HISTOGENESIS OF THE STOMACH OF HELMETED GUINEA FOWL (*NUMIDA MELEAGRIS*)

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ABSTRACT

The histogenesis of the stomach (proventriculus and ventriculus) of helmeted guinea fowl (Numida meleagris) was studied using light microscopy and histochemical techniques. Fifteen (15) embryos were utilized for this study. The result showed that at 10th and 13th days of embryonic development, the primordial proventriculus and ventriculus were lined by pseudostratified columnar epithelium surrounded by mesenchyme connective tissue. At 19th day of embryonic development, the epithelium of the proventriculus and ventriculus as well as the proventricular glands was lined by simple cuboidal epithelium. Tunics; tunica muscularis and serosa were evident at this stage. At 23rd day of embryonic development, tubular glands of the ventriculus became canalized. The 27th day of embryonic develoment of the primordial proventriculus showed an organized glandular lobules, central cavity and prominent muscle layer while the ventriculus showed the presence of cuticle, simple tubular glands, loose connective tissues of the lamina propria and muscle layer. This study has shown that primordial stomach appeared to be completly differentiated to definitive stomach by the 27th day with the potential of commencing functional role.

Keywords: Embryo, Proventriculus, Ventriculus, Guinea fowl, *Numida meleagris*

INTRODUCTION

Avian stomach developed from a simple gastric primordium which gives rise to glandular proventricular and muscular ventricular stomach (McGeady *et al*., 2006). The glandular proventriculus is a short, thick walled, spindled-shaped organ lying above the liver and between the esophagus and the gizzard. It contain glands that opens at the apex of the papillae via several ducts to elaborate pepsinogen, hydrochloric acid and mucus discharge into the stomach lumen (Liman *et al*., 2010; Tadjallz *et al.,* 2011).

The muscular ventriculus (gizzard) is important for mechanical digestion of food since birds are without teeth (Enoki and Morimoto, 2000). It grinds and prepares food for further digestion in the intestine which is facilitated by the cuticular layer, a water resistant layer appearing brown, green or yellow due to the reflux of bile pigments from the duodenum (Maya and Lucy, 2000). The avian stomachs are characterized by great morphological and functional variability, both between and within

species (Dzia£a-Szczepañczyk**,** 2005). These were established due to several researches that has been carried out overtime including that of the guinea fowl with probably little work done on the histogenesis hence this study.

MATERIALS AND METHODS

Fifteen (15) embryos of helmeted guinea fowl (*N. meleagris*) at incubation days 10, 13, 19, 23 and 27 were utilized for this study. The embryos were removed from the egg shells then euthanized using 0.1 ml of phenobarbitone 200 mg/ml via jugular vein (Igwebuike and Eze, 2010). The extra-embryonic membranes were removed and the umbilical stalks were severed close to the body wall (Salami, 2009). Embryonic stomachs were removed through the ventral incision into the thoraco-abdominal cavity and preserved in 10 % formalin. The collected stomachs were dehydrated in alcohol, subjected to paraffin wax embedding and sectioned. Sections were stained using Haematoxylin and Eosin (H and E) to analyze tissue structure. Periodic Acid Schiff (PAS) was used histochemically to analyze the activity of glycosaminoglycans.

RESULTS

The primordial stomach of guinea fowl was observed gradual changes from 10^{th} to 27^{th} days of pre-hatch periods. For both the proventriculus and the ventriculus, the development started with the appearance of the epithelium at the upper layer and the mesenchyme at the lower layer surrounding the epithelium.

Proventriculus: At the 10^{th} and 12^{th} days of embryonic development, preponderance spherical to oval shaped primordial proventricular glands covered by pseudostratified epithelium in mesenchymal connective tissue were observed (Figures 1a, b, c and d). The primordial glands became elongated, branched and joined at its tip during the 13^{th} day of the embryonic development (Figures 1e and f).

