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Results regarding Some Morphometric Features of Spermatozoa in Ram

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Abstract

It is well known nowadays that morphological type classification of spermatozoa is an important component of the modern semen evaluation. Meanwhile, current methods of analysis are subjective and highly diversified; they are also ones of the best tools for artificial insemination using cryogenic preserved semen for the contemporary livestock producer. The aim of the present study was to determine if there are differences associated with sperm head morphometric dimensions in individuals with different sexual maturity ratings. For the present results, there was setting off ram groups depending on age: 12, 36 and 72 months of age. There were used random images from fresh sperm and each head spermatozoon was measured with the aid of a specialized soft 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, $\pi LW/4A$). The analyzed data were statistically processed, being recorded the main population parameters: the average, its error, the standard deviation, the coefficient of variability. There were no significant differences in the sperm head dimension or shape among middle-aged and old individuals ($P > 0.05$). However, significant differences were detected in area, perimeter and width (lower values) and length, ellipticity and elongation (higher values) in old or middle-aged individuals compared with young individuals ($P < 0.01$). As a conclusion, this study confirms that ram age is related to sperm morphometric size. Therefore, the present study provides information regarding the maturation of ram sperm and supports the idea that the dimensions of ram spermatozoa may be taken as an approximate indication of its relative maturity.

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1. Introduction

In the last two decades, following the evolution of the technical devices and investigation equipment in the field of biology there were gone up the studies over the biologic features in the different species of animals.

Starting from the fact that the morphometry researches are not too many and also not very diversified in ovine, we have proposed the comparative assessment of ram semen with the aid of morphometry, depending on the reproducers age.

2. Research Methods

Having in view this proposal, semen from 12 fertile rams with different sexual maturity ratings, 12, 36 and 72 months of age was thawed and prepared on smears for morphometric analysis. Semen collection from each ram was done in the morning hours using artificial vagina. After a few minutes of incubation at 30 degree Celsius a drop of neat semen was taken and mixed with eosin-nigrosin stain. Ram sperm head morphometry was accurately evaluated on at least 100 spermatozoa at x 40 objective magnification, in approximately 3 min. There were made notices and measurements on 120 ram ejaculates.

The results were grouped by age categories, from young, middle-aged and old rams. There were used random images of the samples. The computerized analyzes were made with the aid of the Integrated Sperm Analyze System ISAS Projectes I Serveis R+D S.L. There were determined the primary parameters of the sperm head for each special sperm file: length, area, width and perimeter, *and the derived parameters for head shape:* ellipticity, rugosity, elongation and regularity were calculated as it folows (Hidalgo et al, 2005): Ellipticity = Length (μm)/Width (μm), Rugosity = $4\pi A(\mu m^2)/P^2(\mu m^2)$, Elongation = (Length in μm – Width in μm) / (Length in μm + Width in μm) and Regularity = π x Length in μm x Width in μm / $4A(\mu m^2)$. The analyzed data were statistically processed, being recorded the main population parameters: the average, its error, the standard deviation, the coefficient of variability. There were also calculated the phenotypic correlation values among the analyzed features, and after these the conclusions were recorded.

3. Results and Discussion

The primary data were grouped on age category (young, middle-aged and old) and statistically processed and the results regarding the measured sizes (head length, head width, head area and head perimeter) and the derived calculated parameters of the head shape are shown in Table 1.

One of the analyzed sizes was the length of the spermatozoon head. We notice that between the three analyzed categories appeared a few differences as: the highest value of spermatozoa head length was recorded in the oldest age category, this being as average 8.54±0.24 μm. On the second place, the middle-aged rams are recorded with an average length of the spermatozoon head of 7.95±0.35 μm. They are followed by the youngest rams with an average value of the spermatozoon length of 7.91±0.35 μm. The width of spermatozoon head was the second parameter measured with the aid of the analyzer system and the following mean values for this character were: 4.49 ± 0.15 μm in the youngest category, 4.51±0.18 μm for the middle-aged category and 4.90±0.21 μm for the oldest category.

Table 1. Ram Spermatozoa Head Size and Shape

	Character	Ram age category			Overall Mean	CV
		Young	Middle-aged	Old		
Head size	Length μm	7.91±0.31	7.95±0.35	8.54±0.24	8.13±0.16	2.3
	Width μm	4.49±0.15	4.51±0.18	4.90±0.21	4.62±0.06	1.25
	Area μm^2	28.01±0.41	28.21±0.19	30.20±0.23	28.21±0.23	2.14
	Perimeter μm^2	22.71±0.23	23.01±0.25	24.09±0.22	23.21±0.15	3.12
Head shape	Ellipticity	1.76±0.03	1.76±0.05	1.74±0.03	1.75±0.04	4.25
	Rugosity	0.68±0.01	0.71±0.02	0.64±0.02	0.67±0.02	5.22
	Elongation	0.27±0.01	0.27±0.01	0.27±0.01	0.27±0.01	4.17
	Regularity	0.93±0.01	0.93±0.01	1.02±0.02	0.96±0.02	5.35

