Development of the Rabbit's Respiratory System

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Abstract : **The human respiratory system begins to form about day 26-27 after fertilization. It is first indicated by a median laryngotracheal groove in the caudal and ventral wall of pharynx. The endoderm which lines the laryngotracheal groove gives rise to the epithelium and glands of the larynx, trachea, bronchi and the pulmonary lining epithelium. The connective tissue, the cartilage and the smooth muscle of these structures develop from the splanchnic mesoderm surrounding the forgut. In studying the development of the respiratory system at the Department of Anatomy, Siriraj Hospital, medical students should trace the serial section of 10 mm. pig embryos. In doing this, they will be able to observe the laryngotracheal groove at the floor of the pharynx and when tracing the sections caudally, they will be able to observe the trachea, esophagus and the bifurcation of the trachea forming primary bronchi as well as smaller branches of bronchi. The present study attempts to show the development of the respiratory system of rabbit embryos in order to find the most suitable stage for use as a laboratory model for medical students' embryological studies. A 12 mm. rabbit embryo can be used instead of a pig embryo for studying the development of the respiratory system, as nowadays pig embryos are not available.**

เรื่องย่อ : การเจริญพัฒนาการของระบบทางเดินหายใจของกระต่าย

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ระบบทางเดินหายใจของมนุษย์เริ่มเจริญขึ้นตั้งแต่อายุ 26-27 วันหลังการปฏิสนธิ โดยพบเป็นรอยหว่ำ ลาริงโกเทรเมียล ที่บริเวณพื้นของส่วนปลายของฟาริงซ์ รอยหว่ำนี้เจริญยืดยาวลงมาหน้าต่อหลอดอาหารและปลายแตกแขนงออกไป 2 ข้าง ดังนั้น เอ็นโดเดิมของรอยหว่ำ ลาริงโกเทรเมียล จะเจริญไปเป็นเยื่อบุผิว และต่อมของกล่องเสียง หลอดลมส่วนต้นถึงส่วนปลาย ส่วนเนื้อเยื่อเกี่ยวพัน กระดูกอ่อนและกล้ามเนื้อเรียบ เจริญมาจากมีโซเดิม ที่ล้อมรอบทางเดินอาหารส่วนต้น ในการศึกษาการเจริญพัฒนาการของระบบทางเดินหายใจ ที่ภาควิชากายวิภาคศาสตร์ คณะแพทยศาสตร์ศิริราชพยาบาล ใช้ตัวอ่อนหมูขนาด 10 มม. ตัดเรียงลำดับให้นักศึกษาแพทย์ได้ดูรอยหว่ำ ลาริงโกเทรเมียล ได้ลงมาจนถึงระดับที่มีการเจริญของเนื้อเยื่อปอด แต่เนื่องจากปัจจุบัน ภาควิชาประสบปัญหาในการหาตัวอ่อนหมูมาทำสไลด์ ให้นักศึกษาใช้เรียนการเจริญพัฒนาการของอวัยวะต่างๆ จึงจำเป็นต้องหาตัวอ่อนของสัตว์เลี้ยงลูกด้วยนมชนิดอื่นที่หาง่ายมาใช้แทน จากการศึกษานี้พบว่าการเจริญของระบบทางเดินหายใจของกระต่ายขนาด 12 มม. คล้ายคลึงกับของตัวอ่อนหมูขนาด 10 มม. จึงอาจใช้แทนกันได้ในอนาคต

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INTRODUCTION

The lower respiratory system begins to form during the fourth week of development. It is first indicated by a median laryngotracheal groove in the caudal end of the ventral wall of the primitive pharynx. As this groove deepens to form diverticulum, it becomes invested with the splanchnic mesenchyme. The endoderm, which lines the laryngotracheal diverticulum, gives rise to the epithelium and glands of the larynx, trachea, bronchi and pulmonary lining epithelium. The connective tissue, cartilage and smooth muscle of these structures develop from the splanchnic mesenchyme surrounding the foregut.

