AJBS Asian Journal of Biological Sciences

OPEN ACCESS

Ultrastructural Analysis of Metanephros Development in Dromedary Camel Fetuses: Insights from Scanning and Transmission Electron Microscopy

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ABSTRACT

Background and Objective: The metanephros, the definitive kidney in mammals, undergoes complex morphogenesis and differentiation during fetal development. Understanding its ultrastructure in dromedary camel fetuses provides insights into renal adaptation in arid environments. The objectives of this investigation were to study the morphology of the metanephros in the camel fetus using standard techniques. **Materials and Methods:** A total of 73 fetuses (30 males and 43 females) of dromedary camels (Rashaidi, Bishari, and their crosses) their ages ranged between 71 and 426 days were collected from Tambool slaughter point, preserved in standard international fixatives and divided into three groups according to their age. The curved crown-rump length (CVRL) equation Y = 0.366X-23.99 was used to determine the age of the fetus (x) in days from the known (y) CVRL in centimeters. **Results:** Nephrogenesis in dromedary camel fetuses occurred in the metanephros from 74 to 339 days of age. Ampullae developed from collecting ducts during this period, while podocytes began forming their complex architecture in the first trimester. Distal convoluted tubules differentiated earlier than proximal ones, with the latter developing from the distal tubule's end. Immature podocytes featured a junctional complex (zonula occludens, zonula adherens, and desmosomes), and the basal lamina showed localized thickening in the nephrons. The metanephros of the dromedary camel fetus is characterized by wide medullae, narrow cortex, long loops of Henle, long proximal and distal tubules, long collecting tubules, and small renal corpuscles. The ratio of the thickness of the cortex to the medulla during the first trimester was 1:1, during the second trimester was 1:1.5, and during the third trimester was 1:4 to 1:5. Rich in blood and nerve supply, thickening of the basal lamina at various locations in the nephrons. **Conclusion:** The specialized characteristic structures provide the type of adaptation or mechanical support for the cells, particularly during states of dehydration and sudden rehydration resulting from the sudden consumption of large amounts of water.

KEYWORDS

Metanephros, nephrogenesis, podocytes, ampullae, trimester, fetus

ISSN: 1996-3351 (Print)
https://doi.org/10.3923/ajbs.2025.489.499

Received: 04 Nov. 2024
Accepted: 30 Jan. 2025
Published: 30 Jun. 2025

INTRODUCTION

Knowledge of the development of the mammalian kidney is essential for an understanding of its disorders of growth. Renal mal-development in domestic animals has been well recorded but poorly understood. Knowledge of embryonic kidney development will also form the foundation for developing stem cell-based strategies to treat renal disease. Some of these approaches are based on the directed differentiation of embryonic stem or other multipotent cells into kidney cells using cocktails of growth factors followed by transplantation into embryonic or adult kidneys[1]. The glomerular morphogenesis proceeds through several well-defined stages in embryonic development, beginning first as a renal vesicle, followed by the comma-shaped body, S-shaped body, a capillary loop stage, and then the mature glomerulus[2]. The epithelial components of the glomerulus (the parietal epithelial cells and podocytes) derive from the metanephric mesenchyme. These mesenchymal cells are found adjacent and inferior to the tips of the branching ureteric bud and begin to condense at 11.5 days in the mouse or after 5 weeks of gestation in humans. This mass of cells is known as the peritubular aggregate[2].

In response to inductive cues from the ureteric bud and surrounding stroma, the aggregates undergo a mesenchymal-to-epithelial transition forming the renal vesicle and then the comma-shaped body. The glomerulus develops from the most proximal end of the renal vesicle which is far from the bud tip. Distinct cell types in the glomerulus are first identified in the S-shaped stage, where presumptive podocytes appear as a layer of columnar epithelial cells. A vascular cleft develops and separates the presumptive podocyte layer from more distal cells that will form the proximal tubule. Vascular endothelial cells migrate first and then followed by mesangial cells and enter the cleft[2].

The ureteric bud induces the mesenchyme to form tubular and glomerular epithelia[3]. In turn, the surrounding mesenchyme induces the ureter to continue to grow and branch inside the renal mesenchyme. As the ureter branches, the mesenchymal cells condense around each ureteric tip. The condensate develops into a vesicle, followed by a comma-shaped body that subsequently develops into an S-shaped body. Simultaneously, the glomerular cells differentiate until they acquire their adult features. In humans, nephrogenesis is complete between 34 and 35 weeks of gestation. In mice and rats, however, nephrogenesis continues after birth for about 3 and 7 days, respectively.

