



Histochemical study of proventriculus in pre-hatch and post-hatch days in northern bobwhite quail (*Colinus virginianus*)

S.M. Al-Kafagy¹ , A.K. Al-Jebori¹  and Y.Y. Alseady² 

¹Department of Anatomy and Histology, College of Veterinary Medicine, Al-Qasim Green University, Babylon, ²Department of Dentistry, Al-Hadi University College, Baghdad, Iraq

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Correspondence:

S.M. Al-Kafagy
drsirajmoner@vet.uoqasim.edu.iq

Abstract

Present study was designed to investigate the histochemical developmental changes of the proventriculus at pre-hatching and post-hatching in the northern bobwhite quail at periods 11- and 15-days pre-hatching in addition to 14- and 21-days post-hatching. The proventriculus composed of four tunics are tunica mucosa, tunica sub-mucosa, tunica muscularis and tunica serosa. The tunica mucosa lined with simple columnar epithelium and the height of epithelium increases with the age also the thickness of mucosa, the tunica submucosa filled with proventricular glands at 14- and 21-day post-hatch and the gland was height in diameters while in 11- and 15-day pre-hatch the gland less in diameter and the sub mucosa not fill with the gland. Tunica muscularis thicker in post-hatch from that of pre-hatch, at 11- and 15- day pre-hatch composed of thin inner circular layer and thick outer longitudinal layer but in 14- and 21-day post-hatch the tunica muscularis was composed of thin inner and outer circular layer and thick middle longitudinal layer. The mucosa and proventriculus glands give positive reaction for Alcian blue stain and weak to moderate reaction for periodic acid Schiff stain at pre-hatch, while at post-hatch the mucosa gives positive reaction for Alcian blue stain and negative reaction for periodic acid Schiff stain and the proventricular glands give positive reaction for Alcian blue and periodic acid Schiff stains.

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Introduction

The proventriculus and ventriculus forming the avian stomach (1,2). These portions of the stomach are differentiated on the sixth day of incubation (3). The transformation of the lining epithelium of the proventriculus from pseudo stratified to simple columnar type on day 12 of incubation in quail (4), but on day 17 of incubation in Dandarawi chicken (5). The development of complex glands that produce digestive enzymes distinguishes the proventriculus. The epithelium starts to invade the surrounding mesenchyme on day 6 of incubation, forming small glands. Significant anatomical and functional changes to the embryonic gastrointestinal system occur as a result of

mesoderm recruitment, endoderm induction, and patterning (6,7). The digestive system of the chicken actively transports food to the stomach. The gizzard and proventriculus are the first significant sites of enzyme activity. Generally, the digestive systems of birds differ from one species to another based on their diets and the strains to which they belong. Compared to mammals, birds' stomachs are adjusted based on their diet (8,9). The proventriculus, which has a glandular internal surface, is distinguished by elevated gastric papillae on the upper side. These papillae released gastric secretions that included a combination of mucin, hydrochloride acid, and a digesting enzyme (10,11). According to the type of food consumed, the proventriculus divides into four tunics: tunica mucosa, tunica sub-mucosa, tunica muscularis, and

tunica serosa, as well as the diet's composition and feeding habits.

Materials and methods

Ethical approval

Ethical approval was sought and accepted from the medical research ethical committee of Al-Qasim Green University, Babylon, Iraq numbered UOQASIM /COM/MREC/23-24(10).

Histological protocols

For the light microscopic investigation, thirty-two samples were used that divided into two groups, pre-hatching group (16 samples) and post hatching group (16 samples) each group were subdivided into 8 for 11-day pre-hatch and 8 for 15-day pre-hatch, as well as 8 for 14-day post-hatch and 8 for 21-day post-hatch. The samples were taken from the proventriculus and were washed by tap water then fixed in 10% neutral buffered formalin, the specimens were dehydrated in ascending grades of alcohol then cleared in methyl benzoate and embedded in paraffin wax serial sections 4-5 μm were cut by microtome and stained by the following stains: Hematoxylin and eosin stain for general histological structures, Periodic Acid Schiff technique (PAS) for demonstration of neutral mucopolysaccharide and Alcian blue stain for demonstration of acidic mucopolysaccharides (11,12). Light microscopy was used to analyze tissue slices. Future Win Joe microscopic camera was used for microphotography which provided with analysis software to achieve the micromorphometric measurements that include the height of epithelium, thickness of mucosa, sub-mucosa of the proventriculus as well as the thickness of four layers tunica mucosa, tunica sub-mucosa, tunica muscularis, tunica serosa. The photos were evaluated and graded using the color USB 2.0 digital camera (Scope Image 9.0- (China) which was provided with image processing software (13).

