Histomorphological study of tubular system and collecting tubules in domestic rabbit's fetuses (*Oryctolagus cuniculus*)

Jafar Ghazi Abbas* Farhan Owda Rabie**

*Collage of Veterinary Medicine, Babylon University
**Collage of Veterinary Medicine, Baghdad University

Abstract:

The developmental study of tubular system and collecting tubules has been done in the local rabbit's fetuses, which including detection the timing of appearance of metanephros collecting tubules. The study revealed that the differentiation and development of the collecting tubules in metanephros began in the rabbit at 14 day from pregnancy. Also the histological examination showed at the end of pregnant, the tubular system and collecting tubules visible in medulla and less degree in cortex with clear renal pyramids and well developed long sharp pointed papillae. The purpose of our study to provide a more complete quantitive description of the histomorphology of the tubular system and collecting tubules in rabbits during prenatal development.

الوصف الشكلي النسيجي للجهاز القنوي والنبيبات في أجنة الأرانب المحلية الجامعة

جعفر غازي عباس* فرحان عودة ربيع**

* كلية الطب البيطري، جامعة بابل
**كلية الطب البيطري، جامعة بغداد

الخلاصة:

أجريت الدراسة على مراحل تطور النبيبات الجامعة والجهاز القنوي في الكلية القبلية للأجنة الأرانب المحلية والتي تضمنت تحديد زمن ظهور النبيبات الجامعة للكلية القبلية. الدراسة اظهرت ان تطور وتمايز النبيبات الجامعة يبدا في اليوم الرابع عشر من الحمل. كذلك اظهر الفحص النسيجي في نهاية فترة الحمل ان النبيبات الجامعة والجهاز القنوي واضحة في منطقة اللب وبدرجة اقل في القشرة مع وجود هرم كلوي واضح وحليمة كلوية مدببة حادة وطويله متطورة بشكل جيد. الغرض من الدراسة هو لتوفير وصف تشريحي نسيجي كمي شامل للجهاز القنوي والنبيبات الجامعة عند الارانب خلال فترة التطور الجنيني.

Introduction:

Domestic rabbits in Iraq are descended from the European rabbit, ***Oryctolagus cuniculus***, which are characterized by the presence of a second small pair of upper incisors or peg teeth (1).The length of gestation in rabbits is 30-32 days; ovulation occurs about 10-12 hours post-coitus and ovulation is induced response to copulation (2, 3).

Implantation in rabbits begins on day 7^{th} or 8^{th}; estrus duration is prolonged about 28 days and litter size 4-12 kits (4). Among the laboratory animals the rabbits have been used as an experimental model for research (5), it is a means of research in the field of biology, physiology, medicine, toxicology, pharmacology and surgery (6). Also used as animal husbandry (7, 6). In mammals, Collecting ducts of permanent kidney develop from the ureteric bud, an outgrowth of the mesonephric duct close to its entrance to the cloaca. The bud penetrates the metanephric tissue, which is molded over its distal end as a cap. Subsequently the bud dilates, forming the primitive renal pelvis and splits into cranial and caudal portions, the future major calyces. Each calyx forms two new buds while penetrating the metanephric tissue. These buds continue to subdivide until 12 or more generations of tubules have formed. Meanwhile, at the periphery more tubules form for different times of pregnancy. The tubules of the second order enlarge and absorb those of the third and fourth generations, forming the minor calyces of the renal pelvis. During further development, collecting tubules of the fifth and successive generations elongate considerably and converge on the minor calyx, forming the renal pyramid (8, 9). Because there is no sufficient studies about development of the tubular system in rabbit's mesonephros so we suggest studying histomorphological description and the timing of first appearance of the components of tubular system and collecting tubules at intrauterine life because it has economical importance in our country and in scientific research.

