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Assessment of physiological disturbances during pre- and early postnatal development based on microscopic analysis of human deciduous teeth from the Late Epipaleolithic site of Shubayqa 1 (Jordan)

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ABSTRACT:

Objectives: To study pre- and early postnatal tooth formation and to analyze the effects of physiological disturbances (stress episodes) on enamel and dentin formation in deciduous teeth of infants from the Late Epipaleolithic (Natufian, ~14,400 – 11,400 cal BP) site Shubayqa 1.

Materials and methods: Ten deciduous teeth from six infants (ages at death between 21 and 239 days) were analyzed by light and scanning electron microscopy.

Results: Marked prism cross-striations and an abnormal wavy course of the prisms were recorded in pre- and postnatal enamel of all analyzed teeth. Single or multiple accentuated incremental lines were observed in prenatal enamel of nine teeth and in postnatal enamel of eight teeth. Accentuated Andresen lines and broader zones exhibiting an enhanced calcospheritic pattern were recorded in the pre- and postnatally formed dentin of nine teeth.

Discussion: The structural abnormalities in the pre- and postnatally formed enamel of the Shubayqa infants are considered indicative of chronic stress that negatively affected secretory ameloblast activity. Abnormalities in pre- and postnatal dentin denote that odontoblasts were also affected by this stress. The presence of single or multiple accentuated incremental lines in pre- and postnatal enamel is interpreted as reflecting (short-term) impacts of higher intensity superimposed on the chronic stress. Our findings suggest compromised maternal health affecting the late fetus and compromised health in newborns. Although limited by the small number of analyzed individuals, the present study contributes to the knowledge of maternal and early infant health conditions in Late Epipaleolithic populations.

Keywords: Accentuated incremental lines, dental development, infant skeletons, maternal stress, Natufian, neonatal line

1 **Introduction**

Due to their preferential preservation and lack of remodeling, teeth are important sources of information on normal and impaired development in bioarchaeological studies addressing the living conditions of past human populations. Microscopic analysis of the incrementally formed dental hard tissues allows the retrospective analysis of periods of physiological disturbances (stress episodes) by recording deviations from a normal microstructure (Hillson, 2014).

The Late Epipaleolithic (Natufian) period in the Levant was characterized by significant changes in subsistence strategies, settlement patterns and social life that occurred during the transition from hunter-gatherer to agricultural societies (Bar-Yosef, 1998; Munro & Grosman, 2018). A decrease in health status has been linked to the transition to food-producing societies, but little is known about the impact of these changes on human growth and development (Larsen, 2006). We currently lack adequate detail: current datasets are solely based on the macroscopic identification of the presence or absence of stress episodes, as marked by enamel hypoplastic defects in permanent teeth (Ash et al., 2016). Here, we assess the health status of the young infants from the Natufian site of Shubayqa 1 (Jordan) through an assessment of enamel and dentin microstructures in deciduous teeth.

Dental enamel forms the outer part of the tooth crown and is the most highly mineralized and hardest substance of the vertebrate body (Boyde, 1989; Nanci, 2018). Enamel is formed by specialized cells, the ameloblasts, and amelogenesis can be subdivided into two main stages (Boyde, 1989; Nanci, 2018). During the secretory stage, a proteinaceous matrix is secreted and initially mineralized. In the subsequent maturation stage, the enamel matrix is enzymatically degraded and the breakdown products and water are resorbed. Concomitant crystallite growth leads to the formation of the highly mineralized (~ 96% mineral by weight) mature enamel (Smith, 1998).

In mammals, fully active secretory ameloblasts possess a cellular extension (Tomes' process) at their distal pole with two sites of matrix production (Boyde, 1989, 1997; Nanci, 2018; Warshawsky, 1988). Along a rim located around the proximal portion of the Tomes' process, the matrix of the interprismatic (interrod) enamel is secreted. The distal portion of the Tomes' process protrudes into a pit whose walls consist of interprismatic enamel. Mineralization of the matrix secreted at the secretory pole of the distal portion of the Tomes' process leads to the formation of enamel prisms or rods. These are tightly packed bundles of hydroxyapatite crystallites that extend from the enamel-dentin junction (EDJ) to the outer enamel surface (OES). Crystallite orientation in prims differs from that in interprismatic enamel (Boyde, 1989; Nanci, 2018; Warshawsky, 1988).

The activity of secretory ameloblasts shows a circadian oscillation with alternating periods of higher and lower intensity of matrix formation. In primate enamel this rhythmic fluctuation is reflected by the presence of regular daily incremental markings along the prism course. (Antoine, Hillson and Dean, 2009; Boyde, 1989; Dean, 2006; Gustafson & Gustafson, 1967; Hillson, 2014; Smith, 2006). In ground sections viewed in transmitted light, these markings appear as alternating light and dark bands oriented perpendicular to the prism long axis, referred to as prism cross striations (Boyde, 1989; Hillson, 2014). Most authors agree that these cross striations correspond to regular variations in prism width that in the scanning electron microscopy (SEM) can be demonstrated as alternating varicosities and constrictions (Boyde, 1989, 1997; Hillson, 2014). However, a strict correspondence of the dark and light bands seen in the light microscope and the prism constrictions and varicosities seen in the SEM was questioned by Li & Risnes (2004). Based on etching experiments, they assume that the incremental pattern visible in mature enamel reflects periodic changes in crystal concentration, orientation or composition within the prisms. Periodic variation in the mineral composition has been reported to occur already in the initially mineralized enamel (Boyde, 1989; Gustafson & Gustafson, 1967). Clearly more research is needed to characterize the differences in the appearance of the enamel incremental pattern depending on the techniques used for its visualization.

