

## **STUDY BY SCANNING ELECTRON MICROSCOPY OF THE MORPHOGENESIS OF FILIFORM AND FUNGIFORM PAPILLAE IN THE RABBIT, *ORYCTOLAGUS CUNICULUS F. DOMESTICA***

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**Abstract.** Rudiments of fungiform papillae were observed at day 18 of prenatal development. They were arranged only on the dorsal surface of the apex of the tongue and its margins. The fungiform papillae looked like a hemispherical, dome-shaped eminences. From day 22 of prenatal development, scanning electron microscope showed also that rudiments of fungiform papillae appeared on the body of the tongue at the front of forming torus linguae, and on its both sides. No rudiments of filiform papillae were observed at day 22 of prenatal development. However, after removal the epithelium, the connective tissue cores of rudiments of fungiform and filiform papillae were visible. Scanning electron microscopy showed on the surface of the tongue numerous filiform papillae at day 26 of prenatal development. They were arranged on the entire dorsal surface of the apex and body of the tongue, and on its margins. During morphogenesis two types of filiform papillae were noticed. One type of filiform papillae was similar to separated cones, another had 3–4 processes more. The similar changes was with the shape of connective tissue core. The fungiform papillae were located among filiform papillae. Scanning electron microscope showed three types connective tissue cores of developing fungiform papillae. One type was mace-shaped, another one was bowl-shaped and yet barred-shaped. At day 30 of postnatal development on the surface of the connective tissue cores of fungiform papillae, from its top towards its base there were parallel and longitudinal folds.

**Key words:** domestic rabbit, lingual papillae, morphogenesis

## INTRODUCTION

In literature some structure of filiform papillae and keratinization of their epithelium was frequently described in adult mammals [Boshell et al. 1982, Iwasaki 1990]. Scientists provided research results on the structure of fungiform papillae, their topography as well as their quantitative changes [Ojima 1998, Kilinc et al. 2010]. Several articles have been published in the scientific literature regarding the morphogenesis of papillae of the tongue in various species of animals [Fujimoto et al. 1993, Iwasaki et al. 1999, Igbokwe, Okolie 2009] and in human [Witt, Reutter 1997].

The general structure of a rabbit tongue was published in Barone's atlas [1973]. Later, articles gave some more details about the structure and angioarchitecture of the tongue papillae in adult rabbits [Kobayashi 1992, Ojima et al. 1997, Kulawik, Godynicki 2007]. Development of the rabbit tongue and fungiform papillae investigated by light microscope and presented in literature [Kulawik, Godynicki 2005, 2009]. There is a deficiency of results which describes the development of filiform and fungiform papillae on the rabbit tongue using scanning electron microscope. For this reason, we aimed to examine the morphogenesis of filiform and fungiform papillae by this microscope in this species. The additional factor which induced us to conduct this research was the fact that the rabbit is a popular laboratory animal, which is often bred and kept at home similarly to pets.

## MATERIAL AND METHODS

The tongues of 30 rabbits, *Oryctolagus cuniculus f. domestica*, of both sexes were used in this study. Tongues were removed from oral cavity at four different prenatal stages (E) on the: 18th (3 tongues), 20th (3 tongues), 22nd (9 tongues) and 26th day (5 tongues). The tongues came also from animals at postnatal stages (P): just after birth (4 tongues); on the 15th (3 tongues) and 30th day (3 tongues). The age of fetuses was determined on the basis of the CRL (Crown-Rump-Length) value [Evans and Sack 1973]. Studies on animals were conducted with approval by the Local Ethics Committee, permission N. 4/2000.

The samples of filiform and fungiform papillae from different areas of the tongues were chosen and fixed in the Karnovsky solution (4°C, pH 7.2) for 24 hours. Then the samples were dehydrated through a graded series of ethanol, critical point dried, mounted on aluminum stubs and coated with gold. For the purpose of observations of the connective tissue core of filiform and fungiform papillae, some samples after being fixed in the Karnovsky solution were treated in 10% NaOH solution for 14–20 days at room temperature. The samples were observed at various angles under a scanning electron microscopes: Hitachi S-4200 and LEO 435VP.

