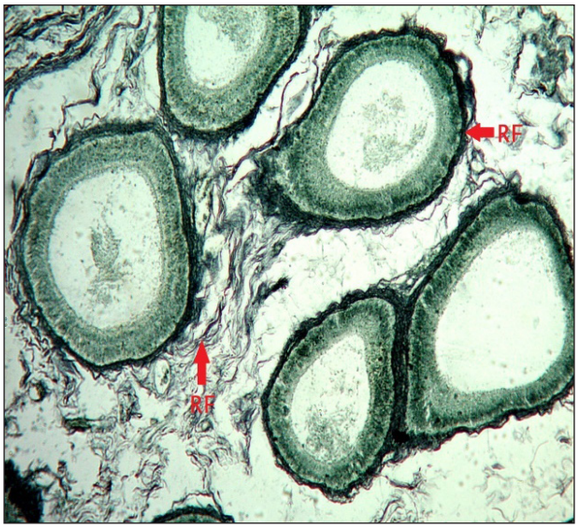

Fig. 5: Photomicrograph of the epididymis of Zovawk, arrow showing reticular fibers (RF). (Gomori's silver stain, 100X).

The parenchyma consists of tubules with distinct boundaries of connective tissues. A thin layer of smooth muscle cells surrounds the epithelium of entire epididymal tubules. The epithelium of epididymis tubule consists of several cell types PC (Principal cells), the BC (Basal cells) and halo cells. The principal cells are the tall columnar cells that extend the full thickness of the epithelium basal lamina to the lumen. The apical surface of PC is covered with long stereocilia, which often branched near their base (Fig. 7). The lumen of the epididymis contains spermatids and breakdown products in addition to spermatozoa (Fig. 8) as also reported in rat (Hoffer *et al*., 1973), monkey (Nabeyama and Leblon, 1974) and bull (Eurell and Brian, 2006). However, the epithelium of the epididymis in hamster is composed of several cell types (Flickinger *et al*., 1978). The Principle cells are the most numerous and are accompanied by the small BC (Basal cells) with few organelles and by halo cells (Flickinger *et al*., 1978).

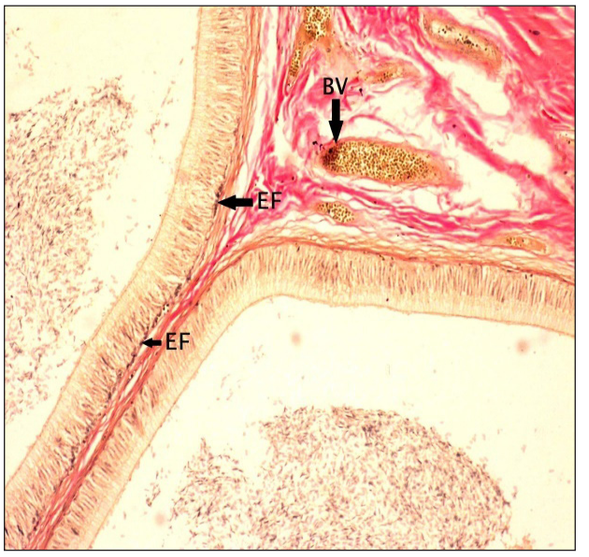

Fig. 6: Photomicrograph of the epididymis of Zovawk, arrow showing elastic fibers (EF) and Blood vessel (BV). (Verhoeff's, 100X).

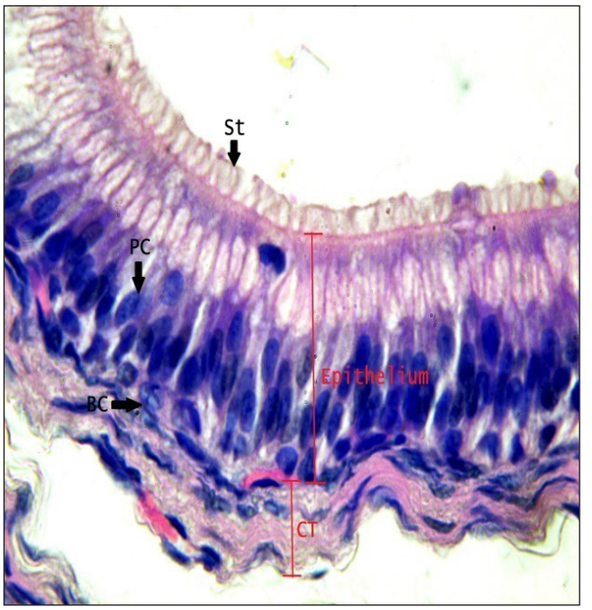

Fig. 7: Photomicrograph of an epididymal tubule of Zovawk, showing connective tissue (CT), epithelium, Principal cells (PC), Basal cells (BC) and Stereocilia (St). (Mayer's hematoxylin and eosin, 1000X).

