

## Developmental study of small intestine in local chicken (pre hatch) (*gulls gulls domesticate* )

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### Abstract

**Aims:** The aim of this research was to found developed small intestine mucosa pre hatching and first two weeks of the life local domestic chicken, also the importance of carbohydrates in various chemical reactions, to study their distribution using special dyes, such as: combined AB-PAS (PH-2.5).

**Methods:** The study is performed on 90 commercial of fertile eggs obtained from birds market, where it is place in a small incubator and incubated with (37.5°C) and humidity (60%).

**Result:** There is a strong correlation between the intestinal epithelium's early development and the growth and feed efficiency of local chicken. This study's goal was to record the morphological, histological, and histochemical evolution of the (duodenal, jejunum, and ileum ) mucosa in local chicken prehatch from days 15 to 21 of incubation. One hundred embryos or poults had their intestinal samples taken, and these were examined under both light and dissecting microscopy. The villus height increases significantly during which time, the junctional complex progressively tightens the epithelial cells' apical end, and mature goblet cells become evident near the tip of the apical of the villi. The Villus height rises gradually till it reaches a plateau after 21 days. Before hatching, the shape of the villi gradually converts projections that resemble fingers to those that resemble leaves around 21 day. The significant morphological changes caused by consuming amniotic fluid before to hatch, as well as nutritional variables, in the local chicken gut epithelium.

**Key words :** small intestine , local chicken , embryo, pre hatch

## Introduction :

Local fowl belongs to the family Phasianidae , order Galliformes and Species *G. domesticus* . Local chickens are a type of bird that evolved in a particular place and then adapted to the natural conditions there. (1)These birds have endured for a very long time thanks to a process called natural selection, which has produced a diverse population with unique morphologies and environmental adaptations. (2).

It is well known that these hens are extremely resilient to diseases and adverse environments in the area. The value of regional chickens meat and eggs from poultry, particularly hens, are among the most widely consumed foods worldwide. Many nations have expanded their commercial production of these necessary goods during the last ten years in order to satisfy rising consumer demand. The World Resources Institute reports that over the past several years, there has been a significant increase in the amount of food produced globally per person .( 3), (4).

The anatomical structures of digestive system of the Birds are not the same as mammals. Also, Depending on how they eat, different bird species have different body types. (5, 6, 7). that total length of the gastrointestinal tract (GIT) is shorter than mammals . The pancreas is located between the duodenal loop and the bile ducts, and the pancreatic ducts

enter the duodenum and go to the jejunoileal region. (8 , 9).

The large intestine consists of the same four layers as the other sections of the small intestine: mucosa, submucosa, muscularis externa, and serosa. Histologically, this basic constructional pattern of the tiny bowel internally with tunica mucosa, which separated into three sublayers: a thin layer of longitudinal muscle fibers, the lamina propria, also known as the tunica propria, and the superficial epithelium. This study's goals were to use histochemical techniques to look at changes in goblet cell density, secretion, and lymphatic nodules, as well as to analyze the histomorphological development of the small intestine of local fowl. While there were more lymphocytes in the duodenal region of the gut, there were more goblet cells in the distal portion. The mucopolysaccharide secretions of Lieberkühn's goblet cells and crypts are acidic and highly sulphated. The ileum's brush boundaries and goblet cells both had significant levels of polysaccharide material. (10) .

## Material and study design

This research was designed to described the sequential Histomorphological and Histochemical development pre-hatch Of small intestine in local chicks (*Gullus Gullus domesticusa* ) and through this study development layers ,glands and villi of small intestine and development layers. The study is performed on 90 commercial of fertile eggs obtained from

birds market, where it is place in a small incubator and incubated with (37.5°C) and humidity (60%). was examined by candling the chicks, who were then split into two groups and had 90 samples taken from their embryos at the ages of 15 and 21 days before hatching, and each sample consist of 45 chicks will be examine small intestine and fixed in fixative formalin .

**Embryos of pre hatching periods :**The embryos of pre hatching periods have distributed on two groups: first group at day 15 in stage 41 in (11) staging system , second group day 21 in 46 in (11) staging system , by taps of the scissors or scalpel broke the upper part of the shell, and the pieces of shell were removed with fine forceps. As the sharp-pointed bits of shell are prone to turning inward and cutting the embryo, the whole embryos were washed by 0.9% of normal slain, then we dissected with the midline of the celomic cavity the embryos for the removal of the tiny bowel, fixations in Ten percent formalin for 48 hours, and processed for routine histological techniques (12).

## Result

### Morphological result pre hatch for small intestine

The results of anatomy displays, position and the connection between the small intestine appearance and differentiated into three parts including duodenum, jejunum, and ileum prehatch.( Fig.1). Each part has distinct anatomical features and functions that contribute to the process of nutrient absorption and

digestion. The gross examination of pre-hatch local chicken embryos at 15 days revealed creamy in color and a subtle distinction amid the jejunal and duodenal, with no clear demarcation amid the ileum and jejunal (Fig. 2). However, by 21 days pre-hatch, distinct demarcations amid the duodenal, jejunal, and ileum were evident (fig.3). Additionally, the duodenal exhibited a creamy coloration and featured a characteristic U-shape, comprising descending and ascending limbs that formed loops accommodating the pancreas between them, as observed in the present study." (Fig.2&3).

The duodenum weight about  $0.091 \pm 0.0004$  gm, while length  $25.21 \pm 0.24$  mm ,  $0.318 \pm 0.09$ gm,  $37.12 \pm 3.85$ mm at age (15 ,21) pre hatch respectively table (4-1 &4-2).

"The JeJunal, the longer and seconds segment of the tiny bowel, commences at the termination of the ascending limb of the duodenal loop. This portion is characterized by creamy in color , multiple short garland-like coils along its dorsal border and is distinguished by a clear demarcation via the Ileum. The diverticulum Meckel no limitation the transition between the jejunum and ileum, The morphometric in table (4-1 ), showing of jejunum the length  $34.78 \pm 0.15$ mm at (15,21) prehatch while weight about  $0.422 \pm 0.003$  gm , $50.90 \pm 4.68$ mm,  $0.378 \pm 0.07$ gm respectively table (4- 2 ).

