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ORIGINAL ARTICLE

Morphological study of the rabbit gustatory lingual papillae during postnatal life by light and scanning electron microscopy

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Abstract

This study aimed to investigate the postnatal morphological features of rabbit's lingual gustatory papillae using histological, histochemical, morphometrical and scanning electron microscopical studies. A total of 48 New Zealand rabbits (1, 7, 15, 23, 30, 60 days postnatal) were used as the material. Tongue consisted of an apex, body and root with three types of gustatory papillae fungiform, vallate and foliate. Rounded to oval fungiform papillae were distributed on lingual apex among filiform papillae. Two foliate papillae on lateroposterior side have parallel folia increased progressively in number (14–20) with age advancement. Two oval vallate papillae on lingual root surrounded by annular grooves. Histologically, the gustatory papillary epithelium was thin at birth then increased in stratification and cornification from third to fourth week. Vallate and foliate grooves were shallow in newborns then grew deeply by desquamation of their lining epithelium which completely opened and connected with lingual excretory ducts at 23 days. Developing serous von Ebner's glands appeared at 23 days and became lobulated form 1–2 months. They gave a negative reaction with Periodic Acid Schiff–Alcian blue stain, while mucous Weber's glands showed Alcian blue positive reaction. Taste buds were firstly seen at 15 days old, increased in number and size and became mature with taste pores from third to fourth week. They distributed dorsally on fungiform and on lateral sides of vallate and foliate. This structural adaptation and maturity of gustatory papillae to meet the functional demands of food ingestion during the transition from suckling to dry matter feeding.

Keywords Light microscopy · Lingual papillae · Morphology · Rabbits · Scanning electron microscopy

Introduction

Domestic rabbits (*Oryctolagus cuniculus*) are small mammals in the family of Leporidae of the order Lagomorpha and they are widely distributed all over the world (Quesenberry and Carpenter 2012). Rabbits are raised for their economic importance, experimental and educational purposes. The fact that the digestive system of rabbits is adapted to herbivorous diet with modified teeth (Gidenne and Lebas 2006) suggested that their tongues might also exhibit particular modifications related to its specialized feedstuffs. In primates, some carnivorous and rodents, the use of tongues for food prehension has been lost, since these animals use their paws to bring food into their mouth (Iwasaki 2002). The mechanism of feed intake in vertebrates is clearly an important factor that determines the success of their tongue to the environment (Iwasaki 2002). Therefore, the rabbit is adapted to a wide variety of feeding environments including; desert to temperate or even cold climates, and is able to beneficially consume variable feeds, from seeds to herbaceous plants (Gidenne and Lebas 2006).

The mammalian tongue possesses a characteristic structure of lingual papillae (Iwasaki 2002; Abumandour 2014). Since the general structure of the tongue in rabbit was published (Barone et al. 1973), several articles have been published regarding the morphogenesis and angioarchitecture of the lingual papillae in various animal species including the rabbit (Abumandour and El-Bakary 2013; Abumandour 2014; Elnasharty et al. 2013; Fujimoto et al. 1993; Kulawik and Godynicki 2006, 2007, 2008, 2009; Kulawik et al. 2013; Nonaka et al. 2008).

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Generally, four types of lingual papillae are distinguished on the surface of tongue in mammals; filiform, fungiform, foliate and vallate (Massoud and Abumandour 2019). Three types of these papillae are gustatory, i.e., vallate, foliate and fungiform. The fungiform papillae were observed on tongues of amphibians (Ojima et al. 1997), reptiles (Shimada et al. 1990) and mammals (Kulawik et al. 2013; El-Mansi et al. 2019; Massoud and Abumandour 2019). The tongues of herbivorous animals have filiform, fungiform and vallate papillae, but lack the foliate papillae (Kobayashi et al. 1995). This specialized gustatory system allows the animals to distinguish varieties of food and greatly affect their food consumption, in consequence this will enhance the animal health and growth (Elnasharty et al. 2013).

Available literatures contain results of developmental studies on fungiform, foliate and vallate papillae located on the tongue of rabbit, which was obtained on the basis of observations under a light microscope and morphometric analysis of selected structural traits of papillae (Kulawik and Godynicki 2006, 2007, 2008; Abumandour and El-Bakary 2013; Elnasharty et al. 2013). Moreover, the microvasculature of fungiform papillae in rabbit was described (Ojima et al. 1997).

Lingual taste buds of the rabbit are located mainly in the fungiform and vallate papillae on the lingual dorsum, and in the foliate papillae on the posterolateral margin of the tongue infront of the sulcus terminalis (Witt and Miller 1992). During the last two decades, the use of scanning electron microscopy promoted detailed investigation of the gustatory organs in various animal species. Even though, little information is available on the development of the vallate papillae (Kulawik and Godynicki 2008). Moreover, the ultrastructural morphology of the vallate papillae and their taste buds were studied with fewer details (Nonaka et al. 2008). There is a deficiency of results which describes progressive postnatal development of the gustatory papillae on the rabbit's tongue using a scanning electron microscope and histochemical techniques. Hence, more investigations are mandatory to clarify the morphogenesis of the lingual papillae in rabbit to elucidate their contribution to the taste perception and giving information to increase food palatability and digestibility that increase the rabbit production efficiency (Elnasharty et al. 2013). As well, in the present study, the temporal and spatial changes of the gustatory papillae during their postnatal development were discussed with those of other animals.

Therefore, the objectives of the current study were: to investigate the surface architecture and morphological changes of the gustatory papillae (fungiform, vallate and foliate) on rabbit's tongue using scanning electron microscopy; and to examine their histological and histochemical characteristics from the day of birth till 2 months old, where the rabbit completely changed its diet from milk to dry matter feeding.

Materials and methods

Animals

A total number of 48 newly born New Zealand rabbits (*Oryctolagus cuniculus*) of both sexes, purchased from Animal Resources Unit at Faculty of Agriculture, Cairo University, Egypt. The animals were divided into six groups according to their ages (1 day, 7 days, 15 days, 23 days, 1 month and 2 months old). Each group consisted of 8 animals (male $n=4$ and female $n=4$). The ages of animals were assigned by the rabbits' source.

All animal procedures implemented in the present study were approved by the Institutional Animal Care and Use Committee of Beni-Suef University, Egypt (2019-BSUV-35, adopted on January 15, 2019).

Histological technique

Samples

The tissue specimens of lingual papillae were collected from the rabbits ($n=36$) at 1, 7, 15, 23 days, 1 month and 2 months, 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 tongues containing the papillae were dissected out and washed with normal saline solution and sectioned into three parts (apex, body, and root). Some of these specimens were fixed immediately in neutral buffer formalin (10%) and others in Bouin's fluid, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraplast. Serial and step serial sections (3–5 µm thick) of transverse, sagittal and horizontal planes were obtained by rotatory microtome and stained with Hematoxylin and Eosin (H&E) to demonstrate the general histological structure, Masson trichrome for collagen fibers and muscles, combination of Periodic Acid Schiff reaction (PAS) and Alcian blue (AB) to demonstrate neutral and acid mucins (Suvarna et al. 2019).

Scanning electron microscopy

For scanning electron microscopy (SEM), the rabbits tongue specimens ($n=12$), two animals (male $n=1$ and female $n=1$) 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 h at 4 °C. Thereafter, the specimens were dehydrated through graded series

of ethanol and critical point dried (with carbon dioxide). Then the specimens were attached to aluminums stubs facing upwards, covered with colloidal carbon tabs and then the specimens were sputtered with gold–palladium. The specimens were examined and photographed with a JEOL/EO-JSM-6510 LV SEM at Faculty of Science, Beni-Suef University, Egypt.

Results

Grossly, the rabbit's tongue is relatively narrow and long. It composed of three areas; apex (the rostral free tip of the tongue with lingual groove on its dorsal surface), body (middle part with lingual prominence on its dorsal surface) and root (the smooth caudal part of the tongue beyond the lingual prominence). Three types of gustatory papillae were identified; the fungiform papillae at the anterior region, and the foliate and vallate papilla were located behind the lingual prominence at the root of the tongue (Fig. 1). The anteroposterior length of the tongue and width of the apex, body and root were measured and recorded (Table 1). According to the obtained data in Table 1, there was a marked increase in the length and width of the tongue with its different parts from birth to 2 months age.

Histological and scanning electron microscopical investigations of rabbit's lingual surface revealed no sexual difference could be identified and three types of gustatory papillae (vallate, foliate and fungiform) could be easily distinguished on the lingual surface epithelium and the diameter of the lingual papillae was measured using SEM images (Table 2). According to the obtained data in Table 2, the diameter of the three gustatory papillae markedly increased from birth to 2 months age, the fungiform papillae (92.53 ± 14.34 µm to 188.92 ± 28.95 µm), the vallate papillae (249.78 ± 59.36 µm to 831.04 ± 77.96 µm) and the foliate papillae (921.98 ± 129.10 µm to 2229.41 ± 334.46 µm).