Statistical analysis

The data were represented by Mean \pm SE and statistical analysis was performed using SPSS, version 24.0.

Results

The proventriculus of northern bobwhite quail was composed histologically of four layers (tunica mucosa, tunica sub-mucosa, tunica muscularis, tunica serosa). The tunica mucosa was lined with simple columnar epithelium with lamina propria and there is no muscularis mucosa, the connective tissue of lamina propria was occupied the fold of the mucosa. The height of epithelium was increased at post hatch from that in pre hatch. at 11- and 15- days pre hatch the height of epithelium measured about 6.23 \pm 0.68 μm , 11.14 \pm 0.42 μm respectively, while at 14- and 21- days post hatch the height of epithelium measured about 28.24 \pm 0.04

μm , 39.41 \pm 0.31 μm respectively (Figures 1 and 2; Table 1). The mucosa at post hatch was more folded than that in pre hatch and the thickness was increased gradually at post hatch than pre hatch, it was measured in 14- and 21- days pre hatch and 14- and 21- days post hatch about 168.78 \pm 0.43 μm , 204.62 \pm 1.43 μm , 439.34 \pm 1.72 μm , 572.64 \pm 1.02 μm respectively. The tunica sub-mucosa was filled with the proventricular glands that separated by loose connective tissue. Proventricular glands at 11 days pre hatch were little in number, deeply located and not filled all the tunica sub-mucosa. At the 15 days pre hatch the proventricular glands became larger in diameter and increased in number than that in 11 days pre hatch. The proventricular glands at 14- and 21- days post hatch has large diameter and completely filled the tunica sub mucosa that separated by loose connective tissue, the proventricular glands were lined with simple cuboidal epithelium that change it shape at the hole. It was measured at pre hatch 11- and 15- days about 818.42 \pm 1.73 μm , 924.31 \pm 1.02 μm respectively and at post hatch 14- and 21- days was measured about 1732.51 \pm 2.31 μm , 2161.45 \pm 3.21 μm respectively (Figures 1 and 2; Table 1). Tunica muscularis was thick layer composed of smooth muscle fibers, at pre hatch 11- and 15- days the tunica muscularis was composed of two layers thin inner circular muscularis layer and thick outer longitudinal layer. At 14- and 21- days post-hatch the tunica muscularis was composed of three layers, thin inner and outer muscularis layer and thick middle longitudinal layer. Thickness of tunica muscularis was increased with the age it was measured at 11- and 15- days pre hatch about 92.88 \pm 0.32 μm , 183.71 \pm 1.42 μm respectively, while at 15- and 21- days post hatch was measured about 312.81 \pm 1.84 μm , 352.62 \pm 1.33 μm respectively (Figures 3 and 4; Table 2). The tunica serosa was the outer thin layer that increased in thickness with the age, it was measured about 8.34 \pm 0.32 μm at 11 days pre hatch and 10.24 \pm 0.43 μm at 15 days pre hatch, while at 15–21-day post hatch was measured about 12.62 \pm 0.68 μm , 18.87 \pm 1.24 μm respectively (Figures 5 and 6; Table 2). At 11 days pre-hatch the mucosa gives positive reaction for alcian blue stain and negative reaction for periodic acid schiff stain this gives indicate that the secretion of mucosa was acidic mucopolysaccharides, while the proventricular glands gave moderate reaction for both stains that reveled presence of both neutral and acidic mucopolysaccharides (Figure 5). At 15 days pre-hatch the mucosa gave positive reaction for Alcian blue stain and moderate reaction for periodic acid Schiff stain. The proventricular glands gave positive reaction for periodic acid Schiff stain with weak reaction for Alcian blue stain (Figure 6). At 14- and 21- days post-hatch the mucosa gives positive reaction for Alcian blue stain and negative reaction for periodic acid Schiff stain, while the proventricular glands gave positive reaction for both stains (Figures 7 and 8).