The precursor structure of the glomerulus can first be appreciated in the 'S-shaped body', so-called because it is shaped like an 'S' when observed in histological sections. There are three major components of the early glomerulus. These are: (1) Layer of primitive podocytes that begins as a columnar epithelium. (2) Thin layer of Bowman's capsule, which appears to be nearly flat, similar to a squamous epithelium and (3) Capillary loop that first enters the glomerular cleft. How the podocytes extend themselves around the capillary loops remains unknown. Early during this process, both the podocytes and the capillary endothelial cells synthesize their basal laminae. As the glomerulus matures these two basal laminae fuse to form a thick basement membrane, known as the glomerular basement membrane[4].

The ultrastructure of dromedary camel metanephros during the three trimesters was not found in the available literature. This research was conducted on the metanephros of the dromedary camel fetus to study the morphology of the metanephros of the one-humped camel (*Camelus dromedarius*) during the three trimesters.

MATERIALS AND METHODS

Study area: The material was collected from the Tambool slaughter point in the Butana Area in central Sudan, between October, and February, for three seasons (2014-2016).

Sample collection: As 73 fetuses were collected and divided into three groups according to their ages as follows:

- 21 fetuses of early age group (first trimester) 14-123 days old
- 2-30 fetuses of middle age group (second trimester) 124-246 days old
- 3-22 fetuses of late age group (third trimester) more than 247 days old

The 73 fetuses of the dromedary camel were collected from the gravid uteri of apparently healthy she-camels to study the metanephros. The curved crown-rump length (CVRL) equation Y = 0.366X-23.99 was used to determine the age of the fetus (x) in days from the known (y) CVRL in centimeters using a tape measure. The age of the fetus was confirmed by using another equation using the vertebral column (VR) Y = 0.324X-24.99[5].

Methodology: Histometrical measurements were carried out in the developing metanephros of camel fetuses of different ages. The diameter of the renal corpuscles and tubules of metanephros were measured. The thickness of the metanephric cortex, medulla, and capsule were also measured. Olympus microscope (CH20-Japan) was utilized in this work, using an Optikam B1 digital camera. After setting the camera with the microscope objective lens ×10, the correct scale for calibration was selected using the micrometric slide on the stage of the microscope. Other measuring tools were used: 1 line to measure the length-diameter. 2-Circles which draw a circle according to the diameter to give the radius and area perimeter of the circle. When the images of the desired histological sections stained with H&E were captured and saved in a file, the selected calibrations were run using mouse button and the measurement then listed. Specimens (~1×1×1 mm) from 10 metanephros of dromedary camel fetuses were fixed in 2.5% glutaraldehyde (15 days), post-fixed in 2% osmium tetroxide, dehydrated in graded ethanol, and embedded in Epon for microscopy. Semi-thin (0.5 μm) and ultrathin (500 Å) sections were stained with Toluidine blue, uranyl acetate, and lead citrate. For SEM, specimens (~2-3 mm^3) were similarly fixed, dehydrated, critical-point dried, gold-coated, and examined using a JEOL JSM 5400 LV microscope.

RESULTS

The immature metanephric renal corpuscle diameter during the first trimester ranged between 110 and 160 μm with an average of 135 μm. The diameter of the metanephric renal corpuscles decreased during the second trimester and ranged between 100 and 140 μm with an average of 120 μm. The diameter of the mature metanephric renal corpuscles decreased even more and ranged between 50 and 70 μm with an average of 60 μm during the third trimester.

Figure 1 illustrates the comma-shaped stage of renal corpuscles in metanephros. The renal corpuscles had a central pore and consisted of primordial columnar podocytes arranged in the form of a spherical mass around the central lumen.

Figure 2 shows the comma-shaped stage of the renal corpuscle in the metanephros of a fetus. Primitive podocytes in the form of large columnar platy or elongated cells with oval nuclei surrounding a central lumen, and small spindle cells.

