A Bioarchaeological Investigation of Fraternal Stillborn Twins from Tell el-Hesi

Jaime Ullinger, Lesley Gregoricka, Rebecca Bernardos, David Reich, Amel Langston, Paige Ferreri, and Brittney Ingram

View of Tell el-Hesi. Photograph by T. Rosen, courtesy of the Joint Archaeological Expedition to Tell el-Hesi.

In the summer of 1981, archaeologists carefully lifted ceramic sherds from a small Ottoman-period burial pit they had been excavating at Tell el-Hesi, and found two infant skeletons lying side-by-side. Initial speculations included that these were twins based on their close proximity to one another within this shared burial space and their similarities in overall size, and that they were stillborn based on their association with a water jug, a practice reserved for perinates among the bedouin burials at Tell el-Hesi. It would be another forty years before we examined their skeletons with a range of bioarchaeological techniques to address whether these two perinates were actually twins, and whether or not they had survived birth.

Ottoman-Period Mortuary Landscapes at Tell El-Hesi

Tell el-Hesi, located roughly twenty-six kilometers northeast of Gaza, is a large, well-known tell site with strata spanning from the Early Bronze Age to the Ottoman Era (fig. 1). The tell, originally excavated between 1890–1892 by William Matthews Flinders Petrie and later by Frederick Jones Bliss, was the first in the region to be excavated stratigraphically (Blakely and Horton 2001). In 1970, archaeologists returned to the site, and large-scale excavations with the Joint Archaeological Expedition to Tell el-Hesi continued until 1983. The primary focus of the Joint Expedition was on the Early Bronze Age and other earlier periods, but the team was committed to the "new archaeology," which meant excavating all areas systematically (Ludvik and Blakely 2020) at a time when other projects may not have done the same (Eakins 1993). A large Ottoman-Era cemetery blanketed the site, and the project decided that the systematic excavation of these burials was as important as uncovering earlier phases. Ultimately, over 800 burials were recorded and excavated from Fields I, V, VI, and IX; of these, 143 were found in 134 graves from Field V, including the 2 infants discussed here (figs. 2 and 3).

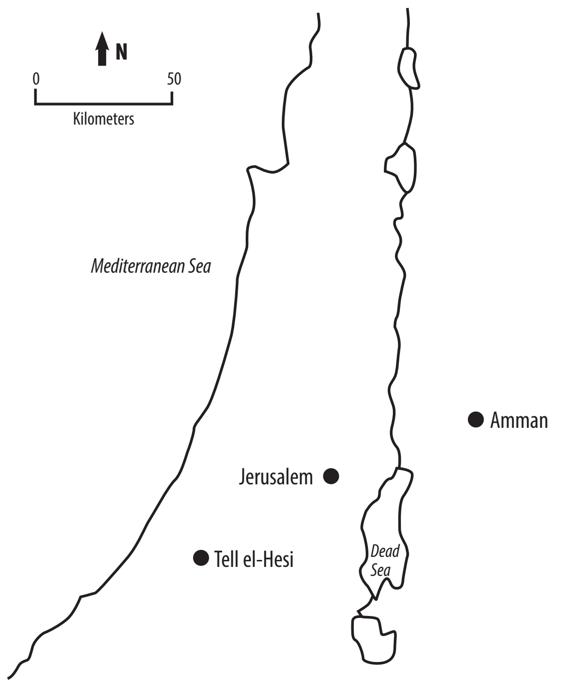

Figure 1. Map of southern Levant.

Figure 2. Aerial photograph of Tell el-Hesi with site of twin burial highlighted. Photograph courtesy of the Joint Archaeological Expedition to Tell el-Hesi.

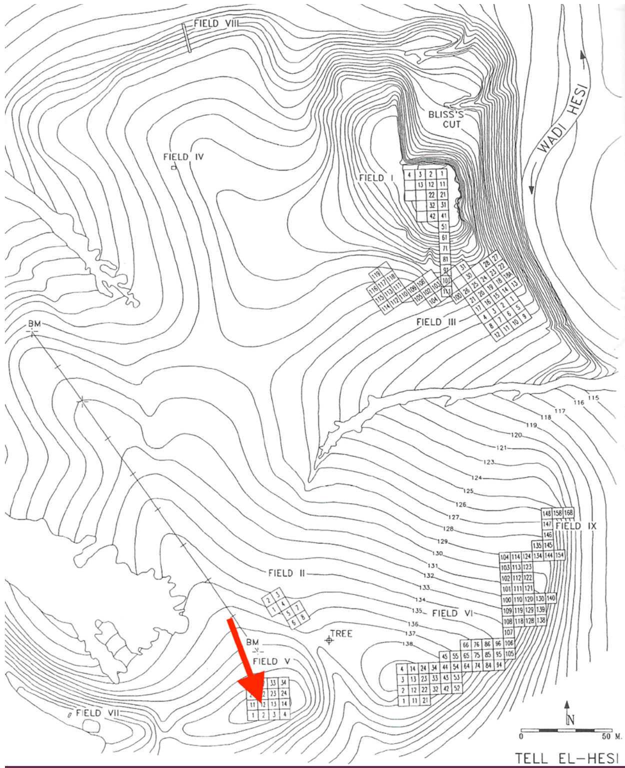

Figure 3. Map of Tell el-Hesi with site of twin burial highlighted. Modified from pl. 2, Eakins (1993).