Materials and Methods:

The study was performed on forty five rabbits fetuses collected from uteri of the local breed pregnant does in estimated ages, five fetuses prepared for every stage beginning from (14,16, 18, 20, 22, 24, 26, 28,30) days by which gestation occurred. All fetuses ages were estimated according to the days assumed to have elapsed from copulation (10). The crown-rump length (CRL) will measure for corrections. CRL (is the measurement from the vertex of the skull to the midpoint between the apices of the buttocks for prenatal only (11). The CRLs at each stage are summarized in the table (1). The body weight was recorded for each prenatal fetuses by using sensitive balance. The body weight was recorded before the fetuses were sacrificed. The mean weight at each stage is summarized in the table (2). Procedure of samples preparation as following: A-the samples were dissected and washed with normal saline solution (0.9%) NaCl and fixed immediately in 10% formalin at room temperature. B-Dehydration: this step was done to remove water from the histological specimens, by using a graded series of ethanol

(50%, 70%, 80%, 90%, and 100%), two changes for each one, and 2 hours for each concentration. C-Clearing and embedded: the free water specimens were transferred from 100%alchohol to xylene. The penetration of xylene was indicated by the clearing effect which accompanies it. Then the specimen transferred to melted paraffin wax (M.P. 58-60 c), and put into the oven where it must remain until it has become completely infiltrated with paraffin. D-Cutting and staining: Sections measured 5-7micrometer thickness were cut by using the rotary microtome and stained routinely with hematoxylin and eosin (12).

Results:

The tubular system and collecting tubules arise from ureteric bud which penetrate the metanephrogenic tissue as a result the reciprocal interaction between wolffain duct (mesonephric duct) derived ureteric bud and the cap of metanephrogenic mesenchyma .

At 14 day rabbit embryo

The distal end of ureteric bud expands to form the primitive renal pelvis which pushes into metanephrogenic tissue then become molded about it as the metanephric cap. The metanephrogenic tissue accompanies the ureter in its ascent always covering the primitive renal pelvis. The cap, which it is forms is at the first thin and small roundish heap of cells and it becomes still more so on the enlargment of the ureter bud to form the primitive renal pelvis (Fig. 1).

At 16 day rabbit embryo

as development proceeds, the first analge of development of tubular system and collecting tubules notice when the wedge-shaped enlargement of the ureteric anlage, the primitive renal pelvis grows in dorsal and cephalic direction and elongates in an antero-posterior direction and at the same time , begins to rotate on it is axis in such away as to make it is dorsal surface have a more lateral position and show central ureteric stalls and peripheral branching ampulla (arrowheads) surrounded by condensing mesenchyma and it is compose of undifferentiating cells (Fig. 2, 3). When the primitive renal pelvis reach metanephrogenic cap divided into interior portion and posterior portion which represent the two major calycas of metanephros giving arise to two or three primary tubules that are the future subdivides and forms secondary tubules and each secondary tubules sequentially continue to subdivide until development approximately ten or more of tubules and collecting duct (Fig. 2, 3).

At 18 day rabbit embryo

When the secondary tubules enlarge absorbs the third and fourth generation tubules and develops into the minor calyces which give arise to papillary duct. The papillary ducts divides into numerous division giving anlagen to collecting tubules which drainage to minor calyces at

papillary pores hereafter, the collecting tubules that converge on the papillary duct forming the renal pyramid and because the kidney of rabbit consist of a single pyramidal structure , they are referred to as unilobar kidneys (Fig.4).

At 20 day rabbit embryo

The collecting tubules well differentiation and relatively visible**.** The larger tubules in developing medulla and those nearer of the renal pelvis, the epithelium is pseudostratified epithelium tissue type, while those nearer the periphery, the tubules is small and lined by simple cuboidal epithelium tissue. Also there is no demarcation line between cortex and medulla (Fig.5).

At 22 day rabbit embryo

The collecting tubules more visible in cortex than medulla and there is invisible demarcation line between cortex and medulla .the collecting tubules adjacent the periphery lined by simple cuboidal epithelium tissue, while these present in medulla translate gradually to become simple columnar epithelium tissue and the nearest to renal pelvic is pseudostratified epithelium tissue.

At 24 and 26 day rabbit embryo

The collecting tubules become visible and show demarcation line between cortex and medulla. Also in this period the first or oldest collecting tubules become enlarged and hereafter taken up into wall of the growing renal pelvis and this reduction of collecting tubules. The reduction begins at center toward the periphery and it is taken up the pelvis forming undivided kidney with single papilla. Large calyx represented by the entire pelvis and finally visible renal pyramid.

