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Exploring prenatal and neonatal life history through dental histology in infants from the Phoenician necropolis of Motya (7th–6th century BCE)

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A R T I C L E I N F O

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A B S T R A C T

The biological life history of infants from archaeological contexts can provide a unique insight into past human populations. Dental mineralized tissues contain a permanent record of their growth that can provide access to the prenatal and early infant life, and mortality, of human skeletons. This study focuses on the histomorphometric analysis of deciduous teeth from the 'Archaic Necropolis' of Motya (7th–6th century BCE, Sicily–Italy). The histomorphometric analysis is conducted on prenatal and postnatal enamel of eight anterior deciduous teeth from seven individuals from this Phoenician population to estimate their chronological age-at-death, health, and enamel growth parameters. Proteomic analysis has been used to determine the sex of the infants.

The presence of the Neonatal Line in all specimens indicates that the seven individuals survived birth. The occurrence of at least one Accentuated Line in prenatal enamel in four out of seven individuals suggests the foetuses and/or their mothers experienced a stress-related event during pregnancy. As expected, there was limited variation in Daily Secretion Rates near the Enamel Dentine Junction. These rates increase toward the outer enamel surface and decrease toward the cervix.

Our findings illustrate the importance of dental histology for reconstructing perinatal and early infancy mortality and morbidity patterns at Motya, which sheds light on the socio-cultural perception of new-borns and infants in an ancient Phoenician community.

1. Introduction

Foetal and neonatal death rates, along with infants and child mortality profiles, carry a wealth of information on past human populations (Lewis 2018). The study of an infant's odontoskeletal remains can provide insight into their biological and social life, through their rate of growth and development, diet, health, mobility, and age-at-death (e.g., Li et al. 2020; Lugli et al. 2022; Müller et al. 2019; Nava et al. 2019). Indeed, social and economic factors may have exposed infants to trauma and/or physiological stresses experienced at different stages of their brief lives (Bode et al. 2020; Halcrow et al. 2020). As vulnerable members of society and wholly dependent on the care of others, understanding neonatal and infant biological life histories provides an accurate measure of a population's ability to adapt to ecological and cultural contingencies (Barker 2004; Halcrow et al. 2020). In this perspective, infants' health and well-being are amongst the most sensitive indicators of biocultural changes that occurred during recent human evolution (Gowland and Halcrow 2019; Hodgkins et al. 2021;

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Nava et al. 2017b; Rebay-Salisbury and Pany-Kucera 2020). Several epidemiological studies on modern populations have indeed shown that adverse conditions during early individual life – from the intrauterine period to the first years of age – often affect health and fertility and potentially produce a higher rate of early mortality in adulthood (Almond and Currie 2011; Barker 2004). The relationship between early life adversities and poor health outcomes in adults has been conceptualised in light of the Developmental Origins of Health and Disease (DOHaD) theory (Barker 1990; Barker et al. 2002; Gluckman and Hanson 2008). According to the DOHaD, the early life environment – including the maternal womb – induces physiological changes in infants' growth and development that have long-term consequences on later-in-life health and disease risk (Barker 2007; Suzuki 2018). However, as stressed by the Osteological Paradox (DeWitte and Stojanowski 2015; Wood et al. 1992), the reconstruction of health and life conditions in ancient human populations is an extremely difficult task that must consider that archaeological skeletal and dental remains are biased proxies of the reference populations' living conditions.

Looking at the social and cultural significance of neonatal and infant remains from archaeological contexts, it is widely accepted that the mortuary treatment of infants and children's bodies provides information on communities' structure, social organization and sub-adults social status (Halcrow and Tayles 2011; Hodgkins et al. 2021). Cultural attitudes and behavioural choices may have dictated different funerary practices towards infant remains. New-borns, infants, and children could be the object of cultural manipulations through exclusion from burial places or clustered burials within dedicated funerary areas (Bondioli et al. 2020; Fulminante and Stoddart 2020; Perry 2005).

1.1. The treatment of neonatal and infant remains in the Phoenician societies

The multifaceted aspects of life and death of infants and children in the Iron Age societies of the Mediterranean basin have been a topic of intense discussion in the last decades (e.g., Gigante et al. 2021; Romero and López 2018; Tabolli 2018). The Phoenician and Punic attitude toward the youngest individuals of the community has been a focus of intense scrutiny, as it was a source of reproach in the ancient sources and likewise is controversial in modern academia from a biocultural, social, religious, and funerary perspective.

Among factors that have contributed to fostering the debate is the media attention given to pan-Mediterranean studies of the tophet sanctuaries where, according to various scholars (McCarty 2019; Melchiorri 2013; Xella et al. 2013), some of the youngest individuals were cremated and buried after being possibly deliberately sacrificed, while other researchers suggest (Schwartz et al. 2012; Schwartz et al. 2017) that tophet just contained mostly infants who died around birth. The relative elusiveness of pre-adult mortuary remains within proper Phoenician and Punic burial grounds contributes to this debate, especially if related to the expected mortality rates of pre-industrial societies (Ceballos 2016; Gómez Bellard and Gómez Bellard 1989; Guirguis et al. 2017; Pla Orquín 2017; Secci 2012). Finally, there lacks a synthesis of research in capturing the multitude of records scattered across the broad Mediterranean basin (Hernández 2021).

Recent discoveries from some of the main cemeteries of the Phoenician colonies (and later Punic sites), alongside the application of new and more sophisticated methods to analyse human remains, have allowed us to better address funerary customs and practices in Phoenician and Punic societies (Guirguis et al. 2020).

In this framework, the present study reports the first histomorphometric analysis of deciduous teeth from infant burials from Motya, Sicily (Fig. 1), one of the main Phoenician sites of the central Mediterranean, for determining the chronological age-at-death, the growth trajectories and health status of these individuals. This study aims to provide an initial picture of the infants' life histories on the island at the time of its maximum floruit (7^{th}-5^{th} century BCE).

