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SCANNING ELECTRON MICROSCOPICAL STUDIES OF DEVELOPING OF VALLATE PAPILLAE IN THE RABBIT (*ORYCTOLAGUS CUNICULUS* F. *DOMESTICA*)*

SKANINGOWE BADANIA MIKROSKOPOWE ROZWOJU BRODAWEK OKOLONYCH U KRÓLIKA (*ORYCTOLAGUS CUNICULUS* F. *DOMESTICA*)

Summary. This study was conducted on 30 tongues of the rabbits (*Oryctolagus cuniculus* f. *domestica*) of both sexes, which were collected at day 18, 20, 22 and 26 of prenatal development (E) and at day 1, 15 and 30 of postnatal life (P). Developmental changes of the rabbit vallate papillae were studied using scanning electron microscopy (SEM). The study showed that the first primordia of vallate papillae were observed at E18. At E20, SEM revealed that primordia of this papillae could be recognized as a circular structures. The furrow of vallate papillae started to form from E22 and finished at P30. After removal the epithelium of the developing vallate papilla, round connective tissue core was exposed. From P1, connective tissue cores of these papillae were formed by varied in length and shape folds. There were irregular hollows between folds. Around connective tissue core there was a circular depression and elevation. Starting from E26 on the surface of developing papillae exfoliating epithelium cells were observed.

Key words: connective tissue core, morphogenesis, scanning electron microscope, vallate papillae

Introduction

Vallate papillae are one of three types of gustatory papillae found on the rabbit tongue. They were described in adult rabbits by KULAWIK and GODYNICKI (2007). These papillae were examined also in many other animal species, e.g. dog (IWASAKI and

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SAKATA 1985), bat (AZZALI et AL. 1992), rat (IWASAKI et AL. 1997) and in human (JUNG et AL. 2004). There are also animal species with no vallate papillae observed on the tongue, e.g. penguin (KOBAYASHI et AL. 1998) and lizard (IWASAKI 1990).

Numerous studies are devoted to the development of lingual papillae, including also vallate papillae, in sheep (TICHÝ 1992), pig (TICHÝ 1991, 1992), cat (TICHÝ 1993), mouse (AHPIN et AL. 1989, IWASAKI et AL. 1996), rat (IWASAKI et AL. 1997) and in human (WITT and REUTTER 1997). Scientific literature reports results of research on the dynamics of the development of vallate papillae on the rabbit tongue (KULAWIK and GODYNICKI 2008). Moreover, in the recent literature, the authors described the morphogenesis of vallate papillae on the basis of studies in the light microscopy (KULAWIK et AL. 2013). However in the literature there is lack of description of the development of these papillae using scanning electron microscope. Supplementary studies are needed to be conducted in this respect. The present paper describes scanning electron microscopic features of morphogenesis of the vallate papillae in the rabbit (*Oryctolagus cuniculus* f. *domestica*) from 18th day of prenatal development to 30th day of postnatal period.

Material and methods

The material for the analysis consisted of 30 tongues of rabbits (*Oryctolagus cuniculus* f. *domestica*), of both sexes. Tongues for this study were collected on 18th (3 tongues), 20th (3 tongues), 22nd (11 tongues) and 26th (5 tongues) day of prenatal development (E) and 1st (3 tongues), 15th (3 tongues) and 30th (2 tongues) day of postnatal period (P). The age of fetuses was determined on the basis of the CRL value (EVANS and SACK 1973). Studies on animals were conducted with approval by the Local Ethics Committee, permission No. 4/2000.

For observations, using a scanning electron microscopy (SEM), tongues were fixed in the Karnovsky solution (4°C, pH 7.2) for 24 h. Then the tissue samples with the vallate papillae were dehydrated through a graded series of ethanol (30-100%), critical point dried, mounted on aluminum stubs and coated with gold.

For the purpose of observations of the connective tissue core of vallate 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 two primordia of vallate papillae were observed. They were located on the root of the tongue symmetrically, on the both sides in the sagittal plane of the tongue. The surface of the tongue was uneven in places where the primordia were found. Higher magnification showed that some superficial epithelial cells of developing vallate papillae were relatively big (Figs. 1, 2).