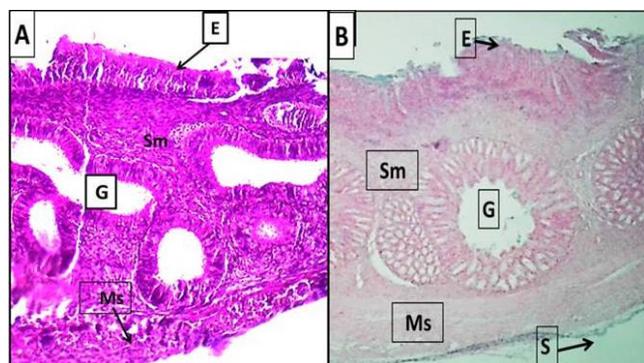


Figure 1: Cross section of northern bobwhite quail proventriculus (A) at 11 days pre-hatch, x100; (B) at 15 days pre-hatch, x100. (E) simple columnar epithelia, (Sm) tunica sub-mucosa, (G) proventricular glands, (Ms) tunica muscularis, (S) tunica serosa. H&E stain

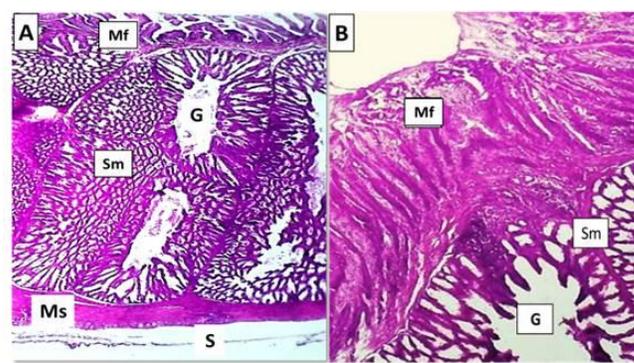


Figure 2: Cross section of northern bobwhite quail proventriculus (A) at 14- day post-hatch H&E stain. x200. show: (Mf) mucosal fold, (Sm) tunica sub-mucosa, (G)proventricular glands, (Ms) tunica muscularis, (S) tunica serosa. H&E.

Table 1: The height of epithelium, thickness of (mucosa, sub-mucosa) of the proventriculus in northern bobwhite quail

Age	Mean \pm SE (μ m)		
	Height of epithelium	Thickness of mucosa	Thickness of sub-mucosa
11 Pre hatch	6.23 \pm 0.68	168.78 \pm 0.43	818.41 \pm 1.73
15 Pre hatch	11.14 \pm 0.42	204.62 \pm 1.93	924.31 \pm 1.02
14 Post hatch	28.24 \pm 0.04	439.34 \pm 1.72	1732.51 \pm 2.31
21 Post hatch	39.41 \pm 0.31	572.64 \pm 1.02	2161.45 \pm 3.21

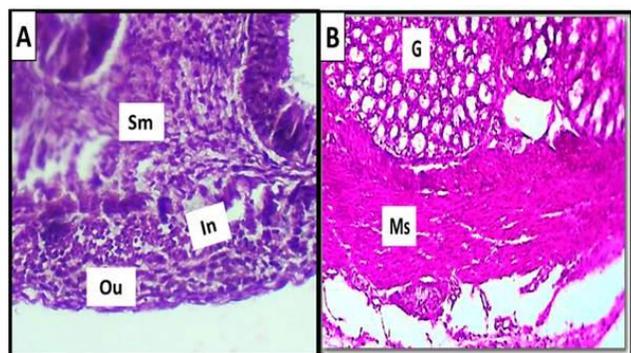


Figure 3: Cross section of northern bobwhite quail proventriculus (A) at 11 days pre-hatch and (B) at 15 days pre-hatch. (Sm) tunica sub-mucosa, (G) proventricular glands, (Ms) tunica muscularis, (In) inner layer, (Ou) outer layer. x200. H&E.

