

FETAL AGE AT DEATH ESTIMATION ON DRY BONE: TESTING THE APPLICABILITY OF EQUATIONS DEVELOPED ON A RADIOGRAPHIC SAMPLE

ESTIMACIÓN DE LA EDAD FETAL EN HUESOS SECOS: INVESTIGANDO LA APLICABILIDAD DE ECUACIONES DESARROLLADAS EN UNA MUESTRA RADIOGRÁFICA

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ABSTRACT The paucity of identified skeletal collections that include fetuses entails the need to pursue unconventional approaches and resources in order to investigate fetal anatomical variation. Radiographic analyses are being considered as a good alternative to data obtained in osteological collections. In a previous work, we developed equations to estimate gestational age (GA) at death by measuring fetal long bones on x-rays. This study aims to test the applicability of these equations in dry bones, and to assess its accuracy and bias. A test sample of 17 fetuses with known gestational age at death from the osteo-

logical collection of the Department of Legal Medicine, Toxicology and Physical Anthropology, Faculty of Medicine of the University of Granada (Spain) was employed. Examined bones comprised the femur, tibia, humerus and radius. The proposed models show high accuracy and low bias in the assessment of gestational age at death in a sample of fetal dry bones. The new equations, especially those obtained with classical calibration, are a valuable tool to estimate fetal gestational age at death in both forensic and archeological contexts. *Rev Arg Antrop Biol* 21(2), 2019. doi:10.24215/18536387e008

PALABRAS CLAVE: restos fetales; estimación de la edad en el momento de la muerte; bioarqueología; antropología forense; paleodemografía

RESUMEN La escasez de colecciones osteológicas que incluyen fetos identificados impone la búsqueda de recursos no convencionales para investigar la variación anatómica fetal. En un trabajo anterior, desarrollamos ecuaciones para estimar la edad gestacional (EG) en el momento de la muerte mediante la medición de huesos largos fetales en radiografías. Este estudio tiene como objetivo demostrar la aplicabilidad de esas ecuaciones en huesos secos y evaluar su precisión y sesgo. Se empleó una muestra de 17 fetos de edad gestacional conocida en el momento de la muerte, perteneciente a la colección osteológica del Departamen-

to de Medicina Legal, Toxicología y Antropología Física de la Facultad de Medicina de la Universidad de Granada (España). Los huesos examinados fueron el fémur, la tibia, el húmero y el radio. Los modelos propuestos exhiben una precisión alta y un sesgo bajo en la evaluación de la EG en el momento de la muerte en una muestra de huesos fetales secos. Las nuevas ecuaciones, especialmente las obtenidas mediante calibración clásica, son una herramienta valiosa para estimar la edad gestacional en el momento de la muerte, tanto en contextos forenses como arqueológicos. *Rev Arg Antrop Biol* 21(2), 2019. doi:10.24215/18536387e008

The accurate estimation of fetal gestational age (GA) is a fundamental procedure in different circumstances, including clinical, forensic and archaeological contexts (Butt & Lim, 2014; Carneiro, Curate, Borralho & Cunha, 2013; Cho *et al.*, 2010; Olsen *et al.*, 2002; Scheuer, Musgrave & Evans, 1980; Sherwood, Meindl, Robinson & May, 2000; Warren, 1999). Gestational age is commonly the only biologi-

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cal profile feature accurately obtainable from fetal remains and is relevant in forensic settings to support identification or evaluate fetal viability (Adalian *et al.*, 2001; Carneiro, Curate & Cunha, 2016). During the past 25 years, research interest in the archaeology of children and childhood has increased noticeably (Crawford & Lewis, 2008; Lewis, 2007; Lillehammer, 1989; Lillehammer, 2015; Ortiz, Paz, Zenteno, Zuñiga & Nieva, 2018) but there is still a relative dearth of bioarcheological studies focusing on fetal remains. Notwithstanding, the proper assessment of fetal age is valuable in the context of broader bioarcheological theoretical questions, including growth studies, demographic analyses, infanticide, maternal-fetal health and funerary rituals (Curate *et al.*, 2015; García-Mancuso, 2014; Gowland & Chamberlain, 2002; Halcrow & Tayles, 2011; Halcrow, Tayles, Inglis & Higham, 2012; Hillson, 2009; Lewis & Gowland, 2007; Lieverse, Bazaliiskii & Weber, 2015; Mays & Evers, 2011; Moore, 2009; Owsley & Bradtmiller, 1983).

