



Research Article

HISTOMORPHOLOGICAL STUDY ON PRENATAL DEVELOPMENT OF FIRST DECIDUOUS INCISOR TOOTH IN GOAT (*Capra hircus*)

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Abstract: The present prenatal histomorphological study on development of first deciduous incisor tooth was carried out at weekly interval from 6 to 11 weeks (CR 2.5 cm to 16.6 cm) in fetal goat (*Capra hircus*). The whole heads and the incisive portion of lower and upper jaw of fetuses were processed for paraffin embedding. Serial paraffin sections were stained by the Harri's Haematoxylin and Eosin stain and the Masson's Trichrome stain. By the 6th week of fetal age, dental lamina was first seen at future dental arch region. bud stage of first deciduous incisor tooth was seen at 7th week, early cap stage at 8th week, late cap stage at 9th week, bell stage at 10th week and advanced bell stage at 11th week goat fetuses. Among all three components of tooth germ, enamel organ and dental papilla were appeared at 7th week and dental sac at 9th week goat fetus. The enamel knot was observed as transitory structure at 8th week and disappeared at 9th week. The histodifferentiation of the odontoblasts and the ameloblasts begun by 10th week at incisal edge and extended progressively towards cervical loop. The successional dental lamina for first permanent incisor was observed at 10th week fetus. The dentinogenesis first begun at 11th week goat fetus. The histomorphological stages for development of Di₁ were reported first of its kind in goat fetuses.

Keywords: Niger, variety, topping, seed yield, economics

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Introduction

The dentition is a major component of the mammalian craniofacial complex. The development of an individual tooth is characterized by an extensive series of reciprocal epithelial-ectomesenchymal interactions [1]. The incisive apparatus plays an important role in small ruminant husbandry, since the condition of the teeth and supporting tissue influences in buying, selling and culling of animals. Schour and Massler were the pioneer to describe the tooth development [2,3]. Age dependent sequential chronology of various histomorphological stages and deposition of hard dental structures for tooth development in human fetuses have been extensively documented [1,3-6]. Garlic was the first to report the histomorphological stages of incisor teeth development, irrespective of fetal age, in bovine [7]. Weinreb and Sharav [8] observed histomorphogenesis of cheek teeth in sheep. Age dependant chronological observations on histomorphological stages of deciduous incisors teeth were already reported for fetuses of sheep [9] [10] buffalo [11] and dog [12]. Standard textbooks on veterinary histology [13-15] and embryology [16-18] describe, in general, histomorphogenesis of teeth without mentioning species and fetal age. Radiographic observations on deciduous incisor teeth development has been recently documented [19]. It seems that no attempts have been made on histomorphogenesis of teeth development in goat fetuses. Hence, to elucidate the scientific information on histomorphological stages of first deciduous incisor tooth in goat fetuses, the present investigation was planned.

Materials and Methods

A total number of 65 goat fetuses from 6 to 11 weeks (CR 2.5 cm to 16.6 cm) were taken from collection repository of Department of Veterinary Anatomy and Histology, College of Veterinary Science and Animal Husbandry,

Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, 385 506. Representative fetuses at weekly interval from 6 to 11 weeks were selected for histomorphological study for right first deciduous incisor tooth (Di₁) development. The whole heads of fetuses of 6 weeks and the right incisive portion of lower and upper jaws from 7 to 11 weeks were processed for routine paraffin embedding method by automated tissue processor (Leica TP 1020, USA). Coronal and sagittal serial paraffin sections were cut at 7 µ by microtome machine (Leica RM 2255, USA). Paraffin sections were stained by the Routine Harris' Haematoxylin and Eosin stain and the Masson's Trichrome stain [20]. The stained sections were examined for various histomorphological stages for right first deciduous incisor tooth (Di₁) development on lines of Kumar [1], Hatt [10] and Patel [11].

Results and discussion

The chronology of various histomorphological stages and its features observed during development of right Di₁ are given in [Table-1,2].

Dental lamina stage

At 6th week of goat fetus, the first histomorphological stage, dental lamina was detected throughout the future dental arch region [Fig-1][Table-1]. At this age, the oral epithelium consisted fewer layers of superficial polygonal cells and a basal layer of cuboidal cells. By 37 days, at future dental arch of lower incisor region, the basal cells of oral epithelium proliferated more rapidly into underline ectomesenchyme and formed epithelial cord called the primary epithelial band [Fig-1].

