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Morphological and histological study of kidney in sheep fetus at first trimester of prenatal period(*Ovis Aries)*in AL-Muthanna

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Abstract

The study focused on kidney development in Awassi sheep fetuses that were collect from Al-Muthanna province. The fetuses were taken from pregnant slaughtered in Al-Muthanna slaughterhouses for different age of pregnant period and fetus were disscted at the college of veterinary Medicine, Al-Muthanna University . To study the embryonic description and the changes that occur in the kidney tissue and developments during the fetus's life period inside the uterus in the first trimester at the age of 40 days, it was noted that the fetus was at a weak and incomplete growth level in terms of the external description of the body. Also, the study found that at the level of the internal organs of the fetus, they were structures

in a developmental stage and were still thin in structure and creamy in color. The kidney was lobed, oval in shape and small in size. At the level of the histological study, the kidney had primitive structures and the cortex surrounded the medulla area and there was no dividing line between them. The study also found clusters of mesenchymal cells in the renal tissue at this age stage.

Keywords: kidney, fetus, prenatal, Histological, Morphological

I. INTRODUCTION

The urinary system is considered one of the most important systems in the body, which is a system shared with the male and female reproductive systems. The urinary system consists of the kidneys, ureters, urinary bladder, and urethras. These organs form urine , store it ,and transport it to the outside through a precise and somewhat complex mechanism in kidney (Biga et al., 2020)

The kidneys are one of the main organs in the urinary system that regulate fluid concentration, acid-base balance and electrolyte Components (David, 2010 and Thotakura & Anjum, 2020). Histological structure:

The kidneys are composed of several layers, including the renal cortex and

renal medulla. The renal cortex contains the glomeruli , the proximal and

distal convoluted tubules, while the renal medulla consists of the renal

pyramids and the loops of henle (Al-Jebory, 2012 and Bello, 2016).

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II. MATERAL AND METHOD

Collection of specimens:

Five fetuses of 40-day were obtained from slaughterhouses in Al-Muthanna Governorate from slaughtered pregnant females. Samples were carefully collected, cleaned, and the kidneys were extracted for histological studies. These steps were carried out at the College of Veterinary Medicine, Al-Muthanna University.

Sample Fixation

The samples were fixed in 10% formalin for 48 hours, then embedded in paraffin, and the inclusions were cut out. The specimen were trimmed and then washed by distal water tap for two to three hour for removing of the formalin.

Dehydration : This process was done to remove the extractable water by passing the samples through series of ethyl alcohol (70% -80%-90%-100%) twice in each run .These specimens were allowed to stand in each grade for two hours , in order to remove all the extractable water from it.

Clearing

Clearing was achieved by passing the specimens through xylene in two steps for 30 minutes to 1 hour for each step.

Infiltration and Embedding (Blocking)

The specimens were transported to melted paraffin in two steps. Each step rest for four hours in electron oven at 58ºc.After that the specimens were put in blocks of pure paraffin (Suvarna et al., 2012).

Sectioning

The sectioning was made by using the rotary microtome thickness of sections (5-6 µm).The histological sections were transferred to water bath (52 ºc) to plain the tissue.

Mounting

The sections were carried out from bath and fixed on a slide contained mayers albumin (Mixture of egg albumin with glycerin)and the ratio of which was 1:1 .A little thymol was added to it to prevent decay (growth of fungi and bacteria) The slides were dried by an oven with 40ºc for 24 hours(Suvarna et al., 2012).

Staining

The samples were regularly stained with Mayer Hematoxylin and eosin to clarify the general structures(Junqueira & Carneiro, 2005), and Masson's trichrome stain was used to stain connective tissue and collagen.

III. Results and Discussion

The present study demonstrated that the crown-rump length (CRL) of sheep fetuses at 40 days of gestation was 6.9±2.08 mm, while the fetal weight was 61.8±33.3 grams. These findings supported the hypothesis that CRL served as a primary indicator for estimating gestational age during early embryonic development. This observation aligned with the conclusions of Evans and Sack (1973) and Silver (1994), who emphasized the reliability of CRL in determining fetal age during early stages of gestation in ruminants. Moreover, recent studies, such as Fathi et al. (2023), reaffirmed the accuracy of CRL measurements using advanced imaging and anatomical techniques. A progressive increase in both fetal weight and CRL with advancing gestational age was observed, consistent with the

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findings of Dyce et al. (2010) and Noakes et al. (2001), who reported a strong positive correlation between gestational age and physical growth parameters in domestic animals.

Externally, the fetus exhibited a creamy to whitish coloration with some rosy areas on the body wall, likely reflecting the onset of vascularization and skin perfusion. This pigmentation pattern was in agreement with the descriptions provided by Getty (1975) and Noakes et al. (2001), who attributed such changes to the development of the circulatory system during mid-gestational stages.

