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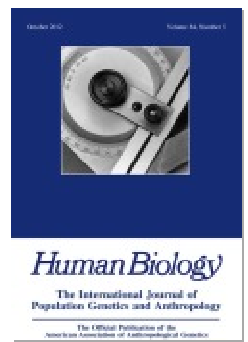


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# *Gestation Length, Mode of Delivery, and Neonatal Line-Thickness Variation*

CLÉMENT ZANOLLI,[1*] LUCA BONDIOLI,[2] FRANZ MANNI,[3] PAOLA ROSSI,[2] AND ROBERTO MACCHIARELLI[1,4]

*Abstract* The transition from an intra- to extra-uterine environment leaves its mark in deciduous teeth (and first permanent molars) as an accentuated enamel incremental ring called the neonatal line (NL). This prominent microfeature separates the enamel formed during intrauterine life from that formed after leaving the womb. However, while the physical structure of this scar is well known, the bases of its formation are still a matter of investigation. In particular, besides the influence of the birth-related abrupt environmental and dietary changes and the role played by physiological factors such as hypocalcaemia, a direct relationship between NL thickness variation and the physical was trauma implied by the birth dynamics, the Caesarean, and the operative modes are apparently associated with the thinnest and the thickest lines, respectively.

By using the histological record from a deciduous dental sample (exfoliated crowns) of 100 modern healthy school-aged children (47 males and 53 females) of reported birth histories (normal delivery mode: 55 cases; Caesarean: 40; operative: 5), we investigated the relationships between birth dynamics and NL thickness variation. The Tukey Honest Significant Difference method was used to test the differences between the means of the grouping levels.

The results of our histo-morphological investigation do not support the suggestion that Caesarean-born children display, on average, a thinner enamel scar compared to children associated to a normal delivery mode. Rather, our study points to the influence exerted by factors intimately related to gestational length variation on the degree of expression of the line.

The birth process leaves its mark on dental enamel in the form of a ring, an accentuated incremental line called the "Neonatal Line" (NL) (Rushton 1933; Schour 1936). This prominent microfeature, occasionally visible also in the

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dentine (Schour 1936; Skinner 1992), separates the enamel formed during intrauterine life from that formed after leaving the womb. In modern humans, the NL is usually present in all crowns forming at birth, that is, all the elements of the primary dentition and the first permanent molars (mesiobuccal cusp) (Christensen and Kraus 1965, Deutsch and Pe'er 1982; Deutsch et al. 1985; Kraus 1959; Levine et al. 1979). Occasionally, a macroscopic hypoplasia is detected in correspondence with the NL, but it is usually worn away (Massler et al. 1941; Moss-Salentijn and Hendricks-Klyvert 1985; Schroeder 1991; Skinner 1992).

At birth, the enamel is only partially mineralized and continues to increase in thickness by apposition of additional centripetal layers secreted by the ameloblasts at the level of the enamel-dentine junction (EDJ) to a circadian rhythm of 4—5 $\mu$m in extant humans (Antoine et al. 2009; Shellis 1984), while in extant great apes and early fossil hominins this rhythm accelerates rapidly (Dean 2006, 2010; Dean et al. 2001; Lacruz et al. 2008). The circadian growth process produces a repetitive microstructural pattern of the enamel, called prismatic cross-striations (short-period lines). In longitudinal crown sections (physical or virtual at high resolution), it is possible to identify another enamel striation mode linked to the crown formation, but with a longer periodicity (on average, seven days), parallel to the EDJ and crossing the prism decussation, called Retzius lines, which terminate on the tooth surface as perikymata (Boyde 1989; Guatelli-Steinberg 2009; Hillson 1996; Simmer et al. 2010). Each perturbation in "normal" development exceeding a critical individual threshold remains imprinted in the enamel as a larger stria of Retzius, called Wilson band (Hillson 1996; Risnes 2001; Rossi et al. 1999). This accentuated line corresponds to the position of the developing enamel (or dentine) front that relates to a stressor experienced during tooth development, as opposed to an intrinsic rhythm (Smith et al. 2010a). Marking the transition from an intra- to extra-uterine environment, the NL can be recorded as the first accentuated enamel microstructure (Gustafson and Gustafson 1967; Risnes 2001).

This scar can be distinctly recognized because of the differences in quality between pre- and postnatal enamel (Humphrey et al. 2007; Kodaka et al. 1996; Thomas and Lee 2003; Wilson and Beynon 1989), as well as due to its characteristic location, specific for each tooth class (Rossi et al. 1999; Skinner 1992; Skinner and Dupras 1993). The NL, whose variable thickness in humans may reach or even exceed 30 $\mu$m (Rossi et al. 1999; Schour 1936; Weber and Eisenmann 1971), is distinguishable from a Wilson band because it is more prominent and displays a more clearly discernible prism disturbance (Kodaka et al. 1996; Rushton 1939; Skinner 1992; Skinner and Dupras 1993; Thomas and Lee 2003; Wilson and Beynon 1989). High-resolution histological investigations reveal that it corresponds to an abrupt change in prism orientation and to structural changes within each prism (Mishra et al. 2009; Weber and Eisenmann 1971; Whittaker and Richards 1978; Wilson and Shroff 1970).

Regarding its topographic position, which is affected by the gestation length (Kronfeld and Schour 1939; Skinner 1992; Skinner and Dupras 1993), in incisors the NL usually extends from the cervical third of the EDJ toward the enamel surface, leaving only a small portion of postnatally formed enamel, while in canines and molars it is located more toward the incisal/occlusal part of the enamel, with only a small portion of prenatally formed enamel present (Rossi et al. 1999; Rushton 1939; Schour 1936; Teivens et al. 1996).

