Histomorphometric and Histochemical Finding of the Proventicular and Ventricular Stomach between the African Grey Parrot (*Psittacus erithacus*) and Black Francolin (*francolinus*) in South Iraq

Eyhab R.M. Al-Samawy[1], Shaima Khazaal Waad[2], Wissam Sajid Hashim Al-Uboody[3], Mustafa Salah Hasan[4]

[1]Assist. Prof., Department of Anatomy & Histology, College of Medicine, Al- Muthanaa University, [2]Assist. Prof., Department of Anatomy & Histology, College of Vet. Med., Al- Muthanaa University, [3]Assist. Prof., Department of Physiology, College of Medicine, Al- Muthanaa University, [4]Assist. Prof., Department of Internal and Preventive Medicine, Col. of Vet. Med.4

Abstract

The study was done on sixteen specimens including: eight Parrot and eight francolins of both sexes. The current study aims to discover the structures of proventiculi and ventriculi belonging to these birds specifically their histologic and histochemical textures. The anatomical studies revealed that stomach of both birds consists of: proventiculi & ventriculi. Histological exam was showed that the wall of proventiculus of the Parrot and francolin composed of four layers including: mucosa, submucosa, muscularis and serosa. The epithelia of proventiculi and venticulus were simple columnar cells for each bird.

The obtained means of thickness of layers of proventiculus and venticulus appear different between male and female of both birds.

For histochemical studies there were some different reaction of PAS- stain which were detailed in the results.

Keywords: *Francolin, Parrot, proventriculus, ventriculus, histochemistry.*

Introduction

Birds fall into about 8948 species and they are allocate din about 27 orders. Each order contains vast varying number of species. Passeriformes is considered the largest one as it possess about 5243 species. As containing one species only, Struthioniformes is considered the smallest. Francolin belongs to the order Anseriformes which possess five species(1). Black francolin belongs to Phasianidae family of pheasants and in the order Galliformes, the gallinaceous birds and it is called *Francolinus francolinus*. Comsidering it as a native bird to Asia, it was referred to as black partridge(2). The structure of the bird stomach was studied by different researchers including(3) in pigeon(4) in Japanese quail, (5) in red-capped cardinal, and(6) in partridge. Due to lack of information about the histological and histochemical structures of the stomach in francolin and Parrot, this study was accomplished to reveal it in both genders. The stomach of the birds is considered the most important part of the digestive system and it possess two parts; the proventiculus and venticulus or what is called gizzard. The latter contains the grit or gravel which has an importance with the aid of the muscles in facilitating the grinding up the food. To fulfil their requirements of energy, birds have a high rate of metabolism and they consume large quantities of food(7).

Corresponding Author:
Eyhab R.M. Al-Samawy
Department of Anatomy, Histology & Embryology, College of Medicine, Al Muthanaa University, Al-Muthanaa/Iraq
e-mail: ehyabrazzaq3@gmail.com

Materials and Method

Bird's Collection: Eight Parrot (*Psittacus erithacus*) and eight black francolin (*francolinus*) both sex were collected to perform the ongoing study. Birds were fetched from local markets at Al-Muthanaa and Al-Basra provinces.

Morphological Study: sodium pentobarbitone (140 mg/kg) was injected i.v. to make birds euthanized before accomplishing dissection[8]. After that, they were dissected by the use of a board for dissecting. Coelomic viscera were viewed by making an incision in the mid – line. By using a digital camera, stomach was photographed. A well illustrating figures were depended to exert the locations of these organs and their relationship. Debris and blood were washed out by the use of normal saline. Stomach was eviscerated by pressure to expel its content and then additional normal saline washing was carried out.

Histological Processing: neutral buffered formalin 10% was used to fix the specimens. Dehydration of the specimens was done by the use of ascending ethanol series to pass them in as a period of two hours each depending the concentrations (70, 80, 90, 95 and 100). Specimens were embedded in paraffin wax and then cleared with xylene till two hours. The prepared blocks were being sectioned on thickness of 6 μm and the below stains were used to perform staining: eosin – hematoxylin Mayer`s routine stain was applied to identify general characteristics, while collagen and smooth muscles were stained with the stain of Masson trichrome.

