pISSN 2466-1384 eISSN 2466-1392
大韓獸醫學會誌 (2018) 第 58 卷 第 4 號
Korean J Vet Res(2018) 58(4) : 171~175
https://doi.org/10.14405/kjvr.2018.58.4.171

<Original Article>

Scanning electron microscopy of filiform papillae development in Korean native goats (*Capra hircus*)

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(Received: September 6, 2018; Revised: November 6, 2018; Accepted: November 9, 2018)

Abstract: The aim of this study was to investigate morphological development of filiform papillae (FP) in Korean native goats by using scanning electron microscopy. Tongues were removed from goat fetuses (days 60, 90, and 120), neonates, and juveniles (days 30, 60, 90, 120, 150, and 180 after birth). During the prenatal period, primordia of FP appeared at fetal day 60 and were observed to be developed at day 90. At fetal day 120, the FP were observed like flower leaves of a double flower bud. In neonates, FP were shaped like an obliquely sectioned cylinder with secondary papillae irregularly arranged in a saw blade-like manner. In 60-day-old juvenile goats, the FP were densely distributed at the inner base of 1/3–1/2 degrees. In 90-, 120-, and 150-day-old goats, FP were compacted at the inner base of 1/2–2/3, 3/4, and 4/5 degrees, respectively. In 180-day-old goats, FP were found to be completely compacted on the inner surface with complete morphogenesis. Microridges, microplicae, and micropits were well-developed on the epithelial surface of lingual papillae from embryonic day 120 to juvenile day 180. These results indicate that FP of goats have different shapes and sizes during development both before and after birth.

Keywords: Korean native goat, development, filiform papilla, morphogenesis, tongue

Introduction

The dorsal surface of the tongue contains numerous lingual papillae with different shapes. They are named as mechanical and gustatory papillae according to their morphological characteristics [17]. Distribution and disposition of mechanical papillae have many differences depending on mastication and prehension habits of animals [6, 7, 16]. Mechanical papillae can be divided into filiform, conical, and lentiform papillae according to their shapes [5, 9] while gustatory papillae are divided into fungiform, vallate, and foliate papillae depending on their morphological features [2, 8]. Foliate papillae have not been found in ruminants [2, 4, 9] or other animals [7, 10, 14].

Using scanning electron microscopy (SEM), lingual papillae have been studied from a variety of animals, including mouse [12], rat [13], weasels [11], cat [8], dog [18, 19], sheep [9], horse [2], cow [5], and human [1, 20]. Concerning lingual papillae of goats, Kumar *et al*. [15] and Lee *et al*. [17] have studied filiform, conical, lentiform, fungiform, and vallate papillae.

Although ruminants such as Korean native goat have been studied, little is known about morphological changes in tongue of goat during development. We have reported on the morphological changes of vallate papillae, which is one of the gustatory papillae during the development of Korean native goats [3]. However, no studies have examined the morphological features of filiform papillae (FP) as representative mechanical papillae. Therefore, in this study, we investigated changes in the shape and size of FP in Korean native goat during pre- and postnatal developments to characterize the development of tongue in ruminants.

This study on the tongue development of Korean native goats could provide basic data for the study of native goats and contribute to the establishment of biological information by providing basic data for preservation of biological resources.

Materials and Methods

Experimental animals

Tongues from three fetuses (two males and one female), four neonate males, and three males (30, 60, 90, 120, 150, and 180 days) of Korean native goat were used in each group. Tongues of fetuses examined in this study were removed from 60-, 90-, and 120-day-old fetuses of Korean native goats aged 2 to 4 years old with body weight ranging

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from 23 to 33 kg by caesarean section under general anesthesia using xylazine hydrochloride (10 mg/kg, intravenously; Bayer Korea, Korea). FP of tongues were examined for morphological development. We also removed tongues of neonates and juveniles at six different postnatal stages: days 30 (P30), 60 (P60), 90 (P90), 120 (P120), 150 (P150), and 180 (P180). All animal experiments were performed according to the protocol set out in the guidelines of the Ethics Committee for Animal Experiments at Gyeongsang National University (Approval No. GNU-LA-10).

SEM examination of FP during development

Tongue tissues used for SEM observations were fixed with 2.5% glutaraldehyde for 4 h at room temperature. Areas appropriate for inspection were dissected and osmicated in 1.0% osmium tetroxide at 4ºC for 2 h. After washing with phosphate buffered saline three times, these tissues were dehydrated using a graded series of ethanol solutions. These specimens were subjected to critical point drying. They were then placed on spinner stubs and coated with gold to a depth of 100 μm as SEM coating unit. These specimens were observed under a scanning electron microscope (SEM-AL 300; Philips, the Netherlands) operated at 15 KV.