Motya, in particular, offers a unique opportunity for a deeper understanding of the Phoenician funerary practices as it hosted mortuary (Falsone and Sconzo 2017; Sconzo 2016; Sconzo 2020) and ritual sites, including a tophet (Ciasca 1992; Ciasca 2002; Ciasca et al. 1996) documented from its earliest phases.

The 'Archaic Necropolis', located on the northern shore of the site, is the earliest Phoenician funerary site known so far in Sicily. Discovered at the beginning of the last century, since 2013 it has been investigated by

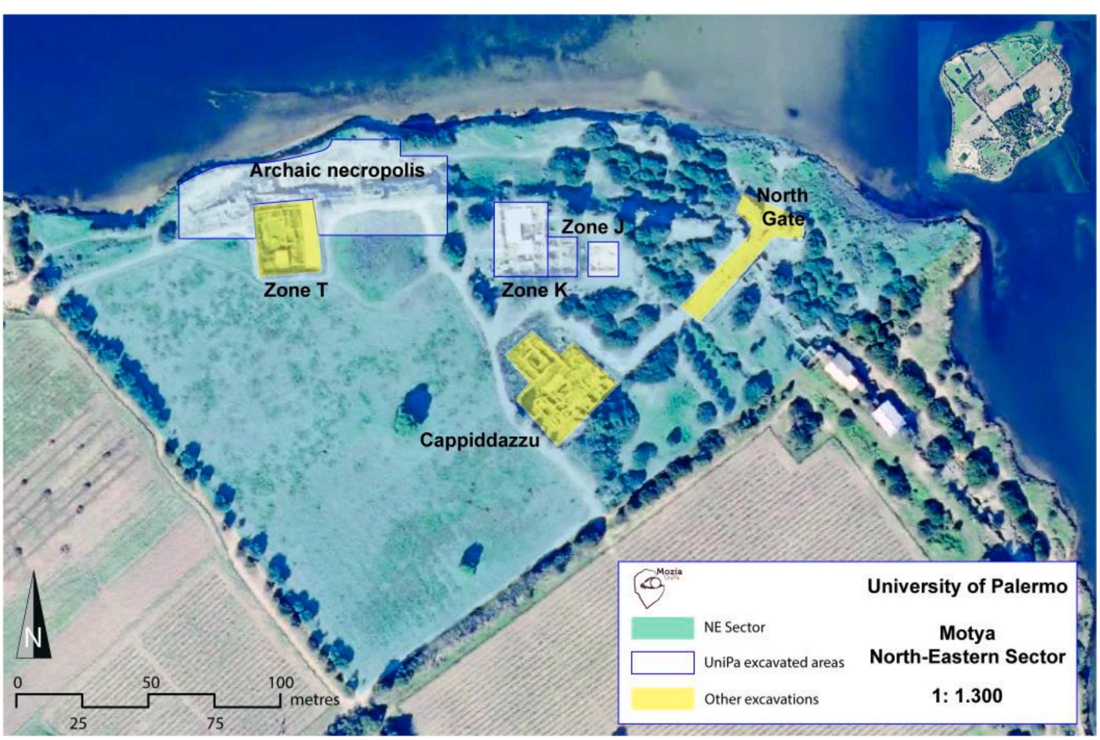

Fig. 1. The geographic location of Motya site and the burial areas.

a joint team of the Palermo University and the Soprintendenza BB.CC. AA. (Beni Culturali e Ambientali) of Trapani using modern techniques (Falsone and Sconzo 2017; Lauria et al. 2018; Lauria et al. 2017; Lauria et al. 2020; Sconzo 2016; Sconzo 2020). It features over 500 graves diachronically distributed over 250 years (late 8th-5th century BCE). The most recent results show that from the very beginning, the burial ground served the entire community living on the island, as it hosted both adult and child interments. While adult individuals were mainly (though not exclusively) cremated and deposited inside pits, stone cists or transport amphorae, the sub-adult segment of the population (including foetuses, perinatal, infants and children) were mainly buried inside large torpedo jars, usually laid horizontally inside a pit (i.e., the so-called *enchytrismós* type) (Lauria et al. 2020; Sconzo 2020). A few cases of primary cremations have also been recently detected.

This funerary evidence suggests a high degree of infant mortality and extreme care by the Motya community towards the society's most fragile population segments (Lauria et al. 2020).

2. Materials and methods

The present study includes eight deciduous teeth from seven individuals (Table 1) from the Motya Archaic Necropolis, excavated by Palermo University in 2017 (Sconzo 2020).

The material analysed here comes from six different *enchytrismói*, namely T.268, T.275, T.278, T. 284, T.288 and T.300, all located in the southernmost sector of the necropolis. These burials date from the 7th to the 6th century BCE, just before and during Carthaginian hegemony in Sicily, when the city was experiencing its maximus prosperity.

Histological analysis was performed on deciduous central and lateral incisors (n = 6), and canines (n = 2), both maxillary and mandibular. Individual 1 from grave 275 was sampled for both the LRdi2 (lower right deciduous lateral incisor) and the LRdc (lower right deciduous canine).

2.1. Dental histology

Before sectioning, a photographic record of each sample was taken. Teeth were sectioned along the central labiolingual plane using standard histological methods (Mahoney et al. 2022; Nava et al. 2019) and prepared at the Human Osteology Laboratory of the University of Kent (UK). The samples were embedded in resin (Buehler, Epicure 2) and were cut using a Buehler, IsoMet 1000 Low-Speed Saw. After the first cut, a microscope slide was attached to the exposed surface using the same resin. A single longitudinal buccolingual thin section, averaging 250 μm thick, was cut from each sample. Each ground section was reduced to a thickness of ~ 80–100 μm using water-resistant abrasive paper of different grits (Buehler, Eco-Met 300). Finally, the sections were polished with a micro-tissue (Buehler, Micro-Polish II μm) and an aluminium oxide powder (Buehler, MicroPolish II 1 μm). Each section was cleaned in an ultrasonic bath, dehydrated in 95–100% ethanol, cleared (Histoclear, National Diagnostic) and mounted with a coverslip using a xylene-based mounting medium (DPX, Thermo Fisher Scientific). Thin sections were digitally recorded through a camera (Olympus DP25) paired with a transmitted light microscope (Olympus BX51) under polarised light, with different objectives (4x, 10x, and 20x). The use of the transmitted light microscope in polarised light is proven to be the most effective tool for observing and counting the variation in prisms' orientation related to the description of amelogenesis (Guatelli-Steinberg 2016; Hillson 2014; Nava et al. 2017b; Nava et al. 2019).