Field V is located on the southern edge of the site, bounded by an Early Bronze Age mud-brick wall. It was this interest in underlying Early Bronze Age structures that led to the discovery of burials in this area in 1975, with formal excavation of the cemetery occurring in 1979 and 1981. Similar to other graves on site, those in Field V were pits dug into topsoil, with bodies placed at the bottom without coffins. There were some variations on this pattern—some graves narrowed at the bottom, while others had stones lining the sides or capstones at the top. Several skeletons were also buried inside of water jars within a grave (fig. 4). All were very young infants, some perinatal and others clearly fetal. This led to the hypothesis that these jar burials may represent infants that were not born alive, or who died shortly after birth.

While most of the excavated Ottoman-period burials were reburied at an off-tell site (Toombs 1985; Eakins 1993), skeletons from 111 burials were retained for further analysis and shipped to the United States. Some of these are represented by nearly complete skeletons, others by as little as a fragment of one bone. Initially residing at the Golden Gate Baptist Theological Seminary in Mill Valley, California, as part of the Marilyn Eakins Archaeological Collection, they are currently curated at the Center for Anthropological Research at Quinnipiac University.

The skeletons kept for further analysis were not randomly selected. Instead, they were retained because of a suspected pathological condition or other "curiosity" of note. Therefore, as a biased assemblage, they cannot answer larger questions about health or daily life during the Ottoman Era. Nevertheless, individual burials allow us to develop osteobiographies that connect us to particular lives in the past (Stodder and Palkovich 2012). One such burial contained two small infants who were believed to be stillborn twins (Eakins 1993; fig. 5). The babies, identified as V.12.020a and V.12.020b by the excavation team (referred to here as Infants A and B), were found lying on their backs, side-by-side, underneath a broken ceramic vessel (fig. 6). Although Eakins (1993) hypothesized that they were twins, multiple infant burials were not uncommon at the site. In Field I, for example, there were seven burials that contained multiple non-adults (Toombs 1985). Some of these were infants of a similar age, while others contained children of different ages. Four additional graves contained both an adult and an infant. Lawrence Toombs (1985) hypothesized that two of them may have been mothers and stillborn babies, but sex could not be estimated for the adult skeletons due to poor preservation. In both cases, the infant bones were scattered on top of the adult's skeleton.

We therefore sought to assess two hypotheses: (1) that the infants were twins, and (2) that they either did not survive birth, or died shortly thereafter. In order to test these hypotheses, we used multiple lines of evidence and a variety of bioarchaeological techniques, including age estimation via bone measurement, stable isotope analysis, assessment of bioerosion via microCT, and ancient DNA (aDNA) analysis.

Twins in the Archaeological Record

Twins have been the subject of anthropological study in the past, and are increasingly discussed in the bioarchaeological literature (Halcrow, Tales, and Elliott 2018); however, there are relatively few confirmed cases of twins from archaeological settings.

Figure 4. Photograph of water jar burial in Field I. Photograph by T. Rosen, courtesy of the Joint Archaeological Expedition to Tell el-Hesi.

David Owsley and Bruce Bradtmiller (1983) and Angela Lieverse and colleagues (Lieverse, Bazaliiskii, and Weber 2015) discussed adult female burials with infants *in utero*, found in the Northern Plains of North America and Neolithic Siberia, respectively. Other examples of possible twins rely on strong circumstantial evidence, which often includes age estimates and mortuary context (Crespo, Subirà, and Ruiz 2011; Halcrow, Tales, and Elliott 2012; Wheeler 2012; Flohr 2014; Dupras et al. 2015; Cavazzuti et al. 2021). Recently, aDNA confirmed a case of Upper Paleolithic monozygotic twins in what is now Austria, where one twin apparently died around the time of birth and the other survived for roughly fifty days (Teschler-Nicola et al. 2020). At times, this same evidence has been used to suggest that dual burials may not be twins at all, and that caution is warranted. For example, Zahi Hawass and Sahar Saleem (2011) used CT imaging to refine the ages of two fetal individuals (twenty-five and thirty-seven fetal weeks) previously believed to be possible twins from the tomb of King Tutankhamun. Although it is not uncommon for twins to have discordant fetal growth trajectories (Blickstein and Goldman 2003), their significant difference in fetal development made this relationship unlikely (Hawass and Saleem 2011). In another example, mtDNA analysis found that individuals from a double burial at Angel Mounds in North America once believed to be conjoined twins did not even share maternal relatives (Marshall et al. 2011).