"this Ileum constitutes The last segment of the tiny bowel, distinguished by its shorter length compared to the preceding parts. In gross appearance, it exhibits a

variable color ranging from creamy to red tones (Fig. 2,3). Continuously extending from the jejunum, the ileum terminates at the ileocecal junction, marking the transition into the large intestine." The ileum length  $6.31 \pm 0.40$  mm,  $21.60 \pm 0.33$  mm at age (15,21) pre hatch respectively while weight about  $0.028 \pm 0.0002$  gm,  $0.073 \pm 0.03$  gm table (4-1 & 4-4-2).

At end the jejunum present structural very well crossly called Meckel's diverticulum, which forms during the embryonic stage of a chicken 21 days before hatching, indicates the beginning of the ileum and the close of the jejunum. (Fig.3).

#### **Histological & Histochemical Finding of small intestine pre hatch**

The local chicken's entire tiny bowel, which is composed of the jejunum, ileum, and duodenum, revealed that presence of four tubular organ layers, specifically the mucosa, submucosa, muscularis and serosa. The intestinal crypts of Liebkühn and villi of different sizes, forms, and shapes were among the tiny intestine's unique characteristics.

The four traditional layers that comprise the mucosa, submucosa, muscularis and serosa, in a local chicken structure, make up the duodenum, jejunum, and ileum microscopically. (Fig.4)

Three elements made up the tunica mucosa of the local chicken's duodenum, jejunum, and ileum: the spreading crypts between villi known as Lieberkühn crypts, and the lamina propria, which described the villi's core beneath the epithelium. The muscularis mucosa

becomes evident in fifteen days, pre hatch (Fig.4,6).

The mucosal properties were framed by the villi and crypts of the lieberkühn. Villi well developed clearly as fingers per villus, with mesenchymal lining each villus and varying in shape and size along the tiny intestine. Goblet cells are not prominent within the duodenum, jejunum and ileum epithelium while in 21 day pre well developed, loose connective tissue made up the lamina propria (Fig.5,6,7).

The base villi of the slightly appear of crypts lieberkühn gland are positioned in the referred muscularis mucosa, the height of the villi of duodenum was  $56 \pm 2.91$   $\mu$ m,  $408 \pm 5.7$   $\mu$ m, the depths of the crypts were  $24 \pm 0.72$   $\mu$ m,  $64.25 \pm 1.15$   $\mu$ m, the width of the villus was  $32 \pm 0.93$   $\mu$ m,  $64.5 \pm 1.01$   $\mu$ m, the height epithelium was  $8 \pm 0.93$   $\mu$ m,  $10.25 \pm 0.72$   $\mu$ m, indicating thickness of the tunica mucosa was  $53 \pm 2.54$   $\mu$ m,  $113.25 \pm 1.15$   $\mu$ m of duodenum at age (15,21) pre hatch table (4-3).

Villi, depth of the crypt, and mucosa layer thickness notably greater in the jejunum than in the duodenal and Ileum, as well as mucosa layer thickness higher than these two regions (Fig.5,6), demonstrating the height of the villus  $76 \pm 2.91$   $\mu$ m,  $131 \pm 2.91$   $\mu$ m, the depths of crypts were,  $15.75 \pm 1.15$   $\mu$ m,  $64.5 \pm 1.34$   $\mu$ m, width villi were  $39.25 \pm 1.15$   $\mu$ m,  $65.75 \pm 1.15$   $\mu$ m, high epithelium were  $9.25 \pm 0.63$   $\mu$ m,  $19.75 \pm 0.91$   $\mu$ m. At age (15,21) days pre hatch, the indicating a significant difference in age (15) table (4-4 & 4-7).

The sort of epithelial cells that border the villi and the depth of the crypt is called

simple columnar epitheliumThe depth of a crypt, which is determined by the lamina propria, a connective tissue that is localized by blood vessels, may play a significant role in determining the crypts' ability to maintain the structure of the villus and to increase its height. This is linked to enhanced absorption, a rapid rate of conversion, and quincker development (Fig.5,6,7).

In contrast to the other two preceding segments, the crypts and villi of lieberkühn of ileum were lined by basic epithelial cells in columns containing a lot of goblet cells. the villi's length progressively shrank in the direction of the ileum to A little smooth muscle bundle and collagenous fibers make up the connective tissue that makes up the villi's core. The lieberkühn crypts, which were basic tubular glands located at the bases of the villi, are examples of connective tissue elements that cover the lamina propria (Fig. 6,7)

115±3.53µm, 170±7.58 µm are the heights of villi. The average depths of the crypts were 12.5±0.88 µm, 31.25±0.88µm, and 36.5±1.07µm, 55±1.53 µm were the mean villus widths; the high epithelium measured 9.25±0.63µm, and 20.25±0.72µm. At age (15,21 ) days prehatch, the mean thickness of this tunica mucosa was 122±1.15µm, and120.5±1.51 µm. table (4-5&4-8) .

The submucosa in duodenum did slightly develop 15day prehatch, while in21 days prehatch clear developed (fig. 4, 6,8 ), displaying thicknesses of 5.75± 0.63µm,

18.25±0.84 µm , table (4-6) in the current current study.

In jejunum sub mucosa a connective tissue made up of blood vessels and Meisner's plexus nerves formed this layer (Fig. 8,9). The showing thickness was 8.25± 0.84µm, 18.5±0.82µm at age (15&21) days prehatch respectively table (4-7).

In ileum sub mucosa a connective tissue progressively developed in days (15, 21 ) prehatch . It is made of tissue that connects. How thick a tunica this is was 6.25±0.88µm 13±1.15µm at age (15,21) days prehatch, respectively, as shown in table (4-8).

In the duodenum , the layer under study consists of two levels: A dense internal round level additionally a similar slender external layer that is longitudinal. (fig.8,9). The thickness at 15, 21 days pre hatch was 87± 1.25µm and 30.75±1.15 µm, respectively, as shown in table (4-6).

Also in jejunum, this layer was created by the longitudinal and circular inner and outer layers of fibers in smooth muscle (Fig. 8,9). The a smooth muscle fibers in The local hens' jejunum make up this tunica.. At age (15&21) days prehatch, the thickness of this tunica was 39.25± 1.15µm, and 40.75±1.15µm, respectively, table (4-7).