Histochemical Processing: Bouin`s solution was used to fix the specimens. Olympus microscope was used to exam and photograph the sections. PAS – alcian combined blue was used to determine the neutral mucin. Epithelial linings basement membranes of were illustrated by the use of PAS [9].

Results/Discussion

Morphological Finding: Figure (1) reveals that the stomach of parrot and francolin included like other birdsproventiculus and venticulus. Of these birds are domestic fowl[10], partridge[11], and Japanese quail. In contrast to that[12] has reported that there are three parts included in the stomach those are; proventiculus, venticulus and in addition to pylorus. Cranially to the esophagus and caudally to the gizzard, the proventiculus of the birds of our study situates as it is clear in (figure 1). Similar to the site in other birds like ostrich as was mentioned by [13] and [14], the proventiculus was vailed partially by hepatic left lobe. Figure (1) also reveals the venticulus of francolin takes a form of spindle and there is an amount of adipose tissue surround it. In other side, ventriculus of parrot appeared ovoid and muscular of a lesser size and there is a thick adipose tissue surround it. Might be due to the nature of food stuff of birds, the shape besides the size of the ventriculus could be differ as considering it as the muscular stomach part. In a line with other species like red –winged tinamou [6] and pigeon [14], the parrot ventriculus shape comes spherical as it I appear in (figure 1).

Histological Finding:

Proventriculus: Four layers were seen in the francolin proventriculus wall. These layers were mucosa, submucosa, muscularis and serosa layers as it is clear in figures 2, 4, and 5. [15] and [16] have reported the same structures of proventriculus in Struthiocamelus and Numidameleagris respectively. Figure 2 reveals that mucosa of francolin consists of folds that are branched and longitudinal and lined with an epithelium that is simple columnar in nature. Infiltrated with lymphocytes and blood vessels, a loose connective tissue was the structure belongs to the lamina properia as it is obvious in figures (2, 3, and 6). Also it was seen that there is a simple mucous tubular gland possessed within the lamina properia as it is obvious in figures (2, 4, and 5). The mean of thickness of this tunic was 2332 mm in male francolin, whereas in the female was higher up to 2521 mm.

In the parrot, the proventriculus characterized by folded mucosa with the presence of surface invaginations of regular intervals (Fig. 2,3). Similar to that in francolin, the lining epithelium was simple columnar and the lamina propria possessed simple tubular mucosal glands lined with the same epithelial lining, invested in loose connective tissue rich with blood vessels. The mean of thickness of this tunic was 1258 mm in male parrot, whereas in the female was higher up to 1431 mm.

The presence of simple columnar epithelial lining of the mucosa in the studied birds were similar to mucosal lining of the most avian species [17], guinea fowl [16] and pigeon [14]. Coming in line with what was reported by [18] and [19], the francolin`s and parrot`s mucosal glands were lined with simple cuboidal epithelium.Figures 2, 4, and 5 reveal that most thickness of the organ is occupied by

submucosa. What is being called proventriculus glands, were seen forming it.

The current findings of proventriculus glands were not in line with those of [20] and [21] who reported the lack of submucosal glands in the proventiculi belonging to chicks. Other findings relating to francolin were not in line with what reported by [22] and [23] who made their studies in aves and gray parrotwhere the submucosa contains tubular glands of proventriculus which are surrounded by a fibrous tissue and they are round in shape found in dense connective tissue while the findings of the researchers above have mentioned the glands to be pearl in shape lined up with columnar epithelium.

The mean of thickness of this tunic was 9860 μm and 9910 μm in male and female francolin, respectively. Whereas, in parrot the mean of thickness of this tunic was 13256 μm (in male) and 13588 μm (in female). The increased size of this tunic occupied most of the real area of the proventriculus wall containing numerous deep proventricular glands. This finding was disagreed with [25] in the jungle fowl who mentioned that the tunica submucosa was very thin in the proventricular wall or poorly developed in birds.

The mean diameter mean of these glands were 4355 μm in male francolin, whereas in the female was higher up to 4990 μm. The mean of diameters of the submucosal glands were 3922 μm and 3260 μm in the male and female parrot, respectively.

