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An Overview of Early Development of Mesonephric System in Camels (*Camelus dromedarius*) with Special Emphasis on Mesonephric Kidney: Structure and Function

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ABSTRACT

This study aimed to examine the features of the mesonephric system in fourteen (14) dromedary camel fetuses. Standard anatomical, histological, histochemical and scanning electron microscopic procedures were applied to investigate the role of the camel's mesonephros as a secretory system and hematopoietic center. In early development, the mesonephros occupied a large field of the abdominal cavity, expanded by lateral symmetry from the diaphragm to the pelvic cavity in the dorso-sublumbar regions. At 112 days of gestation, the mesonephros regressed at the cranial extremities. In the early stages of development, the mesonephros was enclosed by a thin capsule of mesenchymal connective tissue; irregular mesonephric tubules with large lumens lined with simple cuboidal cells interspersed with tiny blood vessels; and a few hemopoietic cells were discovered near the periphery of the mesonephros. With advanced development, giant glomeruli with a parietal layer lined with a simple columnar epithelium were seen in the ventral and associated boundary between the mesonephros and the developing gonads. Glycogen and alkaline phosphatase elicited an intense response in the capsule, the mesonephric tubule epithelium, renal tubule system, blood vessels, and hemopoietic cells, which was expected and contributed to water conservation and urine concentration. Based on the findings, the camel's mesonephric system had a secretory function and served as a hematopoietic center. The morphology and development of the camel's mesonephric system were comparable to those of other mammalian species, however, with unique traits and characteristics.

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INTRODUCTION

Camels (*Camelus dromedarius*) are unique livestock adapted to hot, arid environments due to their specific morphological, anatomical and physiological characteristics **(Schwartz, 1992)**. Furthermore, a comparative analysis of the dromedary camel genome revealed complex features related to desert adaptations, including fat and water metabolism, stress responses to heat, aridity, intense ultraviolet radiation and choking dust **(Wu *et al.*, 2014).**

Water conservation is a physiological adaptation mechanism that allows the camel to decrease or endure severe water loss **(Gebreyohanes and Assen, 2017).** The kidneys of the camel are widely known to be specialized organs responsible for water conservation mechanisms by raising urine osmolality **(Abdalla, 2020).** It is well known that the camel kidneys have a well-developed medulla and a long renal loop **(Abdalla and Abdalla, 1979)**, which contribute to and are successful in concentrating urine and limiting its flow **(Ouajd and Kamel, 2009).** During dehydration, the kidneys control water loss by

maintaining a lower glomerular filtration rate and elevating tubular reabsorption of water **(Etzion and Yagil, 1986; Kataria *et al*., 2007).**

The kidneys are derived from the intermediate mesoderm **(Sainio and Raatikainen-Ahokas, 1999; Mcgeady *et al*., 2017).** During mammalian foetal development, three types of kidneys were identified: pronephros, mesonephros, and metanephros **(Mcgeady *et al*., 2017).** Mesonephros is a transitory stage of the mammalian kidney that differs in terms of structure, location, function and cellular regression **(Oyer, 1992; Sainio *et al*., 1997 ;Sainio and Raatikainen-Ahokas, 1999; Nishino, 2001; Mcgeady *et al*., 2017; Mario, 2018).** Prenatal development of the kidneys has been studied by many investigators in camels and other mammalian species **(Zamboni and Upadhyay, 1982; Merchant-Larios *et al*., 1993; El-Harairy *et al*., 1998; Merchant-Larios and Moreno-Mendoza 1998; Elgozouli and Osman, 2022).** During the early foetal development in camels, the mesonephric kidney served as a hemopoietic system and as a source of numerous stem cells, including ovarian stroma, interstitial and follicular cells, and adrenal cortex precursors **(Aly, 2007; Salehi and Morovati-Sharifabad, 2012; Hidaia and Osman, 2021).**

The development of the camel mesonephros is poorly described **(Aly, 2007; Osman *et al*., 2008; Salehi and Morovati-Sharifabad, 2012; Jaji *et al*., 2022).** Furthermore, a strong emphasis on prenatal kidney development is desirable. As a result, the primary goal of this study was to investigate if the morphology and early development of the dromedary camel's mesonephric system were similar to those of other mammals, with a focus on the structure and function of the mesonephric kidney.

MATERIALS AND METHODS

Sample collection and preparation

A total of fourteen (14) camel embryos (*Camelus dromedarius*) during the first trimester of gestation (age: 71–112 days) were used. The embryos were obtained from Tamboul and Nyala Slaughterhouse (Sudan), where the animals were slaughtered under official licence and supervision for meat production. The approximate age of the fetuses was estimated by using:

The following equation was adopted by **Elwishy *et al*., (1981).**

$$AG = \frac{CVRL + 23.99}{0.366}$$

Where GA: Gestational Age in days; CVRL: Crown vertebral-rump length.

