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Histological study of rabbit lungs in different age groups (Oryctolagus cuniculus)

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ABSTRACT

The aim of this study was to investigate the histology of the lungs in local rabbits (Oryctolagus Cuniculus) at three different stages of development. A total of eighteen rabbits were used in the study, with lung tissue samples collected at 17 and 27 days in utero, as well as at 6 months after birth. The lung undergoes various histomorphological changes during embryonic development, including the embryonic, Pseudoglandular, canalicular, saccular, and alveolar stages. The objective of this research was to examine the histological characteristics of the normal lung tissue in local rabbits. Histological analysis revealed the presence of alveoli, as well as fibrin and individual red blood cells in some of the lung tissue samples. Additionally, a few alveolar macrophages, as well as type I and type II cells, were observed in the alveolar spaces.

1. Introduction

Different kinds of animals have been employed in experimental investigations exploring organ maturity in prenatal and postpartum life, including rats and rabbits. Actually, rabbits are usually chosen because they have big litters and the benefit of being able to identify the gestational age fairly perfectly.(Karnak et al., 1999)

The lungs are a delicate, brittle, and sponge-like mass of tissues located in the pleural space of the thoracic. Each lung is shielded by a folded layer of coelomic epithelium made up of two layers: visceral pleura that wraps around the organ, and the parietal Pleura, that runs along the chest wall. Between both of these layers of pleura, there's is a pleural gap between them, which is a potential region that holds a little quantities of fluids. The lungs are separated into lobes. The right lung has four chambers, while the left has just two. The vascular framework of the tree of the tracheobronchial tract supports a range of functions, distinct from those which are typical for many vascular beds, such as nutrition of tissues, disposal of waste products, and the delivery of migratory cells and mediators, as well as performing important functions related to the conditioning of inhaled and exhaled air (Autifi et al., 2015) (Autifi et al., 2015). The lungs are a vital part of the pulmonary circulation, where the oxygen-depleted blood pumped by the right ventricle of the heart is directed through the pulmonary arteries to the alveolar-capillary beds in the lungs for gas exchange. The oxygen-rich blood from the lung capillaries is then transported back to the left atrium of the heart through the four pulmonary veins. (Khan and Lynch, 2018)

The respiratory (gas-exchange) area of the lung. These alveoli, numbering in the millions, are covered by an incredibly thin layer of simple squamous epithelium, enabling the effortless exchange of oxygen and carbon dioxide. Furthermore, there are also cuboidal cells called Type II pneumocytes present, which secrete surfactant, and they can be found lining the alveolar walls. Surfactant, consisting mainly of dipalmitoylphosphatidylcholine, plays a crucial role in reducing the surface tension of water to facilitate efficient exchange of gases.(Kia'i, 2019)

Bronchioles: The bronchioles, which are smaller branches of the intrapulmonary bronchus, undergo significant changes in their structure. The pseudostratified epithelial cells transform into a simpler columnar epithelial layer, and the mucous glands and cartilage are no longer present. The bronchioles continue to divide multiple times until they reach the respiratory duct. These walls have a smaller diameter and undergo various modifications. The epithelial cells change from simple columnar cells with cilia and small goblet cells to shorter columnar cells that lack cilia and goblet cells. (Senthamilselvi, 2007)

The epithelial tissue of the respiratory system is pseudostratified, mainly found in the upper respiratory tract, including the trachea and pulmonary bronchus, as well as the intrapulmonary bronchus within the lung. The transition of this tissue begins with a gradual change from tall, simple columnar epithelial cells. As it progresses, the cilia are gradually lost in the bronchioles, and it transforms into a superficial cuboidal epithelial tissue at the terminal bronchioles. Finally, it becomes a simple squamous epithelial tissue within the respiratory portion.(Robinson and Furlow, 2007).

