Correlations Between Morphology and Evolution in the Structure of the Tooth Germ

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Abstract

Aims: The aim of this study was the microscopic morphologic assessment of the development of deciduous tooth germs to investigate whether it correlated with the biologic age. ***Methods:*** The study material consisted of oral tissue from 20 fetuses with a minimum gestational age of two months or newborns deceased at birth, cases autopsied in the Vaslui County Forensic Service and in the Pathology Laboratory of the Municipal Hospital Barlad, Romania. Legal harvesting, manipulation and conservation conditions were respected; the collection of the specimens was performed only after ethical approval and with the written consent of the family concerned. The investigation methods consisted of routine histological examination of the specimens after they had been processed through special decalcification techniques and stained with hematoxylin-eosin and three special stains. ***Results:*** The microscopic evaluation of deciduous tooth germs in cap and bell stage allowed the characterisation of the three main components—enamel organ, dental papilla and dental sac—through the cellular details contributing to the development of the typical architecture of the tooth. ***Conclusions:*** In the cap and bell stage, the tooth germs had different morphologic features, without a direct correlation with the gestation age. The marked diversity in the shapes and sizes of the identified tooth germs supports the influence of genetic factors and of the specific interactions with the micro-environment in tooth development.

Key Words: Tooth Germ, Development, Histology

Introduction

The generic term used to describe a developing tooth is "tooth germ"; the expression "tooth bud" should be avoided because of the overlap with the name of the first stage of tooth development [1]. For an accurate definition of the tooth germ throughout its development, it is necessary to use a double terminology that ensures a correlation between the functional phases and the morphological stages [1]. The phases of the development (initiation, proliferation, differentiation and apposition) follow one another dynamically and at times they partially overlap, the development being a continuous process without clear demarcation lines [2]. The names of the stages are based on the morphology of the epithelial structure of the tooth germ: bud, cap, and bell. Consequently, the tooth development process has the ensuing sequences: (a) crown formation, (b) root formation and concomitantly, (c) support tissue formation.

During tooth histo-morphogenesis changes occur:

- In the composition of the basement membrane (involving the metalloproteinases and their inhibitors) [3-5].
- In the signalling molecules mechanisms [6].
- In the localisation of cell surface elements (integrins [7,8], cadherins [9], signalling molecules receptors [10]).

The regulation of the number of cells and their arrangement is achieved through complex processes including proliferation, apoptosis and plasticity in cell-cell and cell-matrix interactions [11,12].

Although similar studies have previously been performed by other groups, the study that is reported in this paper is the first on this topic designed and carried out in Romania.

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Aim

The aim of this study was the microscopic morphologic assessment of the development of deciduous tooth germs to investigate whether it correlated with the biologic age.

Methods

The study material came from 20 human embryos and fetuses with a minimum gestational age of two months, resulting from medical or spontaneous abortions, and newborn babies that had died at birth. They were investigated in the Vaslui County Forensic Service and Pathology Laboratory of the Municipal Hospital Barlad, Romania. The gestational age of each embryo and fetus was established from the relevant medical records. Specimens were harvested, processed and stored according to the ethical guidelines for these procedures. Collection was performed only with the written consent of the family concerned. The study was approved by the ethics committees of the medical institutions mentioned above, and of the Grigore T. Popa University of Medicine and Pharmacy, Iasi, Romania.

The embryos were fixed in 10% neutral buffered formalin, fully embedded in paraffin, and 4 μm-thick serial sections were cut. The cephalic extremities (fetuses aged from 10-16 weeks) and the maxillas and mandibles (fetuses aged from 18-40 weeks) were carefully removed and dissected, fixed in 10% neutral buffered formalin, and decalcified in 5% formic acid and 5 g sodium citrate. The decalcification time was variable (minimum three days, maximum three weeks), depending on the gestational age and on the degree of mineralisation of the dental tissues. After demineralisation, the fragments were processed before being embedded in paraffin. Finally, cross-sections were cut to a thickness of 4 μm. All sections were stained with haematoxylin and eosin (HE) for standard histological examination, as well as with special stains (trichrome Masson, trichrome Azan-Heidenhain, trichrome Szekelly, PAS). The special stains were used in order to permit a clearer identification and differentiation of epithelial and mesenchymal structures within the tooth germs.

