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Research Article

DEPOSITION PATTERN OF HARD DENTAL STRUCTURES DURING DEVELOPMENT OF FIRST DECIDUOUS INCISOR TOOTH IN GOAT (*Capra hircus*)

JOSHI N.H.*[1], CHUDASAMA M.M.[1], PATEL K.B.[1], DESAI M.C.[1], RAVAL S.H.[2], PARMAR R.S.[2], MENAKA R.[3]

[1]Department of Veterinary Anatomy, College of Veterinary Science and A.H., Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, 385506, Gujarat
[2]Department of Pathology, College of Veterinary Science and A.H., Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, 385506, Gujarat, India
[3]Department of Veterinary Anatomy, Vanbandhu College of Veterinary Science and A.H., Navsari Agricultural University, 396450, Navsari, Gujarat, India
*Corresponding Author: Email - niravvety1993@gmail.com

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Abstract: The detailed exploration on deposition pattern of hard dental structures for first deciduous incisor tooth was carried out on 30 goat fetuses at weekly interval from 11 week to 21 weeks (Full term). From 12th week onwards, all deciduous incisors were radiographically showing calcification, were decalcified, processed for paraffin sectioning and stained by the Routine Harri's Heamatoxylin and Eosin and the Masson's Trichrome stain. At 11th week goat fetus, deposition of dentin, the dentinogenesis, and at 13th week deposition of enamel, the amelogenesis was started for first deciduous incisor tooth. At 20th week-crown of Di_1 was found completely formed and the dentin formation of root begun. The cementoblasts differentiation and the cementogenesis could not be detected till the full term goat fetus.

Keywords: *Goat, Fetus, Tooth development, Dentinogenesis, Amelogenesis*

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Introduction

Teeth are another component of mammalian body which get calcified other than bone. Appositional growth is characterized by regular and rhythmic layer like deposition of hard dental structures *viz*. dentin, enamel and cementum during odontogenesis. Apposition is fulfilment of the plan outlined at the stages of histo differentiation and morpho differentiation. Deposited hard dental structures forms the permanent biological kymograph for the tooth. In continued sequence of earlier published work of this department on prenatal dental development [1, 2], the present investigation on deposition of hard dental structures during development of first deciduous incisor tooth in goat (*Capra hircus*) was planned.

Materials and Methods

For present study, total 30 goat foetuses at weekly interval from 11th to 21st weeks (CR 14.7 to 42 cm, Full term) were utilized from collection pool of Department of Veterinary Anatomy and Histology, College of Veterinary Science and Animal Husbandry, Saradarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, 385 506. Tissues of foetuses from 12 weeks onwards, radiographically showing calcification of deciduous incisors, were decalcified before processing for histology on the lines of [3]. After complete decalcification the dental tissues were processed for the paraffin embedding by automated tissue processor (Leica TP 1020). The sagittaly oriented serial paraffin sections were cut from 12 to 14 µ by Leica RM 2255, USA microtome machine. The serial paraffin sections were stained by the Routine Harri's Haematoxylin and Eosin stain and the Masson's Trichrome stain. The sequential deposition pattern of hard dental structures (*viz*. enamel, dentine and cementum) from 11 week to full term was observed on lines of [4-6].

Results and discussion

At 11th week of foetal age, all the components of tooth germ of Di_1, *viz*. enamel organ, dental papilla and dental sac were visible [Fig-1].

