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Research Article

HISTOMORPHOLOGY AND HISTOCHEMISTRY OF RESPIRATORY BRONCHIOLE DURING POSTNATAL DEVELOPMENT OF LUNGS IN GOAT

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ABSTRACT

The study was conducted on 20 goats (birth to 12 months of age) irrespective of sex difference. In all age groups, the respiratory airways included respiratory bronchioles, alveolar ducts, alveolar sacs and alveoli. The respiratory bronchioles forming the first segment of the respiratory airways were further sub-divided into 1st and 2nd parts called terminal bronchiole and true respiratory bronchiole, respectively. The 1st part was lined with a single layer of low columnar or high cuboidal epithelial cells bearing apical secretory blebs whereas 2nd part was lined with a single layer of typical cuboidal epithelial cells with some clara cells protruding into the lumen. The epithelium was interrupted with out pocketing alveoli. There was no distinct basement membrane but the lining cells were supported by fine collagenous, reticular, few elastic and smooth muscle fibres. The cytoplasm of the lining epithelial cells in all age groups contained mucopolysaccharides with traces of glycogen but failed to demonstrate any other histochemical component studied during the present investigation.

Key words: Histomorphology, histochemistry, respiratory bronchiole, postnatal development, goat, lungs

The Indian Council of Agricultural Research (ICAR) is laying more stress on improvement of goats for milk, meat and fibres which constitute an important segment of animal wealth. Heavy mortality is reported due to respiratory tract diseases. Developmental deformities such as agenesis of lungs, faulty branching pattern of bronchial tree and defective development of respiratory alveolar system are also responsible for high death toll of kids in early age. Distal airways which are hereby defined as regions of respiratory exchange system are of physiological and clinical importance in gaseous exchange mechanism. Infact, the developmental anomalies of either hereditary or acquired have become the challenging problems in growth of goat husbandry. In order to gain complete understanding of causes and their possible elimination, the knowledge of histomorphological and histochemical picture of airways is essential. Very few reports are available on histomorphology and histochemistry of respiratory bronchiole of domestic animals in general and goat in particular. Hence, the present investigation has been undertaken to study the histomorphology and histochemistry of respiratory bronchiole in detail during postnatal development of lung from birth to one year of age.

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MATERIALS AND METHODS

In all, 20 goats (*Capra hircus*) of either sex (birth to 12 months of age) were used. These were divided into 4 age groups of 5 animals each from birth to 3 months, 4 to 6 months, 7 to 9 months and 10 to 12 months of age on the basis of eruption of teeth. The 1st age group was later on sub-divided from birth to 1 month, and 2 to 3 months of age in Table 1 only to show the maximum changes noticed in goats between 2-3 months of age as compared to those upto 1 month of age. The respiratory bronchiole tissues were collected from each goat and processed for histomorphological and histochemical studies with paraffin and frozen sectioning techniques The sections were stained with routine Harris' Haematoxylin and Eosin stain (Luna, 1968), Gomori's stain for reticulum (Luna, 1968), Weigert's stain for elastic fibers (Luna, 1968), Modified Mallory's triple stain (Crossman, 1937) and Van Gieson's stain for collagen fibers (Luna,

1968). The histochemical techniques used were: 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-red-'O' 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 for various microscopic parameters was done with the help of a linear calibrated ocular micrometer.

RESULTS AND DISCUSSION

Both the lungs of goats in all age groups were divided into different lobes and lobules. The respiratory airways included respiratory bronchioles, alveolar ducts, alveolar sacs and alveoli (Fig. 1). The same components of respiratory airways were reported by Bhattacharyya and Baishya (1995) in goats and Dellmann and Brown (1976) in domestic animals. Contrary to these findings, Iovannitti *et al.* (1985) in calf and Pirie *et al.* (1990) in horse revealed the absence of distinct respiratory bronchiole and sudden transition of terminal bronchiole to alveolar duct. Banks (1983) reported that respiratory bronchioles were infrequently observed in ruminants and swine, poorly developed in horse and man, while well developed in monkey and carnivores. Whereas, in the present study, the respiratory bronchiole in goats of all age groups was well developed and further sub-divided into two parts: 1st part (terminal bronchiole) lined with a single layer of low columnar or high cuboid epithelial cells without goblet cells and the 2nd part (true respiratory bronchiole) lined with a single layer of typical cuboidal epithelial cells (Fig. 2). The epithelium of the latter part was interrupted with outpocketing alveoli also from its walls (Fig. 3). Similarly, Bloom and Fawcett (1978) in mammals reported the same two parts of respiratory bronchioles lined in the 1st part with a ciliated simple columnar epithelium devoid of goblet cells and non-ciliated simple cuboidal epithelium in the 2nd part. While, Kahwa and Purton (1996) in goats classified bronchioles separately as terminal bronchiole lined with simple columnar or simple cuboidal epithelium, and respiratory bronchiole lined by simple cuboidal epithelium interrupted with outpocketing alveoli.