The lining epithelium of efferent ductules was simple cuboidal to low columnar with scattered basal cells. The lining epithelium (Fig. 7) of ductus epididymis and ductus deferens was pseudostratified columnar epithelium as also reported in bovines (Eurell and Brian, 2006). However, the ductus epididymis was lined by pseudostratified columnar epithelium surrounded by a small amount of loose connective tissue in buffalo (Singh and Dhingra, 1971), ram (Naidu, 1991) and horse (Gaykee *et al*., 2008). The epididymal duct was simple cuboidal to low columnar cells, which differentiated later on into the characteristic tall columnar cells with stereocilia in bovine fetus (Russe and Sinowatz, 1991).

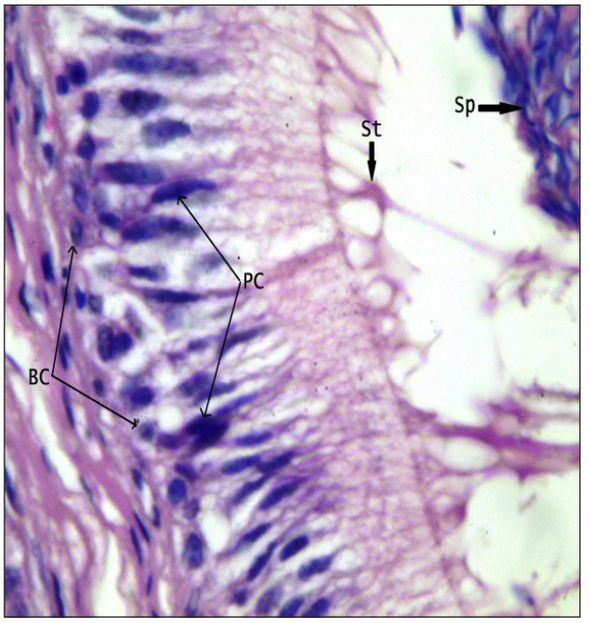

Fig. 8: Photomicrograph of an epididymal tubule of Zovawk showing principal cells (PC), basal cells (BC), stereocilia (St) and spermatozoa (Sp) in the lumen. (Mayer's hematoxylin and eosin, 1000X).

Histochemical observations

Periodic Acid Schiff staining: The carbohydrates activity was detected showing the various intensity of magenta colour. The basement membrane of the tubule, blood vessel and stereocilia shows the intense activity of carbohydrates. Weak activity of carbohydrates was seen in tunica albuginea and the lining epithelium (Fig. 9).

Fig. 9: Photomicrograph of the epididymis of Zovawk, Arrow showing carbohydrates activity in the blood vessel (BV), Basement membrane of the tubule (BMT) and Stereocilia (St). (Periodic acid Schiff, 100X).

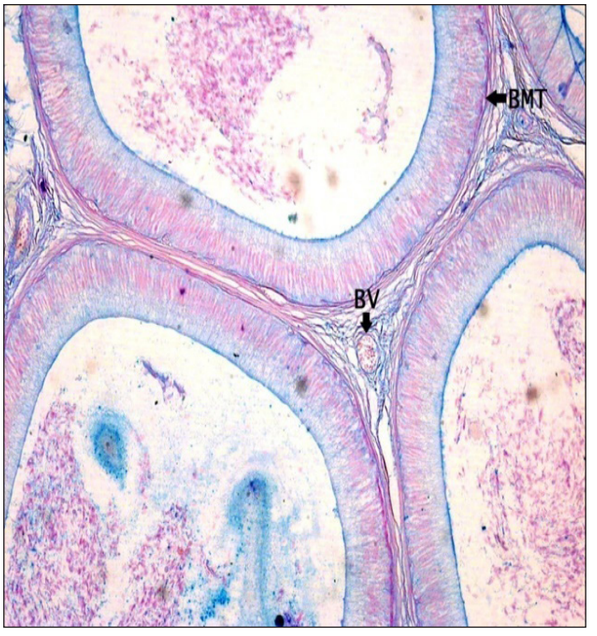

Fig. 10: Photomicrograph of the epididymis of Zovawk, arrow showing acidic mucopolysaccharides activity in Basement membrane of the tubule (BMT) and blood vessel (BV). (Alcian blue, 100X).

However, the basement membrane of epididymis shows strong activity, lining epithelium and blood vessel shows moderate activity, tunica albuginea and peritubular connective tissue shows the weak activity of PAS in Gaddi sheep (Shukla, 2015).

Alcian blue (AB) staining: This stain was used for demonstration of acidic mucopolysaccharide, which stains blue in colour. The basement membrane of tubule and stereocilia shows the moderate activity of acid mucopolysaccharides. The weak activity was observed in tunica albuginea and blood vessels (Fig. 10). However, the basement membrane of the epididymis and peritubular connective tissue shows moderate activity. The tunica albuginea and blood vessel showed the weak activity of Alcian blue in Gaddi sheep (Shukla, 2015).

CONCLUSION

The knowledge of spermatogenesis, especially in the male pig is important for protection against extinction. The knowledge of the basic morphology of reproductive organs is a vital tool in the assessment of breeding soundness and fertility potential in domestic animals. However, there is no literature pertaining to these reproductive systems in Zovawk. Therefore, the present study is aimed for promotion and advancement of the anatomical knowledge at the gross morphological, histological and histochemical studies on the epididymis of Zovawk. The results of the present study can be used as research baseline in other animals.

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