Developmental Study of Metanephrose in Local Bovine's Foetuses during 1st and 2nd Trimester of Pregnancy

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Abstract:
The present work was designed to investigate the histological developmental characteristic of metanephros has been done in the local bovine's fetuses in different stages of pregnancy. The study was performed on 24 bovine´s fetuses distributed at six groups (1, 2, 3, 4, 5, 6) months gestational age collected from the slaughtered pregnant cows in the slaughterhouses in Babylon province. The present study showed that the metanephrose at 1st month appear bean – shape composed of primary excretory duct, primitive renal calyex and not yet at the nephron forming stage. The histological study for different stages showed that differentiation and formation of the renal corpuscle, began in the bovine kidney before 2nd month of pregnancy and it is passed through sequential and complex morphological stages in embryonic life, while the juxtaglomerular apparatus is absent and the Henle loops are undifferentiating relatively in 2nd month of gestation. The purpose of our study to provide a more complete quantitative histolodevelopmental description of the metanephros formation in bovine fetuses.

Introduction:
The kidney is an important organ involved in the removal of unwanted nitrogenous substances, excess water and relative maintenance of osmotic concentration of the blood (Salehi and
Morovati, 2012). The urinary system are derived from intermediate mesoderm, the nephrogenic plate and the permanent mammalian kidney begins to develop when the ureteric bud extends into the surrounding metanephric mesenchyme. The ureteric bud arises from an offshoot of the mesonephric duct invading the mesenchyme around it and ultimately forms the ureter, renal pelvis, major and minor calyces and collecting ducts, while the nephroblastic cells of the metanephric blastema, on contact with the ureteral bud, eventually develop into the nephron: renal corpuscle, proximal convoluted tubule, Henle loop and distal convoluted tubule (Shah et al., 2004; Hyttel et al., 2010). Because there is no sufficient studies about the kidney histogenesis in cow’s fetuses so we suggest studying histomorphological description and the timing of first appearance of the components and parts of metanephrose at intrauterine life because it has economical importance in our country.

Materials and Methods:
The bovine foetuses were collected from the adult clinically healthy pregnant cows slaughtered in the abattoirs of Babylon province. The study was performed on 24 bovine’s fetuses distributed at six groups (1, 2, 3, 4, 5, 6) months of gestational age. Their ages were estimated according to the crown rump length (CRL) as described by (Evans and Sack, 1973). The CRL were measured for all fetuses (CRL is the measurement from the vertex of the skull to the midpoint between the apices of the buttocks according to (Arthur et al., 1989). The CRLs at each stage are summarized in the histogram (1) by using verniae callaper. The body weight was recorded for each prenatal fetus by using sensitive balance are summarized in histogram (2). The weight, length, width and thickness of metanephros at each stage is summarized in table (1). Samples were taken directly from bovine’s fetuses and fixed in 10% formalin to left for 72 hours. After fixation, the specimens were washed by tap water for 3-4 hours to remove the formalin solution and transferred to the following steps: dehydration, clearing and embedded and finally cutting and staining by using the rotary microtome and stained with H & E, Van-Geson and PAS stains (Luna, 1968). A computerized program, the Statistical Package for Social Sciences (SPSS) was used to calculate the statistics (Joda, 2008). Finally, the results of the study were recorded of different histological sections by light microscope and (MEM1300) digital eyepiece for microscope installation instructions.
Histogram: 1. Show relationship between CRL of embryo and time of pregnancy

Histogram: 2. Show relationship between weight of embryo and time of pregnancy
Table (1) show weight, length, width and thickness of right and left kidney at each embryonic stage

<table>
<thead>
<tr>
<th>Parameter period</th>
<th>Right kidney</th>
<th>Left kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight (gram)</td>
<td>Length (mm)</td>
</tr>
<tr>
<td>2nd month</td>
<td>0.09±0.004 A</td>
<td>7.07±0.063 A</td>
</tr>
<tr>
<td>3rd month</td>
<td>0.73±0.038 B</td>
<td>14.6±0.129 B</td>
</tr>
<tr>
<td>4th month</td>
<td>7.96±0.022 C</td>
<td>37.9±0.263 C</td>
</tr>
<tr>
<td>5th month</td>
<td>10.5±0.290 D</td>
<td>45.5±1.692 D</td>
</tr>
<tr>
<td>6th month</td>
<td>17.5±0.221 E</td>
<td>47.2±1.852 E</td>
</tr>
</tbody>
</table>

