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RESEARCH ARTICLE

Bioarchaeological Research in the Al-Qusais Necropolis (Dubai, Uae): Funerary Dynamics, Chronology and Sex Determination Through Dental Proteomics

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Abstract

The Al Qusais necropolis (Dubai, UAE) is one of the most significant funerary complexes in southeastern Arabia, with a chronological sequence spanning from the Late Bronze Age to the Early Iron Age (c. 1400–750 BCE). The archaeological campaigns carried out by the Sanisera Institute in collaboration with the Dubai Culture & Arts Authority have applied advanced bioarchaeological methodologies, including bioapatite dating and palaeoenvironmental reconstruction through malacological analysis. The results confirm that the original environment of the site was a coastal mangrove ecosystem, now disappeared, located barely 700 metres from the shoreline, compared to the current nine kilometres. Faced with the impossibility of determining biological sex by conventional osteological methods—due to the severe fragmentation and poor preservation of the skeletal remains—dental enamel proteomic analysis of the amelogenin protein was implemented for the first time in the United Arab Emirates. This method, applied to samples from seven individuals, allowed for the correct identification of sex in 80% of cases, distinguishing AMELX and AMELY peptides by mass spectrometry using timsTOF Pro II technology. The results obtained correlate with the body orientation of the interred individuals and with the composition of the grave goods, revealing coherent and consistent gender patterns across the different phases of occupation of the site.

Keyword: Bioarchaeology, Sex, AI Qusais, Amelogenin, Proteomics, Grave Goods, Funerary Rituals.

1. Introduction

The Al Qusais necropolis is located in the southeast of the Arabian Peninsula dated between the Late Bronze Age and the Early Iron Age. Recent chronological analyses delimit the Al Qusais period between 1400 and 750 BC, specifically divided into four periods that we can differentiate from the grave goods: Period I (1400 – 1200 BC), Period II (1200 – 1000 BC), Period III (1000 – 900 BC) and Period IV (900 – 750 BC) (Contreras et al.; 2025). That is, Al Qusais covers a chronological framework from the last third of the second millennium to the beginning of the first millennium BC. In addition, the Al Qusais necropolis is divided into three archaeological areas for ongoing research and excavation, designated Area A, Area B and Area C.

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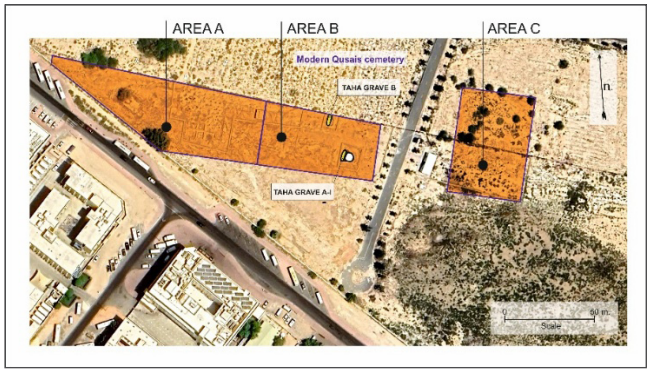

Figure 1. *Defined archaeological areas in the Al Qusais site*

From a geophysical point of view, from the second millennium BC onwards, the landscape surrounding the Al Qusais necropolis has undergone a series of very important changes that would have an influence on the ways of life of the future. One of those most significant changes was the fluctuation of sea level in the Persian Gulf area during the first millennium BC, causing Al Qusais to move about 700 meters from the coast to almost nine kilometers today. This phenomenon resulted in the decline of inland mangrove ecosystems. In this way, the Al Qusais environment changed from being a coastal mangrove ecosystem to a completely desert environment (Fernández-Sánchez *et al*., 2025). Focusing on the appearance of settlements, it is known that since the Iron II period there were stable and provisional settlements both on the coast, in the mountains and even in the oasis areas that were in their interior (del Cerro, 2016). In the case of Al Qusais, the archaeological evidence documented throughout the excavations indicates a continuous presence of human populations during at least the second and first millennium BC (Contreras *et al*., 2020; Valente *et al*., 2023).

