



Original Research

Histochemical Studies on Prenatal Lung of Gaddi Sheep

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Abstract

Detailed histochemical studies were conducted on (n=18) lung samples of Gaddi sheep fetuses. The lung samples were divided into three groups on the basis of their days of gestation as group-I (0-50 days of gestation), group-II (50-100 days of gestation) and group-III (100-150 days of gestation). Histochemical activity showed presence of glycogen, basic protein and fat in basement membrane of tubule, parenchyma/mesenchyme, bronchiole and bronchi basement membrane, blood vessel wall, alveoli, Type I & Type II alveolar cells, trabeculae, capsule and cartilage. A moderate activity was observed for glycogen in parenchyma and basement membrane of bronchioles in group-II. A strong reaction for basic protein was observed in alveoli and wall of blood vessels in group-III and a weak activity for fat was found in the basement membrane of bronchiole in group-III.

Key words: Alveoli, Bronchiole, Gaddi Sheep, Histochemistry, Prenatal Lung

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Introduction

Gaddi sheep is the important breed of Himachal Pradesh, used for fine carpet wool and meat production. A very little work has been done on the histochemistry of the lung of Gaddi sheep. Therefore, present investigation was planned to demonstrate the histochemical activity of glycogen, basic protein and fat in prenatal Gaddi sheep lung which can be used for further investigation and forms basis of further research. Mills *et al.* (1986) observed that the bronchial epithelium generally showed moderate reactions to carbohydrates in pigs. However, the goblet cells showed mild reaction.

Baba *et al.* (2013) studied the histochemistry of carbohydrates and proteins in the pulmonary bronchi of Black Bengal goat (*Capra hircus*) and found that the goblet cells, glands, cartilage and perichondrium manifested intense positive reaction for carbohydrates whereas, connective tissues, muscle fibres and blood vessels showed generally weak to moderate positive reactions. The bronchial epithelium, glands, cartilage,



matrix and muscle layers manifested strong reactions for acid mucopolysaccharides. Reaction for proteins was strong to intense in the bronchial epithelium, glands, perichondrium of bronchial cartilage and muscle fibres. Vikas (2015) conducted studies on prenatal goat fetuses and reported that a weak activity of glycogen, basic proteins, neutral and acidic mucopolysaccharides was observed in group I. The basement membranes were reported to be moderately glycogen and basic protein positive. A weak activity for basic proteins was observed in walls of blood vessels.

Materials and Methods

For present investigation (n=18) Gaddi sheep fetuses were collected from the slaughter houses in and around Palampur region and were then divided into three groups based on their estimated ages *viz.* Group-I (below 50 days), Group-II (between 50-100 days) and Group-III (above 100 days of age) containing 6 number of fetuses in each group. The age was obtained by the formula, $W^{1/3}=0.096(t-30)$ Singh *et al.* (1979), where, W is body weight of fetus in g, t is age of fetus in days. The lung was infused by intra tracheal instillation of 10% neutral buffered formalin solution (Luna, 1968) until it was inflated (of life size), then ligated on the trachea and immersed in the same fixative in a container for proper fixation (Drury and Wallington, 1980). Tissue pieces were collected from apical, cardiac, diaphragmatic and intermediate lobes of the fixed lungs of the sheep fetuses. These tissues were processed for paraffin block preparation by alcohol- benzene schedule (Luna, 1968). Tissue sections of 5-6 μm were obtained from these blocks on clean glass slides with the help of rotary microtome. The sections were then subjected to Best Carmine method for glycogen, Sudan Black B method for fat (Luna, 1968) and Bromophenol Blue method for proteins (Pearse, 1972).

Statistical Methods

No statistical method was used for the present study.

Results and Discussion

Glycogen

In Group-I there was a moderate reaction for glycogen in the mesenchyme, basement membrane of bronchiolar epithelium and blood vessels (Plate 1), whereas the basement membrane of tubule and capsule were moderately to strongly reactive for glycogen. In Group-II a moderate activity was observed for glycogen in parenchyma, basement membrane of bronchiole, wall of blood vessel (Plate 2), but a weak reaction was observed in the capsule. The epithelium of bronchi and alveoli showed moderate amount of glycogen (Plate 3). A moderate reactivity to glycogen was seen in (Table 1). Mills *et al.* (1986) observed that the bronchial epithelium generally showed moderate reactions to carbohydrates in pigs. In contrast,

Suman *et al.* (2005) observed a weak reaction for carbohydrates in respiratory bronchiole in post natal lung in goat.