Table 1 Measurements of the rabbit's tongue length and width in three areas

Anatomical parameters of the tongue areas measurements (cm)				
Age (days)	Length	Width		
		Apex	Body	Root
1	1.65 ± 0.19	0.75 ± 0.13	0.75 ± 0.13	0.85 ± 0.13
7	2.35 ± 0.20	1.0 ± 0.16	1.13 ± 0.13	1.15 ± 0.13
15	2.75 ± 0.19	1.15 ± 0.13	1.30 ± 0.13	1.35 ± 0.13
23	3.0 ± 0.22	1.15 ± 0.13	1.35 ± 0.15	1.50 ± 0.16
30	3.40 ± 016	1.30 ± 0.19	1.55 ± 0.19	1.85 ± 0.19
60	4.17 ± 0.69	1.30 ± 0.19	1.55 ± 019	1.85 ± 019

Values in table are expressed as the mean ± standard deviation error (SD)

The important results of the investigated three gustatory papillae (vallate, foliate and fungiform) in each age were summarized (Table 3).

Vallate lingual papillae

At birth, two developing vallate papillae were located on the dorsal surface of the lingual root slightly caudolateral to the midline of the lingual prominence, surrounded rostrally by the filiform papillae. Each vallate papilla was large and rounded. Its apical part ran in the same level of the dorsal lingual surface. It consisted of a round central bulb separated from a prominent annular pad by a clear

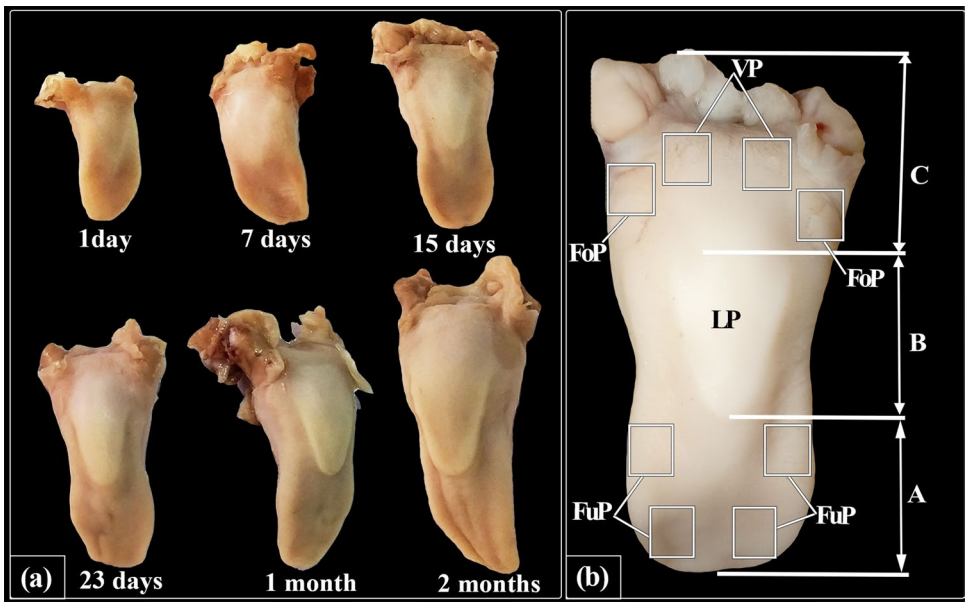

Fig. 1 Dorsal views of rabbits' tongues (**a**) at different postnatal ages (1 day, 7 days, 15 days, 23 days, 1 month and 2 months). Three different areas (**b**) including the apex (A), body (B) and root (C) are visible. Note the lingual prominence (LP) in the body. The areas for collection of histological samples were identified by squares; fungiform papillae (FuP) from dorsal surface and lateral sides of the apex, vallate papillae (VP) from dorsal surface of the root and foliate papillae (FoP) from the posterolateral aspect of the root

Table 2 Diameter of the lingual papillae on SEM images measurements (μm)

Lingual papillae	1 day	7 days	15 days	23 days	30 days	60 days
Fungiform papillae	92.53 ± 14.34	127.92 ± 17.83	144.59 ± 16.24	153.04 ± 20.93	170.72 ± 19.70	188.92 ± 28.95
Vallate papillae	249.78 ± 59.36	321.64 ± 50.91	384.64 ± 81.45	485.73 ± 86.62	758.70 ± 67.52	831.04 ± 77.96
Foliate papillae	921.98 ± 129.10	1260.81 ± 363.48	1410.11 ± 314.49	1829.15 ± 236.72	1897.83 ± 400.25	2229.41 ± 334.46

Values in table are expressed as the mean ± SD

primary groove. The papillary surface was irregular and appeared wrinkly by grooves (Fig. 2/1a, 1d). By LM, each vallate papilla appeared as a cup-shaped structure embedded in between the lingual surface epithelium. Their papillary surface epithelium was thin and keratinized, lined by 2–3 cell layers. The basal cell layer was high columnar cells with oval nuclei and the superficial one was squamous cells with flattened nuclei. The underlying connective tissue was high vascular intermingled with bundles of skeletal muscles (Fig. 2/1c, 1d).

At 7 days old rabbits, the vallate papillae were easily distinguished from the dorsal surface of the tongue. They became larger in size and the surrounding grooves and annular pads were wider than at birth. The papillary surface became more wrinkled with many ridges and grooves (Fig. 2/2a, 2b). By LM, each vallate papilla showed an epithelial cap and a core of connective tissue. The epithelial cap appeared as a typical keratinized stratified squamous epithelium. The connective tissue core with primary papillae increased and composed of collagen fibers. In addition, developing excretory ducts of lingual glands could be recognized (Fig. 2/2c, 2d).

On reaching 15 days of age, a marked increase in the diameter and depth of the surrounding grooves and annular pads (Fig. 2/3a, 3b). By LM, a pronounced increase in the stratification of the epithelial cap. The primary connective tissue core of the developing papilla showed further development of fibrous connective tissue forming secondary papillae. Desquamated cells from the lateral epithelial wall remained in the gustatory groove**.** Few developing taste buds were scattered dorsally and along the lateral and medial sides of the vallate papilla facing the grooves. Each taste bud appeared as ovoid lightly stained epithelial aggregations with rounded and oval nuclei (Fig. 2/3c, 3d).

At 23 days old rabbits, the peripheral parts of vallate papillae were smooth, while the central parts were divided by shallow grooves and had micro papillae and desquamated cells (Fig. 3/4a, 4b). By LM, the gustatory grooves became more deep and associated with serous von Ebner's glands and excretory ducts. The latter opened at the bottom of the papillary grooves. Also, taste buds increased in number on both sides of the vallate papilla**.** The taste buds were formed of few basal, supporting and neuroepithelial cells with distinct taste pores (Fig. 3/4c, 4d).

At 1–2 months old rabbits, the top surface of vallate papillae became more wrinkled with prominent micro papillae (Fig. 3/5a, 5b, 6a, 6b). By LM, a progressive increase in number and size of taste buds became obvious as well as the contained cells per bud**.** The typical structure of vallate papilla was shown at 2 months old rabbits. It had primary, secondary and tertiary papillae. Serous von Ebner's glands and mucous lingual glands appeared in lobulated form which distributed in between the lingual skeletal muscles and dense collagen fibers**.** Serous von Ebner's glands showed small-sized acini lined with truncated pyramidal cells with acidophilic cytoplasm and basally situated rounded nuclei. While the mucous acini were larger in size and lined with large cuboidal cells with foamy cytoplasm containing flattened nuclei which rest on the basement membrane**.** The mucous glands located lateral to the vallate papilla and showed positive reaction with Alcian blue while the serous von Ebner's gland gave negative reaction with PAS technique (Fig. 3/5c, 5d, 6c, 6d).

Foliate lingual papillae

At birth, a pair of developing foliate papillae appeared as slight mucosal protrusions on each side of the lateroposterior portion of the lingual prominence slightly anterior to the vallate papillae, they had parallel projections separated by shallow grooves (Fig. 4/1a). The parallel folia of the foliate papillae progressively increased in number (14–20) with the advancement of the age from the first day to 2 months. By LM, the grooves of foliate papillae were not completely opened. The papillary epithelium formed of 2–3 cell layers. The deepest cell layer was high columnar cells with oval nuclei while the most superficial layer showed flattened cells with flattened nuclei. The surface epithelium appeared slightly keratinized. Numerous newly formed excretory ducts were observed that intermingled with connective tissue and striated muscles bundles. Mitotic figures were observed in the surface epithelium and excretory ducts (Fig. 4/1b, 1c).

