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Short communication

Influence of season and reproductive management on the morphometry of ram sperm head

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ABSTRACT

The aim of the present work was to study the morphometric characteristics of apparently normal ram sperm heads of an ovine insemination centre over a year, using a computer-assisted sperm morphometric assessment system (CASMA) which is commercially available. For this, 383 ejaculates from 9 mature rams kept in non-controlled environmental conditions were used. The morphometry was analyzed in fresh sperm and each spermatozoon was measured for four primary parameters (length, L; area, A; width, W and perimeter, P) and four derived parameters (ellipticity, L/W; rugosity, $4\pi A/P^2$; elongation, $L-W/L+W$ and regularity, $\pi LW/4A$).

A clear seasonal behaviour of both morphometric and derived parameters was observed, with sperm size being larger in autumn and winter. This behaviour was also observed for ellipticity and elongation.

Due to organizational reasons in the insemination centre, the rams' reproductive activity was suspended during the month of August; this fact was reflected, in the ejaculates collected in September, in the decrease of the sperm head size, as well as in the increase of ellipticity.

The procedures used in the present study did not allow for natural separation in sperm subpopulations considering the size and shape of apparently normal sperm heads, but it was possible to divide the ejaculate into two subpopulations, taking rugosity into account. These subpopulations were present in all the rams – and had a clear seasonal distribution.

1. Introduction

Sperm morphology assessment is routinely used for evaluation of fertility potential; however, subjective methods of assessing morphology are highly variable. The need for an accurate objective assessment of sperm morphology has led to the development of systems for computer-aided sperm morphometric assessment (CASMA). The CASMA has been used to relate sperm head morphology and morphometry to fertility in humans (Kruger and Coetzee, 1999) and animals (Casey et al., 1997); it has also been used to determine the effects of cryopreservation on sperm head morphometry of species such as bovine (38) (Gravance et al., 1998b) and dog (Rijsselaere et al., 2004).

Computer Assisted Sperm Analysis (CASA) has been used to classify sperm into subpopulations based on motion or morphological characteristics; although the reason for the existence of these subpopulations in ejaculates is not

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well known yet, the existence of an overall subpopulation structure could be important to maintain the general function of the ejaculate. It has also been suggested that the presence of these subpopulations is due to specific combinations of sperm-related genes carried by each animal, these combinations being the product of meiosis during spermatogenesis (Abaigar et al., 2001).

Apart from these uses, CASMA could be a useful tool to detect the influence of seasons on morphometry, first because rams exhibit a seasonal fluctuation in spermatogenesis (Rosa and Bryant, 2003), and also because the effect of photoperiod on sperm quality, particularly on morphology, varies amongst individuals and breeds (Mandiki et al., 1998).

The objectives of this study were (1) to determine the morphometric characteristics of ram sperm heads using CASMA, (2) explore the variation in sperm head morphometry between individual males and seasons and (3) search for the existence of subpopulations in the sperm head morphometry of fresh spermatozoa.

2. Materials and methods

2.1. Animals and semen collection

Semen was collected from 9 healthy Ile de France mature rams. The rams were in the AI service at the Centro de Reproducción y Selección Animal (CENSYRA), Extremadura, Spain (38°53′ N and 6°49′ W). They were kept in non-controlled environmental conditions and maintained under the same feeding and management programme.

A total of 383 ejaculates were collected using an artificial vagina once a week along a year except for the month of August. Semen from each ram was collected twice with a 15 min interval. For the present study the samples were taken only from the first collection.

2.2. Computerized morphometric analysis

Two microscope slides were prepared from each ejaculate by placing 7 μL of the sperm sample, from a final dilution of 200×10^6/mL. Then they were air-dried for two hours on a warming stage at 37 °C and fixed in a 2% glutaraldehyde solution in medium BL-1 (Pursel and Johnson, 1974). Afterwards they were stained with Harris' Haematoxylin (Panreac S.A., Barcelona, Spain) following the protocol described by Sancho et al. (1998).

