### RESEARCH



# Skeletal remains of human perinatal individuals from the fortified Iberian Period settlement of Ca n'Oliver (6<sup>th</sup> century to 50 years BCE)

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### Abstract

Burial customs in the Iberian Period (Iron Age II) included cremation. Only perinatal and newborn infants were buried directly beneath floor settlement. These infants represent the very few unburned human remains recovered from Iberian sites. The interpretation of these infant burials is in debate, focusing on whether they are unnatural or natural deaths. Our aim is to infer mortality patterns and developmental conditions of these individuals, in order to respond if infanticide was present in these assemblages. A large perinatal human skeletal sample from the Ca n'Oliver site (sixth century to 50 years BCE) from the Iberian Period of the northeast of the Iberian Peninsula was analysed, combining osteological methods together with tooth histology and aDNA analysis. Combining osteological and odontological estimates indicated ages between 22 and 42 weeks of gestation for 47 out of a total of 48 individuals. The remaining individual died at about 6 months after birth. Tooth height and enamel histology indicated in 9 out of a subgroup of 13 individuals a low probability of live birth. The remaining 4 individuals possibly survived birth for less than 2 months. According to morphological and molecular results, the sex ratio of this sample is approximately 1:1 male to female. The mortality distribution is consistent with natural mortality. These perinatal deaths were probably spontaneous abortions and neonatal deaths, reflecting an endogenous mortality profile due to genetic and maternal influences. The present study will serve to broaden our knowledge on perinatal individuals of the Iberian Period.

Keywords Perinatal individuals · Neonatal line · Tooth histology · aDNA

### Introduction

According to the ancient classical historiographers like Strabo, the Iberians (Iron Age II) constituted a group of different communities (e.g. Laietani, Lacetani, Ilergetes, Indigetes, Cessetani), that, during the second half of the first millennium Before Current Era (BCE), populated the coastal

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areas of the Mediterranean from the Herault in today's southern France to Andalusia in the meridional Iberian Peninsula (Sanmartí & Santacana 2005; Sanmartí, 2009; Tirado 2010). This is in agreement with the archaeological and epigraphic evidences like settlement constructions, pottery style, coins, funerary stelae, and lead sheets, left by the Iberians themselves which differentiate them from other groups. The same written language (Iberian) was used in this Mediterranean

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coastal region, which is not currently possible to decipher because it has no relation with any other known language (Sanmartí and Santacana 2005; Sanmartí, 2009). At this point, we should differentiate between the Iberian Peninsula and the Iberian culture, because at this proto-historical time, the centre, north-west, and west of the Peninsula were inhabited by other populations (such as Celts, Celtiberian, and the possibly Pre-Celtic Indo-European Lusitanians, Vettones, and Turdetani), which had different languages and cultures (Almagro-Gorbea 2001).

The Iberian Period represents the ending of the prehistory in this coastal region (Sanmartí, 2004). It was the result of a socio-cultural change from native Bronze Age local groups of the Mediterranean coast (from Andalusia to south of France in the Languedoc region) to stratified societies and regional political entities. This socio-cultural change was due to (i) the increase in population density, (ii) the incorporation of iron technology, and, very importantly, (iii) the commercial interaction with Phoenician-Punic and Hellenic colonial centres in this coastal region. This socio-cultural change led to an administrative system whose most evident expression is found in (i) the strongly hierarchical structure of the settlement, (ii) the expansion of writing and its use in accounting documents, and (iii) the great proliferation of large grain storage areas (silo fields), which demonstrate control over production. At the same time, it can be considered as large capital reserves by part of the elite (Sanmartí, 2004). Evidence available from the Iberians is either from ancient texts written by foreigners or from the archaeological record. There is no direct bioarchaeological information derived from the remains of the individuals themselves. The Iberians usually cremated the bodies of the dead at temperatures close to 660 °C and all the bones are mostly destroyed (Sanmartí & Santacana 2005). In addition, the necropolises (urns fields) excavated are too small for the sizes of the associated settlements, indicating that only a portion of the population have been buried there (Sanmartí and Santacana 2005; Albizuri et al. 2012). These burial customs represent a major impediment to the study of the biological anthropology of these people.

Only remains of very young infants that could anthropologically be determined as perinatal and newborn individuals were found buried directly beneath the floor of buildings in the settlement. This comprises chambers of houses or workshops, such as areas for metallurgy and metal processing (Gusi and Muriel 2008; Crespo et al. 2011; Albizuri 2011). These infants represent the very few unburned human remains recovered from Iberian sites. Their bones and teeth offer the potential for gathering information on aspects of health and disease of these individuals themselves as well as for aspects of the maternal condition during the final phase of pregnancy. The

biological anthropology of foetuses and neonates together with the intimate physical connection with their mothers has only recently moved to the forefront of bioarchaeology (Halcrow et al. 2017; Lewis 2017; Gowland and Halcrow 2020; Alberto-Barroso et al. 2022). The combination of different indicators from skeletal and dental development is able to provide a more differentiated picture of the developmental conditions and on possible interactions with the health of the mother (e.g. Hodson and Gowland 2020). The use of tooth histology provides an individual record of the incremental growths of teeth that is independent of referring to mean values of cross-sectional data and is better able to document variability (Dean et al. 2020). The presence of accentuated incremental markings in the pre- and postnatal portions of the dental hard tissues of the deciduous dentition is interpreted as stress markers that relate to the development in utero as well as after birth (Witzel 2014; Nava et al. 2017a; Lorentz et al. 2019; Kierdorf et al. 2021).

If the differential treatment in terms of burial customs of this portion of the population is rooted in differences in personhood attributed to it or related to some sorts of demographic factors is a further subject of debate. This debate focuses on whether the causes of death of these infants were unnatural, i.e. the product of sacrifices/infanticides (related to e.g. foundational rituals or natality control), or the result of natural mortality along with a differential burial custom (Muriel et al. 2006). This discussion originated because, in some cases, in the same settlement chambers or workshops, these infant burials coincide with the remains of caprines from possible sacrifices (Barrial 1989). Evidence of animal sacrifices is corroborated by zooarchaeological studies (Belarte and Sanmartí, 1997; Sanmartí and Santacana 2005; Albizuri 2011; Albizuri et al 2012) as well as by Strabo ancient text. However, some authors argued that considering infant burials as evidence of infanticide just by the fact that they lie outside cemeteries or religious areas is perhaps too simplistic and somewhat speculative (Ucko 1969; Harris 1982; Woodburn 1982; Mays 1993). Differential burial treatment of children under 1 year of age is found in native pre- and proto-historic Western Mediterranean populations prior to the Phoenician presence (Gusi and Muriel 2008). Indeed, there is evidence of a long endurance of this differential burial treatment from Late Bronze/Early Iron Ages until the end of the Roman and Medieval period (Fernández-Crespo 2008) in this geographical area. There is also ethnographic evidence from many societies in which infants may have different burial treatment from the rest of the population (Ucko 1969; Harris 1982; Woodburn 1982; Mays 1993).

Referring to historical and ethnographical sources, infanticide was a common practice and has been tolerated

in many different societies (references have been compiled e.g. by Mays 2014 and Gilmore and Halcrow 2014). In the absence of specific traces indicative of a violent death, age at death distributions indicating a tight clustering at gestational ages of 38-40 weeks had been used as an indicator for infanticide and were demonstrated in the Roman samples from Ashkelon and several Romano-British sites (Smith and Kahila 1992; Mays 1993; Mays and Eyers 2011). However, the use of regression-based methods has been shown to emphasize the peak at 38-40 weeks while a method based on Bayesian inference yields a more even distribution of gestational ages (Gowland and Chamberlain 2002; Lewis and Gowland 2007; Mays and Eyers 2011). Another line of arguments from a bioarchaeological perspective is centred around whether there are indications that the buried individuals were born alive (Smith and Avishai 2005; Schwartz et al. 2010; Witzel 2014), by histologically observing the presence of a specific accentuated incremental enamel mark, the neonatal line (NNL). This mark forms in relation to the disturbances of foetal physiology caused by the birth process in the deciduous teeth and the first molars that commence their crown formation prior to birth (e.g. Hillson 2014). Only individuals that survived birth could have been at risk for being killed or sacrificed. A certain amount of time of survival beyond birth in the range of several days to about a week is needed for the NNL to become detectable (Whittaker and MacDonald 1989; Boyde 1990; Witzel 2014) and incompletely mineralized enamel is more prone to taphonomic loss (Antoine et al. 2009). Thermal alteration might potentially also obscure the NNL, attested by the controversy on the interpretation of the cremated infant remains from the Tophet of Cartharge (Schwartz et al. 2010, 2017; Smith et al. 2011, 2013).

A sex bias in an assemblage of very young infant skeletons might also yield clues on the likelihood that those individuals had been killed, since offspring not of the desired sex was at higher risk of infanticide (Kumm et al. 1994). Given large enough sample sizes, a biased sex ratio (Faerman et al. 1998; Mays and Faerman 2001), as well as the overrepresentation of the developmental age typical after a full-term gestation (Mays 1993; Gowland and Chamberlain 2002), has been controversially discussed as indirect evidence that a certain proportion of victims of infanticide were likely present in death assemblages (Gilmore and Halcrow 2014; Gowland et al. 2014; Mays 2014, 2021). However, studies on relatively large samples of Iberian babies are lacking. The few studies that exist (Agustí 2000; DeMiguel 2002, 2005; Crespo et al. 2011; Merino et al. 2014) are based on two or three individuals; because of the small sample size, these studies are unable to furnish significant information on the conditions of child mortality in the Iberian Period. Understanding infant mortality patterns and related factors such as gestation length can assist a more accurate interpretation and reconstruction of very early life health conditions in relation to cultural practices such as burial rituals (Schwartz et al. 2010; Kinaston et al. 2009).

Here we present the bioanthropological study of the large perinatal human skeletal sample from the site of Ca n'Oliver (Cerdanyola del Vallès, Barcelona) (2° 08' 12" E; 41° 28' 57" N), a large fortified Iberian Period settlement dated from sixth century to 50 years BCE and located in the north-east of the Iberian Peninsula in Catalonia (Fig. 1). In order to overcome limitations of conventional osteological and dental ageing methods that always make reference to populationbased growth standards, we also used tooth histology with the aim of documenting the presence of a neonatal line (NNL) as an independent indicator of birth survival (Witzel 2014; Siebke et al. 2019; Kierdorf et al. 2021). Combining the results from both approaches offers a broader basis to infer developmental conditions and mortality patterns of foetuses and newborns of the Ca n'Oliver Iberian Period site, to reconstruct identity at the earliest stages of life in this population and better understand Iberian funerary rituals in order to respond to the question if infanticide was likely present in these death assemblages. It will also prepare the ground for a larger bioanthropological study involving further analytical methods like stable isotope, ancient DNA (aDNA), and more detailed histological and developmental analyses.

### Ca n'Oliver archaeological site (Francès and Guardia 2012)

In the Iberian Period, the most outstanding in terms of architecture are the fortifications and town walls. Regarding the settlements, three types are described in relation to their complexity and size (Sanmartí and Santacana 2005): (i) small settlements without fortification located in flat areas and with an economic, agricultural, and livestock functionality. (ii) Fortified enclosures that used to be in high areas with surfaces between 2 and 4 hectares. They had certain structural complexity and strong defences. Examples of these type of settlements are Turó d'en Boscà (Badalona), Torra dels Encantats (Arenys de Mar), and Ca n'Oliver (Cerdanyola). (iii) Oppida (largest towns) were centres that controlled a region or territory. These oppida are the capitals mentioned by Strabo and Pliny the Elder in the written ancient sources. This last type of towns used to be surrounded by stone and rammed earth walls, on which were placed watchtowers and the gates to the city. The main Iberian oppidum on the coast of Catalonia (northeast of the Iberian Peninsula) was el Puig de Sant Andreu and Illa d'en Reixac in Ullastret (Baix Empordà), with an extension of 17 hectares (Sanmartí and Santacana 2005).

Ca n'Oliver was a large fortified settlement (2 hectares), dedicated to gathering and redistributing production, which

**Fig. 1** Localisation of Ca n'Oliver in Catalonia (Spain), north-east of Iberian Peninsula



had an important artisan component. The social importance of this settlement is evidenced by the prominent social elements found such as weapons and sculptures. Ca n'Oliver is situated in the current region of Catalonia in the north-east of the Iberian Peninsula, specifically located in Cerdanyola del Vallès (Barcelona, Spain). The settlement was erected on a hill (138 m above sea level) of the Collserola mountain range (Fig. 1), which forms part of the Catalan Coastal Range. The settlement extended on an east-west axis and was situated at a distance of 6 km from the Barcelona coast and bordered in the north by the plain of Vallès (coordinates 2° 08' 12" Long. E 41° 28' 57" Lat. N. (C. UTM: 31DF277927)). Geographically, it is located in the area that classical texts named Laietania (Barberà and Dupré, 1984). Its position represented a vantage point on Cerdanyola del Vallès and all the plains of Vallès, Montseny massif, and the Pre-Coastal Range. The site is connected with inland areas and the coast by the Besos River and Sant Cugat stream, a tributary to Besos River. The site was inhabited from 550 to 50 years BCE, becoming an important Iberian Period trade centre from the fourth century BCE onwards.

The first occupation of the settlement, called phase 0 (550/535 BCE), corresponds to the Early Iberian Period. This phase was previous to proto-urban occupation of the site and it is represented by a group of huts made of wood and clay (zone 2), seated directly on bedrock, of which only some post holes and cuts are conserved.

Phase I (525-425 BCE) already corresponds to an urban occupation during the Early Iberian Period. The settlement increased its size and town walls were built. The construction in this period combined elements linked to the previous building tradition, using deep cuts in the rock, as well as hard construction, now with stone walls and the structuring of domestic spaces and common public areas of passage. The houses are defined by the construction of a common wall, divided by perpendicular walls which delimit the different houses' spaces. Houses have one chamber with a floor levelled by compacted clay. Archaeological data indicates that at this phase most of the activities were practiced outdoors and houses were used exclusively as a shelter. In this phase the presence of domestic rituals signs started, specifically there are three deposits of caprine remains and one infant inhumation (Fig. 2).

