Section: Anatomy

Research

HISTOGENESIS RESEARCH OF LUNG IN HUMAN FOETUSES IN DIFFERENT WEEKS OF GESTATION A LIGHT MICROSCOPIC STUDY

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Abstract

Background: This paper focuses on Lung histogenesis study in normal human foetuses ranging from 12 to 40 weeks of gestation age. The process of histogenesis is nothing but a series of events that occur during the development of a tissue. In this process undifferentiated cells attain full functional maturity. **Materials and Methods:** The present study included 42 foetuses and abortuses received to the anatomy department from which the lung tissue was collected after they were fixed with formalin and observed for the cytoarchitecture during development. Tissues were stained with Haematoxylin and Eosin and was observed under light microscope. **Result:** In earlier weeks of gestation around 12 weeks mesenchymal tissue are abundant. Development Bronchial duct system well observed from 12 to 14 weeks onwards. At 14-16 weeks bronchi of varying sizes were seen. The lining epithelium in the bronchi varied between low columnar to pseudostratified ciliated columnar and bronchioles showed simple cuboidal epithelium. The Glandular and lymphatic elements were seen developing around bronchial wall by 24 weeks. Respiratory bronchioles appeared by 24-28 weeks and their further division into alveoli by 32 weeks. Multiple Acini were seen packed with cells. Well-developed hyaline cartilage is observed surrounding the secondary and tertiary bronchi by 24weeks. Around 36-40 weeks alveoli were invaded by capillaries and hence was similar to the structure of an adult lung. **Conclusion:** Abnormal or delay in embryogenesis of the lung has significant correlation with structural abnormalities. This knowledge is important to clinicians during their clinical procedures.

INTRODUCTION

There is a specific and unique embryological process in the development of the important respiratory organ lungs.[1]

The maturation of the lung is the histogenesis that deals with significant independency of fetal survival. Lungs develop as a ventral diverticulum from Foregut during 3-4 weeks of gestational period.[2]

The respiratory tract develops from two sources, the upper respiratory tract which includes larynx, trachea, bronchi, bronchioles until respiratory bronchioles develop from Foregut diverticulum and rest of respiratory bronchioles, alveolar ducts, alveolar sacs and alveoli develop from the adjacent mesenchyme.[3]

According to Jeffery, mucosal cells in the airway completely mature at 24 weeks of gestation and by 18th week, the branching of airway is completed.[4]

This present work was taken up for a comprehensive Histogenesis study of various aspects of lung development in foetuses ranging from 14 weeks to 40 weeks. Our study is significant for the neonatologist in the treatment of premature infants.

MATERIALS AND METHODS

The present study was carried in Department of Anatomy, over a period of one year. The clearance from institutional ethical committee (Registration No. ECR/58/Inst/AP/2013/RR-16) to preform autopsy and carry out the Histogenesis study.

The current study consists of 42 unclaimed foetuses (22 male and 20 female foetuses) between 14 weeks

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to 40 weeks of gestation received from the OBG department for routine autopsy.

Exclusion Criteria

1. Twins and foetuses with congenital anomalies.
2. Foetuses with history of maternal infections.

Estimation of gestational age was done by measuring the crown rump length (CRL) than correlated with the actual gestational age of foetuses as per the records, to rule out the possibility of IUGR. Weight of foetuses was also noted.[5] The foetuses were dissected after embalming with 10% formalin for 24 hours.

The lung specimen collected from 42 fetuses were fixed in 10% formalin for 3-5 days and observed for any gross malformations. All the specimens were categorized into 7 groups based on gestational age. (A,B,C,D,E,F &G)

CRL of the 42 foetuses (male 22 and female20) was noted beside the gestational age. [Table 1 & 2] The lung specimen were categorized into 7 groups (A,B,C,D, E,F & G) of 12-16 weeks(A group), 16-20 weeks (B group), 20-24 weeks (C group), 24-28 weeks (D group), 28-32 weeks (E group), 32-36 weeks (F group) and 36-40 weeks (G group) according to the gestational age. From each group a sample of lung specimen was processed for histological examination.