The laryngotracheal diverticulum soon becomes separated from the primitive pharynx by longitudinal tracheoesophageal folds. These folds fuse to form a tracheoesophageal septum which divides the foregut into a ventral portion, the laryngotracheal tube and a dorsal portion, the esophagus. The opening of the laryngotracheal tube into the pharynx becomes the inlet of the larynx or laryngeal aditus.

The bulb-shaped lung bud at the caudal end of the laryngotracheal tube soon divides into two knob-like bronchial buds which grow laterally into the pericardioperitoneal canals, the primordia of the pleural cavities. Together with the splanchnic mesenchyme, the bronchial buds differentiate into the bronchi and their ramifications in the lungs.

Early in the fifth week, each bronchial bud enlarges to form the primordium of the primary bronchus. The embryonic right bronchus is slightly larger than the left one and is oriented more or less vertically; this embryonic relationship persists after birth. The primary bronchi subdivide into secondary bronchi. Each secondary bronchus subsequently undergoes progressive branching to form the tertiary or segmented bronchi, ten in the right lung and eight or nine in the left lung. Each tertiary bronchus with its surrounding mesenchyme is the primordium of a bronchopulmonary segment. By 24 weeks, about 17 orders of branches have developed. An additional seven orders of airways develop after birth.

As the bronchi develop, cartilagenous plates develop from the surrounding splanchnic mesenchyme. The bronchial smooth muscle, connective tissue and capillaries are also derived from this mesenchyme.

The terminal sacs, which develop about 26 weeks after conception and were initially lined with cuboidal epithelium, begin to attenuate into squamous epithelium. By this time, the capillary networks have proliferated close to the alveolar epithelium. The lungs are usually sufficiently well developed to permit survival of the fetus if they are capable to function immediately at birth. To be capable of respiration, the lungs must acquire an alveolocapillary membrane that is sufficiently thin and have an adequate amount of surfactant[1-3].

In understanding how the lungs form, the Embryology Division, Department of Anatomy, Siriraj Hospital prepares serial section slides of a 10 mm. pig embryo for use as the laboratory models. Students should trace the slides cephalocaudally from the pharyngeal level down. They will become familiar with the laryngotracheal groove, the separation of trachea and esopahgus by the tracheoesophageal septum, then the bifurcation of the right and left lung buds. The pig embryo is a very good model for laboratory study and enhances understanding of organ formation. It is regretable that pig embryos are not easily available nowadays for making new slides. Thus, the study of embryo development in other mammals in order to find a substitute for the use of pig embryos may be useful for the future. This study was therefore done to illustrate the development of the lower respiratory system in the rabbit embryo. If rabbits and pigs develop in a similar manner, the rabbit embryo can also be use as a laboratory model for medical education of Embryology.

MATERIALS AND METHODS

The maternal rabbits (*Oryctolagus cuniculus*) with their embryos were obtained from the Department of Animal Laboratory, AFRIMS. They were bred and later fed until 12 and 15 days following conception. At these respective stages, the maternal rabbit was injected with an overdose of an anesthetic drug. A low midline incision was done. The uterus with its proper stage of embryos was dissected from the abdominal cavity. Each embryonic mass was separated from the other and placed in Bouin's solution for fixation for at least 24 hours. The process of removing the excess fixative was done by placing it in 70% ethyl alcohol. The solution was

changed daily until the fixative was entirely removed, which was determined by observing the color of specimens which gradually changed from yellow to white. The embryos were dissected from the uteri and placed in 70% ethyl alcohol. They were then dehydrated, cleared, embedded and serially sectioned. The mounted sections were stained with hematoxylin. The laryngotracheal groove was observed and traced downward under a light microscope. The lung development of the 12 mm. rabbit embryo was compared with that of a 10 mm. pig embryo.

RESULT

1. The 12 mm. rabbit embryo

At the caudal part of the pharyngeal region, the pharyngeal cavity converges so that it becomes smaller than the upper part, broadens side to side and narrows anteroposteriorly. The pharynx situates ventrally to the notochord and is surrounded by the splanchnic mesoderm. A groove forms in the floor of the pharynx and is called the laryngotracheal groove. The groove lengthens caudally, while the dense splanchnic mesenchyme surrounding the pharynx and the groove form a fold, known as the tracheoesophageal fold. The mesenchyme surrounding the pharynx appears to be densely arranged in a circular area. Lateral to this area, the sixth aortic arches branch from the pulmonary trunk to join the paired dorsal aorta. The left one joins the left dorsal aorta, which becomes the ductus arteriosus. In a more lateral direction to both sides are the common cardinal veins. Ventral to the pharynx and the laryngotracheal groove is the heart in the pericardial cavity.