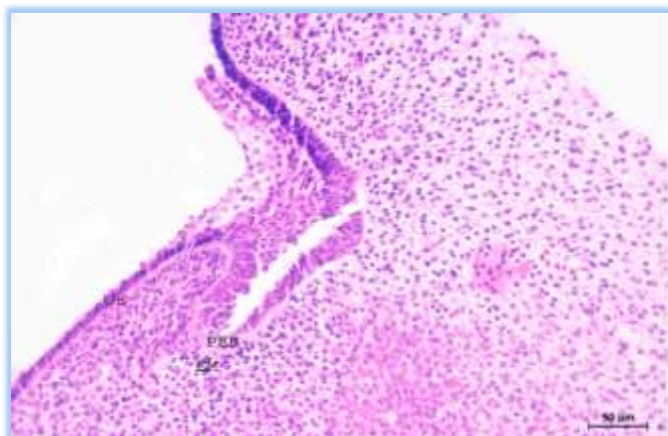


Fig-1 Photomicrograph of coronal section of lower jaw at 6th week (37 days) goat fetus showing the primary epithelial band, H. and E. stain, 400X

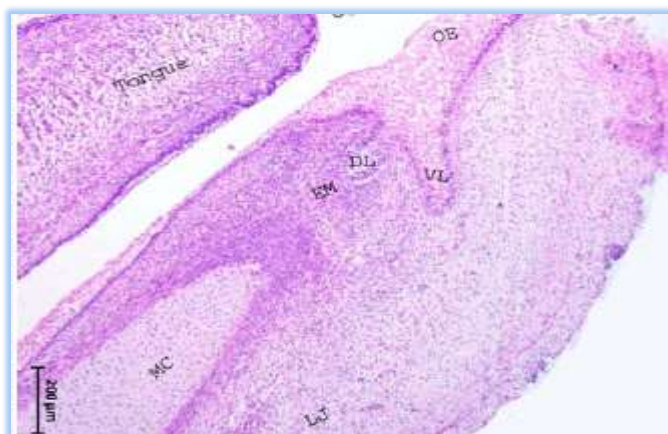


Fig-2 Photomicrograph of sagittal section of head at 6th week (42 days) goat fetus showing bifurcation of the primary epithelial band into the dental lamina for Di₁ and the vestibular lamina, H. and E. stain, 50X

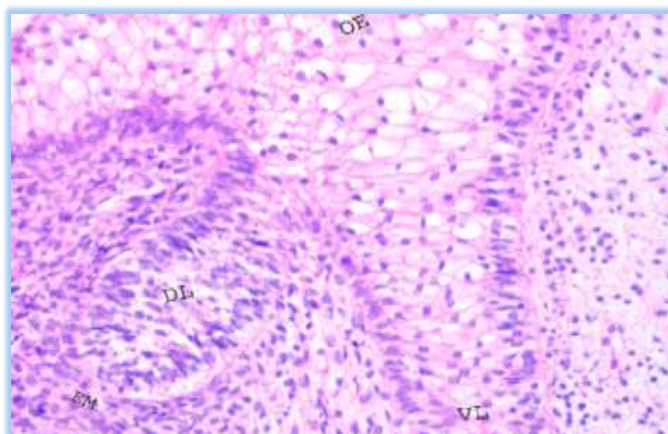


Fig-3 Photomicrograph of lower jaw at 6th week goat fetus showing bifurcation of primary epithelial band into the dental lamina for Di₁ and the vestibular lamina, H. and E. stain, 400X

and E. stain, 400X

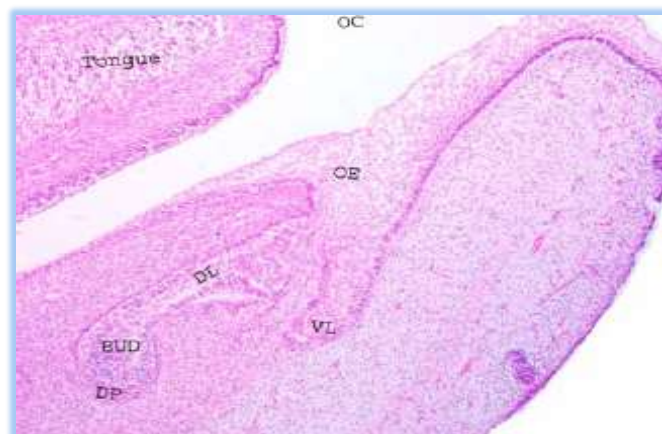


Fig-4 Photomicrograph of sagittal section of head at 7th week goat fetus showing the bud stage of Di₁, H. and E. stain, 50X

