

## Histomorphological Development Study of Cecum in Local Awassi Sheep Fetuses (*Ovis Aris*): Prenatal Periods

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**Abstract:** This study was conducted at Al-Qasim Green University/College of Veterinary Medicine, Anatomy and Histology Branch. The experiment was designed to study the cecum of Awassi sheep fetuses: prenatal periods. Samples (30 male and female samples) were collected from healthy females uterine and divided according to age groups into four groups by crown rump length formula (CRL) for the prenatal stage. In the first age group (50-55), the cecum was in the process of development, as it was not distinguished visually. In the second age group (70-75 days), it was a small protrusion located between the ileum and the beginning of the ascending colon. With advancing age, the third group, which includes two ages in the third trimester of pregnancy, we found that the cecum was completely formed in (100-105) days and at (130-140) days of gestation. Also, upon histological study, we found that the wall of the cecum consists of four layers, as in the rest of the parts of the digestive system, and is lined with an epithelial layer surrounded by a layer of connective tissue. It is supportive of the epithelial tissue. We also found tunica submucosa constantly devolving with age, as it is mesenchymal cells in the first stage of intrauterine life and begin to transform into connective tissue with the presence of fibers and collagen (tunica submucosa) as a result of these cells transforming into spindle cells to form the inner muscle layer, as well as tunica muscularis externa. The latter is surrounded by loose connective tissue that contains a few collagen and elastic fibers surrounded by mesothelial cells (tunica serosa) that contain blood vessels to support development in the inner layers.

**Keywords:** Development, fetuses, cecum, prenatal, sheep.

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### INTRODUCTION

Sheep are a significant producer of milk, wool, and meat. About 65% of all the animals in Iraq were sheep. Iraq has a total of five breeds of sheep. (Hamadani, Karradi, Arabi, Naeimi and Awassi), which consider a very economic ruminants for different productions. *Awassi* sheep (*Ovis Aries*) a species distribution in the middle of Iraq fit in to the family Ovidae, Subfamily Ovinae, Genus *Ovis*. (Al-Jebori, J. G. A., Kazem, A. M., 2022).

The digestive tube starts differentiating by the 3rd week of gestation. The buccopharyngeal membrane, that closes the cephalic end of the digestive tube, The digestive tube is divided into the anterior gut, the midgut and the hindgut. The anterior gut extends from the pharyngeal membrane to the duodenum. The cranial

segment or the pharyngeal gut gives rise to the pharyngeal structures while the caudal segment or foregut will result into the esophagus, stomach and duodenum. The midgut starts distal to the entrance of the bile duct into the duodenum and ends at the beginning of the last third of the transverse colon (Cloete, J. H. L., 1939), The hindgut gives rise to the left third of the transverse colon up to the rectum. All major steps of development are completed by the 18th week of gestation. (Huisman, T. A., Kellenberger, C. J., 2008). The midgut is initially in open connection with the yolk sac. However, body folding gradually narrow this connection down to the vitelline duct, and that is later obliterated as the yolk sac regresses. The midgut grows more rapidly than the body overall, and soon it forms a loop suspended in a dorsal mesentery consisting of a cranial descending and a caudal ascending limb. The

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caudal part of the foregut, which, as already mentioned, develops into the oral portion of the duodenum, is positioned more or less horizontally and is connected to the descending limb of the intestinal loop. The first portion of the descending loop develops into the aboral portion of the duodenum, the subsequent portion into the jejunum, and the tip of the loop, where it connects to the yolk sac, into the ileum (Sadler, 2018).

Along with general tubular elongation, the following morphogenic events occur, the elongating loop rotates 360° around the right vitelline a. (adult cranial mesenteric artery), the caudal part of the loop develops a diverticulum, the future cecum. The loop coils forming a spiral or coiled colon. The hindgut terminates in a cloaca, i.e., a chamber that communicates with digestive, urinary and genital systems (Fletcher, T. F. and Weber, A. F., 2004).

The established large intestine is composed of the cecum, colon and rectum. Embryologically, the large intestine is a part of the developing gastrointestinal (GI) tract and shares the same progenitor tissues with the other organs of the GI tract, as it arises during the development of the endoderm. Later during embryogenesis, it incorporates tissue from all three germ cell layers of the trilaminar embryo (Kostouros, A, *et al.*, 2020).

Caecum is commonly termed the first portion of the large intestinal tract. It is a blind ending tube, which is defined from the colon by the entering of the Ileial part of small intestinal tract. In ruminants, carnivores and in the horses the cecum located in the right part of the abdominal cavity (Bryden, M. M *et al.*, 1972).

