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RESEARCH

Prenatal and postnatal development of New Zealand white rabbit (*Oryctolagus cuniculus*) teeth: histological and computed tomography aspects

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Abstract
The study reveals a lack of histomorphogenesis in New Zealand white rabbit teeth. The teeth development was examined through sequential histological segments in 24 rabbits from prenatal ages (E19, E21, E23, E25, and E28), neonates (E30), and postnatal age (1 week and 2 weeks); (three animal specimens at each age stage). Rabbit teeth first appeared at 19 days of prenatal life (E19) as an ectodermal epithelial thickening on each side of the mouth opening. At E21, the bud of upper incisor tooth appeared as an epithelial bud, which composed of many condensed epithelium cells, was simply identified from the larger with less condensed vestibular lamina, and was surrounded by mesenchymal connective tissue while the lower incisor took the cap stage. At (E23), tooth regular construction is formed from enamel, dentine, and pulp cavity. Peg incisor appearance (supplementary and assistant incisors) is visible at the lingual surface of the upper major incisor. Teeth prenatal development went through successive stages like initiation, bud, cap, late bell, maturation, and crown stages. The first initiation phase of tooth formation was seen as ectodermal epithelial cell collection at (E19). Bud stage saw on upper incisor tooth, while in cap structure in lower incisor teeth at (E19). A cap-formed tooth is composed of the enamel organ and fundamentally dense mesenchymal tissue. Enamel organs are segmented into three distinct layers: the external tooth enamel epithelial, the internal tooth enamel epithelial, and finally the stellate reticular layer. The cement layer covered teeth all around on enamel on both the labial and lingual sides while not contacting the dentine on the lateral side, forming enamel space. Teeth develop consistently all through life; they have expanded enamel thickness; they are diphyodont teeth; they have two continuous dentitions; they are deciduous and perpetual, with long crown teeth and an open root.

Keywords Histomorphogenesis · Rabbit · Teeth · Cementum layer · Enamel organ

Introduction

Lagomorphic mammals known as rabbits can be distinguished from rodents by their two sets of upper incisive teeth, in which the second pair is located shortly beyond the large incisor, as well as their two designated stake incisors, known as the assistant and beneficial incisors. There are no sources in the current document. Moreover, unlike felines, canines, ferrets, and hedgehogs, rabbits lack canine teeth (Meredith 2006). The rabbit teeth is heterodont teeth of various sorts and different sizes just as assorted shapes (incisors, premolar, and molar) types (Crossley 2000; Zoba and Rabab 2012). The rabbits are most appropriate as the favored exploratory creature for early-stage research because of their high rearing rate, the number of springs, and fast proliferation cycle (Haddad et al. 2021; Lerner and Kuhn 1997).

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The mammalian teeth is fundamental for their life. In the herbivorous mammals, the dental adaptations to accessible eating regimens also archived, for example, a morphological transformation of a finish edge course of action or biting muscle size to amplify biting adequacy (Clauss et al. 2008; Fritz et al. 2009; Kaiser et al. 2010). The development of teeth is extremely planned in: mice (Balic and Thesleff 2015), fish (Rasch et al. 2016), or reptiles (Whitlock and Richman 2013). Bertin et al. 2018 explore recent research on tooth implantation, attachment, and replacement in amniotes, revealing evolutionary and developmental mechanisms and providing insights into tooth structure and function diversity across different species.

The rabbit is an appropriate model to contemplate mammalian tooth substitution as opposed to the mouse as the mouse has a solitary, irreplaceable (permanent) tooth set (Bertonnier-Brouty et al. 2020). Moreover, rabbits are alluring model creatures to contemplate tooth development since they are benefiting from wide sorts of vegetation. Besides, they have ceaselessly developed teeth all through life, as every lasting tooth in the hare has a collection of the mesenchymal and epithelium undifferentiated cells in its developing portion. As in all hypselodont animals (Tummers and Thesleff 2003), their dental hygiene was significantly examined in the animal science research (Jekl and Redrobe 2013; Van Caelenberg et al. 2011); the constantly ever-increasing (hypselodont) teeth of numerous rodents and lagomorphs (Ungar 2010), by increased enamel thickness (Rabenold and Pearson 2011). The rabbit is extraordinary compared to other comprehensively utilized reenactments for surveying dental embed (Campillo et al. 2014).

The rabbit teeth has certain unmistakable mammalian highlights. They are diphyodont teeth, and their teeth is replaced only once along with their life, (Bertonnier-Brouty et al. 2020) for example, two continuous dentitions (deciduous and permanent dentition) and a radicular hypsodont (long crown but no true root) open root (Damuth and Janis 2014; Zoba and Rabab 2012).

The aim of the current investigation is planned to briefly demonstrate and inspect the development of the rabbit teeth using various morphological approaches and some histological staining techniques due to the lack of data illustrating the histomorphogenesis development of rabbit teeth. Therefore, the obtained results may serve as a tool for determining the tooth age of the rabbit embryo and fetus.

Materials and methods

Collection of the different examined age stages of animals

Twenty-four embryos and fetuses of NZW rabbits were examined. Three embryo rabbits of each prenatal stage (E19, E21, E23, E25, and E28), three neonates or birth day (E30), and also three fetuses of postnatal age (1 week and 2 weeks) were obtained from the pregnant rabbits acquired from the Public Service Center for Veterinary Inspection in the University of Sadat City (USC), Egypt. The day after mating between male and female rabbits has been indicated as embryonic day (E0) reported in (Abdo et al. 2017) as the ovulation in rabbits occurred only after mating by 10–12 h (Sirotkin et al. 2010). Apparently healthy rabbit embryos at days (19, 23, 25, 28), neonates (day 0), and the postnatal age at 1-week and 2-week age stages were subjected to euthanasia, in which the rabbits were sedated by anesthesia using sevoflurane (United States Pharmacopeia (USP)) as an inhalational agent to lose consciousness and then slaughtered in the cervical region following euthanasia techniques.

This study was conducted in accordance with ethical guidelines and approved by the Institutional Animal Care and Use Committee. All animal control procedures were carried out following the Rules of the Institutional Animal Care Committee (Alexandria University and the University of Sadat City, Egypt), the Egyptian Ethical Code for studies on experimental animals, and the Use Committee (IACUC), with prior approval for using the animals.

For histological examinations using light microscope

Eighteen specimens were collected from (embryos and head of fetuses (pericranium) from examined developmental age of the rabbits (E19, E21, E23, E25, and E28) along the length of the nasal cavity, i.e., the frontal sutures of E28, neonates, and postnatal age were achieved and fixed in 10% neutral-buffered formalin for examination by light microscopic. The collected tooth specimens from different examined ages of rabbits were pounded briefly in tap water, then fixed in 10% formaldehyde for 48 h at pH 7.4, then dehydrated in a graded series of ethanol, cleared with xylene, and immersed in melted paraffin. The paraffin-embedded tissues were cut into 4-µm sections using a Leica rotatory microtome (RM 20352035; Leica Microsystems, Wetzlar, Germany) and stained with hematoxylin and eosin (Haddad et al. 2021). Then, the specimens were grouped: group stained with Hematoxylin and

Eosin (H&E) (Suvarna et al. 2019) for examination of the general histological features; group stained with Masson's trichrome (Masson 1929) for collagen and muscle fiber investigations; and group stained with toluidine blue stains (Suvarna et al. 2013). All stained sections were inspected on the Olympus CHS light microscope (Olympus Optical Co., Ltd., Tokyo, Japan).