Results

Identification of tooth germs

The study allowed a global assessment of each case. Excluding artefacts due to the difficulties of the harvesting and processing the human embryonic and fetal material (including the orientation of the specimens, and the particularities of the decalcification time), only tooth germs that were completely sectioned and had preserved their structural integrity underwent morphological analysis. In these tooth germs, the histological stages of development reported in *Tables 1* and *2* were seen.

Qualitative analysis of the identified tooth germs: defining histological elements

Bud stage

The tooth germs at the bud stage presented a classical pattern, including three types of cells:

- Epithelial cells, which were compactly organised, maintaining the continuity with oral epithelial and forming the epithelial bud.
- Ectomesenchymal cells, which were arranged in a crowded manner around the epithelial bud and initiating the future dental papilla.
- Ectomesenchymal cells, which were localised more externally, as an accessory condensation that ensures the development of the future dental follicle.

Cap stage

The tooth germs in cap stage had various shapes and sizes, without a direct correlation with the gestational age *(Figures 1-3).* The morphological structure included the three main components: the enamel organ, the dental papilla and dental sac. The microscopic morphological evaluation of these components revealed cell structure details contributing to the establishment of the typical architecture. In the enamel organ, the three epithelia—inner, outer and stellate reticulum—were identified *(Figure 2).* The outer enamel epithelium consisted of a single layer of small cells, most frequently cuboidal with a curved spatial arrangement rather than a straight one. The inner enamel epithelium was characterised by the tall columnar shape of its cells. In the initial stages (incipient cap stage), the arrangement of the cells was rather crowded, suggesting a possible stratification; only later (advanced cap stage) did it become a single-layer structure. The stellate reticulum, situated between the inner and the outer ones, revealed different evolutionary aspects: in the initial stages, the extracellular matrix had a more mucoid consistency, the cells dissipated in the matrix and were without conspicuous cytoplasmic expansions, and their identification was supported by the presence of their nuclei. During development, the extracellular

Table 1. *Synopsis of the identified tooth germs in maxillae and the corresponding stage of development*

Specimen	Age (weeks)	Maxillae: Number of tooth germs/stage of development				
		I_1	**I_2**	**C**	**M_1**	**M_2**
1	8th i.u.	2 / **Bd**	2 / **Bd**	2 / **Bd**	2 / **Bd**	NO
2	8th i.u.	2 / **Bd**	2 / **Bd**	1 / **Bd**	1 / **Bd**	NO
3	10th i.u.	1 / **Cp_1**	1 / **Cp_1**	1 / **Cp_1**	1 / **Cp_1**	NO
4	10th i.u.	2 / **Cp_1**	2 / **Cp_1**	1 / **Cp_1**	1 / **Cp_1**	NO
5	10th i.u.	1 / **Cp_1**	1 / **Cp_1**	NO	NO	NO
6	14th i.u.	1 / **B_1**	2 / **Cp_2**	1 / **Cp_1**	1 / **Cp_1**	NO
7	14th i.u.	2 / **B_1**	2 / **Cp_2**	1 / **Cp_1**	1 / **Cp_1**	NO
8	14th i.u.	1 / **B_2**	2 / **Cp_2**	2 / **Cp_2**	NO	NO
9	16th i.u.	2 / **B_2**	2 / **B_1**	1 / **Cp_2**	1 / **Cp_2**	NO
10	18th i.u.	1 / **B_2**	2 / **B_1**	1 / **B_1**	1 / **Cp_2**	1 / **Cp_2**
11	18th i.u.	2 / **B_2**	1 / **B_2**	1 / **B_1**	1 / **B_1**	NO
12	22nd i.u.	1 / **B_2**	1 / **B_2**	1 / **B_2**	1 / **B_2**	1 / **B_1**
13	22nd i.u.	2 / **B_2**	1 / **B_2**	NO	1 / **B_2**	1 / **B_2**
14	22nd i.u.	1 / **B_2**	2 / **B_2**	1 / **B_2**	NO	NO
15	24th i.u.	2 / **B_2**	1 / **B_2**	1 / **B_2**	1 / **B_2**	1 / **B_2**
16	24th i.u.	NO	1 / **B_2**	1 / **B_2**	1 / **B_2**	1 / **B_2**
17	24th i.u.	2 / **B_2**	2 / **B_2**	2 / **B_2**	1 / **B_2**	1 / **B_2**
18	24th i.u.	1 / **B_2**	2 / **B_2**	1 / **B_2**	NO	NO
19	40th / birth	2 / **B_2**	1 / **B_2**	1 / **B_2**	1 / **B_2**	NO
20	40th / birth	2 / **B_2**	2 / **B_2**	1 / **B_2**	2 / **B_2**	1 / **B_2**

