Original Article

Developmental Series of the Foliate Papillae in New Zealand Rabbit during the Postnatal Life

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Abstract

The present study aimed to give a series on the postnatal development of the foliate gustatory lingual papillae in rabbits using scanning electron microscopy, histological, and histochemical techniques. A total of 30 New Zealand rabbits (1 day, 1 week, 1 month, 3 months, and 5 months postnatal) were used as material. Several staining methods were applied including Hematoxylin and Eosin (H&E) for general features of the papillae; Masson's trichrome and Weigert's elastic stain for demonstration of the fibrous core of the papillae as well as Periodic Acid Schiff technique (PAS) and Alcian blue for demonstration of developing Von Ebner's gland as methods for investigations. The papillary epithelium increased in stratification and keratinization from birth to the fifth month of age. The gustatory furrow increased in depth and width by desquamation of the lining epithelium and connected with excretory ducts of the lingual glands at the first month of age. Developing Ebner's glands appeared with a positive reaction of Periodic Acid Schiff and Alcian blue stains. Taste buds appeared on the first day, increased in number and size, and became mature at the first month of age. The structural adaptation and maturity of foliate papillae owing to the transformation of food ingestion from suckling to dry matter feeding.

Keywords: Developmental Series, Foliate Papillae, Postnatal Development, Rabbits, Scanning Electron Microscopy

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1. Introduction

Rabbits are widely distributed animal species that are frequently employed economically and in laboratories (Nath et al., 2016). It is widely used in education and has served as a basis for many medical experiments. Because rabbits are utilized as breeding animals and provide meat, wool, and leather, they have been the subject of several literary sources (Kulawik and Godynicki, 2006). According to (Mohammed, 2013) and (Gidenne et al., 2010) rabbits' digestive systems are accommodated to their herbivorous diet, and their tongues also show unique alterations associated with their specific feedstuffs (Iwasaki, 2002; Yoshimura et al., 2002)

The shape, position, and types of lingual papillae in many mammalian species were reported in the early research on the morphology of the tongue surface carried out by (Sonntag, 1923) Subsequently, the morphogenesis and angioarchitecture of the lingual papillae in a number of animal species, including rabbits, have been the subject of many published articles (Fujimoto et al., 1993; Kulawik and Godynicki, 2006, 2007, 2009; Abumandour and El-Bakary, 2014; Nabil and Tawfiek, 2020; Haddad et al., 2021).

Four different types of lingual papillae can be identified on the lingual surface of rabbits' tongues: According to (Nonaka et al., 2008) there are three gustatory (fungiform, foliate, and circumvallate) and one mechanical (filiform). One of the three varieties of gustatory papillae, together with fungiform and vallate papillae, are foliate papillae (Hassan et al., 2018). According to Nonaka et al. (2008), the foliate papillae are oval-shaped and situated on both sides of the posterolateral margin of the lingual prominence. According to Silva et al. (2002), these are short protrusions with a number of parallel ridges interrupted by grooves on the lateral surface and many taste pores. There are about fifteen ridges and grooves on the foliate papillae. Examination of the lingual mucosa using a light microscope reveals parallel ridges with many taste buds on the grooves' epithelium (Kobayashi, 1990; Nonaka et al., 2008). The connective tissue of the foliate papillae is parallel laminar in shape, and each projection or ridge has three laminar sheets of connective tissue that are classified into groove folds, which are close to the grooves and a septal fold (Kobayashi, 1990; Silva et al., 2002). While, the connective tissue core of each folium of the foliate papillae is made up of one central and two

lateral folds. These folds are situated in the spaces between streaks of epithelium. Moreover, individual folia of foliate papillae are separated and organized parallel to each other. The number of developing foliate papillae, which are made up of 15–21 folia, is gradually increased with the advancement of the age (Yoshimura et al., 2008; Kulawik and Godynicki, 2013).

The papillary epithelium is arranged in the form of stratified squamous epithelium less keratinized by proceeding of age. The papillary covering epithelium's stratification is increased, and its keratinization is noticeable (Kulawik and Godynicki, 2013; AL-Mahmodi, 2016; Nabil and Tawfiek, 2020).

Gustatory grooves of the foliate papillae's aren't entirely open in the first week of age and these grooves increase in width and depth by increasing of age to reach its maximum width and depth by the 2nd month of age (Toprak and Yılmaz, 2007; Yilmaz et al., 2013; AL-Mahmodi, 2016; Nabil and Tawfiek, 2020).

