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Sperm morphometric subpopulations are differentially distributed in rams with different maturity age in cryopreserved ejaculates

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Abstract

It is widely accepted that sperm morphology is a good indicator of fertility and it has been proposed that sperm quality may be related to subtle changes in sperm head morphology. However, a precise estimation of the morphology of ram sperm would be very useful to improve reproductive success in ovine. Computer-assisted morphometric analysis and clustering analysis have been important tools to study sperm subpopulations in domestic animals. However, to the best of our knowledge, no data exist studing morphometric differences regarding to sperm subpopulations within the ovine ejaculate. The aim of this study was to test the presence and distribution of sperm morphometric subpopulations in cryopreserved ejaculates from yearling and mature rams using an objective method by computer analysis system and to establish the relationship between the distribution of the subpopulations found and sperm quality in each individual ram. Principal component analysis revealed that three principal components for yearlings and four components for mature rams that represented more than 84% of the cumulative variance in both cases. After cluster analysis, three sperm morphometric subpopulations for yearlings (CLY) and four for mature (CLM) rams were identified with defined sperm dimensions and shapes. CLY1 included big, round and short sperm (37%), CLY2 included average size and slightly elliptical and elongated sperm (48%), CLY3 included small, long, elliptical and elongated sperm cells (15%). CLM1 consisted of average size and moderate elliptical and elongated (26%), CLM2 consisted of small, long, elliptical and elongated (31%), CLM3 consisted of small and round (32%) and CLM4 included big, short and round (8%) spermatozoa respectively. There were significant differences in the distribution of the three subpopulations ($P < 0.001$) as well as in the sperm concentration, total motility (%), sperm viability (%) and the overall ($P < 0.05$) in the ejaculates among the four yearling rams tested. Same results were found for the four subpopulations and the different sperm quality parameters in the ejaculates among the four mature rams tested. In conclusion, cryopreserved ram semen showed a specific structure with regard to sperm morphometric subpopulations. In addition, the distribution of these subpopulations seems to be related to stud maturity age and the ejaculate quality which would be a very important indicator of sperm function. Thus, analysis of sperm morphometric subpopulation structure together with functional tests could provide valuable information to assess the cryoresistence of ram spermatozoa.

Keywords: Sperm subpopulations; Sexual maturity; Morphometry; Ram; Cryopreservation

1. Introduction

The use of cryopreserved sperm for artificial insemination (AI) in the sheep industry has raised the interest in improving the quantitative analysis of ram sperm samples, in order to estimate the fertility potential of studs. Selection for precocious sexual maturation in

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rams for AI programs, can decrease production costs, reduce generation interval accelerating benefits, and increase genetic gains and overall productivity. However, the age of the ram at semen collection affects the ejaculate concentration, viability, motility and morphometry [1]. In general, the literature shows that all of these ejaculate quality parameters increase as ram age [2,3]. In fact, the flock fertility can be affected by ram age [4]. However, there is not a single test included in the ram sperm semen quality analysis that fulfills an appropriate stud selection, and only some functional tests, can be partially used as single and weak predictors. The existence of well-defined sperm subpopulations within mammalian ejaculates is now widely accepted by the scientific community [5,6]. The identification of sperm subpopulations by using computer sperm analysis has become an issue of utmost interest and has allowed determining the existence of a structure of separate subpopulations in a whole ejaculate. The classical approach considering the whole ejaculate as a homogeneous population with a normal distribution to assess the sperm quality or bio-physiological factors is now considered erroneous [7]. The development of computer-assisted sperm analysis systems has allowed to obtain interesting and objective information of morphometric characteristics of a semen sample [8]. However, the high number of sperm parameters, the lack of information regarding the biological meaning, and thus the practical importance of each, make using the obtained information difficult. Most of these studies have used kinematics properties to disclose these subpopulations [9,10], however, very few studies used other sperm parameters such as sperm morphology [11,12], although sperm morphology is considered as one of the better indicators of quality [13]. The origin of these subpopulations is not yet clear, but it has been hypothesized that their origin maybe due to differences in the assembly of individual spermatozoa during spermatogenesis as well to differential maturational status and age through mixing in the epididymis [9]. The presence of subpopulations of spermatozoa with specific morphometric characteristics and the consequences of cryopreservation process on its structure have been reported in several domestic species such as the pig and bull [12,14]. These works gave relevant new information on the biological characteristics of the mammalian ejaculate. The existence of separate morphometric subpopulations in an ejaculate open up new ways to improve semen analysis techniques, since this structure has to be taken into account when applying morphometric analysis as a functional test of ram semen quality, avoiding to consider that sperm morphology is distributed in a ram semen sample in a uniform, normal distribution. However, there is a substantial loss of information when statistical procedures are applied to the results, since the real distribution of the sperm morphometry sperm is not uniform and normal, but rather structured in separate subpopulations [15]. These analyses could allow classifying heterogeneous semen samples into homogeneous subpopulations, grouping spermatozoa with similar morphometric characteristics. In fact, subpopulations may act as markers for good or bad sample quality [5], and some authors have found relationships between the presence of determined subpopulations and sample fertility [16,17]. Although sperm head morphometry can be considered a good indicator of semen quality in domestic species, the investigation of morphometric sperm subpopulations in cryopreserved ram sperm has received little attention, there being no studies dealing with this issue. Thus, studies about cryopreserved sperm subpopulations could be interesting to reach a better definition of semen quality. This could also have significant economic importance, as better semen analysis will lead to the improvement of sperm doses for AI. Applying the computer sperm analysis and multivariate cluster analyses, it has been possible to determine that discrete subpopulations of spermatozoa with different morphometric characteristics coexist in mammalian ejaculates [12].

Thus, this study was designed to investigate the following related aims; (i) the evaluation of the presence of separate sperm subpopulations, with specific morphometric characteristics, in cryopreserved ejaculates by the clustering of the morphologic parameters to define the ram sperm quality, (ii) to analyse the relationship among sperm subpopulations in cryopreserved semen and the traditional parameters used for semen quality assessment of cryopreserved ejaculates and finally (iii) to determine the influence of ram sexual maturity and sperm quality on the relative frequency distribution of spermatozoa within the different morphometric subpopulations in rams used for AI programs.

2. Material and methods

2.1. Reagents and media suppliers

All chemicals 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 CITA (Centro de Investigación y Tecnología Agroali-