The small cecum of the sheep relatively is slightly bland and has not Haustra or Taeniae also the cecum is lining in the right part of the abdominal cavity inside the supra-omental cavity a combined with the blind cecal upper end that which caudally oriented (Kirk, X. V., 2017).

In sheep, the histomorphology of the wall of cecum has contained of 4 tunicae; Tunica Mucosa, Tunica Submucosa, Tunica Muscularis Externa and serosal layer. The mucosal layer of caecum was containing of several leaf-folds shaped lining by single layer of columnar epithelial cells with great numbers of Goblet cells. The lamina propria was occupied with glands or crypts of lieberkuhn rest on smooth-muscular layer (lamina muscularis). The submucosal layer of caecum was formed from condensed connective tissues. The lymphatic nodules were distributed all through the mucosal layer. The tunica muscularis was composed of outer longitudinal and inner circular layer of smooth muscle detached via a narrowest connective tissues layer (Sultana, N *et al.*, 2021).

## MATERIAL AND METHODS

This study was designed to described the histomorphological and developmental study of cecum in local awassi sheep (*Ovis aris*) at prenatal and. A samples are collected in (September to November) of year (2023). The study is performed on (Bass, L. M., Wershil, B. K., 2016) sheep fetuses, that are collected from pregnant ewes slaughtered in the abattoirs of Najaf provinces for prenatal study. A total of (30) sheep fetuses collected from uteri of the awassi sheep pregnant females for prenatal stage were used in current study, which is determined depending on the crown rump length using of following formula ( $Y=2.74X+30.15$ ) where 'Y' is developmental age of fetus in days and 'X' is the "crown-rump length in cm.

The cecum of sheep's fetus were fixed in (10%) buffered formalin, dehydrated in a graded series of alcohol, cleared in xylene then embedded in paraffin wax. The blocks were sectioned at 5- 6 μm thickness of slice using a rotary microtome histological sections were stained with Haematoxylin and Eosin (H&E), periodic acid schiff (PAS) and trichrome masons stain (Abd-El-Hady, A. A. A *et al.*, 2013). The sections were studied using Olympus light microscope with digital camera USB which connected with the computer slides and attachment at different magnification.

## RESULTS AND DISCUSSIONS

### First Trimester of Gestation (50-55) days

The body weight of sheep embryos in the current stage at (50-55) days of gestation is about (41.6± 0.927) grams, gastrointestinal tract weight about (2.17±0.050) gram and crown rump length in about (10.4 ± 0.509) cm (Figure 1).

Morphologically, at this age of embryo, the current study of large intestine appear elongated translucent tube in appearance, A lot of turning around and whitish in color extended from ending of small intestine (illio-colic junction) without marked appearance of cecum (Figure 2).

At this stage of gestation the cecum not be Differentiated at this age of gestation, This current study different with (Verma, A *et al.*, 2021). Age related variation in gross anatomy of caecum in prenatal goat (*Capra hircus*). Who showed that cecum very small bulge located between the ilium and colon.

At second trimester of gestation (70-75) days the body weight of sheep embryos in current stage at (70-75) days of gestation about (266.6 ± 2.088) gram, gastrointestinal tract weight about (67±1.760) gram and crown rump length about (19.6 ± 0.678) cm.

Cecum relatively formed, appear cylindrical in shape, smooth wall and short in length about (1.04±0.092) cm, ileo- cecal fold extend along between ileum and cecum and only small part from cecum apex

was free while; the other fold called ceco-colic fold less in length than the first one with no longitudinal bands (Figure 3). This study is consistent with a previous study

by (Verma, A *et al.*, 2021) which described the development of cecum in goat at same age.



Figure 1: Photograph of sheep fetus and using vernier caliper to measuring the crown rump length to detect the age of fetus

