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HISTOLOGICAL AND HISTOCHEMICAL STUDY OF THE CECUM AND COLON OF CAPE HARE LEPUS CAPENSIS DURING DIFFERENT AGES

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ABSTRACT

The goal of the current study was to describe the cecum and colon's histological architecture and histochemical characteristics in cape hares at various ages. From 15 healthy cape hare, samples of the cecum and colon were taken: five at one day old, five at fourteen days old, and five at adulthood, Ten samples from each area were collected and fixed in 10% formalin for 24 hours before being subjected to standard histological procedures and having the sections stained with H&E, PAS, AB, and Masson's Trichrome to identify the various components. At one day old, simple columnar epithelium which lined the mucosa of the cecum and colon, were with less goblet cells and vacuolated cells, and no crypts. The cecum's wall was thin. A lengthy spiral fold that extended into the ampulla caecoli substantially expanded the interior surface of it. The appendix, which made up the remainder of the cecum, had a thick wall, and extensively covered in lymph aggregates. At 14 days old, disappeared the vacuolated cells and the villi developed into its mature form and the wall thickness had increased, revealing the early crypts and an accumulation of lymphocytes in the lamina propria. The crypts were fully grown and had thickened all of the structural levels of the wall at ages 40 day as adult. Both the proximal colon's lamina propria and submucosa were found to have highly developed lymphoid tissue, The four tunicae made up the cecum and colon wall (mucosa, submucosa, muscularis and serosa or adventitia). The muscularis mucosa, lamina propria with glands, and epithelium form tunica mucosa, which was the inner layer bordering the lumen, were all visible. These tunica's average thickness in the colon was higher than in the cecum. Large amounts of Lieberkuhn crypts and goblet cells were in the colon, as well as more of them than in the cecum. The submucosa is made up of many dense connective tissues. These tunica's average thickness in the cecum was greater than that in the colon. The tunica muscularis in the colon was thicker on average than in the cecum and was made up of circular and longitudinal smooth muscle layers. The loose connective tissues that make up the tunica serosa or adventitia were thicker on average in the colon than in the cecum. In one and 14 days ages, the goblet cells reacted moderately with PAS, while strongly with AB. In adult age, the goblet cells were moderate reaction with PAS and AB.

KEYWORDS: Cape hare, Cecum, Colon, Histochemical

INTRODUCTION

The Cape hare, also known as the desert hare or the wild rabbit, is a species of nocturnal herbivore that originated in Africa and Arabia and now ranges into India, belong to the family *Leporidae*. They are widely used as laboratory animals by modern biomedical researchers and are also kept as pets and are economically significant due to their meat and fur (1). The oral cavity, oesophagus, stomach, intestine, liver, and pancreas make up the hare's digestive system. It aids in the reception and digestion of food, as well as the transportation through the body and explosion of the unabsorbed portion (2-6), there are three parts consists the large intestine of rabbit; cecum, colon and rectum. Rabbits have a big cecum, its capacity is approximately 40% of the entire digestive tract and is ten times bigger than the capacity of the stomach (7,8). (9) Mention how the autochtanous bacteria present can thrive in an anaerobic environment in the rabbit's cecum. The amount of mucosubstances present in the digestive secretions of vertebrates varies depending on the cell type, anatomical area, functional status, age, pathological condition, sex, and species (10). Numerous studies were done to investigate the developmental changes in the small and large intestine sections' walls. Different species exhibit various patterns of cell growth after birth. In general, suckling rodents' intestinal crypt cell growth was shown to be modest until weaning (11,12). In one week old lambs, the highest migration of enterocytes and the migration rates decreased with age in all parts of the intestine (13), referred to the fact that distinct epithelial lineages can form from primitive, immature cells and that the developing intestine is able to establish and maintain the variations in the differentiation programs in each of these lineages (14). Previous studies examined how the gastrointestinal tract develops after birth depending on a variety of circumstances, including age, food, and hormones generated by the intestine or even by other organs connected to it, such the pancreas and liver (15.16). In mammals, the transition from an amniotic fluid-based diet to a milk-based one occurs around the time of birth. Following that, weaning is regarded as another phase when the diet changes, with milk gradually being replaced by solid food, which then becomes the main source of sustenance (17). In fact, researchers classified the gastrointestinal tract's postnatal development into three phases: birth and early breastfeeding, breastfeeding and the weaning stage (2,18). There is no information available regarding the histological and histochemical changes that occur during the advanced postnatal period in the colon and cecum of the cape hare in Iraq, so that this study to describe the development of cecum and colon in cape hare.

