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RESEARCH ARTICLE

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Developmental morphological analyses on the preglottal salivary gland in Japanese quails (*Coturnix japonica*)

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Abstract

To understand the development of the mucous preglottal salivary gland in *Coturnix japonica* (Japanese quail), morphological and histochemical studies were performed on 20 healthy *Japanese* quail embryos (aging from 10th to 17th incubation days) and 25 healthy quail chicks (aging from 0th to 60th days). The primordia of preglottal salivary gland were observed as an epithelial bud at the early embryonic stage, which then elongated and differentiated into secretory units by the end of this stage. In Japanese quails, the preglottal salivary gland was a mucous polystomatic tubuloalveolar unpaired gland composed of two lateral portions and a middle one embedded into the submucosa of the lingual root. The gland openings accompanied taste pore (8.17 μm) of taste buds associated salivary glands type; some skeletal muscle fibers embedded among secretory lobules extended from muscle cricohyoideus at 14th day-old quail chick. Also, both herbts corpuscles and secretory motor plexus could be detected among secretory lobules. Based on our investigations, the development of the preglottal salivary gland could clearly be distinguished in the embryonic stage into pre bud and bud stages at 10th day old, cord and branching stages ended by cavitation at 11th day old, canalization stage at 13th day old, lobulation and secretory stages by the 17th day old. The secretory materials showed different histochemical reactions ended with highly alcinophilic mucous indicated highly sialomucin (acidic) content. Myoepithelial cells could be demonstrated at a 17-day old quail embryo and thereafter surrounded the secretory endpieces of the preglottal salivary gland.

KEYWORDS
myoepithelial cells, preglottal salivary gland, quails, sialomucin, tubuloalveolar

1 | INTRODUCTION

The salivary glands are characteristic features of the upper digestive tract of birds and these glands have been identified in numerous avian species: Japanese quail (Warner, Mcfarland, & Wilson, 1967); Avian (Mclelland, 1975; Mclelland, Baumel, & Lucas, 1979); birds (King & Mclelland, 1984); chicken (Samar, Avila, De Fabro, & Centurion, 1995); The little egret (Al-Mansour & Jarrar, 2004); The White-tailed eagle (Jackowiak & Godynicki, 2005); the black African ostrich (Porchescu, 2007); and domestic poultry (Calhoun, 1954; Hodges, 1974; King & Mclelland, 1984; Nickel, Schummer, & Seiferle, 1977; Saito, 1965; Ziswiler & Farner, 1972). Salivary glands are well-developed in birds, which consume a dry diet (King & Mclelland, 1984). In some species, glandular tissue is absent while others display a seasonal change in gland size (Mclelland et al., 1979). The main functions of the salivary glands were to produce the saliva,

which can facilitate feed moisten and lubrication (Bell & Freeman, 1971; Gargiulo, Lorvik, Ceccarelli, & Pedini, 1991; Nickel et al., 1977; Reece, 1996

). In addition, the saliva had an antimicrobial effect particularly in the protection of the mucosal surface of the tongue against pathogenic micro-organisms. Furthermore, the saliva forms a sensation necessary hydrophilic environment on the tongue (Gargiulo et al., 1991). Several investigations on the histology and histochemistry of the salivary glands of the birds have been made. However, the knowledge about the histogenesis and the mucins histochemistry of the salivary glands is not known during the pre- and posthatching period. The present investigation focused on the studying the histology and histochemistry of the preglottal salivary glands of the tongue in female and male *Japanese* quails during the pre- and posthatching period in addition to determine alcinophilic changes accompanied the secretion.

2 | MATERIALS AND METHODS

2.1 | Collection of specimens

This research was done in compliance with the ARRIVE guidelines (https://arriveguidelines.org). The Ethics Committee of Veterinary medicine, Assiut University, Egypt approved this study. The quails were obtained from a poultry farm, faculty of agriculture, Assiut University. A total of 45 healthy Japanese quails (Coturnix*coturnix japonica*) were used starting from the sixth day prehatching till hatching day then at 7, 14, 30, and 60th day posthatching old. The quails were slaughtering according to local ethical board guidelines of the animal ethics committee of Veterinary medicine, Assiut University (20152016). After slaughtering, the heads were cut out and rinsed in running tap water and then by phosphate buffer saline to remove traces of blood after that both right and left commissures of the beak were incised. Finally, the oropharyngeal floor immersed in 10% neutral-buffered formalin for 24 hr. Three specimens from each age 7- and 14-days old quail chicks were fixed in 10% neutral buffered formalin and stained by alizarin red and alcian blue stains after maceration (KOH 1%) then photographed using stereomicroscope (LEICA S6D).

2.2 | Paraffine sections

For histological investigations three specimens of each age were used after proper fixation, the samples were kept in 10% formic acid/formol saline for the process of decalcification then histological processing. The specimens are embedded in paraplast (Sigma Aldrich). Serial 5–6 μm cross, longitudinal and frontal sections from the oropharyngeal floor was cut by a LEICA 2155rm automatic microtome. Some of these sections were routinely stained using:

1. Harris haematoxylin and eosin stain for general histological studies (Harris, 1900).
2. Crossmon's triple technique (Crossmon, 1937) to study general structure.

While the other sections were subjected to various histochemical techniques described below:

1. Periodic acid-Schiff (PAS) technique for detection of neutral mucopolysaccharides (Mc Manus, 1946).
2. Alcian blue technique for demonstration of acidic mucopolysaccharides (Bancroft & Stevens, 1982).
3. Combined alcian blue-PAS technique (Mc Manus, 1946).
4. Grimelius–Pascual's modified silver stain technique for demonstration of muscle fibers (Pascual, 1976).

2.3 | Negative image analysis using CMEIAS color segmentation

CMEIAS Color Segmentation is an Improved Computing Technology to Process Color Images for Quantitative Microbial Ecology Studies at Single-Cell Resolution (Gross, Reddy, & Dazzo, 2010). Negative image analysis was performed using CMEIAS color segmentation to assess the complex color micrographs that were obtained and give more details (Abd-Elkareem, 2017; Abd-Elkareem, Abou Khalil, & Sayed, 2020).

2.4 | Semithin sections

Three specimens of 30 days old quail chicken from the preglottal part of the tongues were used for preparation of semithin sections (0.5 μm thickness) cut and stained with toluidine blue.

All stained techniques were adopted after (Bancroft & Gamble, 2002). The histological sections were examined by OLYMPUS BX51 microscope and the photos were taken by OLYMPUS DP72 camera adapted into the microscope.

2.5 | Scanning electron microscope

For the scanning electron microscope (SEM) investigations of a 60-day old quail:

Three samples from each age were used. The components of the floor of the oropharynx were washed several times in 0.1 M phosphate buffer at pH (7.2 ± 0.1). Post hatching samples rinsed with acetic acid 2%, then fixed in 4% glutaraldehyde solution for 24 hr. Post fixation was made in 1% sodium tetroxide solution for 2 hr at 4°C. After that, the fixed samples were washed in 0.1 M phosphate buffer at pH = (7.2 ± 0.1), then dehydrated in ascending grades of ethanol followed by critical point-dried in liquid carbon dioxide. All specimens were mounted on aluminum stubs covered with carbon tabs, sputtered with gold. The prepared specimens were examined and photographed using JEOL SEM (JSM-5400) at an accelerating

voltage of 15 kv in the electron microscope unit of Assiut University (Abou-Elhamd, Abd-Elkareem, & Zayed, 2018). The nomenclature used in the present study was coped with the (Nomina Anatomica Avium) as well as that was synonymized and homologized with names in previous and recent studies of the chicken and other avian species by different authors.

3 | RESULTS

The present study revealed that at 10 days prehatching the primordia of the preglottal salivary gland was observed as a thickening in the dorsal epithelium of tunica mucosa of the lingual root (epithelial placode; pre bud stage; Figure 1a). Then a solid bud-like cellular mass was formed (bud stage) (Figure 1a). By mitotic division, the epithelial bud was elongated and invading the underlining mesenchyme (submucosa) forming elongated solid epithelial cords (Cord stage) surrounded by concentric layers of mesenchymal cells (Figure 1b,c). At 11 days prehatching, the initial epithelial cords of the gland had undergone branching and cavitation (branching and cavitation stage) where numerous central small spaces appeared. These spaces coalesced to form the lumina of the gland tubules (Figures 1d,e).

