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Seasonal dynamics of sperm morphometric subpopulations and its association with sperm quality parameters in ram ejaculates

J.I. Martí[a,*], I.M. Aparicio[b], C.L.V. Leal[c], M. García-Herreros[c,*]

[a] Centro de Investigación y Tecnología Agroalimentaria de Aragón, Zaragoza, Spain
[b] Departamento de Fisiología, Universidad de Extremadura, Cáceres, Spain
[c] Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, Brazil

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Abstract

Sperm morphologic assessment is considered an irreplaceable part of standard laboratory routine analyses in the diagnosis of male fertility. Thus, in an attempt to quantify the effects of season on sperm morphology and its functional significance in relation to sperm quality parameters, sperm head morphometric traits were analyzed by using an objective computerized analysis combined with principal components analysis (PCA) cluster analysis to establish the relationship between the distribution of the subpopulations found and sperm quality in each season. There were slight variations on sperm motility and sperm membrane integrity indexes ($P > 0.05$). However, the mean values for sperm concentration substantially changed among seasons in all individuals studied ($P < 0.01$). There were significant differences in sperm morphometric parameters ($P < 0.01$) as well as in the distribution of morphometric subpopulations between seasons ($P < 0.001$). In conclusion, this study confirmed that there was an important seasonal effect on sperm morphometric traits. In addition, the distribution of these subpopulations seems to be related to the season studied and the ejaculate quality which would be a very important indicator of sperm function. The substantial information derived from these morphometric subpopulations has provided new knowledge which can be used in future studies using sperm morphometry as a seasonal indicator in ram ejaculates.

Keywords: Season; Principal component analysis; Sperm subpopulations; Sperm morphometry; Ram semen

1. Introduction

Sperm morphology assessment has been regarded as one of the most important factors for determining sperm quality, and therefore, has been considered one of the best predictors of male fertility potential. Previous studies have shown a relationship between sperm quality and morphometric characteristics and biophysiological factors, such as ram sexual maturity [1,2]. Environmental factors, such as climatic temperature, humidity, and photoperiod, may alter the quality characteristics of the ejaculate in mammals, particularly in small ruminants and other seasonal ungulates located in temperate latitudes of Southern and Northern Hemispheres [3,4] which typically results in superior sperm motility, concentration scores, and lower semen pH in Autumn [5,6]. This phenomenon is due to complex hormonal interactions, ultimately based on day length, which differ between breeding and nonbreeding periods of the year [7].

* Corresponding author. Tel.: +34 976716471; Fax: +34 976713503.
E-mail address: jimarti@aragon.es (J.I. Martí).
* Alternate corresponding author. Tel.: +551935654067; fax: +551935654117.
E-mail address: herrerosgm@gmail.com (M. García-Herreros).

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The existence of well-defined sperm subpopulations within the mammalian ejaculate is now widely accepted by the scientific community [8]. The classical approach considering the whole ejaculate as a homogeneous population with a normal distribution to assess the sperm quality or the impact of environmental factors is nowadays considered erroneous [9]. The development of computer-assisted sperm analysis systems has facilitated the collection of detailed and objective information regarding morphometric sperm characteristics [8]. However, when these data are subjected to statistical interpretation there is a substantial loss of information as the real distribution of sperm morphometry does not "fit" the uniform normally distributed model applied in statistical programs [10] and could result in the grouping of heterogeneous semen samples into homogeneous subpopulations with similar morphometric characteristics. In fact, subpopulations may act as markers of ejaculate quality [11,12]. Thus, studies on seasonal sperm subpopulations could be interesting to reach a better definition for semen quality throughout the year. This could also have significant economic importance, as better semen analysis will lead to the improvement of sperm doses for AI.

Although there are some references regarding the influence of seasonal effect on different ram sperm quality factors [3,5], to the authors' knowledge, there is no report evaluating the influence of the seasonal effect on the dynamics of sperm morphometric subpopulations and its relationship with sperm quality parameters, despite considering sperm morphology as one of the best indicators of semen quality [13,14]. Thus, this study was designed to investigate the following aims: (1) to evaluate specific sperm quality parameters and sperm head morphometric characteristics during the breeding and nonbreeding season in ram ejaculates; (2) to determine and characterize the presence of ram sperm morphometric subpopulations in fresh semen during breeding and nonbreeding season; and finally (3) to analyze the relationship between sperm quality parameters and sperm morphometric subpopulations during breeding and nonbreeding season in rams used for AI programs.

2. Materials and methods

2.1. Chemicals and experiment location

All reagents used in this study, unless otherwise stated, were of analytical grade and purchased from Sigma-Aldrich Chemical Company (Alcobendas, Madrid, Spain). The experiment was carried out at the Centro de Investigación y Tecnología Agroalimentaria de Aragón (C.I.T.A.) experimental farm in Zaragoza, Spain (41° 39′ N/0° 53′ W/41.65, −0.883 333).

2.2. Animals and semen collection procedure

The experiment was carried out using 45 ejaculates collected from 5 healthy and reproductively mature fertile Rasa Aragonesa rams (aged >36 months, 70–90 kg of body weight and genetically heterogeneous). Two ejaculates were collected and pooled from the same animal per day to obtain homogeneous samples during breeding season (September through December), early nonbreeding season (January through May) and finally during late nonbreeding season (June through August). These ejaculates (three ejaculates per ram per season in duplicate; total six ejaculates per ram per season) were collected on a regular basis (on different and nonconsecutive weeks during the corresponding midseason) using a prewarmed artificial vagina. All rams were maintained under uniform nutritional conditions and housed under the same environmental conditions (outdoor access) in isolated enclosures to avoid herd conditioning effects on semen quality (e.g., ewe effect).

2.3. Sperm quality analyses: concentration, motility, and membrane integrity

After collection, ejaculates were kept at 37 °C in a water bath and the sperm quality of each sample was evaluated (volume, sperm concentration, motility, membrane integrity, and sperm abnormalities) using phase-contrast microscopy. Sperm concentration in each ejaculate was determined using a Neubauer hemacytometer (Marienfeld, Lauda-Königshofen) after a 1:2000 dilution in 1% formaldehyde solution in phosphate-buffered saline (PBS). Progressive individual motility scores were assessed under a coverslip (18 × 18 mm) on a warm stage (37 °C) by phase-contrast microscopy (10× objective) examined under a Leitz Ortholux II microscope (Wetzlar, Germany) after a dilution (1:100) in a Tris-buffered diluent (300 mM Tris, 94.7 mM citric acid, 27.8 mM fructose, pH 7.4, maintained at 37 °C). Sperm membrane integrity was assessed by fluorescence microscopy by using a dual fluorescence staining with carboxyfluorescein diacetate (CFDA) and propidium iodide (PI) as described by Martí et al. [1]. Briefly, 100-μL aliquots of sperm suspensions (10×10^6 spermatozoa [spz]/mL) were combined with 1 μL of the following stock solutions: CFDA (10 μM working stock in DMSO), PI (2.4 mM), and formaldehyde (1% solution in PBS). Spermatozoa were then examined under a Nikon Labophot-2 fluo-