2.2. Histomorphometric analysis of dental enamel

Teeth are individual biological archives, which, during their formation permanently record enamel growth trajectories (e.g., Aris et al. 2020; Dean 2006; McFarlane et al. 2021; Nava et al. 2017b), physiological stresses experienced during development (e.g., Nava et al. 2019; Witzel et al. 2008), and – in individuals who died before crown completion – the chronological age-at-death (e.g., Nava et al. 2017b; Smith et al. 2006). The deposition of the enamel matrix relates to a daily rhythm, which produces Daily Cross Striations (Lacruz et al. 2012; Zheng et al. 2013). Stress events exceeding a certain threshold can disrupt dental development, producing Accentuates Lines. The visibility of the Accentuated Line is related to the variation of prism orientation during amelogenesis (Nava et al. 2019). Following FitzGerald et al. (2006) and Nava et al. (2019), we classified a marker as Accentuated Line if it is visible for at least 75% of its length – from the dentin enamel junction to the crown surface, in the imbricational area of the crown; and/or (ii) is visible for at least 75% of the total distance from buccal to labial sides – from the Dentin Enamel Junction around the dentinal horn, in the cuspal area. The first Accentuated Line is usually the Neonatal Line, which is present in all deciduous teeth and the first permanent mandibular molar in individuals who survived the perinatal stage (Dean et al. 2019; Sabel et al. 2008). The Neonatal Line separates the tissues formed *in utero* from tissues that formed after birth.

Accentuated Lines in prenatal enamel indicate physiological stresses that affected the mother, such as maternal dietary deficiencies or maternal infectious diseases, and/or the foetus, during pregnancy (Gowland and Halcrow 2019; Hodgkins et al. 2021; Nava et al. 2017b; Rebay-Salisbury and Pany-Kucera 2020). Histomorphometry of dental enamel allows the estimation of growth parameters such as the Daily Secretion Rate, i.e., the speed at which ameloblasts – the enamel-forming cells – moves towards the outer surface of the tooth (Dean et al. 2020; Mahoney 2012; Nava et al. 2017a), the Enamel Extension Rate, i.e., the rate at which ameloblasts differentiate along the Enamel Dentine Junction between the dentin horn and the cervix (Guatelli-Steinberg et al. 2012; Shellis 1984), and – in uncomplete growing crowns – the chronological age-at-death (Antoine et al. 2009).

The topographic variation of the Daily Secretion Rates across the crown was established by measuring prism segments' length throughout the crown and dividing the length of each segment by the number of counted Cross Striations (Nava et al. 2017a). The segments were

Table 1

Stages of crown formation, metric parameters and sex of each individual. Mesiodistal diameter (MD), buccolingual diameter (BL), and the height of the tooth crown (CH); [mm] millimetres. Abbreviations: UR = upper right; UL = upper left; LR = lower right; LL = lower left; di1 = deciduous first/central incisor; di2 = deciduous second/lateral incisor; dc = deciduous canine. M = male; F = female. Cr ½ = crown half completed with dentine formation; Cr ¾ = crown three quarters completed; Crc = crown completed with defined pulp roof. *Two teeth from the same individual.

Id	Tooth	Sex (Amelogenin based assessment)	Crown formation stage (Moorrees et al. 1963)	MD [mm]	BL [mm]	CH [mm]
T268	URdi1	M	Crc	6.34	4.80	5.96
T275 ind1a	LRdi2	M	Crc	4.39	3.77	5.85
T275 ind1b	LRdc	M	Cr ½	4.80	3.00	3.42
T275 ind2	ULdi1	F	Crc	6.20	4.90	6.15
T288	LLdi2	F	Crc	4.16	4.13	5.60
T300	ULdc	M	Cr ½	5.54	2.90	3.70
T278	ULdi2	M	Crc	5.41	3.48	3.90
T284	ULdi2	F	Cr ¾	5.98	3.83	5.11

measured from the apex to the cervix and throughout the crown. The number of segments measured varies from a minimum of 96 to a maximum of 146 and each segment counts from a minimum of two Cross Striations to a maximum of eight. The Cross Striations count was performed by two observers (BP and AN) independently. Additionally, the Prenatal Crown Formation Time was calculated for all individuals following Birch and Dean (2014) and Nava et al. (2017a).

2.3. Sex determination through proteomic analysis

Dental proteins were extracted from the teeth following published protocols (Lugli et al. 2020). Specifically, the resin-embedded teeth with the cut surface exposed were immersed in 1.2 M HCl for ca. 30 min at room temperature. Peptides were then purified by using in-house StageTips with C18 resin. Measures were performed with a nano-liquid-chromatography mass spectrometer (nLC-MS; Nano UHPLC Ultimate 3000 coupled to an Exploris™ 480 Hybrid Quadrupole-Orbitrap™ Mass Spectrometer) following Lugli et al. (2019). Chromatograms were manually searched for sex-specific amelogenin peptides, namely AMELX (SIRPPYPSY $[M + 2H]^{+2}$ 540.2796 m/z) and AMELY (M(ox)IRPPY $[M + 2H]^{+2}$ 396.7073 m/z; SM(ox)IRPPY; $[M + 2H]^{+2}$ 440.2233 m/z), following Stewart et al. (2017) and Lugli et al. (2019). No peaks were detected in the extraction blank.