The alveoli: The alveoli in the lungs are like small airbags located within the lung tissue. When the diaphragm contracts, it creates a negative pressure in the chest cavity, allowing air to enter the lungs. The alveoli fill up with air, causing the lungs to expand. The alveoli are connected to tiny blood vessels called alveolar capillaries. At this point, red blood cells pick up oxygen from the air through a process called oxyhemoglobin, which they then transport to nourish the body's cells. The red blood cells also carry carbon dioxide, which is released into the external environment when we exhale. Additionally, the diaphragm creates a positive pressure in the chest cavity, helping to push out carbon dioxide from the alveoli. (Kovar et al., 2002)

The alveolar walls are mainly made up of two typ es of cells, alveolar cells type I from the lining of 95% of the alveolar surface and alveolar cells type II. Alveolar cells type I, also known as pneumocytes or squamous alveolar cells, appear as severely weakened lining cells with a shape akin to squamous epithelial cells. The cell's nucleus is located in the center and extends into the alveolus. The alveolar cells type II cover remaining 5% of the alveolus, they have a cuboidal pattern and are often seen in the alveolar corners. The free surface contains microvilli. The

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most obvious characteristic of those cells is the various intracellular inclusion, the lamellar bodies that produce surfactants from the cells by exocytosis, to prevents the alveoli from collapsing (Abd Al-Hussan and AL-Hashemi, 2014).

Other types of cells found in the alveolus include fibroblasts, which are located in the alveolar septum and may have some ability to contract (known as myofibroblasts). Alveolar macrophages, on the other hand, are large round cells that are typically found freely floating within the alveolus. These cells contain material that they have engulfed through a process called phagocytosis, which appears dark when viewed in sections.(Payne and Wellikoff, 2012).

The primary unit of the respiratory system is called the acinus or primary lobule. It is essentially the end of the bronchiole with all its branches. The acinus is approximately the size of a millimeter and is divided into two halves by its first order respiratory bronchiole. These halves, known as hemi acini, differ in size and shape, as they compete for space within the respiratory system. (Karnak et al., 1999)

Rabbit lungs lack partitions, with the right lung consisting of four lobes and the left lung having three lobes. The left lung exhibits a greater volume of air flow. When rabbits are at rest, their respiration primarily relies on the diaphragm's function. (Ferreira Matos Gomes, 2001)

2. MATERIALS AND METHODS

This study was carried out at the University of Tikrit-College of Veterinary Medicine department of Anatomy and Histology; Eighteen rabbit were using in the present study, classified in to the following group:

Group A: at age seventeen days' fetus.

Group B: at age twenty-seven days' fetus.

Group C: at age six months after birth.

The whole animals were accommodated before the study, Pregnant animals were cared for and fed under normal conditions, as well as non-pregnant animals at the age of 6 months. The anatomical technique was done to dissect the skin of each one by doing a median sternotomy incision in the chest by the aid of surgical blade and chisel.The animals were distributed according to age into 3 groups and housed in a normal room (10-meter length, 8-meter width, and 2.5-meter-high), six rabbits in each group at room temperature (20- 25) °C.

