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Histogenesis of Rabbit Vallate Papillae

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Abstract**—**The gustatory system allows animals to distinguish varieties of food and affects greatly the consumption of food, hence the health and growth of animals. In the current study, we investigated the histogenesis of vallate papillae (VLP) in the rabbit tongue using light and scanning electron microscopy. Samples were obtained from rabbit embryos at the embryonic days 16-30 (E16-30), and from newborns until maturity; 6 months. At E16, the first primordia of vallate papillae were observed as small pits on the surface epithelium of the tongue's root. At E18, the caudal part was prominent with loose mesenchymal tissue core; meanwhile the rostral part of the papilla was remained as a thick mass of epithelial cells. At E20-24, the side epithelium formed the primitive annular groove. At E26, the primitive taste buds appeared only at the papillary surface and reached their maturity by E28. The annular groove started to appear at E26 became more defined at E28. The definitive vallate papillae with substantial number of apparently mature taste buds were observed by the end of the second week. We conclude that the vallate papillae develop early and mature during the early postnatal life.

Keywords—Rabbit, vallate papillae, histogenesis, taste buds.

I. Introduction

The domestic rabbits are raised due to their economic value and experimental purposes [1], [2]. Taste perception is a critical sense for basic food appraisal and confers the organism with valuable discriminatory power. This process is an oral chemical sense actuated by the receptor cells congregated within different subpopulations of taste buds in the gustatory papillae of the tongue and palate [3], [4].

Lingual taste buds of the rabbit are contained in three different kinds of papillae with distinct locations on the dorsal and lateral surfaces of the tongue [5]. These include the foliate, vallate and fungiform papillae. Little information is available on the development of VLP [6]. In addition, the ultrastructural morphology of the vallate papillae and their taste buds were studied with fewer details [7], [8], [9].

Nevertheless, more investigations are required to clarify the ontogeny of VLP in rabbit to elucidate the relationship of their contribution to the taste perception and giving information to increase palatability and digestibility of food that increase the productivity of rabbit. As well, the spatial and temporal changes of these papillae during development will be discussed with those of other animals.

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II. Materials and Methods

A. Animals

Timed-pregnant V-line white rabbits at embryonic days 13-30 (E16-30) as well as newly born rabbits (day 1) and 2, 5, 8 days old, 2 weeks, 1 month, 3 and 6 months old rabbits were purchased from the Faculty of Agriculture, Alexandria University for this study. The days of successful mating were designated as E1; the day of labor was 1 while the ages of postnatal animals were assigned by the rabbits' source.

B. Histological Technique:

1. Samples

We collected tissue specimens from the rabbit embryos at E16, E18, E20, E22, E24, E26, E28, E30 in addition to animals aged 1, 2, 5, 8 days old, 2 weeks, 1 month, 3 and 6 months old. Three animals were used for each age (n=48). Under ethyl anesthesia, pregnant animals at various stages were sacrificed by an overdose intraperitoneal injection of chloral hydrate (600mg/kg) and the embryos were collected in phosphate buffered saline (PBS; pH 7.4). Whole rabbit embryo heads at E16-20 and the tongues of E22-30 as well as tongue tissue blocks of postnatal rabbits containing the vallate papillae were dissected out and immersed in 4% paraformaldehyde in a 0.1M phosphate buffer (PBS; pH 7.4) for 3-7 days. Procedures involving animals and their care were conducted in conformity with the standards for animal experiments and are in compliance with the NIH Guide for the Care and Use of Laboratory Animals (1996).

2. Processing and Staining

The samples were dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin. Thin sections (3-7 µm thick) were prepared and mounted on egg albumin-glycerin coated glass slides, dried and stained with Hematoxylin and Eosin (HE) for general inspection, Van Gieson and Masson trichrome for collagen fibers and muscles, combination of Periodic Acid Schiff reaction (PAS) and Alcian blue (AB) to demonstrate neutral and acid mucins [10].