At 13 days prehatching, the epithelial cords of the salivary gland had undergone more branching and formation of the secretory endpieces. The epithelial cords and the secretory endpieces were surrounded by a concentric layer of mesenchymal cells (Figure 1f). In this age, the canalization began within the epithelial cords (Figure 1f). The canalization process proceeded from the proximal part to the distal part where the secretory endpieces were located (canalization stage; Figure 2a). The lining epithelium of secretory endpieces showed a desquamation phenomenon, where it converted from stratified to monolayered or simple epithelium. The lumen contained a secretory-like substance, which represented a desquamated epithelial lining (Figure 2b,c).

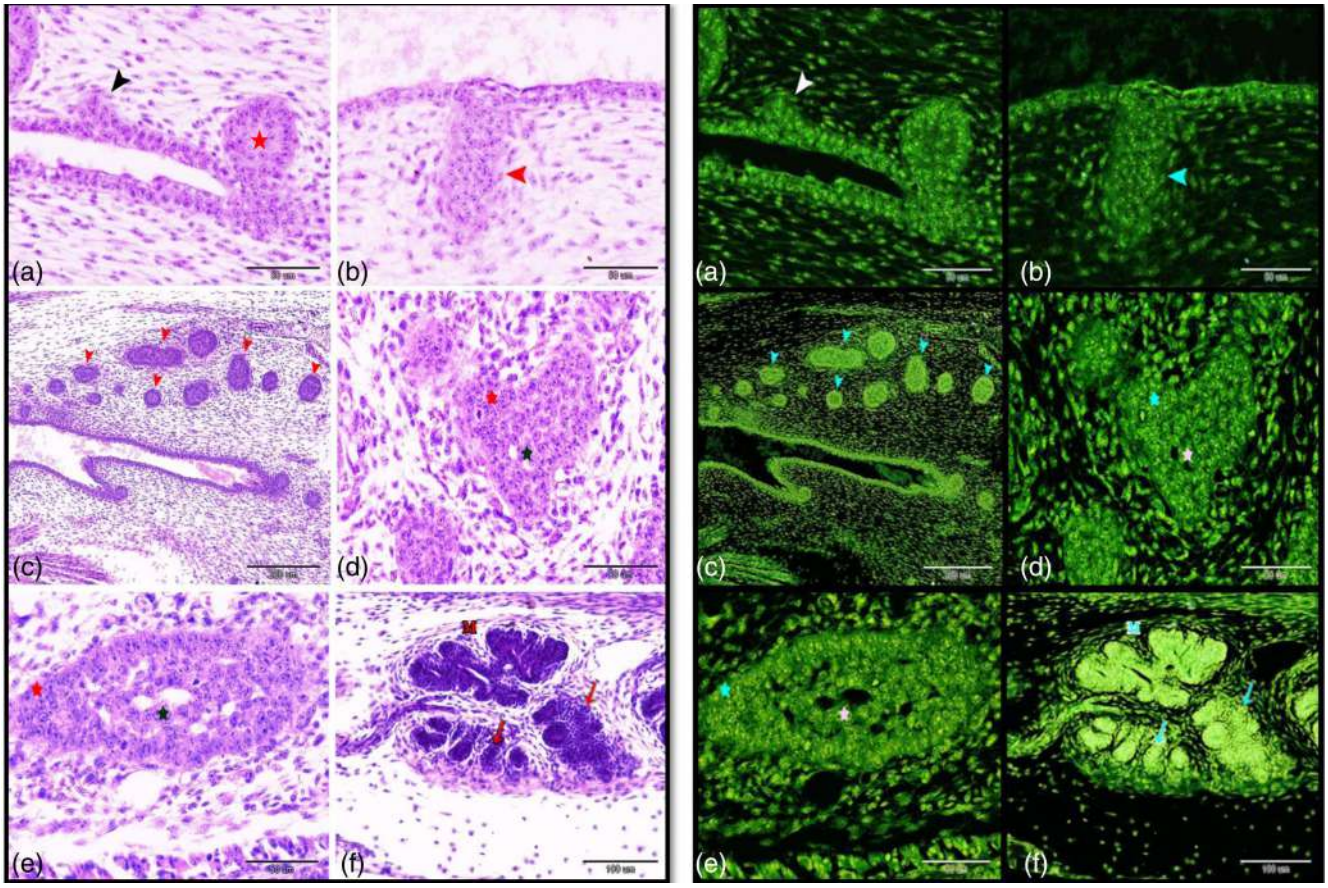

FIGURE 1 (1-left); (a–c): Photomicrographs of frontal sections of 10-day old quail embryo showing the primordia of the preglottal salivary gland. a: Showing the epithelial thickening of the lingual preglottal mucosa (epithelial placode) (black arrowhead) and epithelial bud (red star). (b): Showing the elongating epithelial cord of the gland extended in submucosa and surrounded by mesenchyme (red arrowhead). (c): Showing the elongating epithelial cords of the gland in different stages extended in submucosa (red arrow heads). (d,e): Photomicrographs of sagittal sections of an 11-day old quail embryo showing the gland undergoes branching with high mitotic divisions peripherally (red star) and central cavitation (black star). (f): Photomicrograph of sagittal section of a 13-day old quail embryo showing the branched primordial with early canalization (red arrows) surrounded by concentric layers of mesenchyme (M). Original magnification, (a,b,d, and e) 400×, scale bar = 50 μm, (c) 100X, scale bar = 200 μm, (f) 200×, scale bar = 100 μm, H & E stains. Figure (1-right): Negative images for Figure (1-left) by using CMEIAS color segmentation

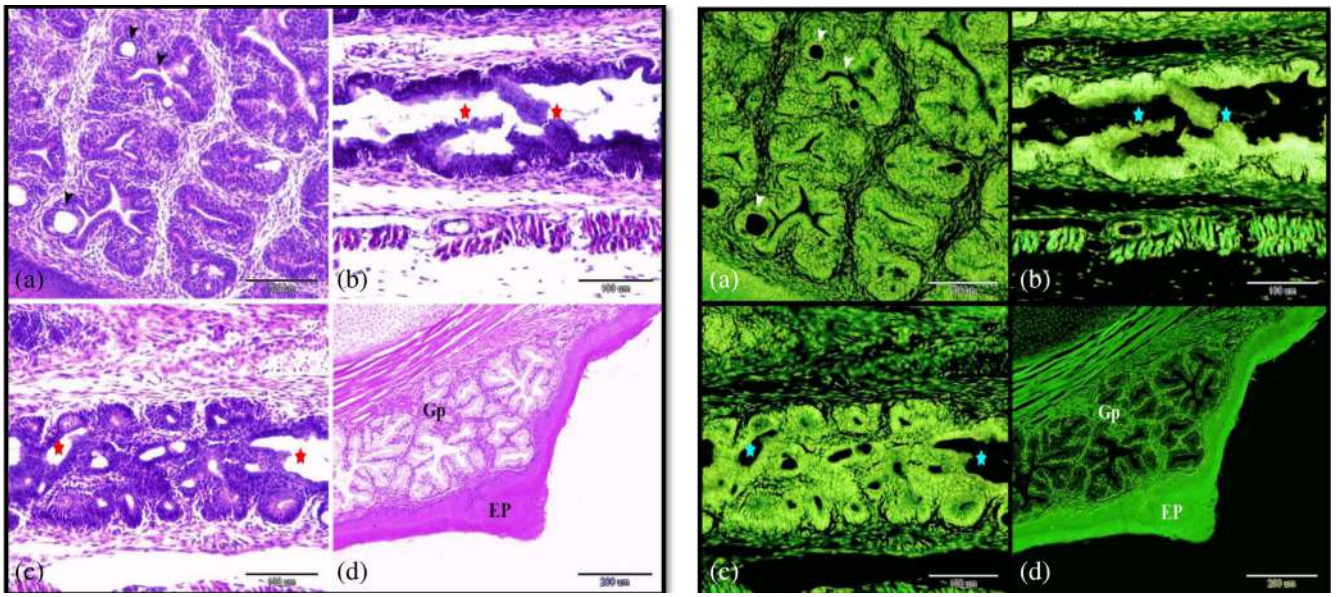

FIGURE 2 (2-left); (a–c): Photomicrograph of a frontal section in the lingual root of a 13-day old quail embryo. (a): Showing the canalization of duct system of the gland prelottalis (black arrow heads). (b): Showing the lumen of the gland prelottalis contains secretory like substances representing a desquamated epithelial lining (red stars). (c): Showing the lumen of the gland prelottalis with more folding and coalescing of small cavities of secretory endopieces. (d): Photomicrograph of a sagittal section in the lingual root of a 17-day old quail chick showing the glandular epithelium of the gland preglottalis (Gp) with more branching and infoldings of the mucous secretory end pieces. Note the branched tubuloalveolar structures of the gland could be demonstrated at this age and the lamia epithelialis was formed of stratified squamous epithelium (EP). Original magnification, (a–c) 200×, scale bar = 100 μm, (d) 100×, scale bar = 200 μm, H & E stains. Figure (2-right): Negative images for Figure (2-left) by using CMEIAS color segmentation

At 17 days prehatching and hatching day (0 day), the all glandular epithelium showed more branching and infoldings in secretory endpieces. The ducts and alveoli are finally hollowed out so that the terminal cytodifferentiation stage ended. During this stage, the lining epithelial cells of the ducts and alveoli proceeded to differentiate both morphologically and functionally. The branched tubuloalveolar structures consisted of a single layer of tall prismatic cells. The nuclei were oval to rounded darkly stained bodies located at the base of the cells. The cell size, shape, and nuclear arrangement were very homogeneous (cytodifferentiation; Figure 2d).