At 11th week; the development of Di_1 was at advanced bell stage. The differentiation of odontoblasts and ameloblasts extended more distally on lateral margin of advanced bell stage, in which the odontoblasts started deposition of dentin, dentinogenesis, at future dentino - enamel junction on tip and it continued uniformly down towards the cervical loop on either side of advance bell stage [Fig.1-3]. The thickness of dentin was found the highest at tip and slowly reducing towards the cervical loop [Fig-1-3]. The odontoblasts deposited first predentin which took light pink colour under H and E [Fig-1]. The predentin subsequently mineralized and formed dentin. The dentin took dark blue colour under H & E stain [Fig-1-3]. Because of continues production of dentin, the odontoblasts moved away towards the dental papilla. At this age, the outer enamel epithelium was wavy in outline [Fig-1-3]. The stellate reticulum was reduced in thickness at the tip. The progressive differentiation of ameloblasts from the tip to cervical loop was observed [Fig-1-3]. Once the dentin formation started, the ectomesenchymal tissue of dental papilla enclosed by dentin was named as the dental pulp. The enamel organ enclosed major part of proliferating dental pulp [Fig-1]. The dental pulp was proliferated by blood vessels [Fig-2]. The progressive deposition continued in similar pattern at 12th week of goat foetuses. At 12th week the differentiation of odontoblasts and ameloblasts extended more cervically [Fig-4-6]. The dentinogenesis continued and the thickness of dentin increased steadily with advancing foetal age. The dentin matrix took blue colour in Masson's Trichrome stain [Fig-4]. The average thickness of deposited dentin at just below to the incisal edge of Di_1 was measured 27 µ and 36µ at 11th and 12th weeks respectively [Fig-1, 4] [Table-1]. Upto 12th week, deposition of enamel was not started [Fig-1]. By 13th week, the deposited dentin induced the ameloblasts to deposit enamel, amelogenesis, over the earlier existing dentin [Fig-7]. Because of continued deposition of enamel over dentin, the ameloblasts, moved away towards the lateral margin of the bell [Fig-7]. The odontoblasts and the ameloblasts moved apart from each other from dentino- enamel junction, by continued and steady

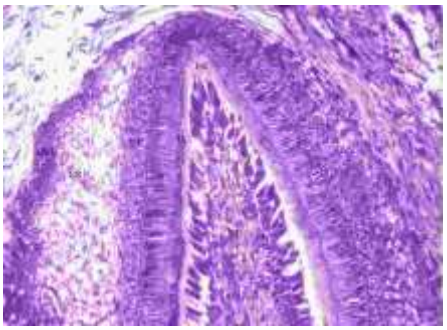

Fig- 1 Photomicrograph of the advance bell stage of Di1 at 11 week of goat foetus showing first deposition of dentin, H and E stain 100X

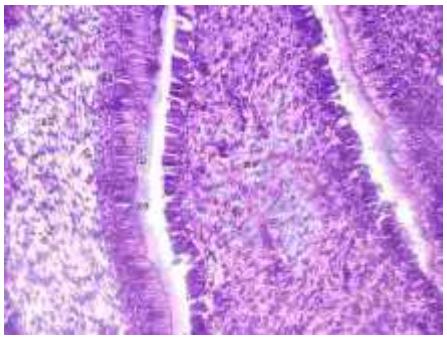

Fig-2 Photomicrograph of lateral margin of the advance bell stage of Di1 at 10 week goat foetus showing deposition of predentin at lower part
H and E stain 100X

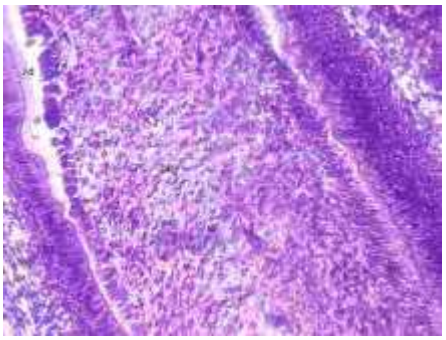

Fig-3 Photomicrograph of the advance bell stage of Di1 at 10 week goat foetus. Showing no deposition of predentin near to cervical loop, H and E stain 100X

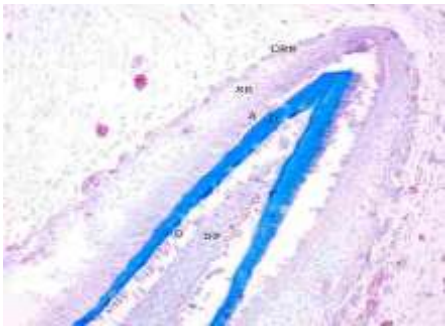

Fig-4 Photomicrograph of Di1 at 12 week goat foetus showing progressive deposition of dentin Masson's Trichrome stain 100X

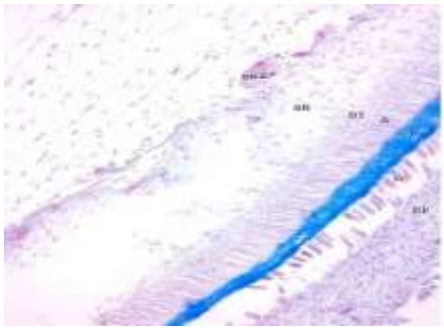

Fig-5 Photomicrograph of Di1 at 12 week goat foetus showing progressive deposition of dentin on side, Masson's Trichrome stain 100X

