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Staircase-pattern neonatal line in human deciduous teeth is associated with tooth type

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Abbreviations¹

¹ Abbreviations:

NNL: the neonatal line

Sc: staircase

FDT: Finnish Deciduous Tooth collection

cs: cross-striation

dm1: first deciduous molar (third deciduous premolar respectively)

dm2: second deciduous molar (fourth deciduous premolar respectively)

CFT: total crown formation time

ml: mesio-lingual

mb: mesio-buccal

EDJ: enamel–dentin junction

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Abstract

Objective: The staircase (Sc) pattern enamel microstructure is an expression of an impaired ameloblast function. It has been reported to appear in the neonatal line (NNL), the accentuated stria evincing live birth in deciduous tooth enamel. Our objective was to investigate the prevalence of Sc NNL in deciduous tooth types and its possible association with perinatal circumstances.

Design: Sc in the NNL of 88 teeth, a collection derived from a long-term, prospectively followed population cohort, was recorded with linear polarised transmitted light and analysed for tooth type, duration and mode of delivery, and pain medication used during labour.

Results: Sc prevalence in the NNL differed highly significantly between tooth types ($p < 0.0001$). An increase in Sc prevalence was significantly associated with an increased ratio of prenatal to total crown enamel ($p < 0.001$), when buccal and lingual crown walls were analysed separately. No significant association was found between Sc prevalence and duration or mode of delivery or pain-relieving medication ($p = 0.57$, $p = 0.65$, $p = 0.58$, respectively).

Conclusion: This research indicates that the NNL location within tooth crown enamel has a strong impact on microstructural changes along the NNL. Considering our results of Sc prevalence, deciduous canines, having the least Sc appearance, could be used in studies that aim to investigate factors associated with NNL width. In addition, Sc prevalence variation in first deciduous molars might enable to investigation of physiological stressors strong enough to cause ameloblast impairment, such as Sc.

Keywords: enamel structure; birth stress; impaired ameloblast function; neonatal line; population study; prevalence of staircase pattern

1. Introduction

A detectable neonatal line (NNL) in the enamel of sectioned deciduous teeth is the representation of live birth, and ongoing enamel formation thereafter (Rushton, 1933; Schour, 1936). This incremental marking in axial sections intersects with the enamel-dentine junction (EDJ) at about the position expected at term birth, outlining the tooth germ in its height. It is shifted more cusally in individuals born preterm and more cervically in individuals born post-term (Norén, 1983; Skinner & Dupras, 1993). The results are controversial, however, regarding how the occurrence of peri-natal circumstances relates with NNL width or the degree of microstructural aberrations (Eli, Sarnat, & Talmi, 1989; Zanolli, Bondioli, Manni, Rossi, & Macchiarelli, 2011; Canturk, Atsu, Aka, & Dagalp, 2014; Kurek, Źądzińska, Sitek, Borowska-Strugińska, Rosset., & Lorkiewicz, 2015, 2016; Hurnanen, Visnapuu, Sillanpää, Löyttyniemi, & Rautava, 2017; Hassett, Humphrey, & Dean, 2017). Therefore, it is unclear which factors affect amelogenesis, disturb the enamel formation, and result in a microstructurally and individually variable NNL emergence.

Weber and Eisenmann (1971) investigated tooth sections by progressively reducing section thickness. In 150 μm thick sections, the appearance of the NNL was blurred and broad, while in 4–20 μm thick sections, the NNL became lighter and narrower. They further reported a distinctive feature through the course of the NNL, called a “staircase” (Sc) configuration. Using a scanning electron microscope (SEM), Whittaker and Richards (1978) and Sabel et al. (2008) confirmed the existence of Sc as a structural feature of the NNL. Sc can also be observed along the postnatal brown striae of Retzius in permanent

teeth (Risnes, 1990, Wilson & Shroff, 1970). Based on SEM views, Risnes (1990) drew a three-dimensional model illustrating the behaviour and size of the different portions of the Tomes' process, the enamel matrix-secreting organ, and thus the proportion between intra- and interprismatic enamel. The Sc consisted of decreased intraprismatic horizontal steps connected by increased interprisms as vertical steps. The horizontal steps varied from accentuated cross-striations, like crested prisms, to even stronger defects interrupting the prism continuity (Risnes, 1990; Whittaker & Richards, 1978; Weber, Eisenmann, & Glick, 1974; Wilson & Shroff, 1970). The latter microstructure, called the prism cleft, was explained to result from a possible delay or pause in intraprismatic cell function in enamel formation. In general, the occurrence of Sc can be related to a more severe impairment of the secretory function of the ameloblasts, compared to normal or accentuated striae of Retzius not exhibiting Sc.

