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Research Article

HISTOLOGY, HISTOCHEMISTRY AND SCANNING ELECTRON MICROSCOPY OF THE ETHMOTURBINATES OF YOUNG PIGS

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ABSTRACT

The present study was conducted on 12 young pigs of 8-10 months age of local mixed breed to determine the structural features of the ethmoturbinates. Major portion of the ethmoturbinates was lined by pseudostratified columnar ciliated epithelium with PAS positive goblet cells. The caudal portion of the ethmoturbinates adjacent to the cribriform plate of the ethmoid bone was lined by olfactory epithelium. The olfactory mucosa contained sero-mucous type of Bowman's glands and increased number of nerve bundles. Predominantly occurring mucous glands were strongly PAS positive especially for mucopolysaccharides, hyaluronic acid and sialomucins. Scanning electron microscopy revealed that the rostral most portions of ethmoturbinates were characterized by presence of tufts of cilia which formed a dense mat like arrangement. The caudal part was having additional microvillus cells studded with microvilli of different shapes and a few brush cells. The olfactory region presented different arrangements of olfactory vesicles, dendrites and axons.

Key words: Ethmoturbinates, histology, olfactory, pig, scanning electron microscopy

The ethmoturbinates in their caudal part carry receptor cells responsible for detecting and discriminating between odors of different substances. Olfactory epithelium also plays an important role to discriminate between oestrus and anoestrous female through Flehman's reflex. Dorries *et al.* (1997) demonstrated role of olfactory epithelium in detection of androstenone mediated sexual behavior in blocked vomeronasal organ in pigs. The maternal recognition of sow to identify its piglets in the early post-partum is also mediated by olfactory epithelium (Maletinska *et al*., 2002). The histology and histochemistry of ethmoturbinates had been studied in goat (Kumar *et al.,*1992; 1993), camel (Suman *et al.,* 1998), horse (Kumar *et al.,* 2000; Lee *et al*., 2016), sheep (Ganganaik *et al.,* 2009), pigs (Kalita *et al*., 2014), dog and sheep (Kayoi *et al*., 2010). The literature is also available on scanning electron microscopy (SEM) of ethmoturbinates in goat (Kumar *et al.,* 1999), horse (Kumar *et al.,* 2000), sheep (Ganganaik *et al.,* 2007), dog and sheep (Kayoi *et al*., 2010) and buffalo calves (Kumar *et al.,* 2011). Keeping in view the importance of ethmoturbinates, the present study describes light and ultrastructural details of ethmoturbinates in young pigs and its comparison with other domestic animals.

MATERIALS AND METHODS

The present study was conducted on 12 young male pigs of 8-10 months age, of local mixed breed. The heads were procured from local slaughter house immediately after decapitation and fixed in 10% neutral buffered formalin solution for 48 hours. Six heads were used to study the histomorphological and histochemical studies of ethmoturbinates.The tissues were collected from cranial, mid and caudal portions of ethmoturbinates. The fixed tissues were processed for routine paraffin technique of light microscopy. The paraffin sections of 5-6 µ were cut and stained by routine Harris' hematoxylin and eosin method, PAS-Alcian blue, Alcian blue, colloidal iron method, performic acid Alcian blue method and Bielschowsky's method (Luna, 1968). Fresh tissues from six pig's heads for SEM were fixed in 2% glutaraldehyde solution for 6-8 hours after thorough washing in chilled 0.1M phosphate buffer (pH 7.4). The tissues were again washed twice with 0.1M phosphate buffer and rest of the procedure was carried out at EM Lab., AIRF, JNU, New Delhi. The tissues after sputter coating with gold were viewed under scanning electron microscope (Zeiss EVO-40).

RESULTS AND DISCUSSION

Histology and Histochemistry: The ethmoturbinates in pig were divided into outer and inner surfaces by an irregular osseous plate. The rostral and mid portions of the ethmoturbinates were lined by pseudostratified columnar ciliated epithelium with goblet cells (Fig.1 & 2). The epithelium presented six to eight rows of nuclei

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placed at different heights. The goblet cells showed positive reaction to the acidic mucopolysaccharides as demonstrated by PAS-Alcian blue, colloidal iron and Mayer's mucicarmine methods (Fig. 3). The Alcian blue method demonstrated stronger activity in goblet cells for the presence of weakly sulfated mucopolysaccharides, sailomucins and hyaluronic acid. A few lymphoid cells especially lymphocytes were infiltrated in between the epithelial cells. Similar type of epithelium had been observed in rostral portion of ethmoturbinates in goat (Kumar *et al.,* 1993), dog (Kumar *et al.,* 1994; Barrios *et al.,* 2014), camel (Suman *et al.,* 1998) and horse (Kumar *et al.,* 2000; Lee *et al*., 2016). However, the ethmoturbinates were lined by pseudo-stratified columnar epithelium having dendrites or the sensory cilia in mithun and zebu (Kalitha and Bhattacharya, 2004), sheep (Ganganaik *et al.,* 2009), pigs (Kalita *et al*., 2014), dog and sheep (Kayoi *et al*., 2010). The propria submucosa had few sero-mucous glands, venous caverns of varying shapes (Fig. 2) and nerve bundles. The sub-epithelial portion was having more distribution of lymphoid tissue which was almost uniform and regular in nature. Follicle associated epithelium was also observed where the goblet cells were absent (Fig. 2 & 3).