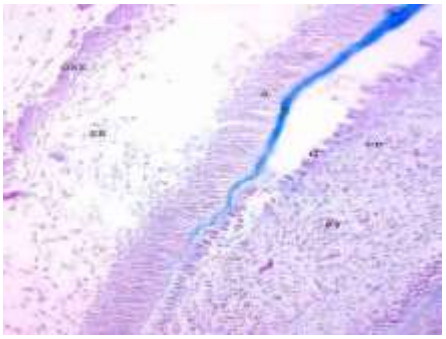

Fig-6 Photomicrograph of Di1 at 12 week goat foetus showing tapering pattern of dentin deposition towards cervical loop, Masson's Trichrome stain 100X

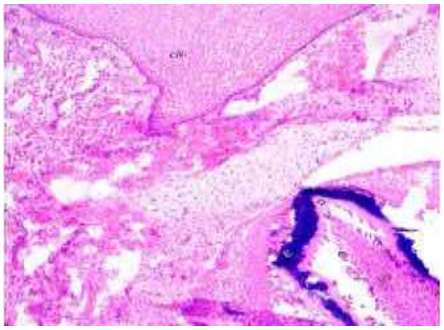

Fig-7 Photomicrograph of Di1 at 13 week goat foetus showing first deposition of enamel, H and E stain 100X

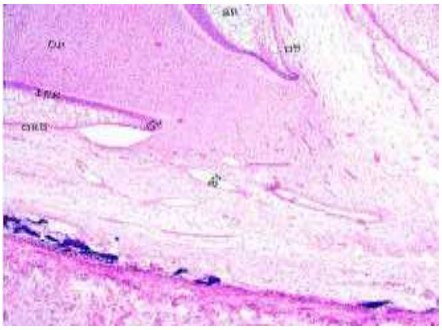

Fig-8 Photomicrograph of Di1 at 13 week goat foetus showing proliferating cervical loop, H and E stain 50 X

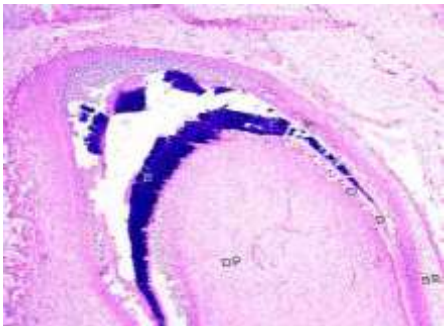

Fig-9 Photomicrograph Di1 at 14 week goat foetus showing progressive deposition of enamel, H and E stain 50X

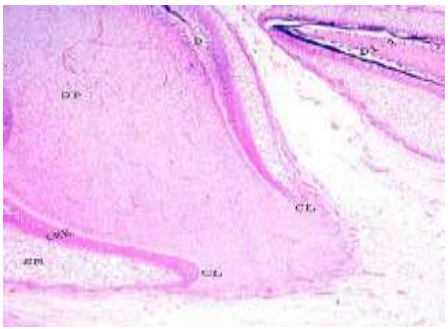

Fig-10 Photomicrograph of Di1 and Di2 at 14 week goat foetus showing proliferating cervical loop of Di1, H and E stain 50X

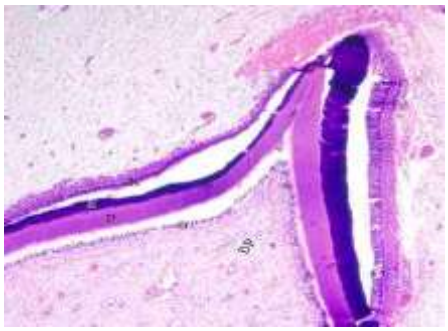

Fig-11 Photomicrograph of tip of developing Di1 at 15 week goat foetus showing deposition of enamel and dentin, H and E stain 100X

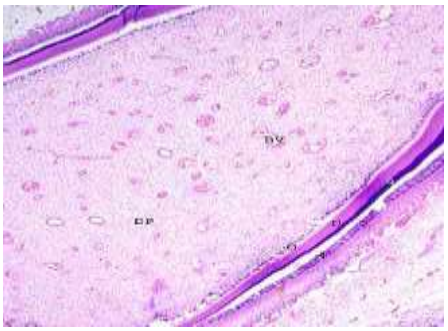

Fig-12 Photomicrograph of tip of developing Di1 at 15 week goat foetus showing deposition of enamel and dentin, H and E stain 100X