*$\mathbf{I_1}$: central incisor, $\mathbf{I_2}$: lateral incisor, **C**: canine, $\mathbf{M_1}$: first molar, $\mathbf{M_2}$: second molar, **Bd**: bud stage, $\mathbf{Cp_1}$: incipient cap stage, $\mathbf{Cp_2}$: advanced cap stage, $\mathbf{B_1}$: incipient bell stage, $\mathbf{B_2}$: advanced bell stage, **NO**: non-sectioned tooth germ(s)

Table 2. *Synopsis of the identified tooth germs in mandibles and the corresponding stage of development*

Specimen	Age (weeks)	Mandible: Number of tooth germs/stage of development				
		I_1	**I_2**	**C**	**M_1**	**M_2**
1	8th i.u.	2 / **Bd**	1 / **Bd**	2 / **Bd**	2 / **Bd**	NO
2	8th i.u.	1 / **Bd**	2 / **Bd**	1 / **Bd**	1 / **Bd**	NO
3	10th i.u.	1 / **Cp_1**	1 / **Cp_1**	NO	1 / **Cp_1**	NO
4	10th i.u.	2 / **Cp_1**	1 / **Cp_1**	1 / **Cp_1**	1 / **Cp_1**	NO
5	10th i.u.	2 / **Cp_1**	2 / **Cp_1**	1 / **Cp_1**	1 / **Cp_1**	1 / **Cp_1**
6	14th i.u.	1 / **Cp_2**	2 / **Cp_2**	1 / **Cp_1**	1 / **Cp_1**	1 / **Cp_1**
7	14th i.u.	1 / **B_1**	2 / **Cp_2**	2 / **Cp_2**	NO	NO
8	14th i.u.	2 / **B_1**	2 / **Cp_2**	NO	1 / **Cp_2**	NO
9	16th i.u.	1 / **B_1**	2 / **Cp_2, B_1**	1 / **Cp_2**	1 / **Cp_2**	NO
10	18th i.u.	2 / **B_1**	2 / **B_1**	1 / **Cp_2**	1 / **Cp_2**	1 / **Cp_2**
11	18th i.u.	1 / **B_2**	1 / **B_2**	NO	1 / **Cp_2**	1 / **Cp_2**
12	22nd i.u.	1 / **B_2**	2 / **B_2**	1 / **B_2**	1 / **B_1**	1 / **B_1**
13	22nd i.u.	2 / **B_2**	1 / **B_2**	1 / **B_2**	NO	NO
14	22nd i.u.	2 / **B_2**	2 / **B_2**	1 / **B_2**	NO	NO
15	24th i.u.	1 / **B_2**	1 / **B_2**	1 / **B_2**	1 / **B_2**	1 / **B_2**
16	24th i.u.	NO	1 / **B_2**	1 / **B_2**	1 / **B_2**	1 / **B_2**
17	24th i.u.	2 / **B_2**	2 / **B_2**	2 / **B_2**	1 / **B_2**	1 / **B_2**
18	24th i.u.	2 / **B_2**	1 / **B_2**	NO	1 / **B_2**	1 / **B_2**
19	40th / birth	1 / **B_2**	1 / **B_2**	1 / **B_2**	1 / **B_2**	1 / **B_2**
20	40th / birth	2 / **B_2**	1 / **B_2**	1 / **B_2**	2 / **B_2**	1 / **B_2**

*$\mathbf{I_1}$: central incisor, $\mathbf{I_2}$: lateral incisor, **C**: canine, $\mathbf{M_1}$: first molar, $\mathbf{M_2}$: second molar, **Bd**: bud stage, $\mathbf{Cp_1}$: incipient cap stage, $\mathbf{Cp_2}$: advanced cap stage, $\mathbf{B_1}$: incipient bell stage, $\mathbf{B_2}$: advanced bell stage, **NO**: non-sectioned tooth germ(s)