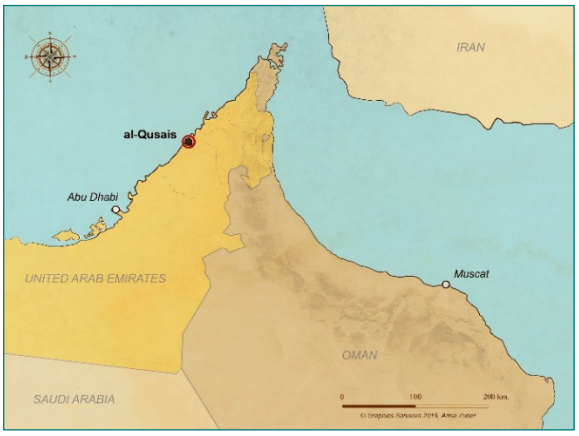

Figure 2. *Location of the Al Qusais site (Dubai, UAE)*

The main objective of this study is to determine the sex of the individuals buried in the necropolis of Al Qusais, regardless of their time or the characteristics of their burial. This is essential to expand the information of the archaeological record that we find about the site and, above all, to publicize this new methodology in the United Arab Emirates. Therefore, it allows us to clarify one of the most important points in archaeology: to understand the forms of burial of a society from the biological point of view through the analysis of amelogenin from dental samples.

Under this approach, if we look at the human remains found in the necropolis of Al Qusais, it has been observed during the three archaeological campaigns that the determination of sex through the traditional and visual form is practically impossible. This type of technique consists of being able to examine certain parts of the body that have different characteristics in each sex, as would be the case of the coxales. In the case of Al Qusais, this fact is unfeasible since it is completely impossible to find intact coxals due to the poor preservation that exists in the place. The same happens with the skull, this being another key element for the estimation of sex; Most of the skulls found in the necropolis are either fragmented or crushed by taphonomic and decomposition factors typical of tombs.

With such a background, due to the degradation of the bones by various environmental and taphonomic processes, it is unattainable to determine the sex of

the population that inhabited Al Qusais by means of these techniques. Therefore, in these cases, in order to estimate sex, we must resort to a completely different method and, as mentioned above, unheard of in the United Arab Emirates: proteomic analyses.

2.Material and Methods

The burials of the necropolis of Al Qusais are presented as an exceptional opportunity to develop an analysis of the estimation of sex given to the variability of grave goods. For the present study, seven tombs have been selected that, due to their state of conservation and representativeness, constitute the basis of this bioarchaeological analysis.

Despite the presence of double or triple burials in some of the tombs, the single tombs of Al Qusais allow a contextual association between the skeletal remains and the recovered teeth. If we start from the hypothesis that the dental features present a significant sexual dimorphism, the data obtained from the analysis of the dental samples of these seven selected graves show us a timely estimate of the specific individual. By correlating the results of the dental analysis with the composition of the grave goods and the specific time frame of each burial, a conclusive reference standard is established. This relationship between biological indicators and material culture allows us to validate the correspondence between biological sex and the funerary practices of the time, providing a reliable basis for extrapolating the characteristics obtained from this analysis to the rest of the individuals in the necropolis, regardless of whether the tomb is single or multiple.

As an initial reference for the study of sex estimation, seven tombs were selected: 83, 309, 335, 345, 349, 350 and 356, all located in Area A of the necropolis, located on the westernmost side of the archaeological site. These tombs were found intact with a good preservation of human remains, both skeletal and dental, and contained a wide and varied collection of objects.

From these seven tombs, seven samples of teeth were selected for the estimation of the sex of the individuals (Table 1). These samples were processed at the CAI of Biological Techniques of the Proteomics Unit of the Faculty of Pharmacy located at the Complutense University of Madrid (Spain). Proteomic analyses of tooth enamel have established themselves as a revolutionary tool due to their resilience over time. Unlike DNA, which disappears rapidly in hot climates and is susceptible to hydrolytic degradation (Taurozzi *et al*., 2024), the amelogenin protein remains protected within the mineral structure of the tooth. Thanks to this stability of the protein, in cases where DNA is impossible to obtain, proteomic analyses become the only reliable scientific way to determine the biological sex of individuals.

