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Histogenesis of Lung in human fetuses at different weeks of gestation in eastern India

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Abstract
Histogenesis of Lung was studied using 40 normal human fetuses ranging from 8 to 26 weeks of gestation, under Light Microscopy after sectioning the lung and staining with Haematoxylin and Eosin. The Bronchial buds undergo repeated division to form bronchial tubes that differentiate into different parts of intrapulmonary bronchial tree. In earlier weeks of Gestation Mesenchymal tissue are more. Developing Duct lined by Cubical Epithelium, Acini and Blood vessels are visible. Developing Bronchus lined with Pseudo stratified columnar ciliated epithelium surrounded by hyaline cartilage. In High Power Bronchus surrounded by hyaline variety of cartilage which extend into lobar and segmented bronchus. But cartilage actually appear in 4th Week. Terminal bronchiole is developing with acini packed with cells are seen. In high power bronchus is thick walled and bronchiole is thin walled lined by epithelium. Multiple acini packed with cells are seen. Ducts were having tubular lumen. Well-developed hyaline variety of cartilage surrounded by pieces of bronchus. Bronchiole was visible. Acini are packed with cells. In later weeks of Gestation Bronchus with cartilage on High power. Bronchioles and acini are visible. Mucosal glands are developed on bronchial wall. Bronchioles were having simple columnar non-ciliated epithelium infolding. Mucosal glands were developed on wall of bronchiole. Bronchiole and Bronchi are in between well-developed septae and blood vessels were seen to be developing. Lymphatic element normally developed. Bronchioles were having low columnar to cuboidal epithelium. Serous acini was increased. Respiratory bronchiole and alveoli were increased. Each terminal bronchi were dividing into respiratory bronchiole which were seen to be dividing into alveoli.

Keywords: human fetus, bronchial tubes, lung, haematoxylin and eosin stain, alveoli

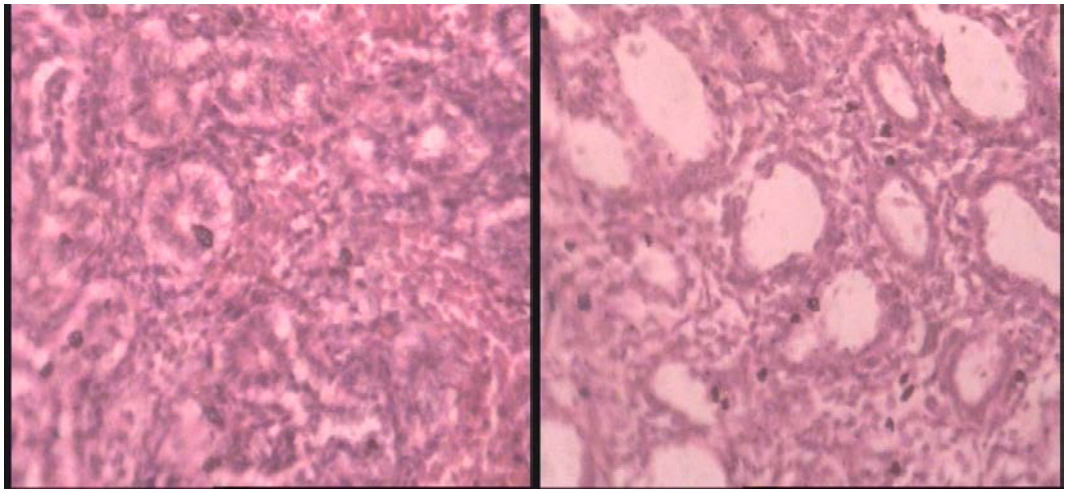
Introduction
Process of histogenesis of lung is very significant as it determines independent survival of the fetus. Although development of human lung has been widely studied and illustrated, most of the articles deal with few aspects of histogenesis [1]. This work has been taken up to comprehensively study the various aspects of histogenesis of lung in fetuses of Indian origin. This prenatal developmental morphology and histology of human lung is expected to be helpful for the management of premature infants. Lungs are major respiratory organs in the human body (Peter Williams, 1931) and they appear to be the lost among the large organs of human body about which a complete unquestioned embryological story has been built [2]. They develop as a diverticulum of the foregut during 3-4 weeks of gestational age (Keith L. Moore & Persaud, 1992) [3]. Lungs have a dual origin like Kidney, according to this larynx, trachea bronchi, bronchioles and part of respiratory bronchioles lined with cuboidal epithelium arises from foregut diverticulum and remaining respiratory bronchioles, alveolar ducts, alveolar sacs and alveoli arise from adjacent mesenchyme (Rose, 1953) [4]. Congenital lung diseases most often from acquired developmental defects arising during gestation. Agenesis, accessory lung, ectopic lung, persistence of foetal lobulation, congenital lung cysts are some of the common developmental abnormalities [5].

