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## THE POSTNATAL DEVELOPMENT OF THE TONGUE IN THE RABBIT (*ORYCTOLAGUS CUNICULUS*): GROSS AND SCANNING ELECTRON MICROSCOPICAL STUDIES

(With 27 Figures)

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تطور اللسان في الأرنب في فترة ما بعد الولادة: دراسات عينية  
وميكروسكوبية إلكترونية ماسحة

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استخدم في هذه الدراسة عدد واحد وعشرين أرنباً تمثل الأعمار المختلفة في فترة ما بعد الولادة وحتى البلوغ، وذلك لرصد التغيرات الشكلية العينية والمجهورية الماسحة في اللسان وحلماته خلال هذه الفترة. كان اللسان في الأرانب حديثة الولادة رقيقاً وطوله حوالي 1.5 سم ولم يلاحظ وجود أي حلمات بالعين المجردة. مع تقدم العمر زاد اللسان في الطول وأمكن تمييز حشوية لسانية واضحة على معظم المسطح العلوي لجسم اللسان، هذا وقد أمكن تمييز الحلمات الميزابية والورقية بالعين المجردة عند عمر أربعة أسابيع. أما على مستوى الميكروسكوب الإلكتروني الماسح فقد كانت الحلمات الخيطية في الحيوانات حديثة الولادة موجودة فقط على مقدمة اللسان وعلى أسطحه الجانبية، ثم امتدت هذه الحلمات لتغطي ظهر اللسان كلياً في الأرانب البالغة. كذلك وكانت هذه الحلمات مختلفة في الشكل من مكان إلى آخر حيث كانت لسانية الشكل في مقدمة اللسان بينما كانت مخروطية ومنثنية للخلف في الجزء الخلفي منه. أما فيما يخص الحلمات الفطرية فقد كانت قبية الشكل على مقدمة اللسان وظهر بها براعم تدوق في الأرانب حديثة الولادة وحتى مرحلة البلوغ، بينما كانت الحلمات الفطرية الموجودة على أسطح اللسان الجانبية زرارية الشكل ويتقدم العمر فتدت هذه الحلمات الميزاب الحلمي بإتحادها مع بعض الحلمات الخيطية المجاورة. هذا وقد أظهرت الدراسة أن الحلمات الميزابية والورقية كانت أقل تطوراً في الحيوانات حديثة الولادة حيث كانت الميزاب الحلمية غير منتظمة ثم يتقدم العمر تميزت هذه الحلمات أكثر وأكثر. يستخلص من هذه الدراسة أن الأرانب تولد بلسان ذو وظائف حركية وتنوقية قليلة والتي تزداد بتقدم العمر نظراً لزيادة عدد الحلمات الخيطية على ظهر اللسان وتطور الحلمات الميزابية والورقية كذلك.

## SUMMARY

Twenty one rabbits, representing various postnatal ages from newly born to four months old (mature rabbits), were used to elucidate the postnatal changes in the morphology of the rabbit tongue grossly and scanning electron microscopically. The tongue of newly born rabbits is thin, measuring 1.5cm long, no papillae could be demonstrated at this age. In the growing rabbits, the tongue increases in length and thickness, an intermolar prominence (Torus linguae) starts to occupy most of the dorsal aspect of the body of the tongue in two weeks old rabbits. The filiform and fungiform papillae are difficult to be observed grossly, where the vallate (two in number) and foliate papillae could be demonstrated in the fourth postnatal week. Scanning electron microscopy reveals that the filiform papillae (the only mechanical type found in rabbit) in newly born rabbits are found only on the tip and lateral surfaces, but absent on the dorsum of the tongue. By advancement of age, they spread on the dorsum of the tongue until the later is completely covered by papillae in mature rabbits. The filiform papillae exhibit regional variations in shape being tongue-shaped on the apex, but they are conical and bent backward on the body. Concerning the fungiform papillae, they are demonstrated on the tip and lateral surfaces of the tongue. The fungiform papillae on the tip of the tongue are dome-shaped, have taste pores and supposed to perform the gustatory function in suckling rabbits. The laterally placed fungiform papillae are button-shaped having no taste pores, they also lose their papillary groove and fuse with the surrounding filiform papillae by advancement of age. The vallate and foliate papillae are weakly developed in newly born rabbits. The papillary grooves are not well defined and the taste pores could not be detected at this stage. By advancement of age, these two kinds of papillae become well developed and demonstrate taste pores on their surfaces. The surface epithelium of the different lingual papillae demonstrates many stubby microvilli in newly born that change gradually to microplicae in adult animals. In conclusion, the mechanical and gustatory functions of the rabbit tongue seem to be weakly developed in newly born rabbits due to the absence of filiform papillae from its dorsum and the weakly developed vallate and foliate papillae at this stage. This weak gustatory function is supposed to be undertaken only by fungiform papillae, but in mature rabbits, the well developed vallate and foliate papillae may improve this function.

