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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(8): 1483-1486

www.thepharmajournal.com
Received: 09-05-2022
Accepted: 12-06-2022

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Histomorphological studies on the lungs of Black Bengal goat and Garole sheep

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Abstract
A study was conducted on gross anatomical studies of lungs in black Bengal goat and Garole sheep. The bronchi were lined by psudostratified ciliated columnar epithelium. Goblet cells, basal cells, and migratory cells were identified in different order of bronchus but the number of goblet cells decreased from primary to tertiary. The height of the epithelium also decreased gradually from primary to tertiary. Similarly the thickness of the propria and submucosa also progressively decrease. The propria and submucosa contained mixed submucosal gland in case of the sheep but no gland was found in case of goat. The frequency of the bronchial gland gradually diminished in case of sheep. The hyaline cartilage appeared in the form of regular plate in case of both the species in primary bronchus. In first order of bronchiole simple ciliated columnar epithelium was found and in the second order the lining epithelium was simple cuboidal. Besides the epithelial cells calara cells were also identified in both the species. Respiratory bronchiole was identified infrequently in the initial part of the lung in the form of out pocketing walls. The alveolar wall was lined by simple squamous epithelium. Two types of alveolar pnumocytes were identified 1) membranous pnumocytes (type-1 pnumocytes) 2) granularpnumocytes (type-2 pnumocytes) in both species.

Keywords: Membranous pnumocytes (type-1 pnumocytes) 2) granularpnumocytes (type-2 pnumocytes)

Introduction
The respiratory and circulator systems work together to deliver oxygen to cells and remove carbon dioxide in two-phase process called respiration. The first phase of respiration begins with breathing in, or inhalation. Inhalation brings air from outside the body into the lungs. Oxygen in the air moves from the lungs through blood vessels to the heart, which pumps the oxygen-rich blood to all parts of the body. Oxygen then moves from the bloodstream into cells, which completes the first phase of respiration. In the cells, oxygen is used in a separate energy-producing process called cellular respiration, which produces carbon dioxide as a byproduct. The second phase of respiration begins with the movement of carbon dioxide from the cells to the bloodstream. The bloodstream carries carbon dioxide to the heart, which pumps the carbon dioxide-laden blood to the lungs. In the lungs, breathing out, or exhalation, removes carbon dioxide from the body, thus completing the respiration cycle.Each lung was divided into a number of lobes and each of it was subdivided into the lobules by interlobular tissue. There after the bronchus was getting into smaller and smaller branches as primary, secondary and tertiary bronchi. Each lobule had a respiratory structure that was arising from the terminal bronchiole (Khyalia *et.al.,* 2019) [1]. The present study was undertaken to investigate the histomorphology in black Bengal goats and Garole sheep.

Materials and method
For histological study, small pieces (5-6mm thick) of lungs, (NBF fixed) were washed under slow tap water for 12 hours. Then these were passed through ascending grades of alcohol(one hour each) for dehydration. Afterwards, the tissues were kept in Xylene for 20 to 30 minutes till the tissues were semitransparent. The tissuewere keptin melted paraffin at a temperature of 58-60°C. Afterwards paraffin blocks were prepared by standard procedure. The section were cut with the help of rotary microtome and stained.The sections (horizontal & Vertical) of 5µ thickness were obtained and stained with routine haematoxylin and eosin (Luna, 1968) [2]. 10% N.B.F. fixed sections were treated for staining collagen fibers as per standard technique of Masson, 1929 [3]. The collagen fibers stained blue colour and cytoplasm, muscle fibres and inter cellular fibers stained red. The nuclei took black stain.

Standard technique of Mallory (1961) [4] for staining elastic fibres. The fibers stained black and collagen stained pink colour. Formalin fixed sections were stained for polysaccharides as per standard technique of McManus1946 [5]. Glycogens and other per iodate-reactive carbohydrates took magenta colour.

