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Microanatomical Studies on the Broncho-pulmonary Segments of Lungs in Domestic Pig (*Sus scrofa domesticus*)

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ABSTRACT

The study was conducted from December, 2020 to May, 2021 in the Department of Veterinary Anatomy, College of Veterinary Science, Rajendranagar, Hyderabad, India to provide a thorough description of the histological characteristics of the broncho-pulmonary segments of domestic pig lungs, given the structural similarities between human and pig lungs and the significance of this species as an experimental model for biomedical research. A typical broncho-pulmonary segment was composed of the following histological structures: segmental bronchi, primary, secondary, and tertiary bronchioles and their branches, all of which terminated in an alveolar duct or a cluster of alveoli. Each broncho-pulmonary segment was accompanied by a matching artery to the tiniest divisions of the bronchial tree. Lamina mucosa, sub-mucosa, and outer adventitia were the three layers of secondary (lobar) bronchi. Mucosal layer was lined by pseudo-stratified ciliated columnar epithelium; the sub-mucosal layer was made up of a hyaline cartilage layer and a stretch of sub-mucosal glands and blood vessels. Segmental bronchi epithelium was simple columnar, stratified cuboidal while cuboidal in terminal bronchioles. The terminal bronchioles had a folded mucosa, formed of small strands of collagen and smooth muscle fibres. Terminal bronchioles were linked to alveolar ducts, which in turn were linked to a spate of alveoli with squamous epithelium.

KEYWORDS: Broncho-pulmonary segment, secondary bronchi, tertiary bronchiole

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1. INTRODUCTION

The pig is an omnivorous, monogastric species with many advantages to serve as an animal model for human diseases. They serve as an appealing model for studying lung diseases because they have many traits in common with human lungs, which makes them a useful model for both normal human lungs and disease abnormalities and treatments. Surfactant composition, function, and therapy have all been studied using porcine lungs (Jeffrey, 2010). The porcine model has contributed significantly to biomedical research over many decades. The similar size and anatomy of pig and human organs make this model particularly beneficial for translational research in areas such as medical device development, therapeutics and xenotransplantation (Judge et al., 2014). Even though mouse models have been widely used in scientific research, they usually fall short in their ability to represent the mechanisms underlying human disease accurately. Large animal models, like pigs, are therefore desperately needed because they closely resemble many aspects of human anatomy and physiology (Aigner et al., 2010). The porcine lung has been used for extracorporeal preservation and immune engineering (Pabst, 2020). In recent years, a major limitation with the porcine model was overcome with the successful generation of gene-targeted pigs and the publication of the pig genome. As a result, the role of this model is likely to become even more important (Judge et al., 2014). The respiratory system performs vital functions in the body (Baba et al., 2008). The respiratory organs provide exchange of gases between the blood and atmosphere (Jayachitra et a., 2021). The lungs are a pair of primary respiratory organs located in the thoracic cavity on either side of the mediastinum. These organs are covered by a thin, double-layered serous membrane called the pleura (Pandey, 2025). The pig lung has the dorsal, ventral, medial and lateral bronchiole systems on either side. In addition, a tracheal bronchiole (bronchus) arises from the right side of the trachea (Nakakuki, 1994). The respiratory system consists of two components-the conducting and respiratory portions. The conducting portion transports air from the external environment to the site of respiration, whereas the respiratory portion facilitates gas exchange and blood oxygenation (Khan, 2025). The conducting portion of the respiratory system includes the nose, nasopharynx, larynx, trachea, and a series of progressively narrowing bronchi and bronchioles, terminating at the terminal bronchiole (Warren, 2024). The respiratory portion starts at the respiratory bronchiole, extends through the alveolar ducts and alveolar sacs, and culminates in the alveoli, where the primary gas exchange occurs. The mammalian airway consisted of cartilaginous tube which begins from larynx continued by trachea and bronchi which are then divided into several generations of bronchioles and ultimately terminates in air sacs. In the field of respiratory medicine, the animal respiratory model gains more importance to understand the ventilation mechanisms (Judge et al., 2014). The branching pattern of these conducting passages looks like the branching of a tree and is hence called the tracheobronchial tree (Patwa, 2015). The goal of the current study was to provide a thorough description of the histological characteristics of the broncho-pulmonary segments of domestic pig lungs, given the structural similarities between human and pig lungs and the significance of this species as an experimental model for biomedical research. Good knowledge of the topographic anatomy of the tracheobronchial tree and of its blood supply is necessary to reduce the operative morbidity, whereas that of the oncological anatomy of lung cancer forms the theoretical basis for the operation and improves the potential for better long-term results (Frechette, 2006).

