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Chick embryo tongue development: A histological study

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Abstract

The purpose of this study was to explore the developing histological structure of the chick embryo tongue at 10,15 and 19 embryonic days (ED), to understand tongue tissue differentiation and histogenesis during development. Fertilized chicken eggs were incubated under identical standard conditions. The tongue was extracted on the specified embryonic days. The tongue sample gross morphology was examined, then samples were fixed, and tissue sections were made using hematoxylin and eosin staining. The results of this study showed that: the tongue of ED10 contained the beginning of the lingual tissue's differentiation (Muscle tissue, cartilage tissue and epithelial tissue). The conical papillae appeared as a bud in ED10. The tongue tissues of ED15 and ED19 were more differentiated compared to ED10. The lingual gland appeared in ED10 and progressed in ED15 and ED19, and the length of the conical papillae increased with age. Mesenchymal cells appeared in ED10, these cells seemed to decrease in number and become differentiated at the proximal part of the tongue. Conclusion: The differentiation of the chick embryo tongue took place from the root to the apex. The tongue of chicken embryos seemed characterized by very different tissues. The tongue elongation rate was at its highest level between 15 and 19 days of incubation.

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Introduction

The tongue is a muscular organ located on the floor of the mouth of most vertebrates. It has a coating of sensors on the dorsal surface for tasting, temperature, pain, and tactile sensations. Additionally, the tongue is necessary for mixing, controlling, and propelling food boluses toward the oropharynx (Sturkie, 2012). The chicken tongue is quite fit with the oral cavity and possesses a characteristic beak-like structure. The tongue consists of three parts: apex, body, and root, the body and the root are separated from each other by a row of giant conical papillae (Ertaş and Erdoğan, 2019).

Chick embryos develop their lingual primordia around day 4 of incubation and continue developing until hatching (Lillie *et al.*, 1952). As the tongue elongates, conical papillae gradually develop between the body and the root of the tongue, towards the tongue root. In a scanning electron microscopic study, the conical papillae appeared on the 8th day of incubation (Bryk *et al.*, 1992).

The chicken embryo is a good model organism for scientific experiments in developmental biology, as it is available all year around. It has a cheap price and rapid development as it needs only 21 days for hatching. All stages of the development of the embryo can be dealt with. It has a relatively large size. It also seems to have considerable similarity to the human embryo at the molecular, cellular and anatomical levels (Wolpert *et al.*, 2002; Vergara and Canto-soler, 2012; Ribatti and Annese, 2023).

Our objective in this study is to understand the spatial and temporal histology structure of the chicken tongue during different developmental stages as this was not covered in the literature on the level of the chicken tongue tissue differentiation.

Materials and methods

All experimental procedures were approved by the Biology Department at King Abdulaziz University. Fertilized chicken eggs were obtained from a private farm in Al-Khamra, Jeddah/ Saudi Arabia. Egg Incubators were purchased from Al-Hakeem Foundation, model number WQ –(56 egg incubators) incubation Specification, automatic temperature, automatic egg turning (every 2 hours), 220 V, power 80 watt.

Experimental design

10 Fertilized chicken eggs for each age 10, 15 and 19 were incubated under identical standard conditions: temperature 37.5 C° and humidity 80%. All the collected embryos were without any abnormalities.

Sample collection

Embryos were extracted on the following incubation days 10, 15 and 19. The egg was opened by knocking the eggshell on the wide side using scissors, then the top of the eggshell was removed. The embryo was extracted by holding it from the neck with blunt forceps inside the egg and placing it in a Petri Dish, then it was washed in warm saline solution. Embryos were decapitated, the jaw was opened, then the cheeks of the left and right sides of the embryos were cut. In the open jaw, the tongue was held with forceps. The tongue was cut from the end of the posterior part and fixed in a 10% formalin solution for histological studies**.**

Photographing

Each tongue was photographed using an iPhone12 Pro Max camera 12 Megapixel, the iPhone was attached to a holder. A ruler was put near the tongue to be used as a scale when preforming morphometrics using the photos. The camera zooming and the distance between the camera and specimens were the same for all whole tongue photos.

Histological sections were photographed by Olympus Bx51m Compound Microscope at King Fahad Medical Research Centre connected to camera DP70.