C. Scanning Electron Microscopical Examination

For scanning electron microscope (SEM), samples from roots of tongues of rabbit fetus at E18, E20, E22, E24, E28, E30, P1, and 6 months (three of each age; n=24). The samples were immediately immersed in 4F1G fixative (2% formaldehyde, 1.25% glutaraldehyde in 0.1 M sodium cacodylate) at pH 7.2 and stored at 4 ºC. The fixed samples were washed in 0.1 M cacodylate containing 5% sucrose processed through tannic acid, dehydrated in graded ethanol series. The critical point dried samples (with carbon dioxide) were then attached to stubs with colloidal carbon and coated with gold palladium in a sputtering device. The samples were examined and photographed with Jeol SEM operating 15Kv at the Faculty of Science, Alexandria University (Egypt).

III. Results

At E16, the earliest VLP primordia appeared as a pair of small pits on the surface oral epithelium (OE) formed of few cell layers at the root of tongue. Each papilla was formed of a small epithelial (with mitotic activity) down growth into the underlying mesenchymal tissue (Fig. 1A). SEM observations at E18 revealed that the VLP appeared as a shallow rounded mass (~20µm in diameter) with a caudal part slightly protruded above the lingual surface while the rostral part was slightly invaginated. The papillary surface presented minute rounded elevations representing the apical part of superficial epithelial cells. (Fig. 1B). With LM, at the rostral part, the developing vallate papilla remained as a placode-like structure formed of a thick mass of epithelial cells dipped into the lingual surface. Meanwhile, a dome-shaped structure with a stratified epithelial covering and loose mesenchymal tissue core could be seen at the caudal part of the papilla (Fig. 1B inset). At E20-24, the VLP were elongated oval in shape, 35x65µm in size, surrounded by a shallow depression and almost at the same level of the lingual surface. The papillary surface had small elevations (Fig. 1C). With LM, the papillary OE became well-defined, 2-3 cells-thick, and the side epithelium (SE) was invaginated into the underlying connective tissue on each side of the papilla forming the primitive annular groove. The upper surface of the papilla was slightly higher than the rest of tongue. The upper surface of papillae became more flattened and nearly at the same level of the upper tongue surface. At this stage, no taste buds were observed in either OE or SE. The papillary core became well-defined (Fig. 1D). At E26-28, the developing VLP became rounded in shape, 130x130µm, more prominent with shallow grooves at the margins of the papillary surface (Fig. 1E). The primitive annular groove ended by canalization at the top of the epithelial bands. The papillary epithelium was formed of stratified squamous with the upper layer made up of one or two strata of small flat cells. Primordia of taste buds were firstly observed at E26 in the papillary surface epithelium. They were formed of small ovoid aggregation of cells, which were not sharply delimited from the surrounding epithelium. They appeared as aggregation of ovoid to elongated cells with lightly stained ovoid nuclei covered by several layers of flattened cells. Taste pores of apparently mature taste buds were firstly observed at E28 (Fig. 1F). Occasional epithelial bands of the OE grow deeply into the lamina propria, and the lower tips of these bands separated to form the future secretory parts of Ebner's glands.

At birth, the VLP was large, rounded of about 150 µm in diameter and surrounded by a narrow and deep annular groove. The papillary surface was wrinkly by deep grooves (Fig. 2A), while the OE formed of thick keratinized stratified squamous epithelium with remarkable degree of connective tissue interdigitations. Taste buds were clearly noticed at the dorsal surface and began to appear on the lateral surface (data not shown). At 4-8 days after birth, the taste buds appeared at both medial and lateral papillary epithelium as well as on the dorsal surface of the papilla (Fig. 2B). At 14-30 days, the morphological features of the VLP became similar to those of the adult rabbits. The taste buds became more frequent along the lateral and medial walls of the papillary groove, and rarely at the dorsal surface. Taste buds were oval pale-staining bodies that extend through the thickness of the epithelium (Fig. 2C). In the fundus of the papilla, numerous excretory ducts of Ebner's glands opened in the annular groove, while Weber's glands were found more lateral and opened into the lingual surface. The mucous acini of Weber's glands stained strongly with Alcian blue and PAS, while the serous acini of von Ebner's glands (VEG) stained weakly with PAS and not stained with Alcian blue (Fig. 2D). The VLP was prominent with several folds on the surface and surrounded by a very deep groove (Fig. 2E). The taste buds had different types of cells. Large cells with spherical nuclei, presumably the supporting or light cells, were the most numerous cells of found in the taste bud. Among these cells, neuroepithelial cells, presumably dark cells with longitudinal dark nuclei were observed. At the base of taste buds, small basal cells were noticed (Fig. 2F).

