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Review Article

HISTOGENESIS AND MOLECULAR DEVELOPMENT OF THE LUNG IN NEW ZEALAND WHITE RABBIT (*Oryctolagus cuniculus*)

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Abstract: In investigating fetal organ development, fetus of the rabbit was one of the furthermost frequently handled as a model in animal investigational research. During the second half of gestation in rabbit fetuses thorax volume (TV) was the predictive factor for assessment of normal development. Concerning these fetuses, evaluation of lung development was more applicable in the 20th–29th gestational days for experiments. A large internal surface area in which the inspired air and capillary blood got in close contact to each other was specified by the lung tissue. Consequently, an efficient gas exchange was occurred. A cycle of separate but overlapping developmental processes happened to reach this target. The anlage of the left and right lungs appeared as an outpouching of the foregut endoderm during organogenesis. A repetitive process of outgrowth and branching pattern (branching morphogenesis) was occurred in each lung bud. Consequently, all of the future airways were formed mainly during the pseudoglandular stage. Repetitive airspace septation would lead to tremendous increase of the gas exchange surface area in both late fetal and postnatal lung development leading to the formation of alveoli. The first air-blood barriers were appeared and surfactant production began during the canalicular stage.

Key words: rabbit fetus; lung development; branching morphogenesis; alveoli

Introduction

Concerning investigation of fetal physiology and maturation, many models of different animals were handled for the intrauterine experimental studies. The most commonly used animals in these studies, were monkeys, sheep, goats, rats and rabbits. Because of large litters of the rabbits in addition to, the advantage of determining gestational age almost accurately, rabbits were selected (1- 4).

Anatomically, inside the thoracic cavity the two lungs were smooth compact and elastic masses of tissues covered by the visceral pleura. A fold of coelomic epithelium formed of two layers covered the surface of each lung. The arrangement consisted of a visceral pleura which was closely adherent to the surface of the lung and parietal pleura, that lined the chest wall. A possible pleural space containing small amount of fluid was formed between these two layers of pleura. The adjustment of lobation showed that four lobes and only two lobes were presented on the right and left lung respectively (5,6).

Embryologically, the respiratory primordium developed as an outpouching from the floor of the foregut endoderm. Organization of certain respiratory cell line in the primitive foregut initiated this development. The improvement of a tree-like tubular system of epithelial airways and vascular structures followed this action giving rise to the mature airways and alveoli. Many epithelial cell types, which lined the inner surface of the developing lung and trachea would differentiate in

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the foregut endoderm. From the lateral mesoderm, the lung mesenchymal tissue originated and gave rise to several structures such as its connective tissue, endothelial cell precursors, the smooth muscle that surrounded airways and blood vessels. In addition to the tracheal rings cartilage, the lymphatics, and the mesothelial cells that lined the outer surface of the lung (7).

At an early stage of embryogenesis, regional specification determined where formation of future respiratory tract structures would occur. Such process was attributed to the expression of different combinations of Hox genes along the cranial–caudal axis of the developing embryo. The initial specification of the lung endoderm was marked by the expression of the epithelial homeodomain transcription factor Nkx-2.1. The dorsoventral patterning of this region was influenced by Wnt-2 and Bmp-4, which promoted the expression of Nkx-2.1(8).

This review summarizes the currently available scientific data on lung developmental stages in rabbit. It arranged chronologically into embryonic, pseudoglandular, canalicular, saccular and alveolar stages with reference to: (1) The histological changes of each stage (2) Molecular determinants of the lung.

Lung development

Lung development was partitioned to three main phases; embryonic, fetal and postnatal. Appearance of lung just as an organ was considered as a division of the embryonic one. Whereas lung at the fetal phase consisted of the pseudoglandular, canalicular and saccular stages respectively. Concerning postnatal phase, lung comprised the degrees of classical alveolarization, as well as, maturation of microvascular system (Table 1) (9,10 and 11).

Concerning 18th and 20th gestational days in rabbit fetuses, lung was in pseudoglandular stage of improvement. In the course of the 22th gestational day, a temporary time in between this section and the canalicular one was diagnosed. At 23th and 24th days of gestation, the canalicular stage was once surely viewed. The saccular phase was revealed on days 25 and 26 of gestation. Throughout the 27th gestational day an overlapping between saccular and alveolar segment used to be diagnosed. The lung was considered in the crucial stage of development (alveolar phase) and lung maturation regarded to be full at day 28 (1).