Morphology identification method

Tongue general morphological features were identified by comparing the morphological picture to several previous studies. The general structure, apex, body, root (Iwasaki and Kobayashin, 1986; Ertaş and

Erdoğan, 2019), and conical papilla (Kadhim *et al.*, 2011; Sabry, 2015).

Morphometry of the chick tongue during development

The full embryonic tongue length was measured using the free software Fiji (https://wsr.imagej.net/distros/win/ij154-win-java8.zip) (Schindelin *et al.*, 2012).

The tongue length growth rate calculation

The tongue length growth rate was calculated according to the following equation (Lugert *et al.*, 2016).

Tongue length growth rate = (final length-initial length)\(time interval)

Histology

Depending on the stiffness of the tissue, different times of histological process were used for each age treatment. All embryo tongues were fixed in 10% formalin solution. Embryonic tongue samples were dehydrated in successive ethanol baths for 15 minutes for 10 and 15 days, and 30 minutes for 19 days.

The samples were cleared in xylene two times for 15 minutes for 10 and 15 days and 30 minutes for 19 days. Paraffin wax was used to replace the xylene by putting the samples in melted paraffin for 30 minutes at 60°C. This was repeated 3 times. A paraffin wax embedding process was performed. Chicken tongue blocks were cut into 4-µm longitudinal vertical sections from apex to root using a microtome.

The slides were put in xylene for 10 minutes. They were then passed in a series of different concentrations of alcohols (95%, 95%, 100%, and 100%) for 2 minutes each. Slides were put in hematoxylin for 12 minutes. Then the slides were washed in water to remove the rest of the pigment, the slides were then put in eosin for 7 minutes. Then they were passed in a series of concentrations of alcohols (95%, 95%) 10 dips then (100%, 100%) 20 dips. The slides were then put in xylene for 2 minutes. After that, a drop of DPX was placed on the sections that were then covered by a slide cover. The slides were then dried on the hotplate.

Histological identification method

Tongue section general and histological features were identified by comparing the histological micrographs to several previous studies. The general structure, epithelia layer, mesenchymal cell (Skieresz-Szewczyk *et al.*, 2021; Khaleel *et al.*, 2023), cartilage (Zhu *et al.*, 2018; Skieresz-Szewczyk *et al.*, 2021; Khaleel *et al.*, 2023), muscles (Bandiera *et al.*, 2014), lingual gland, lingual papillae and connective tissue (Khaleel *et al.*, 2023; Igbokwe *et al.*, 2024).

Statistical analysis

Data was analyzed using SPSS 26. The test used with normal distribution was Anova, Student-Neuman Keul test. Significance was at $p < 0.05$.

Results

General morphology of the tongue during development

In all studied developmental stages, the chick embryo tongue was composed of three parts from outside to inside which were: the apex, body and root.

The chicken embryo tongue at ED10 was quite fit with the oral cavity. The apex was wide in the anterior part with a soft structure. The body was separated from the root by the presence of small ridge-like papillae. The root was connected with the floor of the mouth with the frenulum of the tongue while the body and the apex were free (Fig. 1A).

The chick embryo tongues at ED15 and ED19 were thin and had a sharp tip with a white pointed structure which was longer, sharper and more keratinised in ED19. The tongue body seemed thinner and longer compared to ED10. The conical papilla was clearly seen in the posterior part of the body as outward arrows emerging to the opposite direction of the tongue growth. These conical papillae in ED19 were more in number having white tips, and they formed a ridge like structure separating the body from the root. The root seemed wider than what was seen in ED10 (Fig. 1B &C).

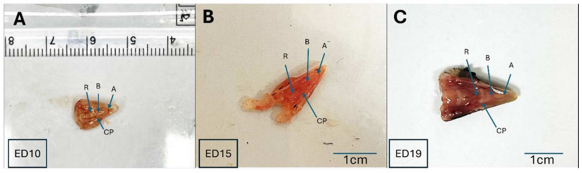

Fig. 1. Showing the chick embryo tongue morphology. (A): The tongue of ED10. (B) The tongue of ED15. (C) The tongue of ED19. A: Apex, B: Body, R: Root, CP: Conical papilla, ED: embryonic incubation day

Morphometric studies

Tongue length growth rate

The mean chick embryo tongue length growth rate was 0.43538mm\day in the period between ED10 and ED15. The growth rate slightly increased to 0.47135mm/day in the period between ED15 and ED19. However, there was no significant difference in the tongue length growth rate between the two periods.