The Sc structure along the NNL has not been comprehensively reported on thus far. The aims of this study were to document the presence of Sc in the NNL in human deciduous teeth and to investigate its relationship to the known mode and duration of birth and the use of pain-relieving medications as proxies of the level of physiological stress affecting ameloblasts during birth.

2. Materials and methods

2.1 Materials

The Finnish Deciduous Tooth (FDT) collection comprises 129 human deciduous teeth from Finnish children born in 1986–87 (Hurnanen et al., 2017). The teeth were collected by one author (M-LM) during 1995–96, as part of a representative, prospectively followed longitudinal population study (Finnish Family Competence study; Rautava & Sillanpää,

1989). Altogether, 1443 young families expecting their first child were recruited with a stratified, randomised cluster sampling from the source population of southwestern Finland. There were no significant differences in sociodemographic or socioeconomic parameters in the dropout analysis between participants in the FDT collection and the original population cohort (Hurnanen et al. 2017). The Finnish Family Competence study design was approved by the Ethical Committee, the Institutional Review Board of the University of Turku and the Turku University Hospital (DNO 540/582/85), covering the present study.

The FDT collection consists of deciduous teeth of all types (n=129: 6 deciduous incisors, 66 deciduous canines, 30 deciduous first molars, and 23 deciduous second molars). Crown walls were poorly preserved in 41 (32%) of the teeth, which had to be excluded from the study. Therefore, we investigated 88 teeth of the FDT collection: 3 incisors (2 maxillary, 1 mandibular), 58 canines (10 maxillary, 48 mandibular), 22 first deciduous molars (15 maxillary, 7 mandibular) and 5 second deciduous molars (1 maxillary, 4 mandibular). Comprehensive medical data starting from the 10th week of pregnancy until the child was 15 years old was available for each of the 88 investigated cases.

2.2 Methods

Tooth specimens were processed as described previously in Hurnanen et al. (2017). In brief, they were run through a rising alcohol series and embedded in polyester resin (Technovit®, Heraeus Kulzer, Hanau, Germany). Teeth were sectioned axiobuccolingually through the mesial lobes, mounted on Plexiglas slides, and ground sections of 20 µm were processed.

The 20 µm ground sections were investigated with linear polarised transmitted light using a Leica DM6000B microscope (Leica, Solms, Germany) with 10x, 20x and 40x objective

lenses and digital micrographs were obtained with a Leica DMC2900 camera (2048x1536 pixels). The NNL in buccal/labial and lingual/palatal walls was divided into two parts: lower and upper (Fig 1) We recorded both parts separately to observe disparities along the NNL and because in samples with heavy wear, the NNL was only partially applicable for investigation. Sc absence (Sc0) and presence (Sc1) (Fig 2 and 3) were recorded from buccal/labial and lingual/palatal crown walls. The character was qualified only when both horizontal and vertical steps were visibly clear enough to create a Sc configuration. In addition, strong/crested cross-striations (cs) were recorded, where Sc-related vertical steps were missing (Fig. 4). For recording, Sc was required to be dominant in each half, in places where prisms were running parallel with the plane of the section, equalling the parazonal zones of the Hunter-Schreger pattern (Lynch, O'Sullivan, Dockery, McGillicuddy, Sloan, 2010). In places, where prism direction was not parallel with the plane of section and the prisms were cross-sectioned, Sc was not always visibly clear enough to be scored.

One observer (JH) analysed all samples with x10 and x20 objective lenses the first time. For validation of the Sc configuration, 11 randomly selected samples were evaluated by another observer (CW). For validation of the intra-observer variation, one observer (JH) analysed the samples a second time with x10, x20 and x40 objective lenses with an interval of one month or more. Observer variation was evaluated with Simple Kappa Coefficient: 0.73 for intra-observer 0.73, 0.84 for inter-observer. All samples (n=88) were included with the data evaluated in the second analysis.

