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ORIGINAL ARTICLE

Evaluation of Obturator Foramen Suggests No Differences Between Sexes in Young Bovines

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Summary

Among the numerous bovine pelvic traits displaying sex differences, no detailed studies of bovine obturator foramen are available in the veterinary literature. The purpose of this work was to study quantitatively this structure using lineal biometrics, shape indexes and Fourier analysis. The material consisted of 60 hemicoxae belonging to young bovines of approximately 1 year of age. Although significant differences were found in area, perimeter and length of obturator foramen, none of the analyses used were able to differentiate between sexes and thus cannot be used as a natural trait for determining sex, at least in young bovines.

Introduction

Sexual size dimorphism has been studied intensively in mammals (Schutz et al., 2009). Generally, males are larger than females ('male-dominant'), although a small number of 'female dominant' cases also exist (Ralls, 1977; Leutenegger, 1978; Abouheif and Fairbairn, 1997; Weckerly, 1998; Loison et al., 1999). Rensch (1960) observed that in male-dominated species, the magnitude of sexual size dimorphism is greater in larger versus smaller species. This allometric pattern by which sexual dimorphism is greater among larger species if males are the larger sex has become known as Rensch's rule (Fairbairn and Preziosi, 1994). Studies of Rensh's rule are common in both mammals and the broader animal literature, and examples of Rensch's rule include primates, pinnipeds and artiodactyls (Sutter et al., 2008).

Primarily, studies to measure size are based on univariate proxies such as skull length, or directly as body length or body mass. Although these descriptors of size are useful when discussing overall body size dimorphism, they do not represent shape and therefore do not fully describe differences between males and females, nor do they typically address sexual differences occurring in specific body regions (Schutz et al., 2009).

The anatomical morphology of bone is used as an important tool for archaeologists to identify animal species, as well as to distinguish human from animals (Sajjarengpong et al., 2003). Several specific bones can be also used for determining the sex of animals, one being the pelvic bones (Sajjarengpong et al., 2003; Bierry et al., 2010). The pelvis is considered to be a part of the skeleton, which presents a large sexual dimorphism (De Panafieu, 2011). Many parts of the human pelvic bones have been studied, such as the great sciatic notch (Jovanovic and Zivanovic, 1965; Jovanovic et al., 1973; Singh and Potturi, 1978; Hager, 1996), the cranial border (Gomez and Fernãndez, 1992), the obturator foramen (Bierry et al., 2010) and the entire bone (Milne, 1990). Pelvic studies have been also carried out in non-human anthropoids (Gingerich, 1972; Hager, 1996) and also in species such as the dog (Sajjarengpong et al., 2003), grey foxes (Schutz et al., 2009), the northern water vole (Ventura et al., 1991), the mouse (Uesugi et al., 1992a,b, 1993), whales (Bejder and Hall, 2002) and the bat (Nwoha, 2000), but no detailed studies of bovine obturator foramen are available in the veterinary literature.

The obturator foramen (*foramen obturatum*) is a large opening in the pelvis, between the ischium and the pubis, that gives passage to vessels and nerves and is partly closed by a fibrous obturator membrane. This large foramen lies below, and slightly cranial to, the acetabulum, between the pubis and ischium. It is bordered by: (1) the caudal border of the pubis (cranial margin), (2) the cranial border of the ischium forms (caudal margin) and (3) the antero-internal angle or symphyseal branch (*ramus symphyseos*) and the pubis form (inner boundary). In the ox, the obturator foramen is large and elliptical, having a thin and sharp inner border (Sisson et al., 1975).

Material and Methods

A random sample of 60 bovine hemicoxae from a commercial abattoir were collected from deboned carcasses of young bovines that were approximately 12 months old (mean age, 380 days) and which presented neither abnormal general appearance nor signs of clinical lameness. Hemicoxae were then dissected and cleaned. Animals belonged to pure and crossed European taurine meat-type breeds ('Bruna dels Pirineus', Charollais, Limousine and their crosses). All pieces were undamaged and showed no pathological appearance that might lead to errors in measurement. Forty-four samples were from males and 16 from females. For the study, no distinction was made between right ($n = 28$) and left ($n = 32$) samples.