## RESULTS

On the 18th day of prenatal development, study by scanning electron microscope revealed rudiments of fungiform papillae as hemispherical, dome-shaped eminences. The rudiments were located irregularly on the dorsal surface of the apex of the tongue and on its margins. No rudiments of filiform papillae were observed at this stage (Fig. 1). At E20, scanning electron microscope showed, that the outline of the surface of the rabbit tongue was similar as at E18.

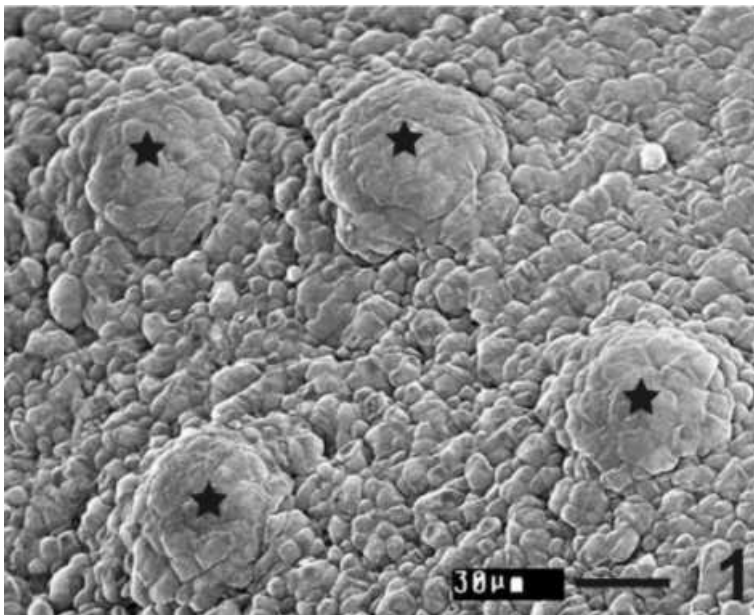


Fig. 1. E18: the rudiments of fungiform papillae (stars); scale bar = 30  $\mu\text{m}$

Rys. 1. E18: zawiązki brodawek grzybowatych (gwiazdki); skala = 30  $\mu\text{m}$

At E22, rudiments of fungiform papillae were additionally located in the anterior part of the dorsal surface of the body of the tongue. They were arranged irregularly. The rudiments of these papillae were observed also on both sides of forming torus linguae. The arrangement of these rudiments was linear. Also at this stage no rudiments of filiform papillae were seen. After removal the epithelium using NaOH maceration method, connective tissue cores of developing fungiform papillae were observed. Arrangement of the connective tissue cores corresponded to the topography of rudiments of fungiform papillae on the tongue, which

was displayed during examination of the surface of the tongue with the usage of scanning electron microscope. Moreover, connective tissue cores of rudiments of filiform papillae were observed. They covered completely surface of the apex, its margins, and the dorsal surface of the body of the tongue. Connective tissue cores of fungiform and filiform papillae were in form irregular protrusions (Fig. 2).

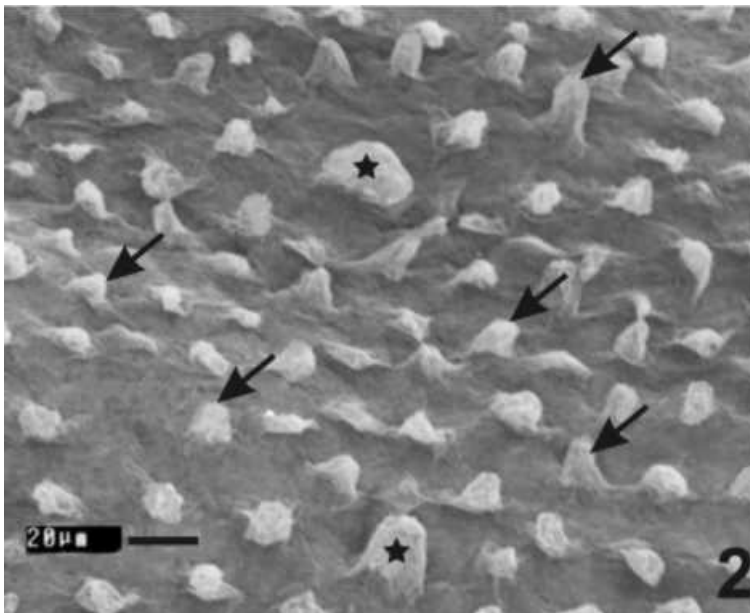


Fig. 2. E22: the connective tissue core of fungiform papillae (stars) and filiform papillae (arrows); scale bar = 20  $\mu$ m