Besides human and nonhuman primates, such as the chimpanzee (Smith et al. 2010b) and the macaque (Bowman 1991; Smith 2004), for example, the NL is reported in different terrestrial, semi-aquatic, and also exclusive marine mammals, including the sika deer (*Cervus nippon*; Iinuma et al. 2004), the elephant seal (*Mirounga leonina*; Laws 1952) and the ringed seal (*Phoca hispida; Stewart et al. 1998),* the bottlenose dolphin (*Tursiops sp.*; Perrin et al. 2008), the h*arbour porpoise* (*Phocoena phocoena*; Perrin et al. 2008), the sperm whale (*Physeter macrocephalus*; Hillson 2005).

This marker is routinely used in forensic investigations (e.g., Gustafson 1966; Skinner and Dupras 1993; Stavrianos et al. 2010; Whittaker and Richards 1978), and its presence/absence, position and variation patterns are also increasingly considered in studies on population samples from archaeological sites (e.g., Alexandersen et al. 1998; Antoine et al. 2009; Bondioli and Macchiarelli 1999; FitzGerald and Saunders 2005; FitzGerald et al. 1999, 2006; Macchiarelli and Bondioli 2000; Macchiarelli et al. 2006a; Rossi et al. 1997, 1999; Schwartz et al. 2010; Smith and Avishai 2005; Smith et al. 2011). However, likely because of methodological constraints related to the only recent availability of noninvasive high-resolution investigative methods in paleobiology (e.g., Macchiarelli et al. 2004, 2008; Mazurier et al. 2006; Smith and Tafforeau 2008; Tafforeau 2004; Tafforeau et al. 2006;), its use in the study of the human fossil record is still very limited (e.g., Macchiarelli et al. 2006b; Smith and Tafforeau 2008; Smith et al. 2010a; Tafforeau and Smith 2008; Zanolli et al. 2011).

While the physical structure of the NL is now well-known (rev. in Simmer et al. 2010), the bases of its formation are not yet fully understood and, as rightly pointed out by Smith (2004), "The study of the NL will benefit from additional longitudinal studies of individuals with known records of birth, illness, stress, life history events, and environmental factors" (Smith 2004: 77).

**Goals of the Study.** The NL formation was at first assumed to directly result from the birth-related abrupt environmental and dietary changes (e.g., Massler et al. 1941). Bouyssou and co-workers (1958, cit. by Wilson and Shroff 1970) were probably the first to hypothesize that also some systemic influences could affect its appearance, even if the first studies did not produce evidence of a clear relationship between NL width and accentuated enamel lines related to systemic perinatal disturbances (Godt 1963; Wilson and Shroff 1970). Later on, the role played by some physiological parameters, notably by the decrease in plasma calcium occurring during the first 48 to 72 hr after birth (hypocalcaemia), was taken into account (Norén 1983, 1984; Ranggard et al. 1994; Seow 1986). In facts, infants born from diabetic mothers prone to hypocalcaemia show thicker and less mineralized NLs compared to those from healthy mothers (Norén 1984).

A relationship between NL thickness variation and the physical trauma implied by the birth dynamics was suggested by Gustafson and Gustafson (1967). Eli and co-authors (1989) investigated the relationships between the method of delivery and NL thickness in a sample of 147 children of known birth history. Their results showed that: (1) operative deliveries (17 cases) were associated with wider lines (13–24 $\mu$m); (2) normal deliveries (125 cases) resulted in intermediate thickness values (7–17 $\mu$m); and (3) Caesarean sections (5 cases) resulted in thinner lines (6–9 $\mu$m). Even if the last category was the least represented in their investigated sample, in principle these results support the conclusion that, in addition to the physiological drop in blood-serum calcium, both environmental changes and the birth process itself significantly contribute to the growth disruption responsible for the formation of the NL (Eli et al. 1989).

By using a series of enamel histological sections from a representative deciduous dental sample of modern healthy school-aged children of reported birth histories, here we investigate the relationships between gestation length, delivery mode, and NL thickness variation. More specifically, because of the composition and characteristics of the available sample, we test the hypothesis that, on average, Caesarean sections result in thinner scars.

## Materials and Methods

**The Fatina Sample.** The histological dental sample used in this study consists of 100 deciduous crown sections representing as many individuals of both sexes (47 males, 53 females) selected from the so-called *Fatina* ("tooth fairy") modern human reference collection. *Fatina* includes a whole of 250 exfoliated primary teeth from 225 healthy children, most of European (Italian) origin, aged 6–10 yrs, sampled in 1996–1997 in four primary schools of Rome ("Agatarco," "C. Corradi," "C. Forlanini," "Pirgotele"). The realization of this collection was conceived and set by the Section of Anthropology of the National Prehistoric Ethnographic "L. Pigorini" Museum, Rome, with the specific aim to create a modern reference record based on controlled and standardized histological sections to be used in paleobiological studies dealing with tooth microstructural growth markers and infant health assessment (e.g., Antoine et al. 2009; Dean 2006; FitzGerald and Rose 2000; FitzGerald et al. 1999, 2006; Geusa et al. 1999; Goodman and Rose 1990; Levine et al. 1979; Rose 1979; Rose et al. 1978; Rossi et al. 1999; Shellis 1984).