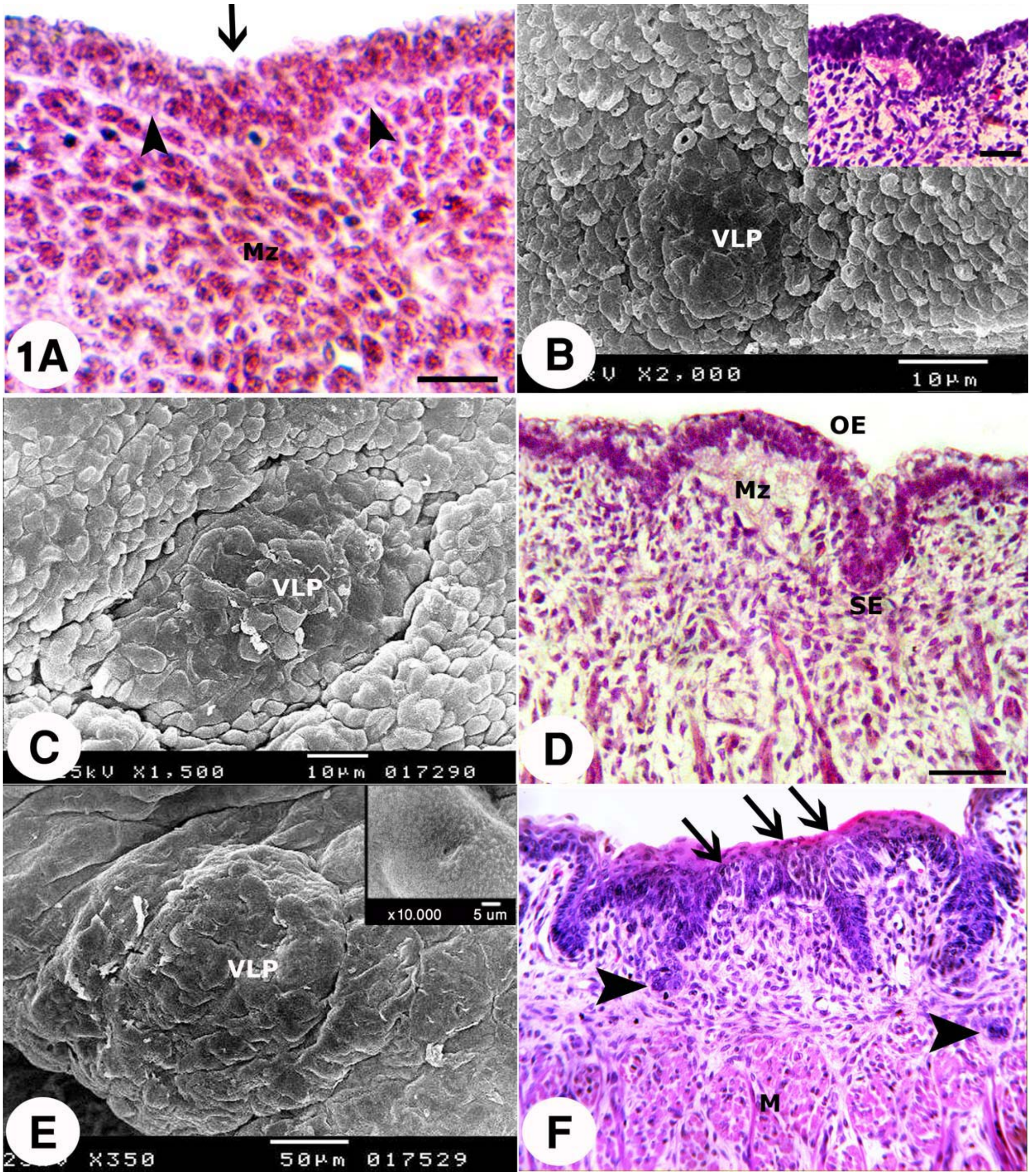

Fig. 1 Prenatal histogenesis of the vallate papilla (VLP) of rabbit. A) VLP at E16 appeared as an epithelial downgrowth (arrow) with mitotic divisions (arrow head). B) SEM of E18 showing the VLP as a shallow rounded mass of which the caudal part was slightly protruded above the lingual surface while the rostral part was slightly invaginated. The inset shows a thick mass of epithelial cells (3-4 cell layers) dipped into the lingual surface. Meanwhile, the caudal part was prominent with a loose mesenchymal tissue core. C) SEM of E22, the VLP was elongated oval in shape, almost at the same level of the lingual surface, and was completely surrounded by a shallow annular groove. The peripheral parts of the papillary surface had small folds similar to those of the surrounding lingual surface. D) A section of the papilla at E20 showing the OE and SE as well as the mesenchymal core. E) SEM of E28 papilla, which is prominent and the oral surface is divided by shallow grooves. The inset shows what looks like a taste pore in the OE. F) A section of E28 papilla showing connective tissue indentations and well-developed taste buds with taste pores in the OE (arrows). Notice the lower tips of the epithelial bands that separated to form the future secretory parts of Ebner's glands (arrow heads). Scale bar: A & D X10µm; E & F X40

