Development of the respiratory acinus in the rabbit lung

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*Abstract:-*The present study was carried out on 38 New Zealand White rabbit fetuses and 10 newborn New Zealand White rabbits. The fetuses were collected on gestational days 23, 25, 27 and 29, while the neonates were sacrificed at the first and third days of postnatal life. The present study showed that at 25th Gestational day, the fetal rabbit lung was in the canalicular stage. At 27th Gestational day, the fetal rabbit lung was in the saccular stage. Alveolar stage extended from 29th Gestational day to the third postnatal day.

Key words:- Lung development, Fetal rabbit lung, Respiratory acinus, Respiratory portion.

1- Introduction

The rabbit fetus is one of the most commonly used animal models in experimental studies investigating fetal lung development **[1]** However, the available literature of the morphological features of developing rabbit lung during fetal and early neonatal life are relatively few. The aim of the present study is to give more information about the normal development respiratory portion in rabbit lung during late fetal and early neonatal life and to determine whether it may be a suitable model for human lung development or not.

2- Materials and Methods

Specimen collection

38 New Zealand White rabbit fetuses were collected on gestational days 23, 25, 27 and 29. 10 newborn New Zealand White rabbits (the first and third days of postnatal life) were also collected. The fetal chest was incised under a dissecting microscope and both lungs were carefully exposed and observed.

Each newborn rabbit was sacrificed. The animal chest was incised and both lungs were exposed

Light microscopy

The entire lung of each fetal and neonate rabbit was immediately fixed in Bouin's fluid for 7- 22 hours. The fixed materials were dehydrated, cleared and embedded in paraffin wax. Step serial sagittal and transverse sections were obtained at 5-7 µm and stained with the following histological stains:- 1- Harris's Haematoxylin and Eosin for general histological examination **[2]**. 2- Weigert's Resorcin Fuchsin stain for demonstration of elastic fibers **[3]**. Other small specimens of lung tissue were fixed in a mixture of 2.5% paraformaldehyde and 2.5% glutaraldehyde in 0.1M Na-cacodylate buffer, pH 7.3 for 4 hours at 4 Cº. They were washed in the same buffer used and then post-fixed in 1% osmic acid in 0.1M Na-cacodylate buffer for further 2 hours at room temperature. The samples were then dehydrated in ethanol and embedded in Araldite-Epon mixture. Semithin sections (1µm in thickness) were cut and stained with Toluidine blue.

Morphometry

Radial alveolar count: it provides a reliable index of lung growth during intrauterine and early postnatal life **[4]**. Perpendicular line was drawn from the lumen of terminal bronchiole to the nearest connective tissue septum and the number of saccules or alveoli interrupted by this line was counted **(Fig1).**

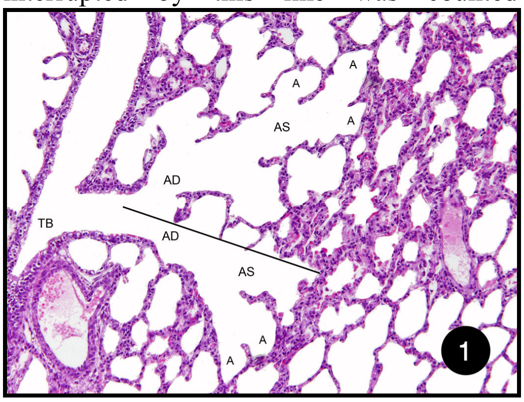


Fig. 1:- Radial alveolar count estimation in rabbit lung at the first day of life. Terminal bronchiole (TB), alveolar ducts (AD), alveolar sacs (AS), alveoli (A).

3- Results

In 25-day-old rabbit fetuses, all airway generations of fetal rabbit lung became greatly widened and elongated; resulting in a marked reduction of the pulmonary interstitial connective tissue **(Fig. 2)**.