Values represent mean ±S.E
Different capital letters mean significant (P≤0.05)

Results and discussion:
The first gestational month, The prenatal development of the bovine kidney in foetuses of (0.17- 0.19) cm CRL (30 to33) post coitus day, the metanephros appears located in pelvic cavity dorsally to mesonephrose composed of primary excretory duct, primitive renal calyx and metanephrogenic cap with no to the composition of renal units, nephrons (fig.1). This confirms the stated of (Hyttel et al., 2010) who stated that metanephros development begins at 25 days cow embryo. On the other hand, our results were different from the facts of (Horster et al., 1999) who recorded that the metanephros originate during 21 days to 28 days of gestation in pig, while (Gaeth et al., 1999) who mentioned that the metanephros in elephant embryo develop between 139 to 166 days and (Tiedemann, 1976) who mentioned that kidney starts to develop in sheep by 27 to 30 days and (Suman et al., 2010) mentioned that the develop of metanephros of buffalo foetii started at 47 days of gestation. These differences go back to the difference in the length of periods of pregnancy.
The second gestational month, at the foetal age of (7 – 8) cm CRL (60 to 65 p.c.d). The metanephros appears located relatively in the sacral region, small in size, having a smooth surface and covered by a thin fibro-muscular capsule and bean – shape like structure located adjacent closely with testes, mesonephros and consider immature organ(fig.2). Histologically, metanephrose is not yet differentiated into a cortex and a medulla with many vesicle stage, comma-shaped, S-shaped bodies and immature glomeruli with collagen fiber are relatively few and poorly development in metanephros parenchyma with more mount covering the external surface (fig.3). Active nephron formation occurs across the developing renal cortex in a band, known as the nephrogenic zone which is thick and evident about 8-10 um in thickness under (4X) as source of nephrons, the nephron arises from metanephric mesoderm and during gestation, the fetal nephron in bovine pass through several morphological changes to reach a structure capable of maintaining volume and the concentration of body fluids. The metanephric mesenchyma has an internal dense medulla which forms the secretory tubule and nephrons, while an external cortex, light loose layer which forms the interstitial connective tissue and capsule.

The beginning of nephron formation occur before second month of gestation in bovine as result the subsequent interaction between the two primordia uretic bud and the metanephrogenic mesenchyma. The metanephric mesenchyme responds to the invading bud by proliferating, then condensing to form coronas of cells around each branch of the bud and within the condensed mesenchyme, cells convert into epithelial cells. These epithelia form oval shape structure called renal vesicles which consider the first step in nephrons development (fig.4) which gradually elongates and convolutes to forms an elongated tubule, the comma-shaped body which consider second stage of nephrogenesis (Fig.5). As development progressive, the comma-shaped body transfrom into next stage of nephron development called S-shaped tubule stage, The upper and middle part of the S-shaped tubule remains tubular and elongates, generates the proximal tubule, loop of Henles, and distal tubule that join the ureteric branch to develop into the collecting ducts. The lower limb of S-shape tubule situated furthestmost from the branch of collecting tubule just below the lower cleft with vascular mesenchyma contained within the concavity of the lower S-curve has long been recognized as glomerulus. The double walled hemispherical structure, which develops from the greater part of the lower S-curve, forms the inner and the outer layer of Bowman’s capsule; the space between the two layers, continuous with the lumen of the tubules, forms Bowman’s space (fig.6) and finally, the maturing glomerulus stage called oval or rounded stage (fig.9).

