

Original Research Article

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Micrometrical Studies of Bronchi and Bronchiole in Prenatal Gaddi (Breed) Sheep Lung

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ABSTRACT

The present study was conducted to investigate various micrometric parameters of bronchiole and bronchi in prenatal Gaddi sheep lung. A total of n=18 Gaddi sheep fetuses were collected from various slaughter houses in and around Palampur town for one year. The prenatal lung samples were divided into three groups which included group-I (0-50 days of gestation), group-II (50-100 days of gestation) and group-III (100-150 days of gestation) based on their age in days. The overall diameter of bronchiole increased from group-I to group-III. However, the diameter of bronchi decreased significantly in right apical, left apical and left cardiac lobe but it increased significantly in right cardiac and left diaphragmatic lobe from group-II to group-III. The epithelial height of bronchiole decreased from group-II to group-III in left apical and right diaphragmatic lobes. While, the height of nucleus of bronchiole decreased significantly from group-II to group-III in left apical, left cardiac and left diaphragmatic lobes. The epithelial height of bronchi decreased significantly from group-II to group-III in apical and accessory lobes of right lung. However, the nucleus height of bronchi decreased significantly from group-II to group-III in right apical and right diaphragmatic lobes.

Keywords

Bronchiole, Epithelium, Gaddi sheep, Prenatal lung, Micrometry

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Introduction

According to 19th livestock census (2012) the total sheep population of Himachal Pradesh is 8,04,334 and Gaddi sheep comprises 70 percent of the total sheep population. Gaddi sheep is a medium sized breed and is one among forty two well-defined sheep breeds of India, mainly distributed in Kangra, Shimla, Chamba, Kinnour and Kullu districts of Himachal Pradesh. The main objective of the

study was to establish histogenesis and organogenesis of lung in prenatal period of Gaddi sheep.

Review of literature reveals that the studies on micrometrical parameters of bronchiole and bronchi are very little in Gaddi sheep. Keeping this fact in mind present investigation was carried out to elucidate the micrometrical details of bronchiole and bronchi in Gaddi sheep lung.

Materials and Methods

Collection and estimation of age of Gaddi sheep fetuses

Eighteen 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), Group-III (above 100 days of age) containing 6 number of fetuses in each group. The age of the fetus was obtained by the formula given by Singh *et al.*, (1979),

$$W^{1/3}=0.096(t-30)$$

Where, 'W' is body weight of fetus in grams, 't' is age of fetus in days.

Collection and histological processing of tissue for micrometrical study

The tissue pieces were collected from apical lobe (at the middle point of cranial and caudal part), cardiac lobe (at the middle point), diaphragmatic lobe (cranially at 0.5 cm behind the interlobular fissure between the cardiac and diaphragmatic lobes) in both right and left lungs. Whereas, in accessory lobe which is present only in the right lung, the tissue was collected at the middle point of the formalin fixed lung lobe 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 stained with Haematoxylin and Eosin.

The parameters which were measured include diameter of bronchiole, diameter of bronchi, epithelium height of bronchiole, nucleus height of the epithelium of bronchiole, epithelium height of bronchi and nucleus height of the epithelium of bronchi. The lungs of group-I of sheep fetuses were embedded as a whole due to very small size of the lobes. So the readings of group-I were taken as a whole.

The comparison is done between various lobes of group-II and group-III. Bronchi are absent in group-I as it depicts tubular stage of lung development which indicates mainly presence of tubules scattered in the loose mesenchymal tissue. Contrary to which bronchi are present in group-II and group-III which depicts the higher stages of lung development. So, in the present study the first comparison is done between the overall values of various parameters between the three groups. However, the second comparison is made between different parameters of bronchiole and bronchi which are present in group-II and group-III and various lobes of both right and left lungs.

The mean values of all the micrometrical parameters were subjected to statistical analysis for comparison between the different lobes of lungs using one way ANOVA and T test (SAS Statistics-9.2). A probability of $p < 0.05$ was considered as statistically significant.

Results and Discussion

Diameter of bronchiole

In the sheep fetuses of group-I the overall diameter of bronchiole was recorded as $168.25 \pm 13.02 \mu\text{m}$. While, in group-II and group-III the overall diameter was recorded as $196.80 \pm 13.68 \mu\text{m}$ and $203.57 \pm 7.66 \mu\text{m}$. The diameter of bronchiole in various lobes of both right and left lung of group II and group III has been listed in Table 1. The diameter of bronchiole was significantly highest in the left diaphragmatic lobe in group II.

