Journal of Applied Veterinary Sciences, 8 (4): 83-94 (October, 2023)
ISSN: Online: 2090-3308, Print: 1687-4072
Journal homepage : https://javs.journals.ekb.eg

Histological Study of Small Intestine Development in Local Chicken (*Gallus gallus domesticus*) and Duck (*Anas platyrhynchos domesticus*) Embryos

Sura M. N. Othman, Saffanah Khuder Mahmood*
Department of Anatomy, College of Veterinary Medicine, University of Mosul, Mosul, Iraq
***Corresponding Author:** Saffanah Khuder Mahmood, **E-Mail: saffanhjeber@uomosul.edu.iq**

ABSTRACT

Growth of the avian small intestine initiates during embryogenesis through simultaneous and compound histogenesis proceedings. The histological study of the small intestine development in local chicken and duck embryos followed a protocol of paraffin embedding technique, and the tissues were stained by Hematoxylin and Eosin stain. The histological study was divided into three age periods, which showed that the walls of the three parts of the small intestine were similar with some differences. The first period in chickens showed that the mucosa had small folds, while the duck had very close folds. The second period in chickens showed that the villi had equal height and width with an elongated columnar epithelium and the presence of Paneth cells; tunica muscularis consisted of two thin muscular layers, the middle circular and outer longitudinal, interspersed with Auerbachian plexuses and tunica serosa consisted of mesothelial cells. While in the duck, the folds' epithelium had a brush border, interspersed with goblet cells, and the presence of Auerbachian plexuses between the middle and outer layer of muscularis. At the end of the second period in chickens, the duodenal mucosa contained finger-shaped villi, while in ducks, the submucosa contained the Meissner plexuses, which were elongated oval in chickens and circular in ducks, and there were no Brunner glands in both bird types. The jejunum's villi were finger-shaped with equal length but shorter than the duodenum's villi, and there were plicae in its wall. The intestinal crypts formed in two ways: either from undifferentiated embryonic cells or by dividing the crypt into two by bifurcation. The ileum's villi were shorter and wider in chickens, while in ducks, they were hook-shaped, with the presence of Beyer's batches. The third period in chicken and duck arrangements an efficient small intestine by the completion of embryogenesis. In conclusion, this combined examination offers a roadmap for researchers to estimate varied investigational data that have gotten at the histogenesis of small intestine growth within the two bird types.

Keywords: Embryos, Histology, Local chickens, Local ducks, Small intestine development.

Original Article:
DOI:https://dx.doi.org/10.21608/javs.2023.223223.1257
Received : 15 July, 2023.
Accepted :03 September, 2023.
Published in October, 2023.

J. Appl. Vet. Sci., 8(4): 83-94.

INTRODUCTION

Growth of the avian small intestine initiates during embryogenesis through simultaneous and compound histogenesis proceedings. The histological study of the small intestine development in local chicken and duck embryos followed a protocol of paraffin embedding technique, and the tissues were stained by Hematoxylin and Eosin stain.

Poultry farming has made great achievements over the past decades through continuous genetic improvement of commercial crosses and great attention to the obstacles of modern breeding, especially with regard to raising chickens and ducks, which constitute one of the most advanced protein production systems in the world **(Adeola, 2006; Kshash and Oda, 2019)**.

Ducks are among the important animals that provide eggs and meat in addition to chickens. Humans have domesticated these birds since ancient times and used them as food and a source of entertainment during the practice of hunting. Ducks live in ponds, swamps, rivers, and coastal areas in oceans and seas and on all continents of the world **(Brown and Tuinen, 2011).**

The digestive system of any animal is important in converting the food it eats into nutrients that the body needs for growth and production (such as eggs). The animal's body breaks down food in

chemical and mechanical ways. In most animals, the mechanical action involves mastication, and because birds do not have teeth, their bodies use other mechanical actions. The chemical action involves the release of digestive enzymes and fluids from different parts of the digestive system, and then the nutrients are absorbed and distributed throughout the body. The organs of the digestive system are distinct as they derive from the common primary intestine **(Gelis, 2005; Denbow, 2015; Ravindran and Abdollahi, 2021).**

The embryonic development of the digestive system is characterized by morphological and functional changes on a large scale, as it begins with the invasion of the anterior and posterior intestinal portal from both ends of the fetus, which begins to form the primitive gut, which is also called the primitive alimentary canal lined with the endoderm, and this primary tube is divided into three sections, namely the foregut, the midgut, and the hindgut. The foregut gives the oesophagus, the proventriculus, and the gizzard. The middle intestine gives rise to the small intestine (duodenum, jejunum, and ileum), while the hindgut gives rise to the large intestine (colon, two cecae, and rectum) **(Spence *et al.*, 2011; Le Guen *et al.*, 2015).**

In view of the lack of research related to the embryonic study of the small intestine in local chickens (*Gallus gallus domesticus*) and ducks (*Anas platyrhynchos domesticus*), this study was planned to study the standard histological functional differences of the small intestine between both types of birds using H&E stain as a general tissue stain.

MATERIALS AND METHODS

One-Hundred Four (104) fertilized chicken eggs and 140 fertilized duck eggs were collected from the city of Mosul and its neighboring villages during the period from (1/9/2022) to /1/2023,, where the eggs were placed in an automatic movement, ventilated, and humidity (60%) and the temperature of the incubator was (37.7 °C) for chicken and (37.5 °C) for duck. The egg turning was stopped for the first three days of incubation; after that, the turning was started to prevent adhesion of the embryonic membranes. For the last three days, the egg turnover was stopped to prepare the embryos for hatching.

Separation, fixation, and microscopic examination of embryos

The Animal Ethical Committee of the College of Veterinary Medicine, University of Mosul, Iraq, has approved the present study under permission No. UM.VET.2022.061. Embryos were separated according to **(Kulesa and Feaser, 2011; Ainsworth *et al.*, 2013)**, examined by a dissecting microscope (Huma scope stereo 14900/5, Germany), and fixed in neutral buffer formalin. After 72 hours, the embryos were dissected, the intestines were separated, and they were placed again in the neutral buffer formalin for 72 hours **(Tripathi, 2013).** The small intestine specimens were processed according to the protocol of the paraffin embedding technique (58–60 °C), where the small intestine specimens were embedded vertically to obtain all the layers of small intestine tissue when cutting **(Dey, 2022)**, and the tissue blocks were sectioned at five µm by an automatic rotary microtome **(Johnson *et al.*, 2019)**. The sections of the tissue were stained with Hematoxylin and Eosin (H&E) stain **(Suvarna *et al.*, 2013; Smith *et al.*, 2018).**

RESULTS

The current study included an investigation of the histological structures of the small intestine development of local chicken (*Gallus gallus domesticus*) and local duck (*Anas platyrhynchos domesticus*) which was divided into three age periods: the first age period in chicken was (one-seven) days of incubation, and the first age period in duck was (one-nine) days of incubation. The seventh-day incubated chicken embryo showed that the intestine consists of the tunica mucosa, which is in the form of small folds, and the intestinal lumen is small too. The intestinal cavity is formed by the gathering of embryonic cells in the form of masses, and through apoptosis, the intestinal canal is formed, and the undifferentiated mesenchymal cells are organized in the form of aggregates under the tunica mucosa. While a ninth-day incubated duck embryo showed that the lumen of the intestine was very small and crescent-shaped, the tunica mucosa was in the form of very close folds, which were lined with stratified cuboidal epithelium, and there were no connective tissues or the muscular layer around the intestinal tube in both types of birds (Fig.1).