On reaching 7 days, the foliate papillae appeared as oval shaped and had further down the growth of the lateral depressions and the grooves started to open forming deep grooves. Some folia were divided into two secondary folia (Fig. 4/2a). By LM, some folia were separated by narrow grooves but others still fused (Fig. 4/2b). While in other

Table 3 A summary of important obtained results for gustatory papillae of rabbits in each age

Papilla	1 day	7 days	15 days	23 days	1–2 months
Vallate papillae	Large and rounded. Its apical part ran in the same level of the dorsal lingual surface	Larger in size and the surrounding grooves and annular pads were wider than at birth. Easily distinguished from the dorsal surface of the tongue	Increased diameter and depth of the surrounding grooves and annular pads	Its peripheral parts were smooth, while the central parts were divided by shallow grooves and had micro papillae and desquamated cells	Its top surface became more wrinkled with prominent micro papillae
	By LM, the papilla appeared as cup shaped structures	By LM, Developing excretory ducts of lingual glands could be recognized	By LM, a pronounced increase in the stratification of the epithelial cap. Few developing taste buds	By LM, the gustatory grooves became more deep and associated with serous von Ebner's glands and excretory ducts. Taste buds increased in number on both sides	By LM, a progressive increase in number and size of taste buds
Foliate papillae	By LM, the grooves of foliate papillae were not completely opened. The papillary epithelium formed of 2–3 cell layers. Numerous newly formed excretory ducts	By LM, oval in shape, had further down growth of the lateral depressions and the grooves started to open forming deep grooves. Some folia were divided into two secondary folia	By LM, Grooves were completely opened. Aggregations of developing serous von Ebner's glands and their excretory ducts intermingled with the striated muscles. Taste buds were ovoid, light stained cellular aggregations on the lateral side of some papillae	By LM, a prominent increase in thickness and stratification of the papillary epithelium and their epithelial streaks. Taste buds increased in number and became scattered on both lateral sides that facing the grooves. The excretory ducts became elongated and connected with the bottom of the gustatory grooves	By LM, von Ebner's glands proliferated and appeared in lobulated form. Excretory ducts were branched off and opened at the bottom of papillary furrows. One row of 2–3 mature taste buds with distinct taste pores. While at 2 months old, taste buds increase in number (5–15 buds per lateral side)
Fungiform papillae	Small round to oval and scattered in a linear form among the filiform papillae. They were numerous with different sizes on the lateral sides of the apex and body of the tongue	Larger in size. The top surface is concave with elevated borders, and have numerous micro ridges and microgrooves. The surrounding filiform papillae were arranged in the form of a rosette with pointed ends	Oval and more flattened separated from the filiform papillae by a layer of flat mucosal pad with a distinct narrow groove. The top surface had elevated areas beside the taste pores	Increased in number appeared as cup shaped with a deep concave top surface, distinct longitudinal micro ridges and surrounded by a thick inter papillary ridge	Large clusters of papillae at the apex of the tongue. On the top surface of the taste pores there were micro ridges and microgrooves, they were surrounded by a thick annular ridge
	By LM, few developing papillae were randomly distributed in between the spiny–shaped filiform papillae	By LM, they appeared as dome shape with a typical epithelial cap and a connective tissue core	By LM, the epithelial covering became more keratinized. Few developing ovoid taste buds	By LM, the papillae had a typical mushroom-like structure with a broad, rounded apex and a narrow base. Most of them had 1–2 well developed taste buds with prominent taste pores	By LM, down growth of the lining epithelium into the underlying connective tissue forming secondary papillae

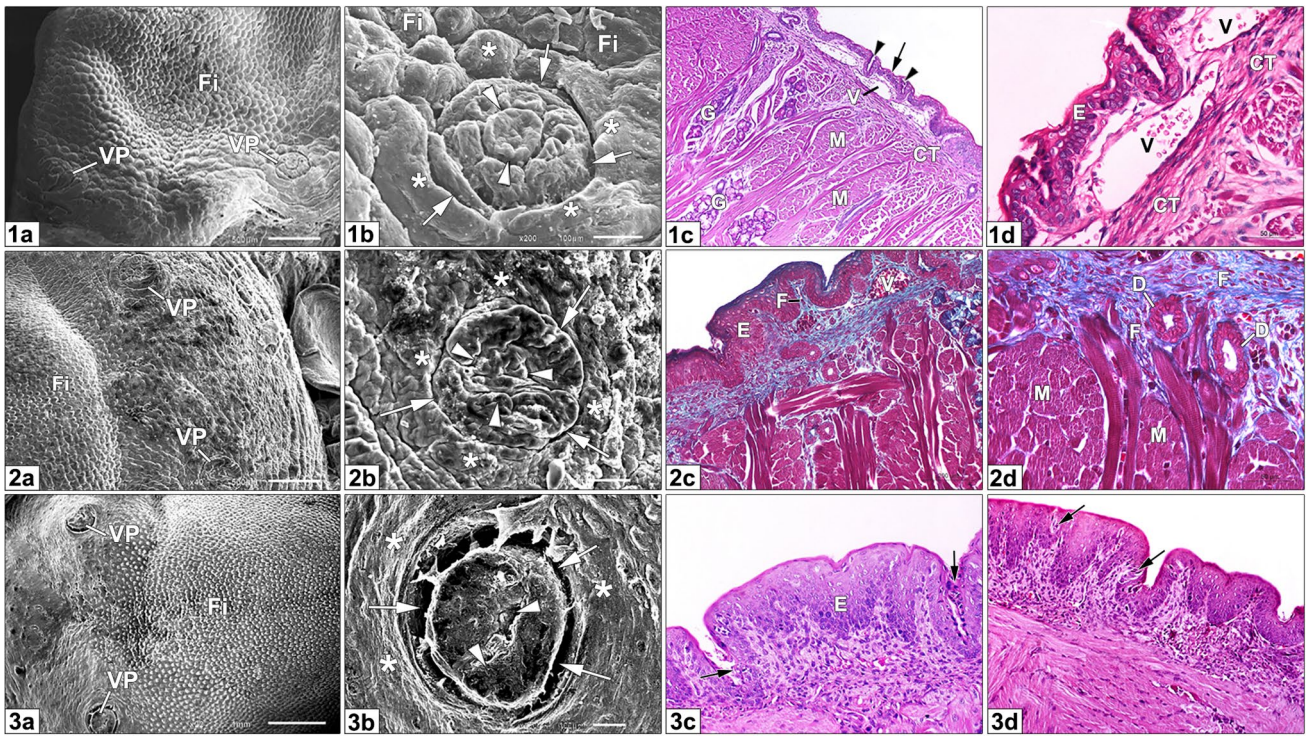

Fig. 2 Postnatal development of the rabbit's vallate papillae as shown by SEM and LM at 1 day (**1a**–**1d**), 7 days (**2a**–**2d**) and 15 days (**3a**–**3d**). **1a**, **1b** SEM at birth showing two developing vallate papillae (VP) located on the dorsal surface of the lingual root slightly lateral to the midline of the lingual prominence, surrounded rostrally by the filiform papillae (Fi), each vallate papilla consisted of a round central bulb separated from a prominent annular pad (asterisks) by a clear primary groove (arrows), the papillary surface was highly irregular and appeared wrinkly by grooves (arrowheads). **1c** LM of a photomicrograph at birth showing a cup-shaped developing vallate papilla (arrow) surrounded by narrow grooves (arrowheads), aggregations of mucous lingual glands (G) in between striated muscle bundles (M), H&E staining. *1d* LM of a higher magnification of **1c** showing the papillary surface lined with a thin layer of keratinized epithelium (E), underlying connective tissue (CT) housing blood capillaries (V), H&E staining. **2a**, **2b** SEM at 7 days showing the vallate papillae (VP) became larger in size and the surrounding grooves (arrows) and annular pads (asterisks), the papillary surface became more wrinkled with many ridges and grooves (arrowheads). **2c** LM, a photomicrograph of vallate papilla at 7 days showing the epithelial cap with a typical keratinized stratified squamous epithelium (E), dense core of collagen fibers (F) with blood capillaries (V), Masson's trichrome staining. **2d** LM of a higher magnification of **2c** showing newly formed excretory ducts of lingual glands (D) with 2–3 cell layers surrounded by dense collagen fibers (F) and striated muscle bundles (M), Masson's trichrome staining. **3a**, **3b** SEM at 15 days, a marked increase in the diameter and depth of the surrounding grooves (arrows) and annular pads (asterisks), with micro ridges and grooves (arrowheads). **3c** LM, a photomicrograph of vallate papilla at 15 days showing prominent increase in the stratification of the lining epithelium (E) with primary and secondary papillae, desquamated cells remained in the gustatory groove (arrows), H&E staining. **3d** LM at 15 days showing newly formed taste buds (arrows) distributed dorsally and on the lateral sides of papilla, H&E staining

examined sections, most of these grooves were opened and the folia were slightly organized into apical epithelial cap and central connective tissue core (Fig. 4/2c). There was an increase in the stratification of the papillary lining epithelium with a pronounced keratinization. In addition, the underlying vascular connective tissue invaded some foliate papillae forming future connective tissue core (Fig. 4/2b, 2c).