All the sections were taken on Rotatory microtome of 5-7 microns followed by embedding & paraffin block preparation. Staining was done with Haematoxylin and Eosin (H&E). Each section was observed for pulmonary maturation under light microscope and photomicrograph were taken relevantly.

RESULTS

The observations in our study are as follows [Table 1 & 2):

1. Fetus was12-16 weeks of gestation: Weight of Baby-200grms; CRL-80-100mm.

The youngest foetus studied was 12 weeks. Mesenchymal tissue was abundant appeared as clumps around 12 weeks of gestation slowly decreasing by 16 weeks. The Large Bronchi lined by tall columnar /pseudostratified columnar ciliated epithelium with few goblet cells and few smooth muscles, were visible by 14 weeks of gestation. Terminal Bronchi were lined by cuboidal epithelium. Few Acini and blood vessels were visible in between the bronchi.

2. Fetus was16-20 weeks of gestation: Weight of Baby-400grms; CRL-110-125mm. The lung section resembled to an exocrine gland. The Mesenchymal tissue was seen around bronchi. Numerous Bronchi with in foldings and lined by tall columnar cells to Pseudostratified columnar ciliated epithelium were visible. Formation of cartilage plates were also identified around 18 weeks. Small bronchioles were present lined by simple cuboidal to flat epithelium associated with packed multiple Acini.
3. Fetus was 20-24 weeks of gestation: Weight of Baby-600grms; CRL 130-180mm. Mesenchymal tissue has decreased. Small bronchial tubes of varying sizes seen lined by simple cuboidal to low columnar epithelium and Large bronchi lined by pseudostratified columnar ciliated epithelium in their walls showed 2 to 3 islands of cartilage. In b/w bronchi and bronchioles well developed collagen fibers in septa were very were identified. Blood vessels were also seen in the septae. Lung appeared like compound racemose gland (Pseudoglandular phase) with 1 to 2 primordial mucous/serous glands in developed in bronchial wall, some no lumen.
4. Fetus was 24-28 weeks of gestation: Weight Of Baby-800 gms; CRL- 190-210mm. Amount of mesenchyme b/w bronchial tubes are reduced. Numerous bronchial tubes with large lumen and branching, glands in the lamina propria bronchi and matrix of cartilaginous plates were identified. The Lymphatic tissue were also well developed around bronchi and bronchioles.
5. Fetus was 28-32 weeks of gestation: Weight of Baby- 1kg; CRL-220-250mm. Mesenchymal tissue almost negligible and with large bronchi lined by pseudo stratified ciliated epithelium was identified. Respiratory bronchiole and developing Alveoli were seen increased with no glands around them.
6. Fetus was 32-36 weeks of gestation: Weight Of Baby -1250kg; CRL -260-300mm. Large bronchus with numerous mucous glands few serous glands and Bronchi lined with ciliated pseudo stratified epithelium with infoldings, and cartilage plates were noted in its wall. Respiratory bronchioles showed numerous outpouchings of primitive alveoli which were lined by flats cells with numerous capillaries were observed.
7. Fetus was 36-40 weeks of gestation: Weight Of Baby- 2kgs; CRL-380-400mm. There was increase of respiratory bronchioles, terminal bronchioles & alveolar ducts. The wall of alveoli was extremely thin resembling that of adult lung. Increased vascularization in the parenchyma of lung was observed.

