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Title:

Validating dental histology for perinatal age estimation using human deciduous teeth

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Abstract

Objectives: This study tests the accuracy of dental histology for perinatal age estimation using deciduous teeth from modern human infants of known age. We evaluate whether enamel and dentin incremental structures, particularly the Neonatal Line (NNL), can be used to provide a reliable estimate of chronological age.

Samples and Methods: Seventeen unerupted deciduous teeth from fourteen full-term infants (0–164 days old) were analyzed from the Granada identified skeletal collection in Spain. Thin sections were examined under transmitted light and confocal microscopy to identify the NNL, cross-striations, and von Ebner's lines. Daily secretion rates (DSR) were measured across crown and root to calculate crown formation time (CFT) and root formation time (RFT), which were compared with the documented ages at death.

Results: Mean enamel DSR was 3.38 $\mu\text{m}/\text{day}$, and mean root dentin DSR was 3.24 $\mu\text{m}/\text{day}$. Enamel DSRs varied between tooth types and crown regions. The NNL was identified in all individuals who survived birth and absent in one stillbirth, confirming its reliability as an indicator of live birth. Prenatal CFTs fell within ranges reported for full-term gestation, supporting the interpretation that all individuals were born at term. Histologically derived ages differed from recorded ages by an underestimation of 8 to an overestimation of 1 day, with a mean difference of 1 day.

Conclusions: Deciduous dental histology provides an accurate estimate of chronological age for human juveniles in the year after birth. These findings suggest that perinatal age estimates derived from dental histology can be applied in bioarchaeological and forensic contexts.

Keywords: Deciduous Teeth; Neonatal Line; Dental Histology; Crown Formation Time; Age Estimation

1. Introduction

Biological rhythms, ranging from circadian to annual cycles, are widespread in living organisms and often leave structural imprints in hard tissues, such as tree rings, marine organism shells, and dental tissues (Scrutton, 1978). In teeth, incremental features in enamel and dentin have long been recognized as key indicators of biological rhythms, offering insights into the development, chronology, and life history of extant and fossil hominins (Boyde, 1963; Bromage & Dean, 1985; Dean et al., 2001; Macchiarelli et al., 2006; Mahoney, 2015; Mahoney et al., 2021; Nava et al., 2017b, 2019, 2024; Peripoli et al., 2023). These incremental structures form through the regular deposition of dental enamel by ameloblasts, which results in microscopic features such as cross-striations and Retzius lines (RLs) that reflect rhythmic variations in matrix secretion, composition, and growth dynamics (Boyde, 1989; Dean, 2000; Smith & Avishai, 2005). Cross-striations represent circadian rhythms corresponding to daily increments in enamel prism growth (Boyde, 1963), and are matched by von Ebner lines in dentin (Dean, 2012b; Smith, 2008). Studies have shown that clock genes such as *Bmal1*, *Clock*, *Per1*, and *Per2* oscillate in ameloblasts with a 24-hour periodicity (Lacruz et al., 2012; Zheng et al., 2011, 2014), regulating stage-specific enamel proteins like amelogenin (*Amelx*) and kallikrein-related peptidase 4 (*Klk4*) (Zheng et al., 2013). RLs reflect longer periodicities, typically 6–12 days in human permanent teeth, which are thought to be associated with about weekly biological patterns (Reid & Dean, 2006; Smith et al., 2007). RLs are paralleled in dentin by and Andersen lines (Dean, 2012b; Smith, 2008).

Experimental evidence has confirmed the circadian timing of cross striations. Bromage (1991) demonstrated through fluorescent labeling in macaques that enamel cross-striations correspond to daily increments of matrix deposition, providing direct support for circadian periodicity in enamel formation. Later, Smith (2006) applied the same technique to developing macaque teeth and found that both cross-striations and the longer-period RLs appeared at predictable intervals, further supporting their use in reconstructing aspects of enamel formation time. These findings were consistent with earlier observations of periodic structures in primate enamel (e.g., Risnes, 1986; Schour & Poncher, 1937) and underscored their potential for calculating crown formation time (CFT).

Accentuated lines (ALs) appear outside these rhythms and signal temporary interruptions in normal enamel formation due to systemic stress, thereby impacting ameloblast activity. Such disruptions can be recorded histologically as more prominent, irregular incremental features, which do not follow the regular rhythmic deposition of enamel and are thought to reflect episodes of systemic physiological stress such as illness or nutritional deficiency (FitzGerald & Saunders, 2005; Guatelli-Steinberg & Huffman, 2012; Witzel et al., 2008).

The Neonatal Line (NNL) is a notable AL that appears in primary teeth and the mesiobuccal cusp of first permanent molars after birth (Antoine et al., 2009; Mahoney, 2008; Nava et al., 2017a). It reflects biological stress at birth, during the transition from intrauterine to extrauterine life, including changes in nutrition and physiology (Janardhanan et al., 2011; Kurek et al., 2016). Characterized by changes in enamel prism size, direction, and mineralization, the NNL provides a critical chronological marker of birth and is especially valuable for distinguishing prenatal from postnatal growth (Birch & Dean, 2009). Research indicates that the NNL becomes visible in individuals who survive at least 10–15 days postnatally, though it may form

even earlier (Fitzgerald & Hillson, 2009; Schwartz et al., 2010; Smith & Avishai, 2005). The NNL allows researchers to estimate biological age in teeth that are still forming by counting cross-striations from the line to the end of enamel formation (Witzel, 2014). Therefore, unerupted primary teeth crowns with still-forming enamel provide a unique material for determining how long an infant survives by quantifying enamel deposition after birth (Martirosyan et al., 2024).