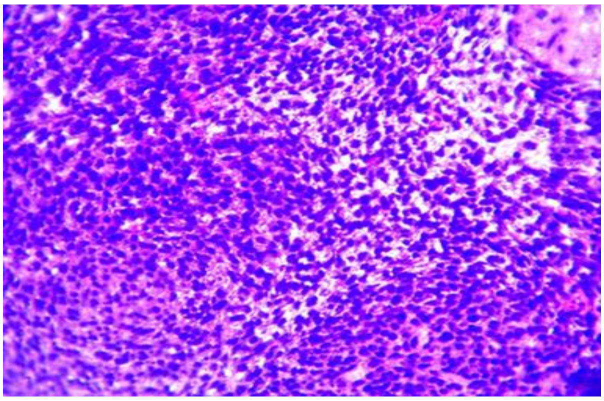
2.1. study sampling

The lung was removed carefully immersed in distilled water for 10 minute to remove the blood clot and debris of tissues. Following this, the lungs were treated by introducing 10% Neutral buffered formalin into the trachea at a consistent pressure of 10 cm water (Nielson and Griffith, 1979). Once the lungs were adequately inflated, the trachea was tied off, and the lungs were submerged in a container filled with 10% formalin for the duration of the night. The lung that had been preserved in formalin solution was dissected so as to separate the segmental, subsegmental, secondary lobules until reach the primary lobule(acinus), by using splitting dissecting maneuver. The acinus was placed in dehydrated alcoholic solutions then cleared in xylene for one hour and embedded in paraffin wax using the master block unit. The tissue blocks were sectioned at 5µm thickness using Leitz microtome so as to make histological slides ready for staining. The staining materials were hematoxylin and eosin. At least 3 specimens were taken from each lung for the histological analysis. Formalin is the fixative: Formalin, known as such when it is mixed with water, is frequently utilized as an industrial disinfectant and a preservative in funeral homes and medical laboratories. Additionally, it can serve as a preservative in various items like antiseptics, medicines, and cosmetics. Due to its ease of use, precise results, and remarkable versatility, formalin is the preferred fixative in pathology laboratories across the globe (Autifi et al., 2015). stained with following histological stains 1-Hematoxylin and eosin stain for enhance contrast and visualize cellular and tissue components (Feldman and Wolfe, 2014) ,2- Masson trichrome stain for highlights collagen fibers (Al-Mahmood, 2020).

3. Results and Discussion:

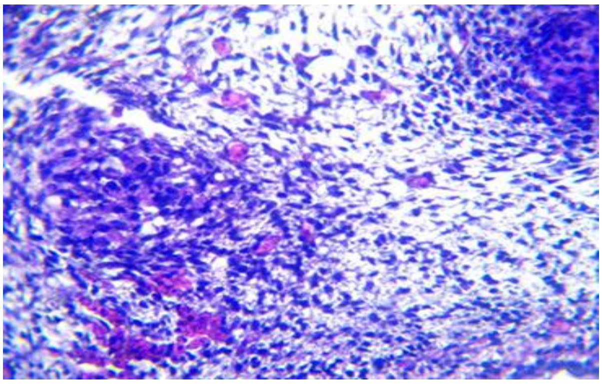
The lungs of rabbits are bilaterally symmetrical parenchymal organs situated within the thoracic cavity. They are relatively small in size and exhibit a pale pink coloration. The lungs are divided into right and left lobes. The internal morphological composition of lung tissue is characterized by the presence of pulmonary lobules, which possess a conical or pyramidal shape. These lobules are discrete regions of lung parenchyma that are separated by connective tissue septa. These septa collectively form the connective tissue framework of the lungs. The stroma, which is made up of loose connective tissue, forms the connective tissue framework and includes elastic fibers, as well as blood and lymphatic vessels. The findings of the recent study revealed that a substantial portion of the lung tissue consists of their functional parenchyma, as observed under a light microscope, in age of 17-day-old rabbit fetus composed of undifferentiated cells and there is on presence of saccular development (fig 3.1), and in age of 27-day-old rabbit fetus the lung composed of alveoli, alveolar sacs and ducts, great respiratory duct lined with cuboidal and alveolar cells also demonstrated adjacent to the pulmonary blood vessel engorged with blood mass (fig 3,3). As maturation proceeds, and in age of six months the interstitial connective tissue was containing thickening of collagen bundles associated with cultured white blood cells, the alveolar sacs where lined by a viola epithelial cells squamous type **I** and few cuboidal type **II**, cellular debris was seen in individual space of alveoli (fig 3,6).

fetus 17 days (A): The histological section of lung tissue was demonstrated the presence of multiple undifferentiated cells at early stage of prenatal development and this was concerning with scanty formation of delicate un well developed connective tissue, the undifferentiated cells appeared as syncytial mass with dark nuclear chromatin. No indication for the presence of saccular development (fig 3.1).