Figure 3 demonstrates the "S' shape stage of renal corpuscle with cleft and capillary loop situated in the cleft in a fetus. The podocytes attached to the capillary wall were star-shaped and extended numerous primary processes while the podocytes away from the capillary wall were spindle in shape with short processes.

As shown in Fig. 4 Dome shape podocytes with numerous long primary processes and many fine secondary processes having foot processes in metanephros from a fetus of 13 cm CVRL (101 days of age).

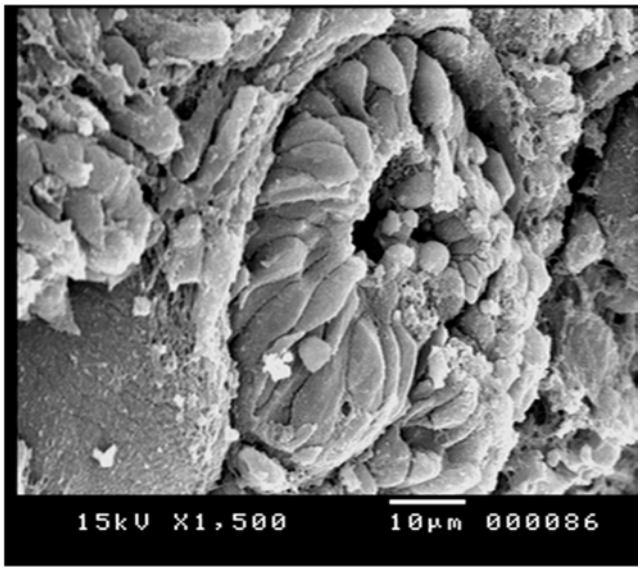

Fig. 1: Comma shape stage of renal corpuscles in metanephros of 7 cm CVRL (85 days of age)

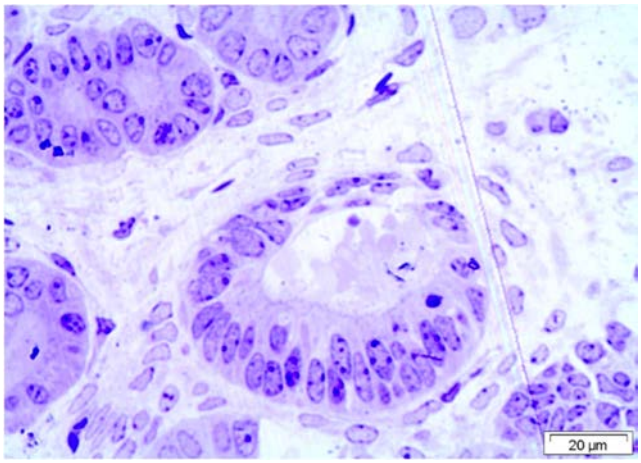

Fig. 2: Comma shape stage of the renal corpuscle in metanephros of a fetus of 13 cm CVRL (101 days of age)
Toluidine blue stain

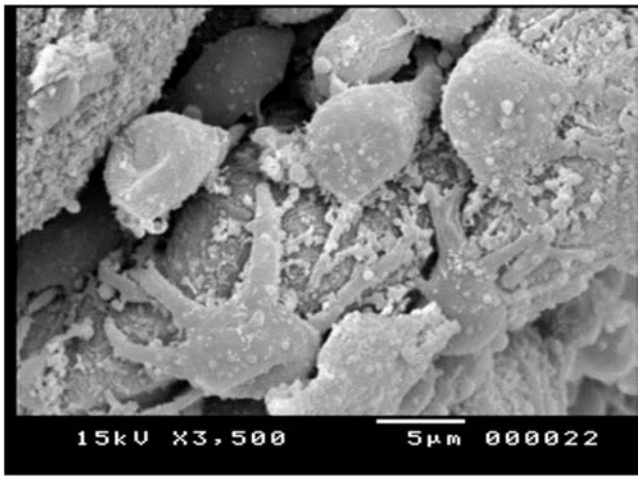

Fig. 3: Demonstrates "S" shape stage of renal corpuscle with cleft and capillary loop situated in the cleft in a fetus of 13 cm CVRL (101 days of age)