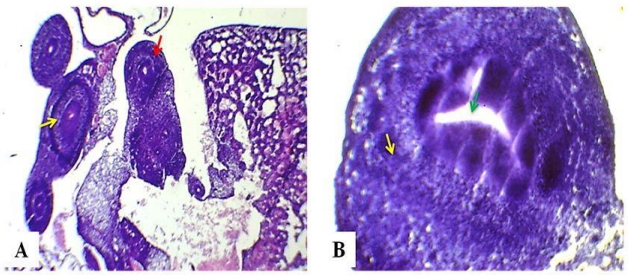

Fig. 1: (A) cross histological section of a chicken embryo incubated for seven days, the red arrow indicates the beginning of the intestine formation. (B) duck embryo, incubated for nine days, the green arrow indicates the intestinal lumen and the yellow arrow indicates the mesoderm cells. H&E stain, magnification (A) 100X, (B) 400X.

While the second age period in chicken (8^{th} - 14^{th}) days and duck (10^{th} -18^{th}) days, showed that in the 10^{th} and 11^{th} days of incubated chicken embryo, was the beginning of the differentiation of the middle circular muscle layer only (Fig. 2&3)

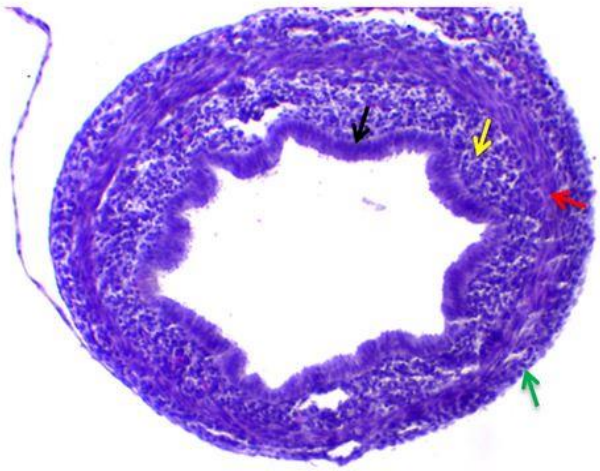

Fig. 2: Cross histological section of a chicken embryo incubated 10th days, the black arrow shows the folds, the yellow arrow shows the undifferentiated embryonic cells, the red arrow shows the middle muscular layer, the green arrow shows the serosa layer, H&E stain, magnification 400X.

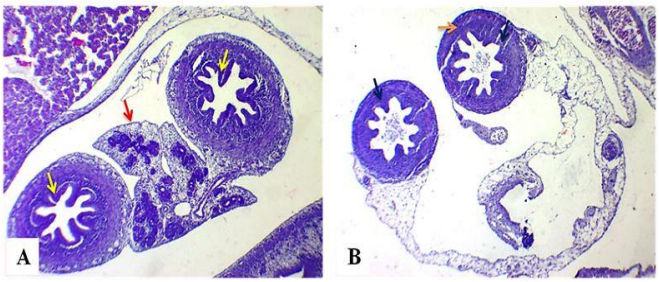

Fig. 3: (A) cross histological section of an 11-day-incubated chicken embryo, the yellow arrow indicates the duodenum, the red arrow indicates the pancreas, and (B) cross histological section of a chicken embryo incubated at 12^{th} days, the black arrow shows the jejunum and the orange arrow shows the beginning of differentiation of the mesoderm, H&E stain, magnification 40X.

While 10^{th} day-incubated duck embryo showed the beginning of the differentiation of the folds' epithelium with brush border, and interspersed with goblet cells, and the beginning of the formation of the middle muscular layer in the form of a ring (Fig. 4).

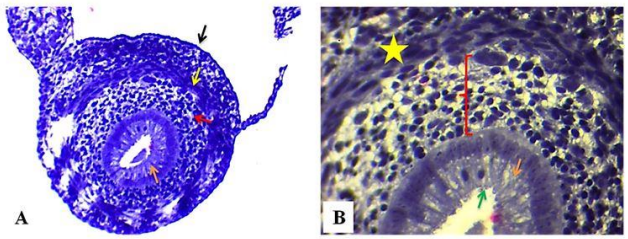

Fig. 4: (A) cross histological section of a duck embryo, incubated for 10^{th} days, the orange arrow shows epithelium, the red arrow shows undifferentiated embryonic cells, the yellow arrow, and asterisk show the middle muscular layer, the black arrow shows serosa layer, the green arrow shows the brush border, H&E stain, magnification (A) 40X, (B) 400X.

On the 12^{th} and 13^{th} day of incubated chicken embryo, the tunica mucosa formed villi of equal height and width, the epithelium was an elongated columnar epithelium containing an elongated ovoid nucleus, and the presence of Paneth cells in the epithelium which is located at the base of the villi, where they were distinguished by their shape. The tunica muscularis consisted of two thin muscular layers, middle circular and outer longitudinal interspersed with nerve plexuses (Auerbachian plexuses). As for the tunica serosa, consisted of the mesothelial cells (Fig. 5).

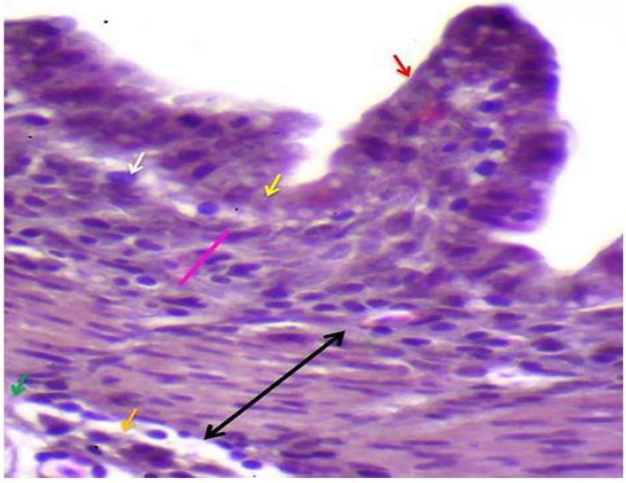

Fig. 5: Cross histological section of a chicken embryo incubated for 13 days, the red arrow indicates the epithelial cells, the yellow arrow indicates the Paneth cells, the white arrow indicates the mesenchymal cells, the pink line indicates the submucosal layer, the black two head arrow indicates the middle circular muscularis layer, the green arrow indicates the outer longitudinal muscularis layer, the orang arrow indicates the Auerbachian plexus, H&E stain, magnification 400X.