Only non-ciliated columnar or high cuboidal cells having secretory blebs on their apical surface were seen lining the terminal bronchiole of the 1st part respiratory bronchiole of goats in all age groups (Fig. 2) during the present investigation. Contrary to the presence of ciliated columnar cells forming the major cell population in terminal bronchiole of dog (Wright *et al.*, 1983), calf (Iovannitti *et al.*, 1985), horse (Pirie *et al.*, 1990), sheep (Bouljihad and Leipold, 1994) and in goats (Kahwa *et al.*, 1997) as well as other cells like few mucus cells noted in the epithelium lining terminal bronchioles of goats (Kahwa *et al.*, 1997) and few goblet cells in the epithelium lining the bronchioles of pig (Baskerville, 1970) could not be demonstrated in any part of the respiratory bronchiole in goats during the present study. Further, it is opined that the columnar cells having the secretary blebs on their apical surface might resemble the brush cells reported by Baskerville (1970) in terminal bronchiole of pig.

The 2nd part, true respiratory bronchiole in goats of all age groups was lined with simple cuboidal epithelium and interrupted by alveoli that outpocketed from the walls of this bronchiole (Fig. 3). It might be responsible for exchange of gases which is in consonance to the reports of Banks (1983) in domestic animals and Kahwa and Purton (1996) in goats. The specialized cuboidal cells known as Clara cells lining the true respiratory bronchiole of goats had protruding surface into the bronchiolar lumen (Figs. 3 and 4) which might have been responsible for the production of lung surfactant to protect the alveolus from collapsing, particularly at low lung volumes. Presence of non-ciliated bronchiolar epithelial Clara cells were also recorded in the respiratory bronchioles of domestic animals (Banks, 1983), sheep (Bouljihad and Leipold, 1994) and in goat (Kahwa *et al.*, 1997).

The average diameter as well as epithelial height in both the parts of respiratory bronchioles

recorded an abrupt increase in goats of 2 to 3 months of age over those below one-month of age and became static with further advancement of age up to 12 months of age (Table 1). Whereas Castleman and Lay (1990) noticed significant increase with age in the mean bronchiolar cross-sectional area in calves and noticed that the bronchial epithelium was well developed by the time the calves were 30 days old. Bhattacharyya and Baishya (1995) reported that age associated increase in the diameter and epithelial height of respiratory bronchioles and neonatal lungs of 30 days old kids almost resembled to that of adult goats. Whereas, Bhattacharyya *et al.* (1996 a) observed a positive correlation of cross-sectional diameter of the terminal bronchiole to age and body weight in Assam goats.

The basement membrane in both the parts of respiratory bronchioles in goats of all age groups was indistinct and the cells lining the two were supported by smooth muscle fibres (Fig. 4), collagen, reticular and few elastic fibres present in the bronchiolar wall which was in agreement to the reports in large ruminants (Dellmann and Brown, 1976) and dog (Banks, 1983). Lymphocytic infiltration in the form of lymphatic nodules was found in the connective tissue forming the wall of only 1st part of respiratory bronchiole in goats of all age groups (Fig. 2), which simulated to that in buffalo (Singh and Mariappa, 1981) as aggregations of lymphoid tissue in the connective tissue wall of the bronchi and bronchioles. Anderson *et al.* (1986) reported that the lymphoid tissue (BALT) was absent in neonatal lungs but increased progressively with age until it declined in aged adult cattle. The widely scattered lymphoid aggregates were the predominant morphological type of BALT in the bovine lungs.

