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Evaluation of ram semen quality using polyacrylamide gel instead of cervical mucus in the sperm penetration test

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Abstract

Fertility is a very complex biological function that depends on several properties of the spermatozoa, including sperm motility. Two objectives are analyzed in this study: (1) Replace the cervical mucus by a synthetic medium in a sperm penetration test, and (2) evaluating the results of this test objectively analyzing the sperm number that migrates. In experiment 1, we have tested eight concentrations of acrylamide (1%–2%). Rheological properties of media were analyzed. The plastic straws, loaded with acrylamide, were placed vertically on the semen sample tube for 15 min at 39 °C. After, the acrylamides were placed, by segments of 5 mm, into wells of a 24-well plate, dyed with Hoechst 33342 and the number of spermatozoa were calculated by automated microscopy analysis. The 1.55% and 1.6% acrylamide gel showed a number of spermatozoa emigrating closer to that seen with natural mucus. In experiment 2, we applied the sperm penetration in acrylamide 1.6% and 1.55% using fresh semen and cooled semen at 15 °C and 5 °C. The spermatozoa counts were performed for each segment of 10 mm. Semen chilled at 15 °C presented intermediate values of sperm counts in comparison with fresh semen (higher) and 5 °C chilled semen. The sperm counts do not differ between acrylamides but the rheological properties of acrylamide 1.6% were more similar to those of the natural cervical mucus. In experiment 3, we have observed significant correlations between the number of spermatozoa and several sperm quality parameters (positive: progressive motility and velocity according to the straight path; negative: damaged acrosomes and apoptotic cells) in 1.6% acrylamide media. We conclude that the size of the cell subpopulation, objectively calculated, that migrate beyond 20 mm in 0.5-mL straws filled with acrylamide is a useful parameter in ram sperm quality assessment and further studies are needed to evaluate its relationship with field fertility.

Keywords: Ram; Sperm quality; Penetration test; Motility; Mucus

1. Introduction

The passage of sperm through the female reproductive tract is regulated to maximize the chance of fertilization and ensure that sperm with normal morphology and vigorous motility will be the ones to succeed [1]. Cervical mucus filters out sperm with poor morphology and motility and as such only a minority of ejaculated sperm actually enter the cervix [1]. Thus, mucus is considered a means of sperm selection in many species. Taking into account the effect of cervical mucus on sperm transport, the evaluation of the ability of spermatozoa to progress through natural mucus (cervical

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mucus penetration test [CMPT]) or mucus substitutes has been proposed as an analysis of sperm quality [2–9]. This penetration test has been applied in several animal species and is accepted by the World Health Organization [10] as a means of analyzing human semen.

Generally, the test is based on the visual assessment of the linear distance covered by the foremost sperm cell (vanguard spermatozoa) in the capillary tube. Another method using the number of spermatozoa accumulated in different segments of the capillary tube as a parameter of analysis. Visual sperm counts at certain distances (10, 20 mm, etc.) from the base of the tube in flat capillary tubes has been used for this kind of assessment [11,12]. Tas et al. [13] have developed a new CMPT technique in which transparent plastic straws are used instead of capillary tubes and the total number of spermatozoa penetrating to predetermined distances in cervical mucus are measured on slides. Ola et al. [7] reviewed the accuracy of in vitro sperm penetration into cervical mucus or substitutes in evaluating sperm motility in human semen, and they showed that vanguard distance as a diagnostic criterion has a low accuracy while sperm concentration is more accurate.

A number of diagnostic studies into the usefulness of the CMPT technique have been developed. Fertilizing capacity of spermatozoa has been shown to be strongly related to the parameters observed in the cervical mucus penetration test (human [14]; bull [13,15]). In other studies, the correlation between sperm migration capacity and fertility was not observed [2–4,16,17]. However, it is generally accepted that penetration of spermatozoa into cervical mucus in vitro provides important information predictive of sperm function [11].

The major problem with cervical mucus as a component of any test system is the difficulty encountered in standardizing the quality of this material. It is difficult to obtain large volumes of natural cervical mucus and the variation among lots of natural mucus is large, even between batches from the same female [18]. Thus, it is desirable to formulate a synthetic medium free of these problems, simple to prepare and with easily reproducible rheological properties. Acrylamide, methylcellulose, and hyaluronic acid have previously been used as a natural cervical mucus substitute for in vitro sperm penetration tests (human [11,12,19]; bull [20]; ram [21]).

In ram, few studies have been performed to analyze the relationship between the penetration test and sperm quality. A modified sperm penetration test was used by Suttiyotin et al. [22], noting that sperm penetration distance in Tris-glucose solution was correlated with a 48-day nonreturn rate and a 60-day conception rate. Robayo et al. [9] studied the relationship between sperm migration in ruminant cervical mucus (distance traveled by the vanguard spermatozoa) and motility patterns observed by computer assisted semen analysis (CASA). Continuous line velocity and average path velocity were the only kinematic parameters that presented significant positive correlations with the migration in sheep cervical mucus. O'Hara et al. [21] assessed the penetrating ability of fresh ram semen using flat capillary tubes and aiding visibility to cells with Hoechst 33342. These authors showed that the penetrating ability of fresh ram semen into artificial mucus was influenced by diluents and storage duration.

The aim of this study was to automate the quantitative analysis of the ram sperm population that migrates in a column of ovine cervical mucus or substitutes (acrylamide) into a plastic straw. We propose to evaluate the straw content by segments, placing each segment onto a slide or a plate, to stain spermatozoa with cell permeable nucleic acid stains and to analyze these samples automatically by a microscope to count the spermatozoa in each sample. This method is more objective than visually counting of the number of unstained spermatozoa in the straw and opens the possibility of assessing the physiological status of spermatozoa using other fluorescent probes.

2. Materials and methods

2.1. Experimental design

2.1.1. Experiment 1: assessment of the suitability of eight synthetic media for in vitro evaluation of sperm progression by a mucus penetration test

To formulate a synthetic medium as an ovine cervical mucus substitute, eight concentrations of acrylamide (1%, 1.5%, 1.55%, 1.6%, 1.65%, 1.7%, 1.85%, and 2%) were compared in the sperm penetration test performed at 39 °C for 30 min. A test with ovine cervical mucus was used as a control assay. The sperm count of the migration assay was measured for each 5 mm of plastic straw (12 segments in total). The tests were conducted over 4 wk (in February and March). Ejaculates from four rams were collected twice a week by artificial vagina, and pooled. Two concentrations of acrylamide, the sperm count of which is more similar to that observed for the natural mucus, were selected for experiment 2.