While on the 12th day incubated duck embryos, showed differentiation of the muscularis layer with the presence of Auerbachian plexuses between the middle and outer layer (Fig. 6).

A 14th day incubated chicken embryo showed that the duodenum wall in the chicken embryo was composed of the mucosa, submucosal, muscularis, and serosa layer. The mucosa layer contained fingers-shaped villi lined with simple columnar cells with goblet cells scattered between them (Fig. 7). While 18th day incubated duck embryo showed that the villi were in the domes shape, and the core consists of loose connective tissue and contains many blood vessels and goblet cells were scattered among the epithelial cells and had transparent or pale cytoplasm and basal nucleus.

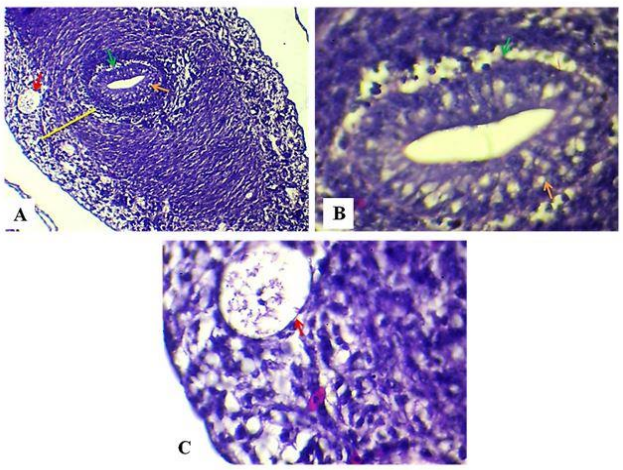

Fig. 6: A cross histological section of a 12th day incubation duck embryo, the orange arrow shows the goblet cells, the yellow line shows the muscularis layer, the red arrow shows the Auerbachian plexus, and the green arrow shows the undifferentiated embryonic cells, H&E stain, magnification (A) 40X, (B&C) 400X.

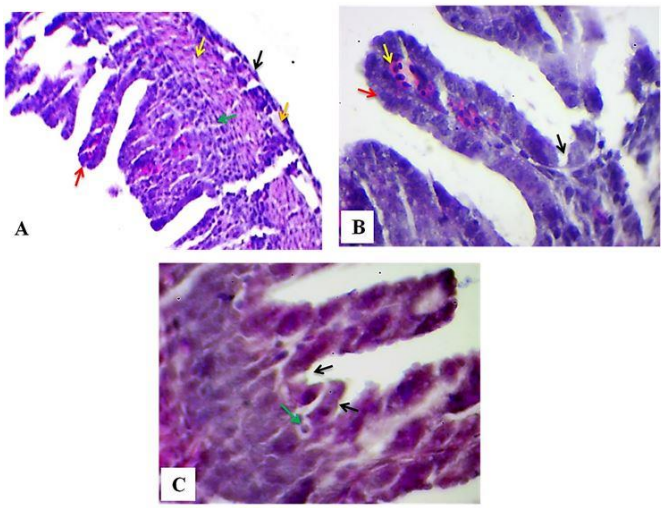

Fig. 7: A cross histological section of the duodenum of a chicken embryo incubated at 14th days, (A) the red arrow shows the villi, the green arrow shows the submucosal layer, the yellow arrow shows the muscularis layer, the black arrow shows the serosa layer, the orange arrow shows the Auerbachian plexus, (B) the red arrow shows the epithelial cells, the yellow arrow shows the leukocytes, (C) the black arrow shows the intestinal crypts, and the green arrow shows endocrine cells, H&E stain, magnification (A)100X, (B&C) 400X.

At this age also, the submucosal layer in the duodenum was thin and consisted of loose connective tissue rich in blood vessels and mesenchymal cells, and contained the Meissner's plexuses, which were elongated oval in chickens and with a circular shape and large size in ducks, and located at the bottom of the submucosal layer. There were no Brunner glands in the duodenum and jejunum of local chicken and duck embryos (Fig. 8).

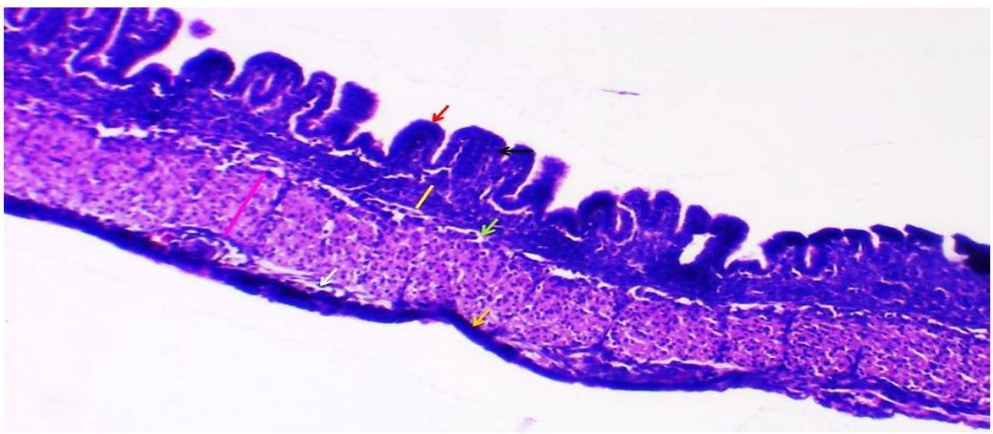

Fig. 8: A cross histological section of the jejunum of an 18th day incubation duck embryo, the red arrow indicates the villi, the black arrow indicates the epithelial cells, the yellow line and indicates the submucosal layer, the green arrow indicates the Meissner's plexus, the pink line indicates the muscularis layer, the white arrow indicates the Auerbachian plexus, the orang arrow indicates the serosa layer, H&E stain, magnification 100X.

As for the muscularis layer, which consisted of two layers of smooth muscle, inner and outer longitudinal and middle circular, which contained many blood vessels and Auerbachian plexuses, which when stained with H&E, the neurons appeared to have a transparent cytoplasm with dark blue nuclei and contained either one or two nuclei, and the glial cells were dark in color (Fig. 9). The serosa layer, which was the outer layer of the intestinal wall, consisted of a thin layer of regular connective tissue that contains mesothelium cells and many blood vessels (Fig. 10).