Histological results

Examination of the histological sections obtained from the tongue of the chick embryo at 10, 15 and 19 days of incubation, showed that it consisted of three part (apex, body and root) and was formed of several types of cells, tissues, and structures; (epithelial layer, mesenchymal cells, cartilage tissue, lingual glands, vascular tissue and muscularis tissues) (Fig. 2A, 3A, 8A, 12, 13A, 13B, 14 and 15).

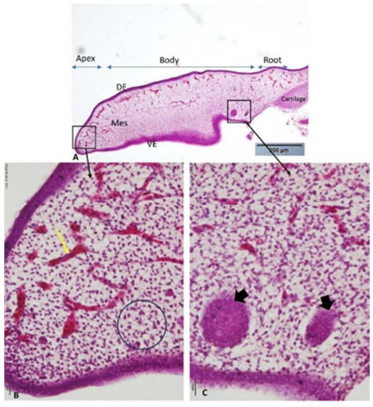

Fig. 2. Showing longitudinal vertical section of 10-day chicken embryo tongue. (A) The tongue was composed of the apex, body, and root. Inside the tongue the epithelial layer, mesenchymal cells, cartilage tissue and lingual glands were seen. (B) The apex of the tongue contained a large number of mesenchymal cells that appeared disordered in purple (black circle), and some of them have begun to differentiate into vascular tissue (yellow arrow) (C) The body of the tongue contains masses of cells that will become lingual glands (black arrow head) (H&E)

On ED10 the apex of the tongue seemed wide at the anterior end of the tongue and consisted of mesenchymal cells, these were star-like shaped cells with round nuclei. These cells were loosely arranged (Fig. 2B). The sections also showed some of the mesenchymal cells beginning to differentiate into vascular tissue (Fig. 2B). Also, the apex at this stage was surrounded with an epithelial layer from the dorsal, ventral and anterior sides. It consisted of stratified squamous epithelium covered by a very thin layer of keratinized cells (Fig. 3B). Some blood vessels not fully developed were also seen (Fig. 4A).

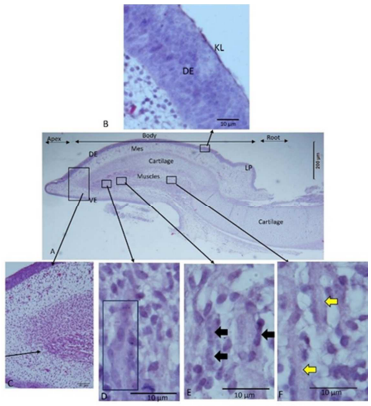

Fig. 3. Showing longitudinal vertical section of 10-day chicken embryo tongue. (A) The tongue is composed of the apex, body, and root. Inside the tongue the epithelial layer, mesenchymal cells, cartilage and muscles tissue were seen. Note the dorsal lingual papilla is seen in this section, also cartilage and muscle differentiation stages are noticed in this section. (B) High magnification of dorsal epithelial layer with the keratinized layer. (C-F) stages of muscles differentiation. (C) The first stage of muscle formation (cell multiplication (black arrow)). (D) Cell alignment. (E) Cell fusion (black arrows). (F) Myotube maturation (yellow arrows). Dorsal Epithelium (DE). Ventral Epithelium (VE). Mesenchymal cells (Mes). Lingual Papilla (LP). keratinized layer (KL) (H&E)

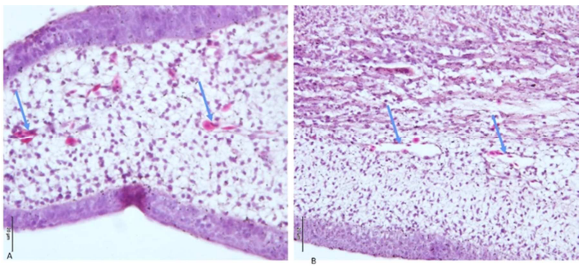

Fig. 4. Showing longitudinal vertical section of 10-day chicken embryo tongue. (blue arrow in A and B): The difference between vascular tissue differentiation in the apex of the tongue (A) where blood vessels not fully developed are seen (blue arrow), and the body of the tongue (B) where fully developed blood vessels are seen (blue arrow). The body seemed more differentiated than the apex (H&E)