In phase II (425–300 BCE; High Iberian Period), the settlement expanded, accompanied by an increase in population density. A significant remodelling of the existing houses is observed, as well as the occupation of new terraces. The houses of this period have at least two chambers. The internal space of the houses became more specialized since the room comprises multiple tools and instruments built in different materials (paved floor, hearth, etc.). This is interpreted showing that different areas of the houses had different roles (cereals processing, warehouse, etc.). Artisanal production is documented for the first time in Ca n'Oliver during this



Fig. 2 General plan of the Can'Oliver settlement, showing the localisation of the inhumations of infants found in this site dating to the Iberian period

period. It is necessary to highlight the sectors numbers 25 and 27 where great quantities of forge remain; two metallurgical ovens and several fireplaces were found, indicating that these spaces were dedicated to metalworking. During this phase, presumable animal sacrifices as well as infant inhumations (Fig. 2) became much more frequent. In this phase, terracottas in the form of the head of Demeter appear, which is related to domestic cults (Francès and Guardia 2012).

During phase III (300-200 BCE), situated in the High Iberian Period, some important reforms took place in Ca n'Oliver. The urban planning initiated at phase II takes more strength and manifest itself in a profound transformation of the town that affects both houses and collective spaces. There was a reordering of the houses of the neighbourhoods of phase II. In many cases, houses were extended following the scheme of the fourth century BC rooms. This transformation also involved the placement of a new sidewalk with paving stones and paving of the street with clay and slate crust. However, the greatest transformation took place in the external defences of the town (Francès and Guàrdia 2011). A trapezoidal tower attached to a new settlement gate was built partially on the old wall and on an old house (Fig. 2). This expanded the foundations of the tower to support the weight of a structure of this size, while projecting towards the interior of the town to maintain the line of the wall defined in the previous phase. In addition, a system of pits, that surrounded the town, were constructed to reinforce the external defences. Furthermore, in this period, an extended group of silos was also created outside the town near the south gate. These deposits had a considerable capacity, which oscillates between 3 and 5 m of depth. It was a period of great accumulation of surplus. In fact, phase III corresponds to a very prosperous period of Ca n'Oliver as an Iberian town, in which it was the Iberian core settlement of an extensive territory. At the end of phase III, the town is abandoned, probably forcefully due to events related to the Second Punic War.

Phase IV (200/175–50 BCE) is situated in a Roman context (Final Iberian Period), dating from the early decades of the second century BCE. During this phase the settlement was reorganized without regard to the previous constructions. The settlement was finally abandoned around the year 50 BCE.

### **Material and methods**

The material of this study comes from different archaeological campaigns carried out at Ca n'Oliver site, specifically the campaigns 1991 to 2007 and 2014 to 2015. A total of 30 burials and several isolated bones were obtained (Table 1). As usual in the Iberian Period, these inhumations were found under pavement structures of the settlement chambers, not always domestic in nature, but also associated with craft activities such as tannery and metallurgy (Albizuri 2011; Francès and Guardia 2012). Figure 2 shows the specific zones of the site from where the analysed sample was exhumed.

The anthropological analysis of this material was carried out at the human bone laboratory at the Unit of Anatomy and Human Embryology in the University Rovira i Virgily, Reus (Tarragona, Spain). The material arrived at the lab in boxes

Table 1 Burial count in each archaeological campaign

Campaign	Burials number
1991	4
1998, 1998–1999	5
1999	5
2000	5
2002	3
2003, 2006, 2007	3
2014	1
2015	4
	Total 30

containing each burial and the isolated bones separately. The skeletal material was cleaned dry by soft brushes and wooden chopsticks. To avoid contamination, we renewed the cleaning material for each individual and used latex gloves and masks. We also inventoried, and if necessary, restored the osteological material. When more than one individual was contained in a burial, it was necessary to attribute the different skeletal elements to each individual. To do this, we took into account the doubled occurrences of skeletal parts, along with differences in size, robustness, pathology, sex, and degree of maturation of these elements (Duday 1990; Villena 1997; Duday et al. 2000). We numbered the different individuals by keeping the numeration received at the excavation. In the case of isolated bones, we calculated the minimum number of individuals (MNI), taking into account the number of repeated bones and their morphology, size, robustness, pathology, sex, and degree of maturation (Brothwell 1987; Bradley and Byrd 2014).

Funerary ritual and spatial relationship of the individuals were described by considering the archaeological documentation (i.e. the drawings and photographs along with the archaeological observations collected during the excavation).

The bioanthropological study of this material consisted of the calculation of the preservation index, the anthropometric study, the paleopathological analysis, and sex and age estimation of the individuals. For a better comprehension, this section has been split into three different subsections according to the different bioanthropological analysis applied and these are set out below.

### Preservation index and anthropometrical analyses

The preservation status of the sample was calculated according to the preservation index of Alesan (1990), which shows the percentage of preserved elements [IP<sub>3</sub>=(Number of present bones/22)×100]. The considered bones are 22 bones: long bones plus both girdles and the cranium and mandible. The presence of a part of a bone is considered as the entire bone, and because of this, it does not inform us about the physical state of the bone but it is useful to inform us about the number of preserved bones.

The anthropometrical study was based on the classical measures of Fazekas and Kósa (1978), Rissech et al. (2001, 2013a, b), and Rissech (2016).

### Sex estimation

Sex estimation was based on the morphological criteria of Schutkowski (1993) based on angle and depth of the greater sciatic notch and the curvature of the iliac crest. However, to account for potential misclassifications, we also extracted and sequenced aDNA in a subsample of 7 individuals (Nen 4, IH 84, INH 105, IH 228, IH 456, IH 90, UE 1257). The selection of these 7 individuals was based on good chemical preservation based on elevated ultra-filtered collagen yield (>2%).

DNA extraction and preparation of genomic libraries were carried out at aDNA facilities located at the Archaeological Research Laboratory (AFL), Stockholm University. The DNA was extracted from 80-120 mg of bone powder from different long bone fragments representing each of the eight tested individuals (Table 2). The surface of the samples was mechanically removed, and the cleaned samples were decontaminated by UV irradiation at 265 nm, 1 J/cm<sup>2</sup> on each side. Thereafter the samples were powdered using Dremel®4000 drill. The bone powder was digested overnight (buffer 0.5 M EDTA pH 8, 1 M urea, 100 µg/mL Proteinase K), and the obtained extract was purified with silicabased spin columns (Yang et al. 1998; Svensson et al. 2007; Malmström et al. 2009) - MinElute PCR Purification Kit (Qiagen), following manufacturer's protocols. Thereafter, 20 µL of each of the DNA extracts was used for preparation of blunt-end Illumina genomic libraries (Meyer and Kircher 2010). The libraries were amplified with single-indexed primers and AmpliTaq® Gold DNA Polymerase (Applied Biosystems<sup>TM</sup>) in seven separate PCR reactions which were pooled, and purified with Agencourt AMPure XP magnetic beads (Beckman Coulter). Obtained genomic libraries were quantified on Agilent 2100 Bioanalyzer Instrument (Agilent Technologies) and screened (shotgun sequenced) on a single Illumina HiSeqX lane (v2.5, PE  $2 \times 150$  bp) at the Science for Life Laboratory Sequencing Centre in Stockholm. Thereafter, six of these seven individuals underwent additional sequencing of either the same (Nen004, IH456, IH901, UE1257) or of a second freshly prepared genomic library (IH084 and IH105). Obtained raw DNA data de-multiplexed based on individual indexes, guality-controlled, and delivered to UPPNEX (UPPmax NEXt Generation Sequence Cluster & Storage) (Lampa et al. 2013).

Sequence analysis computations were performed on resources provided by SNIC through Uppsala Multidisciplinary

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Centre for Advanced Computational Science (UPPMAX). The 150-bp pair-end sequence reads were merged and trimmed using M. Kircher's "MergeReadsFastQ\_cc.py" script (Kircher 2012) and mapped thereafter to the human reference genome 19 (build 37) with BWA v. 0.7.13 (Li and Durbin 2010) (aln with non-default parameters: -1 16,500 -n 0.01 -o 2). Finally, sequences shorter than 35 bp, those with more than 10% mismatches to the human reference genome, and the PCR duplicates were disposed of with FilterUniqueSAMCons.py (Li et al. 2009). Bam files representing different sequencing runs were merged using SAMtools v.0.1.19 (Li et al. 2009).

Obtained DNA fragments were tested for the presence of 3' and 5' misincorporation patterns using PMD tools (Skoglund et al. 2014). The presence of 3' and 5' misincorporations is associated with ancient DNA degradation (Briggs et al. 2007; Hansen et al. 2001; Hofreiter et al. 2001; Orlando et al. 2011; Sawyer et al. 2012). Levels of contamination were estimated by assessing the patterns of distribution of polymorphic sites in the mtDNA consensus sequences of our samples compared to a reference panel (Green et al. 2008). As a second measure, we use the likelihood method (Fu et al. 2013), estimating levels of contamination by testing the probability of mapping of individual sequences to different templates. The consensus sequence was called with SAMtools v.0.1.19 (Li et al. 2009), only using reads and bases with a minimum mapping and base quality of 30.

The biological sex of the individual was estimated based on the ratio of sequences aligning to male and female sex chromosomes, XY and XX respectively. The analyses of the ratio ( $R_Y$ ) between the number of sequences aligning to the Y chromosome ( $n_Y$ ) and the sum of sequences aligning to both X and Y chromosomes ( $n_X + n_Y$ ) are useful in individual sex identification (Skoglund et al. 2013). As a rule, individuals with  $R_Y \le 0.016$  are females, while those with values  $R_Y \ge 0.077$  are recognized as male (Skoglund et al. 2013).

### Age estimation and neonatal line

For age estimation, we used the skeletal measurements and maturation stage process of the different skeletal elements (i.e. the pars basilaris and pars lateralis of the occipital, ischium length, ilium length, femoral diaphysis length, tibial diaphysis length, cranial and long bones, among others) based on the information of Scheuer and Black (2000) and Fazekas and Kósa (1978). When in an individual both leftand right-side measurements from the same element could be obtained, we averaged them for age estimation.

From these methods, we chose as estimated age the midpoint where the age intervals overlapped. The use of bone length and maturation stages are useful for estimating age in perinatal individuals because development is very rapid

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Archeological sample ID	Bone	Genome coverage	MtDNA genome cover- age	NchrY and NchrX	NchrY	Ry	SE	95%CI	Molecular sex assignment	MtDNA contamina- tion estimate (95%CI)	MtDNA authen ticity likelihood
Nen 4	Humerus	0.11	39.48	153,570	11,701	0.0762	0.0007	0.0749-0.0775	XY*	10.89 (8.53–13.25)	0.9432468
IH 84	Fibula	0.04	15.37	53,572	4,488	0.0838	0.0012	0.0814 - 0.0861	ХХ	3.29 (0.45-6.12)	0.9831192
IH 105	Radius	0.03	21.33	51,443	3,577	0.0695	0.0011	0.0673-0.0717	NA	10.38 (5.96–14.8)	0.9840446
IH 228	Ulna	0.01	1.92	19,571	1,233	0.063	0.0017	0.0596 - 0.0664	NA	NA	0.9730365
IH 456	Cranium	0.39	74.95	1,060,835	656	0.0006	0	0.0006 - 0.0007	XX	5.12 (3.24–7.0)	0.996163
106 HI	Radius	0.34	111.57	685,315	475	0.0007	0	0.0006 - 0.0008	XX	5.12 (3.89–6.35)	0.9972555
UE 1257	Femur	0.4	154.95	1,192,913	706	0.0006	0	0.0005-0.0006	XX	8.79 (7.14–10.43)	0.9981968
*Consistent wit	th XY but not	XX									

 Table 2
 Summary statistics and mitochondrial contamination estimates in molecular analysis

during this perinatal period (Jeanty and Romero 1984; Lewis 2007). However, these classical methods are based on regression. It has been argued that the use of regression methods for age estimation may introduce a systematic statistical bias in the estimated ages. This is because of the non-uniform nature of the age distribution of individuals of the reference samples that these methods are based on (Gowland and Chamberlain 2002). With the intention to counter this problem, Gowland and Chamberlain (2002) re-evaluated the age of perinatal skeletal remains from different Romano-British sites by using Bayesian inference, incorporating both uniform and model prior probabilities of foetal and perinatal mortality. Their method is based on diaphyseal length data of femurs collected from various clinical studies of foetuses and infants of known gestational age (Gowland and Chamberlain 2002). A prior probability model incorporates prior knowledge of mortality risks at a given age-at-death based on a life table of modern perinatal mortality (Gowland and Chamberlain 2002). Therefore, to further validate the age of the individuals at Ca n'Oliver, we also applied the method of Gowland and Chamberlain (2002) when a complete femur was present. The probability density matrix of Gowland and Chamberlain (2002) can be applied in two ways: (1) the individual age at death is attributed to the age range with the highest probability or in case of similar values between different age ranges as the mean of them; (2) the entire probability density distribution is taken into account and an individual is split up and distributed according to the respective probabilities. We used approach 1 to compare ages between classical and Gowland-Chamberlain methods. We chose to calculate the average age and call it osteological age. In the subset of the sample where femur length was measurable, we also used approach 2 and compared the resulting mortality profiles with that of Butler and Alberman (1969).

In addition to these age estimation methods that analyse an individual case in comparison with growth standards derived from modern populations, the presence or absence of a neonatal line in the enamel of deciduous tooth crowns was used to detect a "self-contained" marking of birth (Boyde and Jones 1983; Boyde 1990). The neonatal line (NNL) was described as a regularly occurring accentuated incremental line in enamel (and sometimes also dentin) marking the position where the forming fronts of tooth formation are expected to be found around the time of birth (Schour 1936, Rushton 1933 and 1939; Kronfeld and Schour 1939). The NNL, which separates the enamel and dentin formed during intrauterine life from that formed after birth, is regularly observable in individuals who survived for at least 7 to 15 days ex-utero (Weber and Eisenmann 1971; Whittaker and Richards 1978; Levine et al. 1979; Skinner 1992; Witzel 2014). In the present study, the approach outlined by Witzel

(2014) for analysis of the NNL by examining more than one tooth per individual (if possible) with different microscopic techniques was employed.

We used the crowns of deciduous incisors, canines, and deciduous molars found in the individuals of Ca n'Oliver that showed the best preservation. A total of 13 individuals were analysed, 11 of which are represented by two teeth per individual (Table 3) but in one case (NEN 5) the second tooth was present in fragments only, which precluded height measurement. Dental specimens were photographed and no further preliminary treatment was employed in order to prevent further loss of tooth substance. Later, teeth were carefully placed in dichloromethane (as intermedium) and then embedded in epoxy resin (Biodur E12/E1) following the procedures outlined in Witzel et al. (2008) and Kierdorf et al. (2012). Axial ground sections from either labial or buccal to lingual or palatinal (section thickness below 50 µm) were prepared through the centre of the incisors or as close as possible to the tip of the dentin horn of the canines or in case of deciduous molars the dentine horns of the mesial cusps.

