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Sperm survival and heterogeneity are correlated with fertility after intrauterine insemination in superovulated ewes

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Abstract

Efficient animal production involves accurate estimations of fertilizing ability. One key factor is the plasma membrane of the sperm cell, which is actively involved in the cascade of events before oocyte fusion. Many methods are used to analyze the characteristics of this membrane, including partition in aqueous two-phase systems which is an efficient method to analyze sperm surface changes accounting for loss of viability and different functional states. Centrifugal countercurrent distribution (CCCD) analysis can also be used in an aqueous two-phase system to determine the relationship between sperm parameters and in vivo fertility in ewes. In a previous work, we found a significant correlation between two post-CCCD parameters (heterogeneity and recovered viability) and field fertility when the same sample was used after cervical AI. The present study was intended to find out whether the control of several external factors that affect reproductive efficiency is able to increase the correlation coefficient between post-CCCD parameters and fertility. Thus, 90 *Rasa aragonesa* ewes were controlled on the same farm and received intrauterine inseminations using the same technical equipment. The fertilizing ability of the raw semen and sperm samples selected by a dextran/swim-up process was compared using a low number of spermatozoa per insemination (7×10^7) to enhance possible fertility differences. A new post-CCCD parameter was considered; the loss of viability (LV) occurred during the CCCD process. This variable denotes the sperm surviving ability and corresponds to the difference between the total number of viable cells loaded and recovered after the CCCD run. The mean fertility of eight sperm control samples was 60% (range: 25–76%), and there was no significant correlation between standard parameters and in vivo fertility. LV ranged from 2 to 69% (average 27%) and was negatively correlated with fertility ($r = -0.914$,

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$P < 0.01$). Ejaculate heterogeneity (H) ranged from 20 to 47% and was positively, but not significantly, correlated with fertility ($r = 0.391$). A predictive equation for fertility was deduced by multiple analysis with a very high correlation coefficient ($r = 0.967$), and level of significance ($P < 0.005$): predictive fertility PF = 52.546 − 0.594 LV + 0.665 H. The mean fertility of 13 swim-up selected samples was 63% (range: 25–86%). Again, only parameters derived from the CCCD analysis were highly correlated with fertility, especially LV and H ($P < 0.05$).

Keywords: Ram spermatozoa; Intrauterine insemination; Fertility

1. Introduction

One of the main problems in artificial insemination is to develop efficient methods to accurately estimate the fertilizing ability of sires. Several techniques have been developed over the years to correlate different aspects of semen quality with field fertility. They can be divided into basic semen parameter analyses and sperm function assays. The former include assessment of sperm motility [1–3], morphology [4–6], and membrane integrity [7–9]. Other less commonly used techniques involve detecting defective sperm organelles and DNA [10,11] and the analysis of seminal plasma [12,13]. Most parameters are poorly correlated with in vivo fertility when taken individually but results have improved using computer-assisted techniques or new imaging technology [14–17]. Recently, several attempts have been made to analyze how well these simple tests predict fertility [18–21].

Other assays have been developed to evaluate sperm function, such as the sperm penetration assay [22,23], the zona binding assay [24–26] or the hemi-zona assay [27,28], in addition to analyses of the acrosome reaction or capacitation status [29–31]. Although these techniques are still quite time-consuming and technically demanding, they have been used to predict in vivo fertility in several species [32,33].

Despite rapidly developing molecular, genomic and computer techniques, our understanding of fertility is still far from complete. Since fertilization requires several sperm functions, it seems reasonable to use a combination of assays to help predict fertilizing ability more precisely, as suggested by Amann and Hammerstedt [34]. These combinations could include standard semen parameters or others based on sperm function.

The plasma membrane of the sperm cell has several specializations which play unique roles in the cascade of events before fusion with the oocyte. Many methods have been developed to analyze the characteristics of this membrane, including aqueous two-phase systems, which are based on the affinity of the cell surface for immiscible aqueous solutions of polymers, such as dextran (hydrophilic) and polyethylene glycol (PEG, hydrophobic) [35,36]. The extent of partition between the cells in the interface and the PEG-rich upper phase depends on the cell surface properties.

The selectivity and separation resolution can be greatly improved by multistep partitions. Countercurrent distribution (CCD) is a chromatographic process with a stationary (lower) phase and a mobile (upper) phase. The cell sample is partitioned in one system, and the two phases are systematically brought into contact with opposite fresh phases. The loss of viability as a result of dilution [37] and washing during the separation process can be