Biological age reconstructed from dental histology is not identical to chronological age, but the two are closely correlated. Two studies have been undertaken on permanent teeth. Antoine et al. (2009) tested the reliability of prism cross-striation counts in developing permanent teeth from known-age human juveniles in the Spitalfields crypt (London). Their analysis of three permanent teeth from an individual in which a NNL was identified revealed a minimum underestimation of 14 days when compared with the known chronological ages. Smith et al. (2006) applied standard histological methods to identify the NNL, count incremental lines, and reconstruct the duration of crown and root formation in five pig-tailed macaques (*Macaca nemestrina*) of known age at death. Their results corresponded with the known documented ages, with estimates showing an overestimation of at least 8 days to an underestimation of up to 10 days.

Our understanding of the accuracy of histologically derived age estimates for deciduous teeth from human infants is limited. Recent research by Ramirez Rozzi et al. (2025) highlighted potential applications for human infants. Using an estimated rate of enamel secretion, they reported that the age-at-death determined from central incisor crowns for two infants was within two weeks of the known chronological age for one infant, and a few days for the other.

The aim in this study is to estimate the age-at-death for 14 human infants using histological analyses of deciduous enamel and roots. We focus on unerupted deciduous teeth from individuals with documented birth and death records, all of whom were full-term at birth. We calculated daily secretion rates (DSRs), CFTs, and root formation times (RFTs) directly from the teeth of these 14 infants. Our histologically derived estimates were then compared with the known chronological ages of each infant to evaluate the precision of this methodological approach.

2. Materials and Methods

2.1 Samples

We analyzed 17 unerupted deciduous teeth from 14 individuals, aged between 0 (birth) and 164 days, obtained from the Granada identified skeletal collection of infant individuals housed at the Department of Legal Medicine, Toxicology, and Physical Anthropology at the University of Granada, Spain (Alemán et al., 2012). The protocol for studying these remains complied with national legislation (Law 14/2007 on Biomedical Research, 3rd July), and was approved by the ethics committee of the University of Granada. Law 14/2007 is the primary legislation in Spain governing research on human biological samples that ensures protection of personal privacy and confidential treatment of personal data in biomedical research.

The main inclusion criterion was the selection of still-forming teeth. Because the histological procedure is destructive, sampling was limited to fractured specimens that were unsuitable for morphometric analysis but could provide a complete mid-labiolingual section. The final sample comprised 12 central deciduous incisors, 3 lateral deciduous incisors, 1 first deciduous molar, and 1 second deciduous molar (Table 1). Their developmental stages ranged from three-quarters of crown completion (Cr 3/4) to initial root formation with diverging edges (Ri) (AlQahtani et al., 2010; Table 1). Tooth identification and nomenclature followed the FDI World Dental Federation notation (ISO, 2016).

Table 1. Study sample details, including the individuals analyzed, their teeth, tooth maturational stage based on Alqahtani (2010), sex, and age at death.

Individual	Tooth	Tooth maturational stage (AlQahtani, 2010)	Sex	Age at death
G-189	51	Ri	Male	135 days
	61	Ri		
G-215	81	Ri	Female	129 days
G-225	71*	Cr 3/4	Female	44 days
G-309	52	Ri	Female	164 days
	54*	Cr 3/4		
G-346	81*	Cr 3/4	Male	8 days
G-362	71	Ri	Male	62 days
G-363	55*	Cr 3/4	Female	25 days
G-371	71*	Cr 3/4	Female	0 days
G-380	61	Ri	Male	116 days
G-393	52	Crc	Male	91 days
	62	Crc		
G-399	71	Ri	Female	60 days
G-405	61*	Cr 3/4	Male	65 days
G-414	61	Ri	Male	78 days
G-512	81*	Cr 3/4	Female	31 days

2.2 Histological sections

The thin sections were obtained using an approach based on previous methodologies (Martirosyan et al., 2024). Teeth were embedded in ExpoxiCure™ 2 mounting resin (Buehler) and sectioned labiolingually using a Minitom equipped with a diamond cut-off wheel (Struers). The resulting sections were then attached to the histological slides using the same mounting resin (ExpoxiCure™ 2), and thin sections of 450-350µm were obtained with the Minitom (Struers). To achieve an optimal thickness of 80-100µm, 2000µm P silicon carbide abrasive paper was used (Silva et al., 2011). The resulting section was dehydrated in ethanol (96%, and 100%), immersed in a histological clarifying agent (Histolemon, Carlo Erba Reagents S.A.S), and mounted with a coverslip using DPX mounting media (PanReac AppliChem ITW Reagents ®).

The histological sections were observed under a transmission petrographic optical microscope with a Leica DM2700 P dual polarization system at 5X, 10X, and 20X magnifications, using Micrometrics SE Premium Software. Additionally, a confocal Olympus FluoView FV1000 microscope equipped with a Hamamatsu ORCA-Flash4.0 LT monochrome camera at 20X magnification was used for better visualization of the cross-striations. Photocompositions of the images were created using MosaicJ (Schindelin et al., 2012).