Embryonic stage (Organogenesis)

At 12- day - old lung rabbit fetuses, the anlage of the lung showed on the ventral aspect of foregut primitive endoderm. It started as a laryngotracheal groove on the fronto ventral wall of foregut, which prolonged and shaped a respiratory diverticulum (12). This finding agreed with (13) who reported that, the respiratory diverticulum originated from ventral surface of the foregut into the mesoderm that surrounded it, forming the lung bud in human at 4thweek of gestation, while the first establishment of laryngeo tracheal tube demonstrated in cow embryo at 20-21 days of gestation (14) and the lung appeared at 9.5 day of mice fetuses (15) and at 18 day of pregnancy in cat (16), while began at the 17th day of pregnancy in sheep (17).

In mammals, the respiratory primordium was developed as a ventral groove in the ground of the foregut. This groove referred to the laryngotracheal groove. The latter groove was deepened and made an elongated outgrowth, which prolonged in a caudal direction. It was separated from the foregut by the formation of two tracheo-oesophageal grooves on the right and left sides. When these grooves met and fused forming the tracheo-oesophageal septum. The septum separated the dorsal portion of the foregut (the primordium of the oesophagus) from the ventral portion (the primordium of the laryngo-tracheal tube). The laryngo-tracheal tube extended and divided into two bronchial buds; the primordia of the left and right lungs (Fig. 1) (8).

Evagination of the mouse lung starting point from the floor of foregut endoderm and lung branching morphogenesis commenced at 9.5th days of gestation. The dichotomous branching accompanied the developed trachea and two lung buds branching patteren (18). This was alike that in bovine lung by (14) and (19) in rabbit lung.

Concerning micromorphology, the bud was arranged in only three cell layers from interior to exterior: epithelium, mesenchyme and mesothelium. The branched airways that would conduct air to the formed alveoli and gas-exchange units of the lung were developed from the epithelial layer.

Table 1: Different lung developmental stages of different species with their duration

Period	Stage	Duration	Characteristics
Embryonic	Embryonic	Rabbit: n.d.–E18 Sheep: E17–E30 Mouse: E9.5–E12 Rat: E11–E13 Human: 4–7 weeks	Anlage of the two lungs; organogenesis; formation of major airways and pleura
Fetal	Pseudoglandular	Rabbit: E18–E24 Sheep: E30–E85 Mouse: E12–E16.5 Rat: E13–E18.5 Human: 5–17 weeks	Formation of bronchial tree and large parts of prospective respiratory parenchyma; birth of the acinus even if the acinar epithelia are not yet differentiated.
	Canalicular	Rabbit: E23–E27 Sheep: E80–E120 Mouse: E16.5–E17.5 Rat: E18.5–E20 Human: 16–26 weeks	Formation of the most distal airways leading to completion of branching morphogenesis; first air-blood barrier; appearance of surfactant, acini are detectable due to epithelial differentiation.
	Saccular or terminal sac	Rabbit: E27–E30 Sheep: E110–E140 Mouse: E17.5–P4 Rat: E21–P4 Human:24–38 weeks	Expansion of (future) airspaces.
Postnatal	Alveolarization, classical alveolarization (first phase)	Rabbit: E30–term (E31) Sheep: E120–term (E145) Mouse: P4 – P21 Rat: P4 – P21 Human: E252 (36 weeks* preterm) – 3 years	Formation of secondary septa (septation) resulting in the formation of the alveoli; most of the alveolar septa are still immature and contain a double layered capillary network. Depending on the species alveolarization starts before or after birth..
	Alveolarization, continued alveolarization (second phase)	Rabbit: term (E31) – n.d. Sheep: term (E145) – n.d. Mouse: P14 – young adulthood (∼P36) Rat: P14 – young adulthood (∼P60) Human: 2 years – young adulthood (17–21 years)	Formation of secondary septa (septation) but now lifting off of mature alveolar septa containing a single layered capillary network.
	Microvascular maturation	Rabbit: n.d. Sheep: n.d. Mouse: P4 – young adulthood (∼P36) Rat: P14 – young adulthood (∼P60) Human: ∼term – ∼3–21 years (timing uncertain)	Remodeling and maturation of interalveolar septa and of the capillary bed (the double layered capillary network is transformed to a single layered network). In a first approximation it takes place in parallel to alveolarization.

