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Seminal plasma proteins modify the distribution of sperm subpopulations in cryopreserved semen of rams with lesser fertility

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ABSTRACT

Any physiological mechanism involved in sperm selection and semen improvement has effects on heterogeneous sperm populations. This is mainly due to the fact that sperm populations within a single ejaculate have considerable heterogeneity for many variables, such as motility which is meaningful in terms of understanding how some sperm cells possess fertility advantages as compared with other cells. In the present research, initially there was a multivariate and clustering analysis used to assess sperm motility data from cryopreserved ram semen to identify subpopulations and compare the distribution of these clusters between rams with lesser and greater fertility. There were four classifications made of sperm subpopulations (clusters): CL1 fast/linear/progressive sperm; CL2 fast/non-linear sperm; CL3 very fast/linear sperm with vigorous beating and CL4 slow/non-linear sperm. Rams with greater fertility had a lesser proportion of sperm considered as "hyperactivated" (CL2) and a greater proportion of slow and non-linear sperm (CL4) than sperm of rams with lesser fertility. In addition, the effects were assessed for the capacity of seminal plasma (SP) and interacting SP proteins (iSPP) that were present during different seasons of the year to improve the distribution of sperm within subpopulations of semen from rams with lesser fertility. The iSPP and SP were obtained by artificial vagina (AV) and electroejaculation (EE) during breeding and non-breeding seasons and added to thawed semen. All the aggregates had a significant effect on the distribution of sperm subpopulations and effects differed among seasons of the year and depending on collection method used. Even though, future studies are needed to assess the contribution of each subpopulation on ram sperm fertility, it is important that a multivariate analysis be used to evaluate the effect of a treatment on sperm quality variables.

1. Introduction

Ram sperm cryopreservation induces both a decrease in overall sperm motility and alterations in sperm motility patterns (Watson,

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2000; Batellier et al., 2001; Medeiros et al., 2002). This is one of the causes underlying the poor fertilising potential of frozen semen when placed in the female reproductive tract by artificial insemination. Seminal plasma (SP) has been evaluated for improving the quality of frozen-thawed semen, however, its effects are inconsistent (Baas et al., 1983; Graham, 1994; Bernardini et al., 2011). Inconsistencies among studies regarding the effects of SP might be related to the variability of its composition due to males from which collections occur, and/or ejaculate variability within the same male (Muiño-Blanco et al., 2008), season of year when ejaculates were collected (Domínguez et al., 2008) and collection method used to procure the semen (Ledesma et al., 2014). In an attempt to address these inconsistencies of findings among studies, a methodology was developed to obtain the fraction of SP proteins that can bind to the sperm membrane, termed interacting SP proteins (iSPP; Bernardini et al., 2011). The addition of iSPP after thawing can improve the quality of frozen semen by reducing some undesirable effects of cryopreservation, such as premature capacitation (Ledesma et al., 2016).

The development of CASA systems (Computer-Assisted Sperm Analysis) have improved the ability to analyse sperm motility, allowing for assessment of individual sperm cells, thus, allowing for the identification and quantification of different sperm subpopulations with specific patterns of movement. In the last 20 years, multivariate statistics have been applied to identify sperm subpopulations, making it possible to determine how specific treatments affect these subpopulations or to evaluate male differences is sperm subpopulations. Different sperm subpopulations have been identified, therefore, in mammalian ejaculates on the basis of the motility characteristics of individual sperm cells (Quintero-Moreno et al., 2003; Martínez-Pastor et al., 2005; Martínez-Pastor et al., 2011).

The purpose of the present study, therefore, was to use the multivariate cluster analysis as a technique to identify sperm subpopulations in frozen ram semen based on its motility characteristics to: a) compare the relative sizes of the different sperm populations in rams with greater and lesser fertility and b) evaluate the ability of SP and iSPP collected by different methods and in different seasons of the year, to modify the relative sizes of these sperm populations from rams with lesser fertility.

2. Materials and methods

2.1. Frozen semen of greater and lesser fertility rams

All experiments were performed using frozen sperm from 10 Assaf rams divided in two groups of five rams each, according to their fertility (lesser, 35.3% ± 3.0; greater, 60.4% ± 1.5). Fertility was estimated after routine assessments of the semen doses in the field (obtained by artificial vagina and utilized with standard AI procedures), corrected for the effects of farm, date and inseminator. Frozen semen was supplied by OVIGEN (Centro de Selección y Mejora Genética de Ovino y Caprino de Castilla y León, Toro, Spain).

2.2. Seminal plasma and interacting seminal plasma proteins

Semen samples from another eight Assaf rams were collected either by artificial vagina (AV) or electroejaculation (EE) and were used in this study to obtain seminal plasma (SP) and interacting seminal plasma proteins (iSPP). Collections were conducted during the autumn (breeding season) and spring (non-breeding season). Ejaculates were pooled by collection method and then divided in two aliquots. One aliquot was used to obtain SP and the other was used to obtain the iSPP (Ledesma et al., 2016).

2.3. Experimental design to evaluate the effect of iSSP and SP of sperm of rams with lesser fertility

Four independent replicates were conducted with frozen sperm from rams with lesser fertility, dividing the samples so as to apply five experimental treatments, adding SP or iSPP obtained by EE or AV collected during breeding or non-breeding season (plus a control). The experimental design was similar to that in a previous study of Ledesma et al. (2016). Briefly, three straws of semen were thawed, layered over Androcoll-O™ and centrifuged to remove dead cells and SP. The use of colloids is very effective to remove SP and allows for obtaining a population of viable sperm (Martínez-Alborcia et al., 2013; Anel-López et al., 2015). The resulting sperm pellet was re-suspended in PBS and an aliquot of sperm suspension (10×10^6 sperm) was added to tubes with five different media and incubated at 37 °C for 1 h. After this period, samples were analysed by CASA. The incubation media were supplemented with 40 µl of SP or iSSP: 1) SP collected by AV (corresponding to 0.6 mg protein for spring SP or 1.4 mg for autumn SP) in PBS supplemented with 0.5% fructose (PBSF); 2) SP collected by EE (corresponding to 0.48 mg protein for spring SP or 1.2 mgfor autumn SP) in PBSF; 3) iSPP collected by AV (corresponding to 7 µg for spring collection or 23 µg for autumn collection) in PBSF; 4) iSPP collected by EE (corresponding to 3.2 µg for spring collection or 29 µg for autumn collection) in PBSF; and 5) Control: PBSF without supplementation.

The amount of iSPP was selected on the basis of the proportional amount of proteins provided by an equivalent volume of complete SP. The concentration of proteins in SP was 35.0 mg/ml for AV and 30.0 mg/ml for EE for the autumn sample and 15.0 mg/ml for AV and 12.0 mg/ml for EE in the spring sample. The concentration of proteins in iSPP in the autumn was 7.8 mg/ml for samples collected with an AV and 5.8 mg/ml for samples collected by EE, and in spring it was 0.9 mg/ml for samples collected with an AV and 0.8 mg/ml for samples collected with use of an EE (Ledesma et al., 2016).