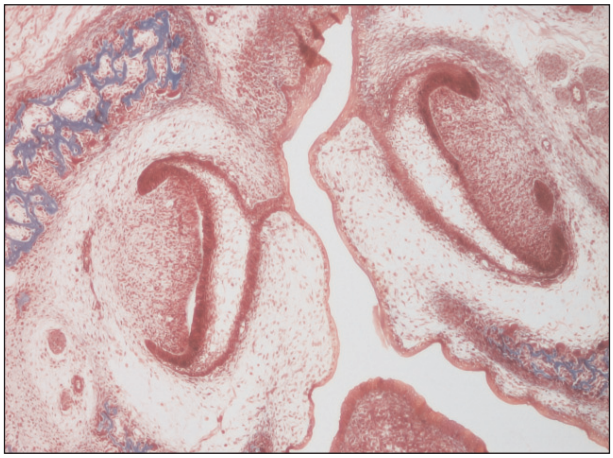

Figure 1. *Developing oral cavity: tooth germs (central incisors) in cap stage, connected to the oral epithelium (Masson, x 2).*

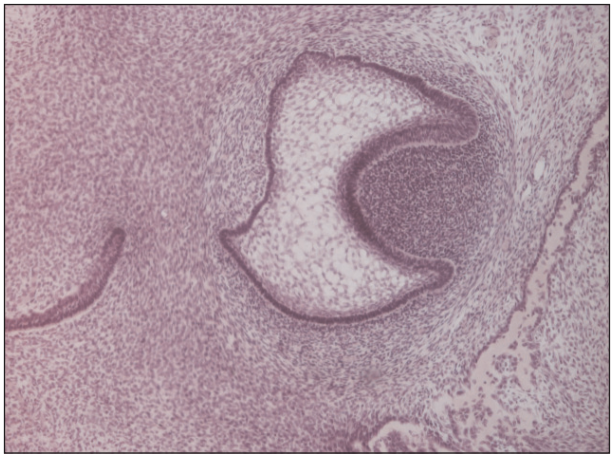

Figure 2. *Tooth germ in incipient cap stage (first molar): the enamel organ, the dental papilla, the dental sac (HE, x 4).*

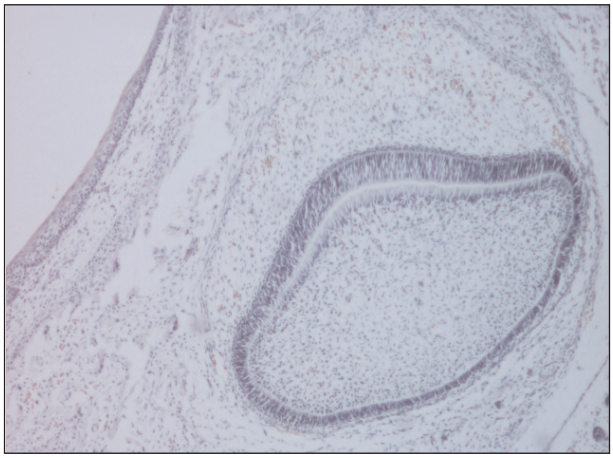

Figure 3. *Tooth germ (lateral incisor) in advanced cap stage: obvious condensation of the ectomesenchymal cells at the periphery of dental papilla (HE, x 4).*

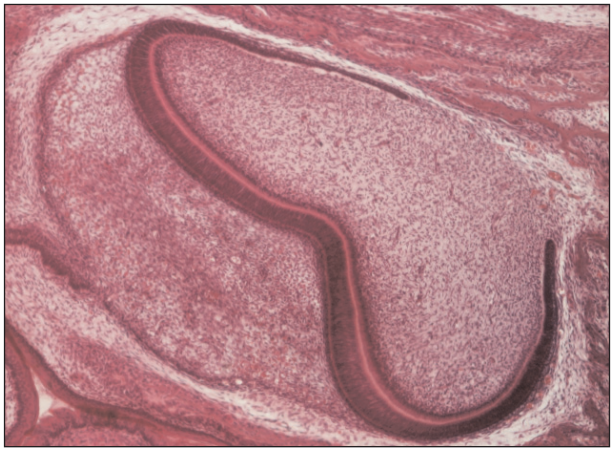

Figure 4. *Tooth germ (first molar) in incipient bell stage: first dentine layer, Hertwig epithelial sheath in different stages of development (mesial vs. distal) (HE, x 4).*

matrix became more transparent and clear and its cells achieved their stellate shape with multiple interconnecting expansions defining an obvious aspect of a cell network. In the dental papilla, the absence of homogenous features characteristic to the stage of incipient cap was noted. The convex area of the papilla, corresponding to the concavity of the enamel organ, showed distinct areas of cell densification typical of the advanced cap stage *(Figure 3).* The dental sac was evident, the ectomesenchyme was concentrically organised and supported the other two components (*Figure 3).*

