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Morphometrically-distinct sperm subpopulations defined by a multistep statistical procedure in ram ejaculates: Intra- and interindividual variation

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Abstract

The existence of sperm subpopulations within the mammalian ejaculate has now been widely recognized. However, to the best of our knowledge, no data exist regarding the existence of sperm morphometric subpopulations within the ovine ejaculate. Computer assisted sperm morphometry analysis (ASMA) data and clustering methods were used in this study to identify sperm-head subpopulations in ram semen. Two experiments were carried out. In Experiment 1, ejaculates from 226 mature rams of the Manchega breed belonging to 36 different herds were used. A minimum of 100 sperm heads were analyzed from each male and eight morphometric characteristics for each individual sperm were recorded. Subpopulation analysis was performed in sequential steps: variable group analysis and correlation analysis to select which morphometric characteristics to use in cluster analyses; nonhierarchical clustering analysis using sperm head length and p2a (also known as roundness) shape factor as initial classificatory variables; and hierarchical clustering analysis to obtain the final number of clusters. The clustering analyses, based on 26 306 individual cells, revealed the existence of four sperm subpopulations (SP1, SP2, SP3 and SP4) with different morphometric characteristics. Significant differences in the proportion of spermatozoa in the SP1 and SP3 were found between rams belonging to different herds. In Experiment 2, the intra- and intermale variability on the distribution of sperm subpopulations was assessed. Three ejaculates from each of 21 rams were collected and the same multistep clustering analysis was performed. For all subpopulations defined, the intermale variability resulted in high values, being the intramale variability much lower. This fact would allow the use of sperm head morphometry to characterize a male and might provide valuable information to asses its fertility. In conclusion, our results show that using computer assisted sperm morphometry analysis and multivariate cluster analyses, four sperm subpopulations with different head phenotype were identified in ram ejaculates.

Keywords: Sperm morphometry; ASMA; Multistep cluster; Sperm subpopulations; Ram

1. Introduction

The classical approach, considering the whole ejaculate as a homogenous population with a normal statistical distribution, and the use of mean values to

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classify the ejaculates, or to assess the effect of a treatment or a biotechnological procedure is considered incomplete [1–3]. Nowadays, the existence of sperm subpopulations in the mammalian ejaculate is widely recognized [4]. There is evidence that the heterogeneity among these subpopulations may have functional significance [5–7]. Although the origin of these subpopulations and their physiological role are not yet clear [8], it has been found that the ability to undergo capacitation and fertility may vary depending on the subpopulation under consideration [5–9]. Thus, identification of subpopulations has been carried out based on different sperm characteristics, such as biochemical parameters [10–12], functional tests [13–15] or sperm motility descriptors [16–18]. Different statistical procedures to identify and characterize sperm subpopulations have been used [19]. Some of them have been focused on a comparison of different methods to obtain subpopulations, while others have used simple methods providing useful information about the subpopulations identified. These studies have also explored relations between subpopulations identified and sperm quality [20], fertility [21], or their ability to survive cryopreservation [3,22].

Improvements in computer assisted sperm morphometry analysis (ASMA) have also enabled individual sperm to be distinguished in morphometry analysis and subpopulations characterized by morphometric parameters to be identified by multivariate statistical analyses [23–25]. Thus, the existence of sperm-head morphometric subpopulations has been identified in different mammalian species [8,23,25–27]. To our knowledge, little attention has been paid to the study of sperm-head morphometric subpopulations in sheep using ASMA. To date, there has been only one study describing the use of ASMA in the ram [28], and no information is available about the sperm subpopulation structure in fresh ram ejaculates. Previous work [28] has characterized morphometrically the frozen-thawed spermatozoa of this species. Furthermore, this study used a rather small number of animals (i.e., n = 8).

Manchega sheep is an autochthonous dairy breed from Spain, which includes a white and a black variety. The white Manchega sheep variety is one of the most important Spanish dairy breeds, widely distributed in the central area of Spain. In this breed, males have not yet been genetically selected for fertility. The average fertility of Manchega rams following artificial insemination at an induced estrus is about 42%, ranging from 8 to 90% [29]. Therefore, males selected for traits, such as milk production are expected to exhibit considerable diversity in sperm characteristics and in fertility, thus being an excellent model to study the sperm morphometric subpopulation distribution in this species.

Some studies have pointed out the existence of this variability between males according to different sperm characteristics [6,30]. However no study has yet deal the intramale variability in the distribution of the sperm subpopulations. The study of sperm subpopulations distribution could be of utmost biological importance to improve our knowledge about sperm physiology and cryobiology.

Considering this background, the purpose of the present work was mainly to develop a simple multistep statistical procedure to identify, in a large-scale study, sperm-head morphometric subpopulations in an ejaculate based on data gathered with ASMA (Experiment 1), as a basis for future analyses of the relationships between sperm quality, freezability and male fertility. A further aim was to explore if the differences between male variability was affected by the herds of origin of males. Furthermore, we studied the degree of variation in the sperm subpopulations distribution between and within individual sires (21 rams; Experiment 2).

2. Materials and methods

2.1. Animals and reagents

Computer-assisted sperm head morphometry analysis was performed on fresh ejaculates from 226 rams of Manchega sheep breed belonging to 36 herds. Animals were manipulated in accordance with the Spanish Animal Protection Regulation, RD1201/2005, which conforms to European Union Regulation 2010/63. Adult rams were maintained at the Regional Centre of Animal Selection and Reproduction (CERSYRA) located in Valdepeñas, Ciudad Real, (Spain). Ram calves were purchased based on their expected genetic value for milk production. At approximately 3 to 4 mo of age, these rams were transferred from 36 different herds to the AI Centre (CERSYRA), where, after quarantine and training periods of 4 mo, semen was collected. Thus, all males were maintained under same environmental conditions since they were 3 to 4 months old.

All chemicals were of reagent grade and were purchased from Sigma or Merck (both of Madrid, Spain).

2.2. Semen collection

All semen samples were collected with an artificial vagina. The males were all regularly collected (twice a week) in the weeks preceding this study. Semen vol-