The overall diameter of bronchiole increased from group-I to group-III. The diameter of bronchiole increased significantly in left apical, right cardiac and right accessory lobe whereas it decreased significantly in left diaphragmatic lobe from group-II to group-III.

Diameter of bronchi

The overall diameter of bronchi was $389.09 \pm 20.57 \mu\text{m}$ in group-II which decreased significantly to $334.30 \pm 16.47 \mu\text{m}$ in group-III. The diameter of bronchi in various lobes of group II and group III of both right and left lungs has been recorded in Table 1. The diameter of bronchi decreased significantly in right apical, left apical and left cardiac lobe but it increased significantly in right cardiac and left diaphragmatic lobe from group-II to group-III.

Epithelium height of bronchiole

In the Gaddi sheep fetuses of group-I the height of epithelium of bronchiole was recorded as $39.40 \pm 2.33 \mu\text{m}$ which decreased significantly to $9.67 \pm 0.26 \mu\text{m}$ and $8.35 \pm 0.15 \mu\text{m}$ in group-II and group-III respectively. In group-II, the epithelium height of bronchiole was significantly higher in right diaphragmatic lobe as compared to right apical and right cardiac lobe of lung. Whereas, in rest of the lobes epithelial height of bronchiole did not vary significantly (Table 2). While, in group-III the epithelium height of bronchiole was highest in left diaphragmatic lobe and lowest in left apical lobe. However, there was no significant variation in the epithelial height within various lobes of left and right lung (Table 2).

The epithelial height of bronchiole decreased from group-II to group-III in left apical and right diaphragmatic lobes. Between group-II and III a non-significant decrease was observed in epithelium height in the rest of the lobes of right and left lung respectively.

Nucleus height of epithelium of bronchiole

In the Gaddi sheep fetuses of group-I the overall height of nucleus of bronchiole was $8.51 \pm 0.32 \mu\text{m}$ which decreased to $5.84 \pm 0.11 \mu\text{m}$ and $5.39 \pm 0.07 \mu\text{m}$ in group-II and III respectively. In group-II, the diaphragmatic

lobe of left lung had significantly higher nuclear height as compare to apical lobe of right lung, which had lowest nuclear height of epithelium of bronchiole among all the lobes of lung. Whereas, in rest of the lobes epithelial height of bronchiole did not vary significantly (Table 2). In group-III there was no significant difference within the lobes of left and right lung. In general, the nucleus height of bronchiole decreased with increase in gestational age except for right apical lobe in the present study. The height of nucleus of bronchiole decreased significantly from group-II to group-III in left apical, left cardiac and left diaphragmatic lobes (Table 2).

Epithelium height of bronchi

The overall height of epithelium of bronchi of group-II was recorded as $12.98 \pm 0.64 \mu\text{m}$, which decreased to $10.94 \pm 0.33 \mu\text{m}$ in group-III in the Gaddi sheep fetuses. In Group-II, the epithelial height in right apical lobe was significantly higher than all the lobes of lungs followed by left diaphragmatic lobe. Left apical, left cardiac and accessory lobes did not varied significantly from left and right diaphragmatic lobes. Thereafter, the epithelial height reduced significantly in bronchi present in cardiac lobe of the right lung (Table 2). While in group-III, the epithelial height of bronchi epithelium was significantly higher in cardiac lobe of left lung as compared to that of apical, diaphragmatic and accessory lobes of right lung (Table 2).

The epithelial height of bronchi decreased significantly from group-II to group-III in apical and accessory lobes of right lung. In rest of the lobes change was non-significant.

Nucleus height of epithelium of bronchi

In the Gaddi sheep fetuses of group-II the overall height of nucleus of bronchi was recorded as $12.98 \pm 0.64 \mu\text{m}$ which reduced to $10.94 \pm 0.33 \mu\text{m}$ in group-III. In group-II,

the height of nucleus was recorded to be significantly highest in right apical lobe as compare to that of left apical & right cardiac lobe (Table 2). While the other lobes of right and left lung had intermediate values of nuclear height which did not vary significantly. While in group-III, there was no significant difference in the values of nucleus height within the various lobes of right and left lung respectively (Table 2).