Photomicrography

The digital images were captured with a digital camera (Olympus DP71 camera) attached to a dissecting stereomicroscope with white adjustable (Olympus VM VMF 2x, Eyepiece 10x Stereo Microscope, Japan). An image analysis program, DP Controller, and DP Manager Software were used. There was no adjustment in the captured images except for the adjustment of contrast and brightness in Adobe Photoshop Elements (Adobe Systems, Tokyo, Japan) (Gabr 2015; Haddad et al. 2021).

Computed tomography (CT scan)

Six heads of examined rabbits' fetuses (pericranium) at (E0) or birth day), 1 week, and 2 weeks of postnatal age were achieved and applied using a 64-detector row CT scanner (Somtam Sensation, Siemens, Forcheim, Germany) at 120 kV and 117 mAs, a 10 cm FOV with a slice thickness of 1 mm. The computed tomographic images were reconstructed in dorsal and sagittal planes via software (Syngo CT 2006G, ICS VB28B, Siemens, Munich, Germany) (Özkadif and Eken 2012; Gabr 2015).

Results

Histomorphological examinations

Rabbit teeth implicated the ectoderm, which gave enamel, and the mesodermal, which gave rise to dentine and pulp. Rabbit teeth appeared first as an ectodermal epithelial thickening on each side of the mouth opening entrance appeared firstly at embryonic age (E19), as shown in (Fig. 1). At embryonic age (E23), the typical structure of a tooth was formed: an enamel layer, dentine, and pulp cavity, encircled by trabecular bony plates of the maxilla and mandible (which appeared within the alveolar bone, either of the maxillary bone or the mandible), as shown in Figs. 2 and 4. The rabbit teeth developed consistently all through life. They have increased enamel thickness; they were diphyodont teeth; they had two consecutive dentitions (deciduous and permanent), just as they were portrayed by a long crown tooth with an open root. Rabbits had four upper incisors (major and minor), as shown in (Fig. 3). The minor one was located at the lingual surface of major upper incisors and was called assistant incisors (peg teeth), as appeared in (Fig. 3).

Embryos of rabbit at 19 days of gestation

At the 19-day prenatal rabbit embryo (E19), the tooth primordial showed up as an ectodermal epithelial thickening observed on both sides of the entrance opening to the mouth opening (Fig. 1A–B). These areas were composed of undifferentiated epithelium cellular collections that constituted the initial indication of tooth development in rabbits (Fig. 1A–D).

Embryos of rabbit at 21 days of gestation

At the 21-day prenatal rabbit embryo (E21), the upper incisor tooth developed and advanced as a tooth bud. To create the tooth bud, the lower dental layer appeared broader and more aggregated. This type of tooth bud, which was surrounded by mesenchymal connective tissue and was made up of many condensed epithelium cells, could be simply differentiated from the larger, less condensed vestibular layer (Fig. 2A). However, the lower incisor tooth first emerged as a cap phase in the second phase of tooth formation. The enamel layer and the underlying thick mesenchymal tissues made up the cap-shaped tooth. The exterior enamel epithelial cells, the interior enamel epithelial cells, and the stellate reticulum were distinguished as the three layers of the enamel layer. From cuboidal to columnar epithelial cells, exterior and internal enamel epithelium cells were produced. Polygonal, faintly pigmented epithelium cells made up the majority of the stellate reticulum. The dental papilla was created from the tooth mesenchyme that was located beneath the internal enamel epithelial and appeared to be condensed. The tooth follicle was created by the mesenchyme that covers the tooth germ (Fig. 2B).

Embryos of rabbit from 23- to 25-day of gestation

At the 23-day prenatal rabbit embryo (E23), the differentiation of the tooth germ happened at this age compared to previous age stages. Two upper roots of major and minor incisor teeth (peg incisor) that were termed assistant incisor teeth emerged in the rostral part of the embryo head embedded in the alveolar socket of the maxilla, separated by maxillary symphysis. The peg incisor appeared only on the lingual surface of the upper major incisor teeth (Fig. 3A). According to our histological investigations, the incisor tooth had the appearance of the late bell phase. The outside enamel epithelium was connected to the long, pointed dental papilla. It seemed to be rotating into the tooth pulp and becoming highly vascularized.