At 14-, 30-, and 60-days old quails, the preglottal salivary gland was an unpaired gland located in the submucosa of the preglottal part of the tongue (radix linguae) extended longitudinally from the caudal process of the paraglossale (mid-length) to the apical end of the larynx. It consisted of two lateral portions, which were extended beyond the level of the caudal end of the glottis and one middle portion. The middle portion was ended at the level of the apical end of the glottis to be continued caudally as laryngeal salivary glands (Figures 3a–c and 5a). The gland had branched tubuloalveolar endpieces, whose cells were characterized by a light foamy cytoplasm and rounded nuclei. The duct of the gland opened onto the dorsal surface of the preglottal part of the tongue with one or more than one opening. (Figures 3d, 4a,b, and 5b). The secretomotor plexus and herbst corpuscles were embedded between secretory lobules (Figure 5d). The openings of salivary glands accompanied by pores of the taste buds associated salivary glands (Figures 4b and 5b,c). The preglottal salivary gland was supported by muscle cricohyoideus whose fibers could be demonstrated inserted among the secretory lobules (Figure 4c,d). The openings of the preglottal salivary gland and the taste bud width were (25.5 and 8.17 μm), respectively at 14 days posthatching age, but the opening of the salivary gland increase in the width at age 30 days old to reach 80.4 μm. At hatching day, 14, 30-, and 60-days old quail the preglottal salivary gland showed the lobulation and secretion characteristic of the gland (lobulation and secretion stage; Figure 5a–d).

By gross staining (Alizarin/Alcian-Blue stains) of a 14-day old quail chick, the preglottal salivary gland has three portions (two lateral and middle one); the middle one extended caudally to divide by glottis into gland laryngealis that extended beyond to the laryngeal papillae, while the lateral portion extended beyond the level of the caudal glottal commissure (Figure 3b,c). By SEM, the preglottal salivary gland had polygonal-shaped openings with mucous secretion (Figure 3d).

Some histochemical techniques as PAS, alcian blue, and combined PAS & alcian blue were chosen to define the histochemical reaction of the mucin of the preglottal salivary glands. Table 1 showed the histochemical properties of the preglottal salivary gland in quails. At a zero-day-old quail chick, the secretory units showed a moderate positive reaction to alcian blue stain. The reaction involved the cytoplasm of secretory cells and the secretion within the lumen (Figure 6a). The secretory units also showed a strong positive reaction to PAS stains (Figure 6b), and a positive reaction to combined AB/PAS stains (purple coloration) (Figure 6c). At 7 day old quail chick, the secretory units of the preglottal salivary glands showed a weakly positive reaction to

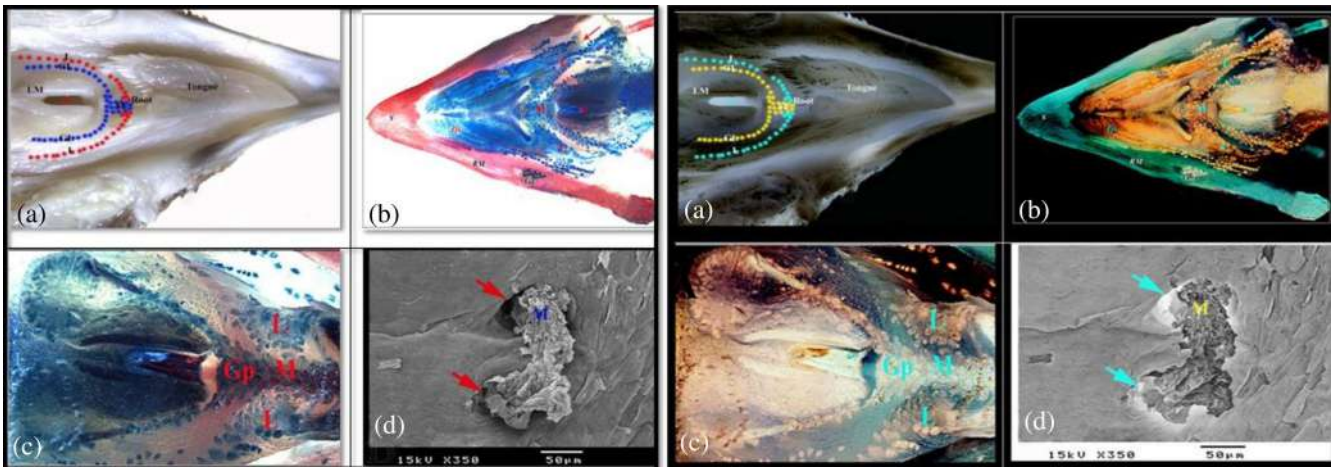

FIGURE 3 (3-left); (a): Photograph of a dorsal view of the oropharyngeal floor of 14-day old quail chick showing the preglottal part of tongue (Lingual Root). Gland preglottalis, middle portion (M) extends caudally as gland laryngealis (GL) (blue dots), lateral portion (L) red dots, laryngeal mound (LM), glottis (G), tongue. (×8). (b,c): Photographs of dorsal views of the oropharyngeal floor of a 14-day old quail chick showing distribution of salivary glands. Notice, gland mandibularis (Gm) lay within bottom of paralingual groove (red arrow), Gland angularis oris (Ga) lay at angles of the mouth, Gland praeglottalis (Gp) (middle and two lateral portions); lateral portion (L) extends till the level of the end of glottis (G), while the middle portion (M) extends caudally to divided by glottis into paired gland laryngealis (Gla), Gland sublingualis (Gs) lay within sublingual floor of the mouth (black arrow), Mandibular ramus (MR). (b,c): (×6.3 and ×10, respectively), Alizarin red & alcian blue stains. (d): Scanning electron micrograph of 60-day old quail showing two polyhedral preglottal salivary gland openings (red short arrows) with mucous secretion (M). ×350, scale bar = 50 µm. Figure (3-right): Negative images for Figure (3-left) by using CMEIAS color segmentation