Table showed different lung developmental stages of different species with their duration and the most characteristics events within each stage based on (9),(10),(11) (E) embryonic or gestational day; (n.d.) not determined; (P) days after delivery and W weeks post coitum

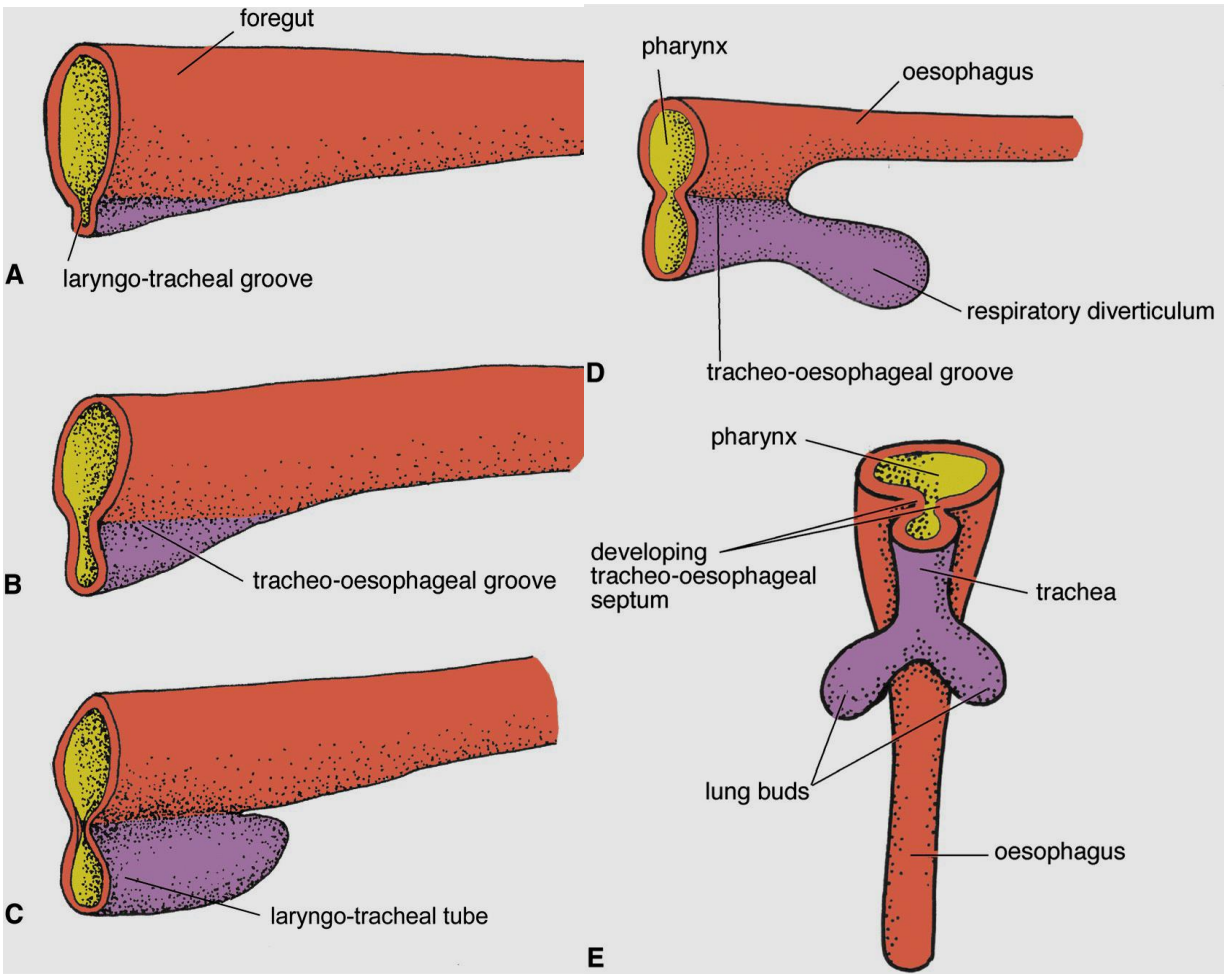

Figure 1: Lateral views, A, B, C and D and a ventral view, E, of sequential stages in the formation of the respiratory diverticulum from the foregut (8)