2. MATERIALS AND METHODS

The study was conducted during December, 2020 to May, 2021 in the Department of Veterinary Anatomy, College of Veterinary Science, Rajendranagar, Hyderabad as part of M.V.Sc Programme.

2.1. Collection of data

Six (6) pairs of fresh lung specimens of either sex were employed for histomorphological investigations. Tissue samples were obtained immediately after slaughter and preserved in 10% NBF prior to paraffin processing (Singh and Sulochana, 1997).

2.2. Processing and staining methodology

On a standard rotary microtome, paraffin sections measuring 6–8 µm were cut and stained using the following techniques: Haematoxylin and Eosin method to study the detailed microarchitecture of lung parenchyma (Singh and Sulochana, 1997); Weigert's method to show elastic fibers (Luna, 1968); Masson's Trichrome method to distinguish between collagen and muscle fibers in lung parenchyma (Luna, 1968) and Safranin O stain for demonstrating cartilage (Luna, 1968).

3. RESULTS AND DISCUSSION

In the present study, it was observed that the lobar or secondary bronchi originated from the respective principal bronchi (Figure 1). Secondary bronchi showed different layers viz., epithelium, lamina propria, and muscularis mucosa, sub mucosa as well as connective tissue elements (Figure 2) and outer adventitia (Figure 3 and 4). A pseudo-stratified ciliated columnar epithelium populated with ciliated columnar, basal, Clara cells, and goblet cells lined the mucosal layer of the secondary bronchus, which sat on a basement membrane. The smooth muscle in the

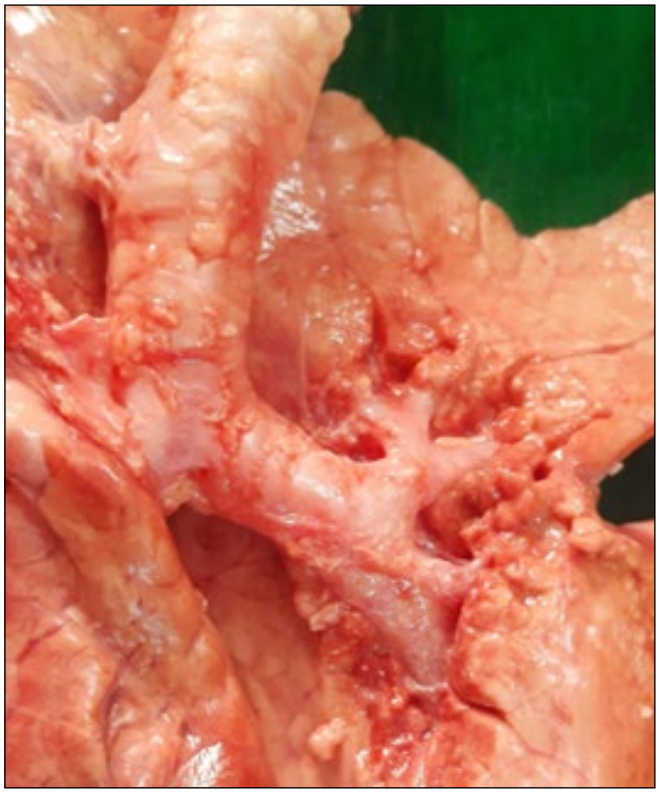

Figure 1: Gross dissection of primary bronchus dividing into lobar bronchi

lamina propria was arranged in a circular pattern. Nickel et al. (1979), in domestic animals observed that the lining epithelium of secondary bronchus contained less mucous and basal cells and more of ciliated or Clara cells (bronchiolar exocrine cells) and the same was reported by Kalita (2014) in mizo pigs. The lining epithelium of secondary bronchi in mammals was pseudostratified ciliated columnar type with basal cells, ciliated, and goblet cells which was in accordance with the reports of Plopper and Adams (1993) in domestic animals; Kahwa et al. (1997), Baba and Choudhary (2016);