The current work revealed that metanephrhos in this stage contain proximal convoluted tubules arises from the middle part of S-shaped tubule while
distal convoluted tubules arises from elongate of terminal end of distal portion to S-shaped tubule to connects between the secretory portion (nephron) and excretory renal components. The proximal and distal tubule consider immature and undifferentiating with absent of Henle loop. The uretic bud in current stage from which the ureter derived, Subsequently elongated and become splits into cranial and caudal evident portions (primary branches), the future major calyces which consider the beginning of duct system induction, from up to ten funnel-shape divisions referred to as minor calyces are forms later (fig.3). The differences in beginning in which nephrogenesis prescribed depend on the length of gestation periods in different species and the time in which occur the reciprocal inductive interactions between the two primordial mesodermal derivates: ureteric bud, which is an epithelial outgrowth of the wolffian duct and metanephric blastema. These similar to that proved in domestic mammals by (Canfield, 1980).

*The third gestational month,* at the foetal age of (16 – 18) cm CRL (90 to 95 p.c.d.). Metanephrose appear thick crescent or rose shaped – structure with beginning of lobulation which appear more visible in histological sections than grossly (fig.7). Histologically, the collagen fiber mount in metanephrose parynechyma are few with nephrogenic zone about (7-8) um at (4X) which continues to add new developing glomeruli, the older being located deeper in metanephros and the newer just under the capsule. For this reason, in a single section of fetal kidney in cow, it is possible to appreciate all the glomerular development stages in a sort of gradient of maturation going from the cortex to the medulla of kidney in para-sagittal sections. The present study shows that there is no demarcation between cortex and medulla (fig.8) and the metanephrogenic cap induces the ureteric bud to branch dichotomously, thus continues of the collecting duct system morphogenesis and the minor calyces are capped by metanephric tissue constitute the renal lobes. The distal end forms an open connection with one of the collecting tubules, establishing a passageway from Bowman’s capsule to the collecting unit and continuous lengthening of the excretory tubule results in formation of the distal convoluted tubule.

The proximal convoluted tubule undifferentiated completely lined by an irregular cuboidal epithelium tissue girdled by thin basement membrane with rounded, oval or irregular large nucleus light in color occupy cell with no brush border and narrow lumen comparatively with distal convoluted tubule which appears immature lined by cuboidal epithelium with wide lumen and the cells of have rounded nucleus in shape with light in color (fig.9) and Henle loop are undifferentiated relatively. The microscopic precise structural configuration in our results is not in the same line with in buffalo embryo, the proximal and distal tubules was first observed in the 4.1 cm crown vertebral rump length foetii (Suman *et al.*, 2010)
found in pig fetuses with 4 cm crown-rump length, highly specialized proximal convoluted tubules cells with microvilli, while (Dudley and Robertson, 1997) in the same animals noticed that the appearance and formation of excretory tubules (proximal convoluted tubule, loop of Henel and distal convoluted tubule) in 8 weeks of pregnancy. In human, differentiation and development of tubules began in 9-10 weeks of gestation in metanephros and (Schmidt-ott et al., 2005) in cats, the metanephros in 38-44 days of cat embryo has well differentiation tubules.

The recent study revealed that the juxtaglomerular apparatus represent by macula densa only. In fetal sheep, the first juxtaglomerular apparatus is detected at 45 days of gestation according to (Wintour et al., 1996), while (Groscurth and Inagami, 1985) in human who mentioned that in 8 weeks old embryo renin granules are seen in the juxtaglomerular epithelioid granular cells of metanephric tissue for the first time.

The para-sagittal section of metanephrose appear visible medullary rays composed of collecting tubule and collecting duct (duct of bellini) which terminate with papilla, the collecting duct in cortex lined by simple cuboidal epithelium tissue and gradually translate to columnar in medulla until become pseudostratified epithelium tissue at papillary duct (fig.8).

**The fourth gestational month.** At the foetal age of (28 – 30) cm CRL (120 to 130 pcd). Metanephrose has taken on a more definite shape resembles the berries and lobulated (fig.10) surrounded with collagen fiber which scarce in hilus and papillae opening. The cortex appears nephrogenic zone about 5-6 um in thickness can be seen along the external boundaries of the metanephros with visible demarcation line from medulla. This result in contrast with (Suman et al., 2010) in buffalo claimed that mean time of appearance of cortex and medulla in metanephros occur between (50-54) days, while (Canfield, 1980) who mentioned that the metanephros by 15–18 weeks gestation, had a well organized internal structure consisting of a definize cortex and medulla in bovine.