Before thin ground sections were prepared, the uncoated surfaces of the tooth blocks exhibiting the best fit with the desired section plane were examined in a scanning electron microscope (Zeiss Evo MA 15) using the backscattered electron (BSE) detector in a low vacuum at an accelerating voltage of 20 kV. Without calibration standards, greylevel differences in the BSE images must be considered a qualitative measure of variation in mineral content of the analysed enamel. Examination aimed to document the position of accentuated incremental lines and the degree of their accentuation.

 Table 3
 Individuals and dental elements in which the neonatal line was analysed

Number of individual	Stratigraphic unit (UE)	Dental elements and most probable FDI code of the element in brackets
NEN 1	498	Upper incisor (51), canine (53)
NEN 4	452	Lower incisor (81 or 82), canine (73)
NEN 5	458	Upper incisor (61), fragments of lower incisor
IH 84	681–682	Lower canine (83), molar 1 (74)
IH 105	733	Upper incisor (51), upper canine (63)
IH 140	817	Lower canine (or molar cusp), upper incisor (62)
IH 225	1027	Upper incisor (61)
IH 228	1035	Upper incisor (51), lower canine (83)
IH 348	1304	Canine (undetermined), molar 1 (74)
IH 456	(Zona 9)	Upper incisor (61), lower canine (83)
IH 651	1952	Upper incisor (51)
IH 672	1968	Lower incisor (81), molar 1 (54)
IH 681	1986	Upper incisor (61), canine (73)

Thin ground sections were viewed and photographed in transmitted light using either a Zeiss Axioskop 2 Plus microscope or a Zeiss AxioImager both equipped with a Axiocam 503c digital camera. Images were stitched together by using the Zeiss Zen Pro Software. Tooth sections were inspected under different transmitted lighting conditions (brightfield, linear polarization, phase contrast) for the presence of accentuated incremental lines that could represent the NNL or had been formed in relation to prenatal stress periods.

In addition, measurements of vertical tooth height were taken from the stitched images of the section in  $\times$  10 magnification with the open-source software package Fiji (http:// fiji.sc). This was done because prior to embedding the teeth were assessed being too brittle for direct measurements. In micrographs exhibiting an untypical cervical margin that deviates from the normal sharp wedge-shaped appearance, a reconstruction of the position of the cervical margin was performed. This was done by elongating the enamel dentine junction (EDJ), a portion of the cervical outer enamel surface (OES), and the mineralizing surface of dentin by auxiliary straight lines. The points of intersection of these lines were assumed to be the original position of the cervical margin of the developing tooth germ at the death of the individual. We used the Kierdorf et al. (2021) correction factor (1.06) for enabling comparison of measurements obtained from micrographs on ground sections with such obtained by direct calliper measurements of tooth germs. Using these partly reconstructed and corrected values, it was first determined if they lie within the range of tooth height at birth given by Liversidge et al. (1998). Additionally, gestational ages in weeks post menses were calculated by using the regression equations for deciduous central and lateral incisors and deciduous canines from Deutsch et al. (1984). Results of individuals contributing two teeth per individual were first checked for internal consistency within the respective methods and all results were checked for consistency between the methods. In cases where individuals contributed a central incisor, stretches measured in prism direction connecting the highest point of the dentine core with accentuated incremental lines (including a potential NNL) and finally the outer enamel surface (OES) were measured to calculate the prenatal crown formation time (pCFT) as outlined by Nava et al. (2017b). Obtained crown formation times in days were then compared to the values of the Imperial Roman sample analysed by Nava et al. (2017b) and to that of modern deciduous teeth analysed by Birch and Dean (2014). Taking into account the high age accuracy obtained when teeth are used as sub-adult age indicator, and in order to more accurately estimate the age of Ca n'Oliver individuals, the estimations obtained by the method of Deutsch et al. (1984) were also taken into account for calculating the final gestational age of the individuals. This was done by averaging osteological age and teeth age. To do this, we used the older age obtained by Deutsch. Both types of methodologies, osteological, and dental for estimating age collectively span the foetal to neonatal and post-neonatal age ranges.

The age estimates are classified based on the duration of the clinically defined gestation in weeks after the last menstrual period (post-menses). This means that they refer to postmenstrual pregnancy duration and not to the true age of the foetus. The true age of the foetus is the gestational age post-fertilization also called fertilization age (embryonic age and later foetal age). It corresponds to the time of development from the fertilization of the ovum. The fertilization occurs within days after ovulation, which, in turn, occurs on approximately 2 weeks after the beginning of the preceding menstruation (Geirsson 1991). Therefore, the gestational age post-fertilization is about equal to the gestational age post-menses minus 2 weeks. In the tables of this study, gestational ages post-menses are indicated because these are the most common specification in the clinical and forensic literature.

According to the mean duration of human gestation 40 weeks (postmenses), the WHO contemporary classification is < 32 weeks immature, 32–36 weeks preterm, 37–42 weeks term, and above 42 weeks post-term. However, these ages and the respective chance of survival take into account a certain modern medical care provided to mother and newborn. This would not have been the case in protohistory. For this reason, we clustered these 48 individuals in immature (younger than 34 weeks); preterm (between 34 and 37 weeks), term (between 38 and 42 weeks), and post-term or babies (between birth and 12 months of postnatal life).

### Results

Thirty burials and 20 isolated skeletal elements scattered in the sediment of the settlement were found at the site of Ca n'Oliver. Twenty-five (Nen 1, Nen 3, Nen 4, Nen 5, IH 84, IH 140, IH 105, IH 203, IH 228, IH 344, IH 225, IH 202, IH 348, IH 356, IH 464, IH 510, IH 503, IH 456, IH 480, IH 681, IH 672, IH 651, IH 755, IH 875, IH 901) of the 30 burials contained exclusively one complete or mostly complete individual (Table 4). According to the photographic and archaeological information, these 25 individuals were found in anatomical connection, indicating primary deposition (Fig. 3). In general, all of them were buried lying on their side, either left or right with no specific cardinal orientation (Fig. 3). The remaining 5 burials (IH1001; IH1051; IH1195; IH1031; IH117) had more than one individual with no anatomical connection (Table 4), specifically, IH1051 and IH1031 contained 3 and 5 individuals respectively (Table 4). In these last 5 burials, the absence of anatomical connection of the bones, the high number of individuals found in small graves (for example, 5 perinatal individuals in a grave with

buried in the	e same bur	141									
Individual	UE	Campaign year	Zone	Gestational age based on classical methods	Gestational age based on Gowland Chamberlain method	Mean gestational age based on bones	Mean age based on bones and teeth	Molecular sex assess- ment	Ostological sex assessment	Ilium	$\mathrm{Pl}_3\%$
Nen 1	498	1991	2	40 weeks	ı	40 weeks	41.6 weeks		Indeterminate		45.45%
Nen 3	451	1991	2	38 weeks		38 weeks	38 weeks		Probable female	Х	9.09%
Nen 4	452	1991	2	33 weeks	33 weeks	33 weeks	33.9 weeks	Male	Probable male	x	86.36%
Nen 5	458	1991	2	40 weeks	40 weeks	40 weeks	37 weeks		Probable female	x	63.63%
IH 84	681-682	1998	6	38 weeks	38 weeks	38 weeks	36.4 weeks	Male	Probable female	X	63.63%
IH 105	733	1998	2	39 weeks	37 weeks	38 weeks	35.7 weeks	ż	Probable male	x	77.27%
IH 140	817	1998	2	40 weeks	ı	40 weeks	38.1 weeks		Probable female	x	86.36%
IH 202	948	1999	2	39 weeks	ı	39 weeks	39 weeks		Probable male	X	54.54%
IH 203	949	1998–1999	2	40 weeks		40 weeks	40 weeks		Indeterminate		13.63%
IH 225	1027	1999	2	40 weeks	ı	40 weeks	41.3 weeks		Probable female	x	72.72%
IH 228	1035	1998–1999	2	40 weeks	41 weeks	40.5 weeks	42 weeks	ż	Indeterminate	х	95.45%
IH 344	1300	1999	2	40 weeks	ı	40 weeks	40 weeks		Probable male	X	90.90%
IH 348	1304	1999	2	39 weeks	ı	39 weeks	37.8 weeks		Indeterminate		77.27%
IH 356	1319	1999	2	32 weeks	31 weeks	31.5 weeks	31.5 weeks		Probable female	х	68.18%
IH 456	ı	2000	6	37 weeks	37 weeks	37 weeks	38.5 weeks	Female	Probable male	x	81.81%
IH 464	1553	2000	6	38 weeks	ı	38 weeks	38 weeks		Indeterminate		22.72%
IH 480	1599	2000		38 weeks	1	38 weeks	38 weeks		Probable male	x	40.90%
IH 503	ı	2000	9	39 weeks	ı	39 weeks	39 weeks		Indeterminate		50%
IH 510	1644	2000	6	40 weeks	ı	40 weeks	40 weeks		Indeterminate		13.63%
IH 651	1952	2002	12	39 weeks	ı	39 weeks	39.3 weeks		Probable male	x	68.18%
IH 672	1968	2002		40 weeks		40 weeks	35.9 weeks		Indeterminate		59.09%
IH 681	1986	2002	12	38 weeks	38 weeks	38 weeks	39.6 weeks		Probable male	x	72.72%
IH 755	2145	2003	19	39 weeks		39 weeks	39 weeks		Probable male	X	63.63%
IH 875	2439	2006	East end of	I	ı	I	ı		Indeterminate		4.54%
			the settle- ment								
106 HI	2510-11	2007		29 weeks	31 weeks	30 weeks	30 weeks	Female	Probable female	x	86.36%
IH 1001–0	2722	2014	ı	38 weeks	37 weeks	37.5 weeks	38.5 weeks		Probable male	x	77.27%
IH 1001–1	2722	2014		38 weeks	1	38 weeks	38 weeks		Indeterminate		4.54%
IH 1031–1	2739	2015	I	41 weeks	41 weeks	41 weeks	41 weeks		Indeterminate		63.63%
IH 1031–2	2739	2015	ı	37.5 weeks	38 weeks	37.75 weeks	37.5 weeks		Indeterminate		22.73%
IH 1031–3	2739	2015	I	26.5 weeks	ı	26.5 weeks	26.5 weeks		Indeterminate		%60.6
IH 1031–4	2739	2015	I	39 weeks		39 weeks	39 weeks		Indeterminate		13.63%
IH 1031-5	2739	2015	I	24.5 weeks	22 weeks	23.25 weeks	23.3 weeks		Indeterminate		50%
IH 1051–0	2796	2015		40 weeks	1	40 weeks	40 weeks		Indeterminate		9.09%

a volume of 6 dm<sup>3</sup>), and the fact that some individuals were represented by one skeletal element only suggest that these burials were secondary burials. In these last 5 burials, we could identify a total of 15 individuals. Additionally, isolated bones (20 skeletal elements, Table 5) were found scattered in the sediment of the settlement chambers and craft activity areas of the site. The calculation of the MNI indicated 8 individuals, which cannot come from the other individuals previously identified.

In total, we identified 48 individuals of which 40 came from burials (25 from individual burials and 15 from multiple burials) and at least eight additional individuals were represented by isolated bones. The state of preservation (IP<sub>3</sub>) was relatively good in the 25 individuals coming from individual burials (Table 4), partly reaching up to 96%. Tables 1S and 2S in supplementary material show the anthropometric measurements of the Ca n'Oliver individuals. In all these individuals, we observed no indications of pathological alterations of the bones with the exception of a right hemi-frontal with a greatly developed cribra orbitalia in an individual belonging to UE 1268 (isolated bones, Fig. 4). The size of the hemi-frontal (30.5 mm) was indicative of an age around 22 weeks of gestation (Table 5). Therefore, this pathological condition reflects some type of intrauterine stress which disturbed the development of this individual.

### Sex identification

From the buried individuals, nineteen ilia were sufficiently preserved for visual sex assessment. From these 19 ilia, results indicate 10 (52.6%) probable males, 8 (36.8%) probable females, and 1 (10.5%) indeterminate individual (Tables 4 and 5). Therefore, according to the osteological analysis, our sample is constituted by males and females with a sex ratio of 1.25 in favour of males.

In the molecular analysis of the 7 individuals, the sex chromosome ratio  $(R_y)$  indicated that three were female, one was male, and another one was more likely a male. In two cases, the sex could not be assigned with confidence. The obtained values are presented in Table 2 and Fig. 5. We produced low-coverage (0.01x-0.4x) genomic data for six of the seven tested individuals. The obtained genomic data was tested for presence of ancient sequences (Fig. 6), as well as contamination using two methods both of which assess contamination levels based on the analyses of mtDNA sequences (Green et al. 2008; Fu et al. 2016). Despite broad contamination point estimates obtained using Green et al. (2008) method (Green et al. 2008) (3.29 to 10.89%), we find that the likelihood method (Fu et al. 2016) in most cases identified between 0.97 and 0.99 probability of mapping sequences being authentic (Table 2). The only exception, Nen4, displayed the probability of

continued)	
ble 4 (	
<u>a</u>	

Individual UE	Campaign year Z	one Gesta based methc	ttional age 1 on classical ods	Gestational age based on Gowland Chamberlain method	Mean gestational age based on bones	Mean age based on bones and teeth	Molecular sex assess- ment	Ostological sex assessment	Ilium	$\mathrm{Pl}_3\%$
H 1051–1 2796	2015 -	41 we	teks		41 weeks	41 weeks		Probable female	x	86.36%
H 1051–2 2796	2015 -	35 we	seks		35 weeks	35 weeks		Indeterminate		31.81%
H 1177–0 2811	2015 -	40 we	seks		40 weeks	40 weeks		Indeterminate		40.90%
H 1177–1 2811	2015 -	38.5 v	weeks	36 weeks	37.25 weeks	36.5 weeks		Indeterminate		13.63%
H1177-2 2811	2015 -	30 we	seks		30 weeks	30 weeks		Indeterminate		4.54%
H1195-1 2837	2015 -	39 we	seks		39 weeks	39 weeks		Indeterminate		45.45%
H1195-2 2837	2015 -	6 mor	ths life		6 months life	6 months life		Indeterminate		13.63%

UE means stratigraphic unit. PI means preservation index

### INFANTILE INHUMATIONS



Fig. 3 Some inhumations of the infants found at the Ca n'Oliver site with the distribution of anatomical elements

0.94. For that reason, we use PMDtools with PMD filtering set to 0 on Nen4 genome, and find that after filtering (resulting in genome coverage reduction from  $0.11 \times to$ 0.06x) the contamination point estimate dropped from 10.89 to 5.38%, and the likelihood of sequences being authentic rose from 0.94 to 0.99. Sex assignment performed on the filtered genome resulted in an unequivocal identification as male.