In addition to the daily incremental markings, regular long-period incremental lines (Retzius lines or striae of Retzius) are present in the enamel. In human permanent teeth, these lines show a periodicity of six to twelve days (Reid & Dean, 2006), while for human deciduous teeth a periodicity between five and nine days has been reported (Mahoney, 2011, 2012). The ultimate physiological cause for the formation of striae of Retzius is unknown, but it has been hypothesized that they represent a regularly repeated accentuation of the process causing prism cross striations, and that they mark the nadir of enamel matrix production within a long-period secretory cycle (Boyde, 1989, Risnes 1998; Hillson, 2014). Newman & Poole (1974) hypothesized that the formation of regular striae of Retzius may be triggered by the action of two independent oscillations that overlap at regular time intervals. The long-period markings in dentin that correspond in periodicity with the striae of Retzius are called Andresen lines (Hillson, 2014).

Secretory ameloblasts are susceptible to physiological disruptions (Goodmann & Rose, 1990), and any impairment of the synthetic/secretory activity of the cells that surpasses a certain threshold will cause aberrations from the normal topography of the Tomes' process and a related deviation from normal enamel microstructure (Boyde, 1989; Warshawsky & Vugman, 1977; Hillson, 2014; Witzel, Kierdorf, Dobney, Ervynck, Vanpoucke & Kierdorf, 2006; Witzel; Kierdorf, Schultz & Kierdorf, 2008). As enamel does not repair or remodel, these microstructural changes remain as a permanent record in the tissue. Their location and extension within the enamel layer can be used to assess the onset and duration of the disturbance of amelogenesis (Dirks, Reid, Jolly, Phillips-Conroy & Brett, 2002; Dirks, Humphrey, Dean & Jeffries, 2010; FitzGerald & Saunders, 2005; Hillson, 2014; Schwartz, Reid, Dean & Zihlman, 2006; Skinner & Byra, 2019).

Externally, periods of impaired enamel matrix secretion manifest as enamel hypoplasia, i.e. as a deficiency in the amount of enamel formed, while internally they manifest as accentuated lines (ALs) or zones of prismless (aprismatic) enamel (Dirks et al., 2002, 2010; FitzGerald & Saunders, 2005; FitzGerald, Saunders, Bondioli, & Macchiarelli, 2006; Goodman & Rose, 1990;Gustafson & Gustafson, 1967; Hillson & Bond, 1997; Hillson, 2014; Kierdorf & Kierdorf, 1997; Kierdorf, Kierdorf, Richards & Sedlacek, 2000; Kierdorf, Kierdorf, Richards & Josephsen, 2004; Lorentz et al, 2019; McGrath et al., 2018; Skinner & Byra, 2019; Witzel et al. 2006; 2008). Different types of enamel hypoplasia (plane, furrow, and pit-type) are distinguished that are considered to reflect varying intensity and duration of impairment of the synthetic/secretory activity of the ameloblasts (Hillson & Bond, 1997; Hillson,2014; Witzel et al., 2008). The relationship between ALs and hypoplastic defects is variable. While plane-type defects are typically associated with a prominent AL, furrow-form defects show a more variable association with ALs which may indicate that this defect type reflects a less intense disturbance (Hillson, 2014; Kierdorf; Witzel, Upex, Dobney & Kierdorf, 2012; Witzel et al., 2008). Typically, ALs in enamel are matched by accentuated markings in contemporaneously formed dentin, present as accentuated Andresen lines or bands showing an enhanced calcospheritic pattern (Hillson, 2014; Witzel et al., 2008).

Regarding the prominence of ALs in enamel, it has been argued (FitzGerald & Saunders 2005) that when a stress impact coincides with the nadir of secretory activity during a long period (stria of Retzius) rhythm it will leave a more distinct marking compared to an impact of the same intensity that occurs between two beats of striae of Retzius formation. Witzel et al. (2008) have proposed a model involving three different thresholds of increasingly compromised activity of secretory ameloblasts to explain varying deviations from normal enamel microstructure. The latter are considered to reflect the combined effect of external (intensity and duration of a disturbance) and internal (susceptibility of the affected ameloblasts) factors. In this model, susceptibility of secretory ameloblasts depends on two factors, first, the previous duration of their matrix producing activity, i.e. whether they represent early, mid or late secretory ameloblasts, and second, their position within a long period secretory cycle. The fact that striae of Retzius are typically best visible in superficial enamel and become less marked or not discernible at all in deeper enamel zones (Boyde, 1989; Hillson, 2014) may reflect the fact that late secretory ameloblasts are more susceptible to physiological disturbance than ameloblasts in earlier stages of their secretory lifespan (Witzel et al., 2008). Independent of, or in addition to, changes in enamel microstructure, the amount or composition of the mineral laid down during phases of disturbed secretory ameloblast activity may deviate from the normal situation (Boyde, 1989; Gustafson and Gustafson, 1967; Hillson, 2014; Hassett et al. 2020).

A specific type of AL is the neonatal line (NNL) that is considered a marker of birth-related stress in teeth forming at birth and identifies individuals that survived birth for a certain period (Antoine et al. 2009; Eli et al. 1989; Gustafson and Gustafson, 1967; Schour 1936; Weber and Eisenmann, 1971; Witzel, 2014a,b; Zanolli, et al. 2011). The micromorphology and the width of the NNL in enamel vary widely (Eli et al., 1989; Gustafson and Gustafson, 1967; Hassett et al., 2020; Rushton, 1933; Schour, 1936; Weber and Eisenmann, 1971; Witzel, 2014a; Zanolli et al., 2011). The extent of disruption of enamel microstructure has been considered to reflect the stress level associated with birth (Eli et al., 1989; Witzel, 2014b). There is also evidence suggesting that the prominence of the NNL varies among the teeth of an individual, reflecting differences in ameloblast susceptibility (Hurnanen et al., 2019).

In a recent study using data from a modern UK birth cohort with known maternal and gestational history, Hassett et al. (2020), however, found no association between the width of the NNL in enamel and long or difficult births. Instead, NNL width was related to parameters reflecting the prenatal environment (e.g. maternal metabolic disruptions or the length of gestation). These authors also observed considerable variation in structural aberrations along the course of individual NNLs suggesting variation in ameloblast susceptibility depending on the stage of their secretory activity, a finding that is in line with the model proposed by Witzel et al. (2006, 2008).