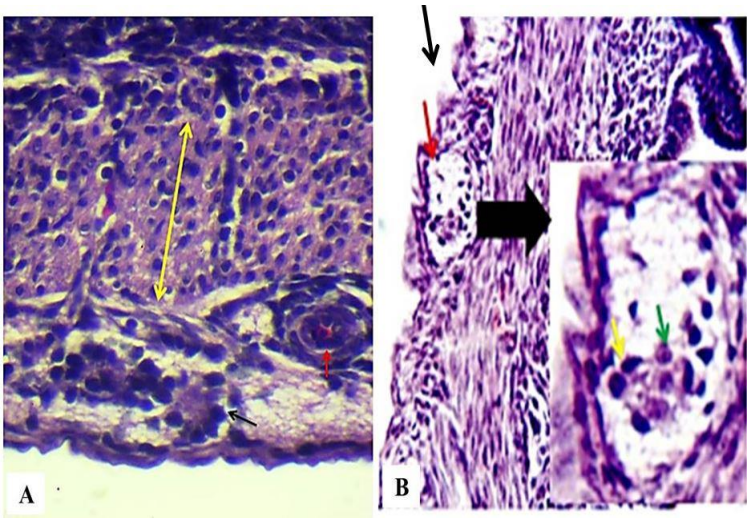

Fig. 9: a cross histological section of the ileum of a chicken embryo incubated 14th days, (A), the yellow two head arrow indicates the arrangement of the smooth muscle layer, the red arrow indicates the blood vessel, the black arrow indicates the Auerbachian plexus, (B), the yellow arrow shows the glial cells, the green arrow shows the nuclei of the neurons, H&E stain, magnification 400X.

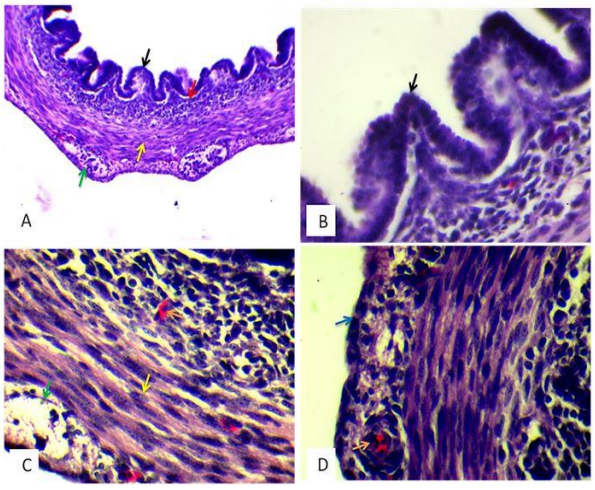

Fig. 10: A cross histological section of the duodenum of an 18th day incubation duck embryo, the black arrow shows the villus and epithelial cells, the red arrow shows the embryonic cells, the yellow arrow shows the muscularis layer, the green arrow shows the Auerbachian plexus, the orange arrow shows the blood supply, the blue arrow shows the serosa layer, H&E stain, magnification (A) 100X, (B, C&D) 400X.

The histological study showed that the wall of the jejunum was similar to the wall of the duodenum and also did not contain Brunner glands, the villi were fingers-shaped with equal length but shorter than the villi of the duodenum, with the presence of plicae in the wall of the jejunum, which is a marked difference from the duodenum (Fig. 11&12).

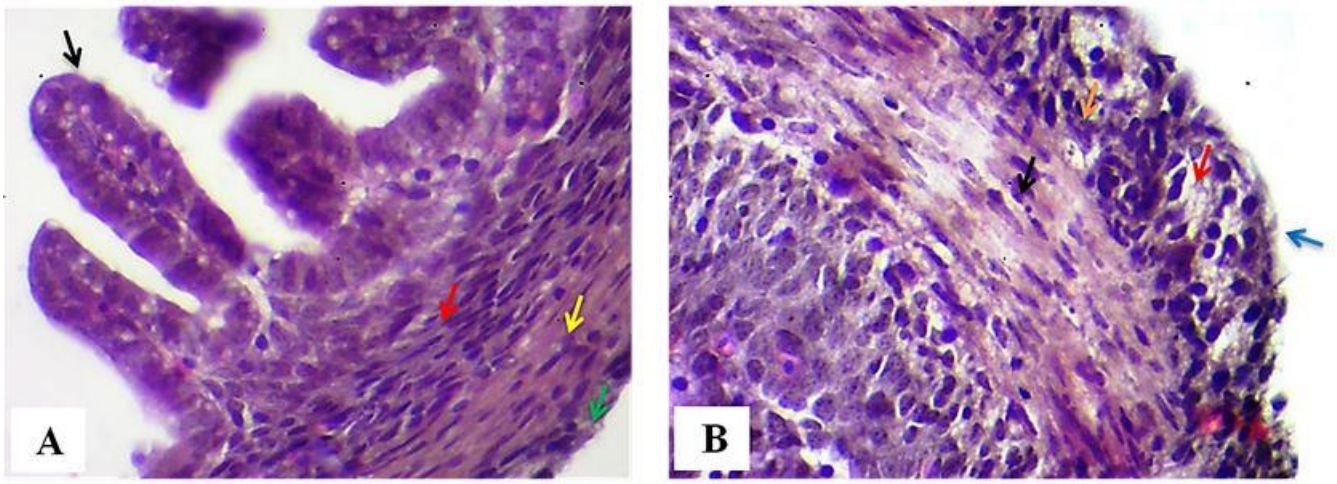

Fig. 11: A cross histological section of the jejunum of a chicken embryo incubated for 14th days. (A) shows the shape of the villus with its four layers, the black arrow shows the villus, the red arrow shows the submucosal layer, the yellow arrow shows the muscularis layer, the green arrow shows the serosa layer, (B) the black arrow shows the circular muscle layer, the orange arrow shows the longitudinal muscle layer, the red arrow shows the Auerbachian plexus, H&E stain, magnification (A) 100X, (B) 400X.