The number of human sequences mapping to sex chromosomes exceeded 100 k in four individuals (Table 2). This allowed for confident sex assignment (Skoglund et al. 2013) of three females (IH456, IH901, UE1257) and one probable male (Nen4) (Table 2 and Fig. 5). As mentioned above, the latter was confirmed male after filtering. Out of the three remaining individuals (with fewer than 100 k sequences mapping to sex chromosomes), one was clearly determined as male (IH84) despite low coverage (0.04x), while in two remaining cases (INH105, IH228) the sex remained unassigned even though the individuals appear as male (Fig. 5). The observed pattern could reflect contamination, which was estimated to be slightly higher in those two children (Table 2); however, the most likely explanation for the lack of clear sex assignment is low genome coverage. Two of these molecular results (Nen4 and IH901) agree and another two (IH84 and IH456) disagree with the visual sex assessment of the respective individuals (see Tables 4 and 5). The remaining 3 cases were molecularly (IH105 and IH228) or visually (IH1257) not possible to be determined. Referring only to confident sex assignments for sex chromosome sequences, the sex ratio is reversed compared with the results obtained from the osteological analysis. However, considering that individuals with low genome coverage were more likely males than females would render the sex ratio of both methods more or less similar in not deviating from 1:1.

## Estimation of age: osteometrics and tooth formation

The results on age based on osteology can be seen in Tables 4 and 5. A comparison of gestational ages obtained by using the classical methods and by using the Bayesian approach of Gowland and Chamberlain (2002) in cases where at least one femur was present revealed a close resemblance of the results. In 7 out of 15 cases, the two approaches revealed the same gestational age. In 5 cases, the individuals were assessed as being 1 to 2.5 weeks younger when using

Table 5 MNI obtained from the isolated bones									
Element	UE Z	one Sid	<ul> <li>Gestational age based classical methods</li> </ul>	Gestational age based Gowland Chamberlain method	Mean age based on bones and teeth	Ostological sex assess- ment	molecular Sex assess- ment		
Humerus, proximal epiphysis + proximal diaphy- sis	1063 10	0 R	41 weeks		41 weeks	Indeterminate		1 individual	Fotal 1
Humerus	1257 2	R	40 weeks	,	40 weeks	Indeterminate	Female		
Humerus	1075 2	Γ	40 weeks		40 weeks	Indeterminate		1 individual	
Femur	1257 2	R	40 weeks	40 weeks	40 weeks	Indeterminate		2 individual	
Femur, distal epiphysis + diaphysis	1075 2	R	40 weeks		40 weeks	Indeterminate			
Femur, proximal epiphysis + proximal diaphysis	1075 2	Γ	40 weeks		40 weeks	Indeterminate			
Femur, distal epiphysis + distal diaphysis	1257 2	Γ	40 weeks		40 weeks	Indeterminate			
Tibia	671 -	R	40 weeks		40 weeks	Indeterminate		4 individual	
Tibia, prox epiphysis + diaphysis	1075 2	R	40 weeks		40 weeks	Indeterminate			
Tibia	671 -	Γ	40 weeks		40 weeks	Indeterminate			
Tibia, proximal epiphysis+diaphysis	1075 2	Γ	40 weeks		40 weeks	Indeterminate			
Tibia	1257 2	Γ	40 weeks		40 weeks	Indeterminate			
Tibia	632 2	Γ	40 weeks		40 weeks	Indeterminate			
Radius, proximal epiphysis + proximal diaphysis	1075 2	Γ	40 weeks		40 weeks	Indeterminate		1 individual	Fotal 4
Fibula	502 7	Γ	39 weeks		39 weeks	Indeterminate		1 individual	
Tibia, proximal epiphysis + diaphysis	753 2	R	39 weeks		39 weeks	Indeterminate		1 individual	Fotal 1
Tibia, proximal epiphysis + proximal diaphysis	793 2	Γ	39 weeks		39 weeks	Indeterminate			
Tibia	2502 -	Γ	30 weeks		30 weeks	Indeterminate		1 individual	<b>Fotal 1</b>
Hemi frontal	1268 10	0 L	22 weeks	ı	22 weeks	Indeterminate		1 individual	Fotal 1

**Fig. 4** Inferior view of the left hemi-frontal of the individual UE1268, showing the *cribra orbitalia* in the orbital roof





**Fig. 5**  $R_{\rm Y}$  ratio in the molecular analysis of the 7 examined individuals.  $R_{\rm Y}$  ratio in the molecular analysis of the seven examined individuals. Error bars indicate 95% confidence interval

the Bayesian approach. In the remaining 3 cases, the gestational age obtained by classical methods was lower by 0.5 to 1 week. Averaging of these age results and using the age according to the classical methods in the remaining cases revealed that 6 individuals from burials were born immature with gestational ages of 33 weeks or below. Another 5 individuals were assessed as being born preterm between 34 and 37 weeks and 27 individuals fall in the term birth range of 38 to 42 weeks. Among the individuals identified from isolated bones, minimally 6 individuals were assessed as being born at term between 38 and 42 weeks of gestational age and 2 others as being immature with 30 weeks and 22 weeks respectively.

The results of age based on tooth formation stages are shown in Table 6. Tooth analyses by employing different methods yielded mixed results (cf. colour codes in Table 6). The consensus within methods when two teeth per individual could be included was better in using the data on tooth height at birth provided by Liversidge et al. (1998). Standard deviations of the mean values yielded rather broad corridors for diagnosing tooth height as compatible with term birth. Accordingly, 7 out of 10 individuals presented as being born term (IH 228 indicated a short-term birth survival on the basis of the incisor) and in three individuals one tooth was classified as preterm. The remaining three individuals with only one analysable tooth were classified as term. In 7 individuals, gestational age could be calculated on the basis of two teeth by the regression formulae of Deutsch et al. (1984). Four individuals showed consistent results within a range of less than 2 weeks (in case of NEN 4 only under the assumption that the second tooth was a lateral instead of a central lower incisor). In one individual, a difference of below 4 weeks was obtained and two individuals exhibited results with above 4 weeks difference. Overall, 7 out of 13 individuals showed indications of immature or preterm birth. Birth around term or the potential of a short-term birth survival was evident in 5 individuals. One individual (IH 681) exhibited a substantial difference of about 13 weeks between the results of the 2 analysed teeth. An explanation could be a misclassification of the deciduous canine that might have been the cusp of a deciduous 1<sup>st</sup> molar. This tooth type starts its mineralization about 2 to 3 weeks earlier than the deciduous canine (Birch and Dean 2014), still leaving a substantial discrepancy. The method of Nava et al. (2017b) could only be employed with central incisors of which 10 were present in the sample. The results provide the period of prenatal and in some case postnatal crown formation in days that could be compared with published reference values (Nava et al. 2017b; Birch and Dean 2014). The identification of a NNL was difficult or impossible (see below); therefore, minor accentuated incremental enamel lines were treated as candidates if applicable. In three cases no indications of surviving birth, after a pCFT compatible with a gestation terminated preterm, could be documented. Two individuals showed no indications of a NNL; however, their pCFT was compatible with term birth. In addition, in IH681 an even longer pCFT was calculated, indicating that he had either a gestation period that lasted post-term, dying in the uterus or at birth or survived birth for an unknown period with an indistinguishable NNL. In 4 individuals, minor accentuated lines were present that could have been the NNL. In one of these (NEN 1), the candidate NNL was in a position that indicated preterm birth with a period of 60 days of post-natal survival. Two of the remaining 3 individuals (NEN 5 and IH 228) exhibited a pCFT consistent with birth at term and showed evidence of about 50 days of postnatal survival. In one case (IH 225), birth was at term, but postnatal survival could not be determined because of diagenetic loss of enamel. In the latter case, according to the tooth height measurements, postnatal survival most probably did not exceed that of the other individuals.

A comparison between the three tooth analysis methods (possible in 10 cases) gave consistent results of all methods in 5 cases. In 2 cases, consistency was limited to only one of the tooth height methods and in 3 cases each method showed a different result. In each individual method, a varying proportion of individuals with preterm and term birth could be documented as well as individuals that survived birth for several weeks.

According to the comparison between the osteological gestational age (Table 4) and the tooth gestational age (Table 6), the trend emerged that individuals where the tooth analyses based on Deutsch et al. (1984) and Nava et al. (2017b) yielded indications of death at or shortly after preterm birth had an advanced osteological age (IH 84, IH 105, IH 140, IH 348, IH 456, IH 672; with the exception of NEN 4 where both osteological and tooth age indicated preterm death). Individuals in which tooth analysis revealed death at term birth or indicated a postnatal survival period of 2 months at maximum the osteological age lagged behind tooth age (IH 225, IH 228, IH 681, NEN 1, NEN 5, with the exception of IH 651 where all methods indicated death at or shortly after term birth).

The results on age resulting from the average of both estimations (osteology and teeth formation, specifically the method based on tooth height after Deutsch) can be seen in Tables 4 and 5. The results indicated that the 47 analysed individuals were classified between 22 weeks of gestation and up to 6 months after birth. The 48<sup>th</sup> individual (IH 875) was only represented by some small skull fragments. According to this combination of our results, most of these individuals were term individuals (Tables 4 and 5). Specifically, 17% (8/47) were younger than 34 weeks (immature individuals), 17.02% (8/47) were between 34 and 37 weeks (preterm individuals), 63.82% (30/47) were between 38 and 42 weeks (term individuals), and 2.12% (1/47) were a 6 months of life baby. In total, there are 16 individuals (36.17%) who were born previous to be in term, and half of these individuals were too immature to survive (8 of 16).

To rule out misclassifying infants of low birth weight (LBW) as pre-term births, Fig. 7 shows the effects of increasing all of the anthropometric measurements of each skeletal element by 5%, 10%, and an extreme 25% (Fig. 7). The intention was to explore any possible presence of LBW infants. This was done because, while mortality is 40% higher in perinates < 2500 g than infants of normal birth weight (Behrman and Shiono 1997), LBW is not reflected in diminished bone length or retarded tooth development (Jaya et al. 1995). The results indicate that even at 25% increase in size, our analyses still classified some individuals as prenates (Fig. 7).

In order to explore whether our results are compatible with to the natural mortality frequencies of perinatal infants, we compared the age-at-death profile of the present study to the mortality distribution produced from a life table based on the 1958 British perinatal mortality survey (Butler and Alberman 1969). This contains gestational age at death data for almost 17,000 births. The results of this comparison are shown in Fig. 8(a) and they indicate that the age-at-death profile of Ca n'Oliver is similar to that coming from natural mortality (Fig. 8(a)).

### Neonatal line (NNL) analysis

In most sections, a certain amount of enamel substance loss at the cervical tooth portion and of subsurface enamel was evident. But at least parts of the original surface enamel were left in places where the location of the NNL was expected (Fig. 9).

In the enamel microstructure, no convincing NNL could have been documented on the basis of different microscopic methods including SEM. The only case of a more marked accentuation was documented in an individual (IH 140) with a tooth height result (after Deutsch et al. 1984) of 36 weeks (Fig. 10). The markings in the form of a more pronounced double line and less pronounced single lines located further internally have the same appearance in both teeth of the individual (Fig. 10(C, D)). The lines are located well underneath the outer enamel surface and the attempt to count of prism cross striations external to the double line accounted for c. 30. This would result in a birth after only about 32 gestational weeks. Therefore, live birth is unlikely and the accentuations more probably represent a sequel of intrauterine stress events. The structural aberrations were only mild as evidenced by the maintenance of prismatic enamel structure. In the SEM micrographs, only slight variations in enamel mineralization in the course of the accentuated incremental lines were visible (Fig. 10(A, B)). Such slightly accentuated incremental lines probably related to intrauterine events were evident in most of the analysed teeth. Two individuals (NEN 4, IH 672) presented an overall accentuation of the incremental pattern (Fig. 9(C)).

### Discussion

The aim of this study was to examine the age-at-death profile of foetuses and newborns from the Ca n'Oliver Iberian site, in order to better understand the developmental conditions during pregnancy and the potential periods of birth survival. Furthermore, it was aimed to shed light on the differential treatment of these individuals within the Iberian funerary rituals in order to reconstruct identity at the earliest stages of life in this population. In addition, all this information will allow us to respond to the question if these perinatal assemblages likely relate to infanticide, as some authors have argued. Significant information on the conditions of foetal and infant mortality in the Iberian Period based on the Ca n'Oliver site has been furnished in this paper. Whereas the analysis of infant mortality rates in a population gives information about the population's capacity to adapt to their environment, the analysis of the ratio of stillbirths, neonatal, and post-neonatal deaths at a site opens the possibility to infer the relatedness of the causes of death of these individuals to factors reflecting (1) individual genetic make-up and



Fig. 6 Misincorporation patterns in molecular analysis of the 7 examined samples

the conditions of pregnancy or (2) conditions of the environment. The former is also termed endogenous mortality and is seen as more closely related to death already during pregnancy or early thereafter. The latter termed exogenous mortality is interpreted as acting cumulative and leading to death later after birth (Lewis 2002; Lewis and Gowland 2007). Different definitions have been proposed to classify the timing of a fatality with relation to either the duration of gestation or the period of birth survival. Foetuses who die before or at birth are referred to as miscarriages or early stillbirths and stillbirths. The period within the first 27 days of extra-uterine life reflects the neonatal mortality, and death between 28 days and 1 year is classified as post-neonatal mortality (Lewis and Gowland 2007). A stillbirth is the birth of an infant that has died in the uterus; strictly speaking, after having survived through at least the first 28 weeks of pregnancy, earlier instances being regarded as abortion, miscarriage, or early stillbirth (Gusi and Muriel 2008). A foetus is the stage that goes from the 9<sup>th</sup> week after fertilisation (11<sup>th</sup> week of gestation post menses) to the moment of birth; however, from the Ca the n'Oliver site, we can only observe individuals equal to or older than 22 weeks of gestation, because at mid-foetal life is when most of the skeletal elements are nearly recognizable (Scheuer and Black 2000; Rissech 2016).