MATERIALS AND METHOD

The study was performed using fifteen of the healthy local breeds of cape hare *Lepus capensis*, Five at birth, five at 14 days old, and five at adulthood, during May and November 2022. They were put to anesthesia via a ketamine overdose (administered intramuscularly), except at birth without anesthesia. Animal's ventral abdominal wall was removed, From the apex to the caeco-colic junction, the cecum was removed. The proximal and distal portions of the colon were found when the colon was sampled from ileocaeco colic junction for start of the sacculation at the colo-rectal junction. They could tell them apart from one another because of the fusus coli, samples were collected from various regions of the (cecum and colon), Five samples were obtained from each area and fixed in 10% formalin for 24 hours before being processed using standard histology procedures, the stains that used; Harris hematoxylin and eosin (H&E) stain for highlighting the tissue's general histological components; Periodic acid Schiff (PAS) stain for show the basement membrane, mucoprotein, mucopolysaccharides and glycoprotein, and Masson's trichrome stain; and collagen and smooth muscles (19). Mean (x-) ± standard error (S E) was calculated for each the measurement, Ten slides of each part of the cecum and colon (proximal and distal) (20).

RESULTS

According to histological discoveries, the four tunicae; mucosa, submucosa, muscularis, and serosa which make up the wall of the cecum and colon (Fig.1,2), At one day of age, simple columnar epithelium with less goblet cells and vacuolated cells lined the tunica mucosa of the cecum and colon (Fig.1,2). At 14 days old, the colon and cecum walls were thicker and revealed the earliest crypts as well as an accumulation of lymphocytes in the lamina propria and disappear the vacuolated cells and the villi were taking the mature shape (Fig.3,4), in adult hare the lengthy spiral fold significantly enlarged the interior surface of the cecum, continuing into the ampulla caecoli (Fig.5). In ages fourteen day and adult, the crypts were well-developed and grew thicker in all of the wall's layers. Both the colon's lamina propria and submucosa have generated lymphoid tissue (Fig.5,6). Instead of the villi, the colon's epithelial lining is structured into crypts and cuffs on surface (Fig. 5), at one day age; demonstrated brief villi-like structures in wall of cecum and colon and the crypts were not obvious; instead, they appeared as aggregation of immature cells nearby the bases of these structures and has deep circular folds (Fig.1,2,5). Auerbach's nerve plexuses were noticeable and well-developed structures found in the tunica muscularis at all studied ages, even in those of one-day-old pups. The tunica muscularis was divided into an inner circular layer and an outer longitudinal layer by connective tissue that was continuous and well-developed (Fig.2,7).

The distal colon crossed the flexure that separates the duodenal and jejunal portions of the small intestine from one another, With the exception of the absence of the villi-like structures seen in the mucosa and the absence of lymphoid follicles in the lamina propria, the histological structure of the distal colon's wall was identical to that of the proximal colon. Additionally, the crypts in the distal colon's wall were closer together than those in the proximal colon's wall (7,8). Micromorphometric analysis of the colons of one day age revealed that the muscularis thickness was greater in the distal region of the colon; while the epithelial height was larger in the proximal colon. Epithelial height measures of the proximal and distal colons at 10 days old were equal. The thickness of the muscularis layer increased slightly compared to the previous age, with the distal colon exhibiting the highest thickness of this tunic (Table 1) (Fig. 1,7, 9). With the exception of the epithelial height of the proximal colon, which was somewhat lower than that of the previous age, measurements of the colon at fourteen days showed an increase in both the parameters in all segments of the colon compared to those recorded at ten days. The proximal colon had the most crypt depth, width, and muscle thickness at this time, whereas the distal colon had the highest epithelium (Table 1). The goblet cells in the cecum gradually rose with age (Fig. 10). In the colon of adult hare, the tunica muscularis thickness was larger in the proximal colon at this age, and micromorphometric studies revealed an increase in both parameters in all segments of colon compared to those of prior ages (Table 1) (Fig.8,9,11). At adult age Three flat layers of longitudinally arranged muscle were created from the outer longitudinal muscle as teniae coli (Fig.11), and thicker than that in cecum (Table 1) (Fig.12-14).

In the distal colon at one, ten-, and fourteen-days postnatal age, goblet cell percentage was higher than in the proximal colon at adult age. The number of goblet cells in the proximal colon gradually rose with age, reaching its peak at forty days, while the number of goblet cells in the distal colon peaked at fourteen days (Table 1). Micromorphometric analyses of the proximal colon at all analyzed ages showed that the epithelium's height gradually increased with age, with the exception of a minor decline at the age of fourteen days. With advancing age, the tunica muscularis grew thicker. For all studied ages, measurements of the proximal parts of colon grew larger with age (Table 1). When the goblet cells were stained with histochemical stains at one and 14 days old, it was discovered that they strongly reacted with PAS-AB (pH2.5), no stain with AB (Fig.14) and only modestly with PAS (Fig.2,4,8,9). These responses more strongly suggested the existence of neutral mucin than of mucin (non-sulphated) type. The goblet cells displayed a modest response to PAS and AB (pH2.5) after fourteen days, indicating the existence of an equal proportion of neutral and sulfated acidic mucin (Fig.7,9).