Gross anatomy

For gross anatomy investigation, the camel fetuses aged 71, 73, 107 and 112 were processed after being fixed with 10% formalin. The fetuses were carefully dissected. The anatomy and topography of the mesonephric system were examined.

Histology

For the histological investigation, the specimens of camel fetuses aged 73, 75, 85, and 93 were obtained and then fixed with 10% buffered formalin. For general histological observations, the specimens were taken either the entire fetus or specimens of mesonephros with a size of the specimens was about 0.5 cm^3. They were prepared using standard histological techniques and stained with hematoxylin and eosin (H&E), and photomicrographs were photographed using an Olympus microscope **(Bancroft and Stevens, 2008**; **Suvarna *et al*., 2019).**

Histochemistry

Glycogen particles

Small pieces of tissue up to 0.5 cm^3 thick were fixed in 10% buffered formalin for glycogen investigation. Then, the specimens were processed for paraffin wax sections and then stained with the periodic acid Schiff (PAS) technique **(Suvarna *et al*., 2019).** The control-positive sections for glycogen were treated with 0.1% malt diastase for 30 minutes.

Alkaline phosphatase enzyme

The calcium phosphate method adopted by **Suvarna *et al*., (2019)** was used for the detection of alkaline phosphatase as described by **Drury and Wallington, (1980); Suvarna *et al*., (2019)**. Fresh frozen sections were fixed in cold action at 4ºC before being processed by routine histochemical procedures **(Drury and Wallington, 1980; Suvarna *et al*., 2019).**

RESULTS

Gross anatomy

At the stage of 2cm CVRL (71 days of gestation), the mesonephros occupied a large portion of the abdominal cavity, expanded by lateral symmetry and curved-cylindrical-shaped from the diaphragm to the pelvic cavity in the dorso-sublumbar regions, constituting the second largest foetal organ after the liver (Fig. 1a). At the stage of 2.8cm CVRL (73 days of gestation), the mesonephros extremities had an oval shape that was related ventrally to the abdominal cavity and pelvic floor (Fig. 1b). The genital ridge occupied the medial border of the mesonephros, while the mesonephric duct was found on its ventral surface (Fig. 1b).

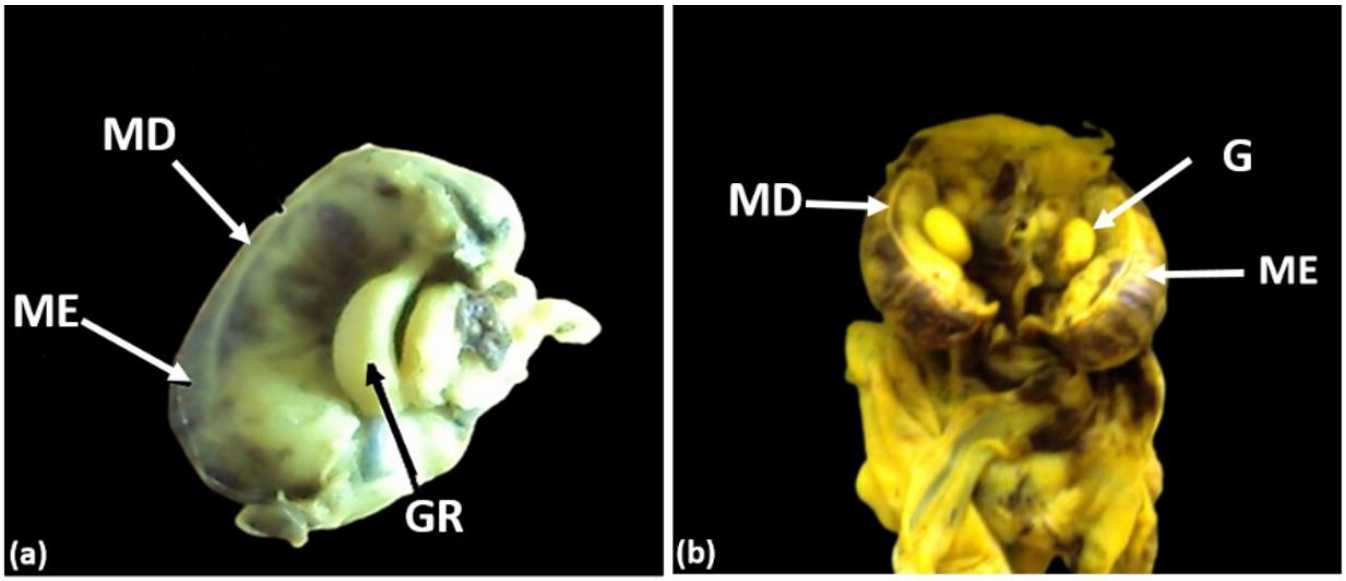


Fig.1a: a photograph of the dissection of a 2cm CVRL camel fetus (71 days of gestation) showing the mesonephros (ME): the genital ridge (GR) is found at the medial border of ME and the mesonephric duct (MD) is found at its ventral surface. Fig. 1b: a photograph of the dissection of a 2.8cm CVRL camel fetus (73 days of gestation) showing the mesonephros (ME), the gonads (G) found at the medial border of ME and the mesonephric duct (MD) is found on its ventral surface.