Aims and objectives
This study is done to know the chronological pattern of histogenesis of human foetuses in this eastern geographical region of India.

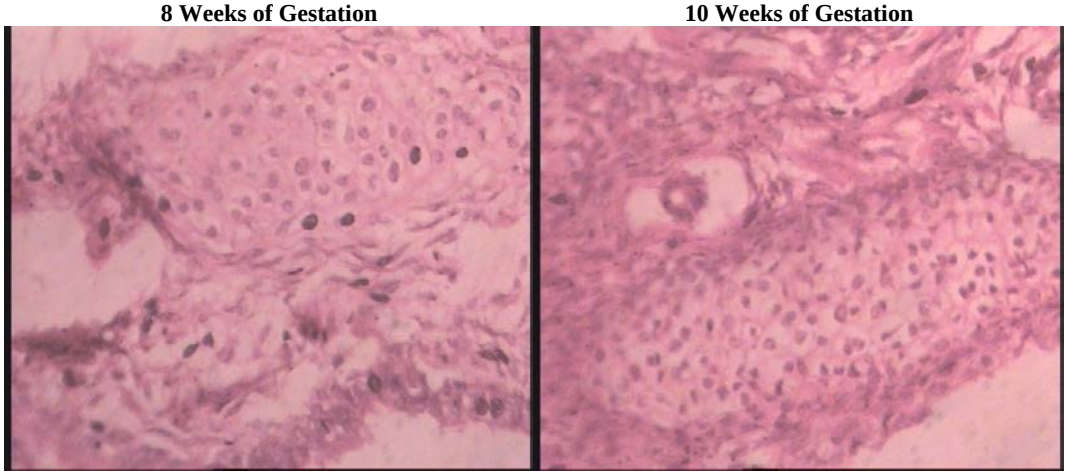
Materials and Methods
This study was done to correlate the chronological pattern of lung development in this geographical eastern region of India, Odisha & compare the results from other researchers nationwide & worldwide. This is a hospital based, observational, cross sectional study conducted at Hi- Tech Medical Colleges & Hospital, Bhubaneswar, India by the Department of Anatomy in collaboration with Department of Obstetrics & Gynaecology from November 2011 to June 2013 on twenty aborted human foetuses without obvious congenital anomaly of gestational age between 8 weeks and 26 weeks collected within 6 hours of delivery by spontaneous miscarriages & therapeutic legal abortions. Study samples were arbitrarily divided into groups of biweekly gestational age by duration of amenorrhoea from medical records & ultrasound fetometry after receipt of informed consent from mother and legal guardians. Fetuses were immediately fixed in 10% Formalin for 1-2 hrs. Lung was dissected by Dissecting Microscope, fixed in 10% Formalin for 48-72 hrs. After fixation by formalin, the tissues were transferred to 30%,

