The Development of Rabbit's Urinary System Serial Sections Study of 12-14 mm Rabbit Embryos and a 10 mm Pig Embryo

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Abstract : **The urogenital system develops from the intermediate mesoderm, the coelomic epithelium and the endoderm of the urogenital sinus. The urinary system of mammal is characterized by three sets of kidneys, i.e. the nonfunctional pronephros, the mesonephros and the functional metanephros. The metanephros or the permanent kidney develops from the ureteric bud and metanephrogenic tissue. At the beginning the kidney is located in the pelvic region but later gradually ascends to the abdomen. The urinary bladder develops from the urogenital sinus and the surrounding splanchnic mesoderm. In studying the development of human urinary system in the Department of Anatomy, Siriraj Hospital, we have employed serial sections of 10-15 mm pig embryos as laboratory models. This method of laboratory study is helpful in understanding and recognizing how the kidney forms. It is quite regrettable that nowadays the pig embryos are no longer available. Thus, it is necessary to find another suitable laboratory model and study the details of its normal kidney development. In this study, we demonstrated that 12-14 mm rabbit embryos can be used instead of pig embryos as the development of the metanephros is very similar. Although the rabbit's mesonephros is smaller than that of the pig, it resembles the human mesonephros more closely. For this reason, the rabbit embryo is suitable for use as a laboratory model for embryology education of urinary system.**

Key words **:** Rabbit embryo's urinary system.

เรื่องย่อ : **การเจริญพัฒนาการของระบบขับถ่ายปัสสาวะของกระต่าย**

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ระบบขับถ่ายปัสสาวะของสัตว์เลี้ยงลูกด้วยนมส่วนใหญ่มีการเจริญของไต ๓ ชุด ไต ๒ ชุดแรกจะเสื่อมสลายไป และไตชุดที่ ๓ จะเจริญเป็นไตถาวร ไตถาวรเจริญมาจากติ่งที่ยื่นออกมาจากท่อไตชุดที่ ๒ รวมทั้งเนื้อเยื่อมีเสนไคม์รอบ ๆ ในระยะตัวอ่อนไตเจริญขึ้นในระดับเชิงกราน ต่อมาจึงค่อย ๆ เคลื่อนขึ้นไปอยู่ในช่องท้อง นักศึกษาแพทย์คณะแพทยศาสตร์ศิริราชพยาบาล ศึกษาการเจริญพัฒนาการของระบบขับถ่ายปัสสาวะ ที่ภาควิชากายวิภาคศาสตร์ โดยใช้ตัวอ่อนหมูขนาด ๑๐-๑๕ มม.ที่ตัดสีเรียล และให้นักศึกษาเลื่อนดูแต่ละเซ็คชั่น วิธีเรียนแบบนี้ช่วยให้เกิดความเข้าใจและเกิดจินตนภาพเป็นภาพ ๓ มิติ และทำให้จดจำได้ดีขึ้น แต่เป็นที่น่าเสียดายว่าปัจจุบันเราไม่สามารถหาหรือซื้อตัวอ่อนหมูมาทำสไลด์ได้เหมือนเมื่อก่อน จึงจำเป็นต้องศึกษาการเจริญของระบบนี้จากตัวอ่อนของสัตว์เลี้ยงลูกด้วยนมชนิดอื่นที่หาได้ง่ายกว่า เช่น กระต่าย จากการศึกษานี้พบว่าตัวอ่อนของกระต่ายขนาด ๑๒-๑๔ มม. สามารถใช้ศึกษาการพัฒนาการของระบบขับถ่ายปัสสาวะได้ ถึงแม้ขนาดของไตชุดที่ ๒ จะเล็กกว่าของตัวอ่อนหมู แต่ก็ไม่น่าแปลกถ้าจะเปรียบเทียบกับของมนุษย์ เพราะตัวอ่อนมนุษย์ก็มีขนาดของไตชุดที่ ๒ เล็กกว่าของตัวอ่อนหมูเช่นเดียวกัน