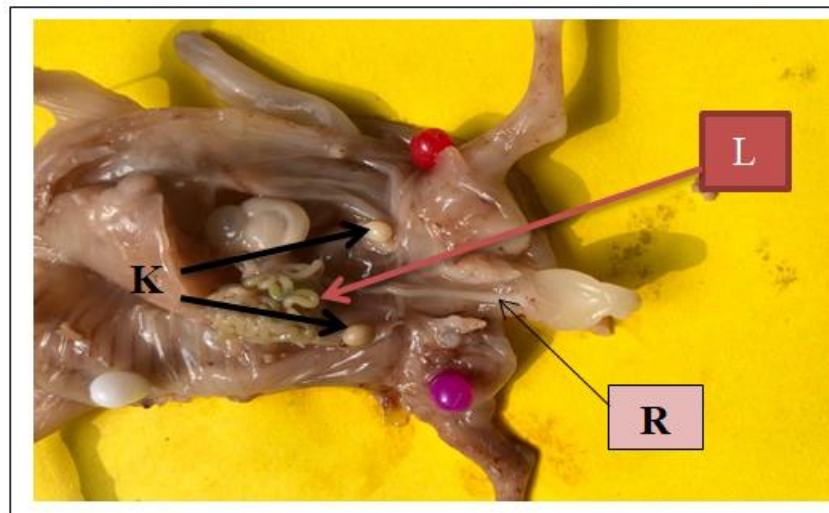


Figure 2: Photograph showing the large intestine(L) of sheep fetuses at (50-55) days of gestation, Rectum (R) and right & left kidneys

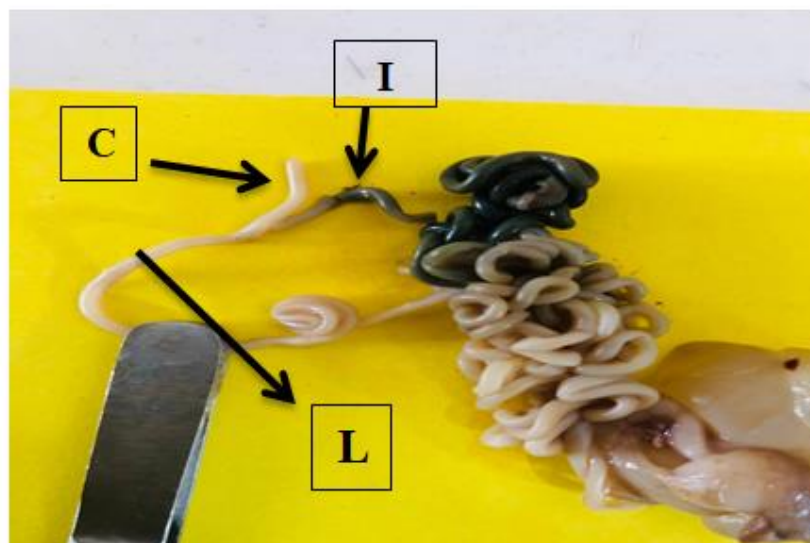
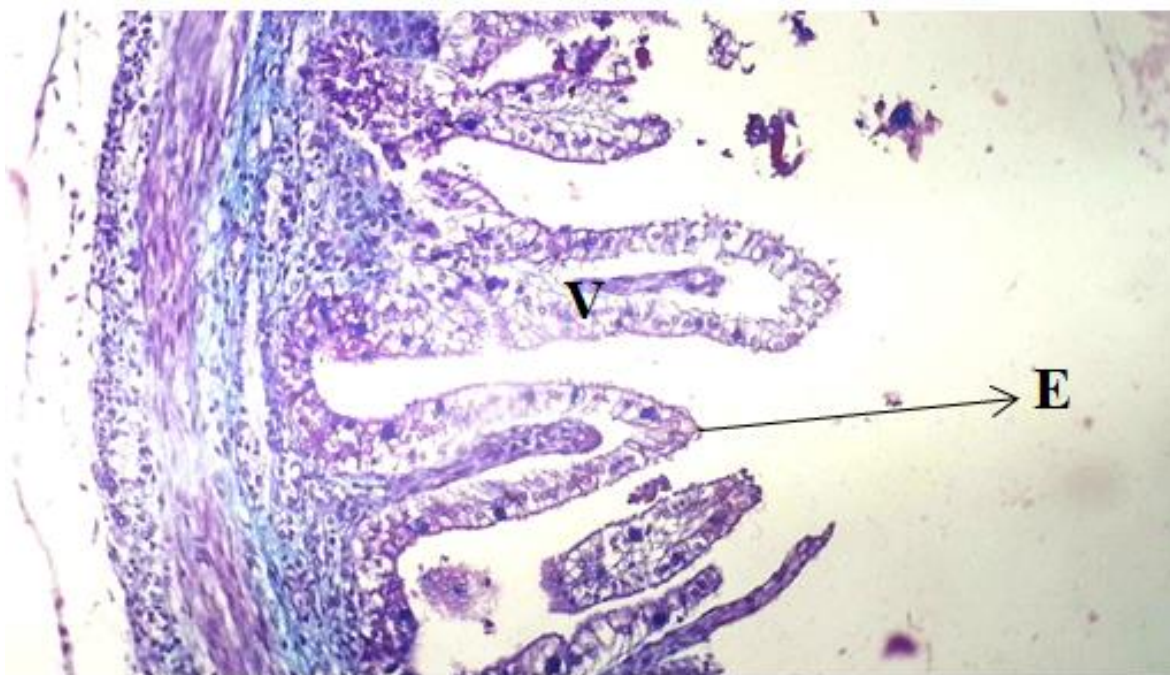


Figure 3: Photograph showing the cecum(C), Ileum and first part of ascending colon(L)

Histologically, the tunica mucosa lining the cecal lumen composed of simple columnar epithelial cells which rest on delicate basement membrane with very few goblet cells, about  $(8.16 \pm 0.120 \mu\text{m})$  and this current study agreement with (Asari, M *et al.*, 1986) which showed the epithelial cells that were covered the cecum villi be simple columnar at same gestational period in cattle fetuses.