At the last part of the small intestine, this layer, which resembles the duodenum and jejunum, consists of fibers in smooth two levels of muscle organization: an outside longitudinal layer and an inner circular layer. At ages 15 and 21prehatch , respectively, the tunica's thickness was



shown to be  $76.5 \pm 1.07 \mu\text{m}$  and  $56.25 \pm 0.88 \mu\text{m}$  of table (4-8).

Tunica serosa in duodenum, jejunum and ileum were made up of collagen fiber bundles surrounded in one layer of cells called mesothelium within loose connective tissue. At 15 also 21 days of age prehatch, respectively, the thickness of this thin layer that demonstrated a high statistically significant difference development was,  $37.2 \pm 1.44 \mu\text{m}$ , and  $12.5 \pm 1.25 \mu\text{m}$  (Fig.11,12) table (4-6).

According to table (4-7) in jejunum, the layer's thickness at age (15,21) days prehatch was,  $26.25 \pm 0.88 \mu\text{m}$  and  $17.5 \pm 0.55 \mu\text{m}$ , respectively

According to table (4-8) in ileum, displays the thickness of the tunica serosa at 15 and 21 day prehatch, which were  $14.25 \pm 1.28 \mu\text{m}$ , and  $15 \pm 1.42 \mu\text{m}$  respectively.

Using the stains combining AB((PH-2.5)) and Periodic Acid-Schiff, The jejunum, ileum, and duodenum were examined to reveal different types of mucins (neutral & acidic mucins).

Goblet cells were found in the duodenal, jejunum and ileum mucosa layer's villi and depth crypt using AB and PAS stain. goblet cells were not found in small intestine at 15 days prehatch (Fig.10). In contrast, both goblet cells in villi and the crypt, they strongly positively reacted to the stain and appeared red in 21 day prehatch (Fig.11), indicated presence of acidic and neutral mucin. While Connective Tissue of the small intestine demonstrated slightly response for Periodic Acid-Schiff stain and negatively

for AB(PH-2.5) Fig ( 10,11). Due to mild presence of neutral polysaccharide.

By using Masson's trichrome stain to expression for collagen fiber in tunica mucosa and submucosa and serosa in small intestine in all ages (Fig.13) except 15 day prehatch was absent of collagen fiber. (Fig.12)

### Discussion

The small intestine of local chickens, comprising the duodenum, jejunum, and ileum, represents a vital organ system essential for nutrient absorption and overall health. Microscopically, The four layers that make up this organ system are the muscularis, serosa, submucosa, and mucosa. Understanding the detailed anatomy of these layers is crucial for comprehending the physiological adaptations that support efficient metabolism and assimilation of nutrients in chickens, the characteristic structures of tiny bowel were presence of the various shape, size and of villi and the intestinal crypts of lieberkühn,

The jejunum, ileum, and duodenum microscopically structured state in this investigation The four classic layers of local chicken were found to be the Mucosa, Submucosa, Muscular is, and Serosa. This finding is in line along with research carried out through (13). in African pied crows *Corvus albus* and by (14) in guinea fowl.

The local chicken's tunica mucosa consisted of three components: the lamina propria, which referred to the villi's core, the Lieberkühn crypts, which extended between the villi, and the epithelium, which was simple columnar. In Uttara

fowl, this conclusion is concurring with (10). The results of the present investigation findings on the muscularis mucosa in duodenal mucosa were comparable to those of (15) in yellow and blue macaws, but they differed from those of (13) in African pied crows, who reported that the muscularis mucosa was lacking. However, (16) noted that in Kestrel and Bulbul, this layer of The mucosa muscularis is lacking Located with the the actual layer additionally submucosa tunica.

The villi also crypts of the lieberkühn served as a framework for the characteristics of the duodenal mucosa. Simple columnar cells of varying shapes and sizes lined the villi, which developed into finger-like shapes per villus. Goblet cells, on the other hand, were not prominent in the Duodenal, Jejunal, and Ileum. Instead, They were established at the crypt's depths and between its villi., growing in number as the villus grew older. Within the villi, The predominant components of the lamina propria were loose connective tissue and collagen fibers.. The lamina propria contents of the present Ross-308 research were similar to those of previous studies on the majority of avian species, including those on broiler chickens, ducks, and geese by (17), Geese by (18) and broiler chicks by (19).

Jejunal mucosa layer thickness significantly taller than duodenum and ileum mucosa, villi, depth crypt, and mucosa thickness higher than these two parts together. These epithelial cells, known as simple columnar epithelium,

border the villi and the crypt's depth. According to (20) research in native ducks, the lamina propria of the jejunum lies between the intestinal gland and the core of the jejunum villi. These results are consistent with the current findings. The Lamina Propria is referred to as a connective tissue localized by blood vessels. According to (21), a key factor in determining a crypt's ability to accommodate villus growth is its depth and maintain the villus structure, which is linked to increased absorption, a high conversion rate, and accelerated development.

Ileum's tunica mucosa, Villi's central component was made of connective tissue., including collagenous fibers and tiny smooth muscle bundles. According to (22), villi in chickens gradually shrank in length as they approached the ileum, which is in line with the findings of the current investigation. Villi and the lieberkühn crypts were surrounded by simple goblet cells surrounded by columnar epithelial cells, more than other two previous segments. In the lamina propria, connective tissue is found. components such as collagenous fibers and lieberkühn crypts, which are basic tubular glands situated at the bases of the villi and resembling those present in the jejunum and duodenum.

Crypts lieberkühn gland located referred muscularis mucosa and localized of base villi, the tubular glands lined by simple epithelial cells same covering the villi As similar cited in the avian literature by (23).

The submucosa was only partially developed at ages 15, 21, in prehatch. In the current study, Brunner's glands were not found in the intestinal submucosa, contrary to (24) reported in the duodenum of ostriches. Moreover, Brunner's glands were lacking from the submucosa layer, according to (25) ,who also found that Kestrel and Bulbul lacked Brunner's gland submucosa. Jejunum and ileum's submucosa It is not well developed in the first 15 days before hatching, but it develops better at age 21 when it hatches because it consists of blood vessels and connective tissue. This conclusion is consistent with a previous investigation in mallards by (26).

Musclaris layer was made up of two layers in the current study: A slender outside extended layer also A dense inner ring layer. This is consistent with other research on Japanese quail that mentioned (27). According to (28, 33), Two layers of smooth muscle fiber make up this layer. — two layers: an exterior longitudinal layer and an inner circular layer —and it corresponds with the thickness of the jejunum's smooth muscle fibers rock doves and sparrow hawks.