In agreement with the school authorities, on a voluntary basis, the parents of the children available to provide for invasive analysis at least one exfoliated tooth from their primary dentition were requested to fill out an anonymous information form about the original "owner" of the specimen and his/her mother. In the forms, basic questions concerned: sex, gestational age, delivery mode, weight and length at birth, health problems occurred during pregnancy and/or along the first six months after birth, lactation, and weaning time. Following a preliminary gross screening for the preservation quality of the collected specimens (consisting at least of the entire crown, even if partially worn), only a portion of the original sample was subsequently considered for histological analysis. However, even in this selected sample currently forming the *Fatina* collection, not all the tooth sections are systematically accompanied by an exhaustive individual information record. Besides few exceptions where confidentiality was not requested by the interviewed parents, of course in no case a quality control on the reliability of the data accompanying the collected specimens was possible. In a limited number of cases, more than one tooth (from 2 to a maximum of 13 specimens) is available from a single individual.

**Sectioning Procedures.** The sectioning of the *Fatina* sample followed the conventional procedures for dental thin-section preparation extensively detailed in Caropreso et al. (2000), FitzGerald and Saunders (2005), FitzGerald et al. (1999, 2006), and Rossi et al. (1997, 1999). At least two longitudinal sections, approximately 70–150 $\mu$m thick, passing through the buccolingual (labiolingual) plane and cutting the tip of the dentin horn (for the molars, that of at least one mesial cusp) were taken from the midsection of each crown preliminarily embedded in an epoxy resin block using a diamond blade microtome (Leica 1600, Leica AG). As noted by Antoine et al. (2009: 46), the exact section plane varies between tooth types; it is strictly a radial plane in incisors and canines, while in molars it does not include the axis of rotation of the tooth but is a tangential section centered through the tips of both the buccal and lingual cusps and the underlying dentine horns.

**Digital Image Processing.** To evaluate the general quality of the enamel microfeatures and the presence and position of the NL on both buccal (labial) and lingual crown aspects, each section was first scrutinized under polarized light at 100× (Figure 1a), and then the NL was observed at a higher magnification (400×) (Figure 1b). While many teeth had two or more sections cut from them, only one slide from each tooth showing the best discernibility of the NL was selected for analysis. Images at 100× and 400× were captured with a high-resolution digital camera (Polaroid Digital Microscope Camera, DMC 1) attached to an optical transmitted light microscope (Laborlux S, Leica AG) and exported into Adobe Photoshop, which was used to assemble montages of relevant areas of tooth sections from adjacent images. Contrast enhancement convolution filters (3×3 and 5×5 kernels) achieved sharper detail, and a change in the look-up table function increased site-specific contrasts of intensity profiles (Rossi et al. 1999; Schwartz et al. 2010). Depending on individual tooth size, each cross-section was reconstructed in a digital photomosaic of 10 or so (up to 15) partial images. Spatial resolution is 0.971 $\mu$m/pixel for the images captured at 100× and 0.241 $\mu$m/pixel for the digital record at 400×.

The ImageJ 1.43 (National Institutes of Health, USA) software was used for digital image processing and measurements.

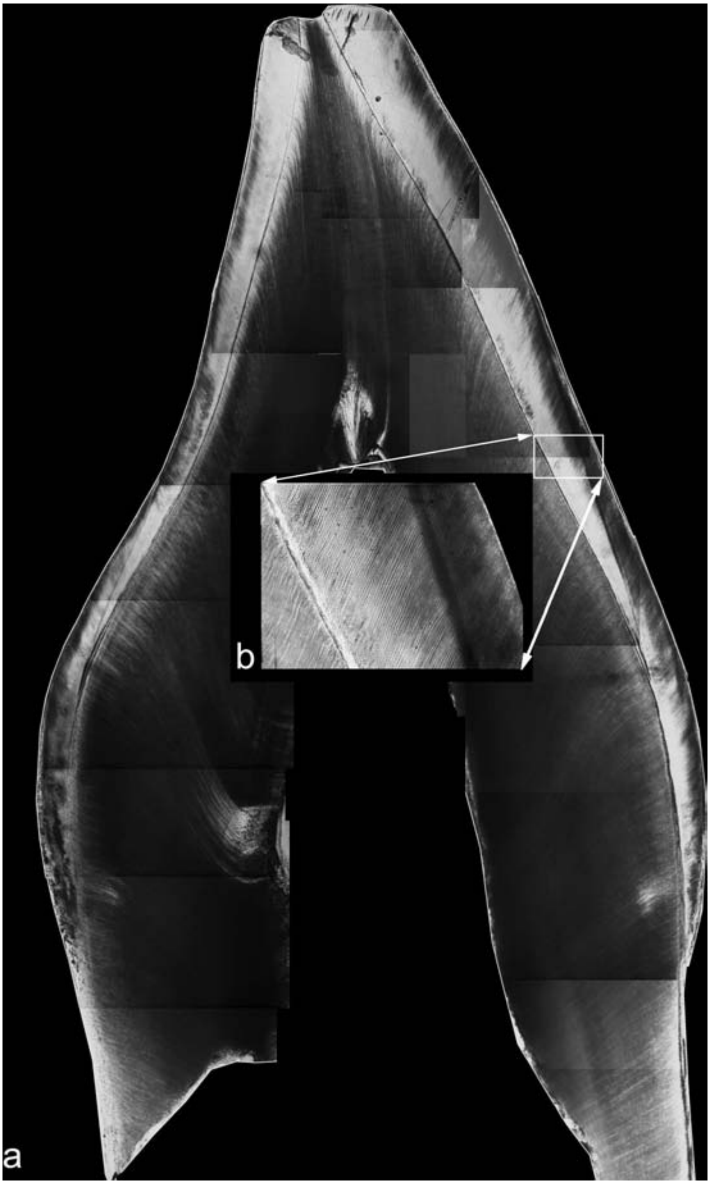


**Figure 1.** (a) Labiolingual section of a poorly worn lower lateral deciduous incisor (Li2) from the *Fatina* collection (100×), and (b) detail of the middle portion of the enamel on the labial aspect of the crown showing a distinct neonatal line (dark, to the right) and the enamel-dentine junction (white, to the left) (400×).