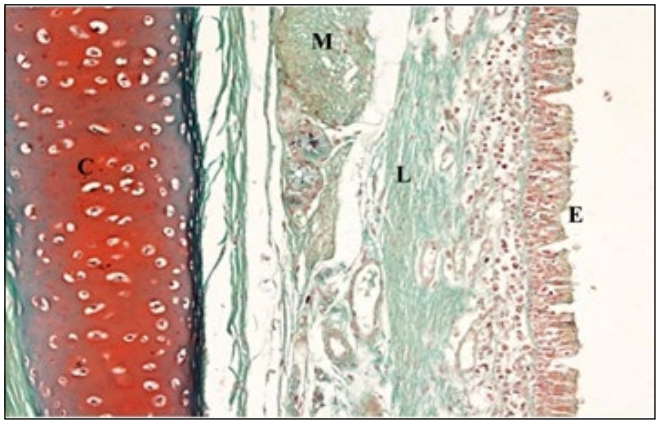

Figure 2: Cross sectional microphotography of secondary bronchus showing hyaline cartilage using safranin O stain under 20X (E: Epithelium; L: Lamina propria; M: Muscularis mucosa; C: Cartilaginous tissue)

and Khyalia et al. (2019) in goats and Wanhong et al. (2019) in Bacitran camels.

The submucosal layer of the secondary bronchus was primarily composed of hyaline cartilage plates. A stretch of submucosal glands existed between the mucosal lamina muscularis and the cartilage layer (Figure 5). Collagen fibre streaks were identified in the mucosa's sub epithelium and loose connective tissue. Submucosal glands were made up of huge pyramid-shaped cells with a spherical nucleus in the basal region of the cell. In the submucosal layer of secondary bronchi, typical hyaline cartilage plates with an intense cartilage matrix were seen in a circular way (Figure 3 and 4). Their edges were defined by collagen fibre strands. Blood

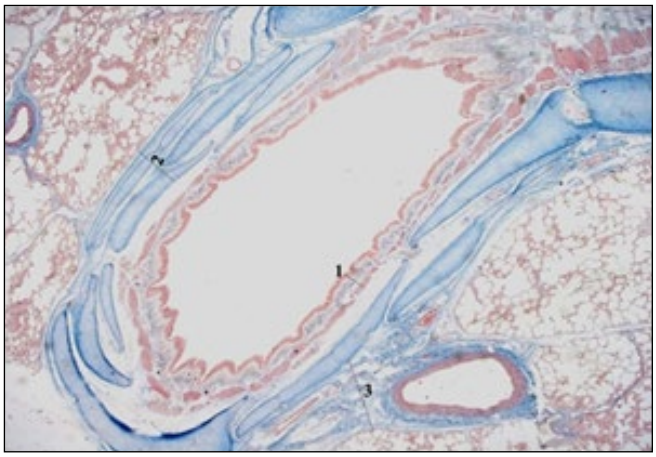

Figure 3: Cross sectional photomicrograph of secondary bronchus (1: Mucosa; 2: Sub-mucosa; 3: Adventitia), Masson's trichrome stain under 4X

vessel branches, such as bronchial and pulmonary arteries, were observed in the submucosal layer of secondary bronchi. In the lamina propria of the secondary bronchus, they found less mucous and basal cells and more ciliated or Clara cells (bronchiolar exocrine cells) and fine collagenous and elastic