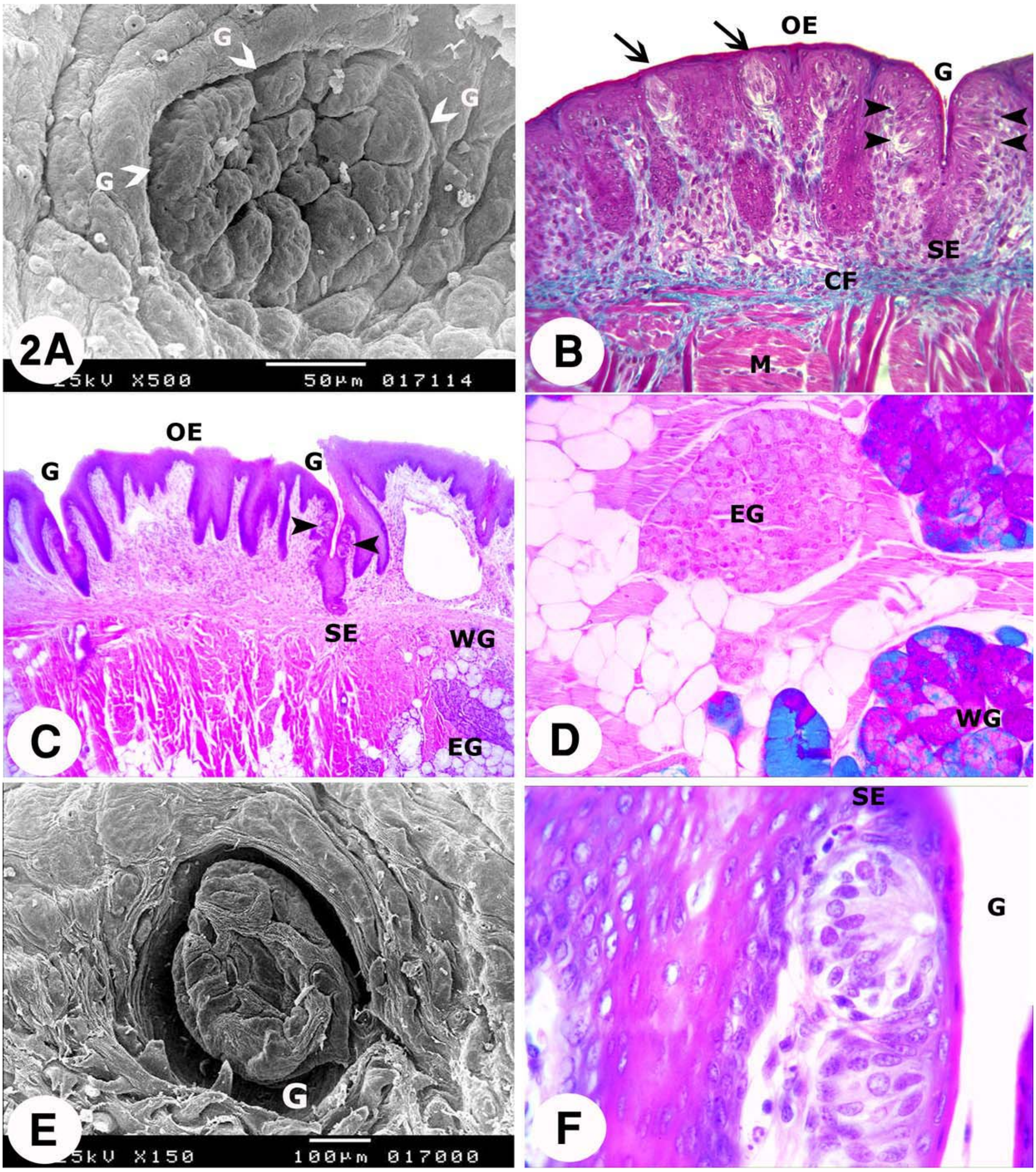

Fig. 2 Postnatal histogenesis of the VLP as shown by SEM (A & E) and LM (B, C, D and F). **A)** SEM of the VLP at birth showing a wrinkled surface divided by deep grooves. **B)** A section of one week-old papilla stained with Masson trichrome. Taste buds appeared in the OE (arrows) and in both sides of the SE (arrow heads). Notice the dense bundles of collagenous fibers in the core and the deeper parts of the papilla. X10. **C)** A section of 1 year old stained with H&E showing a mature VLP X10. **D)** A section at the same age showing the mucous acini of Weber's glands (WG) stained strongly with Alcian blue and PAS, while the serous acini of Ebner's glands (EG) stained very weak. X40. **E)** SEM of the adult papilla (one year's old) with several folds surrounded by a very deep groove (G) and irregular and folded surface. **F)** A typical taste bud in the SE in the adult VLP stained with H&E. X100

IV. Discussion

Fig. 3 illustrate 8 distinct developmental stages of the vallate papilla in rabbit. 1) At E16, a pair of VLP primordia appeared as small pits on the surface oral epithelium formed of 4-5 epithelial layers at the middle of the root of tongue (3A). 2) At E18, the caudal part was prominent with a mainly acellular loose mesenchymal tissue core and formed of 4-5 epithelial layers. Meanwhile, the rostral part of the papilla was remained as a thick mass of epithelial cells dipped into the lingual surface (3B). 3) At E20-24, the papillary OE became well-defined, 2-3 cells-thick, and the side epithelium was invaginated into the underlying connective tissue on each side of the papilla forming the primitive annular groove. Primordia of mucous glands were observed (3C). 4) At E26, immature taste buds were observed in the papillary OE. They appeared as aggregation of ovoid to elongated cells with lightly stained ovoid nuclei, and were covered by about 4-5 layers of polyhedral shaped cells and flattened cells. The annular groove began to form (3D). 5) At E28, taste pores of taste buds were firstly observed, where the surface epithelium was covered by a developing keratin layer, and the annular groove became more defined. Separated epithelial clusters separated from epithelial bands to form primitive Ebner's glands (3E). By E30, mucous glands (green gland) became well developed and their openings were observed in the adjacent epithelium. 6) At birth, the papillary oral epithelium (OE) was formed of thick keratinized stratified squamous epithelium with abundant number of taste buds and remarkable degree of interdigitation of the connective tissue, and a well-developed annular groove. In addition, Ebner's glands were found in the deeper parts of the tongue, and the cells of secretory end pieces were clear but lacked the lumen (3F). 7) At P4-8 days, large numbers of taste buds were noticed on medial and lateral side epithelium of the VLP (i.e. on both sides of the annular groove). In addition, they were also frequently found at the OE. Well-developed Ebner's glands were observed at this stage (3G). 8) By P14-30 days, large numbers of taste buds were found in the SE of the papilla, however they were occasionally found in the OE (3H).