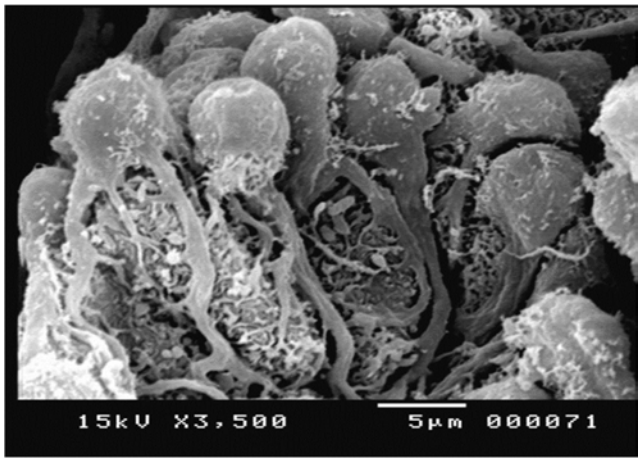

Fig. 4: Dome shape podocytes from a fetus of 13 cm CVRL (101 days of age)

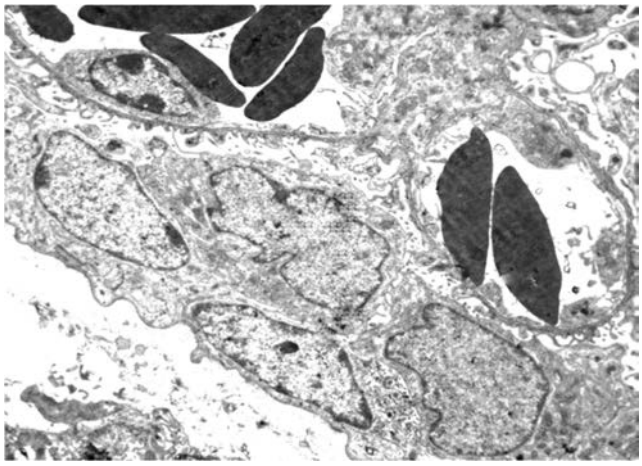

Fig. 5: Nuclei of parietal layer cells, podocytes, and capillary endothelium in immature glomeruli in a fetus of 18.5 cm CVRL (116 days of age)
Capillaries contain red blood cells (X3600)

Figure 5 shows the nuclei of the cells of the parietal layer of Bowman's capsule, podocyte, and the endothelium of capillaries of immature glomeruli with elongated nuclei, and heterochromatin attached mainly to the internal aspect of the nuclear membrane in a fetus. The capillaries contain red blood cells.

Figure 6 illustrates the vesicular stage of renal corpuscle demonstrating elongated columnar podocytes in a fetus of 43 cm CVRL (183 days of age). The podocytes during this stage do not form any processes and are arranged circularly around a central pore. Adjacent podocytes are joined by a junctional complex.

Figure 7 illustrates S" shape stage with an arteriolar loop entering the cleft in a fetus.

Figure 8 demonstrates in a fetus of dome-shaped principal cells of distal convoluted tubules with micro plicae and some cells lacking the cap-like microplicae and under these microplicae are found short microvilli.

Figure 9 illustrates a proximal convoluted tubule cell in a fetus. The cells are dome-shaped containing many elongated dark mitochondria. The apical cytoplasm of the cells contains many small and large vacuoles and numerous long microvilli.

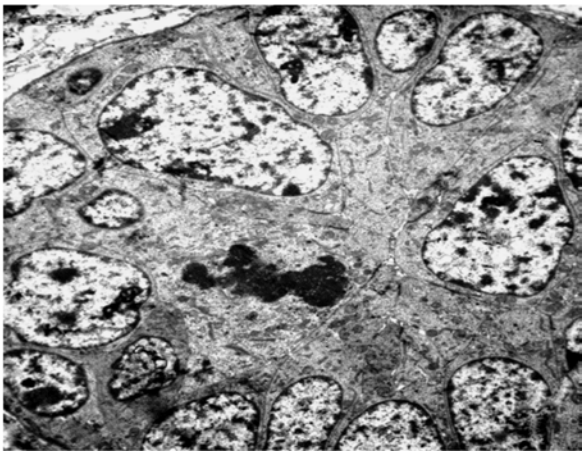

Fig. 6: Vesicular stage of renal corpuscle demonstrating elongated columnar podocytes in a fetus of 43 cm CVRL (183 days of age)
Adjacent podocytes are joined by a junctional complex (X2900)