INTRODUCTION

Three different sets of urinary excretory organs develop in human embryos, the pronephros, the mesonephros and the metanephros. The third set becomes the permanent kidney and begins to produce urine when the fetus is 11-13 weeks old. The pronephros is a transitory, non-functional structure and develops in humans in the fourth week with a few cell clusters or a tubular-arranged structure in the cervical region. The pronephric duct runs caudally and opens into the cloaca. The pronephros degenerates but its duct is utilized by the next set of kidneys. The mesonephros is a large organ which appears later in the fourth week, caudally to the pronephros. The mesonephros functions as the interim kidney until the permanent kidney is established. The mesonephros consists of glomeruli and tubules not unlike those in the permanent kidney, but more simple than those found in the adult kidney. The metanephros or permanent kidney begins to develop early in the fifth week and starts to function about six weeks later. It develops from two sources, the ureteric bud and the metanephrogenic tissue. The metanephric mesoderm is in the caudal part of the nephrogenic cord, both primordia are mesodermal in origin.

The ureteric bud or metanephric diverticulum begins as a dorsal outgrowth from the mesonephric duct near its entry into the cloaca. It elongates and penetrates the metanephric mesoderm, inducing the formation of a metanephric mass or cap over its expanded end. The stalk of the ureteric bud becomes the ureter and its expanded cranial end forms the renal pelvis. Each collecting tubule undergoes repeated branching, forming successive generations of collecting tubules. The end of each arched collecting tubule induces a cluster of mesenchymal cells to form small metanephric vesicles, which become the metanephric tubules. The proximal ends are invaginated by the glomeruli. Thus, the kidney develops from two different sources, the ureteric bud which gives rise to the collecting system and the metanephric mesoderm which provides the excretory units.[1-3]

The study of the normal development of the urinary system can be achieved in a one-hour lecture and one laboratory period. The lab study is completed by tracing the serial sections of a pig embryo 10 mm in length. The sections reveal the large mesonephros with its duct coursing caudally to open into the urogenital sinus. A little more cephalic, the ureteric bud originates from the mesonephric duct and grows cephalically with a cap of metanephrogenic tissue. Serial sections of the pig embryo bring about a more detailed understanding of the system. Because nowadays pig embryos are not available, this study was done to observe the normal development of the rabbit's kidney and also to compare it with the development of the pig's and human's kidney. Since the rabbit's kidney develops in the same manner as those of pig and human, in the future it is possible to use rabbit embryos instead of pig embryos.

MATERIALS AND METHODS

Maternal rabbits (*Oryctolagus cuniculus*) with their embryos were obtained from the Department of Animal Laboratory, AFRIMS. They were bred and later fed until 15 days after conception. The maternal rabbit was injected with an overdose of an anesthetic drug. A low midline incision was done. The uterus with the embryos were 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 performed by placing the specimens in 70% ethyl alcohol. The solution was changed daily until the fixative was entirely removed. Removal of the fixative was determined by observing the color of specimens which gradually changed from yellow to white. The embryos were then dissected from the uterus, placed in 70% ethyl alcohol. They were dehydrated, cleared, embedded and serially sectioned. Then the mounted sections were stained with hematoxyllin. The mesonephric region was observed under a light microscope. The excretory systems of the 12-14 mm rabbit embryos were compared with those of the 10 mm pig embryos.

RESULTS

Vertebrates go through three distinct stages in the development of kidneys. The kidney of each stage is an improvement over the preceeding type. As might be anticipated, the embryos of the higher vertebrates indicate this progress by repeating the same kidney sequence during development. Nowhere can there be found a better illustration of the theory of recapitulation.

1. The urinary system of 12-14 mm rabbit embryo (Figure 1, 2, 3)

The 12-14 mm rabbit embryos exhibit only the second and the third sets of kidney, the mesonephros and the metanephros, respectively, while the pronephros is absolutely degenerated. The mesonephros is not prominent and occupies the caudal segment of the body. The mesonephros appears as a whole bulge in paired longitudinal ridges along each side of the mesentery. Along the ventral aspect of each mesonephros, there is a thickening of the peritoneal epithelium which gives the first definite indication of a future gonad. At this stage, the metanephros or the permanent kidney, is just making an appearance. Its ureteric bud is growing from the mesonephric duct near the cloaca. Its secretory tubules will differentiate from adjacent nephrotome tissue which is located just caudally to the lowest mesonephric tubules.