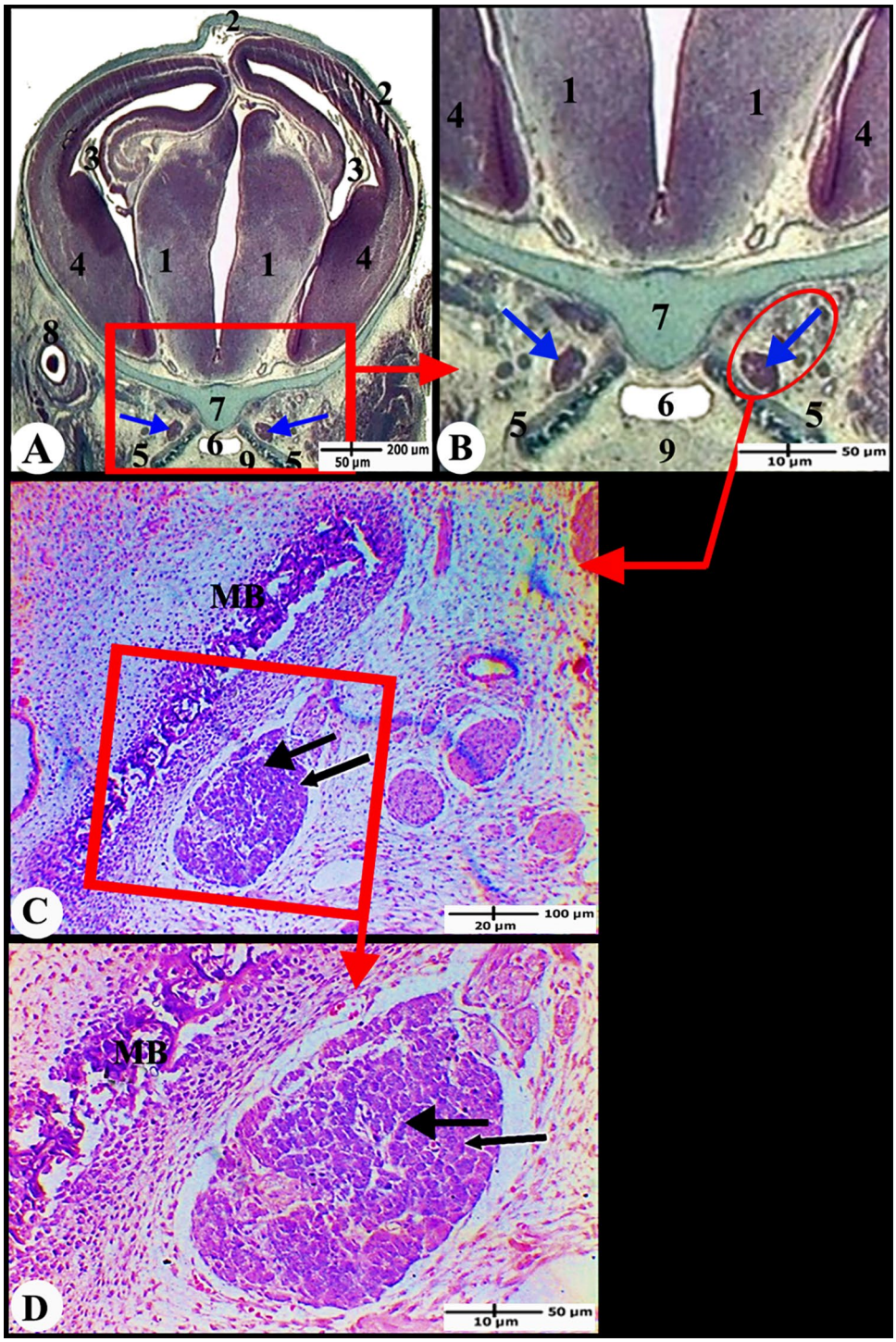

Fig. 1 Photomicrograph of prenatal rabbit embryo, at E19 day: **A** and **B** (magnification of **A**): represents the cross section of 19-day prenatal rabbit embryo showing the primordia of the tooth (blue arrows) on both caudal sides of the upper jaw: medial nasal process (1), cartilaginous lateral nasal capsule (2), primitive nasal campers (3), lateral nasal process (4), separated palatine process (5), primitive stomodaeum (6), ventral pear-shaped cartilaginous nasal capsule (7), optic disk (8), maxillary symphysis (9) Masson trichrome stain. stereomicroscope. **C** and **D** (magnification of **C**): show the primordia of the tooth that appeared as a thickening area of epithelial cells (black arrow), bone of maxilla (MB). Light microscope H&E X40 (**C**) and X100 (**D**)

In consequence of the overproduction of tooth enamel at this phase, the inner enamel epithelial cells were differentiated into ameloblasts and became darkly stained. The odontoblasts were formed by cuboidal or low-columnar epithelial differentiation of the tooth pulp's external layer. As a result of the cells' dentin secretions, these cells had a faintly stained color. The enamel matrix was noticeable all around the tooth. The dental pulp seemed to have good blood flow. Dental hard tissue growth was linked to the late bell phase of tooth development (Fig. 3A–F).

In addition, at these ages, at the cross section in the middle part of the head of the embryo, the molar tooth appeared in the form of the crown stage. The tooth crown established the final form and shape in which it expanded, condensed, and made up around 2/3 of the length of the tooth. Also, the dentin was thick. It was thicker closer to the tooth's crown

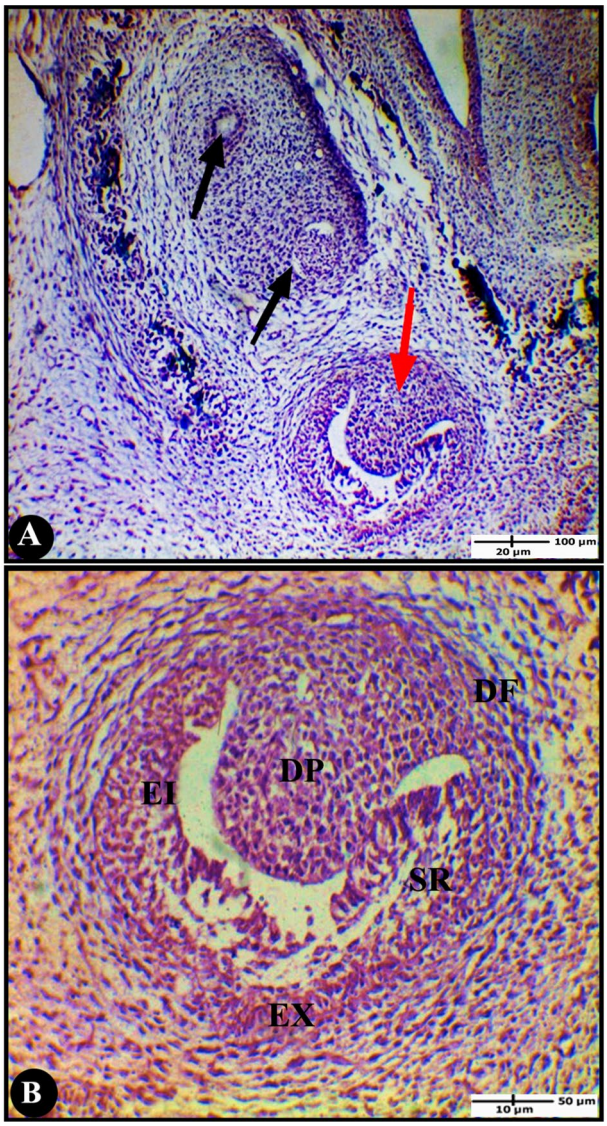

Fig. 2 Photomicrograph of rabbit teeth at E21-day prenatal age **A** represent the upper incisor tooth in the form of the bud stage (black arrow) and the lower incisor tooth in the form of the cap stage (red arrow). Masson trichrome stain, X40 (light microscope). **B** represent the structure of the tooth in the cap stage to show: external enamel epithelium (EX), internal enamel epithelium (EI), dental papilla (DP), dental follicle (DF), stellate reticulum (SR). H&E stain X100

(proximally), and it was thinner downhill till it completely vanished at the tooth's neck. Near the proximal part of the crown, enamels were extremely thick and dense and grew thinner farther away (distally). The pulp of the tooth seemed narrow throughout the crown tooth length while it compressed distally, generating a very short neck at the crown apex (Fig. 4A–C).

The molar tooth germs also achieved their most advanced development around the 25th day of pregnancy (E25), taking the form of cusps in a cross section in the posterior part of the embryo's head (Fig. 5A). The distal fissure was missing its earlier obliquity and currently extended in a straight pattern across the tooth, while the fissures between the cusps were deeper and more upright. There have been instances where the central cusp has been fragmented into two or three smaller cusps (Fig. 5B). The exterior enamel epithelium's cells have begun to lose their cuboidal form and flattened appearance, penetrating the stellate reticulum as the blood vessels now pierce it (Fig. 5B).

Embryos of rabbit from 28 to 30 day of gestation

In a 28–30-day prenatal rabbit embryo (E28–30), the stereomicroscopic observation of the cross section of the rabbit embryo head revealed the presence of two upper molar teeth and two lower molar teeth (Fig. 6A–B). Both upper and lower teeth were symmetrical to each other in shape (Fig. 6A–F). It consisted of a crown and a root. The root was bifurcated into two halves. By light microscopic examination, the upper molar tooth was formed from a dentine core. The enamel organ covered the dentin on both the labial and lingual sides, while it was absent on the lateral surfaces. A layer of cementum covered the tooth all around on the enamel on both the labial and lingual sides while not contacted the dentine on the lateral side, forming a distance called enamel space (Fig. 7). The lower molar tooth was similar to the upper molar tooth. It was shown that in the lower molar teeth, well-developed ameloblasts were detected with a thin layer of enamel matrix, while the ameloblasts were not present on the other side (Fig. 8).

One- to 2-week-old rabbit

Teeth grew continuously throughout life. They increased enamel thickness, and they were diphyodont teeth. Besides, they have two consecutive dentitions; they are deciduous and permanent; and they had long crown teeth with open roots due to enamel space. At 1 week, the stereomicroscopic examination revealed that the root of the upper incisor teeth was separated by the maxillary symphysis (Fig. 9A). The upper major and minor incisor check teeth (premolar and molar) were curved (Fig. 9B). The root and the crown of the upper incisor is displayed in the figure (Fig. 9C). In a 2-week-old rabbit, the computed tomographic examination revealed that upper and lower incisor teeth, and check teeth (premolar and molar) were well-developed (Fig. 10A). The stereomicroscopic examination revealed the root of the upper molar teeth (Fig. 10B).

