The Correlation Between Bull Sperm Head Dimensions and Mitochondrial Helix Length

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Abstract: The objective of this study was to find the relationship between bull sperm head dimensions, elongation, ellipticity and mitochondrial helix length in order to find a parameter for the measurement of sperm motion. Sperm cells were obtained from Holstein bulls and supravital stained with Nigrosin-Eosin then smeared on replicate slides. Sperm head length, head width, elongation, ellipticity and mitochondrial length were measured as 14.25 ± 0.17, 7.27 ± 0.23, 0.32 ± 0.09, 1.98 ± 0.18 and 20.30 ± 0.15, respectively. But no statistically important correlations were found between these traits and mitochondrial lengths.

Key words: Bull, sperm, head, mitochondria, correlation, dimensions

INTRODUCTION

Sperm head dimensions (width and length) are vary between and within species (Van Duijn, 1974; Cummins and Woodall, 1985; Amann, 1989; Verstegen et al., 2002; Kathiravan et al., 2008).

Important differences have been reported in sperm head dimensions between and within breeds of stallions (Ball and Mohammed, 1995; Hidalgo et al., 2008), canines (Dahlbom et al., 1997), bulls (Boersma et al., 1999), buffalo (Aggarwal et al., 2007), rams (Sancho et al., 1998), red deer (Soler et al., 2005) and boar (Garcia-Herreros et al., 2006; Saravia et al., 2007).

Sperm head dimensions have been correlated with fertility in various species including humans (Chan et al., 1999), horses (Casey et al., 1997), boar (Hirai et al., 2001; Pena et al., 2005), Iberian red deer (Esteso et al., 2006) and canines (Nunez-Martinez et al., 2007).

The midpiece is formed by the axoneme surrounded by the outer dense fibers and contains numerous mitochondria. Increased midpiece size, resulting from more or larger mitochondria, results in greater amount of energy available to achieve higher swimming speeds (Anderson and Dixon, 2002; Cardullo and Baltz, 1991). Thus, the volume of mitochondria determines the flagellar beat frequency (Cardullo and Baltz, 1991).

Therefore, the size of the sperm midpiece may be important in determining the outcome of sperm competition in polygamous species. Males of polygamous species have sperm with larger midpieces and presumably higher densities of mitochondria compared with monogamous species, suggesting that increases in midpiece volume may translate to greater swimming velocities and thus lead to an advantage in sperm competition (Anderson and Dixon, 2002). According to a report, there is a positive relations between head length and sperm swimming speed in red deer (Malo et al., 2006). It has also been reported by Malo et al. (2006) that sperm with longer heads and shorter midpiece swam more quickly than sperm with long midpieces and shorter heads. Also many studies have shown the existence of relationships between head and the midpiece (Cardullo and Baltz, 1991; Gage, 1998; Piasecka and Kawiak, 2003; Malo et al., 2006). A negative correlation between midpiece length and head length was reported (Humphries et al., 2008).

A measure of ratio between head dimensions and mitochondrial lengths can represent a useful parameter for the measurement of sperm motion. Therefore, the aim of this study was to find a correlation between Holstein bull sperm head dimensions and mitochondrial helix lengths.

MATERIALS AND METHODS

Semen collection: Semen was collected from 2 years old Holstein bulls housed at a farm of the School of Agriculture, Selcuk University, Konya, Turkey. It was collected 5 times between 9:00 and 11:00 over a 9-mount time period using an artificial vagina. Collected semen samples were pooled in a 50 mL centrifuge tube (C-8296, Sigma, Steinheim, Germany).

Seminal plasma was removed by washing the semen with phosphate buffered saline (PBS, P5493, Sigma-Aldrich, Steinheim, Germany) containing 0.06 mg mL^{-1} penicillin-G (Potassium salt, P7794, Sigma-Aldrich, St Louis, USA).

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Preparation of Nigrosin-Eosin Stain: One gram Nigrosin (1.15924, Merck, Darmstadt, Germany) and 6.7 mL Eosin (Yellow, 45242, Fluka, Steinheim, Germany) was mixed. Then, 6 mL ultra pure water was added and dissolved for 20 min in a water bath at 100°C and filtered. To the filtered stain, 0.68 mL of a glucose solution (100 g L^{-1} solution, G8644, Sigma-Aldrich, St Louis, USA) was added and rinsed with 6 mL of tartrate buffer. The stain was placed in a dark glass container and kept at 4°C.

Staining: For staining, 100 μL semen samples from each petri dish were places in a test tube and fixed with 100 μL formaldehyde (FI/01FS/060929, Kimetsan, Turkey) for 5 min in a water bath at 37°C. Then 100 μL of the NE stain was added and left for 10 min at 37°C.

Measurement of head dimensions and mitochondrial Lengths: For each experiment 8 slides were prepared for the measurements and counted at 100x magnification, using an oil immersion lens under a light microscope (Leica, DM 2500) equipped with a camera (Leica, DFC-280) connected to a computer. Sperm heads and mitochondries were quite visable as in Fig. 1.

On each slides, around 50 sperm cells was measured according to their head length, head width and the mitochondrial length of by using Manufacturers software (Leica IM50, version 4, copyright 1992-2004) have been installed on the computer connected to the camera. The scale was 5 μm and already calibrated by Leica.

Fig. 1: The vision of sperm cells were clear and sharp. The borders of mitochondries and heads were easily seen. The border of the middle piece and principal piece was clearly seen due to the termination of the thick mitochondrial helix (d). The distances between a-b were accepted as head Length (L); c-d accepted as head Width (W) and b-d were accepted as Mitochondrial Length (ML). According to these measures, ellipticites (L/W) and elongations [(L-W)/(L + W)] were calculated

Statistical analysis: Data were analysed by linear regression. Mitochondrial length was accepted as dependent variable and head length was accepted as independent variable. Regression coefficient, the intercept of the regression line and the equation of the regression line were calculated by using microsoft office excel.