The nucleus height of bronchi decreased significantly from group-II to group-III in right apical and right diaphragmatic lobes. The nucleus height of rest of the lobes

decreased non- significantly with increase in gestational age except that of right cardiac lobe.

The diameter of bronchiole increased significantly in left apical, right cardiac and right accessory lobe whereas it decreased significantly in left diaphragmatic lobe from group-II to group-III. The diameter of bronchi decreased significantly in right apical, left apical and left cardiac lobe but it increased significantly in right cardiac and left diaphragmatic lobe from group-II to group-III.

Table.1 Diameter of bronchiole and bronchi of different lobes

LOBES	Diameter of bronchiole		Diameter of bronchi	
	Group II	Group-III	Group II	Group-III
Apical lobe				
Right lung	156.98 ^B ± 33.41 (114.36-323.39)	185.48 ^{AB} ± 19.68 (113.44-263.74)	598.31 ^{Aa} ± 5.88 (574.94-613.52)	265.39 ^{BCb} ± 14.42 (217.21-300.66)
Left lung	152.85 ^{Ba} ± 11.13 (112.59-181.21)	201.09 ^{ABb} ± 17.91 (170.92-288.48)	403.41 ^{Ca} ± 1.23 (398.69-407.35)	311.12 ^{BCb} ± 22.84 (254.29-369.75)
Cardiac lobe				
Right lung	130.38 ^{Ba} ± 10.99 (96.19-156.91)	204.09 ^{ABb} ± 6.26 (193.43-232.68)	211.23 ^{Ea} ± 15.01 (177.61-244.81)	333.11 ^{BCb} ± 28.12 (229.78-391.84)
Left lung	231.69 ^{AB} ± 52.65 (129.99-452.41)	161.81 ^B ± 15.75 (98.42-193.83)	513.33 ^{Ba} ± 0.08 (512.98-513.55)	347.18 ^{Bb} ± 28.17 (259.69-414.81)
Diaphragmatic lobe				
Right lung	239.58 ^{AB} ± 28.47 (171.80-347.41)	209.10 ^{AB} ± 10.31 (161.15-231.68)	294.26 ^D ± 40.46 (199.82-471.51)	221.98 ^C ± 21.06 (168.55-312.07)
Left lung	312.62 ^{Aa} ± 19.73 (228.11-343.73)	203.29 ^{ABb} ± 19.01 (138.41-278.78)	414.12 ^{Ca} ± 15.52 (364.73-439.56)	514.21 ^{Ab} ± 0.81 (510.45-516.04)
Accessory lobe				
Right lung	153.53 ^{Ba} ± 12.81 (94.25-175.46)	260.13 ^{Ab} ± 28.99 (183.07-359.18)	289.03 ^D ± 1.09 (285.58-291.51)	347.12 ^B ± 51.89 (274.81-588.79)

Values having different superscripts in each row (a, b) and column (A, B, C, D, E) differ significantly (P<0.05).

Values within parenthesis denote the range.