Discussion

The rabbits are considered a decent model in embryological examination (Haddad et al. 2021). The normal developmental studies on in NZW rabbit are crucial for mammalian

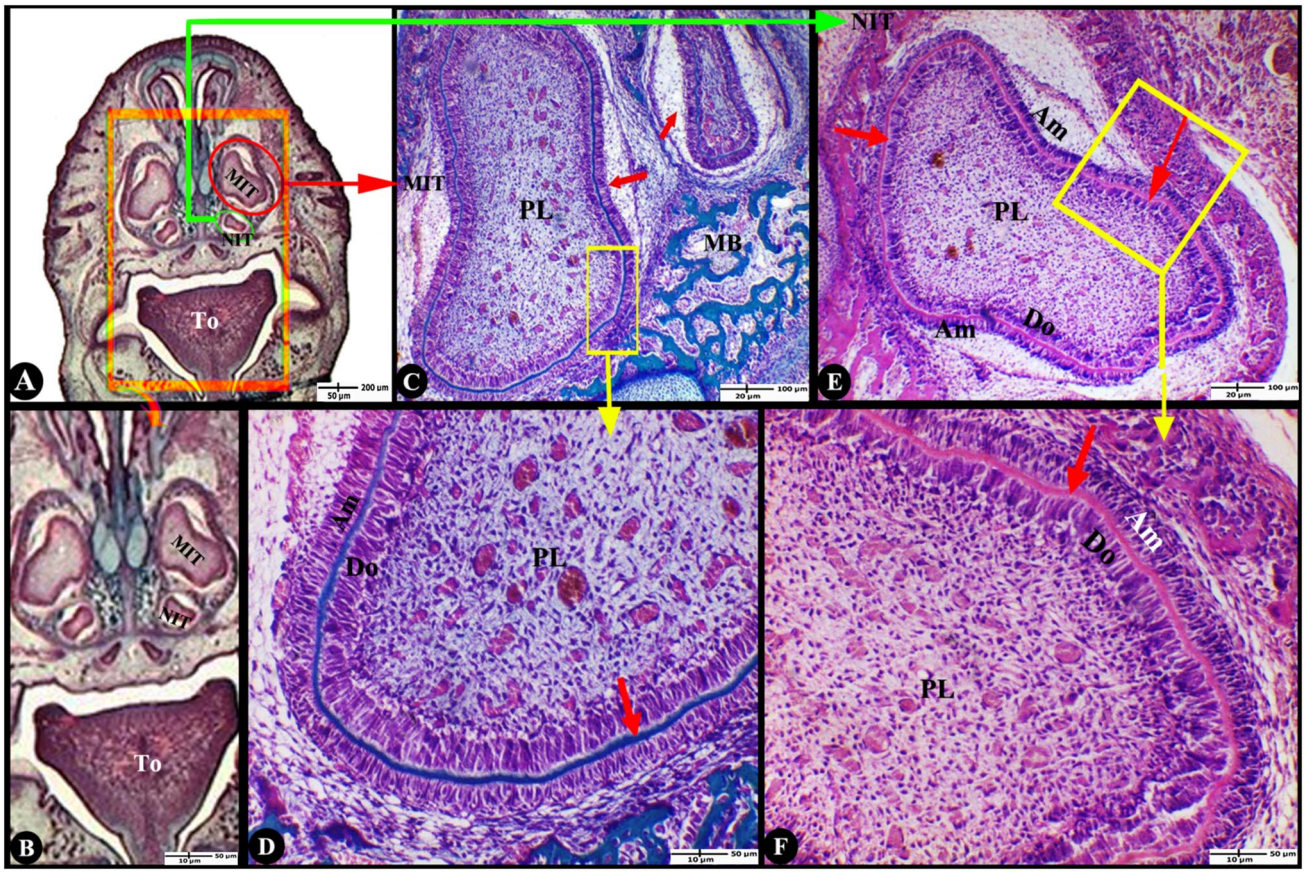

Fig. 3 Photomicrograph of prenatal development of rabbit teeth at a E23-day prenatal rabbit embryo **A** and **B** represents the rostral part of the nasal cavity at (E23), showing two upper roots of the major (MIT) and minor (NIT) upper incisor teeth (peg incisors) or assistant incisors tooth, tongue (To). Masson trichrome stain stereomicroscope. **C** represents the histological image of major (MIT) upper incisor teeth in their late bell stage to show the enamel matrix (red arrow), maxillary bone (MB), the enamel matrix (red arrow), and dental pulp (PL). Masson trichrome stain X40 (light microscope). **D** represent the magnified histological image of major (MIT) upper incisor teeth in their late bell stage to show ameloblasts (Am), odontoblasts (Do), the dental pulp (PL), enamel matrix (red arrow), and maxillary bone (MB). Masson trichrome stain X100. **E** represent the histological image of the minor (NIT) upper incisor tooth (peg incisors) or assistant incisor tooth to show the structure of the developing tooth: ameloblasts (Am), odontoblasts (Do), dental pulp (PL), and enamel matrix (red arrow). H&E stain X40 (light microscope). **F** represent the magnified histological image of the minor (NIT) upper incisor tooth (peg incisors) or assistant incisor tooth to show the structure of the developing tooth: ameloblasts (Am), odontoblasts (Do), the dental pulp (PL), and the enamel matrix (red arrow). H&E stain X100

teratological investigations and abnormalities as their monitoring their health and embryo stage (Gabr 2015) particularly in dental research. The tooth formative investigations in rabbits were vital to knowing and understanding the components of tooth substitution and improvement in vertebrates (Bertonnier-Brouty et al. 2020; Campillo et al. 2014; Martin et al. 2020). Rabbit teeth lacks true roots and has a long anatomical crown. These teeth samples have open roots and frequently erupt. Molar teeth samples have two laminae and two apical apertures. Zoba and Rabab (2012) stated that as a countermeasure to ongoing tooth damage, there are different forms of dentine precipitate in the pulp chamber of continually growing teeth. According to Navarro et al. (1976), the mandibular premolars erupt 23 days after birth, the mandibular permanent molars erupt 9 days later, and the mandibular deciduous molars erupt 4 days after birth. However, (Hirschfeld et al. 1973) claimed that they typically lose their deciduous teeth before or soon after birth and are born with their permanent teeth. Rabbit teeth lacks true roots and has a long anatomical crown. These teeth samples have open roots and frequently erupt. Molar teeth has two laminae and two apical apertures. Histologically, morpho-differentiation and histo-differentiation occur, and the tooth germs develop to the late bell phase and the cusps phase. However, it is important to note that the timing of tooth eruptions can vary among individual rabbits. Additionally, the lack of true roots in rabbit teeth allows for continuous growth throughout their lifespan, which necessitates regular dental care to prevent overgrowth and related health issues.