Rys. 2. E22: zrąb łącznotkankowy brodawek grzybowatych (gwiazdki) i nitkowatych (strzałki); skala = 20  $\mu$ m

At E26, scanning electron microscope revealed that rudiments of filiform papillae were compactly and densely distributed over the entire dorsal surface of the apex, the body and on the margins of the tongue. Their tips were round and covered by desquamating epithelial cells. Rudiments of fungiform papillae were larger and were among developing filiform papillae on the areas described in the earlier stages (Fig. 3). After removal of epithelium, numerous palmate protrusions could be seen, forming connective tissue cores of rudiments of filiform papillae. The connective tissue cores of rudiments of fungiform papillae were of two forms. One of them was mace-shaped and the other one was bowl-shaped. On the surface of connective tissue cores of these rudiments some hollows were visible which were left by taste buds. (Figs. 4, 5).

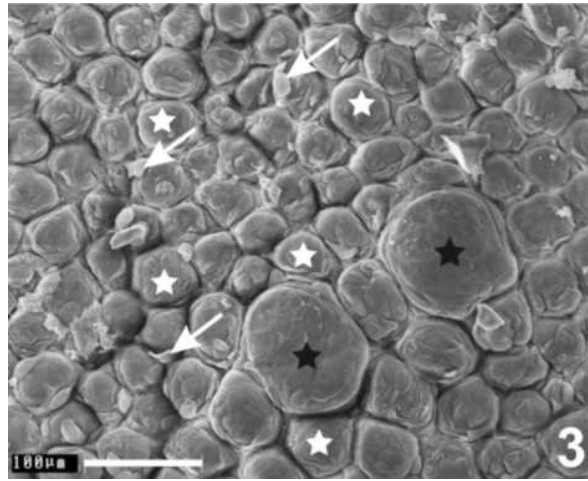


Fig. 3. E26: the fungiform papillae (black stars) and filiform papillae (white stars). Arrows indicate desquamating superficial epithelium cells; scale bar = 100  $\mu\text{m}$

Rys. 3. E26: brodawki grzybowate (czarne gwiazdki) i nitkowate (białe gwiazdki). Strzałki wskazują złuszczone komórki nabłonkowe; skala = 100  $\mu\text{m}$

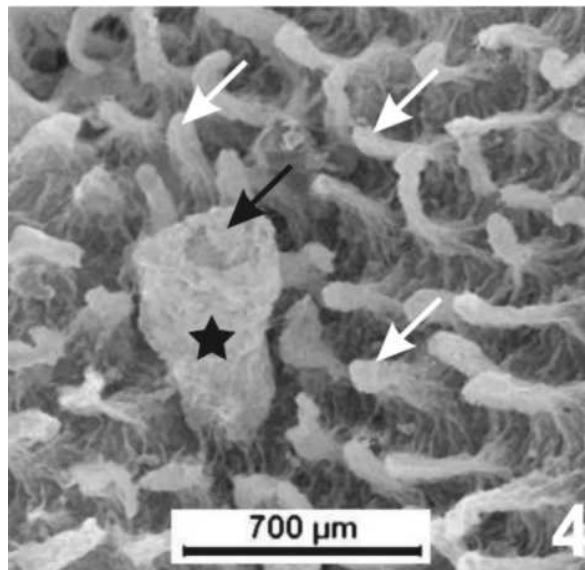


Fig. 4. E26: the mace-shaped connective tissue core of fungiform papilla (star) and connective tissue core of filiform papillae (white arrows). Black arrow indicates hollow which was left by taste buds; scale bar = 700  $\mu\text{m}$

Rys. 4. E26: buławkowaty zrąb łącznotkankowy brodawki grzybowatej (gwiazdka) i zrąb łącznotkankowy brodawek nitkowatych (białe strzałki). Czarna strzałka wskazuje dołek, który pozostawiły kubki smakowe; skala = 700  $\mu\text{m}$