The present study reports the results of a microscopic analysis of aberrations from normal enamel and dentin microstructure in deciduous teeth of young infants from Shubayqa 1 to assess the timing and intensity of pre- and early postnatal stress episodes in this hunter-gatherer population. By this, we aim to contribute to the knowledge of health conditions in mothers and infants from a southwest Asian Epipaleolithic population during the transition from hunting and gathering to agriculture (Bar Yosef, 1998; Munro and Gosman, 2018).

2. Materials and Methods

2.1 Study site

Shubayqa 1, a hunter-gatherer site located in the Harra basalt field c. 22 km north of the town of Safawi in northeast Jordan, has yielded archaeological materials from a sequence of deposits spanning the Early to Late/Final Natufian, between ~14,400 − 11,400 cal BP (Richter et al., 2012; Richter, Arranz Otaegui, House, Rafaiah, & Yeomans, 2014; Richter, Arranz-Otaegui, Yeomans, & Boaretto, 2017). The site was identified in 1993 and briefly tested in 1996 (Betts 1993; 1998). More recently, four excavation seasons (2012 to 2015) were conducted at the site by the University of Copenhagen, under the auspices of the Department of Antiquities of Jordan (Richter et al., 2017, 2019).

The settlement is situated atop a low natural mound that rises about 2-3m above the surrounding area. The site has seven stratigraphic phases ranging from the Early Natufian (Phases 7-4), over the Late Natufian (Phases 3-2) to the Final Natufian (Phase 1) (Richter et al., 2017). During the excavation, two superimposed and well-preserved structures were exposed: Structure 1 belongs to the oldest Phase 7, while Structure 2 belongs to Phase 3. Structure 1, a semi-subterranean oval shaped structure with a flagstone pavement made of local basalt stones, was established at the very beginning of the Early Natufian and occupied continuously for 200-300 years (~14,400 – 14,100 cal. BP). After an extended period of abandonment, Structure 2, a similar semi-subterranean stone structure, was superimposed on top of Structure 1 and occupied during the Late Natufian (~13,317 – 13,055 cal. BP).

2.2 Study sample

Articulated skeletons and isolated human bones, amounting to a minimum number of 23 individuals (including 14 young infants up to one year of age), were recovered at the Shubayqa 1 site. The burials fall into two groups: six graves were associated with Phase 2 and 3, while four graves were associated with Phase 4 (Richter et al., 2019). The human remains were mainly interred individually beneath stone pavements, and some graves are associated with red ochre (Richter et al., 2019). Colorants have been found on human remains in several Natufian sites, but to date, only adult individuals show evidence of this type of pigmentation in the Near East (Bocquentin & Garrard, 2016, Webb & Edward, 2013).

Of the available young infants, six with at least one well preserved deciduous tooth were included in the histological study (Table 1). The burials are described in detail elsewhere (Richter et al. 2019). Briefly, all six individuals were found in a flexed position in primary burials, with four individuals (51b, 85, 108, 112) originating from a Late Natufian context (Phase 2) and two individuals (136, 189) from an Early Natufian context (Phase 4). The four Late Natufian individuals were found under the stone pavement of Structure 2. The skeletons of two individuals (51b and 108) were associated with a red colorant. The two Early Natufian individuals (136 and 189) were recovered from underneath an area of paving that re-used some of the partially buried Structure 1.

2.3 Methods

We collected ten deciduous teeth from the six individuals (Table 1). The teeth were embedded, sectioned, ground and polished according to previously described protocols (Kierdorf, Kierdorf, Frölich, & Witzel, 2013; Kierdorf, Breuer, Richards, & Kierdorf, 2014; Witzel et al. 2008). Uncoated surfaces of the sectioned and polished blocks were examined in a scanning electron microscope (SEM, Zeiss Evo 15 MA) operated in the backscattered electron (BSE) mode. In two teeth (Individual 112, dUI1; Individual 108, dLC), the block surfaces were subsequently etched for 5 seconds with 5% phosphoric acid, sputter-coated with gold-palladium and viewed in the secondary electron (SE) mode in the SEM. For light microscopy, thin ground sections (thickness ~ 50µm) were prepared from the unetched blocks. Sections were photographed in plain transmitted light with phase-contrast using either a Zeiss Axioskop 2 Plus or a Zeiss AxioImager microscope. Images were captured using 10×, 20× or 40× objectives, and high-resolution digital photomontages were produced using either ImageJ freeware (NIH) or the ZENpro (Zeiss) stitching tool.

Identification of the NNL was based on its typical location in the respective tooth (Skinner, 1992; Skinner & Dupras, 1993), its structural qualities (Gustafson & Gustafson, 1967; Weber & Eisenmann, 1977; Sabel et al. 2008; Witzel 2014a), its occurrence in both enamel and dentin (Rushton 1933; Schour, 1936), and published data on (macroscopic) crown height at birth (Liversidge, Herdeg & Rösing, 1998). Identification of the NNL was mostly also possible in the BSE-SEM images, based on grey level variation that reflected differences in mineral content (Figure 1). Typically, the NNL exhibited a darker grey level, indicative of a lower mineral content, compared to the adjacent pre- or postnatal enamel portions.

Crown height at birth was measured on scaled micrographs using the intersection of the NNL with the enamel-dentin junction (EDJ) as a baseline (Figure 2). Crown height at death was measured directly with a sliding caliper as well as on the micrographs. Typically, the latter values were slightly lower than those obtained by direct measurement which is attributed to the difficulty of macroscopically measuring the height of the tiny tooth crowns. As most literature data on crown height at birth report direct measurements, we calculated a correction factor (×1.06) to make our microscopic measurements comparable with the literature data. The distinction between individuals born pre- or full-term was based on the reconstructed crown height at birth, using the data by Liversidge et al. (1998) as reference.