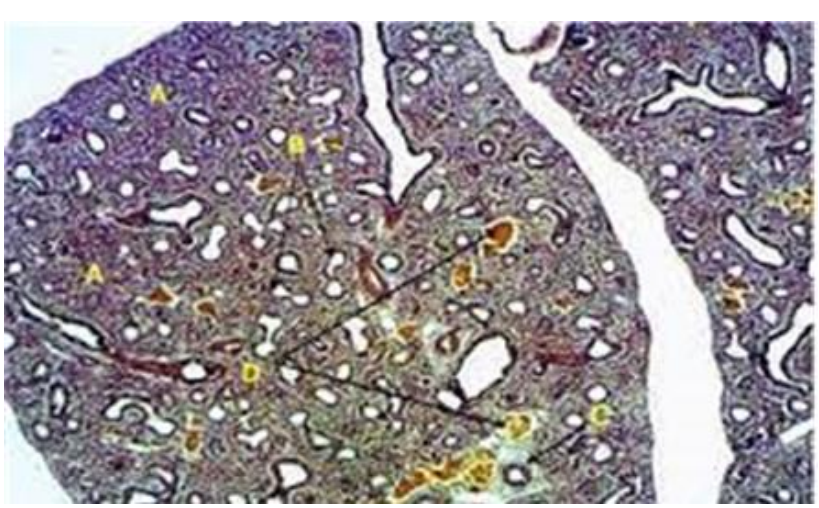

Figure 2: Parasagittal section at 20th day of gestation in rabbit showing pseudoglandular stage of lung :A-intestinal mesenchyma, B-terminal buds, C-bronchioles, D-blood (H&E.X4) (12)

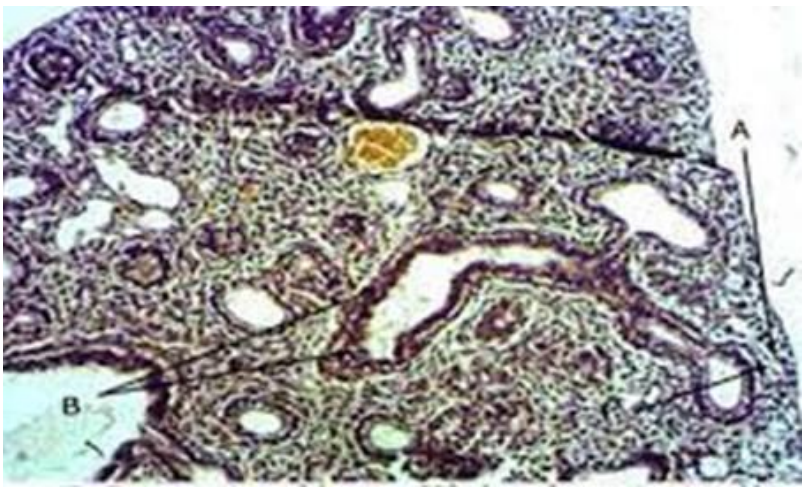

Figure 3: Cross section at 20th day of gestation in rabbit showing zones surrounds the tubular sprouts: A-first zone, B-second zone, C-third zone (H&E.X4) (12)

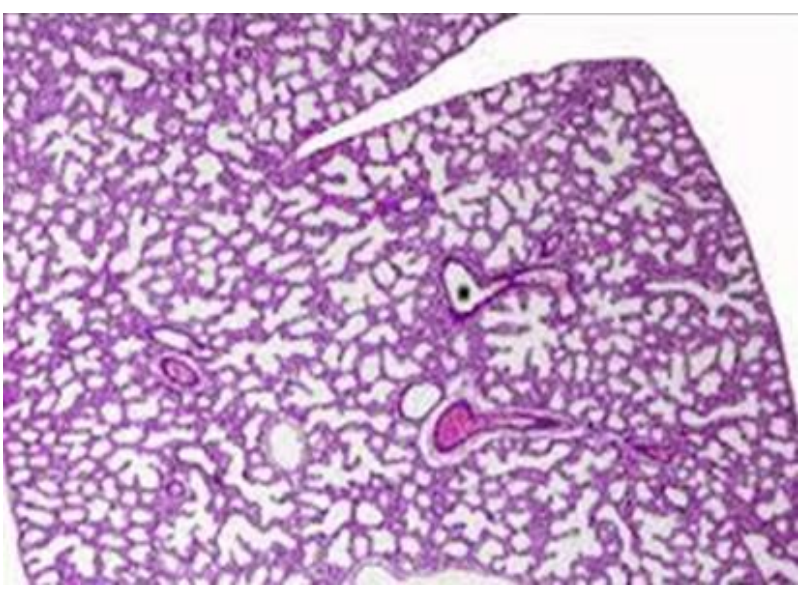

Figure 4: Cross section of fetal rabbit lung showing canalicular stage and several enlarged and elongated canaliculi in the shape of star (Haematoxylin and Eosin stain, X 40) (27).

