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The effect of seminal plasma on alpaca sperm function

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Abstract

In order to advance the development of assisted reproductive technologies in alpacas and other Camelids, the objective of this study was to explore the role of seminal plasma concentration on motility and functional integrity of alpaca sperm. Sixteen male alpacas > 3 y of age were used. In Experiment 1, epididymal sperm were incubated for 0 to 6 h in 0, 10, 25, 50, or 100% seminal plasma and motility was assessed. In Experiment 2, epididymal sperm were incubated in 0, 10, or 100% seminal plasma for 3 h and motility, acrosome integrity and DNA integrity were assessed. In Experiment 3, ejaculated sperm were incubated in 10, 25, 50, or 100% seminal plasma for 0 to 6 h and motility assessed. In Experiment 4, ejaculated sperm were incubated in 10 or 100% seminal plasma for 3 h and motility, acrosome integrity, DNA integrity, and viability were assessed. Epididymal and ejaculated sperm maintained motility longer when incubated in the presence of 10% seminal plasma compared to 0, 25, 50, or 100% seminal plasma ($P < 0.001$). The mean ± SEM percentage of epididymal sperm with intact acrosomes was less ($P < 0.001$) in samples incubated in 0% seminal plasma (39.4 ± 3.73) compared to 10% (75.3 ± 1.20) or 100% (77.4 ± 0.90) within 1 h after incubation. However, DNA integrity of ejaculated and epididymal sperm was not significantly affected by seminal plasma concentration. The mean viability of ejaculated sperm was reduced in the presence of 100 (12.7 ± 2.33) compared to 10% (36.2 ± 4.68) seminal plasma ($P < 0.001$) within 1 h of incubation. We concluded that alpaca semen should be diluted to a final concentration of 10% seminal plasma to prolong motility, preserve acrosome integrity, and maintain viability of sperm.

Keywords: Alpaca; Sperm; Seminal plasma; Motility; Acrosome; DNA integrity

1. Introduction

The development of semen cryopreservation protocols in camelids, particularly alpacas, is currently limited by the viscous nature of alpaca seminal plasma and a lack of understanding of its role in sperm function. In numerous species, seminal plasma is routinely diluted or removed during processing of semen for cryopreservation; this can have either positive or negative effects on sperm function and fertility [1]. Therefore, development of cryopreservation protocols in alpacas will be hindered until the role of seminal plasma in sperm function is determined.

It is not known whether dilution or removal of seminal plasma prior to freezing will inhibit or stimulate function of alpaca sperm. It is generally considered that excessive dilution of seminal plasma during processing is detrimental to sperm function [2], although the effect differs among species [1]. During cryopreservation of stallion semen, seminal plasma was detrimental to sperm function and therefore was either removed by centrifugation or diluted to less than 5% of the freezing medium prior to processing [3]. Conversely, in the boar [4] and ram [5], the presence of 20 or 10% seminal

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plasma, respectively, during cooling or incubation at 37–39 °C was beneficial to the function and integrity of sperm, maintaining both motility and viability.

Understanding the role of seminal plasma in the function of sperm in livestock species has advanced the development of assisted reproductive technologies, e.g., cryopreservation and AI. The addition of seminal plasma to sperm following cryopreservation increased post-thaw motility and fertility in the ram [6] and enhanced post-thaw sperm function in the boar [7]. Moreover, although detrimental during processing, the addition of seminal plasma to stallion sperm post-thaw increased fertility following AI [8]. In camelids, viscous seminal plasma prevents successful cryopreservation; therefore, research has focussed on the treatment of semen with non-specific enzymes [9,10] to reduce viscosity prior to processing. Whilst enzyme treatment of Camelid seminal plasma reduced viscosity, it also adversely affected sperm function and integrity [9], most likely due to non-selective digestion of seminal plasma proteins. Collagenase successfully reduced llama seminal plasma viscosity whilst maintaining sperm function [10]; although these sperm retained their fertilising ability [11], the effect of this enzyme on the success of cryopreservation is unknown. Conversely, in the alpaca, collagenase impaired sperm function [12] and therefore further research is necessary. In other studies, camelid semen was diluted with cryodiluents at rates ranging from 1:1 to 1:4 in an attempt to moderate the effects of seminal plasma [13–16]. In these studies, reasonable post-thaw motility was achieved, but fertility was low and not commercially viable after insemination of frozen-thawed sperm.

The objective of the current study was to explore the role of seminal plasma concentration on the motility and functional integrity of alpaca sperm. To determine whether alpaca seminal plasma is beneficial or detrimental to sperm, as well as the optimal dilution rate to maintain sperm function and integrity, the effect of various seminal plasma concentrations on motility, acrosome integrity, DNA integrity, and viability of epididymal and ejaculated alpaca sperm were studied.

2. Methods

2.1. Animals

This study was performed from February to May 2010 using 16 male alpacas under authorization from the University of Sydney animal ethics committee. All males were > 3 y, since at that age, 100% of alpacas loose their preputial adhesions, testes size reaches a maximum, and this is the recommended minimum age for breeding [17]. All males had a body condition score ≥ 3 (mean 3.4 ± 0.1), weighed > 70 kg (mean 80. ± 2.1), were routinely vaccinated and treated for parasites, were in sound health, had sound genitalia as determined by visual and manual assessment, and had testes more than 3 cm long.

2.2. Experimental design

This study was performed as four experiments. Experiments 1 and 2 determined the effect of seminal plasma on epididymal sperm function, whereas Experiments 3 and 4 determined the effect of seminal plasma on ejaculated sperm function.

In Experiments 1 and 2, epididymides and testes were collected following castration of 10 male alpacas throughout Australia. Six alpacas were used for Experiment 1 (age 43.8 ± 2.5 mo) and four were used for Experiment 2 (age 85.0 ± 24.1 mo). Following castration, epididymides and testes were wrapped in gauze soaked in 0.02 M PBS (Sigma-Aldrich, St Louis, MO, USA) then left to cool to room temperature for approximately 2 h prior to transportation to the laboratory overnight at 4 °C. Epididymal sperm were then harvested and washed as described below prior to incubation in seminal plasma collected from male alpacas as described below.

In Experiment 1, epididymal sperm were harvested and washed and their motility was assessed (time 0 h). Samples were then resuspended to 50×10^6 sperm/mL in 0, 10, 25, 50, or 100% seminal plasma (four replicates/epididymal harvest) containing 0.02 M PBS (Sigma), 0.1% BSA (Cohns fraction 5; Sigma), 7.5 mg/mL penicillin (Sigma-Aldrich, St Louis, MO, USA), and 5 mg/mL streptomycin (Sigma; herein referred to as PBS-BSA). Samples incubated in 100% seminal plasma contained 0.1% BSA, 7.5 mg/mL penicillin, and 5 mg/mL streptomycin, but no PBS. Following dilution, samples were incubated in a water bath at 37 °C and motility was assessed after 0.5, 1, 2, 3, 4, 5, and 6 h. To prevent separation of seminal plasma and PBS-BSA during incubation, incubation tubes were flicked at 15 min intervals and samples were pipetted before motility was assessed.

In Experiment 2, epididymal sperm were harvested and washed, and their motility, acrosome integrity and DNA integrity were assessed (time 0 h). Samples were then resuspended to 50×10^6 sperm/mL in 0, 10, or 100% seminal plasma (four replicates/harvest) containing PBS-BSA and incubated in a water bath at 37 °C. Motility and acrosome integrity of sperm were assessed after 1, 2, and 3 h,