Using the NNL as a landmark, age at death was assessed microscopically by counting prism cross-striations in the postnatal enamel, which was still forming at the time of death. Presence of prism fracture planes at the enamel surface was noted in three teeth, indicating taphonomic loss of outermost enamel. In these , the exact age at death could not be determined. Cross-striation counts were also used to estimate the duration of prenatal enamel formation. As it was not possible to make these counts along an individual prism path from either the EDJ to the NNL or from the NNL to the outer enamel surface (OES), we used either ALs as additional natural landmarks or placed auxiliary lines running parallel to the secretory front as artificial landmarks between which cross-striations were counted.

Deviations from the normal enamel microstructure (i.e. abnormal bending of the prism course, formation of aprismatic enamel; marked constrictions of prism diameters, and disruption of prism continuity) were used to identify stress episodes impairing enamel matrix formation (FitzGerald and Saunders 2005, FitzGerald et al., 2006; Schwartz et al. 2006; Skinner & Byra, 2019; Hillson, 2014). Identification of ALs in enamel was based on previously described criteria (Gustafson & Gustafson, 1967; Goodman and Rose, 1990; FitzGerald & Rose, 2000; FitzGerald & Saunders, 2005; Hillson, 2014). Briefly, ALs were identified by an abnormal microstructure traceable over a larger extension of the affected secretory front. ALs were often further characterized by a reduced mineral content. Originally, Goodman & Rose (1990) considered an incremental line as pathologically accentuated (i.e., as a so-called Wilson band) if it was visible for at least 75% of its length from the EDJ to the OES. This definition was adopted in later studies by FitzGerald & Saunders (2005) and FitzGerald et al. (2006). We found that by strictly applying this definition some clearly traceable ALs in our sections would have escaped registration because they could not be followed over more than 75% of the respective secretory front. We therefore distinguished two types of ALs. The first type (1st order ALs) could be followed over more than 75% of the respective secretory front, while the second type (2nd order ALs) was traceable along 50% to 75% of the respective secretory front. The rationale for this classification is that in both cases ameloblasts over a considerable range of their secretory lifespan were negatively affected, denoting a relatively strong physiological disruption causing both types of ALs (Witzel et al., 2008).

Assessment of enamel microstructure and counting of prism cross-striations were performed independently by two observers (CW, HK). Cross-striation counts in individual teeth varied by less than five percent between these observers. For calculation and tabulation, we used the mean values of the two independent cross striation counts. In a few cases where prism cross striation counts could not be performed with the necessary certainty due to poor visibility, a range of days is given (Tables 2, 3).

We also recorded histologically visible alterations in dentin mineralization (accentuated Andresen lines, enhancement of the calcospheritic pattern) to match them with contemporaneously formed enamel aberrations.

3. Results

The NNL was identified in the enamel of all teeth based on the following criteria. It was either the most prominent incremental line or, if multiple lines of similar prominence were present, it was identified as the line matching a likewise prominent line in the dentin. Typically, the enamel prisms exhibited a marked bending, distinct constriction or even a structural discontinuity along the course of the NNL (Figures 3,4). In BSE-SEM images, the NNL typically appeared darker, i.e. hypomineralized, compared to the adjacent enamel (Figure 1). In the dentin of teeth from individuals 85, 51b, 112 and 189 the NNL appeared as a markedly accentuated Andresen line or as a broader band exhibiting an enhancement of the calcospheritic pattern (Figure 5, Table 2). The fact that the NNL was visible in the enamel of all studied individuals indicated that they had survived birth for a certain period. In four of the ten analyzed teeth, the NNL was not the most interiorly located AL, and in one tooth (dLP4 of individual 51b) it was not the most prominent line in the enamel. In these cases, the identification of the NNL relied on the other criteria listed above.

The enamel in all studied teeth of individuals from Shubayqa 1 exhibited a prominent incremental enamel pattern with marked prism cross-striations in prenatal and postnatal enamel. The prisms also exhibited an abnormal wavy course over large stretches of their extension from the EDJ to the OES (Figures 4b, 5). This contrasts with the typical finding in human teeth where the incremental pattern in prenatal enamel is less distinct than that in postnatal enamel and the prisms follow a rather straight course. This normal situation is illustrated in Figure 4a, showing the enamel of a deciduous incisor of an infant from the Iron Age site Tell Halaf in Syria and regarded to reflect an undisturbed prenatal enamel formation (Witzel, 2014a).

An additional finding in the deciduous upper central incisor of individual 112 was the presence of an extended area of postnatally formed aprismatic surface enamel (Figure 3). Formation of this enamel had started in the second week after birth and the disturbance had apparently affected only late secretory ameloblasts.

In addition to the prominence of the cross striation pattern and the abnormal wavy course of the prisms in pre- and postnatal enamel of all ten analyzed teeth, presence of ALs (1st or 2nd order) was recorded in the prenatal enamel of nine and in the postnatal enamel of eight teeth (Table 2, Figure 6). Frequency of ALs per tooth ranged between zero (dLC of individual 136) and four (dUP4 of individual 51b) in prenatal enamel, and between zero (dLI1 and dUP3 of individual 136) and three (dLI1 and dLC of individual 108, dLP4 of individual 189) in postnatal enamel.

In individual 85 from which an upper and a lower deciduous canine were analyzed, we found a good match between the reconstructed timing of ALs in pre- and postnatal enamel. In the other three individuals (108, 112, 136) from which two teeth were available these belonged to different tooth types. Here a match in formation times of ALs between the two teeth was only recorded for postnatal enamel of individual 108. In the prenatal enamel of this individual and that of individuals 112 and 136, the deciduous incisors exhibited more ALs than the second tooth (deciduous canine or deciduous premolar) (Table 2, Figure 6).

Reconstructed tooth heights at birth are given in Table 3. By comparing these values with literature data, three individuals (51b, 112, 136) were classified as full-term births, while the other three (85, 108, 189) were identified as pre-term births. The reconstructed start of crown mineralization is given in Tables 2,3 and Figure 6.

