

## HISTOMORPHOLOGY AND HISTOCHEMISTRY OF BLOOD AIR BARRIER DURING POST-NATAL DEVELOPMENT OF LUNG IN GOAT (*CAPRA HIRCUS*)

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### ABSTRACT

The study was conducted on 20 goats irrespective of sex varying from birth to 12 months of age. The blood air barrier consisted of five layers in goats below one month of age while in goats of second months onward, it comprised of only three layers. The thickness of this barrier was also more in goats below one month of age which reduced with advancement of age. The alveolar lining cells forming blood air barrier were of two types type-I and type-II categories out of which the former cells dominated the latter in cellular population. Both the cells contained mucopolysaccharides along with a small amount of glycogen only while the endothelial cells forming the barrier failed to demonstrate any histochemical material studied in the present investigation.

**Key words:** Histomorphology, histochemistry, blood air barrier, lung, goat

The respiratory diseases in animals especially in goats due to their grazing habits are the most challenging tasks in the universe. The blood air barrier is the most common factor to regulate the normal respiration. Normal histoarchitecture and development of blood air barrier are quite necessary to know any deviation of these during the development of the lungs which may lead to serious respiratory disorders. Hence, the present study was aimed at to provide the detailed histomorphology and histochemistry of the various components involved in the blood air barrier during the postnatal development of lungs in goats varying from birth to 12 months of age.

### MATERIALS AND METHODS

In the present study 5 goats each of either sex in 4 age-groups from birth to 3 months, 4<sup>th</sup> to 6 months, 7<sup>th</sup> to 9 months and 10<sup>th</sup> to 12 months of age were used. The tissues from apical, cardiac, diaphragmatic and accessory lobes of the lungs from all goats were collected and processed for histomorphological and histochemical studies with paraffin and frozen sectioning techniques. The sections were stained

with routine Harri's haematoxylin and eosin stain (Luna, 1968), Gomori's stain for demonstration of reticulum (Luna, 1968), Weigert's stain for demonstration of elastic fibers (Luna, 1968), modified Mallory's triple stain (Crossman, 1937) and Van Gieson's stain for demonstration of collagen fibers (Luna, 1968) for histomorphological studies. The histochemical techniques included the McManus' method for glycogen without and with saliva treatment (Luna, 1968), Alcian blue method for mucosubstances at pH 2.5 (Luna, 1968), PAS-Alcian blue method (Luna, 1968), Best's carmine stain for glycogen (Luna, 1968), Sudan-black-B and oil-'o'-red in propylene-glycol methods for fat (Luna, 1968), Nile blue sulphate for acidic and neutral lipids (Carleton, 1967) and Gomori's revised methods for alkaline and acid phosphatases (Gomori, 1946). The micrometry was done with the help of linear calibrated ocular micrometer.

### RESULTS AND DISCUSSION

In goats below one month of age the blood air barrier consisted of five layers including alveolar lining epithelial cells, alveolar basal lamina, septal space, endothelial associated basal

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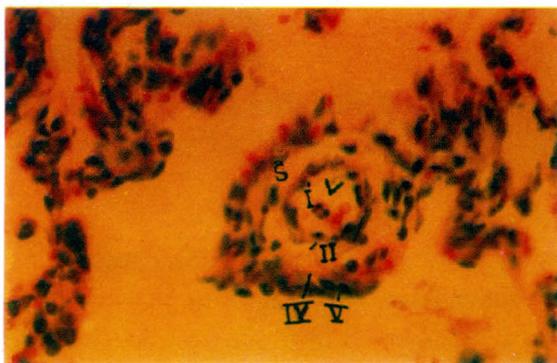


Fig 1. Goat lung of less than 1 month of age showing blood air barrier formed by vessels (V), endothelium (I), endothelial associated basal lamina (II), septal space (S), alveolar basal lamina (IV) and alveolar lining cell (V). (H. & E. x 200)

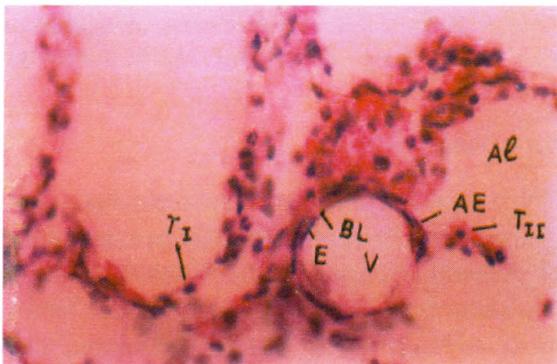


Fig 2. Goat lung of 2 to 3 months of age showing blood air barrier by blood vessel (V), endothelium (E), alveolar epithelial cells (AE) and common basal lamina (BL). Type-I and Type-II pneumocytes lining the alveoli can also be seen. (H. & E. x 200)