Tracing the sections caudally, the pharynx is narrower and the tracheoesophageal folds of both sides fuse to form the mesenchymal septum, the tracheoesophageal septum. The septum separates the esophagus dorsally from the tubular trachea ventrally. Both esophagus and trachea are longitudinal tubes which are lined by rather columnar epithelium. The splanchnic mesenchyme surrounding both tubes is rather dense. The vagus nerve, which should be situated between both tubes is not clearly distinct. Dorsolaterally to the esopahgus is the dorsal aorta and more laterally is the large common cardinal vein. The heart occupies the pericardial cavity and the pleural cavity is not formed at this level.

Tracing the sections more caudally, the trachea bifurcates to form the right and left main bronchi. The right one soon develops more branching to form the secondary bronchi. The esophagus situates between both bronchi and attaches to the dorsal body wall by the mesoesophagus. The developing lung situates in the pleural cavity which still joins with the peritoneal cavity by the pleuroperitoneal communication. Ventral to the forming lung is the liver in the peritoneal cavity.

2. The 10 mm. pig embryo

At the level of the caudal part of the pharynx, the floor of the pharyngeal cavity shows a diverticulum, the laryngotracheal groove. The mesenchyme surrounding the pharynx and the groove is condensed, forming the mesenchymal folds which soon fuse to form the septum, the tracheoesophageal septum, separating the gut from the air tube.

Tracing the sections caudally, the trachea bifurcates to form the right and left main bronchi. The esophagus situates dorsally between both bronchi. The epithelial lining of the bronchi is composed of columnar shaped cells surrounded by splanchnic mesenchyme which attache to the dorsal body wall by the mesoesophagus and ventrally fuse with the septum transversum. The main bronchi, or lung buds, enlarge laterally into the pleural cavity, which is incompletely separated from the peritoneal cavity by the pleuroperitoneal canal. The septum transversum which is a dense mass of mesenchyme forming the major component of the thoracoabdominal diaphragm locates ventrally to the pleural cavity. The pericardial cavity with the developing heart locates more ventrally to the pleural cavity

DISCUSSION

The development of the lower respiratory system of the pig and the rabbit are very similar. A groove, called the laryngotracheal groove, originates at the floor of the caudal end of the pharynx. This groove deepens caudoventrally. The mesenchyme condenses to form bilateral longitudinal folds which soon fuse to form the tracheoesophageal septum. This septum separates the esophagus dorsally and the trachea ventrally. The trachea is lengthened caudally and its end bifurcates to form the right and left main bronchi, or the lung buds. As development proceeds,

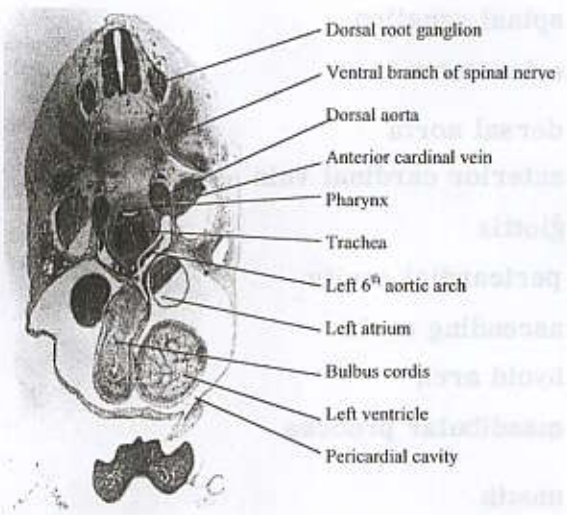

Figure 1. The 12 mm. rabbit embryo at the level of laryngo-tracheal groove.

