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STAGES OF ODONTOGENESIS IN THE FIELD VOLE (*Microtus agrestis*, Rodentia) - A PILOT STUDY

K. WITTER[1], I. MÍŠEK[1], M. PETERKA[2], R. PETERKOVÁ[2]

[1]Institute of Animal Physiology and Genetics CAS, Laboratory of Genetics and Embryology, Brno,
[2]Institute of Experimental Medicine CAS, Department of Teratology, Prague

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Abstract

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The aim of this study was to obtain basic morphological data on prenatal development of this dentition and its timing using serial histological sections and computer-assisted three dimensional (3D) reconstructions. Twenty nine embryos and fetuses were classified into nine Štěrba's comparable stages (SCS) and their ontogenetic age was estimated within 13th and 20th day of ontogenesis (DO). Dental lamina was formed on DO-13. During the next stage, dental cap arose for the first incisor and the first molariform tooth. On DO-16, the dental caps deepened and their histodifferentiation was evident. Measuring of the thickness of the dental epithelium revealed transient rudimental tooth primordia in both the upper and lower jaw quadrant, apart from the functional teeth: one rudiment anterior to the incisor anlage and three rudiments in the the future diastema (margo interalveolaris). Later however, each of these vestiges disappeared. On DO-17, dental caps for the incisor and first molariform tooth transformed into bell-shaped enamel organs. A dental cap was formed for the second molariform tooth from the peg-shaped dental epithelium, which projected posteriorly from the enamel organ of the first molariform tooth into the mesenchyme. On DO-20, bell-shaped enamel organs for the incisor and first molariform tooth, as well as the bell-shaped enamel organ for the second molariform tooth, were well differentiated. In 3D computer-assisted representations, the enamel organs for the molariform teeth exhibited a typical plicident structure. An epithelial peg-shaped expansion grew out of the posterior end of the second molariform tooth primordium. This expansion was predestined to give rise to the third molariform tooth postnatally. Contrary to the second and third molariform tooth, which originated posteriorly from the subepithelial peg-shaped protrusion of the dental epithelium, the incisor and first molariform tooth in the field vole developed on the labial side of the dental lamina arising directly from the oral epithelium. The free terminal margin of the dental lamina projected lingually from the base of the later tooth primordia.

Tooth development, 3D computer-assisted reconstruction, ontogenesis, rodents

The dentition of muroids (subfamily Arvicolinae) is described to be monophyodont and heterodont. The hypothetical original dentition of eutharians is assumed to include three incisors, one canine, four premolars and three molars (Peyer 1937; Ziegler 1971). Voles have only one incisor and three molars in each quadrant of their functional dentition, similarly to other members of the muridae family (Ziswiller 1976; Krebs 1985; Thenius 1989). It follows that during the evolution of muroid rodents, a marked reduction and functional specialization of the dentition took place. This process has been verified by numerous paleontological and comparative anatomical data (Lavocat 1962; Guthrie 1970; Niethammer 1980; Chaline 1987; Viriot et al. 1990). More detailed embryological studies have occurred only sporadically (Štěrba 1981; Štěrba and Míšek 1982; Peterková et al. 1995). In order to comprehend a wider continuity of vole dentition evolution, the results of paleontological and anatomical studies should be correlated with detailed embryological data on vole dentition development. The aim of this pilot study was, therefore, to aquire fundamental knowledge about prenatal odontogenesis and its timing in one of our free - living Arvicolinae representatives - the field vole *(Microtus agrestis)*. The data were achieved from series of histological sections and computer-assisted

3D reconstructions. Because embryos and foetuses of free-living females were used, it was necessary to solve also the problem of standardization of embryos staging on the basis of external morphological criteria.

Materials and Methods

A total of 29 embryos and fetuses were fixed in a 10% formaldehyde solution and classified into age groups via assessment of their morphological characteristics according to Štěrba (1995). In this way, the age of specimens was estimated within days of ontogenesis (DO) 13-20.

The heads were processed using the routine histological method and series of frontal sections (7um thick) were stained with alcian blue-hematoxyline-eosin (modified according to Bancroft et al. 1994).

A microscopic examination of the sections was combined with a 3D computer-assisted reconstructions and morphometry. In selected specimens, projection drawings of the dental and adjacent oral epithelium were made under magnification 100x - 200x using an AMPLIVAL (Carl Zeiss) microscope equipped with a drawing chamber. The drawings were memorized on a personal computer, which was connected to a graphic tablet (GENIUS 1212). The best fit procedure (Gaunt and Gaunt 1978) was employed for superposition of the drawings. The 3D reconstructions were made using the ANATRECON program. In each drawing, the height of the dental epithelium was measured (Fig. 1) and the values were plotted.