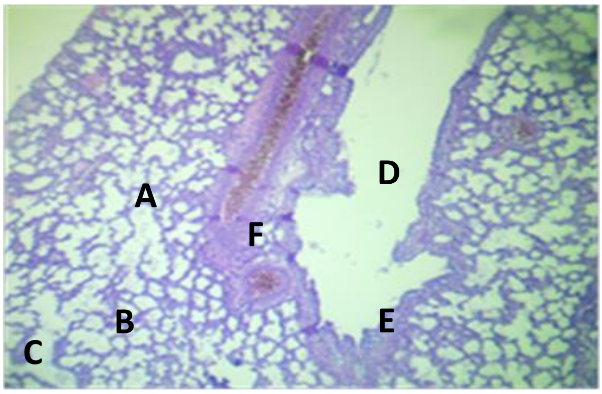
(fig 3.1)

fetus 17 days (B): The lung tissue of prenatal development was indicated by presence of mesenchymal connective tissue associated with the presence of mesenchymal cells of star shaped and foci of micro blood capillaries which were demonstrated and saccular development was indicated in certain areas of mesenchymal connective tissue (fig 3.2).



(fig3.2)

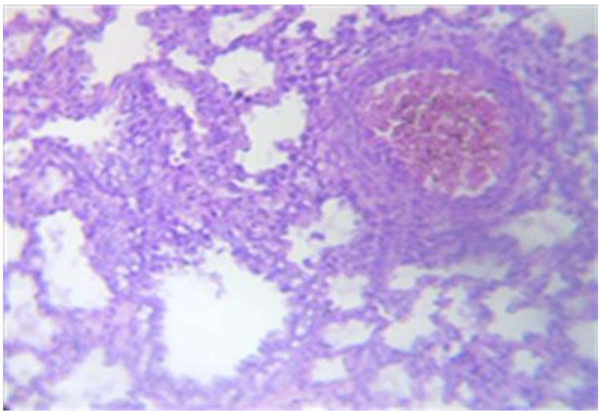
Fetus 27 days (A): The lung tissue had containing the alveoli, alveolar sacs and ducts, great respiratory duct lined with cuboidal and alveolar cells also demonstrated adjacent to the pulmonary blood vessel engorged with blood mass (fig 3.3).



(fig 3.3): lung tissue(A)alveoli, (B)alveoli sac, (C)alveolar duct, (D)respiratory duct lined by(E)cuboidal and squamous cells, (F)pulmonary blood vessel. (H&E*10).

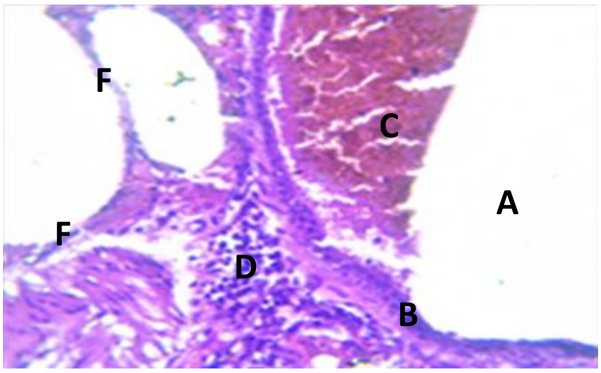
Six month (A): The intra pulmonary bronchiole was wide lumen lined with simple cuboidal epithelium and a mass of blood was present and the lumen of bronchiole, focal aggregation

Fetus 27 days (B): The alveoli and respiratory bronchioles were lined with cuboidal and squamous alveolar cells and presence of intrapulmonary artery which had engorged blood and thick muscular layer, those structures were infiltrated with multiple number of WBCs in the interstitial C.T (fig 3.4).



