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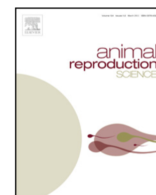

Predictive capacity of sperm quality parameters and sperm subpopulations on field fertility after artificial insemination in sheep

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ABSTRACT

This study was designed to evaluate the relevance of several sperm quality parameters and sperm population structure on the reproductive performance after cervical artificial insemination (AI) in sheep. One hundred and thirty-nine ejaculates from 56 adult rams were collected using an artificial vagina, processed for sperm quality assessment and used to perform 1319 AI. Analyses of sperm motility by computer-assisted sperm analysis (CASA), sperm nuclear morphometry by computer-assisted sperm morphometry analysis (CASMA), membrane integrity by acridine orange-propidium iodide combination and sperm DNA fragmentation using the sperm chromatin dispersion test (SCD) were performed. Clustering procedures using the sperm kinematic and morphometric data resulted in the classification of spermatozoa into three kinematic and three morphometric sperm subpopulations. Logistic regression procedures were used, including fertility at AI as the dependent variable (measured by lambing, 0 or 1) and farm, year, month of AI, female parity, female lambing-treatment interval, ram, AI technician and sperm quality parameters (including sperm subpopulations) as independent factors. Sperm quality variables remaining in the logistic regression model were viability and VCL. Fertility increased for each one-unit increase in viability (by a factor of 1.01) and in VCL (by a factor of 1.02). Multiple linear regression analyses were also performed to analyze the factors possibly influencing ejaculate fertility ($N = 139$). The analysis yielded a significant ($P < 0.05$) relationship between sperm viability and ejaculate fertility. The discriminant ability of the different semen variables to predict field fertility was analyzed using receiver operating characteristic (ROC) curve analysis. Sperm viability and VCL showed significant, albeit limited, predictive capacity on field fertility (0.57 and 0.54 Area Under Curve, respectively). The distribution of spermatozoa in the different subpopulations was not related to fertility.

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1. Introduction

There is no single laboratory test able to accurately predict the true fertilizing potential of a semen sample, although the combination of different parameters can improve predictive accuracy (Rodríguez-Martínez, 2003; Oliveira et al., 2013). The incomplete information provided by the analyses lies behind this restricted ability to achieve accurate predictions. In the ovine, different studies have found relationships between in vivo fertility and in vitro measures of sperm quality using fresh (Hulet and Ercanbrack, 1962; Hulet et al., 1965; Suttiyotin et al., 1992; Pérez-Pé et al., 2002; Vicente-Fiel et al., 2014) and frozen-thawed semen (Choudhry et al., 1995). However, other studies have described that the relationship between the fertility and several sperm quality parameters is not consistent (Sanchez-Partida et al., 1999; O'Meara et al., 2008). Furthermore, although the presence of sperm subpopulations in the ejaculate is widely acknowledged, the majority of studies have addressed the relationship between sperm traits and male fertility based on average values of sperm parameters (Yániz et al., 2015a).

The distribution of spermatozoa in the different subpopulations may have functional relevance. It has been proposed that the level of heterogeneity is meaningful in terms of understanding how spermatozoa from some individuals possess fertility advantages over spermatozoa from their rivals in sperm competition (Holt and Van Look, 2004). Theoretically, a greater heterogeneity of spermatozoa would ensure greater potential to fertilize an oocyte at some unpredictable interval after ejaculation (Curry, 2000). Another possibility is that specific subpopulations may have higher potential success in the fertilization process. Relations between ejaculate heterogeneity and fertility have been found (de Paz et al., 2011; Ramón et al., 2013; Yániz et al., 2015a). However, males in these studies were classified as high and low fertility based on retrospective data, and differences in sperm subpopulations were checked subsequently. To our knowledge, there are no studies evaluating the predictive capacity of the sperm kinematic and morphometric population structure on the potential field fertility of a given semen sample used for artificial insemination (AI).

In two previous studies, we described differences in several sperm quality parameters, and sperm population structure between rams of high and low field fertility (Vicente-Fiel et al., 2014; Yániz et al., 2015a). Using logistic regression procedures and epidemiological approaches, this study was designed to assess the relevance of several sperm quality parameters and sperm population structure on the reproductive success after cervical AI with cooled semen in sheep.

2. Materials and methods

2.1. Reagents

Unless otherwise stated, all chemicals used were obtained from Sigma–Aldrich Chemical Company (Alcobendas, Madrid, Spain), and the diluents were prepared using Milli-Q water (Millipore Ibérica S.A., Barcelona, Spain).

2.2. Animals

All animal procedures were performed in accordance with Spanish Animal Protection Regulation RD223/1988, which conforms to European Union Regulation 86/609. The animals belonged to the Rasa Aragonesa breed selection scheme followed by UPRA-Grupo Pastores. Fifty-six proven rams, age ranging from 2 to 7 years, kept at an insemination centre (ATPSYRA, Movera, Spain) and fed a standard diet with water ad libitum were used in this study. Primiparous ($n = 332$) or multiparous ($n = 987$), 2–8 years old, dry ewes were included in the AI programme. Only healthy ewes with a body condition score between 3 and 4 in a 5-point scale (Russel et al., 1969) at the beginning of hormonal synchronization treatment were included in the study.

2.3. Semen processing

Ejaculates were collected individually using sterilized artificial vaginas and glass tubes (IMV L'Aigle. France). Semen was analyzed by technicians from the centre on the same day as programmed AI. Minimum seminal characteristics were established to process the ejaculates: volume $\geq$0.4 ml, concentration $\geq 2 \times 10^9$ spermatozoa per ml (spectrophotometry), mass motility $\geq$4 (in a scale 0–5, manual analysis, $\times$100 magnifications) using an Olympus BX40 (Olympus, Optical Co., Ltd, Japan) microscope. Ejaculates were diluted to 1.6×10^9 spermatozoa/ml in a milk-based extender (ultra-heat treated milk, 0.7% fat, plus 2000 IU/ml penicillin and 0.4 mg/ml streptomycin, Yániz et al., 2005), packaged in 0.25 ml straws (IMV L'Aigle. France), and stored at 15 °C until AI or sperm quality re-evaluation. One straw per ejaculate were transported at 15 °C to the TECNOGAM Research Laboratory at the University of Zaragoza (Huesca, Spain) in less than 2 h, and immediately processed for sperm quality assessment. The remaining semen straws were used to inseminate the ewes, as detailed below.

2.4. Sperm quality assessment

2.4.1. Sperm motility and concentration determination by computer-assisted sperm analysis (CASA)

Sperm motility and concentration were measured after placing a diluted semen sample in a pre-warmed Makler (Sefi-Medical Instruments, Israel) chamber or in a Burker chamber in duplicate, respectively (Palacín et al., 2013). For this purpose, a computer-assisted sperm analyser (CASA) (ISAS®, Version 1.0; PROISER, Paterna, Spain) and an Olympus BX40 (Olympus, Optical Co., Ltd, Japan) microscope equipped with a negative phase-contrast 10x objective and heated stage set at 37 °C were used. Semen samples were carefully mixed, and sample aliquots were diluted to 50×10^6 sperm/ml using INRA96® for sperm motility assessment (Vicente-Fiel et al., 2014).

The motility variables measured included the sperm cell motility percentage (MS, %), progressive motility percentage (PS, %, VAP $\geq$25 μm/s) curvilinear velocity (VCL, μm/s), straight line velocity (VSL, μm/s), average path velocity

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