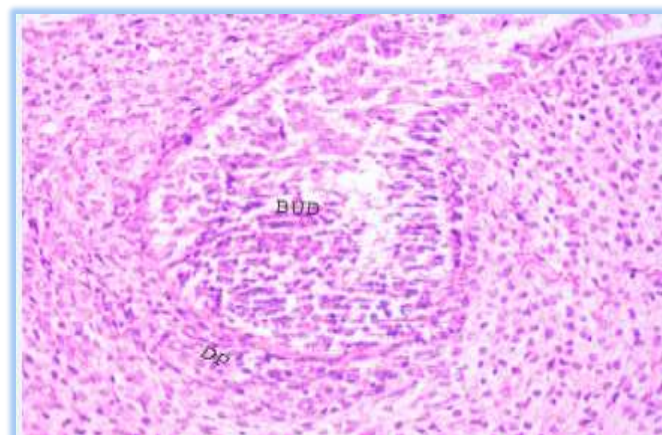


Fig-5 Photomicrograph of bud stage of Di₁ at 7th week goat fetus showing two components of tooth germ, H. and E. stain, 400X

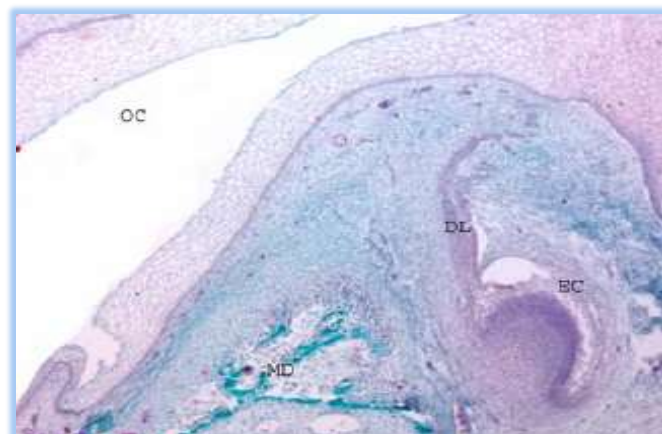


Fig-6 Photomicrograph of sagittal section of head at 8th week goat fetus showing the early cap stage of Di₁ Masson's Tricome stain, 50X

Table-1 Chronology of various histomorphological stages and features observed during development of Di₁

| Foetal age (Week) | Histomorphological stages of Di ₁ | Histomorphological features |
|-------------------|--|---|
| 6 | Dental lamina stage | Initiation Primary epithelial band, Dental lamina, Vestibular lamina |
| 7 | Bud stage | Proliferation, Morphodifferentiation Enamel organ, Dental papilla |
| 8 | Early cap stage | Proliferation, Histodifferentiation, Morphodifferentiation Enamel organ, Dental papilla, Cervical loop |
| 9 | Late cap stage | Proliferation, Histodifferentiation, Morphodifferentiation Stellate reticulum, Cervical loop, Dental papilla, Dental sac |
| 10 | Bell stage | Proliferation, Histodifferentiation, Morphodifferentiation Differentiation of ameloblasts and odontoblasts, Cervical loop, Successional dental lamina |
| 11 | Advance bell stage | Proliferation, Histodifferentiation, Morphodifferentiation, Deposition of dentin Differentiation of ameloblasts and odontoblasts, Dentinogenesis, Cervical loop |

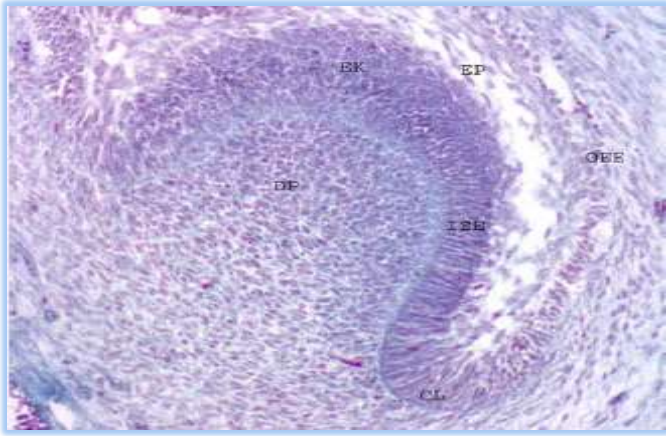


Fig-7 Photomicrograph of the early cap stage at 8th week of goat fetus showing different components of the early cap stage of Di₁, Masson's Trichrome, 400X

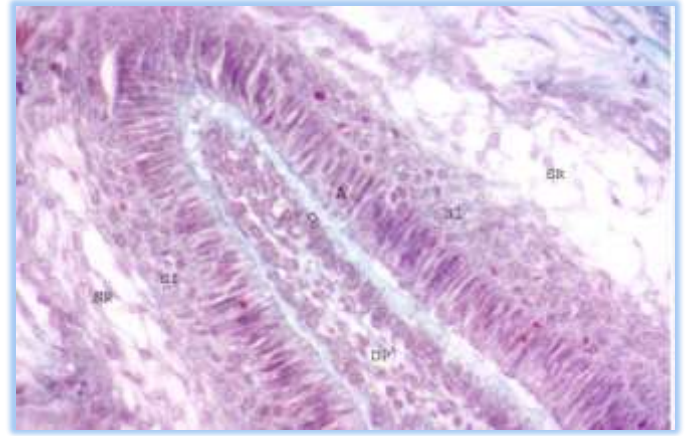


Fig-10 Photomicrograph of the tip of bell stage of Di₁ at 10th week goat fetus showing differentiated ameloblasts and odontoblasts. Masson's Trichrome stain, 100X