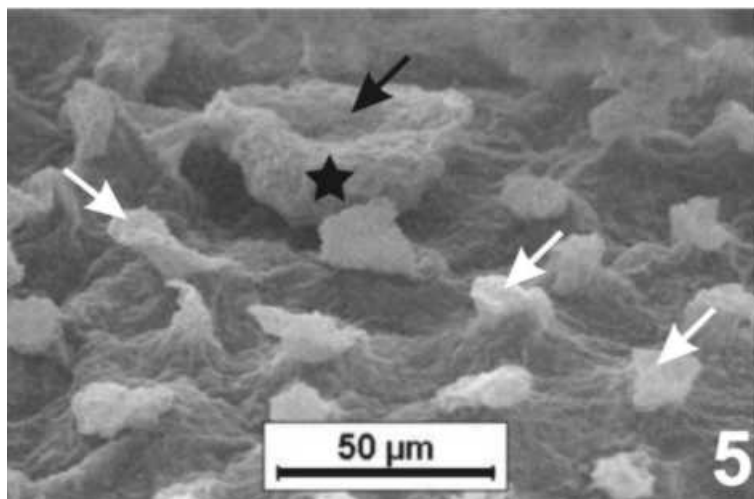


Fig. 5. E26: the bowl-shaped connective tissue core of fungiform papilla (star) and connective tissue core of filiform papillae (white arrows). Black arrow indicates hollow which was left by taste buds; scale bar = 50  $\mu$ m

Rys. 5. E26: miseczkowaty zrąb łącznotkankowy brodawki grzybowatej (gwiazdka) i zrąb łącznotkankowy brodawek nitkowatych (białe strzałki). Czarna strzałka wskazuje dołek, który pozostawiły kubki smakowe; skala = 50  $\mu$ m

At P1, developing filiform papillae still had round tips. The connective tissue core of each filiform papilla consisted of single protrusions, wider at the base and narrower at the tip (Fig. 6). Filiform papillae which were in the posterior part of the body of the tongue were tightly arranged. The connective tissue cores of these papillae were domed-shaped (Fig. 7). Developing fungiform papillae were scattered among the filiform papillae. They were numerous on the tip of the tongue and on its margins. There were desquamating epithelium cells on the surface of these papillae. Their connective tissue cores were mace-shaped and bowl-shaped.

At P15, distribution of filiform and fungiform papillae was the same as in the previous stage. The tips of filiform papillae were more slender, directed towards the throat. But the tips of the filiform papillae on the side parts of the dorsal area of the body of the tongue, nearby fungiform papillae, formed a rosette (Fig. 8). The most compact order of filiform papillae was still observed on the dorsal surface of the body of the tongue on its back. After removing the epithelium, the connective tissue cores of filiform papillae were seen as single protrusions as scanning electron microscope revealed. They were wider at the base and had round or spikey tips. The connective tissue cores of fungiform papillae were of three forms: mace, bowl and barrel-shaped. On its top surface there were hollows left by taste buds (Fig. 9).

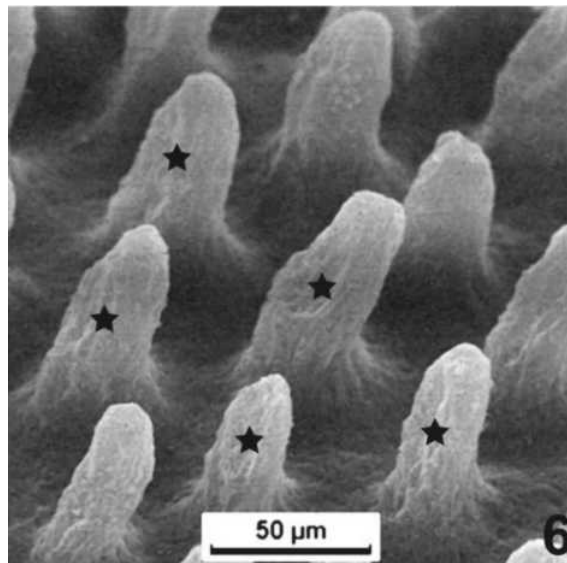


Fig. 6. P1: the connective tissue core of filiform papillae (stars); scale bar = 50 μm