2.4. Statistical analysis

Statistical analyses were conducted using the statistical software R version 4.2.2 (R-Core-Team 2022). The Crown Formation Time variation profiles were calculated with a locally weighted polynomial regression fit (Cleveland et al. 1992) of the lengths on the Enamel Dentine Junction against the Crown Formation Times. The maps of the topographic Daily Secretion Rate variation in the dental crowns were obtained from the sampling of Daily Secretion Rate local estimates collected across the exposed enamel in the thin sections. Six teeth were selected according to their readability of Cross Striations across the whole dental crown section. For each tooth, several sampling points (ranging between 90 and 140) were randomly selected taking care to obtain a homogeneous spatial distribution. The corresponding spatial distribution maps of the Daily Secretion Rate were calculated from the raw data using a surface obtained from a Generalized Additive Model fit using the R package mgcv (Wood 2017).

3. Results

Table 1 provides the stage of crown formation and some metric parameters of the tooth samples, namely the mesiodistal diameter, buccolingual diameter, and the height of the tooth crown and the results of the sex assessment through proteomic analysis. All the individuals show the presence of AMELX peptides, as expected for endogenous enamel

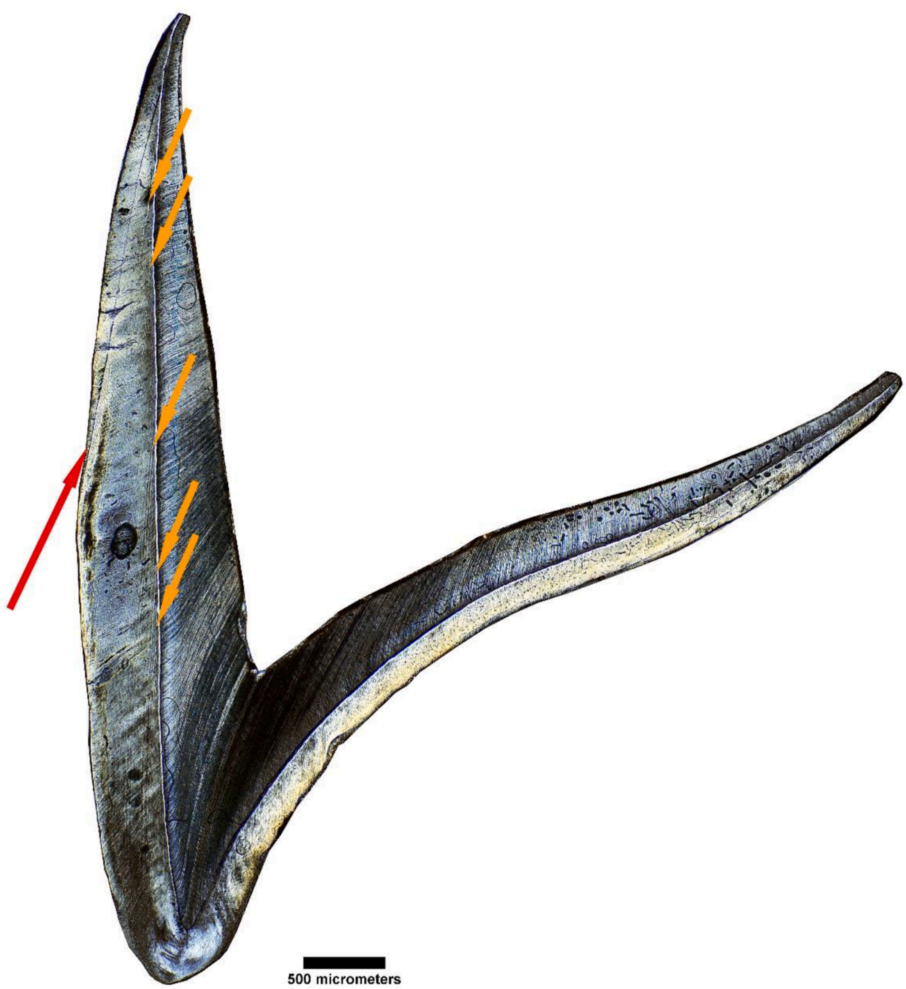

Fig. 2. Micrograph of T278: upper left deciduous lateral incisor (ULdi2); magnification 10x; The Neonatal Line is marked by a red arrow; the five Accentuated Lines in prenatal enamel are marked by orange arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

proteomes. Four of them show the additional presence of AMELY peptides and can be identified as male individuals (see Supplementary Fig. 1).

The Neonatal Line is visible in all thin sections (e.g., Fig. 2), therefore all individuals survived at least a few days after birth. The Neonatal Line allowed for the quantification of the enamel produced before death in the incomplete crowns. Table 2 reports the chronological age-at-death of the individuals based on the histological assessment compared with the age-at-death estimated through the dental developmental stages (AlQahtani et al. 2010). Overall, the youngest individual (T300) died 5 days after birth, whilst the oldest individual (T288) died at ~ 4 months of age (130 days). Due to the lack of the cervical portion of the tooth, individual T284 histological age-at-death has been estimated using the thickness of the postnatal enamel visible in the buccal aspect divided by the observed Daily Secretion Rate.

The histological age-at-death supports the morphological diagnosis in four (T268, T275 ind1b, T300, T284) out of seven specimens (Table 2). Conversely, in the remaining three individuals histological and morphological age-at-death differ.

Table 3 reports the number of Accentuated Lines detected in the prenatal and postnatal enamel for each individual. The Accentuated Lines in prenatal enamel reveal stress episodes affecting the foetus and/or the mother during pregnancy in four out of seven individuals (e.g., Fig. 2).

The Crown Formation Time of the prenatal portion of the crowns is reported in Table 4 for six out of eight specimens (T275 ind1b and T288 do not show enough clear incremental lines in the prenatal enamel).

The topographic distribution of the Daily Secretion Rates in the prenatal and postnatal enamel of the buccal aspect of each tooth is illustrated in Fig. 3.

Table 5 reports the basic statistical parameters of the Daily Secretion Rate values for each tooth.

Table 6 reports the mean Daily Secretion Rates calculated for each tooth, divided into prenatal and postnatal enamel and in the inner, middle and outer portions of the crown.

