The Development of Rabbit Embryo's Heart : Serial Sections of 4-14 mm Rabbit Embryos Compared to a 10 mm Pig Embryo

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Abstract : **Serial sections of 4-14 mm rabbit embryos were carefully studied in relation to the serial sections of 10 mm pig embryos under a light microscope in order to compare the development of the heart and related blood vessels. The heart of the 4-14 mm rabbit embryo has incompletely divided chambers comprising two atria and two ventricles. The partition of the atrium by the septum primum can be seen at the 4 mm stage and still incompletely attaches to the endocardial cushion even at the 12 mm stage. The rupture of the septum primum brings about the communication between the two atria, the foramen secundum, can be seen only at the 12 mm stage. Communication between the two ventricles is also via the interventricular foramen. The right atrium receives venous blood from the right horn of the sinus venosus which is larger than the left side. The bulbus cordis communicates with the right ventricle and brings blood to the aortic sac which extends branches to the branchial arches to join the dorsal aorta. The heart of the rabbit embryo is very similar to that of the pig embryo. The pig embryo is nowadays no longer available for medical student to use in laboratory education. Since rabbit embryos are easy to obtain for slide preparation, there will be no problem in using rabbit embryos instead of pig embryos for the study of the development of the heart.**

Key words **:** Rabbit embryo's heart

เรื่องย่อ : การเจริญพัฒนาการของหัวใจกระต่ายเปรียบเทียบกับหมู

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การเจริญและพัฒนาการของหัวใจของตัวอ่อนกระต่ายขนาด ๔-๑๔ มม. ไม่แตกต่างจากหัวใจของตัวอ่อนหมูขนาด ๑๐ มม. กล่าวคือในระยะดังกล่าวหัวใจประกอบด้วยช่องหัวใจ ๔ ช่อง คือ เอเตรียม ๒ ช่อง และเวนตริเคิล ๒ ช่อง การแบ่งช่องเอเตรียมเริ่มเห็นได้ตั้งแต่ตัวอ่อนกระต่ายมีขนาด ๔ มม. โดยเสปตัมปริมัมเจริญลงมาสู่เอ็นโดรคาร์เดียลคูชัน และยังไม่มีการเชื่อมกันแม้ในตัวอ่อนขนาด ๑๒ มม. รูเล็คคันดัมเริ่มเกิดขึ้นในระยะนี้ เวนตริเคิลยังคงมีรูติดต่อถึงกันอยู่ ไซนัสวีโนสัสด้านขวามีขนาดใหญ่กว่าด้านซ้าย และเปิดสู่เอเตรียมเฉพาะช่องด้านขวา บัลบัสคอร์ดิสติดต่อมาจากเวนตริเคิลช่องขวา นำเลือดต่อไปยังเออออร์ติคแสค ซึ่งให้แขนงสู่แบรงเคียลอาร์ชแต่ละคู่ ในการเรียนภาคปฏิบัติวิชาเอ็มบริย์โอของนักศึกษาแพทย์ศิริราช ขณะนี้ใช้ตัวอ่อนหมูในการศึกษา พัฒนาการของหัวใจเทียบกับมนุษย์ แต่เนื่องจากขณะนี้ไม่สามารถหาตัวอ่อนหมูมาทำสไลด์ให้นักศึกษาแพทย์ใช้ในห้องปฏิบัติการเอ็มบริย์โอวิทยาได้อีกแล้ว และจากการศึกษานี้พบว่าหัวใจของตัวอ่อนกระต่ายมีพัฒนาการคล้ายหัวใจตัวอ่อนหมูมาก ในอนาคตอาจใช้ตัวอ่อนกระต่ายมาทำสไลด์แทนตัวอ่อนหมูได้ เพื่อให้การเรียนการสอนเอ็มบริย์โอวิทยาทำได้อย่างมีประสิทธิภาพต่อไป

INTRODUCTION

The cardiovascular system is composed of the heart, blood vessels and blood cells; and originates from the mesodermal germ layer. At the beginning of the third week following conception, the two heart tubes fuse to form a single, slightly bent heart tube. The heart tube comprises an inner endocardial tube and a surrounding epimyocardium mantle. During the fourth to the seventh week the heart becomes divided into a typical four-chambered structure.