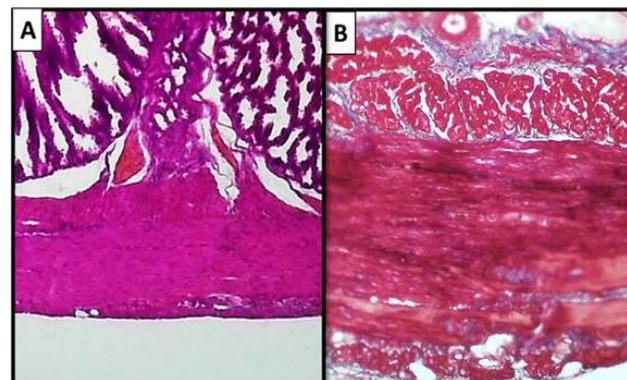


Figure 4: Cross section of northern bobwhite quail proventriculus (A) at 14-day post-hatch, x200. (B) at 21-day post-hatch. x400. H&E.

Table 2: The thickness of tunica muscularis and tunica serosa of the proventriculus in northern bobwhite quail

Age	Mean \pm SE (μ m)	
	Thickness of muscularis	Thickness of serosa
11 Pre hatch	92.88 \pm 0.32	8.34 \pm 0.32
15 Pre hatch	183.71 \pm 1.42	10.24 \pm 0.43
14 Post hatch	312.81 \pm 1.84	12.62 \pm 0.68
21 Post hatch	352.62 \pm 1.33	18.87 \pm 1.24

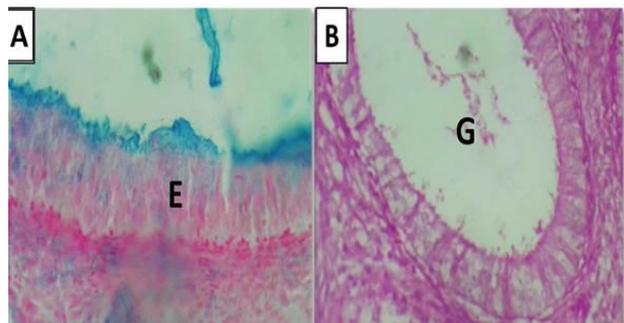


Figure 5: Cross section of northern bobwhite quail proventriculus at 11 days pre-hatch (A) shows the tunica muscularis gave positive reaction for Alcian blue stain, x400. (B) shows the proventricular gland gave moderate reaction for AB stain and PAS stain, x400.

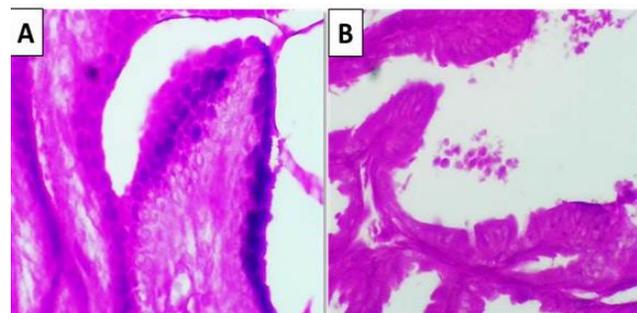


Figure 8: Cross section of northern bobwhite quail proventriculus at 21- day post-hatch (A) shows the mucosa gave positive reaction for Alcian blue stain and (B) shows the proventricular gland gave positive reaction for Alcian blue stain and PAS stain. x400.