The apex of the tongue in ED15 seemed thin at the anterior end of the tongue and was also surrounded by an epithelial layer that consisted of stratified squamous epithelium covered by a very thin layer of keratinized cells. This epithelial layer seemed different from the one seen in ED10 as it had spherical structures containing mesenchymal cells. At the ventral epithelial edge of the apex, there were four layers of large spherical and spindle-shaped cells (Fig. 6B).

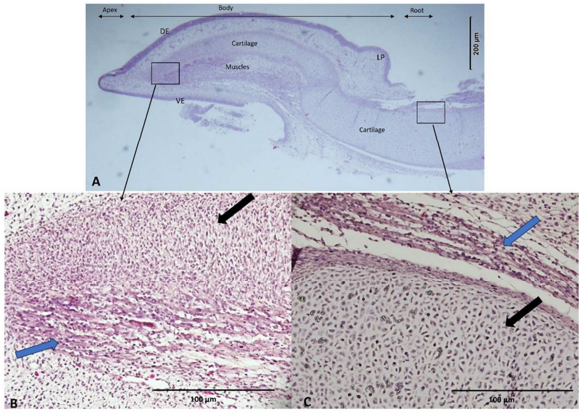

Fig. 5. Showing longitudinal vertical section of 10-day chicken embryo tongue. (A) The tongue is composed of the apex, body, and root. Inside the tongue the epithelial layer, mesenchymal cells, cartilage and muscles tissue. (B and C) shows the difference between muscle (blue arrow) and cartilage (black arrow) differentiation in the anterior part (B) and the posterior part of the tongue (C). The posterior part seemed more differentiated than the anterior part. (H&E)

On ED19 the apex of the tongue seemed very thin at the anterior end compared to ED15. It was surrounded by a stratified squamous epithelium layer covered by a thick keratinized layer. The Dorsal epithelium seemed to be much thicker than the ventral epithelium. The lamina propria was very narrow with connective tissue (Fig. 10).

In all studied ages the body of the tongue began after the anterior tip. In ED10 the body of the tongue had a dorsal and ventral epithelial layer composed of stratified squamous epithelium covered by a very thin keratinized layer (Fig. 3B). At the end of the body segment, ridge-like papillae appeared, representing conical papillae. These papillae separated the body from the root (Fig. 4A). The stages of muscle tissue differentiation were very visible in this part of the tongue such as: cell multiplication (Fig. 3C), cell alignment (Fig. 3D, cell fusion (Fig. 3E), and Myotube maturation (Fig. 3F). The beginning of the differentiation of chondrocytes to form cartilage was also seen as chondroprogenitors and chondroblasts were noticed. On the ventral side of the tongue lingual glands were seen (Fig. 2C). Blood vessels were not fully developed in this part (Fig. 4B).

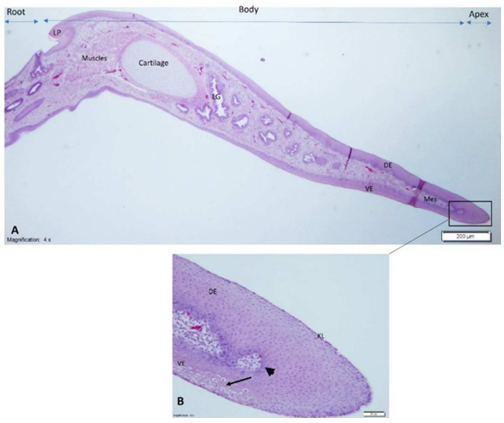

Fig. 6. Showing longitudinal vertical section of 15-day chicken embryo tongue. (A) The tongue is composed of the apex, body, and root. The histological structure of the tongue is composed of the epithelial layer, mesenchymal cells, cartilage tissue, muscles, lingual papilla and lingual glands. (B) The apex of the tongue contains stratified squamous epithelium cells. Black arrow shows four layers of large cells that are located at the end of the

ventral epithelial layer. Black arrow head shows spherical structure with its own mesenchymal core. Dorsal Epithelium (DE). Ventral Epithelium (VE). Mesenchymal cells (Mes). Lingual Papilla (LP). keratinized layer (KL). Lingual gland (LG) (H&E)