At 15 days old rabbits, the grooves were totally opened separating the parallel arranged folia from each other (Fig. 4/3a). By LM, aggregations of developing serous von Ebner's glands and their excretory ducts were intermingled with the striated muscles (Fig. 4/3b). The foliate papilla had a typical epithelial cap and a vascular connective tissue core. The epithelial cap consisted of a keratinized stratified squamous epithelium. The papillary epithelium deeply proliferated forming two lateral epithelial streaks separated by three papillary connective tissue. In addition, the first appearance of developing taste buds was observed as ovoid, light stained cellular aggregations on the lateral side of some foliate papillae (Fig. 4/3c).

A progressive development of the foliate papillae was noticed at 23 days old rabbits. They showed the typical structure of adult papillae and were easily identified on the dorsal surface of the tongue (Fig. 5/4a). By LM, a prominent increase in the thickness and stratification of the papillary epithelium and their epithelial streaks. The latter grew deeper throughout well-developed collagen fibers.

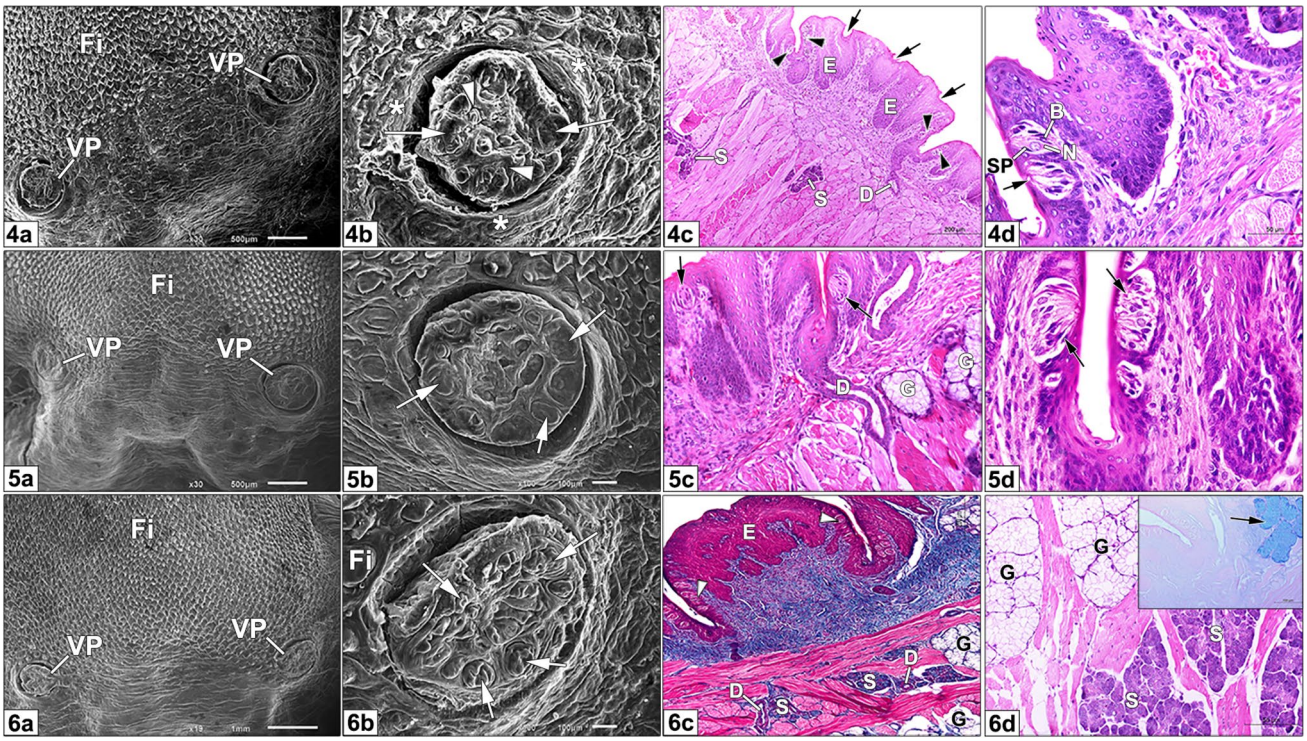

Fig. 3 Postnatal development of the rabbit's vallate papillae as shown by SEM and LM at 23 days (**4a**–**4d**), 1 month (**5a**–**5d**) and 2 months (**6a**–**6d**). **4a**, **4b** SEM at 23 days, the developed vallate papillae (VP) among the filiform papillae (Fi) were surrounded by smooth annular pads (asterisks), central parts were divided by shallow grooves (arrowheads) and had numerous micropapillae (arrows). **4c** LM a photomicrograph of vallate papilla at 23 days showing wrinkled papillary surface by secondary grooves (arrows), multiple and deep epithelial streaks (E) with marked mitotic figures, numerous taste buds distributed on the lateral and medial sides of the papilla (arrowheads), clusters of von Ebner's glands (S) and excretory ducts (D) connected with the papillary grooves, H&E staining. *4d* LM of a higher magnification of the lateral side of vallate papilla at 23 days showing taste buds with distinct taste pores (arrow) with basal cells (B), supporting cells (SP) and neuroepithelial cells (N). H&E staining. **5a**, **5b** SEM at 1 month and **6a**, **6b** SEM at 2 months showing the top surface of the vallate papillae **(VP)** became more wrinkled with prominent micro-papillae (arrows). **5c** LM of a photomicrograph of vallate papilla at 1 month showing well developed dorsal and lateral taste buds (arrows), enlarged excretory ducts (D) opened at the bottom of the groove, mucous lingual gland (G) located lateral to the vallate, H&E staining. **5d** LM of photomicrograph of lateral wall of vallate papilla showing a progressive increase in number and size of taste buds (arrows) as well as their taste cells, *H&E staining*. *6c* LM of a photomicrograph of vallate papilla at 2 months showing the epithelial cap (E) with primary, secondary and tertiary papillae, taste buds (arrowheads), von Ebner's glands (S), excretory ducts (D) and mucous lingual glands (G), Masson's trichrome staining. **6d** LM of a higher magnification of the lobulated form of lingual glands showing von Ebner's glands (S) with small-sized serous acini lined with truncated pyramidal cells with rounded nuclei, mucous lingual glands with large sized mucous acini (G) lined by cuboidal cells with foamy cytoplasm containing flattened nuclei resting on the basement membrane, H&E staining. A photo insert showing positive reaction with Alcian blue (arrow) for the mucous lingual glands while the serous gland were negatively reacted with PAS, (PAS-AB) staining

In addition, the developing taste buds increased in number and became scattered on both lateral sides that facing the grooves. The excretory ducts elongated and connected with the bottom of the gustatory grooves (Fig. 5).

On reaching 1 month (Fig. 5/5b, 5c), the von Ebner's glands were progressively proliferated and appeared in lobulated form. The excretory ducts branched off and opened at the bottom of foliate papillary grooves. Each lateral side of the foliate papilla showed one row of 2–3 mature taste buds with distinct taste pores. While at 2 months old rabbits (Fig. 5/6b, 6c), a pronounced increase in a number of taste buds (from 5 to 15 buds per lateral side) was recognized. Serous secretory acini and excretory ducts showed negative reactivity to PAS staining technique while the mucous gland stained blue with Alcian blue method.

Fungiform lingual papillae

At 1 day old rabbit**,** the developing fungiform papillae appeared as small rounded to oval papillae and scattered in a linear form among the filiform papillae. They were numerous with different sizes on the lateral sides of the apex and body of the tongue. There were desquamating epithelial cells and numerous grooves on the surface of these papillae. The filiform papillae were higher than the fungiform papillae. The size of fungiform papillae on the body is larger than