Result and Discussion

The bronchi were lined by psudostratified ciliated columnar epithelium. Goblet cells, basal cells, and migratory cells were identified in different order of bronchus but the number of goblet cells decreased from primary to tertiary (Fig.1). The height of the epithelium also decreased gradually from primary to tertiary. Similarly the thickness of the propria and submucosa also progressively decrease. The propria and submucosa contained mixed submucosal gland in case of the sheep but no gland was found in case of goat. (Fig.2). The frequency of the bronchial gland gradually diminished in case of sheep. The hyaline cartilage appeared in the form of regular plate in case of both the species in primary bronchus. From secondary to tertiary irregular plates and smooth muscles was interspersed between the luminal side of the plates. The muscular components were arranged in circular fascicles. The thickness and width of the cartilage gradually diminished where as muscular component relatively more abundant from primary to tertiary bronchus in both the species. The adventitial connective tissue was found to be loose with huge amount of collagen fiber and few elastic fibers (Fig.3&4). Fiber orientations were perpendicular to the long axis of the airway. The mucosal fold gradually increased from primary to tertiary bronchus. Dellmann and brown (2006) [11] stated similar observation and also mentioned that the absence of bronchial gland in case of goat. Which in full agreement with our findings. Banks (1993) [6] stated that the lamina epithelial is was a psudostratified ciliated columnar epithelium which contained numerous goblet cells. The tunica mucosa also consists of areolar connective tissue and contained branched, coiled tuboalveolarmucose gland. These glands diminished in number towards the tertiary bronchi. This is in full agreement of our present investigation. Bacha (1990) [7] narrated that in the histological section, the mucosa of large bronchi had few folds. Folds increased as the bronchi decrease in diameter.

Abundant elastic fibers were found within the propria and submucosa area after the treating the slides of the tertiary bronchus with the special stain. The fiber was more towards the submucosal area and around the cartilaginous plates (Fig. 4).

On histological observation different orders of bronchioles were identified in both the species. Two orders of bronchioles were identify in both the species. The shape of the bronchioles were mostly round to elliptical on cross sectional view. In first order of bronchiole simple ciliated columnar epithelium was found and in the second order the lining epithelium was simple cuboidal. Besides the epithelial cells calara cells were also identified in both the species. PAS reaction was negative in the calara cells of the species. PAS reaction was positive towards the border of the lining epithelium. In the propria glands and cartilaginous plates were absent. A thin layer of loose connective tissue was identified in the propria. The smooth muscle fibers were found to be present in circular and oblique manner. The adventitia was made up of loose connective tissue fibers with abundant elastic fiber. Small dome shaped lymphoid follicle was found close to the bronchiole in some cases (Fig. 5&6).

Respiratory bronchiole was identified infrequently in the initial part of the lung in the form of out pocketing walls. The lining epithelium was simple cuboidal which was interrupted in some place by the alveolar epithelium. Lamina propria was indistinct. The smooth muscle was arranged in the form of fascicles beneath the epithelium and in between the fascicles there was opening for alveoli in both the species. The respiratory bronchioles were found to be divided in to numerous alveolar ducts and in few cases it was continued as alveolar duct. It was lined by alveolar epithelium. However, in few cases muscular orientation was revealed towards the luminal border between the adjacent alveoli. Scanty amount of elastic fibers and collagen fibers were found in the wall of the alveolar duct.

The alveolar ducts were found to be divided and expended into number of small sacs which were lined by the alveolar epithelium. The sacs were resembled as saccule. The common opening of the saccule formed the atrium. The alveolar wall was lined by simple squamous epithelium. Two types of alveolar pnumocytes were identified 1) membranous pnumocytes (type-1 pnumocytes) 2) granular pnumocytes (type-2 pnumocytes) (Fig.7&8).

The membranous pnumocytes was the predominant cell type found on the alveolar lining. The nucleolus of the type1 pnumocytes was apparent and protruded in to the lumen with a continuous basal lamina. The type 2 granular epithelial cells were found as cuboidal cell with a central nucleus and was found occasionally among the type 1 cell in the alveolar epithelium. The nucleus found centrally placed. A few free alveolar macrophages were observed in the lumen of the alveoli. Inter alveolar septum was composed of collagen, elastic fibers and few reticular fiber. It contained phagocyte, fibrocytes and macrophages. The outer most covering of lung parenchyma was made up of collagen and elastic fiber. The PAS activity was intense to moderate in all the components of the lung parenchyma in both the species.