SEM measurements for the length of FP

Measurement of FP was performed for three to five different regions by measuring the minimum and maximum diameters of papillae using SEM from neonates to 180-day-old goats. Statistical differences were tested by analysis of variance (ANOVA) with Newman-Keulspost *hoc* test as appropriate using GraphPad Prism 5.0 (GraphPad software, USA). Statistical significance was considered during the next 30 days when p value was less than 0.05. Data are average ± SD.

Results

Morphological characteristics of the developing FP

In prenatal developing goats, the primordia of FP appeared in day 60 fetus (Fig. 1A) while undeveloped microplicae were seen on the epithelial surface (Fig. 1B). The primordia of FP were more developed in day 90 fetus (Fig. 1C). In day 120 fetus, primordia of FP shaped like flower leaves of double flower bud while their top surfaces were concave in shape (Fig. 1D). The FP in day 120 fetus had microplicae developed on the epithelial surface of papillae (Fig. 1E). FP of neonates shaped like an obliquely sectioned cylinder. Secondary papillae were irregularly arranged as saw blade-like processes in neonates. They were placed around the margin (Fig. 1F).

In postnatal developing goats, FP had a feature of obliquely sectioned cylinder with irregular height and rough sow blade shape. They had both big and long primary papillae with narrow and slightly lower secondary papillae in 30-day-old goats (Fig. 2A). The number of secondary papillae was increased up to 6 to 8 in 30-day-old goats compared to 3 to 5

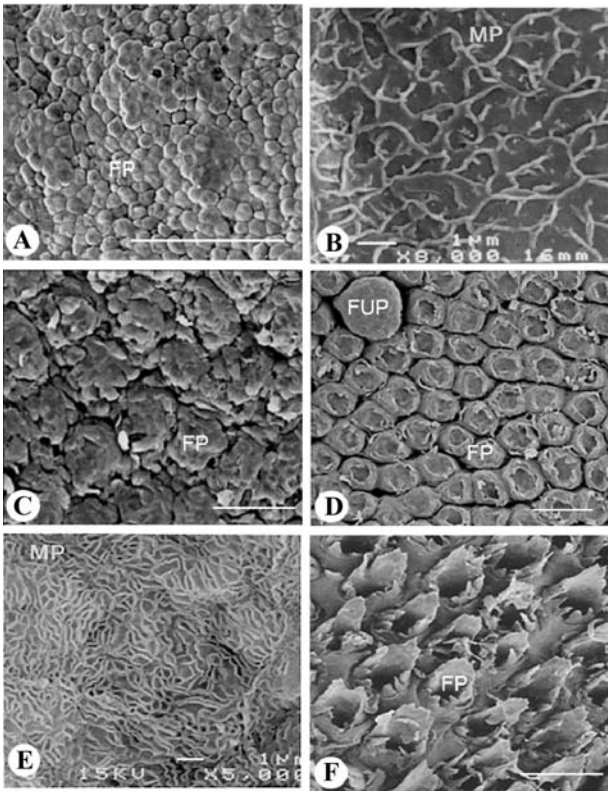

Fig. 1. Scanning electron micrographs showing prenatal filiform papillae (FP) of Korean native goats. (A) Undeveloped primordia of FP appeared in 60-day-old fetus (embryonic day [E] 60). (B) High magnification view on the surface of FP in E60. (C) Developing primordia of FP are seen in 90-day-old fetus. (D) FP shaped like a double flower bud in 120-day-old fetus (E120). (E) High magnification view on the surface of FP in E120. (F) FP have empty inside and irregularly arranged saw blade-like processes in neonates. FUP, fungiform papillae; MP, microplicae. Scale bars = 100 μm (A, B, D and F), 1 μm (B and E).

at the time of birth. They gradually became bigger. A number of developed microplicae were observed on the epithelial surface of 30-day-old goats (Fig. 3A).

In 60-day-old goats, many long and slender leaf-shaped processes of primary papillae were observed on the FP (Fig. 2B). Saw blade-like secondary papillae with irregular height did not show significant differences between 30-day-old goats and 60-day-old goats. However, some of them filled 1/3 to 1/2 in shape compared to those of 30-day-old goats. Microplicae were long with distinct linear shape like a thread while microridge was observed in 60-day-old goats (Fig. 3B).

In 90-day-old goats, inner spaces of FP were packed approximately 1/2 to 2/3 and processes of papillae were sticking out higher than those of 60-day-old goats (Fig. 2C). Microplicae and microridges were very well-developed on the surface of FP (Fig. 3C).