Results

Odontogenesis in the field vole progressed similarly in the upper and lower jaw. The dental lamina was formed (Figs. 2A and 2B) on the 13th day of ontogenesis (DO-13). The dental lamina was not continuous along the whole dental quadrant. An interruption was apparent, separating the dental lamina in the incisor region from the more laterally emerging dental lamina in the prospective diastema and molar region (Fig. 2A). The morphometry revealed a thickening of the epithelium also in front of the first molar anlage (Fig. 2B).

At DO-14, the dental cap formation for the incisor and first molariform tooth was evident. Apart from these tooth anlagen, a small area of the thickened epithelium was present in front of the incisor primordium, and three rudiments could be detected in the prospective diastema region. During DO-15, further deepening of enamel caps for the incisor and first molariform tooth took place.

On DO-16, histodifferentiation of the cap of the incisor and the first molariform tooth was evident. Both these caps (Fig. 3A) had a small protuberance on the lingual side of their base, which was well apparent on histological sections. The existence of the epithelial primordia in front of the developing incisor and in the diastema region was evident (Fig. 3B). The dental epithelium submerged subepithelially

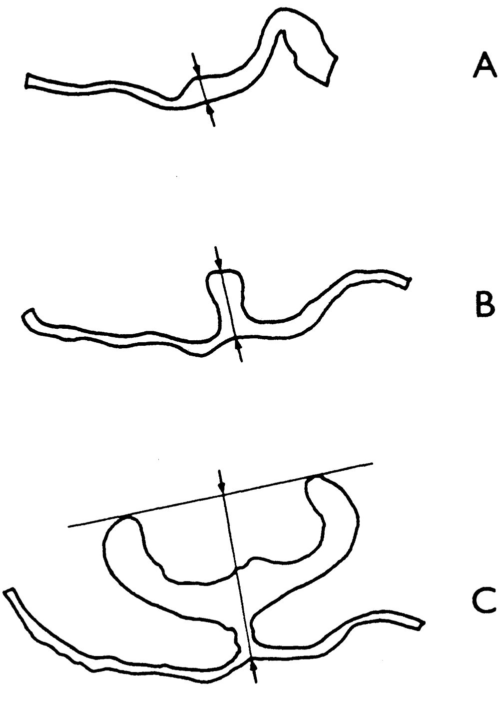
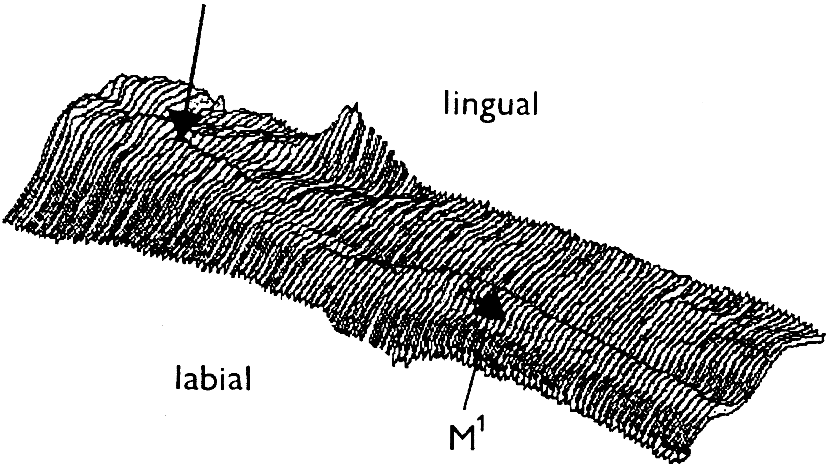
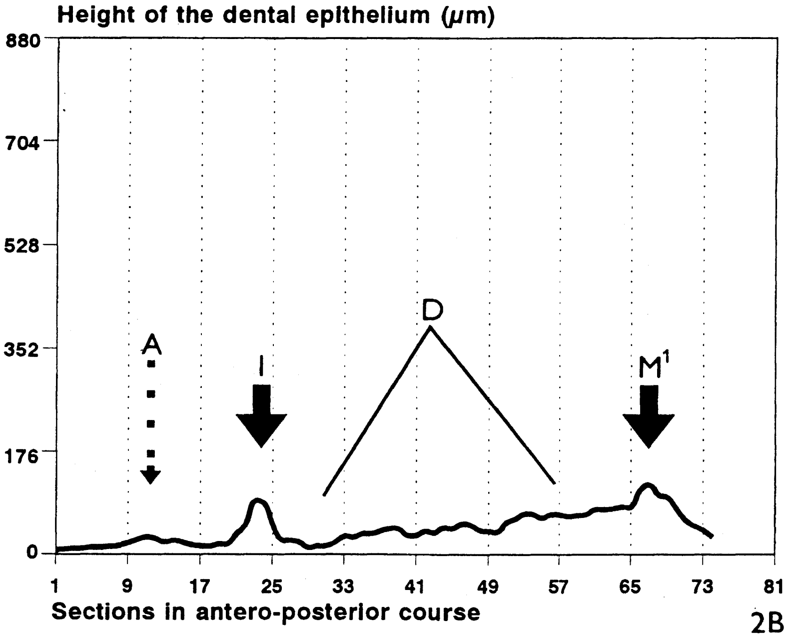


Fig. 1. Diagram representing the method of measuring the height of dental epithelium at different stages of tooth development. A. Dental lamina; B. Dental bud; C. Dental cap or bell.