The available data on the development of vallate papillae in rabbits concentrated mainly on morphometric analysis and essentially lacks the progress of their differentiation [6]. In the present study, the earliest sign of papilla formation was found at E16, somewhat later than previously reported [6], who first observed a change in the surface morphology at E15. This may be due to the difference of calculation of the beginning of gestation. Papilla formation was first seen as a downgrowth of epithelial cells from the dorsal surface of the tongue. This is similar to that reported in mice [12] and human [13]. There are three possibilities to explain how epithelial thickening forms a placode: 1) cell division, 2) cell migration, or 3) mesenchymal-epithelial transformation [14]. Cell migration likely plays a role during VLP placode formation [12].

In the present study, a dome-shaped structure with a loose mesenchymal tissue core, mainly acellular, was formed two days after the solid epithelial downgrowth was firstly observed, and was associated with the formation of a furrow, which surrounded the central part of the papilla. This finding is in close agreement with the observations in mice [12] but contrasts [6] in rabbit. They observed downgrowth of epithelial cells only at E22. This appearance may be due to the rapid growth of epithelium than the underlying mesenchyme. At E20-E22, a dome-like structure with two epithelial cords growing down on either side into the mesenchyme was distinct. These cords are the formed circular trench or vallum that gives the VLP its name as recently observed in mice [15]. A E26, the encircling furrow begins to arise from the underlying solid epithelial band through the formation of large fissures or round spaces. These occur most likely due to unequal cohesion between the epithelial cells. The whole process of the encircling furrow formation in the rabbit is comparable with that in sheep [16] and cat [17].

Our observation in rabbits showed that the most developmental changes in the taste system taken place prior to birth. Probably, the first sign of development of the taste bud, similarly to cat [17], is the presence of light elongated cells on the dorsal surface of the primitive vallate papilla in the E26. The cells are found single or in small clusters and are thought to be implemented in the initial stages of taste bud formation. Taste buds in the typical elongated spindle-shaped forms with taste pore were seen in the dorsal surface epithelium of vallate papillae at E28. Their localization there supports the earlier view [16] and [17] that the buds are first formed on the dorsal surface and travel to the wall epithelium later in association with the growth of papillae of cat and sheep tongue. In our study, most of the critical developmental changes have also taken place prior to birth. Similar findings were reported in dog [11], cat [17], sheep [18 & 19], camel [20] and human [21]. The morphogenesis of feline lingual papillae is going on after birth [17] while the immature taste buds in rat were first observed at E20 but mature buds were first seen at 1.5 day after birth [22]. A delayed maturation process of lingual mucosa in some animals may be a species-specific phenomenon [17].

We found the earliest taste buds in the dorsal surfaces of the papillae, and then they additionally appeared within the trenches. The change in taste bud position may correlate with the progressive growth of the vallate papilla in this period [16]. Similar results were reported in the VLP of dogs [11], sheep [19] and camels [20] and nasoincisor papilla of rat [23]. In contrast, taste buds in the VLP of mice and rat develop only in the lateral epithelium of the trench wall [3] and [24]. The similarities between rabbit, dog and camel in taste buds distribution during development need more investigation to determine the factors that determine this pattern. The presence of taste buds among the papillary epithelium has a significant value since it facilitates the direct contact of saliva and food materials with the gustatory epithelium of the tongue [25].

We compared the prenatal development of taste buds in relation to the lengths of gestation period in rabbit, dog and human. Thus, while mature taste buds are observed by E28 in the rabbit (third trimester of gestation period), E47 in the dog (second half of gestation period), and not until E67 in human (first quarter of gestation period).

