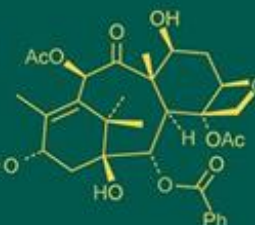
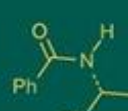
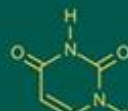
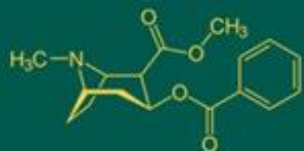


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Histomorphological and histochemical studies on the trachea of uttara fowl during post-hatching development

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Abstract

The present study aimed to characterize the age-related histological, histochemical, and histomorphometric changes in the trachea of post-hatch birds at 0, 7, 28, and 112 days of age, with 6 birds per age group. Histological examination revealed that the tracheal wall was composed of the lamina epithelialis mucosae, lamina propria-submucosa, hyaline cartilaginous rings, bundles of skeletal muscle, and an outer tunica adventitia. The mucosal lining consisted of ciliated pseudostratified columnar epithelium containing numerous intraepithelial mucous glands. The height of the ciliated pseudostratified columnar epithelium increased significantly ($p < 0.05$) with advancing age. Likewise, the number of intraepithelial alveolar glands progressively increased during post-hatch development. The lamina propria-submucosa comprised loose connective tissue rich in collagen and reticular fibres, numerous blood vessels, and scattered lymphocytes, and was continuous with the perichondrium surrounding the tracheal cartilaginous rings. No evidence of ossification was observed in the tracheal cartilage up to 112 days of age. The mean width of the cartilaginous rings increased progressively from $46.79 \pm 5.70 \mu\text{m}$ at day 0 to $69.85 \pm 3.20 \mu\text{m}$, $107.95 \pm 8.50 \mu\text{m}$, and $155.06 \pm 14.66 \mu\text{m}$ at 7, 28, and 112 days of age, respectively. The tunica adventitia consisted of connective tissue containing numerous blood vessels and scattered adipocytes surrounding the bundles of skeletal muscle. Histochemical analysis demonstrated strong positive reactions to Periodic Acid-Schiff (PAS) and Carmine staining in both the cartilaginous rings and intraepithelial glands. Furthermore, the intraepithelial glands exhibited intense PAS-Alcian Blue positivity, indicating the presence of both neutral and acidic mucopolysaccharides (mucins).

Keywords: Uttara fowl, Histology, Histochemical, Trachea

1. Introduction

Indigenous chicken breeds play a pivotal role in sustainable backyard poultry production owing to their adaptability to diverse agro-climatic conditions and their efficient utilization of locally available feed resources, including kitchen waste, crop residues, and other low-cost scavenging materials. These attributes enable them to produce nutritionally valuable meat and eggs under resource-limited production systems (Pal *et al.*, 2020) [15]. Among the indigenous poultry genetic resources of the Indian Himalayan region, the Uttara fowl is widely reared in the hilly terrains of Uttarakhand as a dual-purpose breed for both meat and egg production (Kaur *et al.*, 2010) [11].

The avian respiratory system possesses several unique anatomical and functional adaptations that distinguish it from that of mammals. These specializations primarily facilitate the high metabolic demands associated with flight, efficient thermoregulation, and vocalization (King, 1975) [12]. Anatomically, the respiratory tract is divided into the upper respiratory tract, comprising the nostrils, nasal cavity, and pharynx, and the lower respiratory tract, which includes the larynx, trachea, syrinx, bronchi, and lungs. Together, these structures ensure efficient air conduction and gaseous exchange (Al-Mussawy *et al.*, 2010) [3]. During respiration, inspired air passes sequentially through the nasal cavity, larynx, trachea, syrinx, and bronchi before reaching the pulmonary gas-exchange surfaces (Corral and Pastor, 1995) [6]. Unlike mammals, the trachea of birds is supported by complete hyaline cartilaginous rings that overlap and interlock with adjacent rings, providing both structural rigidity and flexibility while maintaining airway patency (Dellmann and Eurell, 1998) [8].

The Uttara fowl inhabits high-altitude regions where reduced atmospheric oxygen concentration imposes unique physiological challenges. Such environmental conditions may induce structural adaptations within the respiratory tract that enhance respiratory efficiency and facilitate survival under hypoxic conditions. However, detailed information regarding the posthatch development

and microscopic architecture of the trachea in this indigenous breed remains scarce. Therefore, the present study was undertaken to investigate the age-related histological, histochemical, histomorphometric characteristics of the trachea in Uttara fowl, thereby providing baseline anatomical information that may contribute to a better understanding of respiratory adaptation in high-altitude indigenous poultry.

2. Materials and methods

The Uttara fowl were reared at the poultry farm of the College of Veterinary Sciences and Animal Husbandry, GB Pant University of Agriculture and Technology (GBPUAT), Pantnagar, under standard management practices. The birds were provided with adequate feed, clean drinking water, and appropriate health care throughout the study period.