The mesonephros of the rabbit embryo consists essentially of a series of curved tubules, but the tubular structures are not numerous. The mesonephric tubules bear no significant relation to the body's segmentation. The tubules are canalization and appear as having an S-shaped bend. The medial ends of the tubules are associated with blood capillaries forming small glomeruli covered by the thin Bowman's capsules. The mesonephric duct is situated laterally to each mesonephros. Few small branches of the dorsal aorta can be traced to supply the mesonephros. The veins associated with the mesonephros are the posterior cardinal vein and the subcardinal vein, the former is dorsally located while the later is medial to the organ. The right subcardinal vein is larger than the left. The supracardinal vein is not conspicuous enough to be identified at this stage of development. Although the mesonephros of the rabbit embryo is relatively small, it may perform the excretory function as we can see the glomeruli.

The mesonephric duct curves just before joining the cloaca or urogenital sinus. The ureteric bud is an epithelial bud from the mesonephric duct and takes the form of a hollow bud which first grows dorsally and then turns cephalad. The proximal, rapidly elongating stalk of this diverticulum is the future ureter. The distal blind end dilates and pushes into a mass of condensed mesodermal tissue, the metanephrogenic blastema. Such are the early primordia that jointly give rise to the permanent kidney.

2. The urinary system of a 10 mm pig embryo (Figure 4, 5)

The mesonephros is large and complex, composed of large, abundant glomeruli, long and tortuous tubules and one mesonephric duct. The mesonephric duct can be traced along the ventrolateral margin of the mesonephros and opens into the

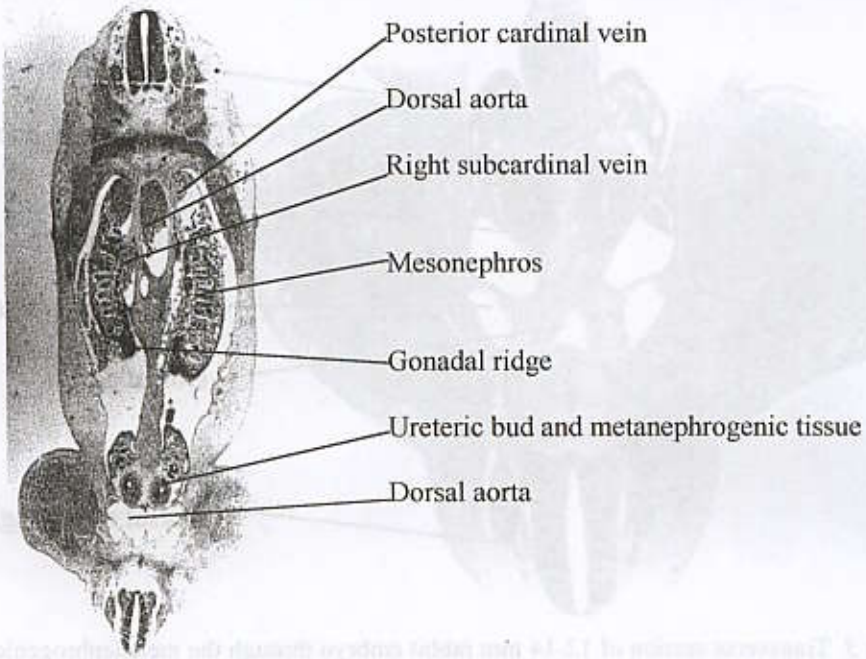


Figure 1. Transverse section of 12-14 mm rabbit embryo through the gonodal ridge.