Prism cross-striation counts in postnatal enamel gave ages at death between 21 days (individual 136) and 239 days (individual 189) (Table 2, Figure 6). In three teeth from two individuals (112 and 136), exact determination of the age at death was not possible due to taphonomic loss of the outermost enamel. In these cases, we considered the age at death to be underestimated to a certain extent. The deciduous canine of individual 108 showed post-mortem damage. However, the enamel surface of its deciduous incisor was undamaged, so that the age at death for this individual was based on cross-striation counts of this tooth.

The prenatally formed dentin of five (51b, 85, 108, 112, 189) and the postnatally formed dentin of four individuals (51b, 108, 112, 189) showed accentuated Andresen lines and broader zones exhibiting a pronounced calcospheritic pattern (Table 2, Figure 5a). Due to diagenesis, the dentin of individual 136 could not be assessed.

4. Discussion

The NNL is considered a marker of birth-related stress and/or negative influences prevailing during the gestational period that is present in teeth forming over the birth period (Hassett et al., 2020). It identifies individuals that survived birth for a certain period. The NNL was recorded in the enamel of all teeth available from the six Shubaqya 1 individuals. A problem with NNL identification can occur when birth is survived for only a few days and very little postnatal enamel is formed (Hillson, 2014; Whittaker & Richards, 1978; Witzel, 2014b). In such cases, a distinct NNL may not be discernable and a distinction between stillbirth and early neonatal death not possible (Witzel 2014b). In the youngest individual (136) of our sample, a postnatal survival period of approximately three weeks was, however, sufficient for assessing age at death based on cross striation counts in postnatal enamel.

The structure of prenatal enamel has been described as reflecting the "apparently smooth course of prenatal enamel matrix formation" (Antoine et al., 2009:49), with less prominent incremental markings compared to postnatal enamel (Antoine et al., 2009; Birch, 2012; FitzGerald & Saunders, 2005; Hillson, 2014; Massler, Schour & Poncher, 1941; Weber and Eisenmann, 1971). This situation is illustrated in Figure 4a. However, in the Shubayqa 1 sample, prominent prism cross-striations and an abnormal wavy course of the enamel prisms were observed not only in postnatal but also in prenatal enamel of all teeth. In consequence, we found no differences in microstructure between their pre- and postnatally formed enamel. (Figures 4b, 5). We interpret this finding to indicate a chronic disturbance of slight to moderate intensity that had impaired ameloblast activity during most of the secretory stage of amelogenesis. The presence of ALs in prenatal enamel of six and postnatal enamel of five individuals is regarded to denote short-term impacts of higher intensity on secretory ameloblasts that were superimposed on this chronic disturbance The co-occurrence of structural aberrations in the enamel and mineralization defects in the dentin of the teeth indicates a generalized impairment of development.

The presence of an extended surface zone of aprismatic enamel in the dUI1 of individual 112 is likewise seen as a manifestation of chronic stress that affected late secretory ameloblasts. These are considered to be more susceptible to disturbances than ameloblasts in earlier stages of their secretory lifespan (Witzel et al., 2006, 2008), and this finding is therefore regarded to reflect a minor physiological perturbation that had occurred during the final days of the life of this individual.

Previous studies (e.g. Dirks et al., 2002, 2010; FitzGerald & Saunders, 2005; FitzGerald et al., 2006; Goodman & Rose, 1990; Lorentz et al, 2019; Schwartz et al., 2006; Skinner & Byra, 2019) employing microscopic analysis of microstructural alterations to assess disruptions of amelogenesis exclusively focused on the registration of ALs. In the present study we broadened this perspective and, in addition to ALs, also considered less marked although clearly discernible deviations from a regular enamel microstructure as indications for a perturbation of enamel matrix formation. In our view, this allows for a more graded assessment of deviations from normal enamel microstructure that better reflects the graded response of secretory ameloblasts to stress of varying intensity. Further research on larger sample sizes is needed to evaluate this approach.

FitzGerald & Saunders (2005) argue that atypicality of prism structures is not a valid criterion to distinguish between normal striae of Retzius and ALs (Wilson bands), but that only the length over which these structures are discernible should be used for distinction. However, in our opinion the length over which an incremental line can be traced in a section is a consequence of the degree of aberrations in microstructure and mineralization occurring along a secretory front.

It has recently been questioned that the width of the NNL or other ALs is a valid indicator of the intensity of disruption of ameloblast secretory activity (Hassett et al., 2020). Actually, a complete stop of matrix secretion can result in the formation of a narrower NNL/AL than a slowing down of secretory activity caused by a less severe impact. Previously, a model of a gradually increasing impairment of secretory ameloblast function and related graded changes in enamel microstructure has been proposed (Kierdorf & Kierdorf, 1997; Kierdorf et al., 2000, 2004; Witzel et al., 2006, 2008). The intensity of ameloblast reaction to a given impact also depends on the susceptibility of the cells. There is evidence to suggest that young ameloblasts are less susceptible to stress than ameloblasts in later stages of their secretory lifespan (Witzel et al., 2006, 2008). This reasoning has recently been used to explain the variation in appearance of the NNL along the cuspal to cervical crown axis (Hurnanen et al., 2019; Hassett et al., 2020).

A good match in the reconstructed timing of ALs in the enamel of the upper and lower canine was recorded in individual 85. A corresponding match was found for the postnatal enamel of the two analyzed teeth (dLI1 and dUC) from individual 108. Due to the earlier start of crown formation in the deciduous incisors compared to the deciduous canines, a matching of ALs in the prenatal enamel was not possible in individuals 108 and 112.