2.3 Calculation of Daily Secretion Rate

The buccal side of the tooth was prioritized for analysis due to better preservation and clearer visualization of the NNL. When significant taphonomic alterations were observed, the lingual side was used instead. Enamel DSR was determined by measuring the distance between cross-striations in enamel prisms across three regions of the crown obtained by dividing the length of the EDJ into three equal segments. Throughout this study, we refer to these regions as upper, middle, and inferior when describing the full sample, because not all teeth have fully formed crowns. When referring specifically to teeth with a complete crown, these same regions correspond to the cuspal, lateral, and cervical portions of the crown (Fig. 1a). This approach follows established methods (Birch & Dean, 2014; Mahoney, 2012, 2015; Mahoney et al., 2016). The linear distance along the path of the enamel prism, spanning three to five cross-striations, was measured and then divided by the number of cross-striations within that span to calculate the average DSR (Fig. 2). To standardize comparisons across regions, measurements focused on the mid-enamel portion within each of the three crown regions (Mahoney et al., 2024). Between 15 and 20 measurements were made per region, resulting in a total of 45–60 DSR values per tooth. For each region, the mean DSR and its associated 95% confidence interval (95% CI) were calculated (Martirosyan et al. 2024).

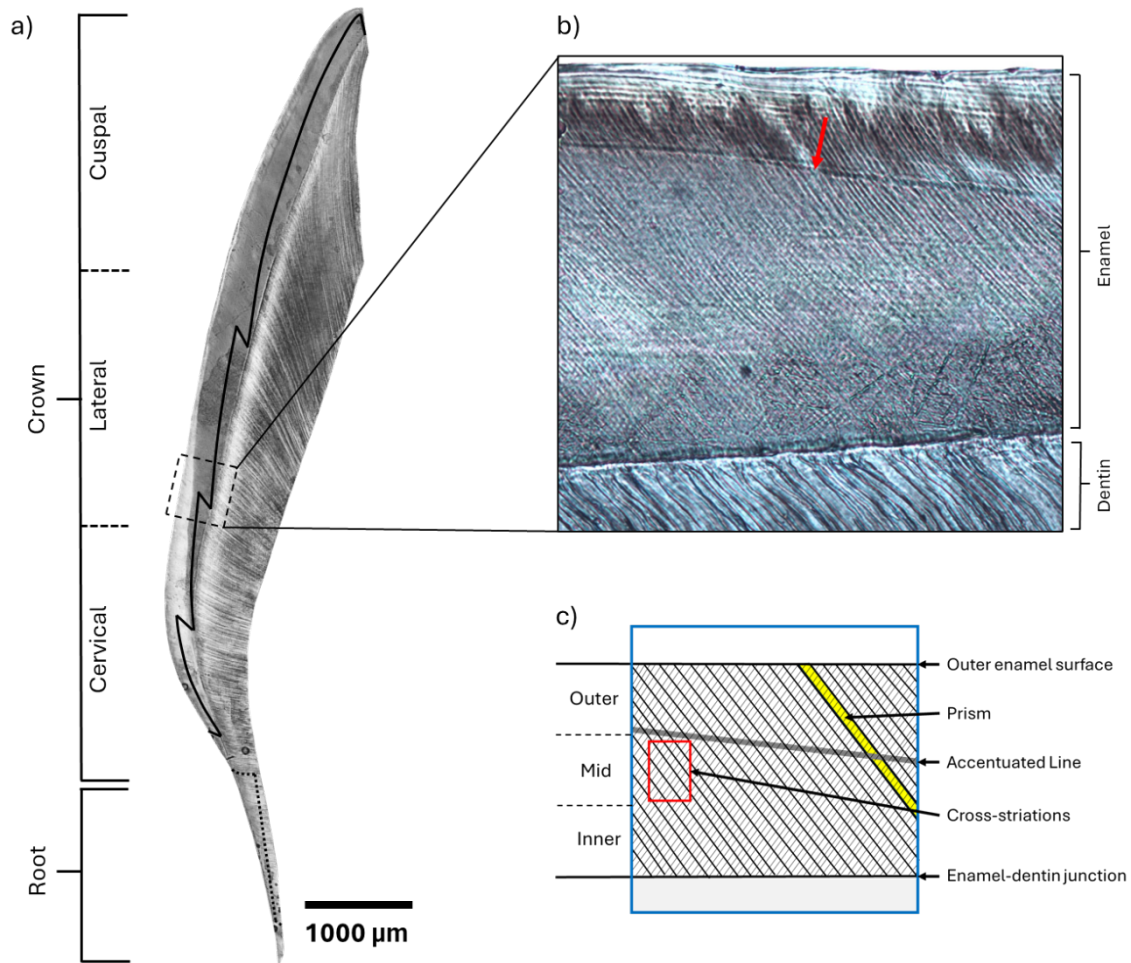


Figure 1. a) Photocomposition of the upper deciduous central incisor from individual G-189, illustrating the root and the division of the dental crown into three regions: cuspal, lateral, and cervical. Within the crown, a continuous line traces the prism path from the enamel-dentin junction (EDJ) towards the surface, then continues back to the EDJ along the direction of an accentuated line, or Retzius Line (RL). The segment traced up to the neonatal line (NNL) represents the prenatal crown formation time (pCFT), while the entire trace indicates the total crown formation time (CFT). A dashed line along the cement-dentine junction (CDJ) marks the trajectory followed to reconstruct root formation time (RFT), extending from the cervical margin towards the apex along accentuated incremental markings. b) Micrograph of a section from the same tooth showing the NNL indicated by an arrow, along with the dentin and enamel dental tissues. c) Schematic representation of enamel structure, showing its three zones: outer enamel, mid-enamel, and inner enamel. The EDJ separates dentin from enamel. Individual enamel prisms are shown, with one highlighted in yellow and indicated by an arrow. A red inset box displays the cross-striations.

For the statistical comparisons, only teeth with fully formed crowns were included to ensure that all regions were equally developed and directly comparable. In these analyses, the three segments correspond to the cuspal, lateral, and cervical portions of the crown. Differences in DSR between tooth types and between crown regions were evaluated using ANOVA with post-hoc comparisons in R (version 4.1.3). Statistical significance was set at $p < 0.05$.