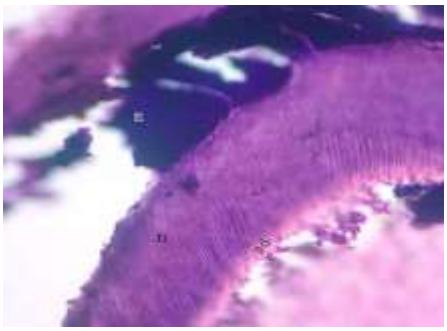

Fig-13 Photomicrograph of developing Di1 at 16 week goat foetus showing dentinal canaliculi and enamel prisms, H and E stain 100X

Fig-14 Photomicrograph of side of tip of developing Di1 at 17 week of goat foetus showing deposition of dentin and enamel, H and E stain 100X

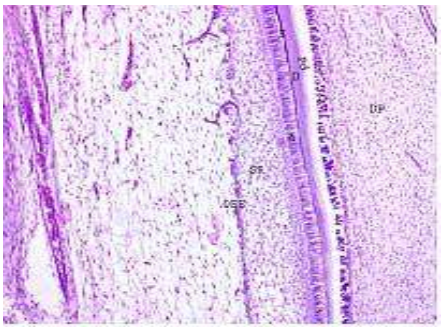

Fig-15 Photomicrograph of developing Di1 at 17 week goat foetus showing tapering pattern of deposition of dentin and enamel towards cervical loop, H and E stain 50X

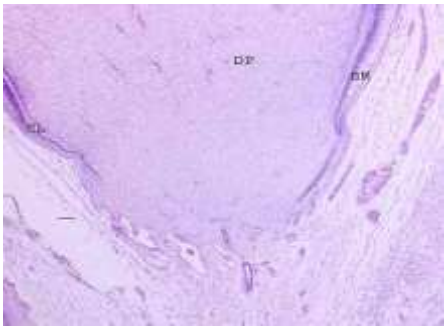

Fig-16 Photomicrograph of developing Di1 at 17 week goat goat foetus showing proliferating cervical loop, H and E stain 50 X

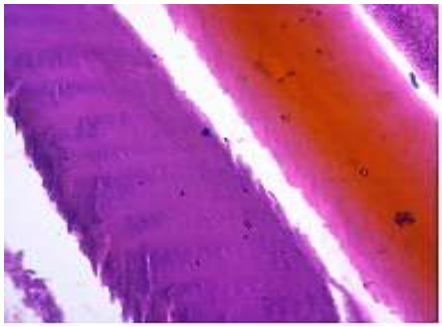

Fig-17 Photomicrograph side of tip of Di1 at 20 week of goat foetus showing deposition of dentin and enamel, H and E stain 100X

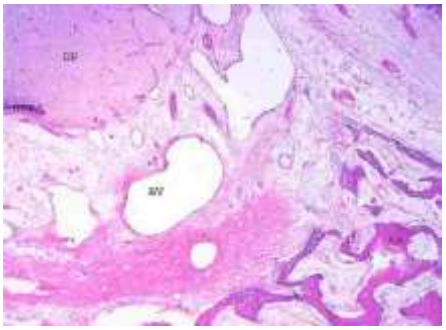

Fig-18 Photomicrograph at 20 week of goat foetus, developing Hertwig's Epithelial root sheath of Di1 close to base of alveolar crypts, H and E stain 50X

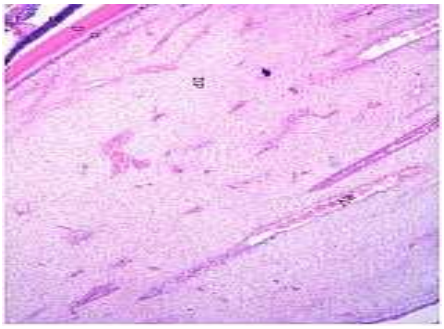

Fig-19 Photomicrograph of developing Di1 at 20 week of goat foetus showing high proliferation of blood vessels in the dental pulp, H and E stain 50X