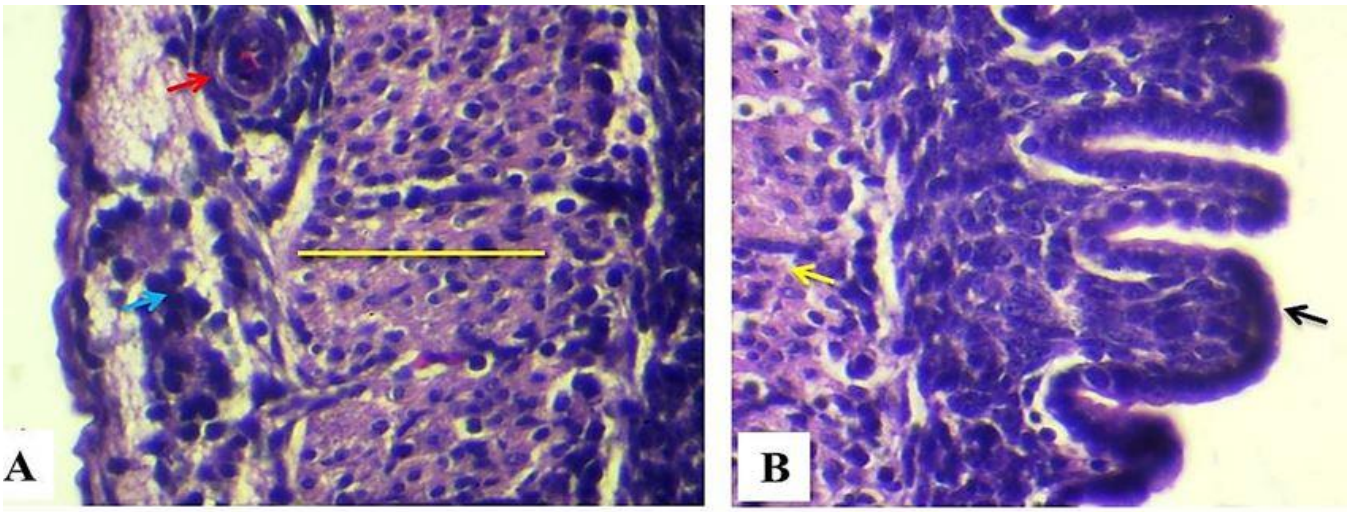

Fig. 12: A cross histological section of the jejunum of an 18th day incubation duck embryo, the black arrow shows the villi, the yellow line and arrow show the muscularis layer, the red arrow shows blood vessel, the blue arrow shows Auerbachian plexus, H&E stain, magnification (A &B) 400X.

As for the intestinal crypts (Lieberkühn crypts) appeared in 14th days incubated chicken embryo and 18th days incubated ducks embryo, the formation of intestinal crypts occurs in two ways, either from undifferentiated embryonic cells or by dividing the crypt into two crypts by bifurcation, and the goblet cells were small in size and few in number, unlike in chickens. The muscularis layer was in the form of bundles surrounded by connective tissue, and the epithelium is simple cuboidal in duck and chicken embryos (Fig. 13).

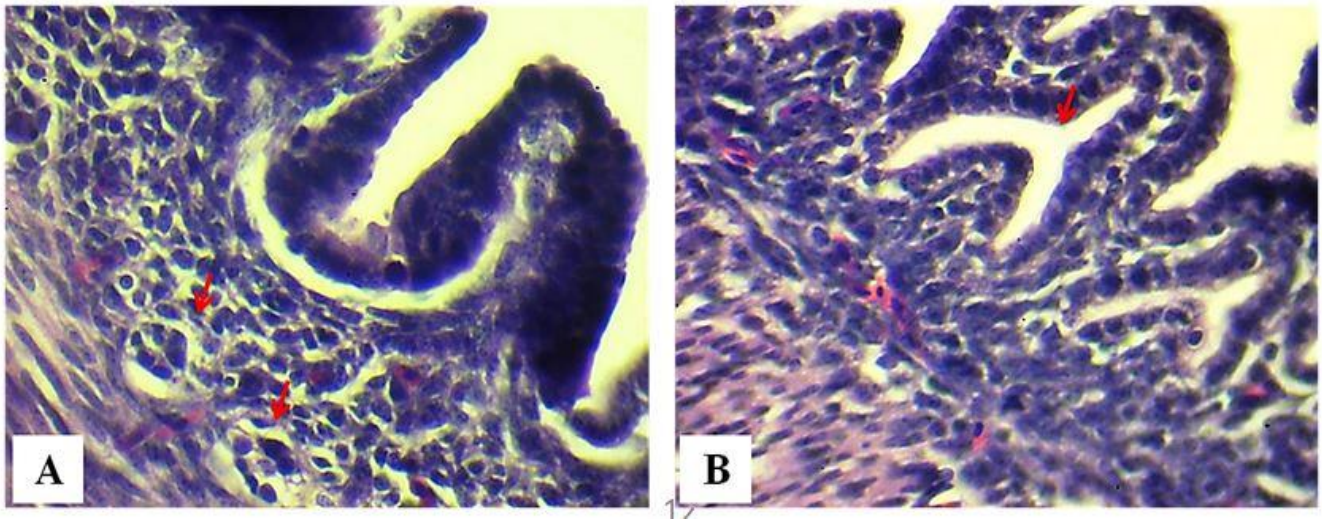

Fig. 13: A cross histological section of the intestine of an 18th day old duck embryo, (A) the red arrow shows how the intestinal crypts form from embryonic cells, (B) the red arrow shows how the crypt divides into two crypts, H&E stain, magnification 400X.

The histological study of the ileum wall showed that was similar to the duodenal and jejunum wall, except for the villi were shorter and wider in chickens. In ducks, the ileum villi were hook-shaped, with the presence of scattered lymphocytes in the submucosal layer that differentiates later into Beyer's batches that did not appear in the chicken embryo at the age of 14th days, and the presence of a middle circular muscle layer only. As for the nerve plexuses in the ileum, they were more than the duodenum and jejunum (Fig. 14&15).