In terms of organogenesis, the fetus at 40 days of gestation had not yet developed a complete external ear, the eyelids remained closed, and the eyeball appeared prominent and protruding. These characteristics corresponded well with observations made by Kaufman (1992), who noted that external ear and eyelid formation in sheep fetuses progressed gradually between days 40 and 60 of gestation, while the eyeball became prominent around day 40. Despite the general agreement with existing literature, some discrepancies were noted in comparison with other studies. For instance, Ahmed et al. (2014) reported a significantly higher CRL value of 8.5 ± 1.2 mm at the same gestational age, which may have been attributed to breed differences, environmental conditions, or measurement techniques. Similarly, Almubarak et al. (2017) documented more advanced morphological development of the external ear and eyelids at 40 day, suggesting methodological differences, such as fixation techniques or fetal preservation protocols.

Furthermore, recent studies such as Rocha et al. (2023) highlighted the influence of maternal nutrition on fetal growth, indicating that the nutritional status during early gestation could significantly affect both CRL and fetal weight. These findings suggested that variations among studies may also reflect differences in maternal diet and management practices, which were not controlled variables in all comparative studies.

In summary, the results of the current study were largely in agreement with both classical and contemporary literature regarding fetal growth and external morphology at day 40 of gestation in sheep, with minor differences that could be attributed to genetic, environmental, and methodological factors.

Histological Findings of the Kidney at 40 Days of Gestation

The current histological examination of the kidney in a 40-day-old sheep embryo revealed early developmental features. Primary differentiation in both the cortex and medulla was observed fig(2), while the renal pelvis appeared structurally incomplete in comparison to that of a mature kidney. The lumen of the renal pelvis was narrow and irregular in shape, with triangular projections extending into it. The inner surface was lined by stratified squamous epithelium, and the underlying connective tissue contained abundant collagen and elastic fibers fig(3). The inner medullary region appeared narrow and extended into the cortex through several longitudinal projections. It contained clusters of proliferating cells, while fully developed tubular structures were absent, except for a few tubules located near the Corticomedullary junction fig(4).

These findings aligned with those reported by Hashem and El-Shafey (2020), who noted that the kidneys of sheep embryos at this stage exhibited initial differentiation of the cortex and medulla, while the renal pelvis remained immature. Similarly, Kumar et al. (2018) stated that the renal pelvis in early embryonic stages may initially be lined by stratified squamous epithelium, which later transitions into transitional epithelium. Abdel-Magied et al. (2019) also confirmed the presence of dense collagen and elastic fibers in the developing renal pelvis, suggesting their role in providing structural support during early morphogenesis.

In contrast, the current findings differed from those of Singh et al. (2021), who observed the formation of distinct renal tubules as early as 35–40 days of gestation. Moreover, Rahman et al. (2022) reported that the renal pelvis in goat embryos was already lined by transitional epithelium at early stages of gestation, which did not match the stratified squamous epithelium observed in this study. These discrepancies may have been attributed to interspecies differences

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(sheep versus goats), individual embryonic variability, or slight deviations in embryonic age. Additionally, differences in histological preparation techniques and interpretation may have influenced the observed structural features.

