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Histological developmental and immunohistochemistry study of the lung in rat(Rattus rattus)

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Abstract

The goal of the current study, which involved 21 fetuses, was to describe the developmental structure of the rat fetus lung. The embryos were taken on days 17, 19, and 21 of pregnancy. According to the current study, the lung was in the canalicular stage during the 17th gestational day. At this stage of development, all lung airways had significantly lengthened and expanded. The terminal bronchiole divides into two, then into four acinar canals, which are large, straight canals. The cuboidal epithelial cells that line these acinar canals have also started to flatten and transform into potential type I pneumocytes. The lung was in the saccular stage on the 19th day of pregnancy. The terminal bronchioles split into several possible alveolar ducts, which terminated In the typical collections of enlarged airspaces called terminal sacs, there is a single layer of cells with cilia on the epithelial surface, a blue nucleus with a gap in the cells, and a terminal opening on the air vesicle with several capillary networks that transform the lining cells into alveolar cells. Close contact between some capillaries and the flattened cells resulted in the emergence of the first blood-air barriers. From the 21st gestational day through the first postnatal day, there was an alveolar stage. The cartilage, which had largely grown by day 21 of pregnancy, contained collagen. Collagen II was discovered on the tracheal hyaline cartilage ring's periphery, inside the bronchi body, and across the whole cartilage ring.

Key words: Rats, lung, Development, Immunohistochemical

I. INTRODUCTION

One of the most often used animal models in experimental investigations looking at prenatal lung development is the rat fetus; yet, there is currently limited information on the morphological traits of rat lung development throughout fetal and early neonatal stages. Life is not abundant [1]. The trachea, bronchi, bronchioles, and lungs are all parts of the lower respiratory tract [2]. The embryonic events that take place during the key stage of the rat's development allow the foregut to transform from a single tube

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to a distinct trachea and well-developed bronchi in less than 24 hours. On day 11 of pregnancy, the earliest respiratory primordium began to emerge [3]. The whole lung development that occurs prior to delivery in the 20–21 day pregnancy of the rat is compressed into the period from around E11 to birth [4]. In a man, the process takes longer. Lung development begins about 3.5 weeks and lasts until birth and beyond [5]. The principal period of alveolar growth in terms of size and number occurs after birth in both species, and neither the lungs of the rat nor the man are fully developed at birth. Based on their findings, the following overview is provided. The developmental stage is E11.5–E13. The lung develops from the ventral surface of the heart. The ventral surface of the upper esophagus is where the lung grows, and elastin, collagen I, and collagen II distributions in the rabbit trachea determined that the earliest signs of development can be seen around the 11th day of pregnancy [6]. These matrix elements were chosen because they are pertinent to tissue engineering, have an impact on mechanical performance, and can potentially be validated using measurements of relative abundance and dispersion [7].

II. MATERIALS AND METHODS

The animals were housed in cages in the animal house of the University of Veterinary Medicine in Basra, and they were fed during the course of the study using equipment as described by [8]. When mating was confirmed the next morning, the females were separated by noting the vaginal plug in order to determine the age of the fetus. Females that were ready for fertilization were put with males in the ratio of one male to three Females in each cage during the night hours. The day the tampon was discovered was regarded as day 0 of pregnancy, and the following day was regarded as day 1 [9].

On gestational days 17, 19, and 21, 21 rat fetuses were obtained. Under a dissecting microscope, the fetal thorax was gently cut open, exposing both lungs, preserving their whole structure, and washing them with normal saline solution to eliminate any blood or other clinging debris. The tissue samples were fixed in 10% formaldehyde for 24 hours before being dehydrated in a graded alcohol, washed in xylol, and embedded in paraffin wax. Each paraffin block was cut into six six-micron-thick sections, which were then stained with (H and E), Masson's trichrome stain, to show the collagen and smooth muscles, and immunohistochemical stain to Collagen II to show the organ's histological components [10].

III. RESULTS AND DISCUSSION

The bronchial tree stage and pseudo glandular stage, which correspond to the seventeenth day of lung development in rats, respectively, acquire their names from the way the lung looks at this time, which resembles a tubular gland. The subsequent generation of future airways develops as the terminal bud extends into the surrounding mesenchyme; by the time this stage is complete, the initial generations of alveolar ducts have already begun to take shape. These findings are consistent with [11], who claimed that the pseudo glandular stage is when the first 20 generations of the developing human airways are