Rys. 6. P1: zrąb łącznotkankowy brodawek nitkowatych (gwiazdki); skala = 50 μm

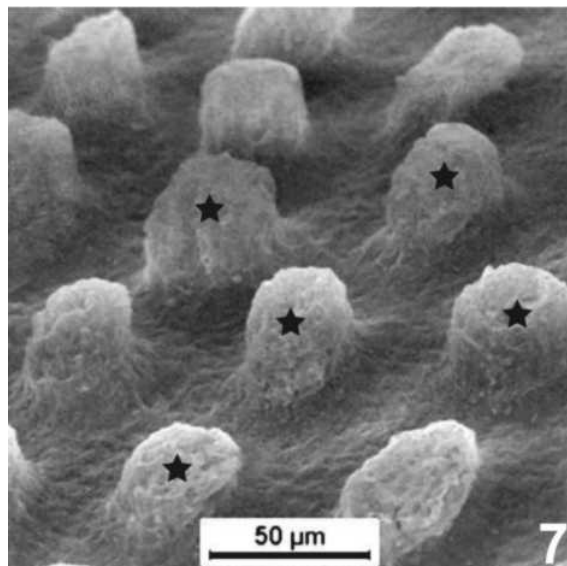


Fig. 7. P1: dome-shaped connective tissue core of filiform papillae (stars); scale bar = 50 μm

Rys. 7. P1: kopulastego kształtu zręby łącznotkankowe brodawek nitkowatych (gwiazdki); skala = 50 μm

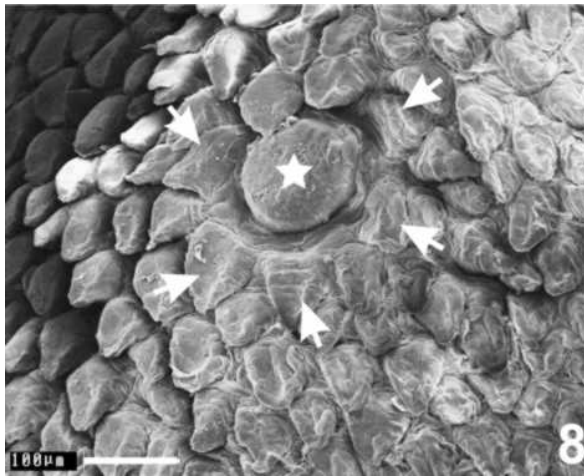


Fig. 8. P15: the fungiform papilla (star) and filiform papillae which forming rosette (arrows); scale bar = 100  $\mu\text{m}$

Rys. 8. P15: brodawka grzybowata (gwiazdka) i brodawki nitkowate które formują rozetę (strzałki); skala = 100  $\mu\text{m}$

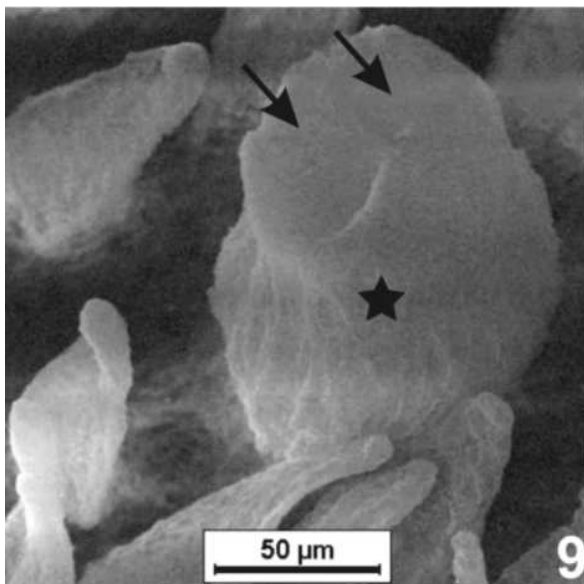


Fig. 9. P15: the barrel-shaped connective tissue core of fungiform papilla (star). Black arrows indicate hollows which were left by taste buds; scale bar = 50  $\mu\text{m}$