At the middle stages of the first trimester (15cm CVRL, 107 days of gestation), the mesonephros shriveled at its cranial extremities, reduced in size and extended caudally to position itself near the developing metanephros and gonad regions (Fig. 2a). The mesonephros continued to shrink, forming the shape of a tiny kidney (Fig. 2b).

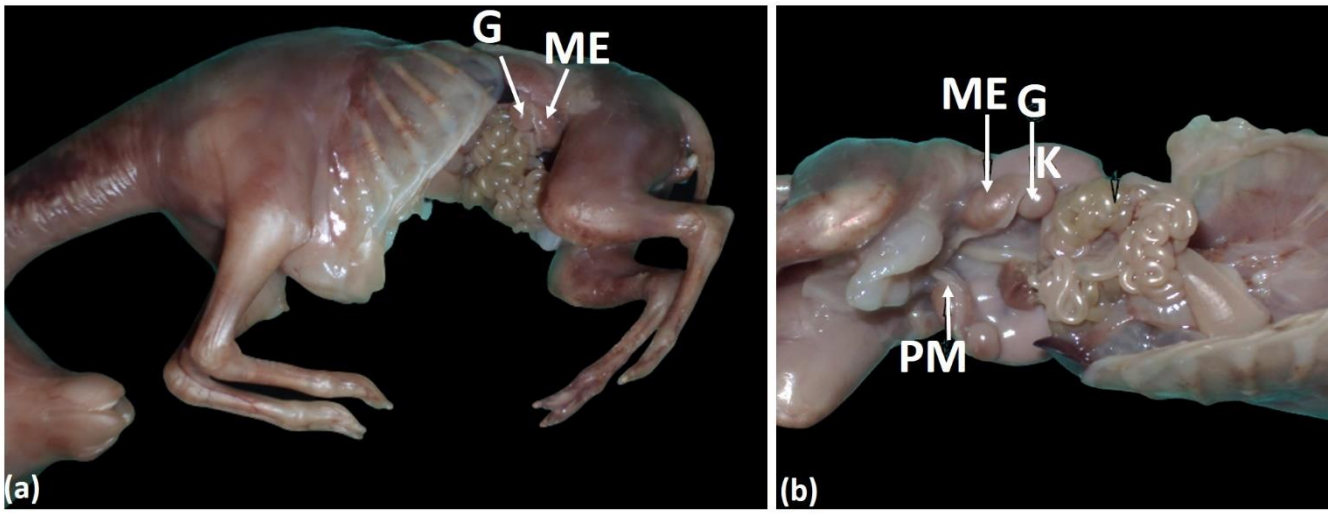


Fig. 2a and b: photographs of the dissection of a 15cm CVRL camel fetus (107 days of gestation) showing the mesonephros (ME), and its relation between the metanephros kidney (K), gonad (G), and the paramesonephric duct (PM).

At 17cm CVRL (112 days of gestation), the mesonephros had a shallow fisher surface and was located caudoventrally to the developing metanephros (Figs. 3a and b). By the end of the first trimester, the mesonephros was fully degenerated, and its remnant was visible as a small prominent triangular shape.