In teeth with crowns that are fully completed but have evidence of root growth, dentin DSRs are needed to calculate RFT (Mahoney, 2019; Smith, 2008). For those specimens, root dentin DSRs were obtained

following the same protocol used for enamel, with measurements taken along clearly visible von Ebner's lines oriented parallel to the dentinal tubules (Fig. 2). Between 15 and 20 measurements were collected per tooth, and the mean DSR, with its 95% confidence interval (95% CI) was calculated for each specimen. For comparison, indirect dentin DSRs were estimated from enamel prism lengths (Mahoney, 2019; see Table S1).

2.5 Calculating formation time

CFT was calculated from the time taken to form the EDJ (eg., Dean, 2012a). We traced consecutive 200 μ m lines along enamel prisms in a zig-zag path guided by long-period RLs or accentuated markings (Fig. 1a), starting at the dentin horn and continuing until the cervix and the end of crown formation (Dean, 2012a). The number of days required to form each 200 μ m segment was reconstructed using a local mean DSR, and its 95% confidence interval (95 CI), for the respective crown regions (upper, middle, inferior). The total CFT was then obtained by summing the durations of all segments. Additionally, we calculated the prenatal crown formation time (pCFT), which is the interval between the initiation of enamel formation until the NNL is reached.

The presence of the NNL was identified when at least 75% of its length was visible and/or it was the first and most marked AL, as seen in Fig.1 (Adserias-Garriga & Visnapuu, 2019; FitzGerald & Saunders, 2005). In teeth lacking a recognizable NNL, the entire crown was traced to estimate total CFT, which in these cases corresponded to the pCFT (95% CI). For teeth with a visible NNL, postnatal survival age was estimated by tracing the NNL to its intersection with the EDJ. From this point, the process used to calculate CFT was repeated until the end of enamel growth, summing the time required to form each 200 μ m segment to obtain the postnatal CFT (95% CI).

To calculate RFT in individuals with fully formed crowns, we followed the procedure applied by Dean and Cole (2013) and Mahoney (2019) for reconstructing root growth. Consecutive 200 μ m lines were traced along the cement–dentin junction (CDJ) in a zig-zag trajectory guided by ALs, starting at the cervical margin and continuing toward the apex (Fig. 1a). For each segment, the number of days required to form 200 μ m of dentine was estimated using the mean root dentin DSR. The cumulative RFT was reconstructed by summing the time intervals required to form all successive root segments, yielding a total duration from the onset of root dentine formation at the CDJ to the most apical preserved point.

All specimens except one formed less than 200 μ m of root dentine. Therefore, only a single individual allowed the application of the full zig-zag trajectory along the CDJ (Fig. 1). In individuals with developing roots, the estimated value represents the duration of root formation up to the preserved apex, which, when added to the postnatal CFT, yields the total postnatal survival age.