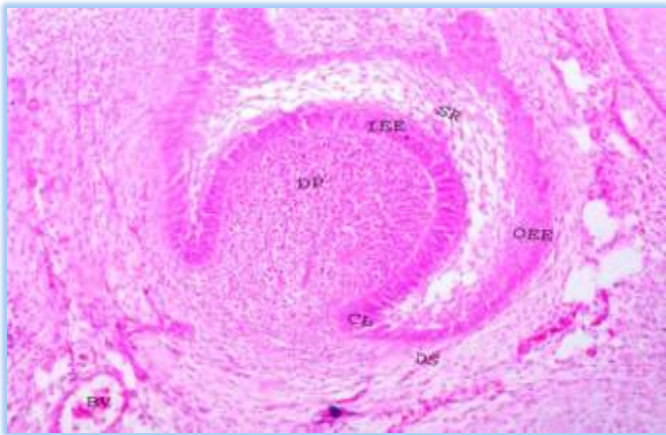


Fig-8 Photomicrograph showing the late cap stage of Di₁ at 9th week goat fetus. H. and E. stain, 100X

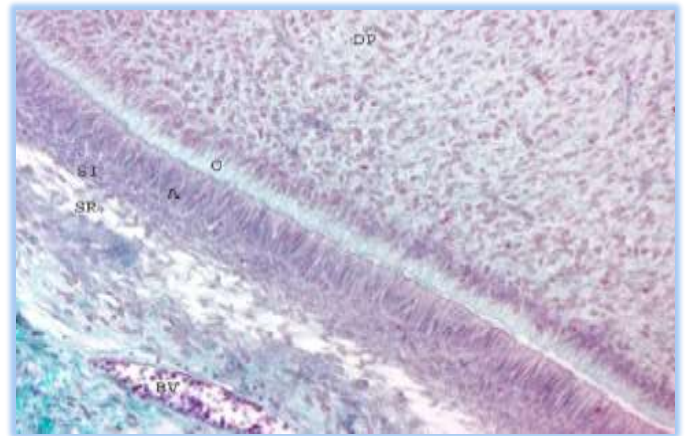


Fig-11 Photomicrograph of lateral margin of the bell stage of Di₁ at 10th week goat fetus showing differentiated ameloblasts and odontoblasts. Masson's Trichrome stain, 100X

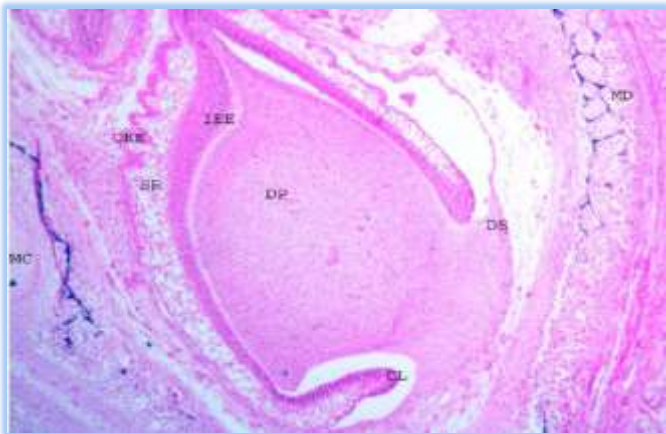


Fig-9 Photomicrograph of the bell stage of Di₁ at 10th week goat fetus showing different components of the bell stage. H. and E. stain, 50X

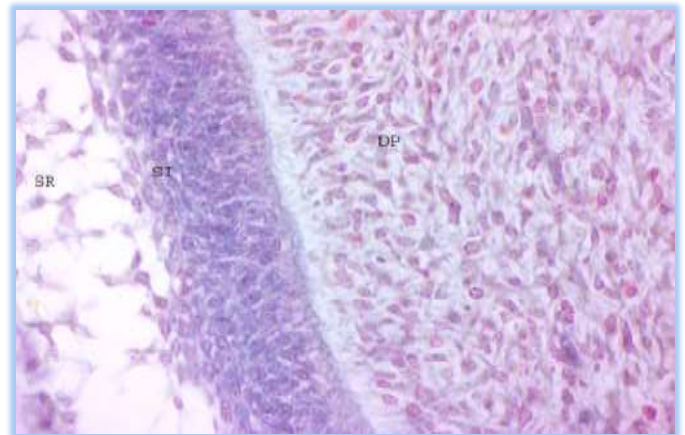


Fig-12 Photomicrograph of lateral margin of the bell stage of Di₁ towards cervical loop at 10th week goat fetus showing no differentiation of ameloblasts and odontoblasts. Masson's Trichrome stain, 100X