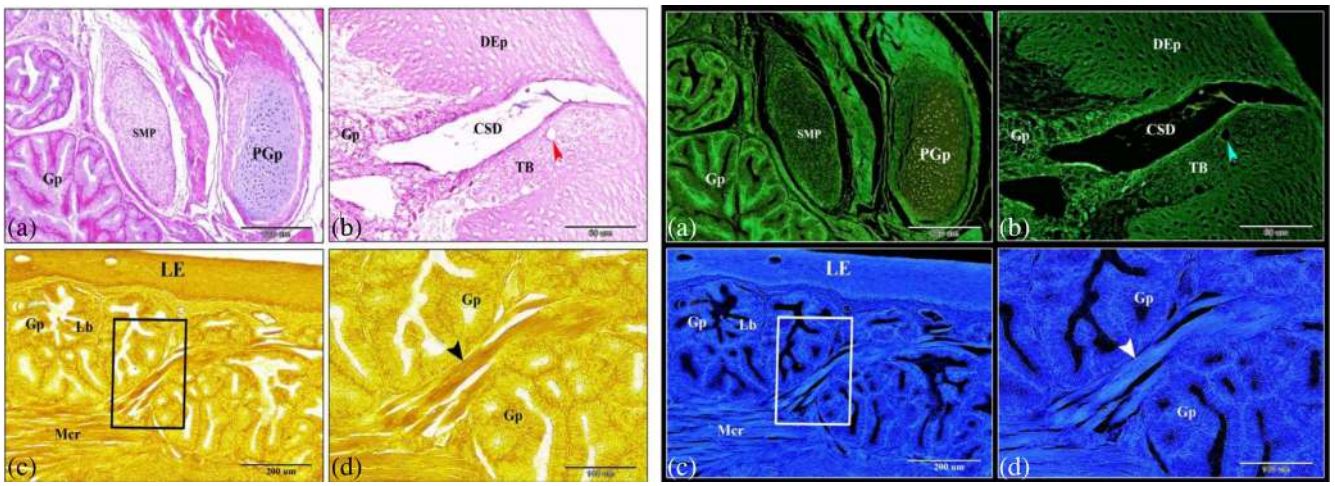

FIGURE 4 (4-left); (a,b): Photomicrograph of a sagittal section in the tongue of 14-day old quail chick. (a): Showing secretomotor plexus (SMP) is embedded between secretory lobules of gland praeglottalis (Gp). Note the processus paraglossale posterior (PGp). (b): Showing common secretory duct (CSD) of gland praeglottalis (Gp) opens into dorsal epithelium (DEp) of the lingual root adjacent to taste bud (TB) and taste pore (red arrowhead). (c,d): Photomicrographs of frontal sections in the tongue of 14-day old quail chicks showing skeletal muscle fibers inserted between secretory lobes (inset and black head arrow) of the gland praeglottalis (Gp). Muscle cricohyoideus (Mcr) and secretory lobes (Lb). Original magnification, (a)100×, scale bar =200 µm, (b) 400×, scale bar =50 µm, (c) 100×, scale bar =200 µm, (d) 200×, scale bar =100 µm (a,b) H & E stains, (c,d) Grimelius silver stain. Figure (4-right): Negative images for Figure (4-left) by using CMEIAS Color Segmentation

alcian blue, PAS, and combined AB/PAS stains (Figure 6d–f, respectively). At 14-day old quail chick, the secretory units of the glands showed a moderate positive reaction to both AB and combined AB/PAS stains, but negative results to PAS stain (Figure 7a–c, respectively). At 30 day old quail, the secretory units of the glands showed a strong positive reaction to AB stain with high alcianophilic basal portion of cells (Figure 7d) and negative results to PAS stain (Figure 7e), However, strong positive result to combined AB/PAS stains (purple coloration; Figure 7F). At 60 day old quail, the secretory units of the glands showed a strong positive reaction to AB stain with high alcianophilic basal portion of cells (Figure 8a,b) and alcianophilic results only to combined AB/PAS stains (blue coloration only), but negative results to PAS (Figure 9). Our study revealed that myoepithelial cells could be demonstrated at 17-day old quail embryo

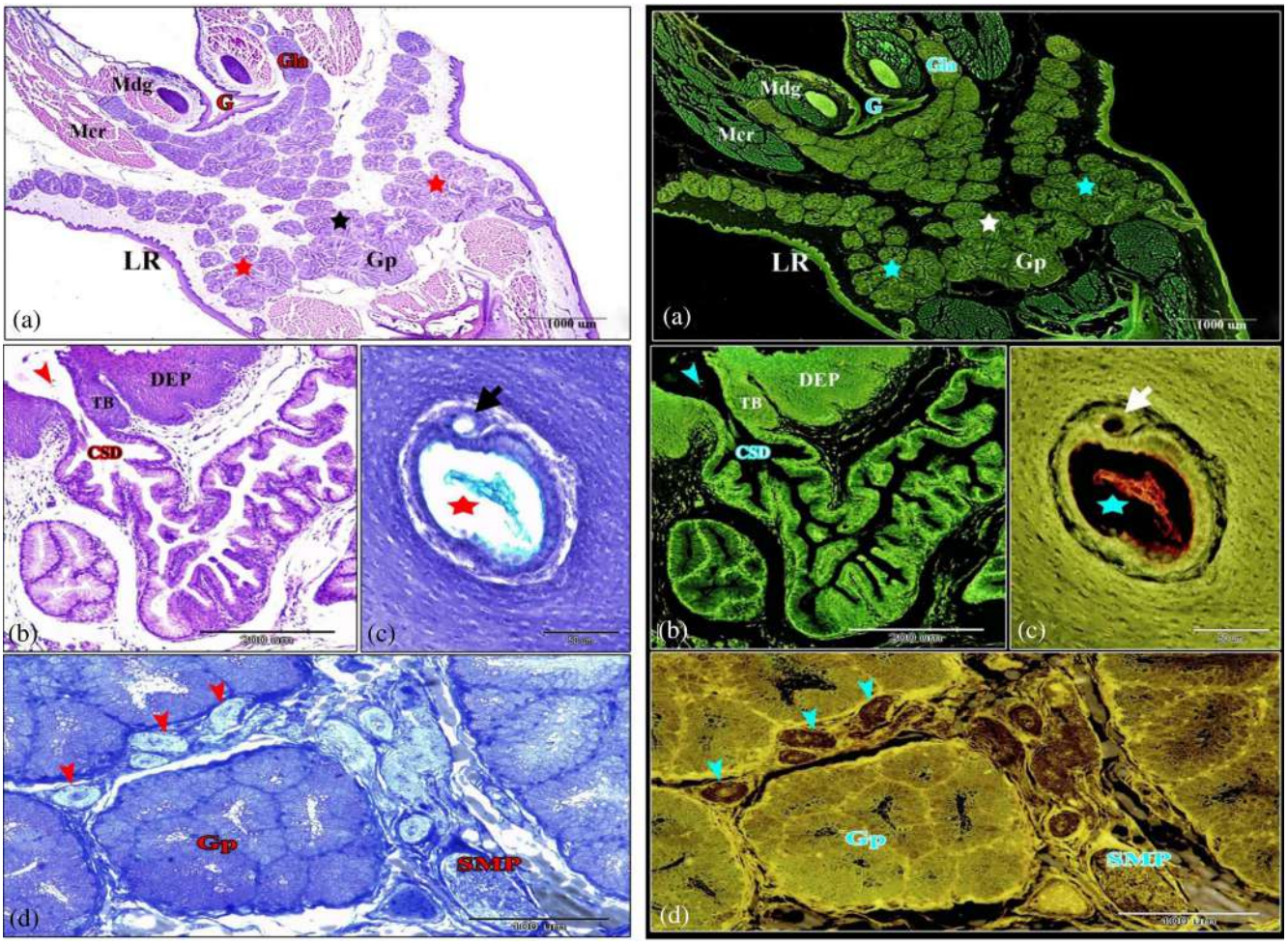

FIGURE 5 (5-left); (a): Photomicrograph of a frontal section in the tongue of 60 day old quail showing the gland praeglottalis (Gp); lateral portions (red stars) and middle portion (black star). Lingual root (LR), glottis (G), muscle cricohyoideus (Mcr), gland laryngealis (Gla) and muscle dilator glottides (Mdg). (125×, scale bar = 1,000 μm, H&E). Figure (b): Photomicrograph of a sagittal section in the tongue of a 30-day old quail showing branched tubulealveolar gland praeglottalis opens into lingual root dorsal epithelium of the tongue (DEp). Common secretory duct (CSD), taste bud (TB) and gland opening (red arrowhead). (100×, scale bar =200 μm, H&E). Figure (c): Photomicrograph of a frontal section in the tongue of a 30-day old quail showing the opening (red star) of the gland praeglottalis (with the secretion) adjacent to taste pore (short black arrow). (400×, scale bar = 50 μm, AB stain). Figure (d): Photomicrograph of a cross semithin section in the tongue of 30-day old quail showing the herbst corpuscles (red arrow heads) between secretory lobules of gland praeglottalis (Gp), secretory motor plexus (SMP). (200×, scale bar =100 μm, Toludine blue). Figure (5-right): Negative images for Figure (5-left) by using CMEIAS color segmentation

TABLE 1 Showing the histochemical properties of the preglottal salivary gland in quails

Age stains	Zero day old chick (hatching)	7 days old chick	14 days old chick	30 days old quails	60 days old quails
AB	Moderate	Weak	Moderate	Strong	Strong
PAS	Strong	Weak	−ve	−ve	−ve
AB/PAS	Positive (purple)	Weak	Moderate	Strong	Strong (alcinophilic only)

surrounded the secretory endpieces of the preglottal salivary gland (Figure 10a). They surrounded all the mucous acini at 1-month post-hatching (Figure 10b). They were star-shaped cells with a central region that contained small oval darkly- stained nuclei surrounding with a small amount of cytoplasm. These cells lie between the secretory cells and their basal lamina and had many cytoplasmic processes. The lobules of the preglottal salivary gland were present in the lamina propria and separated by loose connective tissue trabeculae. The ducts of the preglottal salivary gland open in the surface of the epithelium (Figure 11a–d). Figure 12 drawing diagram explored the different pre-and posthatching developmental stages of the preglottal salivary gland.