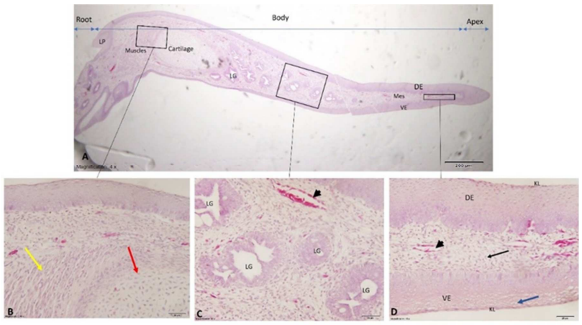

Fig. 7. Showing longitudinal vertical section of 15-day chicken embryo tongue. (A) The tongue is composed of the apex, body, and root. Inside the tongue the epithelial layer, mesenchymal cells, cartilage, muscles tissue, lingual gland and lingual papilla. (B-C) different parts of the tongue body. (B) High magnification of muscles (yellow arrow) and cartilage (red arrow). (C) lingual gland. Arrow head shows the blood vessels. (D) the lamina propria with mesenchymal cells (black arrow), large squamous cells (blue arrow) and blood vessels (arrow head). Dorsal Epithelium (DE). Ventral Epithelium (VE). Mesenchymal cells (Mes). Lingual Papilla (LP). keratinized layer (KL). Lingual gland (LG)

In ED15 the body of the tongue had a dorsal and ventral epithelial layer formed of stratified squamous epithelium covered by a very thin layer of keratinized cells. It was noticed that this layer was thick in the anterior part of the lingual body; the thickness was gradually reduced posteriorly. It was also noticed that the dorsal epithelium seemed thicker than the ventral epithelium (Fig. 7D). At the end of the body segment, finger-like papillae appeared, representing the conical papillae. These papillae define the boundary of the body from the root. Within The conical papilla, a spherical structure containing mesenchymal cells was seen. Two stages of mesenchymal spherical mass present in the conical papilla were noticed in the different sections. In the first stage it was emerging from the conical papillae, while in the second stage, it was forming a new mass in a papilla bud (Fig. 8A-B). Muscularis tissue has also not yet been fully differentiated. However, it was at the myotube maturation stage which seemed more differentiated compared to ED10 (Fig. 7B). The cartilage tissue appeared at the chondroblasts stage (Fig. 7B). Lingual glands were seen in the lamina propria in the anterior part of the lingual body (Fig. 7A and C). Blood vessels were seen fully developed (Fig. 7C-D).

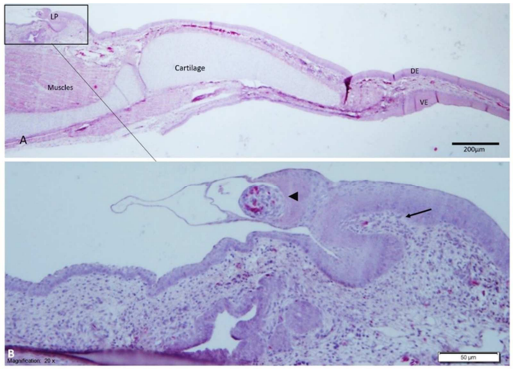

Fig. 8. Showing longitudinal vertical section of 15-day chicken embryo tongue. (A and B) shows the lingual papilla. The arrowhead shows the stage of exit of the mass of mesenchymal cells from the lingual papillae. The arrow shows the stage of formation of a spherical cell mass of mesenchymal cells in the lingual papilla. (H&E). Dorsal Epithelium (DE). Ventral Epithelium (VE). Lingual Papilla (LP)