Table-2 Components of tooth germ and its appearance during odontogenesis of Di₁ in goat foetuses

| Embryonic origin | Component of Tooth germ | Appearance (week) | Histomorphological stage |
|-------------------------------------|-------------------------|-------------------|--------------------------|
| Oral ectoderm | Enamel organ | 7 | Bud stage |
| Neural crest derived ectomesenchyme | Dental papilla | 7 | Bud stage |
| | Dental sac | 9 | Late cap stage |

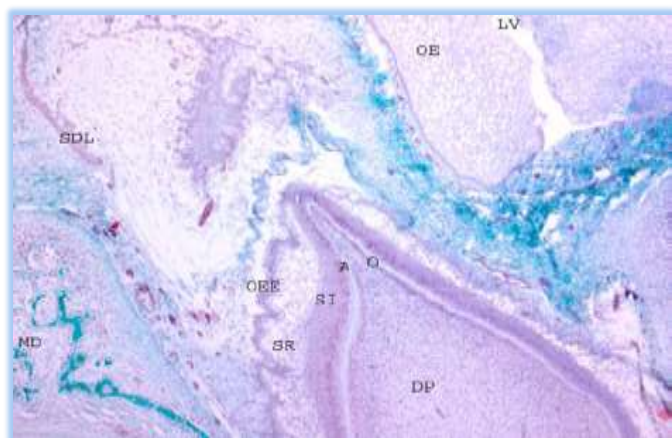


Fig-13 Photomicrograph of the bell stage of Di₁ at 10th week goat fetus showing appearance of successional dental lamina for first permanent incisor. Masson's Trichrome stain, 50X

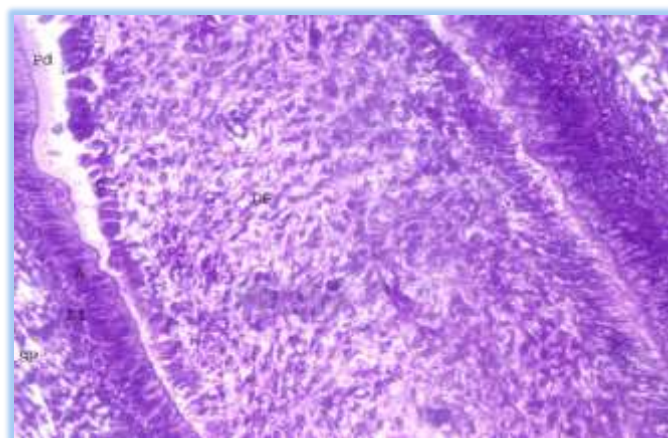


Fig-16 Photomicrograph of the advance bell stage of Di₁ at 11th week goat fetus showing no deposition of dentin near to cervical loop. H. and E. Stain, 100X

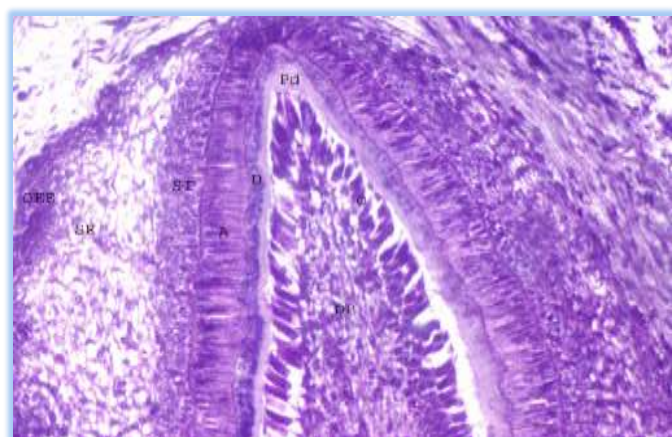


Fig-14 Photomicrograph of the advance bell stage of Di₁ at 11th week of goat fetus showing first deposition of dentin at tip. H. and E. stain, 100X