Rys. 9. P15: beczułkowaty zrąb łącznotkankowy brodawki grzybowatej (gwiazdka). Czarne strzałki wskazują dołki, które pozostawiły kubki smakowe; skala = 50  $\mu\text{m}$



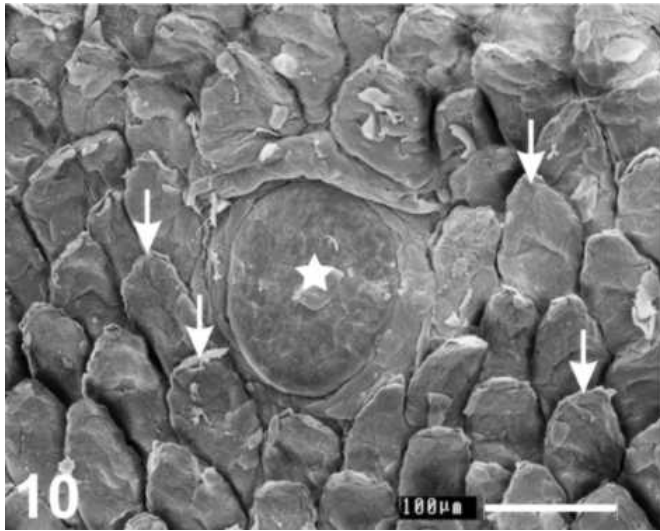


Fig. 10. P30: the fungiform papilla (star) and filiform papillae (arrows) with slightly round tips; scale bar = 100  $\mu$ m

Rys. 10. P30: brodawka grzybowata (gwiazdka) i brodawki nitkowate (strzałki) z lekko zaokrąglonymi końcami; skala = 100  $\mu$ m

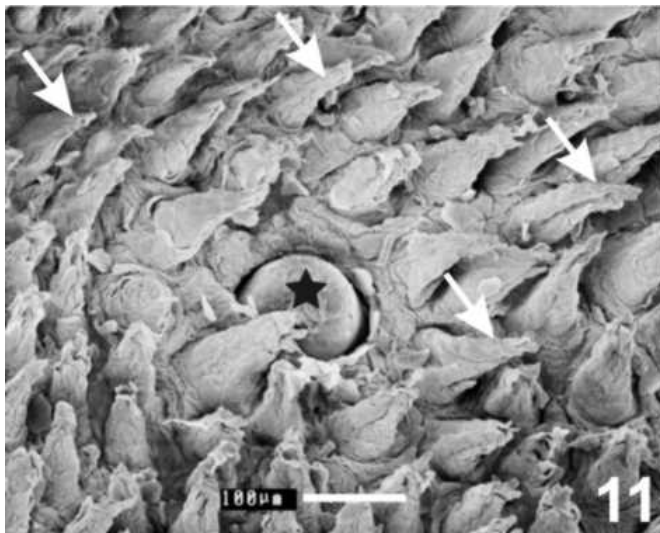


Fig. 11. P30: the fungiform papilla (star) and filiform papillae (arrows) with spiky tips; scale bar = 100  $\mu$ m

Rys. 11. P30: brodawka grzybowata (gwiazdka) i brodawki nitkowate (strzałki) z ostrymi końcami; skala = 100  $\mu$ m

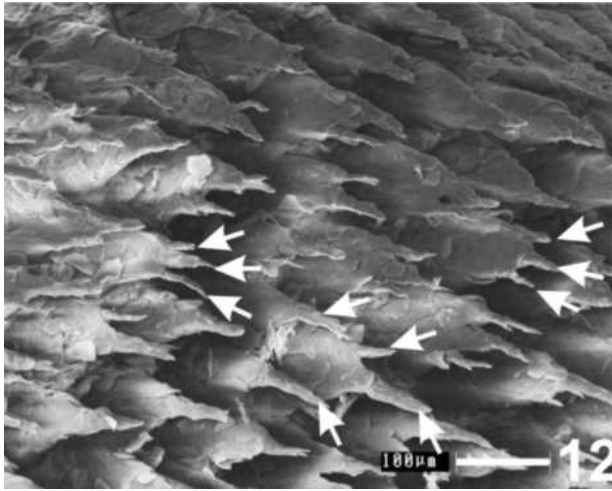


Fig. 12. P30: the filiform papillae with 3-4 processes (arrows); scale bar = 100  $\mu\text{m}$