The results showed that the epithelium are appeared in the cecum were elongated and had acquired the shape of true villi, covered only with one layer of the epithelial cells with centrally placed nuclei with few goblet cells; while the inter-villus areas were multilayers epithelium cells with a central core of mesenchymal tissue (Figure 4).



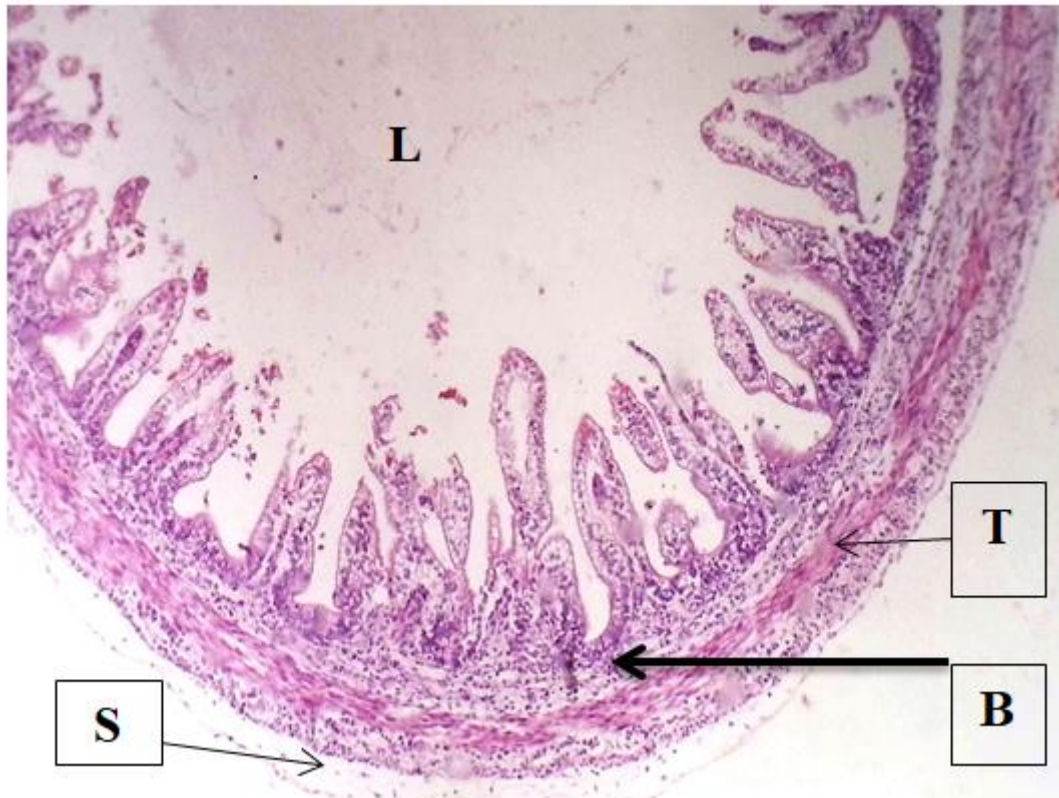
**Figure 4: Cross section of cecum showing the epithelial layer(L), cecal villi(V) at age (70-75) days of gestation (PAS & Alician blue stain, 10X)**

The length of villi in cecum from base of villi to apex about long  $(97.57 \pm 1.56 \mu\text{m})$ , middle  $(70.31 \pm 1.12 \mu\text{m})$  and short  $(33.7 \pm 0.95 \mu\text{m})$  agreement with (Ramkrishna, V., & Tiwari, G. P., 1979). Also, the results of the current study appeared that pluripotential balsmatic tissue about  $(12.30 \pm 0.18 \mu\text{m})$  in thickness were highly vascularize composed of mesenchymal undifferentiated cells, Lamina propria was still in developmental stage and not clearly observed at this age of gestation. Approximately, different description was recorded (Bello, A., 2016) which mentioned the tunica mucosa forming by three layer (epithelial, lamina propria and lamina muscularis) at this stage of gestation and the muscularis layer forming by inner circular and outer longitudinal muscle fibers.