Table 1: Prenatal Lungs specimens according to gestational age arranged according to groups & sex: (42 foetuses)

Serial number	Gestational age (weeks)	Group	Lung specimens (male: 22)	Lung specimens (Female: 20)
1.	12-16	A	2	2
2.	16-20	B	4	3

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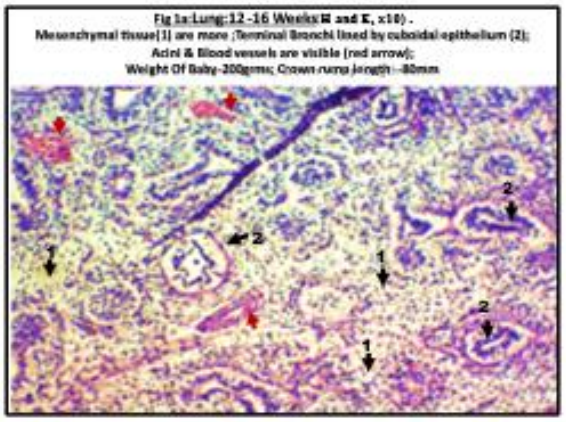
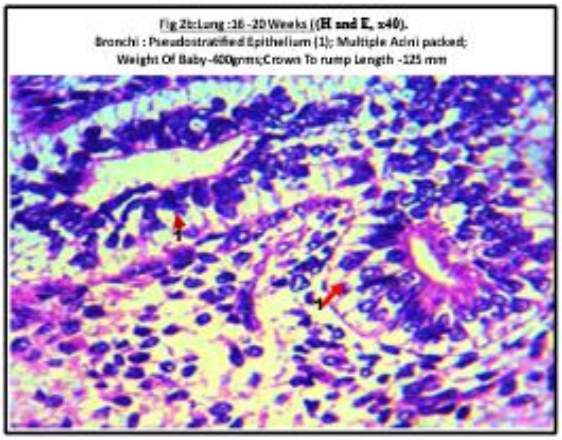
3.	20-24	C	3	4
4.	24-28	D	2	2
5.	28-32	E	3	3
6.	32-36	F	4	3
7.	36-40	G	4	3
Total :7 groups			22	20

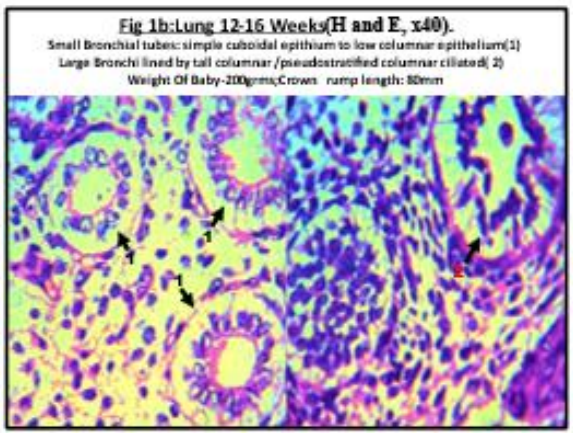
Table 2: Prenatal Lungs specimens according to gestational age according to Crown rump length Textbook of Human Embryology by Hamilton, Boyd and Mossman5

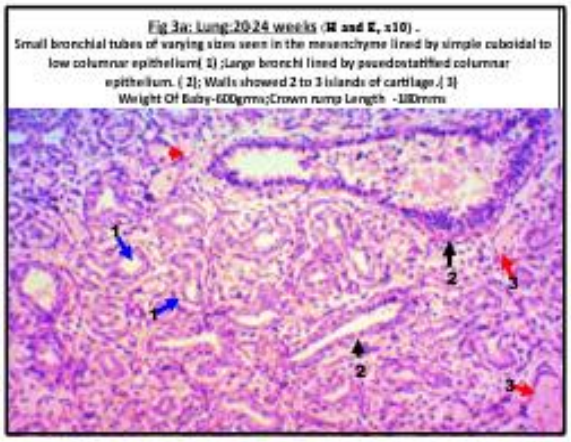
Serial Number	Group	Gestational Age	Crown Rump Length
1.	A	12-16 Weeks	80-100 mm
2.	B	16- 20 Weeks	110-125mm
3.	C	20-24 Weeks	130-180mm
4.	D	24-28 Weeks	190-210mm
5.	E	28-32 Weeks	220-250mm
6.	F	32-36 Weeks	260-300mm
7.	G	36-40 Weeks	380-400mm

