Cryopreservation of alpaca epididymal sperm using commercial equine semen extenders: preliminary investigation

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Abstract

Cryopreservation of epididymal sperm enables saving genetic material in cases of unexpected death or castration. Objective of this preliminary study was to evaluate alpaca epididymal sperm viability and motility following cryopreservation using 3 commercially available equine semen extenders. We hypothesized that equine semen extenders yield postthaw results comparable to those reported for alpaca epididymal sperm cryopreservation. Epididymal sperm samples were collected from 4 adult intact male alpacas at castration. Sperm samples were extended at a 1:1 ratio with 3 commercial equine semen extenders. Extended semen was loaded into 0.5-ml straws and frozen in liquid nitrogen vapor. Straws were immediately thawed in a circulating 38°C water bath. Following thawing, viability and total and progressive motility were evaluated. Postthaw viability and motility was less than what has been reported for alpaca epididymal sperm cryopreservation. Additional research is needed to determine if poor results observed are related to the semen extender or the freezing/thawing method used.

Keywords: Camelid, castration, freezing, motility, straw, viability

Introduction

Cryopreservation of ejaculated sperm has been used for years to permit the dissemination of genetic material in many species.[1-5] In alpacas, most cryopreservation extenders used for ejaculated sperm contain tris, citrate, glycerol, and egg yolk.[3-7] However, various extender components (such as skim milk) have been tested in attempts to improve postthaw sperm motility.[4]

Cryopreservation of epididymal sperm facilitates saving genetic material in cases of unexpected death.[8] For male alpacas that are being castrated to facilitate animal management and fleece production, epididymal sperm can also be cryopreserved at castration.[9] In addition, collection of epididymal sperm avoids handling viscous seminal plasma that allows for a more refined cryopreservation method.[6,9] Similar to cryopreservation of ejaculated sperm, various extender components (such as lactose) have been investigated in alpaca epididymal sperm in attempts to improve postthaw motility.[3]

General practitioners who work with alpacas often also work with horses and have access to commercial equine semen freezing extenders. Objective of this preliminary study was to evaluate alpaca epididymal sperm viability and total and progressive sperm motility following cryopreservation using [3] commercially available equine semen extenders. We hypothesized that equine semen extenders yield postthaw results comparable to reported for alpaca epididymal sperm cryopreservation.

Materials and methods

Four adult intact male alpacas (mean ± SD: 7.5 ± 2.1 years) that were formerly herd sires and recently sired crias were used in this preliminary study. Males were routinely castrated under general anesthesia using intravenous tiletamine and zolazepam (Telazol®, Zoetis). Briefly, a scrotal incision was made over each testis and the fascia surrounding the testis was stripped. Next the spermatic cord was crushed using an emasculator such that a section of vas deferens could be obtained. The skin incisions were allowed to heal by second intention.

Immediately following castration, vas deferens and cauda epididymides were dissected free from each testis (Figure 1). For each side, the ductal lumen was slowly flushed with 0.5 ml of sterile saline (0.9%) in retrograde direction and the sperm-containing effluent was collected into an Eppendorf tube. Epididymal sperm sample was evenly aliquoted into 3 groups (Gent extender [GE], Lactose EDTA extender [LE], and Modified French equine extender [MF5]). Each group was extended at a 1:1 ratio with 1 of 3 commercially available equine semen extenders (Table).

Extended semen was loaded into labeled 0.5-ml cryopreservation straws, sealed, and frozen in liquid nitrogen vapor for 10 minutes before plunging into liquid nitrogen for storage. Semen was thawed at 38°C for 60 seconds and examined under phase microscopy at 400 x magnification to determine postthaw total and progressive motility. In addition, semen was stained with

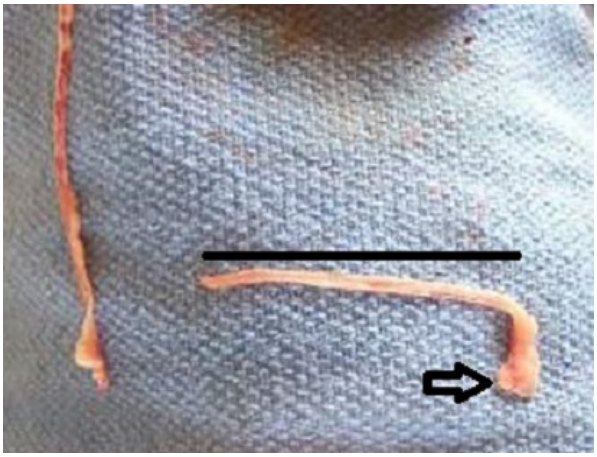

Figure 1. Vas deferens (below bar; bar = 2 cm) and cauda epididymides (arrow)

eosin and nigrosin to determine postthaw viability. All procedures were approved by an Institutional Animal Care and Use Committee [protocol #3664].

Mean ± SD postthaw viability, total motility, and progressive motility were determined for each extender and compared among extender groups using a one-way analysis of variance (GraphPad Prism 9, San Diego, CA). Significance was defined as $p < 0.05$.