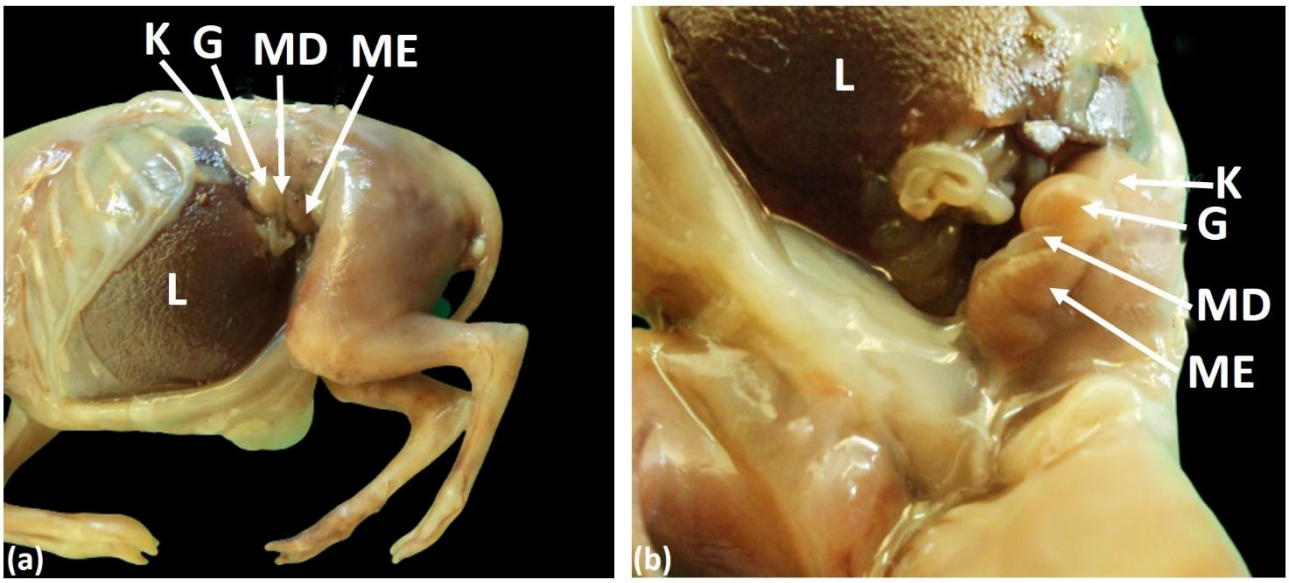


Fig. 3a and b: photographs of the dissection of a 17cm CVRL camel fetus (112 days of gestation) showing the position of the degenerated mesonephros (ME) with the metanephros kidney (K), the gonad (G), the mesonephric duct (MD) and the liver (L).

In the early stages of the first trimester (2.8 cm CVRL, 73 days of gestation), the mesonephros was enclosed by a thin capsule of mesenchymal connective tissue (Fig. 4a). There were irregular mesonephric tubules with large lumens interspersed with tiny blood vessels (Fig. 4a). All of the mesonephric tubules were lined with simple cuboidal cells, with only a few hemopoietic cells, which were discovered near the periphery of the mesonephros (Fig. 4a). At the stage of 3.5 cm CVRL (75 days of gestation), the capsule of mesenchymal connective tissue became more visible (Fig. 4b). The mesonephric tubules appeared in different sizes (small, medium, and large) and shapes (oval, spherical, and irregular), accompanied by increased numbers of blood vessels and hemopoietic cells (Fig. 4b). The stroma of mesenchymal connective tissue appeared.

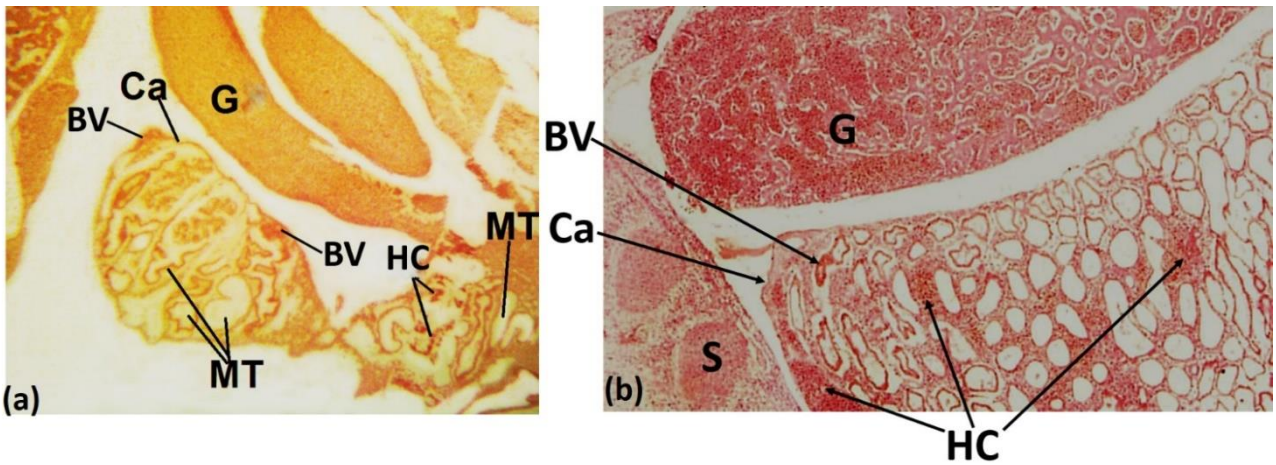
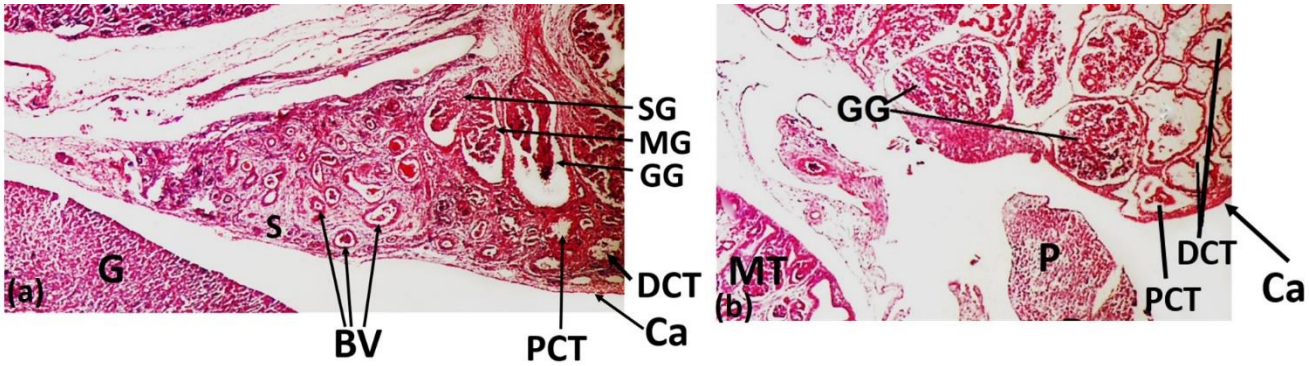