The present study was conducted on 24 Uttara fowl, which were randomly divided into four groups comprising six birds each at 0, 7, 28, and 112 days of age. Prior approval was obtained from the Institutional Animal Ethics Committee (IAEC) before the commencement of the experiment. Following humane slaughter, the thoracic cavity of each bird was opened by careful dissection, and the trachea was excised and fixed in neutral buffered formalin. For histological examination, the fixed tissue samples were dehydrated through ascending grades of alcohol, cleared in xylol, and embedded in paraffin wax. Paraffin blocks were sectioned at a thickness of 6 μm and stained with haematoxylin and eosin, Masson's trichrome, Van Gieson stain for collagen fibres, Periodic Acid-Schiff (PAS) stain, modified Mowry's (1958) colloidal iron stain for acid mucopolysaccharides, Alcian Blue-Periodic Acid-Schiff (AB-PAS; pH 2.5) for histochemical demonstration of mucosubstances (Luna, 1972) [13], and the acetone-Sudan Black method for bound lipids (Bancroft and Stevens, 1977) [4]. Micrometrical parameters evaluated included the mean height of the epithelial cells, width of the lamina propria-submucosa, and thickness of the tracheal cartilages. The micrometrical data obtained were subjected to one-way analysis of variance (ANOVA) to evaluate age-related differences and test the level of statistical significance, following the procedure described by Snedecor and Cochran (1994) [21].

3. Results and discussion

Histological examination of the trachea in Uttara fowl demonstrated a well-organized wall comprising the lamina epithelialis mucosae, lamina propria-submucosa, complete cartilaginous rings, bundles of skeletal muscle, and an outer tunica adventitia. The mucosal lining consisted of ciliated pseudostratified columnar epithelium containing numerous intraepithelial mucous glands. Comparable histological architecture has previously been described in male turkey (Al-Mussawy *et al.*, 2010) [3], domestic fowl (Bello *et al.*, 2018) [5], partridge (Rajathi *et al.*, 2021) [18], and other avian species (Sakr *et al.*, 2022) [19]. In contrast, Pourlis *et al.* (2018) [16] reported the presence of non-ciliated epithelial cells in the trachea of Japanese quail, indicating species-specific variation in tracheal epithelial organization.

Two distinct epithelial cell populations were identified within the mucosal lining. The predominant cell type consisted of ciliated columnar cells with apically located elongated nuclei, whereas the second population comprised smaller basal cells possessing centrally located round nuclei. A similar cellular arrangement has been reported in partridge (Rajathi *et al.*, 2021) [18]. Notably, greater epithelial height and increased density of

intraepithelial mucous glands were observed in regions where adjacent cartilaginous rings overlapped, suggesting enhanced secretory and mucociliary activity in these mechanically stressed areas. Furthermore, the number of intraepithelial alveolar glands increased progressively with age, corroborating observations in Japanese quail (Alan and Liman, 2010) [2]. Histologically, the glandular alveoli were lined by elongated mucous-secreting cells characterized by broad basal portions containing oval nuclei and apically narrowed cytoplasm exhibiting a foamy appearance following haematoxylin and eosin staining.

Histochemical analyses demonstrated strong Periodic Acid-Schiff (PAS) and Carmine positivity within the intraepithelial glands, indicating abundant glycoconjugates. Intense PAS-Alcian Blue staining further confirmed the presence of both neutral and acidic mucins, consistent with observations in chicken (Hodges, 1974) [10]. Similarly, Dar *et al.* (2018) [7] reported strong PAS and Alcian Blue reactivity in the mucous glands and goblet cells of the respiratory tract in Kuttanad duck. The intensity of mucin staining increased with advancing age, suggesting progressive maturation of the tracheal secretory apparatus, a finding that agrees with Alan and Liman (2010) [3, 2]. Moderate Sudan Black B reactivity within the epithelium indicated the presence of lipid-containing cellular components. The mean height of the ciliated pseudostratified columnar epithelium increased significantly with advancing age. The epithelial height at 0, 7, 28, and 112 days was 16.39 ± 0.58 , 24.10 ± 0.98 , 39.90 ± 1.50 , and 50.09 ± 1.80 μm , respectively. In comparison, the mean height of the pseudostratified ciliated columnar epithelium in chicken has been reported to range from 20 to 60 μm (Pal, 1968) [14]. In pigeon, the epithelial height ranged from 9.18 to 12.20 μm (Rajathi *et al.*, 2018) [17], whereas in geese, cattle egret, and house sparrow, it was reported as 225.06 ± 1.50 , 185.20 ± 1.10 , and 216.60 ± 1.30 μm , respectively (Sakr *et al.*, 2022) [19].