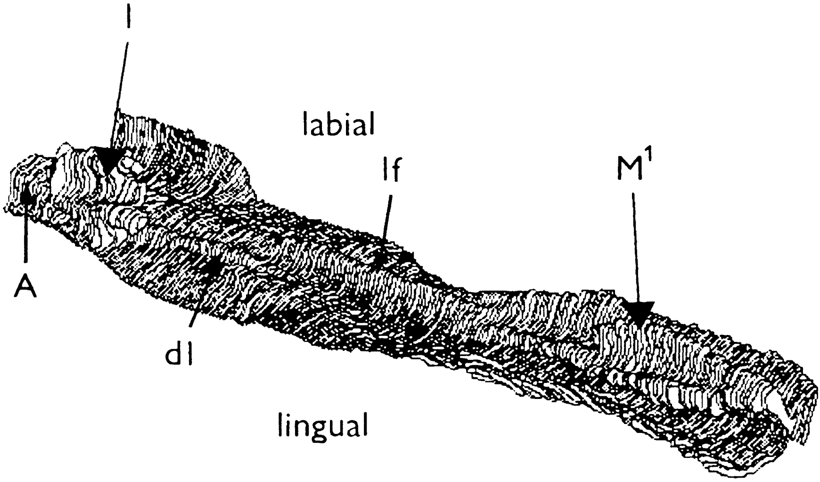
2A

Fig. 2A. The 3D computer-assisted reconstruction of the dental and adjacent oral epithelium in the upper jaw quadrant of an embryo at DO-13 (viewing the mesenchymal side). Prominences representing primordia of the incisor (I) and the first molariform tooth (M^1) are clearly visible.



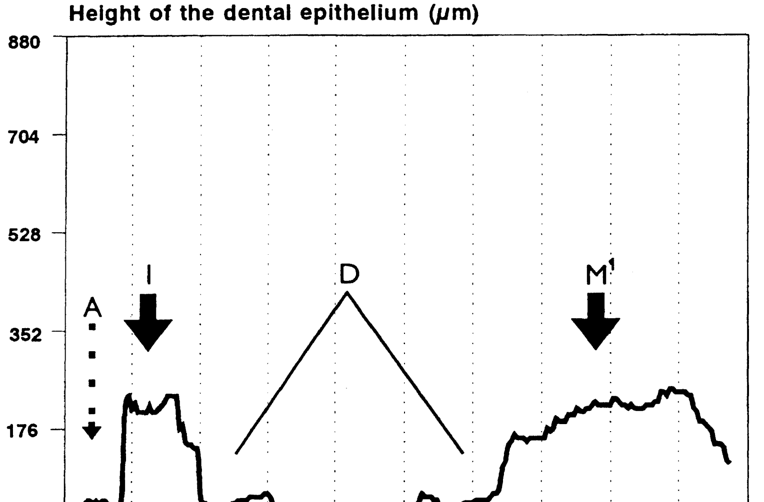
2B

Fig. 2B. Graphic illustration of changes in the height of the upper jaw dental epithelium in an antero-posterior direction at DO-13. A - the area of the thick epithelium in front of the incisor primordium (I). D - diastema region; M^1 - the first molariform tooth primordium.



3A

Fig. 3A. The 3D computer-assisted reconstruction of the dental and adjacent oral epithelium in the upper jaw quadrant of an embryo at DO-16 (viewing the mesenchymal side). A - the area of the thick epithelium in front of the incisor primordium (I). I - incisor primordium; dl - dental lamina; lf - lip furrow; M^1 - primordium of the first molariform tooth.



3B

Fig. 3B. Graphic illustration of changes in the height of the upper jaw dental epithelium in an antero-posterior direction. A - assumed vestigial tooth primordium in front to the functional incisor anlage (I); D - diastema region; M^1 - first molariform tooth primordium.