The primitive atrium is divided into two by the septum primum, a sickle-shaped membrane which descends from the roof of the atrium towards the endocardial cushion. Later the septum secundum is formed at the right side of the septum primum. Only at birth, when the pressure in the left atrium increases, are the two septa pressed against each other and the communication between the two atria is closed. Abnormalities in the atrial septum may range from the total absence of the septum to a small opening known as the oval foramen.

The fused endocardial cushion divides the atrioventricular canal into the right tricuspid and the left bicuspid, or mitral canals. Persistence of the common atrioventricular canal or abnormal division such as atresia of the tricuspid canal are well-known defects.

The interventricular septum consists of a thick muscular part and a thin membranous part. The membranous portion is formed by the endocardial cushion and both bulbar ridges. In many cases, these three components fail to fuse, resulting in an open interventricular foramen. Although this abnormality may be isolated, it is frequently combined with other compensatory defects.

The truncus arteriosus is divided into two blood channels by the spiral-shaped aorticopulmonary septum or earlier bulbar ridges. The bulbar ridges divide the outflow tracts of the aortic and the pulmonary channels and close the interventricular foramen. Many vascular abnormalities such as transposition of the great vessels and the pulmonary valvular atresia result from abnormal division of the bulbotracal region[1-3].

Understanding the normal development of the heart brings about a better understanding of several congenital abnormalities. The Embryology Division, Department of Anatomy, Siriraj Hospital, has tried its best to help medical students recognize this. A three-hour lecture and two laboratory periods are devoted to the development of the heart and its blood vessels. The heart of the pig embryo is very similar to that of a human being, and therefore is an excellent laboratory model. In the past pig embryos were easier to obtain and were commercially available, but currently they are no longer available. Therefore it is important to study the normal development of other mammal embryos in order to find a suitable replacement for the pig embryo in the near

future. Rats and mice are well-known laboratory animals, and their embryos have been studied to determine their normal development and specifically the development of their hearts[4-11]. From these studies, it has been concluded that rats and mice can be used as another comparative model for human heart development. In this study, we examine the embryos of another laboratory animal, the rabbit.

MATERIALS AND METHODS

The maternal rabbits (*Oryctolagus cuniculus*) with their embryos were obtained from the Department of Animal Laboratory, AFRIMS. They were bred and then fed until their embryos reached 12 and 15 days old following conceptions. 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 cardiac region was observed under a light microscope. The hearts of the 4-14 mm rabbit embryos were compared to that of a 10 mm pig embryo.

RESULTS

1. The 4-5 mm rabbit embryo (Figure 1,2,3)

The heart occupies the pericardial cavity, comprises four chambers, two atria and two ventricles. The atrium is situated dorsally to the ventricle and the wall is thinner. The atrium and ventricle are separated externally by the deep sulcus, the coronary sulcus, and internally they are separated incompletely by a mass of endocardial tissue, the endocardial cushion. There are two openings between the atrium and ventricle, the right and left atrioventricular foramens. The partition of the atrium begins to form as a sickle-shaped sagittal fold extending from the dorsocranial wall towards the endocardial cushion, the septum primum. This septum does not reach the endocardial cushion at this stage but leaves an opening named the foramen primum. The right horn of the sinus venosus opens into the right atrium through the sinu-atrial orifice guarded by the right and left valves of the sinus venosus. The left horn of the sinus venosus drains blood to the transverse portion which is situated slightly caudal to the right sinu-atrial orifice. There is no evidence of other venous blood opening into the left atrium. The wall of the ventricle is thick and spongy, forming a meshwork of muscular trabeculae, separated by small sinusoidal spaces. The ventricle is separated into the right and left sides externally by the ventricular sulcus and internally by a muscular fold, the muscular interventricular septum. This septum grows dorsalward towards the endocardial cushion, leaving an opening between the right and left ventricles, the interventricular foramen. This foramen will later be closed by the septum membranaceum which has not developed by this time. The right ventricle continues to the bulbus cordis. The bulbus cordis wall is not spongy but shows thickening of subendocardial tissue to form the right and left bulbar ridges. The bulbar ridges continue to the ridges of the truncus arteriosus, the truncal ridges. The bulbar and truncal ridges will later form the spiral fusion and separate the lumen into the ascending aorta and the pulmonary trunk. The truncus arteriosus drains the blood to the aortic sac which connects to the aortic arches passing through the mesenchyme of the corresponding pharyngeal arches.