Table (1): Measurement of thickness of the wall layers of cecum and colon of cape hare at different ages µm (X- ± S.E)

Tunica / Part	Mucosa	Submucoa	Muscularis externa	Serosa
Cecum 1 day 14 day adult	219.3±1.3 267.2±1.1 359.2±2.3	73.7±0.1 89.3±0.3 124.2±1.2	115.1±0.8 141.3±1.3 185.4±1.2	19.2±0.1 20.4±1.2 22.1±1.3
Proximal colon 1 day 14 day Adult	251.5±1.6 376.2±1.9 452.4±2.4	81.2±0.3 122.5±0.9 150.2±0.7	128.2±0.3 191.4±1.2 225.6±2.3	20.5±1.5 23.1±1.6 24.7±0.2
Distal colon 1 day 14 day Adult	240.6±2.1 351.3±2.4 392.5±4.1	80.2±0.7 105.5±1.3 130.9±1.2	121.3±2.5 170.2±1.6 221.4±2.1	20.1±0.2 22.1±0.3 23.4±0.1

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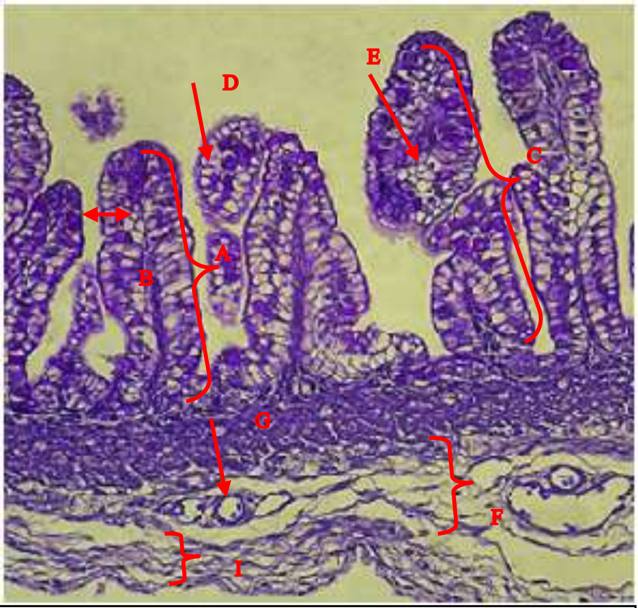

Fig (1): Cross section of the cecum of cape hare, at age 1 day; mucosa (A), epithelium (B), structure like villi (C), goblet cells (D), lamina (E) submucosa, (F), connective tissue (G), muscularis (I), H@E X100.

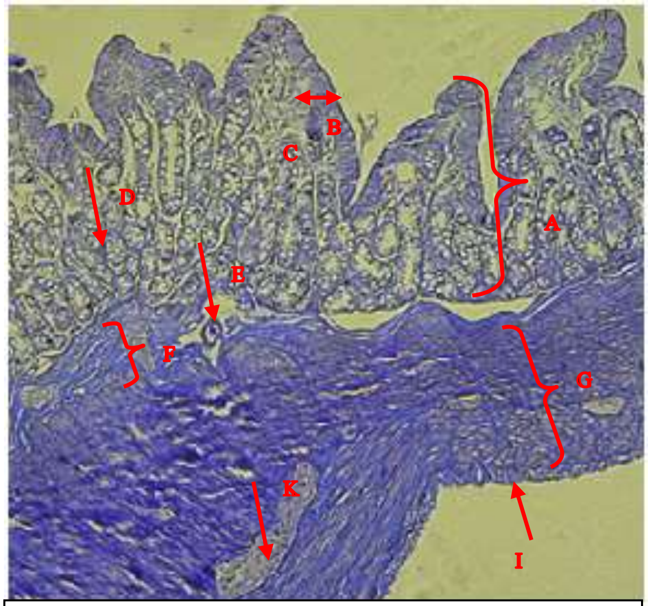

Fig (2): Longitudinal section of the proximal colon of cape hare, at age 1 day; mucosa (A), epithelium (B), lamina propria (C), crypt (D), blood vessel (E), submucosa (F), muscularis (G), nerve (K), adventitia (I), AB-PAS X100.