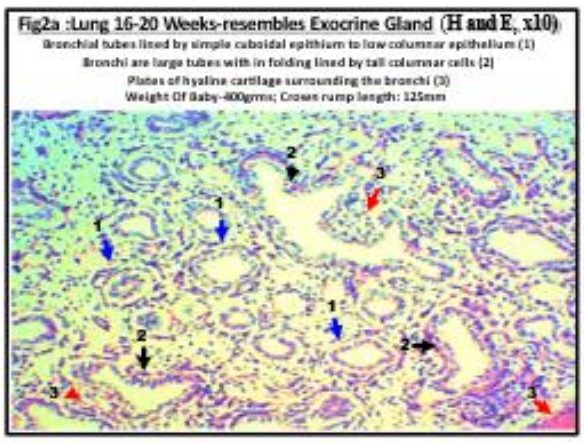
Histogenesis of Lung

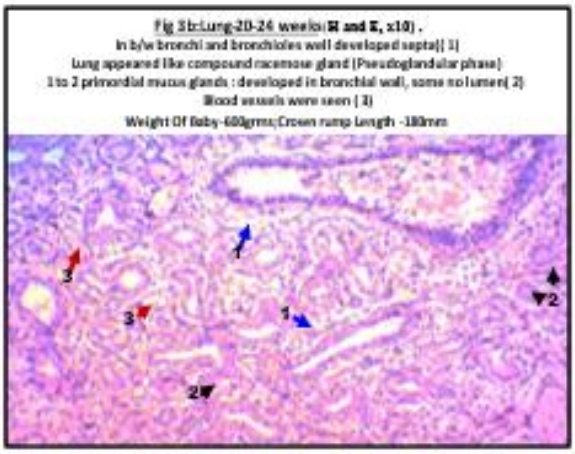












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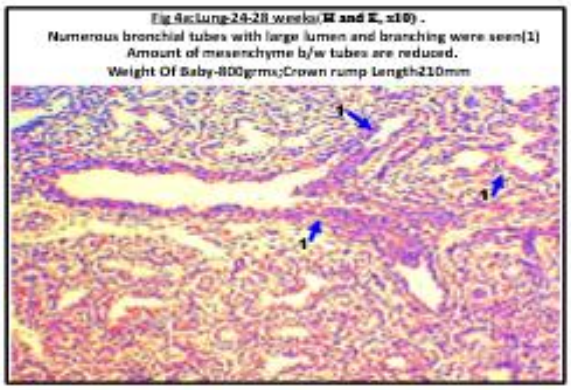