The thickness mean of this tunic was 920 μm in male francolin, whereas in the female was higher up to 1120μm and compared with parrot the mean of this tunic was 1120 μm and 1130 μm in male and female, respectively. Structures like adipose tissue, nerves, and blood vessels were the feature of serosa that was built up with loose connective tissue (Fig. 2,4,5). These findings were also observed by [12] in Japanese quail and [18] in Coot bird (Fulica atra). The mean of thickness of this tunic was 140 μm and 160μm in the male and female francolin, respectively. While the thickness mean of this tunic was 170 μm in male and female parrot.

The ventriculus (Gizzard): Figures (7, and 8) show the structure of this organ. Like other avian species, the structures of this organ was similar to those like red-capped cardinal and guinea fowl [5] and[16] respectively. The simple columnar cells lined the ventriculus in studied birds. Similar observations were recorded in other species for instance partridge [11], Red jungle fowl [25] and in the Blue and Yellow macaws [27]. This epithelium thrown into long folds in parrot (Fig. 7, 8) but not in francolin when they were parallel each to other and revealed between their bases the gastric pits which constitute more than the half of the mucosal thickness (Fig. 7). Mean of diameters belonging to these glands was 560.1 μm in the male francolin, while in the female was higher up to 722.3μm. The means of glands diameters were 344 μm and 321 μm in male and female parrots, respectively.

Muscularis mucosa appeared as circularly arranged smooth muscle bundles interrupted by the presence of mucosal glands in the lamina propria in francolin (Fig. 8), but in parrot appeared thinner layer of circularly arranged smooth muscle fibers separating the mucosa from the underlying submucosa. (Fig. 7).

The mean thickness of this tunic was 1910 μm and to 2100 μm in male and female francolin, whereas in the female, it was higher up to 2100 μm. In the parrot, the mean thickness of the mucosa was 2320 μm and 2540 μm in male and female, respectively.

Figures. 8, 9, and 10 reveal that the submucosa belonging to ventriculus is built up of vast dense connective tissue which is enriched with nerves and blood vessels. The mean of thickness of this tunic was 1280 μm in male francolin, whereas in the female was higher up to 1410 μm. But the thickness was higher in parrot in which the mean thickness of this tunica was 1820 μm and 1580 μm in male and female, respectively.

The above outcome agreed with those observed in the Red-Capped Cardinal (Paroaria gularis gularis)[8], in most avian[29] and in Rock dove (Columba livia)[30].

In the parrot, muscularis extera was formed by two thin layers of smooth muscle fibers. There were fine collagenous fibers distributed between the bundles of these smooth muscles (Fig. 7, and 9).

Three layers of muscles fibers existence in francolin were in line with the findings of[31] in the Urolоncha domestica[22] in the Fulica armillata (granivorous species) and[18] in the ventriculus of the red jungle fowl which is one of the seeds, fruits and insects eaters bird.

While the two layers of muscles fibers existence in the ventriculus of parrot was similar to the findings of[24],[25] and[32] in the organs belonging to red-capped cardinal, Coot bird and most avian species, respectively.

The thickness of this tunica was 11910µm 11840 µm in male and female francolin, respectively, but it was thinner in the parrotventriculus in which the mean thickness was 4410 µm and 4330 µm in male and female. The thickness of this layer in the ventriculus may concerned with mechanical grinding capability of the birds to the ingested food.

The microscopic examination of the serosa layer revealed similar structure of this tunic in the ventriculus of francolin and parrot. For instance, Ostrich [33], and turkey[34] the structures also were seen. The mean of thickness of this tunic was 160 µm in both sex of francolin and 120 µm in both sexes of parrot.

Histochemical Findings: The stomach birds such as (proventriculus and ventriculus) were well studied histochemically by applying three stains: PAS, PAS-AB (pH 2.5) and PAS-AB (pH 1.0). These staining techniques were conducted to view the presence or absence of neutral mucins, acidic mucins and sulfated mucin respectively.