**The Investigated Sample.** The sample selected from *Fatina* for the specific purposes of the present study is detailed in Table 1. As a whole, it consists of the sections from 58 maxillary and 42 mandibular deciduous teeth. Having been

**Table 1.** The *Fatina* Deciduous Dental Sample

| | *Tooth Type* | *Number* | *Males* | *Females* |
|---|---|---|---|---|
| Maxillary teeth | i1 | 38 | 16 | 22 |
| | i2 | 18 | 7 | 11 |
| | c | 2 | 1 | 1 |
| | | 58 | 24 | 34 |
| Mandibular teeth | i1 | 19 | 13 | 6 |
| | i2 | 20 | 9 | 11 |
| | c | 2 | — | 2 |
| | m2 | 1 | 1 | — |
| | | 42 | 23 | 19 |
| Total sample | | 100 | 47 | 53 |

originally selected because of their relatively good preservation conditions and the lack of macroscopic pathological lesions (e.g., enamel decay), their tooth class, position, and side (not considered here), individual attributions were performed before sectioning on comparative morphological and dimensional grounds (Hillson 1996, 2005). In the sample, the central (i1) and lateral (i2) incisors are the most represented teeth (57 and 38 crowns, respectively), while only a single lower m2 represents the molar tooth class.

Information about sex, delivery mode, and gestation length are available for all the individuals represented in this study (Table 2),while data on weight at birth only concern 61 cases. Related supplementary information (mostly, medical data) about pregnancy (cases of miscarriage threats, pregnancy-induced maternal hypertension and hyperemia, maternal isoxsuprine treatment, and temporary myoma) and/or the perinatal period (undernourished placenta, placenta abruption or marginally patent at term, asphyxia at birth) are here available for 12 individuals, but in no case were they accurately detailed by the parents and appear in the anonymous record as telegraph notes only.

To get from the parents simple and unambiguous answers suitable for reliable elaborations, in our record the delivery modes were schematically categorized as follows: “normal”—no special medical care or physical intervention was necessary; “Caesarean”—typical surgical section following anaesthesia;

**Table 2.** Delivery Mode and Gestational Age Categories Represented in the *Fatina* Sample

| *Delivery Mode* | *Number* | *Males* | *Females* |
|---|---|---|---|
| Normal | 55 | 26 | 29 |
| Caesarean | 40 | 18 | 22 |
| Operative | 5 | 3 | 2 |
| Gestational age | | | |
| Preterm | 14 | 6 | 8 |
| Term | 71 | 34 | 37 |
| Post-term | 15 | 7 | 8 |
| Total sample | 100 | 47 | 53 |

"operative"—some kind of active outside intervention had taken place (e.g., breech, forceps, or vacuum delivery) because of labor complications (malpresentation, failure of descent of the fetal head through the pelvic brim or the interspinous diameter, poor uterine contraction strength, active phase arrest, cephalic-pelvic disproportions, shoulder dystocia, etc.). In this respect, the present sample mostly represents normal[55] and Caesarean[40] deliveries, while complicated deliveries apparently occurred in 5 cases only (Table 2).

With reference to the gestational age, the deliveries were categorized has follows: at "term"—occurred within 37–41 wk after fertilization; "preterm"—before the beginning of the 37th week; and "post-term"—over the beginning of the 42th week. In our sample, the first category is largely the most represented (71 cases), while the remaining cases are almost equally distributed between preterm[14] and post-term[15] deliveries (Table 2).

A synthetic picture of the investigated sample in terms of sex, delivery mode, and gestational-age representativeness is provided by the mosaic plot shown in Figure 2. A mosaic plot is basically an area-proportional visualization of observed frequencies, composed of tiles (corresponding to the cells) created by recursive vertical and horizontal splits of a rectangle. Thus, the area of each tile in Figure 2 is proportional to the corresponding cell entry given the dimensions of previous splits (Friendly 1994).

**Neonatal Line Thickness.** The NL is an optical phenomenon due to alterations in height and degree of mineralization of the enamel prisms (Sabel et al. 2008). In all cases used in the present work, its identification and outline assessment at 100× was unambiguous, as this scar clearly divides the smooth course of prenatal enamel matrix formation from the "normal" structure of postnatal enamel matrix (Antoine et al. 2009; FitzGerald et al. 2006; Kodaka et al. 1996; Mishra et al. 2009; Risnes 2001; Rossi et al. 1999; Rushton 1939; Sabel et al. 2008; Schour 1936; Schour and Massler 1937; Skinner 1992; Skinner and Dupras 1993; Szpringer-Nodzak 1984; Weber and Einsenmann 1971; Whittaker and Richards 1978). To measure the NL width, three images were originally taken at 400× in three different positions, preferably along the buccal (labial) aspect: (1) close to the EDJ; (2) in the middle of the dental crown; and (3) close to the apex. After having carefully marked the outline of the NL on the digital photomontages, at least six measurements were realized at each spot by two to three independent expert observers, and the site-specific average thickness value calculated. However, as the enamel secretion rate slightly varies depending on crown topography (Birch and Dean 2009; Mahoney 2008), here we specifically used the mean estimates pertaining only to the middle portions (Figure 1b). Following a quantitatively significant experience developed in the NL assessment on recent and archaeological samples and the realization of repeated tests for intra- and interobserver concordance in analyzing the histomorphometric dental record (e.g., FitzGerald et al. 2006; Macchiarelli and Bondioli 2000; Rossi et al. 1999; Schwartz et al. 2010), in none among the 100 thin sections used here did the discrepancy among the repeated measures exceed 6%.