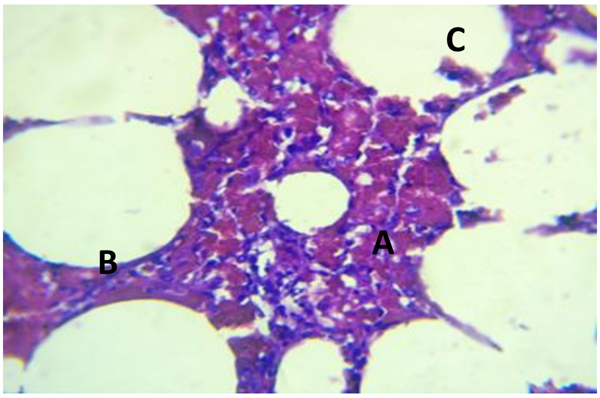
(fig 3.4): (A)alveoli, (B)respiratory bronchiole, lined with(C)cuboidal cells, (D)pulmonary blood vessel congested with blood, (E)WBCs in the interstitial C.T. (H&E*40).

of white WBCs was present around the wall of a bronchioles and around the smooth muscle fibers, the adjacent alveoli were lined with alveolar epithelial cells type **I** and **II** pneumocytes with presence of small blood capillaries around it (fig 3.5).



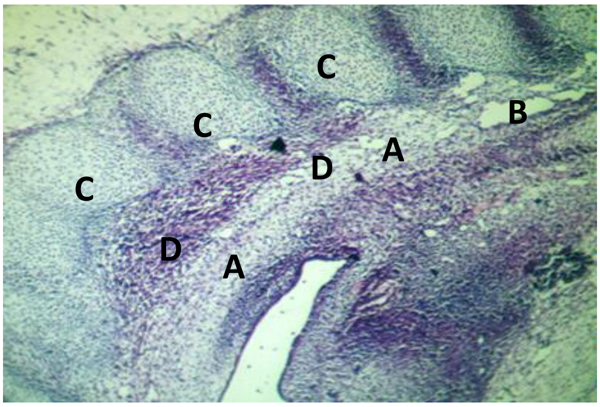
(Fig 3.5): (A) intrapulmonary bronchiole, (B)simple cuboidal cell lining, (C)blood mass, (D)interstitial C.T with WBCs, (E)blood capillary, (F)alveoli with alveolar cells. (H&E×40).

Six month (B): The interstitial connective tissue was containing thickening of collagen bundles associated with cultured white blood cells, the alveolar sacs where lined by a viola epithelial cells squamous type **I** and few cuboidal type **II**, cellular debris was seen in individual space of alveoli (fig 3.6).



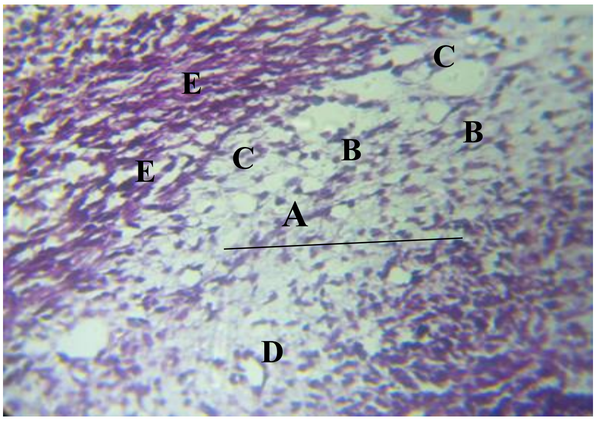
(Fig 3.6): lung tissue, (A) interstitial C.T with collagen bundles and WBCs, (B)alveoli with epithelial cells lining type **I** and **II**, (C)cellular debris. (H&E×40).

Embryo 17 days (A): The lung tissue was formed by delicate mesenchymal tissue and minute alveoli adjacent to the primordia of thoracic vertebrae, small primitive bronchioles was demonstrated (fig 3.7).



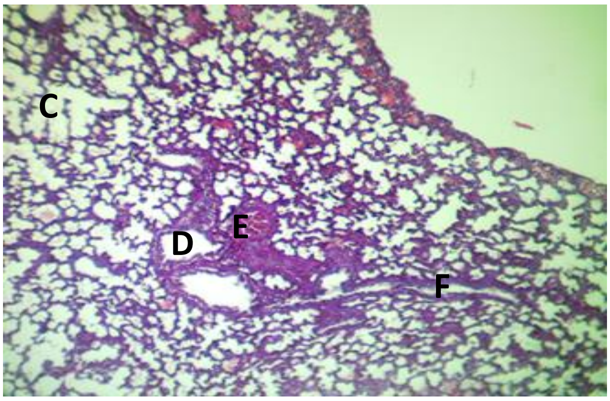
(Fig 3.7): (A)mesenchymal tissue of primitive lung bud, (B)primitive bronchiole, (C)masses of primordial thoracic vertebrae, (D)intermediate mesoderm of primitive muscles. (MTC*4).