Fig.4a: a photomicrograph of the histological features of the mesonephros of a 2.8 cm CVRL camel fetus (73 days of gestation) showing a thin capsule (Ca), the mesonephric tubules (MT), the precursors of the hemopoietic cells (HC) and blood vessels (BV) with the gonad (G). H&E (X 4).
Fig.4b: a photomicrograph of the histological features of the mesonephros of 3.5cm CVRL camel fetus (75 days of gestation) showing visible capsule (Ca), hemopoietic cells (HC) and blood vessels (BV), somite (S). H&E (X10).

At the stage of 7 cm CVRL (85 days of gestation), the stroma of mesenchymal connective tissue became denser, particularly at the connected border with gonads. Small, medium, and large (giant) glomeruli, the proximal and distal convoluted tubules (PCT and DCT), and Bowman's capsule were seen (Figs. 5a and b). The developing mesonephric corpuscles were seen at the sites of both developing gonads and metanephros (Figs. 5a and b).



Figs. 5a and b: photomicrographs of the histological features of the mesonephros of a 7 cm CVRL camel fetus (85 days of gestation) with the metanephros (MT), the gonad (G) and the pancreas (P): showing a dense capsule (Ca), stroma (S), glomeruli in three size small (SG), medium (MG) and giant glomeruli (GG); proximal convoluted tubule (PCT), distal convoluted tubule (DCT), and numerous blood vessels (BV) with various sizes. H&E (X10).

At the stage of 10 cm CVRL (93 days of gestation), the giant glomeruli were discovered with advanced development and occupied the ventral and associated boundary between the mesonephros and the developing gonads (Figs. 5 and 6). The parietal layer of the giant glomeruli capsule and Bowman's capsule were lined with a simple columnar epithelium (Fig. 6). The mesonephric tubules (PCT and DCT) were located near the giant glomeruli or at the periphery of the mesonephros; some of them showed secretion in their lumina (Figs. 5b and 6). At this stage, the mesonephric tubules became thicker; some of them were lined with a simple columnar epithelium. The blood vessels and the hemopoietic cells were present (Fig. 6).