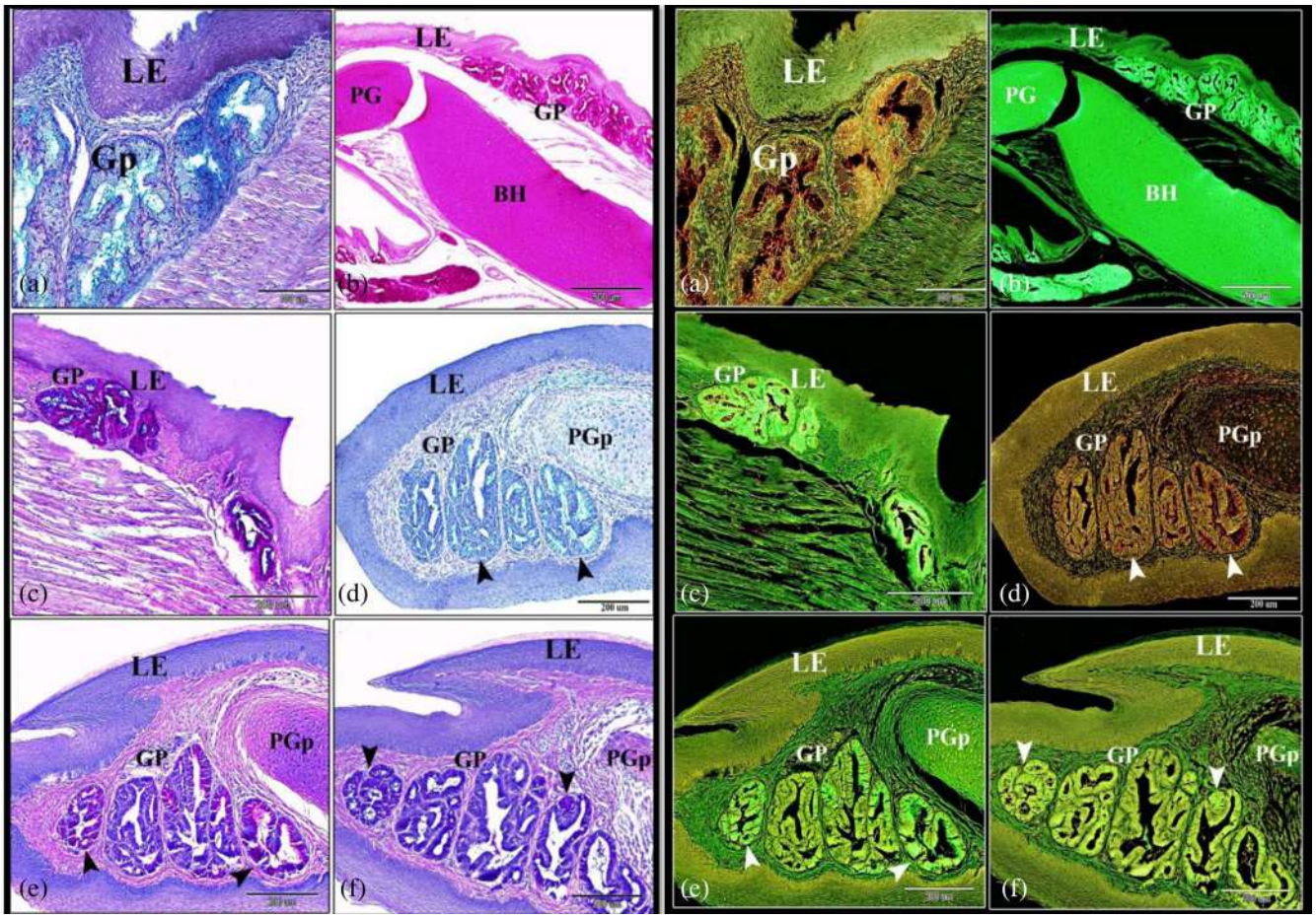

FIGURE 6 (6-left); (a–c): Photomicrograph of a sagittal section in the tongue of zero-day old quail chick. (a): Showing the moderate positive reaction of the gland praeglottalis (GP) to AB stain and this was restricted to secretion and secretory border. Note the lamia epithelialis (LE). (200×, scale bar =100 μm, AB stain). (b): Showing the strong positive reaction of the gland praeglottalis (Gp) to PAS stain. Note the basihyale (BH), paraglossale (PG) and lamia epithelialis (LE). (40×, scale bar =500 μm, PAS stain). (c): Showing the positive reaction of the gland praeglottalis (Gp) to combined AB/PAS stains (purple coloration). Note the lamia epithelialis (LE). (100×, scale bar = 200 μm, AB/PAS stains). (d–f): Photomicrographs of sagittal sections in the tongue of 7-day old quail chicks showing the gland praeglottalis (Gp) weakly positive to AB, PAS and combined AB/PAS stains, respectively (black arrow heads). Note the processus paraglossale posterior (PGp) and lamia epithelialis (LE). (d–f; 100×, scale bars = 200 μm, AB, PAS, & AB/PAS stains, respectively). Figure (6-right): Negative images for Figure (6-left) by using CMEIAS color segmentation

4 | DISCUSSION

The anatomical nomenclature that is used in this study follows of (Homberger & Meyers, 1989). The salivary glands of the tongue in quails are classified as lingual and preglottal glands. In contrast to us, earlier authors (Bryk, Gheri, Sgambati, & Orlandini, 1992; Gargiulo et al., 1991; Hodges, 1974; Mclelland, Baumel, Breazile, & Evans, 1993; Menghi, Scocco, & Ceccarelli, 1993; Nalavade & Varute, 1977; Zaccone, 1977) have named the salivary glands of the tongue as the anterior and posterior lingual glands in several birds.

Our results revealed that the pre- and posthatching development of the preglottal salivary gland is divided into the following stages: prebud stage, bud stage, cord stage, branching and cavitation stage, canalization stage, cytodifferentiation stage, and lobulation and secretion stage. All these stages occurred prehatching except the last one occurred posthatching. Similar results were observed in human (de Mello Gomes, Nagai, Lourenço, & Coutinho-Camillo, 2019; Quirós-Terrón et al., 2019), in pig (Zhou et al., 2010), in rat (Hakami & Hand, 2018), in quail (Liman, Bayram, & Koçak, 2001), and in duck (Skieresz-Szewczyk, Cornillie, & Jackowiak, 2018). The pattern of the development of the lingual gland in birds is like that described for mammals but the terminal buds are formed at the same time as the lumen of the glands (Skieresz-Szewczyk et al., 2018). In humans, salivary gland development is a complex process divided into the following stages, prebud, initial bud, pseudoglandular, canalicular, and terminal bud, to form the final lobular structure of the organ. This process requires coordination of cell proliferation and apoptosis. Both intrinsic and extrinsic apoptosis pathways were important in the early phases of ductal cavitation and luminisation (de Mello Gomes et al., 2019).