### Sex bias: baby girls and boys differential treatment

According to the results of this study (see Tables 2, 4, and 5), biomolecular techniques do not suggest a sex bias in Ca n'Oliver sample, but include too few individuals to be truly informative ( $3^{\bigcirc}_{\rightarrow}$  and  $2^{\bigcirc}_{\rightarrow}$ ). Osteological methods do not suggest a sex bias either ( $8^{\bigcirc}_{+}$  and  $10^{\bigcirc}_{-}$ ), but the lack of agreement with biomolecular methods suggests we cannot rely on osteological methods (Lassen et al. 1997; Waldron et al. 1999). Therefore, whilst we can say that both female and male infants were present in the Ca n'Oliver sample, it is not possible to explore the sex ratio further. These observations do not support the hypothesis, which suggests strict unequal treatment of baby girls or boys at the site. This observation is in accordance with the observations of Subira and Molist (2008) in the Sant Miquel d'Olèrdola site in Olèrdola (Barcelona). However, our observations contrast with those of Afonso et al. (2019). At the late Iberian site of Camp de les Lloses, they identified a dominance of females in the foetal remains found under similar archaeological circumstances. These authors interpret their sample not as unwanted female offspring that became victims of infanticide but as female individuals who had died of natural causes, and suggested that their special burial treatment was related to their high social value. In addition, other aDNA studies of samples from different time periods and geographic locations also failed to reveal a systematic sex bias, indicative of a practice of preferentially killing individuals of the unwanted sex (Lassen et al. 1997; Waldron et al. 1999; Mays and Faerman 2001; Hassan et al. 2014). Despite the well-documented more recent practice of sex bias towards females in abortion and infanticide (e.g. Hrdy 1990), compelling bioarchaeological evidence for this practice is absent so far.

### Age-at-death profile

The age-at-death profile of the Ca n'Oliver sample is consistent with natural mortality profiles (Fig. 8(a)). With the exception of one individual that died at an age of about 6 months and 4 additional individuals that were identified by means of enamel histology as having survived birth for less than 2 months, the Ca n'Oliver sample is exclusively constituted by individuals with a gestational age between 22 and 42 weeks. It is clear that the majority of the individuals are under 42 weeks gestational age. The majority of these individuals (34.04%, 16/47) were too immature to easily survive in this period and, 17.02% (8/47) had no chance of survival because they were younger than 34 weeks of gestational age. This observation is reinforced by the fact that classical osteological methods overestimated the age of the individuals in relation to the methods of Gowland and Chamberlain (2002) based on Bayesian inference. Taking a Bayesian approach gives an age profile even more heavily dominated by pre-term foetuses. When the individuals in our sample that preserved a complete femur were distributed according to the entire probability density of the measured femur length, the age-at death profile presents a broader base and a peak skewed further to the left (Fig. 8(b)). Of course, each individual died only once at a very specific age but by ruling out possible ages at death with a lower probability, these less probable outcomes are cut off from the age-at-death profile and produce a narrower distribution with a more pronounced mortality peak (Mays and Eyers 2011; Gowland et al. 2014). Despite the lack of a distinctively developed NNL, tooth analyses by the method of Nava et al. (2017b) showed post-birth survival in four individuals that have been classified as born at term. Such cases will contribute to this narrower distribution, no matter what the reason of this effect was. It could have been a slower foetal growth with reaching the typical bone length for term birth at a later stage or an effect of health problems of a neonate or its mother after birth. A potential scenario of the latter could be an excess postpartum weight loss of the newborn. Even in the analysis of a group of contemporary Californian woman giving birth in a hospital without major complications, 12% of the infants show excess weight loss ( $\geq$  10% of birth weight) mainly due to a late onset of lactation (Dewey **Table 6** Gestational ages postmenses and postfertilization inferred from tooth crown heights in weeks on the basis of values of knownage teeth given by Deutsch et al. (1984) and pCFT based on cumula-

tive prism stretches between the onset of enamel formation and the potential NL or the outer enamel surface

				reconstructed	d		gestational age (weeks	l Birth timing		cumulative prism length	enamel formation from cum-	(minimal) threshold		
	tooth	preserved	reconstructed	tooth height			pm) from	vs.	cumulative	(μm) till	ulative	preterm		birth timing
	(FDI	tooth height	tooth height	corrected	tooth height	birth	tooth	gestational	prism length	candidate	prism	(maximal)	prenatal	(postnatal
	code)	nisto (mm)	(mm)	*1.06 (mm)	at pirth (mm)	timing	neight	age	(µm)	ININL	length	pcFT (days)	interval	survival)
IH 84	74	2.7	2.8	3.0	3.1 +/- 0.55	term							122 to 113	3
IH 84	83	2.6	2.6	2.8	3.4 +/- 0.94	term	34.68	-					135 to 121	-
IH 105	51	3.9	4.3	4.6	5.4 +/- 1.04	term	33.4	-	510		110	(67)120(147)	152 to 137	preterm
IH 105	63	1.9	2.1	2.2	3.4 +/- 0.94	preterm	29.9	+					135 to 121	-
IH 140	62	3.6	4	4.2	4.5 +/- 0.82	term	36.12	-						
IH 140	dc	2.6	2.8	3.0	3.4 +/- 0.94	term	35.8	-					135 to 121	
IH 225	61	5.3	6	6.4	5.4 +/- 1.04	term	40 + 2.6	+		695	[150]	(67)120(147)	152 to 137	term + x
IH 228	51	5.8	6.1	6.5	5.4 +/- 1.04	(post) term	40 + 3.5	+	812	574	175 [124]	(67)120(147)	152 to 137	(term) + 50 days
IH 228	83	3.5	3.7	3.9	3.4 +/- 0.94	term	40 + 2	+					135 to 121	
IH 348	74	3.2	3.4	3.6	3.1 +/- 0.55	term							122 to 113	•
IH 348	dc	2.8	2.9	3.1	3.4 +/- 0.94	term	36.5	(+)					135 to 121	
IH 456	61	4.4	5.1	5.4	5.4 +/- 1.04	term	37.5	(+)	494		107	(67)120(147)	152 to 137	preterm
IH 456	83	3.4	3.4	3.6	3.4 +/- 0.94	term	40	+					135 to 121	-
IH 651	51	5	5.5	5.8	5.4 +/- 1.04	term	39.6	+	607		131	(67)120(147)	152 to 137	term
IH 672	81	3.3	3.6	3.8	5.4 +/- 1.04	preterm	31.8	+	579		125	(67)120(147)	152 to 137	(term)
IH 672	54	2.7	2.9	3.1	3.1 +/- 0.55	term							122 to 113	6
IH 681	61	3.9	4.5	4.8	5.4 +/- 1.04	term	34.4	-		810	[175]	(67)120(147)	152 to 137	(post)-term
IH 681	73	4	4.5	4.8	3.4 +/- 0.94	term	40 + 8	+					135 to 121	
NEN 1	51	5.8	6.1	6.5	5.4 +/- 1.04	term +	40 + 3.1	+	791	521	171 [112]	(67)120(147)	152 to 137	preterm + 60 days
NEN 1	53	3	3.1	3.3	3.4 +/- 0.94	term	37.8	(+)					135 to 121	-
NEN 4	81	3.1	3.3	3.5	5.4 +/- 1.04	preterm	30.2	+	268		58	(67)120(147)	152 to 137	preterm
	if 82	3.1	3.3	3.5	4.5 +/- 0.82	preterm	32.9	+						
NEN 4	73	2.5	2.6	2.8	3.4 +/- 0.94	term	34.7	-					135 to 121	
NEN 5	61	3.8	4.4	4.7	5.4 +/- 1.04	term	33.9		878	661	190 [143]	(67)120(147)	152 to 137	term + 50 days

NEN 5 di not measurable

In the comparison within and between particular methods employed, green cells denote close congruence, yellow cells denote an acceptable congruence, and red cells denote a larger difference. In case of individuals where only one tooth was assessable, cells are not colour coded for comparisons within methods. Additionally, in the comparison of birth timing assessed by pCFT based on cumulative prism length with birth timing or gestational age assessed by tooth height, orange cells denote a consensus with gestational age according to Deutsch et al., and blue cells denote consensus with birth timing according to Liversidge et al

<sup>1</sup>Values fromLiversidge et al. (1998)

<sup>2</sup>Based on the regression formulae given by Deutsch et al. (1984) for deciduous incisors and deciduous canines

<sup>3</sup>Measurements only performed in central incisors in order to employ the regression formula given by Nava et al. (2017a, b)

<sup>4</sup>CFT calculated by the regression formula given by Nava et al. (2017a, b)

<sup>5</sup>Range of prenatal duration of crown formation in the respective tooth types given byBirch and Dean (2014)

\*Values in squared brackets refer to pCFT at the position of the candidate NNL



Fig. 7 Plots of ages-at-death determined by actual (black dots and continuous line) and incrementally increased (white squares 5%, white down-triangles 10%, white up-triangles 25%) size of some

et al. 2003). Among the risk factors were primiparity and long duration of labour (Dewey et al. 2003). Giving birth under the conditions of a large settlement in the Iberian proto-history would have resulted in fewer possibilities for intervention when birth problems occurred and in dealing with their aftermaths. The relatively high proportion of the two youngest age-at-death classes distinguishes our sample from that of Camp de les Lloses (Afonso et al. 2019). At the latter site, the youngest individual reached 36 gestational weeks and almost half of the sample presented evidence for short-term survival after birth. It can be hypothesized that this difference is, at least partly, an effect of the much higher sample size of our sample.

### Neonatal line and birth survival

In forensic and archaeological science, the presence or absence of the neonatal line (NNL) has been used to assess whether a child was stillborn, or was possibly the victim of infanticide (Rossi et al. 1999; Smith and Avishai 2005; FitzGerald et al. 2006; Macchiarelli et al. 2006; Witzel 2014). Given the periodicity of enamel deposition and the

skeletal elements. Discontinuous horizontal lines indicate the normal value of these variables at birth by Fazekas and Kósa (1978). Measurements are in millimetres

fact that prenatal enamel does not normally present accentuated lines, a NNL is the first postnatal hypoplasia (i.e. stressinduced alteration of enamel deposition). It thus marks the brief period of disruption of enamel secretion (decrease in daily rates of enamel formation) that occurs immediately postpartum. However, there is an increasing awareness of the presence of prenatal accentuated incremental enamel lines (e.g. Nava et al. 2017b; Lorentz et al. 2019; Kierdorf et al. 2021). Despite the physiological buffering of the intrauterine environment (Antoine et al. 2009; Hillson 2014), different perturbations of the mother-infant nexus such as infections during pregnancy, maternal dietary deficiency, or maternal diabetes have been linked to microstructural aberrations of teeth (Birch and Dean 2014; Lorentz et al. 2019; Nava et al. 2017b) as well as adverse health effects in later life (Armelagos et al. 2009; Halcrow et al. 2017). Physiological stress during pregnancy is likely to have an impact on neonatal mortality. Without histological assessment, this effect would go unrecognized. Within each tooth class, but independent of an individual's sex, the location of the NNL is an indirect indicator of gestation length (time of initial mineralization in utero through postpartum), with preterm



**Fig.8** (a) Comparison of pattern distributions of perinatal death based on osteometric and teeth data from Ca n'Oliver (Oliver) and those obtained from Britain perinatal mortality (Natural) survey in 1958 (Butler and Alberman 1969). (b) Subsample of individuals with preserved and measurable femurs. Bayesian frequency included the entire probability density range

birth shifting the line more occlusally (Skinner 1992; Skinner and Dupras 1993). In at least one individual from our sample (Nen1), a period of about 60 days of survival after a preterm birth could be documented. Being born preterm is among the major risk factors for dying as neonate or early infant. By conventional methods, only the developmental stage at death can be assessed; however, dental histology gives us the possibility to retrospectively asses the respective birth timing (Witzel 2014).

Applying a set of methods to estimate age at death that analyse different components of bodily development including tooth development will necessarily introduce a certain variation (Uhl and Nawrocki. 2010). Part of this variation is certainly related to methodological issues but part of it might also convey biological meaning. Methodological issues could be exemplified by the variation between the results of the methods of Liversidge et al. (1998) and Deutsch et al. (1984) inferring tooth height and Nava et al. (2017b) using tooth histology. A broad range comprising the mean and the standard deviation of tooth height at birth (Liversidge et al. 1998) in most cases vielded consensus when two different teeth of the same individual were included. In such cases, deviations were slightly more pronounced with the method of Deutsch et al. (1984). In both methods, the proper identification of the tooth as well as the state of preservation was of



Fig. 9 Transmitted light micrographs of tooth 81 from individual IH 672 in different lightings ((A) plain light; (B) linear polarized light; (C) phase contrast of a magnified view of the labial enamel). (E) Enamel; (D) dentin; deb, debris; asterisks, areas with postmortem

enamel loss; arrow heads demarcate area of reddish enamel staining; tooth height was measured perpendicular to the tooth base as indicated by white lines

Fig. 10 Scanning electron micrographs (A, B) and phase contrast light micrographs (C, D) of details of the axiolabiopalatinal section plane of tooth 62 ((A) labial; (B, D) palatinal) and a deciduous canine or molar cusp (C) of individual IH 140 showing the appearance of accentuated incremental lines in prenatal enamel due to systemic stress events. Maintenance of prismatic enamel structure but variable degree of mineralization in the course of the accentuated incremental lines. Note microbial destruction of dentin in tooth 62



major importance. Calculating gestational ages (Deutsch et al. 1984) rather than classifying tooth height as falling within the range for term birth (Liversidge et al. 1998) allowed for a greater differentiation within the sample. In applying the histology method (Nava et al. 2017b), the major obstacle was the identification of the NNL. In most of the cases, the absence of the NNL was compatible with a death after a preterm to term gestational period. But in four cases, the calculated tooth formation period after the development of the NNL would have been too long for a term birth. In these teeth, the presence of more than one incremental enamel line with a minor accentuation indicated physiological disturbance, one of which most likely was due to the birth stress. In all, half of the cases showed consistent results between all three methods. Given a sufficient preservation of the hard tissues of the tooth, the histological approach is judged to enable the greatest amount of differentiation.