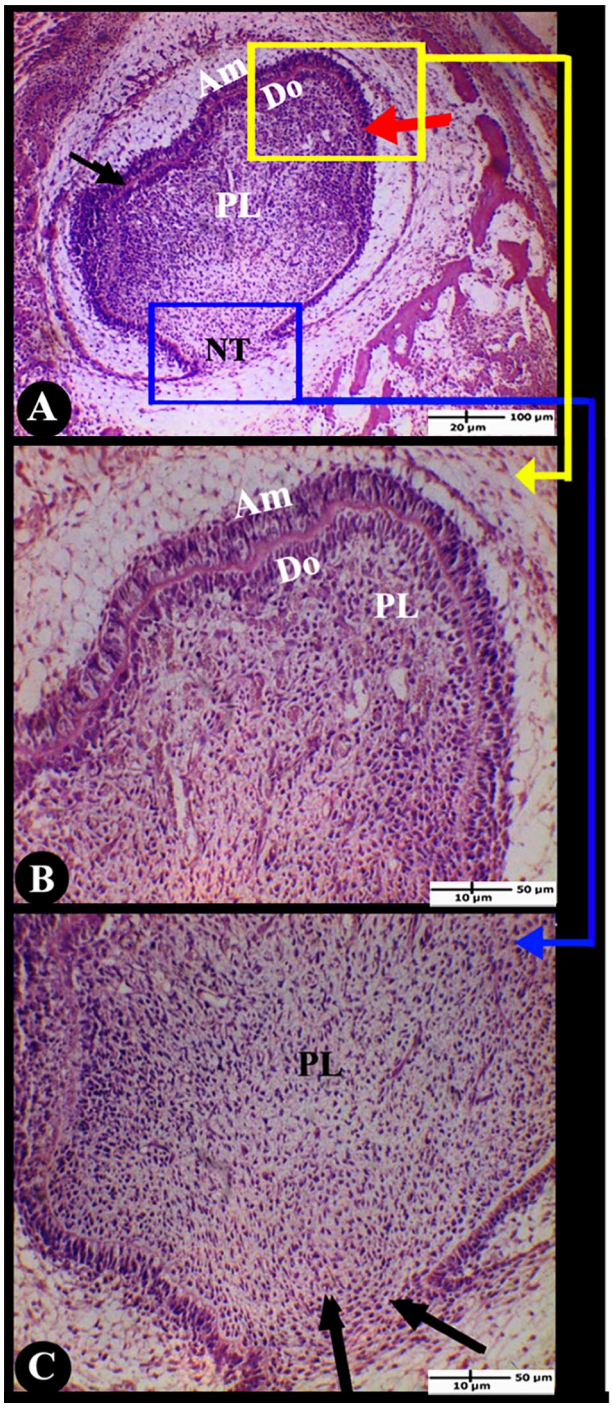

Fig. 4 Photomicrograph of prenatal development of the middle part of rabbit teeth at a 23-day prenatal rabbit embryo. **A** represent the development and structure of the molar tooth in its crown stage (red arrow), ameloblasts (Am), odontoblasts (Do), the dental pulp (PL), enamel matrix (black arrow), and the neck of the tooth (NT). H&E stain X40. **B** and **C** represent the magnification of the crown molar tooth with the constricted neck (black arrow) and the dental pulp (PL). H&E stain X100

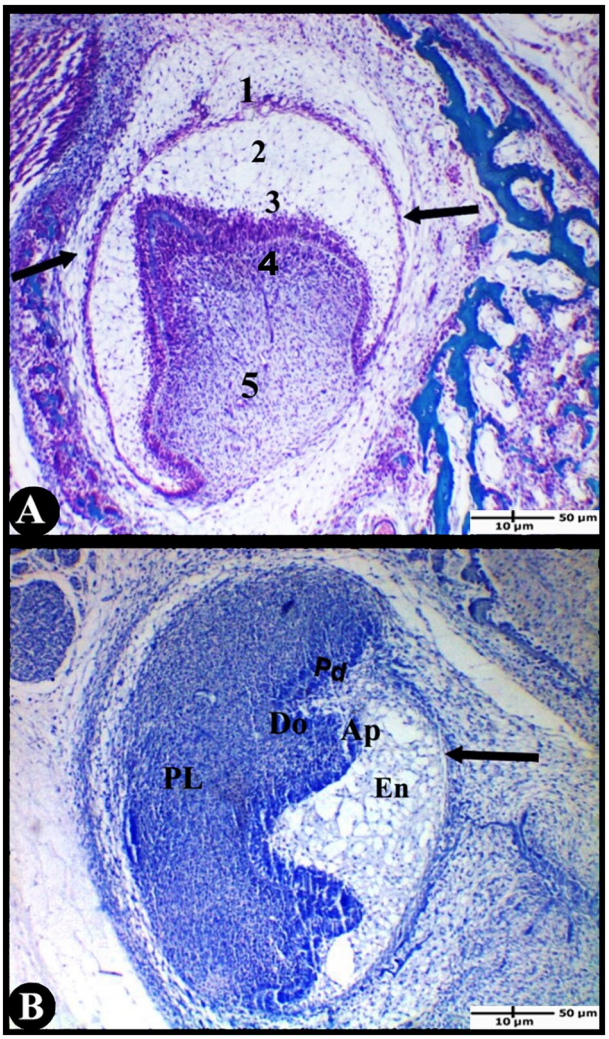

Fig. 5 Photomicrograph of the posterior part of the (E25) embryo head to show: **A** represent the development and structure of the molar tooth in its cusp stage (black arrow), the outer epithelium (1), stellate reticulum (2), ameloblasts (3), odontoblasts (4), and dental pulp (5). Masson trichrome stain X40. **B** represent the cusps of the molar tooth to show predentin (pd), separate odontoblasts (Do) from preameloblasts (Ap) at the cusp, dental pulp (PL), and enamel (En). (toluidine blue stain X40)

Our results revealed that the rabbit teeth implicates the ectoderm, which gives enamel, and the mesoderm, which gives rise to dentine and pulp. Additionally, our results observed that the teeth appears first as an ectodermal epithelial thickening on each side of the entrance opening to the mouth opening. Likewise, the teeth appeared first on the 19th day of prenatal embryonic age (E19), as an ectodermal epithelial thickening on each side of the entrance opening to the mouth consisted of undifferentiated epithelium cellular collections that represented the first sign of tooth development in rabbits. These obtained results came in agreement with (Mostafa et al. 2020) in 2 cm CVRL in the Egyptian