The epithelium invaginates deep into the mesenchymal connective tissue forming several glandular lobules lined by simple cuboidal epithelium at the 19^{th} day of embryonic development (Figure 2a). At this stage, the glands were observed to react positively to PAS (Figure 2d). The lower part of the mesenchymal connective tissue differentiate where lamina propria, thin tela submucosa and tunica serosa separated (Figure 2a). Further more, the mesenchyme appeared to be replaced by loose connective tisue thereby decreasing the spaces between adjacent primordial proventricular glands (Figure 2a). The space between the primordial proventricular glands occupied by connective became reduced drastically at the 23^{th} day of the embryonic development and more reduced at 27^{th} day. The later appeared similar with that at post-hatch showing complete and organized tunics; mucosa, submucosa, muscularis and serosa (Figures 2b, c, e and f).

Ventriculus: The primordial ventriculus is covered by pseudostratfied epithelium surrounded by mesnchymal connective tissue at the early develomental stage. These histological features were observed from the 10^{th} to the 13^{th} day (Figures 3a, b, d and e). By the 19^{th} day of embryonic development, numerous vertical strands observed that happens to be the future simple tubular glands (Figures 3c and f). Lamina propria became evident at this stage. By the 23^{rd} day of embryonic development, the simple tubular glands became evident at this stage (Figure 4a) reacting positively to PAS (Figure 4d). Mesenchymal connective replaced by tunica muscularis a nd serosa. Cuticular layer appeared at the 27^{th} day of the embryonic development. At this stage, the simple tubular glands were observed to be lined by simple cuboidal epithelium (Figures 4b, c, e and f).

DISCUSSION

The secretory and mechanical functions of the avian stomach are essential in digestion with gastric juice pH of around 2.

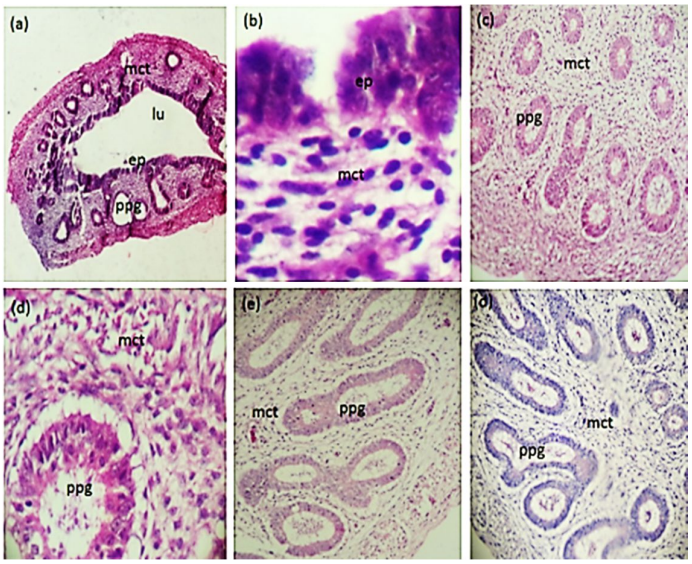

Figure 1: Sections of primordial proventriculus on the 10th (a, b), 12th (c, d) and 13th (e, f) days of developments. The 10th day of development shows preponderance primordial proventricular glands (ppg), within a mesenchymal connective tissue (mct); beneath a pseudostratified epithelium (ep) lining the lumen (lu). H and E x 40 (a) x400 (b). The 12th day of development shows the oval to spherical primordial proventricular glands of pseudostratified epithelium. H and E x 40 (c) x400 (d). Elongation, branching and joining of the primordial proventricular glands on the 13th day of development. H and E x 100 (e). Weak histochemical reaction to PAS x100 (f).