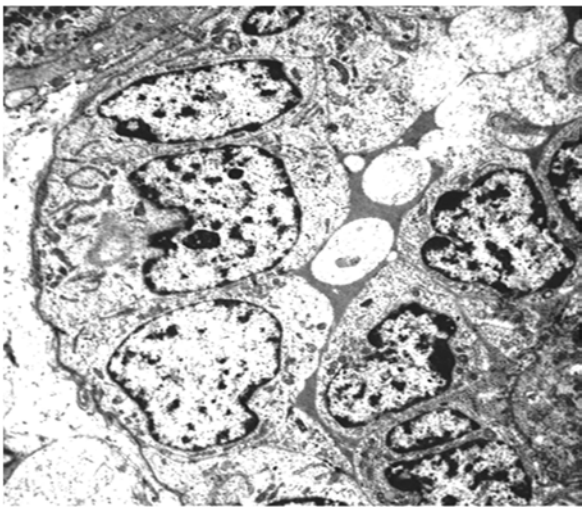

Fig. 7: "S" shape stage with arteriolar loop entering the cleft in a fetus of 48 cm CVRL (197 days of age)
X3600

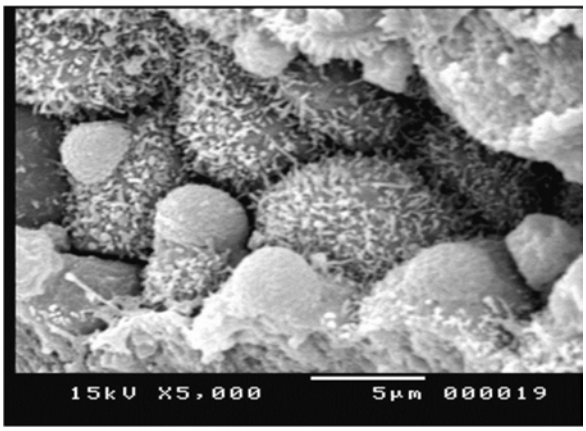

Fig. 8: Fetus of 13.5 cm CVRL (102 days of age) dome-shaped

Figure 10 shows after removing the capsule of the metanephros fetus illustrating ampullae of the collecting ducts occupying the entire subcapsular layer of the metanephros in bud-like tips surrounded by many rows of mesodermal cells as cell nest.

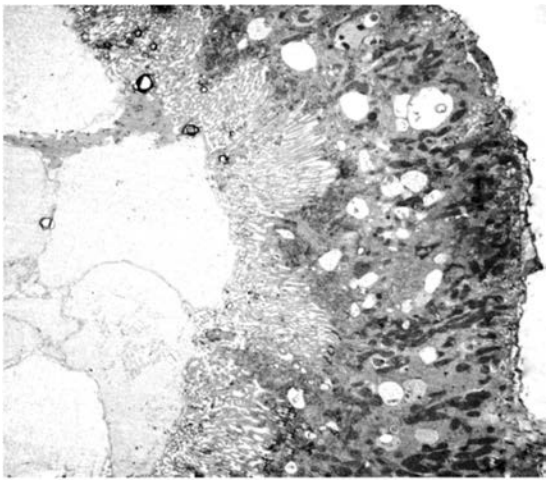

Fig. 9: Proximal convoluted tubule cells in a fetus of 18.5 cm CVRL (116 days of age) X290

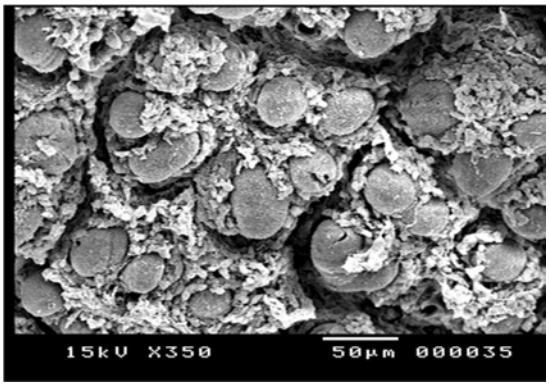

Fig. 10: Fetus after removal of the capsule of the metanephros. 43 cm CVRL (183 days of age)