According to (29), that layer is the same as the duodenum and jejunum and is made up includes both the inner also external circular levels of smooth muscular fibers. It is found in common quail and green-winged teal. However, compared to earlier segments, this layer's thickness was higher, which corresponds with findings made in several avian species by (30) and (15) in Macaws, Both Yellow & Blue. The Tunica Serosa

composition Within the duodenum's connective tissue underlay, bundles of collagen fibers were seen, encased in a single layer of mesothelial cells. (31) describes the pigeon (*Columba livia*), and these results are consistent with those seen in other bird species.

The jejunum's Tunica serosas made up of unfastened connective tissue, which is bordered on the outside by mesothelium and contains collagen fibers, blood vessels, and nerves. The majority of bird species, including mallards, showed the same conclusions of the current study (26,34). The mesothelium and blood arteries are covered in a thin layer of collagen-containing connective tissue called the ileum tunica serosa, as stated in Kadaknath fowl by (32).



**Table : 4-1: morphological measurement length in local chicken**

length	duodenum		jejunum		ileum	
	15	21	15	21	15	21
Pre hatch	25.21±0.24	37.12±3.85	34.78±0.15	50.90±4.68	6.31±0.40	21.60±0.33

**Table : 4-2: morphological measurement weight in local chicken**

Weight	duodenum		jejunum		Ileum	
	15	21	15	21	15	21
Pre hatch	0.091±0.000 4	0.318±0.09	0.422±0.00 3	0.378±0.0 7	0.028±0.000 2	0.073±0.03

**Table : 4-3: histological measurement of duodenum in local chicken( μm)**

	Height of villi		Depth of crypt		Height epithelium	
	15	21	15	21	15	21
Pre	56±2.91	408±5.7	24±0.72	64.25±1.1 5	8±0.93	10.25±0.7 2

**Table : 4-4: histological measurement of Jejunum in local chicken(  $\mu\text{m}$ )**

	height of villi		depth of crypt		height epithelium	
	15	21	15	21	15	21
Pre	76 $\pm$ 2.91	131 $\pm$ 2.91	15.75 $\pm$ 1.15	64.5 $\pm$ 1.34	9.25 $\pm$ 0.63	19.75 $\pm$ 0.91

**Table : 4-5: histological measurement of Ileum in local chicken(  $\mu\text{m}$ )**

	Height of villi		Depth of crypt		Height epithelium	
	15	21	15	21	15	21
Pre	115 $\pm$ 3.53	170 $\pm$ 7.58	12.5 $\pm$ 0.88	31.25 $\pm$ 0.88	9.25 $\pm$ 0.63	20.25 $\pm$ 0.72

**Table : 4-6: histological measurement of duodenum in local chicken(  $\mu\text{m}$ )**

	Width of villi		mucosa		Sub mucosa		musclaris		serosa	
	15	21	15	21	15	21	15	21	15	21
Pre hatch	32 $\pm$ 0.93	64.5 $\pm$ 1.01	53 $\pm$ 2.54	113.25 $\pm$ 1.15	5.75 $\pm$ 0.63	30.75 $\pm$ 1.15	87 $\pm$ 1.25	18.25 $\pm$ 0.84	37.2 $\pm$ 1.44	12.5 $\pm$ 1.25

Table : 4-7: histological measurement of jejunum in local chicken(  $\mu\text{m}$ )

	Width of villi		mucosa		Sub mucosa		musclaris		serosa	
	15	21	15	21	15	21	15	21	15	21
Pre hatch	39.25 $\pm 1.15$	65.75 $\pm 1.15$	70 $\pm 0.53$	122.2 $\pm 1.07$	8.25 $\pm 0.84$	18.5 $\pm 0.82$	39.25 $\pm 1.15$	40.75 $\pm 1.15$	26.25 $\pm 0.88$	17.5 $\pm 0.55$

Table : 4-8: histological measurement of ileum in local chicken(  $\mu\text{m}$ )

	Width of villi		mucosa		Sub mucosa		musclaris		serosa	
	15	21	15	21	15	21	15	21	15	21
Pre hatch	36.5 $\pm 1.07$	55 $\pm 1.53$	122 $\pm 1.15$	120.5 $\pm 1.51$	6.25 $\pm 0.88$	13 $\pm 1.15$	76.5 $\pm 1.07$	56.25 $\pm 0.88$	14.25 $\pm 1.28$	15 $\pm 1.42$

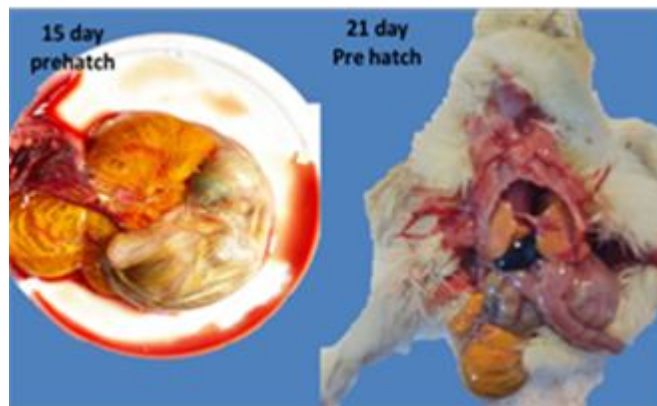
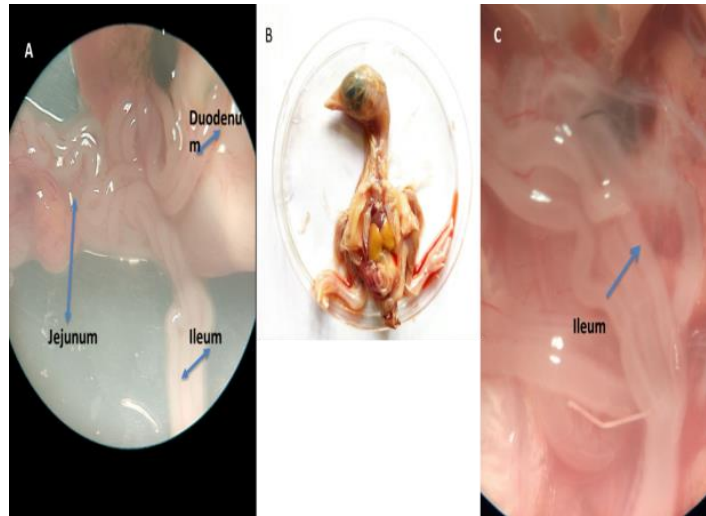
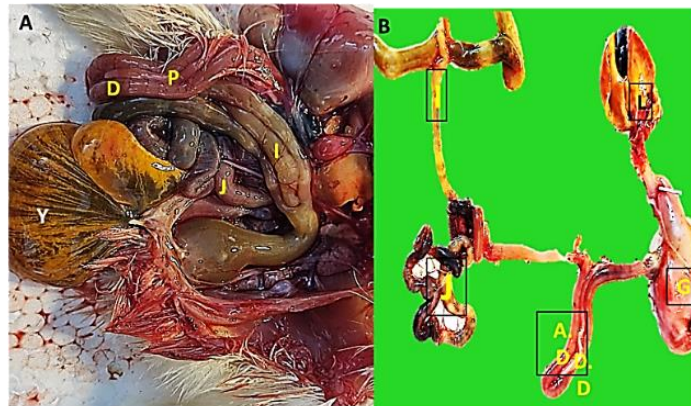