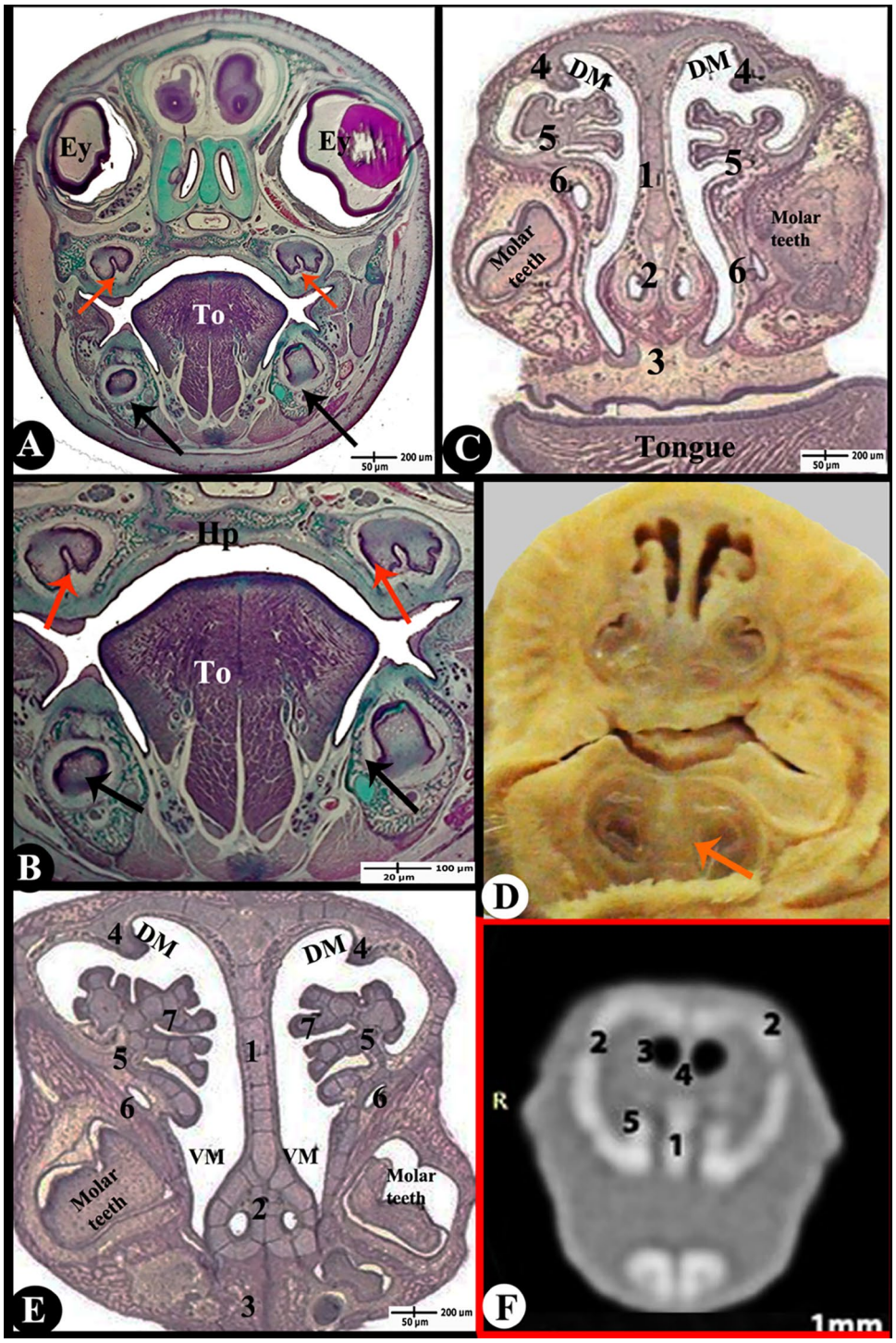

Fig. 6 Photomicrograph of (E28-30) embryo head to show: **A** and **B** (magnification of **A**): represent the cross section at the caudal part of the nasal cavity at (E28) embryo to show the upper molar tooth (orange arrow) and lower molar tooth (black arrow), eye (Ey), hard palate (HP), and tongue (To), Masson trichrome stain, stereomicroscopic. **C** represent the cross section at the middle part of the nasal cavity at (E28) embryo to show the dorsal nasal meatus (DM), cartilaginous septum nasi (1), the vomeronasal organ (2), maxillary symphysis (3), dorsal nasal conchae (4), branches of ventral nasal conchae (5), maxillary sinus (6), H&E stain stereomicroscope. **D** represent the gross image of the cross section of the cranial part of the nasal cavity at (E28) embryo to show the upper molar tooth (orange arrow), lower molar tooth (black arrow), and tongue (to). **E** represent the cross section at the middle part of the nasal cavity at 30 days (neonates) of the embryo to show the dorsal nasal meatus (DM), cartilaginous nasal septum (1), the vomeronasal organ (2), maxillary symphysis (3), dorsal nasal conchae (4), branches of ventral nasal conchae (5 and 7), maxillary sinus (6), H&E stain, stereomicroscope. **F** represent the computed tomographic image of the coronal section at the cranial part of the nasal cavity of the neonate's embryo at 30-day gestation to show the upper molar tooth (orange arrow) and lower molar tooth (black arrow), dorsal nasal meatus (DM), cartilaginous septum nasi (1), the vomeronasal organ (2), maxillary symphysis (3), dorsal nasal conchae (4), and branches of ventral nasal conchae (5)

buffalo embryos and with (Slavkin 1990) the molecular determinants of mouse tooth development. In the interim Navarro et al. (1978), expressed that, the first tooth appeared in the jugal region at 16-day prenatal embryo life (E16), the bud stage appeared from 17- to 19-day prenatal embryo age (E17-19), the cap stage on 20-day prenatal embryo age (E20), the early bell stage at 25-day prenatal embryo age (E25), and the late bell, the cusps on 28–30-day prenatal embryo age (E28-30), and the beginning of dentinogenesis, amelogenesis, and cementogenesis from 15 to 30.

According to Glasstone (1938), the epithelium of the mouth began to thicken locally at the 13-day prenatal embryonic age (E13), and this thickening only results in the formation of a thin continuous band in the molar region. The general dental lamina was formed by the thicker epithelial cells that penetrated the underlying tissue about 24 h later.

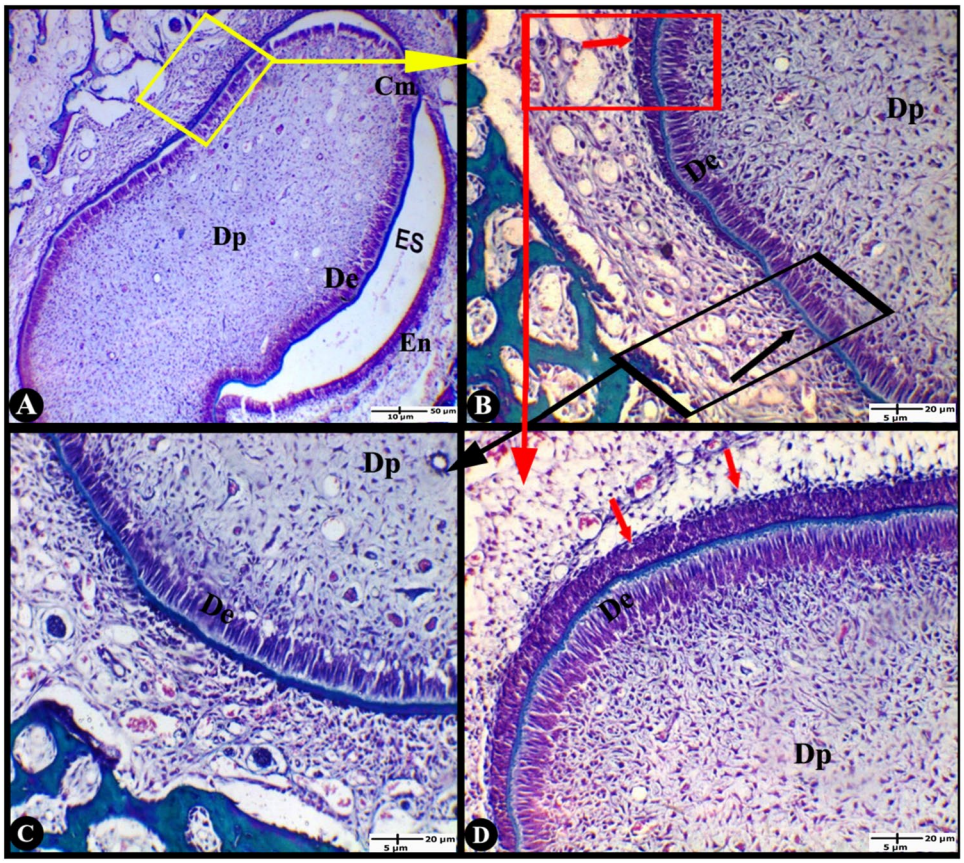

Fig. 7 Photomicrograph of (E28-30) embryo head to show: **A** represent the cross section of the upper molar tooth to show: enamel space (ES), dentin (De), enamel (En), the dental pulp (Dp), and cementum (Cm) (Masson trichrome stain X40). **B**, **C**, and **D** represent the cross section of the lower molar tooth that had well-developed ameloblasts (red arrow) that were detected with a thin layer of enamel matrix, while the ameloblasts were not presented on the other side (black arrow), dentin (De), and dental pulp (Dp). (Masson trichrome stain X100)