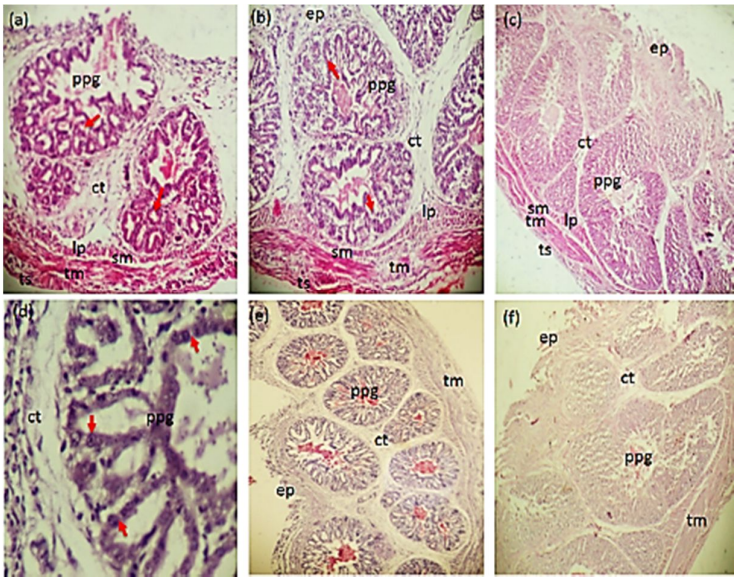

Figure 2: Sections of primordial proventriculus on the 19th (a, d), 23rd (b, e) and 27th (c, f) days of developments. The 19th day shows several glandular lobules (red arrows) within the primordial proventricular glands (ppg) lined by simple cuboidal; primordial proventricular glands separated by connective tissue (ct); slit-like submucosa (sm) separating lamina muscularis (lp) from tunica muscularis; beneath is the tunica serosa (ts).It shows postive histochemical reaction to PAS at this stage. H and E x 100 (a), PAS x400 (d). 23rd day shows that the priomordial proventricular (ppg) getting closer and also postive reaction to PAS. H and E x100 (b), PAS x40 (e). The 27th day of shows a an organized tunics of the primordial proventriculus with the epithelium (ep) lining the surface tunica mucosa; within the later are the primordial proventricular glands (ppg) separated by conncetive tissue, lamina muscularis (lp). Slit-like submucosa (sm) proximal to tunica muscularis (tm) and tunica serosa (ts)

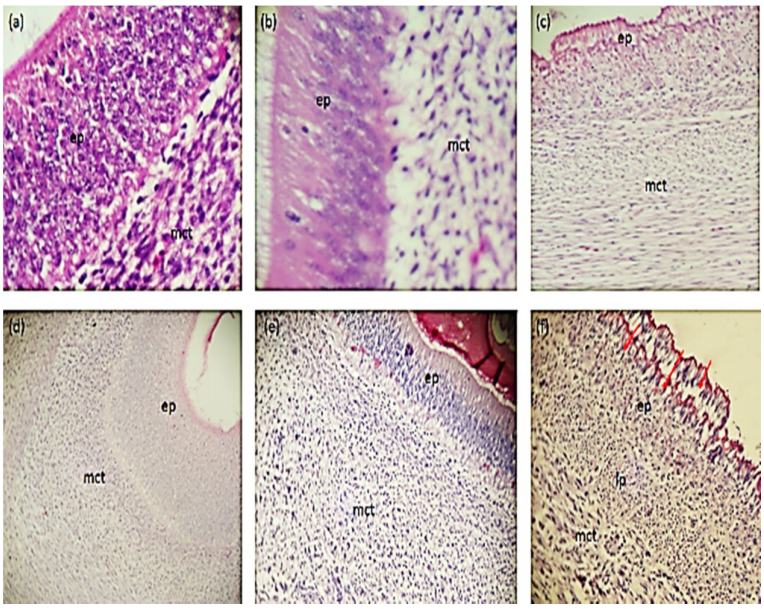

Figure 3: Sections of primordial ventriculus on the 10th (a, d), 13th (b, e) and 19th (c, f) days of developments. 11th and 13th days of development shows the pseudostratified epithelium (ep) surrounded by mesenchymal connective tissue (mct). H and E x 400 (a, b). Weak histochemical reaction to PAS x400 (d, e). The 19th day of development shows the change to simple epithelium (ep) beneath lamina propria (lp) with all surrounded by mesenchymal connective tissue H and E x 400. Positive reaction to PAS x 400.