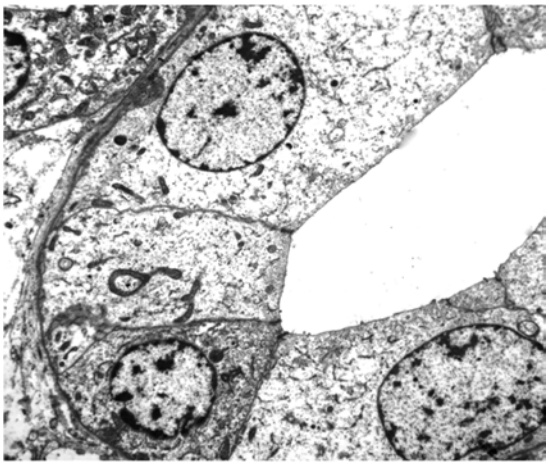

Fig. 11: Metanephros of a fetus of 99 cm CVRL (336 days of age) X3600

Figure 11 shows the metanephros of a fetus. The lining epithelium of the collecting tubule contains two types of cells: The majority of cells have low electron-dense cytoplasm, while a few cells have highly electron-dense cytoplasm.

Figure 12 demonstrates thick electron-dense basal lamina under a distinct basal cell membrane of the lining epithelium of the thin segment of the loop of Henle. The cells overlap each other.

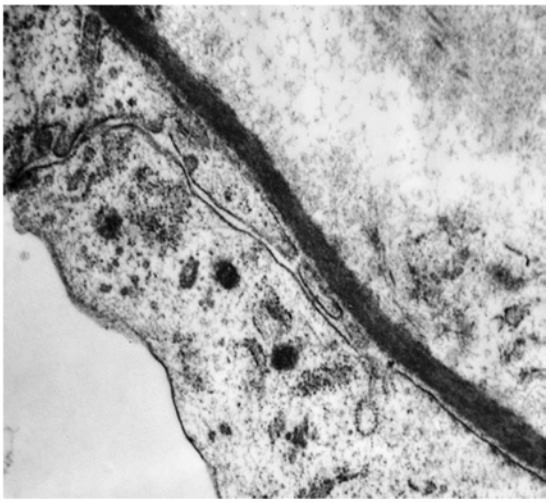

Fig. 12: Thick electron-dense basal lamina under a distinct basal cell membrane of the lining epithelium of the thin segment of the loop of Henle
X14000

The diameter of the metanephric proximal and distal convoluted tubules during first trimester ranged between 8 and 14 μm with an average of 11 μm and between 20 and 40 μm with an average of 30 μm during second and third trimesters.

The metanephric capsule thickness ranged between 10 and 12 μm with an average of 11 μm during the first trimester, between 13 and 37 μm with an average of 25 μm during the second trimester, and between 41 and 47 μm with an average of 44 μm during the third trimester.

The thickness of the cortex during the first trimester ranged between 2 and 3 mm with an average of 2.5 mm, and increased during the second trimester to measure between 3 and 5 mm with an average of 4 mm. During the third trimester the thickness of the cortex ranged between 4 and 5 mm with an average of 4.5 mm. The thickness of the medulla during the first trimester was in the range of 5mm and 8mm with an average of 6.5 mm and increased during the second trimester to measure between 10 and 15 mm with an average of 12.5 mm. During the third trimester, the thickness of the medulla decreased and ranged between 20 and 25 mm with an average of 22.5 mm.

DISCUSSION

The ratio of the thickness of the cortex to medulla during the first trimester was 1:1, during the second trimester was 1:1.5, and during the third trimester was 1:4 to 1:5. The medullary-to-cortical thickness ratio in the metanephros during the third trimester is consistent with the finding in the kidney of the adult camel[6]. This indicates that, the kidney of the camel acquires one of attain anatomical requisites for the production of hypertonic urine in the third trimester of intrauterine life, and that the ability of the camel to produce concentrated urine is not a postnatal adaptation.

Renal corpuscle development in mammals follows a seven-stage morphological progression: condensation, renal vesicular, comma-shaped body, S-shaped body, capillary loop, maturing renal corpuscles, and the mature stage[7,8]. In the present study of dromedary camel fetus metanephros during the first trimester, renal development includes developed collecting ducts, renal corpuscles in various stages (vesicular, comma-shaped, S-shaped body, with cleft, capillary loop, and maturing stages), and proximal and distal tubules embedded in nephrogenic tissue. The loops of Henle first appear around the middle of the first trimester. Continuous generation of renal tubules and corpuscles occurs at the periphery of the metanephros, the tips of ureteric ducts induce mesenchymal aggregation and epithelial conversion[9].