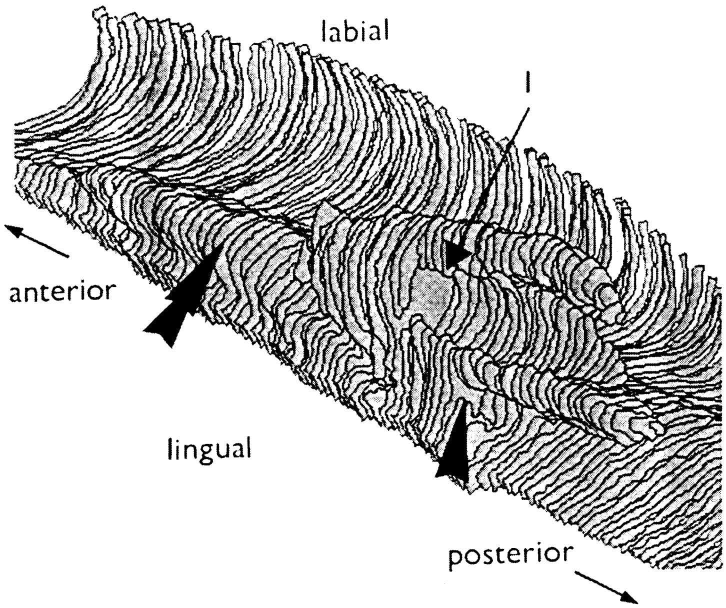


Fig. 4. A detail view on the upper incisor area in 3D reconstruction in a fetus at DO-17. The epithelial thickening (see double large arrow) is evident anterior to the functional incisor primordium. This thickening is related to the vestigial tooth primordium (see „A" on Fig. 3A). A protrusion of the epithelium (see single large arrow) projects lingually from the base of the functional incisor.

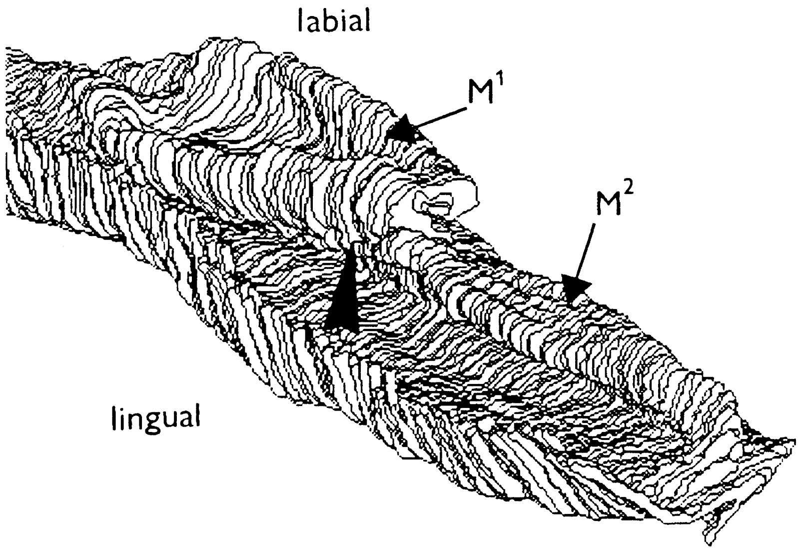


Fig. 5. A detailed view depicting the forming first (M^1) and second (M^2) upper molariform teeth in 3D reconstruction in a fetus at DO- 17. An epithelial ridge is evident (see arrow) lingually at the base of the enamel organ for M^1.

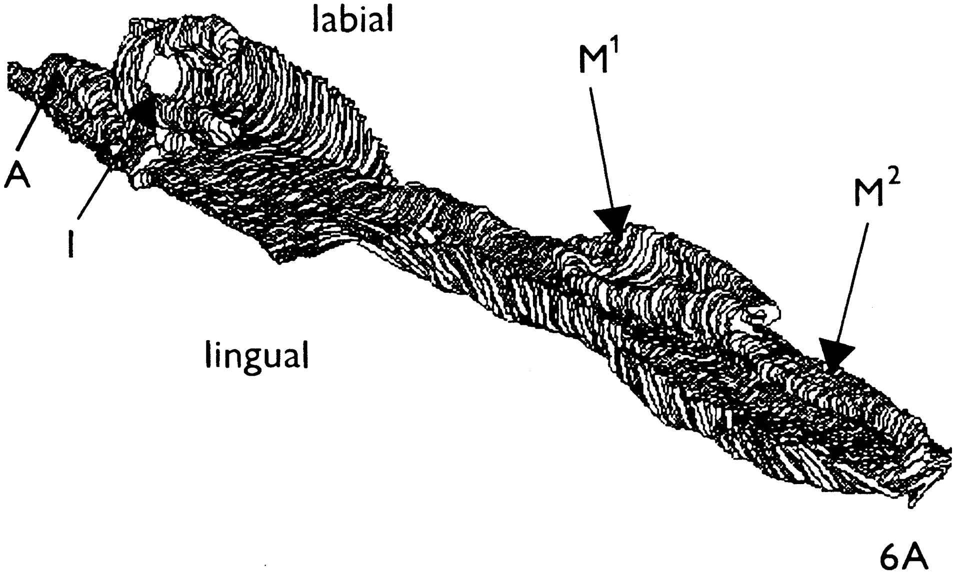


Fig. 6A. The 3D computer-assisted reconstruction of the epithelial tooth primordia in the upper jaw quadrant of a fetus at DO-17 (viewing the mesenchymal side). A - assumed vestigial tooth primordium in front of the functional incisor anlage (I); M^1, M^2 - primordium of the first and second molariform tooth, respectively.