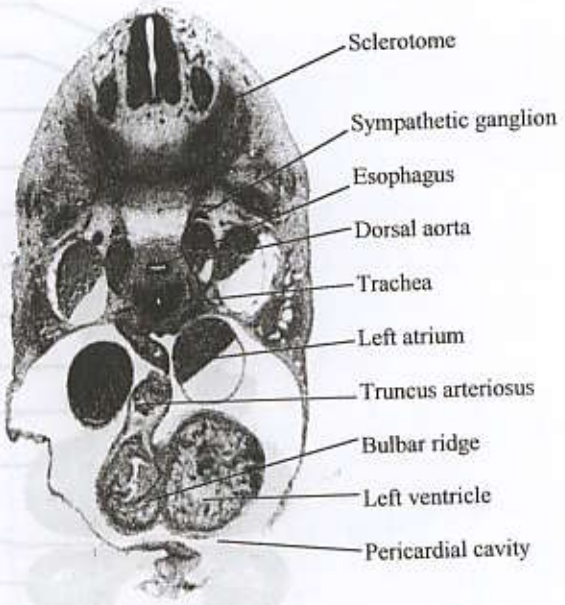

Figure 2. The 12 mm. rabbit embryo at the level of esophagus and trachea.

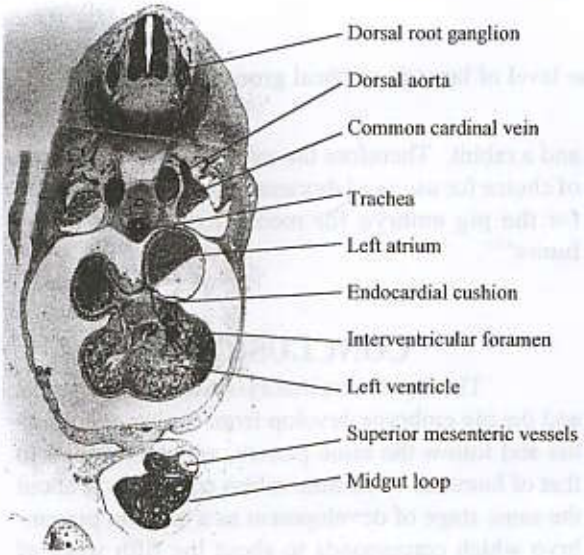

Figure 3. The 12 mm. rabbit embryo at the level of trachea.

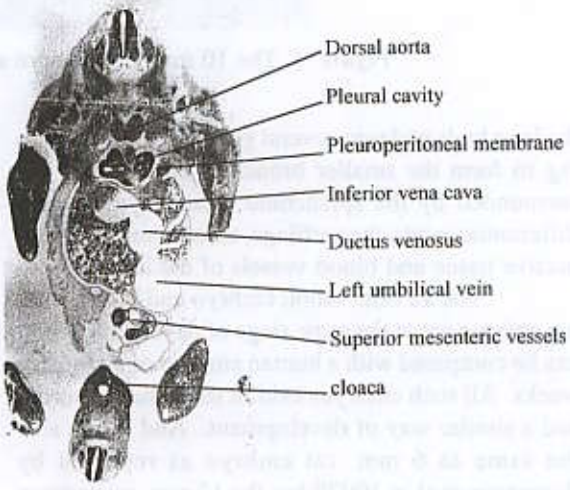

Figure 4. The 12 mm. rabbit embryo at the level of lung bud.