Determining whether individuals were born full-term or pre-term, in conjunction with evidence of pre- and postnatal growth impairments, may provide insights into the overall health situation of past populations (Halcrow, Tayles, & Elliott, 2017). Previous research has shown that the location of the NNL is directly related to the duration of pregnancy, with the NNL in case of pre-term births being positioned closer towards the incisal portion of the tooth compared to full term births (Seow, Young, Tsang, & Daley, 2005; Skinner and Dupras. 1993; Szpringer-Nodzak, 1984). Using published values for crown height at birth (Liversidge et al., 1998), the reconstructed crown heights at birth for the teeth of the Shubayqa 1 sample indicate pre-term birth in individuals 85, 108 and 189, and full-term birth in the three other individuals (51b, 112, 136).

The literature data compiled by Birch (2012) for the start of crown mineralization in human deciduous teeth show a huge variation that can, at least partly, be attributed to the different methods used for establishing the onset of crown formation. For a modern dental sample, Birch and Dean (2014) report a narrower range of variation based on the analysis of histological ground sections and regression equations derived from these data. Here, we used the 95% confidence intervals of the means given by these authors for the onset of prenatal crown formation for the different deciduous teeth (152-137 days for the deciduous central incisor, 142-131 days for the deciduous lateral incisor, 135-121 days for the deciduous canine, 146-135 for the deciduous third premolar, and 122-113 days for the deciduous fourth premolar) to distinguish between pre- and full-term birth. Comparing these values with our data for the start of prenatal crown formation based on prism cross-striation counts, revealed one individual (85) as a pre-term birth and four individuals (51b, 112, 136, 189) as full-term births. In individual 108, the data for the dLI1 indicated full-term birth while those for the dLC indicated pre-term birth. Using the threshold of 120 days prenatal crown formation time to distinguish between pre- and full-term birth in deciduous first incisors (Nava et al., 2017a) would not change any of the three classifications based on this tooth in the Shubayqa 1 sample. A recent paper by Dean, Humphrey, Groom & Hassett (2020) reports a wide range (60-150 days) for the duration of prenatal enamel formation in modern human deciduous canines. However, no information on birth timing (pre- vs. full-term) of the individuals is provided.

In four individuals, the diagnosis of pre- or full-term birth is consistent between the two methods, while in one individual (189) they provide conflicting results. In another individual (108), recorded crown height at birth was only slightly less than the value given for newborns by Liversidge et al. (1998). Our cross-striation counts in the dLI1 of this individual indicated full-term birth, while the value for the dLC indicates pre-term birth when compared to the data of Birch and Dean (2014). Regarding the reliability of the two methods applied for establishing birth timing, it must be considered that in direct measurements on ground sections already a slight deviation of the section plane from the mid-line can cause a relatively large change in recorded crown height. Prism cross-striation counts are less affected by deviations of the section plane, and therefore the latter method is regarded the more reliable one. Based on this reasoning, we consider individual 189 a full-term birth.

Thus far, only few cases of ALs in prenatal enamel have been reported (Birch, 2012; Lorentz et al., 2019; Massler et al., 1941; Nava et al., 2017b; Norén, 1983). Their rarity has been regarded as reflecting the buffered condition in the intrauterine environment (Antoine et al., 2009; Hillson, 2014). However, physiological perturbations in pregnant women can be transmitted to the fetus and affect prenatal enamel (Armelagos, Goodman, Harper & Blakey, 2009; Halcrow et al., 2007). It has been inferred that occurrence of ALs in prenatal enamel denotes physiological stress of an intensity that exceeds the capacity of the intrauterine environment to buffer fetal development (Lorentz et al., 2019). It may thus be hypothesized that the formation of ALs of similar microscopic appearance requires a stronger impact in the case of prenatal compared to postnatal amelogenesis.

Disturbances of prenatal enamel formation have been linked to various conditions including maternal dietary deficiencies, maternal diabetes or infections during pregnancy (Birch and Dean, 2014; Lorentz et al., 2019; Nava et al., 2017b; Norén, 1984). In their study, Hassett el al. (2020) recorded an association between the width of the NNL and season of birth, gestational age and maternal metabolic disturbances during pregnancy, but not with duration and method of delivery or early neonatal life conditions.

Impaired secretory ameloblast function during prenatal enamel formation was established in all Shubayqa 1 infants, both pre- and full-term births. These findings are compatible with the assumption that periods of maternal stress negatively affected enamel formation in the fetuses. Thus, no direct relationship between the timing of birth and the deduced maternal stress during pregnancy is evident from our findings. Evidence of one or multiple episodes of more intense disturbance was observed in the postnatal enamel of five individuals. The early death of the sixth individual (136) leaves only a small time window for assessing postnatal enamel formation.

Previous research using larger samples reported different peak frequencies of AL formation in postnatal enamel (FitzGerald et al., 2006; Lorentz et al., 2019; Nava, Frayer, & Bondioli, 2019). This suggests that the underlying causes may be site and time specific rather than reflecting a universal pattern of increased susceptibility at a certain age. Most individuals from Shubayqa 1 have not survived for long enough to compare the number of ALs with published postnatal AL peak frequencies. In a sample from the Iranian urban site of Shahr-i Sokhta, Lorentz et al. (2019) found that individuals displaying signs of prenatal stress died significantly younger than individuals without such signs. This might also have been the case at Shubayqa 1, but our sample is too small for such an analysis.

Age-at-death profiles provide an insight in a population’s adaptive success, as infant deaths are influenced by the ability to provide biocultural means for a child’s survival (Rousham & Humphrey, 2002; Lewis & Gowland, 2007). Distinguishing neonates from post-neonates is crucial here, as these age categories might indicate different causes of death. Identification of the NNL and analysis of incremental markings in dental hard tissues enable an assessment of duration of birth survival (FitzGerald and Saunders, 2005; Witzel 2014b). In historical demographic studies, stillbirths and deaths occurring in the neonatal period (0-27 days) are attributed to endogenous factors such as maternal or genetic conditions (birth trauma, prematurity, low birth weight, congenital anomalies). In contrast, post-neonatal deaths (28 days-1 year) are more likely to be caused by exogenous factors such as infectious diseases, malnutrition, food shortage or other adverse living conditions (Scott & Duncan, 1999). In the Shubayqa 1 sample, only one individual (136) died at an age of less than 27 days while the others survived for longer. Based on the above reasoning this would suggest death from exogenous causes in the majority of the Shubayqa 1 infants.