Fig. 4 shows the box-and-whisker plots of the prenatal Daily Secretion Rates values in the deciduous incisors by tooth class and sex of the individuals. The central incisors do not significantly differ between male and female individuals, while the lateral upper incisor is significantly different between sexes (Tukey's Honest significance differences test $p < 0.01$; Yandell 2017). The lower lateral incisor is characterised by the slowest Daily Secretion Rates values, and it is significantly different from all the other teeth (Tukey's Honest significance differences test $p < 0.01$). In general, males tend to show higher Daily Secretion Rates than females.

Fig. 5 reports the increase in Enamel Dentine Junction length during the formation of the deciduous crown for six of the Motya individuals compared with 18 deciduous central incisors from the Imperial Roman necropolis of Porta Marina at Velia (Nava et al. 2017a). The general trend shows a clear deceleration from the apex toward the cervix in the recruitment of new secretory ameloblasts along the Enamel Dentine Junction. The Motya individuals tend to occupy the lower portion of the Velia range with the central incisors showing the less pronounced acceleration and thus contrasting with the higher Daily Secretion Rates for the same tooth class.

Table 2
Age-at-death from histological assessment. The histological age estimation is compared with the one based on dental developmental stages.

Id	Chronological age-at-death (dental histology)	Morphological age at death (dental development)
T268	18 days	Perinatal
T275 ind1b	87 days	~ 3 months
T275 ind2	41 days	~ 3 months
T288	130 days	~ 3 months
T300	5 days	Perinatal
T278	28 days	Perinatal
T284	~ 14 days	Perinatal

Table 3
Occurrence of Accentuated Lines (ALs) in prenatal and postnatal enamel.

Id	ALs in prenatal enamel	ALs in postnatal enamel
T268	1	1
T275 ind1a	0	0
T275 ind1b	0	1
T275 ind2	0	0
T288	0	0
T300	1	0
T278	5	0
T284	2	0

Table 4
Individual Prenatal Crown Formation Time (pCFT) in days.

Id	Tooth	pCFT (days)
T268	URdi1	173
T275 ind1a	LRdi2	142
T275 ind2	ULdi1	176
T300	ULdc	96
T278	ULdi2	173
T284	ULdi2	164

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4. Discussion

This research represents the first study of an Iron Age Mediterranean infants' sample analysed via dental histology. While acknowledging the small number of individuals here analysed, this study shows how maternal-foetal and early infancy health status can be derived in ancient skeletal remains using histological analysis of tooth enamel, overcoming some of the limitations associated with the use of mortality samples (Bondioli et al. 2016; DeWitte and Stojanowski 2015; Wood et al. 1992).

In Motya's sample, a discrepancy between the estimates of age-at-death through morphological parameters (developmental stages of teeth) and histological age-at-death estimates has been observed in three out of seven individuals (Table 2). The presence of the Neonatal Line in all Motya specimens indicates that no stillbirths occurred in this sample. According to clinical studies, the occurrence of the Neonatal Line most likely reflects a decrease in blood calcium values within the first 48–72 h *ex-utero* (Ranggård et al. 1994). It also reflects the stressful event of foetal delivery from the mother's uterus (Sabel et al. 2008; Schwartz et al. 2010; Zanolli et al. 2011). Literature reports that a newborn must survive at least 7 to 15 extra-uterine days for the Neonatal Line to emerge completely (Birch and Dean 2014; Witzel 2014). Anyhow, three out of seven individuals died within the first twenty days after birth. Indeed, the birth process and the first few days after birth are the most crucial phases for the newborn's survival. Even nowadays, according to the World Health Organization (World Health Organization 2006), the peak of neonatal death occurs within four weeks of birth. The causes of death – which, we emphasise, can very rarely be determined in past skeletal populations – in the perinatal age may be various: among these are premature childbirth, low birth weight, infections, and trauma related to the expulsion of the foetus (Bakketeig and Bergsjø 2011). In pre-antibiotic societies, general population health conditions are equally important for neonatal survival. Unsanitary sources of water and food could expose people to infections such as cholera, dysentery, gastroenteritis, hepatitis, typhus, intestinal parasites, etc. Some of these result in severe dehydration which is still among the causes of infant death in developing countries (Kourtis et al. 2014). The gradual decrease of maternal antibodies in the first month of new-born life must also play a

