

FIRST MOLAR DEVELOPMENT IN *CALOMYS CALLOSUS*

DESENVOLVIMENTO DO PRIMEIRO MOLAR EM *CALOMYS CALLOSUS*

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ABSTRACT: Due to an increased use of *Calomys callosus* as a laboratory animal, the present study purposed to describe morphologically the embryonic development of the first molar, seeking to determine for this rodent, the age in which each odontogenesis phase occurs. In this experiment fetuses of *Calomys callosus* obtained during a gestational period of 11 to 20 days were used. The heads of the fetuses were removed and fixed in 10% formol and in a 0.1M phosphate buffer for 15 hours, decalcified in 10% EDTA and embedded in glycol metacrilate (Historesin-Leica). Serial frontal sections of the upper first molar were cut at 2 micrometers of thickness and stained with 1% toluidine blue for light microscope analysis. At day 11, no indications of odontogenesis were found. On the 12th day, epithelial ingrowths compatible with primary epithelial bands were observed. On the 13th day of development, the dental lamina and the beginning of the bud stage were seen, becoming well defined in the 14th day. On the 15th and 16th days the cap stage was observed. On the 17th, 18th and 19th days the bell stage were observed. On the 20th day the beginning of the crown stage was seen. The results obtained indicate that the odontogenesis of the *Calomys callosus* is similar to the one of other rodents used in laboratory, which justifies its use as a biological model for studies related to the dental development.

UNITERMS: Odontogenesis, *Calomys callosus*, Molar, Dental germ.

INTRODUCTION

Tooth development involves many complex biological processes, since it correlates epithelial mesenchyme, morphogenesis and mineralization (AVERY; STEELE, 2001; KATCHBURIAN; ARANA, 1999; TEN CATE, 1998). Several rodents such as rats (LEFKOWITZ; BODECKER; MARDFIN, 1953), mice (COHN, 1957; COSTA; CORREA, 1982; HUNT, 1959) gerbils (HIATT; GARTNER; PROVENZA, 1974) and hamsters (GARCIA; RAMALHO, 2001; RUSSIAN; GARTNER, 1987) have been used in studies of dental biology. *Calomys callosus* is a rodent which has been suggested as a very well suited laboratory animal due, to advantages like: easy handling, high reproduction level, high infection level resistance, and its reduced size, not needing large physical spaces and high costs (MELLO, 1984). Due to the increased usage of *Calomys callosus* as a laboratory animal, the present study intended to describe morphologically the embryonic development of

the first molar of this rodent, determining the age in which each dental development phase occurs.

MATERIAL AND METHODS

Ten female and five male rodents were used in this experiment. The animals were coupled in the proportion of 2 females per male. Day 1 of gestation was designated by the presence of the vaginal plug. During the period of 11 to 20 days of gestation, the female rodent was anesthetized with Ketamine (Ketalar, Aché Laboratories) and Xylamine (Rompum, Bayer Laboratories) (1:1, 0.5 ml) and the fetuses were removed by laparotomy, being obtained 6 fetuses per female. The heads of the embryos, were removed and fixed in 10% formol in 0.1M phosphate buffer for 15 hours. After the fixation, the heads were decalcified in 10% EDTA and embedded in glycol metacrilate (Historesin-Leica). Serial frontal sections of upper first molar were cut at 2 micrometers of thickness and stained with 1% toluidine

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blue for light microscope analysis. The histological analysis and the photographic registrations were made with the aid of the Reichert-Jung photomicroscope (Wien, Austria).

RESULTS

With 11 days of development the embryo did not show any indication odontogenesis. On day 12 epithelial ingrowths compatible with the primary epithelial band were

observed in the first molar area (Fig 1 A- B).

On day 13, the dental lamina and beginning of the bud stage of development were observed (Fig 2 A), which became defined in the 14th day (Fig 2 B). The periphery cells of the epithelial bud were constituted by one or two layers of short cylindrical cells. The cells of the central area of the bud showed a polygonal shape. Ectomesenchymal cells were condensed adjacently to the epithelial bud.