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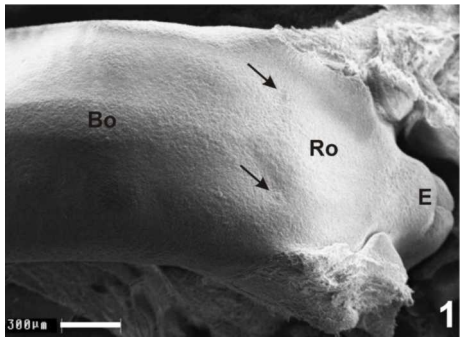

Fig. 1. E18: SEM showing body (Bo) and root (Ro) of the tongue with primordia of vallate papillae (arrows), E – epiglotis; scale bar = 300 µm
Rys. 1. E18: SEM ukazuje trzon (Bo) i korzeń (Ro) języka z zawiązkami brodawek okolonych (strzałki), E – nagłośnia; skala = 300 µm

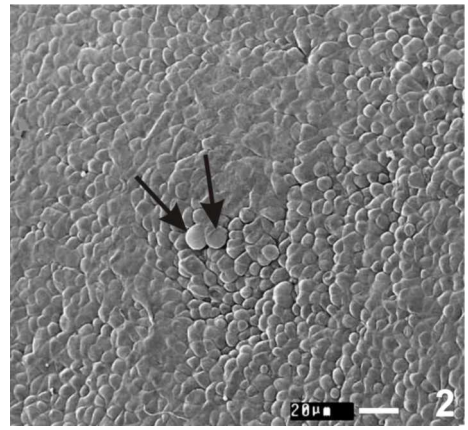

Fig. 2. E18: Primordium of vallate papilla with some big superficial cells of the epithelium (arrows); scale bar = 20 µm
Rys. 2. E18: Zawiązek brodawki okolonej z dużymi komórkami powierzchniowymi nabłonka (strzałki); skala = 20 µm

At E20, SEM revealed that primordia of vallate papillae could be recognised as a circular structures. Primordia of these papillae distinctly were separated from the vicinity. Their surface was covered by nearly flat epithelium (Fig. 3).

At E22, the body of developing vallate papillae was surrounded by longitudinal depressions. It was the first symptom of forming furrows of vallate papillae. On the dorsal surface of papillae, irregular in shape and of different sizes, superficial epithelial cells were observed by the SEM. Some of them had processes, with which were connected. At higher magnification showed that on the superficial cells and on the borders between adjacent cells were scattered microvilli (Figs. 4, 5). After removal, the epithelium of the developing vallate papilla, round connective tissue core was exposed. On the surface of the connective tissue core some pits were visible. The connective tissue core was surrounded by a circural depression (Fig. 6).

At E26, observations using SEM showed that primordia of vallate papillae were covered by the epithelium, which few superficial cells were exfoliating. Developing papillae were round in outline and surrounded by narrow circular depression (Fig. 7). The connective tissue cores of papillae were uneven on the surface. There was a circural hollow around connective tissue core. From its external side there was a discontinuous circural elevation (Fig. 8).

In newborn rabbits (P1), SEM revealed that on the body of vallate papillae there were grooves, so their surfaces were uneven and covered by many exfoliating epithelium cells. On the surface of the tongue, just around examined papillae, was a distinctive unbroken furrow. The furrow of vallate papillae was similar to a narrow fissure (Fig. 9). After removal of the epithelium of vallate papilla, numerous folds of connective tissue

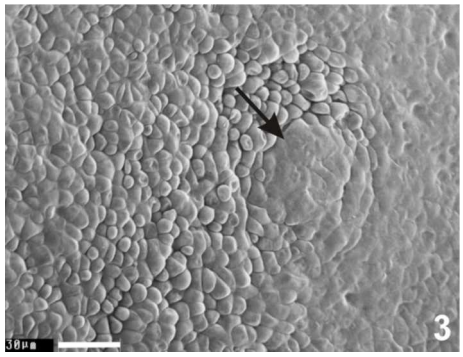

Fig. 3. E20: SEM showing round shaped primordium of vallate papilla (arrow); scale bar = 30 µm
Rys. 3. E20: SEM ukazuje kolisty zawiązek brodawki okolonej (strzałka); skala = 30 µm