The body lingual of ED19 was covered dorsally and ventrally with stratified squamous epithelium covered by a thick layer of keratinized cells. It was noticed that this layer was thick in the dorsal epithelium compared to the ventral epithelium of the lingual body (Fig. 11A-B). While the hind part of the body had a dorsal epithelium layer without the ventral epithelium layer (Fig. 12). At the end of the body segment, finger-like papillae appeared, representing the conical papillae. These papillae defined the boundaries of the body and the root. It was noticed that these conical papillae seemed longer compared to the ones seen in ED15 (Fig. 12). The muscularis tissue appeared not fully differentiated yet. It seemed to be at the myotube maturation stage as ED15. However, it was positioned in the middle part the body under the entoglossal cartilage near the ventral epithelia as ED15 (Fig. 11B).

The muscles in the hind part of the body were more differentiated and intense as they were seen in bundles taking many dimensions compared to ED15 (Fig. 12). Entoglossal cartilage was seen clearly. It appeared less differentiated in the front part compared to the hind part of the body (Fig. 11A-B).

Fig. 9. Showing longitudinal vertical section of 15-day chicken embryo tongue. (A) The root of the tongue is composed of dorsal epithelium, lingual gland, blood vessels, cartilage and muscles tissue. Longitudinal muscles (blue arrow). Circular muscles (black arrow). Blood vessels (arrow head). (H&E) 10X. Dorsal Epithelium (DE). Lingual Papilla (LP). Lingual gland (LG)

Fig. 10. Showing longitudinal vertical section of 19-day chicken embryo tongue. The apex of the tongue is composed of the epithelial layer, blood vessels and connective tissue. The apex of the tongue contained stratified squamous epithelium cells. The lamina propria with connective tissue (black arrow), and blood vessels (arrow head). Dorsal Epithelium (DE). Ventral Epithelium (VE). (KL) keratinized layer (H&E)

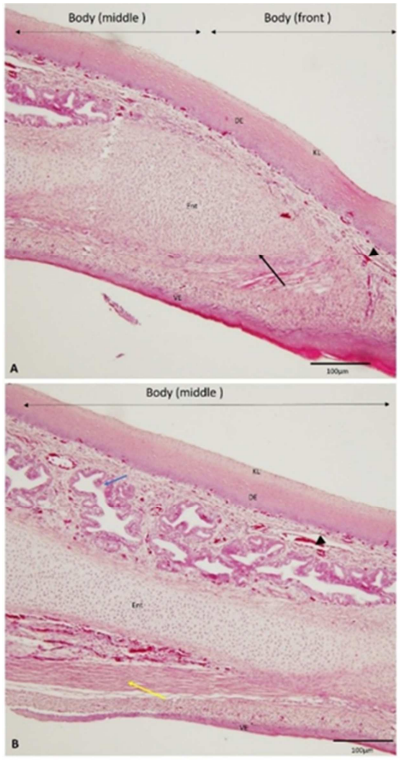

Fig. 11. Showing longitudinal vertical section of 19-day chicken embryo tongue. (A-B) The front and middle part of the body are composed of epithelial layer, blood vessels, lingual glands, cartilage and muscles tissue. (A) Front body. (B) Middle body. muscles (yellow arrow) and entoglossal cartilage (black arrow). lingual gland (blue arrow). Arrow head shows the blood vessels. Dorsal Epithelium (DE). Ventral Epithelium (VE). keratinized layer (KL). entoglossal cartilage (Ent)

However, the tongue body cartilage at ED19 seemed more differentiated compared to ED15. The lingual glands in ED19 tongue body were seen in the lamina propria in the middle part on the dorsal side of the lingual body above the entoglossal cartilage. The glands sections seemed full of secretion (Fig. 11B). Also a gland opening was seen in the dorsal epithelia of the hind part of the body (Fig. 12). The vascular tissue was seen as fully developed blood vessels in all the body parts (Fig. 11 and 12).

In all studied ages the root of the tongue began after the papillae. In ED10 a very thin dorsal epithelium was noticed. The muscles of the tongue root seemed more differentiated compared to the tongue body of the same age. All muscular tissue

was in the myotube maturation stage (Fig. 5C). Also there were clusters of cartilage tissue covered by perichondrium that separated the cartilage tissue from the muscle tissue (Fig. 5C). The cartilage tissue in the root seemed more differentiated compared to the body.