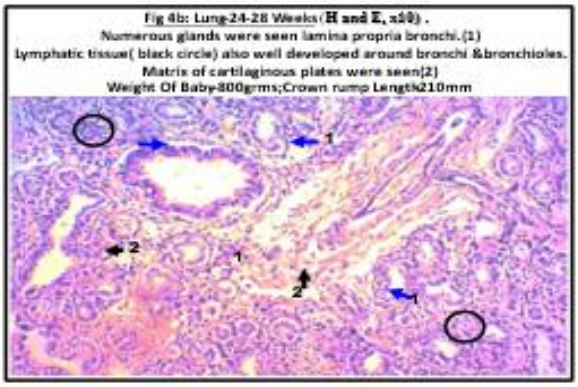

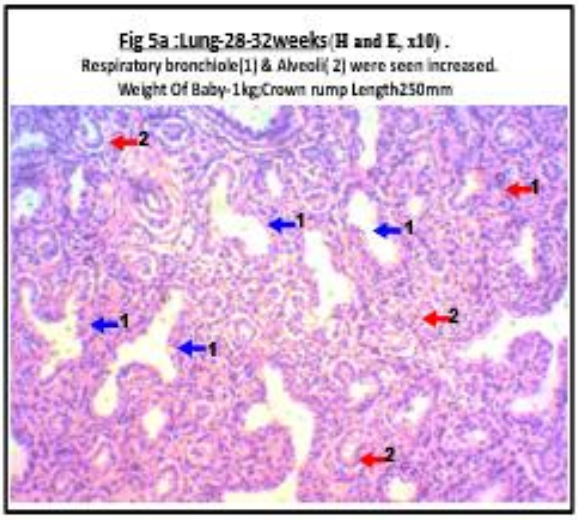

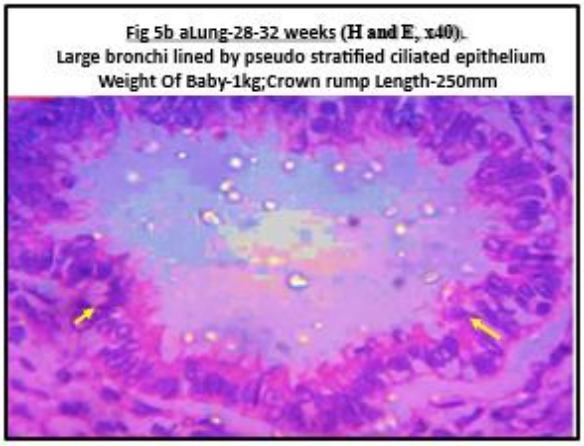

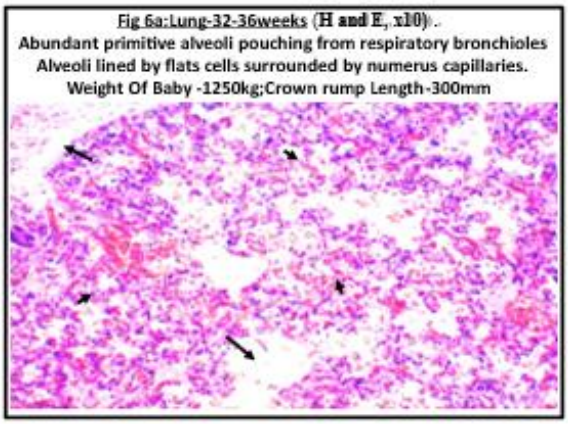

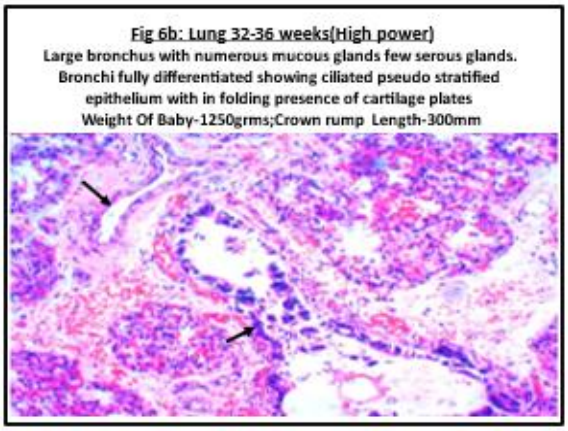

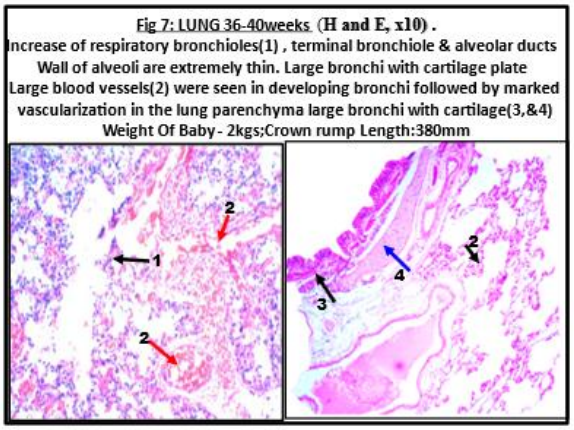

DISCUSSION

Process of histogenesis of lung is very significant as it involves independent survival of the foetus. Prenatal developmental morphology and histogenesis of human Lung is therefore useful to manage the premature infants.