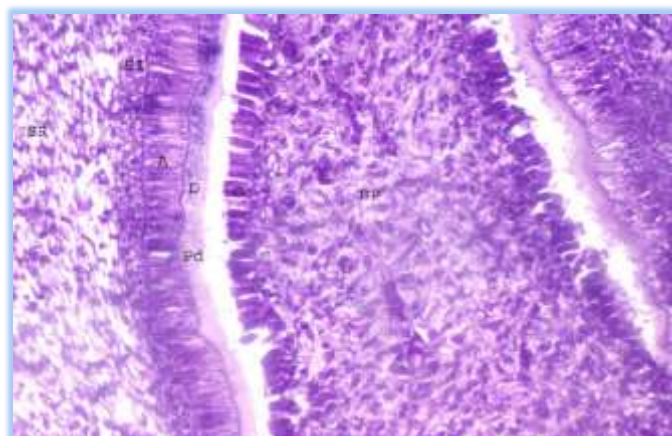


Fig-15 Photomicrograph of lateral margin of the advance bell stage of Di₁ at 11th week goat fetus showing deposition of dentin at lower part. H. and E. stain, 100X

Common legends used for Fig-1-16

- PEB= Primary epithelial band
- EM= Ectomesenchyme
- MC= Mackel's cartilage
- OE= Oral epithelium
- LJ = Lower jaw
- DL= Dental lamina
- VL = Vestibular lamina
- OC= Oral cavity
- BUD= Enamel organ
- EC= Early cap
- EK=Enamel knot
- EP= Enamel pulp
- OEE= Outer enamel epithelium
- IEE= Inner enamel epithelium
- DP= Dental papilla
- CL= Cervical loop
- SR= Stellate reticulum
- DS= Dental sac
- SI= Stratum intermedium
- O= Odontoblasts
- A = Ameloblasts
- BV= Blood vessel
- SDL= Successional dental lamina
- LV= Labial vestibule
- OEE= Outer enamel epithelium
- D=Dentin
- DP= Dental pulp

Table-3 The comparative chronology of appearance of histomorphological stages of Di₁ of goat fetuses in the present study and earlier reports in other domestic ruminants

| SN | Histomorphological stages of Di ₁ | Goat fetuses | Sheep fetuses | Buffalo fetuses |
|----|--|----------------------|--------------------|-------------------|
| | | Present study (Week) | Hatt (1967) (Week) | Patel 1996 (Week) |
| 1 | Dental lamina | 6 | 5 | 7 |
| 2 | Bud stage | 7 | 5 | 9 |
| 3 | Early cap stage | 8 | 9 | 13 |
| 4 | Late cap stage | 9 | 9 | 18 |
| 5 | Bell stage | 10 | 13 | 20 |
| 6 | Advance bell stage | 11 | 13 | 25 |

By 42 days, the primary epithelial band grown deeper into underlying ectomesenchyme and divided into two divisions viz. the dental lamina and the vestibular lamina [Fig-1]. The dental lamina was in the form of epithelial ridge. The epithelial ridge was directed lingually towards Mackel's cartilage, called the dental lamina. The formation of dental lamina was the initial sign of development of tooth called the initiation. The epithelial cells of dental lamina were found separated from underlying ectomesenchyme by a distinct basement membrane [Fig-3]. Concomitantly at 42 days, one more labially directed division of primary epithelial band was found in the form of elongated ingrowth of oral epithelium into the underlying ectomesenchyme called the vestibular lamina [Fig-2,3].

Bud stage

The second histomorphological stage, the bud stage, for Di_1 was detected at 7th week of goat fetus [Fig-4] [Table-1]. At the future location of Di_1 , the basal epithelial cells of dental lamina proliferated more rapidly and formed knob like cellular growth into underlying ectomesenchyme called the bud stage [Fig-4]. The tooth bud was found directed towards Mackel's cartilage and ossifying mandible [Fig-4]. This tooth bud was represented as the enamel organ. The peripheral cells of the tooth bud were low columnar and intensely mitotic [Fig-5]. The centrally located cells of tooth bud were less mitotic and polygonal in shape [Fig-5]. Adjoining to the tooth bud, ectomesenchymal cells proliferated and condensed to form the dental papilla [Fig-4, 5]. The two components of the tooth germ (tooth primordium), enamel organ and dental papilla were appeared at 7th week of goat fetus [Fig-5] [Table-2]. The vestibular lamina got widened and invaginated more into ectomesenchyme separating the developing lip and the dental arch [Fig-4].