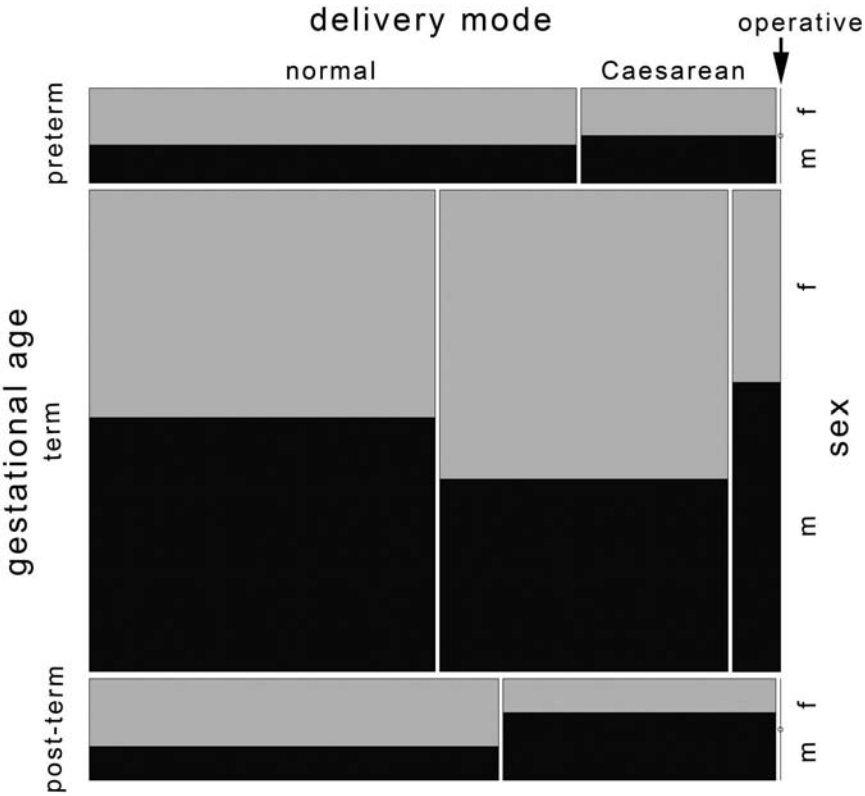


**Figure 2.** Mosaic plot describing the composition of the investigated deciduous dental sample from the *Fatina* collection in terms of sex, delivery mode (normal, Caesarean, operative), and gestational age representativeness (preterm, term, and post-term). m: males; f: females.

**Statistical Analyses.** To compare the NL width and the weight at birth means among the groups defined by the delivery mode, the sex, and the gestation length, we used the analysis of variance with an incomplete model with three factors (sex, delivery mode, and gestation length) and two interactions (sex by gestation length and sex by delivery mode). The Tukey Honest Significant Difference (HSD) method was used to test the differences between the means of the grouping levels. We used this method because, when comparing the means for the levels of a factor in an analysis of variance, a simple comparison using *t*tests would inflate the probability of declaring a significant difference when it is not in fact present (Yandell 1997).

Statistical analyses and graphs were realized with the R v.2.13.1 language (R Development Core Team 2011), with the support of the package vcd (Meyer et al. 2006). The box-and-whisker plots in Figures 3, 4, and 5 systematically show (from bottom to top): the minimum value, the 1st quartile, the median, the 3rd quartile, and the maximum value recorded in our series of observations.

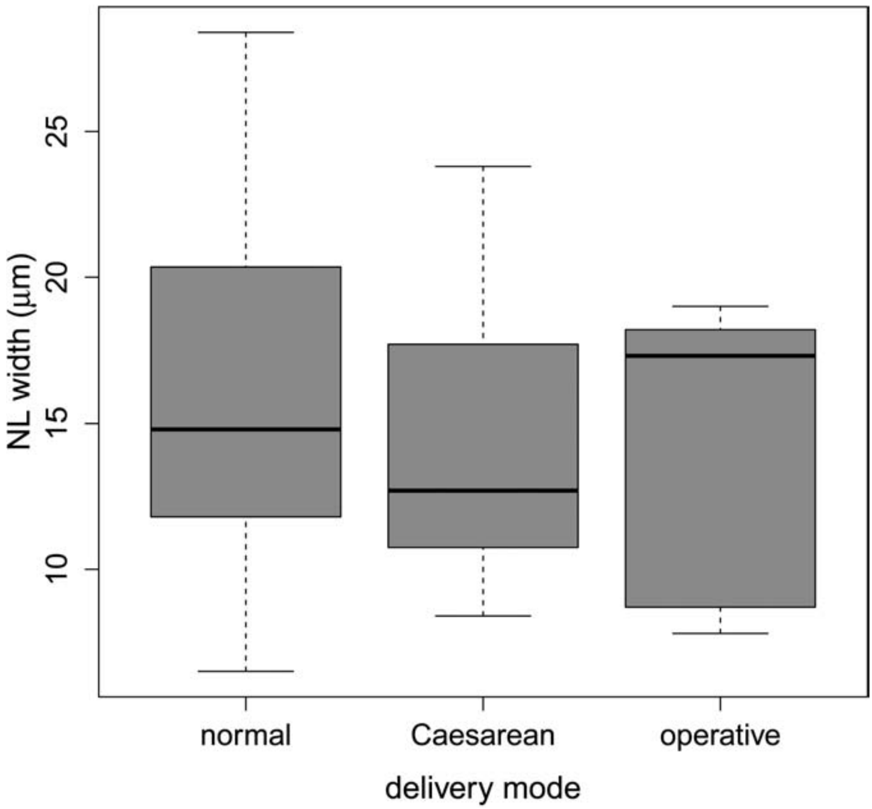


**Figure 3.** Box-and-whisker plot of the neonatal line thickness (NL width) variation in the *Fatina* sample according to the delivery mode.