What had caused the impairment of pre- and postnatal enamel formation in the Shubayqa 1 individuals cannot be inferred from the recorded enamel changes as these represent unspecific reactions of secretory ameloblasts that can be triggered by wide variety of factors (Goodman & Rose, 1990; FitzGerald & Rose, 2000; FitzGerald & Saunders, 2005; Hillson, 2014; Kierdorf & Kierdorf, 1997; Kierdorf et al. 2004; Schwartz et al., 2006; Skinner & Byra, 2019; Witzel et al., 2008).

Archeological studies (Bocquet-Appel 2002, 2011) reported an abrupt increase in the proportion of 5- to 19-year-old juveniles in Late Epipaleolithic/Neolithic burial grounds from Europe and North Africa and related this to the transition from a hunter-gatherer to an agricultural lifestyle. A corresponding situation has also been observed in other areas of the world (Bocquet-Appel & Naji, 2006; Bellwood & Oxenham, 2008; Guerrero, Naji, & Bocquet-Appel, 2008; Kohler, Glaude, Bocquet-Appel, & Kemp, 2008). For a North African site (Taforalt, Morocco) that is contemporaneous to Shubayqa 1, Humphrey et al. (2014, 2019) reported high numbers of perinates and infants in the burial assemblages of a population that had adopted a more sedentary lifestyle.

It has been hypothesized (Bocquet-Appel, 2008, 2011) that an increasing population growth rate during the transition from a hunter-gather to a sedentary agricultural lifestyle was achieved through a reduced interbirth interval. This requires increased maternal investment into reproduction and is associated with higher costs for feeding and care of multiple dependent children (Gurven and Walker, 2006). This could lead to compromised maternal health and a rise in infant mortality. Support for the above hypothesis is provided by an ethnographic study from the Philippines that demonstrates a trade-off between increased maternal fertility, higher reproductive success and impaired overall maternal health as well as reduced offspring survival and health (Page et al., 2016). A novel evolutionary life history model proposed by Wells & Stock (2020) assumes that in human populations the limited energy available is allocated in competition between four basic life functions (maintenance, growth, immune defense and reproduction). Natural selection favors energy allocation strategies that maximize the reproductive fitness of individuals. Wells & Stock (2020) suggest that transition to a more sedentary lifestyle favors increased energy allocation to reproduction and immune defense. The latter reflects higher parasite loads and increased exposure to infectious disease agents in sedentary agriculturalists compared to foragers. The findings in the Shubayqa 1 sample that indicate a stressful fetal and early post-natal life are in principle consistent with such a scenario. However, in order to further substantiate our suggestions on the health effects associated with the change from a hunter-gatherer to a sedentary lifestyle, further studies using larger samples from societies undergoing this transition are required.

In conclusion, the histological findings in the teeth of the six Shubayqa 1 individuals indicate periods of physiological perturbations both before and after birth, suggesting compromised health conditions of pregnant women and newborns. Our findings, although limited by small sample size, thereby contribute to the assessment of maternal and early infant living conditions in a Natufian population. Finally, the present study highlights the value of histological assessment of microstructures in deciduous teeth for reconstructing critical episodes during late fetal and early postnatal life. This opens a new perspective on the reconstruction of health conditions in Late Pleistocene populations in southwest Asia from which little data have thus far been available.

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Figure legends

Figure 1. Presence of prism cross-striations and variation of mineral content in pre- and postnatal enamel of a deciduous upper first incisor from individual 112. a) BSE-SEM image. The white line represents an approximate prism course along which grey values were measured. White arrow: NNL in enamel, asterisk: enamel-dentin junction (EDJ). b) Graph of grey level variation along the white line indicated in Figure 1a (higher values correspond to brighter grey levels). Black arrow: position of the NNL. Note hypomineralization of the NNL compared to the adjacent pre- and postnatal enamel portions. OES: outer enamel surface.

Figure 2: Micrograph of ground section of a deciduous lower canine of individual 108 demonstrating the measurements of crown heights at birth (1) and at death (2). The neonatal line in enamel is marked by arrows. D: dentin; E: enamel. Section viewed in transmitted light with phase contrast.

Figure 3. SE-SEM image of enamel of the acid-etched block surface of a deciduous upper first incisor from individual 112, demonstrating marked constriction of prism diameter and partial disruption of prism continuity along the NNL (white arrow). Note presence of surface zone with aprismatic enamel (asterisk).

Figure 4. Micrographs of ground sections viewed in transmitted light with phase contrast demonstrating the microstructure of prenatal (pre) and postnatal (post) enamel in the mesial crown portions of a deciduous first incisor of an infant from Tell Halaf (a) and a deciduous lower canine of individual 85 from Shubayqa 1 (b). Note indistinct incremental pattern in prenatal and regular prism cross-striations in postnatal enamel of the individual from Tell Halaf. The prism course is almost straight in this tooth. By contrast, in the individual from Shubayqa 1, pre- and postnatal enamel exhibit marked prism cross-striations and a wavy prism course. White arrows: NNL. Arrowheads in b: ALs in prenatal enamel. EDJ to the lower right in both images.

Figure 5. Micrographs of ground sections viewed in transmitted light with phase contrast demonstrating irregular microstructure of enamel (E) and dentin (D) in teeth from Shubayqa 1. a) Palatal enamel of a deciduous upper first incisor from individual 112. Arrows: NNL in enamel; Arrowheads: AL in postnatal enamel; Double-headed arrow: area of prenatally formed dentin exhibiting an enhanced calcospheritic pattern. b) Buccal enamel of a deciduous lower fourth premolar from individual 189. Pre- and postnatal enamel show a marked incremental pattern and a wavy prism course. White arrows: NNL in enamel. Black arrows: NNL in dentin.