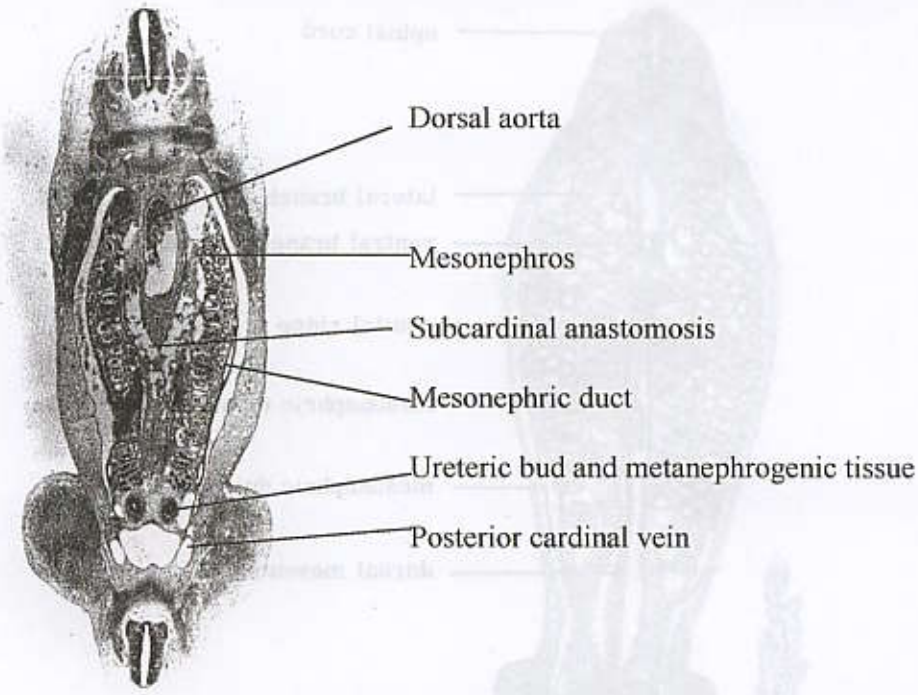


Figure 2. Transverse section of 12-14 mm rabbit embryo through the subcardinal anastomosis.

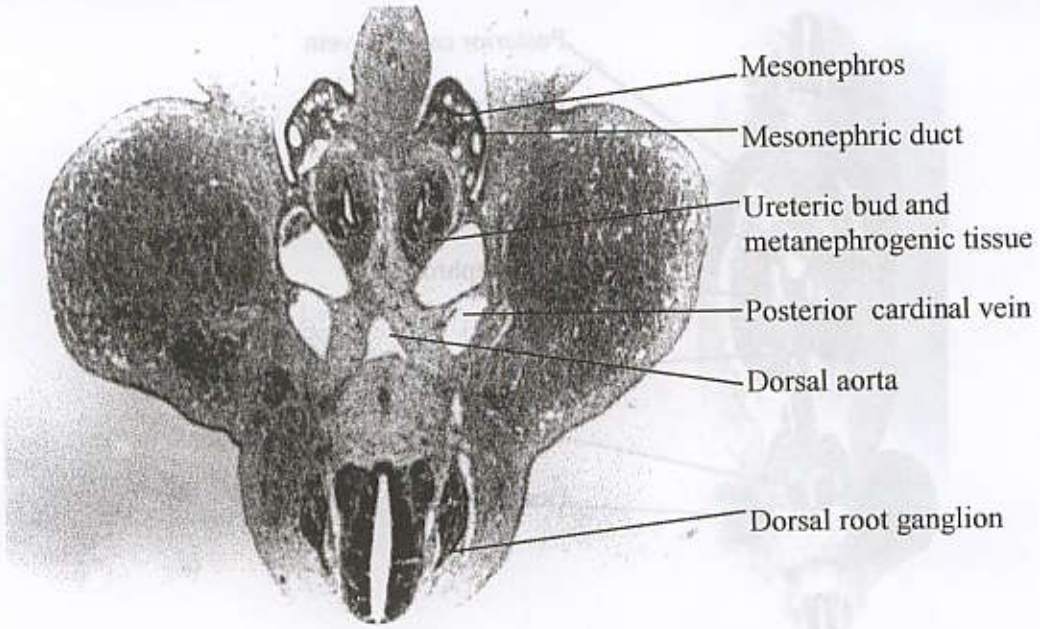


Figure 3. Transverse section of 12-14 mm rabbit embryo through the metanephrogenic tissue.