The lamina propria-submucosa consisted of connective tissue containing collagen fibres (Fig. 5), elastic fibres (Fig. 4), and reticular fibres (Fig. 3), along with numerous blood vessels and a few lymphoid cells. Similarly, Waad (2015) reported the presence of loose connective tissue with dense aggregations of lymphocytes in the trachea of coot birds. In contrast, large bundles of collagen fibres accompanied by heavy lymphocytic infiltration were observed in the lamina propria-submucosa of indigenous male turkey (Al-Mussawy *et al.*, 2010) [3]. The presence of elastic fibres within the submucosa has also been documented in the Japanese quail (Demirkan *et al.*, 2007) [9]. In the present study, the lamina propria-submucosa lacked both mucous and serous glands; however, numerous seromucous glands were reported in the lamina propria-submucosa of adult Iraqi pigeon (Suhaib, 2021) [22]. The width of the lamina propria-submucosa at 0, 7, 28, and 112 days was 28.13 ± 1.70 , 44.95 ± 1.50 , 67.98 ± 2.50 , and 125.74 ± 9.40 μm , respectively. In comparison, the width of the lamina propria-submucosa has been reported to range from 220 to 316 μm in pigeon (Rajathi *et al.*, 2018) [17] and from 323 to 405 μm in partridge (Rajathi *et al.*, 2021) [18].

The lamina propria-submucosa was continuous with the perichondrium of the tracheal cartilaginous rings, which is in accordance with the observations of Sakr *et al.* (2022) [19] in avian species. The perichondrium of the tracheal cartilage comprised an outer connective tissue layer containing fibrocytes with flattened nuclei, along with collagen and reticular fibres, and an inner layer consisting of chondroblast

cells with elongated nuclei. Chondrocytes embedded within the intercellular matrix were also observed beneath the perichondrium (Fig. 1). The presence of the perichondrium and chondrocytes has similarly been reported in pigeon (Rajathi *et al.*, 2018) ^[17]. The chondrocytes were located within lacunae and were oval to elongated in shape with centrally placed oval nuclei. In some lacunae, more than one chondrocyte was observed. Histological examination also revealed overlapping of adjacent tracheal cartilaginous rings (Fig. 1), a finding comparable to that reported in indigenous male turkey (AL-Mussawy *et al.*, 2010) ^[3]. The width of the tracheal cartilaginous rings at 0, 7, 28, and 112 days was 46.79 ± 5.70 , 69.85 ± 3.20 , 107.95 ± 8.50 , and 155.06 ± 14.66 μm , respectively, whereas the mean width of the cartilaginous rings in pigeon has been reported to be 115 μm (Rajathi *et al.*, 2021) ^[18].

The tracheal cartilage exhibited an intense positive reaction to Periodic Acid-Schiff (PAS) staining (Fig. 7), indicating the presence of glycogen, which is consistent with the findings

reported in chicken by Pal (1968) ^[14]. In contrast, Carmine staining demonstrated a moderate positive reaction for glycogen (Fig. 9). Sudan Black B staining showed a mild to moderate positive reaction in the lamina propria-submucosa, indicating the presence of bound lipids (Fig. 10).

External to the tracheal cartilage, the tunica adventitia was composed of connective tissue containing numerous blood vessels and a few adipocytes, which is in agreement with the findings of AL-Ahmed and Sadoon (2020) ^[1]. The tracheolateralis muscles (skeletal muscles) were firmly attached to the adventitia on both the left and right lateral aspects of the proximal part and approximately the distal half of the trachea. These observations are consistent with the reports of Waad (2015) in coot birds and Sakr *et al.* (2022) ^[19] in avian species. The adipose tissue present within the tunica adventitia exhibited an intense positive reaction to Sudan Black B staining, indicating the presence of bound lipids.

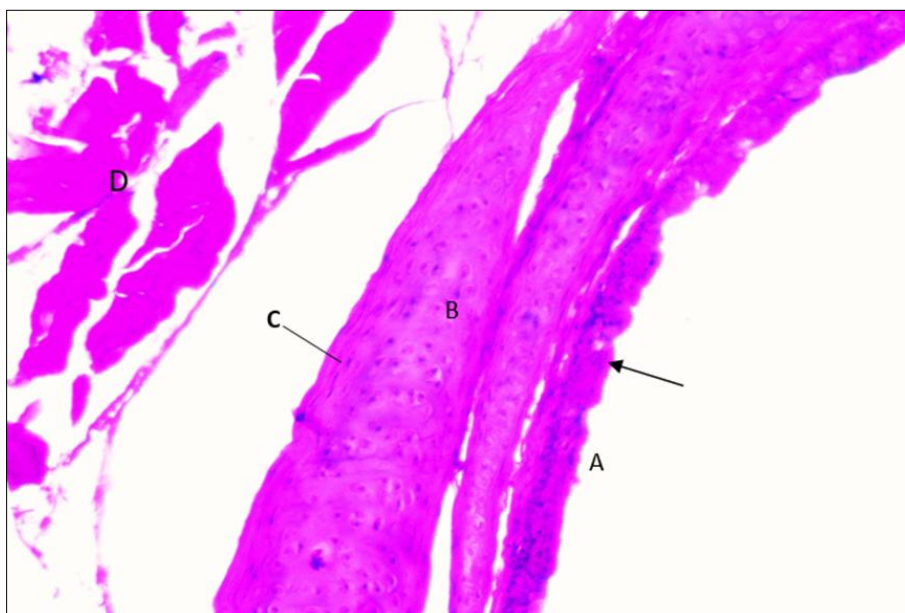


Fig 1: Photomicrograph of trachea in 112days old Uttara fowl showing, A. Pseudo stratified ciliated columnar epithelium, arrow show intraepithelial gland, B. Overlapping Hyaline cartilage, c. Perichondrium, D. skeletal muscle. H&E; X100