RESULTS AND DISCUSSION

The mean measurements for mitochondrial length, sperm head length, head width, ellipticity and elongation were 20.30±0.15, 14.25±0.17, 7.27±0.23, 1.98±0.18 and 0.32±0.09 (Fig. 2). There were no correlation between head dimensions (length and width), elongation, ellipticity and mitochondrial lengths (Fig. 3). Mammalian sperm are highly specialized cells and their heads vary greatly in their size and shape within and between species (Van Duijn, 1974; Cummins and Woodall, 1985; Bedford and Hoskins, 1990).

An experiment conducted on 18 bulls of different ages and breeds by Gravance *et al.* (1998). In their study, mean sperm head length and width were measured as 8.63±0.08 and 4.48±0.05 μm) but in this study, the mean sperm head length and width measured as 14.25±0.17 and 7.27±0.23 μm. The mean head size measurements reported by Gravance *et al.* (1998) and his cliques was different than the study because of the difference in source of semen and the procedure applied. Here, these huge differences in estimated head dimensions are also present among published papers. And this appears to reflect differences in the way sperm are prepared for measurement as well as the methods used for the measuring head dimensions (Foote, 2003).
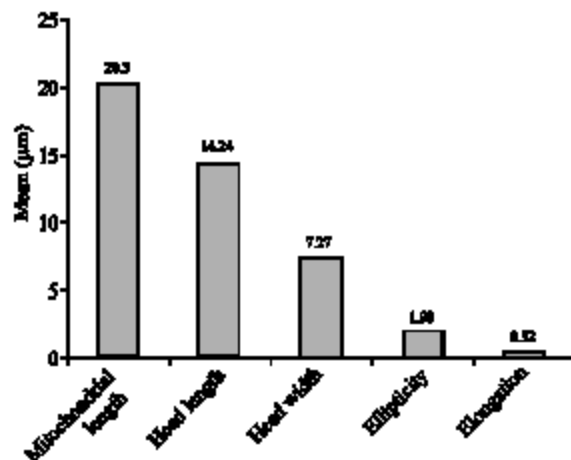

Fig. 2: Measured mean values of bull sperm head dimention (length and width) and calculated mean values of sperm ellipticity and elongation
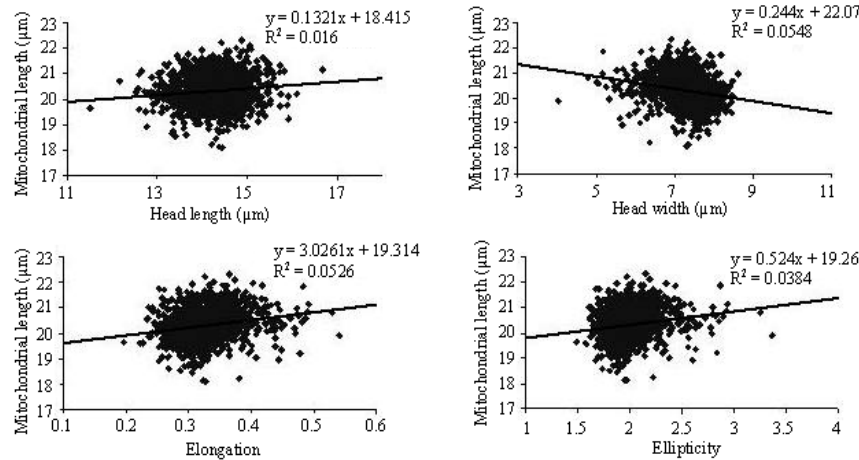
Fig. 3: The correlations between head lengths, head width elongations, ellipticities and mitochondrial lengths were not statistically important

Volume of live sperm cells differs according to the osmotic characteristics of the buffers used with supravital stains (Foote, 2003). Different stains and solutions used for fixing and staining of sperm cells and fractions of live versus dead sperm may affect the estimated size of sperm heads (Van Duijn, 1974). That was why the measurements of head dimensions were different from the measurements of Gravance and his cliques.

Mitochondrial helix defines distinctively the middle piece, which is located between the neck and the annulus and surrounded by numerous mitochondria. It is possible that an increase in midpiece length associated with the quantity of mitochondria could increase higher rate of energy production facilitating sperm motility.

Bull sperm midpiece length is heritable and it varies among bulls of same breed. Sperm midpiece length also differs in the ejaculate of the same bull in different times (Lukeahr and Hohenboken, 1981). According to the result of a study conducted by Lukeahr and Hohenboken (1981) on Holstein bulls, the mean sperm midpiece length was reported as 13.63±0.36 μm long. This was quite shorter then the mean length measured in this study. It seems that the major cause of this difference was the difference in the calibration of equipment used in measurements. But this not affects the result of the study.

Because we were just looking for the correlation between head dimension and midpiece length and the same calibration was employed for these measurements. Also this study, semen from 3 bulls were pooled and it was collected over a 9-mount time period. Thus, bull effect and the seasonal effect are not important sources of variation in this study. According to the result of an experiment on porcine, a positive correlation between sperm head size and midpiece length and a negative correlation between ellipticity and elongation were reported (Gill *et al.*, 2009), while experiment on Iberian red

deer revelled no relationship between sperm head size and midpiece length (Malo *et al.*, 2006). It seems that the degree of correlation depends on animal breed. Here, we have not find any significant correlation between sperm head dimensions, elongation, ellipticiy and midpiece length.

CONCLUSION

Presently, it is not possible to use the ratio between head dimension and mitochondrial length as a parameter for the measurement of sperm motion.

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