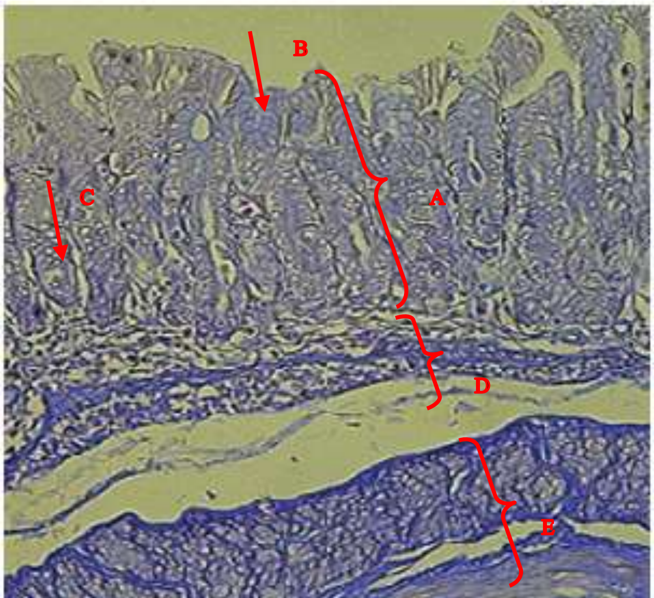

Fig (3): Cross section of the distal colon of cape hare, at age 14 day; Show; mucosa (A), goblet cell (B), crypt (C), submucosa (D), muscularis (F), Masson X100.

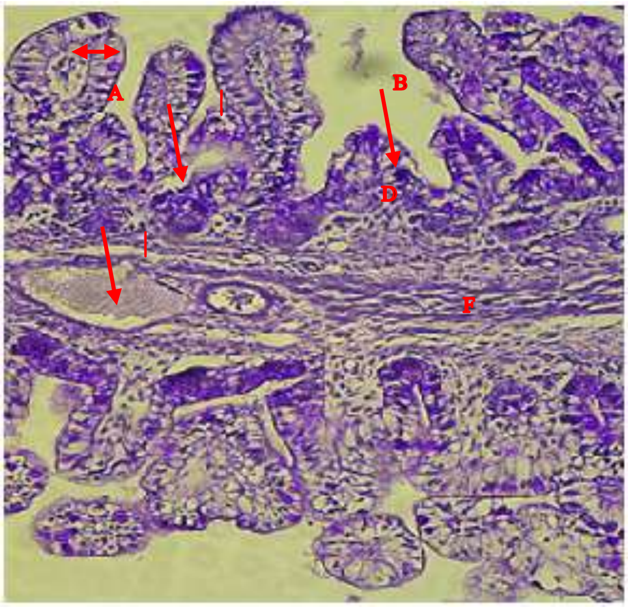

Fig (4): Longitudinal section of the distal colon of cape hare, at age 14 day; show: epithelium (A), goblet cells (B), crypt (C), lamina propria (D), blood vessels (E), sub mucosa (F), PAS X100.

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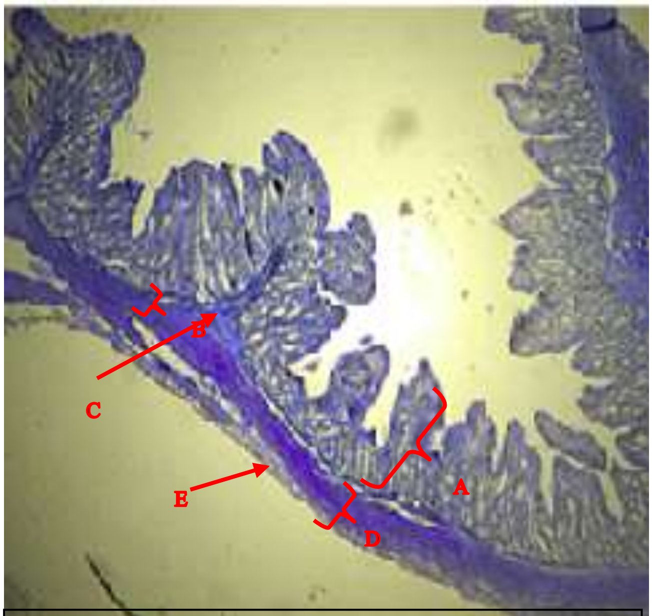

Fig (5): Cross section of the distal colon of cape hare, at adult age; mucosa (A), submucosa (B), plicae circularis (C), muscularis (D), serosa (F), PAS-AB X100.