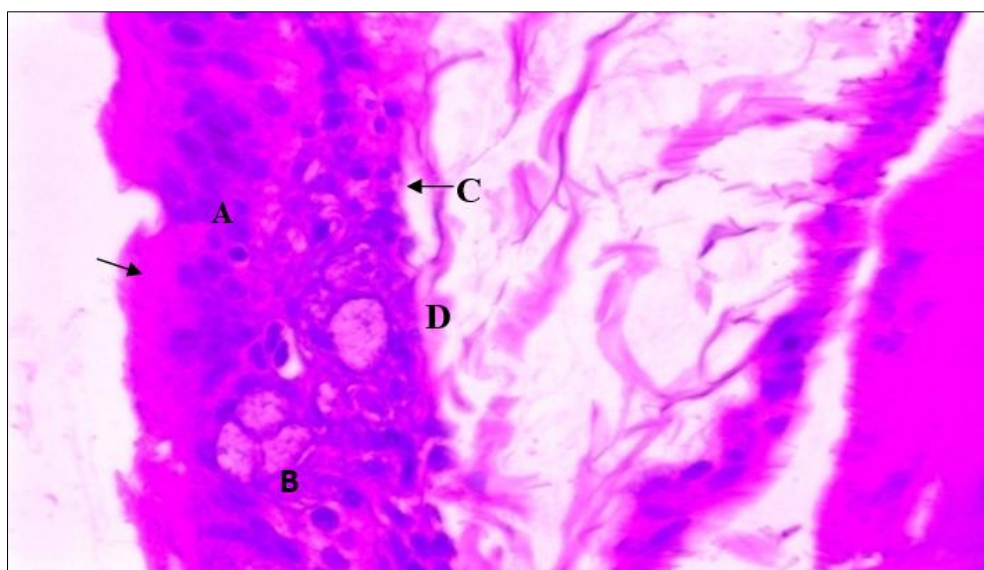


Fig 2: Photomicrograph of trachea in 112days old Uttara fowl showing Arrow. Cilia, A. Pseudo stratified columnar epithelium B. intraepithelial gland C. Basal cells, D. lamina propria submucosa. H&E; X400

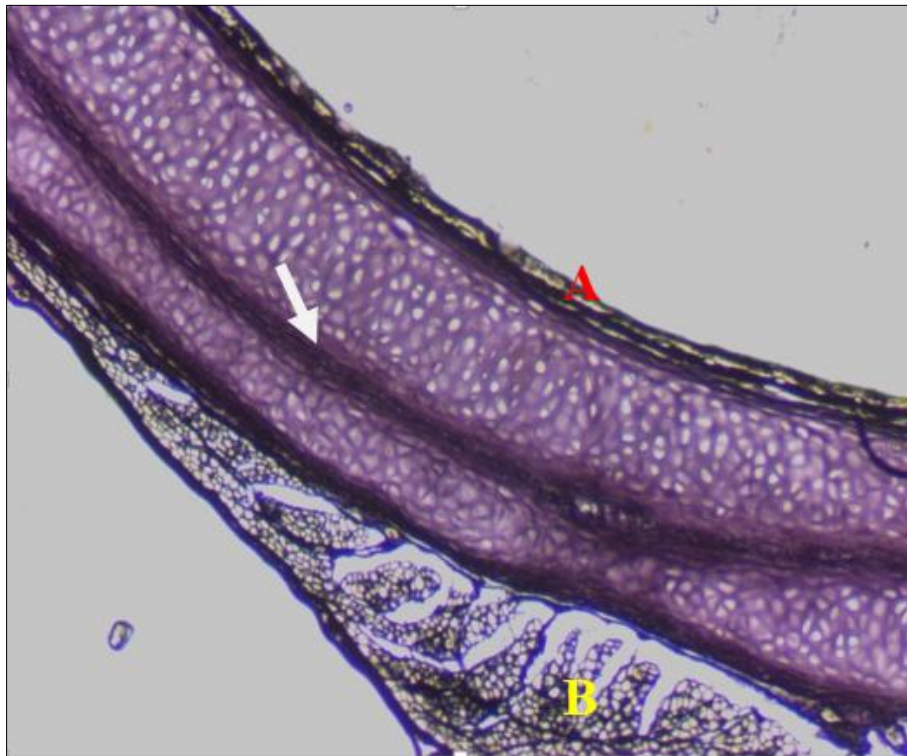


Fig 3: Trachea from 0 day old Uttara fowl showing reticular fibers in (A) lamina propria submucosa (arrow) perichondrium, (B) Bundle of striated muscle. Gordon & Sweet's method; X100