Embryo 17 days (B): The mesenchymal membrane was present with mesenchymal cells and minute alveoli were present in association with primitive bronchioles, the mesenchymal tissue of lungs was surrounded by primitive muscular tissue of intermediate mesoderm (fig 3.8).



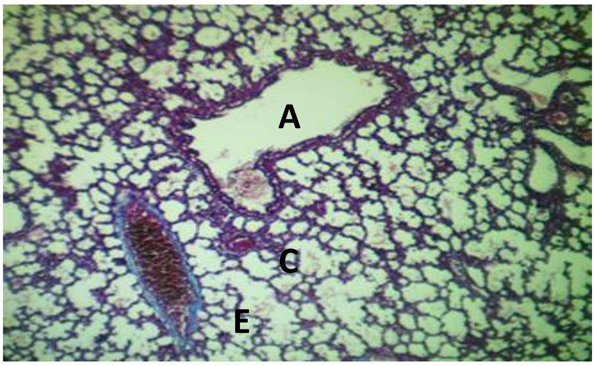
(Fig 3.8): (A)mesenchymal membrane of lung bud, (B)mesenchymal cells, (C)minute alveoli, (D)primitive bronchiole, (E)intermediate mesoderm of primitive muscles. (MTC*40).

Fetus 27 days (A): The lung parenchyma was covered by pleural visceral membrane composed of delicate C. T, the lung had great number of alveoli, alveolar ducts and alveolar sacs. intrapulmonary bronchioles and congested blood vessels also present (fig 3.9)



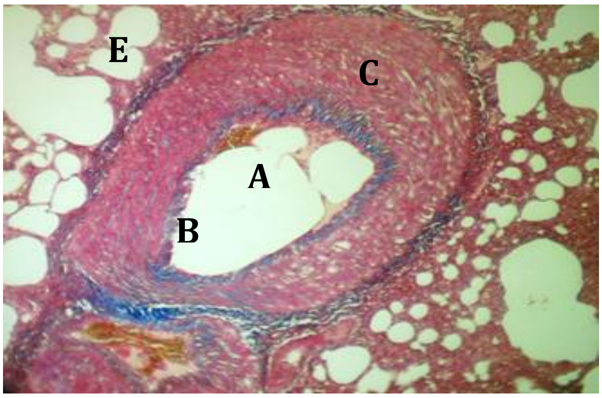
(fig 3.9): lung parenchyma (A)pleural visceral membrane, (B)alveoli, (C)alveolar sac, (D)terminal bronchioles, (E)blood vessel, (F)alveolar duct. (MTC*10).

Fetus 27 days (B): Intrapulmonary bronchiole lined with simple cuboidal-columnar epithelium, the alveoli and other alveolar sacs and ducts were surrounded by delicate C.T as well as the different blood vessels (arteries and veins), were present with blood mass in its lumens (fig 3.10).



(fig 3.10): lung parenchyma (A)intrapulmonary bronchiole, (B)congested blood vessel, (C)alveoli, (D)respiratory bronchiole, (E)alveolar duct. (MTC*10).

Six month (A): The lung tissue was demonstrated which have pulmonary artery formed by tunica intima with internal lamina, also tunica media formed by smooth muscle fibers invested by delicate fibers continuous with interstitial connective tissue which have multiple alveoli of small size surrounding the artery (fig 3.11).



(Fig 3.11): (A)lung tissue, intrapulmonary artery, (B)endothelium with internal elastic lamina, (C)tunica media with smooth muscle fibers, (D)interstitial C.T, (E)alveoli. (MTC×40).