Image capture was performed with a Nikon® (Barcelona, Catalunya, Spain). D70 digital camera (image resolution of 2240 × 1488 pixels) equipped with a Nikon AF Nikkor® 28–200 mm telephoto lens. The focal axis of the camera was parallel to the horizontal plane of reference and centred on the obturator foramen. A computerised image analysis system was then used to measure the obturator foramen using digital picture analysis software (UTHSCSA Image Tool® 3.0, available at ftp://maxrad6.uthscsa.edu) (Wilcox et al., 2002). The boundary of the foramen was delimited digitally. As spatial calibration is available in UTHSCSA to indicate real linear and area dimensional measurements, all the images included a measuring piece to facilitate calibration by the software. For each sample, the following measurements were obtained: dorsoventral maximal diameter (height), and perpendicular to this, craneocaudal maximal diameter (width), perimeter and area. The area was calculated in square millimetres by the corresponding option in the UTHSCSA software and recorded as the obturator foramen area.

For the shape visualisation, the processing of images was performed using the GIMP® v. 2.6.11 software package (available at http://the-gimp.softonic.com/?gclid=CN_Sk-ugna0CFYEmtAodEnarWA). After the outline of interest was manually extracted, a threshold transformation of the grey-level image into a binary image was applied. The outline was described by an ordered series of harmonics defined each by four parameters called coefficients. Stepwise reconstructions of outlines were performed using an increasing number of harmonics. Reconstructions were made point by point and corresponded to a summation of vectors. Fourier analysis was performed using Shape, developed by Iwata and Ukai (2002; available at http://lbm.ab.a.u-tokyo.ac.jp/~iwata/shape/).

Each variable was measured by the author. Normality was tested with the D'Agostino–Pearson test. Data were analysed using PAST® version 1.94b (Hammer et al., 2001; available at http://nhm2.uio.no/norlex/past/download.html).

Obturator foramen shape indices

The shape indices were obtained by combining linear parameters in various ways (Table 1). Size parameters used in the study were form factor, roundness, circularity, rectangularity and ellipticity and were adapted from Pothin et al. (2006). Form factor is a method for estimating surface area irregularity, giving a value of one for a perfect circle. Roundness and circularity give information on the similarity of various features to a perfect circle, giving a minimum value of one and four, respectively. Rectangularity describes the variation of length and height with respect to area, one being a perfect square. Ellipticity indicates whether changes in axis length are proportional.

Fourier shape analysis

Fourier shape analysis (FSA) is a technique for generating from a poor outline, a set of shape-representative variables suitable for use in statistical comparisons between samples. It has been applied to many bones, but there are few papers dealing with foramen analysis (Bierry et al., 2010).

Fourier shape analysis deconvolution has been shown to be a powerful taxonomic descriptor and has several advantages over other methods. It does not require the points to be spaced equally and can fit arbitrary closed contours. FSA coefficients are independent of the obturator foramen position on the digitisation grid. The shape evaluation method is a powerful tool for analysing biological shapes and involves complex procedures such as derivation of the descriptors. In general, Fourier expansions of shape data, such as these outlines, are sensitive to information that is usually considered irrelevant, such as location, size and orientation of the object; this has

Table 1. Obturator foramen indices considered.

Lineal biometric variable	Shape indices
Foramen area (Ao)	Form factor = $(4\prod Ao)Po^{-2}$
Foramen perimeter (Po)	Roundness = $(4Ao)(\prod FL^2)^{-1}$
Foramen length (FL)	Circularity = $P^2\ Ao^{-1}$
Foramen height (FH)	Rectangularity = $Ao\ (FL \times FH)^{-1}$
	Ellipticity = $(FL{-}FH)(FL + FH)^{-1}$

limited the practical use of Fourier descriptors. The discovery of reasonable normalisations that can remove these sensitivities was a very significant achievement by Kuhl and Giardina (1982).