## Results and Discussion

The descriptive statistics of the NL thickness variation in our tooth sample of 100 individuals (data pooled and distinct per sex) is summarized in Table 3, which also details the results distinctly for each delivery mode and gestational age category. As a whole, NL width widely varies from 6.5 to 28.4 $\mu$m (av. thick. = 15.2 $\pm$ 5.19 $\mu$m), a range which approximates the estimates provided for other recent and archaeological population samples (Macchiarelli et al. 2006a; Rossi et al. 1997, 1999). In the series, no significant differences appear between males and females (14.7 $\pm$ 5.28 vs. 15.7 $\pm$ 5.12 $\mu$m) nor, as expected (cf. Rossi et al. 1999), between the average values from the upper and lower crowns, or between those of the central and lateral incisors, which are the most represented tooth elements (95/100).

As shown in Figure 3, the results indicate a substantial overlap among the thickness distributions of the normal (av. thick. = 15.9 $\pm$ 5.74 $\mu$m), Caesarean (14.4 $\pm$ 4.26 $\mu$m), and operative delivery modes (14.2 $\pm$ 5.47 $\mu$m), even if the last

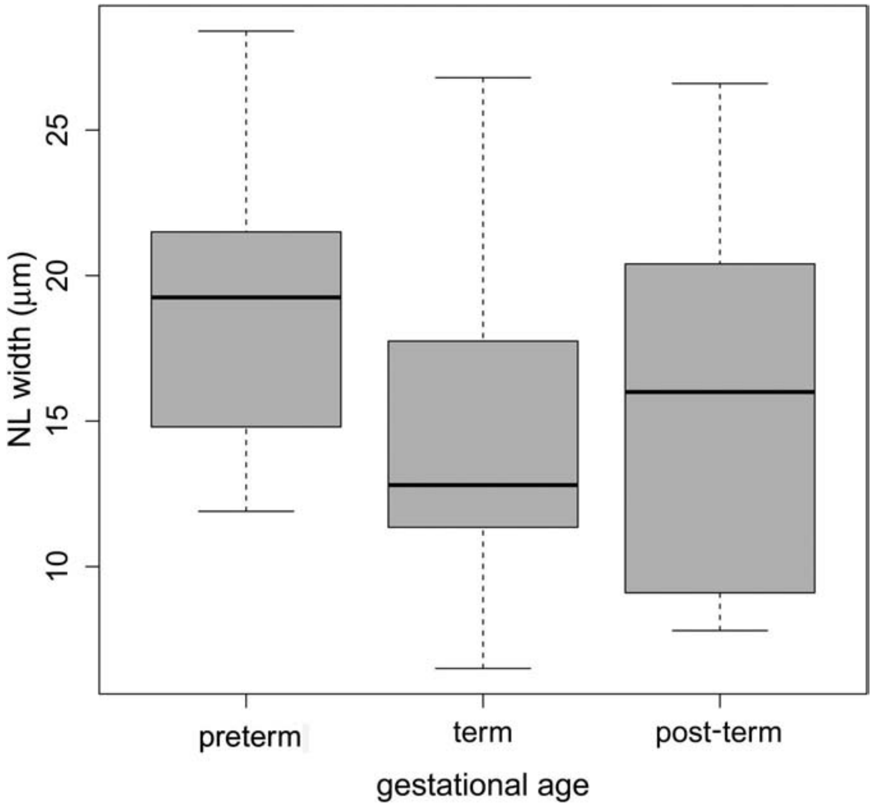


**Figure 4.** Box-and-whisker plot of the neonatal line thickness (NL width) variation in the *Fatina* sample according to the gestational age.

category is only represented by 5/100 cases (Table 3). In this regard, noteably, according to our record, the individuals showing a NL width ≥24 $\mu$m (7 cases, 3 males/4 females) were apparently all born without active external intervention ("normal" delivery). However, two among them, a preterm and a post-term child, correspond to a case of asphyxia at birth and of marginally patent placenta, respectively. Among the 11 cases of minimal (≤9 $\mu$m) NL thickness measured in our series (7 m/4 f), none being associated to a particular condition or medical record available to us, all three delivery categories are represented (6 normal, 2 Caesarean, 2 operative).

The results illustrated above differ from the analytical figures reported by Eli et al. (1989) in their study of a similar sample of school-aged children, where a distinct trend of relative NL thickness increase was found from the category elective Caesarean sections (6–9 $\mu$m vs. 8.4–23.8 $\mu$m in *Fatina*) to that operative (13–24 $\mu$m vs. 7.8–19.0 $\mu$m) through the normal delivery pattern (7–17 $\mu$m vs. 6.5–28.4 $\mu$m). One reason for these differences likely relates to the heterogeneous proportions of delivery modes represented in the two samples. In