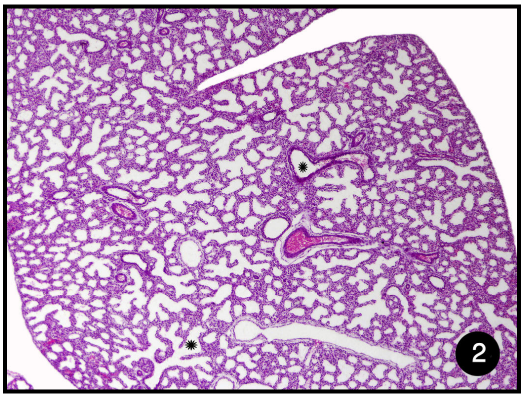


Fig. 2:- Transverse section in fetal rabbit lung at 25th gestational day, showing the massive widening and lengthening of all airspace generations (stars); resulting in a marked reduction of the mesenchymal tissue **(Haematoxylin and Eosin, X 40)**

At this gestational age, the fetal rabbit lung was in canalicular stage of development. Each terminal bronchiole branched into 2 - 4 wide and straight canals termed acinar canals or canaliculi **(Fig. 3)**.

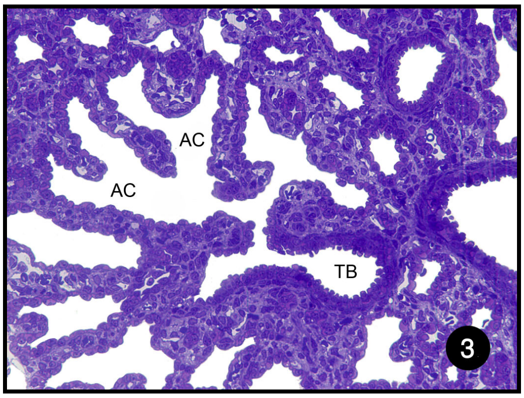


Fig. 3:- Transverse section in fetal rabbit lung at 25th gestational day showing terminal bronchiole (TB) branched into several acinar canals (AC). **(Semithin section, Toluidine blue, X 200)**

Some cells of the epithelial lining of these acinar canals started to flatten and differentiate into prospective type I pneumocytes**(Fig. 4)**. Other epithelial cells remained cuboidal and showed metachromatic reaction in semithin section stained with toluidine blue. Vascularization of the surrounding mesenchymal tissue was progressively increased. Numerous dilated thin-walled blood capillaries were demonstrated in close contact to the acinar canals or canaliculi. Some capillaries displayed an intimate contact to the flattened cells resulting in the formation of first air-blood barriers.

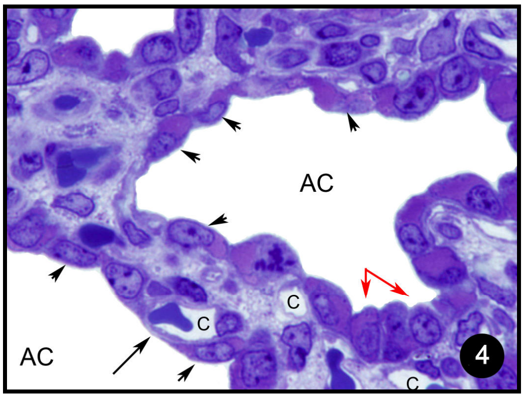


Fig. 4:- Transverse section in fetal rabbit lung at 25th gestational day, showing the acinar canals (AC). Some cells start to flatten (short arrows), some capillaries (C) show an intimate contact to the flattened cells resulting in the formation of first air-blood barriers (long black arrow), and other cells remain cuboidal (red arrows)**(Semithin section, Toluidine blue, X1000)**

Step serial paraffin sections stained with Weigert's Resorcin Fuchsin demonstrated fine elastic membranes in inter-canalicular septa **(Figs. 5)**.