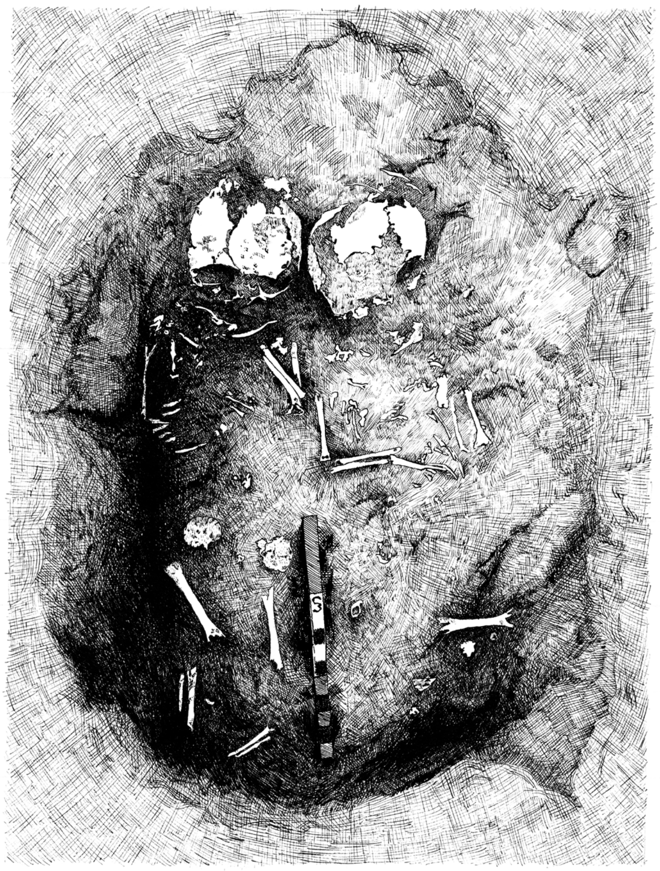

Figure 5. Illustration of the excavated burial. Illustration by Nasreen Abd Elal.

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Morphometrics

Measurements were taken from the bones of both skeletons to estimate age. Each measurement was taken twice by two

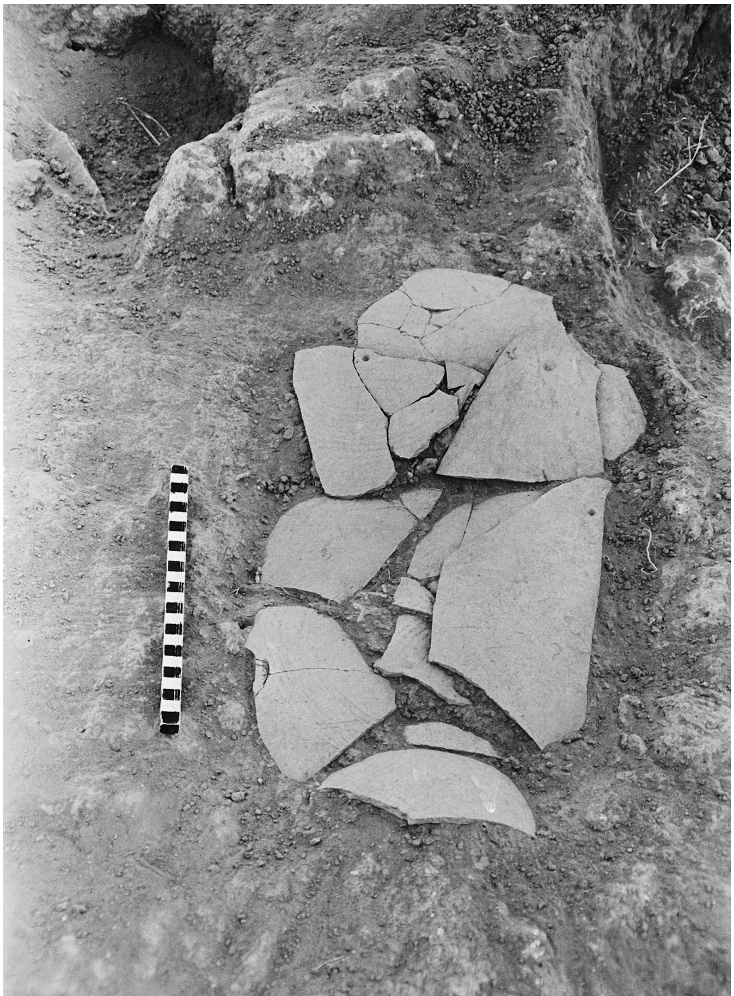

Figure 6. Photograph of twin burial with jar fragments on top. Photograph by R. Adams and M. Rose, courtesy of the Joint Archaeological Expedition to Tell el-Hesi.