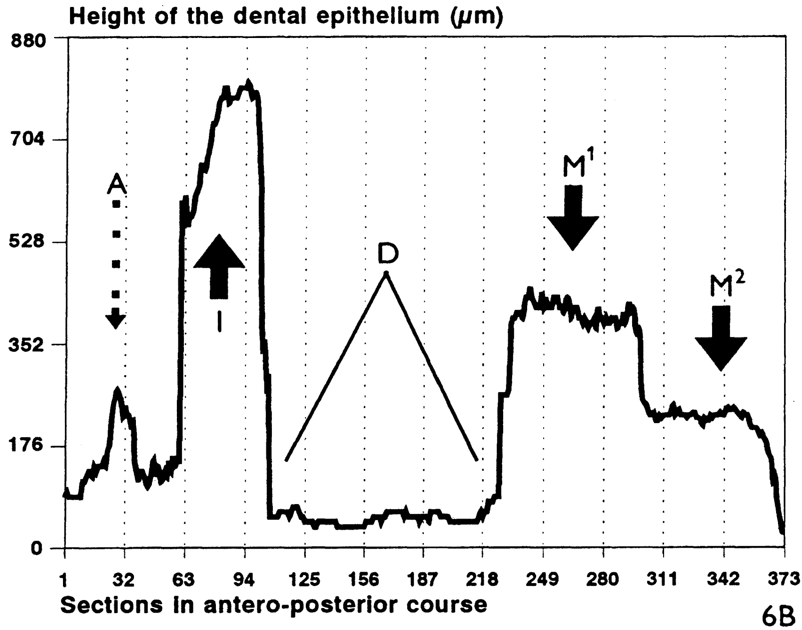


Fig. 6B. Graphic illustration of changes in the height of the upper jaw dental epithelium in an antero-posterior direction in a fetus at DO-17. A - assumed vestigial tooth primordium in front of the functional incisor anlage (I); D - diastema region; M^1, M^2 - primordium of the first and second molariform tooth, respectively.

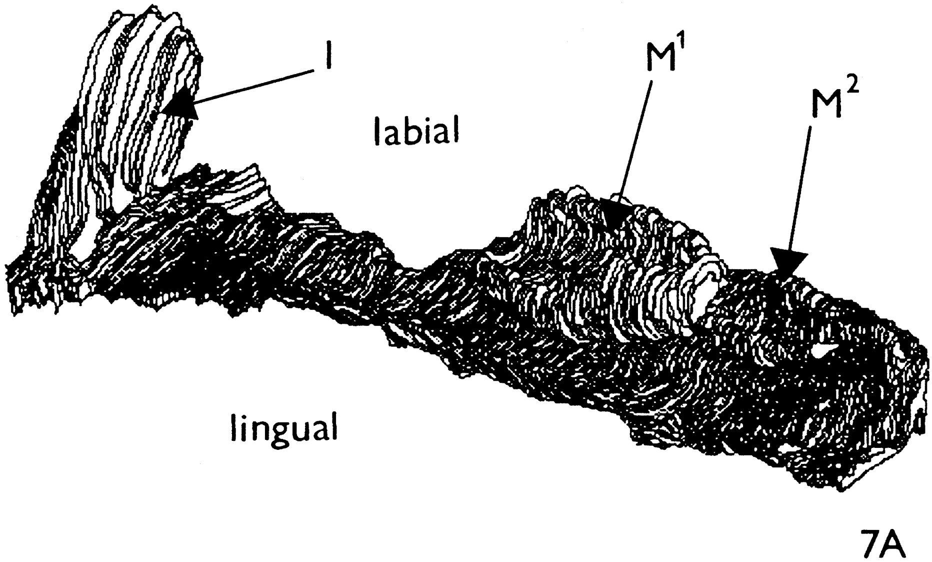


Fig. 7A. The 3D computer-assisted reconstruction of the epithelial tooth primordia in the upper jaw quadrant of a foetus at DO-18 (viewing the mesenchymal side). I - functional incisor primordium; M^1 - primordium of the first molariform tooth; M^2 - primordium of the second molariform tooth.