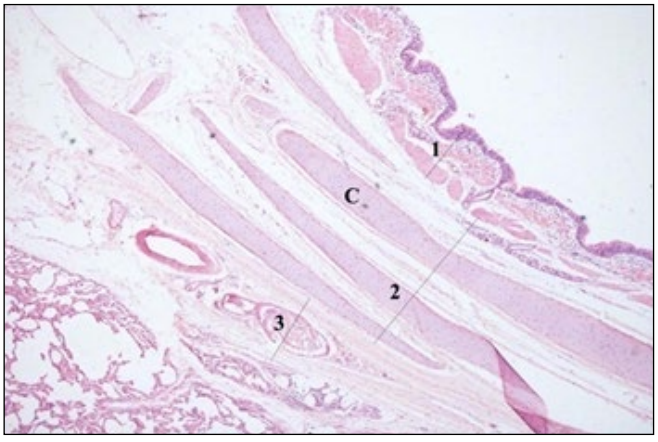

Figure 4: Cross sectional microphotography of secondary bronchus (C: Overlapping cartilage; 1: Mucosa; 2: Sub-mucosa; 3: Adventitia), Haematoxylin and Eosin stain under 4X

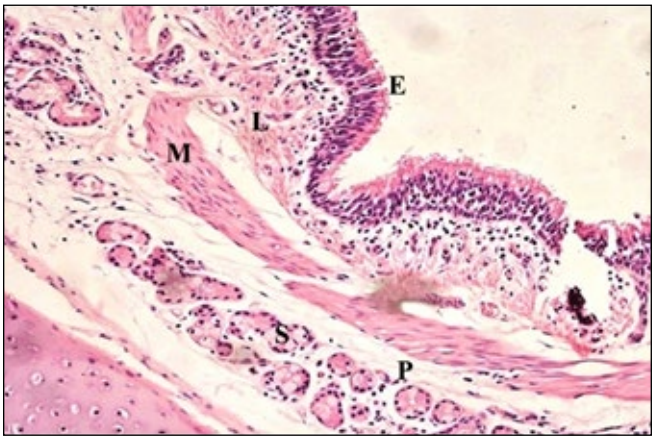

Figure 5: Cross sectional photomicrograph of secondary bronchus (E: Epithelium; L: Lamina propria; M: Muscularis mucosa; S: Submucosal glands; P: Pyramidal cells), Haematoxylin and Eosin stain under 40X

fibres. The smaller divisions of secondary bronchi in this investigation supported the theory that the cartilage layer was composed of tiny, irregular plates of hyaline cartilages that gradually shrunk in size with the relative expansion of smooth muscle. The submucosa of the secondary bronchi in goats was composed of loose connective tissue with small tubule-alveolar, mucous or seromucous glands (Khyalia et al., 2019 and Nabi et al., 2021). Outer adventitia was made up of loose connective tissue with cells, fibres, and blood vessel branches. Adventitia was made up of loose connective tissue with collagen and elastic fibres corresponding to Nickel et al. (1979) in domestic animals, Kalita (2014) in Mizo pigs, and Baba and Choudhary (2016) in goats.

Bronchus Associated Lymphoid Tissue (BALT) was identified as noticeable aggregations of lymph nodes within the trachea-bronchial junction (Figure 6). This type of lymphoid tissue was distinguished by a huge mass of DLT and clear little spherical lymph nodules visible as separate

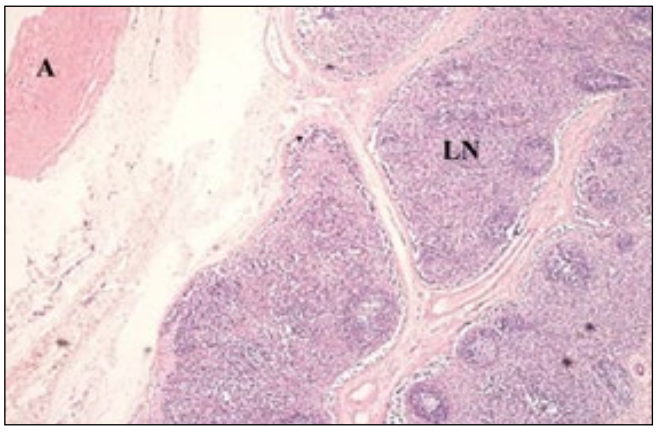

Figure 6: Cross sectional photomicrograph of trachea-bronchial junction showing BALT (A: Artery; LN: Lymph node), Haematoxylin and Eosin under 10X

small patches in interalveolar septae. BALT masses were enclosed in connective tissue and had a thin layer of densely packed lymphocytes on the outside. Dellmann and Brown (1987) witnessed solitary aggregations of lymphoid cells in the wall of pulmonary bronchioles in large ruminants, as well as the existence of lymphatic tissue in goats. Nabi et al. (2021) reported in goats that the lamina propria of terminal bronchioles had few lymphocytes and elastic fibres.