In 120- and 150-day-old goats, FP had many small peaked papillae processes. Their bottoms were filled up to levels of 3/4 and 4/5, respectively (Fig. 2D and E). In 180-day-old

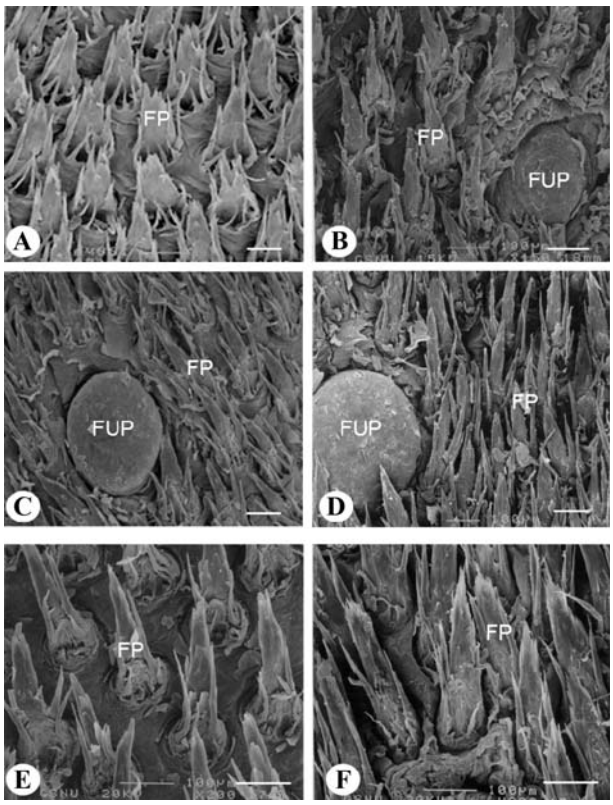

Fig. 2. Scanning electron micrographs showing postnatal FP from juveniles of Korean native goats. (A) FP have big and long primary papillae with narrow and low secondary papillae in 30-day-old goat. (B) FP of 60-day-old goat and (C) FP of 90-day-old goat showed well-developed secondary papillae. (D) FP of 120-day-old goat and (E) FP of 150-day-old goat had many small peaked papillae processes. (F) FP of 180-day-old goat had completed morphogenesis. Scale bars = 100 μm.

goats, FP had completed morphogenesis (Fig. 2F) while the surface of epithelia of FP had straight microplicae (Fig. 3D).

Length measurement of FP after birth

Height and oblique length of FP from neonates to 180-day-old goats are shown in Fig. 4. The height and oblique length of newborn papillae were 47.0 ± 3.53 and 67.0 ± 3.72 μm, respectively. Heights and oblique lengths of 30-day-old goats were 76.0 ± 8.69 and 95.0 ± 10.65 μm, respectively. In weaning period of 60-day-old goats, heights and oblique lengths were 107.0 ± 12.15 and 123.0 ± 14.18 μm, respectively. Heights and oblique lengths of 90-day-old goats were 126.0 ± 7.54 and 148.0 ± 7.13 μm, respectively. In 120-day-old goats, they were 131.0 ± 8.48 and 151.0 ± 10.57 μm, respectively. Heights and oblique lengths of 150-day-old goats were 127.0 ± 9.51 and 156.0 ± 12.45 μm, respectively. During maturing period of 180-day-old goats, they were 133.0 ± 11.52 and 158.0 ± 10.47 μm, respectively. Sizes of FP were rapidly increased in height and oblique length until 90-day-old goats. They then gradually increased in 90 to 180-day-old goats. There were

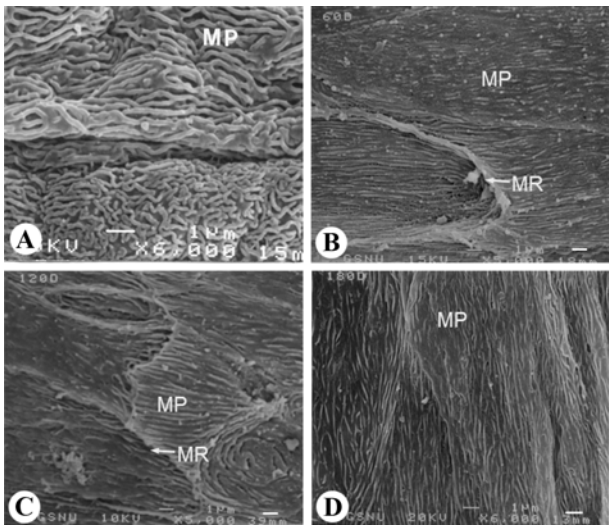

Fig. 3. High magnification view on the surface of postnatal developing FP in Korean native goats. (A) Many MP are developed on the surface of FP in 30-day-old goat. (B) MP are long and straight shaped like thread while microridge (MR) is observed in 60-day-old goat. (C) MP and MR are well-developed in 90-day-old goat. (D) The surface epithelium of FP has straight MP in 180-day-old goat. Scale bars = 1 μm.