The caudal portion of the ethmoturbinates close to the cribriform plate of the ethmoid bone was lined by pseudostratified columnar epithelium having dendrites or the sensory cilia (Fig. 4). The epithelium was consisted of basal, supporting and olfactory cells. The lymphoid cells particularly lymphocytes were infiltrating in between the cell types. Some of the lymphocytes reached up to the free surface of the epithelium. The free surface of epithelium was having uniform border because of the presence of the olfactory dendrites. The ethmoturbinates with olfactory epithelium had similar distribution in the goat (Kumar *et al.,*1992, 1993), buffalo (Gupta *et al*., 1994), dog (Kumar *et al.,* 1994; Barrios *et al.,* 2014), camel (Suman *et al.,* 1998), horse (Kumar *et al.,* 2000; Lee *et al*., 2016), mithun and zebu (Kalita and Bhattacharya 2004), sheep (Ganganaik *et al.,* 2009), pigs (Kalita *et al*., 2014), and dog and sheep (Kayoi *et al*., 2010). The propria submucosa had vertically oriented clusters of sero-mucous type of Bowman's glands, nerve bundles and a few blood capillaries. The Alcian blue method demonstrated stronger activity in the mucous acini for the presence of weakly sulfated mucopolysaccharides, sailomucins and hyaluronic acid (Fig. 5). These glands also presented positive reaction for colloidal iron and Mayer mucicarmine methods indicating presence of acidic mucopolysaccharides (Fig. 6). Performic acid-Alcian blue method showed positive reaction in mucous acini showing presence of less than 4% of cysteine (Fig. 7). Similar type of glands had been observed in ethmoturbinates of pigs (Kalita *et al*., 2014). However, the glandular acini were serous in nature in buffalo (Gupta *et al*., 1994), goat (Kumar *et al*., 1992, 1993) and camel (Suman *et al*., 1998). In between the glandular clusters, large numbers of nerve bundles oriented in different profiles were also observed (Fig. 8) as reported in buffalo (Gupta *et al*., 1994), camel (Suman *et al*., 1998) and goat (Kumar *et al*., 1993).

Scanning Electron Microscopy: The rostral and mid portions of the ethmoturbinates were characterized by the presence of tufts of cilia which formed a dense mat like arrangement (Fig. 9 & 10). The densely packed cilia were directed caudally and these were of uniform size and shape with smooth surface except towards the tip where bulb like arrangement was observed (Fig.11). At some places, the pattern of cilia beating in different waves was also observed. The densely arranged tufts of cilia masked the appearance of other cell types. The microvillus cells having microvilli of different size and a few brush cells were observed in areas where density of the ciliated cells was comparatively lesser (Fig. 9,12 &13). In between these microvillus cells, a few brush cells were also present. The microvilli of some cells were very extensive and intermingled with that of adjacent cell forming small bridge like pattern. The goblet cells were visible only in the region where density of ciliated cells was less. The opening of glandular ducts was seen in between ciliated and microvillus cells towards free surface of the epithelium (Fig. 12 & 13) as reported in goat (Kahwa and Balemba, 1998; Kumar *et al*., 1999), horse (Kumar *et al.,* 2000), sheep (Ganganaik *et al*., 2007) and buffalo calves (Kumar *et al.,* 2008). The olfactory region was confined to the cribriform plate of ethmoid bone and presented different arrangements of olfactory vesicles with sensory cilia, dendrites and axons (Fig.14 & 15). The olfactory vesicles were numerous presenting dendrites in different directions and formed a dense interwoven network. In some regions, these vesicles were lesser and the density of olfactory dendrites