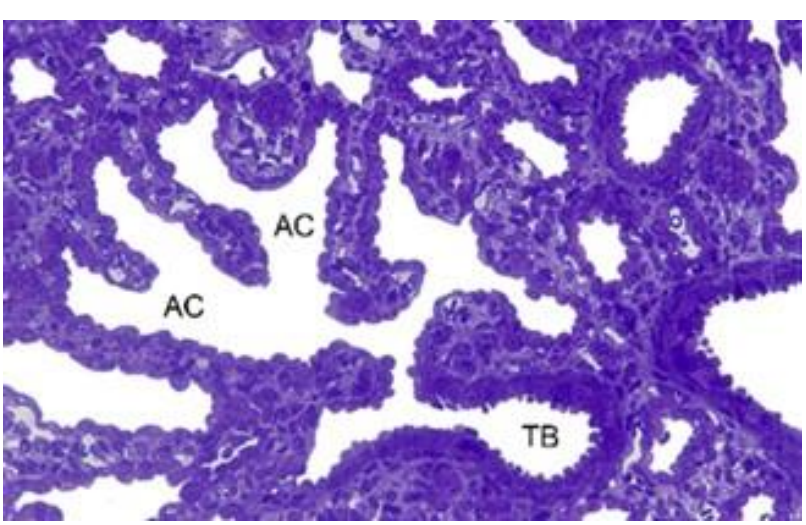

Figure 5: Transverse section of fetal rabbit lung showing division of the conducting terminal bronchiole (TB) into several enlarged and elongated canaliculi or acinar canals (AC) (Semithin section, Toluidine blue stain, X 200) at 25th gestational day (27)

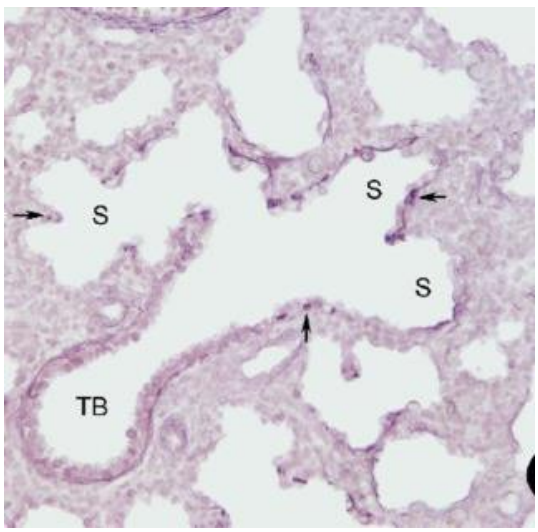

Figure 6: Showing division of terminal bronchiole (TB) into small air saccules (S). Arrows of fine elastic strands were revealed in the mesenchymal tissue surrounding the airway at 27-day-old rabbit fetuses lung on cross section (Weigert's Resorcin Fuchsin special stain, X 200) (27)

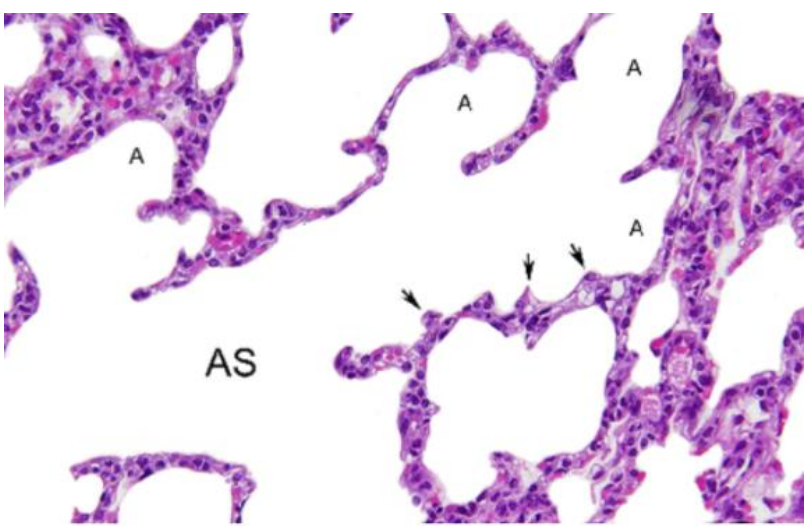

Figure 7: Showing alveolar ducts (AD), alveolar sacs (AS) ended by alveoli (A). Some arrows of secondary septal ridges were demonstrated on cross section of neonate rabbit lung at the first postnatal day (H&E, X 400) (27).