Medico-legal and anthropological research of the fetus has a long history, with the Italian Gaetano Corrado providing one of the first comprehensive descriptions of fetal and neonatal anatomy (Corrado, 1899). Before that, the systematic weighing of neonates was partially justified by the clinical necessity to assess chances of survival. Also, fetal viability (*i.e.*, the competence of a fetus to survive outside the uterus after birth) and the possibility of infanticide gained relevance in the medico-legal context since the 19th century (Tanner, 1981). As such, growth curves for the fetal period began to be constructed by anatomists, obstetricians and embryologists (Calderini, 1875; Stratz, 1910; von Hecker, 1864).

The use of fetal length to estimate GA also became pervasive during this period (Tanner, 1981). Carl von Hecker (1864) conveyed the weights and lengths of 486 stillborns, using the length of the fetuses to diagnose the month of gestation. In 1875, Haase published the length statistics for the Berlin Charité, also providing a rule for estimating the month of gestation. Later, Scammon and Chalkins (1923, 1929) updated and improved earlier methods for the determination of gestational age from the fetal

length, showing that the length of nearly every external dimension was linearly correlated with the fetal crown-heel length. After Scammon and Chalkins, the interest in fetal growth and aging waned until the 1960's. Since then, several studies have established the strong association between gestational age and the diaphyseal length of long bones (Adalian *et al.*, 2001; Carneiro *et al.* 2013, 2016; Chávez-Martínez, Ortega-Palma, Castrejon Caballero, Arteaga-Martínez, 2016; Fazekas and Kósa, 1978; Jeanty, Kirkpatrick, Dramaix-Wilmet & Struyven, 1981; Olivier & Pineau, 1960; Piercecchi-Marti *et al.*, 2002; Scheuer *et al.*, 1980; Olsen *et al.*, 2002).

In clinical or epidemiological settings, age estimates of fetuses are commonly made *in utero* using ultrasound measurements (Butt & Lim, 2014) but, in forensic or bioarcheological contexts, fetal growth research and evaluation of gestational age has been hindered by the scarcity of osteological fetal collections (Fazekas & Kósa, 1978; Kósa, 2000). Notwithstanding, some studies have been conducted in the medico-legal area, either by making measurements directly in fresh bone (Piercecchi-Marti *et al.*, 2002), dry bone (Scheuer & Black, 2000), or using medical imaging (Warren, 1999; Adalian *et al.*, 2001; Piercecchi-Marti *et al.*, 2002; Olsen *et al.*, 2002; Carneiro *et al.*, 2013; Gilbert-Barness & Dedich-Spicer, 2004). Fazekas and Kósa's study (1978), a long-standing reference in the osteology of fetuses, encompasses invaluable information, including measurements of most bones from three lunar months to term. Nevertheless, their work is grounded upon a logical fallacy: the study sample was essentially of unknown age, and gestational age was established as the ratio between the total length of the fetus and GA (Cunha *et al.*, 2009; Scheuer & Black, 2000).

In previous studies (Carneiro *et al.*, 2013, 2016), we have produced and improved a method for estimating GA based on the measurements of the diaphysis of fetal long bones. The models for gestational age at death estimation were created employing a conventional least squares regression (both classical and inverse calibration) approach, from *post mortem* radiographic measurements in an identified hospital sample of Portuguese origin. The proposed equations for GA estimation proved exceedingly accurate

and unbiased in a holdout radiographic sample (Carneiro *et al.*, 2016). In order to verify their consistency in forensic and, especially, bioarcheological contexts, it is important to test the applicability of these equations in dry bones, but the paucity of reference collections that include a reasonable number of fetal skeletons hinders this objective (Franklin, 2010). As such, the models for the femur, tibia, humerus and radius proposed in Carneiro *et al.* (2016) were assessed in an identified test sample of fetal dry bones (from the Granada collection, Spain) to evaluate its accuracy and bias.

MATERIALS AND METHODS

The test sample is part of the osteological collection stored in the Department of Legal Medicine, Toxicology and Physical Anthropology, Faculty of Medicine of the University of Granada, Spain. It comprises individuals who died between 1871 and 2001, most of which are from the 1930’s to the 1970’s. There is a considerable amount of information available for these individuals, namely birth date (except for the fetuses), death date, sex and, in some cases, cause of death. The Granada collection, which is still under construction, has a considerable number of sub-adults. In 2012, from the 542 exhumed skeletons, 230 belonged to individuals aged from 5 months of gestation to eight years (Alemán *et al.*, 2012).