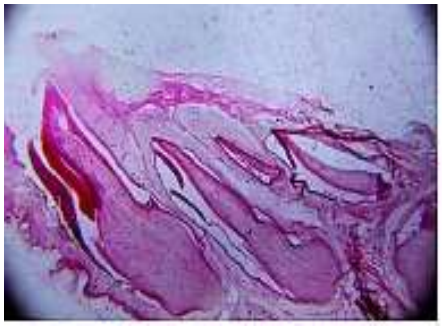

Fig-20 Subgross photomicrograph of lower jaw at 20 week of goat foetus showing all developing deciduous incisors, H and E stain 10X

Fig-21 Photomicrograph showing tip of developing Di1 near to gum line at 21st week goat foetus, H and E stain 100X

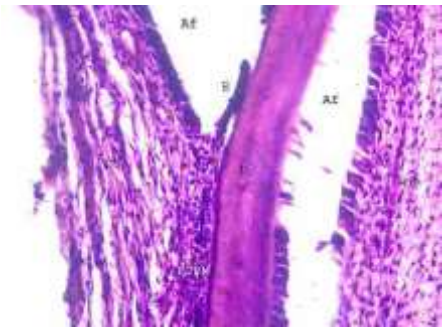

Fig-22 Photomicrograph showing disintegrated epithelial root sheath (epithelial cell rests of Malassaze)at 21 week (Full term goat) foetus, H and E stain 100X

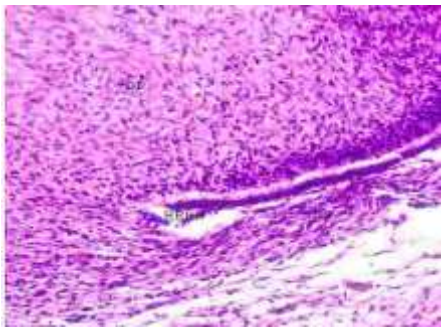

Fig-23 Photomicrograph showing proliferating Hertwig's epithelail root sheath at 21week (Full term) goat foetus, H and E stain 100X

Common legends used for figures

OEE-Outer Enamel Epithelium
SR-Sellate Reticulum
SI-Stratum Intermedium
A-Ameloblasts, O-Odontoblasts
Af-Artifact, D-Dentin
Pd-Predentin, Dp-Dental Pulp
BV-Blood Vessel, CL-Cervical Loop
DS-Dental Sac, E-Oral Epithelium
AB-Alveolar Bone
HERS-Hertwig's Epithelial Root Sheath
ECRM-Epitheilal Cell Rests of Malassaze
GM-Gum line

deposition of dentin and enamel. With advancing foetal age, the thickness of enamel over dentin at just below to the incisal edge of Di_1 was measured as 40 µ and 132µ at 13th and 14th weeks respectively [Fig-7, 9] [Table-1].

Table-1 *Thickness of dentin and enamel just below to incisal edge at various foetal age in goat*

SN	Foetal age in weeks	Thickness of dentin below to incisal edge (in µ)	Thickness of enamel over dentin below to incisal edge (in µ)
1	11	27	-
2	12	36	-
3	13	75	40
4	14	97	132
5	15	126	155
6	16	182	225
7	17	431	388
8	20	472	417
9	21	573	Could not be measured because of artifact

Table-2 *Beginning of deposition of hard dental structures during development of teeth in present investigations and earlier reports*

SN	Beginning of deposition of hard dental structures	Present study Goat Foetuses (Weeks)	Hatt (1967) Sheep Foetuses (Weeks)	Patel (1996) Buffalo Foetuses (Weeks)
1	Dentinogenesis	11	13	25
2	Amelogenesis	13	13	23
3	Cementogenesis	Not detected	17	36