3. Methodology

The methodology used in this study was chosen taking into account different factors such as the speed of the method, the objectives of the study and the preservation characteristics of the dental samples.

Taking these points into account, the only way we have to estimate sex is by extracting the peptides from the enamel. Each of the samples was subjected to the peptide extraction process, adjusting the protocol described by previous researchers (Stewart *et al*., 2017; Hendy *et al*., 2018).

The area of tooth enamel where the sample was to be taken was washed with 3% hydrogen peroxide (H2O2) for one minute, rinsed with two successive washes of double-distilled water (ddH2O) of one minute each and an additional one with 10% trifluoroacetic acid one minute; tooth enamel was etched with 5% hydrochloric acid (HCl) for two minutes.

The extracted peptides were analyzed by liquid mass spectrometry at the Proteome Unit of the Faculty of Pharmacy of the Complutense University of Madrid. In summary, the peptides were loaded into EVOSEP tips following the manufacturer's instructions and loaded into the EVOSEP ONE instrument for reversed-phase liquid chromatography in a 15 cm column (PepSec C18 15 cm x 150 µm, 1.5 µm, Bruker Daltonics) in a gradient of 15 SPD (samples per day) (88 minutes). A tims TOF Pro II mass spectrometer, coupled in-line with an EVOSEP ONE chromatograph, was operated in positive mode using a standard DDA method in an MS scan range of 100 to 1700 m/z using a mobility ramp of 0.6 to 1.6 1/K0. Up to 10 precursors were selected per mobility ramp using a ramp and an accumulation time of 100 ms each.

The raw data files were analyzed using FragPipe, an LC-MS analysis pipeline that integrates identification and validation tools (Hsiao, Y., *et al*., 2024). Protein cleavage was established as nonspecific, as were loading rules, peptide length to 7-20 amino acids and modification to oxidized methionine and tryptophan, deamidation of asparagine and glutamine, hydroxylation of proline, and conversion of arginine to ornithine. As a reference database, we used UniProt entries, which include all annotated isoforms

of the AMELX and AMELY genes, along with a selected list of proteins previously detected by our group in the tooth enamel of modern individuals. The decoy sequences required for FDR estimation were automatically generated and appended using FragPipe software. PSM validation was performed using the Percolator algorithm with an FDR of 0.5%, and protein inference was performed using ProteinProphet.

The selection of the dental samples was a painstaking task, as we needed impeccable preservation of the enamel. Recent studies have managed to identify ancient proteins present in enamel that are preserved in good condition and that can be used to perform phylogenetic analyses in both animals and humans. Specifically, one of the proteins necessary for sex identification is the sequence of the protein called amelogenin (Hendy, 2021), whose gene contains the X (female) and Y (male) chromosomes. In other words, the protein sequences obtained from amelogenin differentiate the X and Y variants, which allows it to be used as a marker of biological sex.

Amelogenin is a hydrophobic protein and is the main protein component found in the organic matrix of enamel and, in addition, the gene that encodes this protein is found on both the X chromosome and the Y chromosome (Fincham *et al*., 1999). In other words, it is one of the main proteins that make up tooth enamel and that support both its morphology and its own structure.

When it comes to being able to identify using this protein, it is simple since we find differences in both the sequences and the alleles encoded in both chromosomes. The result of the presence of these two genes on the X and Y chromosomes is the presence of sex-susceptible proteins: AMELX for women and AMELY for different men (Parker, *et al*., 2019). One thing that must be taken into account about a genetic issue is that, when it comes to inheritance and the transfer of chromosomes parents/children, in the case of men they can inherit both the X chromosome (the mother's) and the Y chromosome (the father's); while females can only inherit the X chromosome. Therefore, in many investigations we will find that results that are positive in males we find AMELX and AMELY proteins present.