2. The 12-14 mm rabbit embryo (Figure 4,5)

By the time the rabbit reaches 12-14 mm, the heart has a little more advance development. The heart is composed of two atria and two ventricles. The atrial wall is thin while the ventricular wall is thick and spongy due to proliferative myocardium. The atrium and ventricle are separated externally by the deep coronary sulcus and internally by the fused endocardial cushion. The endocardial cushion incompletely divides the atrioventricular canal

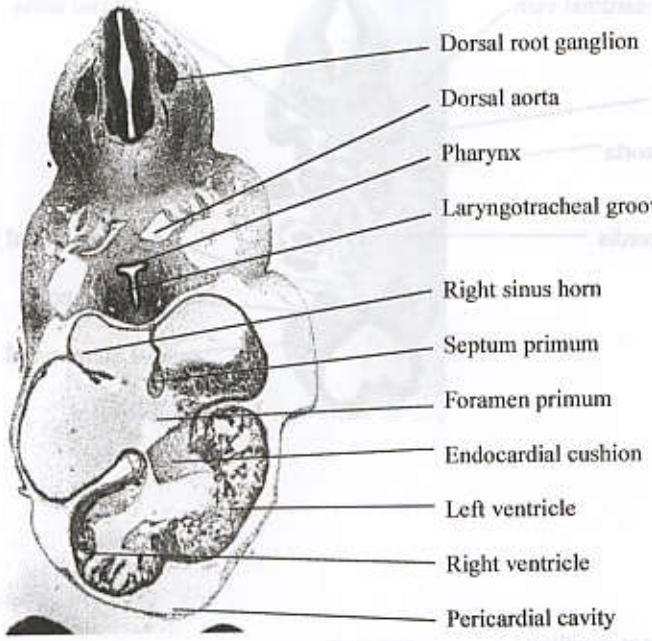

Figure 1. Transverse section of 4-5 mm rabbit embryo through the interatrial septum.

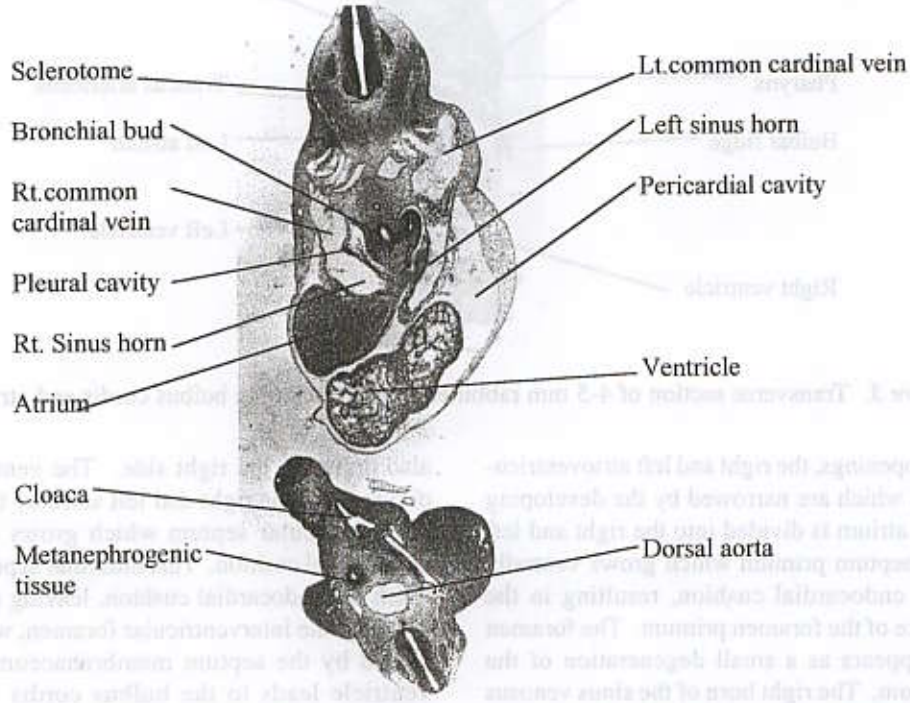

Figure 2. Transverse section of 4-5 mm rabbit embryo through cloaca.