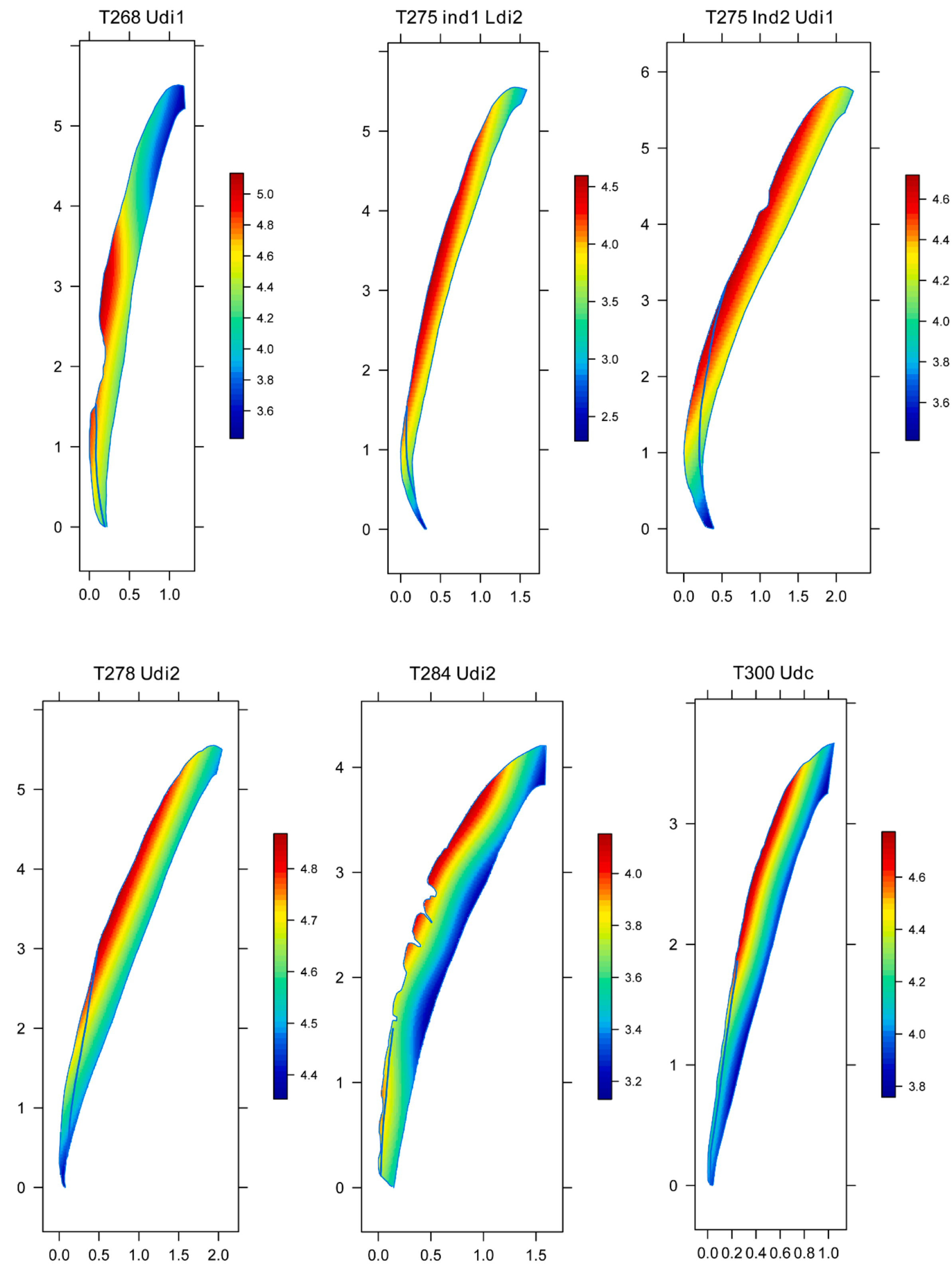

Fig. 3. Maps of the topographic Daily Secretion Rate (DSR) variation in prenatal and postnatal enamel of six out of eight teeth included in this study.

significant role (Hanson et al. 2003).

Physiological stress episodes *in utero* – visible histologically as Accentuated Lines in the enamel microstructure – can have a lasting effect on the health and survivorship of the infants (Harding 2001; Żądzińska et al. 2015). At present, only a few cases of prenatal Accentuated Lines are reported in literature. Accentuated Lines are detected in two out of eighty-three individuals in Żądzińska et al. (2015), in three out of twenty-nine individuals in Sipovac et al. (2023), in four out of twelve individuals in Lorentz et al. (2019); all six individuals in Kierdorf and collaborators' work (Kierdorf et al. 2021) have shown Accentuated Lines in prenatal enamel, while three different Accentuated Lines were detected in the Ostuni foetus analysed in Nava et al. (2017b). The

Table 5
List of the Motya teeth samples selected for the maps of the topographic Daily Secretion Rate (DSR). Six teeth belonging to six individuals were selected according to the optimal readability of Cross Striations across the whole dental crown. Abbreviations: SD = Standard Deviations; Min = minimum; Max = maximum.

Id	Tooth	N sample points	Mean	SD	Min	Median	Max
T268	URdi1	129	4.36	0.57	2.82	4.43	5.46
T275 ind1a	LRdi2	106	3.84	0.60	1.51	3.96	4.92
T275 ind2	ULdi1	108	4.36	0.39	3.33	4.42	5.12
T300	ULdc	140	4.16	0.50	2.98	4.11	5.54
T278	ULdi2	90	4.62	0.48	3.08	4.68	5.75
T284	ULdi2	137	3.55	0.42	2.33	3.60	4.53

paucity of pathological striae in prenatal enamel may be connected to the buffered condition in the intrauterine environment that shields the amelogenesis from the stressful events involving the mother's body (Antoine et al. 2009). However, Accentuated Lines can be detected in prenatal enamel if the intensity of the stressors overcome the defensive capacities of the intrauterine environment and the physiological disturbances are transmitted to the foetus implying a disruption of the amelogenesis (Lorentz et al. 2019).

At Motya, prenatal stress episodes have been detected in all individuals who died within one month from birth (T268, T300, T278, T284).

Interestingly, T278 shows five different stress episodes in the prenatal portion of the crown, depicting a highly stressful intrauterine life, possibly related to problems suffered by the mother and/or by the foetus during pregnancy. It is worth noting that Daily Secretion Rates values of this individual are the highest recorded in the sample, thus suggesting little correlation between the Accentuated Lines occurrence and the speed of enamel secretion. These episodes occurred at 120, 113, 94, 48, and 25 days before birth. Two individuals only (T268 and T275 ind1b) have a stress episode evident in postnatal enamel. The scarcity of Accentuated Lines in postnatal enamel (Table 3) can be attributed to the small amount of postnatal enamel available in the teeth, rather than a real absence of episodes of physiological stresses.

As highlighted by the Osteological Paradox (Bondioli et al. 2016; DeWitte and Stojanowski 2015; Wood et al. 1992), the reconstruction of life histories in ancient skeletal populations may be vitiated by certain biases, such as 'hidden heterogeneity in risk' and individual 'frailty'. These parameters may affect, for instance, individual growth rates and stress responsiveness; but this cannot be specifically tested. While considering these possible biases even in the Motya sample, we found that these infants show a positive correlation between the presence of

Table 6
Mean Daily Secretion Rates (DSRs) by pre- and postnatal enamel, and the inner, middle, and outer part of the crown.