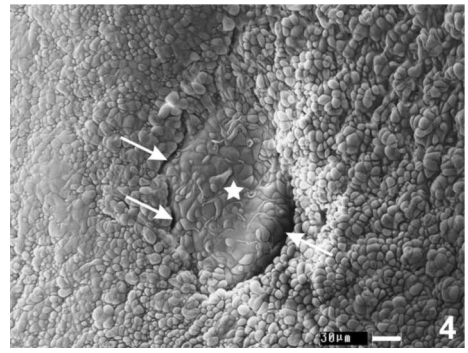

Fig. 4. E22: SEM showing primordium of vallate papilla (star) and longitudinal depressions (arrows); scale bar = 30 µm
Rys. 4. E22: SEM ukazuje zawiązek brodawki okolonej (gwiazdka) i podłużne zagłębienia (strzałki); skala = 30 µm

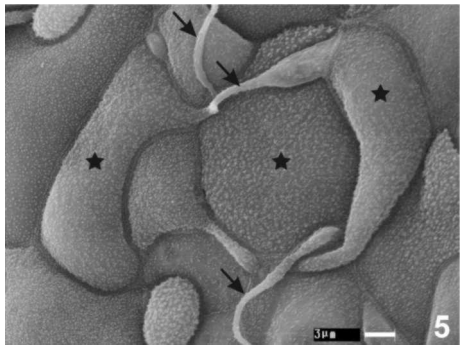

Fig. 5. E22: Irregular in shape superficial cells of primordium of vallate papilla (stars) with projections (arrows); scale bar = 3 µm
Rys. 5. E22: Nieregularnego kształtu komórki powierzchniowe zawiązka brodawki okolonej (gwiazdki) z wyrostkami (strzałki); skala = 3 µm

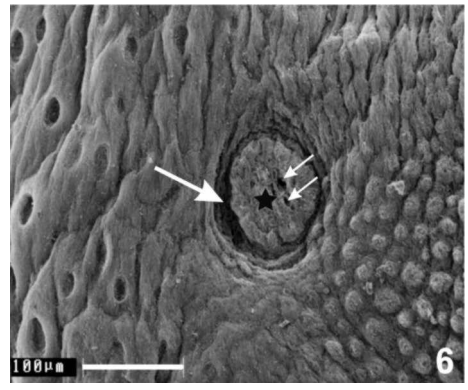

Fig. 6. E22: Connective tissue core of developing vallate papilla (star) with pits on the surface (small arrows) and circular depression (big arrow); scale bar = 100 µm
Rys. 6. E22: Zrąb łącznotkankowy rozwijającej się brodawki okolonej (gwiazdka) z dołkami na powierzchni (małe strzałki) i kolistym zagłębieniem (duża strzałka); skala = 100 µm

core were exposed. The folds were of different shapes. Between them there were some irregular depressions. Around the connective tissue core there was a hollow. On the bottom of the hollow there were numerous orifices. There was also a circural elevation around this hollow. In contrast to the previous stage of development, the circural elevation was unbroken (Fig. 10).

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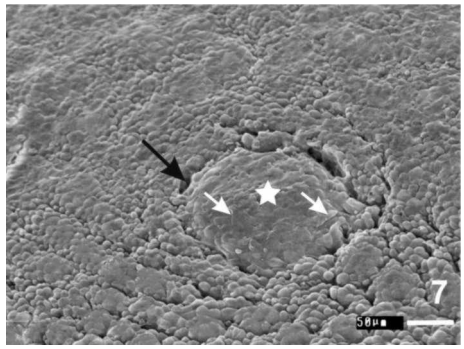