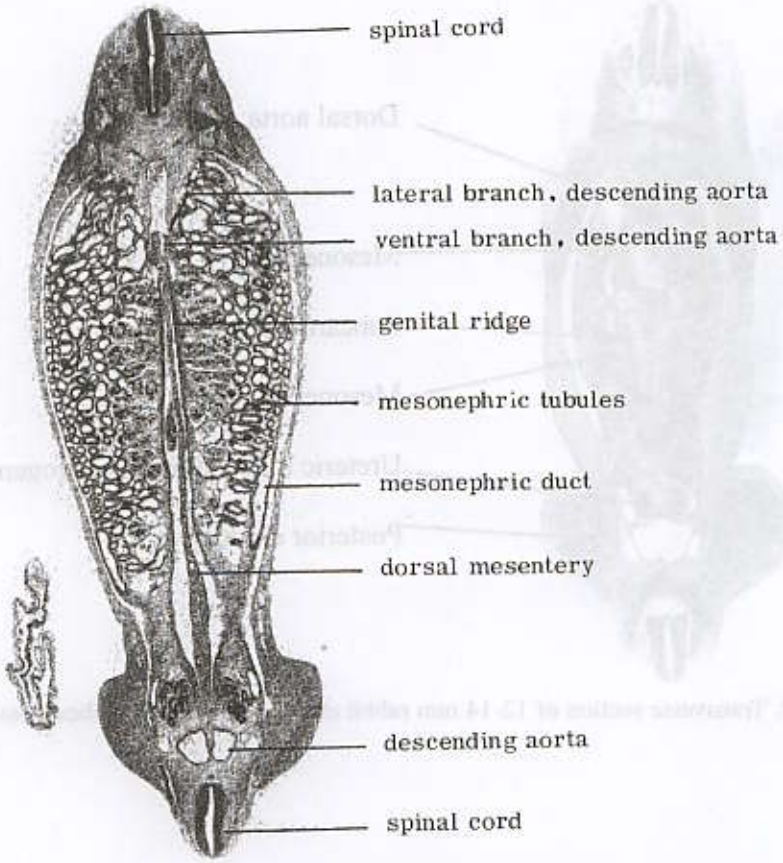


Figure 4. Transverse section of 10 mm pig embryo through the caudal part of mesonephros.

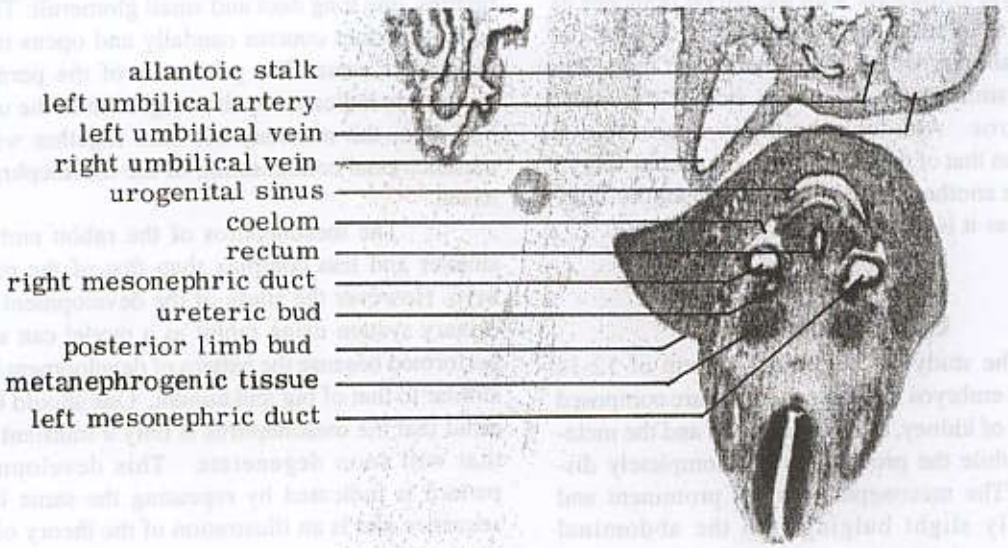


Figure 5. Transverse section of 10 mm pig embryo through the ureteric bud.