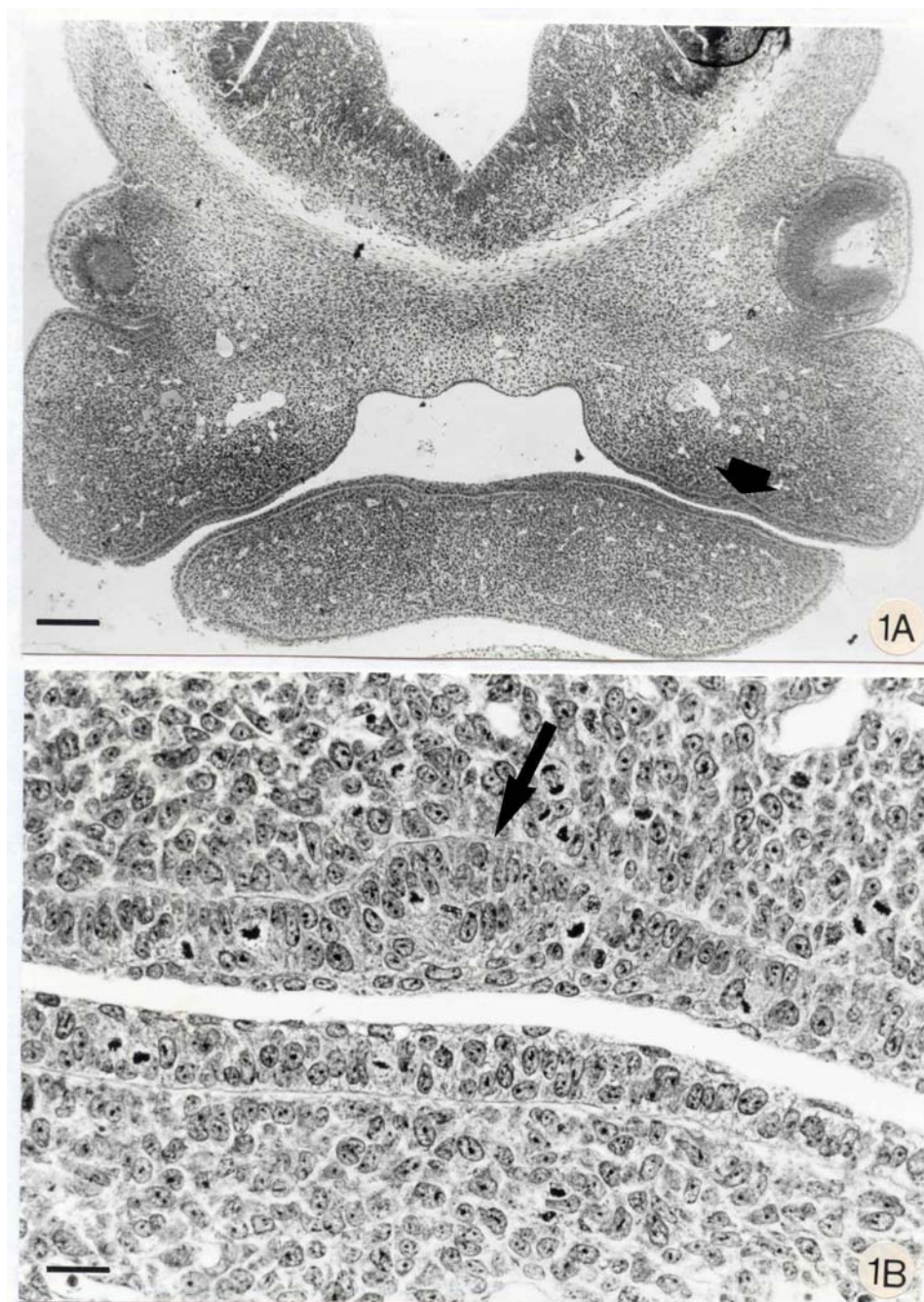


Figure 1. A - Photomicrographs of frontal sections of head of fetuses of *Calomys callosus* with 12 days (arrow), bar= 250 μ m. B - evidencing the area of epithelial primary band (arrow), bar= 25 μ m.