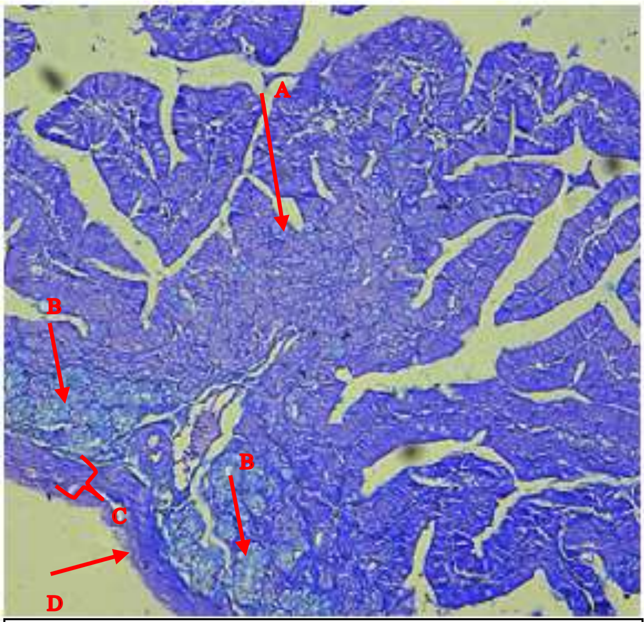

Fig (6): Longitudinal section of the cecum of cape hare, at age 14 day; goblet cell (A), crypt (B), muscularis (C), adventitia (D), PAS-ABX100.

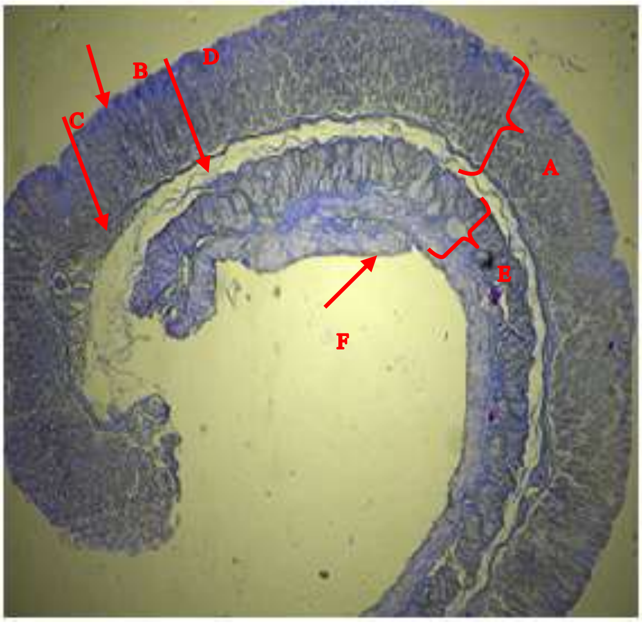

Fig (7): Cross section of the distal colon of cape hare, at adult age; mucosa (A), goblet cell (B), basement membrane (C), submucosa (D), muscularis (E), serosa (F), Masson X100.

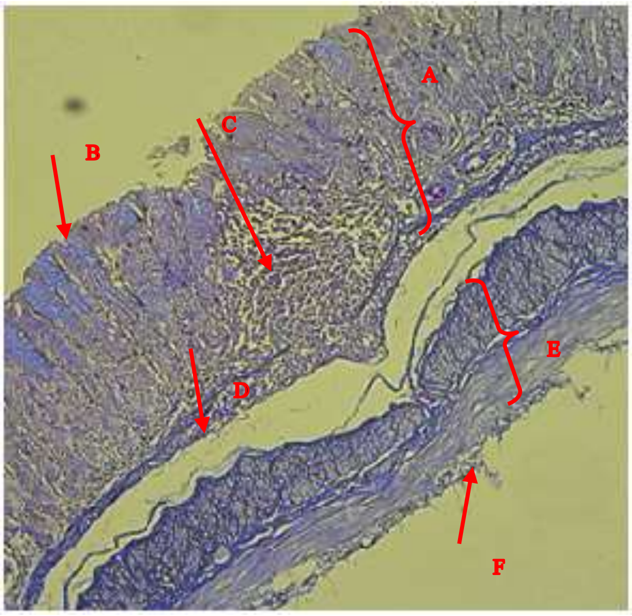

Fig (8): Cross section of the proximal colon of cape hare, at adult age; mucosa (A), goblet cell (B), lymphatic tissue (C), submucosa (D), muscularis (E), serosa (F); Masson X100.