In this study, only fetuses with known GA (in months of gestation) were used. Thus, the test sample included 17 skeletal individuals with GA between five and nine gestational months; eight were females and nine were males (Table 1). GA was converted from months into weeks of gestation. Whenever present and complete, the maximum length of the diaphysis of the femur (Fig. 1), the tibia, humerus (Fig. 2) and the radio were measured, totaling 66 measurements. All measurements were directly performed in the bones, with the aid of a digital calibrated caliper (instrument error: 0.01 mm, repeatability: 0.01 mm). Gestational age at death in the Granada individuals was estimated following the regression equations for the femur, tibia, humerus and radius proposed by Carneiro *et al.* (2016) (Table 2). Gestational age was used as the response variable (*y*) and diaphysis length

TABLE 1. Gestational age (GA) and sex of the fetuses from the identified collection of Granada in months and in weeks

Case	GA in months	GA in weeks	Sex
192	9	40	female
276	9	40	male
279b	6	28	male
289	7	32	female
290	9	40	male
302c	8	36	female
367	7	32	female
371	9	40	female
373	7	32	female
382	7	32	female
390	7	32	male
397	7	32	male
398	9	40	male
408	6.5	29	male
410	5	23	male
412	8	36	male
419	6	27	female

as the predictor variable (*x*) in the linear inverse calibration models. On the contrary, in classical calibration models, *x* is the variable for which estimates are to be generated and not *y*. Generally, the model relating *y* and *x* is assumed as linear, with a true intercept α and a slope β . Inverse calibration – in which gestational age is regarded as the response (i.e., dependent) variable – typically yields an effect called regression toward the mean that underestimates age in older individuals and overestimates it in younger ones (Aykroyd, Lucy, Pollard & Solheim, 1997).

The reliability (accuracy and bias) of the classical and inverse calibration equations was evaluated through the mean absolute error (MAE, a proxy for accuracy) and the mean error (ME, a proxy for bias, Walther & Moore, 2005), as follows:

$$MAE = \sum |estimated\ GA - documented\ GA|/N$$

and,

$$ME = \sum (estimated\ GA - documented\ GA)/N$$



Fig. 1. Maximum length of the diaphysis of the fetal femur (MLDFF).



Fig. 2. Maximum length of the diaphysis of the fetal humerus (MLDFH).

TABLE 2. Inverse and classical regression equations for the estimation of gestational age (GA) in weeks (Carneiro et al. 2016)

Diaphysis	Inverse calibration equations	Classical calibration equations
Femur	$GA=8.525 + (0.372 \times \text{femur [mm]})$	$\frac{\text{femur [mm]} + 18.72}{2.52}$
Tibia	$GA=8.514 + (0.428 \times \text{tibia [mm]})$	$\frac{\text{tibia [mm]} + 15.76}{2.17}$
Humerus	$GA=6.814 + (0.452 \times \text{humerus [mm]})$	$\frac{\text{humerus [mm]} + 10.40}{2.02}$
Radio	$GA=7.003 + (0.542 \times \text{radius [mm]})$	$\frac{\text{radius [mm]} + 8.42}{1.66}$

RESULTS

Error results obtained from the application of the formulae proposed by Carneiro et al. (2016) to the fetal identified collection of Granada are presented in Tables 3 and 4. Accuracy is almost always better when using the classical

calibration models (the exception ensues with the left humerus formula).

As a rule, such models are also less prone to systematic error (Figs. 3 and 4). Regarding the type of bone studied, the femur presents the highest accuracy, while bias is smaller in the equations for the humerus – independently of

TABLE 3. Mean absolute error (MAE) and bias (ME) in the left bones (fetuses, Granada Collection)

		Inverse calibration	Classical calibration
Femur (N=17)	MAE	3.07	2.94
	Bias	-1.74	-1.29
Tibia (N=17)	MAE	3.81	3.25
	Bias	-1.60	-1.05
Humerus (N=16)	MAE	3.58	3.85
	Bias	-1.47	-0.73
Radius (N=17)	MAE	3.61	3.23
	Bias	-1.92	-1.03