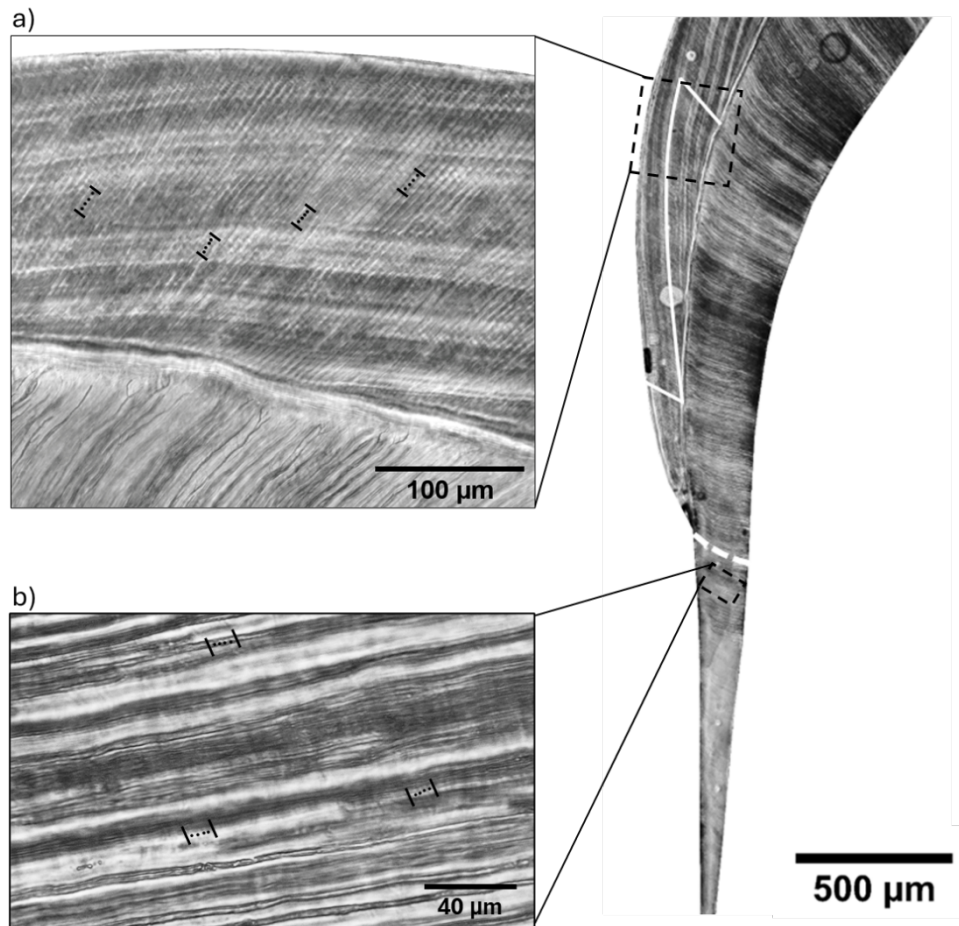


Figure 2. On the right, a detailed photocomposition of the central lower incisor from individual G-380, captured using confocal microscopy. A continuous white line marks the prism path in enamel used to determine postnatal crown formation time. A dashed white line shows the trajectory used to estimate root formation time. In this case, only the first segment could be traced, measuring less than 200 μm . On the left: (a) 20 $\times$ confocal micrograph of the boxed enamel area, showing prism segments with cross-striations used to calculate the daily secretion rate (DSR) in the middle enamel region, and (b) 20X confocal micrograph showing von Ebner's lines in root dentin. For both dentin and enamel, the DSR was calculated by measuring the distance between the two black lines marking the first and last cross-striation or von Ebner's line within a segment.

3. Results

3.1 Enamel Daily Secretion Rate

A total of $n=765$ DSR measurements were obtained across all specimens and crown regions. The overall mean DSR in this study was $3.38 \mu\text{m/day}$ (minimum of $1.58 \mu\text{m/day}$ to a maximum of $6.68 \mu\text{m/day}$), indicating a broad distribution of DSR across the sample. Mean DSRs ($\mu\text{m/day}$) and standard deviations by tooth type and crown region are shown in Table 2 (see Table S2 for further details).

Table 2. Descriptive values (mean DSR in $\mu\text{m/day}$ and standard deviation, SD) by tooth type and crown region (upper, middle, inferior).

Tooth type	Mean upper DSR (SD)	Mean middle DSR (SD)	Mean inferior DSR (SD)
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Upper deciduous central incisor	3.72 (0.743)	3.62 (0.53)	3.68 (0.735)
Lower deciduous central incisor	3.42 (0.858)	3.42 (0.789)	3.39 (0.649)
Upper deciduous lateral incisor	3.38 (0.344)	3.24 (0.355)	3.49 (0.754)
Upper deciduous first molar	2.78 (0.333)	2.30 (0.21)	2.38 (0.308)
Upper deciduous second molar	2.68 (0.21)	2.39 (0.24)	2.59 (0.245)

To assess variation in DSR across crown regions and tooth types, a factorial ANOVA was conducted on the subset of teeth with fully formed crowns (upper incisors: $n = 5$; lower incisors: $n = 3$). The overall model was statistically significant ($p < 0.001$), indicating that both incisor type and crown region, as well as their interaction, significantly affect DSR values (see Table S3 for further details). Significant differences were found between incisor types ($p < 0.001$, $\eta^2 = 0.065$), across crown regions ($p = .002$, $\eta^2 = 0.031$), and in the interaction between type and region ($p = .007$, $\eta^2 = 0.025$).

Post hoc comparisons revealed distinct patterns between upper and lower deciduous central incisors (Fig. 3; Table S4). Overall, upper deciduous central incisors exhibited significantly higher DSR values than lower deciduous central incisors in the cuspal ($p < 0.001$) and lateral crown regions ($p < 0.001$). In lower deciduous central incisors, the cervical crown region exhibited significantly higher DSR values than both the cuspal ($p < 0.001$) and lateral regions ($p < 0.001$). In contrast, upper deciduous central incisors showed no significant differences among crown regions. These results highlight that enamel formation rates vary not only within a crown but also between tooth types.

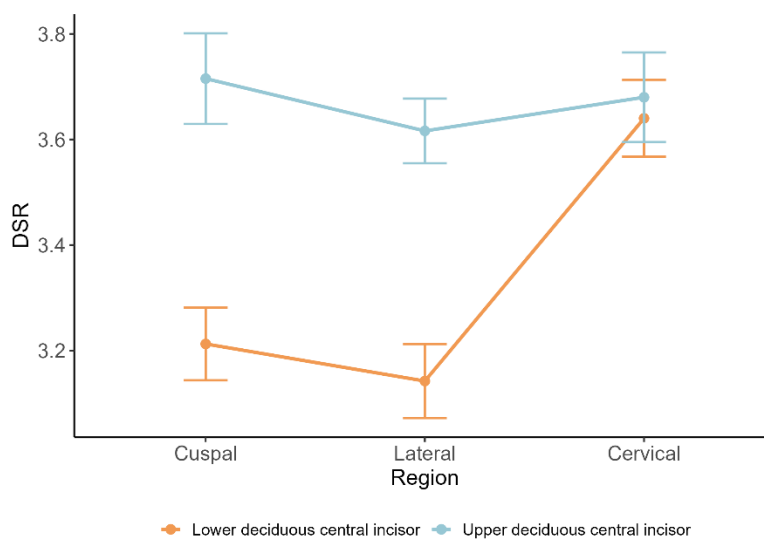


Figure 3. Line plot showing variation in Daily Secretion rate (DSR) across three crown regions (cuspal, lateral, cervical) for deciduous central incisors. The plot compares Lower deciduous central incisor and Upper deciduous central incisor. Error bars represent standard error.

3.2 Crown Formation Time

The pCFT of all samples fell within the documented ranges for full-term birth (Fig.4).>NNL was identified in all teeth except for one (tooth 71 from G-371), supporting the classification of the individual as a full-

term stillbirth. In the remaining individuals, the histological analysis of the pCFT and the presence of the NNL aligned with known records of live birth (Figs. 4 and 5).