The tunica muscularis of the cecum be developed at mid stage of gestation in sheep fetuses about  $(19.7 \pm 0.201 \mu\text{m})$  in thickness, Histologically, the tunica muscularis at 70-75 days of age in sheep fetuses showed developing smooth muscle fibers arranged in

layers with blood vessels within the tunica This current study is identical to the previous studied (Ramkrishna, V., & Tiwari, G. P., 1979) Which showed its results the tunica muscularis of cecum in goat fetuses be formed at mid stage of gestation and be arranged into two layers(inner circular and outer longitudinal smooth muscle fiber. and agreement with (Singh, O *et al.*, 2012) who described the tunica muscularis only the inner circular be observed at same stage of development in the cecum of buffalo fetuses.

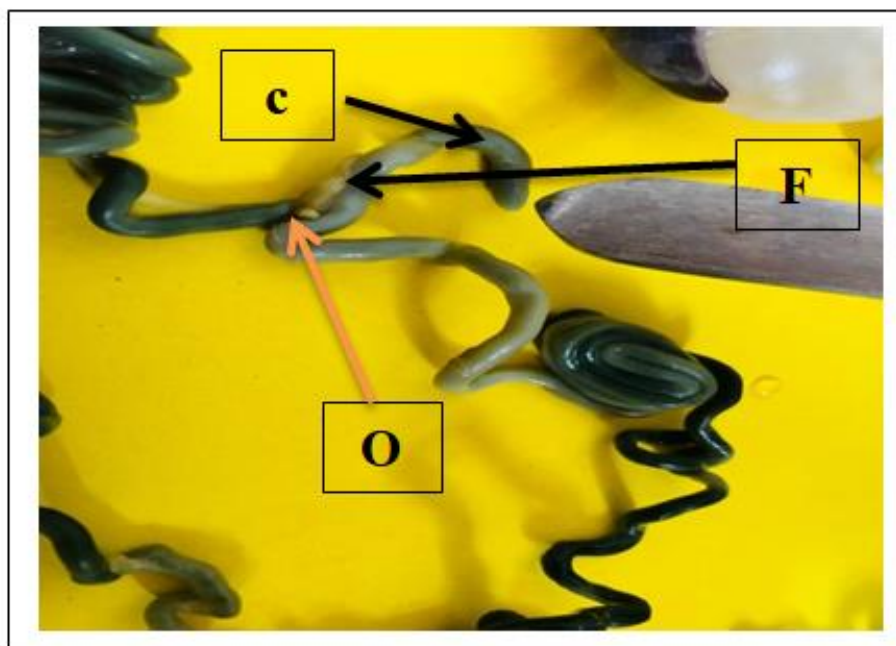
Tunica serosa at this age be more observed and development forming by loose connective tissue made up by fibroblast cells with collagen and elastic fibers and covered by a layer of mesothelial cells which be squamous in shape with flat nuclei and measured their thickness about  $(11.14 \pm 0.199 \mu\text{m})$ . This result agreement with (Singh, O *et al.*, 2012) which described the serosa of cecum formed by fibroblast cells, collagen and elastic fiber which covered by a one layer of mesothelial cells in buffalo fetuses at same age of gestation.



**Figure 5: Cross section of cecum at (70-75) days showing: tunica submucosa (B), tunica muscularis(T) lumen of cecum and tunica serosa(S). (H&E stain, 10X).**