Fig. 7. E26: SEM showing round in outline primordium of vallate papilla (star), surrounded by narrow circular depression (big arrow), small arrows indicate exfoliating epithelial cells; scale bar = 50 µm
Rys. 7. E26: SEM ukazuje kolisty w zarysie zawiązek brodawki okolonej (gwiazdka), otoczony przez wąskie koliste zagłębienie (duża strzałka), małe strzałki wskazują złuszczające się komórki nabłonkowe; skala = 50 µm

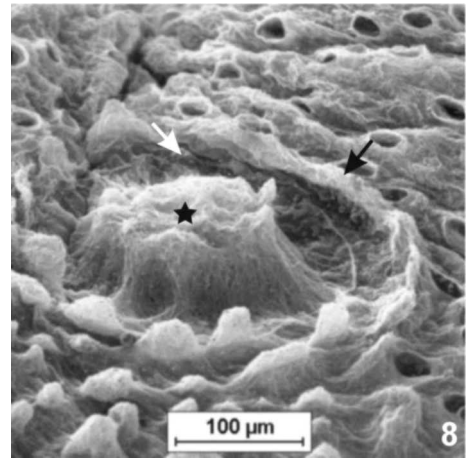

Fig. 8. E26: Connective tissue core of developing vallate papilla (star), surrounded by circular depression (white arrow) and circular elevation (black arrow); scale bar = 100 µm
Rys. 8. E26: Zrąb łącznotkankowy rozwijającej się brodawki okolonej (gwiazdka), otoczony przez koliste zagłębienie (biała strzałka) i koliste wzniesienie (czarna strzałka); skala = 100 µm

At postnatal 15thday, each round shaped vallate papilla was surrounded by the narrow furrow of the papilla. There was also visible an outer wall of the papilla. Higher magnification showed that on the dorsal surface of vallate papillae the superficial epithelium cells were exfoliating. There were taste pores on the surface of papillae (Fig. 11). Connective tissue core of papillae was similar to the one which was observed in P1.

SEM observations showed that at P30, the surface of vallate papillae was very much grooved with single outgrowths on it. The furrow of each vallate papilla did not resemble the narrow fissure as in the previous periods. It was wide and separated the body of papilla from the outer wall of papilla. SEM revealed that the outer wall of some vallate papillae was not visible. This was because it was connected with the surface of the tongue with no distinct border. At higher magnification showed taste pores in the epithelium covering dorsal surface of vallate papillae (Fig. 12). The connective tissue core of vallate papillae had many of different shapes folds. Between the folds there were hollows, simillary to earlier periods. The connective tissue core was surrounded by circular depression and elevation.

In these studies there were no differences in sex and ontogenic in the morphogenesis of vallate papillae of the rabbit tongue.

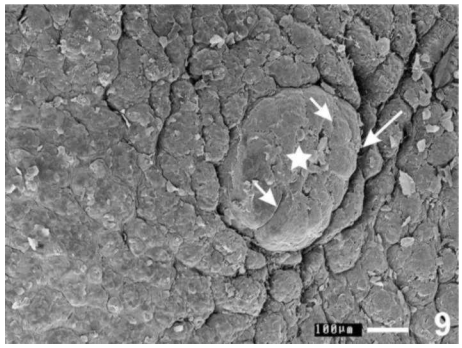

Fig. 9. P1: SEM showing an uneven surface of vallate papilla (star) with grooves (small arrows) surrounded by narrow fissure (big arrow); scale bar = 100 µm
Rys. 9. P1: SEM ukazuje nierówną powierzchnię brodawki okolonej (gwiazdka) z bruzdami (małe strzałki) otoczoną przez wąską szczelinę (duża strzałka); skala = 100 µm