Rys. 12. P30: brodawki nitkowate z 3-4 wyrostkami (strzałki); skala = 100  $\mu\text{m}$

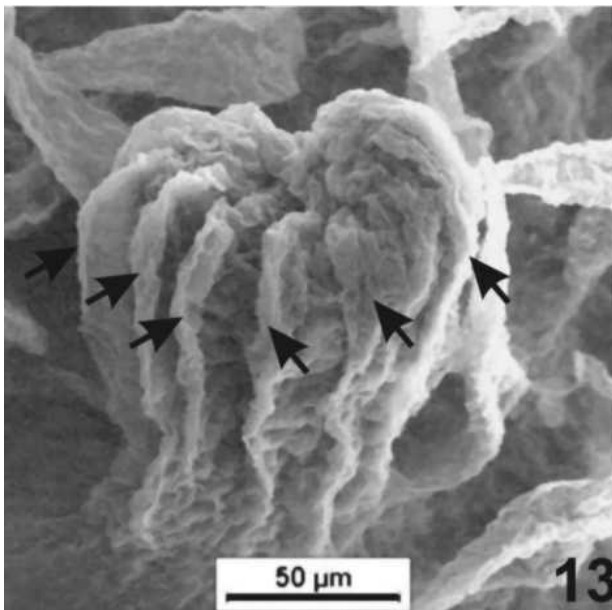


Fig. 13. P30: the connective tissue core of the fungiform papilla with parallel and longitudinal folds (arrows); scale bar = 50  $\mu\text{m}$

Rys. 13. P30: zrąb łącznotkankowy brodawki grzybowatej z równoległymi i podłużnymi fałdami (strzałki); skala = 50  $\mu\text{m}$

At P30, the filiform papillae were distributed on the rabbit tongue less compactly. Filiform papillae were of single cones with a slightly round tips (Fig. 10). But on the posterior part of the tongue, scanning electron microscope revealed filiform papillae with spiky tips (Fig. 11). The connective tissue cores of filiform papillae consisted of single cone-shaped protrusions, likewise at P15. At medial part of dorsal surface of the body of the tongue, filiform papillae had one central cone protrusion with 3–4 processes (Fig. 12). The connective tissue cores some of filiform papillae in this part of the tongue had also additional 3–4 short processes, branching off one central protrusion. Fungiform papillae were scattered among the filiform papillae (Figs. 10, 11). The biggest cluster of these papillae were observed on the top of the tongue and on its margins. On the surface of the connective tissue cores of fungiform papillae, from its top towards its base there were parallel and longitudinal folds. On the top of the connective tissue core some pits were observed likewise in previous stages (Fig. 13).

## **DISCUSSION**

The present study by scanning electron microscope indicates, that the morphogenesis of filiform and fungiform papillae is complicated process. Development of these papillae proceed both in prenatal and postnatal life.

Some studies revealed that the filiform papillae of various species had a diverse structural organization [Kullaa-Mikkonen et al. 1987, Estecondo et al. 2001]. Furthermore, the shape of these papillae varies in different regions of the tongue [Kurtul, Atalgin 2008]. Regional variation of filiform papillae, and their connective tissue cores also was observed in rabbit. The filiform papillae in the rabbit occurred in two forms as single papillae and ramified papillae. Additionally, the endings of filiform papillae were slightly circular or spiky. Some differences also concerned their distribution. On the sides of the dorsal surface of the tongue, the filiform papillae were distributed slightly more loosely than on the remaining areas of the tongue. Some researches point out that structural differences of taste papillae and their sizes depend on food and environmental conditions [Iwasaki 2002, Yoshimura et al. 2002]. These factors were perhaps because of some morphological changes that occurred during morphogenesis of filiform and fungiform papillae in rabbit. Rabbit during prenatal development has contact with amniotic fluid and with chemical substances that are present in it. After birth an animal is fed on mother milk and then regular food. Contact with chemicals contained in amniotic fluid probably stimulates development of the fetal taste buds which were described by Fujimoto et al. [1993]. Development of the taste buds are strictly connected with development of taste papillae such as fungiform papillae. It is important to remember that the crucial condition that effect the development of taste

structures has its innervation [Nakashima et al. 1990, Whitehead, Kachele 1994]. The kind of food that the rabbits eat and processes that undergo in oral cavity, probably causes development different form of filiform papillae. So that kind of papillae, their structure, sizes and distribution are species property.