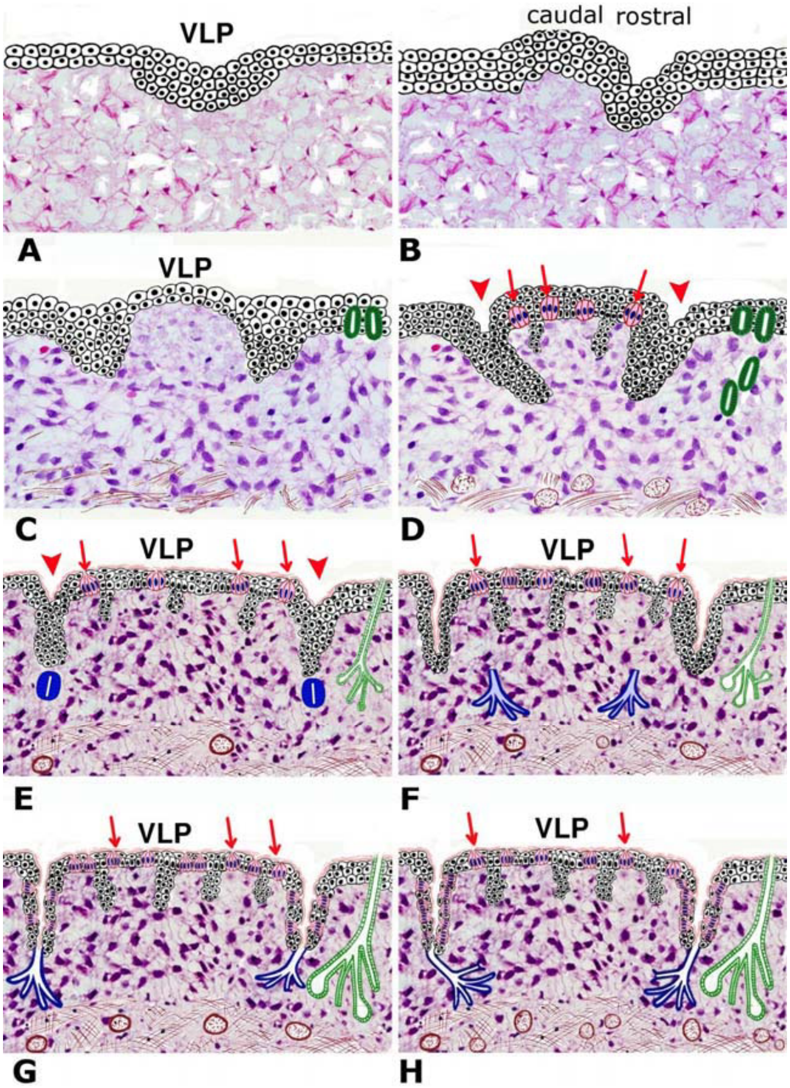

Fig. 3 Illustrative drawing showing eight distinct developmental stages of the vallate papillae in rabbit. 1) At E16, a pair of VLP primordia appeared as small pits of the surface oral epithelium formed of 4-5 epithelial layers at the middle of the root of tongue (3A). 2) At E18, the caudal part was prominent with a mainly acellular loose mesenchymal tissue core and formed of 4-5 epithelial layers. Meanwhile, the rostral part of the papilla was remained as a thick mass of epithelial cells dipped into the lingual surface (3B). 3) At E20-24, the papillary oral epithelium (OE) became well-defined, 2-3 cells-thick, and the side epithelium (SE) was invaginated into the underlying connective tissue on each side of the papilla forming the primitive annular groove. Primordia of mucous glands (green rings) were observed (3C). 4) At E26, immature taste buds (blue arrow) were observed in the papillary OE. They appeared as aggregation of ovoid to elongated cells with lightly stained ovoid nuclei, and were covered by about 4-5 layers of polyhedral shaped cells and flattened cells. The annular groove (red arrow head) began to form (3D). 5) At E28, taste pores of taste buds (blue arrows) were firstly observed, where the surface epithelium was covered by a developing keratin layer (pink line), and the annular groove became more defined (red arrow head). Separated epithelial clusters separated from epithelial bands to form primitive Ebner's glands (blue ring) (3E). By E30, mucous glands (green gland) became well developed and their openings were observed in the adjacent epithelium. 6) At birth, the papillary oral epithelium (OE) was formed of thick keratinized stratified squamous epithelium with abundant number of taste buds (blue arrow) and remarkable degree of interdigitation of the connective tissue, and a well-developed annular groove. In addition, Ebner's glands (blue glands) were found in the deeper parts of the tongue, and the cells of secretory end pieces were clear but lacked the lumen (3F). 7) At P4-8days, large numbers of taste buds (blue arrow) were noticed on medial and lateral side epithelium of the VLP (i.e. on both sides of the annular groove). In addition, they were also frequently found at the OE. Well-developed Ebner's glands were observed at this stage (3G). 8) By P14-30 days, large numbers of taste buds were found in the SE of the papilla, however they were occasionally found in the OE (3H)