Taste buds in the foliate papilla are arranged in the form of a single row of two to three mature buds with distinct pores on each lateral side (Chamorro et al., 1986; Miller and Bartoshuk, 1991). These taste buds increased in number and size by proceeding of animal age (Toprak and Yılmaz, 2007; Yilmaz et al., 2013; Nabil and Tawfiek, 2020). On the contrary Mohammed (2013) recorded that the rabbit's foliate papillae are free from any taste buds.

The von Ebner's glands are appeared firstly as isolated serous acini in the connective tissue beneath the foliate papillae by development of the age, and showed up lobulated growths and gradually multiplied. In addition, their excretory ducts split off and open at the base of foliate papillary grooves (Kock et al., 1992; Toprak and Yılmaz, 2007; Yilmaz et al., 2013; Nabil and Tawfiek, 2020).

This study aimed to give a full histological and ultrastructural picture for the lingual papillae of the rabbit's tongue during postnatal life.

2. Materials and methods

2.1. Animal Selection and Grouping

All animal procedures and sampling were approved by the Institutional Animal Care and Use committee of the Faculty of Veterinary Medicine, Minia University, El-Minia 61519, Egypt (Feb.16.2022). Newly born New Zealand rabbits (*Oryctolagus cuniculus*) with a total number of 50 animals from both sexes were purchased from the animal resources unit at Faculty of Agriculture, Assiut University, Egypt. The animals were divided into five groups by their ages (1 day, 1 week, 1 month, 3 months, and 5 months old). Each group consisted of 10 animals (5 males and 5 females). The animals' age was determined based on records from the source facility.

2.2. Samples and Histological Techniques

Samples of the tongue were collected from the rabbits (n = 30) at 1 day, 1 week, 1 month, 3 months, and 5 months old; six animals (male n = 3 and female n = 3) were used for each age. After the animals were euthanized with a deep inhalation anesthetic liquid (Isoflurane), the tongue samples with lingual papillae were dissected, washed with normal saline solution (NaCl 0.9%), and sectioned into three parts (apex, body, and root) fixed immediately in neutral buffered formalin 10%. The obtained samples were dehydrated in increasing grades of ethyl alcohol (50%, 70%, 80%, 90% , and 100%), washed and cleared in several changes of xylene, impregnated in soft paraffin, and embedded in hard paraffin. Sections (4–6 μm thick) were obtained by rotatory microtome and stained with Harris Hematoxylin and Eosin (H&E) to demonstrate the general histological structure, Masson trichrome for collagen fibers and muscles, Periodic Acid Schiff reaction (PAS), Alcian blue (AB) to demonstrate neutral and acid mucins and weigert's elastic stains for demonstration of elastic fibers (Suvarna et al., 2019).

2.3. Scanning Electron Microscopy

For scanning electron microscopy (SEM), two rabbits tongue specimens for each age were used. The specimens were fixed in 3% Glutaraldehyde solution in phosphate buffer (pH 7.2–7.4), post fixed with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer at pH 7.2 for 1 hr at 4°C. Thereafter, the specimens were dehydrated through a graded series of ethanol and critical point dried (with carbon dioxide). Then, the specimens were attached to aluminum stubs facing upwards, covered with colloidal carbon tabs, and then sputtered with gold–palladium. The specimens were examined and photographed with a JEOL/ EO-JSM-6510 LV SEM at Faculty of Veterinary Medicine, Assiut University, Egypt.

3. Results

Investigations using the histological techniques and SEM of rabbit's lingual surface recording no sexual difference. Three types of gustatory lingual papillae (vallate, foliate, and fungiform) were easily distinguished on the lingual surface of the rabbit tongue. The foliate papillae are one of the gustatory lingual papillae located on both lateral sides of the tongue root.