2.3 Statistical analysis

Sc absence (Sc0) or presence (Sc1) was tabulated in relation to deciduous tooth type (incisor, canine, 1. molar and 2. molar) and perinatal medical data: mode of delivery (spontaneous vaginal, assisted vaginal or caesarean section), duration of delivery and use

of pain-relieving medication (nitrous oxidative, epidural anaesthesia, paracervical injection). The prevalence of Sc NNL was compared between the tooth types with Fisher's exact test and continued with chi-square tests for pairwise comparisons. Furthermore, the Cochran–Armitage trend test was used to analyse if Sc presence increases along with the growing proportion of prenatal enamel in relation to total crown enamel. The percentages were obtained by using tooth type–specific mean amounts of daily enamel apposition for prenatal and total crown enamel, as provided by Mahoney (2011, 2012) and Birch and Dean (2014). With Fisher's exact test, the prevalence of Sc with mode of delivery and use of pain medication was examined. For the measurements of the duration of delivery, the Wilcoxon rank sum test was used because the data was not normally distributed. P values less than 0.05 (two-tailed) were considered statistically significant. The analyses were performed using the SAS System, version 9.3 for Windows (SAS Institute Inc., Cary, NC, USA) and with JMP® Pro, Version 13.1.0. (SAS Institute Inc., Cary, NC).

3. Results

In 19 canines (22% of 88 samples) and 1 buccal crown wall of a first molar, tooth wear had destroyed a part of the NNL, and the appearance was able to be investigated only partially (Table 1). The explorable lower parts in all 19 canines showed Sc0, in both crown walls, and in the 1 first deciduous molar the score was Sc1 elsewhere in crown walls. Analyses in these worn samples were made assuming that NNL configuration in the upper parts continued as it appeared in the lower part. This assumption was made based on the collected data showing that in only 7 of 176 crown walls the recording was not uniform in the lower and upper portions.

3.1 Sc NNL and tooth type

The Sc feature was Sc0 in 68 (77%) and Sc1 in 20 (23%) in the 88 teeth investigated. Prevalence of Sc scored along the>NNL in at least one crown wall, differed between tooth types, $p < 0.0001$ (incisors, canines, 1. molars and 2. molars). In pairwise comparisons, incisors and canines ($p = 0.0027$), canines and 1. molars (< 0.0001) and 1. molars and 2. molars ($p = 0.0005$) were highly statistically significantly different. Between incisors and 1. molars ($p = 0.065$), incisors and 2. molars ($p = 0.17$) and canines and 2. molars ($p = 0.77$), statistically significant differences were not found. In more detailed analysis, crown walls were set in a sequence with an increasing proportion of prenatal enamel in relation to total crown enamel (Table 2). The proportions were obtained from previous studies (Mahoney, 2011, 2012; Birch & Dean, 2014). Only one canine showed the Sc feature, found on the buccal wall. Sc1 in first deciduous molars was 50% (11/22) buccally/labially and 55% (12/22) lingually/palatally. In second molars ($n = 5$), Sc was not present (Table 2). The Cochran–Armitage trend test showed that when crown walls in canines, 1. molars and 2. molars were studied separately, it was more likely that Sc was present as the amount of prenatal enamel increased ($p < 0.0001$). Additionally, strong cs were scored in canines ($n = 1$), 1. molars ($n = 8$) and 2. molars ($n = 1$) (Table 1). The addition of strong cs to recordings of Sc presence (strong cs + Sc1) emphasised that Sc1 prevalence was rising along with the increasing amount of prenatal enamel formed (Table 2.).

3.2 Sc>NNL and perinatal events

The duration of delivery was recorded in 67 (76%) of 88 mothers and varied from 2h 50min to 28h 8min (median 9h). Data on delivery duration were missing for caesarean sections ($n = 17$, 19%) as well as 4 cases of spontaneous vaginal delivery. The duration of vaginal deliveries (spontaneous $n = 47$ and assisted $n = 20$) was tested with the prevalence of Sc. Sc1 showed no effect when analysed with respect to the duration of the deliveries ($p = 0.57$).

The delivery modes were spontaneous vaginal 58% (51/88), assisted vaginal 23% (20/88) and caesarean sections 19% (17/88) (Table 3). The mode of delivery was not associated with an Sc feature of the NNL ($p=0.65$). Background data revealed the use of one or several pain-relieving medications in 72% (63/88) of the deliveries. The pain-relieving medication did not show statistical effect on the Sc feature emergence along the NNL ($p=0.58$) (Table 3.).