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formed. The current study shown that the canalicular stage of the rat fetal lung at the 19th day of gestation, all lung airway generations expanded and elongated greatly at this time of development, leading to a noticeably reduced amount of connective tissue in the lung. Throughout this stage, there were significant alterations to the rat fetal lung. The terminal bronchiole divides into two, then into four acinar canals, which are large, straight canals. The cuboidal epithelial cells lining these acinar canals also started to flatten and transform into potential type I pneumocytes Figure. The current study also discovered that in the saccular stage, the terminal bronchioles split into a number of potential alveolar ducts, which terminate in the typical collections of enlarged airspaces known as air saccules or terminal sacs. This stage of the fetal rat lung is composed of a single layer of cells with cilia on the epithelial surface, the blue nucleus with a gap in the cells, and a terminal opening on the air vesi The initial blood-air barriers appeared as a result of close contact between some capillaries and the flattened cells, which is consistent with the findings of [12,13].

The lungs' blood vessels were many, the airways and alveoli were developed and remarkably transparent, and the canals were overflowing with fluid on the 21st day of pregnancy. Figure (5A,B) (5A,B). The surrounding mesenchymal tissue compressed into thick inter-saccular septa, resulting in few gaps, thickening septa, and immature alveoli. The alveoli are composed of many layers. The large intersaccular septa's interstitial contained a lot of cells. Figure (6). These findings lined up with a rabbit mention in [14-17]. Additionally, type II collagen's immunohistochemistry localisation was examined, as well as its presence in the fetal rat bronchi's growing cartilage. On day 21 of pregnancy, the cartilage that had mostly grown could be seen to contain collagen (Figure 7). At this stage, chondrocyte activity was visible. The current findings are consistent with those found in rabbits by [15-16]. The entire cartilage ring, however, was covered in Collagen II's great discolored tracheal hyaline cartilage. On the outside surface of the cartilage ring, collagen II staining was seen. In addition, the bronchi body's interior had a peculiar staining pattern (Figure 7). This was seen throughout the entire bronchi cartilage, and it agrees with the observations made by[18-19].

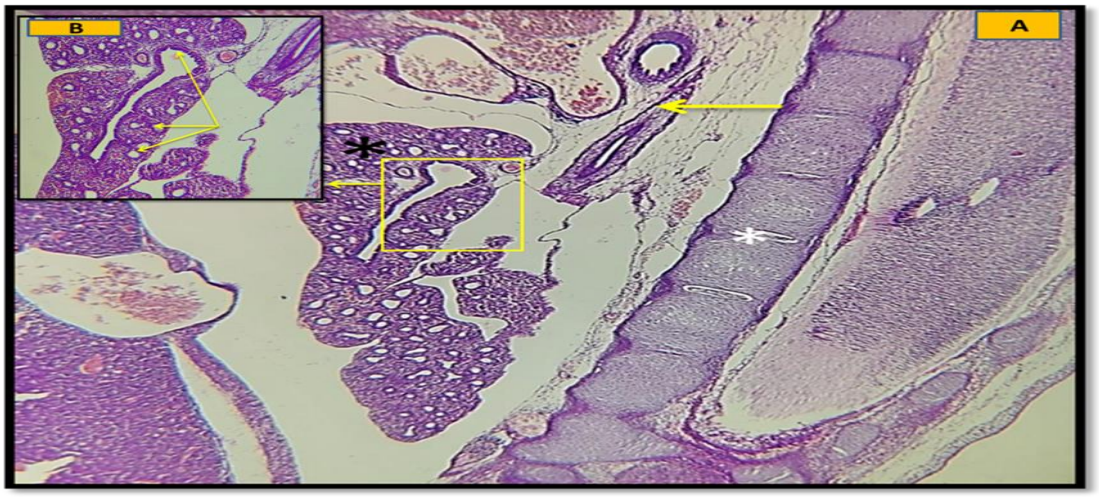

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Figure 1: Cross section in 17th day rat embryo, show the lung development:

A-Trachea (arrow), widening and lengthening of all airspace generations (black stars); reduction of the mesenchymal tissue (H&E Stain 40X)

B-Development the airway (yellow arrow) (H&E Stain 100X)