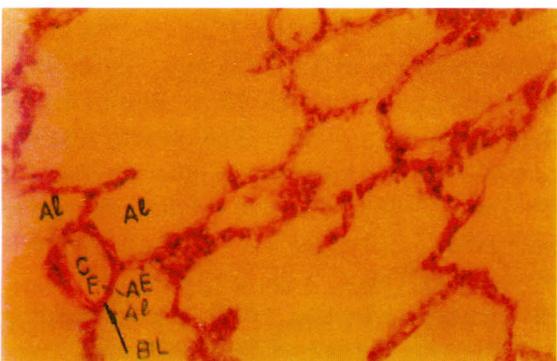


Fig 3. Goat lung between 4 to 6 months of age showing blood air barrier formed by endothelium (E), alveolar epithelial cells (AE), common basal lamina (BL) between capillary (C) and alveoli (Al). PAS+ve reaction in alveolar epithelial cells can also be seen. (PAS x 100)

lamina and endothelial cells (Fig 1). However, in goats of all ages from second month onwards the barrier was comprised of only three layers

such as cytoplasmic processes of alveolar epithelial cells, vascular endothelial cells and a basal lamina common between the two (Figs 2, 3). The average thickness of blood air barrier was the thickest ( $12.44 \pm 0.58 \mu\text{m}$ ) in goats below one month of age which thinned out abruptly ( $8.58 \pm 0.61 \mu\text{m}$ ) in goats at the age of 2 to 3 months and was thinnest ( $7.52 \pm 0.41 \mu\text{m}$ ) in goats of 10 to 12 months age. It is opined that the thickness of this blood air barrier may not allow the rapid exchange of air between blood and alveolar space in new born due to which the breathing is rapid in them whereas, this barrier thinned out with advancement of age providing most efficient diffusion pathway, which results in slow respiration rate in adults. Hence the thickness barrier is inversely proportional to the rate of respiration.

The alveolar lining cells are of two types such as type-I pneumocytes or squamous alveolar cells and type-II pneumocytes or cuboidal alveolar cells where, the former cells dominated the latter amongst the cell population forming the blood air barrier. The type-I pneumocytes resembled endothelial like cells with attenuated acidophilic cytoplasm in the vicinity of nucleus. The nucleus was oval or flat, densely basophilic and protruding into the lumen of alveoli. The type-II pneumocytes were found interposed between type-I and were cuboidal or rounded in shape with lightly acidophilic foamy cytoplasm. Due to foamy appearance of their cytoplasm, these cells may also be called vacuolated cells. The rounded vesicular nucleus located in the centre of the cell was lightly stained. The cytoplasmic extensions of pneumocytes were very thin and joined together with those of adjacent epithelial cells. Both types of cells were supported by a basal lamina composed of fine collagenous, reticular and few elastic fibers. Histochemically the cytoplasm of type-I cells contained high amount of homogenous PAS+ve material while type-II cells were filled with moderate to high amount of fine granular PAS+ve material (Fig 3). The Best's carmine reaction for glycogen was very weak in both the cells.

The capillaries forming the vascular portion of this barrier were distributed in the interalveolar spaces. These vessels had variable diameter

which increased with advancement of age. The endothelium lining these vessels consisted of single layer of endothelial cells having flat squamous cells with elongated cytoplasmic extensions (Fig 2). The cytoplasm was acidophilic and homogeneously distributed. The basophilic nuclei were oval to flat in shape and stained darkly. The basal lamina supporting the endothelial cells contained fine collagenous and reticular fibers along with few elastic and smooth muscle fibers. This lamina was separated from the basal lamina of alveolar epithelial cells by an intervening space in goats below 1 month of age. However, laminae of endothelial and alveolar epithelial lining formed a common basal laminae devoid of space in goats of all age groups beyond 2 to 3 months. The lamina elastica was not observed in the walls of blood vessels of the barrier. The endothelial cells of the vessels had no affinity towards any histochemical technique conducted during present investigation.

Above findings were in complete agreement of the earlier reports in domestic animals (Dellmann and Brown, 1976, Banks, 1983). Bhattacharya and Baishya (1995) observed interalveolar septa in the lungs of goat at birth, was highly vascularised and the capillaries came in immediate contact with basement membranes of the epithelium, establishing the blood air barrier. Similar observations were recorded by Castleman and Lay (1990) in bovine calves and Davies *et al.* (1988) in sheep. Alcorn *et al.* (1981) in sheep lung stated that in regions of close contact between epithelial type-I cell attenuations and capillary endothelial cells, connective tissue was most often absent, the two cells being only separated by a fused basement membrane forming blood air barrier. However, the capillaries showed direct contact with alveolar epithelium in embryos of 145 days onwards in goats (Unni, 1989). Epling (1963) in bovine lung reported that the alveolar epithelium and capillary endothelium were separated by a varying mass of connective tissue and at its thinnest, this separation was accomplished by a fused single basement membrane which was common for both the alveolar epithelium and the endothelium. Mercurio and Rhodin (1984) observed that in cats an increasingly larger

portion of the type-I cells surface area was utilized in early postnatal life for blood air barrier formation while there was an increase in the capillary surface area and the decrease in the blood air barrier thickness with the advancement of postnatal age. The similar increase in capillary surface area, as is evident by the increase in vascular diameter and decrease in the blood air barrier thickness recorded in the present investigation.

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