TABLE 4. Mean absolute error (MAE) and bias (ME) in the right bones (fetuses, Granada Collection)

		Inverse calibration	Classical calibration
Femur (N=16)	MAE	3.03	3.02
	Bias	-1.25	-0.77
Tibia (N=17)	MAE	3.39	3.21
	Bias	-1.44	-0.89
Humerus (N=17)	MAE	3.42	3.36
	Bias	-1.28	-0.53
Radius (N=17)	MAE	3.78	3.42
	Bias	-2.07	-1.27

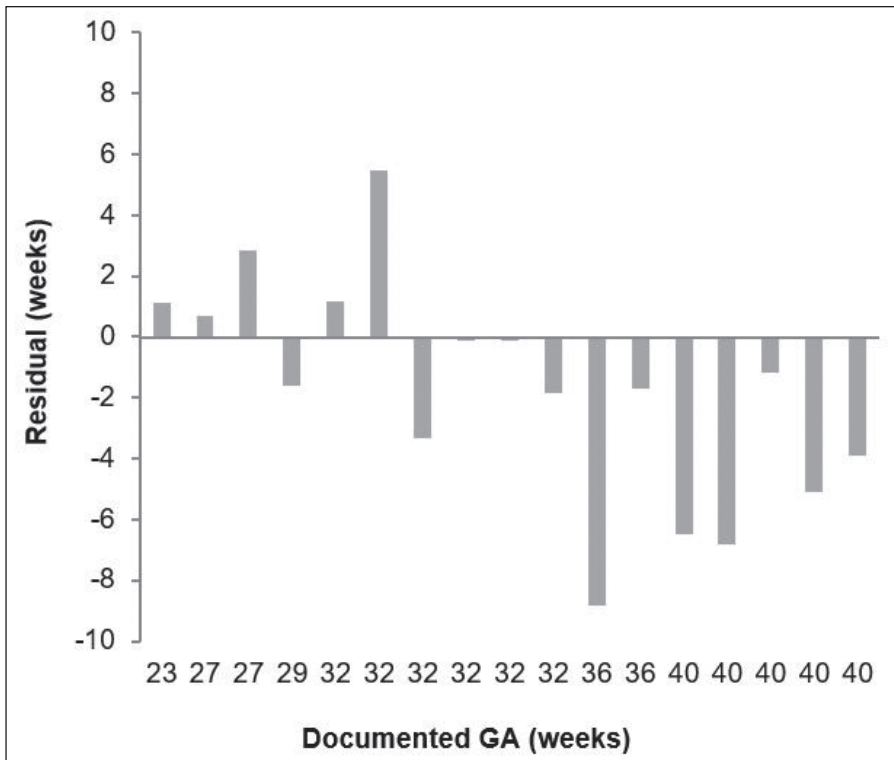


Fig. 3. Difference in weeks between documented gestational age at death and estimated gestational age at death (left femur, inverse calibration).