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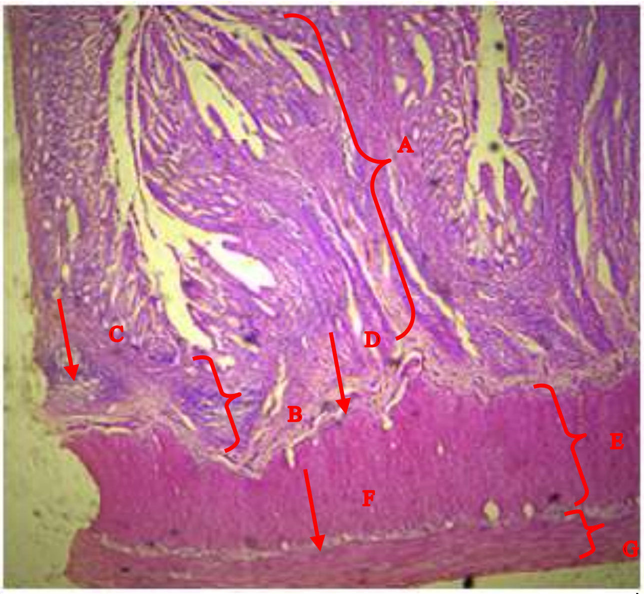

Fig (9): Longitudinal section of the distal colon of adult cape hare, mucosa (A), submucosa (B), lymphatic tissue (C), blood vessels (D), inner muscularis (E), outer muscularis (G), connective tissue (F); H@E X100.

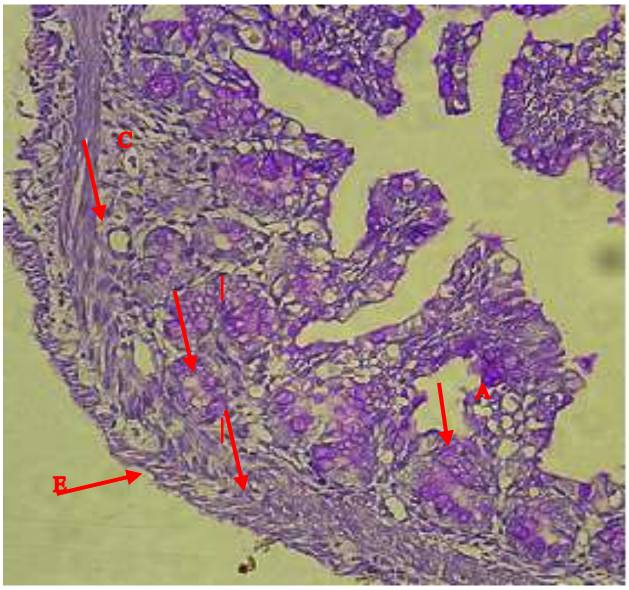

Fig (10): Cross section of the cecum of cape hare, at 14-day age; goblet cell (A), crypt (B), connective tissue (C), muscularis (D), serosa (E), PAS X100.

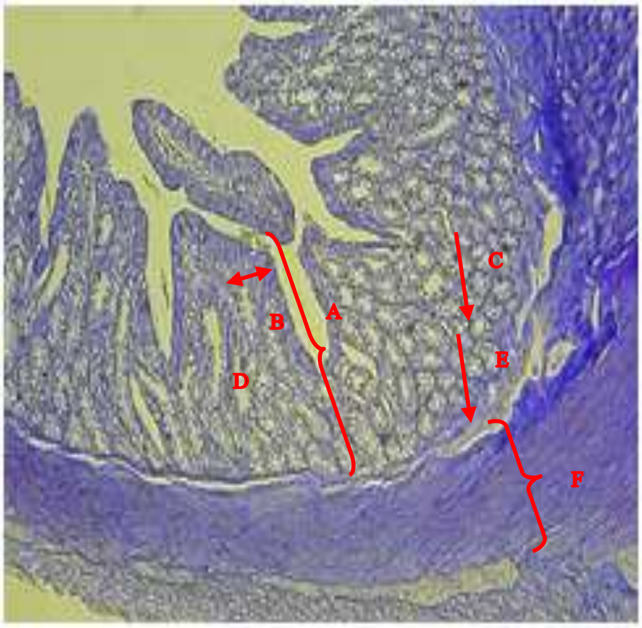

Fig (11): Cross section of the distal colon of cape hare at 1 day age, mucosa (A), epithelium (B), crypt (C), lamina propria (D), submucosa (E), teniae coli (F), Masson X100.