The bronchial tree was closely followed by branches of the pulmonary artery, which had a thick arrangement of elastic fibres in its tunica media (Figure 7). This artery's branches continued into the interstitial tissue area between alveoli to

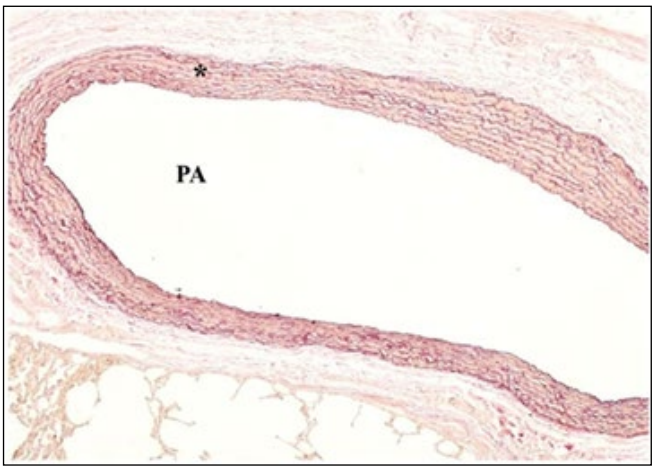

Figure 7: Cross sectional microphotography of pulmonary artery branch using Weigert's satin under 4X (*: Indicating elastic fibres)

facilitate gas exchange. Secondary bronchi epithelium was pseudostratified ciliated columnar, transitioning to stratified cuboidal in segmental bronchi. The latter were divided into smaller bronchioles, such as tertiary bronchioles, which eventually became terminal bronchioles in the lung parenchyma (Figure 8). Terminal bronchioles were made up

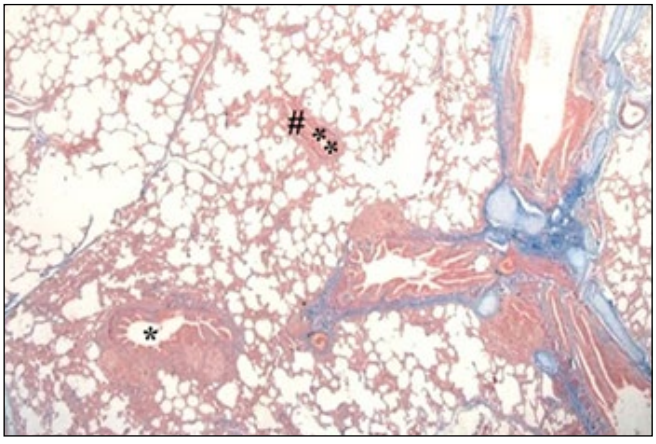

Figure 8: Cross sectional microphotography of segmental bronchus giving smaller bronchioles using Masson's trichrome stain under 20X (*: Teritiary bronchiole, **: Terminal bronchiole)

of thin strands of collagen and smooth muscle fibres, with a folded mucosa and a simple columnar epithelium. According to Kalita (2014) in Mizo pigs, Baba and Choudhary (2016) in goats, and Bacha and Bacha (2000) in domestic animals, the smallest pulmonary bronchi, the tertiary bronchi, were lined by folded simple ciliated columnar epithelium lacking goblet cells. At the bronchiole confluence, the submucosa consisted of a separate smooth muscle layer with sparsely scattered cartilage isolates. In this study, folded mucosa with simple ciliated columnar epithelium was seen in terminal bronchioles with thin strands of collagen and smooth muscle fibres (Figure 9).