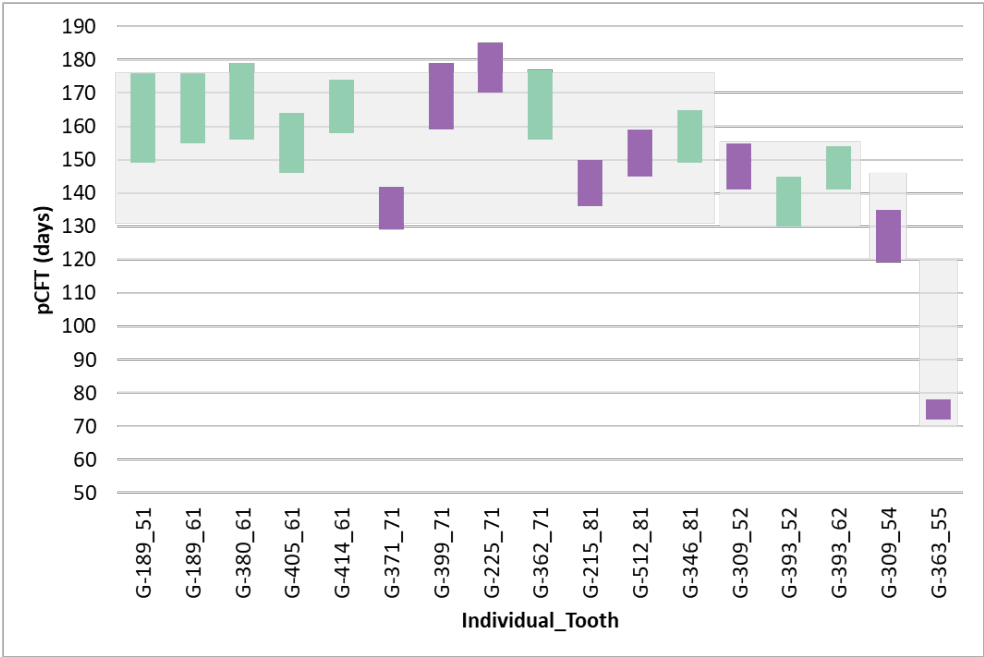


Figure 4. Graphical representation of the prenatal Crown Formation Time (pCFT 95% CI) for the deciduous teeth, indicated in boxes arranged by first appearing the central deciduous incisors (51,61,71,81), lateral deciduous incisors (52,62), first deciduous molars (54), and then second deciduous molars (55). The gray frame encapsulates the published data on pCFT between the 39 to 40 gestational weeks for central deciduous incisors (ranging from 137 to 176 days), lateral deciduous incisors (ranging from 131 to 156 days), first deciduous molars (ranging from 121 to 146 days) and second deciduous molars (ranging from 71 to 122) as reported by Mahoney (2012, 2015) and Birch and Dean (2014). The pCFT (95% CI) for each sample is depicted within boxes. Individual sex is indicated by color, with purple for females and green for males.

For samples with crowns still in formation, postnatal age was estimated using the postnatal CFT, which closely matched the known chronological ages at death (Fig. 5). G-346 (tooth 81) and G-363 (tooth 55) yielded a postnatal CFT of 8 days (7–8) and 23 days (22–24), compared to recorded survivals of 8 and 25 days, respectively. G-512 (tooth 81) and G-225 (tooth 71) produced postnatal CFT of 30 days (29–32) and 44 days (43–46), corresponding to a known age of 31 and 44 days. G-393 (teeth 52 and 62) provided slightly lower values of 83 days (78–89) and 85 days (81–89) than the recorded survival of 91 days. G-309, represented by two teeth, had a known age at death of 164 days, which aligns with the postnatal CFT of 164 days (154–176) from tooth 54, whose enamel formation was still in progress.

For samples with completed crowns, but developing roots, there was a close correlation between the histologically derived age and the documented ages at death (Fig.5). G-399 (tooth 71), G-362 (tooth 71), G-405 (tooth 61), and G-414 (tooth 61) yielded values of 60 days (56–63), 61 days (59–64), 65 days (62–68), and 77 days (73–82), respectively, consistent with their recorded survivals of 60, 62, 65, and 78 days. At older ages, G-380 (tooth 61) provided an estimate of 117 days (109–125) compared to 116 days, and G-

215 (tooth 81) gave 126 days (114–139) compared to 129 days. G-189 (teeth 51 and 61) yielded 135 days (126–146) and 136 days (128–144), both overlapping with the documented survival of 135 days. Finally, G-309 (tooth 52) provided 164 days (157–172), encompassing the recorded survival of 164 days.