In humans the Lung develops during intrauterine period and infancy after which it increases in size but not complexity. Congenital lung diseases most often occur from acquired embryological defects commonly arising during gestational period. Agenesis, accessary lung, ectopic lung, persistence foetal lobulation, congenital lung cysts are some of common congenital anomalies.

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Histogenesis of human foetal lung specimens under light microscope has been studied by various authors. The observations of previous works has defined a timeline for appearance and has been discussed with the present study.

1. Mesenchymal tissue/cells

In the present study at 12 weeks of gestation the lung parenchyma was occupied by abundant mesenchymal cells of varying size. As the gestational age increased more number of bronchi appeared differentiating into various levels of division and alveoli also appeared replacing mesenchymal tissue.[6]

2. Development of bronchi

In the youngest foetus studied in our series at 12-16 weeks generations of bronchi were seen with multiple bronchial tubes at various levels of differentiation. Bucher and Reid,[7] described that at 10 week of gestation cartilaginous plates were found developing around the main bronchi. In the present study precartilage plates were identified around 12-14 weeks of gestation around the main bronchi . At 16 -20 weeks few bronchi were lined by simple columnar to pseudo stratified epithelium with 1-2 goblet cells. At regions the bronchi showed infoldings of lumen.[8]

Some authors described simple columnar epithelium at first half of fourth month followed later part of gestation pseudo stratified epithelium.[9]

In subsequent stages 20-24 weeks of gestation of development there was an increase in number of bronchi and were lined by pseudostratified ciliated columnar epithelium surrounded by cartilage plates

3. Epithelium

In present study lining epithelium of bronchi at 12weeks simple cuboidal to columnar type. However later pseudo stratified epithelium was noticed by 16 weeks of gestation at certain places. Cilia also became evident by 20 weeks of gestation which was compared with previous authors.

Kate et al,[8] described fully differentiated bronchi at 23weeks of gestation and few cuboidal bronchi.

Edward et al,[10] have also described typical pseudostratified epithelium in bronchi as early as 10 weeks . They also noted cilia from 10-13 week of gestation. The present study showed fully differentiated bronchi at 24-28 weeks of gestation.

4. Glands

Brites G and Brenekar,[11,12] in 1941 reported that mucous glands first appeared in the lungs in the 12 week of foetal life.

U.Bucher and L.Reid,[13] observed that foetus of 13 weeks glands could be detected in the bronchial wall and lumen was detected in them at 14 weeks of IUL giving a primitive pattern. The acini at 24 weeks were of mucous type. Serous cells made their appearance by 26 weeks and ducts could be visible by then. In the present study at 16 weeks of gestation glands could been seen emerging from the basal epithelium which coincided with Brites G and Brenekar and U. Bucher and Reid. Our observation included glands with lumen was found later at 20-24 weeks of gestation. By 24 weeks mucus acinar was noted and 28 weeks serous acini could be seen. These findings were slightly later as compared with above authors.

According to them serous glands started decreasing in number after 25 weeks of IUL and not visible by 30 weeks.[7,13,14] Some authors(14) observed male superiority in gland number but other studies described no sex differences in the gland development . The present study was not able to differentiate any such sexual dimorphophism.