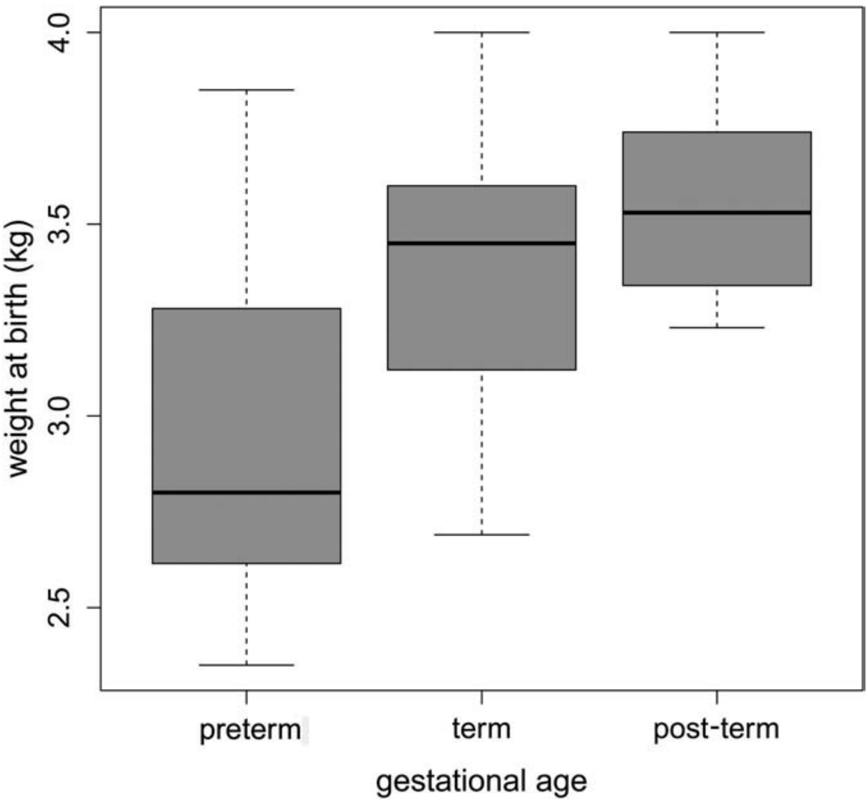


**Figure 5.** Box-and-whisker plot of the weight variation at birth in the *Fatina* sample according to the gestational age.

fact, while in *Fatina* the normal and the Caesarean are the most represented modes (55 and 40 individuals, respectively), the sample examined by Eli and co-workers (1989) mostly includes normal[125] and operative[17] cases. In other words, while our results clearly do not support the existence of a statistically significant difference in NL width between children born "normally" and those having left the uterus following a typical Caesarean intervention, the poor representativeness in our *Fatina*'s subsample of the operative category (5 cases) does not legitimate any conclusion on a comparative basis. In our view, the same should be the case for the sample of 5 teeth representing the category "elective Caesarean sections" measured by Eli et al. (1989). However, we should also evoke the different nature of the two original records among the additional factors potentially responsible for such discrepancies. In fact, while in Eli and co-workers (1989) all technical and medical information details about the birth histories, including type of delivery and the condition of the newborn, were obtained from hospitals, in *Fatina* they are from parental sources, which makes problematic a direct comparison of the two records, notably in the cases of

**Table 3.** Neonatal Line Thickness (width) Variation (in μm) in the *Fatina* Sample

| | *Number* | *Mean* | *SD* | *Min.* | *Max.* |
|---|---|---|---|---|---|
| Total sample | 100 | 15.2 | 5.19 | 6.5 | 28.4 |
| Males | 47 | 14.7 | 5.28 | 6.5 | 28.4 |
| Females | 53 | 15.7 | 5.12 | 7.8 | 26.8 |
| Delivery mode | | | | | |
| Normal | 55 | 15.9 | 5.74 | 6.5 | 28.4 |
| Males | 26 | 15.3 | 5.80 | 6.5 | 28.4 |
| Females | 29 | 16.5 | 5.71 | 7.8 | 26.8 |
| Caesarean | 40 | 14.4 | 4.26 | 8.4 | 23.8 |
| Males | 18 | 14.5 | 4.42 | 8.4 | 23.8 |
| Females | 22 | 14.4 | 4.24 | 9.0 | 21.6 |
| Operative | 5 | 14.2 | 5.47 | 7.8 | 19.0 |
| Males | 3 | 11.6 | — | 7.8 | 18.2 |
| Females | 2 | 18.2 | — | 17.3 | 19.0 |
| Gestational age | | | | | |
| Preterm | 14 | 18.5 | 4.78 | 11.9 | 28.4 |
| Males | 6 | 20.0 | 5.72 | 13.1 | 28.4 |
| Females | 8 | 17.3 | 3.92 | 11.9 | 22.4 |
| Term | 71 | 14.5 | 4.78 | 6.5 | 26.8 |
| Males | 34 | 13.4 | 4.53 | 6.5 | 24.3 |
| Females | 37 | 15.5 | 4.86 | 8.3 | 26.8 |
| Post-term | 15 | 15.8 | 6.45 | 7.8 | 26.6 |
| Males | 7 | 16.4 | 5.71 | 8.4 | 26.6 |
| Females | 8 | 15.2 | 7.38 | 7.8 | 24.8 |

"normal" and "complicated" (operative) deliveries. Additionally, no systematic information is available in *Fatina* about the causal factors related to the Caesarean surgical sections.

Differently from the picture offered by the delivery pattern, in the *Fatina* series some difference in NL thickness emerges when gestational weeks are considered (Figure 4, Table 3). In particular, here the estimates for the preterm-born children (av. thick. = 18.5 ± 4.78 μm) clearly exceed those associated to both term (14.5 ± 4.78 μm) and post-term (15.8 ± 6.45 μm) subsamples. In this case, however, only one preterm individual is included among those showing an NL width ≥24 μm (the already mentioned case of asphyxia at birth), while none is found among the 11 cases of minimal (≤9 μm) NL thickness. So, in our investigated sample, gestational age apparently more strictly relates to NL width than delivery modalities.