50%, 70%, 90% and Absolute alcohol each for 30 minutes. This ascending grading of the dehydrating fluid was done because when alcohol mixes with water, it produces diffusing current which can damage the tissues. Then the tissues were put in xylol for 24 hours to clear the residual alcohol. These tissues were processed for paraffin sections by tissue blocking (Paraffin Embedding). 3 pots of hard paraffin were taken; paraffin was melted in the incubator at 56 degrees, as hard paraffin is ideal for materials which are to be cut in thin sections about 12 mu. The aining only fresh melted paraffin at 90 minutes interval. Then tissue was put in the first pot containing equal parts of paraffin and xylol and then changed to second and third pots contthe tissues were mounted in fresh melted paraffin with L-Block. The L-Block was then trimmed to a rectangular shape. Then the L-Block was fixed with the block holder (choke) and the block holder was clamped in the rotary microtome. 5 mu sections were cut in rotary microtome. The microtome was revolved at 40 per min and ribbon was formed. Then the ribbon was put in tissue flotation bath. Albuminised slide was then made by putting a drop of Mayor's albumin (equal parts of glycerine and egg white) and spreading it uniformly by rubbing with finger. The piece of ribbon was then taken on the slide and dried at room temperature. The slide was then put in the warming table. When the paraffin melted the slide was put into xylol for 2-3 minutes because xylol removes paraffin. Then the tissue was put in decreasing grades of alcohol (Absolute alcohol,90%,70%, 50% and 30%) then was put in the prepared Harris Alum Haematoxylin (nuclear) stain for 7 minutes and lastly washed with distilled water.2-3 drops of 1% acid alcohol (1cc Hcl in 75% alcohol) was added to remove the excess stain beyond the nucleus. The slide was then put in running tap water for 30 minutes to develop haematoxylin colour (bluish). Then the slides were again dipped in ascending grades of alcohol (30%, 50%, and 70%) and then put in eosin Y (cytoplasmic) stain for 30 seconds. Then the slide was washed with absolute alcohol for a few seconds so that excess of eosin was removed and lastly the slide was placed in xylol. The slide was then taken out from xylol and then put in 1-2 drops of DPX (Adhesive agent) and a cover slip was put on it and pressed slightly so that air bubbles were removed. Sections were then seen in light microscope under low power 10X followed by high power 45X magnification. Thereafter photomicrographs were taken by camera using microscope adapter.