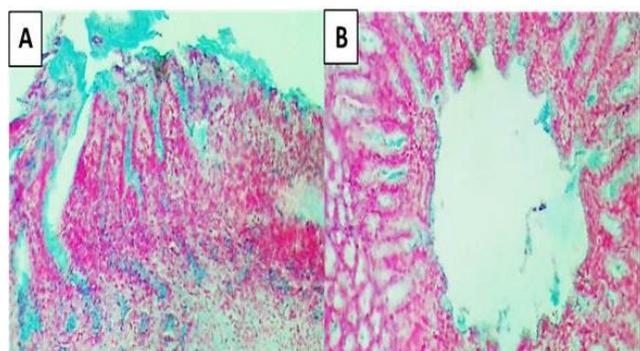


Figure 6: Cross section of northern bobwhite quail proventriculus at 15 days pre-hatch (A) shows the mucosa gave positive reaction for Alcian blue stain with moderate reaction for PAS stain, x200. (B) shows proventricular gland gave positive reaction for PAS stain with weak reaction for Alcian blue stain, x400.

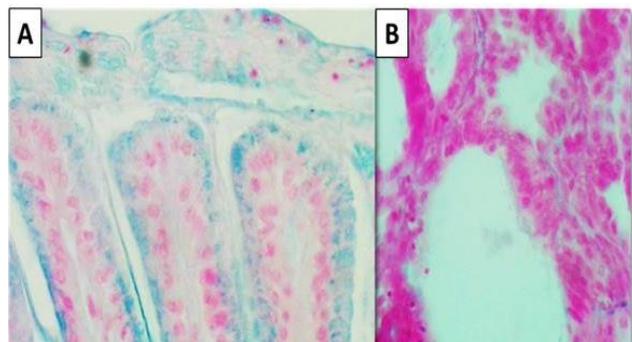


Figure 7: Cross section of northern bobwhite quail proventriculus at 14- day post-hatch (A) shows the mucosa gave positive reaction for Alcian blue stain and (B) shows the proventricular gland gave positive reaction for Alcian blue stain and PAS stain. x400.

Discussion

The proventriculus of northern bobwhite quail was composed histologically of four layers. This finding was similarly documented of most avian species, such as; Ostrich (14), Guinea fowl (15), quail (16), Japanese quail (17) and Coot bird (18). However, Batah *et al.* (19) cited only three tunicae in the wall of proventriculus in which only tunica mucosa, tunica muscularis and tunica serosa were detected. The tunica mucosa was lined with simple columnar epithelium with lamina propria and there is no muscularis mucosa, and the height of epithelium was increased at post hatch from that in pre hatch. This finding was agreeing with de Oliveira Rocha (20) and Islam (21). The mucosa at post hatch was more folded than that in pre hatch and the thickness was increased gradually at post hatch than pre hatch, this finding agrees with finding of Attia (17), Ahmed (18) in quail and Banks (22) who said that the mucosal folds became branched and increased in number by the advancement of age. At the 15 days pre hatch the proventricular glands became larger in diameter and increased in number than that in 11 days pre hatch. The proventricular glands at 14- and 21- days post hatch has large diameter and completely filled the tunica sub-mucosa that separated by loose connective tissue, the proventricular glands were lined with simple cuboidal epithelium that change it shape at the hole. This finding agrees with Madkour *et al.* (23) in duck and Ventura *et al.* (24) in goose who mention that the tunica sub-mucosa get thicker with age and accordance with those observed in the same organ of the Coot bird (18) and common quail (25).

Tunica muscularis was thick layer composed of smooth muscle fibers, at pre hatch 11- and 15- days the tunica muscularis was composed of two layers thin inner circular muscularis layer and thick outer longitudinal layer. this finding agrees with finding of Selvan *et al.* (16) in guinea fowl and Banks (22) who said that the muscularis layer

composed of two layers and disagree with Zaher (26) who mentioned the tunica muscularis mallard constructed of two layers, inner thin longitudinal and an outer thick circular layer. At 14- and 21- days post-hatch the tunica muscularis was composed of three layers, thin inner and outer muscularis layer and thick middle longitudinal layer. Thickness of tunica muscularis was increased with the age progress, this finding disagrees with Al-Saffar (27) who mentions that muscularis layer composed of one layer.