Six month (B): Intrapulmonary vein was indicated which have blood mass in its lumen, its tunica media formed by collagen fibers and few smooth muscle fibers, it is surrounded by different sizes of alveoli (fig 3.12).

(Fig 3.12): (A)intrapulmonary vein, (B)blood mass, (C)degeneration the sarcoplasm of mass smooth muscle fibers of tunica media, (D)alveoli, (E)vacuolation of interstitial C.T. (MTC×40).

4.Discusion:

The results agree with those found by (McGowan, 2014)at the fetus 27, the lung tissue had containing the alveoli, alveolar sacs and ducts, great respiratory duct lined with cuboidal and alveolar cells also demonstrated adjacent to the pulmonary blood vessel engorged with blood mass and the alveoli and respiratory bronchioles were lined with cuboidal and squamous alveolar cells.

Current results agreed with (Hussein, 2020) suggested that lung tissue at six months aged rabbit have pulmonary artery formed by tunica intima with internal lamina, also tunica media formed by smooth muscle fibers.

The present study is in agreement with (Karnak et al., 1999, Salaets et al., 2020) The process of lung maturation was nearly finished by the 27th-28th days of gestation. However, the lungs still showed signs of being structurally immature. This was evident through the presence of thicker partitions and a higher number of cells, as well as less intricate airspaces. These characteristics suggest that by day 28 of gestation, the lungs are in the saccular stage of development.

The results also agreed with (Ramchandani et al., 2000) in Compared to mature rabbits, immature rabbits were found to have a lower proportion of cartilage in their airway walls. and with (Fraser, 2005) that veins contain a varying number of elastic layers, with small groups of smooth muscle cells in between. There are no valves present inside the veins. In the arterial system, the inner layer called the intima is very thin and consists of a layer of endothelial cells and its neighboring basement membrane. The submucosa, which is the layer beneath the intima, is mostly composed of loose connective tissue containing proteoglycans and mature collagen. This connective tissue is continuous with the connective tissue found in the nearby pulmonary arteries, as well as in the tissue surrounding the veins near the hilum, and in the interlobular and pleural interstitium. The interdependence of this connective tissue is crucial for maintaining the overall structure of the lung and providing support for the more delicate connective tissue of the parenchyma.

In our study, it was observed that the lung undergoes incomplete development during the fetal stage at 17 days of gestation, indicating that it is in its early stages of morphogenesis for example (presence of multiple undifferentiated cells, scanty formation of delicate un well developed connective tissue, and no indication for the presence of saccular development).

Conclusion:

The research concluded that at 17 days of gestation, the lungs of rabbits are not completely developed and there is a clear difference in the tissue structures of the lung with the formation of the fetus, after birth, and advancing age of rabbits.

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دراسة نسيجية لرئتي ألارنب في فئات عمرية مختلفة

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الملخص

ان هدف هذه الدراسة هو دراسة أنسجة الرئة في الأرانب المحلية (Oryctolagus Cuniculus) في ثلاث مراحل عمريه مختلفة. تم استخدام 18 أرنبًا في الدراسة، وتم جمع عينات من أنسجة الرئة في عمر 17 و27 يومًا في الرحم، وبعد 6 أشهر من الولادة. تخضع الرئة لتغيرات نسجية مختلفة أثناء التطور الجنيني، بما في ذلك المراحل الجنينية، والغدية الكاذبة، والقناة، والكيسية، والسنخية. هدف هذا البحث هو فحص الخصائص النسيجية لأنسجة الرئة الطبيعية في الأرانب المحلية. أظهر التحليل النسيجي وجود الحويصلات الهوائية والفيبرين وخلايا الدم الحمراء الفردية في بعض عينات أنسجة الرئة. بالإضافة إلى ذلك، لوحظ وجود عدد قليل من الخلايا البلعمية السنخية، بالإضافة إلى خلايا النوع الاول والنوع الثاني، في المساحات السنخية.

الكلمات المفتاحية : (أرنب , رئة , تطور الرئة , جنين , الفئات العمرية).