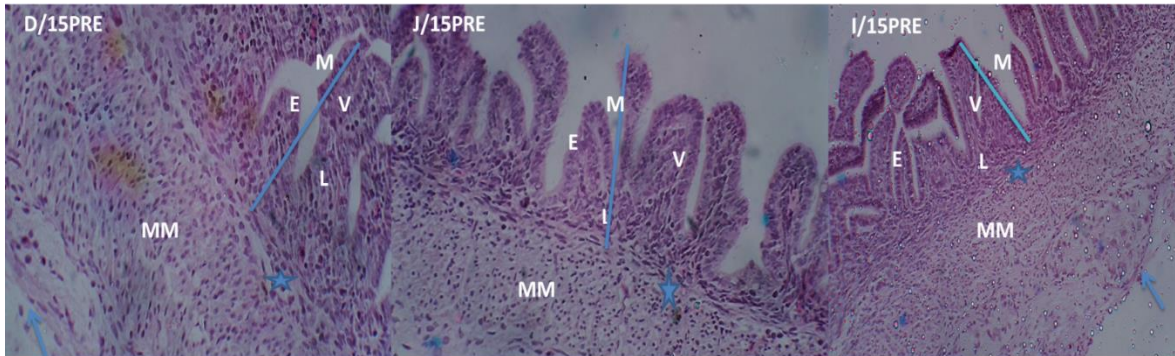
Fig: (1) Crossly embryo pre hatch after remove from shell of egg in 15 day and 21 day pre hatch



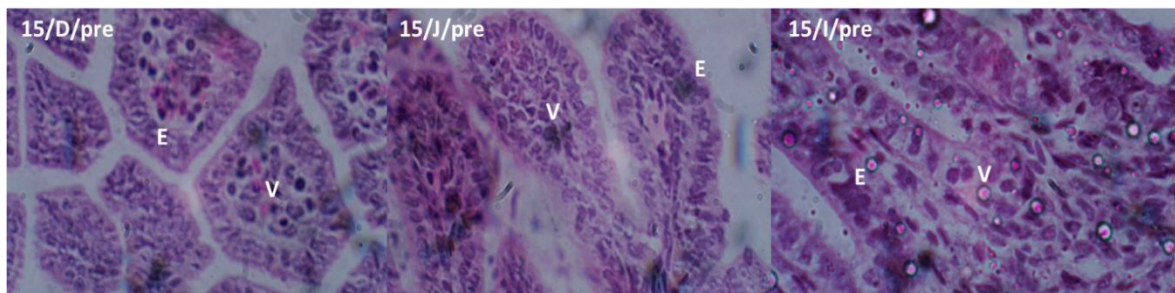
**Fig: (2):**A&C: photomicrograph under dissecting microscope of embryo in day 15 day pre hatch of local chicken showing the duodenum, jejunum, and ileum in the small intestine. The intestine has become longer and wider, and it is more distinct. magnified section :A:10X; C: 20X.



**Fig: (3) A:** embryo pre hatch 21 day after incision of celomic cavity show: duodenum descending and ascending loop(D.D &A.D) between pancreas(P) and attachment with gizzard (G)on left side and attachment with (yolk sac). (y) and fig. B : photograph of viscera of embryo of local chicken in 21 day posthatch : showing Duodenum (D), Jejunum (J), and Ileum (I) are the three small intestines attached with gizzard (G)

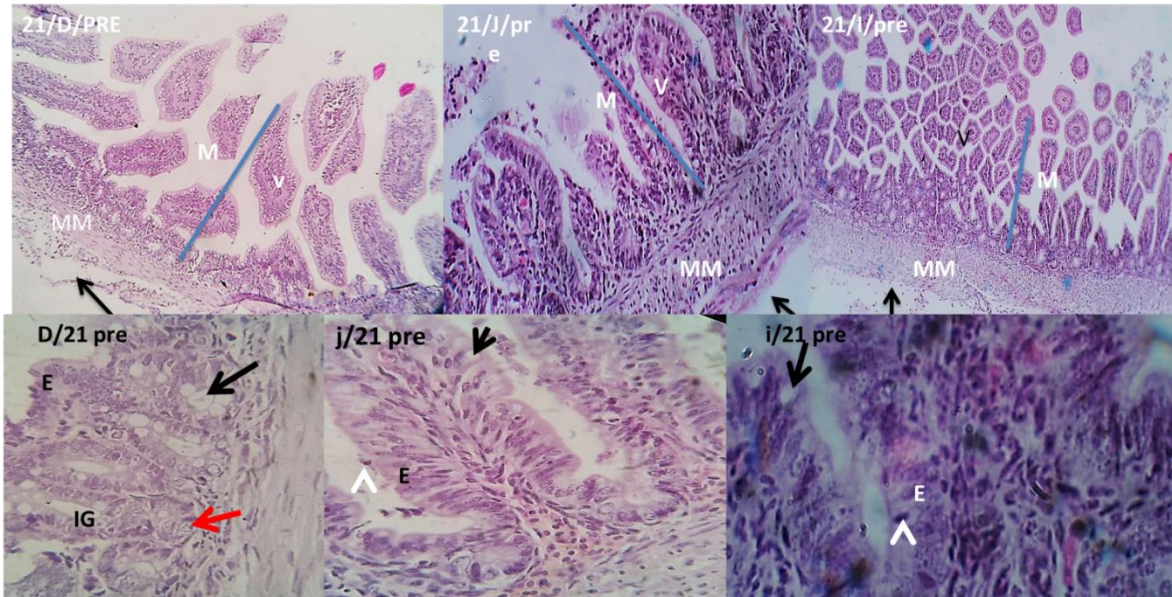


**Fig: (4)** Cross section of the duodenum, jejunum and ileum in 15day pre hatch Of local chicken showing tunica mucosa (M), sub mucosa(blue star) , tunica muscularis (TM) and serosa (S), simple of epithelium (E). H&E stain. magnified section ;200X