At 11 days pre-hatch the mucosa gives positive reaction for Alcian blue stain and negative reaction for periodic acid Schiff stain this gives indicate that the secretion of mucosa was acidic mucopolysaccharides, while the proventricular glands gave moderate reaction for both stains that reveled presence of both neutral and acidic mucopolysaccharides. At 15 days pre-hatch the mucosa gave positive reaction for Alcian blue stain and moderate reaction for periodic acid Schiff stain. The proventricular glands gave positive reaction for periodic acid Schiff stain with weak reaction for Alcian blue stain. At 14- and 21- days post-hatch the mucosa gives positive reaction for Alcian blue stain and negative reaction for periodic acid Schiff stain, while the proventricular glands gave positive reaction for both stains. This result agrees with Ibrahim (5) in Dandarawi chick and Banks (22) who said that the surface and glandular epithelium show height protection of mucin from pre-hatching till maturity and disagree with Denbow (28) who mention that the surface epithelium of the tunica mucosa and the cells lining the proventricular glands showed a positive reaction to PAS and Alcian blue staining's in common starling on the other hand, Rajab *et al.* (29) and Taki-EI-Deen (30) applying PAS-AB (pH 2.5) stain, the esophageal glands were given blue color with Alcian blue stains and no reaction with PAS stain. This reaction indicated the presence of high content of acidic mucin secretions. The mucin substances were playing an important role in the protection of mucosa as a pre-epithelial barrier (31).

Conclusion

This study concluded the proventricular glands were highly packed and fill the submucosa at post hatching period while they were ill developed at pre hatching period. Histochemical, the gland showed positive reaction with Alcian blue and periodic acid Schiff stain in pre and post hatching periods.

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Conflict of interest

Authors declared that there is no conflict of interests.

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دراسة كيميائية نسيجية للمعدة الحقيقية قبل الفقس وبعده في طائر السمان ذي الحجل الشمالي

سراج منير الخفاجي^١، علي كاظم الجبوري^١
ويحيى ياس السعيد^٢

^١ فرع التشريح والأنسجة، كلية الطب البيطري، جامعة القاسم الخضراء، بابل، ^٢ قسم طب الأسنان، كلية الهادي الجامعة، بغداد، العراق

الخلاصة

صممت الدراسة الحالية للتحقق من التغيرات النسيجية التطورية لنسيج المعدة الغدية في مرحلة ما قبل الفقس وما بعد الفقس في السمان ذو الحجل الشمالي في اليومين ١١ و ١٥ قبل الفقس واليومين ١٤ و ٢١ بعد الفقس. تتكون المعدة الغدية من أربع طبقات وهي الغلالة المخاطية، الغلالة تحت المخاطية، الغلالة العضلية والغلالة المصلية. تبطن الغلالة المخاطية بظهارة عمودية بسيطة ولوحظ أن ارتفاع الظهارة يزداد مع تقدم العمر وكذلك سماكة الغشاء المخاطي. لوحظ امتلاء الغلالة تحت المخاطية بالغدد المعوية في اليومين ١٤ و ٢١ بعد الفقس وكانت أقطار الغدد عالية بينما لوحظ في اليومين ١١ و ١٥ قبل الفقس أن الغدة ذات القطر اقل وان تحت المخاطية غير ممتلئة بالغدد. كانت الغلالة العضلية في فترة بعد الفقس أكثر سماكة من تلك الموجودة في فترة قبل الفقس وكانت في اليومين ١١ و ١٥ قبل الفقس مكونة من طبقة دائرية داخلية رقيقة وطبقة طويلة خارجية سميكة ولكن في اليومين ١٤ و ٢١ بعد الفقس كانت الغلالة العضلية تتكون من طبقة دائرية رقيقة داخلية وخارجية وطبقة طويلة متوسطة سميكة. أظهرت مخاطية المعدة والغدد المعوية تفاعلا موجبا مع ملون الاليشيان الأزرق وتفاعلا ضعيفا الى متوسط مع ملون حامض شف الدوري في فترة قبل الفقس في حين أظهرت مخاطية المعدة تفاعلا موجبا مع ملون الاليشيان الزرقاء وتفاعلا سالبًا مع ملون حامض شف الدوري في فترة بعد الفقس بينما أظهرت الغدد المعوية تفاعلا موجبا مع كل من ملون الاليشيان الزرقاء وملون حامض شف الدوري بعد الفقس.