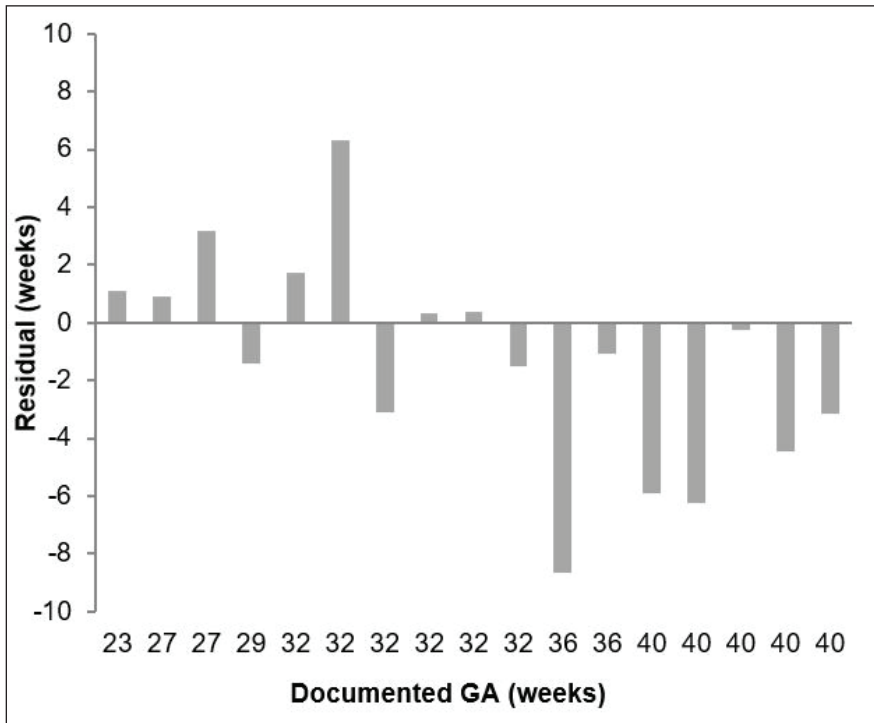


Fig. 4. Difference in weeks between documented gestational age at death and estimated gestational age at death (left femur, classical calibration).

the model employed. The mean absolute error in the femur is 2.92 (left femur) with classical calibration, and 3.03 (right femur) with inverse calibration; while bias in the right humerus is -0.53 with classical calibration.

On the other side, the radius shows the highest mean absolute error and bias (i.e., mean error), both with classical (MAE=3.42; ME=-1.27) and inverse calibration (MAE=3.78; ME=-2.07). Differences between bone sides are negligible. Both calibration approaches – but particularly inverse calibration equations – are less accurate and more biased when estimating gestational age in preterm fetuses (Figs. 3 and 4).

DISCUSSION

The underrepresentation of infantile and juvenile bodies in orthodox funerary spaces has been ascribed to taphonomic factors, sociocultural customs towards death and bioarcheological partiality – establishing a long-lasting bias in paleodemographic research (Chamberlain, 2006). Particularly, there is a non-random fu-

nerary treatment associated with fetuses and newborns, as well as very young children, since many funerary practices in the past led to the exclusion of most of these individuals from formalized burial sites, making its recovery infrequent (Moore, 2009). Also, there are methodological limitations associated with the recovery of perinatal individuals, which can be justified by the fragility of the fetal/newborn skeleton, but also by the inexperience in dealing with this type of skeletal remains.

A further complication arises from the difficulty to obtain an accurate fetal biological profile, as gestational age is commonly the only feature easily available from fetal remains. Therefore, dependable approaches for the estimation of gestational age in fetuses are needed, both in bioarcheological and forensic settings. The existing methods, such as Fazekas and Kósa's (1978), exhibit a number of conceptual flaws that mandated the creation of new references. The regression equations by Scheuer *et al.* (1980) are often employed – mainly in bioarcheology – to estimate fetal gestational age

but they were developed on a reference sample predominantly consisting of older fetuses (GA between 24 weeks and six postnatal weeks). Also, preceding work suggests that this method is less accurate and more biased (Carneiro *et al.*, 2013, 2016; Table 5). Other metric standards for the estimation of fetal age at the time of death in forensic anthropology and bioarcheology are available (e.g., Adalian *et al.*, 2001; Chávez-Martínez *et al.*, 2016; Sherwood *et al.*, 2000).

Carneiro *et al.* (2016) introduced equations for the diaphyses of the humerus, radius, femur and tibia that were established through conventional least squares regression, with both inverse and classical calibration procedures. The results presented here suggest that classical calibration models display slightly better accuracy and considerably less bias than inverse calibration equations – the only exception occurs with accuracy in the left humerus. This is congruent with the previously mentioned regression toward the mean effect observed in inverse calibration that results in an underestimation of age in older individuals and an overestimation in younger individuals (Aykroyd *et al.*, 1997). This attraction of the middle (Masset, 1989) has been recorded when using age estimation techniques both in adults (e.g., Calce & Rogers, 2011; Meinl *et*

al., 2008; Saunders, Fitzgerald, Rogers, Dudar & McKillop, 1992) and non-adults (Liversidge, Smith & Maber, 2010).