5. Cartilage & smooth muscle

According to Edward et al,[10] by 4th week of IUL cartilage was in the trachea and extended to lobar and segmental bronchi by 12-16 weeks . Similar findings were also observed by U.Bucher and L. Reid in developing bronchi.[7]

Some authors described the appearance of smooth muscles fibers around bronchial tubes after 15 weeks and few authors described smooth muscles in the wall of the tubules around 24 weeks.[8,16]

In the present study precartilage plates two to four in number were identified around 12-16weeks of gestation around the main bronchi. Later by 20-24 weeks of gestation few bronchi were lined by pseudostratified columnar epithelium surrounded by cartilage plates was eosinophilic even at 36 weeks of gestation.

The smooth muscles cells around the bronchus was identified by 12-16 weeks of gestation and 20-24 weeks the smooth muscle around bronchus seen increased. These observations coincided with above authors.

6. Lymphatic elements

In present study lymphatic elements in the walls of the bronchi was seen 24-28 weeks as aggregation of cells. Reid and Rubin,[15] observed the lymphatic elements in the wall of bronchi at 20 weeks, slightly early according to our observation.

7. Development of Bronchioles, respiratory bronchioles and primitive alveoli

In the present study around 16-20 week of IUL fetal lung section showed bronchioles were lined by simple cuboidal/columnar non ciliated epithelium devoid of cartilage. By 20-24 weeks we could find few respiratory bronchioles and alveolar ducts lined simple cuboidal epithelium. Around 24-28 weeks of gestation alveoli were lined by flattened epithelium.

8. Respiratory bronchioles and few alveoli arise from terminal bronchioles by 24-26 weeks as quoted by K.Moore.[2] Similar findings were noted in present study wherein respiratory bronchioles were seen branching and at its end, were alveoli lined by flattened epithelium. Thus structure and time of appearance of respiratory bronchiole and alveoli is accordance with U. Buther [7]

These findings also coincide with findings of Mitsuri, who observed the lining epithelium of terminal buds changes from columnar, cuboidal and to flat cells by 4th, 5th and 6th month of gestation respectively.[16]

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8. Development of Blood Vessels

In our study few blood vessels were observed at 16-20 gestational weeks in well appreciated in interlobar septa. Number of blood vessels increased with increasing gestation.

Reid and Hislop,[17,18] observed preacinar blood vessels at 16-17 weeks of gestation and intracinar vessels developed later with the development of respiratory airways. By 34-36 weeks of gestation alveoli had double capillary layer in their walls. These findings were similarly seen in the present study as well the blood vessels were observed in the lung parenchyma in close proximity to squamous cell alveoli 32-36 weeks of gestation.

The contact of epithelial of alveoli and endothelial cells observed by 24 weeks of IUL, by thebulging of capillaries into the developing alveoli.[19,20]

40 week of gestational age specimen shows [Figure 7] shows squamous epithelium lining the alveoli, respiratory bronchioles, terminal bronchioles, alveolar ducts and bronchi. According to John D. Bancroft (2002),[20,21] Squamous epithelium lining the alveoli, respiratory bronchioles, terminal bronchioles and alveolar ducts developed from 32 weeks of gestation to 8 years after birth.

Inderbir Singh 2003 explained the detail development of lung histogenesis in relation to gestational age.[22]

A.K. Datta 2005 also reported the development of lung histogenesis in phases according to gestational age.[23]

We anticipate this new data will clarify categories and may be of value in future studies in treatment of premature infants. In the present study the findings in relation the maturation of lung almost coincided with all the above workers and in agreement with the literature.

Postnatal development of Lung continues, wherein the adult number of alveoli in lungs is observed by 8 years, but not done in our study.

CONCLUSION

In the current study was done to establish the sequential changes of events in the histogenesis of lungs correlating with the gestational age of fetuses. The lung tissue was considered viable after 28 weeks of gestation with time sequence of appearance of various microscopic structure. This assumption is finally based on independent survival of the foetus. Delay in developmental morphology and histogenesis of the lung cells leads to histopathological abnormalities. This knowledge is expected to be helpful for clinicians during the management of premature infants.

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