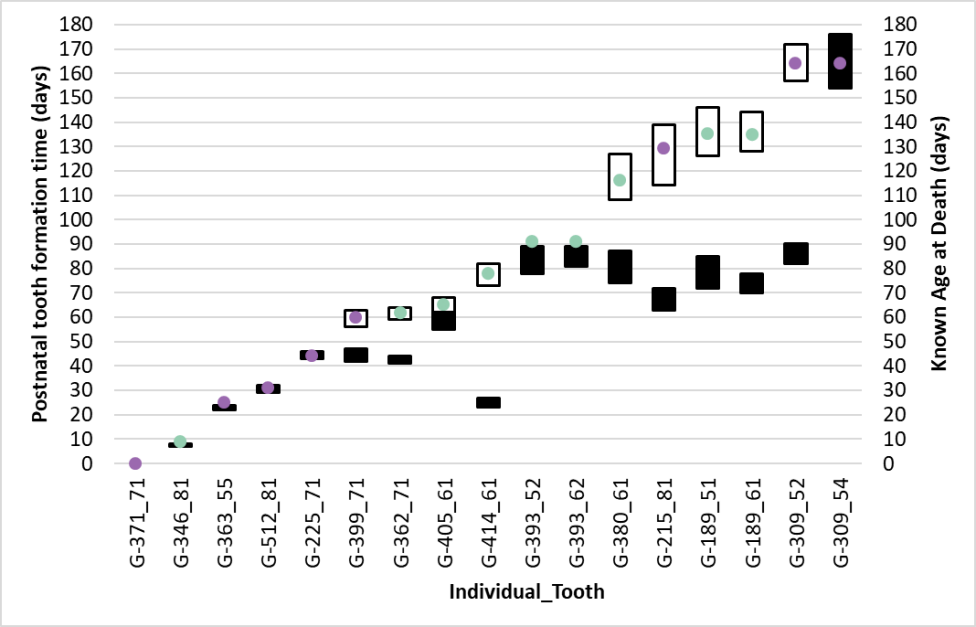


Figure 5. Graphical representation of the Tooth Formation time in days, with black boxes corresponding to postnatal Crown Formation Time in days (postnatal CFT 95% CI) and white boxes Root Formation Time in days (95% CI). The samples are organized by their known age at death, which is represented by a dot, purple for female individuals and green for male individuals.

4. Discussion

In this study, histologically derived age estimates from deciduous enamel and roots differed from known chronological ages by an underestimation of 8 days to an overestimation of 1 day among 14 individuals. This level of accuracy surpasses that of traditional osteological methods, which are often limited by variability in growth rates and cannot reliably distinguish preterm births, stillbirths, or short-term postnatal survival (Saunders & Barrans, 1999). Factors such as poor maternal nutrition, placental insufficiency, or maternal stress can restrict intrauterine growth, resulting in infants who are small at birth but were carried to term (Lewis, 2009; Mulder et al., 2002). Our results confirm that dental histology provides a precise alternative for perinatal age estimation, effectively addressing these limitations. This finding aligns with previous studies on small samples of human teeth, which also reported a strong correlation between histologically derived and documented chronological ages (Antoine et al., 2009; Ramirez Rozzi et al., 2025).

4.1 Daily secretion rates

The mean enamel DSR across all tooth types in our study was 3.38 $\mu\text{m/day}$ (ranging from 1.58 to 6.68 $\mu\text{m/day}$). Our mean and range of values are consistent with previous research (eg., Birch & Dean, 2014;

Dean et al., 2020; Mahoney, 2012, 2015; Mahoney et al., 2016; McFarlane et al., 2021; Nava et al., 2017b, 2017a; Peripoli et al., 2023). For dentin, the mean DSR across all tooth types was 3.24 $\mu\text{m}/\text{day}$ (ranging from 1.28 to 5.65 $\mu\text{m}/\text{day}$), which also falls within the ranges reported in the literature (eg., Mahoney, 2019; Schour & Poncher, 1937). Indirect dentin DSRs yield similar values with a mean of 3.27 $\mu\text{m}/\text{day}$ (ranging from 2.34 to 4.69 $\mu\text{m}/\text{day}$; see Table S1).

Our dataset revealed substantial variation between tooth types and crown regions (eg., Beynon et al., 1991). Overall, comparison of mean enamel DSR values and standard deviations showed that molars consistently displayed lower secretion rates than incisors (Table 2). In addition, post-hoc comparisons showed that upper deciduous central incisors had higher DSR values than lower incisors across crown regions (Fig.3). Previous studies have reported inter- and intra-tooth as well as inter-population differences in secretion rates (e.g., Mahoney, 2015; Nava et al., 2017a; Peripoli et al., 2023), and our results further demonstrate the variation in the rate at which ameloblast cells deposit enamel during the secretory stage of formation. These differences likely reflect the influence of multiple biological and developmental factors, as odontogenesis and enamel mineralization in utero can be interfered by genetic variation, intrauterine improper nutrition, maternal physiological and psychological stress, preterm birth, or delivery complications (Collignon et al., 2022).

In line with earlier studies showing variation by tooth type and crown region (e.g., Aris et al., 2020; Birch & Dean, 2009; Mahoney, 2012), our results confirm that DSR are influenced by sex and individual growth patterns. Therefore, standard average values, whether derived from the literature or from different teeth within the same dentition, are not appropriate for developmental reconstructions. Instead, DSR should be calculated directly from the specific tooth and crown region under study to ensure a robust and biologically meaningful reconstruction of enamel formation, which in this case is age at death.

4.2 Prenatal formation time

The NNL was observed in all the samples from individuals who survived birth (Fig. 5). Three samples (G-393, G-399 and G-414) had been previously analyzed using Synchrotron X-ray Fluorescence, confirming birth through the presence of a Zn peak coinciding with the histological NNL that is associated with the high Zn content of colostrum and liver release at birth (Martirosyan et al., 2024, 2025). The only individual without the presence of the NNL is G-371, who did not survive birth (Fig. 5).

The pCFT for deciduous central incisors ranged from 129 to 185 days, and for deciduous lateral incisors from 130 to 154 days. These values overlap with published pCFT for deciduous incisor teeth, which span 78 to 124 days in modern human samples and fall within the broader ranges of 100 to 179 days documented for a Roman archaeological population (Mahoney, 2012; Nava et al., 2017a). For the deciduous upper molars, the pCFT ranged from 119 to 135 days for the first molar and 72 to 78 days for the second molar. These values are consistent with reported prenatal molar enamel formation ranges of 87–167 days for first molar and 37–135 days for second molar in modern populations (Mahoney et al., 2025). The wide variability observed in deciduous central incisors may reflect individual differences in the timing of the onset of mineralization (Dean et al., 2020), a pattern that cannot be evaluated in deciduous lateral incisors and molars due to the limited sample size of these groups.