4. Discussion

Our results evinced significant association between Sc NNL prevalence and specific deciduous tooth types. This supports FitzGerald and Saunders's (2005) suggestion that the extent of prismatic disruption may be equally regulated according to the tooth type as it may be influenced by the degree of physiological perturbation arising from a stressor. By analysing accentuated striae of Retzius, referring to them as Wilson bands, FitzGerald and Saunders (2005) speculated that there may be a tooth type–related susceptibility to the presence of accentuated striae of the enamel. Systemic physiological stressors were related to disturbances in ameloblast activity and thereby to the formation of accentuated striae (Goodman & Rose, 1990). The NNL is viewed as such a stria, caused by birth-related stress. With the documented microstructural differentiation according to tooth type, our results support FitzGerald and Saunders's (2005) speculation of tooth type–related susceptibility. In addition, in the present study, the absence of a significant relationship between the Sc NNL and perinatal circumstances exemplifies that the severity of the perinatal physiological stressors cannot be interpreted in a straightforward manner from the NNL configuration (FitzGerald & Saunders, 2005).

In our cohort, Sc was absent in almost all canines and 2. molars (dm2). In contrast, the Sc character was repeatedly found in first deciduous molars (dm1). This could be explained by the variation in the ameloblast response being dependent on the location of the NNL within the tooth crown. The location of the NNL has two components: 1) NNL distance from the EDJ (representing the time or lifespan that ameloblasts already spent secreting enamel matrix) and 2) the location where the NNL intersects with the EDJ in relation to tooth crown height (representing the number of previous cell divisions of cells in the inner enamel epithelium prior to terminally differentiating into ameloblasts).

At birth, the size and morphology of deciduous tooth germs exhibit tooth type-specific variation (AlQahtani, Hector, & Liversidge, 2010; Deutsch, Tam, & Stack, 1985). The prenatal enamel is demarcated by the NNL, and its position depends on the initiation time of enamel mineralisation (Mahoney, 2011, 2012; Birch & Dean, 2014; Sunderland, Smith, & Sunderland, 1987). Factors causing variation in the positioning are biological variance in initiation times and being born pre- or post-term (Norén, 1983; Skinner & Dupras, 1993). Based on the accounts of daily enamel formation of prenatal and total crown enamel provided by Mahoney (2011, 2012) and Birch and Dean (2014), a proportion of the prenatal to total crown formation time (CFT) can be created (dm2 mesio-lingual (ml) 15% - > dm2 mesio-buccal (mb) 19–23% -> canine 20–30% -> dm1 ml 29–43% -> dm1 mb 29–43%). We found the highest prevalence of Sc NNL in dm1s, which are the most advanced in mineralisation at birth (AlQahtani et al, 2010; Mahoney, 2011,2012; Birch & Dean, 2014). This demonstrates a higher susceptibility to stress of the older (i.e., with a later terminal differentiation) ameloblasts, which is exhibited by more pronounced microstructural aberrations (Witzel, Kierdorf, Schultz, & Kierdorf, 2008).

Another factor influencing the trajectory of enamel apposition is whether the enamel-secreting front is dome-shaped and continuous above the dentin horn (appositional or

cuspal enamel) or crop out on the enamel surface (imbricational or lateral enamel). These modes can be expressed by the proportion of prenatal enamel to cuspal enamel formation (dm2 ml 39% -> dm1 ml 55% -> dm2 mb 76% -> dm1 mb 100% and canine 100%) (Mahoney, 2011, 2012). It indicates the relative position at which the NNL intersects the EDJ, either towards the dentine horn cuspally, or cervically, towards the cervix of the crown. In the first three cusps of the sequence, the enamel-secreting front is continuous above the dentin horn and the NNL is located inside the cuspal enamel. In contrast, the cuspal enamel formation at birth in deciduous canines and dm1 mb-cusps is about 100% and the NNL is situated either close to the final enamel surface or pushed to end in lateral enamel. There was no Sc NNL in deciduous canines, whereas the configuration was mostly present in dm1 mb-cusp. This could be explained by deciduous tooth morphology, which adjusts the vertical location of the NLL inside the tooth crown. Liversidge et al. (2018) measured maxillary and mandibular full crown heights and in canines, being over 1 mm higher, the NNL, even when demarcating the whole cuspal volume, stays more in the incisal third. In dm1, tooth morphology pushes the NNL to end laterally on the buccal wall and to locate more in the middle third of the crown. In addition to Sc NNL findings in dm1, most of the strong or crested cross-striations were recorded, in the present study, in dm1s. FitzGerald et al. (2006, p. 183) reported the highest frequencies of Wilson bands in the middle crown area, proposing the explanation that "sensitivity to stress events varies in some way through the developmental cycle of the tooth, being lowest early in cuspal development, as well as later towards the cervix, and highest in the middle period of tooth development".