In salivary glands, branching morphogenesis and tubulogenesis is a multistep mechanism requiring coordinated cell proliferation,

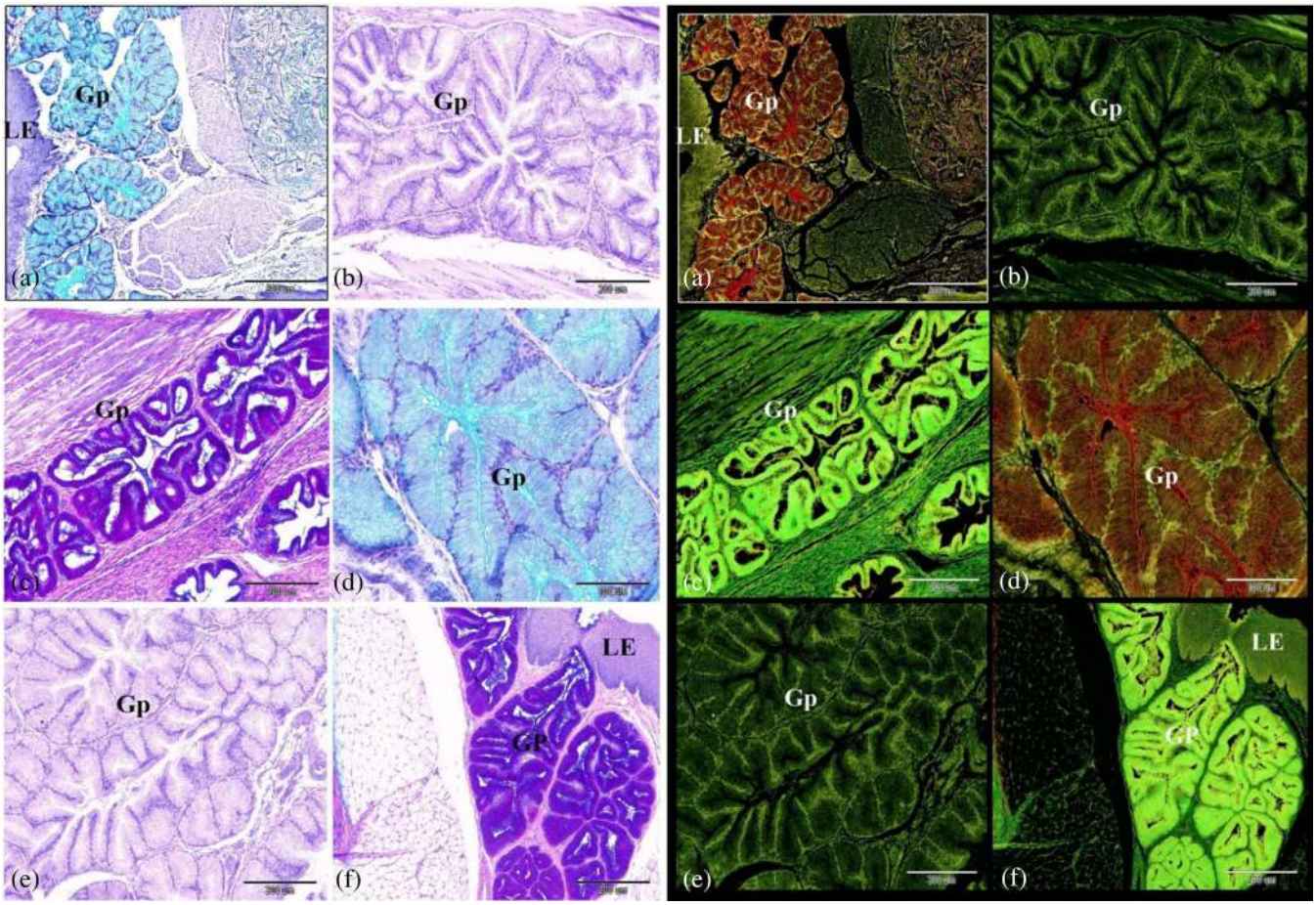

FIGURE 7 (7-left); (a–c): Photomicrograph of a frontal section in the tongue of 14-day old quail chick. (a): Showing the gland praeglottalis (Gp) moderate positive to AB stain. (40×, scale bar = 500 μm, AB stain). (b): Showing the gland praeglottalis (Gp) negative to PAS stain. Note the lamia epithelialis (LE). (100×, scale bar = 200 μm, PAS stain). (c): Showing the gland praeglottalis (Gp) moderate positive to combined AB/PAS stains (purple coloration). (100×, scale bar = 200 μm, AB/PAS stains). (d–f): Photomicrograph of frontal section in the tongue of 30 day old quail. (d): Showing the gland praeglottalis (Gp) strong positive to AB stain. (200×, scale bar = 100 μm, AB stain). (e): Showing the gland praeglottalis (Gp) negative to PAS stain. (100×, scale bar = 200 μm, PAS stain). (f): Showing the gland praeglottalis (Gp) strong positive to combined AB/PAS stains (purple coloration). Note the lamia epithelialis (LE). (100×, Scale bar, 200 μm, AB/PAS stains). Figure (7-right): Negative images for Figure (7-left) by using CMEIAS color segmentation

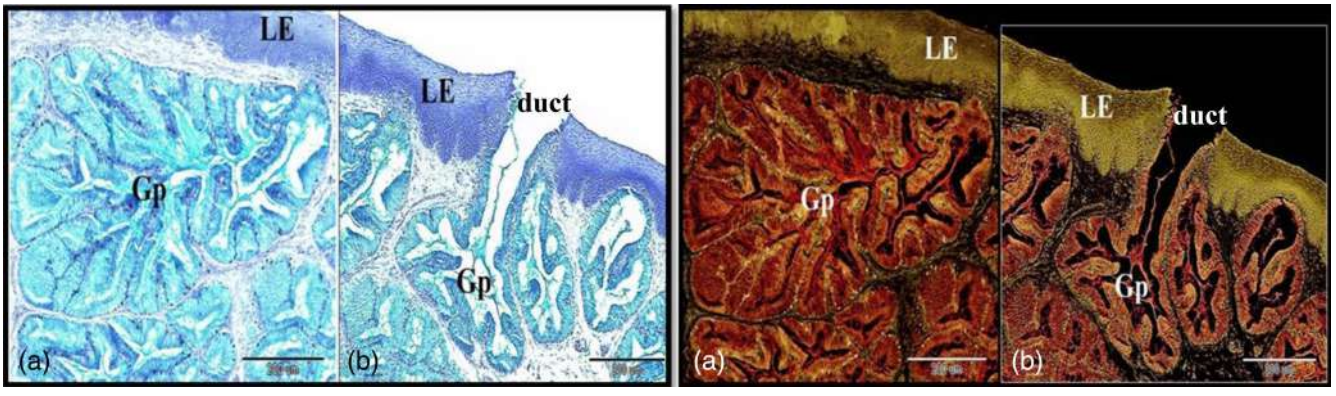

FIGURE 8 (8-left); (a,b): Photomicrograph of a frontal section in the tongue of 60-day old quail showing the gland praeglottalis (Gp)very strong positive to AB. Note the lamia epithelialis (LE) and the duct of the gland open in the surface epithelium. (100×, scale bar = 200 μm, AB stain). Figure (8-right): Negative images for Figure (8-left) by using CMEIAS color segmentation

FIGURE 9 (9-left): Photomicrograph of a frontal section in the tongue of 60-day old quail showing the gland praeglottalis (GP) strong alcinophilic to combined AB/PAS stains. Note the lamia epithelialis (LE). (40×, scale bar = 500 μm, AB/PAS stains). Figure (9-right): Negative images for Figure (9-left) by using CMEIAS color segmentation