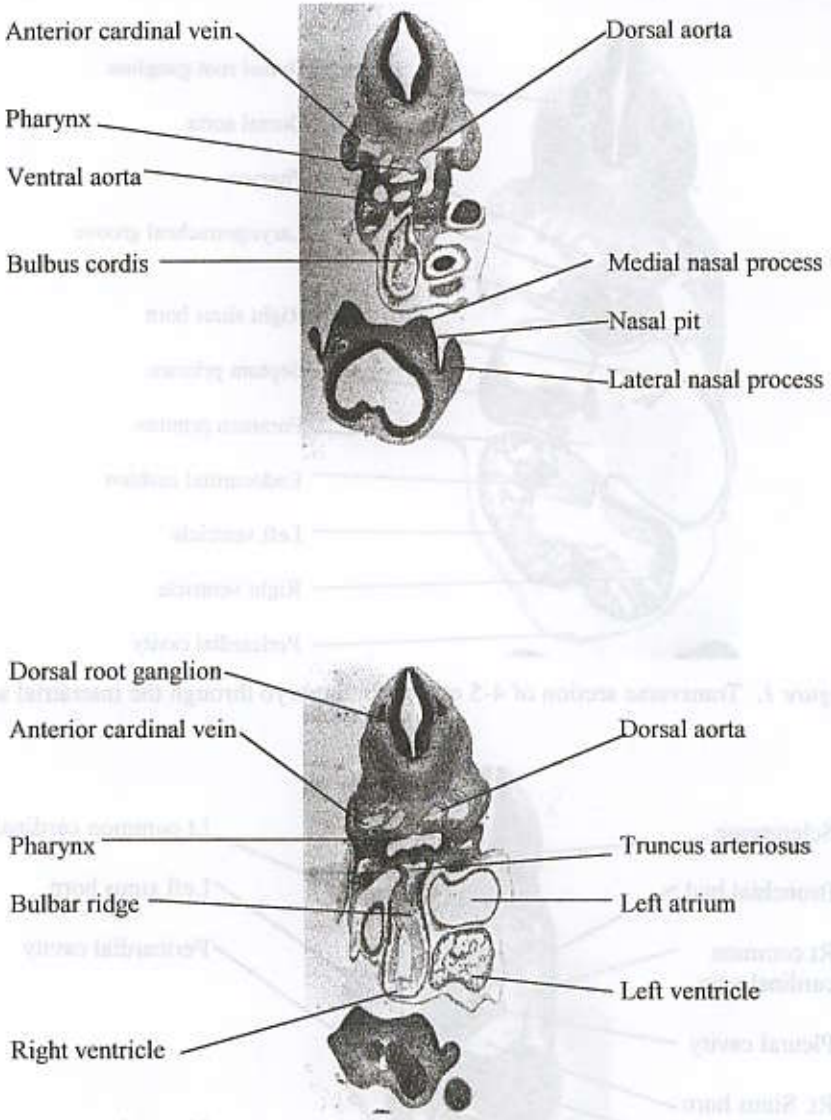

Figure 3. Transverse section of 4-5 mm rabbit embryo through the bulbus cordis and atrium.

leaving two openings, the right and left atrioventricular foramens which are narrowed by the developing valves. The atrium is divided into the right and left atria by the septum primum which grows ventrally towards the endocardial cushion, resulting in the disappearance of the foramen primum. The foramen secundum appears as a small degeneration of the septum primum. The right horn of the sinus venosus opens into the right atrium while the left horn opens into the transverse portion of sinus venosus which also drains to the right side. The ventricle is also divided into the right and left sides by the muscular interventricular septum which grows towards the endocardial cushion. This muscular septum does not reach the endocardial cushion, leaving a very small opening, the interventricular foramen, which is later closed by the septum membranaceum. The right ventricle leads to the bulbus cordis whose wall thickens on the right and left sides to form the right and left bulbar ridges. The bulbar ridges progress to