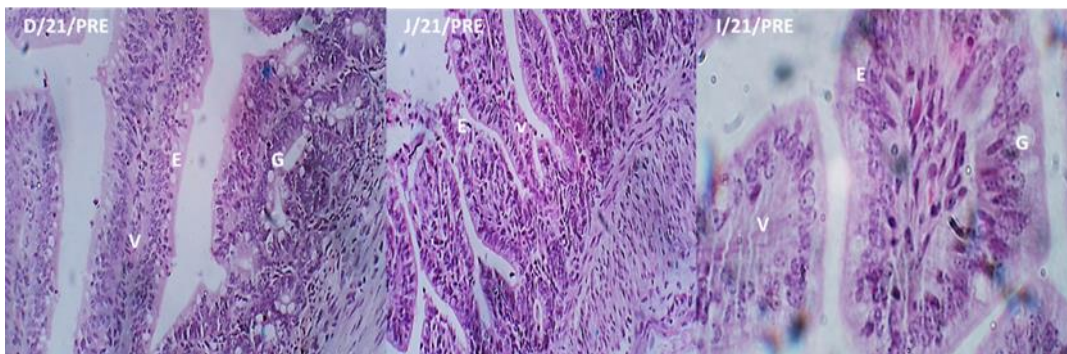


**Fig: (5)** Cross section of the DUODENUM, JEJUNUM and ILEUM in 15day pre hatch Of local chicken showing Villi (v) lining with mesenchymal cells (E) . H&E stain. : magnified section , 40X; 40X; 1000X.



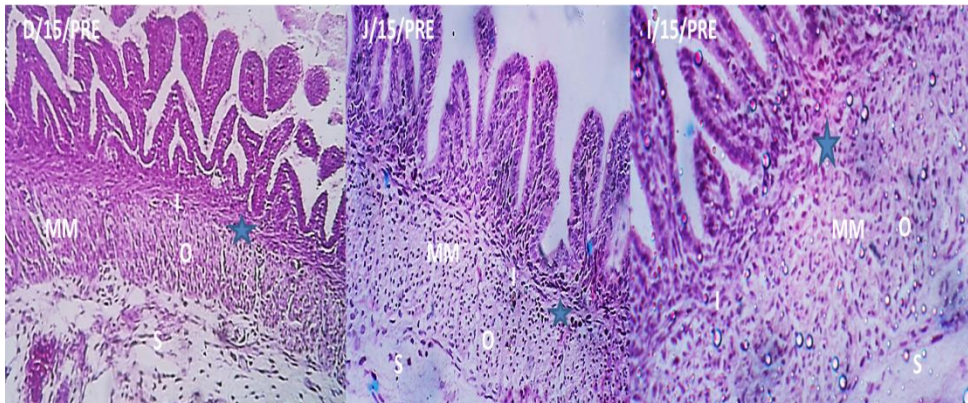


**Fig: (6)** Upper & lower panel Cross section of the DUODENUM, JEJUNUM and ILEUM in 21day pre hatch Of local chicken showing tunica mucosa (M) , tunica muscularis (TM) and serosa (black arrows), villi(v). H&E stain. : upper panel magnified section 100X; lower panel magnified X400

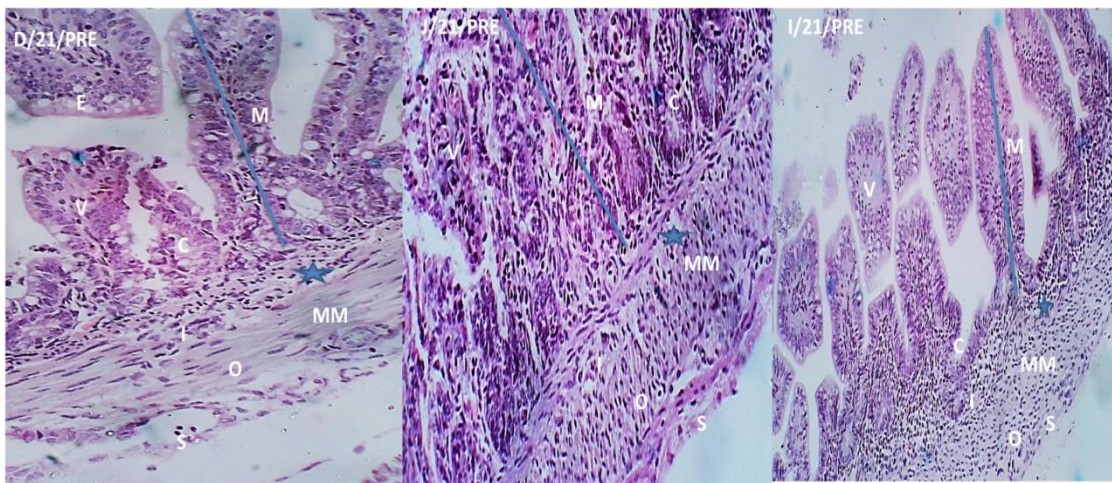


**Fig: (7)** Cross portion Of the in DUODENUM, JEJUNUM and ILEUM in 21day pre hatch of local chicken showing Villi (v) lining with epithelium cells (E) and goblet cells (G) . H&E stain. : magnified section for 400X for (D&J) and magnified section for I: 400X; 200X ;1000X