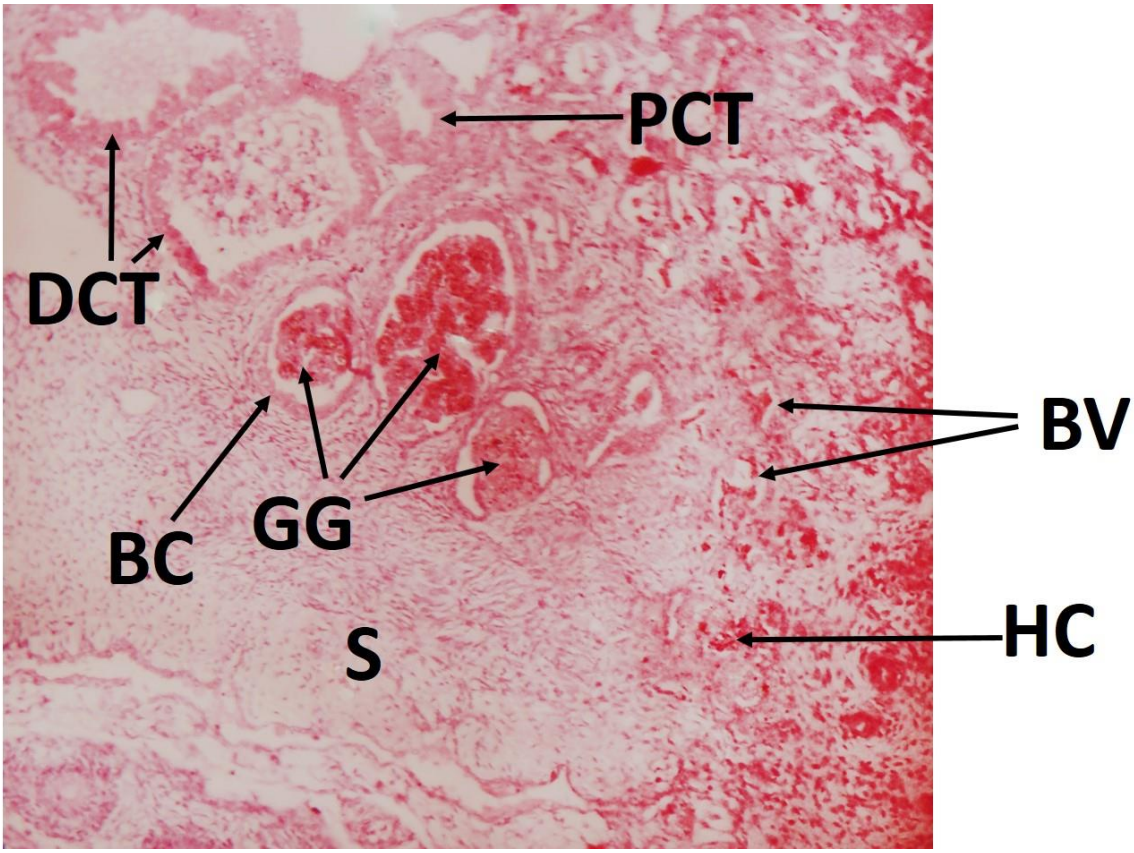


Fig. 6: a photomicrograph of the histological features of the mesonephros of a 10 cm CVRL camel fetus (93 days of gestation) showing a dense mesonephric stroma (S), the giant glomeruli (GG) consist of parietal layer of the Bowman's capsule (BC) composed of a simple columnar epithelium, the proximal convoluted tubule (PCT), distal convoluted tubule (DCT) and clear hemopoietic cells (HC). H&E (X40).

Histochemistry

At the stage of 6 cm CVRL (81 days), the glycogen particles showed a strong reaction in the capsule and the blood vessels, whereas the hemopoietic cells showed a moderate reaction (Fig. 7a). The glycogen particles showed a mild reaction in the wall of the mesonephric tubules, PCT and DCT, and in the wall and lumen of mesonephric glomeruli (Fig. 7b).

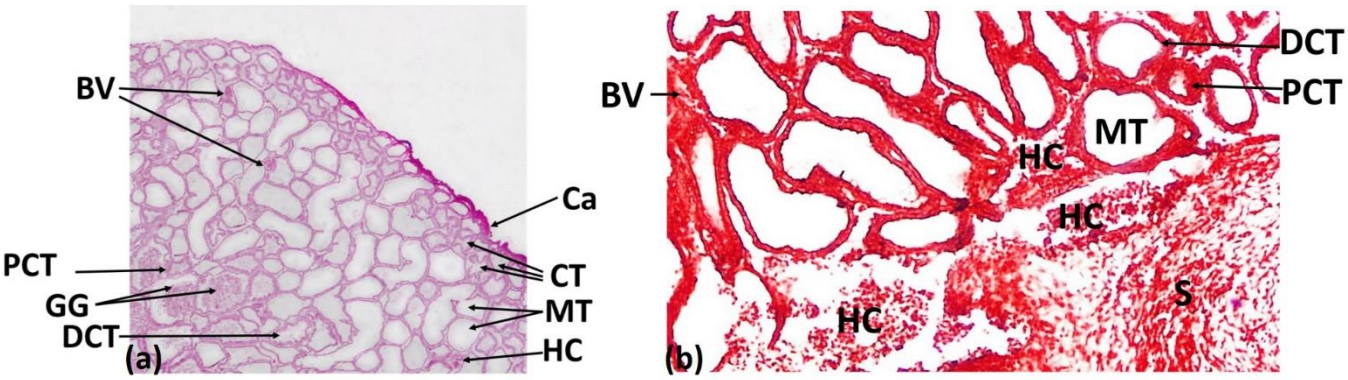


Fig.7a: a photomicrograph of the histochemical features of the mesonephros of a 6cm CVRL camel fetus (82 days of gestation) showing glycogen particles reaction in the developing mesonephros as dark magenta color in the capsule (Ca) and blood vessels (BV), light color in the hemopoietic cells (HC). The giant glomeruli (GG), the proximal convoluted tubule (PCT), the distal convoluted tubule (DCT), the collecting tubule (CT) and the mesonephric tubules (MT) showing moderate reactions (lighter magenta color). PAS (X4).
Fig.7b: a photomicrograph of the histochemical features of the mesonephros of a 3cm CVRL camel fetus (74 days of gestation) showing a strong reaction of the alkaline phosphatase activity in the hemopoietic cells (HC), the blood vessels (BV), the proximal convoluted tubule (PCT), the distal convoluted tubule (DCT), the mesonephric tubules (MT) and the stroma (S). (X10).

DISCUSSION

The main findings of the current study were that the mesonephros at early development, occupied a large portion of the abdominal cavity, constituting the second largest foetal organ after the liver, regressed during the middle stages of the first trimester and extended caudally were situated near the developing metanephros and gonads regions, and were fully degenerated by the end of the first trimester.