*key words: Rabbit, Tongue, Gross Anatomy, Scanning electron microscopy.*

## INTRODUCTION

The rabbit is a non-ruminant herbivore that tends to select low fibre portions of plant material (Van Soest, 1982). Its tongue is large for the size of the animal, it possesses four types of papillae; namely, vallate, foliate, fungiform and filiform papillae (Okerman, 1994). There are many studies on the three-dimensional structure of the tongue and its papillae in rat (Baratz, and Farbman, 1975), goat (Qayyun and Berg, 1975), cat (Boshell *et al.*, 1982), rabbit (Lui and Lee, 1982), Cow (Steflik *et al.*, 1983; Chamorro *et al.*, 1987), horse (chamorro *et al.*, 1986; de Pass Cabello *et al.*, 1988), one-humped camel (Qayyun *et al.*, 1988), black rhinoceros (Emura *et al.*, 2000), bactrian camel (Eerdunchaolu *et al.*, 2001), donkey (Abd-Elnaeim *et al.*, 2002) and koala (Kobayashi *et al.*, 2003). Little work has been published on the development of the lingual papillae, Dougbag (1987, 1988) has studied the morphogenesis of the mechanical and gustatory papillae of the one-humped camel.

The young rabbits start hopping during the third postnatal week and can be weaned at four weeks old, small breeds are sexually mature at three months (Okerman, 1994). Important morphological changes are expected to occur in the tongue of the rabbit during the postnatal period. Studies on the postnatal development of the tongue and its papillae in the rabbit is lacking in the available literature. This study aims to elucidate the postnatal changes in the tongue and its papillae both grossly and scanning electron microscopically.

## MATERIALS and METHODS

Twenty-one Balady rabbits representing different ages ranging from newborn (one day old) through growing (2-10 weeks old) to mature (3-4 months old) were used in this study. For gross anatomy, the rabbits were perfused through the common carotid artery using 4% paraformaldehyde solution. The tongues were then removed, examined grossly, measured and photographed. For scanning electron microscopy, representative tissue samples from all lingual papillae were taken, fixed in paraformaldehyde 2.5% and glutaraldehyde 2.5% solution in 0.1M phosphate buffer for 4 hours at 4°C. After washing in the same buffer, the specimens were post-fixed in osmium tetroxide 1% in phosphate buffer for 2 hours followed by washing in the same buffer. The samples were then dehydrated in ascending grades of ethanol followed by critical

point drying in carbon dioxide then sputter-coated with gold and examined with a JEOL-5400LV scanning electron microscope.

## RESULTS

### **Anatomy:**

The tongue of the newly born rabbits is thin measuring about 1.5cm long with relatively short apex (0.5cm). The dorsal surface of the tongue demonstrates a slight elevation in the body region. At this stage, the lingual papillae could not be observed grossly (Fig.1).

In the growing rabbits, the tongue increases in length and thickness, it reaches about 2.4cm long in 4 weeks old rabbits. In two weeks old rabbits, a clear intermolar prominence starts to occupy most of the dorsal aspect of the body of the tongue. This prominence increases in size with the advancement of age. The filiform and fungiform papillae are difficult to be observed grossly. However two vallate papillae could be demonstrated in the fourth postnatal week (Fig.1).

In adult rabbits (3-4 months old), the tongue (about 3.6cm long) demonstrates a constriction between its apex (about 1.2cm long) and body. The apex is three sided in cross section due to high lateral borders. The intermolar prominence becomes well developed. There are two vallate papillae, one on each side of the midline at the caudal boundary of the intermolar prominence, while the foliate papillae are situated in the caudo-lateral margin in front of the palatoglossal folds (Fig.1).

