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Evaluation of shape variability of stallion sperm heads by means of image analysis and Fourier descriptors

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ABSTRACT

This study quantified and evaluated the variability of sperm head shape for 10 different stallions. Sperm head shape characteristics including sperm head length to width ratio, position of the center of gravity, curvature, and degree of roundness were assessed and analysed from images using elliptic Fourier descriptors and inverse Fourier transformation. The first four principal components accounted for 88.46–92.33% of the total variance and provided a good summary of the overall data. In the case of the ejaculate with defective sperm heads the components accounted for 97.35–98.21% of variation. The study was able to quantitatively confirm that head length to width ratio, which contributed 48.63–53.48% and 71.30–73.34% to the total variance for normal and defective sperm, respectively, was the predominant determining parameter of sperm head shape. There were no statistical significant relationships between Fourier descriptors and values of sperm concentration and/or motility.

1. Introduction

Spermatozoa from otherwise normal individuals differ in shape and dimensions among species (Downing-Meisner et al., 2005) and even between individuals (Roldan et al., 1998; Thurston et al., 2001). Although sperm head shape and relative dimensions are considered reliable indicators of sperm quality, their quantification is most often operator-driven and therefore subjective (Saravia et al., 2007). Thus several computer-assisted methods and procedures have been developed (Esteso et al., 2006; Saravia et al., 2007; Hidalgo et al., 2008). Automated sperm morphometry analysis and other systems have improved the assessment of sperm morphology, with accurate and repeatable measurements of sperm head dimensions of different species (Gravance et al., 1996; Sancho et al., 1998; Thurston et al., 2001; Buendía et al., 2002; Beletti et al., 2005; Hidalgo et al., 2006; Tuset et al., 2008), including stallions (Ball and Mohammed, 1995; Hidalgo et al., 2005). In addition, subtle differences in the sperm head dimensions among normal animals, as well as between fertile and subfertile stallions, have been shown with automated systems (Hidalgo et al., 2008). Automated systems hold the potential advantage of reducing technical variation that is inherent in manual, subjective morphology analysis. However, their use requires careful specimen preparation and staining in order to reduce digitization errors (Davis and Gravance, 1993).

Morphology of spermatozoa has been used in fertility evaluation of stallions and other males (Bielanski et al., 1982; Jasko et al., 1990; Voss et al., 1981; Phillips et al., 2004), and abnormal sperm morphology has been associated with reduced fertility (Jasko et al., 1990). A number of studies (Hingst et al., 1995; Karabinus et al., 1997) indicate that variation of sperm head morphology is a sensitive biomarker of abnormal chromatin structure and thus of fertility. While measuring sperm head morphometry could be considered an easy task, quantitative criteria for the defini-

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Table 1
Stallion details and mean values (three individual semen collections and 100 randomly selected sperm heads evaluated for each collection) of first principal components.

Stallion no.	Name	Breed	Year of birth	First component (proportion %)	First four components (proportion %)
1	Aristo Z	Zangersheide	2001	51.02	89.73
2	Ballast	Russian Holstein	1986	49.47	88.46
3	Cassilius	Holstein	1997	52.76	91.56
4	Catalin	Furioso	1980	48.63	90.05
5	Genius L	Czech Warmblood	1999	50.04	88.91
6	Lancelot	Holstein	2002	53.48	92.33
7	Nesan	Silesian Noriker	1999	49.60	91.27
8	N-Mates	Haflinger	2000	48.71	89.73
9	Oscar	KWPN	1996	50.53	90.40
10	Oušor M	Hucul	1992	51.59	90.59

tion of what constitutes a normal spermatozoon in regard to fertility has only been done in the human (WHO, 1999). What is normal is particularly important in species, such as the stallion, in which several morphological sperm head forms are clearly distinguishable by visual methods. It is possible that sperm quality or fertility levels might be correlated with different sperm morphology classes in each sample (Hidalgo et al., 2008).

This paper is focused on the quantification and evaluation of stallion sperm shape variability, considering its length to width ratio, position of the center of gravity, curvature and degree of roundness. The procedure used for this task is based on the elliptic Fourier descriptors (EFDs) (Kuhl and Giardina, 1982). This method has been applied with success when evaluating different biological shapes (Toyohara et al., 2000; Iwata et al., 2002; Yoshioka et al., 2004, 2006; Havlíček et al., 2008 and other) and even the sperm shape (Thurston et al., 2001; Beletti et al., 2005). The method offers mathematical description of the entire shape through transforming coordinate information concerning the contour into Fourier coefficients. Principal component analysis of the coefficients can then extract the independent shape characteristics, and make it possible to analyze the shape quantitatively by using the component scores as ordinary quantitative characters (Rohlf and Archie, 1984). General review of image analysis as a tool for biological shapes evaluation is given in Horgan (2001).

2. Methods

2.1. Semen collection and evaluation

Semen was obtained by artificial vagina from 10 clinically healthy stallions (Table 1). Stallions were housed in Zemský hřebčinec Tlumačov, s.p. The stallions had frequently been used for semen collection during the breeding season (3–4 times per week) with normal sperm collected (sperm motility, sperm concentration and subjectively assessed sperm morphology). The stallions were considered to have normal fertility based upon previous breeding history. The semen used in this research was collected in the period of March–May 2009. Slides were prepared and examined within 30 min after collection. Samples from each stallion were prepared and evaluated from three individual collections at one-week intervals. Two more ejaculate samples, where increased presence of defective sperm heads was detected by the examining technician, were evaluated as reference samples. Sperm motility and sperm concentration were determined by standard microscopical techniques.

2.2. Image processing and quantitative measurement of the sperm head shape

For examination of spermatozoa, 10 μl of raw semen was placed on the edge of a slide and evenly spread. Preparations were fixed in formaldehyde solution (10% solution of 35% formaline in physiological solution of NaCl – 0.99%) for 15 s. The preparations were washed in water and coloured in the aniline blue solution (5% solution of aniline blue in distilled water) for 15 s and in a crystal violet solution (0.5% solution of crystal violet solution in distilled water) for 6 s.

Microscopic slides were analyzed for sperm head shape. The equipment used consisted of a microscope Olympus BX51 (Olympus Optical Company, Ltd., Tokyo, Japan) equipped with a 100× oil immersion objective (Olympus UPlanFl 100×/1.30 Oil Iris) and a photo-ocular (Olympus WH 10×/22). A camera DP70 (Olympus Optical Company, Ltd., Tokyo, Japan) was mounted on the microscope to capture the images and transmit them to a computer. Resolution of images was 72 dpi in the horizontal and vertical axes. Sperm were randomly identified and 100 sperm heads were captured on several slides. The raw images were converted to full colour (24-bit) bitmap format and subsequently to a grey scale. The grey scale images were converted to binary images in which the objects and background are represented as 1 (white) and 0 (black), respectively. The image analysis software Shape (Iwata and Ukai, 2002) was used to perform all following steps. The closed contours of the sperm heads were obtained through binary images with appropriate thresholds, and were described by a chain-code (Freeman, 1974). Namely, each contour was represented as a sequence of x and y coordinates of ordered points that were measured counterclockwise from an arbitrary starting point. Assuming that the contour between the $(i-1)$th and ith chain-coded points is linearly interpolated, and that the length of the contour from the starting point to the pth point and the perimeter of the contour are denoted by the t_p and T, respectively, then the elliptic Fourier expansions of the