In this study, the FSA descriptor was used in accordance with the procedures suggested by Kuhl and Giardina (1982). Normalised FSA descriptors were calculated using the Shape software. This program performs image analysis, contour recording (shape) and derivation of the Fourier descriptors (Chc2Nef). More details are given by Rohlf and Archie (1984) and Ferson et al. (1985).

Lastly, a principal coordinates analysis (PcoA) (Davis, 1986; Podani and Miklos, 2002) was used to determine differences between sexes. Each of the harmonics is defined by four coefficients and the number of harmonics required can also be estimated from the average Fourier power spectrum. The Fourier power of a harmonic is proportional to its amplitude and provides a measure of the amount of shape information described by this harmonic. To maximise the difference between sexes, a Hotelling's T-squared test (Davis, 1986), in which each sex was placed in the group where its classification function value is largest, was also used for the three analyses.

Results

Analysis by Student's t-test revealed that males and females were not significantly different ($P > 0.05$) in age: 347.8 and 391.6 days for males and females, respectively. Distribution of age was not normal ($P < 0.0001$), so a bias for age difference could be expected.

Size

Perimeter, area, height and width values were normally distributed in both sexes ($P < 0.05$) except for height and width for males. The means of area, perimeter and length for the males were higher than those for the females, but in the Hotelling's T-squared test, only 65% of individuals were correctly classified according to their sex.

Shape indices

Form factor, roundness, circularity, rectangularity and ellipticity appear in Table 2. No statistically significant differences in shape indices were found between sexes (Table 3). In the Hotelling's T-squared test, only 61% of the individuals were correctly classified according to their gender.

Fourier shape analysis

As the first 15 harmonics reached 99.19% of the cumulative power, the FSA indicated that the simple obturator foramen shape could be summarised by 15 harmonics (Fig. 1). Therefore, one obturator foramen was described by 60 variables. Using the Fourier shape analysis, reconstruction of the mean contour shape suggested that there was no sex shape difference. A visual estimate made from a Fourier series reconstruction using increasing numbers of harmonics (empirically from one to five) is shown in Fig. 2.

Table 2. Comparison of the size measurements (mean ± S.D.) of obturator foramen in males (n = 44) and females (n = 16).

Size variables	Males	Females	t
Foramen area (mm^2)	3721.4 ± 408.30	3315.5 ± 405.91	−3.410***
Foramen perimeter (mm)	221.9 ± 12.28	208.3 ± 13.96	−3.651***
Foramen length (mm)	78.0 ± 7.53	72.9 ± 7.53	−2.309*
Foramen height (mm)	66.3 ± 8.17	62.1 ± 6.46	−1.187

***$P < 0.001$.
*$P < 0.05$.

Table 3. Comparison of the shape indices between males (n = 44) and females (n = 16).

Shape indices	Males	Females	t
Form factor	0.94 ± 0.018	0.95 ± 0.035	1.549
Roundness	0.79 ± 0.163	0.80 ± 0.147	0.258
Circularity	13.27 ± 0.265	13.13 ± 0.492	−1.405
Rectangularity	0.72 ± 0.056	0.73 ± 0.039	0.646
Ellipticity	0.08 ± 0.084	0.08 ± 0.074	−0.062

Using each of the 15 harmonics considered, principal coordinate analysis results did not support differences between sexes (Figure 3). The first eigenvalue (38.13%) was similar to the second one (31.91%), indicating that the first coordinate does not represent the largest variation between sexes. In general, the three-first coordinates accounted for 88.46% of the total dispersal of the two groups (sexes). The classification success of the discriminant analysis indicated no clear separation between sexes, with the F-test associated to Wilks' lambda not being significantly different ($F_{20,39} = 0.733$). In the Hotelling's T-squared test, 14 males and six females were incorrectly classified, so overall, only 66.6% of individuals were correctly classified. A supplementary stepwise linear discriminant analysis revealed that the maximum contribution of a variable was 19.39% of the variability between sexes.