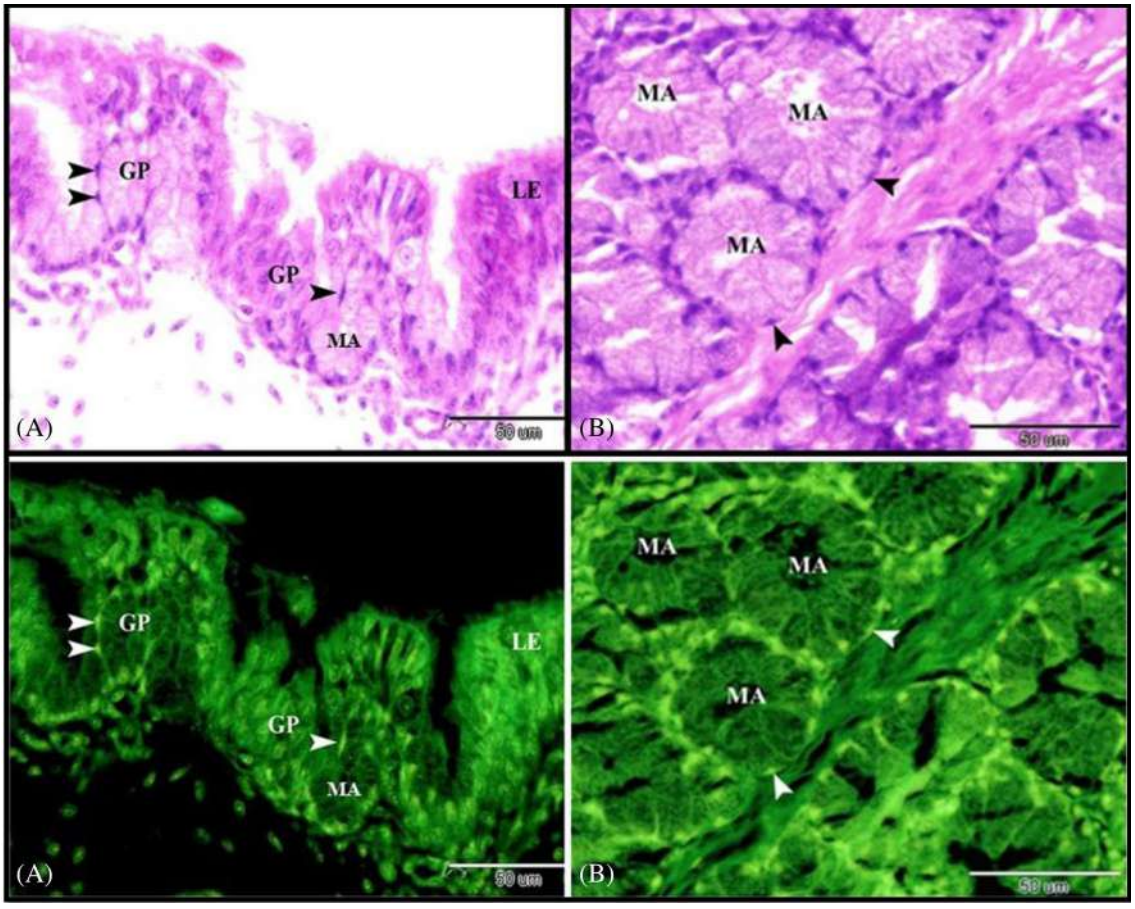

FIGURE 10 (10-upper); (a): Photomicrograph of a sagittal section in the tongue of 17-day old quail embryo. (A): Showing lamia epithelialis (LE) and myoepithelial cells (arrowhead) surrounded the secretory endpieces of the gland praeglottalis (GP). (400×, scale bars = 50 μm, H&E). (b): Photomicrograph of a sagittal section in the tongue of 1-month old quail chick showing myoepithelial cells (arrowhead) surrounded the mucous acini (MA) of the gland praeglottalis (GP). (400×, scale bars = 50 μm, H&E). Figure (10-lower): Negative images for Figure (10-upper) by using CMEIAS color segmentation

clefting, differentiation, migration, apoptosis, polarization, and reorganization of epithelial cells to form a lumen, and lumen expansion. This mechanism was done by reciprocal interactions between the epithelial, mesenchymal, neuronal, and endothelial cells (Nedvetsky et al., 2014; Patel & Hoffman, 2014). At the early stages of salivary gland morphogenesis formation of the duct lumen is driven by apoptosis and is expanded by cell polarization. As the epithelial cells have an intrinsic ability to organize into polarized structures. While the later lumen expansion was achieved by the presence of electrolytic fluid within the developing ducts (Nedvetsky et al., 2014; de Paula et al., 2017). It was demonstrated that parasympathetic nerves regulate ductal tubulogenesis in the developing salivary gland through the secretion of the neurotransmitter vasoactive intestinal peptide (VIP) by the innervating ganglia. VIP activates ductal growth, lumen formation, and lumen expansion (Nedvetsky et al., 2014). Our study revealed that the lining epithelium of the developing gland showed a desquamation phenomenon, where it converted from stratified to monolayered or simple epithelium and this phenomenon may play role in cavitation and luminisation of the gland.

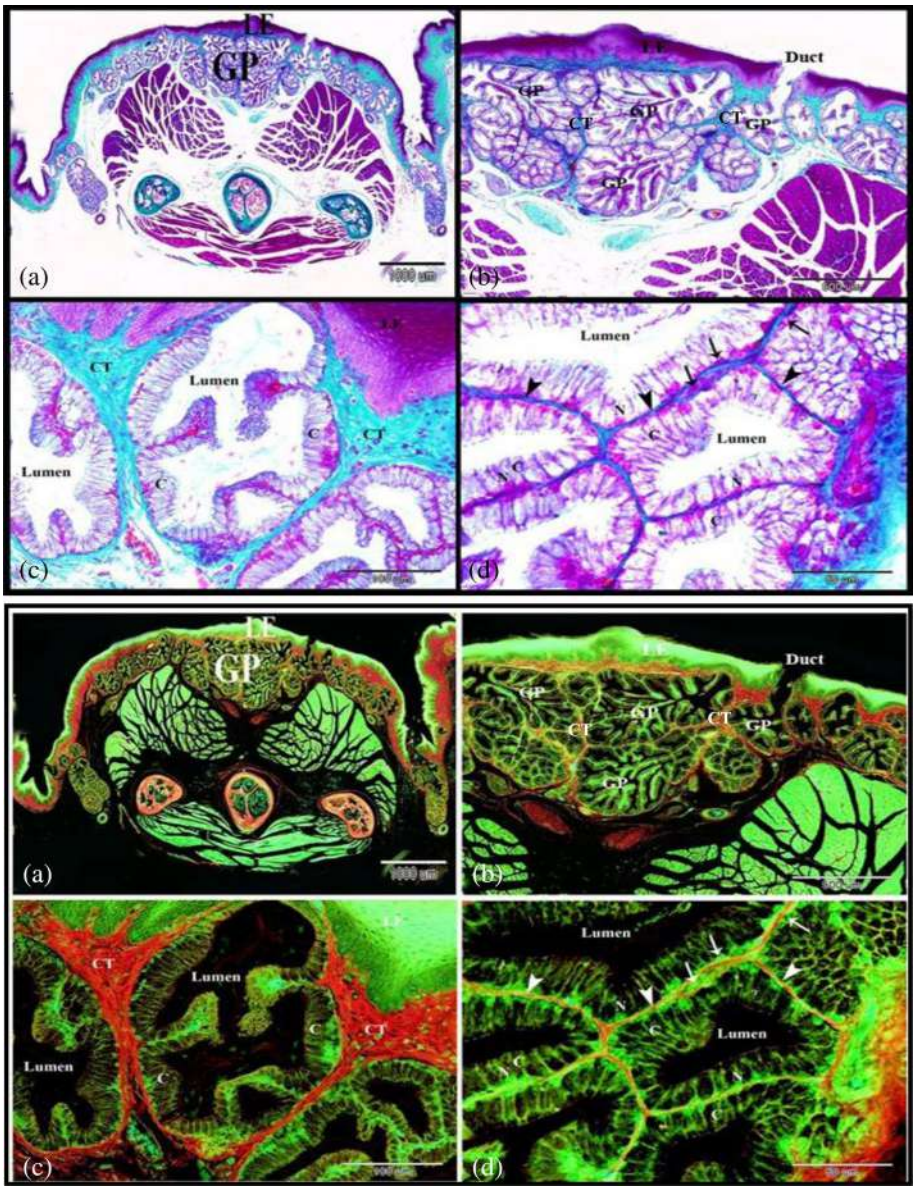

FIGURE 11 (11-upper); (a–d): Photomicrograph of a sagittal section in the tongue of 60-day old quail. (a): Showing the gland praeglottalis (GP) and lamia epithelialis (LE). (125×, scale bars = 1,000 μm, Crossmon's trichrome stain). (b): Showing the lobules of the gland praeglottalis (GP) present in the lamina propria and separated by loose connective tissue trabeculae (CT). Note the duct open in the surface of the epithelium. (40×, scale bars = 500 μm, Crossmon's trichrome stain). (c): Showing the secretory endpieces of the gland praeglottalis had a wide lumen and lined by columnar epithelial cells (c). Note the loose connective tissue trabeculae and lamia epithelialis (LE). (200×, scale bars = 100 μm, Crossmon's trichrome stain). (d): Showing the secretory endpieces of the gland praeglottalis had a wide lumen and lined by columnar epithelial cells (C) with rounded basal nuclei (N). Note the star-shaped myoepithelial cells with a central region, contained small oval darkly-stained nuclei (arrow), surrounding with small amount cytoplasm. These cells lie between the secretory cells and their basal lamina and had many cytoplasmic processes (arrowhead). (400×, scale bars = 50 μm, Crossmon's trichrome stain).
Figure (11-lower): Negative images for Figure (11-upper) by using CMEIAS color segmentation

The preglottal salivary gland is a branched tubuloalveolar unpaired gland within the submucosa of the tongue. It has consisted of two lateral portions and one middle portion. The middle portion of this gland extends from the row of lingual papillae to the front of the glottis. The middle portion of the preglottal gland is divided into two portions by the laryngeal cleft.