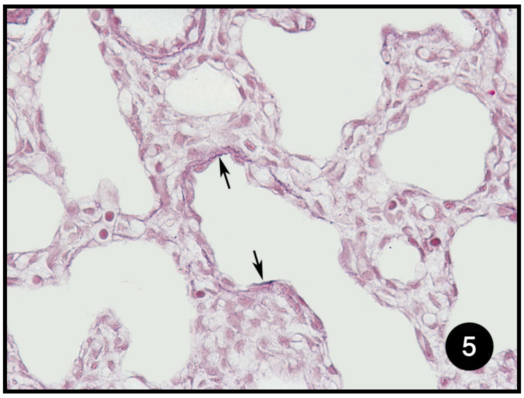
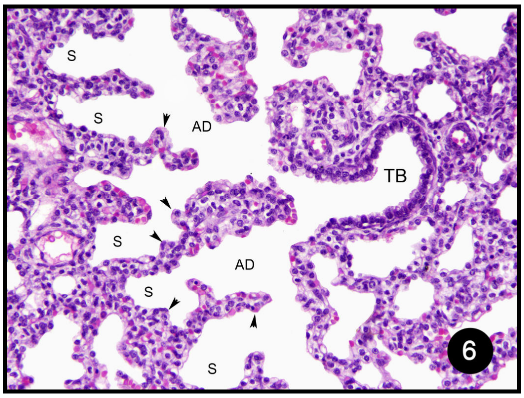


Fig. 5:- Transverse section in fetal rabbit lung at 25th gestational day, showing fine elastic membranes in acinar septa (arrow)**(Weigert's Resorcin Fuchsin, X 400)**

In 27-day-old rabbit fetuses, the fetal rabbit lung was in the saccular stage of development. The terminal bronchioles branched into several prospective alveolar ducts which ended by typical clusters of widened airspaces termed air saccules or terminal sacs. The surrounding mesenchymal tissue condensed to form thick inter-saccular septa or primary septa that contained a double capillary layer. Inter-saccular (primary) septa were relatively thick and their interstitium was highly cellular and contained numerous fine elastic fibers **(Figs. 6 and 7)**. Few low septal ridges (developing secondary septa) were demonstrated protruding from the primary septa **(Fig. 6)**.

ISBN: 978-1-61804-111-1



Figs. 6:- Transverse section in fetal rabbit lung at 27th gestational day, showing prospective terminal bronchiole (TB), prospective alveolar ducts (AD), air saccules (S), and developing secondary septa (arrowheads)**(Haematoxylin and Eosin, X 200)**

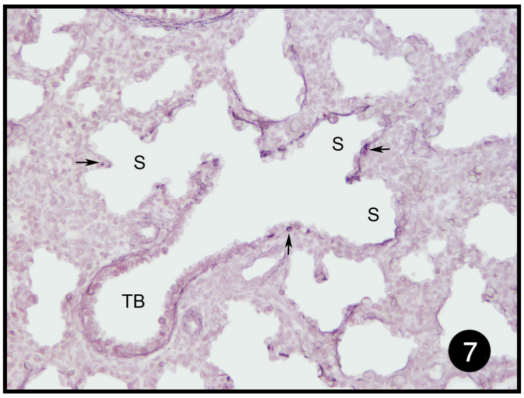


Fig. 7:- Transverse section in fetal rabbit lung at 27th gestational day, showing terminal airway unit started by terminal bronchiole (TB) and ended by air saccules (S). Fine elastic strands are demonstrated in the interstitium surrounding the airway (arrows) **(Weigert's Resorcin Fuchsin, X 200)**

Very fine elastic fibers were recognized in the apical portion of these developing crests **(Fig. 8)**.
Semithin section stained with toluidine blue showed that air saccules were lined largely by squamous type I pneumocytes which characterized by densely stained nuclei. Morphologically mature type II pneumocytes were interspersed singly among type I pneumocytes. Type II pneumocytes were nearly rounded or cubiodal in shape, projected to the airway lumen and contained large vesicular rounded nuclei with distinct nucleolus and peripherally arranged chromatin. Darkly stained intra-cytoplasmic inclusions were demonstrated between the nucleus and the apical cell pole **(Fig.9)**.