On the first day of life, the developing foliate papillae appeared as mucosal projections of the lingual mucosa rest on the developing submucosal tissue, which was formed from fibroelastic connective tissue housing blood vessels and developing serous gland and their execratory ducts. The connective tissue was firmly fixed to the lingual mucosa on the lingual muscles (Figure 1A). The foliate papillae were separated by ill-developed gustatory furrows and covered by stratified squamous epithelium with minimal keratinization

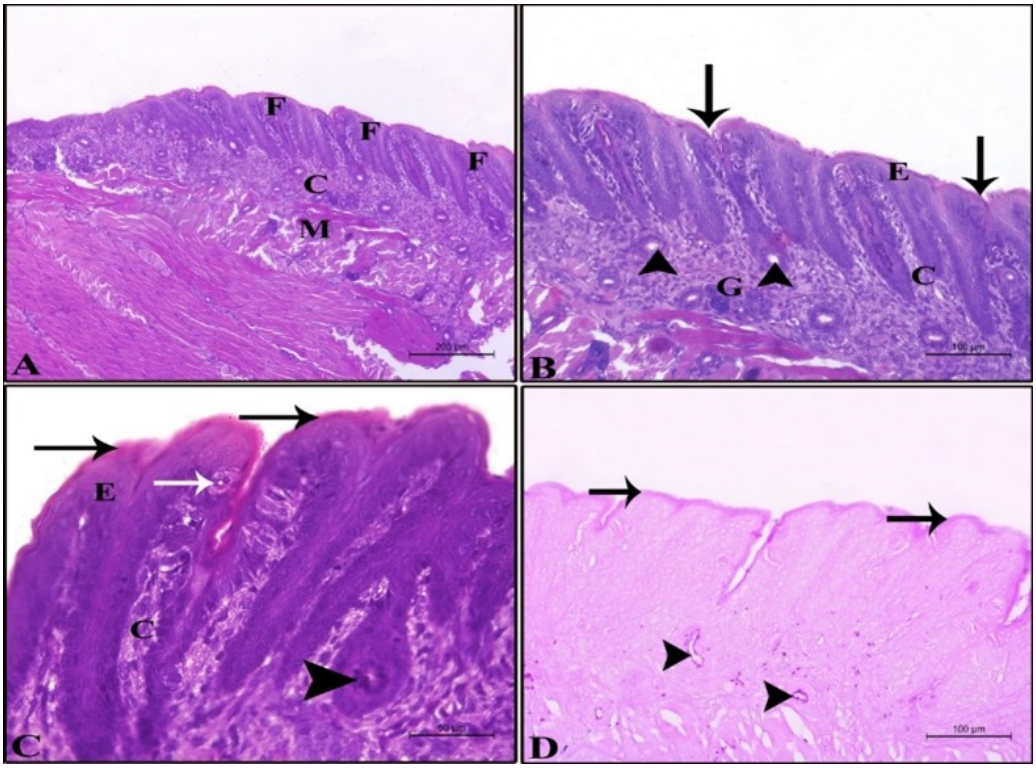

Figure 1: A photomicrograph of rabbit's foliate papillae at the first day of age showing: **A**. A cross-section of the tongue at the level of the foliate papillae with developing papillae as mucosal projections (F) resting on developing submucosal tissue (C) and lingual muscles (M). H&E stain, ×100. **B**. Developing foliate papillae are separated by an ill-developed gustatory furrow (arrow) covered by minimally keratinized stratified epithelium (E) and supported by connective tissue core (C). The connective tissue beneath the papillae housed early developing Ebner's gland (G) with many excretory ducts (arrowhead). H&E stain, ×200. **C.** Higher magnification of foliate papillae showing the epithelial covering of the papillae consisted of groove side epithelium (arrow) and septal epithelial process (E). The connective tissue core was formed by an ill-demarcated groove side fold (C) and an ill-developed septal fold. Note that a few taste buds (white arrow) were located at the lateral sides of the papillae. H&E stain ×400. **D.** Developing foliate papillae showed a strong reaction with PAS staining in the keratinized epithelium (arrow) and excretory ducts (arrowhead) of the Ebner's gland. PAS stain ×200.

supported by connective tissue core. The connective tissue beneath the papillae contained early developing Ebner's gland with many excretory ducts (Figure 1B). The Epithelial covering of the developed papillae consisted of a groove side epithelium and a septal epithelial process. The connective tissue core was formed of ill-demarcated groove side and ill-developed septal folds. Few taste buds were observed at the lateral sides of the papillae (Figure 1C). Developing foliate papillae showed a strong reaction with PAS staining in the keratin layer of the epithelial cap and excretory ducts of the Ebner's gland (Figure 1D).