The cervical loop also extended cervically [Fig-8]. The thickness of dentin below to incisal edge was 126µ, 182 µ, 431 µ, 472 µ and 573 µ at 15th, 16th, 17th, 20th and 21st week goat foetuses, respectively. [Fig-11,13,17, 20] [Table-1]. By rhythmic and steady dentinogenesis, the thickness of dentin increased with advancing foetal age. The dentinal canaliculi were clearly visible at 16th week of goat foetus [Fig-13]. The thickness of enamel over dentin at just below to incisal edge was measured as 155 µ, 225 µ, 388 µ and 417 µ at 15th, 16th, 17th and 20th week of goat foetus respectively [Fig-11,13,17,20] [Table-1]. By rhythmic and steady amelogenesis the thickness of enamel increased with advancing foetal age [Fig-7, 9,11,14,17, 20]. At 15th week of foetal age, the developing Di_1 was found completely enclosed within ossifying alveolar bony crypts. At 20th week of goat foetus, all four deciduous incisors were seems like stack of playing cards in sagittal section [Fig-20]. The deposition of enamel was found already ceased at the distal cornal boundary of Di_1 [Fig-20], indicating complete crown formation. The dentin deposition of root for Di_1 was already started before 20th week of foetus [Fig-20]. At 20th week, the cervical loop proliferated distally and formed double layered epithelial sheath called Hertwig's epithelial root sheath [Fig-18]. The Hertwig's epithelial root sheath was devoid of the stratum intermedium. The Hertwig's epithelial root sheath bended upward and inward and forming an epithelial diaphragm [Fig-18]. The Hertwig's epithelial root sheath was very close to base of alveolar crypts and guided the root formation [Fig-18]. The inner layer of Hertwig's epithelial root sheath induced the peripheral ectomesenchymal cells of dental papilla to differentiate into the odontoblasts. These differentiated odontoblats deposited the dentin for the root. At 21st week, disintegration of the Hertwig's epithelial root sheath was started. The remnant of disintegrated Hertwig's epithelial root sheath were present in the form of isolated group of cells in the region of future periodontal ligaments. The remnants of Hertwig's Epithelial Root Sheath, called the Epithelial Cell Rest of Malassaze, were visible at 21st week foetus [Fig-22, 23]. After disintegration of Hertwig's epithelial root sheath, the ectomesenchymal cells of the dental sac came into contact with the dentin of root. The ectomesenchymal cells of dental sac could not found differentiated into cementoblasts to deposit cementum, fibroblasts to form periodontal ligament and osteoblasts to form alveolar bone even at 21st week of goat foetal age in the present investigation [Fig-22 and 23]. Root length increased by steady and continues deposition of root dentin. With increase in root length, the Di_1 moved away from the base of alveolar crypt. This intraosseous non functional eruption of Di_1 provided space for further growth of root At 21st week of age, the incisal edge was found very close to gum mucosa, due to intraosseous non functional eruption of Di_1 [Fig-21]. The enamel matrix was represented as elongated prism like at full term [Fig-21].

In present investigation the deposition of hard dental structures followed sequential pattern: First the ectomesenchymal cells of the dental papilla induced the inner enamel epithelial cells to differentiate into ameloblasts. Then, these ameloblasts induced peripheral cells of ectomesenchyme of the dental papilla to differentiate into odontoblasts. The odontoblasts secreted predentin and dentin. This deposited dentin induced the ameloblasts to produce enamel. The processes of histodifferentiation and deposition of hard dental structures started first at the tip of bell and slowly progressed towards cervical loop. The inner layer of the Hertwig's epithelial root sheath induced differentiation of odontoblasts from ectomesenchymal cells of dental papilla to produce dentin of root. An overview of deposition pattern of hard dental structure during development of Di_1 is given in [Fig-24]. In the present study, the deposition of dentin was first observed at 11th week goat foetus. However, first dentinogenesis was reported at later ages by 13th week in foetuses of sheep [5] and 25th weeks in foetuses of buffalo [6]. In contrast, beginning of dentinogenesis was reported at earlier ages by 7th week in foetuses of dog [7] [Table-2]. In the present investigation, the deposition of enamel was first observed at 13th week of goat foetuses similar to that reported in foetuses of sheep [5]. However, beginning of amelogenesis was reported very late by 23rd week in buffalo foetuses [6][Table-2]. In the present study, differentiation of cementoblasts and deposition of cementum, the cementogenesis, was not detected till full term. However, beginning of cementogenesis was reported at 17th week in sheep foetuses [5] and at 36th weeks in buffalo foetuses [6] [Table-2]. The sequential pattern of deposition of dentin and enamel observed in the present investigation were in close agreement with that reported in foetuses of sheep [5], buffalo [6], dog [7], cattle [8, 9], human [4,10] [Table-2].