This lamina was composed of two distinct layers of cubical epithelial cells in the transverse section, along with a dense collection mass of erratically organized flattened epithelium cells between them. There was also a bud-shaped thickening structure that constituted the tooth germ's initial appearance by the 15th day of prenatal development (E15). At approximately 18 days of prenatal embryonic age (E18), the cusps started to develop as a modest dipping of the internal enamel epithelial into the dentine papilla. Two cusps—the disto-lingual and the middle-lingual—have separated after 48 h. In a comparable direction, the author looked at the differences between rats and rabbits. The tooth germ in the rat is elliptical, whereas the one in the rabbit has the customary bell shape. In the rat, the enamel cord is better defined and lasts for a day after cusp-formation has started, while in the rabbit, it starts to deteriorate as soon as the cusps start to show. The external enamel epithelial cells in the rat maintain their cubic shape until they start to degenerate, while in the rabbit, they do not appear.

Our findings at the 21-day prenatal rabbit embryo (E21) reported that the upper incisor tooth development appeared in the form of a tooth bud, in which this bud was composed of many condensed epithelium cells, was simply identified from the larger with less condensed vestibular lamina, and was surrounded by mesenchymal connective tissue while the lower incisor took the cap stage. In addition, our study noticed the cap-like tooth consisted of an enamel structure and underlying dense mesenchymal tissue. The enamel consisted of three layers: the external and internal enamel epithelium and the stellate reticulum. These perceptions match (Mostafa et al. 2020) in 11 and 21 cm CVRL buffalo embryos. Our findings at 23-day prenatal embryonic age (E23) demonstrated that the typical tooth structure is formed from the enamel layer, the dentin, and the pulp cavity, which are surrounded by trabecular bony plates of the maxilla and mandible (which appear within the alveolar bone, either the maxillary bone or the mandible).

At the current investigated 28–30-day prenatal embryo age (E28–30), the developed tooth consisted of a crown and root. The root was bifurcated into two halves, and with the help of the examination under the light microscope, it had a dentin core. An enamel organ covered the dentin on both the labial and lingual sides, while it was absent on the lateral surfaces. A layer of cementum covered the tooth all around the enamel on both the labial and lingual sides while not contacting the dentine on the lateral side, forming a distance called enamel space. The current work reported that the lower molar tooth was similar to the upper molar tooth. It was shown that in the lower molar, well-developed ameloblasts were detected with a thin layer of enamel

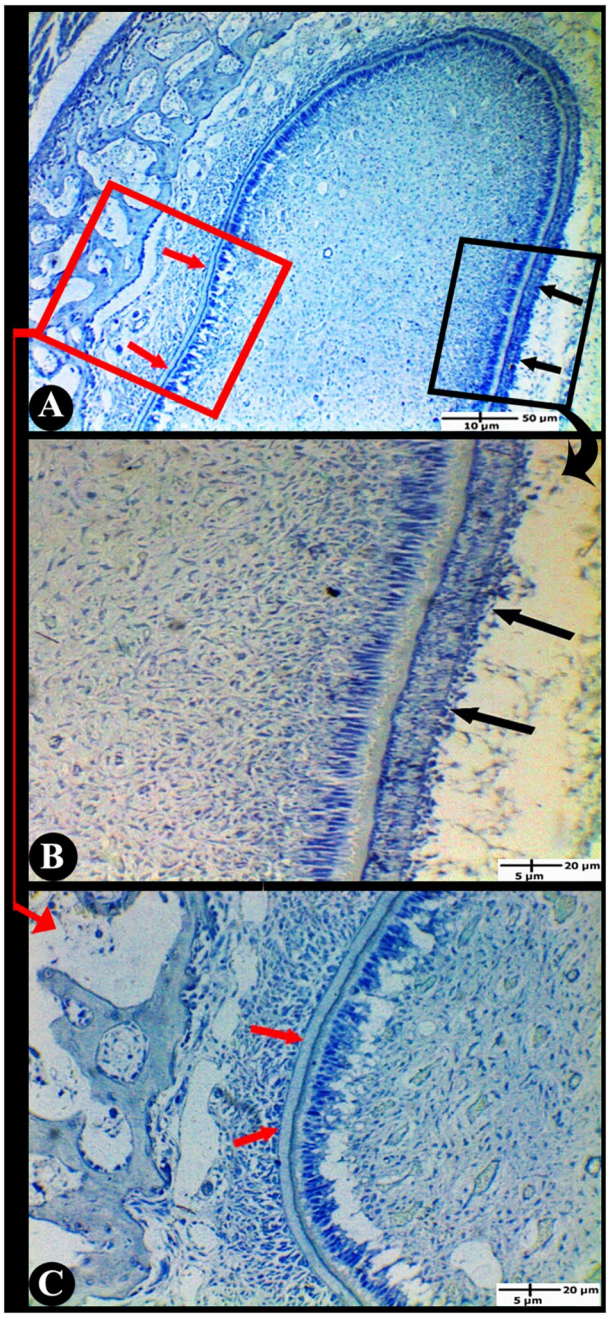

Fig. 8 Photomicrograph of (E28-30) embryo head to show: the cross section of the upper molar tooth to show a well-developed ameloblast (black arrow), while not presented on the other side, but a well-developed dentin layer (red arrow). Toluidine blue stain X40 (A) & (X100 in B&C)

matrix, while the ameloblasts were not present on the other side. These observations were accepted by Hirschfeld et al. (1973), Navarro et al. (1978), Simoens et al. (1995), Zoba and Rabab (2012). The pulp chamber included typical pulp tissue and was lined by an odontoblastic layer that was covered by the dentine and enamel layers from its outside surface. Cellular cementum, enamel, and dentine layers were covered on the side facing the groove between the two laminae. Additionally, it was clear that the enamel layer lining the groove was thinner than the enamel layer covering the cementum-coated exterior tooth surface.

Addison and Appleton Jr (1922) claims that 2 days prior to the enamel formation, at around 21 days into the pregnancy, blood vessels began to enter the rat's enamel organ. Contrarily, the current research found that in rabbits, blood vessels do not enter the enamel structure at any point during tooth formation, despite the fact that many blood vessels exist at the time of enamel development close to the degenerating stellate reticulum. The lingual portion of the deciduous precursor is where new teeth in mammals begin to form (Järvinen et al. 2009). Similar to the ferret and fruit bat, the development of the dental lamina at the lingual side of the tooth initiates the replacement of teeth in rabbits (Jussila et al. 2014; Popa et al. 2016). After then, there may or may not be a gap between the dental lamina replacement, the oral epithelium, and the first primordial teeth. The tooth's link to the oral epithelium was lost first. The rabbit dental lamina replaces the mesenchyme prior to the development of permanent teeth, as was described in pigs (Wang et al. 2014) and with reference to the fruit bat or the ferret (Järvinen et al. 2009; Popa et al. 2016).