### **Scanning Electron Microscopy:**

#### ***Filiform papillae:***

In the newly born rabbits, the filiform papillae are demonstrated only on the tip and the lateral surfaces of the tongue, the dorsum is bare (Fig.2). They are either conical with round or pointed ends, the pointed ones are usually bent backwards (Figs.4, 5). In higher magnification, the surface epithelium of both filiform papillae and the dorsum of the tongue is covered by many stubby-shaped microvilli (Figs.3, 6).

In growing rabbits (2-4 weeks old), the filiform papillae are arranged in compact rows on the tip and the lateral surfaces of the tongue. By advancement of age, they spread caudally, where they cover most of the dorsal surface of the tongue at the 10<sup>th</sup> postnatal week (Fig.7). They are tongue-shaped on the apex, conical laterally and weakly developed on the dorsal aspect of the intermolar prominence (Figs.8, 9). In higher magnification, the surface epithelium of the filiform papillae shows concentrically arranged microplicae (Fig.10).

In the adult rabbits, the filiform papillae are arranged in the form of compact rows distributed over the entire dorsal and lateral surfaces of the tongue (Fig.11). They are thick tongue-shaped on the apex (Fig.12), but they are conical with wide intervening spaces on the caudal part of the lateral surfaces of the tongue (Fig.13). On the intermolar prominence, the filiform papillae are thin with pointed ends and bent backwards (Fig.14). The covering epithelial cells are polygonal with distinct intercellular border, they demonstrate microplicated surface specially on the tips of the papillae (Fig.15).

#### ***Fungiform papillae:***

The fungiform papillae of the newly born rabbits show regional variations in shape. They are dome-shaped (75-100 $\mu$ m in diameter) and carry small taste pores on the tip of the tongue (Figs.16, 17), but they are button shaped (150-200 $\mu$ m in diameter) on the lateral surface of the tongue (Fig.19). At higher magnification the surface of both forms of fungiform papillae is covered by microvilli.

In growing rabbits, the fungiform papillae on the tip of the tongue retain their dome shape, they only increase in diameter where they reach 150-200 $\mu$ m in adult rabbits (3-4 months old). However, the fungiform papillae on the lateral surface of the tongue start to expand and fuse with the surrounding filiform papillae taking mostly star-shape (Fig.20). In adult rabbits, they appear as flat bare areas amidst the filiform papillae (Fig.21). They take different irregular shapes that measure (400-600 $\mu$ m in diameter). Higher magnification demonstrates microplicated surface with some desquamating cells. The microplicae are arranged in different patterns. Taste pores are demonstrated only on the fungiform papillae found on the tip of the tongue (Fig.18).

#### ***Vallate papillae:***

In newly born rabbits, there are two vallate papillae on the caudal third of the tongue, one on each side, situated slightly at a lower level than the lingual surface. They are elliptical with convex surface and measure 200-250 $\mu$ m in diameter. A wide and irregular papillary groove encircles each papilla (Fig.22). The papillary surface and the surrounding areas demonstrate mucosal ridges, but at higher magnification, similar microvilli as those described above cover the surface, taste pores could not be demonstrated.

In the growing rabbits, the vallate papillae become larger with better developed papillary groove. The area surrounding the vallate papillae demonstrates low mucosal folds, but later on small filiform papillae appear in this area (Fig. 23).

The vallate papillae of the adult rabbits are large-sized (400-500  $\mu\text{m}$  in diameter) and elliptical in shape, each papilla carries 8-10 finger-like filiform papillae on its papillary surface. The papillary groove is wide, deep and completely encircling the papilla. The surrounded area is covered by numerous small filiform papillae and mucosal ridges. At higher magnification, a well developed network of microplicae cover the surface cells that are separated by distinct intercellular ridges, taste pores could be demonstrated at this stage (Fig.24).