Id	Tooth	Average DSR (μm/day)	Prenatal DSR (μm/day)	Postnatal DSR (μm/day)	Inner region DSR (μm/day)	Middle region DSR (μm/day)	Outer region DSR (μm/day)
T268	URdi1	4.36	4.35	4.47	4.20	4.41	4.54
T275 ind1	LRdi2	3.84	3.94	2.78	3.92	3.87	3.66
T275 ind2	ULdi1	4.36	4.36	4.34	4.23	4.33	4.66
T300	ULdc	4.16	4.17	3.16	3.90	4.31	4.37
T278	ULdi2	4.62	4.63	4.60	4.53	4.67	4.77
T284	ULdi2	3.58	3.57	4.25	3.32	3.71	3.71

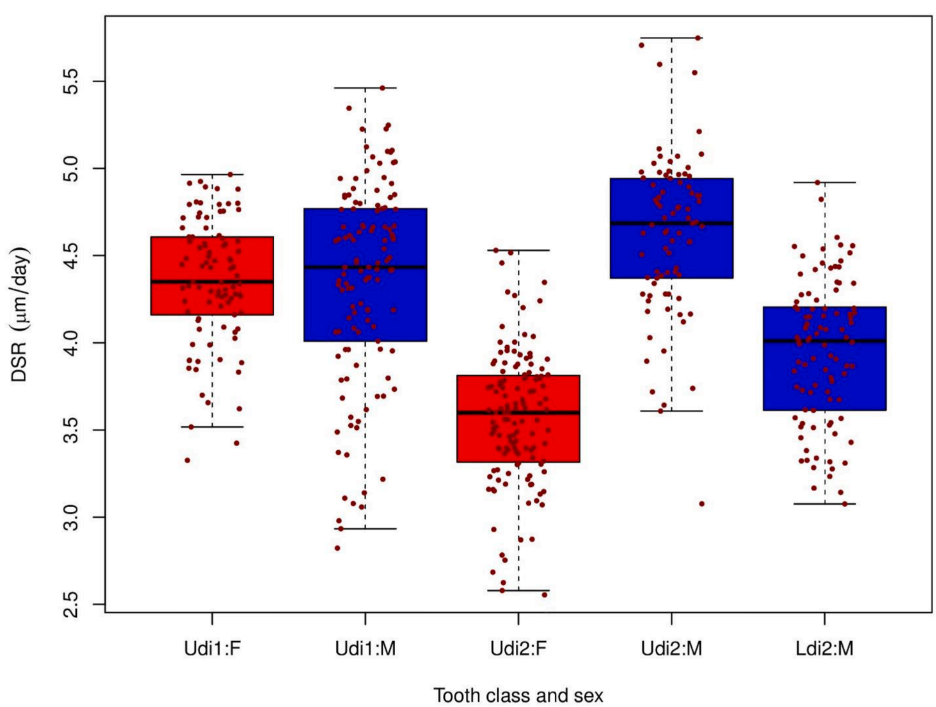

Fig. 4. Box-and-whisker plots of the Daily Secretion Rate (DSR) variation in prenatal enamel of the deciduous incisors included in this study. Bold line = median, limits of the box = first and third quartile, whiskers = maximum and minimum values. Dots outside the whiskers represent outliers according to the Tukey's 1.5x interquartile range criterion.

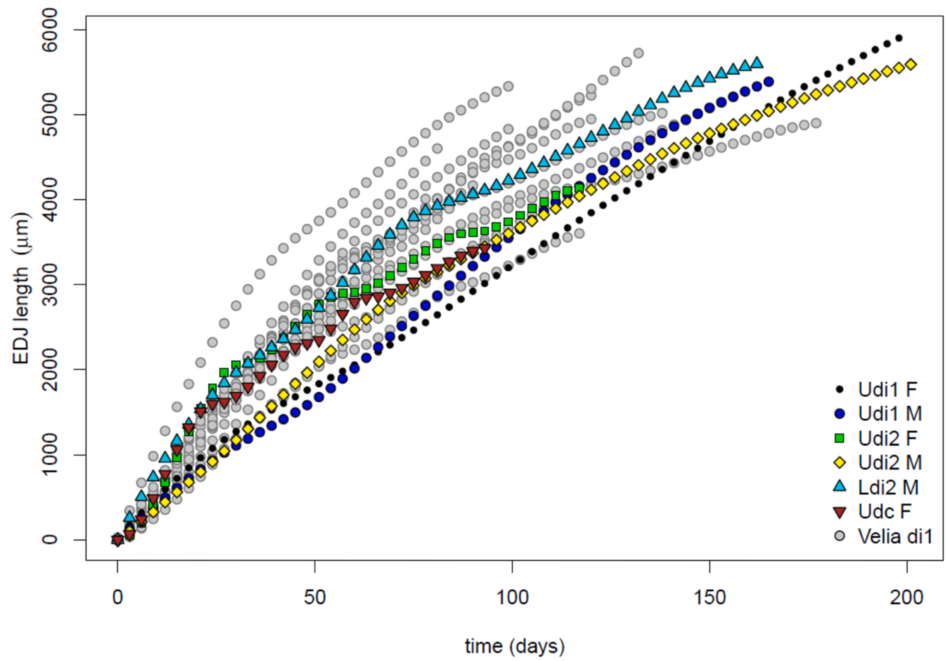

Fig. 5. Individual Crown Formation Time (CFT) variation contrasted with the Enamel Dentine Junction (EDJ) length in the Motya deciduous incisors compared with data from the Imperial Roman necropolis of Porta Marina at Velia (Nava et al. 2017a). Each profile was calculated from the discrete observations along the EDJ interpolated with a locally weighted polynomial regression fit (Cleveland et al. 1992; Nava et al. 2017a).

stress recorded during the prenatal phase and early infant death. Infants who died within two months of life recorded at least one intrauterine stress, possibly due to the poor health condition of the mother. These findings support the DOHaD hypothesis that individuals who experience systemic stress during intrauterine and early childhood life tend to die relatively earlier (Almond and Currie 2011; Armelagos et al. 2009; Barker et al. 2002; Cosmi et al. 2011).