At 28 and 30 day rabbit embryo

The collecting tubules visible in medulla and less degree in cortex with clear renal pyramids and show well developed long sharp pointed papillae which drainage to calyces. The collecting tubules nearest of cortex lined by simple cuboidal epithelium tissue and change gradually until become tall columnar epithelium tissue in medulla and adjacent area of renal pelvis hereafter when papillary duct open at the tip of a renal paplliae become lining by pseudostratified epithelium tissue (Fig.6). As they progress toward the renal papilla, the collecting tubules become wider. The terminal portion of these tubules is lined by a columnar or pseudostratified epithelium and is called the papillary duct (Fig.6).

Table (1) show body weight of embryo in different ages Before birth

parameter / Period	crown-rump length /mm
14	12.008±0.216 B
16	18.350±0.244 C
18	25.981±0.209 D
20	34.576±0.279 E
22	38.598±0.331 F
24	49.082±0.735G
26	17.087±0.721E
28	25.225±1.205F
30	35.500±1.426G

Values represent mean ±S.E
Different capital letters mean significant (P≤0.05) results between different age pups

Table (2) show Crown rump length of embryo in different ages before birth

parameter / period	Weight of fetuses / gm
14	0.283±0.020 A
16	0.537±0.027 A
18	1.407±0.053 A
20	3.156±0.077 B
22	5.773±0.147 C
24	13.17±0.316 D
26	66.757±1.243H
28	80.708±2.197 I
30	90.628±2.346 J

Values represent mean ±S.E
Different capital letters mean significant(P≤0.05) results between different age pups.

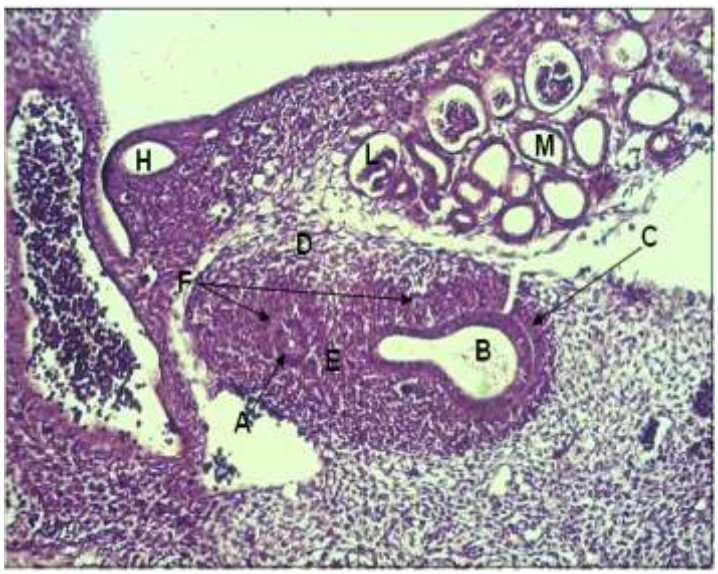

Fig. (1) Show mesonephros & metanephros in 14 day rabbit embryo (parasagittal-section). A- Primary excretory duct B-primitive renal pelvis C- metanephrogenic cap D- external layer(looser) E- internal layer(denser) F-condensing mesenchyma(metanephric tissue) H- mesonephric duct L- mesonephric glmeruli M- mesonephric tubules **(H&E.X4)**

