Plant extracts reduce DNA fragmentation in frozen-thawed stallion sperm

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INTRODUCTION

Most horse breed associations now allow the use of artificial insemination. However, mares inseminated with frozen-thawed stallion sperm have reduced fertility when compared to mares inseminated with fresh sperm (1, 2). Processing mammalian sperm for cryopreservation increases concentration of free radicals and induces oxidative stress, which can result in DNA damage and may lead to lower fertility (3). Plant extracts can be a rich source of antioxidants and it is hypothesized these antioxidants may reduce the concentration of free radicals during freezing and may improve post-thaw motility and DNA quality. The objective of the current study was to evaluate a variety of extracts from one plant genus, a known antioxidant, on post-thaw stallion sperm motility and DNA quality.

EXPERIMENTAL DESIGN

SEMEN COLLECTION

- Ten stallions were housed at the Colorado State University Equine Laboratory
- An artificial vagina was used to collect semen
- Concentration of ejaculates was determined using an spectrophotometer and prepared for freezing

FREEZING PROTOCOL

- Sperm was extended to 200 x 10^6 cells/ml with E-Z Freezin LE (Animal Reproduction System, Chino, CA) alone or in one of three plant oil extracts from two different commercial sources
- Plant oils were added to the extender at 3% vol/vol
- Extended semen was loaded into 0.5 cc straws and frozen over liquid nitrogen vapor for 10 min
- Samples were plunged into liquid nitrogen dewer and stored until analysis

THAWING PROTOCOL

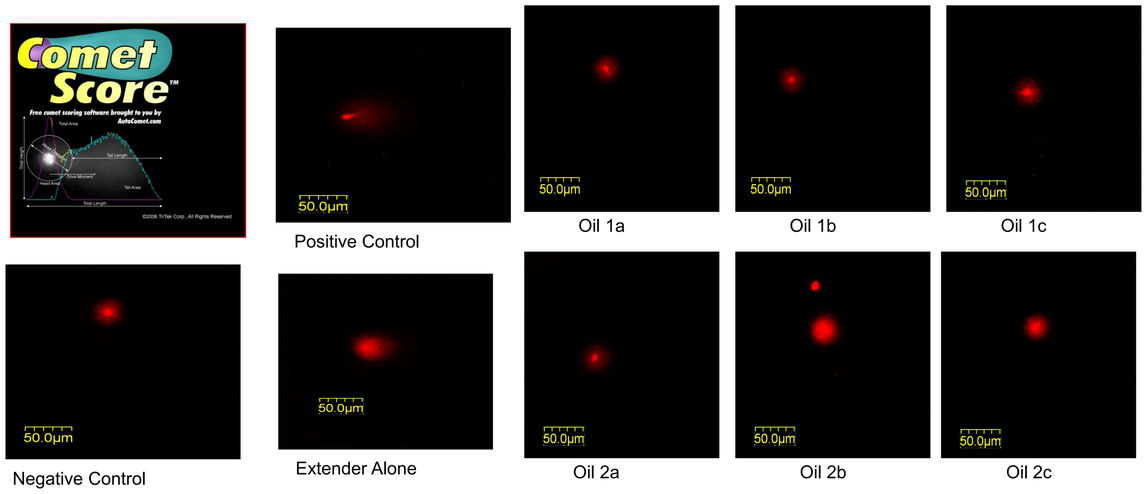
- Straws were thawed in 37^{o}C water bath for a minimum of 45 sec
- Samples were stained with IDENT™ for 10 min at 37^{o}C according to manufacturer's protocol

SPERM ANALYSIS

- Motility and velocity parameters were determined using an IVOS v.12 Sperm Analysis System (Hamilton Thorne Biosciences)
- Motility and velocity were determined at 0, 30, and 60 min post-staining/post-thaw

COMET ASSAY

- An aliquot of cells (~ 10^6 cells) was prepared immediately post-thawing for neutral Comet assays
- Comet assays were preformed according to manufacturer's protocol with slight modification for sperm cells
- DNA was stained with ethidium bromide and visualized using a confocal microscope and images were captured
- Cells were scored for DNA damage with CometScore™ software