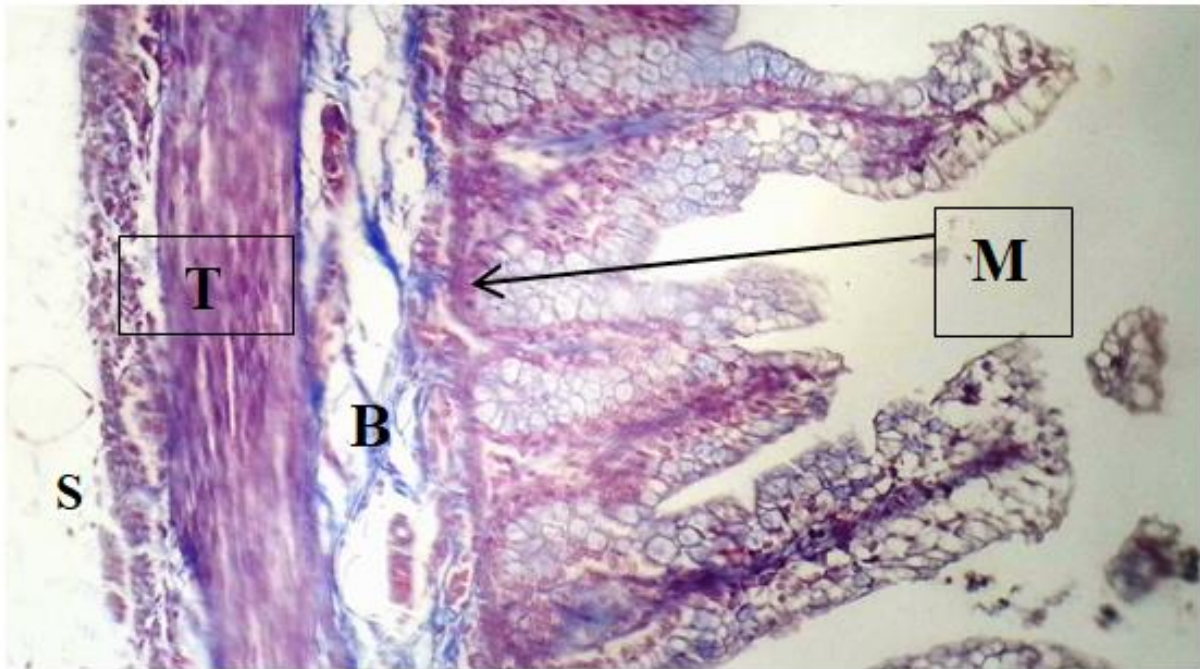
At the starting of third trimester of gestation (100-105) days morphological study of cecum oblique cylindrical in shape located slightly in the right of inguinal subregion, The cecal apex points caudally, its length about  $(3.160 \pm 0.107)$  cm, ileo- cecal fold extend

from the base of cecum to the end of cecal body, only small part of cecal apex was free, ceco- colic fold lesser in length from the first. The common opening (ileo-cecocolic orifice) can be easily identified due to increasing in diameter of both cecum and colon (Figure 6).



**Figure 6: Photograph showing the cecum(C), common ileo-cecocolic opening(O) and ileo-cecal fold at third trimester of gestation (100-105) days**

The wall of cecum histologically at third trimester showed consist from four layer (mucosa, submucosa, muscularis and serosa) (Figure 7).

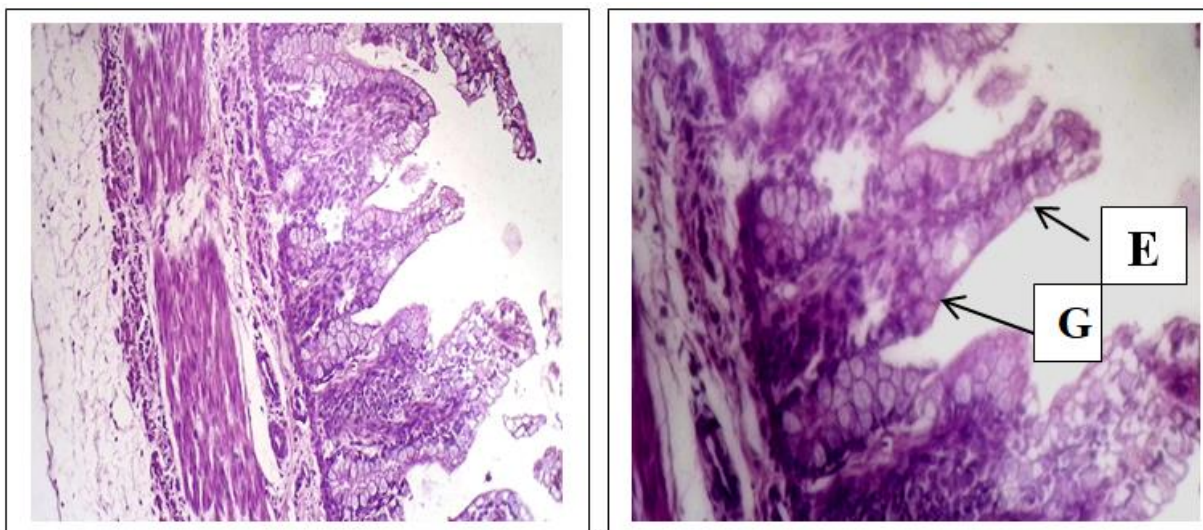


**Figure 7: Cross section of colon showing the tunica mucosa(M), tunica submucosa(B), tunica muscularis(T) and tunica serosa(S). (trichrome masson's stain,10X) at (130-140) days of gestation**

The mucosal layer about  $(11.08 \pm 0.146 \text{ } \mu\text{m})$  and  $(13.45 \pm 0.184 \mu\text{m})$  at (100-105) days and (135-140) days respectively be more developed than the second trimester including epithelial layer, lamina propria and lamina muscularis.