The method – and especially the inverse calibration equations – seems to produce less accurate and more biased results with preterm fetuses (GA close to 40 weeks). This is possibly related with the above-mentioned regression toward the mean effect that is characteristic of conventional least squares regression procedures – even though the high correlation coefficients should entail less bias (Besalú 2013). A Bayesian approach could be appropriate to circumvent this problem, as proposed by Gowland and Chamberlain (2002). Notwithstanding, Mays and Evers (2011) tested both conventional least squares regression and Bayesian approaches in a Roman Britain sample and found that perinatal deaths showed a strong clustering at a gestational age consistent with full-term. We would advise, thus, for the application of both statistical approaches – and for the use of confidence intervals to convey GA estimates. Also, whenever possible, GA estimates based on long bone lengths should be complemented with data from other parts of the skeleton (e.g., García-Mancuso, Inda & Salceda, 2016; Tocheri and Molto, 2002), particularly methods based on dental remains, usually

TABLE 5. Mean absolute error (MAE) and bias (ME) obtained when comparing the classical and inverse regression models by Carneiro *et al.* (2016) with analogous formulae developed by Scheuer *et al.* (1980) and Fazekas and Kósa (1978) in a hospital (radiographic) sample from Portugal (adapted from Carneiro *et al.* [2016])

		Inverse calibration	Classical calibration	Scheuer <i>et al.</i> (1980)	Fazekas & Kósa (1978)
Femur	MAE	0.049	0.056	0.143	0.070
	Bias	0.004	-0.004	0.138	-0.043
Tibia	MAE	0.056	0.060	0.127	0.071
	Bias	0.009	0.002	0.102	-0.050
Humerus	MAE	0.060	0.067	0.102	0.053
	Bias	0.009	0.001	0.078	-0.004
Ulna	MAE	0.068	0.075	0.098	0.070
	Bias	0.008	0.003	0.062	0.001
Radius	MAE	0.070	0.078	0.099	0.111
	Bias	0.011	0.008	0.076	0.098

more accurate and less biased (García-Mancuso, 2014; Nava *et al.*, 2017). In the case of fetal remains, the main problem with dental methods is associated with «availability / accessibility»: crown mineralization ensues relatively late during pregnancy, between the 3rd and 4th months *in utero*, and in part due to their small dimensions, tooth crowns are more likely to not be retrieved during excavation. As such, in many situations, skeletal age will be the only available technique (Sunderland, Smith & Sunderland, 1987).

Anyway, gestational age estimation error is undoubtedly multifactorial. Firstly, there is inter-population variability in skeletal development *in utero*, with the discrepancy mostly mediated through environmental constraints on growth. Fetal growth and development are adversely affected by poor maternal health and nutrition (Kinare *et al.*, 2010). Most fetuses in the past, particularly those that did not survive through the end of pregnancy, would have been subjected to intrauterine growth restriction – resulting in an underestimation of GA based in modern aging standards (Bonsall, 2013). Moreover, the estimation of GA in fetuses with pathological conditions is more inaccurate and biased (Sherwood *et al.*, 2000). In the Granada collection, 90% of the individuals lived and died during the mid-20th century, when social, cultural and health structures were rather dissimilar to the ones observed in the present day. Secular changes in skeletal size were also observed in some studies (Olivier, 1977; Schack-Nielsen, Mølgaard, Sørensen, Greisen & Michaelsen, 2006). Another potential source of error is the calculation of gestational age based on the last menstrual period (Gardosi, 1997). GA in the Granada collection, although considered “known”, was assessed based on fallible scientific information, during a period in which pregnancy was dated in a subjective way (Huxley, 2005).

Finally, it is known that bone shrinkage affects fetal dry bones, due to the loss of organic matter and water, especially in younger fetuses (Huxley, 1998; Huxley and Kósa, 1999). As such, aging methods based on radiographic images, ultrasound or fresh bones (i.e., surrounded of soft tissues, or bones dissected for this purpose) are susceptible to error when used in dry bones (Huxley, 1998), although Warren (1999)

observed that the use of radiographic measurements do not differ from those performed directly on dry bone. In any case, the lack of reference material in this age group hinders the development of studies on the basis of dry bones. Incidentally, Dirkmaat and Cabo (2012) consider that radiographic studies are a good alternative in order to perform original research, or to update old works, when it is not possible to collect data in osteological collection

Final remarks

This study provides evidence on the proficiency of the method by Carneiro *et al.* (2016) in a sample of dry fetuses of non-Portuguese origin, with effective results but below the remarkably accurate and unbiased performance in a radiographic holdout sample of Portuguese origin (i.e., anonymous fetopathological records of recent fetuses with documented GA, in a hospital setting). The application of the new method in this type of sample, i.e., with dry bone, is thus advisable, not only because it is accurate and less biased but also because it is simpler to implement both in forensic and bioarcheological contexts. The method should be complemented, whenever possible, with other statistical approaches and data from different parts of the skeleton.

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