The histological findings of bronchioles, respiratory bronchioles, alveolar duct, and alveoli were in accordance with Dellmann & Brown (2006) [11]. Banks (1993) [6] stated that the respiratory bronchioles in frequently observed in ruminants. In this present investigation respiratory bronchioles were observed in frequently in both the species which in agreement with banks. Regarding the other components the statements made by banks was in full agreement with our present findings. Bacha (1990) [7] narrated that alveoli were lined mainly by exceedingly thin squamous epithelial cell.

Alveoli were separated from one another by a thin, highly vescularized layer of fine collagenous and elastic fiber. This layer, together with the squamous cell lining the adjacent alveoli, formed an alveolar septum which is similar with our present finding. Yadav (2005) [9] reported that dome shaped lymphoid follicle was present close to the small bronchiole indicating presence of frank nodules in case of buffalo. Inter-alveolar septum was composed of collagen and elastic fibers. The outer most covering of lung parenchyma was made up of collagen and elastic fibers. In support of our observation Baba (2008) [8] Reported that the goat alveoli were generally small, roughly spherical structures that opened into the alveolar ducts, alveolar sacs or into the respiratory bronchioles wherever present. The alveolar ducts were found as tubular structures surrounded by alveoli and usually followed a long tortuous course and gave off several branches. The walls of

the alveolar ducts consisted of open sides of alveoli and the terminations of the inter alveolar septa which separated the alveoli. Collagen and elastic fibres and smooth muscle fibres were discernible in the walls of the alveolar ducts at the portions wherefrom the alveolar sacs arose from them. Smooth muscle fibres and elastic fibers were also observed on the margins at the apices between the adjacent alveoli, the alveolar ducts terminated into clusters of saccules termed as alveolar sacs. Mercer and carpo (1990) [10] reported that the collagen-elastin fiber network was major factors in lung parenchymal micromechanics. Quantitative serial section analysis and morphometric evaluations of planar sections were used to determine the spatial location of collagen and elastin fibers in Sprague-Dawley rat and normal human lung samples. Large concentrations of connective tissue fibers were located in the alveolar duct wall in both species. This is in full agreement of our present study.

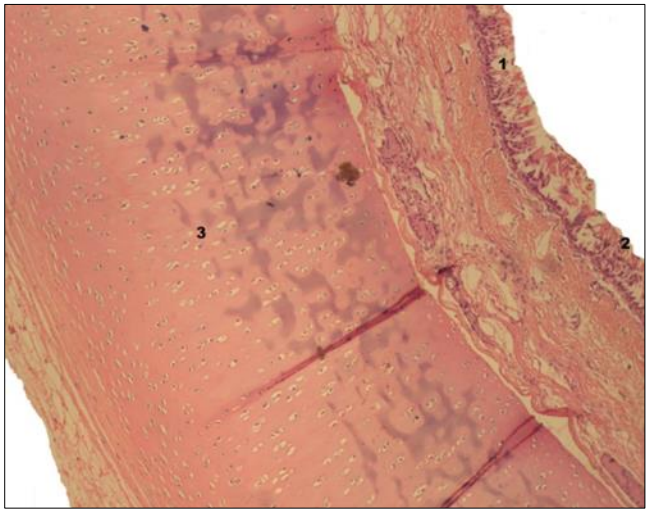

Fig 1: Photomicrograph showing Principal bronchus of goat Goblet cell (1) Epithelium (2) Cartilage (3) H&E, X 100