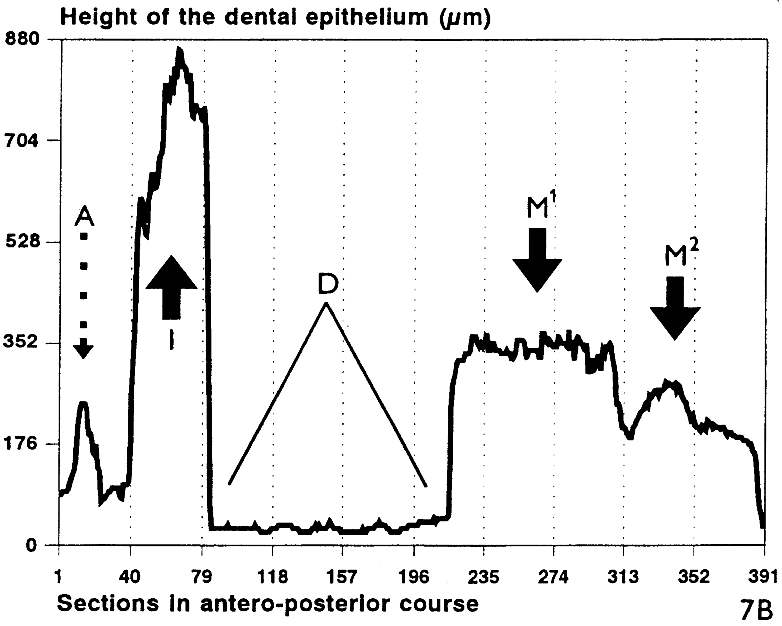


Fig. 7B. Graphic illustration of changes in the height of dental epithelium in an antero-posterior direction in a fetus at DO-17. A - assumed vestigial tooth primordium; I - functional incisor primordium; D - diastema region; M^1 - first molariform tooth primordium; M^2 - second molariform tooth primordium.

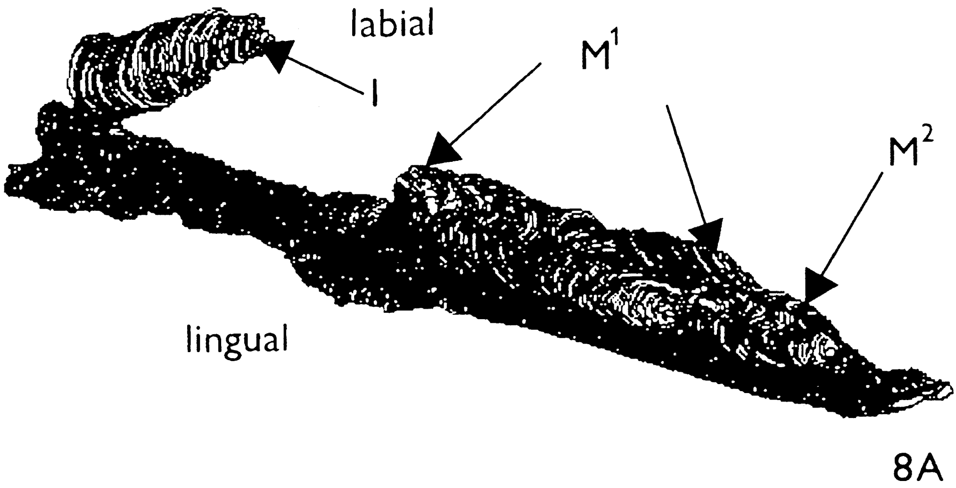


Fig. 8A. Three-dimensional computer-assisted reconstruction of the epithelial tooth primordia in the upper jaw quadrant of a fetus at DO-20 (viewing the mesenchymal side). I - functional incisor primordium; M^1 - primordium of the first molariform tooth; M^2 - primordium of the second molariform tooth.

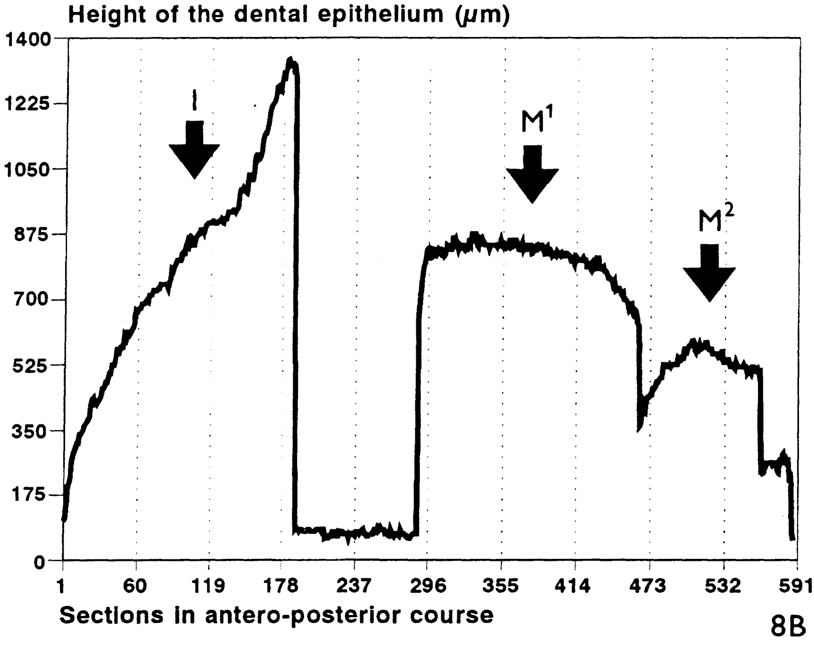


Fig. 8B. Graphic illustration of the antero-posterior changes in the height of the upper jaw dental epithelium in a fetus at DO-20. I - functional incisor primordium; M^1 - first molariform tooth primordium; M^2 - second molariform tooth primordium.

behind the first molariform tooth anlage, forming a short and thick epithelial peg, which bent slightly in a medial direction (data not shown).