4. Results

The discovery of intact funerary contexts belonging to ancient periods is an exceptional phenomenon given the complex taphonomic and environmental variables that condition the preservation of the archaeological record. In this sense, the seven tombs selected for this study stand out for their integrity or, failing that, for presenting an optimal state of conservation that allows a bioarchaeological analysis to be carried out. In this way, from these seven tombs it was possible to describe both the original position of the buried individuals and the objects that surrounded them, and even to define the relationship between them. To this reading of the archaeological record, we must add the ability to define the biological identity of the buried individuals thanks to the results obtained from the proteomic analysis of tooth enamel; In this way, we provide a complete anthropological vision that, until now, the taphonomic limitations of the region had prevented us from obtaining. These seven tombs are briefly described below.Tomb 83 was selected for being one of the best-preserved tombs found during excavations during the 1990s conducted by Dr. Qandil. Even so, it was re-excavated during the 2020 archaeological campaign in which the few bones of a single individual were found that, when exposed and exposed to the elements, were lost over time. The individual follows the characteristics found in most of the Al Qusais burials: flexed position and lateral recumbency, in this case straight. As for the grave goods, neither in the first excavation nor in the re-excavation of 2020, no object associated with it was found as grave goods, only some shell fragments.

Figure 3. *Human remains found in tomb 83 by Dr. Qandil in the 1990s*

In tomb 309, during the 2024 archaeological campaign, the poorly preserved fragments of a single adult individual in a flexed position and in the left lateral decubitus position were found. Thanks to the conservation of the canines, incisors and premolars, it was possible to establish an age range of between 18 and 30 years (Rodríguez Cuenca, 1994; Lovejoy, 1985). It was found associated with a very well-preserved grave goods which consisted of two beaded bracelets on both wrists, a beaded necklace, a copper ring, two copper anklets on both feet and a votive shell with greenish pigment inside. Due to the presence of these ankle bracelets, that is why she was selected for this study since they are often related to female individuals.

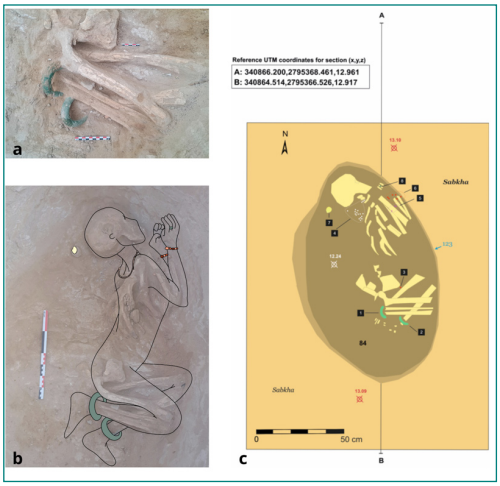

Figure 4. *Grave 309: a) pair of copper anklets, b) physiognomic reconstruction of the individual, c) grave 309 map*

Tomb 335 found the remains of two individuals, Sk1 was intact while Sk2 only found remains of long bones (lower and upper). Sk1 was buried in a flexed position along with a carnelian beads necklace, a copper ring, a button belt and beads around his waist. Grave 345 was the burial of two individuals, the age could not be determined as we only found one Sk2 tooth in poor condition and the fusion states of the epiphyses could not be determined. They were buried simultaneously, Sk1 in a flexed position and lying on his right side while Sk2 presents a position that, most likely, was not the original one and was changed by different factors. Only the age of Sk2 could be estimated since no teeth could be found from Sk1.

Figure 5. *Grave 345: a) three votive shells along with the copper chisel, b) photo of the grave with the skeletal remains (Sk1 and Sk2), c) grave 345 map*

In tomb 349, fragments of long bones were found that could belong to a non-adult individual. What is interesting and unique about this tomb is the discovery of six fish vertebrae that were probably part of the funerary goods. Along with these vertebrae, two arrowheads were also found, one in a context of abandonment and the other in a context of occupation, very close to the fish vertebrae. Grave 350 consists of the burial of an individual in a flexed position and in the left lateral decubitus position. It was associated with a very well-preserved grave goods consisting of carnelian beads and shells, a soft stone vessel, a ceramic vessel, and a shell with greenish pigment inside. Thanks to this complete trousseau, he was selected for the study.

Finally, tomb 356 was discovered this year and consisted of the burial of two individuals almost on the surface. Only two objects were found as part of the trousseau, beads that could be part of a necklace associated with Sk2 and a belt button associated with Sk1. For this study, only the age of Sk1 could be estimated since Sk2 could not find a tooth in good condition.