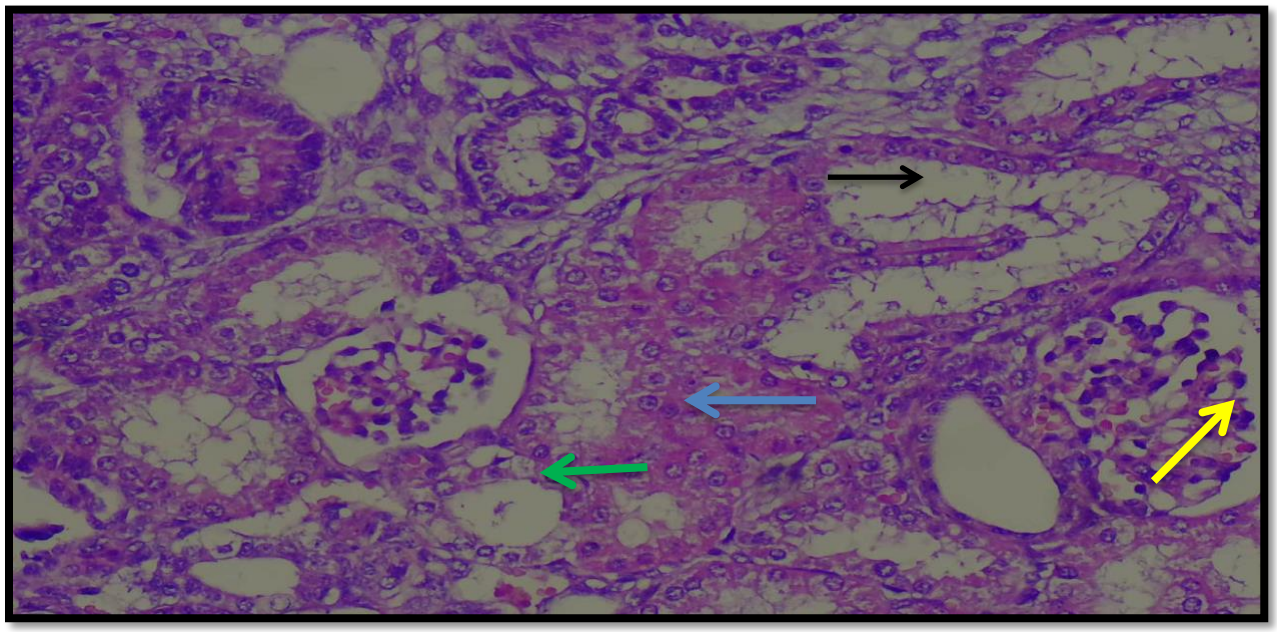

Fig.(1):photomicrograph of the kidney of 40 days old sheep fetuses showing S-shape (black arrow), renal corpuscle(yellow arrow), primitive proximal convoluted tubule(green arrow), primitive distal convoluted tubule(blue arrow), H&E stain ,20x

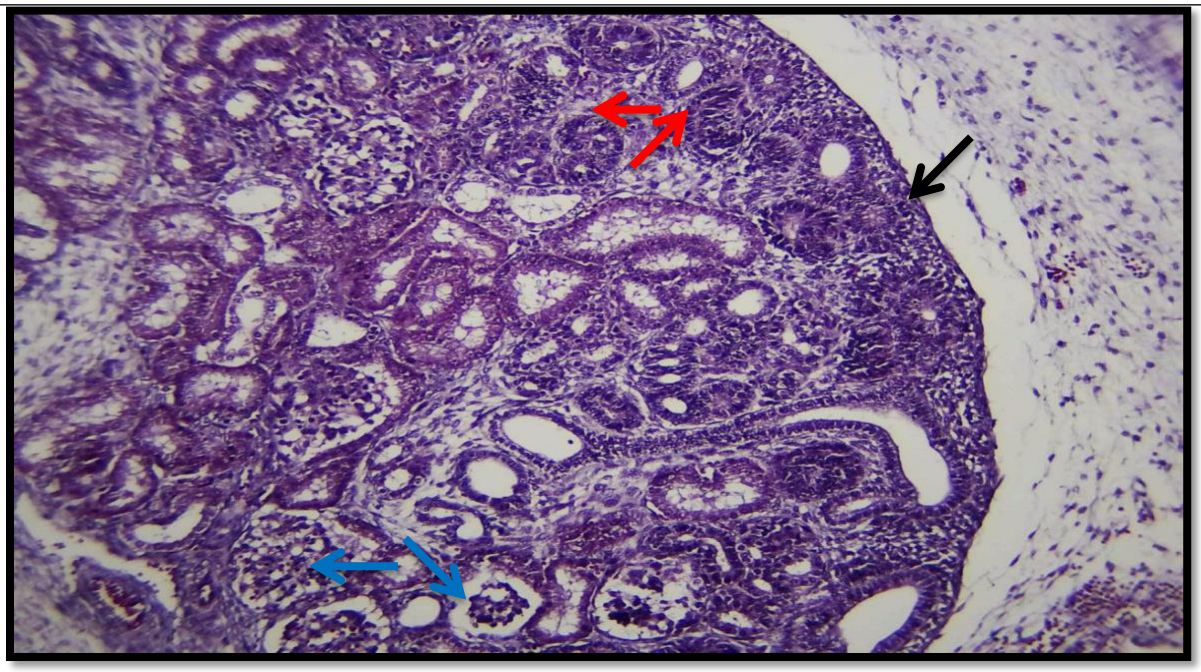

Fig.(2):photomicrograph of the kidney of 40 days old sheep fetuses showing cortex region(black arrow) , coma shape (red arrow), tuft capillary(blue arrow), Masson stain, 10x.