The results of the analysis of variance of the NL thickness variation globally run with respect to the sex, delivery mode, and gestational age factors (Table 4) show a statistically significant contrast ($P = 0.03$) for this latter variable only (pooled sexes). More precisely, as revealed by the Tukey HSD method used to test the differences between the means of the grouping levels, a statistically significant difference exists between the average values of the preterm and term male subsamples (Table 5). Accordingly, preterm-born children, notably of male sex, tend to have a thicker enamel NL, independently from their experienced delivery dynamics. However, the sample size of our

**Table 4.** Analysis of Variance (F) of the Neonatal Thickness in the *Fatina* Sample with Respect to Sex, Delivery Mode (DM), and Gestational Age (GA)[a]

| | *d.f.* | *F* | *P* |
|---|---|---|---|
| Sex | 1 | 0.90 | 0.35 |
| GA | 2 | 3.63 | 0.03* |
| DM | 2 | 0.70 | 0.50 |
| Sex by GA | 2 | 2.02 | 0.14 |
| Sex by DM | 2 | 0.64 | 0.53 |

a. d.f.: degrees of freedom.

currently available preterm subsample (14 cases, 6 m/8 f) does not allow for any reliable conclusion about a possible sex-related influence of the number of gestational weeks on NL thickness, a subject which certainly deserves additional investigations.

While in this study we did not use scanning electron microscopy to detail variations in porosity between pre- and postnatal enamel or prism disruption patterns, some studies on primary teeth from low-birth weight preterm individuals reported the occurrence of a high frequency of mineralization disturbances, notably the presence of a distinct hypomineralized postnatal zone (Norén 1983; Rythén et al. 2008), a condition compatible with the evidence from *Fatina* of a sometime thicker NL. However, in evaluating the possible relationships between NL width and the variables considered in Table 4, take into account that the deciduous tooth sections used in the present histo-morphological study were selected from the *Fatina* collection exactly because of the unambiguous presence of the NL. In other words, the present sample is unable to provide any information about a possible differential lack of this mark, for example, in individuals distributed according to the categories assessed here, nor to any other additional variable available to us.

Another parameter intimately related to gestational age, thus of possible relevance in such kind of studies dealing with NL thickness variation, is represented by the weight at birth. In this respect, our record concerns only 61/100 cases (31 m/30 f). The individual values range from 2.35 to 4 kg (av. = 3.33 ± 0.41 kg), with no significant differences between males

**Table 5.** Results of the Tukey Honest Significant Difference (HSD) Method Used to Test the Differences Between the Means of the Grouping Levels for the Analysis of Variance Presented in Table 4. Only the Significant Differences (*P* 0.05) Are Reported[a]

| | *Diff.* | *Lower* | *Upper* | *P adj* |
|---|---|---|---|---|
| GA | | | | |
| Preterm - term | 3.92 | 0.40 | 7.43 | 0.03 |
| Sex by GA | | | | |
| Males preterm - males term | 6.72 | 0.22 | 13.22 | 0.04 |

a. GA: gestational age; diff.: difference in the observed means; lower: the lower end point of the confidence interval; upper: the upper end point of the confidence interval; *P* adj: the probability value after adjustment for the multiple comparisons.

(av. = 3.44 ± 0.43 kg) and females (av. = 3.23 ± 0.36 kg), nor among the groups referred to here as normal (35 cases: 3.30 ± 0.45 kg), Caesarean (25 cases: 3.39 ± 0.35 kg), and operative (one single case: 3.25 kg). As expected and shown in Figure 5, preterm children (11 cases) on average display the lowest weights (2.97 ± 0.49 kg), while a substantial overlap exists between the figures available to us for the term (42 cases) and post-term (8 cases) subsamples (3.39 ± 0.35 vs. 3.56 ± 0.26 kg, respectively). Of course, given the evidence that at least a few days of life ex utero are necessary for the NL to leave its imprint in the enamel (Levine et al. 1979; Massler and Schour 1946; Schour 1936; Schour and Massler 1937; Weber and Eisenmann 1971; Whittaker and Richards 1978), possibly or even likely, physiological weight fluctuations along the first week may affect NL thickness variation. Nonetheless, again, the heterogeneous quality of our record does not authorize any conclusive statement on this matter.

## Conclusions

The physical trauma implied by the birth dynamics is commonly listed among the factors having a direct impact on the degree of expression (thickness variation) of the neonatal line, the enamel growth disruption marker usually found in all deciduous crowns and the first permanent molars of the individuals having survived at least a few days the perinatal stage. This was corroborated by the quantitative results of the study realized by Eli and co-authors (1989) on a large sample of healthy children of known birth history. Their work suggests an intimate association between operative deliveries and thicker lines, on one side, and Caesarean sections and thinner lines, on the other one, normal deliveries apparently resulting in intermediate values. Accordingly, Eli et al. (1989) have tentatively estimated at about 37% the proportional weight of the birth process itself on the determination of the NL width. However, note that their investigated sample only included five cases associated with elective Caesarean section.

The results from the present histo-morphological investigation performed on a deciduous dental sample of 100 modern school-aged children selected from the so-called *Fatina* collection do not support the suggestion that Caesarean-born children (40 cases in our sample) display, on average, a thinner enamel scar compared to children associated with a "normal" delivery mode (55 cases). Rather than delivery modalities, our study supports the view of an influence exerted by factors intimately related to gestational length variation on the degree of expression of the line (review in Rythén et al. 2008).

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