Proventriculus: The lamina propria extended between the gastric mucosal glands were moderately reacted with same stain. These findings were akin to those observed by [32] in the black- winged kite glandular stomach. The connective tissue and wall of blood vessels of submucosa and serosa give activist reaction with PAS and smooth muscle fiber in muscularis showed poor staining with PAS (Fig.6). Whereas, [16] has reported variant findings in guinea fowl proventriculus in which the submucosal glandular epithelium showed depressing reaction for mucins post staining with the PAS.

The strong PAS-positive reaction which observed in the francolin glandular stomach mucosa was noticed parrot mucous glands (Fig. 6) and such reaction was similarly documented in previous investigations such as[35] and[36]. Positive reaction toward PAS by the mucosal folds of the glandular stomach may have a protective role in avoiding the effect of hydrochloric acid on the mucous membrane of the proventriculus [34]. Conversely to these findings in francolin and parrot, (Fig. 3, and 6).

The tunica muscularis showed mild reaction with the same stain, while the interspersed collagen fibers showed a moderate reaction in parrot, and francolin (Fig.3, and 6). The epithelium of the mucosal folds secreted acidic sulfated and neutral mucins which were well stained with the combined PAS-AB (pH 1) stain (Fig. 3, and 6). These findings agreed with [38] observations in the proventriculus lining of the domestic duck in which mucosal folds surface lining cellsbestowed red and blue colors with the both parts of this combined stain, respectively.

By using combined PAS-AB (pH 1) on the sections of the proventriculus wall to differentiate the sulfated from non sulfated acidic mucins, positive materials were detected in the lumina of the mucosal glands, lining cells of the surface and the secretory duct of the submucosal glands. The reaction indicated the presence of sulfated and neutral mucopolysaccharides in the francolin andparrot proventricular surface epithelial cells (Fig. 3,6). The current responses of this combined stains were similar to the previous records of [40] in the francolin proventriculus glands which showed PAS positive reaction due to the presence of both acidic and neutral polysaccharides. However, strong reaction was observed in Alcian blue part of the stain in this bird.

The ventriculus (Gizzard): The wall prepared sections of the ventriculus that were stained by PAS-AB (pH 1) revealed positively blue and red colors in the epithelium and gastric glands of the mucosal layer in the studied birds because of the presence of the sulfated and neutral mucopolysaccharides, respectively.

The cuticle covering stained with PAS in francolin whereas, negatively did with that of parrot.