Results

Dissecting and catheterizing vas deferens and cauda epididymides for flushing sperm cells was not technically difficult. Three of the 4 alpacas had viable sperm after freezing and thawing. There were no differences in viability, total motility, or progressive motility among extender groups ($p = 0.3873$, $p = 0.1573$, and $p = 0.1496$, respectively; Figure 2). There was a variable amount of red blood cells in each epididymal sample; however, this did not affect the viability or motility. Hyperactivated motility was observed in 1 postthaw sample frozen with the LE extender.

Table. Equine cryopreservation semen extenders and their ingredients

Extender	Ingredients
Gent Extender for Frozen Stallion Semen (Minitube of America, Inc.)	Glucose, glycerol, egg yolk, skim milk, lactose, sodium, potassium citrate, gentamicin
E-Z FREEZIN™ 'Lactose EDTA' Equine Semen Extender (Animal Reproduction Systems)	Glucose, glycerol, egg yolk, lactose, Equex STM, dehydrated sodium citrate, disodium EDTA, sodium bicarbonate, ticarcillin disodium
E-Z FREEZIN™ 'Modified French' Equine Semen Extender (Animal Reproduction Systems)	Glucose, glycerol, egg yolk, nonfat dry milk, lactose, sodium citrate dihydrate, potassium citrate, monohydrate, Hepes free acid, ticarcillin disodium

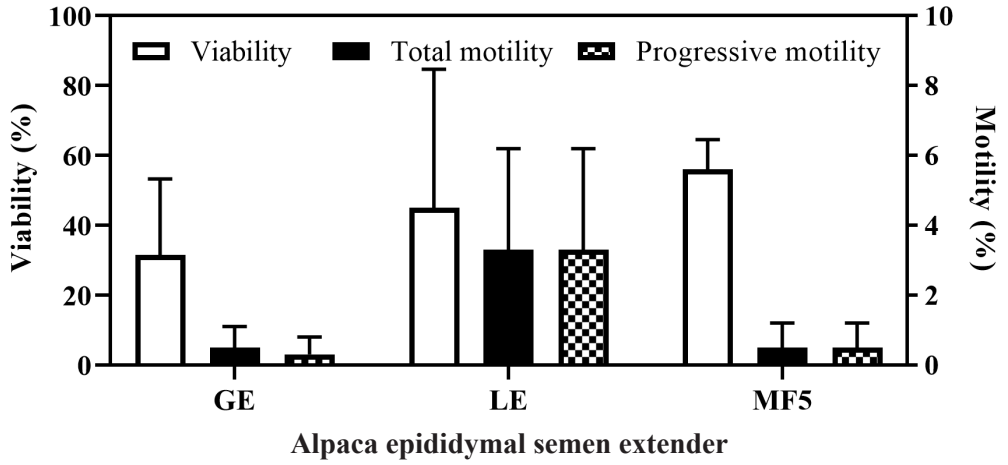

Figure 2. Mean ± SD postthaw viability (left y-axis), total motility (right y-axis) and progressive motility (right y-axis) for 3 alpaca epididymal semen extenders: Gent Extender (GE) for Frozen Stallion Semen, E-Z FREEZIN™ 'Lactose EDTA' (LE) Equine Semen Extender, and E-Z FREEZIN™ 'Modified French' (MF5) Equine Semen Extender for alpaca epididymal sperm.

Discussion

Alpaca cryopreserved epididymal sperm can be stored as pellets or in straws. Sperm pellets are frozen on the surface of dry ice (- 78.5°C), whereas sperm packed in straws are frozen in liquid nitrogen vapor (-140°C to -180°C). Research regarding postthaw motility between the 2 freezing methods had conflicting results. Pelleted frozen alpaca epididymal sperm had higher postthaw motility compared to straws frozen in liquid nitrogen vapor.[3] Postthaw motility when pooled across time was significantly higher when alpaca sperm was frozen in straws compared to frozen in pellets.[10] It is also important to consider the artificial insemination technique when selecting a method for cryopreservation.[3,11] Sperm cryopreserved in straws allows for insemination directly from the straw that can be performed in seconds after thawing.[11] For these reasons, the current research was focused on alpaca epididymal sperm frozen in straws.

Selection of an appropriate semen extender is essential to the success of sperm cryopreservation. Alpaca epididymal sperm has been evaluated for sperm motility following cryopreservation.[2,3,9,12,13] Ejaculated alpaca sperm in skim milk-based extenders yield higher postthaw total motility compared to Tris-based extenders.[4,13] Lactose-based extenders yielded higher postthaw total motility (18.2 ± 5.7%) compared to Tris-based extenders (11.3 ± 3.0%).3 Based on this research, we sought to compare postthaw motility in 3 lactose-based extenders, with 2 having skim milk. Interestingly, postthaw motility for all 3 extenders were lower (< 5%). However, extender that had no skim milk (LE) yielded a numerically higher postthaw total and progressive motility compared to skim milk-based extenders.

The main limitation present in this investigation was lower postthaw total and progressive motility. The 4 alpacas used for this preliminary study had previously sired cria and were assumed to have normal sperm motility, but this was not tested prior to castration and epididymal sperm cryopreservation. Future research in alpacas' epididymal sperm should only be conducted after a complete breeding soundness examination.

Conclusion

Epididymal alpaca sperm can be cryopreserved using commercial equine semen extenders. However, postthaw motility in this preliminary study was less than what has been reported for alpaca epididymal sperm cryopreservation.

Conflict of interest

None to report.

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