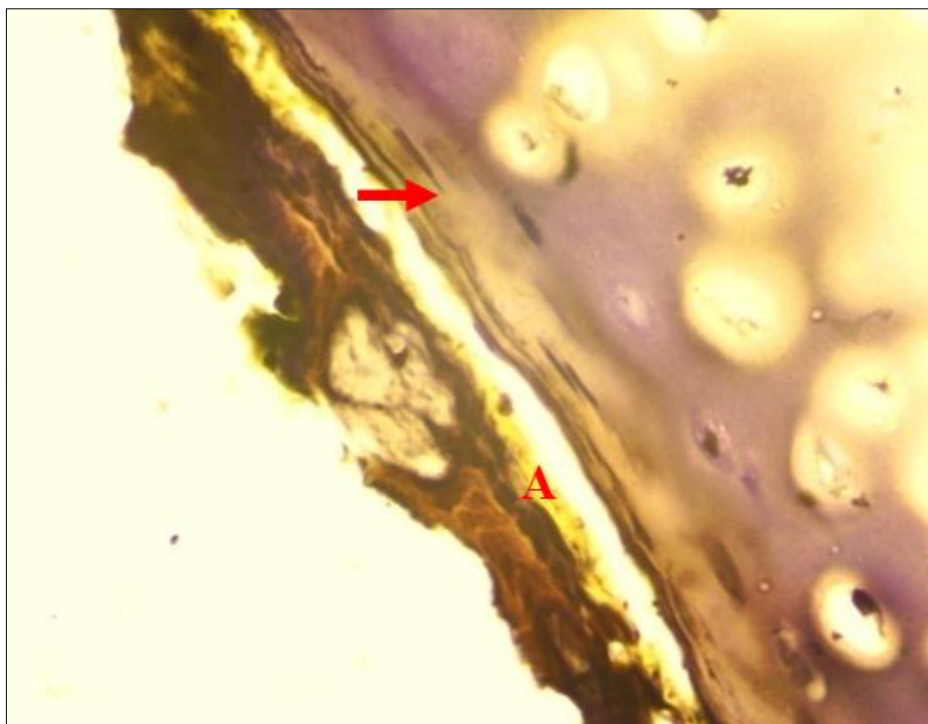


Fig 4: Trachea from 112 day old Uttara fowl showing elastic fibers in (A) lamina propria submucosa, (arrow) perichondrium. Verhoeff's Method; X400

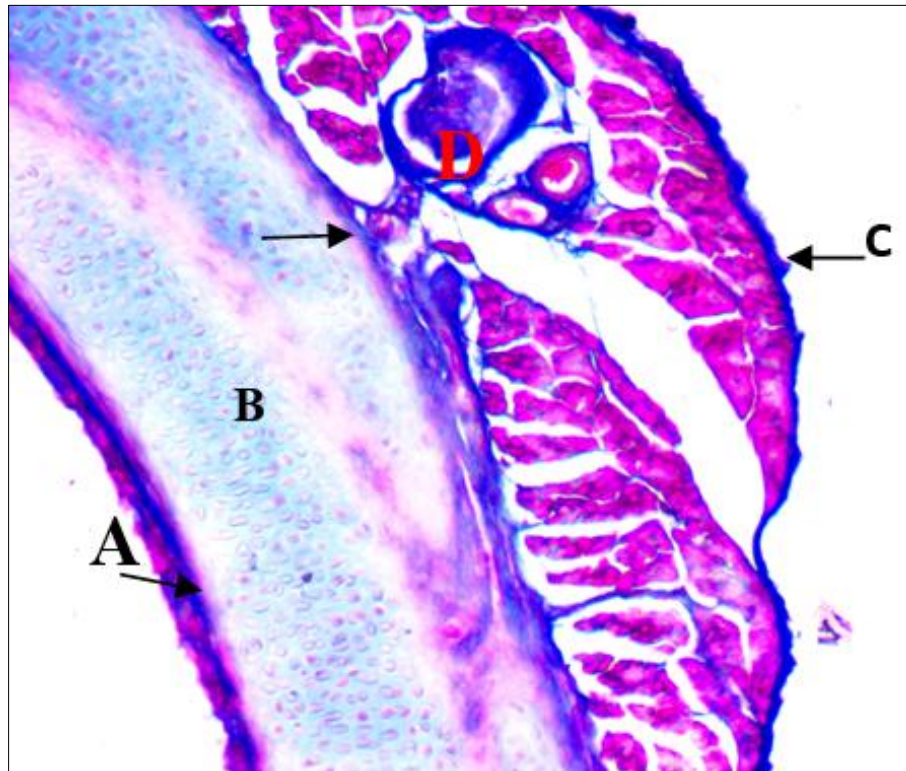


Fig 5: Trachea from 0 day old Uttara fowl showing collagen fibers in (A) lamina propria, (B, Arrow) Hyaline cartilage and perichondrium, (C) Around the Connective tissue of striated muscle (D) Blood vessels. Masson's Trichrome method; X100