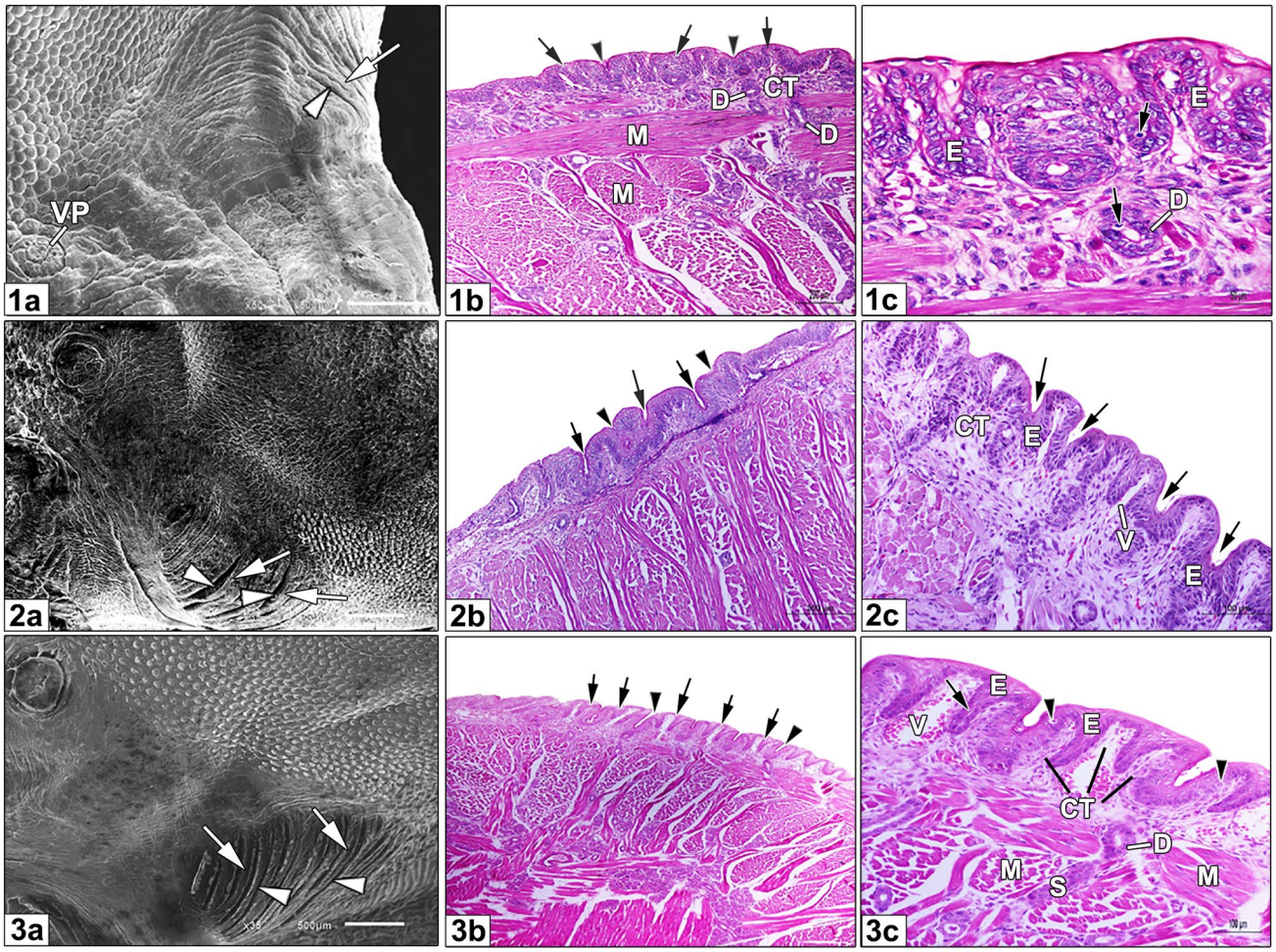

Fig. 4 Postnatal development of the rabbit's foliate papillae as shown by SEM and LM at 1 day (**1a**–**1c**), 7 days (**2a**–**2c**) and 15 days (**3a**–**3c**). **1a** SEM at birth, the developing foliate papillae appeared as slight mucosal protrusions anterolateral to the vallate papilla (VP), each foliate papilla consisted of parallel projections (arrows) separated by shallow grooves (arrowheads). **1b** LM of a photomicrograph at birth showing the developing foliate papillae as mucosal projections (arrows) separated by shallow depressions (arrowheads), newly formed excretory ducts (D) intermingled by connective tissue (CT) and bundles of skeletal muscles (M), H&E staining. *1c* LM of a higher magnification of (**1b**) showing the foliate papillae lined by (2–3) thin layers of slightly keratinized epithelium (E), marked mitotic figures (arrows) in the surface epithelium and excretory ducts (D), H&E staining. **2a** SEM at 7 days showing oval-shaped foliate papillae with many folia (arrows) and further down the growth of the lateral depressions and the grooves started to open forming deep grooves or furrows (arrowheads). **2b** LM of a photomicrograph of the developing foliate papillae at 7 days showing some folia separated from each other by deep grooves (arrows), others still fused (arrowheads), H&E staining. **2c** LM of another section at 7 days showing most of the papillary grooves were opened (arrows), increase in the stratification of the papillary epithelium (E) with the invasion of the underlying connective tissue (CT) accompanied by blood capillaries (V), H&E staining. **3a** SEM at 15 days, the grooves (arrowheads) were totally opened separating the parallel arranged folia (arrows) from each other. **3b** LM of a photomicrograph of the developing foliate papillae at 15 days showing the folia (arrows) separated by distinct grooves (arrowheads), H&E staining. **3c** LM of a higher magnification of **2c** showing typical epithelial cap (E) of the papillary surface and vascular connective tissue core (V), proliferation of two lateral epithelial streaks (arrows) separated by three papillary connective tissue (CT), the developing taste buds oriented on the lateral side (arrowheads), clusters of von Ebner's glands (S) and excretory ducts (D) surrounded by striated muscle bundles (M), H&E staining

that on the apex (Fig. 6/1a, 1b). By LM, in sagittal sections of tongue, few developing fungiform papillae were randomly distributed in between the developing spiny–shaped filiform papillae. They appeared as protrusions, each had rounded, blunt apex and central core of connective tissue which derived from the underlying lamina propria. Horizontal sections of tongue showed larger rounded fungiform papillae than the filiform papillae. They formed of peripheral concentric epithelial layers and central core of vascularized connective tissue. The epithelial layers formed of a basal cell layer of columnar cells with oval nuclei and superficial flattened cells with flattened nuclei (Fig. 6/1c, 1d).

At 7 days old rabbit, the fungiform papillae were larger than that at birth, the top surface of the fungiform papilla