Observations



8 Weeks of Gestation **10 Weeks of Gestation**



12 Weeks of Gestation **14 Weeks of Gestation**

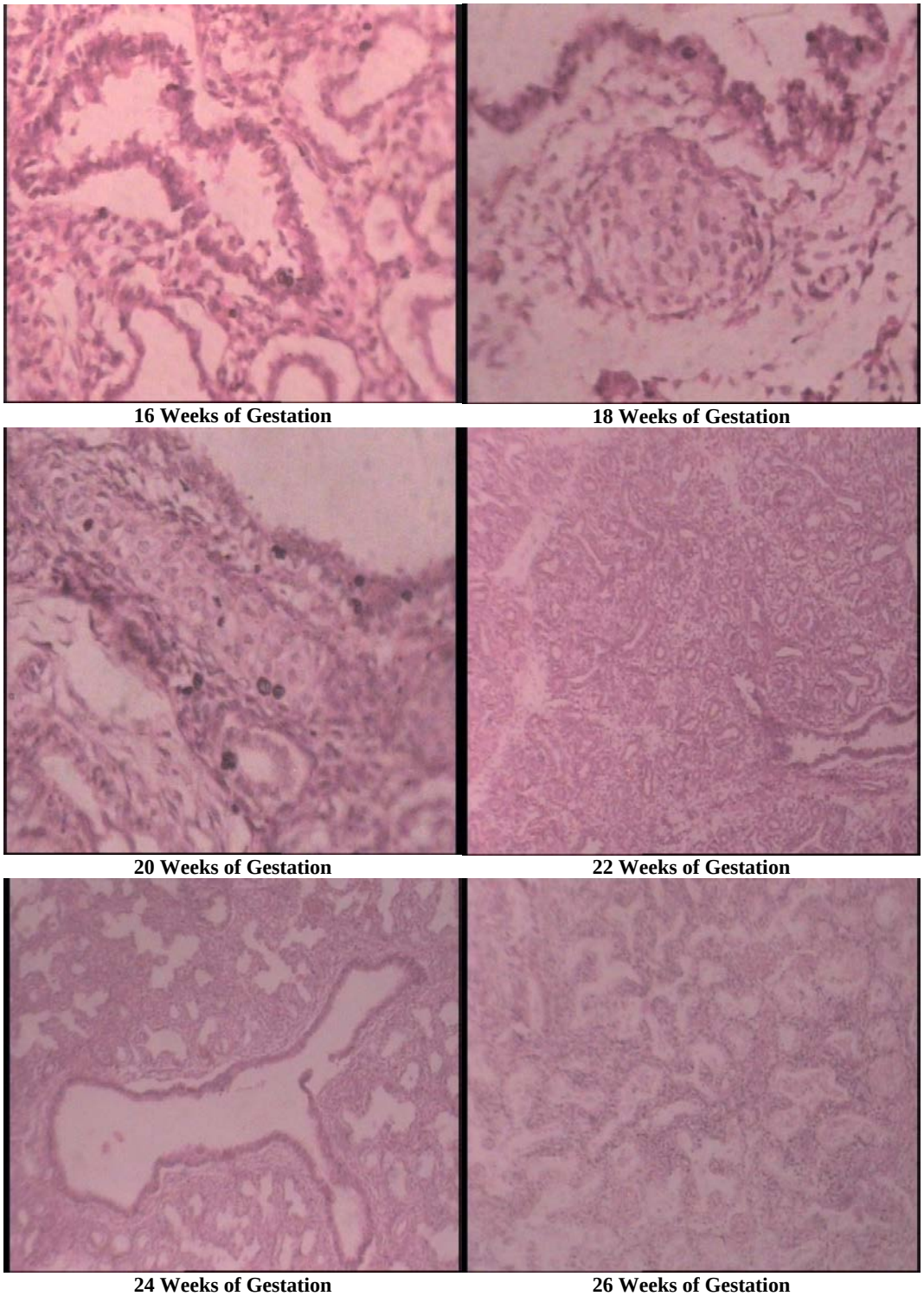


Fig 1

8 Weeks Fetus: Mesenchymal tissue are more. Developing Duct lined by Cubical Epithelium, Acini and Blood vessels are visible.

10 Weeks Fetus: Developing Bronchus lined with Pseudo stratified columnar ciliated epithelium surrounded by hyaline cartilage. In High Power Bronchus surrounded by hyaline variety of cartilage which extend into lobar and segmented bronchus. But cartilage actually appear in 4th Week.

12 Weeks Fetus: Terminal bronchiole is developing with acini packed with cells are seen. In high power bronchus is thick walled and bronchiole is thin walled lined by epithelium. Multiple acini packed with cells are seen. Ducts were having tubular lumen.

14 Weeks Fetus: Well developed hyaline variety of cartilage surrounded by pieces of bronchus. Bronchiole was visible. Acini are packed with cells.

16 Weeks Fetus: Bronchus with cartilage on High power. Bronchioles and acini are visible. Mucosal glands are developed on bronchial wall. Bronchioles were having simple columnar non-ciliated epithelium infolding. Mucosal glands were developed on wall of bronchiole.

18 Weeks Fetus: Bronchiole and Bronchi are in between well-developed septae and blood vessels were seen to be developing.

20 Weeks Fetus: Lymphatic element normally developed. Bronchioles were having low columnar to cuboidal epithelium.

22 Weeks Fetus: Serous acini was increased.

24 Weeks Fetus: Respiratory bronchiole and alveoli were increased.

26 Weeks Fetus: Each terminal bronchi were dividing into respiratory bronchiole which were seen to be dividing into alveoli.

Discussion

Histogenesis involves a series of integrated processes that occur during embryonic development, where in undifferentiated cells assume the characteristics of various tissues contained in human body and attain functional maturity [6]. These undifferentiated cells comprise part of three primary germ layers- ectoderm, mesoderm, and endoderm. Process of histogenesis of lung is very significant as it determines independent survival of the fetus [7]. Although development of human lung has been widely studied and illustrated, most of the articles deal with few aspects of histogenesis[8]. This work has been taken up to comprehensively study the various aspects of histogenesis of lung in fetuses of Indian origin. This prenatal developmental morphology and histology of human lung is expected to be helpful for the management of premature infants.

Conclusion

This prenatal developmental morphology and histology of human lung is expected to be help full for the management of premature infants[9]. The knowledge will also helpful for the Lung Resection surgery. Bronchoscopy for Broncho-Alveolar Lavage can also be useful with knowledge of this study [10].

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