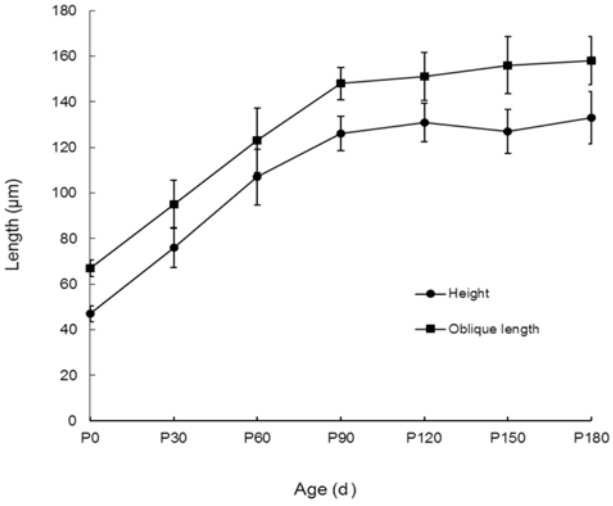

Fig. 4. Scanning electron microscopy measurements of the length of FP. Each height and oblique length were measured from postnatal day 0 (P0) to 180-day-old Korean native goats (P180). There were no statistically significant differences in size of FP during the next 30 days ($p > 0.05$).

no statistically significant differences in size of FP during the next 30 days.

Discussion

Ruminants have five different types of lingual papilla (filiform, conical, fungiform, vallate, and lentiform papillae) on the dorsal surface of the tongue [2, 5, 9]. A scanning elec-

tron microscopic study have measured the length of lingual papillae of the tongue of adult goats [17]. Studies for the most lingual papillae, including ruminants, have mainly reported morphological characteristics in mature animals. However, no studies have examined the morphological changes of the FP in tongue of developmental ruminants. To characterize the development of tongue in ruminants, we previously reported three-dimensional structural changes associated with the development of vallate papillae, one of the gustatory papillae in Korean native goats [3]. In addition, we investigated changes in the structure that occur during morphogenesis of FP, a mechanical papilla, in Korean native goats during prenatal and postnatal development in this study.

In rat fetuses, rudiments of fungiform papillae could be observed at embryonic day (E) 12 and the rudiment of the circumvallate papilla could be recognized at E16. However, FP are formed at P0 after the appearance of rudiments of fungiform and circumvallate papillae [13]. The primordia of fungiform papillae arranged like lattice structure have been observed in 15-day-old mouse fetuses while primordia of FP are not sprouted up [12]. Boshell *et al*. [1] have also reported that fungiform and vallate papillae grow faster than FP in human. In our previous study, primordia of goat's vallate papillae were clearly observed and moat was shallowly spread in E60 fetuses [3]. In the present study, primordia of FP could be observed E60 without showing definite shape. However, primordia showed more development at E90. Moreover, primary papillae of FP were found to be well-developed in 60-day-old goats as in adults and their inner surface were completely compacted in 180-day-old goats. These results confirm that the mechanism of morphogenesis and development of FP might be different from those of gustatory papillae. However, they might have the same trend as gustatory papillae that are developed prior to mechanical papillae shown in other studies.

Concerning fine structure on the epithelia surface of lingual papillae, Iwasaki *et al.* [12] have reported that microridge-like structures are observed on epithelial cells at the base of FP in mouse neonates. In the present study, a number of microplicae with distinct morphologies on FP were found at E120 before birth while long and straight microplicae similar to those in adults were observed in P180. Therefore, surface structures of lingual epithelia of the FP in Korean native goats were somewhat different from those in mouse.

Concerning the size of FP, Lee *et al*. [17] have reported that the size of FP measured in adults of Korean native goat using SEM is 150 μm. In the present study, the height of FP in neonate was 47 μm. It was 107 μm in the weaning period of 60-day-old goats and 133 μm in the maturing period of 180-day-old goats. Therefore, sizes of FP of goats were gradually increased during development, similar to results of other study.

In conclusion, FP of goats had different shapes and sizes during development. Results of this study provide basic data for future studies on morphogenesis of lingual papillae in ruminants.

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