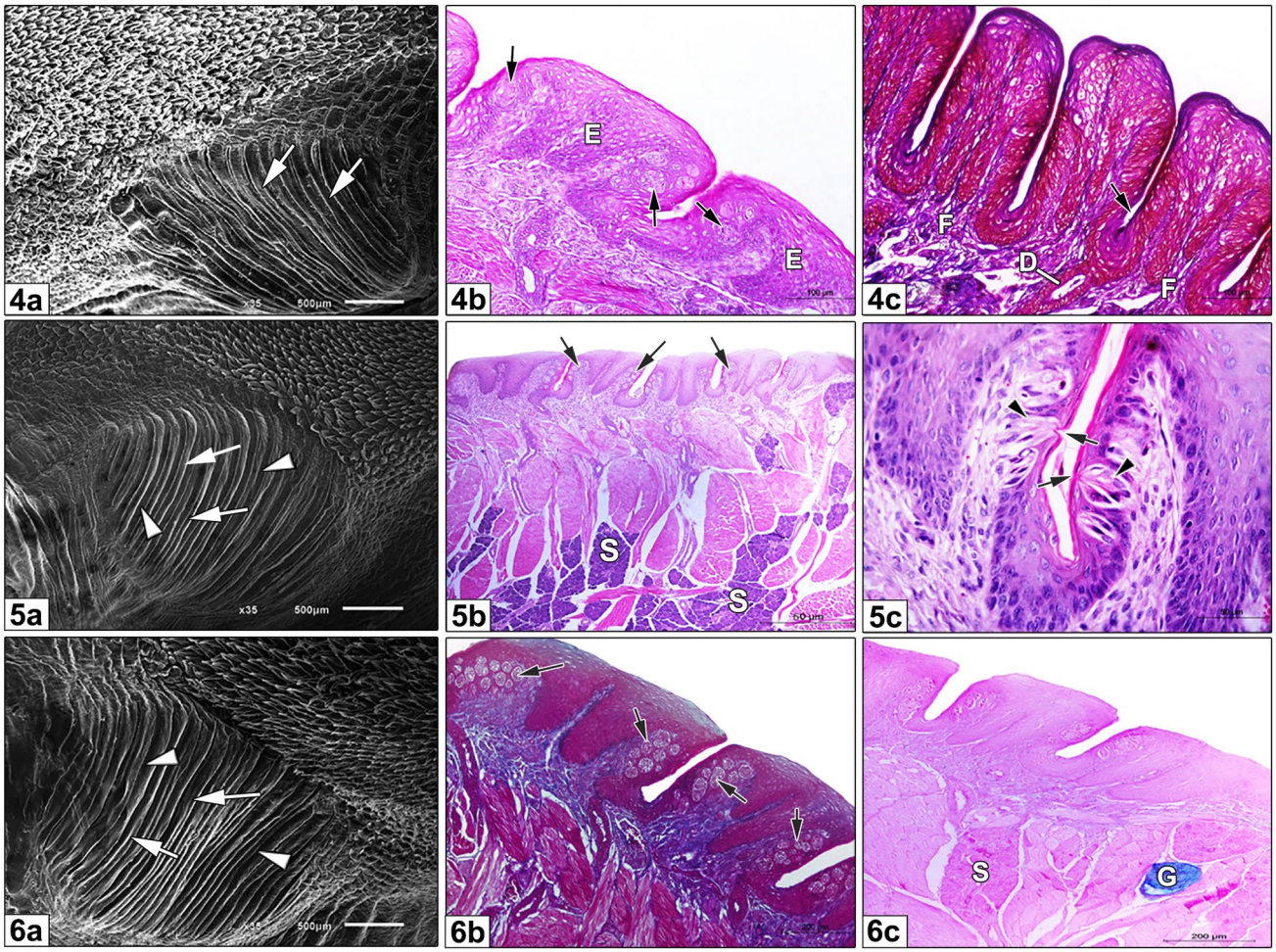

Fig. 5 Postnatal development of the rabbit's foliate papillae as shown by SEM and LM at 23 days (**4a**–**4c**), 1 month (**5a**–**5c**) and 2 months (**6a**–**6c**). **4a** SEM at 23 days showing the typical structure of adult foliate papilla (arrows) on the posterolateral side of the tongue. **4b** LM of a photomicrograph of foliate papillae at 23 days showing a marked increase in thickness and stratification of the papillary epithelium (E) and their epithelial streaks, 1–2 taste buds (arrows) were scattered on each lateral side of the papillae, H&E stain. **4c** LM of a photomicrograph of foliate papillae at 23 days showing the lingual excretory ducts (D) connected with the bottom of the papillary groove (arrow) surrounded by dense collagen fibers (F), Masson's trichrome staining. **5a** SEM at 1 month and **6a** SEM at 2 months showing further increase in size and complexity of folia (arrows) and grooves (arrowheads). **5b** LM of a photomicrograph at 1 month showing progressive proliferation of von Ebner's glands (S), each lateral side of foliate papilla occupied by one row of 2–3 taste buds (arrows), H&E staining. **5c** LM of a higher magnification of the lateral side of foliate papilla at 1 month showing large-sized taste buds (arrowheads) with distinct taste pores (arrows), H&E staining. **6b** LM of a photomicrograph of foliate papillae at 2 months showing increase in number (5–15) taste buds (arrows) on the lateral sides, Masson's trichrome staining. **6c** Mucous glands (G) were positively stained with Alcian blue while serous von Ebner's gland (S) showed negative reaction, PAS/AB method

was concave with elevated borders, and had numerous micro ridges and microgrooves. The surrounding filiform papillae were arranged in the form of a rosette with pointed ends (Fig. 6/2a, 2b). By LM, each papilla appeared as dome-shaped structure with a typical epithelial cap and a core of connective tissue. The epithelial covering was less keratinized stratified squamous epithelium and the core occupied by a large amount of high vascularized fibrous connective tissue (Fig. 6/1c, 1d).

On reaching 15 days old, the fungiform papilla became oval and more flattened separated from the surrounding filiform papillae by a layer of flat mucosal pad (inter papillary epithelium) with a distinct narrow groove surrounded the fungiform papilla. The top surface of the fungiform papilla presented elevated areas beside the taste bud pores (Fig. 6/3a, 3b). By LM, the epithelial covering became more keratinized. Also, few developing taste buds were first appeared on the apical surface of some fungiform papillae. They appeared as ovoid, light stained epithelioid structures with few taste cells per the taste bud (Fig. 6/3c, 3d).

A progressive development of the fungiform papillae was observed at 23 days old rabbit. They showed a marked

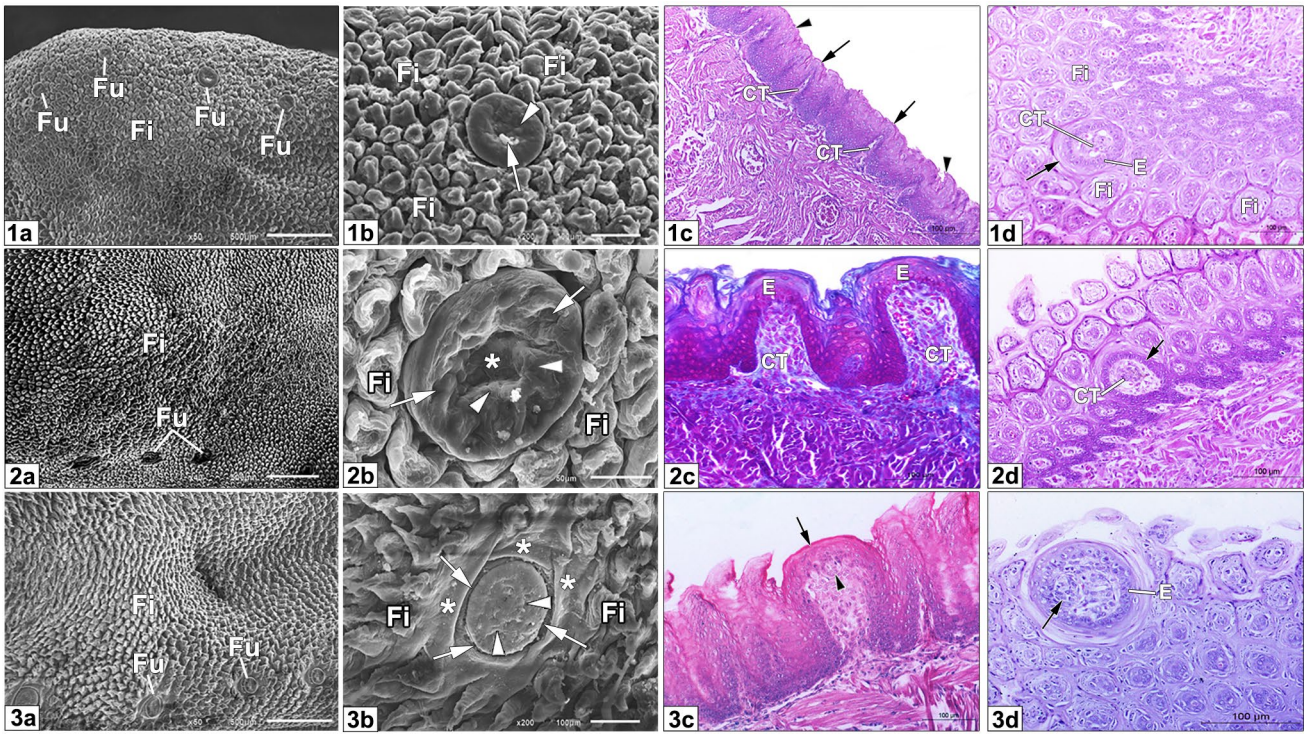

Fig. 6 Postnatal development of rabbit's fungiform papillae as shown by SEM and LM at 1 day (**1a**–**1d**), 7 days (**2a**–**2d**) and 15 days (**3a**–**3d**). **1a**, **1b** SEM at 1 day showing the developing small rounded to oval fungiform papillae (Fu) scattered in a linear form among the filiform papillae (Fi), there were desquamating epithelial cells (arrow) and numerous grooves (arrowheads) on the top surface of these papillae. **1c** LM of a sagittal section of rabbit's tongue at birth showing the developing fungiform papillae as mucosal projections with rounded, blunt apex (arrows), and central core of connective tissue (CT), they were randomly scattered among the spiny-shaped filiform papillae (arrowheads), H&E staining. **1d** LM of horizontal sections of rabbit's tongue at birth showing large rounded fungiform papilla (arrow) between the filiform papillae (Fi), they formed of peripheral concentric epithelial layers (E) and a central core of vascularized connective tissue (CT), H&E staining. **2a**, **2b** SEM at 7 days showing the fungiform papillae (Fu) were larger than at birth, their top surface (asterisk) was concave with elevated borders and numerous microridges (arrowheads) and microgrooves (arrows) and the surrounding filiform papillae (Fi) were arranged in the form of a rosette with pointed ends. **2c** LM of a sagittal section of rabbit's tongue at 7 days showing the fungiform papillae as a dome-shaped structure with a typical epithelial cap (E) and core of connective tissue (CT), the epithelial cap was stratified squamous epithelium less keratinized and the core occupied by large amount of highly vascularized collagen fibers, Masson's trichrome staining. **2d** LM of horizontal sections of rabbit's tongue at 7 days showing the fungiform papilla (arrow) with wide central core of vascular connective tissue (CT), H&E staining. **3a**, **3b** SEM at 15 days showing the fungiform papilla (Fu) became oval and separated from the surrounding filiform papillae (Fi) by a layer of flat mucosal pad (asterisks) with a distinct narrow groove (arrows), the top surface of the fungiform papilla presented elevated areas (arrowheads). **3c** LM of a sagittal section of rabbit's tongue at 15 days showing the keratinized epithelial cap (arrow) of the fungiform papilla and newly formed intra-epithelial taste bud (arrowhead) located on the dorsal surface, it appeared as ovoid, light stained epithelioid structures with few taste cells, H&E stain. **3d** LM of a horizontal section of rabbit's tongue at 15 days showing the large-sized fungiform papilla with thick lining epithelium (E) housing a taste bud (arrow), H&E staining

increase in number and scattered among the elongated and thorn-like filiform papillae on the dorsum and lateral sides of the apex and body of the tongue. They appeared as cup shaped with a deep concave top surface, distinct longitudinal micro ridges and surrounded by a thick inter papillary ridge (Fig. 7/4a, 4b). By LM, the fungiform papillae had a typical mushroom-like structure with a broad rounded apex and a narrow base (stalk). Most of them had 1–2 well-developed taste buds with a prominent taste pore. They had a large number of taste bud cells in the form of basal, supporting and neuroepithelial cells (Fig. 7/4c, 4d).