Discussion

The three methods described and used in this study (conventional lineal biometrics, shape indices and Fourier

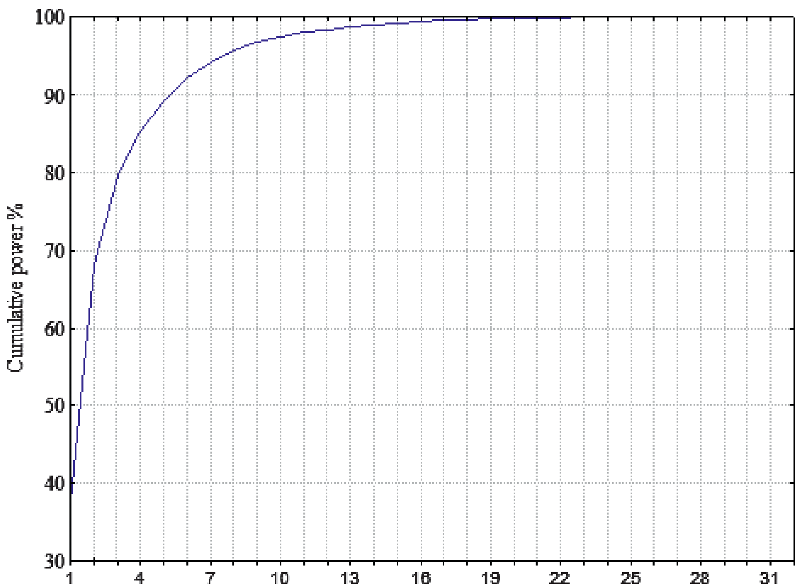

Fig. 1. Cumulative power resulting from the Fourier reconstruction of an outline based in increasing numbers of harmonics.

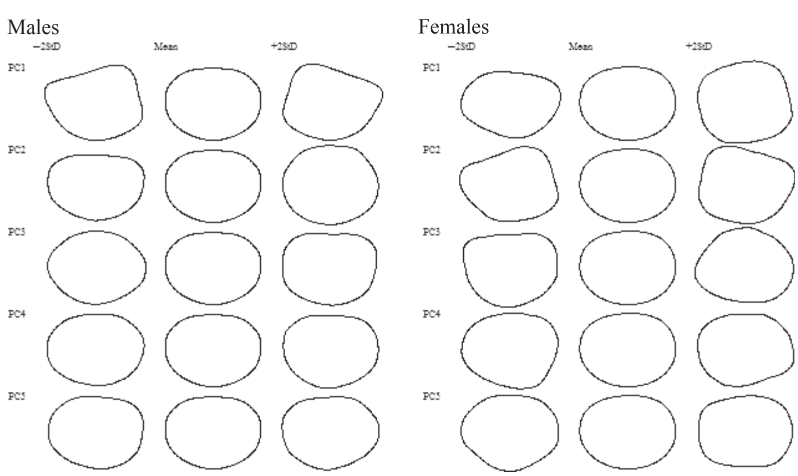

Fig. 2. Cumulative power resulting from the Fourier reconstruction of an outline based in increasing numbers of harmonics.

analysis), do not provide a technique capable of detecting sexual differences in the obturator foramen in young bovines, although they did not yield exactly the same information.

Firstly, lineal biometrics failed to show sexual differences in obturator foramen characteristics, although statistically significant differences in area, perimeter and length appeared. Obturator foramen size cannot therefore be used to efficiently differentiate between sexes. The bovine obturator foramen biometric study found that the length of the foramen was longer than the width in both sexes.