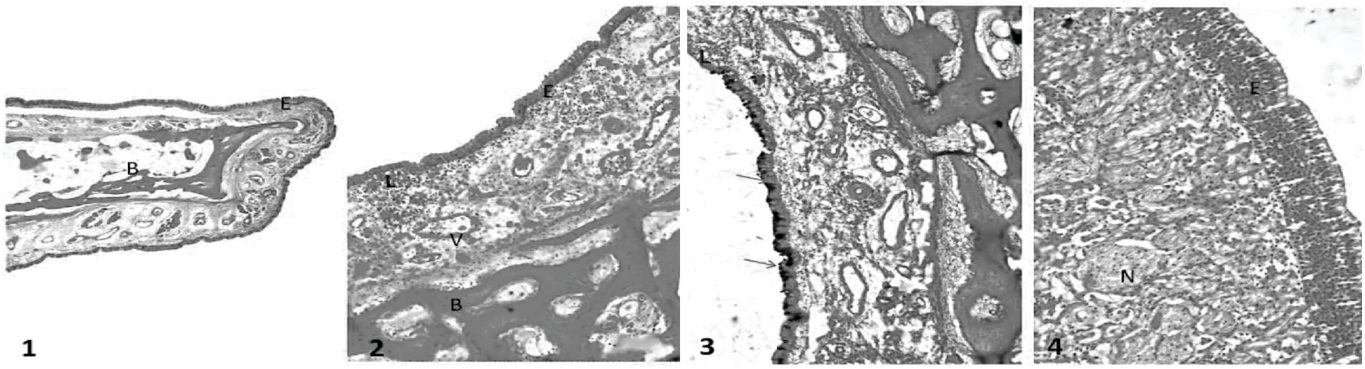

Fig.1. Photomicrograph showing pseudostratified columnar ciliated epithelium with goblet cells (E) and osseous plate (B) at the tip region of ethmoturbinates (H. & E. x 100); Fig.2. Photomicrograph showing pseudostratified columnar ciliated epithelium with goblet cells (E), FAE (L), venous caverns (V) and osseous plate (B) at the mid region of ethmoturbinates (H. & E. x 200); Fig.3. Photomicrograph showing positive activity of acidic mucopolysaccharides in the goblet cells at the tip region of ethmoturbinates (PAS AB x 100); Fig.4. Photomicrograph at higher magnification showing pseudostratified columnar epithelium olfactory cells and nerve bundles (N) at the base region of ethmoturbinates (H. & E. x 400)

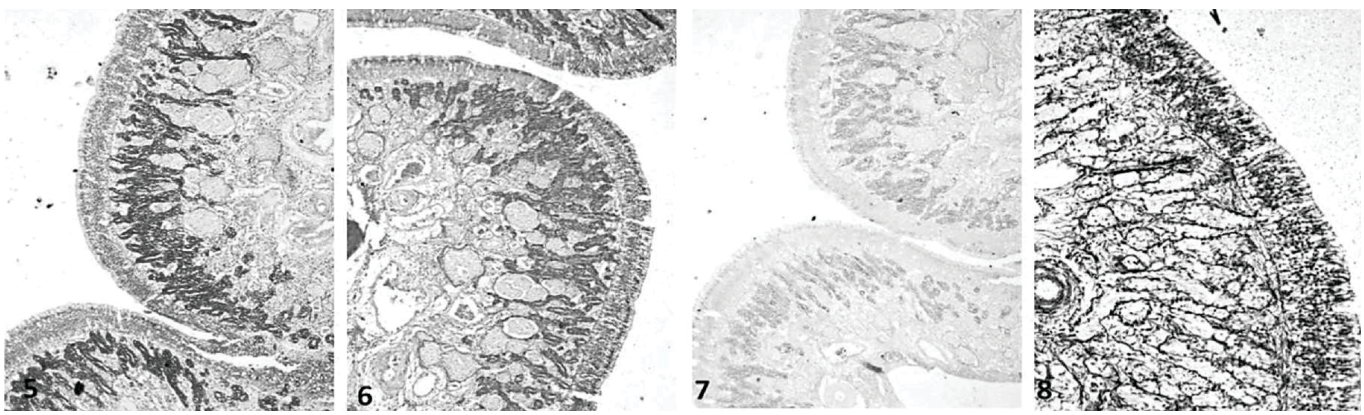

Fig.5. Photomicrograph showing presence of hyaluronic acid and sialomucins in the glands (blue color) at the base region of ethmoturbinates (Alcian blue x 200); Fig.6. Photomicrograph showing presence of acidic mucopolysaccharides in the glands (deep blue color) at the base region of ethmoturbinates (Colloidal iron method x 200); Fig.7. Photomicrograph showing positive activity of 4% cystine in the glands (light blue color) at the base region of ethmoturbinates (Performic acid Alcian blue method x 100); Fig.8. Photomicrograph showing nerve bundles at the base region of ethmoturbinates (Bielschowsky's method x 400).