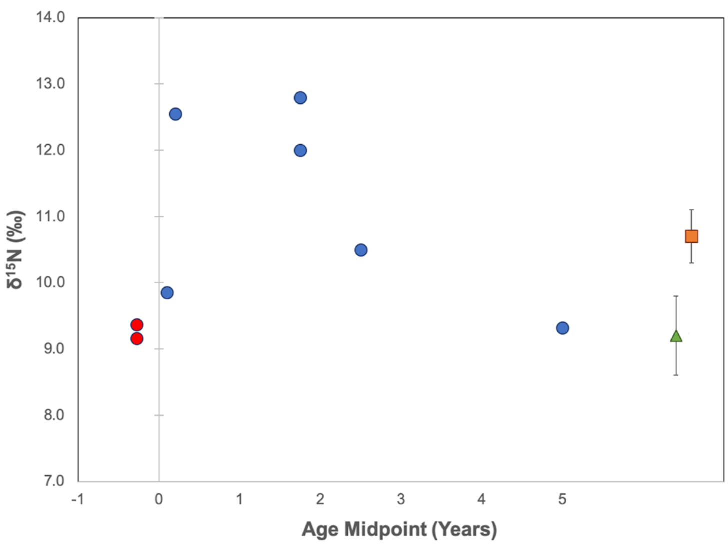

Figure 7. Nitrogen isotope values for Infants A and B (red circles), plotted relative to age alongside other nonadults (blue circles) as well as adult males (mean = orange square, ± 1s) and adult females (mean = green triangle, ± 1s) recovered from the Ottoman-period burial ground at Tell el-Hesi (adapted from fig. 4, Gregoricka and Ullinger 2022).

researchers (authors Langston and Ferreri) using Mitutoyo Absolute digital calipers. Following confirmation of statistically insignificant interobserver error, an average of each measurement was calculated (table 1). Age was estimated using a variety of techniques (Sherwood et al. 2000; Tocheri and Molto 2002; Schaefer, Black, and Scheuer 2009; Nagaoka, Kawakubo, and Hirata 2012; Nagaoka and Kawakubo 2015; Carneiro, Curate, and Cunha 2016). The widest range of possible ages for the infants spanned eighteen to thirty-eight fetal weeks. Overall, the measurements between the two were very consistent, suggesting that they were the same age when they died—between thirty and thirty-six fetal weeks.

Stable Isotopes

Bone collagen from the ribs of Infants A and B was sampled for stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes, broadly reflective of the protein component of diet (Schoeninger and DeNiro 1984; DeNiro 1987). *In utero*, fetuses are generally expected to share the same isotope values as that of their mother (Katzenberg, Herring, and Saunders 1996). While recent studies have shown modern infant $\delta^{13}C$ and $\delta^{15}N$ values from fingernails and hair to be slightly higher than those of the mother at birth (Fuller et al. 2006; de Luca et al. 2012), this difference could reflect short-term changes not evident in maternal bone collagen (Beaumont et al. 2015). Maternal hair nitrogen values generally decrease during pregnancy by approximately 0.5–1 percent, including in a documented case of twin pregnancy (Fuller et al. 2004), but can also increase if the mother experiences debilitating morning sickness or other periods of nutritional stress due to ^{15}N-enrichment from muscle catabolism (Fuller et al. 2005). After birth, with the initiation of breastfeeding, ^{13}C- and ^{15}N-enriched breastmilk elevates infant carbon values up to 1 per mille and nitrogen values between 2.0–3.6 per mille, gradually decreasing only when weaning begins (Schurr 1998; Fuller et al. 2006).

Infants A and B exhibited nearly identical carbon (A: -14.7 per mille; B: -14.8 per mille) and nitrogen (A: 9.2 per mille; B: 9.4 per mille) isotope values (fig. 7), indicating that they received comparable nutrition. While to date, no known bioarchaeological studies on twin (fetal or postbirth) $\delta^{13}C$ and $\delta^{15}N$ values have been conducted, the similar values of the Tell el-Hesi perinates lend support to their status as twins. Moreover, $\delta^{15}N$ values for Infants A and B closely mirror mean adult female values (9.2 ± 0.6 per mille, 1σ; n=4) at the site (Gregoricka and Ullinger 2022), indicating that they had not yet begun to breastfeed due to a lack of ^{15}N-enrichment. As these infant $\delta^{15}N$ values were not significantly elevated over those of adult females, this result may also point to an absence of nutritional stress for the mother.