The vascular and stromal components of the lung were formed by mesenchymal tissue surrounding the epithelial ducts. The lung lobes were enclosed by single layer of cells called outermost layer (mesothelium). Complex signaling networks would control interaction between three cell types (20,21).

Pseudoglandular Stage

Histologically, in 18- and 20-day-old lung rabbit fetuses several branched airways and vessels were distinguished in between relatively loose, extremely cellular, densely formed mesenchymal tissue. Several plaques of cartilage surrounded the presented bronchi that were lined by stratified columnar epithelium in proximal part of airways. Relatively low columnar or cubic epithelium showed in distal part of airways. Within the mesenchymal tissue, recently formed bronchi appeared frequently (1).

In pseudoglandular lung, the proximal portion of airway tubes was lined by tall columnar epithelium and the height of the cells was declined continuously toward the boundaries. These cells became cuboidal in the terminal branches of airways. The terminal buds epithelium were remained lined by cuboidal undifferentiated state until branching of lung was ended. Denser, loose mesenchymal cells surrounding the tubular sprouts, was observed nearly underneath the pleural cavity called first zone. While in the second zone, the bronchi were surrounded by more densely arranged interstitial cells. Sites of the gas exchange region were located between two zones. The third zone containing the future conducting airways which was characterized by epithelial tubes surrounding by peripheral layer of smooth muscle cell precursors (Fig. 2, 3) (12).