On reaching the first week of rabbit's age, by using SEM, the developing foliate papillae appeared as parallel projections separated by very shallow grooves (Figure 2A), these papillae rest on the developing submucosal tissue and lingual muscles (Figure 2B) and are separated by shallow gustatory furrow covered by stratified epithelium less keratinized supported by a connective tissue core with many blood vessels. The connective tissue beneath the papillae housed developed Ebner's glands with many excretory ducts open at the bottom of the gustatory furrow (Figure 2C). The epithelial covering of the papillae consisted of groove side epithelium stratified less keratinized and septal epithelial process. The connective tissue core was formed by well well-demarcated groove side fold and ill-developed septal fold. Several taste buds were located at the lateral sides of the papillae (Figure 2D).

By proceeding of age, at the first month old by the SEM, the developing foliate papillae appeared as parallel ridges separated by deep grooves (Figure 3A). The developing papillae at this age became large and broader, separated by very deep and wide gustatory furrows covered by stratified epithelium less keratinized, housed lateral rows of taste buds and supported by connective tissue core containing many blood vessels (Figure 3B). The foliate papillae are supported by connective tissue cores formed mainly from collagen bundles intermingled with striated muscle. The collagen cores of the papillae were distributed in two parts: groove side and septal folds (Figure 3C). The foliate papillae supported by connective tissue core reached with elastic fibers in both groove side and septal folds (Figure 3D).

At the third month of rabbit age, the developing fo-

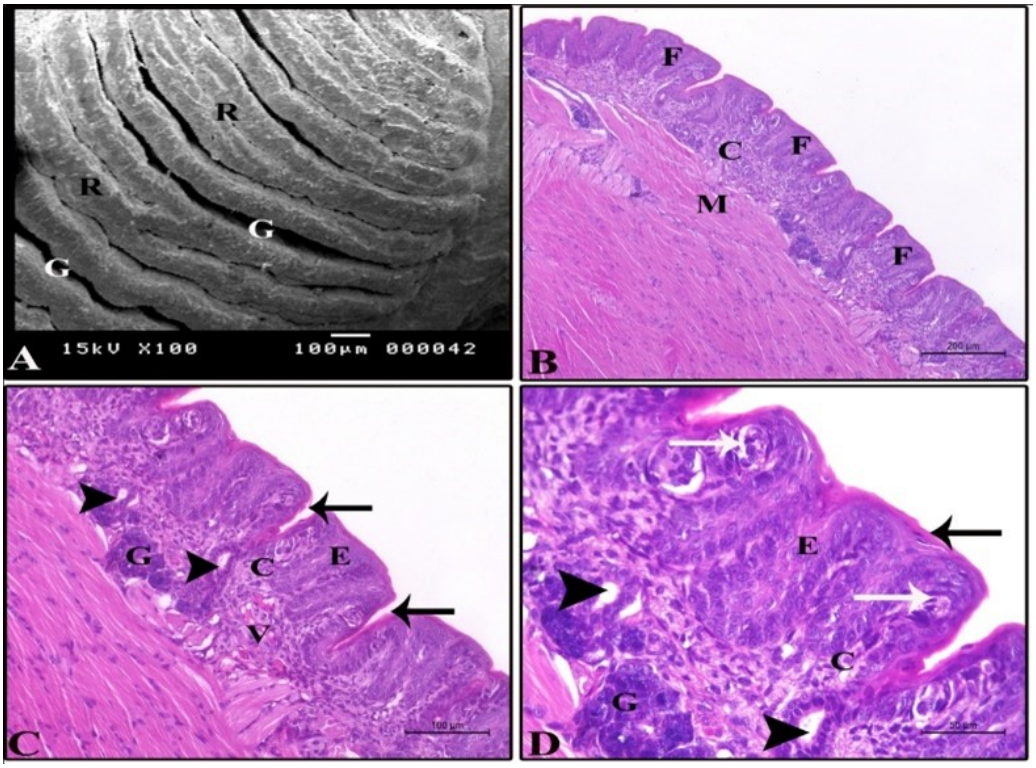

Figure 2: A photomicrograph of the rabbit's foliate papillae at the first week of age showing: **A.** SEM at birth, the developing foliate papillae appeared as parallel projections (R) separated by very shallow grooves (G)**. B.** cross-section of the tongue at the level of the foliate papillae showing developing papillae (F) resting on developing submucosal tissue (C) and lingual muscles (M). H&E stain ×100. **C.** Developing foliate papillae separated by well-developed gustatory furrow (arrow) covered by stratified epithelium less keratinized (E) supported by connective tissue core (C) with many blood vessels (V). The connective tissue beneath the papillae housed a developed Ebner's gland (G) with many excretory ducts (arrowhead) open at the bottom of the gustatory farrow. H&E stain ×200. **D.** Higher magnification of foliate papillae showing the epithelial covering of the papillae consisted of groove side epithelium stratified less keratinized (arrow) and septal epithelial process (E). The connective tissue core was formed by a demarcated groove side fold (C) and an ill-developed septal fold. Note that several taste buds (white arrow) were located at the lateral sides of the papillae. H&E stain ×400.