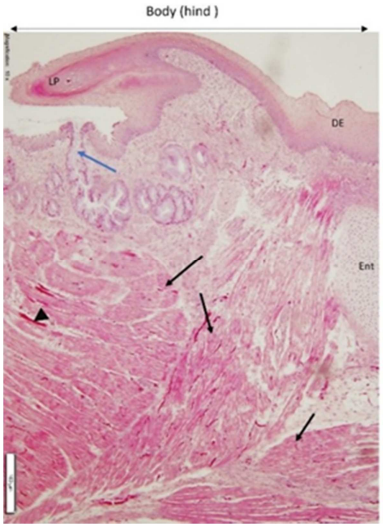

Fig. 12. Showing longitudinal vertical section of 19-day chicken embryo tongue. The hind part of the body is composed of dorsal epithelium, blood vessels, lingual gland, conical papillae, cartilage and muscles tissue. (Arrowhead) shows the blood vessels. (black arrows) shows the different shape of muscles. (Blue arrow) shows the opining lingual gland. (H&E). Dorsal Epithelium (DE). Ventral Epithelium (VE). Lingual Papilla (LP). Entoglossal cartilage (Ent

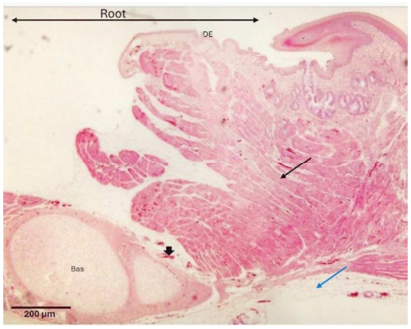

Fig. 13. Showing longitudinal vertical section of 19-day chicken embryo tongue. The root of the tongue is composed of dorsal epithelium, blood vessels, adipose tissue, cartilage and muscles tissue. Adipose tissue (blue arrow). muscles (black arrow). Blood vessels (arrow head). (H&E). Dorsal Epithelium (DE). basihyale bone (Bas).

In ED15 a very thin dorsal epithelium layer was seen. The muscular tissue of tongue root seemed more differentiated in this part of the tongue compared to the body. Two types of muscles were seen, longitudinal muscles and circular muscles (Fig. 9). The adipose connective tissue was seen under the very thin dorsal epithelium, on the ventral side of the tongue, around the lingual glands and the muscular tissue (Fig. 9). Few lingual glands were seen in the root of the tongue, and some had their openings on the dorsal surface (Fig. 9).

In the root of the ED19 an epithelial layer was seen as a thin layer of dorsal epithelium (Fig. 13). The muscular tissue seemed similar to the muscle in the body part (Fig. 12). The connective tissue as the basihyale bone, adipose tissue and some glands were seen in the root part (Fig. 13). It is interesting to mention that no adipose tissue or glands were seen in the tongue root on ED10.

Discussion

To date a limited amount of literature has been published about the complete histogenesis of embryonic tongues in avian species. Observations of spatial and temporal histological changes in chick embryonic tongue help characterize the process of histodifferentiation that has not yet been described.

The tongue primordium is composed of migrating cranial neural crest cells (Han *et al.*, 2012); these cells play an important role in tongue development.

The results of this study found that the lingual mesenchymal stem cell population decreased while lingual structure differentiation increased with embryonic development, this was seen in many studies (Augello and De Bari, 2010). It was also noticed that the differentiation direction of the chicken tongue was from the root to the apex, as it is from the proximal part of the tongue where the primordium has evolved towards the distal part of the tongue, as blood vessels, nutrients and signals help build the path to the new formed tongue tissues. This is also similar to limb development in vertebrates (Glimm *et al.*, 2020).

As shown in this light microscopic study, by day 10 of incubation, mesenchymal cells had invaded the interior of the lingual prominence, extending as far as the entoglossal cartilage. In addition, there were papillae that looked like a ridge between the body and the root of the tongue.

At ED15, the mesenchymal tissue decreased in number, and the entoglossal cartilage fell back towards the lingual root. While on the ED19 star-like cells were not seen in the tongue section. By the 19th day of incubation, the tongue apex had already become triangular, and the mesenchymal cells were already differentiated.