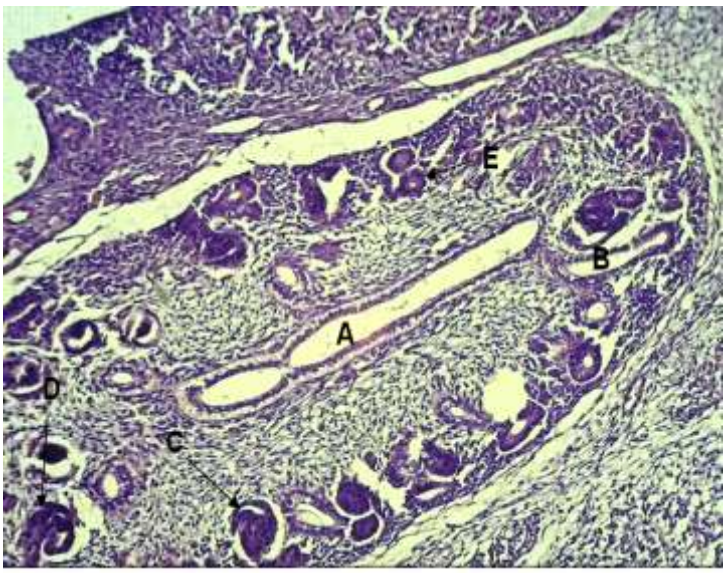

Fig.(2) show metanephros in 16 day rabbit embryo (parasagittal-section).A- uretric bud(central stalk) B- peripheral braching ampulla C- comma shape body D- s-shape body E- vesicle shape body **(H&E.X4)**

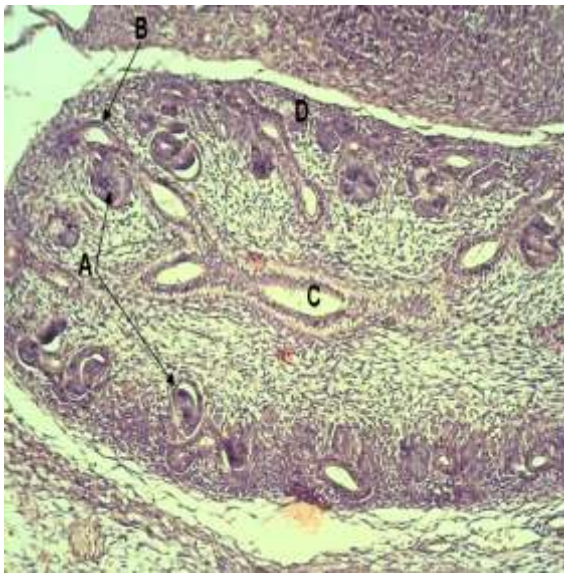

Fig. (3) Show metanephros & diferentition of collecting tubule in 16 day rabbit embryo (parasagittal-section). A- nephron primordium B- collecting tubule primordium C- renal pelvis D- nephrogenic zone **(H&E.X10)**

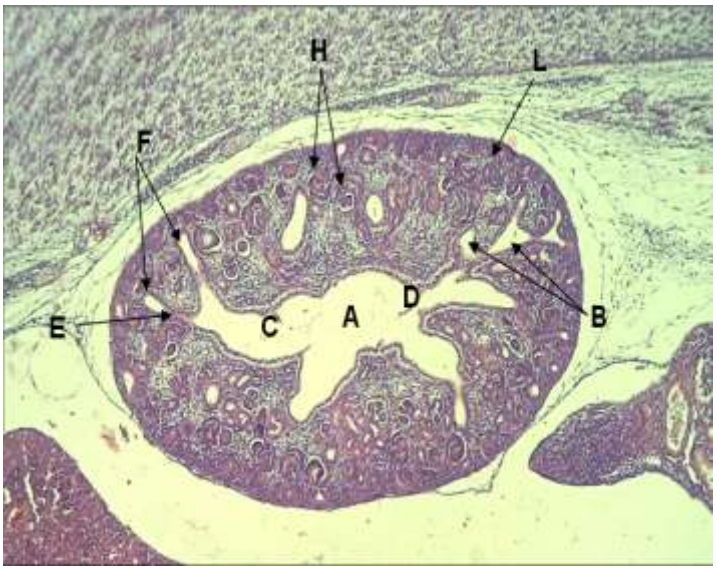

Fig. (4) Show metanephros & diferentition of collecting tubule in 18 day rabbit embryo (parasagittal-section). A- Major calyx B-minor calyx C- anterior portion D- posterior portion E-primordium of papillary duct F-collecting tubule primordium H-nephron primordium L- nephrogenic zone **(H&E.X4)**