liate papillae by SEM appeared as parallel long ridges separated by deep and wide grooves (Figure 4A). The developing foliate papillae became large and broader, separated by very deep and wide gustatory furrows covered by stratified epithelium highly keratinized housed lateral rows of taste buds supported by connective tissue core with many execratory large ducts of secretory Ebner's glands (Figure 4B). The foliate papillae supported by connective tissue cores formed mainly from collagen bundles intermingled with striated muscle bundles. Note, the collagen core of the papillae is distributed in two parts: groove side fold and septal fold (Figure 4C). The foliate papillae with the PAS technique give a strong reaction in the keratin layer, Ebner's gland, and its excretory duct (Figure 4D).

By reaching the fifth month of age, the foliate papillae showed that by SEM appeared as parallel very long ridges separated by very deep grooves (Figure 5A). The papillae became very long, separated by very deep gustatory furrows covered by stratified epithelium less keratinized housed lateral rows of taste buds supported by connective tissue core with many blood vessels (Figure 5B). The papillae are supported by an extensive network of connective tissue core with elastic fibers in both groove side fold and septal fold (Figure 5C). The papillae with Alcian blue technique give sa trong reaction in Ebner's gland (Figure 5D).

4. Discussion

In vertebrates, the tongue's morphological components are performing various tasks, including swallowing, grooming, vocal modulation, water absorption, food capture and manipulation and sucking (Mançanares et al., 2012). The morphological characteristics of mammalian tongues vary from species to species as a result of these lingual function techniques (Iwasaki, 2002; Abumandour and El-Bakary, 2013; Nabil and Tawfiek, 2020).

Based on dietary specialties, food types, and environmental adaptations, the morphological changes and differences that manifest in the tongue occur (Iwasaki, 2002). The distribution of various papillae on its lingual surfaces is one of its defining characteristics; it may even be unique to a species (Jung et al., 2004; Sohn et al., 2011). According to Abumandour and El-Bakary (2013) and Kilinc et al. (2010), the diet