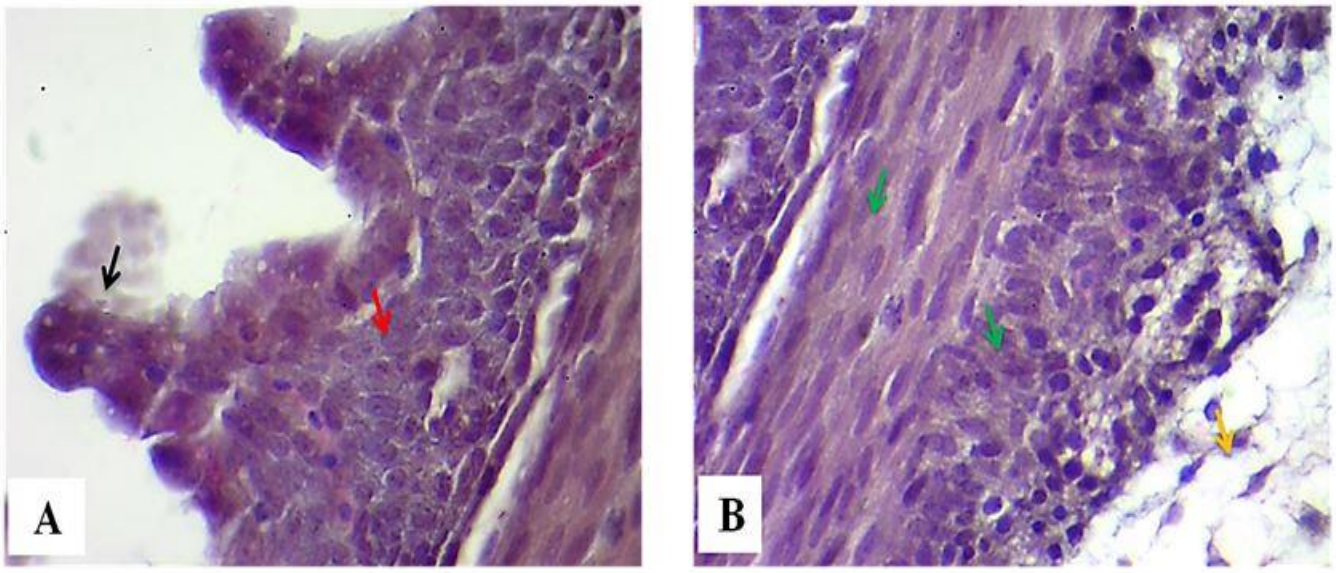

Fig. 14: A cross histological section of the ileum of an 14th day incubated chicken embryo, (A) the black arrow shows the epithelium, the red arrow shows the submucosal layer, (B) the green arrow shows the muscularis layers, the orange arrow shows Auerbachian plexus, H&E stain, magnification 400X.

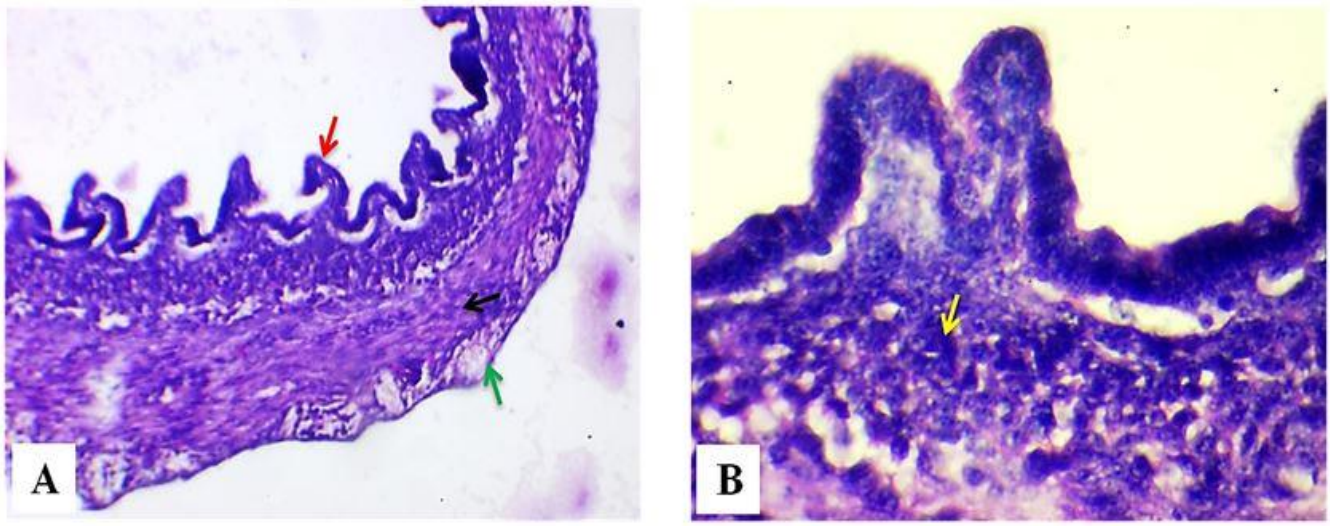

Fig. 15: A cross histological section of ileum of an 18th day incubated duck embryo, (A) the red arrow shows the villus, the black arrow shows the muscularis layer, the green arrow shows Auerbachian plexus, (B) the yellow arrow shows lymphocytes, H&E stain, magnification (A) 100X, (B) 400X.

While the third age period in chicken (15th -21st) days and duck (19th -28th) days: The chicken and duck embryos showed that the villi were fingers-shaped in the duodenum, few numbers of large goblet cells, the villi were lined with simple columnar epithelium, the presence of triangular Paneth cells at the base of the villi, and the muscularis layer consisted of three layers, outer, inner and middle with longitudinal and circular pattern with oblique muscular organization and bundles in chicken. As for the serosa layer, it was thick and contained mesothelium cells. As for the jejunum and the ileum, the villi are shorter than the duodenum, and the wall is similar to the duodenal wall (Fig. 16), except for the muscularis layer in chicken was in the form of bundles.

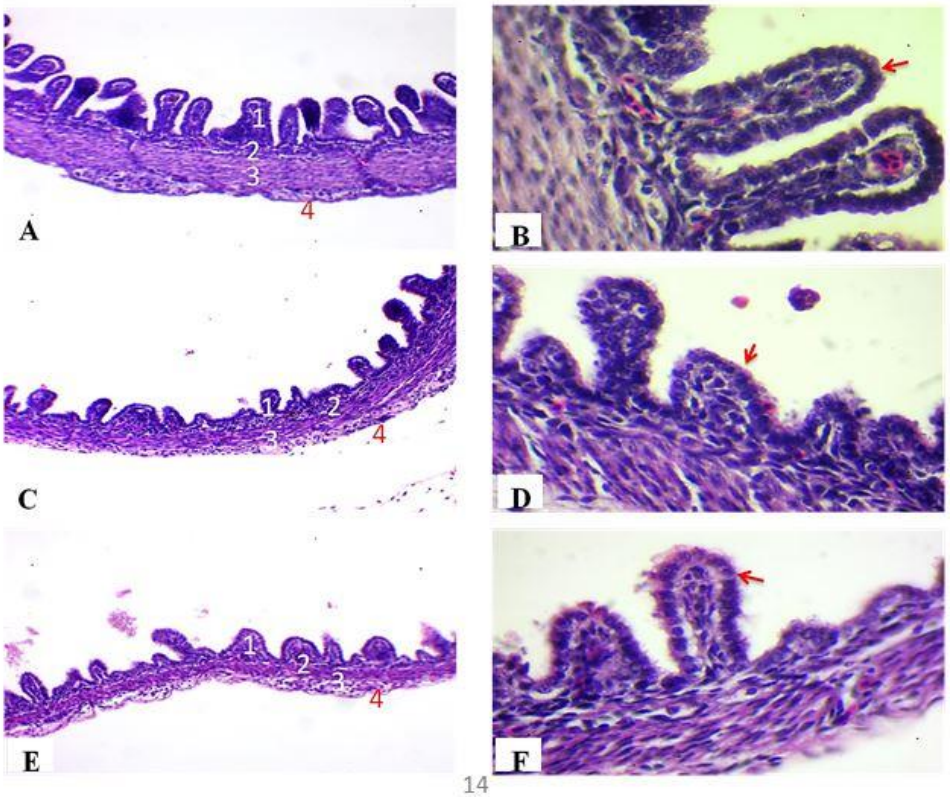

Fig. 16: A cross histological section of the small intestine of a duck embryo incubated for 25 days, (A&B) duodenum, (C&D) jejunum, and (E&F) ileum, the red arrow shows simple columnar epithelium, numbers (1,2,3,4) represent layers of the intestinal wall, H&E stain, magnification (A,C,E) 100X, (B,D,F) 400X.