Bell stage

The tooth germs at the bell stage were of various shapes and sizes, their diversity was independent of the gestational age, making a dynamic evaluation possible for the three main components: enamel organ, dental papilla and dental sac *(Figures 4-9).*

The analysis of the enamel organ allowed the observation of the multiple and successive changes in shape and size that determine the passage from the cap to the bell stage. For the outer epithelium of the enamel organ, the cells—mostly cuboidal with reduced cytoplasm and large conspicuous nucleus—maintained the irregular curved arrangement from the cap stage along the concavity, except at its upper pole where their arrangement was linear. The appearance of the stellate reticulum of the enamel organ was typically that of an interconnected cell network, consisting of stellate cells with multidirectional processes. Between the stellate reticulum and the inner epithelium, the stratum intermedium of the enamel organ was identified; at this stage, it consisted of one to four cell layers, which although extremely differently shaped, suggested a perpen-

dicular disposition on the inner epithelium cells. The analysis of the inner epithelium revealed two different aspects, allowing for the differentiation of the two stages: incipient bell *(Figure 4)* and advanced bell *(Figures 5* and *6).* In the incipient bell stage, the inner epithelium consisted of columnar cells with a basophilic cytoplasm and, extremely importantly, a central position of the nucleus. The advanced bell stage was defined by the presence of dentine deposed by the odontoblasts in the dental pulp, with the consequent occurrence and later apposition of the enamel layer *(Figures 5* and *6).* In the inner epithelium of the enamel organ, the elongation of the columnar epithelial cells was identified: pre-ameloblasts and then ameloblasts, the characteristic morphologic feature being the change in their polarity *(Figure 7).* The process was conspicuous through the specific position of the nucleus and the reorganisation of the cytoplasmic distribution. In the development of the bell stage, the thickness of the enamel layer was variable, clearly greater in the incisal areas and/or at the level of the future cusps and smaller in the lateral areas of the future crown *(Figures 8* and *9).*

The structure characteristic for the enamel organ included the transformation of Hertwig's epithelial sheath *(Figures 8* and *9),* which contributed to the development of the epithelial diaphragm, which was also identifiable in the specimens. The dental papilla showed transformations directly correlated with those identified in the enamel organ. The mesenchymal cells clustered at the periphery of the papilla at the end of the cap stage and evolved morphologically and functional-

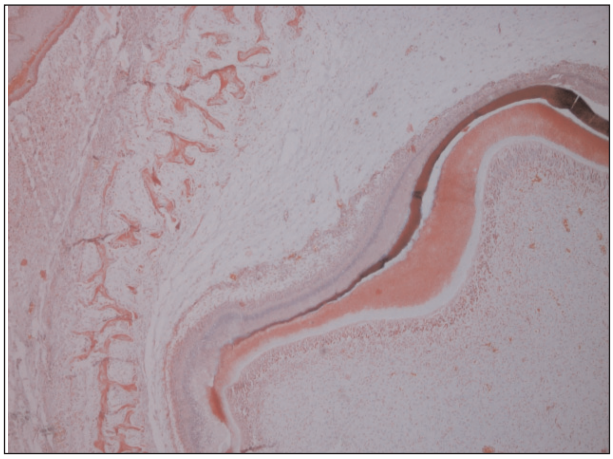

Figure 5. *Tooth germ (central incisor) in advanced bell stage: the enamel organ, the dental papilla, the dental sac (PAS, x 4).*

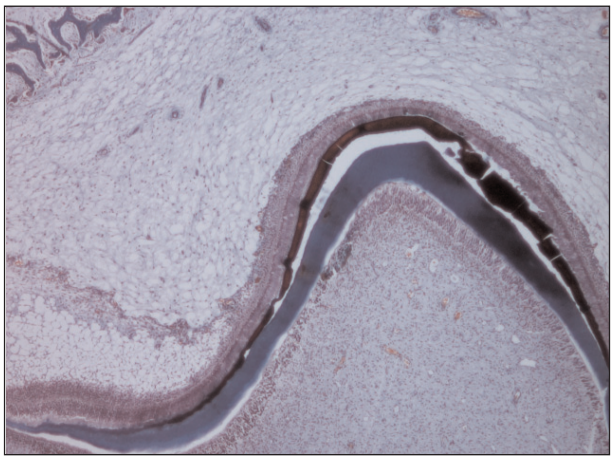

Figure 6. *Tooth germ (central incisor) in advanced bell stage: cusp tip presenting dentine, enamel, and the outer enamel epithelium positioned next to the stratum intermedium in areas of more advanced apposition (Masson, x 4).*