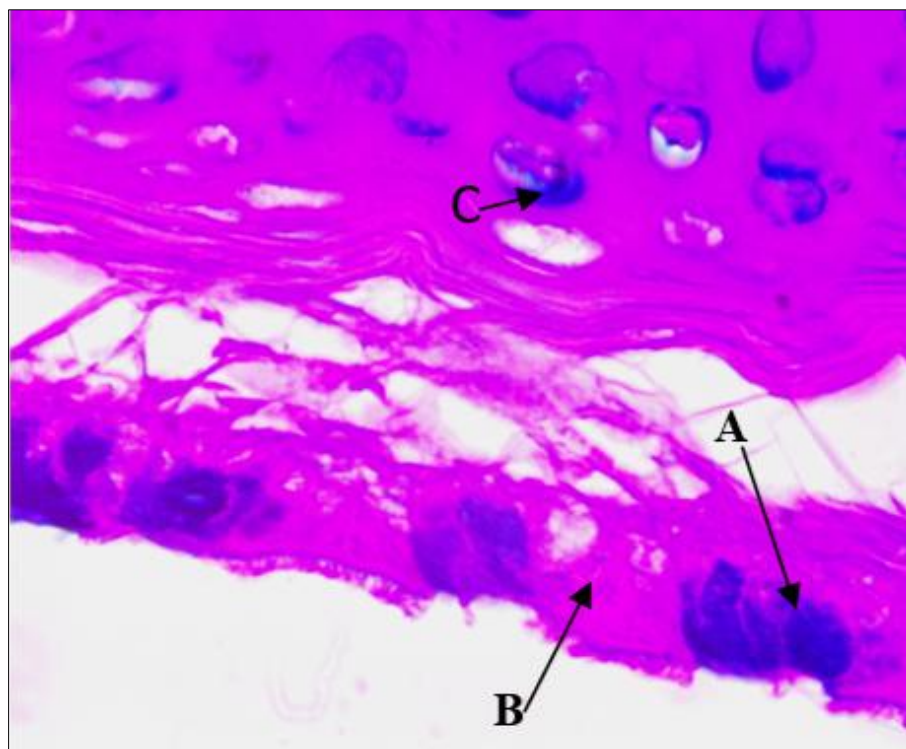


Fig 6: In 112 day old Uttara fowl showing (A) blue color of acidic mucin and (B) red color show neutral mucin in intraepithelial gland of tracheal and (C) inside lacunae. PAS - AB method (Ph- 2.5); X400

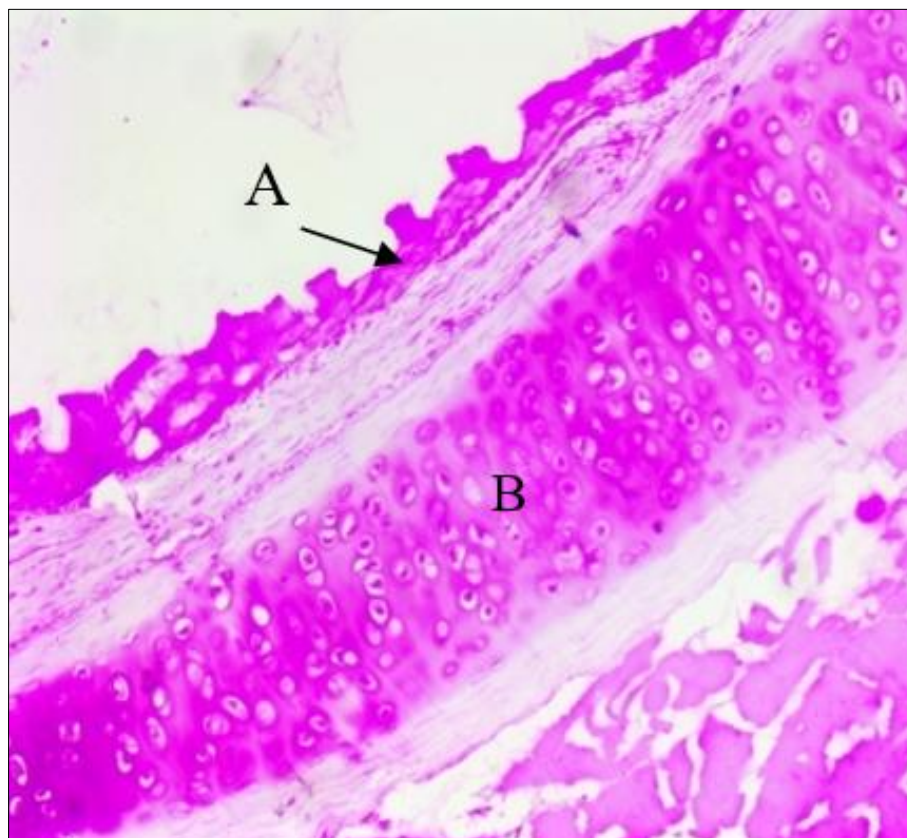


Fig 7: Trachea from 112 days old Uttara fowl showing PAS intense reaction in (A) intraepithelial gland (B) tracheal cartilage. PAS; X100