The ribs and long bones of six additional nonadults from Fields V and VI, all less than five years of age at death, were also sampled so that the isotopic values of Infants A and B might be better contextualized against culturally prescribed childhood feeding trends at the site (fig. 7). These additional nonadult δ^{15}N values (11.2 ± 1.5 per mille, 1σ; n=6), while limited in number and in ages represented, suggest that supplemental breastfeeding continued until at least two years of age (Gregoricka and Ullinger 2022). The breastfeeding and weaning curve observed here further illustrates that Infants A and B did not possess elevated δ^{15}N values indicative of the initiation of breastfeeding, making it likely that they were stillborn.

Bone Bioerosion

It has been observed that after death, decomposition of the skeleton generally occurs by bacterial action from our own microbiome, and not from exogenous bacteria in the burial environment (White and Booth 2014). Bacteria colonize our gastrointestinal tract during the birthing process and breastfeeding (Kuperman et al. 2020). Therefore, if a baby does not live to nurse, its microbiome is not populated, and it is not exposed to bacterial degradation after death. Recent experiments have shown that stillborn infants do not have evidence of the microstructural changes brought about by bacterial bioerosion in their skeletons (Booth 2015), and that these changes can be assessed nondestructively via observation of bone cross-sections using microCT (Booth, Redfern, and Gowland 2016). Bacterial changes cause a cross section of bone to appear less dense (darker) generally, radiating outward from the internal portion of the bone (the medullary cavity) in a gradual, diffuse pattern. The left femur from each perinate was imaged with microCT, and neither shows signs of bacterial tunneling (figs. 8 and 9), indicating that they were likely stillborn or died shortly after birth, prior to breastfeeding. Both images have uniform density throughout the cross section and lack evidence of bacterial tunneling (e.g., see Booth, Redfern, and Gowland 2016 for clear illustrations of no bioerosion [fig. 7.a] and minor bioerosion [fig. 7.c]).

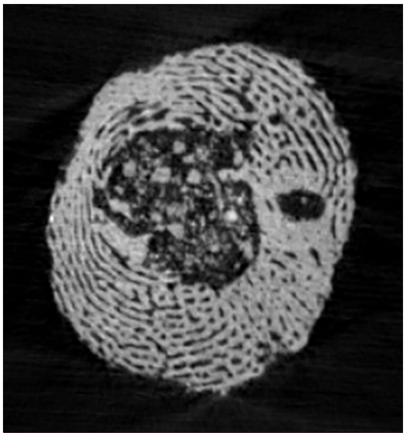

Figure 8. Micro-CT cross section of femur from Infant A, showing uniform density and a lack of bioerosion. Larger holes visible in the image are due to postburial factors that are unrelated to bacterial bioerosion. Image courtesy of Andrew Nelson.

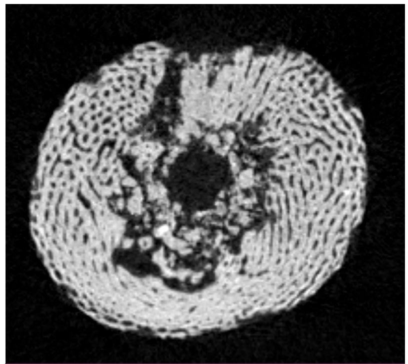

Figure 9. Micro-CT cross section of femur from Infant B, showing uniform density and a lack of bioerosion. Larger holes visible in the image are due to postburial factors that are unrelated to bacterial bioerosion. Image courtesy of Andrew Nelson.

DNA

The right *pars petrosa*, a portion of the temporal bone in the skull, from each individual was analyzed for aDNA. We (authors Reich and Bernardos) isolated the DNA-rich cochlea from each *pars petrosa* using a fine dental sandblasting tool and milled the isolated cochlea into fine powder (Pinhasi et al. 2019). DNA was extracted using a protocol designed to retain short DNA fragments, as are typical of degraded ancient specimens (Dabney et al. 2013; Korlević et al. 2015), and converted into a form that could be sequenced into double-stranded libraries, doing so in the presence of the enzyme uracil-DNA-glycosylase (UDG) to reduce the rate of characteristic ancient DNA degradation (Rohland et al. 2015). We then enriched the libraries for the mitochondrial genome (Fu et al. 2013), and for about 1.24 million single nucleotide polymorphism (SNP) targets in the nuclear genome (Haak et al. 2015; Fu et al. 2015). The resulting enriched DNA as well as unenriched DNA was sequenced from the same two libraries on Illumina NextSeq500 instruments. The individuals were consistent with authentic uncontaminated ancient DNA as assessed by the presence of a high rate of characteristic cystosine-to-uracil deamination at the terminal nucleotide (7.7 percent and 7.8 percent), and by a high rate of matching of mitochondrial sequences to the consensus sequence (95 percent confidence intervals of 98.3–99.6 percent and 99.3–100 percent respectively).