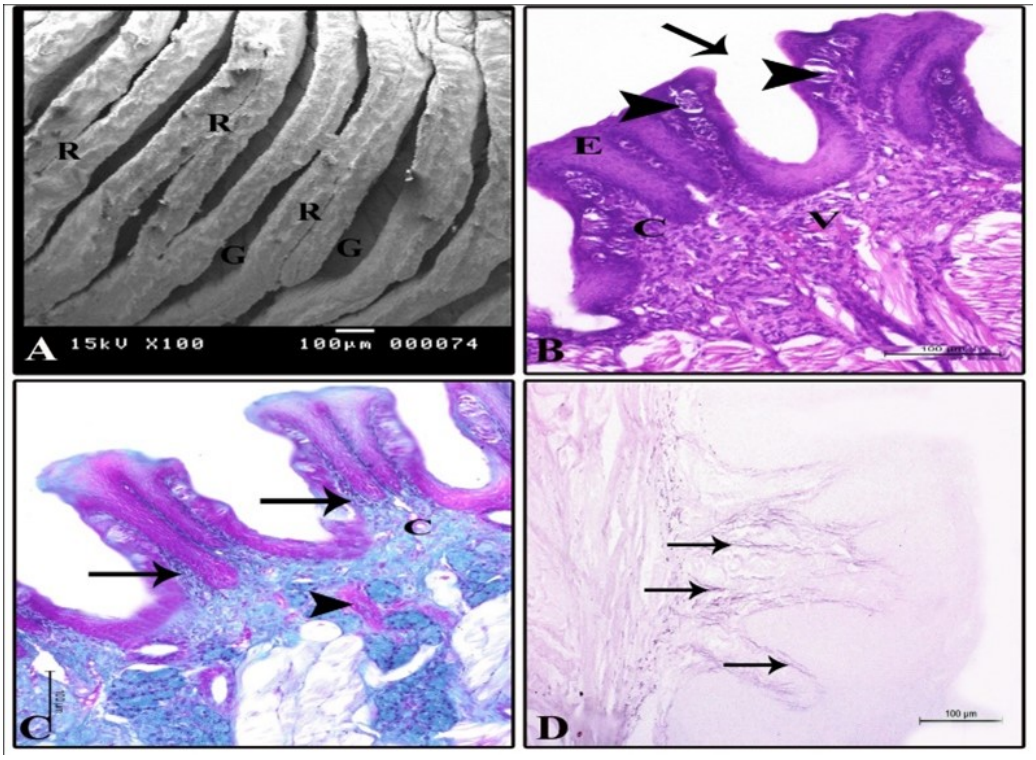

Figure 3: A photomicrograph of the rabbit's foliate papillae at the first month of age showing: A. SEM, the developing foliate papillae appeared as parallel ridges (R) separated by deep grooves (G). **B.** Developing foliate papillae became large and broader, separated by very deep and wide gustatory furrow (arrow) covered by stratified epithelium less keratinized (E), housed lateral rows of taste buds (arrowhead) and supported by connective tissue core (C) containing many blood vessels (V). H&E stain ×200. **C.** The foliate papillae supported by connective tissue cores formed mainly from collagen bundles intermingled with striated muscle (arrowhead). Note that the collagen core of the papillae is distributed in two parts: groove side fold (arrow) and septal fold (C). Masson's trichrome stain ×200. **D.** The foliate papillae are supported by connective tissue core with elastic fibers (arrow) in both groove side fold and septal fold. Wiegert's elastic stain ×200 .

is one of the factors that most influences the morphology, distribution, and type of papillae. In certain animals, particularly those with short gestational periods, such as rabbits (Elnasharty and Elsharaby, 2013; Nabil and Tawfiek, 2020), rats (ASAR et al., 1996), mice (Toprak and Yilmaz, 2016), hamsters (Whitehead and Kachele, 1994), and cats (Haddad et al., 2019), the morphogenesis of the gustatory papillae was extended during the postnatal period. The majority of developmental differentiations in animals with lengthy gestation periods took place throughout the prenatal period as in camels (Abou-Elhamd et al., 2018).

The morphological series of the rabbits' foliate papillae from the first day of life to five months of age were examined in this study using histological, histochemical, and scanning electron microscopy. The obtained results were contrasted with earlier studies on the foliate papillae in other animal species.

We observed that, the foliate papillae at 1st day of life developed as mucosal protrusions in form of parallel small projections separated by very shallow grooves (Kulawik and Godynicki, 2006; Nabil and Tawfiek, 2020); these projections increased in number and size from the first day of life to 5 months old. The foliate papillae of neonates were incompletely separated due to their shallow lateral depressions. The depressions increased in depth and width with the proceeding of age till it became a typical structure at 1 month of postnatal life, and the folia were completely separated from the others. Similar results were observed in rabbits (Nabil and Tawfiek, 2020) and rats (ASAR et al., 1996). Hamed et al. (1980) and Fujimoto et al. (1993) described the process of gustatory groove formation by epithelial cleavage or desquamation. There are two theories about the starting of the epithelial cleavage. The first study mentioned that the beginning is from the proximal part of the epithelial column (Hamed et al., 1980), and the other studies (Fujimoto et al., 1993; Nabil and Tawfiek, 2020) suggested that the cleavage started from the distal part. Our observations coincide with the first theory as the developing of foliate grooves began to be opened proximally at the first week of age and progressed downwards till completely opened at 3 weeks separating the folia from each other and giving a parallel arrangement.

Our observation on papillary epithelium that it arranged in form of stratified squamous epithelium less keratinized in the first day of life and by age development the papillary covering epithelium's stratification increased, and its keratinization was noticeable.