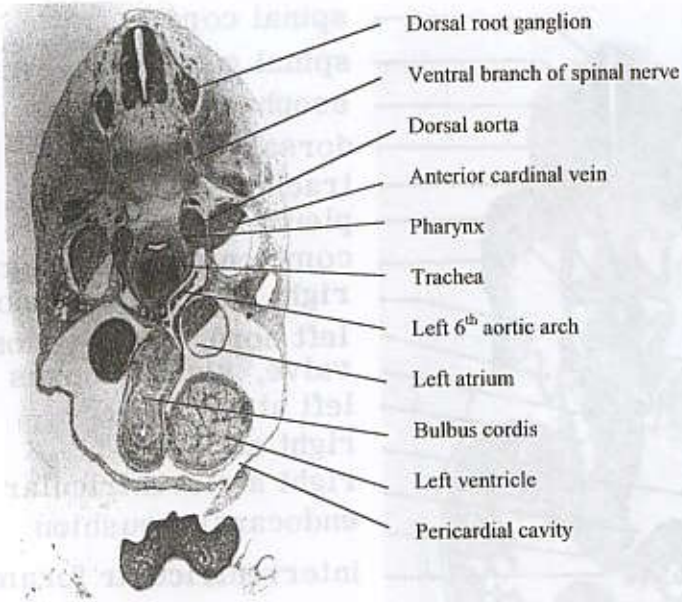

Figure 4. Transverse section of 12-14 mm rabbit embryo through 6th aortic arch.

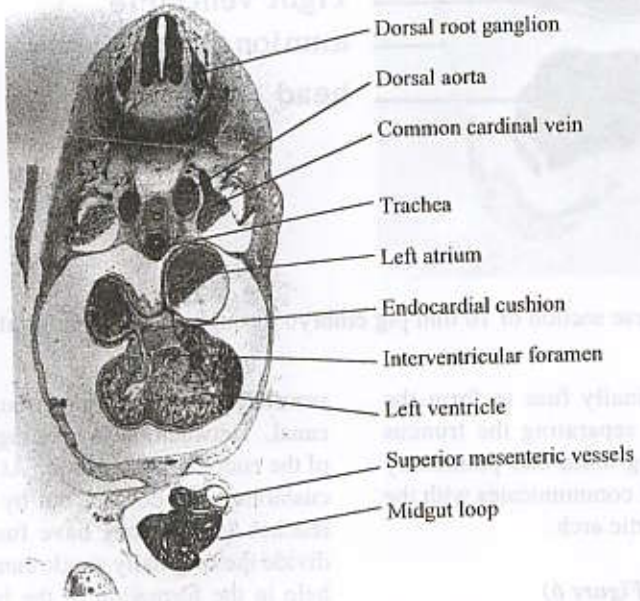

Figure 5. Transverse section of 12-14 mm rabbit embryo through endocardial cushion.