By examining the rate of Y chromosome to the sum of X and Y chromosome sequences we observed very low ratios of 0.7 percent and 0.8 percent which is consistent with an absence of Y chromosomes and female genetic sex assignments for both individuals. Uncontaminated female DNA typically has a ratio of less than 3 percent by this assay, and uncontaminated male DNA typically has a ratio of greater than 35 percent (supplementary table 1).

To determine how these two female infants were biologically related, we represented each SNP on chromosomes 1–22 in each of the individuals for which we had data by one or two randomly selected sequences; we had at least one sequence covering 365,899 SNPs for one individual and 690,545 SNPs for the other individual (supplementary table 1). We then used a previously described technique to measure how they were related (Kennett et al. 2017; van de Loosdrecht et al. 2018; Olalde et al. 2019). First, we measured the rate of mismatch of genetic variants at SNPs for two sequences drawn from the same individual (we did this for both infants). Twice this number is the rate of mismatch expected for unrelated individuals in a population, under the assumption that a person's parents are not close relatives. Second, we measured the rate of mismatch of genetic variants at SNPs for sequences at the same position drawn across individuals. We found that this rate of mismatch was half that expected for unrelated individuals, as expected if the two individuals were first-degree relatives: mother-daughter, or sisters.

Since the two individuals were interred together and were of identical ages when they died, the combined genetic and bioarchaeological data provide compelling evidence that they were twin sisters.

Figure 10. Illustration of the infants being interred at the site. Illustration by Nasreen Abd Elal.

Infant Mortality and Burial

Multiple bioarchaeological lines of evidence for this double burial at Tell el-Hesi, including metric, isotopic, and aDNA, suggest that these two individuals were female fraternal (dizygotic) twins. It also seems likely that the twins were either not born alive or did not live long enough to nurse and promote growth of their microbiome, resulting in a lack of bacterial bioerosion in their skeletons. This supposition is corroborated by low nitrogen isotope values relative to those of other young nonadults at the site.

Infant mortality was relatively high in this area during the Ottoman period. Over 50 percent of individuals excavated at Ottoman-Era Dor were nonadults, many of whom were infants (Faerman and Smith 2008), while nearly three-quarters of all burials from Field V at Tell el-Hesi were nonadults (Eakins 1993). Even those of higher social status experienced complications with pregnancy and childbirth; for instance, the Ottoman princess Kaya Sultan died after giving birth in 1659 CE due to complications with the placenta (Kia 2011). Twin pregnancy further increases the likelihood that babies would die during childbirth (Santana et al. 2018). Giving birth to multiples is one of the leading causes for preterm infant death, with "preterm" defined as less than thirty-seven gestational weeks (Halcrow et al. 2018). The Tell el-Hesi infants were likely younger than thirty-six fetal weeks and, therefore, preterm.

Birth complications, maternal health, and disease are further complicating factors in perinatal/fetal deaths, all of which may have contributed to the twins' outcome (Gilmore and Halcrow 2014). For instance, elevated $\delta^{13}C$ values for these infants (A: -14.7 per mille; B: -14.8 per mille) suggest that their mother consumed more C_4 resources than her adult peers (n=7; -17.4±0.4 per mille, 1σ; Gregoricka and Ullinger 2022). This observation could suggest a prescribed diet that included more millet, sorghum, camel's milk, and/or sugar due to pregnancy or illness, although more recently, the diets of seminomadic, pregnant Bedouin in the Negev did not differ from that of the larger community beyond the consumption of more bread (Groen et al. 1964).

Following death, burying infants of varying ages within ceramic vessels was not uncommon in prehistory in the southern Levant, as documented in the Neolithic (Faerman and Smith 2008; Orrelle 2008), Chalcolithic (Eshed and Bar 2012), Middle Bronze Age (Nakhai 2019), and Iron Age (Birney and Doak 2011; Faerman et al. 2011; Garroway 2018). It was also practiced in Ottoman-period cemeteries at Tell el-Hesi (Toombs 1985; Eakins 1993) and Tell Mubarak (Taha 2018), although jar burials at these Ottoman sites were of small perinates; other, older infants were not placed inside of ceramic vessels. Here, the twins were not placed inside of a complete jar, likely because both bodies could not fit inside of a single vessel, but instead had broken sherds from a single water jar placed atop their remains. A member of their community, possibly a father or other male relative (Granqvist 1965), likely placed the fragments over them symbolically to offer them a burial similar to other babies in the cemetery who suffered the same fate (fig. 10). It is not known whether it was socially important to bury them together, or simply expedient.