On DO-17, well formed bells for the incisor and first molariform tooth were present. The lingual bud at the incisor base increased markedly in size on histological sections and thus gave the enamel organ a very peculiar shape (Fig. 4). The protuberance on the base of the enamel organ of the first molariform tooth became very conspicuous on histological sections. The 3D reconstructions revealed that this protuberance formed a longitudinal, antero-posterior epithelial ridge (Fig. 5). In the bend of the epithelial peg growing posteriorly out of the first molariform tooth enamel organ, a cap for the second molariform tooth developed (Figs. 6A and 6B). The diastemal epithelial rudiments disappeared, whereas the epithelial rudiment in front of the incisor still persisted.

By DO-18, further growth of the incisor and first molariform tooth had taken place. Thin dentin and enamel layers were visible on histological sections. The second molariform tooth primordium transformed into a bell-shaped enamel organ. All vestigial structures described at earlier stages had definitively disappeared, except the epithelial rudiment in front of the incisor tooth germ (Figs 7A and 7B).

Before birth (on DO-21), further growth of the tooth bells occurred. A peg-shaped expansion of the dental epithelium projected posteriorly from the bell for the second molariform tooth, giving rise to the third molariform tooth postnatally.

The epithelial rudiment in front of the developing incisor was no longer detected (Figs. 8A and 8B). Dentin and enamel production was present even in the second molariform tooth. The 3D reconstruction (Fig. 9) showed the plicident shape of the bell for the first molariform tooth, which in fact represented a negative cast of the future functional tooth.

Discussion

The developing teeth of the field vole *(Microtus agrestis)* progressed through the classical odontogenesis stages already described at the end of the last century (Freund 1892; Adloff 1898). Timing of the individual stages of tooth development was similar to that one reported

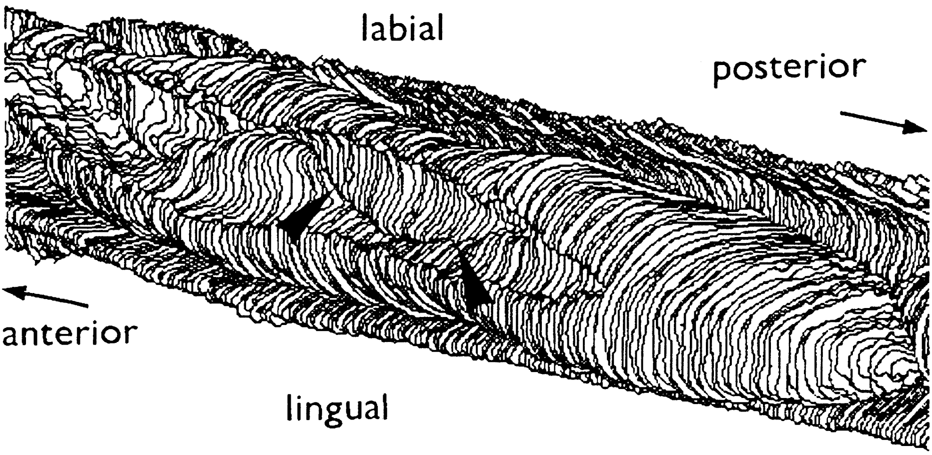


Fig. 9. Detail of the 3D computer-assisted reconstruction of the first molariform tooth primordium (M^1) in a fetus at DO-20. Notice the oblique laminas (arrows) reflecting a characteristic morphology of the so-called plicident tooth.

in other Arvicolinae representatives, eg. *Microtus arvalis*, *Clethrionomys glareolus* and Microtus subterraneus (Štěrba 1981; Štěrba and Míšek 1982). Prenatally, the tooth germs for only the first incisor and the first and second molariform teeth were formed. The tooth germ for the third molariform tooth develops postnatally. A detailed description of the quick development of the functional tooth primordia between the 14th and 17th DO will require a focused study in the future. A more detailed staging of embryos and fetuses, as well as examination of a greater number of specimens, should enable the identification of intermediate odontogenesis stages (Luckett 1993).