The area of the spermatozoon head and the perimeter of the head, the other two head measurements, were established and recorded for each studied ram and analyzed group also by age category. The highest value of the area was recorded in old-aged category, with 30.20±0.23 μm^2 being followed by the last two categories, the middle-aged one and the young ram category, within those last two categories being recorded significant differences. The head shape was expressed by the derived parameters, ellipticity, rugosity, elongation and regularity and recorded for all the groups. The head elipticity values were higher in the young and middle-aged category, 1.76±0.03 and 1.76±0.05, but lower in the older category 1.74±0.03.

Elongation recorded the same value in all three categories 0.27±0.01, but the variability within was 4.17%. Regarding the regularity, the experiment reveals the fact that this derived parameter recorded the highest value in the old rams, being followed by the other two categories with the mean value of 0.93±0.01.

These measures and calculated data are illustrated in Figure 1, for head size and Figure 2 for head shape.

In 2010, Gravance CG, Champion ZJ and Casey PJ, in the study ***Computer-assisted sperm head morphometry analysis (ASMA) of cryopreserved ram spermatozoa***, highlighted the accuracy of ASMA procedures in sperm assessment (Gravance, 2010).

The objective of their study was to develop accurate methods for employing ASMA of ram sperm heads. Frozen semen from 10 fertile rams was thawed and prepared on slides for morphometric analysis. The morphometric measurements of sperm heads for all rams were as follows: length = 8.08 microns, width = 4.80 microns, width: length ratio = 0.59, area = 29.13 micron 2 and perimeter = 23.93 microns.

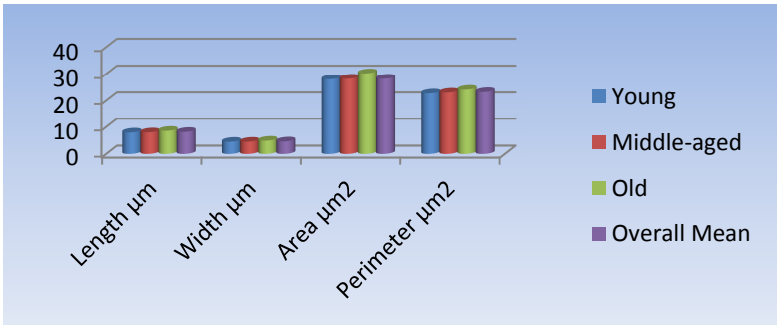

Figure 1. Ram Spermatozoa Head Size Depending on Age

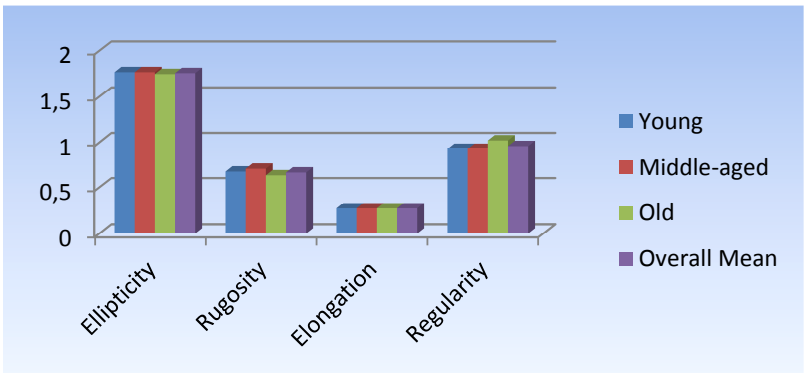

Figure 2. Ram Spermatozoa Head Shape Depending on Age

The mean within analysis coefficients of variation for all individual analyses and parameters ranged from 4.8% for length to 6.0% for area (Tapaloaga, 2003).

Meanwhile et al. studied 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) (Bravo et al., 2011). 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 the same parameters as in our study (Tapaloaga, 2004). A clear seasonal behaviour of both morphometric and derived parameters was observed, with sperm size being larger in autumn and winter.

4. Conclusions and Recommendations

Sperm quality at thawing for all sperm parameters evaluated was significantly higher for old individuals than for middle-aged or young individuals ($P < 0.01$). There were no significant differences in the sperm head dimension or shape among middle-aged and old individuals ($P > 0.05$). However, significant differences were detected in area, perimeter and regularity (higher values) in old individuals compared with middle-aged young individuals ($P < 0.01$). In conclusion, this study confirms that ram age is related to sperm morphometric dimensions. Therefore, the present study provides information on the morphometric maturation of ram sperm and supports the idea that the dimensions of spermatozoa may be taken as an approximate indication of its relative maturity.

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