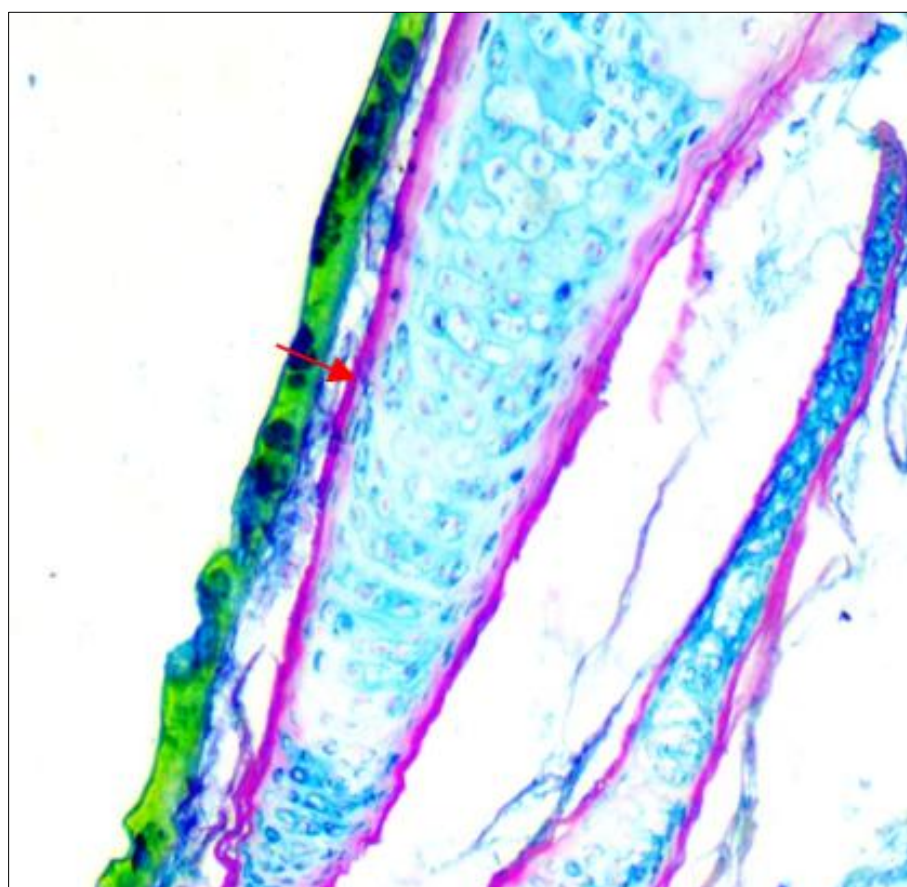


Fig 8: Trachea from 112 days old Uttara fowl Arrow showing intense acid mucopolysaccharids reaction in intraepithelial gland. Modification of Mowry's (1958) colloidal Iron stain acid Mucopolysaccharids; X100