An interesting phenomenon was the presence of vestigial epithelial rudiments in front of the incisor primordium and in the diastema area in both the lower and upper jaw. Similarly localized rudimental structures have been described in various rodents (*Mus*; *Rattus*; *Sciurus*) and in rabbits, and attributed to dental rudiments (e.g. Freund 1892; Moss-Salentijn 1975, 1978; Peterková et al. 1995). Our findings suggest existence of rudimental tooth primordia also in voles. Premolar teeth or rudimental tooth primordia occur in greater numbers in the upper jaw than in the lower one in fossil and recent rodents (e.g. Viret 1955; Wood 1962; Luckett 1993; Peterková 1995). Such a trend, however, was not exhibited in the vole.

The epithelial rudiment present transiently in front of the incisor primordium, could be homologized with the so called vestigial incisor, reported during dentition development in sciurids, muroids, caviomorphs and bathyergids (Freund 1892; Adloff 1898; Woodward 1894; Fitzgerald 1973; Moos-Salentijn 1978; Luckett 1985; Luckett et al. 1989). However, the bud-shaped epithelium in front of vole's incisor never exhibited further differentiation.

According to their morphology and location, the epithelial lamina, situated lingually on the base of the incisor and the first molariform tooth enamel organ, resembles the dental lamina for the permanent teeth described e.g. in the human (Radlanski 1993). The finding of such a structure projecting from the first molariform tooth primordium in the vole might support opinion of those authors, who identify the first molariform tooth in certain rodent groups with the fourth temporary (deciduous) premolar of other mammals (Grassé 1955; see also Wilson 1956). In such a case, the three rudimental tooth primordia in front of this tooth in the field vole might suggest a repetition of the extinct premolars.

Further detailed studies of prenatal development of dentition in the *Microtus agrestis*, including also intermediate odontogenesis stages should contribute to understanding of the tooth patterning and homology in *Microtinae*.

Conclusions

1) In the field vole (*Microtus agrestis*), a discontinuous dental lamina was formed on DO-13. On DO-14, the cap for the incisor and first molariform tooth appeared. These caps progressed to the bell stage on DO-17. Contemporaneously, the cap for the second molariform tooth arose, achieving the bell stage still before birth. The formation of the third molariform tooth germ and functional teeth eruption occurs postnatally.

2) During prenatal development, epithelial rudiments were transiently present in the upper as well as in the lower jaw: one in front of the incisor primordium and three in the prospective diastema. They might represent a developmental repetition of teeth suppressed during phylogenetic development.

3) A free end of the dental lamina existed transiently, projecting lingually from the base of the bell for incisor and the first molariform tooth in the field vole fetuses. This structure resembled the dental lamina of the permanent dentition in diphyodont mammals.

Stadia odontogeneze u hraboše mokřadního (*Microtus agrestis*, Rodentia) - pilotní studie

Cílem práce bylo popsat způsob a časový průběh prenatálního vývoje dentice *M. agrestis* pomocí seriových histologických řezů v kombinaci s počítačovou trojrozměrnou rekonstrukcí. Bylo zpracováno celkem 29 embryí a fetů zařazených do 9 ontogenetických stadií a odhadnuto jejich ontogenetické stáří. Vývoj zubní lišty začal 13. den ontogeneze. V dalším stadiu došlo k formování zubních pohárků pro první řezák (hlodák) a první molariformní zub. V 16 dnech ontogeneze se zubní pohárky prohloubily a byla patrná jejich histodiferenciace. Metodou měření tloušťky epitelu na dásňovém okraji čelistí jsme prokázali v horním i dolním kvadrantu rudimentální epitelové pupeny i mimo oblast funkčních zubů: tzn. 1 pupen anteriorně od základu hlodáku a 3 pupeny v místě budoucího diastematu, resp. v oblasti margo interalveolaris. Později však všechny tyto rudimenty vymizely. V 17 dnech ontogeneze se přeměnily zubní pohárky pro hlodák a první molariformní zub ve zvonkovité orgány skloviny. V místě ohbí epitelového čepu za základem prvního molariformního zubu se vytvořil zubní pohárek pro druhý molariformní zub. Ve 20 dnech ontogeneze byly dobře diferencovány zvonkovité orgány skloviny pro hlodák a první molariformní zub a zvonkovité orgány skloviny pro druhý molariformní zub. Z posteriorního konce základu pro druhý molariformní zub vyrůstal epitelový čep pro pozdější 3.molariformní zub, který se formoval až postnatálně. Orgány skloviny pro molariformní zuby vykazovaly v trojrozměrné počítačové rekonstrukci typickou plicidentní strukturu. Řezák a první molariformní zub se zakládaly na labiální straně dentální lišty, která vycházela přímo z orálního epitelu. Přitom byl prokazatelný volný terminální konec dentální lišty linguálně od zubních základů. Druhý i třetí molariformní zub se zakládaly terminálně na volném okraji subepiteliálního čepu.

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Address for correspondence:
MVDr. K. Witter
Institute of Animal Physiology and Genetics
Academy of Sciences of the Czech Republic
Laboratory of Genetics and Embryology
Veveří 97
602 00 Brno
Czech Republic
Tel.(05) 726 8135