It thus can be hypothesised that ameloblast age has two components: 1) the time period of secreting enamel matrix by a secretory ameloblast (life span, the distance from the EDJ) and 2) the location along the forming EDJ where cells differentiate into secretory

ameloblasts (the differentiation age, the location in relation to crown height). Both processes might lower the capability of ameloblasts to cope with physiological stress. In our sample, occurrence of the Sc pattern appeared to be independent of ameloblast secretory lifespan. In this respect, the NNL seems to represent a special case compared to striae of Retzius, where Sc is more frequently observed in subsurface enamel (Risnes 1990, Witzel et al. 2008). Thus, according to our results, the relationship to total crown height correlates more strongly with Sc NNL than NNL distance from the EDJ.

NNL location within tooth crown enamel obviously has a strong impact on ameloblast reaction under stressful physiological events, as also reported by Schour and Kronfeld (1938). Their published images showed a lower canine (NNL in incisal third) and a lower dm2 (short life span with the least mineralised enamel, NNL locating in incisal third) to be the only tooth types to continue enamel formation after a birth event with a brain injury to infant. In their research, Schour and Kronfeld reported a “pathologically accentuated” NNL, and in other deciduous tooth types, ameloblast arrest was permanent, leaving the NNL exposed, as the surface of enamel, causing a hypoplastic tooth crown. Their study showed that in the canine and dm2 the ameloblasts in the enamel-forming front at birth resisted even such a strong physiological stress as a brain injury. We tested different modes of delivery and pain medications to address a strong enough stressor to cause impairment in ameloblasts, but no correlation was found between Sc prevalence and the tested variables. The lack of Sc presence in our deciduous canine samples supports a strong tooth-related Sc presence. However, since dm1 in the present study showed the highest variation in Sc prevalence, in a larger sample, the effects of strong enough perinatal circumstances might correlate with such variation.

The through-going cleft in Sc configuration denotes an interruption in prism path and has been speculated as a pause in ameloblast function in matrix secretion (Risnes, 1990;

Weber & Eisenmann, 1971; Schour & Massler, 1937; Kronfeld & Schour, 1939). Such a transient cessation expressed as a cleft in the mature enamel microstructure was also suggested by Witzel et al. (2008) as denoting a stronger response to external stress. Thus, Sc pattern could affect NNL width. We have previously shown a correlation between the duration of delivery and the width of the NNL, suggesting that a long delivery, as a greater disturbance, causes a pause in enamel secretion, generating a narrower line (Hurnanen et al., 2017). Hasset et al. (2017) were unable to reproduce these results, nor did the present study support the suggestion of a possible ameloblast pause in long deliveries, in the sense that Sc configuration did not occur more frequently in specimens that had prolonged durations of delivery. Our results show the least Sc NNL in deciduous canines. Thus, it is reasonable to recommend using only canines when varying NNL width is compared with diverse pre-, peri- or postnatal parameters.

One of the limitations is the absence of incisors in our analyses. Thus, we failed to study the cervical crown portion, the region of NNL location, in lower incisors. Furthermore, our study size in dm1s was relatively small to enable further analyses between Sc NNL and associated predictors. Finally, even though previously reported data of deciduous molar cusp enamel mineralisation initiation times includes only mandibular molars, maxillary molars are also included in our recordings and analysis. The strengths include population-based sampling, intensive long-term collection of medical and social data and various deciduous tooth types for study.

5. Conclusions

NNL formation is different in various deciduous tooth types, indicating varying ameloblast susceptibility in different parts of deciduous tooth enamel. Our results generate two

suggestions for NNL analyses: 1) to use canines in NNL width studies to exclude possible transient ameloblast cessation expressed in the form of Sc presence and 2) to test the impact of a physiological stressor in the presence of Sc in a large sample of first deciduous molars.