urogenital sinus. The allantois communicates with the ventral part of the urogenital sinus. Mesonephric corpuscles, the Bowman's capsules with the vascular glomeruli, are situated in a medial position. The glomeruli receive the lateral branches of the dorsal aorta. The mesonephric tubules are lined with thin epithelium and empty into the mesonephric duct. The posterior cardinal vein lies on the dorsal surface while the subcardinal vein is situated at the medial surface of the mesonephros. The mesonephros of the pig embryo is a very large organ, which bulges extensively into the abdominal cavity. The gut, especially when it is elongating and coiling, is unable to occupy itself in the limited space of the abdominal cavity. A physiological herniation of the intestinal loop therefore occurs in the pig embryo as well as in human. The mesonephros performs the excretory function for a period of time as the organ exhibits the filtration structures, the glomeruli.

The mesonephric duct courses downward to open into the urogenital sinus. More cephalically to this opening, the origin of the metanephros is seen as a small evaginated tube, or ureteric bud. The bud takes the form of a hollow tube which grows dorsally and cranially. The condensation of the nearby mesenchyme surrounds the ureteric bud to form the so called metanephrogenic blastema. Both are the primordia of the permanent kidney.

DISCUSSION

We have attempted to find the most suitable laboratory animals, the embryos of which develop in a pattern similar to those of the pig and human for embryology education.[4-10] It was quite regrettable to find a very small size mesonephros in 6 mm rat and mouse embryos.[5,11] Also their mesonephroi showed no evidence of performing excretory function. The mesonephros of rat embryo acquired only slightly elongated and curved tubular structures surrounded by mesenchyme and no glomerulus formed in this organ. The aorta sent no branch to the organ, also the posterior cardinal vein was relatively small. Moreover, the subcardinal vein was not conspicuous enough to be identified. The organ as a whole was relatively small and caused only slight umbilical herniation of the intestinal loops. The arrangement of the other organs showed slight variation in position. All of the points mentioned above describe the features of non-similarity between rat and pig embryos,[11] since the later exhibits very large mesonephros which may fully perform the function of excretion for that period of time.

In this study, we demonstrated that the urinary excretory organ of the rabbit embryo is more similar to the pig and human than those of the rat and mouse. The mesonephros of the rabbit embryo comprises the tubules, the duct and also small

mesonephric corpuscles with the lateral branches of dorsal aorta to form the glomeruli. Moreover the subcardinal vein, which was unidentified in the rat, is clearly situated on the medial side of the rabbit mesonephros. Although the organ as a whole is smaller than that of the pig embryo, the rabbit embryo will still be another animal of choice for embryology education as it is better than the rat and mouse.

CONCLUSION

The study of the urinary system of 12-14 mm rabbit embryos indicates that they are composed of two sets of kidney, the mesonephros and the metanephros, while the pronephros has completely disappeared. The mesonephros is not prominent and causes only slight bulging into the abdominal cavity. The serial sections show only short-curved tubules, one long duct and small glomeruli. The mesonephric duct courses caudally and opens into the urogenital sinus. The primordia of the permanent kidney are indicated by the outgrowth of the ureteric bud from the mesonephric duct together with the mesenchymal condensation of the metanephrogenic tissue.

The mesonephros of the rabbit embryo is smaller and less complex than that of the pig embryo. However the study of the development of the urinary system using rabbit as a model can also be performed because the pattern of development is very similar to that of pig and human. One should bear in mind that the mesonephros is only a transient organ that will soon degenerate. This developmental pattern is indicated by repeating the same kidney sequence and is an illustration of the theory of recapitulation.

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