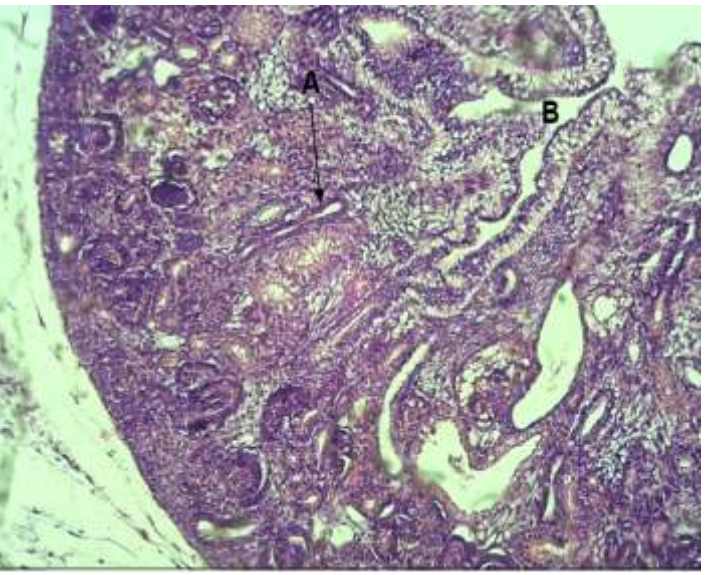

Fig. (5) Show maturation of the collecting duct &tubule in 20 day rabbit embryo A- collecting tubule B-transitional epithelium of collecting duct at renal pelvis (**H&E.X10)**

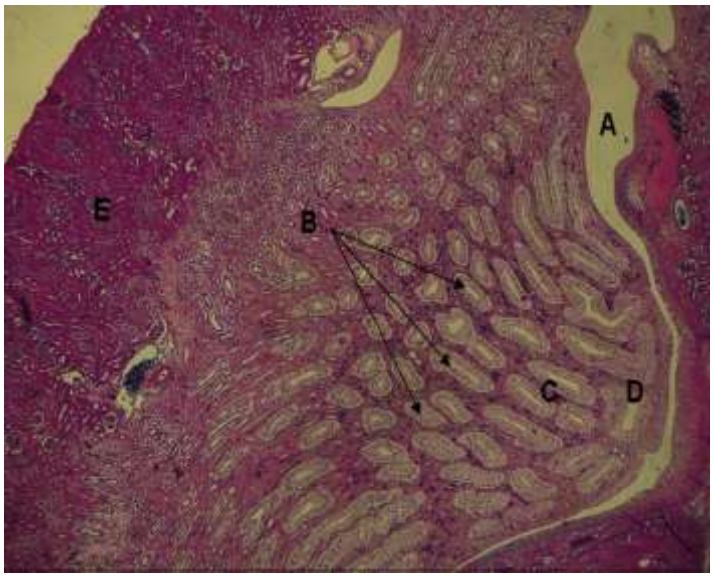

Fig. (6) Show collecting tubule & duct in 30 day rabbit embryo A- renal pelvis B-collecting duct C- renal papilla D- papillary duct E- Cortex **(H&E.X4)**

Discussion:

The first analge of development of tubular system and collecting tubules noticed when the wedge-shaped enlargement of the ureteric anlage, the primitive renal pelvis grows in dorsal and cephalic direction and elongates in an antero-posterior direction. This unlike to results done by (13) in spiny mouse who mentioned that the kidney has taken on a more definitive shape, while the first branch of the ureteric bud is still clearly visible, multiple branching events have taken place and (14, 15) noticed that first differentiation and formation of the tubular system and developmental collective tubules at 12 day of mouse embryo, while in rat at 13 day of embryonic life (16, 17) and in contrast with the findings of (9) in human who mentioned that first appearance of collecting tubules at sixth week of pregnancy. This greater level of development in the spiny mouse compared with other rodent species and rabbit is likely to be advantageous in the hot, dry, arid desert, and rocky environment where these animals naturally inhabit therefore the mean timing of first appearance of collecting tubules consider more early compared with that in tubular system of murine and rabbit. The differences in time of first evolution of collecting tubules in animals also because the difference in gestation periods.