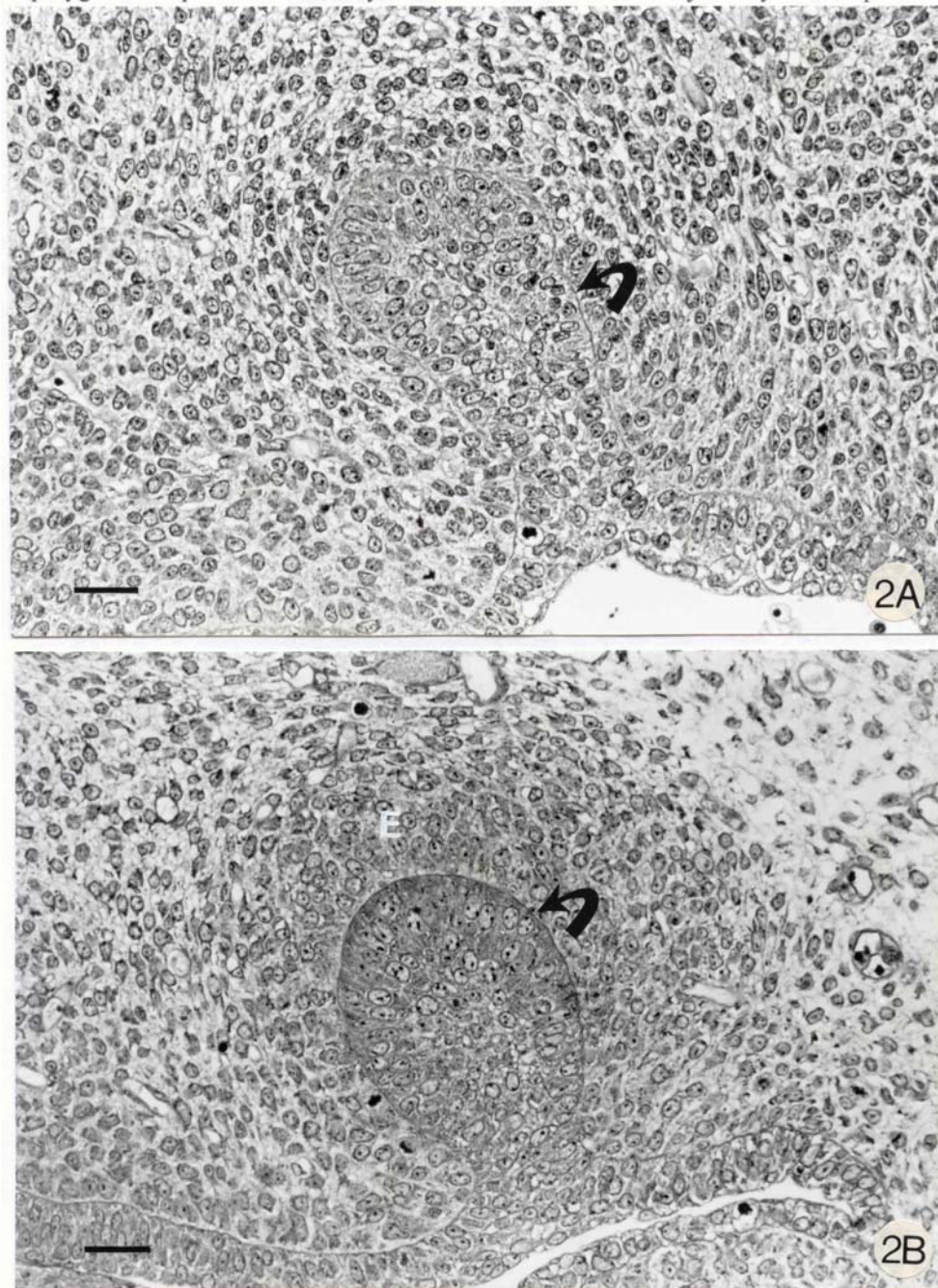


Figure 2. Photomicrographs of head of fetuses of *Calomys callosus*. **A-** with 13 days evidencing the dental lamina (arrow). **B-** with 14 days evidencing the bud stage, epithelial bud (seta) and condensed ectomesenchymal cells (E). Bar= 25 μ m. The beginning of the cap stage (Fig 3 A) was observed on the 15th day, which became defined on the 16th day (Fig 3 B). In this stage it was possible to identify the enamel organ components. The external epithelium, in the convexity of the cap, showed a layer of short cubic cells. The internal epithelium, in the concavity of the cap, showed a layer of juxtaposed cylindrical cells. The stellate reticulum in the central area of the dental organ and the stratum intermedium adjacent to the internal epithelium were observed. The dental papilla stated in the concave area of the enamel organ showed condensed polygonal cells and small blood vessels. The dental follicle showed itself defined, mainly in the adjacent area of the papilla and the cervical loop. These were characterized by presenting about 6 layers of fusiform cells (Fig 3 B).