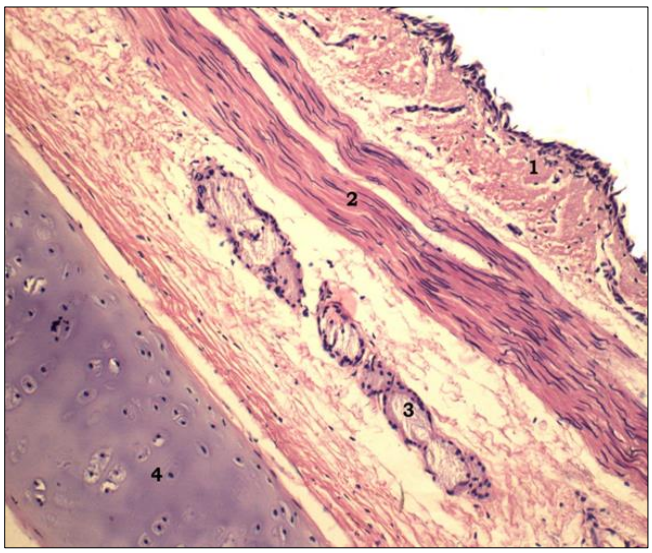

Fig 2: Photomicrograph showing Principal bronchus of Sheep Epithelium (1) Muscle (2) Gland (3) Cartilage (4) H&E, X 100

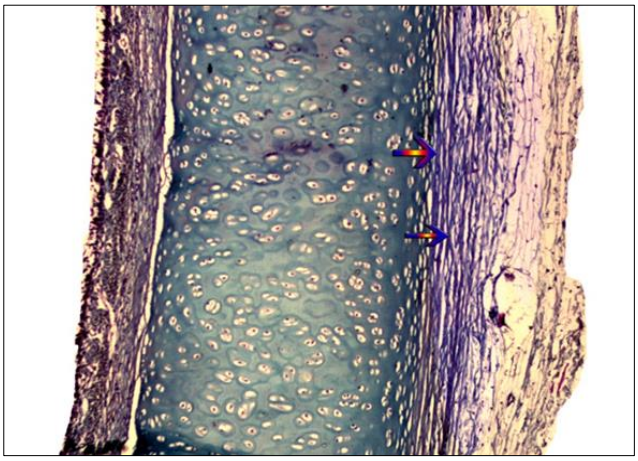

Fig 3: Photomicrograph showing (arrow) collagen fibers in primary bronchus of goat M&T X 100

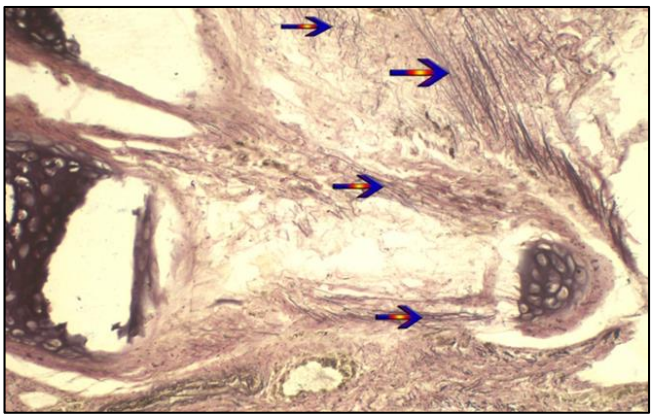

Fig 4: Photomicrograph showing (arrow) elastic fibers in tertiary bronchus of goat. WR&F X 200

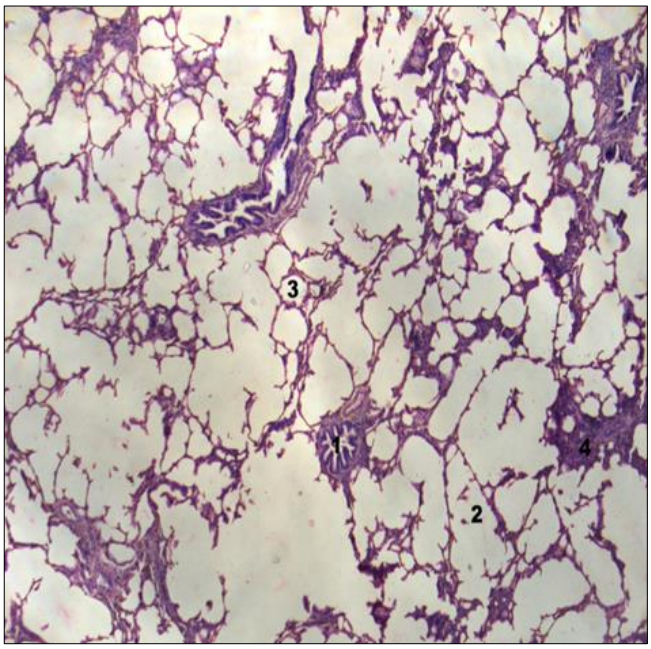

Fig 5: Photomicrograph showing lung of goat Bronchiole (1) Alveolar duct (2) Alveolus (3) Smooth muscle (4) H&E, X 50