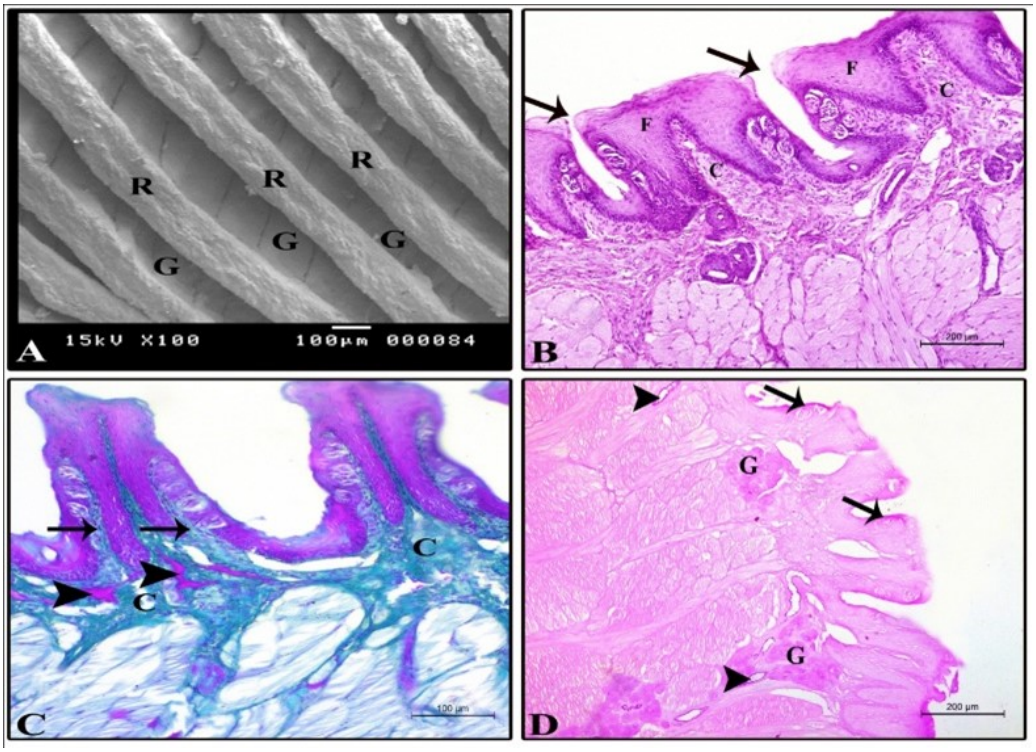

Figure 4: A photomicrograph of the rabbit's foliate papillae at the third month of age showing: **A.** SEM, the developing foliate papillae appeared as parallel long ridges (R) separated by deep and wide grooves (G). **B.** Developing foliate papillae became large and broader separated by very deep and wide gustatory furrow (arrow) covered by stratified epithelium highly keratinized (F) housed lateral rows of taste buds supported by connective tissue core (C) with many execratory large ducts of secretory Ebner's glands (V). H&E stain ×200. **C.** Foliate papillae supported by connective tissue core formed mainly from collagen bundles intermingled with striated muscle bundles (arrowhead). Note that the collagen core of the papillae is distributed in two parts: groove side fold (arrow) and septal fold (C). Masson's trichrome stain ×200. **D.** Foliate papillae with PAS technique give a strong reaction in the keratin layer (arrow), Ebner's gland (G), and its secretory duct (arrowhead). PAS stain ×200. .

Same findings were recorded by Kulawik and Godynicki (2007); Yilmaz et al. (2013); AL-Mahmodi (2016); Nabil and Tawfiek (2020). The connective tissue core of each folium of foliate papillae is made up of one central fold and two lateral folds that are rich in collagen and elastic fibers. These folds were situated in the spaces between streaks of epithelium. Same findings were observed by Yoshimura et al. (2008); Kulawik and Godynicki (2013); AL-Mahmodi (2016).

The taste buds are the main characteristic feature of the gustatory papillae. They arranged on lateral and medial sides of the papillae in rabbits (Elnasharty and Elsharaby, 2013; Nabil and Tawfiek, 2020) and rats (ASAR et al., 1996). The taste buds may be developed prenatally and differentiated after birth as an immature or primitive form. We found that the developing foliate papillae contained taste buds on the birth day. Similar findings were reported in foliate of rabbits (Shalaby, 2011). On the contrary, Toprak and Yilmaz (2016) and Nabil and Tawfiek (2020) observed that the taste buds appeared on the third day of postnatal life. Very small taste buds were developed from anaplastic gustatory cells in suckling animals at 7 days postnatal (Amasaki et al., 2003) in rabbits (Elnasharty and Elsharaby, 2013; Nabil and Tawfiek, 2020) and rats (Hosley and Oakley, 1987), the taste buds in newborn animals were immature due to the odor, rather than taste, being the chemosensory guide in suckling animals.