Declarations of interest: none

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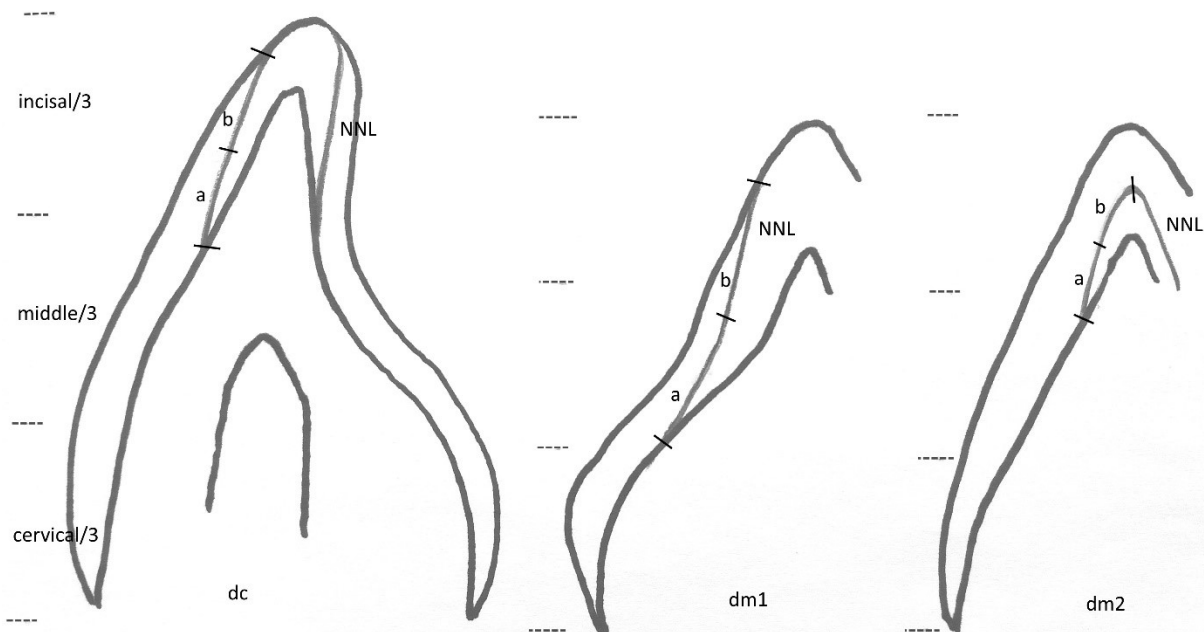


Fig 1. The drawing illustrates different paths of the crown wall neonatal line and the division of the line into lower (a) and upper (b) parts for recording. The dashed lines on the side divide the crown wall into incisal, middle and cervical thirds. The relation in mean crown heights is approximately real.