The mesenchymal cells seen in the ED10 and ED15 histological sections of this study might have evolved from the neural crest cells and myotomal cells that migrated to the tongue site early during tongue development. These mesenchymal cells might be the precursors of the several tissues and structures seen in the tongue later on. As tongue differentiation progresses their numbers would decrease, therefore they were seen mostly in ED10 distal sections while they were reduced in ED10 proximal sections (posterior part of lingual body and root). While in ED15 they were only noticed in the apex, and they were not seen in the ED19 sections. However many of these cells will reside in the different differentiated tissues as specified stem cells for maintenance purposes.

The avian tongue's epithelium comprises multiple layers of undifferentiated cells. At the beginning of tongue formation, the epithelial layer develops into three distinct layers. As the embryo matures, the epithelial cells create a strong cytoskeleton and a protective barrier. In the pre-hatching stage, a cornified structure forms on the tongue (Skieresz-Szewczyk *et al.*, 2018).

Based on microscopic observations of histogenesis of the epithelium layer on the tongue chick embryo, this layer was seen in all embryonic ages of this study. However, the epithelium layer in ED10 was seen as a thin layer, while in ED15 and ED19 the epithelium layer was thicker, especially on the anterior dorsal side of the tongue. Epithelial layers are formed as the epithelium matures toward the adult type.

We also visually noticed that the thickness of the dorsal epithelium increased with development this was mentioned in previous studies (Skieresz-Szewczyk *et al.*, 2021). The lingual papillae in ED15 were elongated posteriorly with a spherical structure seen in it. This was mentioned in a previous study with no explanation of this spherical structure (Skieresz-Szewczyk and Jackowiak, 2017). This structure might act to control the growth of the lingual papillae in the opposite direction of the tongue apex. However, this needs more investigation to be proved.

Cartilage development starts with the aggregation of mesenchymal cells, which then differentiate into chondrocytes (Gilbert, 2010; Humphreys *et al.*, 2022). Our study revealed some interesting observations about how tongue cartilage develops at various stages. By ED10, it was found that cartilage was in the initial differentiation stages. We noticed that the mesenchymal cells were tightly packed in the dorsal part of the tongue body.

However, the cartilage in the root part of ED10 was more differentiated than the cartilage in the body of the chick embryo tongue. This suggests that the root region is developing faster than the apex, which is fascinating in terms of how different parts of the tongue mature.

Further observations revealed that the cartilage showed even more signs of differentiation by ED15 and ED19. It demonstrates how cartilage development in the tongue is dynamic, with each area taking its path and pace during this critical time.

In birds, the lingual gland develops in a similar pattern to mammals; pre bud stage, bud stage, cord stage, branching and cavitation stage, canalization stage and cytodifferentiation stage (Jaskoll and Melnick, 2005).

In our study, we saw the bud stage of the lingual gland in the histological section of the chick embryo tongue in ED10. Similar results were observed in quails (Khalifa *et al.*, 2021). By E15 and ED19, the lingual glands showed more developed structures, including branching of the gland and ducts that were connected to the tongue surface. This connection is essential for the functional aspect of the glands, enabling the secretion of fluids necessary for feeding and digestion.

It is very interesting to see the spatial differences of the lingual gland in the studied ages, as they were located on the ventral side of the tongue body in ED10, then they were seen in the lamina propria in the anterior part of the lingual body in ED15. Lastly in ED19 they were seen in the lamina propria above the entoglossal cartilage in the middle and hind part of the lingual body. Therefore, this study describes the spatial and temporal presence and differentiation of some tissues and structures in the chick embryo tongue. To our knowledge, this was not mentioned in previous studies.

Conclusion

The tongue of chicken embryos is characterized by very different tissues. In day 10 embryos, the tongue is small in size with a wide tip and full of mesenchymal stem cells. We also noticed different stages of muscle and cartilage differentiation. While the embryos tongue of day 15 and day 19 were more differentiated, they showed a large number of lingual glands, different forms of skeletal muscles with reduced numbers of mesenchymal cells compared to 10-day embryo. More investigations are needed to explain the function of the different types of cells and structures noticed in the epithelium and papillae in this study.

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