The histochemical reactions have been summarized in Table 2. A strong PAS +ve reaction was observed in the cytoplasm of epithelial cells lining both parts of respiratory bronchiole in goats of all age groups (Fig. 5), which was slightly reduced on saliva treatment. The PAS +ve material formed secretory blebs (Fig. 6). Though, the cytoplasm of these cells reacted weakly with Best's carmine but it did not stain with Alcian blue dye which indicated that these cells contained traces of glycogen and mucopolysaccharides other than acidic and sulphated mucus substances. Bhattacharyya *et al.* (1996b) recorded an age associated increase in PAS +ve reaction up to 30 days of age in the basement membrane of bronchi and bronchioles in Assam local goats. Whereas, Kahwa and Purton (1996) denied the presence of mucus producing cells from the bronchiolar level distally in adult goats, but Baskerville (1970) reported that the non-ciliated cells lining the bronchioles contained masses of glycogen and a number of secretory granules in young pigs, which aggregated in the 9-week and 16-week old pigs. Any kind of fat or enzyme could not be demonstrated during the present investigation in these cells. The literature was also found silent about the presence of these substances in respiratory bronchioles of domestic animals.

Table 1
Micrometry of respiratory bronchiole in goat at different age groups (mean ± S.E.)

Age (Months)	Diameter (μm)	Epithelial height (μm)
0-1	167.88 ± 08.16	09.07 ± 0.35
2-3	175.53 ± 12.27	11.57 ± 0.49
4-6	180.12 ± 15.95	12.34 ± 0.41
7-9	182.97 ± 13.74	12.86 ± 0.53
10-12	182.80 ± 15.05	12.90 ± 0.48

Table 2
Histochemistry of respiratory bronchiole in goat at different age groups

Technique	Birth to 3 months	4 to 6 months	7 to 9 months	10-12 months
PAS without saliva	++++	++++	++++	++++
PAS with saliva	+++±	+++±	+++±	+++±
Best's carmine	±	±	±	±
Alcian blue at 2.5 pH	-	-	-	-
Nile blue sulphate	-	-	-	-
Sudan black-B	-	-	-	-
Oil-red-o	-	-	-	-
Gomori's revised method for				
(a) Alkaline phosphatase	-	-	-	-
(b) Acid phosphatase	-	-	-	-

- Negative, ± Weak, + Mild, ++ Moderate, +++ High, ++++ Strong

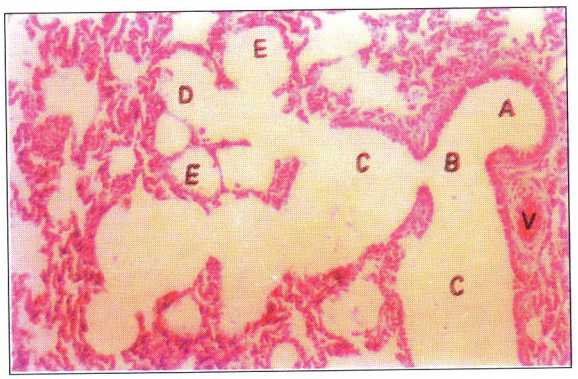

Fig.1 Photomicrograph of the goat lung (below 1 month of age) showing 1° part (A) and 2° part (B) of respiratory bronchiole, alveolar duct (C), alveolar sac (D) and alveoli (E).

(H. & E. x 50)

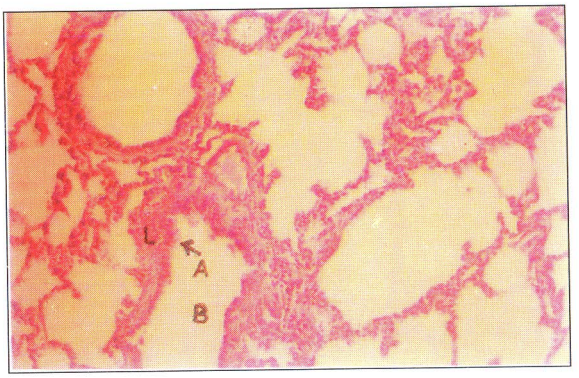

Fig.2 Photomicrograph of the goat lung (below 1 month of age) showing 1° part (A) and 2° part (B) of respiratory bronchiole. Note the secretory blebs (arrow) and lymph nodule (L.).