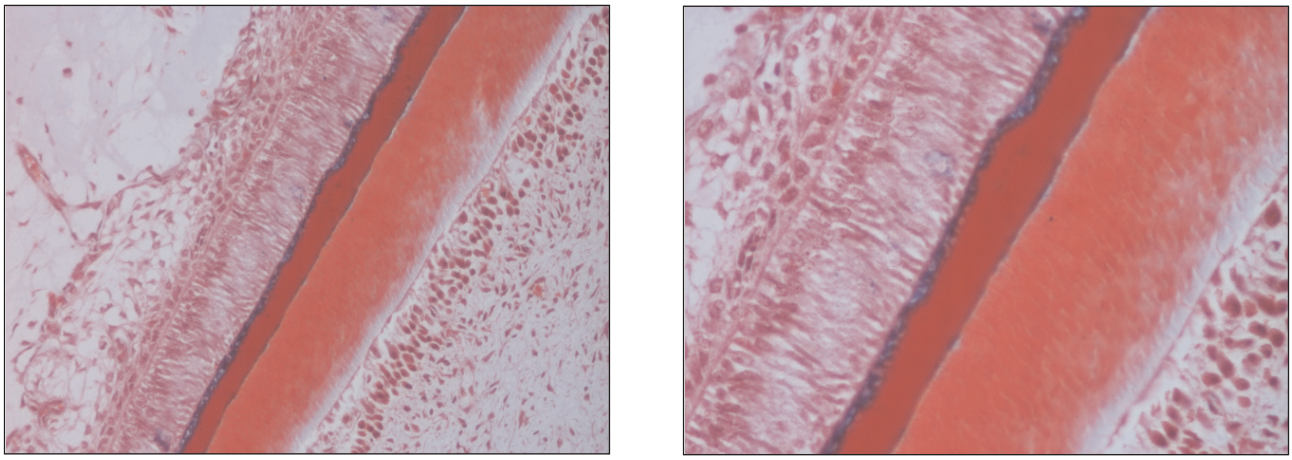

Figure 7. *Tooth germ (central incisor) in advanced bell stage: details for ameloblasts, enamel, dentine and odontoblasts (Masson, x 10(a), x 20(b)).*

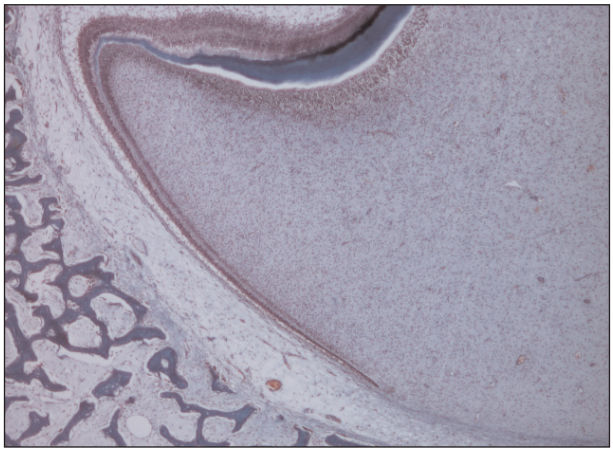

Figure 8. *Tooth germ (central incisor) in advanced bell stage: the Hertwig epithelial sheath (Masson, x 4).*

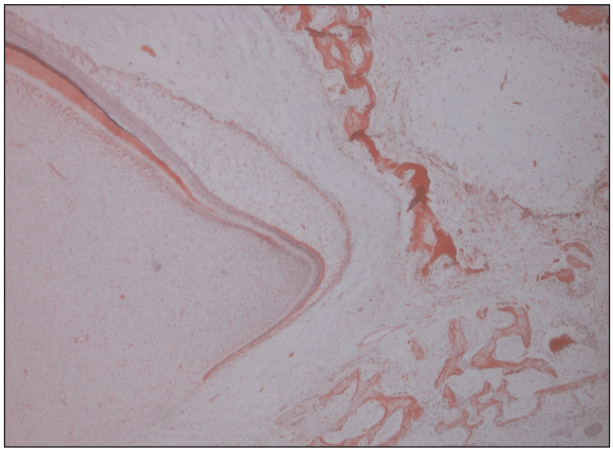

Figure 9. *Tooth germ (central incisor) in advanced bell stage: transformation of the Hertwig's epithelial sheath into the epithelial diaphragm (PAS, x 4).*