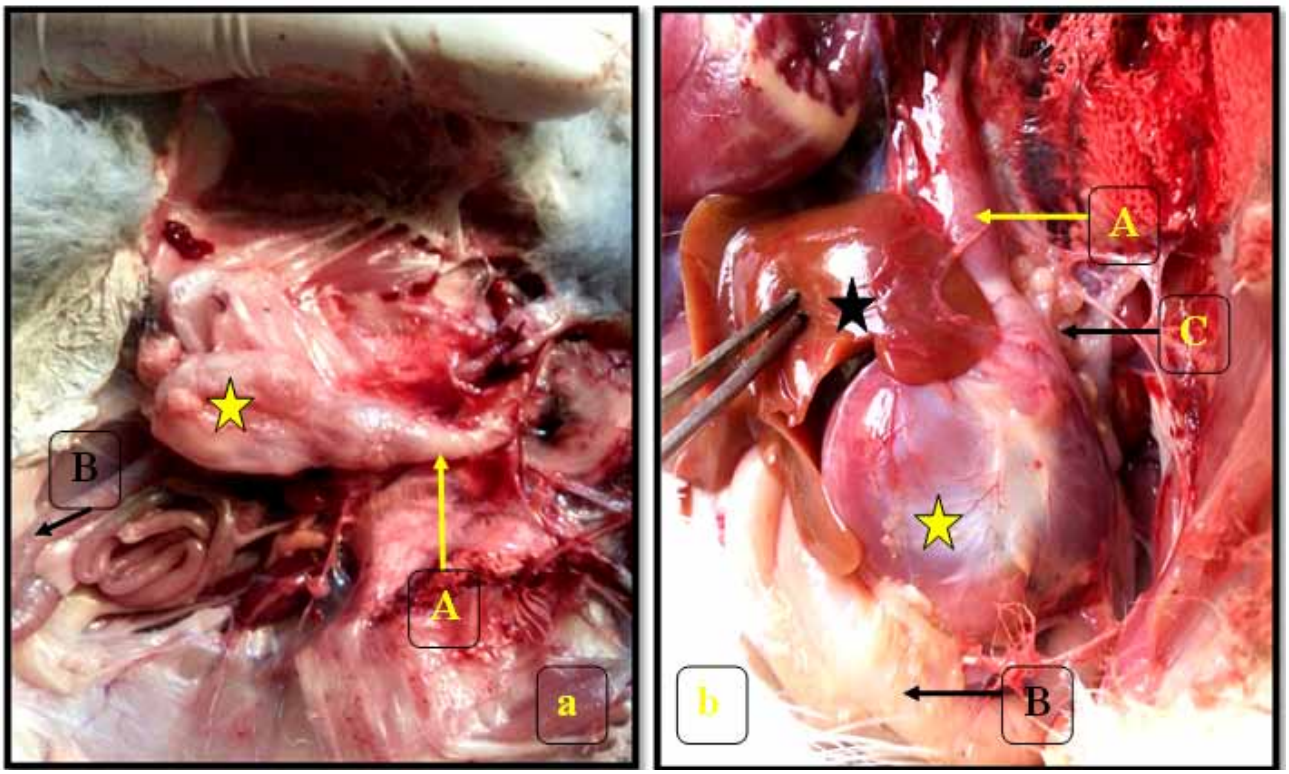

Fig. 1 . Visceral of abdomen showed: proventriculus (A), Ventricular (yellow star), Duodenum (B), Ovary (C), Liver (black star). parrot (a) and Francolin (b).