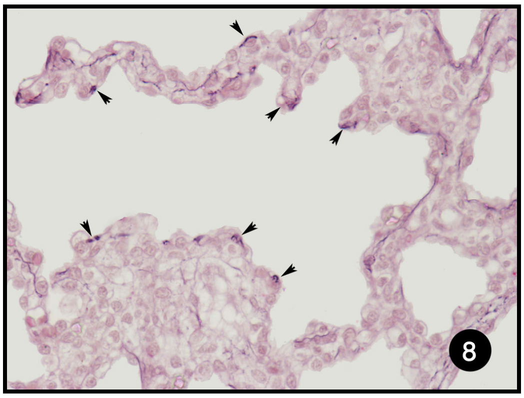


Fig. 8:- Transverse section in fetal rabbit lung at 27th gestational day, showing very fine elastic fibers concentrating in the apical portion of the developing crests (arrowheads)**(Weigert's Resorcin Fuchsin, X 400)**

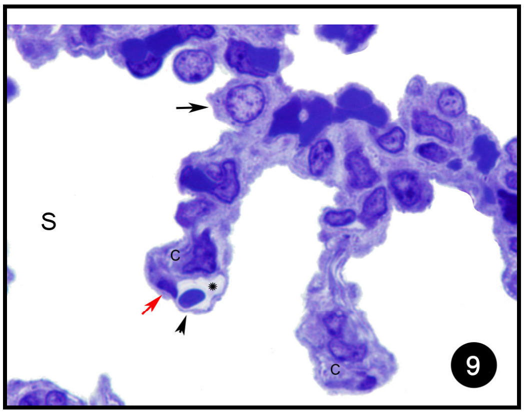


Fig. 9:- Transverse section in fetal rabbit lung at 27th gestational day, showing air saccule (S). Notice the developing crests or secondary septa (C), blood capillary (star), type II pneumocytes (black arrow), and type I pneumocytes (red arrow) that constitute part of the extremely thin blood air barrier (black arrowheads)**(Semithin section, Toluidine blue, X1000)**

The mean value of radial alveolar count at this stage of development was 1.75 ± 0.05 **(Table 1).**

Age	Radial alveolar count
27 GD	1.75 ± 0.05
29 GD	3.8 ± 0.02
1(p.n)	4.75 ± 0.25
3(p.n)	5.4 ± 0.61

Table (1):- The radial alveolar count of the rabbit lung during the fetal and early neonatal life. (Values are expressed as mean ± SE.)

In 29-day-old rabbit fetuses, the fetal rabbit lung was in the alveolar stage of development. Large numbers of developing crests (secondary septa) were recognized along the primary septa giving them a

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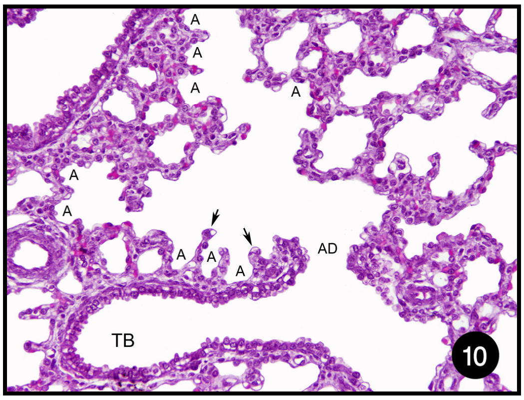
crenated appearance. Secondary septa increased in height and subdivided the airsaccules into smaller units (primitive alveoli) **(Fig. 10)**.



Figs. 10:- Transverse section in fetal rabbit lung at 29th gestational day, showing terminal bronchiole (TB), alveolar ducts (AD), air saccules (AS), secondary septa (arrows) and primitive alveoli (A)**(Haematoxylin and Eosin, X 200)**

All primary and secondary septa showed the double capillary network so that they were considered immature septa **(Fig.11)**.