Table 1: Reaction exhibited by tissue components for glycogen

Parameter	Group-I	Group-II	Group-III
Basement membrane of tubule	++/+++	+++	NA
Parenchyma/Mesenchyme	++	++	++
Bronchiole basement membrane	++	++	++
Bronchi basement membrane	NA	+++	++
Blood vessel wall	++	++	++
Alveoli	NA	NA	++
Type I & Type II cells	NA	NA	++
Trabeculae	NA	++	++
Capsule	++/+++	+	++
Cartilage	NA	+	++

- Absent, + weak, ++ moderate, +++ strong, +++++ intense, NA not found

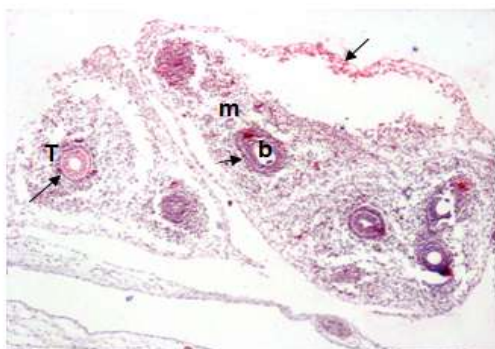


Plate 1. Photomicrograph showing moderate to strong reaction for glycogen in capsule (arrow), basement membrane of tubule (T), bronchi (b) and around the bronchioles (b). Best Carmine, 46 days, 40X

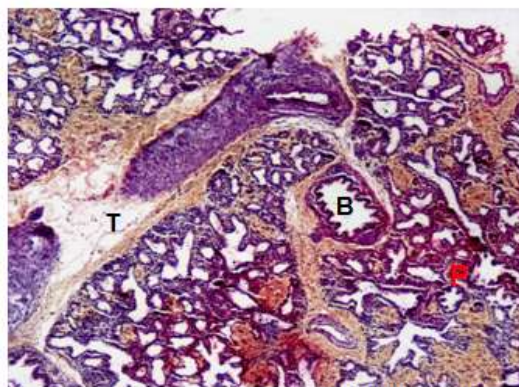


Plate 2. Photomicrograph showing moderate reactivity for glycogen in parenchyma (P), basement of membrane of bronchi (B) and trabeculae (T). Right apical lobe. Best Carmine, 100 days, 40X

Basic Protein

In group-I strong reactivity for basic proteins was observed in basement membrane of tubule (Plate 4) and bronchiole. However, a moderate reaction was observed in the mesenchyme and capsular region.

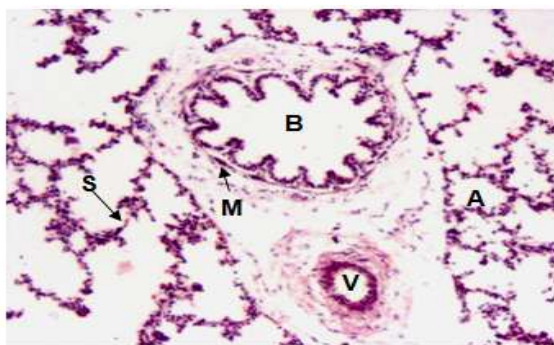


Plate 3. Photomicrograph showing moderate reactivity for glycogen in epithelium of bronchi (B), alveoli (A), interalveolar septae (S), blood vessel (V) and muscularis (M) layer lining the bronchi in right diaphragmatic lobe, Best Carmine, 145 days, 100X

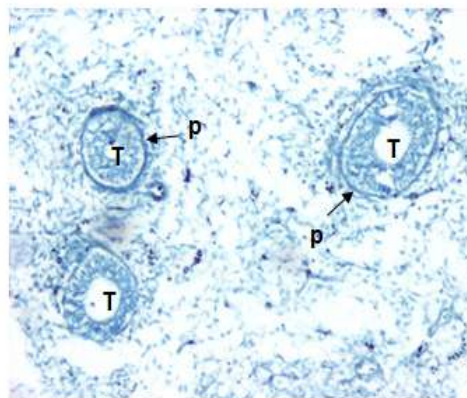


Plate 4. Photomicrograph showing strong reactivity for basic proteins (p) in basement membrane of tubules (T). Bromophenol Blue stain, 42 days, 100X

Table 2: Reaction exhibited by tissue components for basic protein

Parameter	Group - I	Group - II	Group - III
Basement membrane of tubule	+++	+++	NA
Parenchyma/Mesenchyme	++	++	+++
Bronchiole basement membrane	+++	+++	+++
Bronchi basement membrane	NA	++	+++
Blood vessel wall	++/+++	++	+++
Alveoli	NA	NA	+++
Type I & Type II cells	NA	NA	++
Trabeculae	NA	++	++
Capsule	++	+++	++
Cartilage	NA	++	++