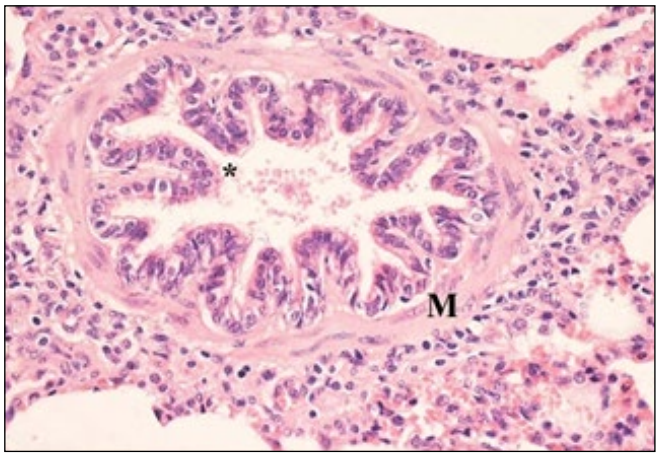

Figure 9: Cross sectional microphotography of tertiary bronchiole, Haematoxylin and Eosin stain under 40X (*: cuboidal epithelium; M: Muscularis mucosa)

Bronchial (nutritive) and pulmonary (functional) arteries were the blood vessels that followed the bronchial tree. The former arteries were observed as having many tiny branches inside the interalveolar septae, while the latter functional pulmonary artery branches were seen as having multiple tiny branches within the interalveolar septae. The terminal bronchioles connected to alveolar ducts, which led to a slew of alveoli (Figure 8). The terminal bronchioles simple, high cuboidal epithelium gave way to a simple squamous epithelium in the alveoli. Thin squamous epithelium bordered the alveolar walls. The tertiary bronchiole has a double layer of smooth muscle in its submucosa (Figure 9). It has a folded mucosal epithelium with a high cuboidal epithelium and a strong spherical nucleus. BALT was detected as tiny aggregates in interalveolar septae other than near bronchioles.

The gas exchange (respiratory) area in pig lungs was characterised by many alveoli, alveolar ducts, respiratory bronchioles, and inter-alveolar connective tissue in the current study. The smallest of the bronchial system's conducting airways opened either into a respiratory bronchiole or directly into the alveoli (Figures 8 and 10). Eurell and Frappier (2006) in domestic animals, Kalita (2014) in Mizo pigs, and Jayachitra et al. (2021) in pigs all supported these findings. The respiratory bronchiole's wall was disrupted by alveolar sacs entering the mucosa, and the bronchiole terminated in alveolar sacs or ducts in the lung parenchyma. Simple cuboidal epithelium lined the terminal bronchioles, which were bordered by smooth muscle. It progressed into an alveolus (Figure 10).

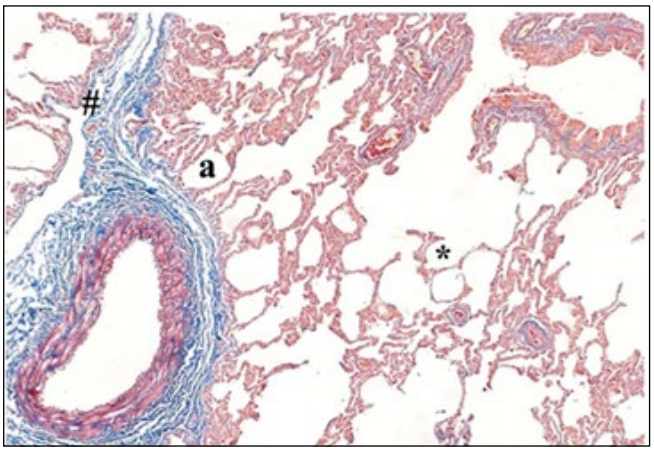

Figure 10: Cross sectional microphotography of terminal bronchiole opening into alveolar duct and alveoli using Masson's trichrome stain under 10X (a: Alveoli, *: inter alveolar septae, #: Capillaries in interstitial connective tissue)

Respiratory bronchioles were observed entering alveolar ducts, which then expanded into numerous alveoli. Kalita (2014) in Mizo pigs and in goats by Khyalia et al. (2019) and Nabi et al. (2021), all of whom stated that the epithelium of respiratory bronchioles was lined by a single layer of cuboidal epithelium without goblet cells and was supported by a smooth muscle layer since a distinct basement membrane was lacking so that cells rested on a composite network of smooth muscle cells, collagen, reticular, and a few elastic fibres. Clara cells with projecting nuclei were also discovered populating between the cuboidal cells in goats (Nabi et al., 2021).