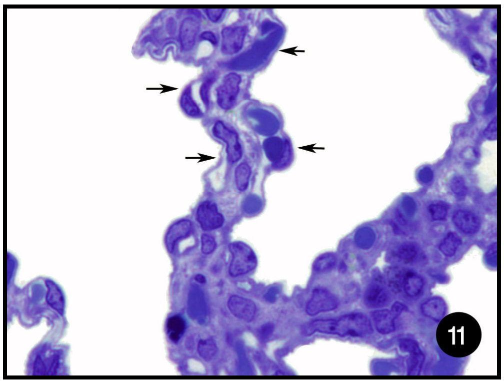


Fig. 11:- Transverse section in fetal rabbit lung at 29th gestational day, showing immature septum characterized by double capillary network (arrows)**(Semithin section, Toluidine blue, X1000)**

Primitive alveoli were lined by flattened type I pneumocytes and morphologically mature type II pneumocytes that showed numerous deeply stained intracytoplasmic inclusions **(Fig. 12)**.

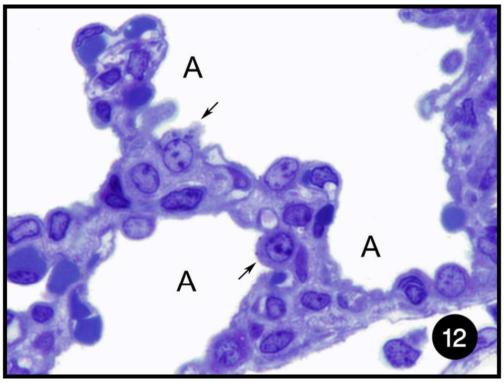
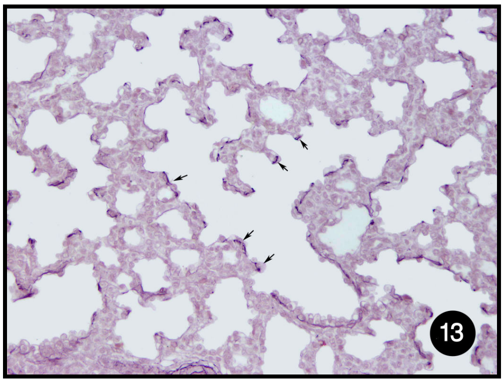


Fig. 12:- Transverse section in fetal rabbit lung at 29th gestational day, showing the structure of primitive alveoli (A). Note type II pneumocytes (arrows) containing deeply stained inclusions **(Semithin section, Toluidine blue, X1000)**

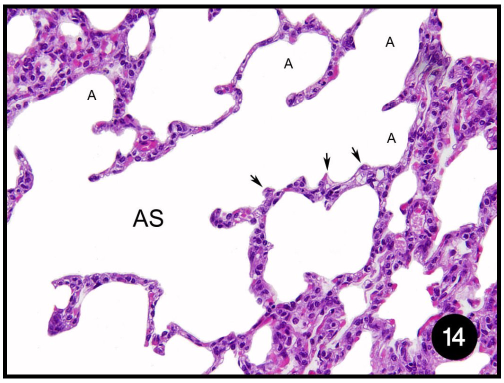
More elastic fibers were demonstrated near the apex of the secondary septa compared to the amount observed in the lungs of 27- days-old rabbit fetuses **(Figs. 13)**.



Figs. 13:- Transverse section in fetal rabbit lung at 29th gestational day, showing the respiratory portion. Elastic fibers are demonstrated near the apex of the secondary septa more than those observed in the lungs at the previous age (arrows) **(Weigert's Resorcin Fuchsin, X 200)**

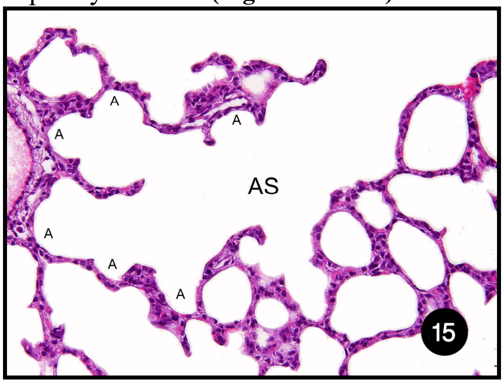
Radial alveolar count increased at this stage of development, reaching a mean value of 3.8 ± 0.02 **(Table 1).**