ly. Consequently, in the incipient bell stage a continuous cell layer consisting of tall cuboidal cells with oval nuclei located at their basal pole—the odontoblasts—was present, covered by the first line of dentine, with variable thickness according to the gestational age and, implicitly, to the age of the tooth germ *(Figures 5-7).* Under the odontoblastic layer, the dental papilla converted into dental pulp, maintaining its clustering of mesenchymal cells, thus constructing the sub-odontoblastic layer with differentiation potential, under certain circumstances, into odontoblasts. The central area of the dental pulp presented the typical features of a loose connective tissue. The advanced bell stage was characterised by the changes from the periphery of the dental pulp, marking the development of the peripheral pulp with an architecture differing from that of the central pulp through the occurrence, under the odontoblastic layer, of the cell-free zone (zone of Weil) and of the cell-rich zone. The dental sac was readily identifiable around the developing tooth germs; its structure was compact *(Figures 8* and *9).*

Discussion

The morphogenesis of the tooth and its supporting tissues is a component of the embryologic development of an individual and is in direct correlation with the development of the head. For many years, researchers have studied the mechanisms involved in the early development of the structures associated with the oral cavity and specifically those building the tooth and its supporting structures. The sequential morphologic development of these structures is already known, so current research (mainly experimental) focuses on a biologic approach at a molecular and genetic level [4,6,9,10], opening broad perspectives for tissue engineering [13,14].

The development of the tooth takes place as a consequence of the interrelations between the oral epithelium and its subjacent mesenchyme [2,15]. The development process is essentially identical for all teeth, both deciduous and permanent. The odontogenesis involves morphogenesis, epithelial histogenesis and cell differentiation [2]. The regional development of the teeth can be translated through modulation—space and time—involving the understanding of the concepts of induction, competence and differentiation [1]. Dental epithelial remodelling results from variations in the cell proliferation speed, apoptosis and changes in the adherence and the shape of the cell, leading to the placement of the cells with different functions [2]. Independent of the signalling molecules, which play a major role in the induction or the modulation of specific stages, cell-cell and cell-matrix interactions regulate the plasticity/rigidity of certain areas in the enamel organ [2].

Within this framework, for the first time in Romanian dental research, the current study has demonstrated the picture of the tooth development phases versus the biologic age, and, concomitantly, comments on the difficulties occurring during the investigation of tooth development.

The value of microscopic examination in the characterisation of the tooth development stages

The entire deciduous dentition is initiated between

weeks 6 and 8 of embryonic development, the permanent successional dentition between week 20 *in utero* and the tenth month after birth, and the permanent molars between week 20 *in utero* (for first molars) and the fifth year of life (for third molars) [1]. In the current study, the ages of the embryos and fetuses enabled an investigation across many stages in the development of the deciduous dentition. However, the study focused on the identification of tooth germs in pre-eruptive stages. Depending on the biologic age, it was possible to observe differences in the position of the developing tooth germs on the inside of the maxillary bone in the areas equivalent to the dental alveoli. The differences in the position of the tooth germs depended on the development stage of the maxillary and constituted an indicator of the eruptive position. The tooth germs identified in the first trimester of pregnancy were of very small size and were located at considerable distance from one another. The tooth germs identified in the second and third trimesters of pregnancy were larger, as a result of fast growth and, consequently, the space between them was considerably reduced. However, as tooth formation occurs over a wide time span, the developmental stage of individual tooth germs is poorly related to gestation time.

The microscopic examination confirmed the asymmetrical growth characteristics of the components of the tooth germs and was representative of the proliferation stage, which leads to the cap stage. There was a transformation of the epithelial bud with a change in its shape from oval to round and, later on, to central curving leading to the appearance of the cap structure placed over a "ball" of condensed mesenchymal tissue.

The microscopic examination also allowed visualisation, in the evolution from cap to bell stage, of the transformation of the cervical loop into Hertwig's epithelial sheath by the expansion of the inner and outer epithelia of the enamel organ, and the disappearance of the stratum intermedium and stellate reticulum.