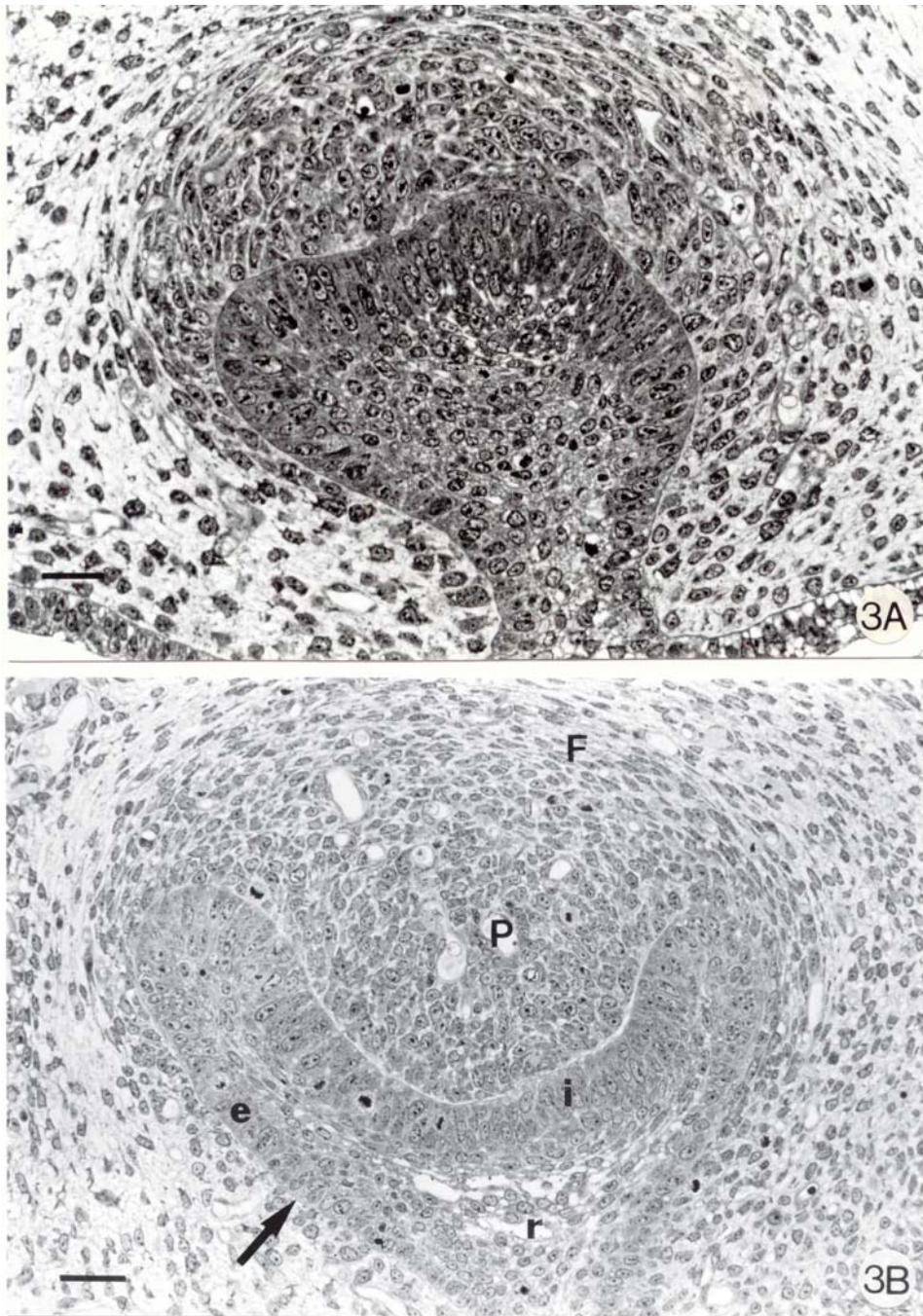


Figure 3. Photomicrographs of head of fetuses of *Calomys callosus*. **A-** with 15 days evidencing the beginning of the cap stage. **B-** with 16 days evidencing the cap stage, enamel organ (arrow), external epithelium (e), internal epithelium (i), stellate reticulum (r), dental papilla (P), and dental follicle (F). Bar= 25 μ m.