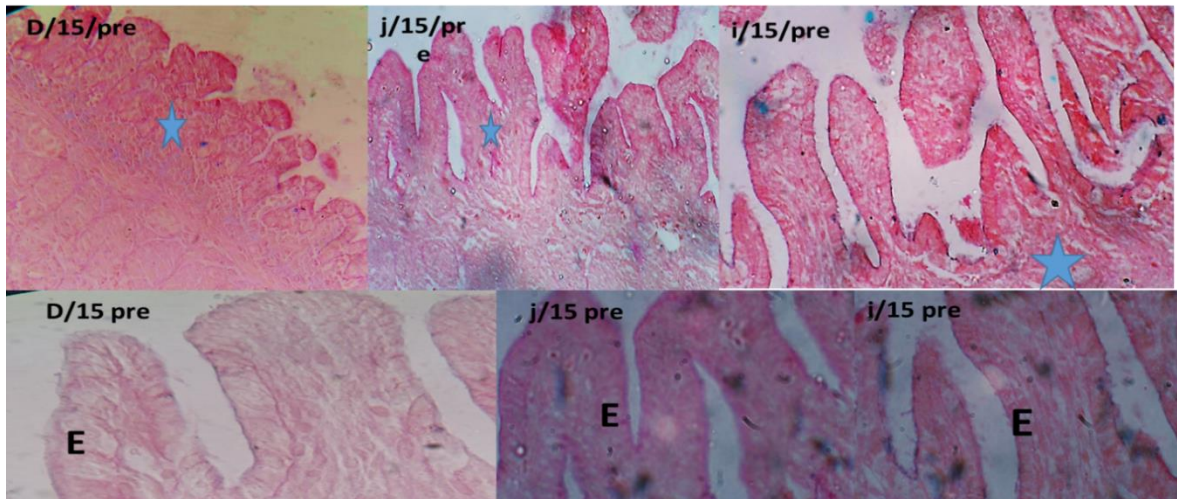


**Fig: (8)** Cross section of the DUODENUM, JEJUNUM and ILEUM in 15day pre hatch of local chicken showing sub mucosa (blue star), tunica muscularis (MM) consist of thick outer layer (O) and thin inner layer (I) and serosa (S), . H&E stain. magnified section 100X

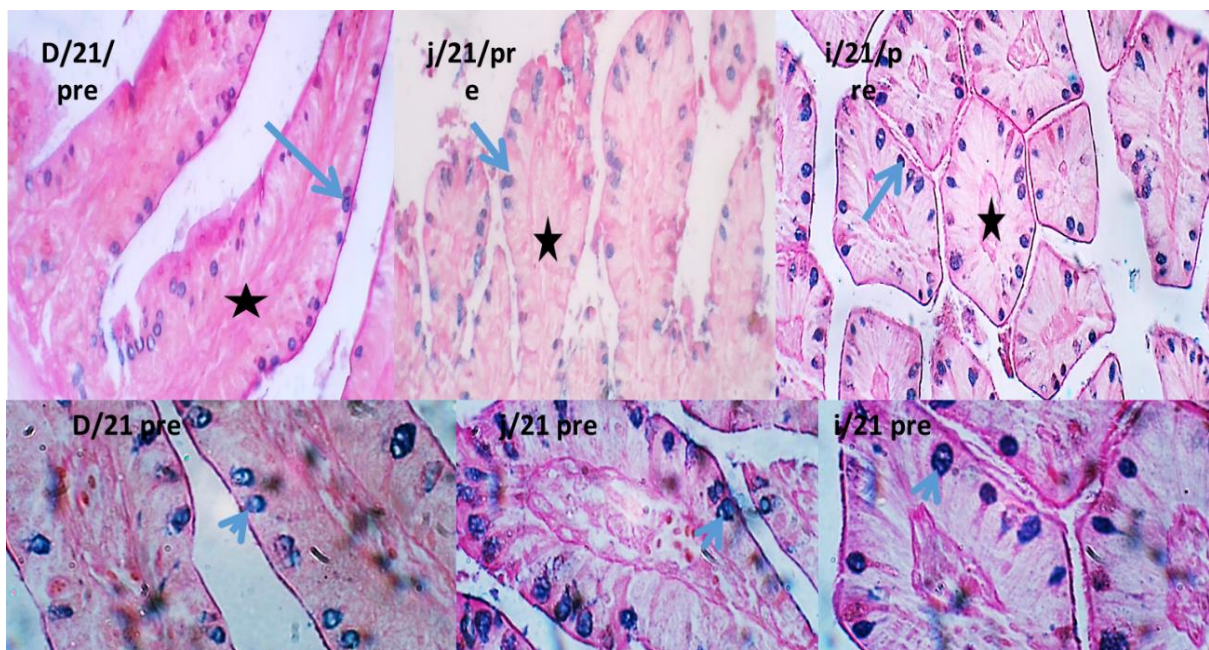


**Fig: (9)** longitudinal section of the DUODENUM, JEJUNUM and ILEUM in 21 day pre hatch of local chicken showing **tunica mucosa (M)** , **sub mucosa(blue star)** **tunica muscularis (MM)** consist of thick outer layer(O) and thin inner layer (I) , serosa (S), villi (V) with simple of epithelium (E).and crypts of leibrkan (C). **H&E** stain. magnified section **100X**