On reaching 1–2 months, continued development of fungiform papillae was observed. They were scattered by a high number among the spoonful conical filiform papillae. The large clusters of these papillae were observed at the upper marginal area of the anterior dorsal part of the tongue and for a short distance on the inferior marginal surface of the lingual apex and in the median lingual sulcus. On the top surface of the taste pores there were micro ridges and microgrooves, they were surrounded by a thick annular ridge (Fig. 7/5a, 5b, 6a, 6b). By LM, a down growth of the lining epithelium into the underlying

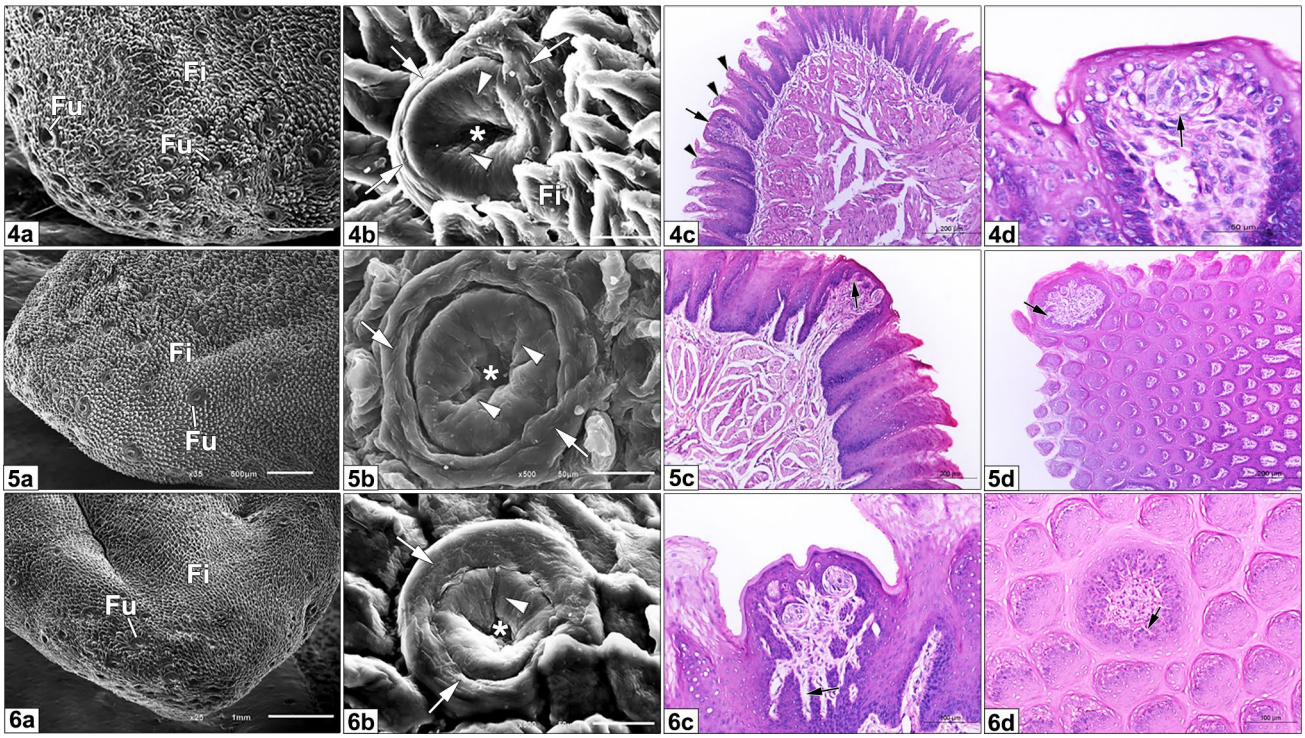

Fig. 7 Postnatal development of rabbit's fungiform papillae as shown by SEM and LM at 23 days (**4a**–**4d**), 1 month (**5a**–**5d**) and 2 months (**6a**–**6d**). **4a**, **4b** SEM at 23 days showing a marked increase in number and distribution of the fungiform papillae (Fu) and scattered among the thorn-like elongated filiform papillae (Fi) on the dorsum and lateral sides of the tongue apex and body, they appeared as cup-shaped with a concave top surface (asterisk), distinct longitudinal microridges (arrowheads) and surrounded by a thick interpapillary ridge (arrows). **4c** LM of a sagittal section of rabbit's tongue at 23 days showing the fungiform papillae (arrow) as a typical mushroom-like structure with broad apex and narrow base scattered among the thorny filiform papillae (arrowheads), the apical surface has well-developed taste buds with prominent taste pores, H&E staining. **4d** LM of a high magnification of the fungiform papilla at 23 days showing a taste bud (arrow) with numerous basal, supporting and neuroepithelial cells, H&E staining. **5a** SEM at 1 month and **5b** SEM at 2 months of the developing fungiform papillae (Fu) among the filiform papillae (Fi) showing the top surface of taste pores (asterisk) surrounded by microridges (arrowheads) and the annular ridge (arrows). **5c** LM of sagittal and **5d** LM of horizontal sections of the rabbit's tongue apex at 1 month showing the fungiform papillary epithelium (arrows) with few and small secondary papillae, H&E staining. **6c** LM of sagittal and **6d** LM of horizontal sections of rabbit's tongue apex at 2 months showing multiple and elongated secondary papillae (arrows), H&E staining

connective tissue forming secondary papillae (Fig. 7/5c, 5d, 6c, 6d).

Discussion

Different morphological structures of the tongue in vertebrates are specialized to fulfill different functions, such as food capturing and manipulation, water uptake, swallowing, grooming, vocal modulation, and suckling (Mançanares et al. 2012). These different strategies of lingual functions cause a diversity in the morphological structures of mammalian tongues from species to species (Iwasaki 2002; Abumandour and El-Bakary 2013). Moreover, the morphological differences and variations appearing in the tongue are directly associated with dietary specializations and food type, as well as adaptations to various environmental conditions (Iwasaki 2002). The distribution of different papillae on the lingual surfaces is a characteristic feature of a genus and may even be distinctive among different species. One of the elements that contribute most to the morphological, distribution, and type of papillae is the diet (Abumandour and El-Bakary 2013).

In this study, the morphological characteristics of the gustatory lingual papillae in rabbits during their postnatal life from the first day to 2 months old were investigated using histological, histochemical and scanning electron microscopical techniques. The findings were compared with previously conducted researches on the lingual papillae in different animal species.

Morphogenesis of the gustatory papillae was completed during the postnatal life of some animals especially that having a short gestational period, such as in rabbit (Elnasharty et al. 2013), in rat (Asar et al. 1996; Hosley and

Oakley 1987), in mice (Toprak and Yilmaz 2016), in hamster (Whitehead and Kachele 1994) and in cat (Haddad et al. 2019). While in animals having a long gestational period, most of their developmental differentiations occurred during the prenatal life, and complete their maturation postnatal as in camel (Abou-Elhamd et al. 2018).

The developmental changes of the gustatory lingual papillae depend on their shape, size, number and distribution. These differences depend on verities in diet, feeding habits and food prehension (Abumandour and El-Bakary 2013; El-Bakary and Abumandour 2017). Firstly, they appeared postnatal as lingual mucosal projections either single as the vallate and fungiform papillae in rabbit, hamster, rat and mice (Hosley and Oakley 1987; Kulawik et al. 2013; Toprak and Tilmaz 2016; Whitehead and Kachele 1994) or multiple projections which parallel arranged as the foliate papillae in rabbit and rat (Asar et al. 1996; Fujimoto et al. 1993). Moreover, the second postnatal unique developmental feature is the change in thickness and keratinization of their lining epithelium which proceeds with growth in parallel with the development of the lingual epithelium. In newborn rabbit, it was observed that the developing papillae lined by thin epithelium formed of 2–3 cell layers and less keratinized. As growth advanced, a marked increase in the stratification and cornification at 23 days old till become typical at 1–2 months. Similar results were recorded in rabbits (Kulawik et al. 2013). The epidermal thickness reached its maximum level on the 60th day in vallate papilla of mice (Toprak and Yılmaz 2016).