At the vesicle stage, mesodermal lenticular cells form spherical masses that differentiate into polygonal columnar cells, becoming primordial podocytes in the comma-shaped stage. These podocytes first appear around the pore and gradually extend in parallel rows toward the renal corpuscle's other pole. This pore likely represents a continuous canal leading to the collecting duct in rat metanephros[8]. Similar observations were reported in the central lumen formation in the comma-shaped renal corpuscles of domestic animal fetuses[9]. In contrast to mouse studies where primitive podocytes start as columnar epithelium at the "S" shape stage, the camel fetus shows polygonal columnar podocytes in the comma-shaped stage, preceding the "S" shape[4].

In the comma-shaped stage, podocytes are cuboidal, with flat surfaces and primary processes extending from them. This aligns with observations in puppy metanephros in human fetuses[10,11], where podocytes form processes and foot processes during this stage. As development progresses to the late "S" shape, before capillary loop entry, the podocytes' surface becomes rounded with foot processes forming at their base. During the "S" shape with cleft stage, the glomerulus develops its characteristic cell architecture, as vascular loops enter the cleft, marking a significant stage in mesangial and glomerular capillary development.

Mesangial cells, found within the cleft above the podocyte layer during the "S" shape stage, support glomerular capillaries and have spinous cytoplasmic processes that adhere to the basement membrane[12,13]. Podocytes develop three subcellular compartments: cell body, primary processes, and foot processes[14,15]. The primary processes extend to form foot processes that interdigitate and adhere to the basal lamina. The differentiation of these processes where primary processes form first, followed by secondary and tertiary processes[16]. In human fetuses, podocytes are dome-shaped with filamentous projections, but no such projections are observed in the camel fetus metanephros[17]. During the "S" shape stage with a cleft, a vascular loop forms a capillary network, entering intercellular spaces between podocytes. Podocytes in contact with capillary walls adopt a star shape with numerous primary processes, while others, away from the capillaries, appear spindle-shaped, also extending primary processes.

Development of proximal and distal tubules is evident by the second trimester. The metanephric blastema differentiates into nephron epithelia and portions of the renal collecting system[18]. In the camel fetus, mesodermal cells form two spherical masses around the collecting duct. One mass, with long interdigitating cells, is likely to become the primordial podocytes, while the other mass forms the distal convoluted tubule, as evidenced by short microvilli on the cell surfaces. Principal cells in the distal tubule display a cilium and microvilli in other species[17].

During the third trimester, nephrogenesis ceases, with no new renal corpuscles formed beyond the 100 cm CVRL stage (339 days of age). At this stage, collecting tubules are composed of polygonal cells with minimal projections[9]. In the mature stages, the podocytes and capillary endothelial cells form their basal laminae, which fuse at some points as the glomerulus matures. This is consistent with observations in the mouse metanephros[4].

CONCLUSION

The developmental stages of renal corpuscles in the dromedary camel fetus follow a pattern similar to other species but with some differences in timing, such as the early formation of polygonal podocytes in the comma-shaped stage. The study highlights the intricate processes of podocyte and tubular differentiation, including the formation of the basal lamina, primary processes, and foot processes, as well as the development of mesangial cells and capillaries, which collectively support nephrogenesis throughout the fetal stages.

SIGNIFICANCE STATEMENT

The metanephros of the dromedary camel fetus is characterized by a wide medulla, narrow cortex, long loops of Henle, long proximal and distal tubules, long collecting tubules, and small renal corpuscles. The important basic information included in this study will lead to a better understanding of any malformation that will result in physiological and pathological disturbances.

ACKNOWLEDGMENTS

Thanks to Professor Allam Nafadi, Head of the Electron Microscope Unit and to all staff members, Assuit University, Egypt. I really appreciate the financial support provided by the German Academic Exchange Service (Deutscher Akademischer Austausch Dienst e.v. DAAD) and by the University of Khartoum which enabled me to carry out this research work.

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