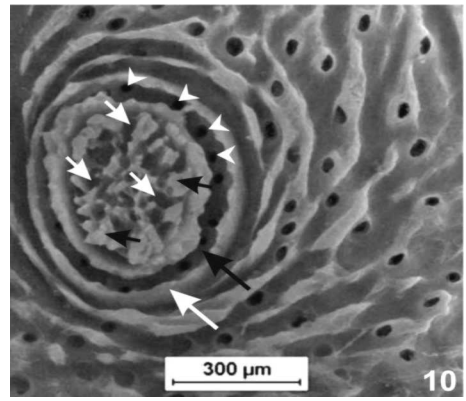

Fig. 10. P1: Connective tissue core of vallate papilla with folds (small black arrows) and hollows (small white arrows), surrounded by round in outline depression (big black arrow) and elevation (big white arrow). At the bottom of depression there are orifices (white arrowheads); scale bar = 300 µm
Rys. 10. P1: Zrąb łącznotkankowy brodawki okolonej z fałdami (małe czarne strzałki) i dołkami (małe białe strzałki), otoczony przez koliste w zarysie zagłębienie (duża czarna strzałka) i wzniesienie (duża biała strzałka). Na dnie zagłębienia znajdują się dołki (białe groty); skala = 300 µm

Discussion

It was shown in the investigation using SEM that as early as on the 18th day of prenatal development in the rabbit, the tongue was formed from its primordia. At that time the symptoms of the formation of vallate papillae were also observed. Their shape and structure were different from those described previously in adult rabbits (KULAWIK and GODYNICKI 2007). In the rabbit primordia of vallate papillae appear simultaneously. However in humans, one central primordium of a vallate papilla appears first, then the next ones (WITT and REUTTER 1997).

Two developing vallate papillae of the rabbit were located simetrically on the root of the tongue. For comparison, in the hamster (MILLER and SMITH 1988), mouse (‚AHPIN et AL. 1989, IWASAKI et AL. 1996) and rat (IWASAKI et AL. 1997) only one vallate papilla was found on the tongue. This papilla was located in the middle of the posterior part of the tongue.

Starting from E22, the developing vallate papillae in the rabbit changes significantly. On the surface of the body of each primordium of papilla there were cells of irregular shapes, which cell processes formed intercellular connections. In other authors publications (IWASAKI et AL. 1996, 1997, WITT and REUTTER 1997, ELNASHARTY et AL. 2013)

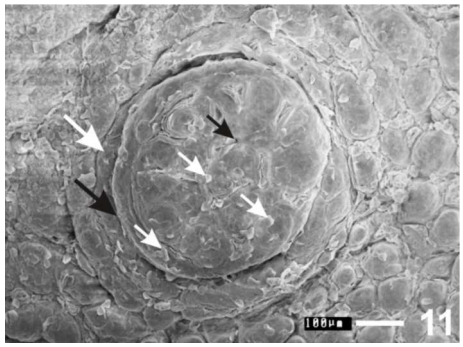

Fig. 11. P15: The vallate papilla with a wall (big white arrow) and furrow (big black arrow) of papilla. On the grooved dorsal surface of papilla SEM showing taste pores (small black arrow) and exfoliating epithelial cells (small white arrows); scale bar = 100 µm

Rys. 11. P15: Brodawka okolona z wałem (duża biała strzałka) i rowkiem (duża czarna strzałka) brodawki. Na pobrużdżonej powierzchni grzbietowej brodawki SEM ukazuje pory smakowe (mała czarna strzałka) i złuszczające się komórki nabłonkowe (małe białe strzałki); skala = 100 µm

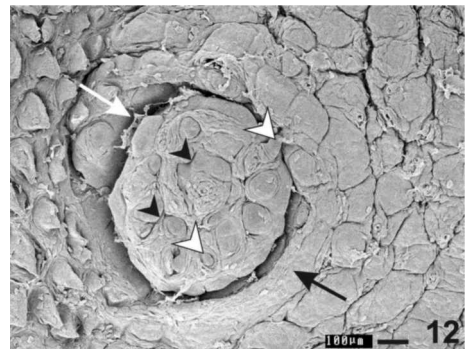

Fig. 12. P30: The vallate papilla with a wall (black arrow) and wide furrow (white arrow) of papilla. SEM showing projections (white arrowheads) and grooves (black arrowheads); scale bar = 100 µm