The available literature on the postnatal development of lingual gustatory papillae in rabbits lack the progress of their differentiation, hence it was difficult to confront the obtained SEM results. Study by SEM revealed that the vallate papillae of rabbits at birth was large rounded masses located on the posterior dorsal surface of the lingual root just behind the lingual prominence and consisted of central bulb surrounded by annular groove and annular pad (Abumandour and El-Bakary 2013; Elnasharty et al. 2013). The diameter of these vallate papillae increased progressively and having wrinkled top surface with many ridges and grooves. At 1–2 months marked micropapillae were observed on their papillary surface. While the foliate papillae were observed at birth as slight mucosal protrusions on each side of the posterolateral aspect of the lingual root slight anterior to the vallate papillae, they had parallel projections separated by shallow grooves (Fujimoto et al. 1993; Kulawik and Godynicki 2006), these projections increased progressively in number and size from the day of birth till 2 months old. The rabbit's fungiform papillae were distributed on both sides of the tongue in a linear form as rounded masses between the filiform papillae on the lingual body and apex (Kulawik and Godynicki 2007; Abumandour and El-Bakary 2013). With the advancement of the age, their number and size increased, they were randomly distributed on the lateral and top surfaces of the lingual body and apex, their papillary surface became grooved at the 23 days old with distinct micro ridges and surrounded by a distinct flat inter papillary epithelium, the papillae appeared as cup-shaped structures.

During the early postnatal life (1–7 days) in rabbits, the primary epithelial streaks were formed in the developing gustatory papillae by extension of the lining epithelium deep into the underlying connective tissue. They increased and branched with growth forming secondary epithelial streaks at 15 days in circumvallate and foliate papillae. Further increase in their length and depth to form two elongated lateral epithelial streaks in foliate papillae which remain in adults (Kulawik and Godynicki 2008). In addition, tertiary epithelial streaks were recognized in vallate papilla at 2 months (Kulawik et al. 2013). While, these secondary epithelial streaks were recognized at 1 month in the fungiform papillae on the rabbit's tongue, and became more elongated at 2 months as recorded in adult cat (Haddad et al. 2019). The protruded epithelium associated with invasion of the underlying vascular connective tissue to form a typical epithelial cap and a core of connective tissue at 7 days in vallate and fungiform papillae as recorded in rats (Shalaby 2011), while in foliate papillae at 15 days as observed in mice (Toprak and Yilmaz 2016).

Morphogenesis of the gustatory grooves and associated von Ebner's glands was similar in the developing vallate and foliate papillae. According to the type of papilla, there was different shapes and number of the postnatal developing furrows. The same results were described in rabbit (Fujimoto et al. 1993).

In neonate rabbits, we observed two developing vallate papillae on the dorsal surface of the lingual root caudolateral to the lingual prominence and surrounded by a narrow circular groove which increased in depth and width with advanced ages. This was observed in rabbit (Kulawik et al. 2013), in mice (Toprak and Yilmaz 2016) and in rat (Hosley and Oakley 1987). While the foliate papillae of neonates were incompletely separated due to their shallow lateral depressions. These depressions increased in depth and width with the advancement in growth till became a typical structure at 23 days postnatal and the folia were completely separated from each other. Similar results were observed in rat (Asar et al. 1996).

Regarding the appearance of desquamated cells from the lateral wall of the gustatory papillae and remained in the grooves, Fujimoto et al. (1993) and Hamed et al. (1980) described the process of gustatory grooves formation by epithelial cleavage or desquamation. There is a difference between scientists about the starting part of the epithelial cleavage. Some of them revealed that it began from the proximal part of the primary epithelial column (Hamed et al. 1980), others (Fujimoto et al. 1993) suggested that

the cleavage started from the distal part. Our observations coincide with the later author as the developing grooves of foliate papillae began to be opened distally at day 7 and progressed upwards till completely opened at day 15 separating the folia from each other and showed a parallel arrangement.

Serous von Ebner's and mucous Weber's lingual glands were located in the mucosa of tongue and intermingled with lingual skeletal muscles. In our study, the development of the serous von Ebner's glands associated particularly with morphogenesis of the gustatory furrows of vallate and foliate papillae. This was observed in rabbits (Elnasharty et al. 2013; Kulawik et al. 2013). Von Ebner's glands developed from the same epithelial down growth forming the gustatory papilla (Elnasharty et al. 2013). Also, they have been linked to taste perception especially in taste buds of vallate and foliate papillae (Li and Snyder 1995). We observed that von Ebner's gland cell cords and ducts appeared around the time of birth which proliferated and branched with age advancement and clearly opened at the bottom of the groove at 23 days postnatal. Moreover, the serous acini increased forming lobulations at 1–2 months. Similar results were recorded in rabbits (Kulawik et al. 2013), in rat (Asar et al. 1996), in hamster (Paliwal et al. 2006) and in camel (Abou-Elhamd et al. 2018). Moreover, the acini of von Ebner's glands are serous. Our results asserted this observation due to the weak reactivity to PAS for the acini. We conclude a low amount of neutral or acid mucosubstances in the von Ebner's glands of rabbit. Nagato et al. (1997) stated that von Ebner's gland facilitates the sensation of taste by rinsing the area of the gustatory papillae.

In this study, Weber's glands were located lateral to von Ebner's glands and opened into the lingual surface, their acini stained strongly blue with AB-PAS. Similar findings were investigated in rabbit (Elnasharty et al. 2013; Kulawik et al. 2013), in rat (Nagato et al. 1997) and in hamster (Paliwal et al. 2006). It was indicated that they are mucous due to having acidic mucopolysaccharides.

Development of the taste buds is the main characteristic feature of the gustatory papillae. They have similar shapes but differ in their number and pattern of distribution according to the type of gustatory papilla. They developed on the dorsal surface of the fungiform papillae in hamster (Whitehead and Kachele 1994) and in camel (Abou-Elhamd et al. 2018), on lateral and medial sides of the foliate and vallate papillae in rabbit (Elnasharty et al. 2013) and in rat (Asar et al. 1996). Sometimes apical taste buds were observed in rabbit vallate papillae (Elnasharty et al. 2013). While only in lateral epithelium of the furrow wall of vallate papillae in rats and mice (Hosley and Oakley 1987; Uchida et al. 2003).

Taste buds may be developed prenatally and differentiated after birth as an immature or primitive form in mice vallate papillae (Toprak and Yilmaz 2016) and in hamster fungiform papillae (Whitehead and Kachele 1994). Conversely, we found that all types of developing gustatory papillae lack taste buds at birth. Similar findings were reported in vallate papilla of rabbit (Kulawik et al. 2013), mice (Toprak and Yilmaz 2016), hamster (Miller and Smith 1988), in vallate, foliate and fungiform papillae of rabbit and rat (Asar et al. 1996; Fujimoto et al. 1993; Shalaby 2011). Fujimoto et al. (1993) observed that foliate fetal taste buds degenerate and disappear soon after birth and regenerated at 5 days postnatal. Immature taste buds were observed in our study in all gustatory papillae at 15 days postnatal. This was supported by the observations of Shalaby (2011) in rabbit fungiform papilla and Silva et al. (2002) in rabbit vallate papillae. While at 3 days in mice foliate papillae (Toprak and Yilmaz 2016), at 4–8 days in rabbit vallate papillae (Elnasharty et al. 2013) and at 5 days in rat foliate papillae (Fujimoto et al. 1993). There were no taste buds in newborn hamsters (Miller and Smith 1988). Very small taste buds were developed from anaplastic gustatory cells in suckling animals at 7 days postnatal (Amasaki et al. 2003). These immature taste buds appeared as small-sized; oval and light stained intraepithelial bodies without taste pores. They had few numbers of supporting, basal and neuroepithelial cells. Similar findings were investigated in rabbits (Elnasharty et al. 2013) and in rat (Hosley and Oakley 1987). Later authors suggested that the immaturity of lingual taste buds in newborn rats supports the view that odor, rather than taste, is the chemosensory signal that guides suckling in altricial rodents. In this study, with the advancement of the growth, taste buds rapidly proliferated, increased in number, size and became mature. Mature taste buds with distinct taste pores were observed at 23 days postnatal. Similar results were recorded by Shalaby (2011) who stated that mature taste buds were clearly detected in fungiform papillae of 3 and 4 weeks old rats. Amasaki et al. (2003) and Asar et al. (1996) observed that the rat vallate and fungiform taste buds were approximately mature after the weaning phase (21 days). They became morphologically similar to those of adult rabbits at 14–30 days (Elnasharty et al. 2013). Also, they increased at 30 days in mice (Toprak and Yılmaz 2016), from 10 and 45 days in rats (Hosley and Oakley 1987). We found a marked increase in a number of mature taste buds ranged from 5 to 15 buds per lateral side of foliate papilla at 2 months old rabbit. While 5–9 mature taste buds in porcupines foliate (Yilmaz et al. 2013).

Conclusion

Investigations in this study postulated that there are a great relationship between the feeding behavior and development of the gustatory papillae. They become mature with increasing in number and size at the third to fourth week

of postnatal life during the transition from suckling to hard feedstuff self-feeding.

Author contribution We declare that all listed authors have made substantial contributions to the research design. Material collection, specimen preparations, interpretation of data and to drafting the paper or revising it critically. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Consent for publication The content of this study has not been published or submitted for publication elsewhere. All authors have contributed significantly, and that all authors are in agreement with the content of the manuscript.

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