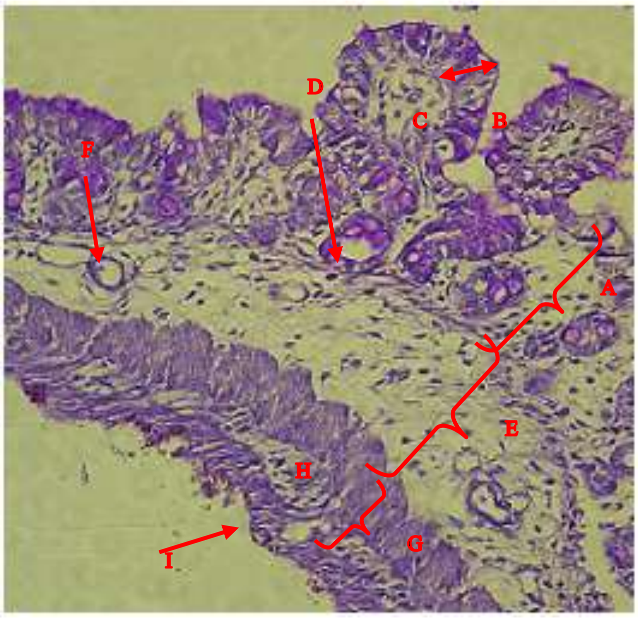

Fig (12): Cross section of the cecum of adult cape hare, mucosa (A), epithelium (B), lamina propria (C), crypt (D), submucosa (E), blood vessels (F), muscularis (G), nerve (H), serosa (I); PASX100.

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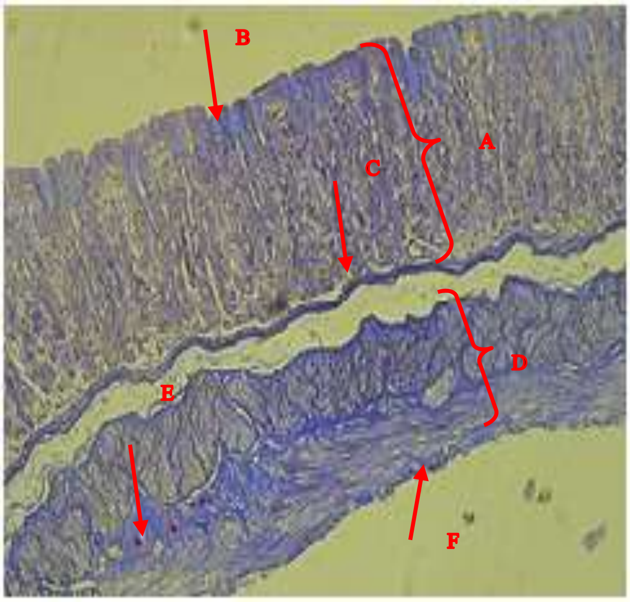

Fig (13): Cross section of the cecum of cape hare, mucosa (A), goblet cell (B), muscularis mucosa (C),muscularis (D), connective tissue (E), serosa (F); Masson X100.

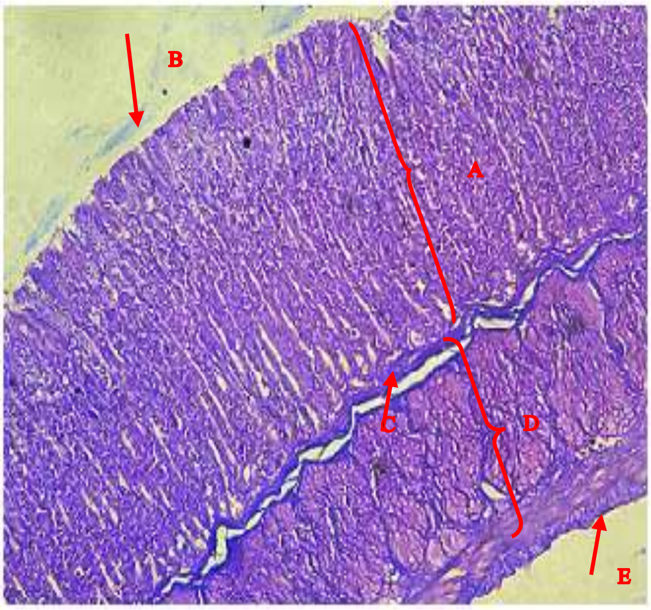

Fig (14): Cross section of the distal colon of cape hare, mucosa (A), epithelium (B), submucosa (C), muscularis (D), serosa (E); AB X100.

DISCUSSION

In all study ages, the intestinal surface is homogeneous and flattened due to the absence of the villi, with the exception of one age has villi like structure; and the outside longitudinal layer of the muscularis of the colon is structured into three distinct bands, as taenia coli, this similar to (10). The type of taenia coli causes sacculations of large intestine (21). The progenitors cells in the lower portions of the crypts continuously supply enterocytes to the crypt plates, these cells are removed from the depths of the crypts, they will each undergo six cell divisions and move quickly toward the mucosal surface, the pace of the cell replacement must parallel for the loss of the cells to maintain appropriate epithelial homeostasis (11). Because they can express alkaline phosphatase, an antimicrobial peptide, enterocytes in the colon are specialized cells for absorbing both electrolytes and water. They also play a role in the epithelial defense mechanism (9,21). In contrast, mucus thickness in the colon consistently increases from proximal part of colon toward end of the rectum (22). The study looked at the mucin profile in the colon at birth, which is similar to that seen in the adult colon when acidic mucins predominate. Animals like mice have a colon at birth (22) and newly borne pig (11) It has been proven the goblet cells produced the sulphated acidic mucins.