(H. & E. x 50)

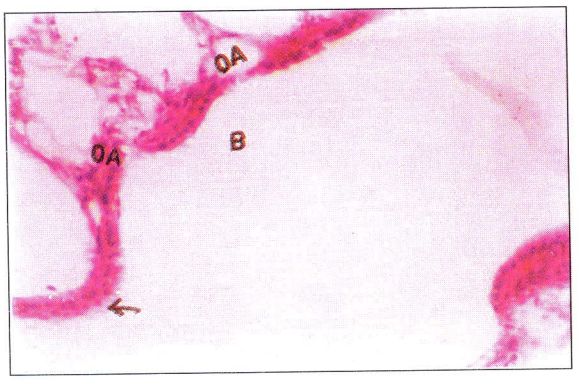

Fig.3 Photomicrograph of the goat lung (2 to 3 months of age) showing 2° part (B) of respiratory bronchiole interrupted with outpocketing alveoli (OA). Note protruding cuboidal (Clara) cells (arrow) lining the respiratory bronchiole.

(H. & E. x 100)

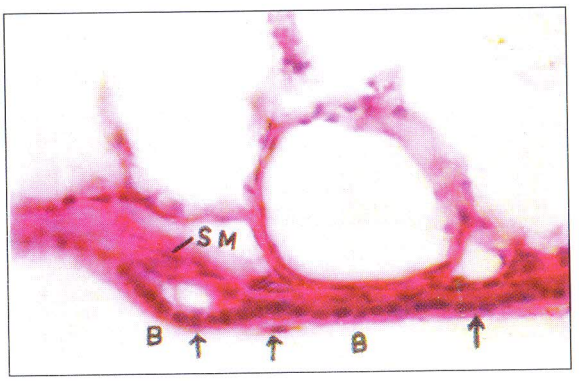

Fig.4 Photomicrograph of the goat lung (2 to 3 months of age) showing 2° part (B) of respiratory bronchiole and protruding surface (arrows) of cuboidal (Clara) cells. Note loosely arranged smooth muscle fibers(Sm.)

(H. & E. x 200)

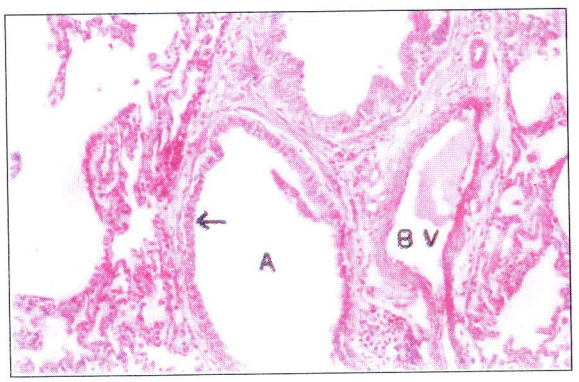

Fig.5 Photomicrograph of the goat lung (below 1 month of age) showing strong PAS +ve reaction (arrow) in the supranuclear zone of cells lining the respiratory bronchiole (A). Note mild homogenous reaction in the wall of blood vessel (BV).

(PAS x 50)

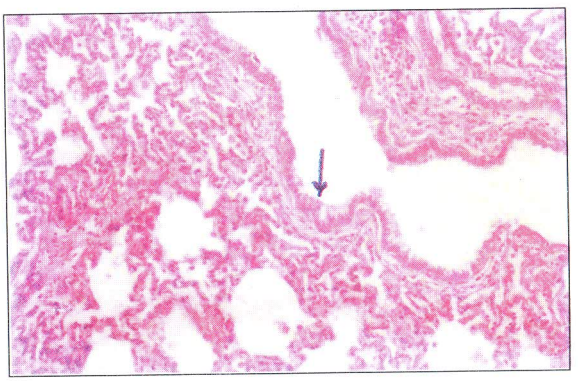

Fig.6 Photomicrograph of the goat lung (below 1 month of age) showing PAS +ve blebs (arrow) at the apical surface of the cells lining the respiratory bronchioles.

(PAS x 50.)

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