Thus, this confirms that the longer the gestation period, the earlier in fetal life the taste system begins to develop [11]. Several questions addressed the biological and behavioral significance of the early development of the taste receptors in the uterus of some mammals. Other sensory systems, such as the eye have shown to be more susceptible to modifications by the environment after birth [26]. It has been speculated that stimulation of the peripheral taste receptors by prolonged exposure to changing levels of chemicals in the amniotic fluid may be necessary to influence the normal formation of central neural connections in the gustatory system [21]. Taste experience in the uterus has also been suggested to play a role in the establishment of taste preference and aversion behavior expressed after birth [27].

The arrangement of connective tissue of the vallate papilla was slightly irregular with several central and lateral prolongations, corresponding to the characteristics described in the Tupaia glis [28]. The connective tissue formed interdigitations, which serve to increase the mechanical connection and blood and nerve supply.

We found distinct ridges and grooves on the surface papillary epithelium especially toward the annular groove, the deepest of which is similar to the secondary groove previously reported in dog [25] and horse [30]. Secondary groove was not reported in previous studies in the rabbit vallate papillae [9 & 29]. The existence of these ridges, depressions and grooves on the papillary surface work together with the primary and secondary grooves for keeping the saliva for more contact with taste buds [31].

In the present study, Weber’s glands were located lateral to the Von Ebner gland as previously reported in rat [32]. The acini of Weber’s glands stained strongly with AB and PAS, which is similar to hamaster [33]. Weber’s glands in the lesser bushbaby contain both a neutral glycoconjugate and a sialomucin [34], which are strongly PAS and AB positive, respectively, this was the same as in rabbit. In a comparative histochemical survey of species in six different mammalian orders, [35] found that neutral glycoconjugates were present in Weber’s glands of the rabbit. Members of the Rodentia (rat, mouse, hamster, guinea pig) also had sulfomucins [32]. The primary function of the mucus produced by Weber’s glands seems obvious; that these glands are strategically placed to aid in deglutition as stated by [32]. The mucus provides a slippery substrate for the easy swallowing of dry food and helps to facilitate tongue movements. In the rat, which lacks lingual tonsils, such a (washout) arrangement is important because the Weber’s glands are large and patent and form potential receptacles for impacted food [32]. A second possible function for the mucous secretions of Weber’s glands is in taste.

The main groups of serous cells in the tongue (von Ebner’s glands) have been linked to taste perception [36], particularly in those taste buds associated with the vallate and foliate papillae. In the present study, the serous lingual salivary glands of Von Ebner's glands of the tongue of the rabbit were located in the mucosa and between bundles of lingual skeletal muscle beneath and around the vallate papilla. This is similar to that in ferret's VEG [37]. The serous acini of the VEG stained weakly with PAS and not at all with AB. This is similar to hamster's VEG [33]. In contrast, in bovine, the cytoplasm of secretory cells showed a diffuse positivity with PAS and AB at pH 2.5. This is due to the presence of a mixture of neutral and acid muco-substances [38]. We conclude low amount of neutral or acid muco-substances in the VEG of the rabbit which gives weak PAS reaction. The rabbits feed on easily digestible forages such as rabbit pellets, kale, or carrot tops and Hay [39]. The above differences may be related to the feeding habits and the kind of food eaten by this animal.

V. Conclusion

We conclude that the general morphogenesis pattern of the VLP of the rabbit in the present study during fetal and postnatal periods was similar to those described in other vertebrate species, although the chronologic sequences were different. The rapid developmental pattern of sensory structures of rabbit VLP can be obviously recognized in the late gestation and in newly born animal, and thus their subsequent maturation is completed during the first month after birth in association with the change of feeding from suckling of milk to dry ration.

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