Although stillborn perinates or young infants seem to have a distinct mortuary pattern at Tell el-Hesi by being interred inside of jars that were then buried, they were treated similarly to adults in other aspects. Their graves were in the same cemetery as older children and adults, and this particular burial was located roughly fifty meters from a *weli* (a burial structure for a holy man). Hamdan Taha (2018) noted that bedouin groups in Transjordan preferred to bury their people on ancient sites near *welis*, while some infants in Palestine and Egypt were buried in different contexts from adults: some in separate cemeteries, others under house floors. An ethnography based on observations in the 1920s of women near Bethlehem suggested that babies would have been washed by midwives before being taken to the gravesite, where only men could inter them (Granqvist 1965), indicating that the babies were afforded similar rituals as adults in the Islamic tradition of burial. According to a hadith, a fetus of 120 days or more gestation has a soul and is therefore afforded human rituals, although there may be local and/or personal differences as to when pregnant mothers feel that their baby is "real" or "human" (Kilshaw 2017). Subsequently, while we cannot know exactly how the twins were viewed by their community, we can surmise that because they were afforded similar mortuary treatments to those granted to older children and adults, they were considered members of their communities.

By applying multiple bioarchaeological and biochemical techniques to this distinctive double burial, we have confirmed that the two infants were sisters of the same age, and while not genetically identical, they were almost certainly fraternal twins. Stable isotope analysis, combined with lack of bacterial bioerosion, reinforces the supposition that the two were either stillborn or did not live long after birth. This supports the idea that while stillborn babies were buried within the same cemetery as other children and adults, they also had their own funerary ritual of jar burial, which may reflect a unique status within the community.

Table 1. Skeletal measurements (R=right; L=left; Max = maximum; wks = weeks; mos=months).

Bone	Feature	Measurement	Side	Infant A Average (mm)	Infant A Fetal Age Estimates	Infant B Average (mm)	Infant B Fetal Age Estimates	Reference
Temporal	Pars petrosa	Length	R	-	-	-	-	-
			L	-	-	27.90	32 wks	Schaefer, Black, and Scheuer 2009
							8.2 mos	Nagaoka and Kawakubo 2015
		Width	R	-	-	-	-	-
			L	-	-	13.12	30-32 wks	Schaefer, Black, and Scheuer 2009
Occipital	Basilar portion	Sagittal Length	-	11.17	32–34 wks	11.02	30–34 wks	Schaefer, Black, and Scheuer 2009
					8.4 mos		8.4 mos	Nagaoka, Kawakubo, and Hirata 2012
		Max Width	-	11.15	30–32 wks	11.27	30–32 wks	Schaefer, Black, and Scheuer 2009
					8.4 mos		8.4 mos	Nagaoka, Kawakubo, and Hirata 2012
		Max Length	-	13.75	30–38 wks	13.69	30–38 wks	Schaefer, Black, and Scheuer 2009
Sphenoid	Lesser wing	Length	R	-	-	-	-	-
			L	11.54	26–28 wks	14.79	30–34 wks	Schaefer, Black, and Scheuer 2009
		Width	R	-	-	9.63	34 wks	Schaefer, Black, and Scheuer 2009
			L	-	-	9.37	34 wks	Schaefer, Black, and Scheuer 2009
	Postsphenoid	Body Width	-	6.62	18–20 wks	-	-	Schaefer, Black, and Scheuer 2009
	Greater wing	Length	R	-	-	22.21	28–30 wks	Schaefer, Black, and Scheuer 2009
			L	-	-	-	-	Schaefer, Black, and Scheuer 2009
		Width	R	15.11	34–36 wks	-	-	Schaefer, Black, and Scheuer 2009
			L	-	-	-	-	-
Rib 1	-	Max Length	-	15.93	24–30 wks	-	-	Schaefer, Black, and Scheuer 2009
Clavicle	-	Max Length	R	34.73	27–32 wks	36.33	28–34 wks	Schaefer, Black, and Scheuer 2009
	-	Max Length	L	-	-	36.35	28–34 wks	Schaefer, Black, and Scheuer 2009
Humerus	-	Distal Width	R	-	-	-	-	-
	-	Distal Width	L	12.81	30–32 wks	-	-	Schaefer, Black, and Scheuer 2009
Radius	-	Max Length	R	-	-	-	-	-
	-	Max Length	L	-	-	44.5	34–36 wks	Schaefer, Black, and Scheuer 2009
							31–35 wks	Sherwood et al. 2000
							29–33 wks	Carneiro, Curate, and Cunha 2016
Ulna	-	Max Length	R	44.92	30–32 wks	-	-	Schaefer, Black, and Scheuer 2009
					28–32 wks			Sherwood et al. 2000
					26–30 wks			Carneiro, Curate, and Cunha 2016
	-	Max Length	L	-	-	-	-	-
Metacarpal 1	-	Max Length	R	7.32	34–36 wks	-	-	Schaefer, Black, and Scheuer 2009
					33–37 wks			Sherwood et al. 2000
	-	Max Length	L	7.15	34–36 wks	-	-	Schaefer, Black, and Scheuer 2009
					32–36 wks			Sherwood et al. 2000

Supplementary table 1 (available online, https://doi.org/10.1086/720748). Technical results on the two ancient DNA libraries.