Rys. 12. P30: Brodawka okolona z wałem (czarna strzałka) i szerokim rowkiem (biała strzałka) brodawki. SEM ukazuje wyrostki (białe groty) i bruzdy (czarne groty); skala = 100 µm

there was no information about similar research results. This might suggest that the connections are for intercellular communication during the developement of vallate papillae. It is known that the morphogenesis of papillae is a complicated process, in which hormones play a big role as well as interaction between the epithelium and the mesenchymal tissue, as it was indicated by MACKENZIE (1984) and SANDERS (1988). Development of taste buds and taste papillae on the tongue depends on sensory nerves (AHPIN et AL. 1989, FARBMAN and MBIENE 1991). It was also confirmed experimentally by denervation of taste papillae (GANCHROW and GANCHROW 1989, NAKASHIMA et AL. 1990).

Vallate papillae observed in the rabbit in all developmental stages were round in contour. The similar shape of papillae was observed in the bat (AZZALI et AL. 1992) and formosan serow (ATOJI et AL. 1998). In contrast in mouse (IWASAKI et AL. 1996), rat (IWASAKI et AL. 1997), blind mole rat (KILINC et AL. 2010) and dasypus hybridus (CIUCCIO et AL. 2010), the vallate papillae found on the tongues were oval in shape. AGUNGPRIYONO et AL. (1995) revealed on the tongue of lesser mouse deer, only in one individual two types of papillae; elongated and round-shaped types.

This investigations showed that the primordia of vallate papillae can be observed as early as at E18. Similar results were demonstrated in the work by ELNASHARTY et AL. (2013). Vallate papillae of the rabbit tongue changed their structure and the shape in the analysed periods of pre- and postnatal development. At P30, the vallate papillae were

similar to those observed in adult rabbit, which were described by KULAWIK and GODYNICKI (2007).

The architecture of the connective tissue core of vallate papillae is different depending on species. This can be the reason why many authors conduct their research on connective tissue core of papillae using NaOH maceration method (WATANABE et AL. 1997, MARTINEZ et AL. 2000, KOBAYASHI et AL. 2004).

In summary, the present report first describes by SEM, that development of vallate papillae in rabbits is a long and complicated process which lasts in prenatal and postnatal periods.

Conclusions

1. The primordia of vallate papillae can be observed as early as at E18.
2. The furrow of vallate papillae started to form at E22.
3. The connective tissue core of vallate papillae was observed from E22.
4. Starting from P1, connective tissue core consisted of folds and hollows surrounded by circular depressions and elevation.

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SKANINGOWE BADANIA MIKROSKOPOWE ROZWOJU BRODAWEK OKOLONYCH U KRÓLIKA (*ORYCTOLAGUS CUNICULUS* F. *DOMESTICA*)

Streszczenie. Badania prowadzono na 30 językach królików (*Oryctolagus cuniculus* f. *domestica*) obu płci, które pobrano w 18., 20., 22. i 26. dniu rozwoju płodowego (E) oraz w 1., 15. i 30. dniu życia postnatalnego (P). Zmiany rozwojowe brodawek okolonych badano, używając skaningowego mikroskopu elektronowego (SEM). Pierwsze zawiązki brodawek okolonych obserwowano w E18. W E20 SEM wykazał, że zawiązki tych brodawek były możliwe do rozpoznania jako struktury koliste. Rowek brodawek okolonych zaczął się formować w E22 i zakończył w P30. Po usunięciu nabłonka z rozwijających się brodawek okolonych ujawnił się okrągły zrąb łącznotkankowy. Od P1 zrąb łącznotkankowy tych brodawek był uformowany przez różnej długości i różnego kształtu fałdy. Pomiędzy fałdami znajdowały się nieregularne zagłębienia. Dookoła zrębu łącznotkankowego znajdowało się koliste zagłębienie i wzniesienie. Począwszy od E26 na powierzchni rozwijających się brodawek obserwowano złuszczające się komórki nabłonkowe.

Słowa kluczowe: zrąb łącznotkankowy, morfogeneza, mikroskop elektronowy skaningowy, brodawki okolone

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