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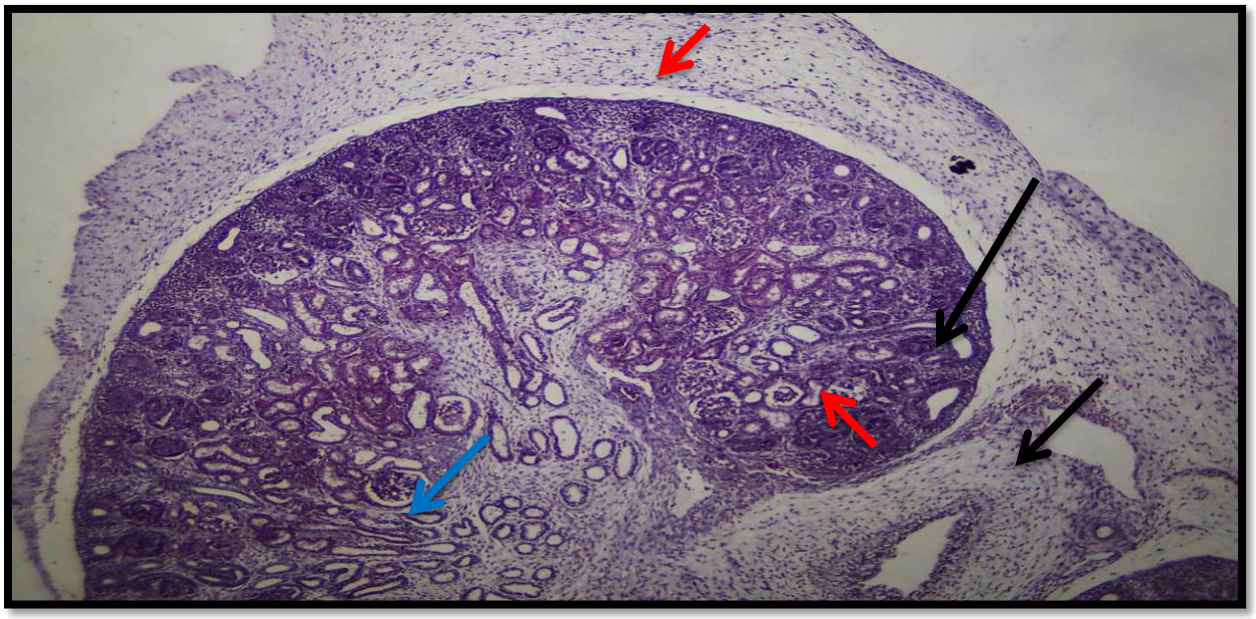

Fig.(3):photomicrograph of the kidney of 40 days old sheep fetuses showing capsule (red arrow) , renal pelvic (black arrow) ,longitudinal structures(blue arrow) Masson stain ,4x.

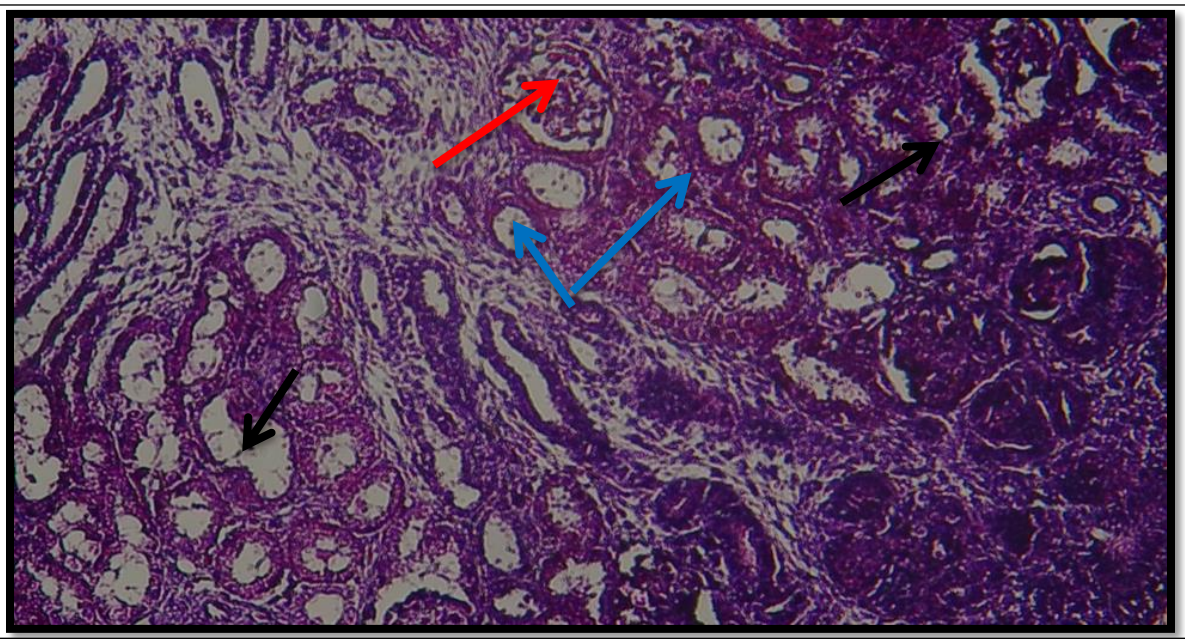

Fig.(3):photomicrograph of the kidney of 40 days old sheep fetuses showing S-shape (black arrow), preemptive glomeruli(red arrow),proximal convoluted tubule(blue arrow) H&E stain,10x

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