All the Daily Secretion Rate maps show a relative stability of the daily rates along the Enamel Dentine Junction in the lateral portion. All teeth in our sample show a pattern of increasing Daily Secretion Rates towards the outer enamel, and a decrease toward the most cervical portion. This same pattern has been reported in deciduous teeth from both modern and archaeological populations (Birch and Dean 2014; Dean et al. 2020; Mahoney 2012; Nava et al. 2017a). Concerning the deciduous canine (T300), the mean Daily Secretion Rate immediately after birth (3.16 µm/day) is considerably slower than the mean Daily Secretion Rate in prenatal enamel (4.17 µm/day), showing a substantial reduction of secretion rate immediately after birth. In the deciduous canines analysed by Dean et al. (2020), a similar decrease in Daily Secretion Rate immediately after birth was also observed. The authors report Daily Secretion Rates within 30 days after birth of 3.09 µm/day, while faster rates were observed near the Enamel Dentine Junction (3.31 µm/day) (Dean et al. 2020) which also fit with the Daily Secretion Rate values in the corresponding region of the deciduous canine (T300) in our sample.

As deciduous crown formation is initiated during the early stages of pregnancy, a considerable portion of the crown is formed *in utero* (Nava et al. 2017b). As shown in recent work, prenatal enamel has the potential to provide a window into the health status of the mother during the later stages of gestation and also into foetal stress events (Guatelli-Steinberg 2016; Hillson 2014; Nava et al. 2017b; Nava et al. 2019). Moreover, the Prenatal Crown Formation Time enables us to discriminate between neonates born full-term (~40th week of gestation) or preterm, assuming a constant crown initiation time in humans (Birch and Dean 2014). In the Motya sample, T268 and T275 ind2 central deciduous incisors present a Prenatal Crown Formation Times of 173 days and 176 days, respectively. These are well within the range of values reported in the literature for central deciduous incisors (Birch and Dean 2014; Mahoney 2015; Nava et al. 2017a).

The Prenatal Crown Formation Times of the three deciduous lateral incisors of Motya's sample (T275 ind1a, T278 and T284) fall within the published range of values (Birch and Dean 2014; Mahoney 2012; Mahoney 2015). Specifically, T275 ind1a (lower di2) has a Prenatal Crown Formation Time of 142 days, in agreement with the range reported by Mahoney (2012) (84–189 days), whilst T278 and T284 (upper di2) have a Prenatal Crown Formation Times of 173 and 164 days, respectively, which compare well with Mahoney (range = 90–216 days, Mahoney 2015), and with Birch and Dean (range = 126–175 days, Birch and Dean 2014) values. The Prenatal Crown Formation Time of T300 (upper canine) is 96 days and is also within the range reported by Mahoney (2012) (13–76 days) and Dean et al. (2020) (90–150 days). Therefore, the Prenatal Crown Formation Time values of the Motya sample are within the range reported in the literature and, consequently, the individuals can be considered as born full-term.

The pace of enamel secretion (Daily Secretion Rate) is similar between the sexes for the central deciduous incisors, as already observed in deciduous molars and in permanent teeth (Aris et al. 2020; McFarlane et al. 2021). Conversely, in the Motya sample the mean Daily Secretion Rates are significantly different between the male lateral incisor (T278) and the female one (T284). This result provides an additional building block for understanding the process of dental enamel apposition and mineralization in the human prenatal stage. Together with possibly sex-related differences in DSRs, we emphasise how other factors may have also contributed to the occurrence of discrepancies between DSRs in the lateral incisors of female and male individuals at Motya. In the literature, physiological and environmental factors – such as hereditary factors, intrauterine malnutrition, maternal medical complications, dietary factors, deficiencies of vitamin D, low calcemia, and maternal psychological stresses – are reported to be impacting the metabolic and functional balance, interfering with odontogenesis and enamel mineralization in utero, slowing or accelerating these processes (Collignon et al. 2022; de Oliveira et al. 2020; Pinho et al. 2012; Tapias-Ledesma et al. 2003; Thomaz et al. 2015).

The growth trajectories along the Enamel Dentine Junction of the

Motya deciduous teeth shown in Fig. 5 suggest that the deciduous anterior teeth at Motya grew more slowly during the prenatal period when compared with the growth profiles from the perinatal and infant individuals from Velia (Nava et al. 2017a), thus suggesting the Motya women may have experienced worse environmental conditions during gestation than Velia's ones.

5. Conclusions

The Motya necropolis has returned, over the years, a significant number of infant and foetal skeletal remains. To contribute to the ongoing debate on the differential funerary rituals between the necropolis and the tophet in the Phoenican-Punic world, it is necessary to accurately estimate age-at-death and, for inhumations, the biological sexes of the infant and foetal remains through aDNA or proteomic analyses.

Despite the limited sample size, this study offers a methodological approach that can be extended to larger investigations from the same or similar funerary contexts.

The high occurrence of Accentuated Lines in prenatal enamel and the possibly reduced enamel extension rates suggests harsh intrauterine life conditions that fit well with the general high mortality rate in early infancy at Motya (Lauria et al. 2018; Lauria et al. 2017; Lauria et al. 2020).

From a historical and cultural-behavioural perspective, this study contributes to a better understanding of the funerary treatment and socio-cultural perception of newborns and infants in Motya's archaic society.

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CRediT authorship contribution statement

Beatrice Peripoli: Data curation, Investigation, Writing – original draft, Writing – review & editing. **Melania Gigante:** Writing – original draft, Writing – review & editing. **Patrick Mahoney:** Funding acquisition, Investigation, Resources, Writing – review & editing. **Gina McFarlane:** Investigation, Writing – review & editing. **Alfredo Coppa:** Funding acquisition, Resources, Writing – review & editing. **Federico Lugli:** Data curation, Investigation, Writing – review & editing. **Gabriele Lauria:** Investigation, Writing – review & editing. **Luca Bondioli:** Conceptualization, Data curation, Formal analysis, Supervision, Writing – original draft, Writing – review & editing. **Paola Sconzo:** Investigation, Resources, Writing – review & editing. **Luca Sineo:** Conceptualization, Resources, Writing – review & editing. **Alessia Nava:** Conceptualization, Data curation, Funding acquisition, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jasrep.2023.104024.

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