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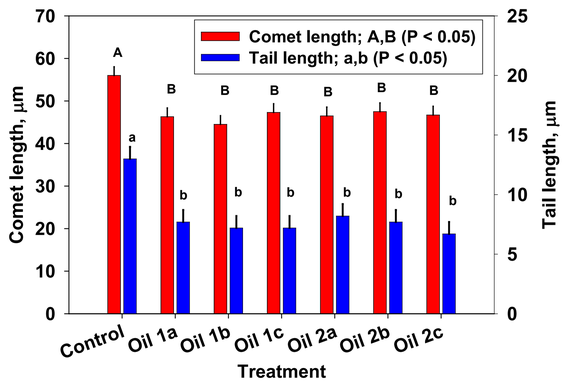
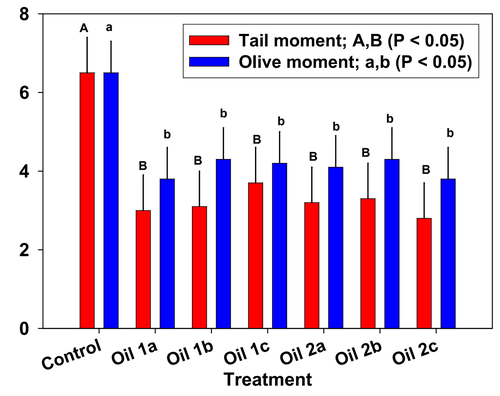
1. Aurich C. Recent advances in cooled-semen technology. Anim Reprod Sci 2008; 107: 268-275.
2. Bedford SJ, Jasko DJ, Graham JK, Amann RP, Squires EL, Pickett BW. Effect of seminal extenders containing egg yolk and glycerol on motion characteristics and fertility of stallion spermatozoa. Theriogenology 1995; 43: 955-967.
3. Ball BA, Vo AT, Baumber J. Generation of reactive oxygen species by equine spermatozoa. Am J Vet Res 2001; 62: 508-515.

RESULTS

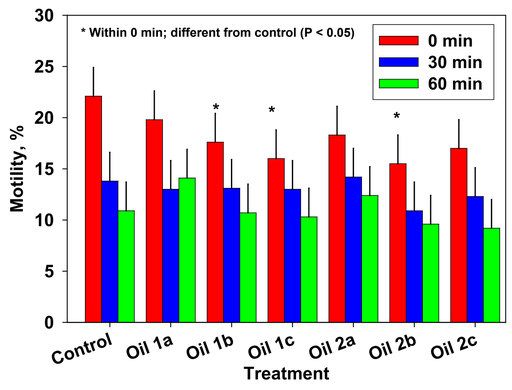
Comet & Tail Length



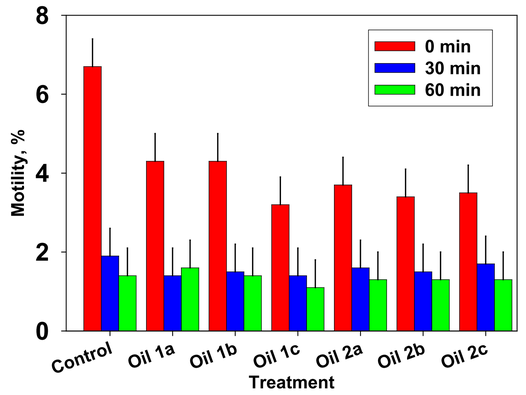
Tail & Olive moment



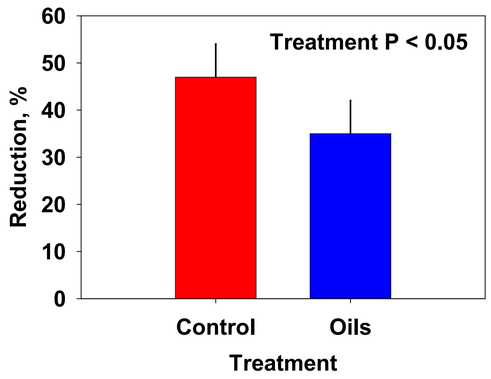
Total Motility



Progressive Motility



Percent Reduction in Total Motility from 0 to 60 min



DISCUSSION & CONCLUSIONS

- Freezing and thawing stallion sperm often induces DNA damage and reduced fertility. Here, we show that the addition of plant oil extracts to a commercially available extender resulted in improved DNA quality
- Tail and olive moment, which are excellent indicators of DNA integrity, were reduced in sperm cells frozen in plant oil extenders
- Total motility was lower for sperm cells frozen in oils 1b, 1c, and 2b immediately post-thaw (0 min), but did not differ at any other time point. The reduction in motility in oil treatments could be due to an increase in viscosity of the extender
- Progressive motility was not different among treatments at any time point post-thaw
- At 60 min post-thaw, there was a 47% reduction in total motility for sperm cells frozen in control extender, while only a 35% reduction for sperm cells frozen in oils
- The addition of plant oil extracts to commercial extenders appears to be a novel, commercially viable way to reduce oxidative stress during freezing thereby leading to superior DNA quality post-thaw and improved fertility
- We anticipate the addition of these plant oil extracts to bovine, human, and boar extenders may improve post-thaw DNA quality as well