These portions extend to the row of the laryngeal papillae on each side of the glottis. The lateral portions of the preglottal gland have been found within the submucosae of the preglottal part of the tongue extends from the mid-length of the caudal process of the paraglossale toward the level of the caudal glottal commissure. In contrast to us (Gargiulo et al., 1991; Hodges, 1974; Menghi et al., 1993; Nalavade & Varute, 1977; Zaccone, 1977) do not define the preglottal or posterior lingual gland but they have only mentioned that the preglottal gland was unpaired as we did, also (Homberger & Meyers, 1989) do not agree with us. However (Liman et al., 2001) agree with us.

Again, in contrast to us, (Homberger & Meyers, 1989) do not declare the continuation of the preglottal gland as the laryngeal gland. Also, Homberger and Meyers (1989) have stated that the laryngeal glands are paired and they consisted of a main globular body and an elongated cranial lobe; the main body extended longitudinally from the level of the caudal end of the glottis to slightly caudal of the row of the laryngeal papilla. While the elongated cranial lobe extends to the anterior end of the glottis in contrast to us, that cranial lobe is represented to be the lateral preglottal part continuation and their orifices opened into the medial part of the paralingual groove. Whereas Liman et al. (2001), is agreed with us. But all previous authors have not focused on the lateral portion of the preglottal salivary glands extension as we did.

The present study has revealed that two elliptical openings were observed on the dorsal lingual epithelium especially the preglottal part with the size of (25.5 and 8.17 μm) corresponding to the openings of the preglottal salivary gland and the taste bud width, respectively at

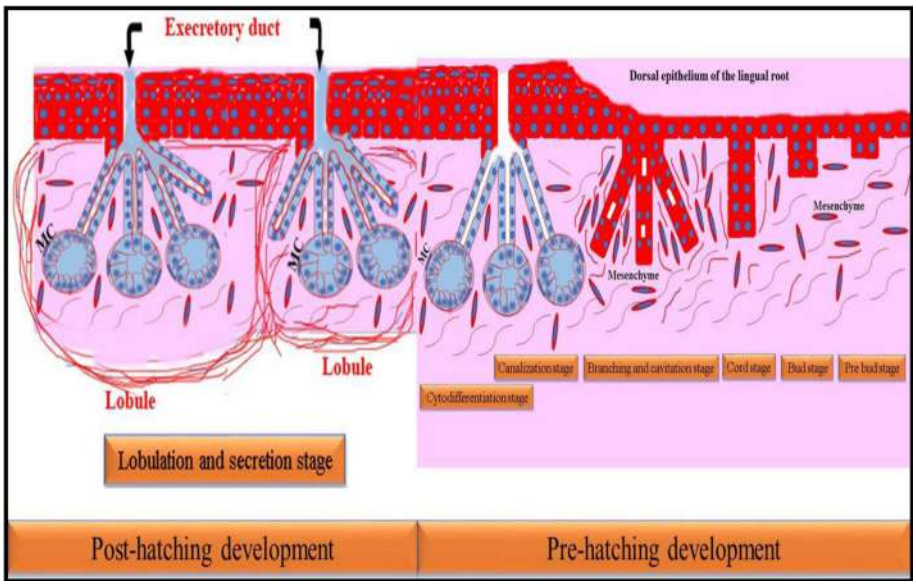

FIGURE 12 Drawing diagram summarized the different pre- and posthatching developmental stages of the preglottal salivary gland. Note the myoepithelial cells (MC)

14 days posthatching age. But, the opening of the salivary gland increases in the width at age 30 days old to reach 80.4 μm. While taste pore's width is still constant that has been explained in the previous reports that have been described the size of taste pores as 5–10 μm (Gentle, 1971) or 6 μm (Saito, 1965) in width. Thus, these two openings are thought to correspond to the openings to the salivary glands and taste pores, respectively. Also, it is corresponding to (Kudo, Nishimura, & Tabata, 2008) who have assumed that the taste pore is 3–7 μm in width and is further observed in the apical surface of the taste bud. Moreover that (Li & Sugita, 2013) have found that the width of the opening of the salivary gland and taste bud in the large-billed Crow is more than 50 μm and less than 10 μm, respectively at the caudal lingual epithelium only. The present investigation indicates that the connective tissue capsule surrounding the entire gland as septa which envelopes each lobe, lobule, and acinus. (Calhoun, 1954) has reported the presence of muscle fibers in these septa of adult glands.

This in agreement with our present studies indicated that some muscle fibers inserted between lobular capsules of the preglottal gland and lymphoid tissues are present between septa also in older ages. However, (Mccallion & Aitken, 1953) do not find any muscle fibers or lymphoid tissues within the capsule.

Our results indicated that the gland was strong positive to PAS stain, while it was weak positive to AB stain at 0 day hatching old then start to be negative to PAS stain and strong to AB stain in 30- and 60-days posthatching old. The present results showed that the quails have well-developed lingual salivary glands even though it feeds on moist food. This finding agrees with the results of (Samar et al., 1995) who have described highly developed salivary glands in penguins. Nevertheless, our results do not agree with the report of (Ziswiler & Farner, 1972) who found that the salivary glands of fish-eating birds are poorly developed. It was emphasized that all the salivary glands in the adult bird are of purely mucous type (Calhoun, 1954; Grossman, 1927; Schauder, 1923). It was reported that posterior salivary glands of the Common Myna are more strongly positive for neutral mucopolysaccharides than the anterior one whereas, both the glands are strongly positive for acid mucopolysaccharides (Kadhim, Hameed, & Abass, 2013). Similar results are reported in the red jungle fowl (Kadhim, Zuki, Babjee, Noordin, & Zamri-Saad, 2011) whereas, the lingual salivary glands of the little egret are considered free of neutral mucosubstance (Al-Mansour & Jarrar, 2004).

The nature of the neutral mucin of the lingual salivary glands may act as a lubricant for the food to facilitate swallowing. Besides, the mucin may also preserve hydration by providing a hydrophilic environment. In quail of all ages, the histochemical reactions showed that the cytoplasm of the secretory cells of the preglottal gland contained sialomucins, which were slightly variable according to the ages (Liman et al., 2001). Mucins have important roles in the maturation and maintenance of the ductal network in developing and mature human salivary glands. It also has many biological functions, such as protection of mucosal surfaces from adverse environmental influences, facilitation of glandular secretion, promotion and modulation of cell adhesion, and regulation of signaling (Teshima et al., 2011). In birds, saliva is produced by the minor salivary glands, which are mostly concentrated in the tongue.(Gabrielli & Tomassoni, 2014). In quails, the dorsum of the tongue coated with mucous fluid seems to be suitable for intaking various foods (Pourlis, 2014).

The present study revealed that myoepithelial cells could be demonstrated surrounded the secretory endpieces of the preglottal salivary gland. These cells lie between the secretory cells and their basal lamina (del Cacho, Gallego, Felices, & Bascuas, 1991). These cells are star-shaped with a central region contain small oval darkly-stained nuclei, surrounding with a small amount of cytoplasm, containing cell organoid around the nuclei and contractile filaments. The cells have many long cytoplasmic processes, which connect by desmosomes. The cytoplasmic processes surround the secretory cells as an octopus to form a basket-like network around the secretory units, hence the name basket cells (del Cacho et al., 1991; Redman, 1994; Tandler, 1965).

They are associated with adenomeres of the most exocrine glands. Their origin is ectodermal (epithelial) but their cytoplasm is rich in contractile elements (actin and myosin filaments), like those of smooth muscle fibers. By contraction, these cells force the secretory substance into duct system (Hand, 2004; Shah, Mulla, & Mayank, 2016; Shear, 1966).

5 | CONCLUSION

In summary, we suggested that such an arrangement and histochemical structure of the preglottal salivary gland in the quail is important for various food intakes and responsible for the reduction of friction and formation of food bites.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Mahmoud Osman Khalifa, Mahmoud Abd-El Kareem, Wafaa Gaber, and Abdelmohaimen M. Saleh designed the research; Mahmoud Osman Khalifa and Mahmoud Abd-El Kareem acquired the data. Mahmoud Osman Khalifa, Mahmoud Abd-El Kareem, Wafaa Gaber, Li, and Abdelmohaimen M. Saleh analyzed the data and wrote the manuscript. All authors discussed the results and commented on the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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