The biggest structural diversity of fungiform papillae in the rabbit was observed after epithelium removal. During morphogenesis there were three types of connective tissue cores. They were mace, bowl and barrel-shaped. Diversity of shapes of connective tissue cores has confirmed the species distinctness in some research results concerning a tongue [Zheng, Kobayashi 2006, Emura et al. 2008]. Every examined species by authors had different form of connective tissue cores of papillae.

Iwasaki et al. [1997] and Witt and Reutter [1997] revealed by scanning electron microscope in mammals, that taste papillae appeared before mechanical papillae. Appearance of fungiform papillae (taste papillae) in rabbit first was confirmed by us.

In the rabbit the centre of fungiform papillae was noticed on the top of its tongue and its margins. The same observation was done in other animal species by Emura et al. [1999], Estecondo et al. [2001] and Ciuccio et al. [2008].

The present study do not revealed papillae on the ventral surface of the apex and on the lateral sides of the tongue. Yet, in other animal species some papillae on these areas can be found [Emura et al. 1999, 2008].

## **CONCLUSIONS**

1. The primordia of fungiform papillae can be observed as early as at E18, but the filiform papillae – at E26.
2. During morphogenesis two types of filiform papillae were noticed. One type of filiform papillae was similar to separated cones, another had 3–4 processes more. The fungiform papillae had three types of connective tissue cores: mace-shaped, bowl-shaped and barred-shaped.

## **ACKNOWLEDGEMENTS**

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**BADANIE PRZY POMOCY MIKROSKOPU ELEKTRONOWEGO  
SKANINGOWEGO MORFOGENEZY BRODAWEK NITKOWATYCH  
I GRZYBOWATYCH U KRÓLIKA, *ORYCTOLAGUS CUNICULUS*  
*F. DOMESTICA***

**Streszczenie.** Zawiązki brodawek grzybowatych obserwowano w 18 dniu rozwoju prenatalnego. Były one rozmieszczone na powierzchni grzbietowej wierzchołka języka i na jego brzegach. Brodawki grzybowate wyglądały jak półkoliste, kopulaste wyniosłości. Od 22 dnia rozwoju prenatalnego, mikroskop elektronowy skaningowy wykazał, że zawiązki brodawek grzybowatych pojawiły się także na trzonie języka przed formującym się wałem języka i po jego bokach. Żadnych zawiązków brodawek nitkowatych nie obserwowano w 22 dniu rozwoju prenatalnego. Jednak po usunięciu nabłonka, zręby łącznotkankowe zawiązków brodawek grzybowatych i nitkowatych były widoczne. Mikroskop elektronowy skaningowy wykazał na powierzchni języka liczne brodawki nitkowate dopiero w 26 dniu rozwoju prenatalnego. Były one rozmieszczone na całej powierzchni grzbietowej wierzchołka i trzonu języka i na jego brzegach. Podczas morfogenezy zanotowano dwa typy brodawek nitkowatych. Jeden typ brodawek nitkowatych był podobny do pojedynczych stożków a drugi miał dodatkowe 3–4 wyrostki. Podobne zmiany dotyczyły kształtu zrębu łącznotkankowego. Brodawki grzybowate zlokalizowane były pośród brodawek nitkowatych. Mikroskop elektronowy skaningowy wykazał trzy typy zrębów łącznotkankowych brodawek grzybowatych. Jeden typ był kształtu buławkowatego, drugi miseczkowatego a kolejny beczułkowatego. W 30 dniu rozwoju postnatalnego, na powierzchni zrębów łącznotkankowych brodawek grzybowatych, od ich wierzchołka w kierunku podstawy przebiegały podłużne i równoległe fałdy.

**Słowa kluczowe:** brodawki językowe, królik domowy, morfogeneza

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