In rabbits, because suckling milk is being replaced by a completely solid feed intake during the time leading up to weaning, this is a crucial stage. Additionally, the digestive tract undergoes physical changes that shift it from an exclusively hydrolytic system powered by chemicals found in the intestine to an internal digestion followed by bacterial fermentation in the cecum. Because young animals are more susceptible to stomach issues around the time of weaning, it is crucial to be aware of their dietary needs (23). In the surface epithelium, colonic crypts, or cecum of the mouse intestine between the ages of 15 and 42 days, paneth cells could not be seen. The peyer's patches in the small intestine, the lymphoid patches in the cecum and appendix, and other solitary and aggregated follicles are all found throughout the mammalian intestine. Recently, (24) documented that the lymphoid tissues of the rabbit cecum are made up of several lymphoid follicles. They reached the bottom of the submucosa and completed the lamina propria. There are thin crevices separating them from one another. They developed dome-shaped structures in the ileocecal patch that were divided from one another by thin mucosal folds. The clefts that separated the follicles from mucosal folds eventually became wide and flat as the follicles grew older. We found tiny dome areas with underdeveloped germinal centers in the newborn hare. In the senior age, these characteristics changed as the follicles grew larger with clearly as dome areas and germinal centers.

In the cecum and distal colon, as well as neutral glycoconjugates and acidic/sulphated glycans, crypt cells were seen. In the tunica muscularis, Auerbach's nerve plexuses were visible and well-developed structures at all analyzed ages, even in pups older than one day was also described by (25), He did not discover the villi-like shape in the distal colon, but rather in the proximal colon. The latter reference discussed these "transient villus-like structures," which have been described as "villi-like structures" in the rat colon and function similarly to enterocytes in the small intestine. The current findings in the large intestinal mucosa revealed a large proportion of the goblet cells that, along with absorptive cells lining the lumen of the intestinal crypts, are in agreement with previous records of (9) in rabbits. These cells produce mucus that helps the contents move more easily through the large intestine's lumen. The cells on the upper portion of the villi, however, seemed vacuolated because of their unique absorptive capabilities. 14 days postpartum is a vital period with obvious changes. In fact, Rabbits consume a lot of dietary fiber during and after the weaning phase. The increased feed intake may accelerate colon and cecum development by triggering microbial metabolism and promoting hindgut fermentation, did not quite match the view of (26) who argued that the amount of solid feed consumed had little bearing on how the colon developed and that ontogenetic variables were more important.

At one day old, each of the cecum and colon had small, villi-like structures that had wide bases and tiny tips. The crypts weren't immediately apparent; rather, they developed as a cluster of immature cells close to bases of these structures. These outcomes concurred with those (11) on of intestine of growing pig. Currently, the mucosa, submucosa, and muscularis and serosa, which make up the proximal colon's wall, are present and deep circular folds. At 14 days old, the colon's wall thickness had increased, revealing the colon's early crypts and an accumulation of lymphocytes in the lamina propria. Evidently, compared to other mammalian species, rabbits had more developed gut associated lymphoid tissue in the current study and the review that was previously mentioned (12). They were often thought to be ileocecal patches, appendix peyer's patches, sacculus rotundus, and lymphocyte aggregation in the proximal colon (9,26), of contrast, the gut-associated lymphoid tissues in ruminants, pigs, and dogs were exclusively visible in the ileocecal patches (12).

The crypts were highly formed and thickened in all structural levels of the wall in the proximal colon of adult hares. Both the lamina propria and the submucosa have lymphoid tissue that is well-developed. However, at 2 weeks, the colonic mucosa of the rabbit showed fold and a compact arrangement (11). However, at 4 weeks, the folds vanished and the muscle grew thinner, which may be related to the increased consumption of high dietary fiber that causes distension in the gut. With the exception of the absence of the villi-like structures seen in the tunica mucosa and the lymphoid follicles in the lamina propria, the structure of the wall of the distal colon was identical to the general histological structure of the proximal section. Additionally, compared to those found in the proximal colon wall, the crypts of the distal colon were closer together. These morphological characteristics agreed with those seen in the distal colons of rabbits, rats, and pigs that documented (12). Conclusion: the majority of the distinctive and characteristic histological and histochemical alterations occur around the age of 15 days, making this a crucial time for developmental changes.

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