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Data Availability

The aligned sequences are available through the European Nucleotide Archive, accession PRJEB53219.

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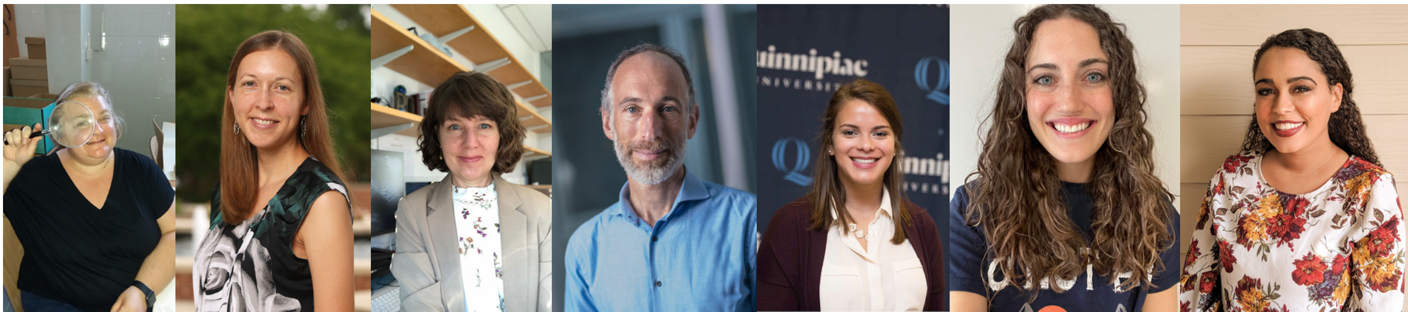

Jaime Ullinger (PhD) is a professor of anthropology and codirector of the Bioanthropology Research Institute at Quinnipiac University. As a bioarchaeologist, she researches health, relatedness, and mortuary contexts in the past, particularly in the Bronze Ages of Southwest Asia and Eastern Europe.

Lesley A. Gregoricka (PhD) is a bioarchaeologist and associate professor of anthropology in the Department of Sociology, Anthropology, and Social Work at the University of South Alabama. Her research focuses on prehistoric mortuary practices and the biogeochemistry of ancient human skeletal remains to examine changing patterns of mobility and social complexity across Arabia and the Levant. She now codirects the Research Experiences for Undergraduates site: Bioarchaeology of Bronze Age Social Systems.

Rebecca Bernardos is the Reich Laboratory Ancient DNA Sample Manager in the Department of Genetics at Harvard Medical School. She has an AS in Physical Therapy, BA and PhD in Neuroscience, MS in Plant and Soil Science, and professional training in curatorial practices and data management.

David Reich is a geneticist at Harvard University and the Howard Hughes Medical Institute. He and his colleagues develop statistical methods and laboratory tools to learn about human population history and natural selection, and applies them in their laboratory to study diverse human populations. He has been a coauthor of a number of recent publications on the population history of the ancient Near East based on DNA data.

Amel Langston is a doctoral student at the University of Vermont. She is completing her Doctor of Nursing practice. During her undergraduate studies at Quinnipiac, she studied anthropology, health science studies, and global public health. This provided a multifaceted understanding of health and wellness based in both biomedicine and an understanding of how anthropology informs and can be used to understand human health.

Paige Ferreri has a master's degree in Secondary Education as well as a Bachelor of Arts in History with a minor in Anthropology from Quinnipiac University. Her undergraduate research focused on American bison conservation efforts during the Progressive Era and its lasting influence on American culture. She is currently teaching American History to middle schoolers in New Hampshire, but has also taught high school psychology, sociology and geography in Missouri.

Brittney Ingram has a Bachelor of Arts in Anthropology from the University of South Alabama. Her undergraduate research focused on stable carbon and nitrogen isotope analysis of human skeletal remains to study diet and behavior among bedouin from the Ottoman period cemetery of Tell el-Hesi. After graduation, she worked alongside the Poarch Band of Creek Indians Tribal Historic Preservation Office to create exhibits, community activities, and educational programs focused on archaeology and cultural historic preservation.