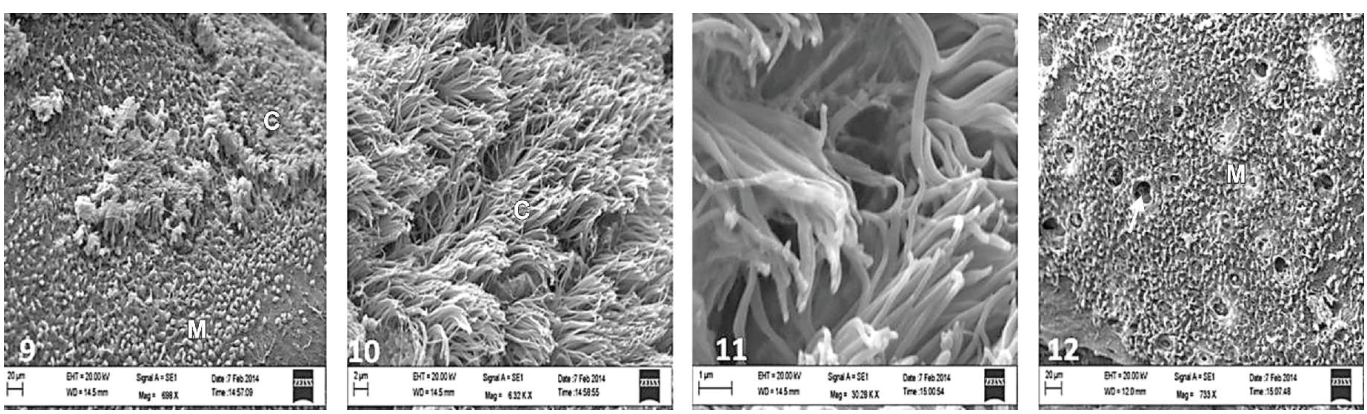

Fig.9. Scanning electron micrograph showing microvillus cells (M) and cilia (C) of respiratory epithelium (x 698); Fig.10. Scanning electron micrograph at higher magnification showing dense carpet of cilia (C) of respiratory epithelium (x 6320); Fig.11. Scanning electron micrograph at higher magnification showing tip of cilia showing bulb like arrangement in respiratory epithelium (x 30280); Fig.12. Scanning electron micrograph of respiratory epithelium showing the microvillus (M), opening of glandular ducts and goblet cells (x 733).

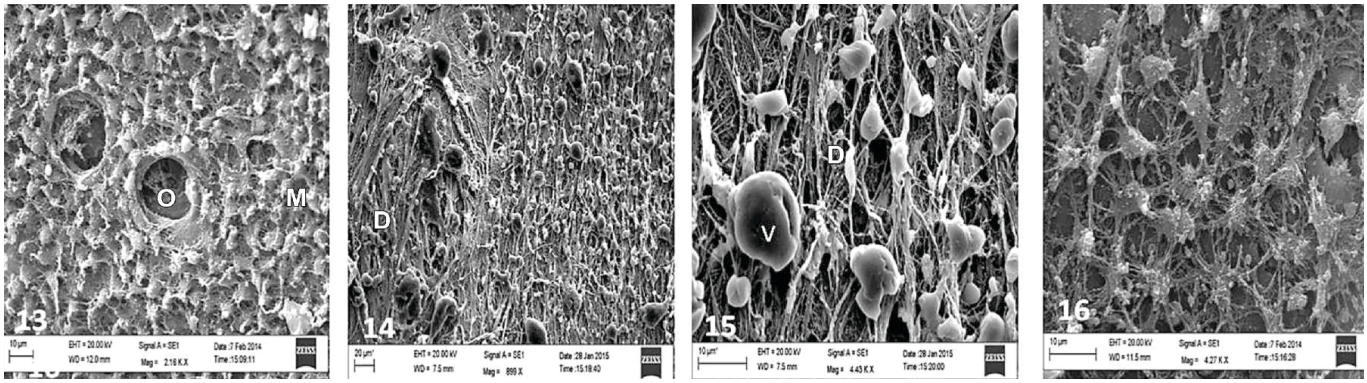

Fig.13. Scanning electron micrograph of respiratory epithelium at higher magnification showingthe goblet, opening of glandular ducts (O) and microvillus cells (M) (x 2160); Fig.14. Scanning electron micrograph showing a dense meshwork of olfactory dendrites (D) and olfactory vesicles (x 899); Fig.15. Scanning electron micrograph at higher magnification showing a dense meshwork of olfactory dendrites (D) and olfactory vesicles (V) (x 4430); Fig.16. Scanning electron micrograph showing a dense meshwork of olfactory vesicles (x 4270).

and axons were more numerous (Fig. 15 & 16). At isolated places, mucous trapped in between dendrites and axon was also observed asreported in goat (Kumar *et al.,* 1999), horse (Kumar *et al.,* 2000), sheep (Ganganaik *et al.,* 2007), dog and sheep (Kayoi *et al*., 2010) and buffalo calves (Kumar *et al.,* 2008).

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