- Absent, + weak, ++ moderate, +++ strong, ++++ intense, NA not found

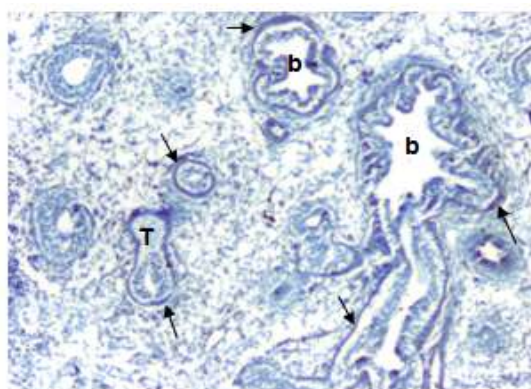


Plate 5. Photomicrograph showing strong reactivity (arrows) in basement membrane of bronchiole (b) and around tubule (T) in right diaphragmatic lobe, Bromophenol Blue stain, 54 days, 100X

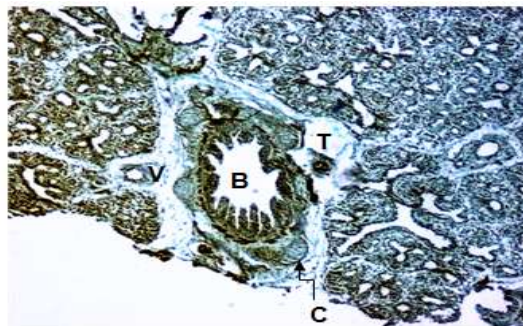


Plate 6. Photomicrograph showing moderate reaction for basic proteins in cartilage (C), trabeculae (T), blood vessel wall (V), basement membrane of bronchi (B) and parenchyma in right diaphragmatic lobe, Bromophenol Blue stain, 91 days, 40X

A moderate reaction was observed in cartilage, trabeculae, blood vessel wall, basement membrane of bronchi and parenchyma of the lung tissue in group-II (Plate 6). Whereas, the basement membrane of bronchiole (Plate 5) and capsule showed strong reactivity for basic proteins. In group-III a moderate reactivity was observed in cartilage, capsule, trabecular region of the lung tissue. While a strong reaction in alveoli, wall of blood vessel, basement membrane of bronchi & bronchiole was observed (Plate 7).

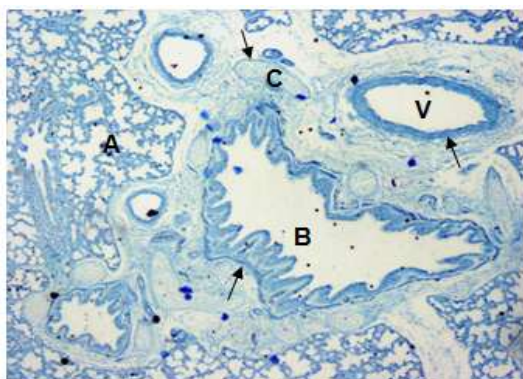


Plate 7. Photomicrograph showing strong reaction in alveoli (A), wall of blood vessel (V), basement membrane of bronchi (B) and cartilage (C) in right accessory lobe. Bromophenol Blue stain, 144 days, 40X

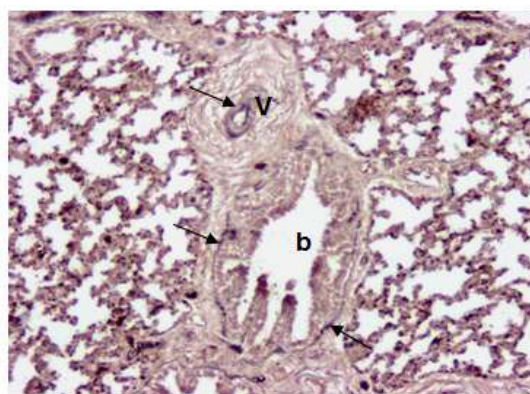


Plate 8. Photomicrograph showing weak reaction for fat around the bronchiole (b) and inside the blood vessel (V) in left cardiac lobe. Sudan Black B stain, 144 days, 40X

In group-III strong reactions to basic protein was observed in the cytoplasm of the lining epithelium of the developing airways (Table 2). Baba *et al.* (2013) reported that the cytoplasm of the lining epithelium of the developing airways showed strong reactions to basic proteins. Similar observation was found in group-III.

Fat

The fat content was not observed in the lungs of fetuses in group I and II. However a weak reaction to fat was observed in group-III around the basement membrane of bronchioles and internal lamina of blood vessels (Plate 8).

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Conclusion

With the advancement of gestational age the parenchyma, basement membrane of bronchiole & bronchi, blood vessel wall, trabeculae, capsule and cartilage exhibited moderate reaction for glycogen except for basement membrane of tubule as tubular stage of lung development is not found in advanced gestational age. The moderate amount of glycogen present in various tissue components pertain to the use of glycogen



reserves by the growing fetus such as in the synthesis of surface active phospholipids in the adult lung. The basic protein content increased in parenchyma, bronchi basement membrane and wall of blood vessels with the advancement of gestational age. As alveoli, Type I & Type II cells are visible only above 100 days of gestation, they exhibited moderate reaction for glycogen. The alveoli exhibited strong protein content whereas Type I & Type II cells exhibited moderate basic protein content above 100 days of gestation.

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