Early cap stage

The third histomorphological stage, the early cap stage, for Di_1 was observed at 8th week of goat fetus [Fig-6] [Table-1]. At this age, early cap stage of Di_1 was found facing towards 'V' shaped ossifying mandible [Fig-6]. At this fetal age, the actively proliferating epithelial cells at distal end of tooth bud invaginated within the bud forming a cap shaped concavity by morphodifferentiation. The concave shaped enamel organ called the early cap stage [Fig-6,7]. The cap shaped enamel organ showed three layered structure consisting of outer enamel epithelium, enamel pulp and inner enamel epithelium [Fig-7]. The convex cellular layer at the periphery of the early cap stage of the enamel organ was called outer enamel epithelium [Fig-7]. This outer enamel epithelium consisted of 2-3 layered stratified cuboidal cells [Fig-7]. The cellular layer lining the concavity of early cap was called the inner enamel epithelium which consisted highly mitotic stratified cuboidal cells. In the middle of concavity, the cell layer of inner enamel epithelium actively proliferated forming condensed cellular mass called the enamel knot [Fig-7]. Distally directed double layered circular rim formed by the outer enamel epithelium and the inner enamel epithelium called the cervical loop [Fig-7]. The condensed cells forming central core between the outer enamel epithelium and the inner enamel epithelium called the enamel pulp [Fig-7]. At this stage, the concavity of early cap stage along with its cervical loop partly enclosed the darkly stained spherical mass of highly proliferating dense ectomesenchyme of the dental papilla [Fig-7]. At this stage the dental lamina was found in the form of elongated epithelial cord [Fig-6]. The early cap stage was completely separated from the developing vestibular lamina by a thick layer of ectomesenchymal tissue [Fig-6].

Late cap stage

At 9th week of goat fetus, the fourth morphological stage, the late cap stage of Di_1 was detected [Fig-8] [Table-1]. The cervical loop of enamel organ further extended distally and enclosed almost 2/3rd part of the proliferating dental papilla [Fig-8]. At this stage, the cells of enamel pulp of enamel organ got disassociated, moved apart and formed network of star shaped empty cells. These stellate shaped faint staining cells of the enamel pulp called the stellate reticulum [Fig-8]. After appearance of the stellate reticulum the developing enamel organ called the late cap stage. At late cap stage, inner enamel epithelium had single layer of mitotically active columnar epithelium and outer enamel epithelium consisted of one or two layers of cuboidal epithelium with less mitotic activity [Fig-8].

Peripherally to the developing enamel organ and dental papilla, concentrated ectomesenchymal cells formed fibrocellular sheath called the dental sac [Fig-8]. The third component of the tooth germ, the dental sac was appeared at 9th week of goat fetus. The dental sac was found proliferated by blood vessels [Fig-8]. All three components of tooth germ viz. enamel organ, dental papilla and dental sac for Di_1 were found at 9th week of goat fetus [Fig-8] [Table-2]. At this stage developing tooth germ of Di_1 was found directed towards concavity of 'V' shaped ossifying mandible. The enamel knot, which appeared at early cap stage, got disappeared at 9th week of goat fetus during late cap stage [Fig-8].

Bell stage

The fifth stage of odontogenesis, the bell stage for Di_1 was observed at 10th week of goat fetus [Fig-9] [Table-1]. The concavity of enamel organ deepened much and developing Di_1 took a morphological shape of the bell called the bell stage [Fig-9]. This stage was characterised by continuation of proliferation, histodifferentiation and morphodifferentiation [Fig-9-12].

At this stage, enamel organ showed four distinct layers viz. outer enamel epithelium, stellate reticulum, stratum intermedium and inner enamel epithelium [Fig-9]. At this stage, the peripheral ectomesenchymal cells of dental papilla induced the differentiation of inner enamel epithelium. The inner enamel epithelium started histodifferentiation into tall columnar cells called the ameloblasts from incisal edge and progressed cervically on lateral margin of the bell stage [Fig-10-12].

These differentiated ameloblasts cells with apically placed nucleus were regularly arranged. At this stage, between differentiated ameloblasts and stellate reticulum, 2-3 layered flattened cells appeared called the stratum intermedium [Fig-10-12]. At the bell stage, the stellate reticulum reduced in thickness at the tip of the bell, though it expanded further on the lateral margin of bell [Fig-10, 11].

The outer enamel epithelium now consisted of 2-3 layered stratified flattened cells with wavy outline [Fig-9,13]. Many small blood capillaries were found close to outer enamel epithelium [Fig-11]. The differentiated ameloblasts induced the histodifferentiation of subjacent ectomesenchymal cells of the dental papilla into columnar shaped odontoblasts cells [Fig-10,11]. These differentiated odontoblasts showed basally placed nucleus and were loose and not regularly arranged. At the bell stage, there was high degree of histodifferentiation from their respective precursor cells to ameloblasts and odontoblasts [Fig-10, 11].

The differentiation of ameloblasts and odontoblasts was progressively extended from incisal edge to cervical loop of developing Di_1 [Fig-10-12]. The differentiation of these cells was reached almost near to middle of the bell [Fig-10,11]. The progression for differentiation of the ameloblasts was at greater extent towards the cervical loop whereas the differentiation of odontoblasts was at lesser extent [Fig-10,11]. At the growing margin of the bell stage, cervical loop showed active mitotic proliferation. At the distal end of cervical loop, the inner enamel epithelium was not differentiated into ameloblasts and also the peripheral ectomesenchymal cells of the dental papilla were not differentiated into the odontoblasts [Fig-12]. Concomitant to histodifferentiation, the ameloblasts and the odontoblasts aligned to each other and outlined as the bell shape for Di_1 . The junction between ameloblasts cell and odontoblast cells formed future dentino-enamel junction. The mitotic activity was not detected in these differentiated ameloblasts and odontoblasts.