The study revealed when the primitive renal pelvis reach metanephrogenic cap divided into interior portion and posterior portion which represent the two major calycas of metanephros giving arise to two or three primary tubules that are the future subdivides and forms secondary tubules and each secondary tubules sequentially continue to subdivide until development approximately ten or more of tubules and collecting duct. the collecting tubules that converge on the papillary duct forming the renal pyramid and because the kidney of rabbit consist of a single pyramidal structure , they are referred to as unilobar kidneys. This coincides with the described results reported by (14) in mouse , (18) in murine and (19) in rabbit who claimed that The ureteric bud penetrates the metanephric tissue, which is molded over its distal end as a cap then Subsequently the bud dilates, forming the primitive renal pelvis, and splits into cranial and caudal portions, the future major calyces. Each calyx forms two new buds while penetrating the metanephric tissue. These buds continue to subdivide until more generations of tubules have formed. Meanwhile, at the periphery more tubules form and the tubules of the second order enlarge and absorb those of the third and fourth generations, forming the minor calyces of the renal pelvis. During further development, collecting tubules successive generations elongate considerably and converge on the minor calyx, forming the renal pyramid.

In contrast with(19) in aquatic mammals(seals , otters and whales) who claimed that the terminal end of ureteric bud gives rise to a number of branches each capped by metanephric tissue forming a kidney lobe , termed a renculus and (20) who mentioned that the uerteric bud, from which the ureter is derived, forms two major branches (primary branches) that subdivided into 12 to 25 minor (secondary) branches. Consequently, the bovine kidney develops 12-25 separate lobes; each retaining it is distinct pyramid-forming papillae. The papillary ducts within each lobe drain into calyx and the bovine kidney have no renal pelvis while in the horse, small ruminant and carnivores no calyces are formed and the papillary duct drains directly into a common pelvis. In the horse, the renal pelvis possesses two long, thin-walled processes terminal recesses in which the urine is collected and this is not in agreement with the results of (8, 9, 21) in human. These differences in the branching of the ureteric bud and the arrangement of nephrons around it lead to viration in macroscopic appearance of the mature kidney in different animals.

The collecting tubules become well differentiation and relatively visible between 24 and 26 day rabbit embryos. This disagreement with (22) in goat who mentioned that collecting tubules become visible and differentiation between 56-63 day of life embryo, while (13) in spiny mouse mentioned that the collecting duct system is visible within the developing medulla at 28 day of pregnancy. This differences in beginning in which tubulogenesis commences relying on the length of gestation periods in species and the time at which the ureteric bud reach into mesenchyma and divide into multiple branches and changing to an epithelial phenotype giving analge of collecting tubules. The collecting tubules nearest of cortex lined by simple cuboidal epithelium tissue and change gradually until become tall columnar epithelium tissue in medulla and adjacent area of renal pelvis hereafter when papillary duct open at the tip of a renal papillae become lining by pseudostratified epithelium tissue (Fig.4-18). This agreement with results of (23, 24) in domestic animals who mentioned that the epithelial cells of the collecting tubules are pale and vary from cuboidal near the distal tubules to columnar close to the papilla. Cell boundaries are normally clearly defined compared with the cells of the proximal and distal convoluted tubules. As they progress toward the renal papilla, the collecting tubules become wider. The terminal portion of these tubules is lined by a columnar or pseudostratified epithelium and is called the papillary duct (Fig.4-18).

References:

1. Flecknell, P. (2002). Textbook of rabbit medicine. Butterworth-Heinemaun. Britain and oxford. P:1-18.

2. Fox, R. R. and Laird, C. W. (1970). Sexual cycles in reproduction and Breeding Techniques for Laboratory Animals. E.S.E. Hafez, ed. Lea and Febiger, Philadelphia. P: 107-122.

3. Richardson, V. (2003). Rabbit: health , husbandry and diseases. Blackwell publishing company. Oxford. P:44-59.

4. Tscheudschilsuren, G.; Hombach-Klonisch, S. ; Kuchenhoff, A. ; Fischer, B. and Klonisch, T. (1999). Expression of the arylhydrocarbon receptor and the arylhydrocarbon receptor nuclear translocator during early gestation in the rabbit uterus. Toxicol. Appl. Pharmacol. 160:231-237.