The results obtained in the present study revealed that during the early stages of development, the mesonephros had a curved-cylindrical shape, occupied a large portion of the abdominal cavity, and expanded by lateral symmetry from the diaphragm to the pelvic cavity in the dorso-sublumbar region. The results agreed with **Jaji *et al*., (2022)**, who observed that the developing kidneys of dromedary camels were retroperitoneally located below the lumbar transverse processes throughout the prenatal developmental period and were symmetrically adhered to the fatty tissues surrounding the first three lumbar transverse processes by the third trimester of gestation. However, the current results contradicted the findings that reported that the mesonephros during its early development exhibits an arrow strip along the roof of the thoracolumbar region of the vertebral column in camels and other mammalian species **(El-Harairy *et al*., 1998; Aly, 2007; Osman *et al*., 2008; Salehi and Morovati-Sharifabad 2012; Gaber, 2017).**

The present study clarified that the mesonephros were observed at 2 cm CVRL stage (71 days of gestation). The results agreed with **Aly, (2007),** who observed a well-developed mesonephros at 1.9 cm in CVRL camel embryos. Other investigators reported that the mesonephros was first observed at 5, 6, 8, 9, and 10 mm CVRL stage camel embryos **(Osman *et al*., 2008).**

In the present study, at the stage of 2.8 cm CVRL camel fetus (73 days of gestation), the extremities of the mesonephros had an oval shape and were related ventrally to the abdominal cavity and pelvic flour, while the mesonephric duct was found on the ventral surface of the mesonephros. The gonads occupied the medial border of the mesonephros. The results disagreed with **El-Harairy *et al*., (1998); Aly, (2007); Osman *et al*., (2008),** who emphasized that the terminal ends of the mesonephros appear as an arrow strip at the early stage of development.

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The current gross anatomical results revealed the mesonephros reduced in size during the middle stages of the first trimester (15 cm CVRL). The shrinkage was initially discovered at the cranial extremity of the mesonephros and then continued caudally. Additionally, the mesonephros migrated caudally to the developing metanephros and gonads regions, which agrees with the previous findings in camels and other mammalian species **(Zamboni and Upadhyay, 1982; Ludwig and Landmann, 2005; Aly, 2007; Bello *et al*., 2013; Gaber, 2017).** On the other hand, the mesonephros was fully degenerated and its remnant was visible as a small prominent triangular shape in a 17 cm CVRL camel fetus. Similar findings were described in camels and other mammalian species **(Zamboni and Upadhyay, 1982; Ludwig and Landmann, 2005; Aly, 2007; Gaber, 2017).**

The present observations demonstrated that the position of the mesonephric ducts varied during the early development of mesonephros in camels. During early development, the mesonephric ducts were found on the ventral surface of the mesonephros. Similar results have been reported by **Aly, (2007)** in camels. The findings disagreed with **Kaufman, (1992),** who mentioned that the mesonephric ducts were located on the lateral aspect of the mesonephros in mice.

The current investigation revealed that mesonephric tubules appeared in different sizes (small, middle and large) and shapes (oval, spherical, irregular) and were lined by two types of epithelial cells (simple, cuboidal, and columnar). The findings agreed with those of **Osman *et al*., (2008)**, who identified two types of mesonephric tubules in camel embryos. The results obtained in the present study demonstrated that the renal corpuscle (giant glomeruli and Bowman's capsule) and renal tubules (PCT, DCT, and CD) were present in association with advanced development. Similar findings were reported in camels **(Aly, 2007 and Osman *et al*., 2008).**

The present study documented that the parietal layer of the giant glomeruli capsule is lined with a simple columnar epithelium with a wider urinary space than that of the permanent kidney glomeruli, while in the adult kidney; the partial layer of the glomerular capsule is lined with a simple squamous epithelium **(Bloom and Fawcett, 1986 and Eurell and Frappier, 2006).** Scanning electron microscopy findings of a 17 cm CVRL camel embryo (**Elgozouli and Osman, 2022)** observed small mesonephric corpuscles in different sizes and shapes and a narrow urinary space lined by thin, elongated fenestrated squamous cells in the parietal layer of Bowman's capsule.

Molecular biology studies on mammalian kidneys indicated that the entire process of mesenchymal– to- epithelial transformation development involves a large number of molecules that are encoded by different genes and regulated by signalling, growth, transcription, the extracellular matrix, and other factors **(Kuure *et al*., 2000; Little, 2001; Wellik, 2011; Mcmahon, 2016).** Therefore, further molecular and genomic investigations are needed to identify the major genes and factors expressed during early mesonephrosis development in camels.