Conclusion

At 11th week goat fetus, deposition of dentin and at 13th week, deposition of enamel was started for first deciduous incisor tooth. At 20th week, crown of Di_1 was found completely formed and dentin formation of root begun. The cementoblasts differentiation and the cementogenesis could not detected till the full-term goat fetus. The deposition of hard dental structure followed a sequential pattern: i) first the ectomesenchymal cells of the dental papilla induced the differentiation of ameloblasts form the inner enamel epithelium ii) these ameloblasts induced adjacent ectomesenchymal cells of dental papilla to differentiate into the odontoblasts, iii) the ododntoblasts deposited predentin and dentin, iii) this deposited dentin induced ameloblasts to produce enamel. vi)The Hertwig's epithelial root sheath induced differentiation of ectomesenchymal cells of dental papilla into the odontoblasts to produce dentin for root.

Future perspective

Though the present investigation is limited to first deciduous incisor tooth, the further study can be extended for the other incisor teeth and cheek teeth in the goat fetuses.

Application of research: Study of odontological research work in goat foetuses

Research Category: Veterinary Anatomy

Acknowledgement / Funding: Authors are thankful to College of Veterinary Science and A.H., Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, 385506, Gujarat.

Abbreviations: Di_1- First deciduous incisor, µ- Micron, cm- Centimetre

***Research Guide or Chairperson of research: Dr K. B. Patel**
University: Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, 385506, Gujarat
Research project name or number: MVSc Thesis

Author Contributions: All author equally contributed

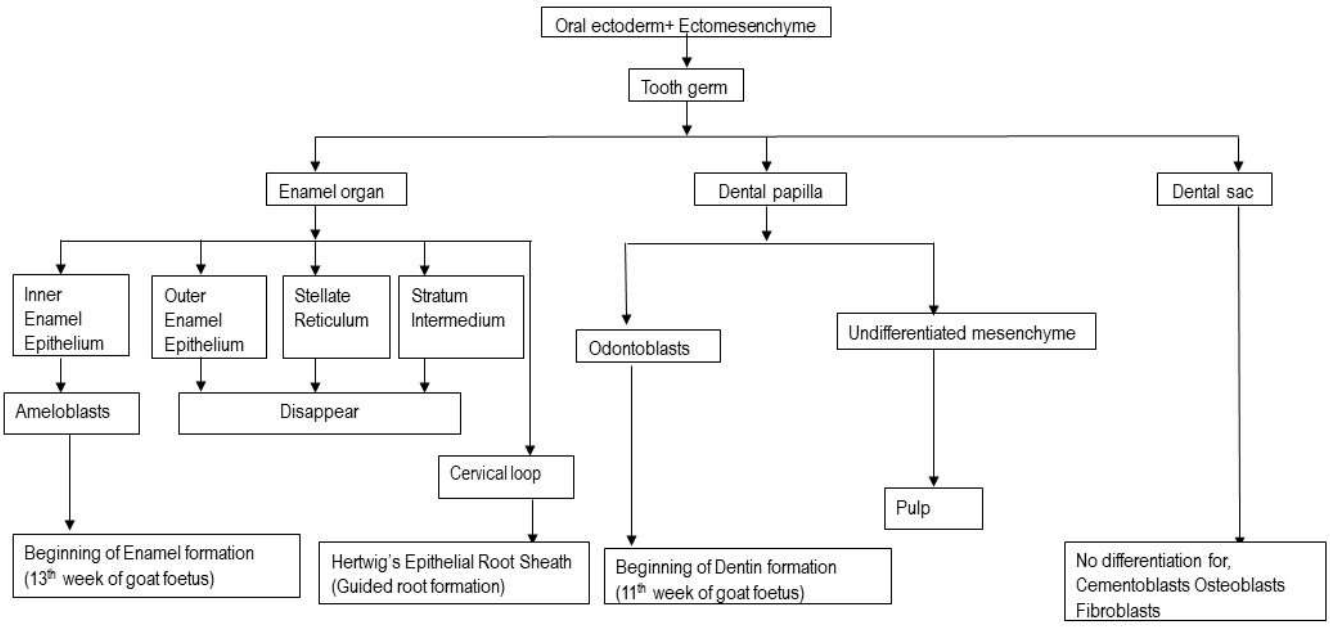

Fig-24 Flow chart showing deposition pattern of hard dental structures during development of Di1

Author statement: All authors read, reviewed, agree and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Department of Veterinary Anatomy and Histology, College of Veterinary Science and Animal Husbandry, Saradarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, 385 506

Animal name: Goat - *Capra hircus*

Conflict of Interest: None declared

Ethical approval: Ethical approval taken from College of Veterinary Science and A.H., Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, 385506, Gujarat.
Ethical Committee Approval Number: Nil

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