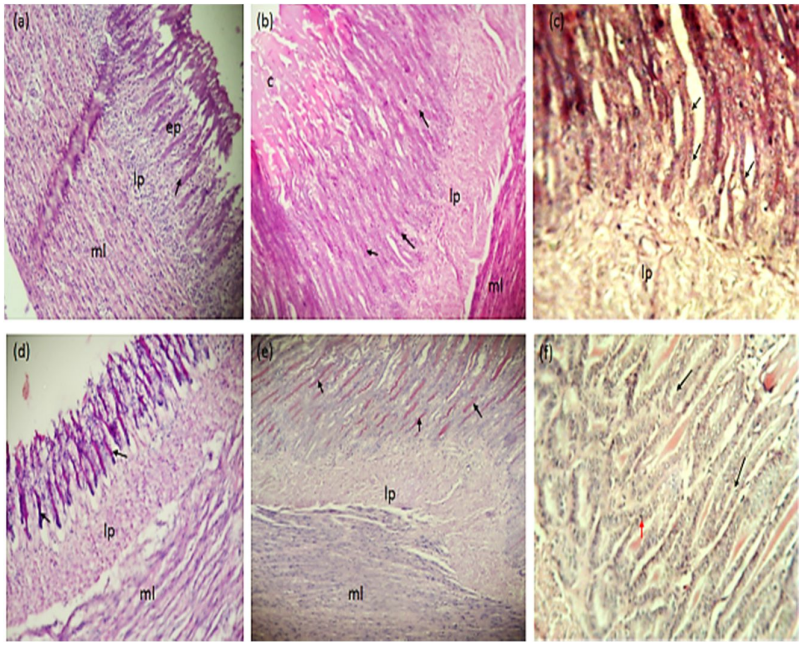

Figure 4. Sections of primordial ventriculus on the 23rd (a, d) and 27th (b,c e and f) days of developments. The 23rd day shows simple tubular glands (black arrow) withing the epithelium (ep); beneath is the lamina propria surrouded by muscular layer (ml). H and E x100 (a). PAS positive reaction. x100 PAS (d); the 27th day shows cuticular layer (c) at the surface of canalised (black arrows) simple cuboidal epithelium. Beneath is a well developed lamina propria (lp) and muscular layer (ml).

This vary with the amount, retention time, and chemical characteristics of the feed in the the stomach (Duke, 1986; Svihus, 2014) and also the thickness of the proventricular glands (Selvan *et al.,* 2008).

Primordial proventriculus of the helmeted guinea fowl appeared as a dilation differentiating it from ventriculus on the 10th day of embryonic development caudal to the oesophagus (Gosomji *et al*., 2015). It histogenesis appeared similar with most avian species with the epithelium appearing pseudostratified at the ealier stage which later transformed to simple columnar as the embryo advanced in age agreed with the findings in chicken (Ventura *et al*., 2013) and quail (Soliman *et al*., 2015) but contradicted the finding of Attia (2008) in quail and Dale (1968) in chicken where they observed stratified columnar. This epithelium at the initial stage of development is surrounded by undifferentiated mesenchymal cells which later differentiated into the subepithelial layers such as tunica submucosa, tunica muscularis and tunica serosa (Fukuda and Yasugi, 2005; Naghani *et al*., 2010).

The gradual changes from pseudostratified to simple epithelium could be due physiological balance between mitotic activity and apoptosis of the cells at each stage of embryonic development (Soliman *et al.,* 2015).

The positive reaction of the primordial proventriculus to PAS as at from 19th to 27th day indicates that the proventricular glands could be functional contradict the findings of (Wali and Kadhim, 2014) who observed PAS-negative reaction but Alcian positive secretion.

It was observed that the primordial ventriculus in this study followed similar pattern of histogenesis with primordial proventriculus. This corroborated Ventura *et al*. (2013) and Pinhiero *et al*. (1989) findings in *Gallus gallus* on the 15th day of embryonic development. The variation in the days of development of the species could be due to difference in incubation period. The presence of cuticular layer at the 27th day of embryonic development is an important indication that the primordial ventriculus is fully prepared for mechanical activities.

The positive histochemical reaction of the epithelial lining of the primordial ventriculus exposed the tubular glands which later became canalized from 23th day and gain prominence at the 27th day indicate that starting from the 19th day there could slight functionality of the ventriculus agreeing with the findings of Soliman *et al*. (2015) that observed in quail from the 12th day and by 14th day it has become canalized.

Conclusion: The histogenesis of the stomach of helmeted guinea fowl (*Numida meleagris*) follows similar pattern with other avian species. The primordial stomach starts differentiating to form the four (4) tunics from the 19th day but become fully organized at the 27th day which appears similar to structures at post-hatch.

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