**Foliate papillae:**

In the newly born rabbits, the foliate papillae are represented by few irregular grooves situated at the caudolateral margin of the tongue on both sides in front of the palatoglossal folds. They are obliquely oriented to the long axis of the tongue (Fig.25). By advancement of the age, the folds and grooves of the foliate papillae increase in numbers and dimensions, they are 6-8 oblique folds in 4 weeks old rabbits (Fig.26). In the adult, the foliate papillae are represented by 18-20 large folds separated by deep grooves arranged obliquely to the long axis of the tongue. Secondary folds are also demonstrated on the primary ones. Higher magnification shows folded surface with some taste pores (Fig.27).

## DISCUSSION

The present study reveals that the rabbits are born with weakly developed lingual papillae. These findings differ from the situation in large mammals with longer gestation period like camel, which is born with a well-developed set of lingual papillae (Dougbag, 1987). It can be postulated that the very short gestation period in rabbits does not allow enough time for advanced development of the lingual papillae.

The sequence of morphological changes in the tongue and its papillae from birth to puberty points to three developmental stages i.e., newly born, growing and mature stages. In the newly born rabbits, the tongue is small with short apex and weakly developed intermolar prominence. Further gross development of the tongue includes increase in the length and development of the intermolar prominence. The later resembles the *Torus linguae* of some herbivorous mammals (Nickel *et al.*, 1979; Dougbag, 1987; Takehana *et al.*, 2001).

Scanning electron microscopy reveals that in newly born rabbits, the filiform papillae (the only mechanical type found in rabbit) are found only on the tip and lateral surfaces, but absent on the dorsum of the tongue. By advancement of age, they spread on the dorsum of the tongue

until the later is completely covered by papillae at the 12<sup>th</sup> postnatal week. Because young rabbits start hopping during the third postnatal week and can be weaned at the age of four weeks (Okerman, 1994), it can be suggested that the development of the filiform papillae on the dorsum of the tongue occurs during the weaning period (from the 4<sup>th</sup> postnatal week on) parallel with feeding on plant materials that would exert mechanical stress on the lingual surface.

The current study supports the previous reports of regional variations in the shape of the filiform papillae. In this concern, Iwasaki *et al.* (1987) assumed that the reason for the distribution of these different types, in different areas of the tongue, is due to functional differences. The rostral part of the tongue (with tongue-shaped papillae) possibly contributes to the touch and prehension, while the caudal part (with conical and bent backward papillae) may facilitate the swallowing of the masticated food.

The fungiform papillae, in the newly born rabbits, are better developed than any other gustatory papilla. They are variably shaped being dome-shaped on the tip and button-shaped on the lateral surfaces. The fungiform papillae on the tip of the tongue have taste pores and supposed to perform the gustatory function in suckling rabbits. Unlikely, the laterally placed fungiform papillae have no taste pores, they also loss their papillary groove and fuse with the surrounding filiform papillae by advancement of age. This suggests that these papillae have no gustatory function in adult rabbits. Fungiform papillae without gustatory function have been recorded in horse (Barone, 1976), goat (Kumar *et al.*, 1998) and donkey (Abd-Elnaeim *et al.*, 2002). However, fungiform papillae with both mechanical and gustatory functions (having few taste pores) exist in horse (Chamorro *et al.*, 1986) and buffalo (Scala *et al.*, 1995).

The vallate and foliate papillae are weakly developed in newly born rabbits. The papillary grooves are not well defined and the taste pores could not be detected at this age. By advancement of age, these two kinds of papillae become well developed and demonstrate taste pores on their surfaces. These observations point to an increasing gustatory quality of the rabbit tongue during the postnatal period until puberty.

Concerning the number of the vallate papillae, the present study supports the previous statement of Chamorro *et al.* (1987) that the rabbit has only two vallate papillae. The number of vallate papillae varies greatly among different mammalian species. Some rodents like rat, mouse and hamster have only one vallate papilla while other species like

several primates and man can have four or more vallate papillae (Kubota and Hayama, 1964). Kubota (1988) stated that there is an intimate relationship between the feeding habits and the development of the vallate papillae. In addition, the presence of finger-like filiform papillae on the papillary surface of the vallate papillae may give them a mechanical quality.