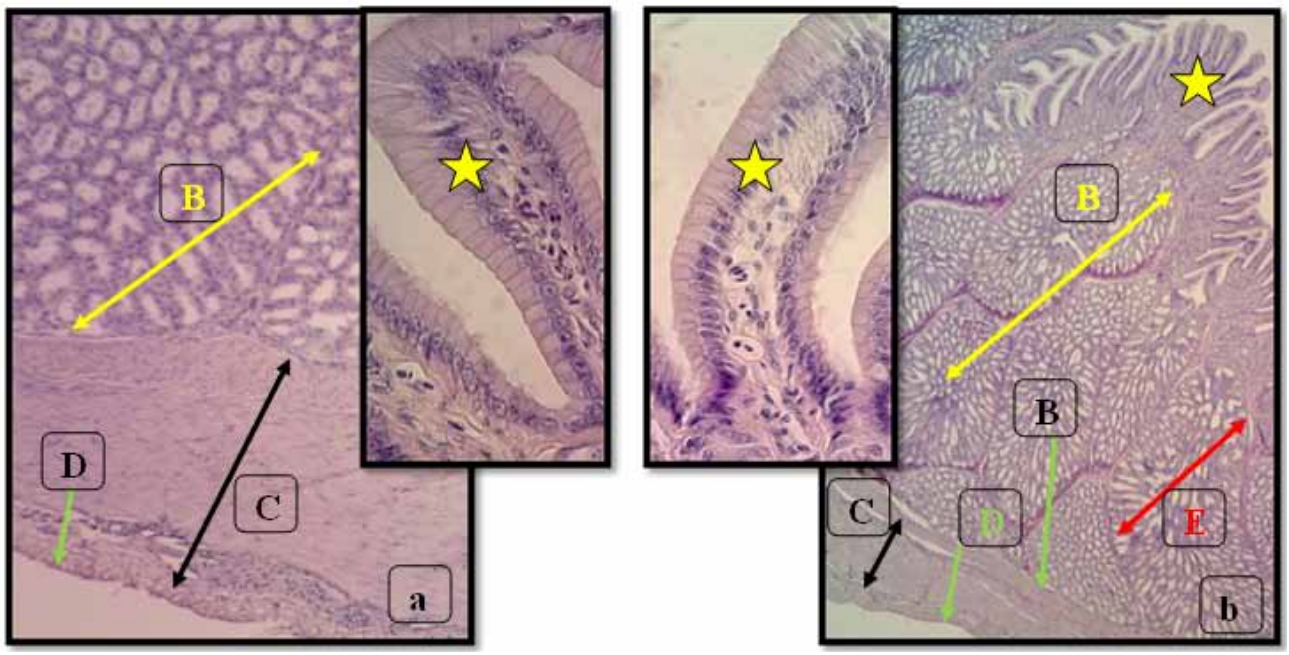

Fig. 2. Showed theproventriculus wall of parrot showed mucosa (yellow star),Submucosa (B), Muscularis (C), serosa (D)and proventricular gland (E), (yellow star) showed epithelial cells in mucosa. (a) Male X400, (b) Female X100 H & E stain.