The results of the sex determination of these burials are presented below (Table 1).

Table 1. *Sex determination from human remains from the Al Qusais tombs (Area A) with their respective chronologies. "N.D.: No Data"*

Grave	Sample ID	Teeth type	Individual	Sex	Calibrated date (95.4%)
83	AQ. S_F7_25_1	Molar	SK1	Male	n.d.
309	AQ. S_F4_84_13	n.d.	SK1	Female	n.d.
335	AQ. S_F3_184_1	Right upper central incisor	SK1	Male	1359 – 1294 cal BC (2σ)
345	AQ.S._E6_210_4	n.d.	SK2	n.a.	1422 – 1271 cal BC (2σ)
349	AQ.S._E6_191_4	Canine	SK1	Male	901 – 806 cal BC (2σ)
350	AQ.S._E6_193_2	Canine	SK1	Female	1402 -1213 cal BC (2σ)
356	AQ.S._E7_185_2	Maxila premolar	SK1	Male	1223 – 1016 cal BC (2σ)

5. Discussion

This study presents a powerful preliminary proposal for the determination of the sex of the individuals buried in Al Qusais. The proposed results show us a completely different paradigm from the one that could be originally thought about the forms of burials and their corresponding grave goods.

Considering that, of the seven samples analyzed, six yielded positive results for the estimation of sex, the success rate of this analysis would be approximately 85.7%. This result suggests sufficient preservation of the protein amelogenin in most of the dental samples provided for analysis. Of these samples studied that were positive, four of them belong to men and only two of them belong to women. The case of the sample corresponding to tomb 345 (Sk2) has not yielded results for two main reasons. The first is that we know that two individuals were buried in tomb 345 and, the interesting thing about this case, was to be able to determine the sex of both. Unfortunately, only one tooth could be extracted from Sk2 and none from Sk1 since no teeth were found. The second reason was the higher concentration of other proteins in the tooth (such as collagen) and the absence of the protein of interest to the study, amelogenin. With this paradigm, a second attempt was requested, as it appeared that the tooth had some sediment underneath which some of the dentin was poking out. Again, the result obtained was the same as on the first occasion. This problem is usually quite recurrent in such old samples, with more collagen than amelogenin.

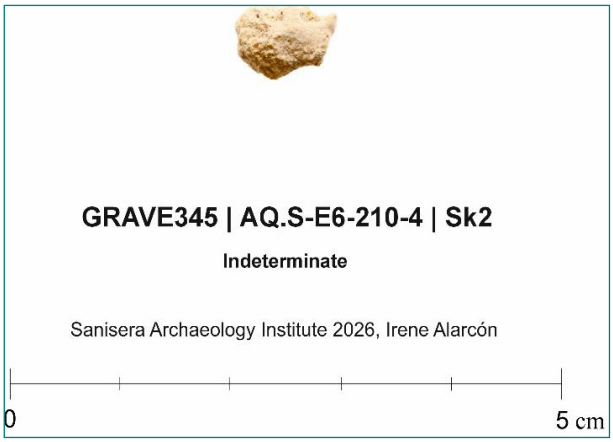

Figure 6. *Dental sample from tomb 345; the image itself show that the tooth emanel is barely visible.*

With regard to funerary practices and objects used as grave goods, both similarities and differences have been observed in terms of the distinction of biological sex. Starting with the differences, if we consider the subsequent chronological analysis carried out in some of the tombs of this study, we can observe that there is no clearly differentiated distribution between sexes throughout the different chronological periods established for Al Qusais. However, the small sample size limits the possibility of identifying a significant trend.

Continuing with the similarities, in the funeral ritual itself we can observe that there are certain tendencies

that mark a clear distinction in sexual differentiation and that may be related to each other.

One of them is the comparison between the estimated biological sex and the anatomical position in which the individuals were buried, which suggests the existence of a possible pattern in funerary practices. In the cases estimated as male and in which we can observe the original position of the individual, that is, tombs 83, 335 and 356, they were buried in the right lateral decubitus position; on the other hand, the female cases, tombs 309 and 350, were buried in the left lateral decubitus position.