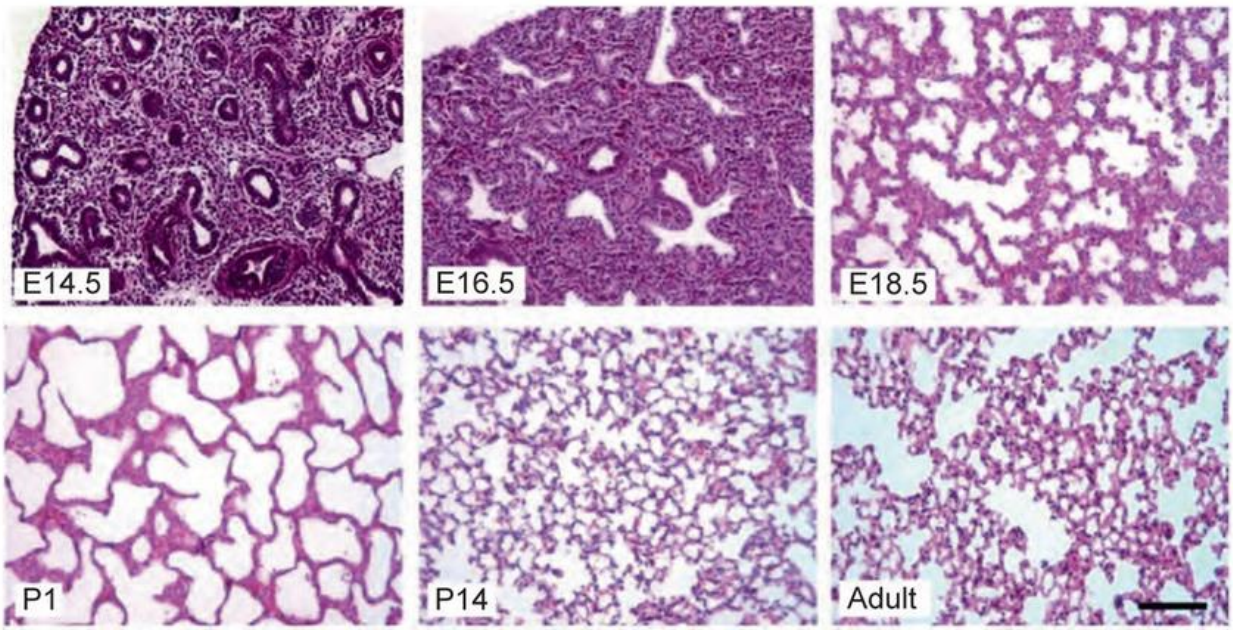

Figure 8: Demonstrating characteristic developmental stages of mouse lung histologically. Lung improved from embryonic stage (9.5 G.d) to morphogenesis of pseudoglandular stage (14.5 G.d) to stage of canaliculi formation (16.5 G.d) then to saccular stage (18.5 G.d and P1). Formation of secondary septal crests was resulted from establishment of lung alveolarization stage in neonatal mouse (P14). In the end, normal pulmonary structure and function was established as a result of appearance of mature honeycomb-like appearance with alveoli surrounding alveolar ducts in the adult lung mouse. (26)

Between 9.5th and 16.5th days of gestation, the mouse lung buds would generate a branched tree-like airways till formation of several terminal bronchioles. During pseudoglandular stage, the branching sample was once tremendously stereotyped and essentially equal between inbred mouse lines (22). During 12.5th and 16.5th days of gestation, the mouse lung buds began pretty organized pattern of branching referred to as branching morphogenesis. The latter branching pattern generated several arrangement of airways with many terminal tubules (23). These findings disagreed with (15) who reported that this stage took place at (5-17) week of pregnancy in human,while in cat occurred between 23-25 days of gestation (24), in sheep between 30-85 day of gestation, in rat between 13-18 day of gestation (25).

Canalicular stage

There was a partial overlapping between canalicular stage and the previous one because of quick development of the cranial part of the lung. Expansion in diameter and length of the respiratory tree would be occurred during this stage. Vascularization and angiogenesis along the airway could be seen. In addition, a great rise in the quantity of blood capillaries was occurred. Respiratory bronchioles and acinar ducts were resulted from division of the terminal bronchioles. As well as, epithelial cells along airways were segregated into peripheral squamous flat cells and proximal cuboidal cells (26).

At the end of the canalicular stage, the remainder distal airways generations had been already arisen. With the aid of branching pattern very few terminal airways could also additionally be formed at the succeeding stage depending on the species (11).

At 25th days of gestation, there was an increase in width and length in all branched airway generations of fetal lung of rabbit in canalicular stage of development. This was leading to obvious reduction of the mesenchymal interstitial tissue. Two - four wide and straight canals termed acinar canals (canaliculi) were resulted from division of each terminal bronchiole. Potential type I pneumocytes would appear due to flattening and differentiation of the epithelial cells lining of these

canaliculi but residual acinar epithelial cells remained cuboidal (Fig. 4, 5) (27). Lung was in canalicular stage at (28-32) days of development in cat (24) and at 16–26 weeks of pregnancy in human (28) and about 22th gestational day in rabbit (12), and sheep about 80–120 gestational day, mouse at 16.5–17.5 day of gestation and rat between 18.5–20days of gestation (25).

The cuboidal epithelial cells of the canaliculi and the vascular pulmonary system became more nearly placed to each other leading to the formation of the future air-blood barrier. Consequently, differentiation between type I and type II pneumocyts was occurred during this stage (29).

The future air-blood barrier would be recognized at lung gas exchange surface area from the conductive tubules and canaliculi of the airways. Birth of the acinus was the most characteristic event at lung canalicular stage of development. A probable conducting terminal bronchiole would branch resulting in formation of several short generations of clustered respiratory acinar canals. Immaturely born child lacked the chance to stay alive due to failure of gas exchange if this developmental stage was missed (10).

Saccular stage

During this stage of development, the differentiation of alveolar epithelial cells (AECs) would lead to formation of two different cell types: mature squamous flat (type I pneumocytes) and surfactant secretory rounded (type II pneumocytes) showing lamellar bodies (26). while in buffallo the cells resembling the type 1 and type 2 pneumocytes demonstrated at the alveolar stage (30)

During 27- days -old rabbit fetuses, enlarged air saccules or terminal sacs would be formed from the terminal portions of the acinar canals or canaliculi. The condensation of the surrounding interstitial tissue would lead to formation of marked inter sacular septa or primary septa that had a double capillary layer fig. Few low secondary septal ridges were established bulging from these primary septa (Fig. 6) (27).