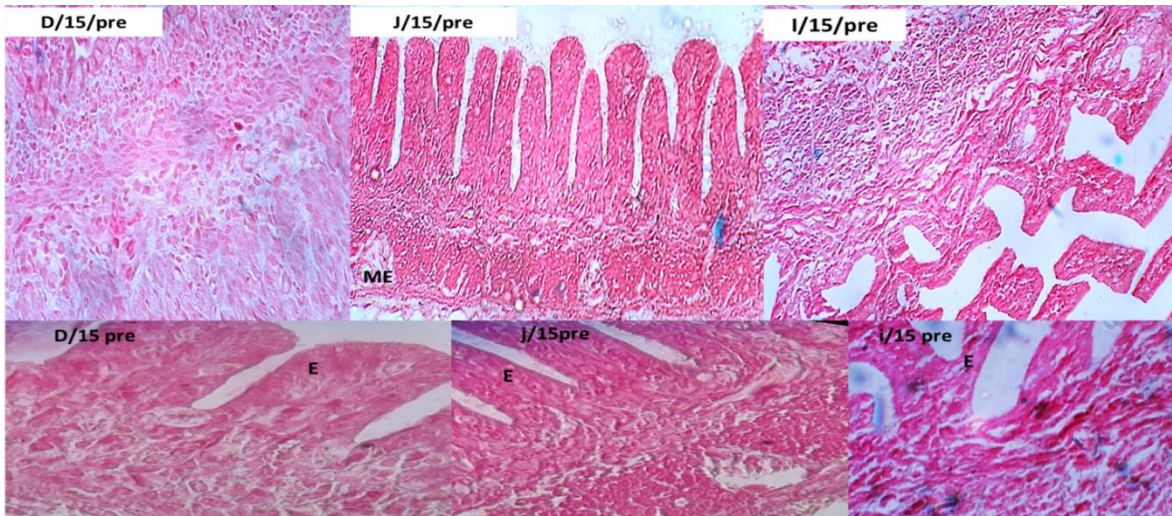


**Fig: (10)** :upper & lower panel **longitudinal section** of the DUODENUM, JEJUNUM and ILEUM in 15 day pre hatch of local chicken showing not present of goblet cells and connective tissue slightly reaction (blue stars) with PAS stain. combined AB-PAS stains. magnified section upper panel 400X. lower panel X1000

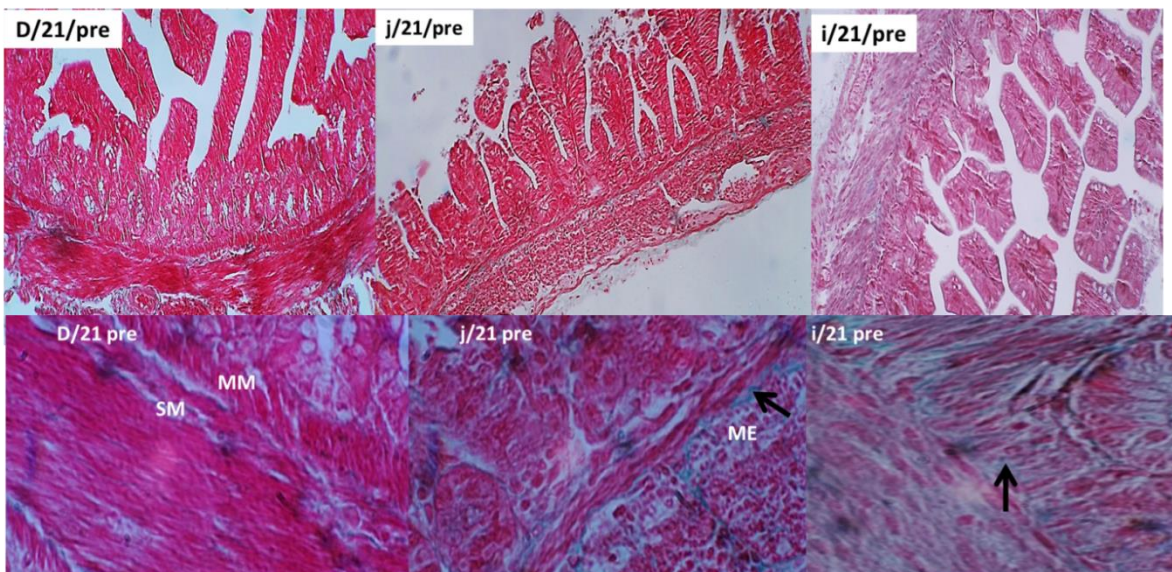


**Fig: (11)** Upper & lower panel **longitudinal section** of the DUODENUM, JEJUNUM and ILEUM in 21 days pre hatch of local chicken showing of goblet cells strong reaction with the combined stain (blue arrows) and connective tissue slightly reaction (black stars) with PAS stain. combined AB-PAS stains. magnified section. Upper panel X400 & lower panel X1000

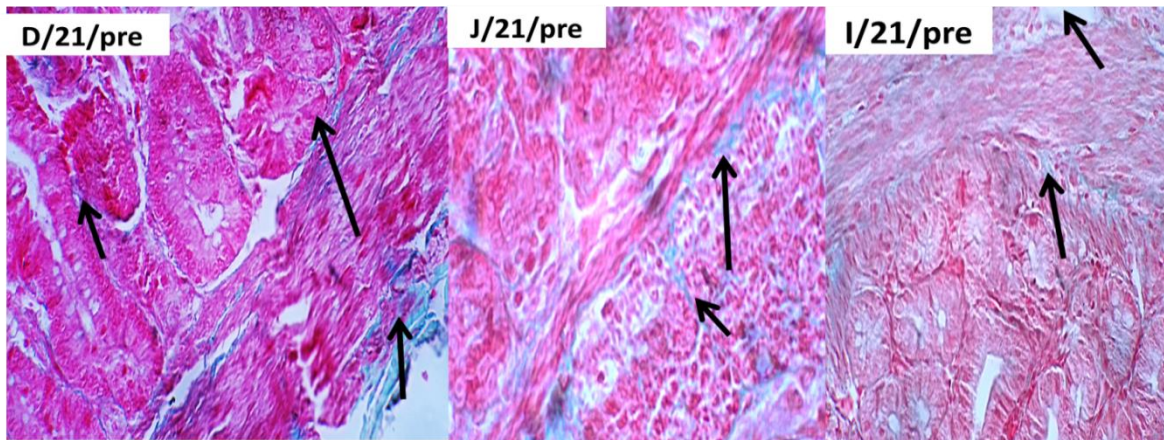




**Fig. 12:**Upper &lower panel Histological section of the duodenum, jejunum and ileum at 15day prehatch shows: absent of collagen fiber in tunica mucosa, submucosa and seronsa . (Masson,s Trichrome stain ) . Magnified upper panel X400&lower panel X1000



**Fig. 13:** Upper &lower panel Histological section of the duodenum, jejunum and ileum at 21day prehatch shows: collagen fiber (black arrows) , musclaris mucosa(MM), sub mucosa(SM), messener plexus(ME)&myentric plexus(MY) . (Masson,s Trichrome stain ) . Magnified upper panel X200 &lowe panel X1000



**Fig. 14:** Histological section of the DUODENUM, JEJUNUM and ILEUM at 21day prehatch shows: collagen fiber in tunica mucosa, submucosa and seronsa (black arrows) . (Masson,s Trichrome stain ) . Magnified D&I : X400 : J:X1000

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