The present study confirmed that lobation begins in the bovine kidney between third and fourth month of gestation with an average of 17-22 lobes. At this stage, generally 14-22 papillae and calyces are present, corresponding with the same number of lobes and deep clefts on the surface separate the lobes. The histarchitecture of metanephros appear more definitive than in the previous stage. The oldest renal corpuscles located in juxtamedullary region and appeared spherical in shape with evident Bowman's spaces surrounded by a double layered cup – shaped Bowmann’s capsule, the outer or partial layer of Bowman's capsule was simple squamous epithelium supported by a basal lamina. The inner or visceral layer was represented by the unclei of podocytes, also there was the mesangial cells which associated with glomerular capillaries. Between the two layers was the bowman space, which at the urinary
pole leads into the proximal tubule (Fig.11). The juxtaglomerular apparatus associated with vascular pole seen in fourth month of gestation composed of juxtaglomerular cells and macula densa only (fig.11).

The Proximal and distal convoluted tubule more mature than the previous stage, the proximal tubule lined by simple cuboidal epithelial tissue with rounded large nucleus light in color composed of thin and thick part (fig.10) with no brush border while distal convoluted tubule lined by tall cuboidal epithelium with cells have rounded nucleus dark in color (fig.11) while, Henle's loop become visible consists of two part, The first called thin segment of Henle's loop lined by squamous cells whose at least two nuclei bulge into the lumen while the second part called thick segment similar to proximal convoluted tubule lined by low cuboidal cells (fig.12). The collecting tubules and duct more evidence than the previous stage (fig.12).

The fifth gestational month, At the foetal age of (38-40) cm CRL (150 to 160 pcd). Metanephrose has a bunch of grapes in shape (fig.13) and lobulated with visible line between cortex and medulla is seen. The outline boundaries of metanephros cortex occupied by a thin nephrogenic zone about 4-5um in thickness. The whole components of juxtaglomerular complex can be seen associated with vascular pole of more mature renal corpuscle and the extraglomerular mesangial cells (lacis cells) appear for the first time situated between the wall of bowman's capsule and macula densa but, with less development and differentiation (fig.14). The hilus, papillae and space between lobes contain considerable amount of collagen fibers. Histological sections showed the presence of thick capsule which becomes thicker with advancement of gestational age was noticed in the kidneys composed of collagen and elastic fibers. The proximal and distal tubules more development and mature than the previous stage with appears of brush border in lumen of proximal convoluted tubule (fig.15) and evident of collective tubules and collecting duct(fig.14, 15). This is enhanced by (Dellmann and Brown, 1987) and (Bacha and Bacha, 2000) 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 renal papilla.

The sixth gestational month, at the foetal age of (55-60) cm CRL (180 to 190 p.c.d.). The nephrogenic zone about 2-3 um in thickness. Metanephrose take a bunch of grapes like structure in shape and has more evident demarcation line between cortex and medulla. The glomeruli in the cortex of bovine fetus are crowded together, immature and small in size, whereas the older, more mature glomeruli deeper in the cortex are more widely separated and few in number with large size. Glomeruli in the maturing stage resemble adult glomeruli by histology but are smaller in diameter (fig.16). The medulla increases both in length and thickness, in thickness by an
increase in the diameter of the individual collecting tubules and by the downgrowth of the loops of Henle, in length by the growth, in length of the individual collecting tubules and collecting duct. The cortex increases both in thickness by an increase of diameter of convoluted tubules and in length by the downgrowth of convoluted tubules. A renal lobe and lobule appear very clearly where renal lobe composed of a medullary pyramid surrounding by cortical parenchyma that covers the base of a pyramid, whereas the septal cortex surrounds the sides of a pyramid. Thus, a septum (column) of Bertin represents the confluence of two layers of septal cortex from two adjacent lobes. Although a lobe does not represent a functional renal unit, it may be viewed as an anatomic organizational unit while renal lobule composed of medullary ray and surrounding cortical tissue (fig.16) and the bovines metanephrose have no renal pelvis.