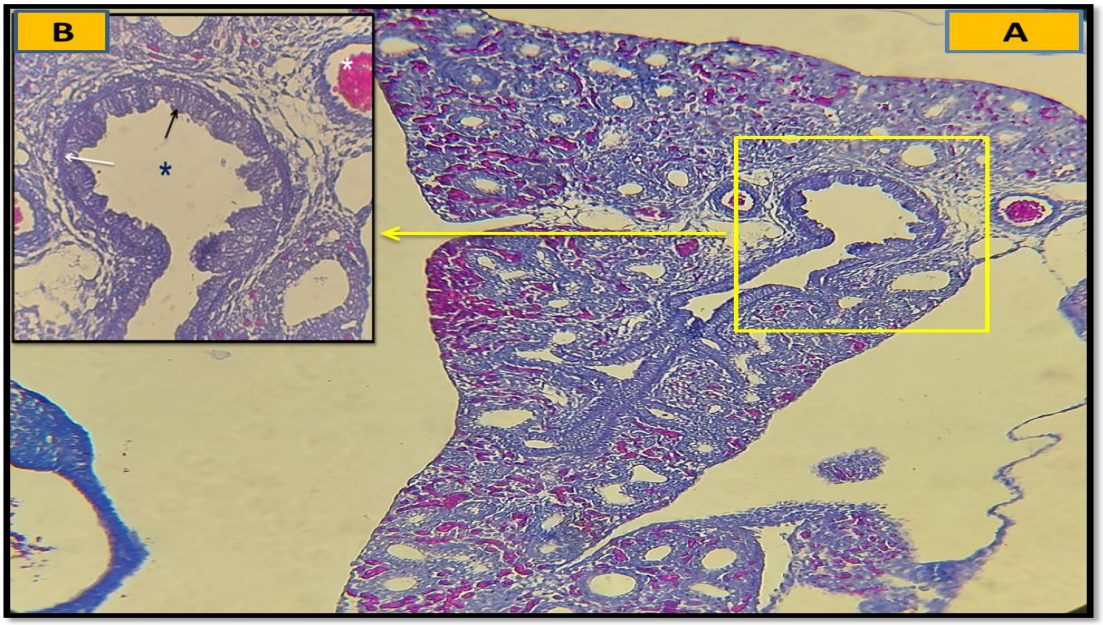

Figure 2: Cross section Show of rat embryo 17th day

A-lung, terminal bronchiole, (Masson trichrome stain 40X)

B- Terminal bronchiole (black*), the collagen bundles (arrow white), respiratory epithelium

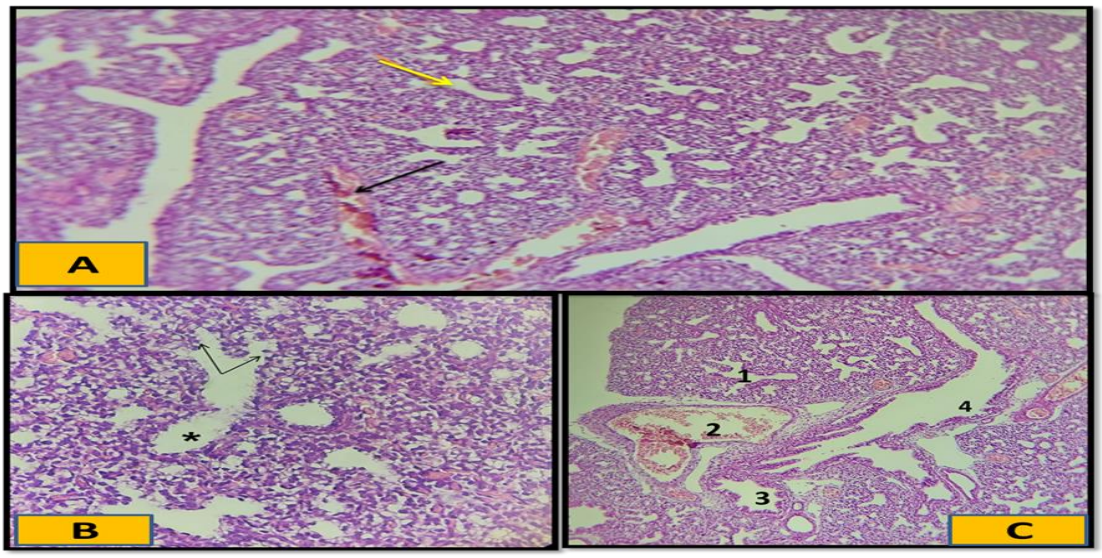

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Figure 3: Cross section in19th day of rat embryo lung, show:

A-lung fetus widening and lengthening of all airspace generations resulting in a marked reduction of the mesenchymal tissue (arrow yellow), Blood vessel (arrow black)(H &E stain 100X)

B- Terminal bronchiole (* black) branched into several acinar canals (black arrow) (H&E Stain100X)

C- Immature alveoli (1), blood vessels in air ways (2), few gaps and terminal bronchus (3), bronchioles (4), (H&E stain100X)

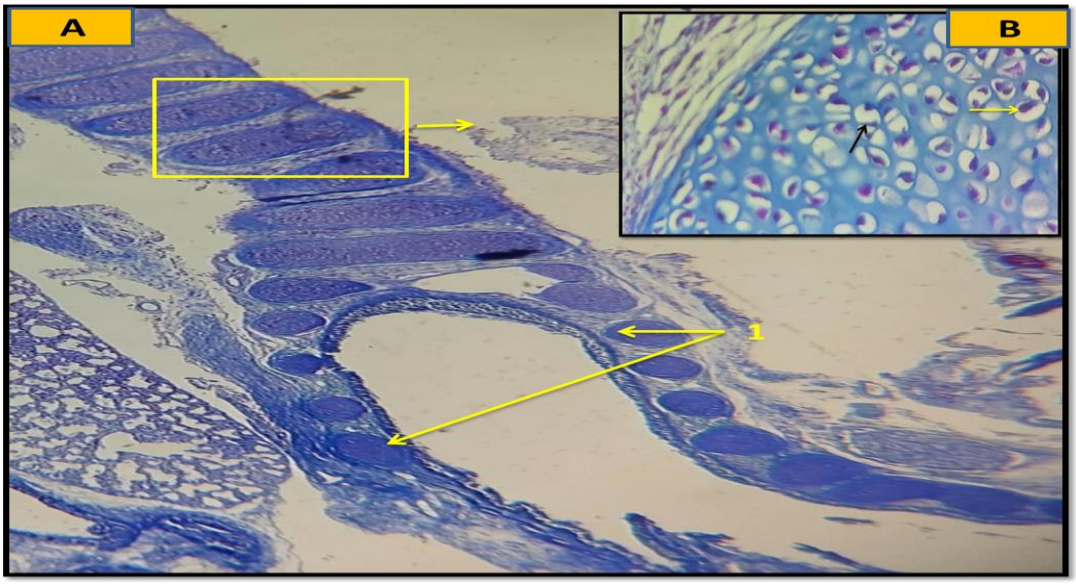

Figure 4**:** Cross section show of 19Th day of rat embryo

A- Bronchus Hyaline cartilage (1), (Masson Trichrome 40X)

B -Hyaline cartilage with chondrocyte inside lacuna (Masson Trichrome 100X)

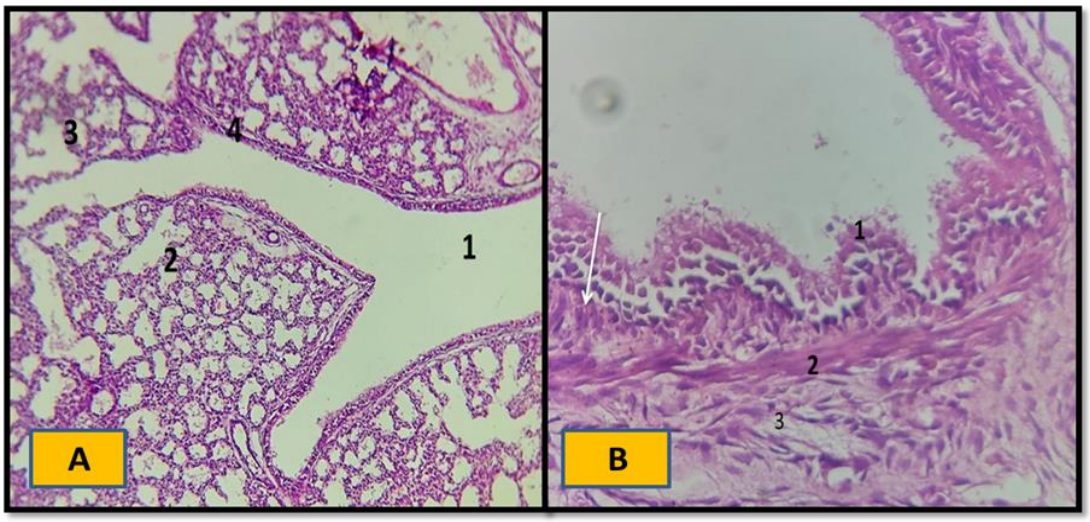

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Figure5:Cross section of 21 day rat embryos, lung of terminal bronchiole: show:

A- Respiratory bronchiole (1), immature alveoli air saccules (2) prospective alveolar ducts respiratory bronchioles(3),conversion epithelium (4)(H&E40X)

B-Respiratory epithelium single layer and folded of cells with cilia (1),Nucleus (white arrow), smooth muscle (2), Sub mucosa (3) (H&E400X)

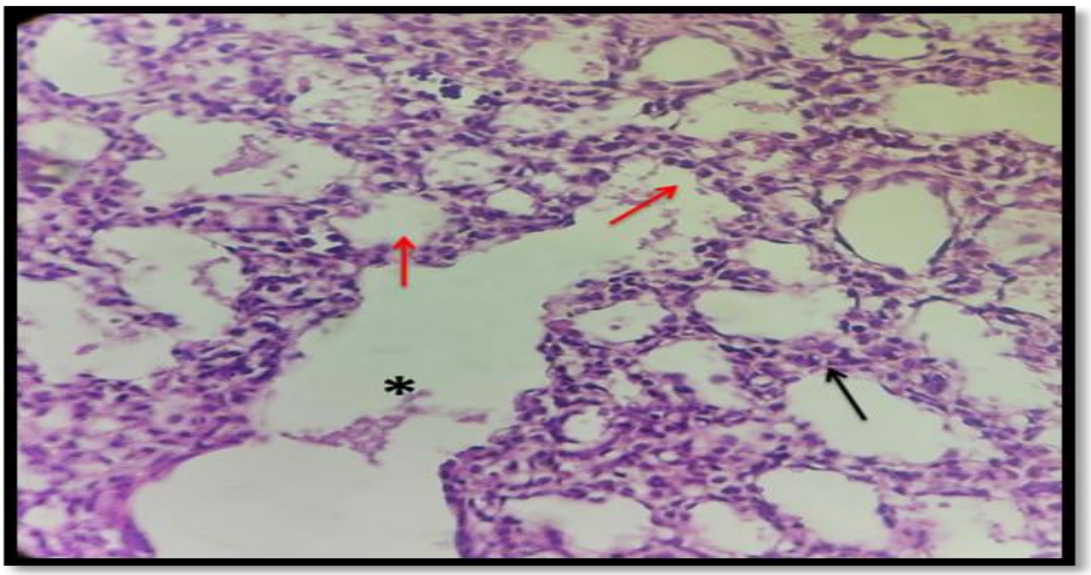

Figure 6: Cross section of 21 day rat embryos, showing terminal airway unit started by terminal bronchiole (*) and ended by air saccules (red arrow), and developing secondary septa (arrow black) (H&E stain 200X)

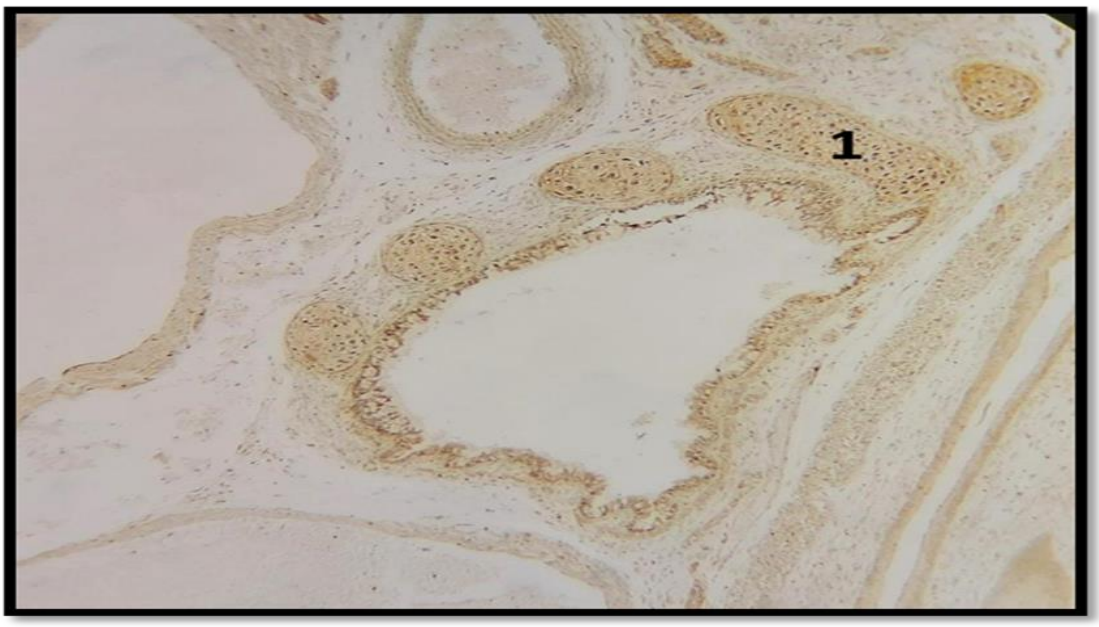

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Figure7: Cross section 21 day embryos rat, show: bronchi cartilage with Collagen II (1) (COL2A1, 200X)

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