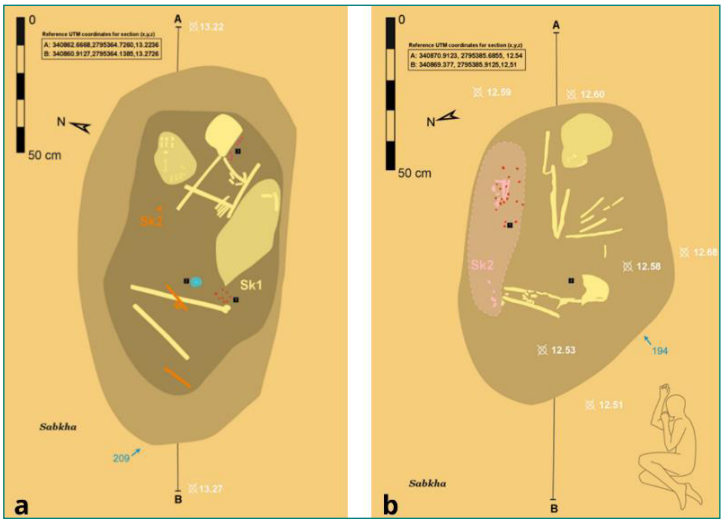

Figure 7. *Anatomical position of the male individuals: a) grave 335, b) grave 356*

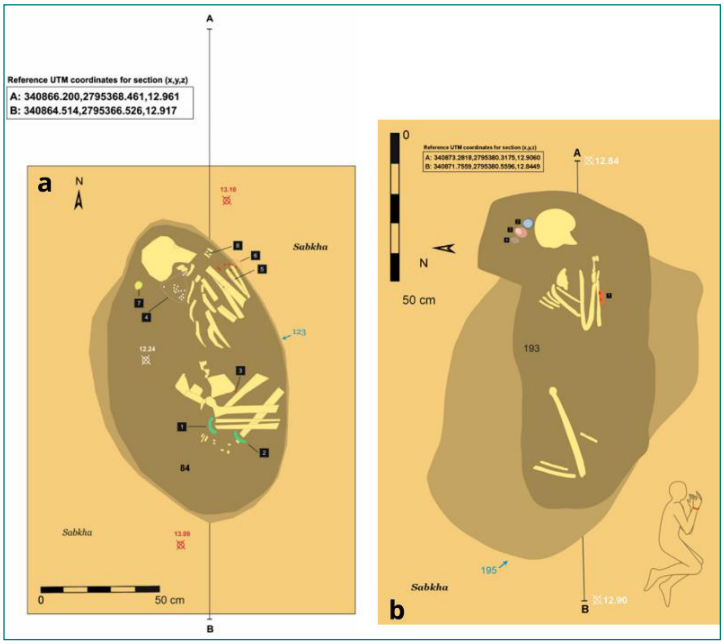

Figure 8. *Anatomical position of the female individuals: a) grave 309, b) grave 350*

It does not appear that the placement of the individual, in one way or another, changes over time. For example, in the case of males we can see that Sk1 from tomb 335 was buried at a certain time during Period I, while Sk1 from tomb 356 was buried during Period II.

Another similarity in funerary practices is the type of grave goods with which they were buried. In the case of men, taking tombs 335 and 356 as examples, belt buttons made of shells were found in both cases. We know that the circular buttons were part of the belt with which certain individuals were buried and, most likely, they carried some kind of object with them. At Al Qusais, numerous evidences have been found of these buttons with decorations of concentric circles associated with daggers that were part of the belt as an object of personal use (Contreras *et al.*, 2025). Likewise, the fact that they were buried with this belt, and possibly with a copper object such as a dagger, is a clear indicator of the social status they would have had in life. These sets of belts are also found in several necropolises in the southeast of the Arabian Peninsula, such as Shimal or Qidfa (Vogt

and Franke-Vogt, 1987; Al Tikriti, 2022) associated with male burials.