These findings coincide with those reported by (Moore and Persaud, 2003) whom they said that the increasing in kidney size resulted from the elongation of tubules as well as the increase of interstitial tissue and in human infant, the increase in renal size during the postnatal period also is due to an increasing in the length and size of established nephrons. The gradual evolution and differentiation advancement of metanephros by formation of the renal corpuscle, tubular system and developmental collective tubules which represent the specialized excretory ducts to complete and facilitate the biological kidney function in the body at sixty month from bovine fetus life, and major calyces, minor calyces, papillary ducts and renal pyramids were clearly showed at this period.

Figure (1) Parasagittal-section at one month of gestation in cow showing
A- Metanephros  B- Mesonephros  C- ureter  D- primitive renal calyces
E- Primary excretory duct  (H&E.X4)
Figure (2) Photograph illustrates kidney in two month of bovine fetuses. A- Left kidney  B- Right kidney  C- testes

Figure (3) Parasagittal-section at 2nd month of gestation in cow showing Metanephros. A- nephrogenic zone  B- mesenchymal tissues  C- vesicle stage, comma-shaped and S-shaped bodies and immature glomeruli  D- minor calyx  E- major calyx (H&E X4)

Figure (4) Cross-section in cortical region of metanephrose at 2nd month of bovine fetuses showing first developmental stage of glomerulus. A- Metanephric cap  B- Branching collecting duct  C- Nephrogenic zone  D- Renal vesicle (Metanephric vesicle) (H&E X40)
Figure (5) Cross-section in cortical region of rabbit kidney at 2nd month of cow fetuses showing second developmental stage of glomerulus (comma-shaped stage). A- Comma-shaped body (H&E X40).

Figure (6) Cross-section in cortical region of kidney at 3rd day of cow fetuses showing third developmental stage of glomerulus (S-shaped body). A- Upper cleft B- Lower cleft C- proximal tubule primordial D- Upper limb E- Lower limb F- Middle limb (P.A.S. X40).

Figure (7) Photograph illustrates location of metanephrose at 3rd month of pregnancy in cow fetuses showing A- left metanephrose B- right metanephrose C- ureter D- testes.
Figure (3) Para-sagittal section at 3rd month of gestation in cow showing cortex and medulla (there is demarcation line between cortex and medulla). A- Cortex  B- Medulla  C- Nephrogenic zone  D- Medullary rays (H&E X4)

Figure (9) Cross-section showing at 3nd month of gestation in cow shows developmental stages of excretory portion in mesonephros. A- Distal convoluted tubule B- Proximal convoluted tubule C- Bowman’s space  E- Parsial layer  F- Visceral layer (H&E X40)

Figure (10) Photograph illustrate left and right metanephrose at bovine fetus at 4th of gestation.
Figure (11) Cross-section showing differentiation of juxtaglomerular apparatus at 4th month of gestation in cow where there is Macula densa and juxtaglomerular cells only. A- Macula densa B- Juxtaglomerular cells C- Distal convoluted tubule D- Bowman’s space E- Parietal layer F- Visceral layer. H- proximal convoluted tubule (H&E.X40)

Figure (12) Cross-section show Henle’s loop of Metanephros at 4th month of gestation in cow. A- Thin segment of Henle’s loop B- Thick segment of Henle’s loop C- Collecting duct D- Collecting tubules (PAS.X40)

Figure (13) Photograph illustrate bovine fetus at 5th of gestation with left & right mesonephros
Figure (14) Cross-section showing juxtaglomerular apparatus at 5th month of gestation in cow. A- Macula densa  B- Juxtaglomerular cells C- Mesangial cells. D- collecting duct (H&E.X40)

Figure (15) Cross-section showing tubules of Metanephros at 5th month of gestation in cow. A- Proximal convoluted tubules  B- Distal convoluted tubules C- Collecting tubules D- brush borders  (PAS.X40)

Figure (16) Parasagittal-section through Metanephros at 6th month of cow gestation showing. A- major calyx  B- Medullary rays C- Nephrogenic zone (H&E.X4)
References:


