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3 AUTHORS, INCLUDING:

Soha Soliman

south Valley University

Yasser Abdel Galil Ahmed

South Valley University

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ORIGINAL ARTICLE

Histogenesis of the stomach of the pre-hatching quail: a light microscopic study

Soha A. Soliman[1] · Yasser A. Ahmed[1] · Mohammed Abdelsabour-Khalaf[2]

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Abstract The current study conducted a careful description of the histological events during the embryonic development of quail stomach. Daily histological specimens from the quail stomach from day 4 to day 17 post incubation were examined by light microscopy. The primitive gut tube of the embryonic quail appeared at day 4 post incubation. The gut tube consisted of an endodermal epithelium of pseudostratified type, surrounded by splanchnic mesenchyme. The prospective glandular epithelium invaginated at day 5 in the proventriculus and gradually developed to prospective proventricular glands. The muscular coat became distinguished at day 7 and day 8 in the proventriculus and gizzard, respectively. Transformation into simple columnar epithelium occurred in both proventriculus and the gizzard at day 12. The gizzard epithelium gave rise to tubular invaginations also at day 12. Canalization of the gizzard tubular glands was recognized at day 14. By day 15, the proventricular surface epithelium invaginated in a concentric manner around a central cavity to form immature secretory units that contained inactive oxyntico-peptic cells. The mucosal folding in the gizzard appeared at day 15 to form plicae and sulci. The wall of the proventriculus and gizzard at day 17 acquired histological features of post-hatching birds.

✉ Yasser A. Ahmed
yasser.ali@vet.svu.edu.eg

1 Department of Histology, Faculty of Veterinary Medicine, South Valley University, Qena 83523, Egypt

2 Department of Anatomy and Embryology, Faculty of Veterinary Medicine, South Valley University, Qena 83523, Egypt

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Introduction

Different animal models, including avian species, are currently used in developmental research. Among avian species, the chick embryo model attracts many developmental biologists. The Japanese quail has recently been introduced as a favorite model in embryological studies (Ainsworth et al. 2010). As suggested by Shiina et al. (2004), the genetic distance between the quail and the chicken is likely to be closer than to other birds. Moreover, quail has many advantages over other avian species as a convenient model for studies of developmental biology, as summarized by Huss et al. (2008). Quail are small birds, easy to grow in a laboratory, while large chickens need farms. Quail also develop quicker than chicken; they hatch in about 16 days, while a chicken hatches in about 21 days. Furthermore, they have short life span and become sexually mature in about 8 weeks versus the 5–6 months needed for chicken. Recently, the quail has proven to be a successful model for the production of a transgenic avian (Poynter et al. 2009; Seidl et al. 2013). Transgenic lines of quail can be produced (Salter et al. 1999) by injection of cloned DNA into fertilized ova.

Similar to other birds, the quail stomach consists of two parts; the glandular part or proventriculus, and the muscular part or gizzard (Ahmed et al. 2011). The proventriculus is located caudal to the esophagus followed by the gizzard, which connected to the duodenum. The proventriculus is responsible for secretion of HCL and pepsin, while the gizzard is needed for food grinding (Caceci 2008).

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The histology of the stomach of different avian species has been studied by many authors (Mogil'naia and Bogatyr 1983; Ogunkoya and Cook 2009; Ahmed et al. 2011; Kadhim et al. 2011; Zhu et al. 2013). However, few reports on the stomach of pre-hatching quail are available. The current study aimed to extend our knowledge of the sequence of histological changes during development of the stomach of quail embryos.

Materials and methods

Egg incubation

Fertile quail (*Coturnix coturnix japonica*) eggs were obtained from the Research Quail Farm, connected to the Department of Histology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt. The eggs were incubated at 37.5 °C with a relative humidity 65 %; eggs were rotated automatically every 6 h from day 3 to day 17 of incubation.

Gross macroscopic examination of the stomach

The abdominal cavity of quail embryos was opened and the topography of the stomach during their developmental stages was reported. The stomach was dissected (whenever that was possible) and grossly examined.

Sample processing for light microscopy

Eggs were opened carefully at the wide part, and at least five embryos were daily collected starting from day 4 until day 17 (few hours before hatching). Embryos were rinsed in phosphate-buffered saline (PBS), and fixed in either 4 % neutral-buffered formalin or Bouin's fluid for at least 24 h at 4 °C. The whole embryos were dehydrated in ascending grades of ethanol and embedded in paraffin wax blocks. Longitudinal and cross paraffin serial sections (3–5 µm thickness) were cut and stained with hematoxylin and eosin (H&E) for identification of general structures, Periodic acid-Schiff's reagent (PAS) and alcian blue staining for the analysis of neutral and acid glycosaminoglycans (GAG), Crossman's trichrome stain for detection of collagen and muscular fiber development, and silver impregnation technique to identify enteroendocrine cells. Some specimens were fixed in 2.5 % glutaraldehyde in 4 % paraformaldehyde, post-fixed in 1 % osmium tetroxide, dehydrated in ascending grades of acetone and embedded in Spur's resin. Semithin sections (0.5 µm thickness) were undertaken and stained with toluidine blue. Paraffin and semithin sections were examined with light microscopy.

Results

Macroscopic development of the stomach of quail embryo

Gross anatomical examination of the abdominal cavity of quail embryos revealed that the quail stomach was completely covered ventrally by the liver lobes until day 9 of embryonic development. Therefore, the stomach at this developmental stage was separated from the ventral wall of the body cavity by the liver. At day 10, part of the gizzard projected caudally behind the level of the liver to reach the vent, thus this projected part was in close contact with the ventral wall of the body cavity. The gizzard projected more caudally than the level of the liver with advancing age by day 17, and terminated just in front of the vent. Interestingly, the gross demarcation between the proventriculus and the gizzard was seen for the first time at day 9, and increased with advanced embryonic age (Fig. 1a–d). Furthermore, the two anatomical parts of the gizzard, the body and blind sacs were more distinct at older ages (Fig. 1c, d).

Microscopic development of the proventriculus

On day 4, the primitive gut of the embryonic quail appeared as a tube located ventromedial to the developing kidney (Fig. 2a). The gut tube was represented by a pseudostratified epithelium surrounded by a layer of condensed mesenchyme underneath a layer of mesothelium (Fig. 2b). At this developmental stage, it was difficult to differentiate the prospective proventriculus from the gizzard.

At day 5 post incubation, a part of the gut tube became enlarged and gained the morphological characteristics of the avian stomach, enabling the proventriculus to be identified by slight invaginations of the epithelium downward to the mesenchyme, which is likely to be considered the first sign of glandular formation (Fig. 2c).

At day 6, the proventriculus could be sharply defined from the gizzard (Fig. 2d). The proventricular epithelial invaginations became canalized and developed into sac-like primitive secretory units lined with pseudostratified epithelium (Fig. 2d–f). Development of the muscular coat in the proventriculus was confined to the cranial part of the prospective proventricular wall (Fig. 2g).

At day 7 of incubation, the proventriculus composed of four layers: mucosa, submucosa, muscularis and serosa (Fig. 2h). By day 8, the surface epithelial cells transformed to GAG-producing cells and produced intracellular metachromatic granules. The secretion covering proventricular epithelia was rich in GAG as it was stained red–purple by toluidine blue (Fig. 3a). Myoblasts began to

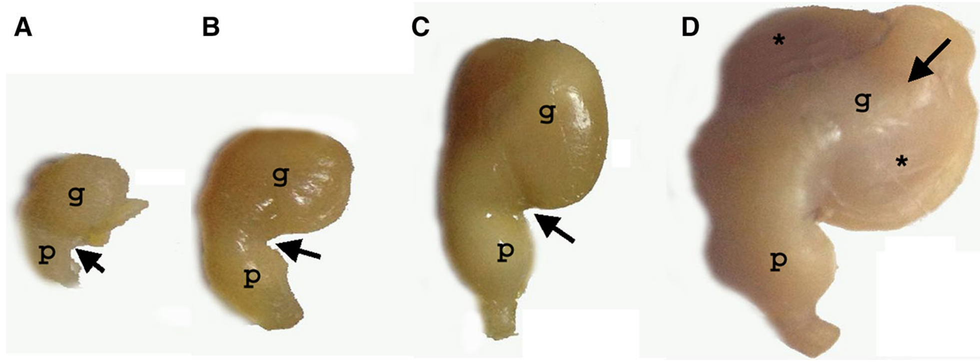


Fig. 1 Macroscopic morphologic development of the quail stomach. Photographs from the stomach of **a** 9-, **b** 10-, **c** 12- and **d** 17-day-old quail embryos. *Arrows* Demarcations between proventriculus (*p*) and gizzard (*g*) in **a**–**c**. In **d**, *arrow* indicates body, and *asterisks* indicate blind sac of the gizzard

aggregate around the primitive glandular units, which developed the muscularis mucosa and the circularly arranged muscular layer, which represented the future outer circular muscularis (Fig. 3b).

At day 9, the development of the primitive glandular epithelium continued in the cranial part of the proventriculus. Some sac-like glandular invaginations lined with pseudostratified epithelium were seen within the submucosa, and the muscularis mucosa along with the circular muscular coat of tunica muscularis became more developed (Fig. 3c, d). At day 10, the number, size and depth of the glands increased, and the compound architecture of the gland began to be found, while the second layer of the muscular coat appeared as inner longitudinal smooth muscle fibers (Fig. 3e).

At day 12, the pseudostratified mucosal epithelium transformed into a simple columnar type. The glands showed secretory units opening into a common central cavity (Fig. 3f, g). The pseudostratified lining epithelium of the glands transformed into a simple columnar type at the location of oxyntico-peptic cells precursors (Fig. 3h). The future ductal epithelia appeared as darkly stained low columnar cells, with some secretions in the proventricular lumen (Fig. 3g).

In addition to the usual mechanism of proventricular glandular development described above, which started at day 5 of incubation, we noticed another mechanism of proventricular gland development, which began at day 15, namely, in the late stage of proventricular development. In this newly observed mechanism, the surface epithelia of the proventricular wall exhibited extensive dynamic features, starting as invaginations of the epithelium, close to the surface and forming immature secretory units. The secretory units formed by concentric lamellae wrapped around a central lumen. The lumen was lined with highly acidophilic cuboidal cells of the ductal epithelium, whereas the peripheral lamellae were lined with inactive basophilic secretory oxyntico-peptic cells (Fig. 4a–d). The lumen developed to a central cavity, in which many secretory units opened and the secretion reached at the surface through the duct system. More newly formed proventricular glands were added by this mechanism.

By day 17 of incubation, the morphology of the proventriculus of the embryonic quail became similar to that of post hatched birds (Figs. 4h, 6a). The surface epithelial cells were of mucous secretory type as shown in toluidine blue-stained semithin sections (Fig. 4f). Oxyntico-peptic cells became activated and showed few cytoplasmic secretory granules (Fig. 4g). The ductal epithelium of the proventricular glands showed no PAS and alcian blue reaction from day 12 to day 17 of incubation (Fig. 4e). Enteroendocrine or argyrophilic cells were detected in the proventricular glands by using the silver impregnation technique (Fig. 4h).

Microscopic development of the gizzard

After 5 days of incubation, and during its course of embryonic differentiation, the prospective gizzard was recognized as two morphological different parts: a thick-walled body and thin-walled sacs (Fig. 5a). The wall of the primitive gizzard was lined with pseudostratified endoderm and enclosed by mesoderm (Fig. 5b).

By day 6, the gizzard enlarged and its wall was distinguished into four layers; ill-developed mucosa, submucosa, muscularis and serosa (Fig. 5c). From day 6 to day 11, most histological changes in the gizzard were noticed in the muscular coat. The mesenchymal cells were condensed at the site of the future tunica muscularis (Fig. 5c) and developed into myoblasts at day 7 (Fig. 5d), and then the myoblasts differentiated into spindle-shaped smooth muscle fibers by day 8 of incubation (Fig. 5e). At this developmental stage, the muscular coat showed histological differences between the sac and the body. The tunica

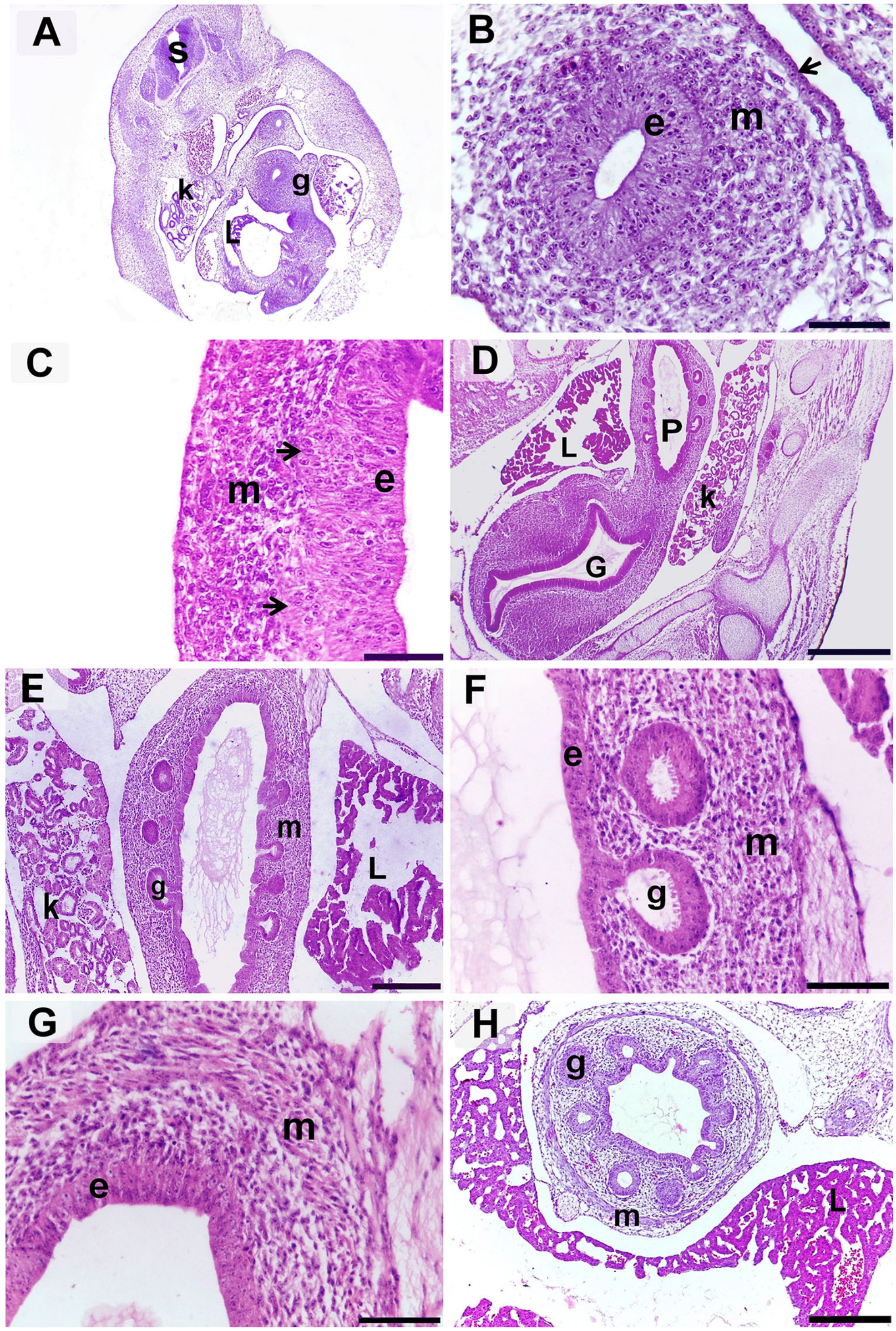


Fig. 2a–h Light micrographs of the gut tube and proventriculus of 4- to 7-day-old quail embryos. Hematoxylin and eosin (H&E)-stained paraffin sections from a whole embryo (**a**), gut tube (**b**), proventriculus (**c**, **e**–**h**) and proventriculus and gizzard (**d**) at day 4 (**a**, **b**), 5 (**c**), 6 (**d**–**g**) and 7 (**h**) of pre-hatching development. Note gut tube (*g*), somite (*s*), kidney (*k*), liver (*L*) in **a**; lining epithelium (*e*), mesenchyme (*m*) and covering mesothelium (*arrow*) in **b**; epithelium (*e*), epithelial invaginations (*arrows*) and mesenchyme (*m*) in **c**; prospective proventriculus (*P*), gizzard (*g*), kidney (*k*) and liver (*L*) in **d**; prospective proventricular glands (*g*) and mesenchyme (*m*), kidney (*k*) and liver (*L*) in **e**; epithelium (*e*), mesenchyme (*m*) and prospective proventricular glands (*g*) in **f**; epithelium (*e*) and muscular coat (*m*) in **g** and proventricular gland (*g*), muscular coat (*m*) and liver (*L*) in **h**. *Bars* **a**, **d** 200 μm; **b**, **c**, **f**, **g** 20 μm; **e**, **h** 80 μm

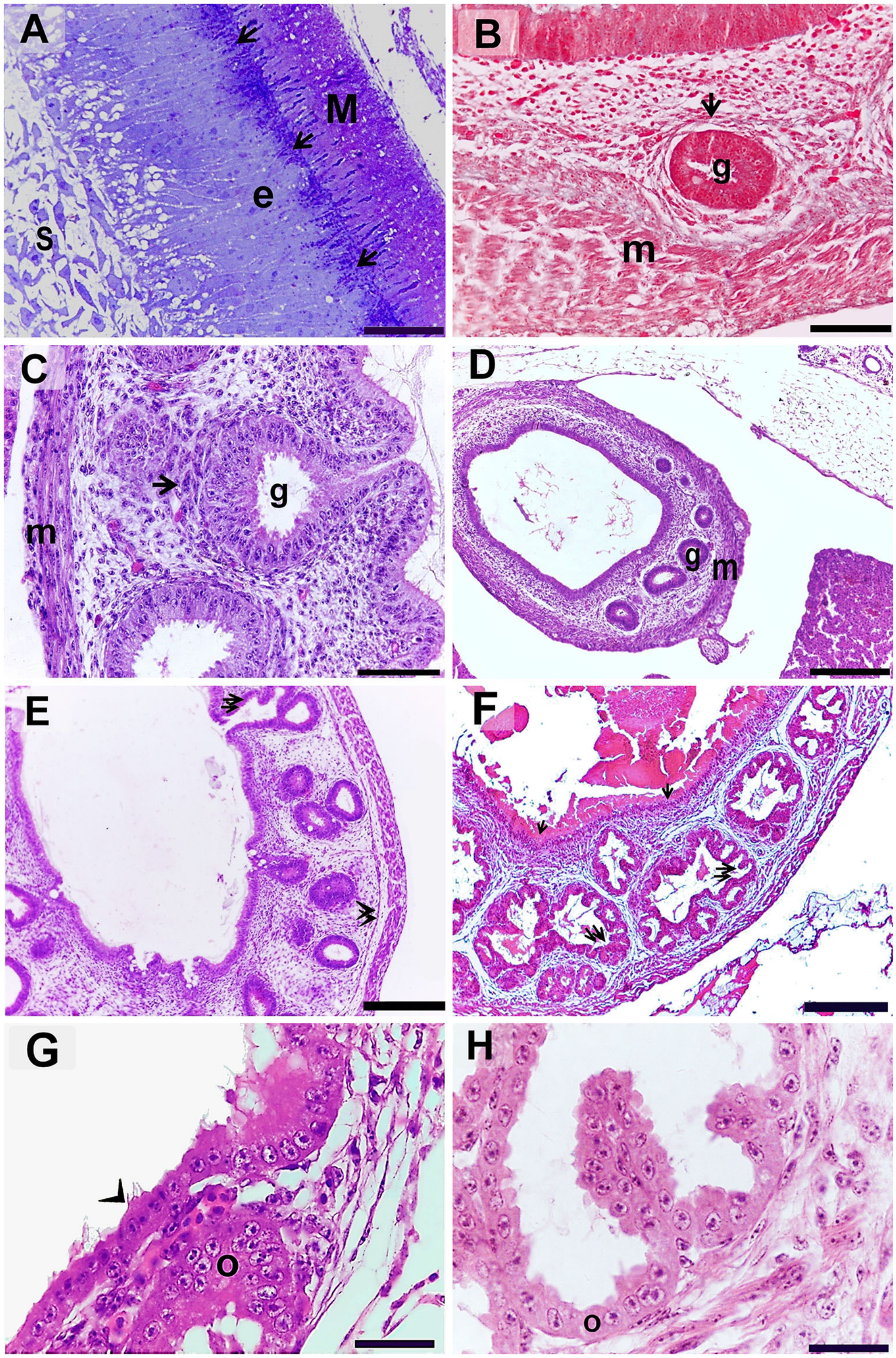


Fig. 3a–h Light micrographs of the proventriculus of 8- to 12-day-old quail embryos. Toluidine blue-stained semithin (**a**), Crossman's trichrome (**b**) and H&E-stained paraffin (**c**–**h**) sections from the proventriculus of 8- (**a**, **b**), 9- (**c**, **d**), 10- (**e**) and 12- (**f**–**h**) day-old quail embryos. Note epithelium (*e*), mucous secretion (*M*), metachromatic granules (*arrows*) and submucosa (*S*) in **a**; muscularis mucosa (*arrow*), developing proventricular gland (*g*) and well-developed muscular wall (*m*) in **b** and **c**; primitive glandular epithelium (*g*) in the cranial part of the proventriculus and muscular coat (*m*) in **d**; branched proventricular gland (*double arrows*) and inner longitudinal muscular layer (*double arrowheads*) in **e**; epithelium (*arrows*) and primitive ductal epithelium of compound proventricular glands (*double arrows*) in **f**; oxyntico-peptic cells (*o*) in **g** and **h**, and primitive ductal epithelium of compound proventricular glands (*arrowhead*) in **g**. *Bars* **a**, **h** 8 μm; **b**, **c**, **g** 20 μm; **d** 200 μm; **e**, **f** 80 μm

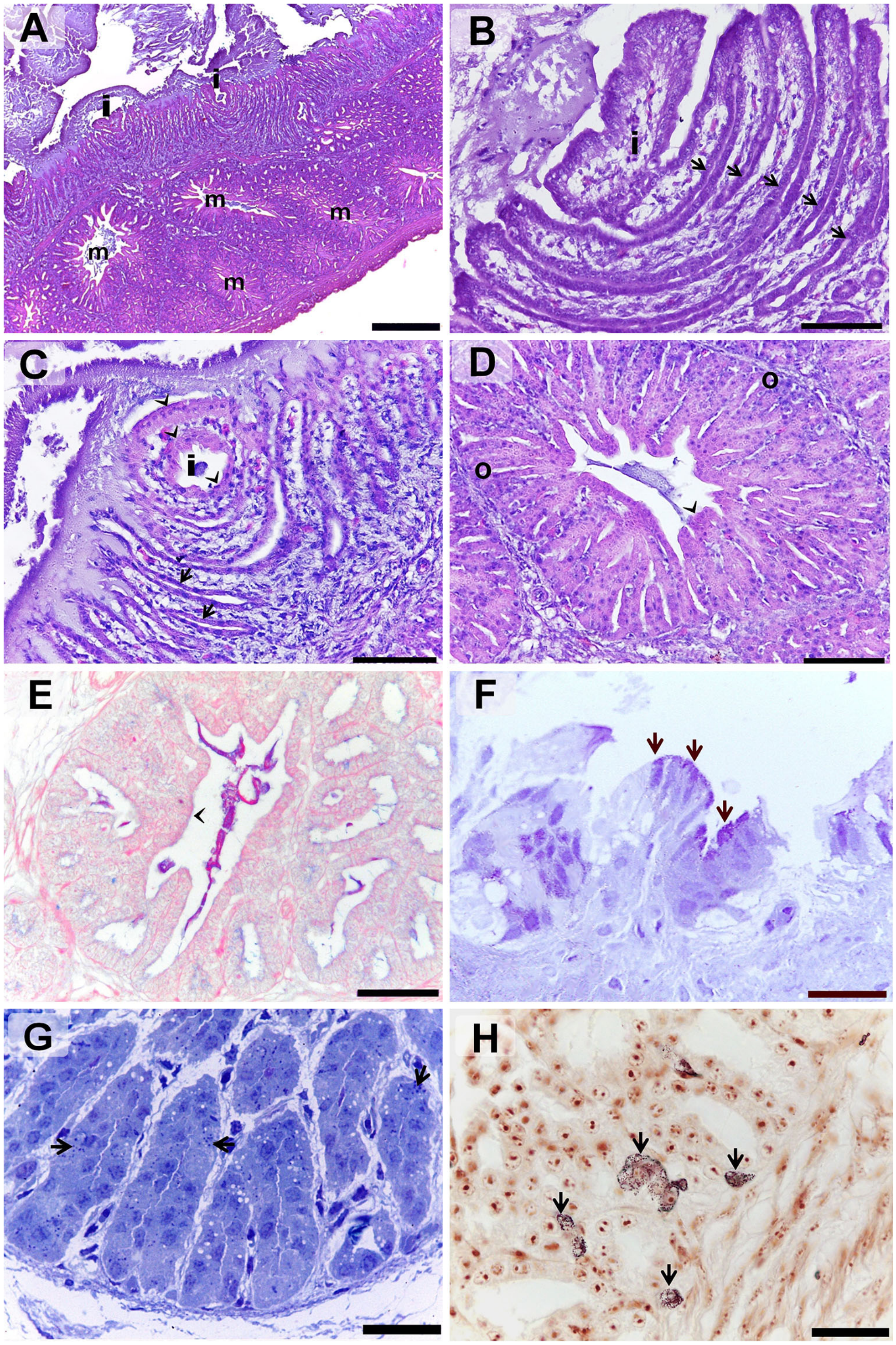


Fig. 4a–h Light micrographs of the proventriculus of 15- and 17-day-old quail embryos. H&E (**a**–**d**), PAS/Alcian blue (**e**), silver impregnation (**h**)-stained paraffin and toluidine blue-stained semithin (**f**, **g**) sections from the proventriculus of 15- (**a**–**e**, **h**) and 17- (**f**, **g**) day-old quail embryos. **a**–**c** Proventricular epithelia developed immature secretory units (*i*). Note inactive basophilic oxyntico-peptic cells (*arrows*) in **b** and **c**, the primitive ductal epithelium "acidophilic cells" (*arrowheads*) in **c** and **d**, mature secretory units (*m*) in **a** and oxyntico-peptic cell progenitors (*o*) in **d**. **e** Ductal epithelium of the proventricular unit stained negative for PAS/Alcian blue (*arrowhead*). **f** Surface epithelial cells were modified to glycosaminoglycans (GAG)-producing cells; *arrows* refer to metachromatic granules. **g** Oxyntico-peptic cells contained few secretory granules (*arrows*). **h** Endocrine cells stained positive for silver impregnation (*arrows*). *Bars* **a**, **f**, **h** 20 µm; **b**–**e** 50 µm

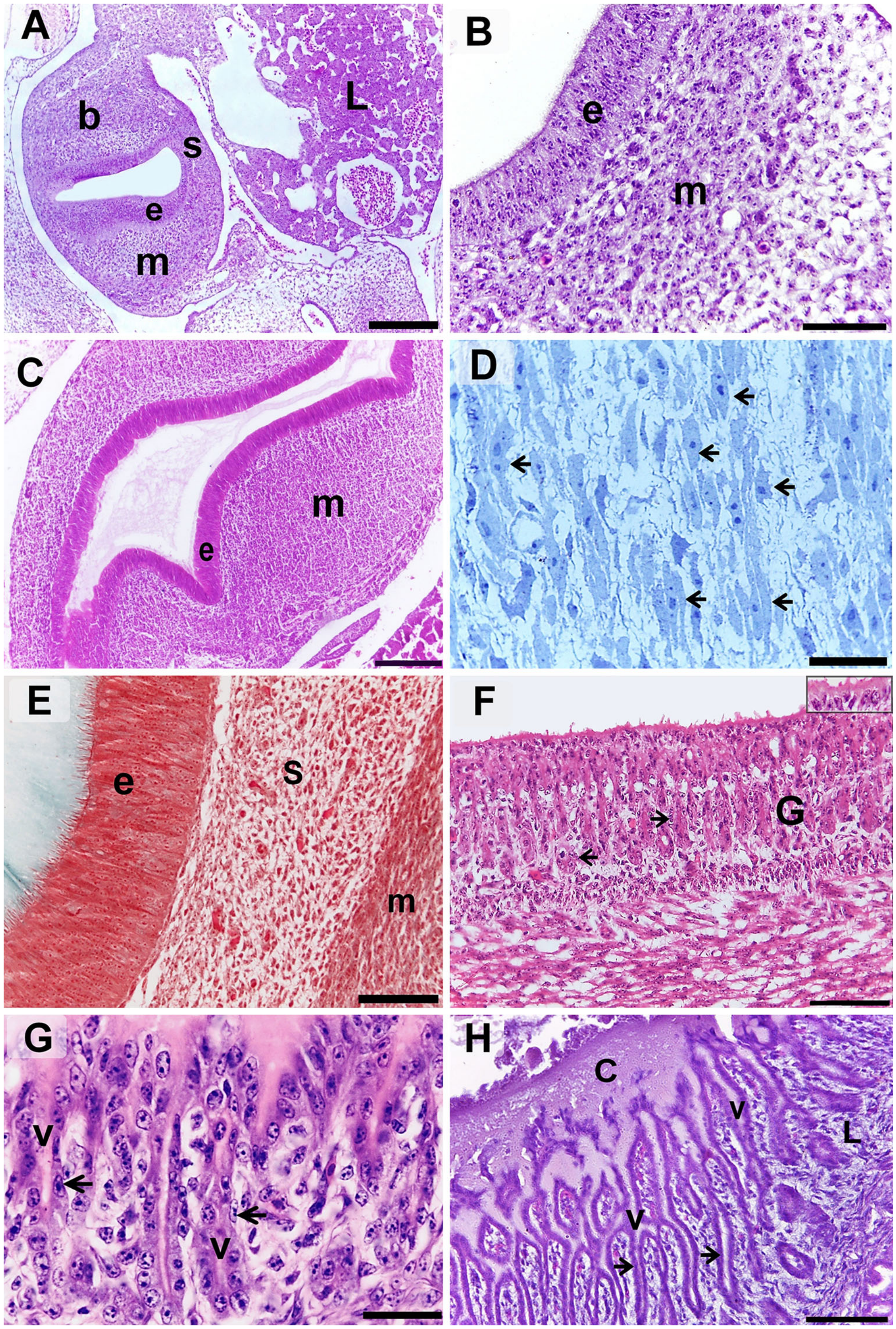


Fig. 5 Light micrographs of the gizzard of 5- to 15-day-old quail embryos. H&E (**a**–**c**, **f**–**h**) and Crossman's trichrome (**e**)-stained paraffin, and toluidine blue-stained semithin (**d**) sections from the gizzard of 5- (**a**, **b**), 6- (**C**), 7- (**d**), 8- (**e**), 12- (**f**), 14- (**g**) and 15- (**h**) day-old quail embryos. **a** Thick-walled body (*b*) and thin-walled sac (*s*), epithelium (*e*) of primitive gizzard, and liver (*L*). **b**, **c** Epithelium (*e*) and mesoderm (*m*). **d** Myoblasts (*arrows*). **e** Epithelium (*e*), submucosa (*s*) devoid of collagen fibers and circular muscular layer (*m*) of the body. **f** Magnified columnar epithelium (*square*), primitive glandular tissue (*G*) and chief cells precursors (*arrows*). **g** Glandular canalization and vertical rods filling tubular glands (*v*) and chief cells precursors (*arrows*). **h** Well-developed gizzard glands (*arrows*), vertical rods (*v*) and cuticle (*c*). *Bars* **a**, **c**, **h** 80 μm; **b**, **e**, **f** 50 μm; **d**, **g** 8 μm

muscularis of the sac developed into two layers: the inner longitudinally and outer circularly oriented smooth muscle fibers, whereas the splanchnic mesoderm in the body consisted of a thick circular layer of smooth muscle fibers. The muscular coat increased in thickness with increasing the age of the embryo.

The first sign of glandular development in the gizzard began at day 12, when the epithelium invaginated into the underlying tissue forming solid tubular glands lined with simple cuboidal cells and the surface epithelia became simple columnar type (Fig. 5f). Canalization of the tubular glands was clearly observed at day 14 and the glandular lumen was filled with vertical rods of the cuticles (Fig. 5g).

By day 15 and later on, the mucosa folded into plicae and sulci and the number, size and depth of the tubular glands increased (Fig. 5h). The muscular coat became well developed. The gizzard established histological characteristics similar to those of post-hatching birds at late embryonic stages (Fig. 6a). The four tunics—mucosa, submucosa, muscular layer and serosa—were clearly differentiated (Fig. 6a, b). The mucosal folds showed well-defined plicae and sulci (Fig. 6c). The surface epithelia cells exhibited features of GAG-producing cells and the granular contents appeared metachromatic by toluidine blue (Fig. 6d). The chief cells lining the tubular glands appeared inactive and were devoid of secretory granules (Fig. 6e).

Fig. 6a–e Light micrographs of the gizzard of 15- to 17-day-old ▶ quail embryos. H&E-stained paraffin (**a–c**) and toluidine blue-stained semithin (**d**, **e**) sections from the gizzard of 16- (**a**, **b**) and 17- (**c–e**) day-old quail embryos. Note in **a**: body (*b*) and sac (*s*) of the gizzard, proventriculus (*p*), immature secretory units (*i*) and lung (*L*). Note in **b**: gizzard tubular glands (*G*), submucosa (*s*), thin inner longitudinal (*L*) and thick outer circular (*c*) muscular layers of the gizzard sac. Note in **c**: plicae (*p*) and sulci (*s*) and single circular muscular of the body (*m*). Note in **d**: metachromatic granules of the surface epithelium (*arrows*). Note in **e**: chief cells of gizzard gland with no secretory granules (*arrows*) and magnification of chief cells (*square*). *Bars* **a** 200 µm; **b** 20 µm; **c** 80 µm; **d**, **e** 8 µm

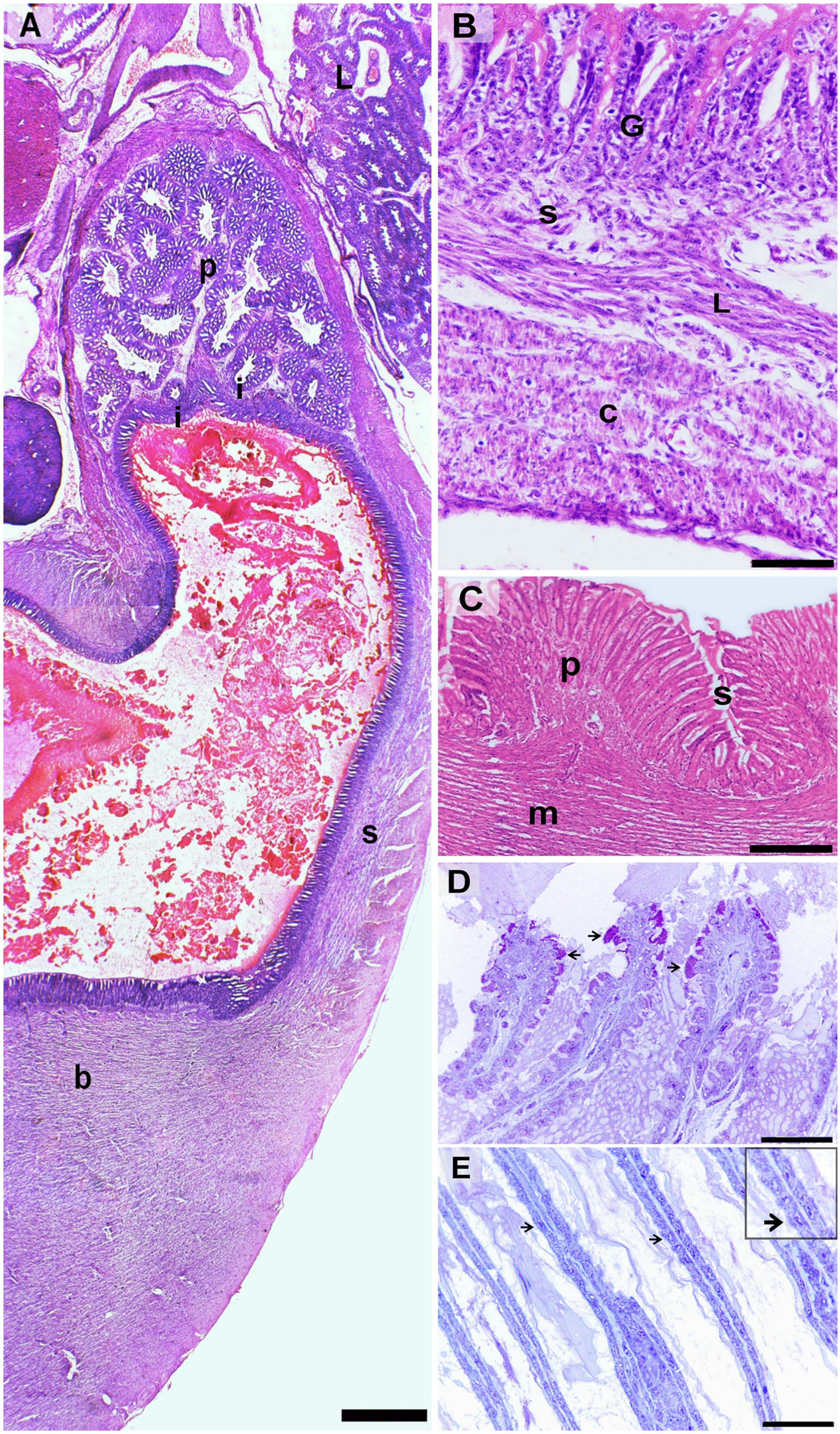
Discussion

Quail embryo is now considered an important model for developmental experiments, although literature reporting histological changes during its embryonic development is scarce. The current study was undertaken with the aim of making a careful description of the main histological events during the embryonic development of quail stomach. To achieve this aim, daily histological specimens from the stomach of quail after 4 to 17 days of incubation were examined with light microscopy. The main histological sequences of stomach development of quail embryo are summarized in Table 1 and Fig. 7.

The present study showed that the primitive gut tube of the embryonic quail appeared from day 4 post incubation and consisted of an endodermal epithelium of pseudostratified type surrounded by undifferentiated mesenchymal tissue. The mesenchymal tissue is the origin of stomach sub-epithelial tunics: submucosa, muscularis and serosa (Fukuda and Yasugi 2005). In the current study, the primitive proventriculus and gizzard appeared as two enlargements in the wall of the primitive gut tube at day 5 post incubation. Attia (2008) observed the structure of the primitive proventriculus of embryonic quail at day 3 of incubation. However, he showed a figure similar to those seen after day 7 in the current study. The current study showed that the lining epithelium of the prospective proventriculus was established first as pseudostratified, and then transformed to simple columnar type by day 12. Unlike the current study, Attia (2008) in quail stated that epithelial transformation occurs after day 9. A reason for such conflicting data may be the different sources of fertilized eggs and the technique of incubation. We collected our samples from the farm connected to our department at specific times as shown in the Materials and methods. However, Attia (2008) did not mention to the farm from which his samples were collected, thus it is likely that embryos had started their development in the period between egg collection and incubation. The stratification and simplification of the epithelium during early and late embryonic stages may be due to the physiological balance between mitotic activity and apoptosis of the cells at each stage of development. It is known that the sites of glandular development are associated with a high mitotic activity in the invading epithelium, and thinning of the basement membrane underlying this epithelium (Hayashi 1987). The simple proventricular glands were lined with pseudostratified epithelium at day 6 and became simple cuboidal epithelium later by day 12. It is likely that the reduction in the epithelium height is due to organ enlargement and surface epithelium increase in relation to mitotic activities of the cells.

In addition to the usual mechanism, we noticed for the first time a new mechanism of proventricular gland development at late embryonic stages by day 15 until hatching. In this mechanism, the superficial epithelium developed into concentric lamellae and made small secretory units. The secretory units formed a central cavity that was surrounded by lamella. The lamellar epithelium represented the inactive secretory oxyntico-peptic cells and the central cavity formed the central ductal system of the developing proventricular glands.

The current study showed that the quail gizzard first appears as an enlargement of the gut tube at day 5 and consists of an internal endodermal pseudostratified epithelium. By day 12, the surface epithelium transformed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from pseudostratified into columnar type. This transformation can be explained by the enlargement of the gizzard and increase in epithelial surface area.

A thin cornified layer was first observed in the gizzard at day 8, and increased in thickness thereafter. The cornified layer stained red–purple by toluidine blue indicating high GAG content. This layer is likely to be derived from surface epithelial cells. The secretory activity was confined only to the covering surface epithelial cells, which contained metachromatic granules. However, no secretory activity was seen in the gizzard glands. Chief cells were devoid of secretory granules. The covering cuticle stained red–purple by toluidine blue. Thus, the intra-luminal secretion appears to provide protection in late embryonic stages. Secretion of carbohydrate-rich cuticle may be considered as a preparatory stage before food ingestion after hatching. The gizzard of the quail embryos after 17 days of incubation showed similar structure to those of post-hatching birds (Ahmed et al. 2011).

In conclusion, the current study explained the histological changes of the quail proventriculus and gizzard during embryonic development. The study presents for the first time another (unusual) mechanism of the compound tubule-alveolar proventricular gland development. The

Table 1 Summary of the main histological events during development of Japanese quail stomach

Embryonic day	Proventriculus	Gizzard
4	Formation of the gut tube (endodermal lining and mesodermal covering)	
5	Slight invaginations of the endoderm. The first sign of glandular formation	Distinction of the body and sac of the gizzard. Mesenchymal condensation at the areas of the prospective muscular coat
6	Formation of sac-like glandular invaginations	
7	Development of the outer muscular layer. Surrounding of the prospective glandular sac by strands of muscularis mucosa	
8	Differentiation of the surface epithelial cells into mucous producing cells	Differentiation of the surface epithelial cells to mucous producing cells. Development of the inner muscular layer of the sac and the circular muscular coat of the body
10	Appearance of the complex architecture of the proventricular glands. Development of the inner muscular layer	Development of the outer muscular layer of the sac, which continued with circular layer of the gizzard
12	Transformation of the surface and the glandular epithelia to simple type. The glandular epithelium distinguished into small cuboidal cells of the ductal epithelium and large columnar to cuboidal inactive oxyntico-peptic cells. The mucosa was unfolded	Formation of tubular invaginations of primitive glandular epithelium. Transformation of the epithelium to simple type
14		Canalization of the tubular glands was completed
15	The mucosal surface became folded and the surface invaginations gave rise to immature secretory units, which could be observed in different stages of development:	
	Stage 1: Appearance of a central cavity, lined by the future ductal epithelium, and the surrounding concentric lamellae, which were lined by the precursors of oxyntico-peptic cells	
	Stage 2: Enlargement of the central cavity and invagination of additional lamellae	
	Stage 3: the lamellar epithelium was enlarged and transformed to inactive oxyntico-peptic cells, while the surface epithelium invaginated to add more lamellae to the superficial part of the developing secretory unit	
	Stage 4: formation of mature secretory units that contained enlarged inactive oxyntico-peptic cells and the ductal system was well-established	
17	The surface epithelial cells were mucous secretory cells. Oxyntico-peptic cells became activated, producing few granules. Entero-endocrine or argyrophilic cells were detected in the proventricular glands	The four tunic's mucosa, submucosa, muscular layer and serosa were clearly differentiated in the body and the sac. The mucosal folds showed well-defined plicae and sulci in the body. The surface epithelia cells exhibited features of glycosaminoglycan-producing cells. The chief cells, which lined the tubular glands, appeared inactive

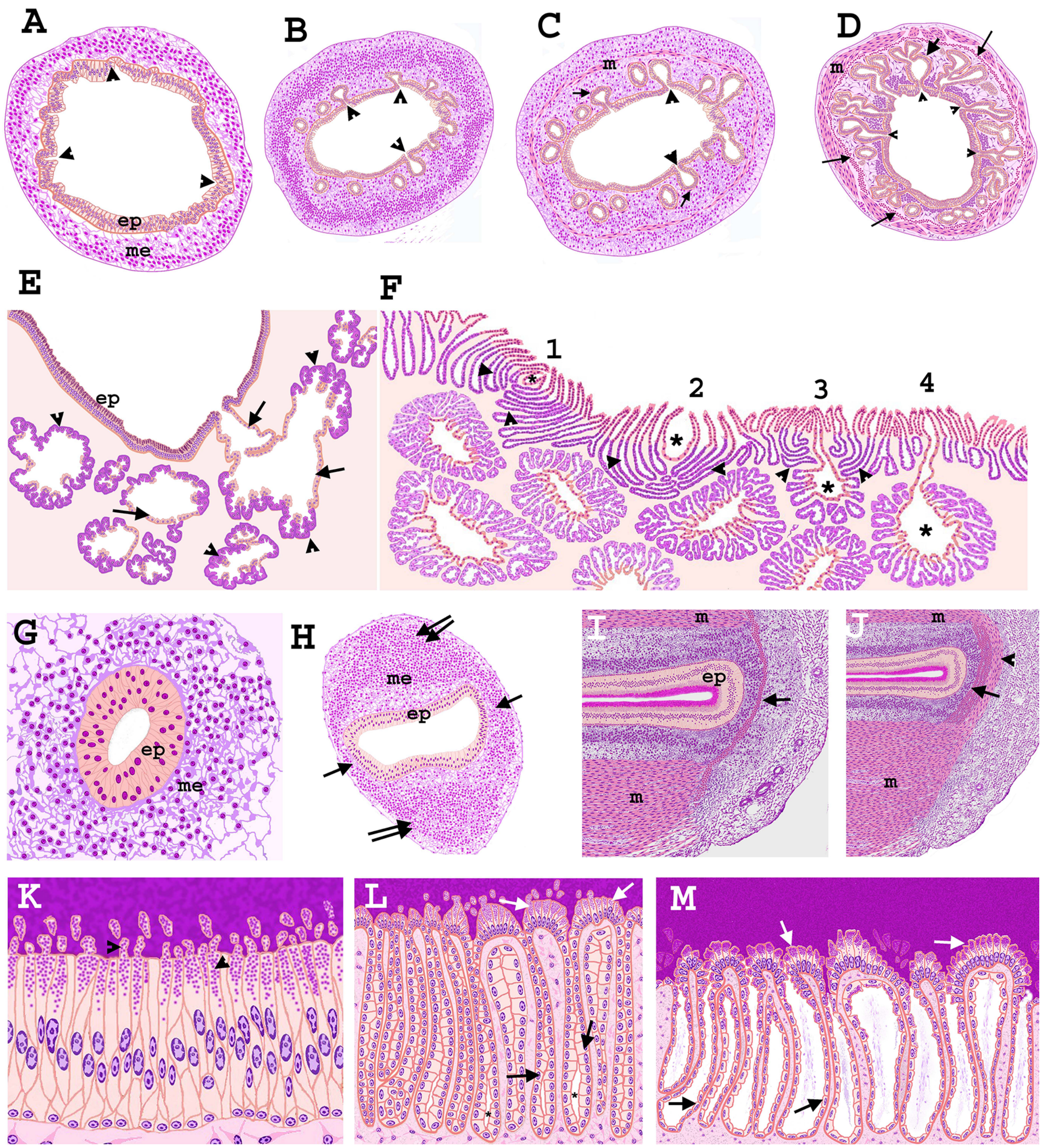

Fig. 7a–m Summary of the sequence of developmental histological changes of the stomach of quail embryos. Illustrations explaining the most dramatic histological events during development of the quail embryo proventriculus (**a**–**f**) and gizzard (**g**–**m**). **a**–**d** *Arrowheads* refer to invaginations of the pseudostratified epithelium (*ep*) in **a** (day 5) and formation of primitive sac-like glands in **b** (day 6) and **c** (day 7). Note in **c**: strands of muscularis mucosa (*arrows*) surrounding the prospective glandular sacs. In **d** (day 10), appearance of the complex architecture of the proventricular glands (*short arrows*) originating from the epithelial invaginations (*arrowheads*) and development of the inner muscular layer (*long arrows*). In **e** (day 12), the epithelium transforms into simple columnar type at the surface (*ep*) and ductal epithelium (*arrows*) with inactive oxyntico-peptic cells (*arrowheads*) at the glands. **f** (day 15) summarizes stages 1–4 of development of immature secretory units at the surface of the proventriculus. Note the development of central cavity (*asterisks*) and invaginating lamellas (*arrowheads*). **g** Gut tube (day 4), and **h** primitive gizzard (day 5). Note pseudostratified epithelium (*ep*) and mesenchyme (*me*) in **g** and **h**, while body (*double arrows*) and sac (*arrows*) of the gizzard in **h**. **i** Gizzard at day 8; mucous secretory epithelium (*ep*), and development of circular muscular coat of the body (*m*) and inner longitudinal muscular coat of the sac (*arrow*). **j** Gizzard at day 10; development of the outer circular muscular layer of the sac (*arrowhead*) and *arrow* refers to inner longitudinal muscular coat. **k**–**m**; development of the gizzard glands at day 8 (**k**), tubular invaginations at day 12 (**l**) and canalization of the glands at day 14 (**m**) of pre-hatching stages. Note the pseudostratified epithelium with apical mucous producing cells (*arrowheads*) in **k**, transformation of epithelium into simple columnar cells (*white arrows*), and inactive chief cells (*black arrows*) of the gizzard glands in **l** and **m**. *Asterisks* Cavitations of the gizzard glands in **l**

results presented in this study should be taken into consideration in future developmental and/or molecular studies of the embryonic quail stomach as well as in chick/quail chimeric studies.

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Compliance with ethical standards

Conflict of interest None.

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