### **Possible growth disruption?**

The observation that many of the individuals with a tooth development indicative of dying after a preterm birth had more advanced osteological ages whereas those with dental indications for birth survival mostly had a delayed skeletal development might be interpreted as conveying biological meaning. A delay of skeletal development compared to tooth development has already been described, especially in samples of individuals that had a low socioeconomic status (e.g. Hodson and Gowland 2020). The lesser chronological variability of tooth formation compared to development of long bone length (that is more sensitive of adverse conditions) had been used to investigate growth disruption (cf. Hodson



Fig. 11 Scatterplot showing the growth of femoral diaphysis length from Ca n'Oliver individuals and the mean values given by Fazekas and Kósa (1978)

and Gowland 2020 and the discussion therein). In our sample, however, there is no evidence of growth disruption because femoral growth of these individuals is similar to that expected by the given values of Fazekas and Kosa (1978); see Fig. 11. In further studies, it should be investigated if the reverse relation of an advanced skeletal development could be related to such conditions like pregnancy diabetes. To do so, more research is also warranted into the actual variability of the gestational age at which deciduous canines begin to mineralize that has been shown to be slightly greater than previously thought (Dean et al. 2020).

### Foetal and neonatal deaths in proto-history

During the Iron Age, babies born between 22 and 36 weeks of gestation were highly exposed to the risks caused by their vital organ immaturity, in particular the lungs and brain. At this period of gestation, the thermoregulatory system is still not completely developed. Because of this, when the newborn's temperature drops below 32 °C, mortality could reach 98% (Dupâquier 1994). During populations where medical care is not provided at birth, the first week of life is the most critical, with the frequency of death gradually falling thereafter (Faerman and Smith 2008). For example, from a rural community of Nigeria, Lawoyin (2001) reported 55.10% of all infant deaths occurred in the first month (neonatal deaths) and nearly 74.1% of these died in the first week (perinatal deaths). About half of stillbirths occur during childbirth, with this being more common in the developing than developed world. Worldwide in 2015 there were about 2.6 million stillbirths that occurred after 28 weeks of pregnancy (about 1 for every 45 births) (Lawn et al. 2016). This rate increases in low-income countries: in South-Asia and Sub-Saharan Africa the rate is one stillbirth at the 28th week of pregnancy or later per 33 births (Cousens et al. 2011). In fact, most stillbirths (early or late stillbirths) worldwide (98%) happen in low- and middle-income countries, where medical care can be of low quality or unavailable (Cousens et al. 2011). In addition, at this proto-historical time, natural miscarriages, mainly due to deficient health conditions, were probably about a quarter of pregnancies in a month (González-Wagner et al. 1996). Therefore, the results of this study indicate that most of these perinatal deaths in Ca n'Oliver were probably miscarriages, stillbirths, and neonatal deaths.

Clinically, the majority of foetal and neonatal deaths are considered to reflect the endogenous state of the infant as the result of genetic and maternal influences such as congenital anomalies, prematurity, low birth weight, birth trauma, maternal infectious diseases, and maternal poor nutrition (Scott and Duncan 1998). The presence of well-developed lesions of cribra orbitalia in individual IH 1268 aged around 22 weeks of gestation indicates the existence of intrauterine development disturbances, at least in this individual. Cribra orbitalia is mainly associated to megaloblastic and haemolytic anaemias (Schultz 2001; Ortner 2003; Walker et al. 2009); however, it can also be associated to scurvy, rickets, and inflammatory processes of the skull and tumorous lesions (Schultz 2001; Ortner 2003; Walker et al. 2009; Brickley 2018). Chronic dietary deficiencies and malabsorption of vitamin B12 and/or folic acid are the most common causes of megaloblastic anaemia. Thalassemia is the most common cause of haemolytic anaemias. The age of this individual indicates that it has grown in the mother womb under the influence of some type of intrauterine stress which disturbed the development of the individual. It could be explained by an illness or bad nutrition of the mother which impedes the access of the baby to get all the necessary nutrients for having a successful and healthy development. In addition, this observation is also in accordance with the observation of sequels of intrauterine stress events observed in the histological analysis of the teeth of the analysed sample. Epidemics (that both newborns and their mothers will be subject to), precarious nutrition in most cases as well as both the poverty of available resources and the scant consideration of the child within the family scale would cause the strongest to survive since they would be the only ones capable withstanding future adversities (Gusi and Muriel 2008).

### **Bioarchaeology: cultural and funerary practices**

In bioarchaeology, one of the key aspects of infant mortality studies is that they provide information on the population's ability to adapt to their environment, their cultural practices, fertility rates, demography, maternal health, disease epidemics, birthing practices, and social attitudes towards children. However, the lack of data about the adult and children population from the Iberian Period (as we explained in the "Introduction" section) does not allow us to analyse most of these topics. Despite this, we can contribute to the knowledge on the possible foetal development of these individuals and contribute to the knowledge of cultural and funerary practices and social attitudes towards perinatal children, reconstructing identity at the earliest stages of life.

The Ca n'Oliver mortality pattern, which is similar to that obtained from a modern British population (first half of the twentieth century), indicates that the development of Ca n'Oliver individuals was within the normal range of growth expected for these foetal ages. This is evident when their femoral diaphysis length growth is compared with the growth data from Fazekas and Kósa (1978) (Figs. 7 and 11). The normal range of growth is in accordance with the social status of the Ca n'Oliver settlement, which corresponds to a second-class settlement (see the "Introduction" section) and if the entire population had equal access to the large grain silos, nutritional deficiencies are not expected. Therefore, it can be assumed that this population was reasonably well-adapted to their environment and that the broad mortality profile could be reflecting an endogenous mortality due to genetic and maternal influences, not being indicative of infanticide as some authors have suggested. It can be hypothesized that the presence of these stillbirths and neonatal individuals underneath the floor of domestic structures of the Ca n'Oliver site and not in a cross-generational cemetery suggests that these individuals died before the age at which they would have been accepted in the society as real individuals.

Ancient sources, such as Pliny the Elder, indicate that burial in domestic areas was the funeral practice on children who had died before the age of 7 months of life (Muriel et al. 2006), the age at which dental eruption occurs. Children who had not erupted their teeth were buried at home and those who had begun to erupt their first teeth received the same treatment as the rest of the population: cremation was practiced. Some authors relate the infant burial in the domestic environment of the Iberian Period with the Mother-Earth concept, capable of generating a new living being from the full return of a prematurely dead person, a belief that is observed in some African tribes (Vincent 1982; Merino et al. 2014). In fact, the foetal position, in which the perinatal and infant individuals of the Iberian Period are found buried, can be related to the condition of the embryo in the maternal body (Eliade 1991; Merino et al. 2014). On the other hand, at these perinatal ages, the babies would not be integrated in the community yet, and for this they would lack existence as such and their death would belong to the intimate sphere (Herthz 1905; Gusi and Muriel 2008). We, probably, are in front of familiar funerary rituals. However, a question arise, why infant inhumations are mostly found in economic activity areas? According to Merryfield (1987), the relationship between economic activity and infant inhumations could be explained through a beginning, ending, or change of activity ritual. Each new burial would explain the foundation of a new space dedicated to a productive activity, although this idea is difficult to be applied to our findings because there is no clear evidence of newborn sacrifices. If we think in a high mortality rate of neonatal individuals at this proto-historic period, we could think in natural deaths which happened in the period of time that followed the construction of a pottery, tannery, or metallurgic craft workshop as the case of Sant Miquel d'Olèrdola site (Subirà and Molist 2008). According to Molist (2005), in these cases, we probably are in front of a selection of buried individuals in these areas of production. This is because probably these areas of productivity would be controlled by the leaders of the settlement; and because of this the buried newborns in these areas could belong to a specific social status. This fact could explain the scarcity/lack of perinatal burials in other smaller settlements with an economic, agricultural, and livestock functionality, as for example Can Xercavins, which was contemporary to Ca n'Oliver, located at a distance of 2 km (Carlús-Martín and Ruiz-López 1991). The foundation of the craft, without a specific date, could be extended in time until the moment of the infant death. In this way, and from the infant death, the ritual of principle by which an offering is made could be practiced to promote success in the activity that was to begin in said space (Gusi and Muriel 2008). This idea is related to the idea which propose the infant burials as prophylactic deposits to promote a good season of agricultural work, sufficient rains for the harvests, or simply, as in this case, a success in the productive activity (Gusi and Muriel 2008). In this sense, a possible parallel is the practice, documented in Ancient Greece, of symbolically sacrificing infants who died at birth to Aphrodite, perhaps to propitiate fertility (Moneo 2003).

This type of burials of perinatal individuals underneath domestic floors was the most widespread in the northeast area of the Iberian Peninsula during the Iberian Period. In this geographical region, their presence has been documented in most of the sites belonging this period such as the sites of Necròpolis de Santa Madrona de Tarragona (seventh century BC), Illa d'en Reixac d'Ullestret (5<sup>th</sup>-fourth century BC), Puig Castellar de Sta Coloma de Gramanet (5<sup>th</sup>-fourth century BC), Necròpolis del Turó dels Dos Pins de Cabrera de Mar (3<sup>rd</sup>-second century BC), Mas Castellar de Pontós (5<sup>th</sup>-third century BC), Molí d'Espígol de Tornabous (4<sup>th</sup>-third century BC), Estinclells de Verdú (third century BC), Els Vilars d'Arbreca (third century BC), and Castell Vell d'Olius (2<sup>nd</sup>-first century BC). They are also documented in Early Iberian as well as in pre-Iberian contexts such as La Pedrera de Vallfogona de Balaguer, El Tossal de les Tenalles de Sidamon, Carretelà d'Aitona, and Zafranales de Fraga, among others (Agustí, 2000; Muriel et al. 2006; Merino et al. 2014). The perinatal individuals of Ca n'Oliver site would be situated within this context.

Therefore, these perinatal deaths were probably spontaneous abortions and neonatal deaths, reflecting an endogenous mortality profile due to genetic and maternal influences. In this paper, biological and social information on foetal and perinatal individuals of the Iberian Period is furnished for first time. The present study will serve to broaden our knowledge on individuals of the Iberian Period, and especially those of the Lands of the North-East Iberian Peninsula.

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Author contributions CR and CW designed the study, performed statistical analysis, Figs. 1, 4, 7, 8, 9, 10, and 11 and Tables 1, 2, 3, 4, 5, and 6, and wrote the main manuscript text.

CW did the dental study.

OL, AG, MK, and EK did the DNA analysis and Figs. 5 and 6.

CR, SM, and MM did the anthropological study.

MG and JF prepared Figs. 2 and 3, excavated the site, and curated the skeletal material in the museum.

All of the authors have reviewed the manuscript.

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### Declarations

**Ethics approval** Not applicable. As we used archaeological individuals from the Iron Age II, the research did not require an approval from the Ethics Committee.

Competing interests The authors declare no competing interests.

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### References

- Afonso C, Nociarova D, Santos C, Martinez-Labarga C, Mestres I, Duran M, Malgosa A (2019) Sex selection in late Iberian infant burials: integrating evidence from morphological and genetic data. Am J Hum Biol 31:e23204. https://doi.org/10.1002/ajhb.23204
- Agustí B (2000) Enterraments infantils i altres restes antropològiques (1987–1990) Monografies d'Ullestret, 1: Excavacions arqueològiques a l'Illa d'en Reixac (1988–1992). Museu d'Arqueologia de Catalunya, Ullestret (Girona), pp 305–306
- Alberto-Barroso V, Delgado-Arias T, Ordóñez AC, Serrano G, Fregel R, Velasco-Vazquez J (2022) Perinatal burials at pre-Hispanic noncemetery sites in Gran Canaria: Tophet, infanticide, or natural mortality? Int J Osteoarchaeol 32:100–110
- Albizuri S (2011) Depósitos de ovicaprinos en ámbito doméstico ibérico. El ejemplo del poblado de Ca n'Oliver (Cerdanyola del Vallès, Barcelona). Archaeofauna 20:85–101
- Albizuri S, Subirà ME, Ruiz J (2012) Ritual Patterns in the deposits of fauna associated with Iberian burials: the necropolis of El Poblado during the fourth to second centuries BC (Archaeological Complex of Coimbra del Barranco Ancho, Murcia). Int J Osteoarchaeol 25:180–200. https://doi.org/10.1002/oa.2274
- Alesan A (1990) Estudi d'una població subadulta de l'Edat del Ferro: demografia, antropometria i creixement. Master thesis dissertation. Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Barcelona
- Almagro-Gorbea M (2001) Aproximaciones a la demografía de la Celtiberia. In: Berrocal-Rangel L, Gardes P (eds) Entre celtas e íberos: las poblaciones protohistóricas de las Galias e Hispania. Real Academia de la Historia, Casa de Velázquez, pp 45–60
- Antoine D, Hillson S, Dean MC (2009) The developmental clock of dental enamel: a test for the periodicity of prism cross-striations in modern humans and an evaluation of the most likely sources of error in histological studies of this kind. J Anat 214:45–55. https://doi.org/10.1111/j.1469-7580.2008.01010.x
- Armelagos GJ, Goodman AH, Harper KN, Blakey ML (2009) Enamel hypoplasia and early mortality: bioarcheological support for the Baker hypothesis. Evol Anthrop 18:261–271. https://doi.org/10. 1002/evan.20239
- Barberà J, Dupré X (1984) Els laietans, assaig de síntesi. Fonaments 4:31–86
- Barrial O (1989) El paradigma de les inhumacions infantils i la necessitat d'un nou enfocament teòric. Cuader Prehis y Arqueo Castello 14:9–18
- Behrman RE, Shiono PH (1997) Neonatal risk factors. In: Fanaroff AA, Martin RJ (eds) Neonatal-perinatal medicine: diseases of the fetus and infant. Mosby, St. Louis, USA, pp 3–12
- Belarte C, Sanmartí J (1997) Espais de culte i practiques rituals a la Catalunya protohistórica. Quader Prehis i Arqueo Castelló 18:7–32
- Birch W, Dean MC (2014) A method of calculating human deciduous crown formation times and of estimating the chronological ages of stressful events occurring during deciduous enamel formation. J Forensic Leg Med 22:127–144. https://doi.org/10.1016/j.jflm. 2013.12.002
- Boyde A (1990) Developmental interpretations of dental microstructure. In: DeRousseau CJ (ed) Primate life history and evolution:

monographs in primatology. John Wiley & Sons, Hoboken, pp 229–267

- Boyde A, Jones SJ (1983) Backscattered electron imaging of dental tissues. Anat Embryol 168:211–226
- Bradley A, Byrd J (2014) Commingled human remains. Methods in recovery, analysis and identification. How many people? Determining the number of individuals represented by commingled human remains. Academic Press, Oxford
- Brickley MB (2018) Cribra orbitalia and porotic hyperostosis: a biological approach to diagnosis. Am J Phys Anthropol 167:896– 902. https://doi.org/10.1002/ajpa.23701
- Briggs AW, Stenzel U, Johnson PLF, Green RE, Kelso J, Prüfer K, Meyer M, Krause J, Ronan MT, Lachmann M, Svante P (2007) Patterns of damage in genomic DNA sequences from a Neandertal. Proc Natl Acad Sci USA 104:14616–14621. https://doi.org/ 10.1073/pnas.0704665104
- Brothwell DR (1987) Desenterrando huesos. Fondo de cultura económica, México
- Butler NR, Alberman ED (1969) Perinatal problems. Livingstone, Edinburgh
- Carlús-Martín X, Ruiz-López FJ (1991) Can Xercavins: un nou assentament ibèric al Vallès. Limes Rev D'arqueol 1:127–133
- Cousens S, Blencowe H, Stanton C, Chou D, Ahmed S, Steinhardt L, Creanga AA, Tunçalp O, Balsara ZP, Gupta S, Say L, Lawn JE (2011) National, regional, and worldwide estimates of stillbirth rates in 2009 with trends since 1995: a systematic analysis. The Lancet 377:1319–1330. https://doi.org/10.1016/S0140-6736(10) 62310-0
- Crespo L, Subirà E, Ruiz J (2011) Twins in prehistory: the case from Olèrdola (Barcelona, Spain; s.IV-II BC). Int J Osteoarchaeol 21:751–756. https://doi.org/10.1002/cpt.1169
- De Miguel MP (2002) Estudio antropológico de la inhumación infantil de la Peña Negra. In: González-Prats A (ed). La necrópolis de cremación de Les Moreres (Crevillente, Alicante, España) (s. IX-VII). Edición a parte del III Seminario Internacional sobre Temas Fenicios, pp 471–475
- De Miguel MP (2005) Muertos y ritos. Aportes desde la osteoarqueología. In Grau-Mira I, Sala-Sellés F, Abad-Casal L (eds) La contestania Ibérica, treinta años después. Serie Arqueología. Publicaciones Universidad de Alicante, Alicante, pp 325–331
- Dean MC, Humphrey L, Groom A, Hasset B (2020) Variation in the timing of enamel formation in modern human deciduous canines. Arch Oral Biol 114:104719. https://doi.org/10.1016/j.archoral-bio.2020.104719
- Deutsch D, Pe'er E, Gedalia I (1984) Changes in size, morphology and weight of human anterior teeth during the fetal period. Growth 48:74–85
- Dewey KG, Nommsen-Rivers LA, Heining MJ, Cohen RJ (2003) Risk factors for suboptimal infant breastfeeding behavior, delayed onset of lactation, and excess neonatal weight loss. Pediatrics 112:607–619. https://doi.org/10.1542/peds.112.3.607
- Duday H (1990) L'étude anthropologique des sépultures à incinération. Nouvelles De L'archéologie 40:28
- Duday H, Depierre G, Janin Th (2000) Validation des paramètres de quantification, protocols et strategies dans l'étude anthropologique des sépultures secondaires à incinération. L'exemple des nécropoles protohistoriques du Midi de la France. Archaéologie de la Mort, archéologie de la tombe au premier âge du fer (Conques 8–11 mai 1997). Actes du XXI Colloque International de l'Association Française pour l'Étude de l'Âge du Fer. MAM 5:7–29
- Dupâquier J (1994) Pour une histoirie de la prématurité. Ann Démograph Histori, pp 187–202
- Eliade M (1991) Mitos, sueños y misterios. Colección Paraísos Perdidos. Grupo Libro 88, Madrid

- Faerman M, Bar-Gal GK, Filon D, Greenbaltt CL, Stager L, Oppenheim A, Smith P (1998) Determining the sex of infanticide victims from the Late Roman Era through ancient DNA analysis. J Archaeol Sci 25:861–865
- Faerman M, Smith P (2008) Has society changed its attitude to infants and children? Evidence from archaeological sites in the Southern Levant. In: Gusi F, Muriel S, Olària C (eds). Nasciturus, infans, puerulus, vovis mater terra. The death in the childhood. Servei d'Investigacions Arqueològiques i Prehistòriques. Diputació de Castelló, Castelló, pp. 211–229
- Fazekas GI, Kósa F (1978) Forensic Fetal Osteology. Akademiai Kiadó, Budapest
- Fernandez-Crespo T (2008) Los enterramientos infantiles en contextos domésticos en la Cuenca Alta/Media del Ebro: a propósito de la inhumación del despoblado altomedieval de Aistra (Álava). Munibe Antropol – Arkeol, 59:199–217
- FitzGerald C, Saunders S, Bondioli L, Macchiarelli R (2006) Health of infants in an Imperial Roman skeletal sample: perspective from dental microstructure. Am J Phys Anthropol 130:179–189. https://doi.org/10.1002/ajpa.20275
- Francès J, Guàrdia M (2011) Les defenses exteriors del poblat ibèric de Ca n'Oliver (Cerdanyola, Vallès Occidental). Rev Arqueol Ponent 21:165–172
- Francès J, Guardia M (2012) El poblat ibèric del Turó de Ca n'Oliver (segles VI-I aC). Balanç de 25 anys de recerca i difusió. Tribuna D'arqueologia 2010–2011:267–291
- Fu Q, Mittnik A, Johnson PLF, Bos K, Lari M, Bollongino R, Sun C, Giemsch L, Schmitz R, Burger J, Ronchitelli AM, Martini F, Cremonesi RG, Svoboda J, Bauer P, Caramelli D, Castellano S, Reich D, Pääbo S, Krause J (2013) A revised timescale for human evolution based on ancient mitochondrial genomes. Curr Biol 23:553–559. https://doi.org/10.1016/j.cub.2013.02. 044
- Fu Q, Yang YR, Dhakal S, Zhao Z, Liu M, Zhang T, Walter NG, Yan H (2016) Assembly of multienzyme complexes on DNA nanostructures. Nat Protoc 11:2243–2273. https://doi.org/10.1038/ nprot.2016.139
- Geirsson RT (1991) Ultrasound instead of last menstrual period as the basis of gestational age assignment. Ultrasound Obstet Gynecol 1:212–219. https://doi.org/10.1046/j.1469-0705.1991.01030 212.x
- Gilmore HF, Halcrow SE (2014) Sense or sensationalism. Approaches to explaining high perinatal mortality in the past. In: Thompson JL, Alfonso-Durruty MP, Crandall JJ (eds) Tracing childhood. Biological investigations of early lives in antiquity. University of Florida Press, Gainsville, pp 123–138
- González-Wagner C, Peña V, Ruiz LA (1996) La mortalidad infantil en el mundo antiguo: causas, biopatologias y conductas culturalmente pautadas. Consideraciones a propósito del debate sobre la incidencia del infanticidio. In: Villalain-Blanco JD, Gómez-Bellard C, Gómez-Bellard F (eds) Actas del III Congreso Nacional de Paleopatologia (Valencia, 1993), Valencia, pp 63–67
- Gowland RL, Halcrow SE (eds) (2020) The mother-infant nexus in anthropology. Springer, Cham, Switzerland
- Gowland RL, Chamberlain AT (2002) A Bayesian approach to ageing perinatal skeletal material from archaeological sites: implications for the evidence for infanticide in Roman-Britain. J Archaeol Sci 29:677–685. https://doi.org/10.1006/jasc.2001.0776
- Gowland RL, Chamberlain A, Redfern RC (2014) On the brink of being: re-evaluating infanticide and infant burial in Roman Britain. J Roman Arch Suppl Series 96:69–88
- Green RE, Malaspinas AS, Krause J, Briggs AW, Johnson PLP, Uhler C, Meyer M, Good JM, Maricic T, Stenzel U, Prüfer K, Siebauer M, Burbano HA, Ronan M, Rothberg JM, Egholm M, Rudan P, Brajković D, Kućan Z, Gusić I, Wikström M, Laakkonen L, Kelso J, Slatkin M, Pääbo S (2008) A complete Neandertal

mitochondrial genome sequence determined by high-throughput sequencing. Cell 134:416–426. https://doi.org/10.1016/j.cell. 2008.06.021

- Gusi F, Muriel S (2008). Panorama actual de la investigación de las inhumaciones protohistóricas del sudoeste mediterráneo europeo. In: Gusi F, Muriel S, Olaria C (eds) Nasciturus, Infans, Puerulus vovis mater terra. SIAP Diputació de Castelló, Castelló, pp 257–329
- Halcrow SE, Tayles N, Elliott GE (2017) The bioarchaeology of fetuses. In: Han S, Betsinger TK, Scott AB (eds) The anthropology of the fetus: biology, culture, and society. Berghahn Books, New York, NY, pp 83–111
- Hansen A, Willerslev E, Wiuf C, Mourier T, Arctander P (2001) Statistical evidence for miscoding lesions in ancient DNA templates. Mol Biol Evol 18:262–265. https://doi.org/10.1093/oxfordjour nals.molbev.a003800
- Harris O (1982) The dead and the devils among the Bolivian Laymi.In: Bloch M, Parry J (eds) Death and the regeneration of life.Cambridge University Press, London, pp 45–73
- Hassan NAM, Brown KA, Eyers J, Brown TA, Mays S (2014) Ancient DNA study of the remains of putative infanticide victims from the Yewden Roman villa site at Hambleden, England. J Archaeol Sci 43:192–197. https://doi.org/10.1016/j.jas.2013.12.017
- Hertz H (1905) La représentation collective de la mort. L'année Sociologique 10:45–137
- Hillson S (2014) Tooth development in human evolution and bioarchaeology. Cambridge, New York
- Hodson CM, Gowland R (2020) Like mother, like child: investigating perinatal and maternal health stress in post-medieval London. In: Gowland R, Halcrow S (eds) The mother-infant nexus in anthropology, small beginnings, significant outcomes. Springer, Las Vegas, pp 39–82
- Hofreiter M, Jaenicke V, Serre D, von Haeseler A, Pääbo S (2001) DNA sequences from multiple amplifications reveal artifacts induced by cytosine deamination in ancient DNA. Nucleic Acids Res 29:4793–4799. https://doi.org/10.1093/nar/29.23. 4793
- Hrdy SB (1990) Sex bias in nature and history: a late 1980s reexamination of the "biological origins" argument. Am J Phys Anthropol Yearbook 33:25–37. https://doi.org/10.1002/ajpa.1330330504
- Jaya DS, Kumar NS, Bai LS (1995) Anthropometric indices, cord length and placental weight in newborns. Indian Pediatr 32:1183–1188
- Jeanty P, Romero R (1984) Obstetrical Ultrasound. McGraw-Hill, London
- Kierdorf H, Witzel C, Upex B, Dobney K, Kierdorf U (2012) Enamel hypoplasia in molars of sheep and goats, and its relationship to the pattern of tooth crown growth. J Anat 220:484–495. https:// doi.org/10.1111/j.1469-7580.2012.01482.x
- Kierdorf H, Witzel C, Bocaege E, Richter T, Kierdorf U (2021) 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). Am J Phys Anthropol 174:20–34. https://doi.org/10. 1002/ajpa.24156
- Kinaston RL, Buckley HR, Halcrow SE, Spriggs MJT, Bedford S, Neal K, Gray A (2009) Investigating foetal and perinatal mortality in prehistoric skeletal samples: a case study from a 3000-yearold Pacific Island cemetery site. J Archaeol Sci 36:2780–2787. https://doi.org/10.1016/j.jas.2009.09.004
- Kircher M (2012) Analysis of high-throughput ancient DNA sequencing data, in ancient DNA. In: Shapiro B, Hofreiter M (eds) Methods in molecular biology. Humana Press, New York: 840–197–228
- Kronfeld R, Schour I (1939) Neonatal dental hypoplasia. JADA 16:18– 20. https://doi.org/10.14219/jada.archive.1939.0041

- Kumm J, Laland KN, Feldman MW (1994) Gene-culture coevolution and sex ratios. The effects of infanticide, sex-selective abortion, sex selection, and sex-biased parental investment on the evolution of sex ratios. Theor Popul Biol 46:249–278. https://doi.org/ 10.1006/tpbi.1994.1027
- Lampa S, Dahlo M, Olason P, Hagberg J, Spjuth O (2013) Lessons learned from implementing a national infrastructure in Sweden for storage and analysis of next-generation sequencing data. Gigascience 2:9. https://doi.org/10.1186/2047-217X-2-9
- Lassen C, Hummel S, Herrmann B (1997) Molecular sex determination in skeletal remains of premature and newborn infants of the Aegerten, Switzerland, burial field. Anthropol Anz 55:183–191
- Lawn JE, Blencove H, Waiswa P, Amouzou A, Mathers C, Hogan D, Flenady V, Føen JF, Qureshi ZU, Cl Calderwood, Shiekh S, Jassir FB, You D, McClure ME, Mathai M, Cousens S (2016) Stillbirths: rates, risk factors, and acceleration towards 2030. Lancet 387:587–603
- Lawoyin TO (2001) Risk factors for infant mortality in a rural community of Nigeria. J RSPH 12:114–118. https://doi.org/10.1177/ 146642400112100213
- Levine RS, Turner EP, Dobbing J (1979) Deciduous teeth contain histories of developmental disturbances. Early Hum Dev 3:211–220. https://doi.org/10.1016/0378-3782(79)90009-4
- Lewis M (2002) The impact of industrialisation: comparative study of child health in four sites from medieval and post-medieval England (850–1859 AD). Am J Physl Anthropol 119:211–223. https://doi.org/10.1002/ajpa.10126
- Lewis ME (2007) The bioarchaeology of children: perspectives from biological and forensic anthropology. Cambridge University Press, Cambridge
- Lewis ME (2017) Fetal palaeopathology. An impossible discipline? In: Han S, Betsinger TK, Scott AB (eds) The anthropology of the fetus: biology, culture, and society. Berghahn Books, New York, NY, pp 112–131
- Lewis ME, Gowland R (2007) Brief and precarious lives: infant mortality in contrasting sites from medieval and post-medieval England (AD 850–1859). Am J Phys Anthropol 134:117–129. https://doi. org/10.1002/ajpa.20643
- Li H, Durbin R (2010) Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics 26:589–595. https:// doi.org/10.1093/bioinformatics/btp698
- Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R (2009) The Sequence Alignment/Map format and SAMtools. 1000 Genome Project Data Processing Subgroup. Bioinformatics 25:2078–2079. https://doi.org/10. 1093/bioinformatics/btp352
- Liversidge HM, Herdeg B, Rösing FW (1998) Dental age estimation of non-adults. A review of methods and principles. In: Alt KW, Rösing FW, Teschler-Nicola M (eds) Dental anthropology: fundamentals, limits and perspectives. Springer, Vienna, pp 419–442
- Lorentz KO, Lemmers SAM, Chrysostomou C, Dirks W, Zaruri MR, Foruzanfar F, Sajjadi SAM (2019) Use of dental microstructure to investigate the role of prenatal and early life physiological stress in age at death. J Archaeol Sci 104:85–96. https://doi.org/ 10.1016/j.jas.2019.01.007
- Macchiarelli R, Bondioli L, Debénath A, Mazurier A, Merceron G (2006) How Neanderthal teeth grew. Nature 444:748–751. https://doi.org/10.1038/nature05314
- Malmström H, Gilbert TPM, Thomas MG, Brandström M, Storå J, Molnar P, Andersen PK, Bendixen Ch, Holmlund G, Götherström A, Willerslev E (2009) Ancient DNA reveals lack of continuity between Neolithic hunter-gatherers and contemporary Scandinavians. Curr Biol 19:1758–1762. https://doi.org/10. 1016/j.cub.2009.09.017
- Mays S (1993) Infanticide in Roman Britain. Antiquity 67:883-888