The stained slides were used to perform a computerized morphometric analysis using the specific module of the system ISAS (Integrated Semen Analysis System, Proiser $R+D$ S.L. Valencia, Spain) The system was equipped with a microscope Nikon Eclipse 50i with a ×40 bright-field objective magnification lens and a video camera Basler A302fs. The array size of the video frame recorder was 782 (H) × 582 (V) × 8 bits, and 256 grey levels. The image resolution was 0.207469 μm per pixel in the horizontal and vertical axes. Before observation, a green filter was applied to the light source and its intensity was standardized. After the treatment of the images, some of the sperm had to be discarded because of defective binarization, as observed by false correspondence between the original image and its overlapped mask.

In this way a minimum of 150 spermatozoa per sample were correctly processed.

Finally the system software provided the size and shape parameters: area (A, μm^2), perimeter (P, μm), length (L, μm), width (W, μm), F1 (ellipticity, L/W), F2 (rugosity, $4\pi A/P^2$), F3 (elongation, $L-W/L+W$) and F4 (regularity, $\pi LW/4A$).

The measurements of each individual sperm head were saved in an Excel program (Microsoft Corporation, Redmond, WA, USA)

2.3. Statistical analyses

The statistical analyses were performed using the SPSS 15.0 (SPSS Inc., Chicago, IL, USA) and R software, and the data were considered statistically significant at $p < 0.05$ level.

Table 1
Mean (±SD) values of motility and morphology parameters obtained from fresh semen.

Sperm morphometric parameters	Overall mean ± SD (n = 60,968)
Length (μm)	8.240 ± 0.346
Width (μm)	4.882 ± 0.198
Area (μm^2)	34.764 ± 1.97
Perimeter (μm)	23.862 ± 0.876
F1 (ellipticity)	1.689 ± 0.081
F2 (rugosity)	0.767 ± 0.030
F3 (elongation)	0.255 ± 0.022
F4 (regularity)	0.909 ± 0.030

F1, L/W; F2, $4\pi A/P^2$; F3, $L-W/L+W$; F4, $\pi LW/4A$. Shape factors are dimensionless, n: number of spermatozoa.

MANOVA, ANOVA and Tukey's HDS multiple comparisons tests were applied to detect differences in morphometric parameters among rams and seasons. Also, a Wilk's Lambda selection algorithm was applied to determine the most useful parameters to distinguish between rams or seasons.

In order to compare the variability of the parameters within-male and between-male, both coefficients of variation (CV) were calculated: for every morphometric parameter, the first, CV_{intra}, was calculated as the mean of the CVs of all the spermatozoa from each ram; the second, CV_{inter}, was calculated as the mean of the CVs of all the spermatozoa corresponding to each date.

We tried to determine and characterize subpopulations according to morphometric parameters by a cluster analysis. Previously, all the variables were standardized to avoid the scale influence in the final decision. The techniques – provided by SPSS or R – applied in order to decide the number of clusters were the following: the Calisnski–Harabasz method, a bietapic algorithm according to Bayesian Information Criterium, the Elbow method and a two-step method beginning with a k-mean clustering with a large enough k, followed by a hierarchical clustering of the k resulting centroids.

It must be pointed out that, due to the vast amount of data (n = 60,968), most of the statistical tests applied provided significant results, although the correlation between variables and the difference of means were small in the samples.

3. Results

Descriptive statistics of the overall sperm population were calculated to characterize Ile de France ram spermatozoa. The results obtained from sperm head morphometric parameters are summarized in Table 1.

All the parameters considered provided significant differences ($p < 0.001$) among the means of the rams. All the parameters except F4 were useful for distinguishing between rams, area being the most important, followed by F2.

In order to compare the variance of the parameters both within-male and between-male, coefficients of variation (CV) were calculated. The results showed that, in both cases, A (5.13 and 5.05%) and F3 (8.28 and 8.70%) were the parameters with the highest CV, whereas F4 rendered the lowest ones (3.37 and 3.35%).

The month and season (Table 2) affected all the morphometric variables analyzed ($p < 0.001$). Again, all the parameters except F4 were useful to distinguish between months, in this case F2 being the most important, followed by area. The evolution of A and F2 throughout the year is illustrated in Figs. 1 and 2