The histological study of Meckel's diverticulum showed that its wall was composed of four layers similar to the layers of the small intestine wall, and the submucosal layer contains large numbers of lymphocytes (Fig. 17).

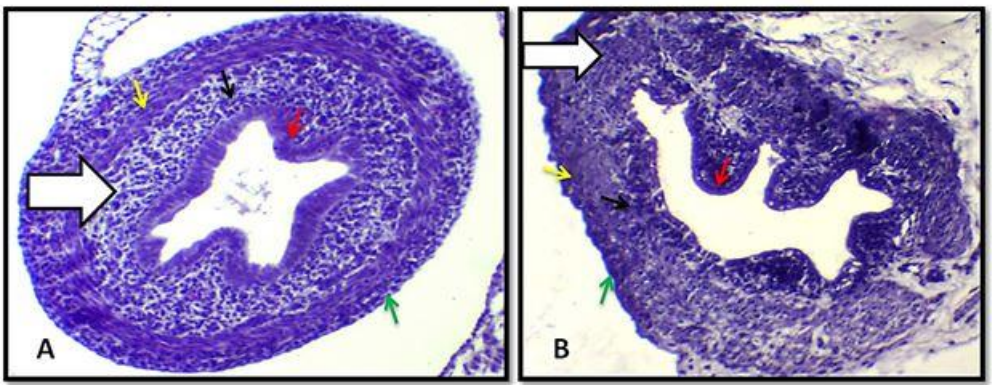

Fig. 17: A cross histological section showing Meckel's diverticulum, (A) chicken embryo incubated for 11 days, (B) duck embryo incubated for 15 days, the red arrow shows the epithelium, the black arrow shows the submucosal layer, the yellow arrow shows the middle muscularis layer only, the green arrow shows the serosa layer and the white arrow shows the lymphocytes, H&E stain, magnification 400X.

DISCUSSION

The current histological study showed that the wall of the small intestine, with its three parts (duodenum, jejunum, and ileum), consists of four layers: the mucosa, the submucosa, the muscularis, and the serosa layer. This result was consistent with **Klasing, (1999)** in his study of chickens and **Calhaun, (1988)** in his study of domesticated chickens, where their study showed that the wall of the small intestine is composed of four main layers.

The mucosa layer consists of three secondary layers; the mucosa is lined with simple columnar epithelium, lamina propria, and muscularis mucosa. This result is consistent with **Ventura *et al.*, 2013; Soliman *et al.*, 2016** in their histological study of the mucous membrane of the intestine of chickens and

quail, which showed that it is composed of three secondary layers.

The first age period of incubation in local chickens and ducks showed that the mucous membrane is in the form of folds, and undifferentiated mesenchymal cells form the outer wall of the primary intestine, and this result is consistent with what was mentioned by **Shyer *et al*., (2013)** in his study of the embryonic formation of villi in chicken embryos, where it was shown that undifferentiated mesenchymal cells form the outer layer of the wall of the alimentary canal, and **Huycke and Tabin,** (**2018)** mentioned that the gastro-intestinal canal developed from the endoderm surrounded by the visceral mesoderm, where the endoderm gives the epithelium lining the intestines and the ducts of the mucous glands, while the mesoderm gives the muscular and connective tissues.
In the second age period of incubation in local chickens and ducks, the mucous membrane is in the form of raised folds to form later villi, and this result is consistent with what was mentioned **(Huycke and Tabin, 2018)** that the intestinal folds start from the duodenum in the day (eighth) of incubation, and these folds grow and increase in number and become narrower and straighter to push the intestinal epithelium towards the villus to be triangular with two opposite edges to form the edges of the villi in the future. After that, the folds increase to reach 50% during the day (9^{th} -10^{th}) of incubation; thereafter, they take a zigzag and wavy shape, turning into long leaf-like villi. After that, vascular tissue appears on day (16^{th}) of incubation, the epithelial cells become simple cuboidal in the basal part of the cell, then the villi increase in length in the last days of incubation and increase in number, and the epithelium turns into simple columnar epithelial cells and remains throughout the life of the chicks. Mitotic activity occurs on the 17^{th} day of incubation of the epithelial cells to form the primary crypts of Lieberkühn, which arise as villi at the beginning of the duodenum.

As for day 10^{th} in chickens and 12^{th} in ducks of the current study, it was the beginning of the differentiation of the middle circular muscle layer and the presence of mesothelium cells, brush borders, and goblet cells at this age in ducks, in contrast to chickens where they were not present, and noting the abundance of blood supply in ducks more than in chickens, this result is consistent with what was observed by **(Shyer *et al*., 2013)** that the differentiation of the smooth muscular layer within the mesoderm into progressive smooth muscle, on the sixth day of incubating the chicks, the first layer of circular smooth muscle is distinguished, which also restricts growth peripherally and leads to the fusion of the epithelium and mesenchyme in longitudinal protrusions that extend along the small intestine. Later, on the 12^{th} day of incubation, the outer layer is distinguished of smooth muscle aligned longitudinally between the peripheral layer and the mesothelium leads to an increase in length with further growth, and with what was noted by **(Van Dijk *et al*., 2002)** that the apical membrane of the intestinal cells shows short protrusions called microvilli, which increase the area of its adhesion with food and sugary substance (glycocalyx) and is called the brush edge, where the microvilli increase the area of the absorbent surface of nutrients and gives areas of support for many enzymes involved in the digestion process in the extracellular milieu, and with what the researchers **(Wilson *et al*., 2018)** observed in the turkey intestine on the 15^{th} day of incubation, longitudinal folds were evident in the small intestine, which developed into single villi during the last stages of incubation with the differentiation of the epithelium and the brush border on the 18^{th} day of incubation onwards.