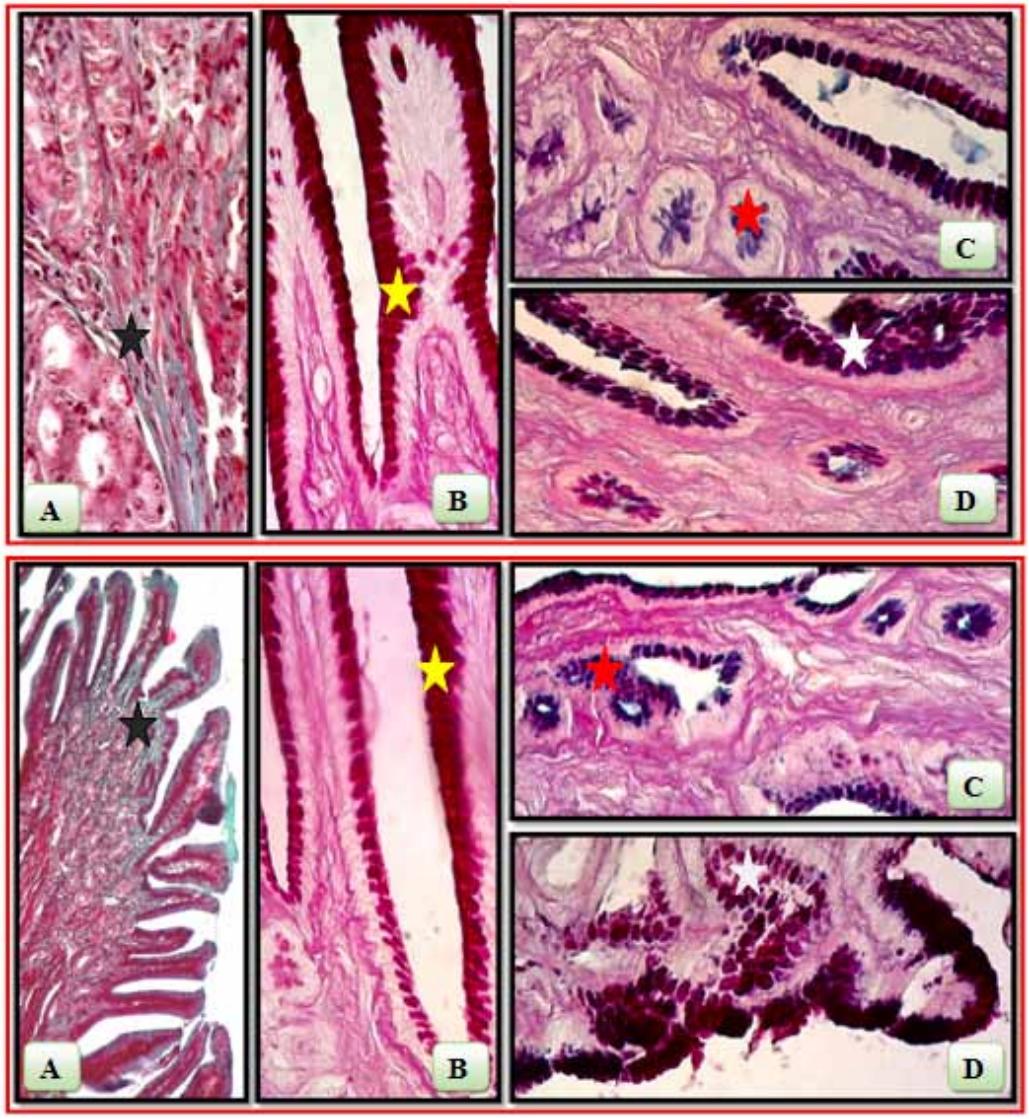

Fig. 3. Showed the proventriculus wall of parrot Upper panel in male and Lower panel in female showed : Connective tissue (Black star) (A), Neutral mucin (Yellow star) (B), Neutral and sulfate mucin (Red star) (C) and Neutral and acid mucin (White star) (D). Masson's Trichrome (A), PAS (B), PAS+AB =pH 0.1 (C) and PAS+AB= 2.5 (D). X400

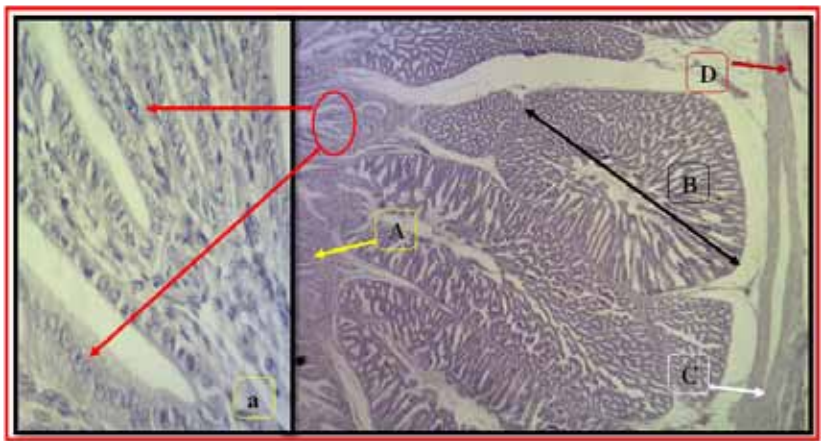

Fig. 4. Showed theproventriculus wall of male Francolin showed: mucosa (A),Submucosa (B), Muscularis (C), serosa (D), (a) showed epithelial cells in mucosa. H & E 400X

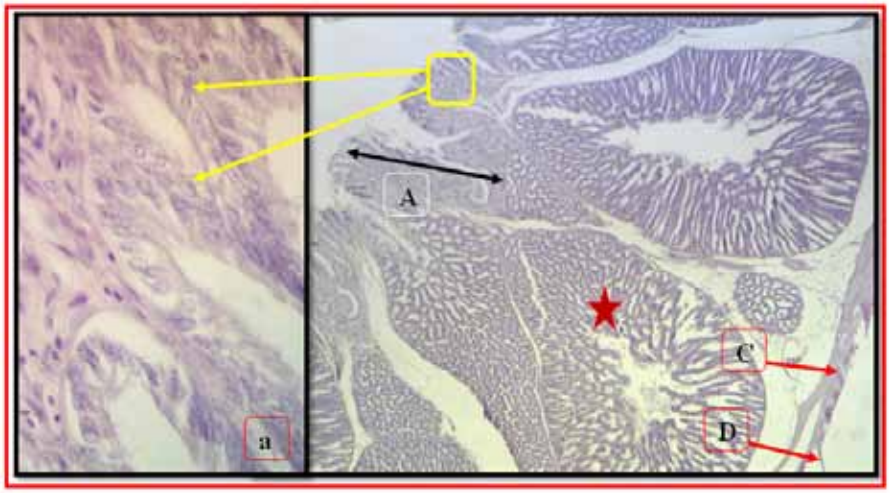

Fig. 5. Showed theproventriculus wall of female Francolin showed: mucosa (A),Submucosa (glands) (Red star), Muscularis (C), serosa (D), (a) showed epithelial cells in mucosa. H & E 400X