The surface epithelium of the different lingual papillae demonstrates many stubby microvilli that are gradually changed to microplicae in adult rabbits. These microplicae are reported on the papillary surfaces of mouse (Utiyama *et al.*, 1995), monkey (Iwasaky *et al.*, 1992), horse and cow (Chamorro *et al.*, 1986) and donkey (Abd-Elnaeim *et al.*, 2002). These microplicae are supposed to enhance the spreading and adhesion of the mucous coat (Sperry and Wassersug, 1976), which protect the superficial cells (Nair and Schroeder, 1981) or as supporting structures for feed uptake, mastication and swallowing (Iwasaki *et al.*, 1987).

In conclusion, our study has shown that the rabbit tongue exhibits striking morphological changes during the postnatal period. The mechanical and gustatory functions seem to be weakly developed in newly born rabbits due to the absence of filiform papillae from the tongue dorsum and the weakly developed vallate and foliate papillae at this age. This weak gustatory function is supposed to be undertaken only by fungiform papillae, but in mature rabbits, the well developed vallate and foliate papillae may amplitude this function.

## LEGENDS

**Fig. 1:** Photograph showing the tongue of the rabbit in newly born, one month old and four months old rabbits arranged from left to right. The *Torus linguae* (T) is clearly demonstrated on the dorsum of the tongue of one and four months old rabbits. Note the vallate papillae (arrows).

**Figs. 2-6:** Scanning electron micrographs showing the distribution of filiform papillae in newly born rabbits. The filiform papillae do not cover the dorsum (D) of the tongue (Fig.2), this dorsum appears covered by microvilli in higher magnification (Fig.3). The filiform papillae are conical with rounded ends on the apex of the tongue (Fig. 4) and conical with pointed ends on the lateral surfaces of the body (Fig. 5). The surface cells of the filiform papillae are covered by stubby microvilli (Fig. 6).



**Figs. 7-10:** The filiform papillae in growing rabbits (one month old). They start to spread on the dorsum (D) of the tongue (Fig. 7). They are variable in shape being tongue-shaped on the apex (Fig. 8) and conical on the lateral surfaces of the body (Fig. 9). The microvilli covering the surface of the filiform papillae start to form concentrically arranged microplicae (Fig. 10).

**Figs. 11-15:** The filiform papillae in mature rabbits (four months old). They cover the dorsum (D) of the tongue completely (Fig. 11). They are variable in shape being tongue-shaped on the apex (Fig. 12), conical on the lateral surfaces of the body (Fig. 13) and short conical and bent backward on the *Torus linguae* (Fig. 14). The surface of the filiform papillae is now covered by more or less concentric microplicae (Fig. 15).

**Figs. 16-21:** The fungiform papillae in different locations and ages. In newly born rabbits, they are dome-shaped on the tip of the tongue (Fig. 16), button-shaped on the lateral surfaces of the tongue (Fig. 19) and are surrounded by papillary groove (arrowheads). The dome shaped papillae show taste pore (arrow) and are covered by abundant microvilli in newly born rabbits (Fig. 17), but they are covered by microplicae and show also taste pore (arrow) in mature rabbits (Fig. 18). The laterally placed fungiform papillae loss their papillary groove and start to fuse with the surrounding filiform papillae in growing (Fig. 20) and mature (Fig. 21) rabbits.

**Figs. 22-24:** Vallate papillae in the three postnatal stages. They are small with irregular papillary groove (arrow) in newly born rabbits (Fig. 22), the inset shows microvillous surface without taste pores at higher magnification. In growing rabbits (Fig. 23), the papilla increases in dimensions and also has papillary groove (arrow), its surface is also covered by microvilli as shown in the inset. In mature rabbits (Fig. 24), the papilla is fully developed with distinct papillary groove (arrow) and short filiform papillae (arrowheads) on the papillary surface. The inset shows higher magnification of the microPLICATED papillary surface with a taste pore (double arrow) in between.

**Figs. 25-27:** Foliate papillae in the three postnatal stages. They are in the form of irregular grooves (arrow) in newly born rabbits (Fig. 25), the inset shows microvillous surface without taste pores at higher magnification. In growing rabbits (Fig. 26), the papillary grooves (arrow) and ridges start to be better developed, surface microvilli transform into microplicae as shown in the inset. In mature rabbits

(Fig. 27), the papillary grooves (arrow) and ridges (arrowhead) are now fully developed. The inset shows higher magnification of the folded papillary surface, which carries a taste pore (double arrow).

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