On the 12^{th} day in chickens and the 13th day in ducks, the intestinal wall consisting of four layers appeared, and goblet cells appeared along with the epithelium in duck embryos and paneth cells in chicken and duck embryos. This result is consistent with what was mentioned by **(Southwell, 2006)** in his study of the stages of intestinal development in fetus chickens and with what they mentioned **(Clevers and Bevins, 2013)** in their study of Paneth cells, where these researchers showed that Paneth cells are formed alongside epithelial cells at the base of the villi and intestinal crypts, and with what was mentioned **(Wilson *et al*., 2018)** that the small intestine in Turkey develops on the fourth day of incubation, the wall of the primitive alimentary canal is lined with a pseudocolumnar layer, and its thickness increases on the sixth day of incubation and undifferentiated mesenchymal cells form the outermost layer of the alimentary canal wall. However, in the chicken embryo **(Huycke and Tabin, 2018)** observed a simple columnar epithelium of the intestine during the third day of incubation, which changed to a pseudo-columnar stratified epithelium on the fourth day of incubation. Further development and differentiation of the alimentary canal wall began on the ninth day of incubation. Whereas in the tunica mucosa, which is lined with a pseudo-stratified epithelium by the submucosa layer (propria submucosa), where the wide irregular longitudinal folds of the intestinal epithelium were evident on the 15^{th} day of incubation and the development of the folds occurred during the eighth or ninth day of incubation in chicks, the relative difference observed

during the evolution of different species can be attributed to the incubation period.

On the 14th day in chickens and the 18th in ducks of incubation, the four layers in the three parts of the small intestine have been completed histologically, and the mucous membrane is lined with simple columnar epithelium interspersed with goblet cells. This result is consistent with what was mentioned by **(Duritis *et al.*, 2013)** in their study on the description of goblet cells in the small intestine before and after hatching in ostrich embryos, where they showed that goblet cells are scattered with epithelial cells in the villi and crypts of the intestine, and it does not agree with what was mentioned by **(Huycke and Tabin, 2018)** that mucous and muscle cells appeared on the day 14th, while goblet cells appeared just before hatching.

The villi in the duodenum also appeared in the current study in the form of fingers and were longer than the villi in the jejunum and ileum, and this result is consistent with what was mentioned by **(Grey, 1972; Jamroz, 2005; Bohórquez, 2011)** in their study on the villi in the duodenum in duck and turkey embryos, where he mentioned that the villi in the descending duodenum were pointed from the top, while the base was thicker in the transverse and ascending duodenum, and with what each of the researchers mentioned **(Van Leeuwen *et al.*, 2004; Incharoen and Yamauchi, 2009; Khambualai *et al.*, 2009)** that the intestinal villi take the form of the tongue, and decreases in length and volume from the duodenum towards the jejunum; they described the intestinal villi in the duodenum as narrower and longer than the villi in the jejunum, with a rounded top, and the height of the villi in the ileum is shorter relative to its height in the jejunum, and the cellular distance is narrower, as these morphological characteristics in the ileum can be explained according to the absorptive function that is concentrated in the upper part of the small intestine in when low-content materials reach the ileal villus.

As the current histological study showed, the intestinal crypts were formed in the embryos of local chickens and ducks in the last third of incubation and increased in depth until the day of hatching. This result is consistent with what was mentioned by **Uni *et al.*, (2000; 2003)** in their study of the development of the small intestine in chicks, where they showed that the intestinal crypts are formed in the last days of incubation, as they were observed at the age of 17th days, and with what was mentioned by **Jamroz, (2005)** in geese, where he found an increase in the depth of the intestinal crypts in the last days of incubation and the first week after hatching.

The current histological study also showed that the submucosal layer in each of the local chicken and duck embryos does not contain the duodenal glands, and this result contradicts what was mentioned by **ELKarmoty, (2014); Kadhim *et al.*, (2018)** in their study of histological and morphological changes in the digestive system in geese and ducks, where they found that the duodenum glands are located in the submucosal layer within the loose connective tissue together with the lymphatic fibres.

The histological study of Meckel's diverticulum showed that its wall consists of four layers similar to the layers of the intestinal wall and is lined with simple columnar epithelium. The submucosal layer contains large numbers of lymphocytes because it is considered a lymphoid organ. This result agreed with what the researchers mentioned **(Besoluk *et al.*, 2002; Mohammadpour, 2006; Igbokwe and Abah, 2009)** . When comparing Meckel's diverticulum with the jejunum and ileum, Meckel's diverticulum lacks villi and contains large amounts of lymphoid follicles in the tunica submucosa in the lamina propria and submucosa, as well as the muscularis mucosa, which is only a circular layer and very thin. The Meckel diverticulum contains a few numbers of crypts in its cavity. These features indicate that Meckel's diverticulum differs from the digestive system, especially in its morphological structure, and that it plays a functional role as a lymphatic organ. Meckel's diverticulum is a secondary lymphoid immune organ and is a lymphoid organ in the gut-associated lymphoid tissue, and finally, it is an intestinal immune system. Currently, Meckel's diverticulum is considered a third pouch of the intestine and an apparent lymphoid organ in birds because it contains a high amount of lymphoid tissue, and according to what was mentioned by **Gofer *et al.*, (2009); Igbokwe and Abah, (2009)** that Meckel's diverticulum contains lymphatic follicles, which are parts of lymphocytes that are more abundant in broiler chickens than in domestic chickens.

CONCLUSION

This study offers numerous important insights into the histogenesis of small intestines in local chickens and ducks. Initially, it provides a complete and exhaustive explanation of the progressive points, proposing new understandings of the histogenesis of the small intestine of these bird species. Furthermore, it clarifies the histological differences between chicken and duck embryos produced in comparable atmospheres, giving a detailed description of these differences. Finally, the formation of an embryonic presentation for the small intestine in chickens and ducks histologically offerings original models for reviewing waterfowl

development. The distinct development periods recognized in this study will help as valued incomes for upcoming studies, comprising molecular examinations into the histological changes of the small intestine in chicken and duck embryos. With frequent doubts still adjacent to the growth of local birds, this study holds the potential to support both local and wild bird investigators by contributing to a profoundly thoughtful and complete histogenesis of small intestine development in chickens and ducks. This combined examination offerings a roadmap for detectives to estimate varied investigational data gotten at the histogenesis of small intestine growth within the two bird types.

Conflict of interests

The authors declare no potential conflict of interest.

Acknowledgement

The authors are thankful to the Department of Anatomy, College of Veterinary Medicine, University of Mosul, Iraq for the cooperation and kind support for the completion of the research work.

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How to cite this article:
Sura M. N. Othman, Saffanah Khuder Mahmood, 2023. Histological Study of Small Intestine Development in Local Chicken (*Gallus gallus domesticus*) and Duck (*Anas platyrhynchos domesticus*) Embryos. Journal of Applied Veterinary Sciences, 8 (4): 83-94.
DOI: **https://dx.doi.org/10.21608/javs.2023.223223.1257**