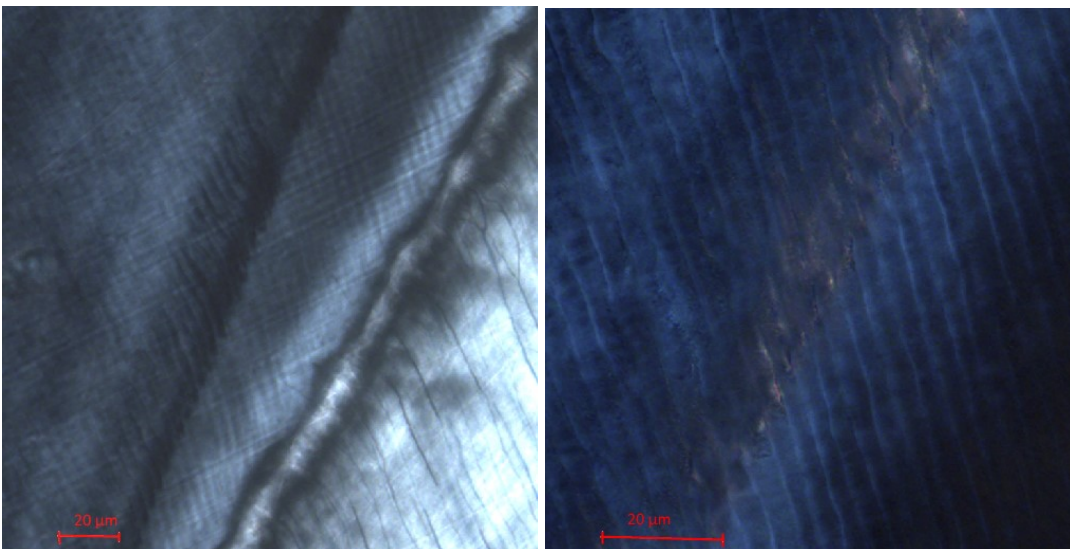


Fig 2. Deciduous canine buccal crown wall neonatal line without staircase-like feature (Sc0) polarised light, viewed with x20 (left) and x40 (right) object lens, scale 20 µm.

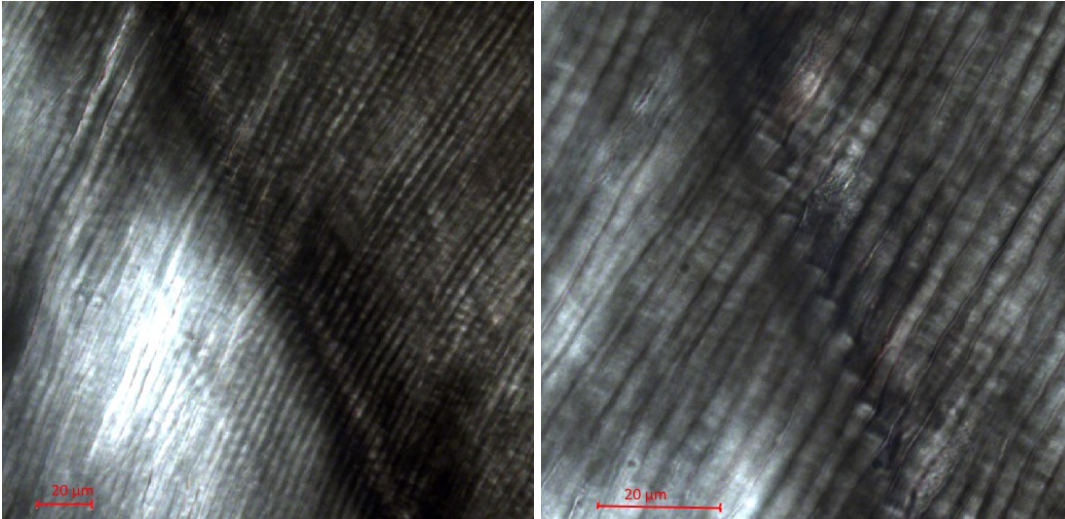


Fig. 3. Staircase-like neonatal line (Sc1) in deciduous 1. molar lingual crown wall, polarised light, viewed with x20 (left) and x40 (right) object lens, scale 20 μm

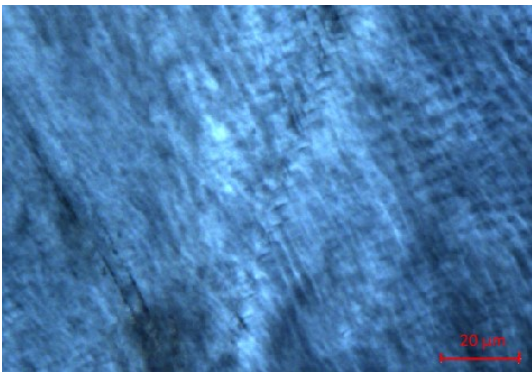


Fig. 4. Strong/crested cross-striations along the neonatal line in 1. deciduous molar buccal wall, polarised light, viewed with x20 object lens, scale 20 μm