At the first day of life, the pulmonary tissue showed a variation in degree of aeration. In well-aerated area, the pulmonary alveoli were dilated, thin-walled giving a cup-shaped appearance. In addition, some developing secondary septa were recognized along the pre-existing primary septa **(Fig. 14)**.



Figs. 14:- Transverse section in neonate rabbit lung at the first postnatal day showing, terminal bronchiole (TB), alveolar ducts (AD), alveolar sacs (AS) and alveoli (A). Some secondary septa (arrows) are also recognized **(Haematoxylin and Eosin, X 400)**

The radial alveolar count increased at this stage of development,reaching a mean value of 4.75 ± 0.25 **(Table 1). ,** the pulmonary tissue was completely aerated. The inter By the third day of postnatal life -alveolar septa were compact and thinner than those observed at the previous stage but still contained double capillary network **(Figs. 15 and 16)**.



Figs. 15:- Transverse section in neonate rabbit lung at 3rd postnatal day, showing terminal bronchiole (TB), alveolar ducts (AD), alveolar sacs (AS) and alveoli (A) **(Haematoxylin and Eosin, X 400)**

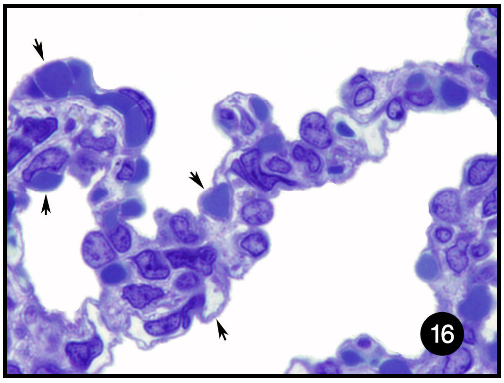


Fig. 16:- Transverse section in neonate rabbit lung at 3rd postnatal day, showing immature interalveolar septum which is characterized by double capillary network (arrows)**(Semithin section, Toluidine blue, X1000)**

The radial alveolar count increased at this stage of development, reaching a mean value of 5.4 ± 0.61 **(Table 1).**

4- Discussion

The present study revealed that at 25th gestational day, the rabbit fetal lung was in the canalicular stage. At this stage of development, all airway generations of fetal lung became greatly widened and elongated; resulting in a marked reduction of the pulmonary interstitial connective tissue. The canalicular stage comprised important steps in the development of fetal rabbit lung. Firstly, the terminal bronchiole branched into 2 - 4 wide and straight canals termed acinar canals. Secondly, some cells of the epithelial lining of these acinar canals started to flatten and differentiate into prospective type I pneumocytes. Other epithelial cells remained cuboidal and showed metachromatic reaction in semithin sections stained with toluidine blue. Finally, some capillaries displayed an intimate contact to the flattened cells resulting in the formation of first air-blood barriers. These findings agreed with **[5]** who reported that during canalicular stage of lung development, the acinar canals widened and elongated resulting in a decrease in mesenchymal tissue mass. As a result of the reduction in mesenchymal tissue mass, the epithelial cells lining the acinar canals and the pulmonary vasculature became more closely positioned to one another. This served as a starting point for the establishment of the future air-blood barrier. It also served as a starting point for initiation of the cuboidal epithelium lining the acinar canals to differentiate into type I and type II pneumocyts. **[6]** Mentioned that at the beginning of the canalicular stage, the future gas exchange region of the lung could be distinguished from the conductive tubules of the airway tree. The authors characterized this step as "birth of the acinus." The early respiratory acini were composed of several short generations of acinar canals arranged in clusters and taking origin from the actual last segment of the conducting airways, a prospective terminal bronchiole. At the end of this stage, the lung had reached a state of development in which gas exchange was possible. Before these developmental steps, a prematurely born infant has no chance to survive. The present investigations showed that at 27th gestational day, the fetal rabbit