The analysis of the structural details of the tooth germs leads to an evaluation of the changes occurring in the ameloblasts, a morphological event essential in the tooth development process. The tooth germs of fetuses in the second and third trimesters of pregnancy showed the elongation of the cells in the inner epithelium of the enamel organ. Thus, in a first stage it was possible to identify the central position of the oval nucleus, occupying almost the entire cell cytoplasm. Later, an increase in cell size, which causes the oval nucleus to become placed at the apical pole (towards the stratum intermedium), was noted. The tooth germs presenting the first dentine layer revealed prismatic ameloblasts with a nucleus that had already migrated to the basal pole (towards the dental papilla). All these modifications provided evidence of the modifications in the polarity of the ameloblasts, an event which precedes the occurrence of an apical cytoplasmic differentiation (the Tomes process), conspicuous in the advanced bell stage and characterised by the deposition of the first enamel layer. It should be stressed that identification of the Tomes process, prominent in newly formed enamel, is extremely difficult due to the characteristics of hard-tissue processing. However, because of the large number of tooth germs that were investigated, this was possible and the typical aspect of "saw-teeth" or "picket fence" created by the Tomes process at the junction between the ameloblasts and the enamel in the process of formation was observed.

Concurrently with the appraisal of modifications in the ameloblasts, it was also possible to observe the transformation of the odontoblasts placed at the periphery of the dental papilla, turning into dental pulp. The first indication of this differentiation was identified in tooth germs at the incipient bell stage. It was the disappearance of the cell-deprived area between the papilla and the inner epithelium of the enamel organ while in the advanced cap stage, the occupation of this territory was achieved by the increase in size of the odontoblast. The microscopic examination allowed identification of tooth germs in the incipient bell stage at the starting point from the area at the top of the dental papilla adjacent to the inner epithelium of the enamel organ, where the first layer of dentine arises. Other tooth germs revealed different stages in the process of dentine deposition on the side of the folding of the zone previously described and indicating the starting point of the development of cusps, advancing downwards and laterally on the cusp slope up to the Hertwig epithelial sheath. Because of the differences in the thickness of the dentine layer, it was possible to separate the peripheral outer dentine (mantle dentine), with a lower mineralisation degree, from the circumpulpal dentine, which already contained dentinal tubules, their slightly woven trajectory being a clear indicator of the arrangement of the odontoblasts in their cen-

tripetal movement towards the inside of the dental pulp.

During examination of the tooth germs, the existing blood supply was also assessed in order to evaluate the differences between the cap and bell stages. The multiplication of the vascular elements in the dental papilla was noted. They were simultaneous with the passage from the cap to the bell stage. The important development of the blood supply in the dental sac responsible for the nourishing support of the ameloblast and the entire enamel organ—after differentiation of the ameloblasts and the beginning of enamel formation, with the consequent interruption of contact with the dental pulp—was also noted.

These descriptive elements offer a picture of the microscopic morphology of tooth germ development. At an international level, the histology described in this paper has previously been reported. However, it seems that it has previously been supported by a relatively small number of images and they appear frequently, with due acknowledgement to their original authors, in a number of books and other publications. This may be because of the difficulties in accessing suitable material. The authors of this paper believe that the number of embryos and fetuses examined and the number of the resulting images will make a contribution to the current state of knowledge.

Difficulties in the tooth development research

Technical considerations

The histological processing of the tooth requires a mandatory phase for demineralisation, because of the hard, inorganic material present in some structures. Consequently, several technical problems can occur. The choice of the best demineralisation solution and the assessment/evaluation of the time needed for this procedure are dependent not only on the instructions available in books, but also on the experience of each laboratory. We consider that our technique leads to specimens of high quality, ensuring the visual support for our results.

Bioethical implications

The research on tooth development in humans is difficult because of bioethical implications. The use of human material, represented by embryos and fetuses, is highly controversial [16] as a result of moral, ethical and religious considerations. However, studies based on cell lines or animal models cannot completely replace the human model. In this context, the authors consider that this study has been valuable and that the investigation of human material should be permitted as long as the ethical principles of the research are respected.

Conclusions

In the cap and bell stage, the tooth germs had different morphologic features, without a direct correlation to gestational age. The marked diversity in the shapes and sizes of the identified tooth germs supports the influence of genetic factors and of specific interactions with the micro-environment in tooth development.

Contribution of each author

- SDS and IDC designed the study.
- SDS collected the specimens.
- VD performed the dissections of the fetuses, before the fixation of the specimens.
- SDS and MT performed the histological examinations and analysed the results. They produced the first draft of the manuscript.
- IDC planned and conducted the study, participated in the interpretation of the results, and drafted the final version of the paper.
- All authors approved its content.

Statement of conflict of interests

The authors are aware of no conflicts of interest.

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