On the 17th day (Fig 4 A), the dental germ was in transition from the cap to the bell stage. On the 18th (Fig. 4 B) and 19th (Fig. 5 A) days, the dental germ was in the bell stage, showing the shape of the future crown pattern. The dental organ increased its size due to the development of all their components. The cell layer of the external dental epithelium became more defined and cubic. The cells of the internal epithelium stayed cylindrical. The

intermediate stratum, that was constituted by two layers of flattened cells and stellate reticulum, showed a clear, reticular area. In this stage the dental papilla's size increased and the cells increased in number (Fig. 5 A). On day 20 of development, the dental germ was in the beginning of the crown stage (Fig. 5 B). The cells of the external epithelium showed a cubic form close to the dental lamina, which progressively flattened as it extended itself

towards the cervical loop. The internal epithelium showed tall cylindrical cells, with a cell nucleus migration toward the pole of the cell, away from the dental papilla. The cells of the stratum intermedium had their volume reduced and became more juxtaposed to the internal epithelium. The stellate reticulum did not present any morphological changes. The dental organ was still linked to the oral epithelium through the dental lamina. The undersurface

cells of the dental papilla were organized in a layer of juxtaposed and polarized cells, the odontoblasts. The observation of a layer of dentin matrix produced by the odontoblast located in the area of the future cusps was possible in this stage. The central cells of the papilla showed a star-shaped aspect and high amounts of intercellular compounds. The dental follicle was reduced in the area of the dental organ (Fig 5 B).