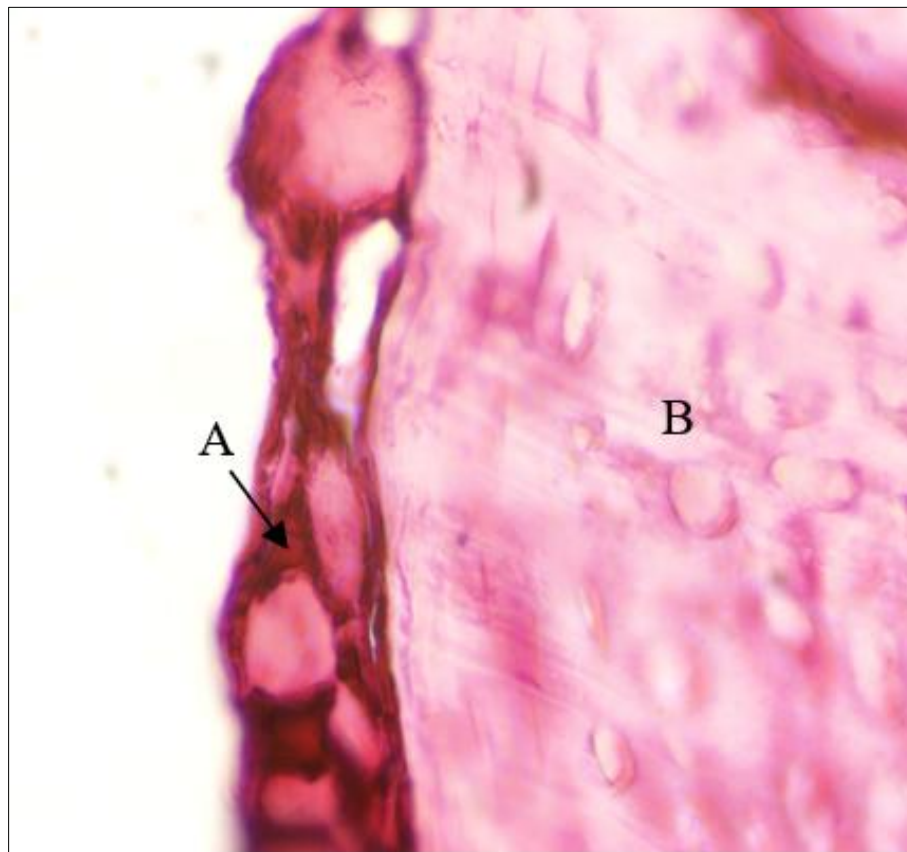


Fig 9: Trachea from 112 days old Uttara fowl showing carmine intense reaction in (A) intraepithelial gland (B) mild reaction in tracheal cartilage. Carmine; X400

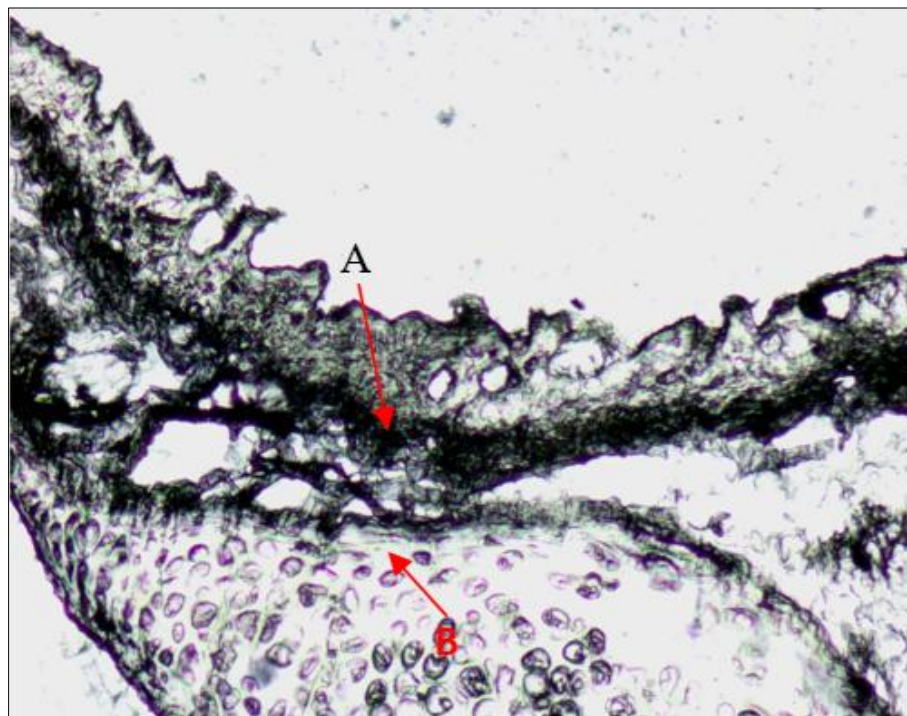


Fig 10: Trachea from 112 days old Uttara fowl showing moderate reaction in (A) Basement membrane of epithelial cell (B) moderate reaction in perichondrium of tracheal cartilage. Acetone- Sudan Black B; X100

4. Conclusion

The tracheal mucosa was lined by ciliated pseudostratified columnar epithelium containing numerous intraepithelial mucous glands. The height of the ciliated pseudostratified columnar epithelium increased significantly ($p < 0.05$) with

advancing age, accompanied by a progressive increase in the number of intraepithelial alveolar glands, indicating post-hatch maturation of the tracheal mucosa and its secretory apparatus. The lamina propria-submucosa consisted of loose connective tissue rich in collagen and reticular fibres, numerous blood

vessels, and scattered lymphocytes, and was continuous with the perichondrium surrounding the tracheal cartilaginous rings. No evidence of ossification was observed in the tracheal cartilage throughout the study period up to 112 days of age. The tunica adventitia was composed of connective tissue containing abundant blood vessels and scattered adipocytes enclosing the bundles of skeletal muscle. Histochemically, the cartilaginous rings and intraepithelial glands exhibited strong positive reactions to Periodic Acid-Schiff (PAS) and Carmine staining, indicating the presence of glycoconjugates and glycogen. Furthermore, the intraepithelial glands showed intense PAS-Alcian Blue (PAS-AB) positivity, demonstrating the coexistence of both neutral and acidic mucins, which are likely to contribute to the protective and lubricating functions of the tracheal mucosa.

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