| | | buccal lower | buccal upper | lingual lower | lingual upper | BUCCAL | LINGUAL |
|-----------------|-------------|-----------------|-----------------|------------------|------------------|------------|------------|
| Incisor | n=1 | Sc1 | Sc1 | Sc0 | Sc0 | Sc1 | Sc0 |
| | n=2 | Sc0 | Sc0 | Sc0 | Sc0 | Sc0 | Sc0 |
| total | n=3 | | | | | | |
| Canines | n=38 | Sc0 | Sc0 | Sc0 | Sc0 | Sc0 | Sc0 |
| | n=18 | Sc0 | <i>wear</i> | Sc0 | <i>wear</i> | Sc0 | Sc0 |
| | n=1 | Sc1 | Sc1 | Sc0 | Sc0 | Sc1 | Sc0 |
| total | n=58 | n=1 strong cs | <i>wear</i> | Sc0 | <i>wear</i> | Sc0 | Sc0 |
| 1.molars | n=1 | Sc1 | Sc1 | Sc1 | Sc1 | Sc1 | Sc1 |
| | n=1 | Sc1 | <i>wear</i> | Sc1 | Sc1 | Sc1 | Sc1 |
| | n=2 | Sc1 | Sc1 | strong cs | strong cs | Sc1 | Sc0 |
| | n=2 | Sc1 | Sc1 | Sc0 | Sc1 | Sc1 | Sc1 |
| | n=4 | Sc1 | Sc1 | Sc0 | Sc0 | Sc1 | Sc0 |
| | n=2 | strong cs | strong cs | Sc1 | Sc1 | Sc0 | Sc1 |
| | n=1 | strong cs | strong cs | Sc0 | Sc1 | | |
| | n=1 | strong cs | strong cs | strong cs | strong cs | Sc0 | Sc0 |
| | n=1 | strong cs | Sc1 | Sc0 | Sc0 | Sc1 | Sc0 |
| | n=4 | Sc0 | Sc0 | Sc1 | Sc1 | Sc0 | Sc1 |
| | n=1 | Sc0 | strong cs | Sc1 | strong cs | Sc0 | Sc1 |
| | n=2 | Sc0 | Sc0 | Sc0 | Sc0 | Sc0 | Sc0 |
| total | n=22 | | | | | | |
| 2.molar | n=4 | Sc0 | Sc0 | Sc0 | Sc0 | Sc0 | Sc0 |
| | n=1 | Sc0 | strong cs | Sc0 | Sc0 | Sc0 | Sc0 |
| total | n=5 | | | | | | |
| TOTAL | n=88 | | | | | | |

Table 1. The combinations of presence (Sc1) or absence (Sc0) of staircase (Sc) and strong cross striations (cs) in the neonatal line. Within each tooth type the number of each score combination in upper and lower parts of both crown walls is shown. The final two columns on the right side show the recordings used in the analyses.

| | 2.molar ML | 2.molar MB | Caninus lingual | Caninus buccal | 1.molar ML | 1.molar MB | <i>Cochran-Armitage trend test</i> |
|---|---------------|---------------|--------------------|-------------------|---------------|---------------|--|
| Prenatal enamel/ total crown enamel* | 15% | 19% | 20% | 20% | 29% | 29% | |
| Absent (Sc0) | 5 | 5 | 58 | 57 | 10 | 11 | |
| Present (Sc1) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (2%) | 12 (55%) | 11 (50%) | <i>p < 0.0001</i> |
| Absent (Sc0) | 5 | 4 | 58 | 56 | 7 | 4 | |
| Present (Sc1+strong cs) | 0 (0%) | 1 (20%) | 0 (0%) | 2 (3%) | 15 (68%) | 18 (81%) | <i>p < 0.0001</i> |

* percentages formed by amounts of daily enamel apposition provided by Mahoney 2011, 2012
ml: mesio-lingual, mb:mesio-buccal

Table 2. Absence (Sc 0)/presence (Sc 1) of staircase (Sc) in the neonatal line in different crown walls of 85/88 deciduous teeth. Crown wall sequence is according to growing percentage of prenatal enamel in relation to total crown enamel. Upper sequences show staircase absence or presence. In lower sequences, staircase present recordings are supplemented with strong cross-striations (cs).

| Perinatal Circumstances | Sc0 n | Sc1 n | Total n | <i>Fisher's exact</i> |
|-------------------------|-------|-------|---------|-----------------------|
| Mode of delivery | | | | |
| Spontaneous vaginal | 41 | 10 | 51 | |
| Assisted vaginal | 15 | 5 | 20 | <i>p = 0.65</i> |
| Caesarean | 12 | 5 | 17 | |
| Medication | | | | |
| Nitrous oxide | 27 | 8 | 35 | |
| Epidural | 34 | 12 | 46 | <i>p = 0.58</i> |
| Paracervical | 5 | 3 | 8 | |

Table 3. Staircase absence (Sc 0)/presence (Sc 1) in the neonatal line in all tooth types in relation to perinatal circumstances.