In the present study, the mesonephric tubules were simple and non-convoluted during the early stages of development, then became more coiled and secreted in their cores with advancing gestation. This result suggested that the mesonephros of the camel served as a secretory organ. The relative thickness of the mesonephric tubules can be considered an index and a predictor of maximum urine-concentrating ability. The current study also documented the presence of numerous hemopoietic cells located between the mesonephric tubules and adjacent to the giant glomeruli. **Osman *et al*., (2008)** noted that the mesonephros acts as a hematopoietic center. Furthermore, **Sainio and Raatikainen-Ahokas, (1999)** have reviewed the role of the mesonephros as a source of hematopoietic stem cells. On the other hand, the role of the mesonephros as a functional kidney in fish, amphibians and during mammalian embryogenesis and the development and regression of mesonephric nephrons have been reviewed by **Seely, (2017). Bertrand *et al*. 2010** stated that the hematopoietic stem cells were derived directly from the haemogenic endothelium lining the dorsal aorta during the embryonic development of zebra fish.

Moreover, **Medvinsky and Dzierzak, (1996)** found that the aorta gonad-mesonephros area represents the source of the hematopoietic stem cells in mouse embryos. Many investigators concluded that the mesonephric kidney served as a hemopoietic

system during embryogenesis in camels and sheep **(Upadhyay Zamboni 1982; Aly, 2007; Salehi and Morovati-Sharifabad, 2012).** Therefore, the present study suggests that the camel's mesonephric kidney acts as a hematopoietic stem cell and primitive hematopoiesis precursor during the early stages of development.

According to the present observations, developing mesonephric corpuscles were found at the site of gonads and in association with metanephros development. The findings in the present study agreed with previous results in camels and other mammalian species, in which the mesonephros has a role in gonadal differentiation **(Zamboni and Upadhyay, 1982; Merchant-Larios *et al*., 1993; Merchant-Larios and Moreno-Mendoza 1998; Hidaia and Osman 2021).** The current investigation demonstrated that blood vascularization increased as gestational age increased. These findings are in agreement with the previous results in camels and humans **(Aly, 2007; Osman *et al*., 2008; Ukey *et al*., 2018).** Moreover, morphological observations in camel's mesonephros reported a high distribution of hemopoietic cells in different stages of development in the tuft of sinuses of the large renal corpuscles in the small renal corpuscles during the first three months of gestation **(Elgozouli and Osman, 2022).**

Despite recent studies on immunohistochemistry in the mesonephric kidney of camels, few data are available regarding the detection of glycogen and alkaline phosphatase (ALP) enzymes **(Osman *et al*., 2008; Abdalla, 2020).** The results obtained in the present study demonstrated that the glycogen particles showed a strong reaction in the capsule, the mesonephric tubules. The findings are in agreement with **Osman *et al*., (2008),** who showed that the mesonephric tubules display a positive reaction to PAS. The ALP usually occurs at the site where PAS-positive diastase-resistant material is demonstrable, which is linked to the possible role of ALP on the membrane transportation of substances **(Eurell and Frappier, 2006; Gartener *et al*., 2011).**

It is well documented that ALP plays a major role in membrane transport and reabsorption **(Sharma *et al*., 2014)** particularly for water and glucose; therefore, the intensity of ALP activity in the camel's kidney has been expected to contribute to water conservation and urine concentration **(Abdalla, 2020).** The present study demonstrated intensive ALP activity in the mesonephric kidney in camels, mainly in the epithelial cells of the mesonephric tubules, renal tubule system (PCT, DCT, CD), blood vessels, and hemopoietic cells. Similar results have been reported by **Abdalla, (2020),** who concluded that the intensity of ALP activity detected in the camel's kidney indicates the first step of water conservation and emphasizes the role of ALP activity in tubular reabsorption mechanisms. In regard to ALP activity throughout the camel's mesonephric kidney, the present findings revealed that the strong ALP activity detected is probably due to the diversity of cellular structures and the function of different parts of the mesonephric kidney that are linked to water conservation and the production of concentrated urine.

On the other hand, **Miner, (2011)** stated that the variation in the components of the basement membranes in mammalian kidneys is thought to be linked to the diversity of cellular structures and activities. Furthermore, the present study suggested that the intensive ALP activity observed in the hemopoietic cells explained that the mesonephric kidney served as a hemopoietic system during the early foetal development of camels. In the present study, the mild reaction of glycogen particles on the wall of the mesonephric tubules and the lumen of mesonephric glomeruli associated with the presence of degenerated mesonephric tubules explained that the time of mesospheric regression may be accompanied by increased activity of the acid phosphate enzyme. It is well known that acid phosphatase is a lysosomal enzyme found in degenerating cells found in sporadic places **(Gartener *et al*., 2011).**

CONCLUSION

The mesonephric kidney of dromedary camels has unique anatomical and histochemical characteristics required for the camel's physiological adaptation to water conservation and the production of concentrated urine. The efficient immune-histochemical activity of mesonephric kidney components (glycogen and ALP responses) allows the camel's kidney to conserve water and adapt to desert and hot environments.

Conflict of interests

The authors declare no potential conflict of interest.

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