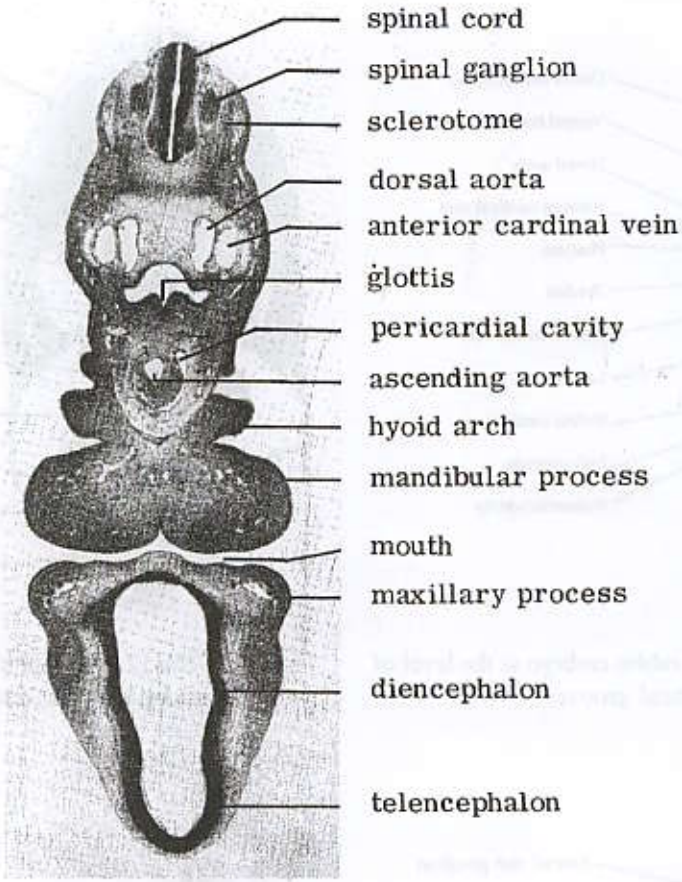

Figure 5. The 10 mm. pig embryo at the level of laryngo-tracheal groove.

the lung buds undergo several generations of branching to form the smaller bronchi. The bronchi are surrounded by the splanchnic mesenchyme which differentiates into the cartilage, smooth muscle, connective tissue and blood vessels of the lung.

The 12 mm. rabbit embryo and the 10 mm. pig embryo are at the same stage of development and can be compared with a human embryo of about five weeks. All such embryos exhibit the same primordia and a similar way of development. And this is also the same as 6 mm. rat embryo as reported by Rojananin et al in 1992[10] but the 12 mm. rat embryo exhibited advanced development of the lungs beyond the embryological stage.

This study shows the similarity of the development of the lower respiratory system of a pig and a rabbit. Therefore the rabbit can be the animal of choice for use as a laboratory model as a substitute for the pig embryo for medical education in the future[4-11].

CONCLUSION

The lower respiratory system of the rabbit and the pig embryos develop from the same primordia and follow the same pattern, which is similar to that of humans. A 12 mm. rabbit embryo is at about the same stage of development as a 10 mm. pig embryo which corresponds to about the fifth week of human embryo development. Therefore rabbits can also be laboratory models in the future for studying respiratory development.

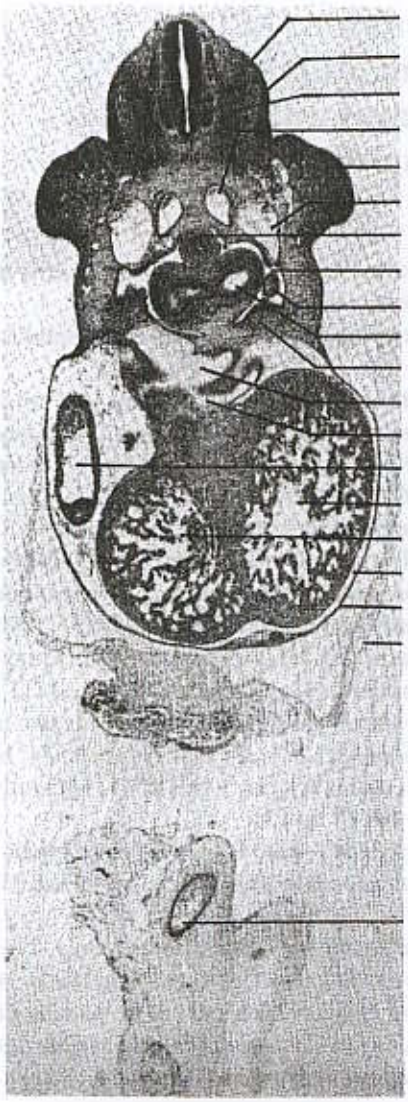

Figure 6. The 10 mm. pig embryo at the level of lung bud.

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