5. Ghoshal, N.G. and Bal, H.S. (1989). Comparative morphology of the stomach of some laboratory mammals. *Laboratory animals.* Vol. 23: 21-29.

6. Abidu- Figueiredo, M.; Xavier-Silva, B.; Cardinot, M.; Babinski, M. and Chagas, M. (2008). Celiac artery in New Zealand Rabbit: anatomical study of its origin and arrangement for experimental research and surgical practice. *Pesq. Vet. Bars.* Vol. 28, No. 5.

7. Hristov, H.; Kostov, D. and Valadova, D. (2006). Topographical anatomy of some abdominal organs in rabbits. *Trakia Journal of Science.* Vol 4, (3). Pp:7-10.

8. Vize,P.D. ; Woolf ,A.S. and Bard, J.B.(2003).The kidney from normal development to congenital disease " Maldevelopment of the Human Kidney and Lower Urinary Tract " Academic press,Inc.U.S.A.p:377-394 .

9. Sadler, T. W.(2008). Langman's medical embryology. 10th ed. Williams and Wilkins, USA. p: 247-280.

10. Oda, S. (1985). Suncus Murinus. Tokyo: Japan Scientific Societies Press (in Japanese). Pp. 102-111.

11. Arthur, G. H.; Noakes, D. and Pearson, H. (1989). Veterinary Reproduction and Obstetrics. 6th edition. Bailliere. Tindall, London. Pp: 59.

12. Luna, L.G. (1968). "Manual of Histological Staining Methods of the Armed Forces Institute of Pathology". 3rd ed. McGraw-Hill Book Company. P:3- 34.

13. Dickinson, H. ; Walker, D.W. ; Cullen- McEwen, L. ; Wintour, E.M. and Mortiz, k. (2005). The spiny mouse (Acomys cahirinus) completes nephrogenesis before birth. Am J. phys. Ren. 289: 273-279.

14. Al- yasery, A. J. (2007). Developmental study for Embryological formation of kidneys in the Embryos of white Swiss mice

.Msc. thesis ,The University of Basra , Iraq.

15. Costantini, F. and Kopan, R. (2010). Patterning a Complex Organ: Branching Morphogenesis and Nephron Segmentation in Kidney Development. Elsevier Inc. Developmental Cell. 18:698-712.

16. Rogers, S.A. ; Talcott, M. and Hammerman, M.R. (2003) . Transplantation of pig metanephroi. ASAIO. J. 49: 48-52.

17. Bernardini, N. ; Mattii, L. ; Bianchi, F. ; DaProtta, I. and Dolfi, A. (2001). Gfalpha mRNA expression in renal organogenesis : astudy in rat and human embryos. Exp. Nephro. (2)90-98.

18. Cebrian, C. ; Borodo, K. ; Charles, N. and Herzlinger, D. (2004). Morphometric index of the developing murine kidney. Dev. Dyn. 231:601-608.

19. Mcgeady, T.A. ; Quinn, P.J. ; Fitzpatrick, E.S. and Ryan, M.T. (2010). Veterinary embryology. Blackwell publishing Ltd. P:233-243.

20. Hyttel, P. ; Sinowatz, F. ; Vejlsted, M. and Betteridge, K. (2010). Domestic animal embryology. Saunders Elsevier. New-york, London and Sydney. P:1-24.

21. Cukuranovic, R. and Vlajkovic, S. (2005). Age related anatomical and functional characteristics of human kidney. Medicine and Biology. 12(2): 61-69.

22. Chaudhury, A. R. ; Farooqui, M. M. and Chandra, P. (2006). Histological and histochemical studies on the metanephros of goat (Capra hircus) in prenatal period – excretory part. The Indian Journal of Small Ruminants. 12(2): 9710-9857.

23. Dellmann, H . D . and Brown , E . M. (1987).Text book of Veterinary histology (Urinary System) 3rd ed ,Lea and Febiger ,Philadelphia.

24. Bacha, W.J. and Bacha, L.M. (2000). "Color Atlas of Veterinary Histology". 2nd ed. D. Balado. Lippincott Williams and Wilkins, Maryland, p: 163-174.