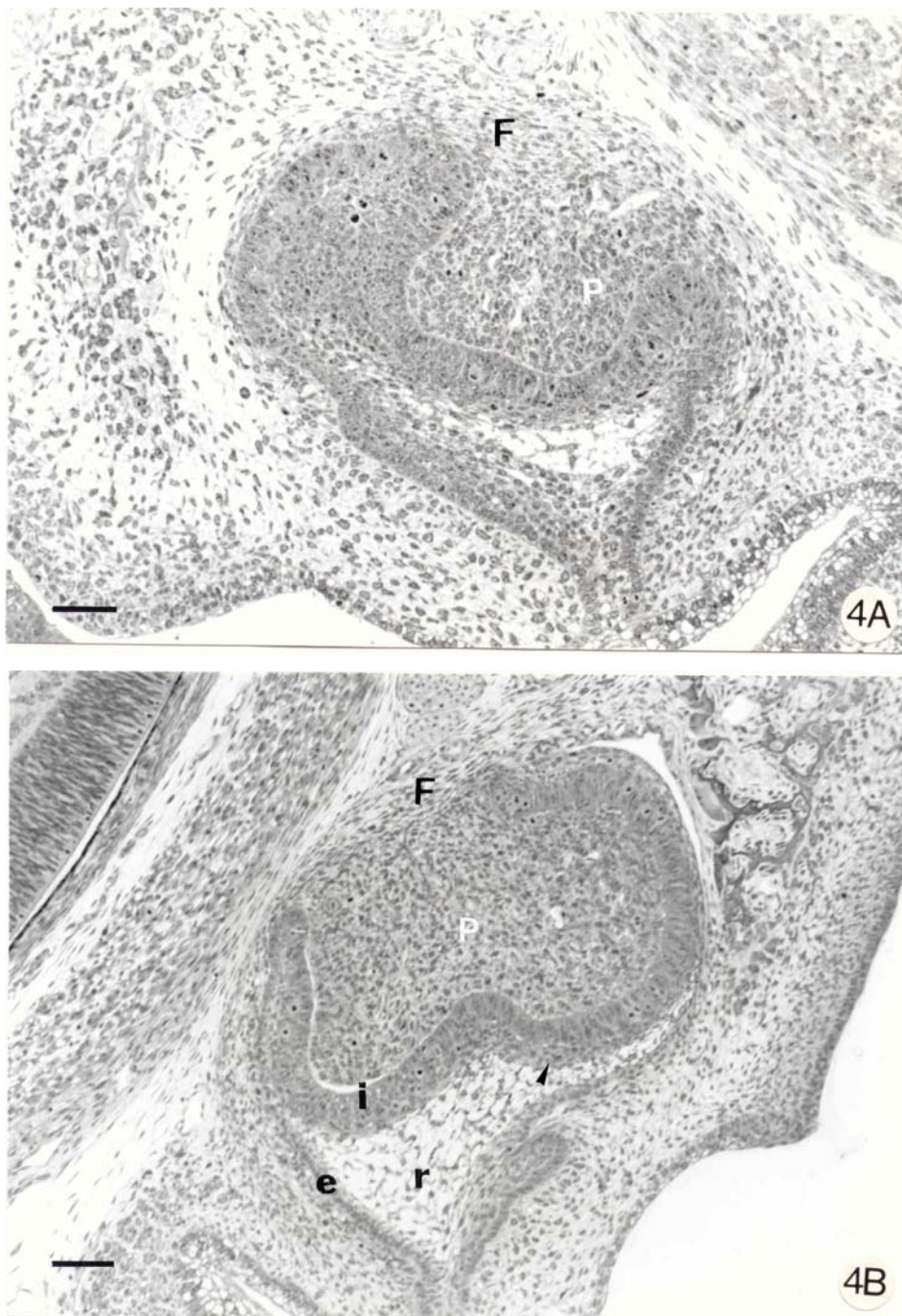


Figure 4. Photomicrographs of head of fetuses of *Calomys callosus*. **A-** with 17 days evidencing the beginning of the bell stage. **B-** with 18 days-evidencing bell stage. Dental papilla (P), dental follicle (F) and dental organ components: external epithelium (e), internal epithelium (i), stellate reticulum (r), intermediate stratum (arrowhead). Bar= 100µm.



Figure 5. Photomicrographs of head of fetuses of *Calomys callosus*. **A-** with 19 days evidencing the defined bell stage. **B-** with 20 days evidencing the beginning of the crown stage. Dental papilla (P), odontoblasts (o), dentin matrix (d), dental follicle (F), dental organ components: external epithelium (e), internal epithelium (i), stellate reticulum (r), stratum intermedium (arrowhead). Bar= 100µm.

DISCUSSION

Studies involving dental biology have been done in several animals, mainly in rodents as rats (LEFKOWITZ; BODECKER; MARDFIN, 1953), mouse (COHN, 1957; COSTA; CORREA, 1982; FINN;

MACLAREN 1967; VIRIOT et al, 2002), gerbils (HIATT; GARTNER; PROVENZA, 1974) and hamsters (GARCIA; RAMALHO, 2001; RUSSIAN; GARTNER, 1987). *Calomys callosus* is an animal easy to handle (MELLO, 1984) that has been used as a laboratory animal.

The development of the dental lamina in the *Calomys callosus* happened on the 13th day of development, just as in the rat (LEFKOWITZ; BODECKER; MARDFIN, 1953), and the mouse (COHN, 1957). The bud stage in *C. callosus* happened on days 13 and 14 of development. The epithelial bud showed one or two layers of cells in the periphery, similar to the gerbil's (HIATT; GARTNER; PROVENZA, 1974), whereas in the hamster (GARCIA; RAMALHO, 2001; RUSSIAN; GARTNER, 1987), rat (LEFKOWITZ; BODECKER; MARDFIN, 1953), and mouse (COHN, 1957), there is a single cell layer. The central epithelial bud cells were distributed in a dispersed manner, contrasting the epithelial bud in the rat which showed denser cellular nuclei (LEFKOWITZ; BODECKER; MARDFIN, 1953). Hiatt; Gartner; Provenza (1974), described this area in the gerbil as presenting a starred shape. In the mouse Cohn (1957), has reported these areas like a mass of slack cells. The cap stage was observed on the 15 and 16 days of development, similar to observations in mice (COHN, 1957). In rats with 16 days, the dental germ was in the bud stage (LEFKOWITZ; BODECKER; MARDFIN, 1953), while the hamsters were in the crown stage (RUSSIAN; GARTNER, 1987). The internal epithelium of *Calomys callosus* consisted of a layer of cylindrical cells similar to the data gathered by Hiatt; Gartner; Provenza (1974), for the gerbil. In the rat (LEFKOWITZ; BODECKER; MARDFIN, 1953), mouse (COHN, 1957), and hamster (RUSSIAN; GARTNER, 1987), however, the internal epithelium is composed by a layer of cubic cells. The external epithelium and the stellate reticulum of the *Calomys callosus* showed cubic cells similar to the other rodents (COHN, 1957; GARCIA; RAMALHO, 2001; LEFKOWITZ; BODECKER; MARDFIN, 1953; RUSSIAN; GARTNER, 1987). The beginning of the bell stage in *Calomys callosus* happened in the 17th day of development, in agreement to the observations in mice (COHN, 1957; FINN; MACLAREN, 1967). The gerbil presented this stage only on the 22nd day (HIATT; GARTNER; PROVENZA, 1954) and the hamster (RUSSIAN; GARTNER, 1987) on the 14th day of development. The intermediate stratum of the *Calomys callosus* was observed on the 17th day of development, being characterized by the presence of two or three cell