Table.2 Epithelial and nucleus height of bronchiole and bronchi

LOBES	Epithelium height of bronchiole		Nucleus height of bronchiole		Epithelium height of bronchi		Nucleus height of bronchi	
	Group II	Group III	Group II	Group III	Group II	Group III	Group II	Group III
Apical lobe								
Right lung	8.50 ^B ± 0.34 (7.79-10.11)	7.89 ± 0.39 (6.45-9.09)	5.14 ^B ± 0.21 (4.34-5.68)	5.36 ± 0.25 (4.67-6.31)	22.62 ^{Aa} ± 0.19 (21.98-23.12)	9.38 ^{Cb} ± 0.38 (8.23-10.87)	6.95 ^{Aa} ± 0.05 (6.75-7.10)	5.92 ^b ± 0.13 (5.68-6.52)
Left lung	9.07 ^{ABa} ± 0.33 (7.72-9.70)	7.81 ^b ± 0.29 (7.07-8.93)	6.23 ^{ABa} ± 0.24 (5.22-6.88)	5.35 ^b ± 0.12 (4.92-5.65)	11.45 ^{BC} ± 0.22 (10.83-12.11)	10.88 ^{ABC} ± 0.57 (9.07-12.89)	5.93 ^B ± 0.23 (5.37-6.97)	5.82 ± 0.21 (5.26-6.54)
Cardiac lobe								
Right lung	8.55 ^B ± 0.14 (8.16-9.11)	8.40 ± 0.28 (7.24-9.37)	5.83 ^{AB} ± 0.32 (4.95-6.85)	5.56 ± 0.25 (4.77-6.22)	^D 9.36 ± 0.15 (8.91-9.65)	10.64 ^{BC} ± 0.66 (8.61-12.14)	5.93 ^B ± 0.14 (5.59-6.61)	6.99 ± 0.66 (5.10-8.90)
Left lung	9.55 ^{AB} ± 0.37 (8.69-11.30)	8.69 ± 0.41 (6.90-9.57)	6.15 ^{ABa} ± 0.11 (5.87-6.58)	5.36 ^b ± 0.15 (5.01-5.93)	11.79 ^{BC} ± 0.09 (11.45-12.01)	13.55 ^A ± 0.94 (11.21-17.40)	6.29 ^{AB} ± 0.19 (5.66-6.87)	6.12 ± 0.35 (5.30-7.77)
Diaphragmatic lobe								
Right lung	11.31 ^{Aa} ± 0.76 (9.20-14.08)	8.18 ^b ± 0.35 (7.08-9.42)	5.53 ^{AB} ± 0.34 (4.34-6.54)	4.86 ± 0.16 (4.32-5.36)	11.02 ^C ± 0.61 (8.91-12.45)	9.04 ^C ± 0.68 (7.00-11.54)	6.83 ^{ABa} ± 0.37 (5.66-7.91)	5.61 ^b ± 0.18 (4.97-6.31)
Left lung	10.96 ^{AB} ± 1.08 (8.52-15.78)	8.86 ± 0.51 (7.86-10.47)	6.34 ^{Aa} ± 0.13 (5.91-6.74)	5.64 ^b ± 0.16 (5.20-6.15)	12.71 ^B ± 0.54 (10.92-14.82)	13.07 ^{AB} ± 0.19 (12.12-13.35)	6.57 ^{AB} ± 0.11 (6.32-6.90)	6.16 ± 0.19 (5.45-6.77)
Accessory lobe								
Right lung	9.74 ^{AB} ± 0.39 (8.88-11.52)	8.64 ± 0.45 (7.41-9.91)	5.70 ^{AB} ± 0.32 (4.80-7.11)	5.65 ± 0.12 (5.36-6.14)	11.88 ^{BCa} ± 0.19 (11.34-12.47)	10.03 ^{Cb} ± 0.68 (8.47-12.00)	6.21 ^{AB} ± 0.24 (5.30-6.90)	5.97 ± 0.29 (5.23-6.95)

Values having different superscripts in each row (a, b) and column (A, B, C) differ significantly (P<0.05). Values within parenthesis denote the range.

Sohi *et al.*, (2006) reported in buffalo fetuses bronchiolar diameter decreased with increase in CRL. Castleman and Lay (1990) stated that the mean bronchiolar cross-sectional area increased significantly with age in calves. Similar observations were found by Bhattacharya (1994) in goat fetuses. The

epithelium height of bronchiole was significantly higher in right diaphragmatic lobe as compared to right apical and right cardiac lobe of lung in Gaddi sheep fetuses of 50-100 days of gestation. In the present study it was observed that with the advancement of gestational age the epithelial height of

bronchiole decreased as reported earlier by Sohi *et al.*, (2006) in fetuses of Indian buffalo. The epithelial height of bronchi decreased significantly from group-II to group-III in apical and accessory lobes of right lung. In rest of the lobes change was non-significant. Sohi *et al.*, (2006) had also reported that the epithelial height decreased with increase in Crown Rump Length in the lungs of prenatal Indian buffalo.

The epithelial height of bronchiole decreased with the advancement of gestational age. While, the nucleus height of bronchiole decreased with increase in gestational age except for right apical lobe in the current study. The epithelial height of bronchi decreased significantly from group-II to group-III in apical and accessory lobes of right lung. However, the nucleus height of bronchi decreased significantly from group-II to group-III in right apical and right diaphragmatic lobes.

Acknowledgements

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