ISBN: 978-1-61804-111-1

lung was in the saccular stage of development.. During this stage, the terminal portions of the acinar canals formed typical clusters of widened airspaces termed air saccules or terminal sacs. The surrounding interstitial tissue condensed to form thick inter-sacular septa or primary septa that contained a double capillary layer. These findings agreed with **[7]** who stated that the term (terminal sac) referred to the airspace in the developing lung which is bounded by thick septa that contain a double-capillary system. **[8]** Reported that the terminal sacs developed as clusters of dilated structures at the end of the respiratory duct of human fetal lung. They suggested that the intraluminal mechanical pressure played a key role to the formation of terminal sacs.

The present study revealed that at 29th gestational day, the fetal rabbit lung was in the alveolar stage of development. In agreement with many authors **[8] [9],** the formation of the pulmonary alveoli as shown in the present study was initiated by developing of secondary septa from the pre-existed primary septa. These secondary septa subdivided the air saccules into smaller units, the primitive alveoli. After birth, the pulmonary alveoli became dilated and thin-walled giving a cup-shaped appearance. In addition, numerous developing secondary septa were recognized. This suggests that the process of alveolarization continued after birth. All the primary and secondary septa showed a double capillary network. By contrast the interalveolar walls of the adult lung contain only a single capillary layer winding around an axial sheet of supporting connective tissue. This striking difference between a lung during alveolarization and the mature adult lung makes it clear that the alveolar stage cannot be the last stage in lung development. It must be followed by a stage of microvascular maturation during which the double capillary network gets reduced to a single one. This stage was not recognized in our study. Such finding supported the statement of **[6]** who stated that after the stage of alveolarization, the interalveolar septa and their capillary networks were remodeled to optimize gas exchange during the stage of microvascular maturation.

The time point of birth as determined relative to lung development showed a great variation between different species. The present findings revealed that the rabbits are born in early alveolar stage. On the other hand, the marsupial quokka wallaby (*Setonix brachyurus*) represents the mammal possessing the most immature lung at birth. These animals are born in the canalicular stage. Rats and mice are born during the saccular stage. Human babies are born in the early alveolar stage while the precocial mammals such as sheep are born during late alveolar stage **[6].**

The present study showed that the radial alveolar count method of Emery and Mithal might be satisfactory applied in the estimation of airspace development whether saccular or alveolar. The present data revealed a progressive increase of the radial alveolar count during intrauterine and early neonatal life. The radial alveolar count method provided a reliable index of lung development during intrauterine and early neonatal life.

[10] Observed that radial alveolar count showed a significant correlation coefficient with fetal body weight, fetal lung volume and gestational age. During canalicular stage, fine elastic membranes were demonstrated around the acinar canals. During saccular stage, elastic fibers were concentrated at the tips of the developing secondary septa. The elastic fibers increased markedly with the advancement of gestational age and reached its peak during alveolarization in full-term fetuses and in neonate rabbits. The present findings suggested that elastic fibers play a critical role in septation and alveolar formation. Similar findings were recognized by **[11]** in mice and by **[12]** in *Macropus eugenii.*

[13] mentioned that elastic fibers is a constituent of connective tissue that is present in all tissue which has vital role in organs that undergo repeated cycle of extension and recoil such as arteries, lung and skin. Elastic fibers are secreted from cells of mesenchymal origin such as smooth muscle cells, fibroblasts, chondrocytes and endothelial cells.

5- Conclusion

The present study revealed that the rabbit lung showed similar findings to that recognized in human fetuses by **[6]** and **[13].** These findings support the statement of **[14]** who stated that rabbit could be an appropriate experimental model for studying lung development in human during intrauterine and early neonatal life.

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