layers, similar to the other rodents (COHN, 1957; HIATT; GARTNER; PROVENZA, 1954; RUSSIAN; GARTNER, 1987).

The dental papilla of the *Calomys callosus* was constituted by ectomesenchyme cells with a fusiform shape. The same happened in the gerbil (HIATT; GARTNER; PROVENZA, 1974), the mouse (COHN, 1957), and the hamster (RUSSIAN; GARTNER, 1987). The dental follicle in *Calomys callosus* has approximately six layers of fusiform cells. These involve the dental organ and papilla similar to that observed in the hamster (RUSSIAN; GARTNER, 1987). The dental germ of the *Calomys callosus* with 20 days of development, showed differentiated ectomesenchyme cells in the odontoblasts, characterizing the beginning of the crown stage. The same happened with the mouse (COHN, 1957) and the rat (LEFKOWITZ; BODECKER; MARDFIN, 1953). The gerbil was in the bell stage on the 20th day of development (HIATT; GARTNER; PROVENZA, 1974). The hamster presented the crown stage with 16 days of development, probably because of its 18 day gestation period.

The dental lamina kept linking the dental germ to the oral epithelium through the odontogenesis stages in *Calomys callosus*, similar to what happens in hamster (GARCIA; RAMALHO, 2001; RUSSIAN; GARTNER, 1987).

The 2nd molar tooth bud of the *Calomys callosus* was observed with 18 days of development, just as in the rat (LEFKOWITZ; BODECKER; MARDFIN, 1953). In mice, it happened with 16 days (COHN, 1957) and in gerbil with 19 days (HIATT; GARTNER; PROVENZA, 1974). The morphological differences observed between the *Calomys callosus* and the other rodents should probably be owed to the differences in the gestational time of each animal and also to the natural differences among the species.

The results obtained indicate that the odontogenesis of the *Calomys callosus* is similar to the others rodents used in laboratory, therefore, this rodent is indicated as a biological model for studies related to dental development. This study determined the age in which each phase of odontogenesis occurs in *C. Callosus*. These findings contribute in this research area, which will allow more advanced studies.

RESUMO: Devido ao aumento crescente de utilização do *Calomys callosus* como animal de laboratório, o presente estudo teve como objetivo descrever morfológicamente o desenvolvimento embrionário do primeiro molar, visando determinar a idade em que ocorre cada fase da odontogênese. Neste experimento foram utilizados fetos de *Calomys callosus* obtidos durante o período gestacional de 11 a 20 dias. As cabeças dos fetos foram removidas e fixadas em formol 10% por 15 horas, descalcificadas em EDTA 10% e processadas para inclusão em glicol metacrilato

(Historesin-Leica). Cortes frontais da região do primeiro molar superior, com 2 µm de espessura, corados em azul de toluidina 1%, foram analisados ao microscópio de luz. Aos 11 dias não houve indício de odontogênese. Com 12 dias de desenvolvimento observou-se crescimento epitelial compatível com a banda epitelial primária. Aos 13 dias observou-se a lâmina dental e início da fase em botão, que se tornou definida no 14º dia. A fase em capuz foi observada no 15º e 16º dias de desenvolvimento. A fase em campânula foi observada no 17º, 18º, 19º dias. No 20º dia foi observado o início da fase em coroa. Os resultados encontrados indicam que as fases da odontogênese em *C. callosus* são similares a de outros roedores usados em laboratório, o que justifica o uso desse animal como modelo biológico para estudos relacionados ao desenvolvimento dental.

UNITERMOS: Odontogênese, *Calomys callosus*, Molar, Germe dental.

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