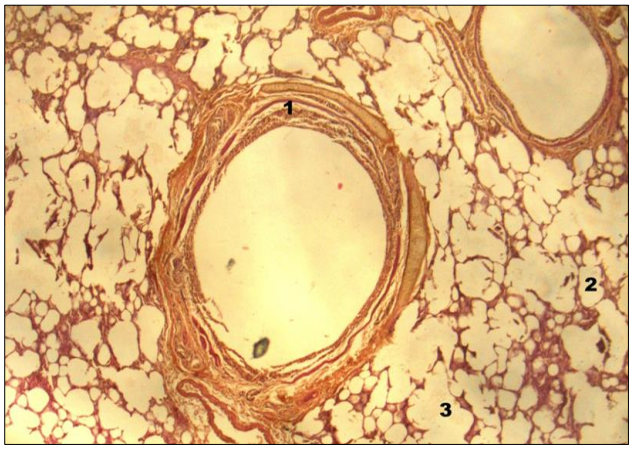

Fig 6: Photomicrograph showing normal Lung of sheep Bronchus (1) Alveoli (2) Alveolar duct (3) H&E, X 50

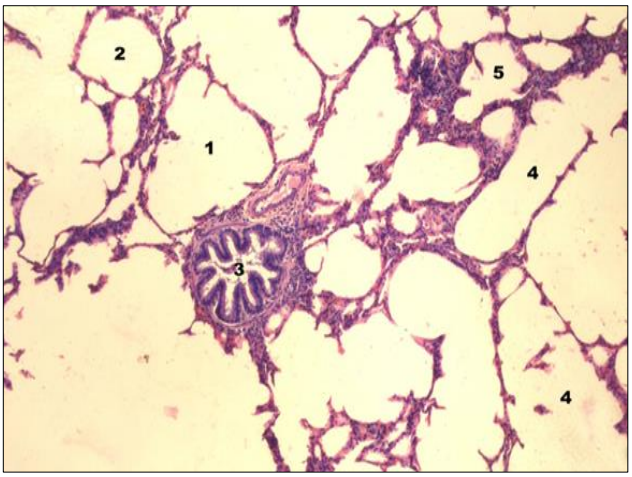

Fig 7: Photomicrograph showing lung of goat Alveolar Sac (1) Alveoli (2) Bronchiole (3) Alveolar duct (4) Respiratory bronchiole (5) H&E, X 100

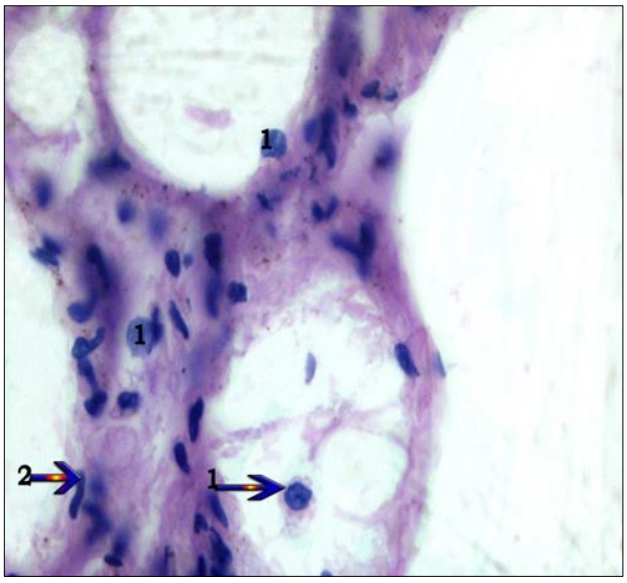

Fig 8: Photomicrograph showing lung. Alveolar microphage (1) granular pnumocyte (2) H&E X 1000

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