Figure 6. Figure summarizing the findings on the estimated onset of enamel formation, the reconstructed age at death and the timing of ALs in the enamel of the teeth from the Shubayqa 1 individuals. The X axis gives days before and after birth. Day of birth = 0.

Table 1: Analyzed teeth and their context

Individual	**Analyzed teeth**	**Period**
51b	dUP4	Late Natufian
85	dLC, dUC	Late Natufian
108	dLI1, dLC	Late Natufian
112	dUI1, dLC	Late Natufian
136	dLI1, dUP3	Early Natufian
189	dLP4	Early Natufian

Table 2: Start of prenatal enamel formation, age at death and timing of first order (1st) and second order (2nd) accentuated incremental lines (ALs) in pre- and postnatal enamel (based on prism cross-striation counts), and additional findings in the dentin of the Shubayqa 1 infants. Age at death in brackets refers to teeth in which some loss of surface enamel has occurred. Day 0 = day of birth.

Ind.	**Tooth type**	**Enamel formation start (day)**	**ALs in prenatal enamel**	**ALs in postnatal enamel**	**Age at death (days)**	**Findings in dentin**
51b	dUP4	-158	AL (1st order): -58 to -56 days AL (1st order): -115 to -113 days AL (1st order): -137 to -136 days AL (1st order): -147 to -146 days	AL (1st order): 44 to 45 days AL (1st order): 102 to 103 days	118	Accentuated Andresen lines in pre- and postnatal dentin
85	dLC	-66 to -65	AL (1st order): -13 to -11 days AL (1st order): -38 to -36 days AL (2nd order): -50 to -47 days	AL (1st order): 37 to 39 days	54	Accentuated Andresen lines in prenatal dentin
	dUC	-72 to -71	AL (1st order): -14 to -12 days AL (2nd order): -35 to -33 days	AL (1st order): 39 to 41 days	52	Accentuated Andresen lines in prenatal dentin
108	dLI1	-145	AL (2nd order): -89 to -92 days AL (1st order): -107 to -110 days AL (2nd order): -134 to -132 days	AL (1st order): 18 to 19 days AL (1st order): 37 to 39 days AL (2nd order): 52 to 54 days	64	Pre- and postnatal dentin with accentuated calcospheritic pattern
	dLC	-105	AL (2nd order): - 20 to -21 days	AL (2nd order): 18 to 19 days AL (2nd order). 25 to 26 days AL (1st order): 37 to 40 days	(54-56)	Pre- and postnatal dentin with accentuated calcospheritic pattern
112	dUI1	-131	AL (1st order): -35 to -37 days AL (2nd order): -92 to -94 days AL (1st order): -96 to -100 days AL (1st order): -119 to -120 days	Aprismatic outer enamel, formation beginning 2nd week postnatally	(68)	Pre- and postnatal dentin with accentuated calcospheritic pattern
	dLC	?	AL (2nd order): -31 to -29 days	Not applicable: late secretory stage not yet reached	(56)	Pre- and postnatal dentin with accentuated calcospheritic pattern
136	dLI1	-153	AL (2nd order): -77 to -78 days	No ALs	(21)	Not evaluated (diagenesis)
	dUP3	-161	No ALs	No ALs	(19)	Not evaluated (diagenesis)
189	dLP4	-120 to -115	AL (2nd order): -24 to -29 days AL (2nd order): -34 to -39 days	AL (2nd order): 160 to 162 days AL (1st order): 206 to 207 days AL (1st order): 221 to 222 days	239	Pre- and postnatal dentin with accentuated calcospheritic pattern and accentuated Andresen lines in late formed postnatal dentin

Table 3: Tooth height at birth and prenatal crown formation times (based on prism cross-striation counts). Corrected values (=measurements on ground sections ×1.06) enable comparison with direct measurements published by Liversidge et al., (1998). A slash indicates a range of days in cases where prism cross-striation counts could not be performed with the necessary certainty.

Ind.	**Tooth**	**Tooth height at birth (mm), measurement on ground section**	**Corrected tooth height (mm) at birth**	**Tooth height (mm) at birth (mean ± SD) (Liversidge et al.,1998)**	**Deduced birth timing based on corrected tooth height**	**Prenatal start of crown mineralization (days)**	**95% confidence intervals (days) for prenatal start of crown mineralization (Birch & Dean, 2014)**	**Deduced birth timing based on assessed prenatal start of crown formation**
51b	dUP4	2.72	2.88	3.1 ± 0.55	Full-term	-158	-122 to -113	Full-term
85	dLC	2.02	2.14	3.4 ± 0.94	Pre-term	-66/-65	-135 to -121	Pre-term
85	dUC	2.15	2.28	3.4 ± 0.94	(Pre-term)	-72/-71	-135 to -121	Pre-term
108	dLI1	3.87	4.10	5.4 ± 1.04	(Pre-term)	-145	-152 to -137	Full-term
108	dLC	2.17	2.30	3.4 ± 0.94	(Pre-term)	-105	-135 to -121	Pre-term
112	dUI1	4.95	5.25	5.4 ± 1.04	Full-term	-131	-152 to -137	(Full-term)
112	dLC	(2.26)[a]	(2.40)[a]	3.4 ± 0.94	nd	nd	-135 to -121	nd
136	dLI1	4.86	5.15	5.4 ± 1.04	Full-term	-153	-152 to -137	Full-term
136	dUP3	3.22	3.41	4.0 ± 0.38	(Full-term)	-161	-146 to -135	Full-term
189	dLP4	2.01	2.13	3.1 ± 0.55	Pre-term	-120/-115	-122 to -113	Full-term

[a] Approximate value because section plane did not pass through the highest point of the tooth crown.

Deduced birth timings in brackets refer to values that deviate from those given by Liversidge et al. (1998) and Birch & Dean (2014).

Green color indicates consistency between the results of the two methods applied, yellow color indicates partially consistent, and red color inconsistent results.