The development of the taste buds rapidly occurred, resulting in an increase in their number and size. Similar results were observed by Elnasharty and Elsharaby (2013); Nabil and Tawfiek (2020). Serous von Ebner's associated with the gustatory furrows of foliate papillae. This was observed in rabbits Elnasharty and Elsharaby (2013); Kulawik and Godynicki (2013); Nabil and Tawfiek (2020). The Von Ebner's glands developed from the epithelial down growth Kock et al. (1992); Elnasharty and Elsharaby (2013). The secretion of these glands help in taste perception (Li and Snyder, 1995; Leinonen et al., 2001; Abou-Elhamd et al., 2018). The von Ebner's glands contained serous acini, the same findings reported by Nabil and Tawfiek (2020). Oomori et al. (1995) and Nagato et al. (1997) stated that the products of the von Ebner's gland aid in the taste sensation by rinsing the area of the gustatory furrow.

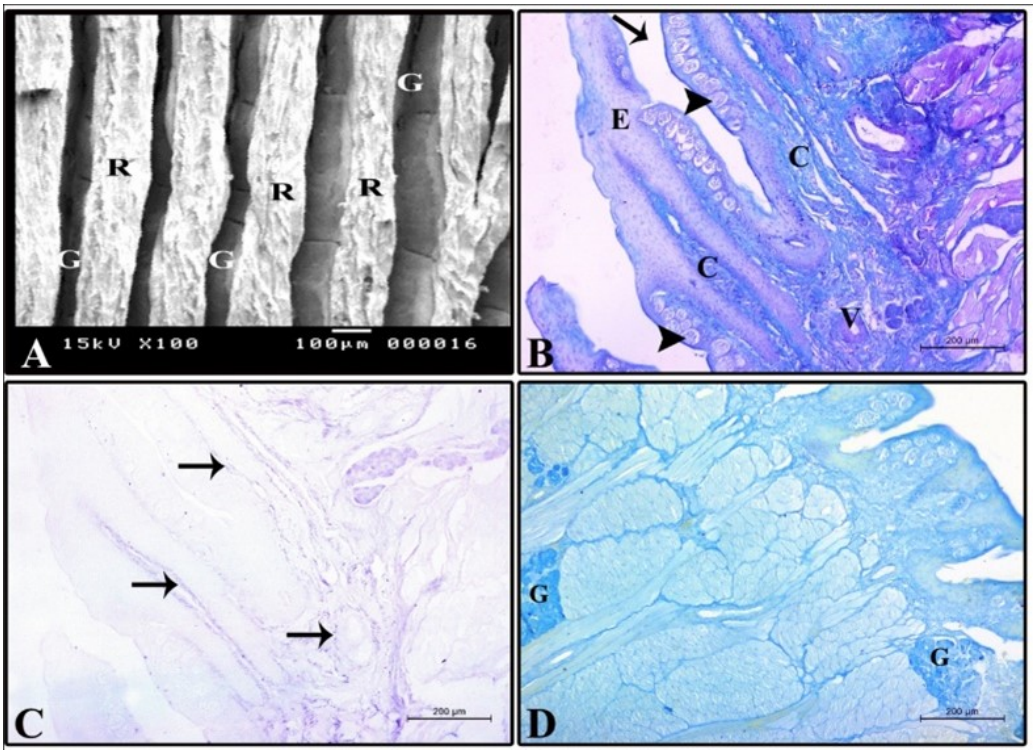

Figure 5: A photomicrograph of the rabbit's foliate papillae at the fifth month of age showing: **A.** SEM, the developing foliate papillae appeared as parallel very long ridges (R) separated by very deep grooves (G)**. B.** Developing foliate papillae became very long, separated by a very deep gustatory furrow (arrow) covered by stratified epithelium less keratinized (E), housed lateral rows of taste buds (arrowhead) supported by connective tissue core (C) with many blood vessels (V). Masson's trichrome stain ×200. **C.** Foliate papillae supported by an extensive network of connective tissue core with elastic fibers (arrow) in both groove side fold and septal fold. Wiegert's elastic stain ×200. **D.** Foliate papillae with Alcian blue technique give a strong reaction in Ebner's gland (G). Alcian blue stain ×200.

4.1. Conclusion

Our investigation suggested that the structural adaptation and maturation of foliate papillae are owing to the transformation of food ingestion from suckling to dry matter feeding.

Article Information

Ethical Approval. All animal procedures and sampling were approved by the Institutional Animal Care and Use committee of the Faculty of Veterinary Medicine, Minia University, El-Minia 61519, Egypt (Feb.16.2022).

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Conflict of Interest. The authors declare no conflict of interest.

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