This bell shaped peculiar arrangement by the proliferation, morphodifferentiation and histodifferentiation formed blueprint for Di_1 [Fig-10,11]. The ectomesenchymal tissue of the dental sac was found between the enamel organ; dental papilla and the ossifying alveolar bony crypt. The dental sac was represented by a fibrovascular ectomesenchymal cell layer [Fig-9]. The proliferation of blood capillaries was found at the base of the bell. At the bell stage, the disintegration of the dental lamina started. Hence, the enamel organ lost its continuous connection with the oral epithelium [Fig-9,13].

At this stage, the dental lamina for first permanent incisor called the successional dental lamina was observed as lingual ingrowth from earlier existing dental lamina of Di_1 [Fig-13]. By 10th week of goat fetal age, disintegration of epithelium of vestibular lamina started and a deepened furrow appeared to form labial vestibule [Fig-13].

Advance bell stage:

The sixth morphological stage of developing Di_1 , the advanced bell stage was observed at 11th week of fetal age by the commencement of deposition of dentin [Fig-14] [Table-1]. At the tip of the advanced bell stage, the odontoblasts started deposition of the dentin, dentinogenesis, at future dentino-enamel junction [Fig-14] and deposition continued uniformly down towards the lateral margin and the cervical loop [Fig-15,16]. The differentiation of ameloblasts and odontoblasts was progressively extended towards the lateral margin and growing cervical loop of Di_1 [Fig-14-16]. The differentiation of these cells was reached almost lower to middle of the advanced bell [Fig-15, 16]. In the present investigation, dental lamina and vestibular lamina developed from earlier existing primary epithelial band which was in agreement with results in fetuses of human [1,6] and buffalo [11]. In contrast, the vestibular lamina and the dental lamina were reported to develop separately in fetuses of sheep [10] and dog [12]. The various histomorphological stages and development of right first deciduous incisor tooth presented in [Table-3]. The present findings on sequential development of various histomorphological stages for Di_1 were in close agreement with the reports in fetuses of sheep [10], buffalo [11], cattle [7], dog [12] and human [1,6]. However, the time programme of appearance of various histomorphological stages for present investigation varies with the species [Table-3]. Hatt [10] reported the dental lamina and the bud stage at earlier ages; cap stage at almost similar ages and bell and advance bell stage at later ages in fetuses of sheep than that of present investigation in goat fetuses [Table-3]. The various histomorphological stages were reported comparatively at later ages in fetuses of buffalo [11] and at earlier ages in fetuses of dog [12] as compare to the present study [Table-3]. The sequence of various histogenetic process viz. initiation, proliferation, histodifferentiation, morphodifferentiation and apposition observed for the first deciduous incisor tooth development in the present study was essentially similar to that reported in fetuses of cattle [7], sheep [10], buffalo [11], human [1, 2, 21] and Dog [12] [Table-3]. Veterinary histology [13, 14, 15] and embryology [16, 17, 22] texts describe very brief account of tooth development supporting to the present findings. However, these texts did not mention the age dependant developmental chronology for any tooth or species. As compare to the present findings for goat fetuses, the successional dental lamina for first permanent incisor reported at later ages in fetuses of buffalo at 20th week [11] and sheep at 13th week [10]. Similarly, the enamel knot was reported as transitory structure during the cap stage in human [1, 6] and buffalo [11].

Summary and conclusion

The various histomorphological stages of first deciduous incisor (Di_1) was observed as: dental lamina at 6th week, bud stage at 7th week, early cap stage at 8th week, late cap stage at 9th week, bell stage at 10th week and advanced bell stage at 11th week goat fetuses. Among all three components of tooth germ, enamel organ and dental papilla were appeared at 7th week and dental sac at 9th week goat fetus. The enamel knot was also observed as transitory structure at 8th week and disappeared at 9th week. The histodifferentiation of the odontoblasts and the ameloblasts begun at 10th week. The successional dental lamina for first permanent incisor was observed at 10th week fetus.

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

The present embryological findings on age dependant histomorphogenesis of first deciduous incisor in goat would be useful as baseline information for future odontological investigation in veterinary field.

Abbreviations:

CR – Length - Crown- Rump length
 Di_1 - First deciduous incisor

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Author statement: All authors read, reviewed, agree and approved the final manuscript

Conflict of Interest: None declared

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Specimen: Not live specimens

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