Alveolar stage

At 29-day-old rabbit fetuses, the lung alveolar developmental stage, crenated appearance of septa was occurred as a result of establishment of huge numbers of secondary septal crests alongside the primary one. The air saccules were subdivided into smaller units known as primitive alveoli by expanded secondary ridges. Flattened squamous pneumocytes type I and morphologically mature type II would line these alveoli. These pneumocytes type II cells contained several deeply stained intracytoplasmic lamellar inclusions bodies (Fig. 7) (27).

At round 16.5 - days - old mouse fetuses, improvement of pulmonary tissue switched from branching pattern of pseudoglandular stage to the formation of canaliculi and saccules. So the alveolization stage, that generated the practical units for gas exchange, would start. In relation to variation between species, alveolar development took place at postnatal day (P5–30) in mice, however in human beings development of these alveoli began then persisted for months postnatal (Fig. 8) (26,31).

Molecular Determinants of Lung Development

The organization of a focused domain of expression of Nkx2-1 in the ventral wall of the anterior foregut endoderm would initiate development of the respiratory system (the trachea and lungs). Specification of the two primary lung buds was performed at E9.5 in the mouse and 28 days in the human concerning this domain. The tracheo-oesophageal septum separated the single foregut tube, just anterior to the lung buds, into two structures a dorsal esophagus that led into the stomach and a ventral laryngeo – tracheal tube that connected to the two primary lung buds during this period (31).

Greatest SRY-Box Transcription Factor 2 (Sox2) expression was once discovered in the future esophagus dorsally, while future trachea was developed ventrally due to greatest expression of Nkx2-1. This manner depended on alerts such as Bmps and their antagonist, noggin, as properly as Fgfs and Wnts located in the mesenchymal tissue. Stoppage of foregut separation and unusual differentiation of the epithelium and mesenchyme was occurred because of deficiencies in some of these genes signals. In addition to , loss of Nkx2-1 expression and extension of Sox2 expression was due to loss of both Wnt2 and Wnt2b, which were located in the mesenchyme surrounding the anterior foregut endoderm, or of beta-catenin (Ctnnb1) in the endoderm (32,33,34,35,36, 37).

Fgf10, a fibroblast growth factor that was essential for budding. The latter was used to be presentd nearby in the mesenchymal tissue beside these Nkx2.1-expressed in endoderm. Major lung bud formation was resulted from setting off Fgf receptor 2b signaling. Just vital respiratory buds formed, epithelial airways beared massive branching pattern. This was ended by the establishment of the tree-like airways and the future alveolar gas exchange area (7,38).

During pseudoglandular stage, the pulmonary endodermal cells additionally started out to enhance different cell lineages along its proximal-distal axis. The endoderm progenitor proximal lineage was manifested by the expression of Sox2 while the endoderm distal progenitor lineage was marked by the blended expression of Sox9 and Inhibitor of DNA Binding 2 (Id2). Significantly, two populations had great derivatives. The former was the proximal endoderm progenitors which gave upward thrust to airway neuroendocrine cells, secretory cells, ciliated cells and mucosal cells.The latter was the distal progenitors which gave upward differentiation to AEC1 and AEC2 (37 ,39).

FoxM1 was known to play important roles in lung development. Such gene regulated the appearance of genes critical for differentiation of mesenchyme, adjusting of extracellular component, and pulmonary vasculature (40). FoxM1 was intimately related not only with essential structural maturation of the lung, but also with the production of surfactant proteins (SP-A, B, C, and D). Consequently, it was an essential feature of neonatal respiratory distress syndrome (41).

Conditionally deleted FoxM1 could not alter lung development, branching morphogenesis of bronchial tubules, or epithelial multiplying in the mouse developing pulmonary epithelium. Though maturation of the lung was blocked, and severe respiratory collapse established after birth. Reduced expression of T1-α and aquaporin 5 was the cause of defects in respiratory maturation and delayed demarcation of alveolar epithelial type I cells. Additionally, the expression of surfactant proteins (SP) was diminished by this deletion (41).

Conclusion

It could be concluded that rabbits may be an applicable animal model for studying the different developmental stages of lung with their time scale and the most characteristics events within each stage. Preterm rabbits revealed high expression of FoxM1 mRNA and protein in the lungs compared to full term rabbits. A relationship between FoxM1 and expression of SP-A/B and lung maturation in preterm rabbits was demonstrated.

The authors declare that they have no competing interests.

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