- Mays S (2014) Bioarchaeology of the homicide of infants and children. In: Thompson JL, Alfonso-Durruty MP, Crandall JJ (eds) Tracing childhood. University Press of Florida, Gainsville, Biological investigations of early lives in antiquity, pp 99–122
- Mays S (2021) The archaeology of human bones, 3rd edn. Routledge, London
- Mays S, Eyers J (2011) Perinatal infant death at the Roman villa site at Hambleden, Buckinghamshire, England. J Archaeol Sci, 38–1931–1938. https://doi.org/10.1016/j.jas.2011.04.002
- Mays S, Faerman M (2001) Sex identification in some putative infanticide victims from Roman Britain using ancient DNA. J Archaeol Sci, 28–555–559. https://doi.org/10.1006/jasc.2001.0616
- Merino M, Rissech C, Turbòn D (2014) El yacimiento Ibérico de Castellvell de Olius (Lleida, Solsonés). Estudio bioantropológico de dos individuos infantiles. In: Alonso S, Hervella M, Izaguirre N, Peña JA, Rebato E, de la Rua C (eds) Investigación en antropología física. Una mirada al futuro. Servicio Editorial de la Universidad del País Vasco, Bilbao, pp 451–458
- Merryfield R (1987) The archaeology of ritual and magic. New Ambsterdam Books, New York
- Meyer M, Kircher M (2010) Illumina sequencing library preparation for highly multiplexed target capture and sequencing. Cold Spring Harbor Protocols, pdb.prot5448. https://doi.org/10.1101/ pdb.prot5448
- Molist N (2005) La funcionalitat dels espais amb inhumacions perinatals múltiples en época ibèrica. In: Mercadal-Frenandez O (ed) Actes del XIII Col·loqui internacional d'Arqueologia de Puigcerdà. Món Ibèric als Països Catalans. Homenatge a Josep Barbera i Ferràs (Puigcerdà, 2003). Institud d'Estudis Ceretans, Puigcerdà, pp 1015–1026
- Moneo, T. (2003). Religión Ibérica. Santuarios, ritos y divinidades (siglos VII-I AC). Biblioteca Arqueológica Hispana 20. Real Academia de Historia, Madrid
- Muriel S, Asencio D, Cardona R, Ferrer C, Morer J, Pou J, Saula J, Saula C (2006) Un enterrament infantil al jaciment ibèric d'Estinclells (Verdú, Urgell). Urtx 19:41–69
- Nava A, Coppa A, Coppola D, Mancini L, Dreossi D, Zanini F, Bondioli L (2017a) Virtual histological assessment of the prenatal life history and age at death of the upper Paleolithic fetus from Ostuni (Italy). Scientific Reports, 7–9427. https://doi.org/10. 1038/s41598-017-09773-2
- Nava A, Bondioli L, Coppa A, Dean C, Rossi PF, Zanolli C (2017b) New regression formula to estimate the prenatal crown formation time of human deciduous central incisors derived from a Roman Imperial sample (Velia, Salerno, Italy, I-II cent. CE). PLoS One, 12-e0180104. https://doi.org/10.1371/journal.pone.0180104
- Orlando L, Ginolhac A, Raghavan M, Vilstrup J, Rasmussen M, Magnussen K, Steinmann KE, Kapranov P, Thompson JF, Zazula G, Froese D, Moltke I, Shapiro B, Hofreiter M, Al-Rasheid KAS, Gilbert MTP, Willerslev E (2011) True single-molecule DNA sequencing of a Pleistocene horse bone. Genome Research, 21–1705–1719. http://www.genome.org/cgi/doi/10.1101/gr. 122747.111
- Ortner DJ (2003) Identification of pathological conditions in human skeletal remains, 2nd edn. Academic Press, USA
- Rissech C (2016) La estimación de la edad de individuos adultos y subadultos a partir de material esquelético. In: Sanabria C (ed) Patología y antropología forense de la muerte: la investigación científico-judicial de la muerte y la tortura, desde las fosas clandestines, hasta la audiencia pública. Forensic Publisher, Bogotá DC, pp 237–292
- Rissech C, Sañudo JR, Malgosa A (2001) Acetabular point: a morphological and ontogenetic study. J Anat 198:743–748. https://doi. org/10.1046/j.1469-7580.2001.19860743.x
- Rissech C, López-Costas O, Turbón D (2013a) Humeral development from neonatal period to skeletal maturity – application in age and

sex assessment. Int J Leg Med 127:201–212. https://doi.org/10. 1007/s00414-012-0713-7

- Rissech C, Marquez-Grant N, Turbon D (2013b) A collation of recently published Western European formulae for age estimation of subadult skeletal remains: recommendations for forensic anthropology and osteoarchaeology. J Forensic Sci 58:163–168. https:// doi.org/10.1111/1556-4029.12011
- Rossi PF, Bondioli L, Geusa G, Macchiarelli R (1999) Osteodental biology of the people of Portus Romae (Necropolis of Isola Sacra, 2nd–3rd Cent. AD). I. Enamel microstructure and developmental defects of the primary dentition. Museo Nazionale L. Pigorini, Rome
- Rushton M (1933) On the fine contour lines of the enamel of milk teeth. Dental Research 53:170–171
- Rushton A (1939) The birifrangence of deciduous tooth enamel formed before and after birth. Br Dent J 67:1–10
- Sanmartí J (2004) DeIs grups locals a l'estat arcaic: el desenvolupament de la complexitat a la Catalunya protohistórica. Pyrenae 35:7–41
- Sanmartí J (2009) From the archaic states to romanization: a historical and evolutionary perspective on the Iberians. Institut d'Estudis Catalans. Barcelona Catalan Histo Rev 2:9–32
- Sanmartí J, Santacana J (2005) Els Ibers del Nord. Rafael Dalmau, Barcelona
- Sawyer S, Krause J, Guschanski K, Savolainen V, Pääbo S (2012) Temporal patterns of nucleotide misincorporations and DNA fragmentation in ancient DNA. PLoS One 7:e34131. https://doi. org/10.1371/journal.pone.0034131
- Scheuer L, Black S (2000) Developmental juvenile osteology. Academic Press, London, UK
- Schour I (1936) The neonatal line in the enamel and dentin of the human deciduous teeth and first permanent molar. JADA 23:1946–1955
- Schultz M (2001) Paleohistopathology of bone: a new approach to the study of ancient diseases. Am J Phys Anthropol Yearbook, 44–106– 147. https://doi.org/10.1002/ajpa.10024
- Schutkowski H (1993) Sex determination of infant and juvenile skeletons: I. Morphognostic features. Am J Phys Anthropol 90:199– 205. https://doi.org/10.1002/ajpa.1330900206
- Schwartz JH, Houghton F, Macchiarelli R, Bondioli L (2010) Skeletal remains from Punic Carthage Do not support systematic sacrifice of infants. PLoS ONE 5:e9177. https://doi.org/10.1371/journal. pone.0009177
- Schwartz JH, Houghton F, Bondioli L, Macchiarelli R (2017) Two tales of one city: data, inference and Carthaginian infant sacrifice. Antiquity 91:442–454. https://doi.org/10.15184/aqy.2016.270
- Scott S, Duncan CJ (1998) Human demography and disease. Cambridge University Press, Cambridge
- Siebke I, Moghaddam N, Cunningham CA, Witzel C, Lösch S (2019) Those who died very young—inferences from δ15N and δ13C in bone collagen and the absence of a neonatal line in enamel related to the possible onset of breastfeeding. Am J Phys Anthropol 169:664–677. https://doi.org/10.1002/ajpa.23847
- Skinner MF (1992) Gestation length and location of the neonatal line in human enamel. J Paleopath 2:41–50
- Skinner MF, Dupras T (1993) Variation in birth timing and location of the neonatal line in human enamel. J Forensic Sci 38:1383–1390
- Skoglund P, Storå J, Götherström A, Jakobsson M (2013) Accurate sex identification of ancient human remains using DNA shotgun sequencing. J Archaeol Sci 40:4477–4482. https://doi.org/10. 1016/j.jas.2013.07.004
- Skoglund P, Northoff BH, Shunkov MV, Derevianko AP, Pääbo S, Krause J, Jakobsson M (2014) Separating endogenous ancient DNA from modern day contamination in a Siberian Neandertal. Proc Natl Acad Sci USA 111:2229–34. https://doi.org/10.1073/ pnas.1318934111

- Smith P, Avishai G (2005) The use of dental criteria for estimating postnatal survival in skeletal remains of infants. J Archaeol Sci 32:83–89. https://doi.org/10.1016/j.jas.2004.06.008
- Smith P, Kahila G (1992) Identification of infanticide in archaeological sites: a case study from the Late Roman-Early Byzantine Periods at Ashkelon, Israel. J Archaeol Sci 19:667–675. https://doi.org/ 10.1016/0305-4403(92)90036-3
- Smith P, Avishai G, Greene JA, Stager LE (2011) Aging cremated infants. The problem of the sacrifice at the Tophet of Carthage. Antiquity 85:859–874. https://doi.org/10.1017/S0003598X0 0068368
- Smith P, Stager LE, Greene JA, Avishai G (2013) Cemetery or sacrifice? Infant burials at the Carthage Tophet. Antiquity 87:1191–1207. https://doi.org/10.1017/S0003598X00049954
- Subirà MA, Molist N (2008) Inhumacions perinatals múltiples i espais de treball en els assantaments ibers. In: Gusi F, Muriel S, Olària C (eds) Nasciturus, Infants, Puerulus, Vobis Mater Terra. The dearh in the childhood. Servei d'Investigacions Arqueològiques i Prehistòriques. Diputació de Castelló, Castelló, pp 365–385
- Svensson EM, Anderung C, Baubliene J, Persson P, Malmström H, Smith C, Vretemark M, Daugnora L, Götherström A (2007) Tracing genetic change over time using nuclear SNPs in ancient and modern cattle. Animals Genetics 38:378–83. https://doi.org/10. 1111/j.1365-2052.2007.01620.x
- Tirado JB (2010) Breve historia de los Íberos, 2nd edn. Ediciones Nowtilus SL, Madrid
- Ucko PJ (1969) Ethnography and the archaeological interpretation of funerary remains. World Archaeology 1:262–80
- Uhl NM, Nawrocki SP (2010) Multifactorial estimation of age at death from the human skeleton. In: Latham KE, Finnegan M (eds) Age estimation of the human skeleton. Thomas publisher, Illinois, Charles C, pp 243–261
- Villena N (1997). Hiérarchie et fiabilité des liaisons ostéologiques (par symetrie et par contiguïté articulaire) dans l'étude des sépultures anciennes. Doctoral Thesis. Université Bordeaux I, Bordeaux
- Vincent LT (1982) La mort africaine. Idéologie funéraire en Afrique noire. Payot, Paris

- Waldron T, Taylor GM, Rudling D (1999) Sexing of Romano-British baby burials from the Beddingham and Bignor villas. Sussex Archaeological Collections 137:71–79
- Walker PL, Bathurst RR, Richman R, Gjerdrum T, Andrushko VA (2009) The causes of porotic hyperostosis and cribra orbitalia: a reappraisal of the iron-deficiency-anemia hypothesis. Am J Phys Anthropol 139:109–125. https://doi.org/10.1002/ajpa.21031
- Weber DF, Eisenmann D (1971) Microscopy of the neonatal line in developing human enamel. Am J Anat 132:375–392. https://doi. org/10.1002/aja.1001320307
- Whittaker DK, MacDonald DG (1989) A colour atlas of forensic dentistry. Wolfe Medical Publications, Ipswich, UK
- Whittaker DK, Richards D (1978) Scanning electron microscopy of the neonatal line in human enamel. Arch Oral Biol 23:45–50
- Witzel C (2014) Echoes from birth mutual benefits for physical and forensic anthropology by applying increment counts in enamel of deciduous teeth for aging. Anthropologischer Anzeiger 71:87– 103. https://doi.org/10.1002/ajpa.20822
- Witzel C, Kierdorf U, Schultz M, Kierdorf H (2008) Insights from the inside: histological analysis of abnormal enamel microstructure associated with hypoplastic enamel defects in human teeth. Am J Phys Anthropol 136:400–414
- Woodburn J (1982) Social dimensions of death in four African hunting and gathering societies. In: Bloch M, Parry J (eds) Death and the regeneration of life. Cambridge University Press, Cambridge, pp 187–210. https://doi.org/10.1017/CBO9780511607646.008
- Yang DY, Eng B, Waye JS, Dudar JC, Saunders SR (1998) Technical note: improved DNA extraction from ancient bones using silica-based spin columns. Am J Phys Anthropo 105:539–43. https://doi.org/10.1002/(SICI)1096-8644(199804)105:4<539:: AID-JPA10>3.0.CO;2-1

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