In the current study, the walls of alveolar ducts were made up of spiral bands of smooth muscle and elastic fibres that gave them a knob-like appearance, i.e., gaping lips of the alveoli (Figure 10). Hare (1975), Banks (1993), Adams and Dellmann (1998), Bacha and Bacha (2000), Aughey and Frye (2001), and Kalita (2014) in Mizo pigs were also reported similar findings about the gaping lips of alveoli. The alveoli were mostly polygonal in form and bordered with simple squamous epithelium (Figure 11). There was continuity from the terminal bronchiole to the tertiary bronchiole to the alveolar ducts and alveoli. These alveoli are the fundamental units of the gas exchange zone. The interalveolar septum was filled by two types of pneumocytes that were distributed throughout the connective tissue. Epling (1964) discovered fibroblasts, a few lymphocytes,

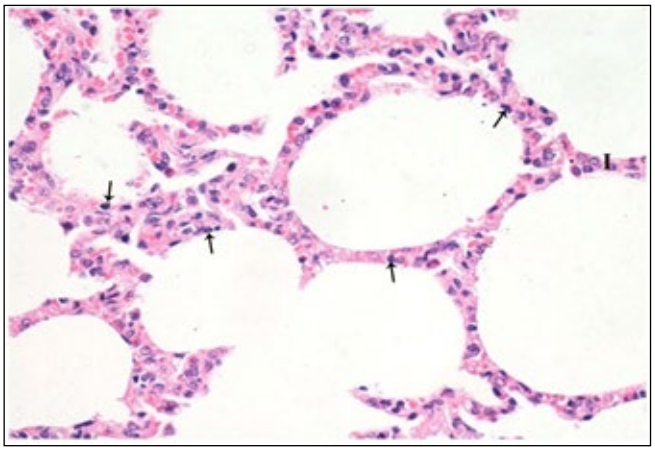

Figure 11: Microphotography of inter-alveolar septae, using Haematoxylin and Eosin under 40X

macrophages, mast cells, and plasma cells in the interalveolar connective tissue of the bovine lung, and Pathak and Rajesh (2020) discovered the same in sheep. In this study, the cell profile in interalveolar septae in pig lungs revealed two types of pneumocytes, Type I and Type II, with the former having a higher proportion of mast and plasma cells. Banks (1993), Eurell and Frappier (2006) in domestic animals, Baba and Choudhary (2008) in goats, and Kalita (2014) in Mizo pigs. According to them, pneumocytes formed the predominant lining epithelium of alveoli, while Type II cells were occasionally seen among the Type I cells.

The histological anatomy of the interalveolar septum seen in the current study included an alveolar lining epithelium, its basal lamina, septal gap, endothelium basal lamina, and capillary endothelium. This description of the five components of the blood-air barrier is comparable to that of Banks (1993), Dellmann and Eurell, (1998) in different species of animals; Suman (2008) in the blood-air barrier of the post-natal lung of goats; and Pathak et al. (2020) in goats, the blood-air barrier is composed of the plasma membrane of alveolar cells, the basal lamina of alveolar cells, interstitial tissue, the basal lamina of endothelial cells, and the thin endothelial cell of the blood capillary.

4. CONCLUSION

Following histological structures were involved in forming a typical broncho-pulmonary segment: segmental bronchi, primary, secondary, and tertiary bronchioles that terminated in an alveolar duct or a cluster of alveoli. One broncho-pulmonary segment was accompanied by arteries to the smallest bronchial divisions as well as the lung parenchyma. In the parenchyma, interalveolar connective tissue was thin and composed of strands of collagen fibres and many blood vessels. Branches of the pulmonary artery were detected in between the alveoli.

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