The epithelial layer forming by single layer of epithelial cells, columnar in shape and the nuclei be at middle or near to the base of epithelial cell, oval or elongated in shape with specialized secretory cells,

goblet cell were distributed among epithelial cells at apices and lateral wall of cecal villi and be increase in number at the base of cecal villi and decrease in number at their apices (Figure 8). (Singh, O *et al.*, 2012) which mentioned the epithelial layer be simple layer at the third trimester of gestation in fetuses of buffalo and goblet cells be starting to appear at the at the ending of first trimester and be increase with the advanced age and be more concentration in rectum from other part of large intestine.

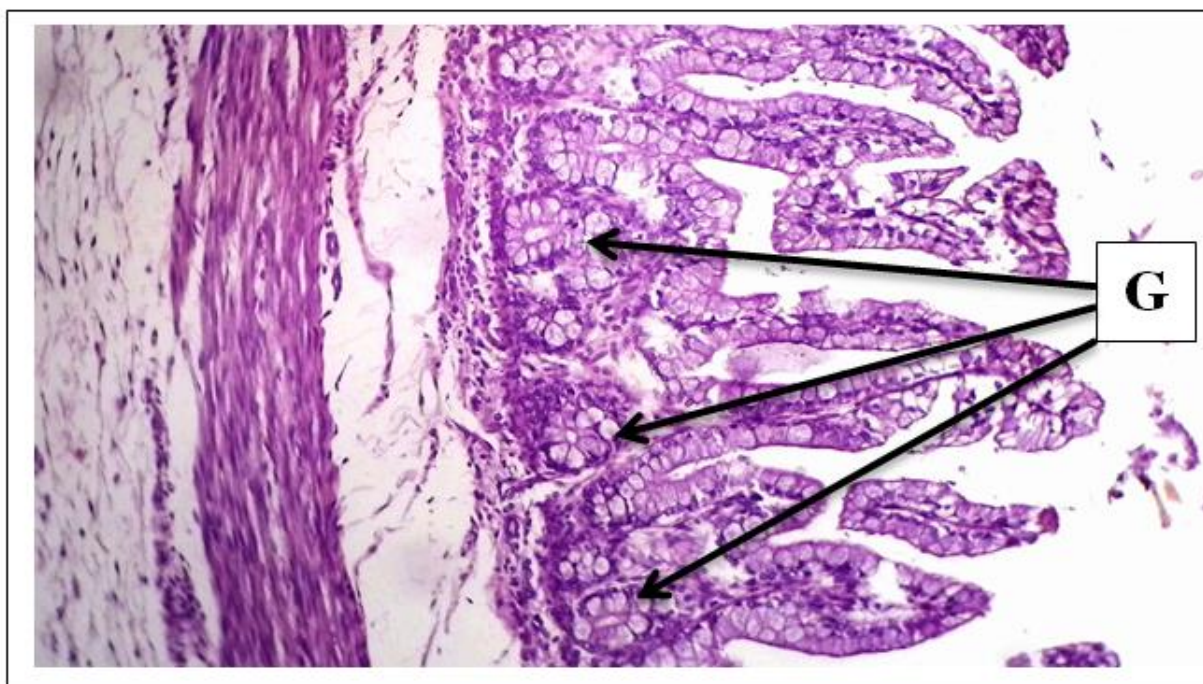


**Figure 8: Cross section of cecum at (130-140) days of gestation showing the simple columnar epithelial cell(E)and goblet cells(G). (PAS stain, 10X right, 20X left)**

Villi of cecum at (100-105) days of gestation appear longer and narrowing than villi at (135-140) days of gestation composed of three types: long villi about  $(150.63 \pm 2.026) \mu\text{m}$ , medium villi about  $(71.30 \pm 1.63) \mu\text{m}$ , and short villi about  $(47.5 \pm 1.45) \mu\text{m}$  in their height with a clear increase in diameter of cecum while cecal villi at (135-140) days of gestation be reduced in height and divided into short villi  $(40.67 \pm 1.34) \mu\text{m}$ , middle villi about  $(45.29 \pm 0.89) \mu\text{m}$  and long villi  $(101.63 \pm 1.023) \mu\text{m}$  covered by simple columnar epithelial cell with distributed of goblet cell that appear more dense and number at (130-140) day of gestation and this current study be agreement with (Ramkrishna, V., & Tiwari, G. P., 1979) who showed

same result in goat fetuses and described the cecal villi be disappear at 119 day of gestation in the cecum and rectum and same result recorded by (Singh, O *et al.*, 2012) who showed the villi of cecum and colon disappeared at the ending of third trimester of gestation in fetuses of buffalo.