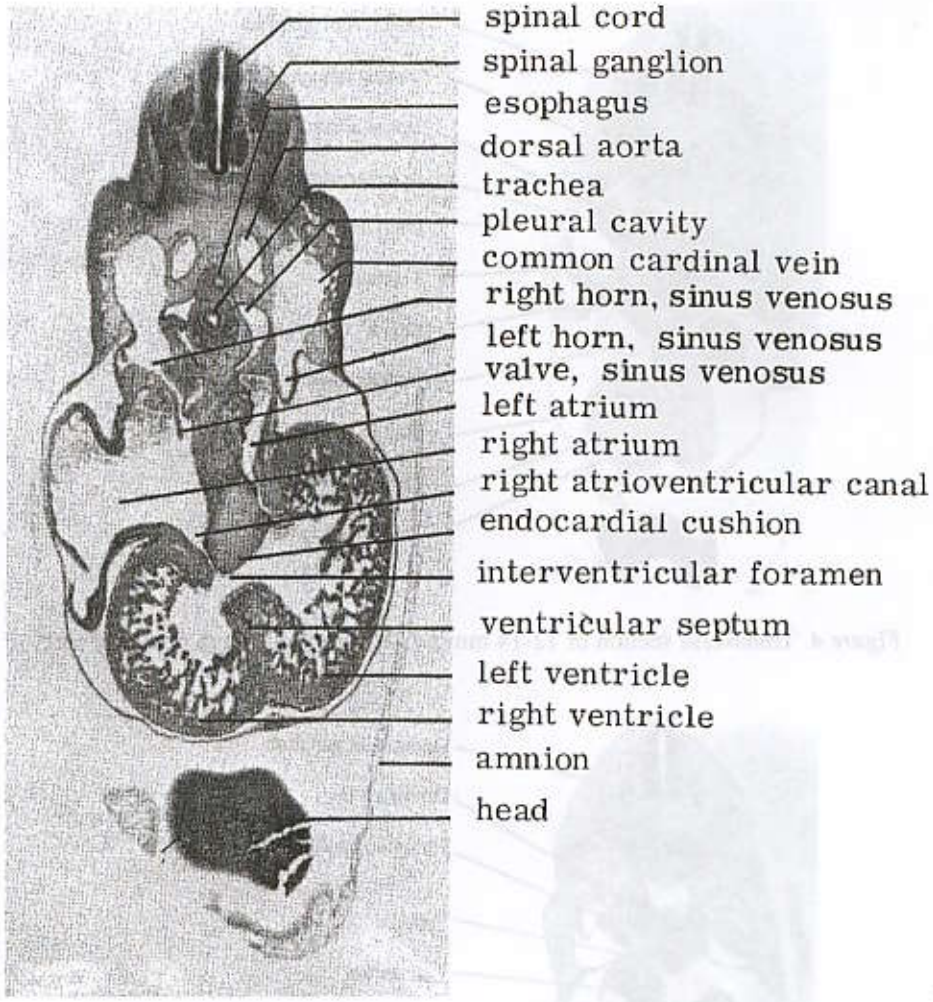

Figure 6. Transverse section of 10 mm pig embryo through the interventricular foramen.

the truncal ridges which finally fuse to form the aorticopulmonary septum, separating the truncus arteriosus into the ascending aorta and pulmonary trunk. The pulmonary trunk communicates with the dorsal aorta via the sixth aortic arch.

3. The 10 mm pig embryo (Figure 6)

All four heart chambers are shown. The atria are partially separated by the septum primum, which is incomplete because of the rupture of the septum primum, the foramen secundum. It is not possible to satisfactorily see the septum secundum in embryos of this size. Each atrium communicates with the ventricle of the same side through the atrioventricular canal. Between these openings is the fused portion of the endocardial cushion. At an earlier stage, these cushions were double, but by the time the embryo reaches 10 mm they have fused midway and thus divide the originally single canal into two. They also help in the formation of the bicuspid and tricuspid valves. Between the two ventricles is the interventricular septum, which grows towards the endocardial cushion leaving a gap, the interventricu-lar foramen. The ventricular wall is thick and spongy, forming a network of muscular trabeculae. Dorsal to the atria are the common cardinal veins. The right vein

empties into the sinus venosus, the left crosses the midplane and connects the sinus at a lower level. The sinus venosus drains into the right atrium through a slit-like opening in the dorsal atrial wall, guarded by the right and left valves of the sinus venosus. The ascending aorta and pulmonary trunks communicate with the truncus arteriosus, while the latter shows the thickened internal ridge on each side. The sixth aortic arches connect the dorsal aortae with the main pulmonary trunk.

DISCUSSION

The heart of a 4-14 mm (crown-rump length) rabbit embryo and the heart of a 10 mm pig embryo show no significant differences, although the 4-14 mm rabbit embryos' hearts show a somewhat lower level of development and the ventricular walls are slightly thinner. This organ lies within the pericardial cavity and comprises four chambers, two atria and two ventricles. In addition, a small chamber, the sinus venosus, receives all the blood returned to the heart and empties into the right atrium while the bulbus cordis still serves as a common arterial outlet. The entrance from the sinus venosus into the right atrium is a nearly sagittal slit, guarded by the right and left valves of the sinus venosus. The dorsal wall of the left atrium shows no evidence of pulmonary blood return at this stage of embryonic development. The partition between the two atria appears only formed by the septum primum while the septum secundum has not yet developed. The endocardial cushions fuse midway and subdivide the atrio-ventricular canal into two. The two ventricles communicate by the interventricular foramen. The bulbus cordis separates distally into an ascending aorta and pulmonary trunk. Because of these highly similar features in heart development of pig and rabbit embryos, the rabbit embryo can be another good model for use in the laboratory studies of medical students.

CONCLUSION

Serial sections of 4-14 mm rabbit embryos are highly similar to those of the 10 mm pig embryos in the region of heart development. There are two atria and two ventricles. The atrium is partitioned by the septum primum, and the ventricle is by the muscular interventricular septum. The right horn of the sinus venosus drains blood into the right atrium while the left horn drains into the transverse portion of sinus venosus. The atrioventricular canal is divided into two by the fused endocardial cushion. The bulbus cordis continues to the truncus arteriosus which is later divided by the truncal ridges to become the ascending aorta and the pulmonary trunk. From this study we conclude that rabbit embryo is an appropriate substitute for pig embryo in the medical students' embryology laboratory studies of heart development.

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