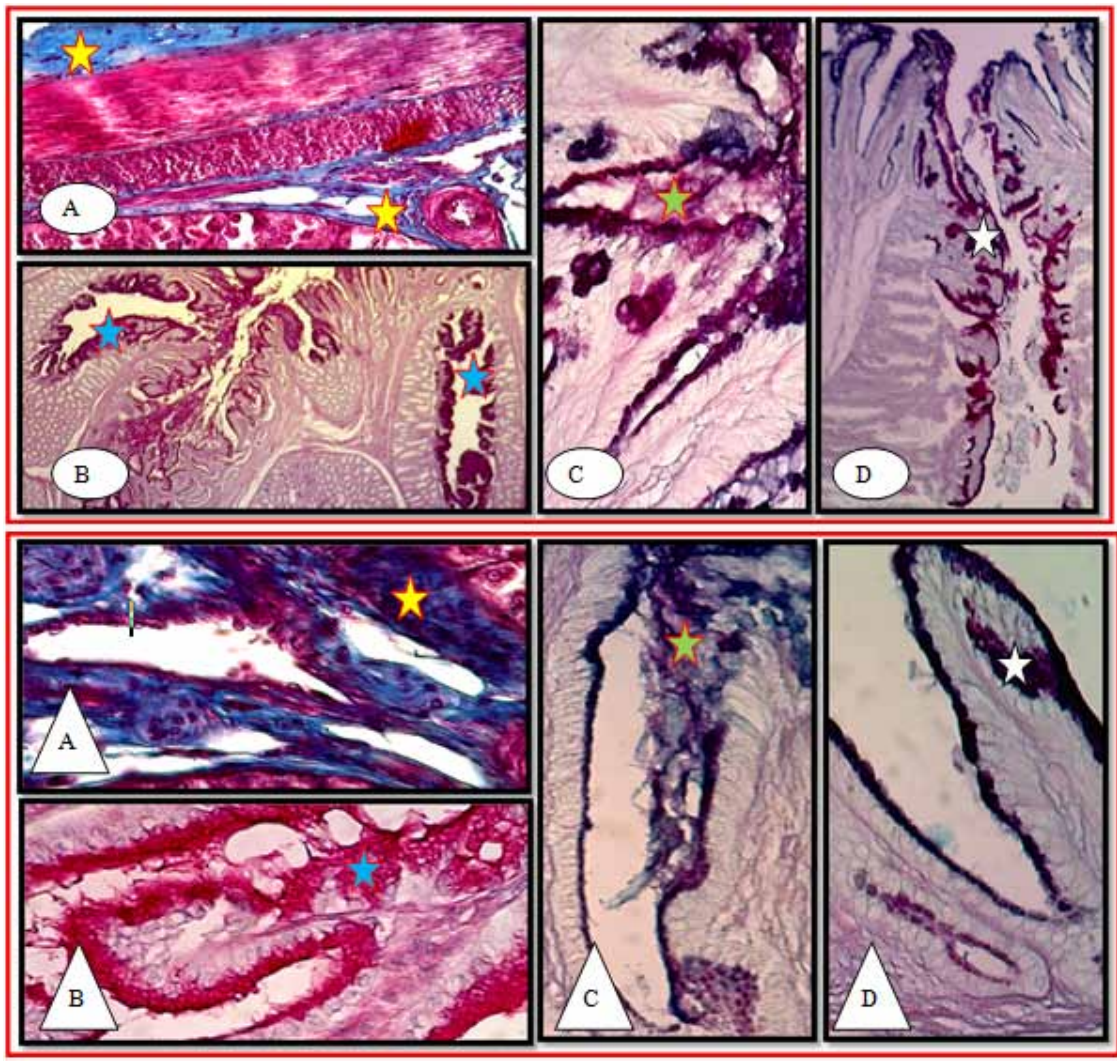

Figure (6). Francolin Proventriculus wallUpper panel in male and Lower panel in female showed : Connective tissue (Yellow star) (A), Neutral mucin (Blue star) (B), Neutral and sulfate mucin (Green star) (C) and Neutral and acid mucin (White star) (D). Masson's Trichrome (A), PAS (B), PAS+AB =pH 0.1 (C) and PAS+AB= 2.5 (D). X400

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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