Intestinal gland (crypt of luberkhan) be more developed and denser at (130-140) day gestation situated at the base of cecal villi and made up columnar epithelial cell (monolayer) with abundant number of goblet cells and some of absorptive cells which play critical role in absorption and secretion within intestinal tract (Figure 9).



**Figure 9: Cross section of cecum at (130-140) days of gestation showing the intestinal gland of cecum (crypt of luberkhan) at the base of villi. (PAS stain, 10X)**

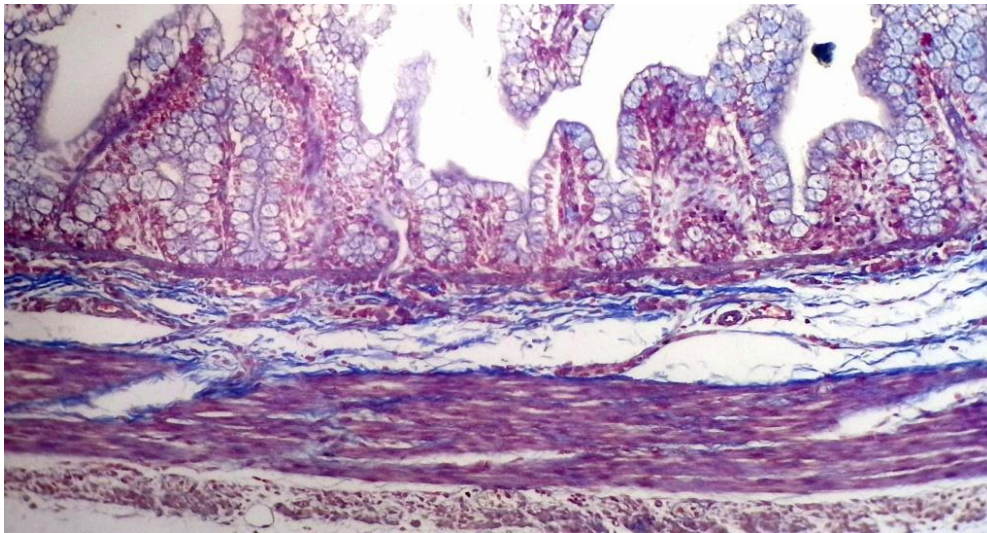
Lamina propria formed by loose connectival cells like fibroblast which responded for producing of extracellular matrix and collagen fiber and another immunological defense cells be founded, and this lamina be extended into the cecal lumen to formed the core of cecal villi.

Lamina muscularis also be observed as dedicated a smooth muscle fibers separated the lamina propria from tunica submucosa which appear more clear at end of gestation.

Tunica submucosa of cecum at this age of gestation be more developed and made up by dense connectival tissue cells like fibroblast which was responsible for forming the extracellular matrix like

collagen, elastic and reticular fibers and another immune cell defense be formed like microphage, mast cell and plasma cell, the measurement of this tunica about  $(14.7 \pm 0.114) \mu\text{m}$  at (100-105) days and about  $(24.88 \pm 0.431) \mu\text{m}$  at (135-140) days. Other structure observed in tunica submucosa like blood and lymphatic vessels be increased to supplied the progressive development of large intestine in sheep fetuses.

Tunica muscularis be more developed in cecum than lamina muscularis and comprised by inner circular be more developed and outer longitudinal smooth muscle fiber, the measurement of this tunica about  $(21.52 \pm 0.159) \mu\text{m}$  at (100-105) days and  $(38.26 \pm 0.467) \mu\text{m}$  at (130-140) days of gestation (Figure 10).



**Figure 10: Cross section of cecum at (130-140) days of gestation showing; the lamina propria(P), lamina muscularis(N), tunica submucosa(B), inner muscularis externa(TI) and outer muscularis externa (TO) (trichrome masson stain, 10X)**

Tunica serosa at this age of gestation measured about (15.70±0.221um), (28.96±0.405um) at (105-110) and (135-140) days of gestation respectively formed by loose irregular connective tissue had isolated collagen, elastic and reticular fibers along with varying amount of fatty tissue and few blood capillaries flat mesothelial cell layer was present

The result agreement with (Singh, O *et al.*, 2012) who described the tunica serosa forming by Fibrocellular lined externally by mesothelium and be well differentiated buffalo fetuses at third trimester of gestation and be increased with gestational age.

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