Conclusion

Rabbit teeth first appeared at 19 days of prenatal life (E19) as an ectodermal epithelial thickening on each side of the mouth opening. The teeth went through stages like initiation, bud, cap, late bell, maturation, and crown stages. The teeth samples are composed of an enamel organ and dense mesenchymal tissue. The enamel organs are segmented into three layers: external, internal, and stellate reticular. The cement layer covers the teeth on both the labial and lingual sides, forming enamel space. Teeth develops consistently throughout life, with expanded enamel thickness, diphyodont teeth, two continuous dentitions, deciduous and perpetual teeth, long crown teeth, and an open root. At E21, the bud of upper incisor tooth appears as an epithelial bud, composed of many condensed epithelium cells, was simply identified from the larger with less condensed vestibular lamina, and was surrounded by mesenchymal connective tissue while the lower incisor took the cap stage. At (E23), tooth regular construction is formed from enamel, dentine, and pulp cavity. Peg incisor appearance (supplementary and assistant incisors) is visible at the lingual surface of the upper major incisor. Teeth prenatal development went through successive stages like initiation, bud, cap, late bell, maturation, and crown stages. The first initiation phase of tooth formation

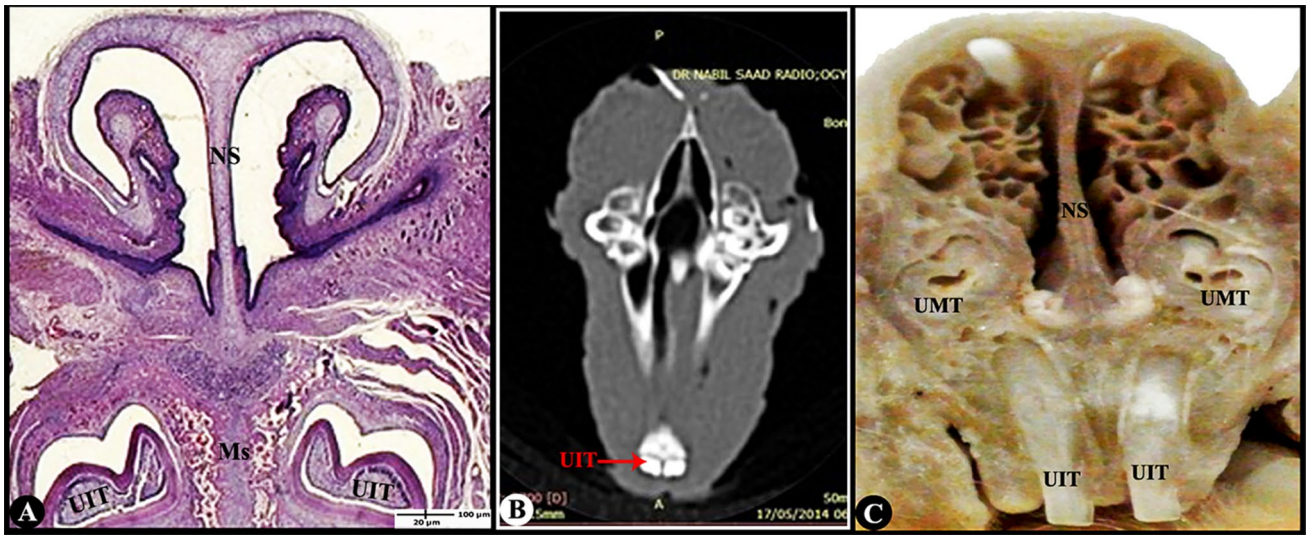

Fig. 9 Photomicrograph of 1 week after the birth of the rabbit head to show: **A** represent of the cross section of the rostral part of the nasal cavity to show root upper incisor teeth (UIT) that are separated by maxillary symphysis (Ms) and nasal septum (NS). (Masson trichrome stain a stereomicroscopic image). **B** represent a sagittal computed tomographic image of the rabbit maxilla to show the upper major and minor incisors, check teeth (premolar and molar), and nasal septum (NS). **C** represent a gross cross-sectional image of the nasal cavity and upper jaw to show the root and crown of the upper incisor

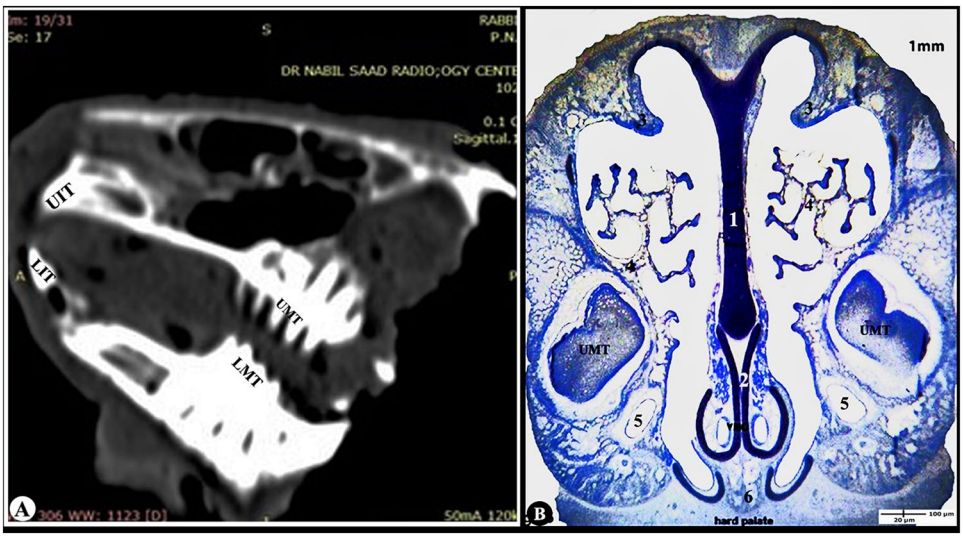

Fig. 10 Photomicrograph of 2 weeks after the birth of the rabbit head to show: **A** represent a sagittal computed tomographic image of the rabbit head to show upper (UIT) and lower incisor teeth (LIT), check teeth (UMT, LMT, premolar, and molar). **B** (toluidine blue stain stereomicroscope) represent the stereomicroscopic image of the cross section of the nasal cavity to show the root of the upper molar teeth (UMT), cartilaginous septum nasi (1), vomeronasal organ (2), dorsal nasal conchae (3), ventral nasal conchae (4), maxillary sinus (5), and maxillary symphysis (6)

was seen as ectodermal epithelial cell collection at (E19). Bud stage saw on upper incisor tooth, while in cap structure in lower incisor teeth at (E19). A cap-formed tooth is composed of the enamel organ and fundamentally dense mesenchymal tissue. Enamel organs are segmented into three distinct layers: the external tooth enamel epithelial, the internal tooth enamel epithelial, and finally, the stellate reticular layer. The cement layer covered teeth all around on enamel on both the labial and lingual sides while not contacting the dentine on the lateral side, forming enamel space. Teeth develops consistently all through life. They have expanded enamel thickness, and they are diphyodont teeth. Besides, they have two continuous dentitions, they are deciduous and perpetual, with long crown teeth and an open root.

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Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Ethical approval This study was carried out with the ethical permission from the faculty of Veterinary Medicine, Alexandria University, and approved by Institutional Animal Care and Use Committee (ALEXU-IACUC) (Approval code: 182/2022/31/10/2022). All methods were performed in accordance with relevant guidelines and regulations by the Basel Declaration and the International Council for Laboratory Animal Science (ICLAS). The anatomical nomenclature was applied according to Nomina Anatomica Veterinaria (NAV 2017).

Consent for publication Not applicable.

Consent to participate Not applicable.

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