Secondly, no differences were observed in all of the shape indices. Obturator foramen appeared to be rather regular, although not a perfect circle, with changes in axis length not being proportional.

Thirdly, although FSA is a methodological approach particularly well adapted to discrimination between different simple shapes (Bierry et al., 2010), it too showed a low rate of accuracy in classification according to sex.

Variation in form and shape of obturator foramen by both sexes are not expressed, perhaps because samples were from young animals, and therefore, they do not represent a phenotypic measurement of gender. The obturator foramen has been traditionally described as larger in males than in females and having an oval form and triangular form, respectively. But the form of the foramen becomes distinct during the period of chondrification, because the tissue that covers them remains membranous while the surrounding tissue is converted into cartilage. Sampled bovines were below the age of total chondrifica-

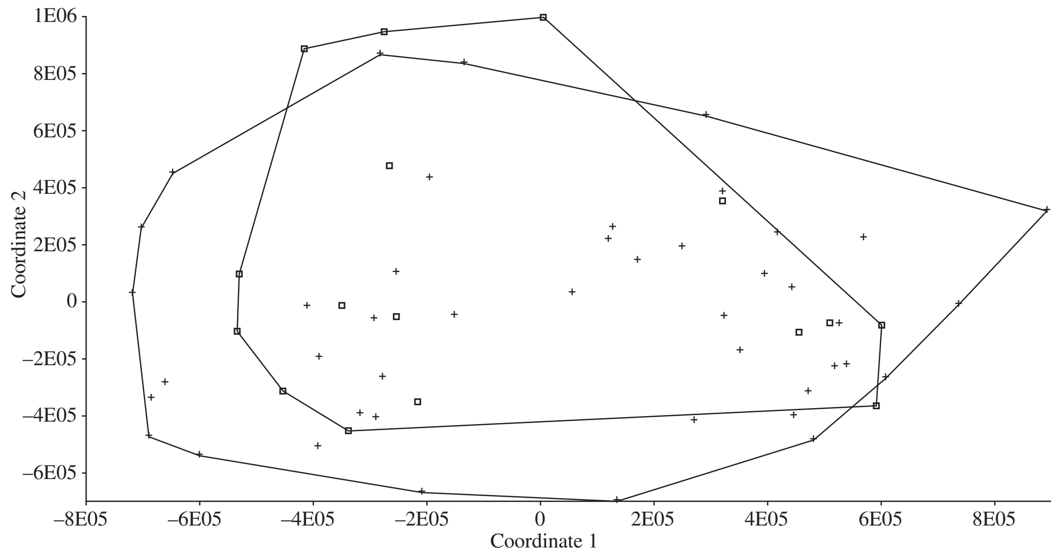

Fig. 3. Principal coordinates analysis using the first 15 harmonics. Crosses: males. Quadrates: females.

tion, so a lack of sexual dimorphism could explain the difficulty to separate sexes according to studied parameters. Similar procedures for more aged bovines might reflect better the discriminative power of those techniques. Analysis of size and shape cannot be considered a suitable technique to separate sexes and thus are not appropriate to be used as natural traits for differentiating sexes, at least in young bovines. Evidently, measurement of the pelvic diameters, the pelvic inclination among others, could give support for describe female or male anatomical constitution. Morphological and physiological characteristics of different breeds has been stabilised (Bellows et al., 1971; Johnson et al., 1988; Donkersgoed, 1991; Weiher et al., 1992; Okuda et al., 1994; Barreto et al., 2008), but none referred to the obturator foramen.

Lack of dimorphism in the obturator foramen was probably due to the fact that most of the studied animals were of approximately 1 year of age. Changes to pelvis conformation could appear as the animal age, and therefore, differences in the shape of obturator foramen could emerge. Moreover, Rensch's rule may not be maintained in domestic bovines in which the proportional size difference between young males and females is essentially the same.

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