The case of tombs 83 and 349, both biologically determined to be male, is somewhat uncertain to associate with the other two male tombs (335 and 356). The case of tomb 83 is due to the fact that no type of material or object such as grave goods was found, so in this case we do not find any possible association. On the other hand, the grave goods in tomb 349 correspond to that of a non-adult whose grave goods consisted of a copper arrowhead and six vertebrae of a fish. These fish vertebrae are new evidence at Al Qusais, being the first time, they have been found and, moreover, intact. Thus, having a single piece of evidence, we cannot formulate a valid hypothesis about its presence in this tomb.

Figure 9. *Six fish vertebrae from tomb 349*

In both cases, the proteomic analysis of amelogenin should be highlighted, as it has made it possible to identify two individuals as male who, otherwise, would have been impossible to identify due to the absence of skeletal remains that facilitate their estimation and the lack of objects that can help us to associate them with the other male graves.

On the other hand, the funerary record of tombs 309 and 350 reveal a pattern of burial associated with women. The fact that both copper anklets and bead bracelets (both cases, pairs) were found, along with elements of personal adornment such as shells and carnelian beads, reinforces the fact that the incorporation of these objects as grave goods acted as markers of gender and their own social status (Green, 2007; Taha, 2009). The presence of votive shells, specifically those with greenish pigments inside, is especially relevant in both tombs, being a finding that has traditionally been linked to cosmetic practices, as we can see in other contemporary sites such as Tell Abraq (Potts, 1992). In addition, according to Masia (2000), those pigments with a darker green tone contained atacamite, this being a compound that was part of the cosmetics of the time. The combination of these elements with pottery vessel and soft stone vessel in tomb 350 may also be related to cosmetics, being perhaps the place where they kept the elements they used for this activity. This association of objects such as grave goods allows us to establish a typological relationship between the two graves and to identify, in the future, similar objects in other tombs that would be part of the female grave goods.

Figure 10. *Pigmented shells from female tombs: a) grave 309, b) grave 350*

In the case of tomb 345 with double burial, whose proteomic analysis did not obtain a determination of the possible sex, the material culture can offer us some significant indicators of this. Sk1 presents a trousseau composed of three votive shells (one with pigment inside), a copper chisel, a fragment of stone and a polished tool; on the other hand, Sk2 presents a trousseau composed of a soft stone cup and a soft stone vessel. The presence of three votive shells, one of which preserves traces of pigment, establishes a direct link with the pattern observed in tombs estimated to be female, suggesting the presence of at least one female individual. However, the presence of the copper chisel and a polished tool introduces a variation in the funerary goods.

Figure 11. *Complete funerary grave goods from the double burial of tomb 345*

Even so, if we look at the anatomical position in which Sk1 was buried in tomb 345 (Figure 5), it presents a left lateral decubitus arrangement, a pattern that is repeated in tombs 309 and 350 (Figure 8). If we add the positional coincidence of Sk1 with the women of tombs 309 and 350 with the presence of the pigmented shell (a very recurrent gender marker in southeastern Arabia (Potts, 1992), we are reinforced by Sk1's interpretation of tomb 345 as a woman. On the other hand, the chisel and polished tools indicate technical or artisanal work functions, which may indicate a new area of research on the division of labor in Al Qusais by gender.

6. Conclusion

The results obtained from the estimation of sex using amelogenin present in tooth enamel allowed the identification of four individuals as men and two as women, while one sample did not yield conclusive results. Despite the limited sample size, the results have been decisive, demonstrating the effectiveness of the technique. This success is particularly relevant in the United Arab Emirates, where aggressive weather often reduces sample recovery. By achieving clear estimates with reduced sampling, it strengthens new avenues of study for future research in similar arid contexts such as Al Qusais, allowing the total reconstruction of ancient populations.

Certainly, the hypothesis put forward about the variation in the anatomical position in which they were buried – men in the right lateral decubitus with their faces to the north and the women in the left lateral decubitus with their faces to the south – must continue to be investigated in the necropolis since it is a very suggestive starting point: Standardized burial pattern at Al Qusais to express the social identity and lineage of each person buried. Gender differentiations have been a constant since the origins of humanity, so it is reasonable to suggest that this may influence burial practices

Similarly, the relationship between the objects that are part of the grave goods and gender can be glimpsed a possible pattern where the shell belt buttons are associated with male individuals, while the pigmented shells and jewelry that come in pairs are associated with female individuals.

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