The individuals included in this study are considered to have been full-term at birth, based on their recorded causes of death, which do not indicate prematurity (Table 1). This interpretation is further supported by the fact that all pCFTs coincide with the established range for a full-term birth (Birch & Dean, 2014; Mahoney, 2012, 2015), suggesting that this calculation may help in validating the presence of the NNL and differentiating it from other prenatal or postnatal ALs.

4.3 Age-at-death

Across all samples, the average range (95% CI) spanned 8 days for postnatal CFT estimates and 6 days for RFT estimates. In individuals with still-forming enamel (G-346, G-363, G-393, G-512, G-225, and G-309), the calculated chronological age was underestimated by up to two days (Fig. 5). This discrepancy may be related to the time required for NNL formation, as noted in previous studies, although our results suggest a shorter duration (Nava et al., 2017a; Witzel, 2014).

In individuals with completed crowns, chronological age reconstructed using both postnatal CFT and RFT consistently matched the known ages at death (Fig. 5). Among these samples (G-189, G-215, G-362, G-380, G-399, G-405, and G-414), postnatal CFTs varied considerably. Deciduous central incisors ranged from 23 to 90 days, with a mean of 41 days, whereas lateral incisors ranged from 82 to 90 days, approaching the 107–119 days reported by Birch and Dean (2014).

Our results indicate that age estimates derived from deciduous enamel and dentine are accurate to within approximately a week of the documented chronological ages. Earlier work on permanent teeth reported substantially larger discrepancies, with reconstructed ages falling short of the known values by at least two weeks and often more (Antoine et al., 2009). Despite these potential deviations, tooth histology appears to be a reliable method for age determination, as conventional anthropological methods primarily estimate developmental age at death but are limited in their ability to address birth-related factors such as preterm birth or stillbirth (e.g., Smith, 2006; Smith et al., 2010).

The use of dental histology in age estimation has applications in both bioarchaeology and forensic anthropology (Aris, 2024; Martirosyan et al., 2024). The identification of the NNL and the calculation of pCFT are essential for distinguishing between individuals born at term, perinatal deaths, and prenatal losses (Martirosyan et al., 2024; Nava et al., 2017b; Peripoli et al., 2023). It can also provide insights into survival at birth, stillbirths, and miscarriages, especially in cases where remains are fragmented, in an advanced decomposition, deteriorated, or skeletonized, since teeth are better preserved (Martirosyan et al., 2025; Peripoli et al., 2023; Witzel, 2014).

In bioarchaeological contexts, histology allows the reconstruction of infant mortality patterns in past populations using age categories comparable to those in demographic studies, providing insights into living conditions and demography (Martirosyan et al., 2024). In forensic cases, it has proven valuable in distinguishing live births from stillbirths, particularly in investigations of neonaticide, where determining whether an infant survived postnatally is critical (Janardhanan et al., 2011). Moreover, NNL width has been shown to be inversely proportional to labor duration, suggesting that prolonged labor may influence amelogenesis and alter the formation of this structure (Canturk et al., 2014; Hurnanen et al., 2017). Other studies have highlighted the interpretive potential of ALs, which have been associated with stress episodes

in ancient populations (Dalen et al., 2025; Fitzgerald et al., 2006). More recently, histological studies of Phoenician and Neanderthal deciduous teeth have reinforced the importance of maternal health and nutrition in shaping infant survival and development, while also revealing that Neanderthals followed growth and weaning trajectories broadly comparable to those of modern humans (Mahoney et al., 2021; Nava et al., 2020; Peripoli et al., 2023).

4. Conclusion

This study demonstrates the utility of deciduous enamel and root histology for estimating age-at-death in perinatal individuals. The consistent presence of the NNL in individuals who survived birth, and its absence in the stillbirth case, confirms its value as a clear marker distinguishing prenatal from postnatal life. Similarly, the obtained prenatal CFT values fall within the established ranges for full-term births, validating the effectiveness of this method in differentiating full-term births from prematurity or prenatal losses. The close correspondence between histologically derived and documented chronological ages, typically within a few days, validates the precision of this approach, confirming that dental histology remains the most precise method for age determination in perinatal individuals, surpassing the limitations of conventional anthropological techniques based on biological age estimation.

By integrating quantitative histological analysis with documented modern reference data, this study reinforces the potential of dental tissues as a biological archive of developmental timing. These findings have broad implications for both bioarchaeology and forensic anthropology, improving reconstructions of perinatal mortality patterns and enabling more accurate differentiation between stillbirth and short-term postnatal survival in past and modern populations.

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Author Contributions

Ani Martirosyan: Conceptualization (lead); methodology (lead); data curation (lead); investigation (lead); formal analysis (lead); visualization (lead); writing original draft (lead); writing review and editing (equal). **Javier Irurita:** Resources (equal); writing review and editing (equal). **Assumpció Malgosa:** Supervision (equal); funding acquisition (lead); project administration (lead); resources (equal); writing review and editing (equal). **Judit Molera:** Supervision (equal); resources (equal); writing review and editing (equal). **Patrick Mahoney:** Conceptualization (equal); validation (equal); supervision (equal); writing review and editing (equal). **Xavier Jordana:** Conceptualization (equal); validation (equal); supervision (equal); writing review and editing (equal).

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article and its Supporting Information.

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