

medication had elevated DFI values, e.g., 48% which following removal of the medication decreased over a period of 2 months to 10%. For boars, a heterospermic trial was conducted to assess fertility. The boars were divided into two groups consisting of three boars of higher fertility and three of lower fertility. Boars siring more piglets than statistically expected had significantly lower %DFI than those that had fewer piglets. In another study, a significant negative correlation was found between average total number of pigs born per litter versus %DFI suggesting that sperm with fragmented DNA fertilized the eggs but the embryos died in utero due to sperm deficient DNA. Bulls with the best heterospermic performance had significantly ($r = 0.94$, $p < 0.01$) lower %DFI. In other bull fertility studies, SCSA data were significantly correlated (-0.65 , $p < 0.01$) with non-return rates.

Many cases have been seen where different species of males have been considered fertile due to an acceptable semen analysis. However, when such males failed to produce offspring, the SCSA has often provided the likely etiology, namely, the presence of live, normal appearing sperm that have fragmented DNA leading to early embryo death.

Keywords: Sperm DNA fragmentation; SCSA; Infertility

CRYOPRESERVATION OF STALLION SEMEN TREATED WITH CHOLESTEROL-LOADED-CYCLODEXTRINS

B.E. Spizziri, J.E. Bruemmer, E.L. Squires, J.K. Graham

Department of Biomedical Science, Colorado State University Fort Collins, CO 80523, USA

Cholesterol-loaded-cyclodextrins (CLCs) were added to stallion sperm in an attempt to improve cryosurvival. Eight stallions were collected three times a week for 5 weeks. Each ejaculate was diluted to 50×10^6 sperm/mL in a modified Tyrode's Medium and split into two equal fractions. One fraction was treated with 1.5 mg/mL CLCs while the other was not treated. Samples were held at 22 °C for 15 min, then centrifuged at 2400 rpm for 11 min, and the sperm pellets suspended to 400×10^6 sperm/mL in a EZ-Freezin "LE" (Animal Reproduction Systems (ARS), Chico, CA, USA). The sperm were packaged into 0.5 mL straws, frozen in liquid nitrogen vapor, and stored in liquid nitrogen for >48 h prior to thawing. Straws were thawed in 37 °C water for 30 s, the sperm diluted to 40×10^6 sperm/mL

in EZ-Mixin' CST (ARS) and maintained at 37 °C for 5 min prior to motility analysis using a computer-assisted sperm analysis system (IVOS, Hamilton Thorne Biosciences, Beverly, MA, USA). Treating sperm, with CLCs prior to freezing, improved cryosurvival of sperm for all stallions and ejaculates within stallions. Samples treated with CLCs exhibited higher percentages of total motile sperm (51%) than the control sperm (37%; S.E.M. = 3; $P < 0.05$). Similarly, progressive motility for CLC-treated sperm was higher than the control sperm (33% versus 22%, respectively, S.E.M. = 2; $P < 0.05$). In conclusion, addition of CLCs to stallion sperm prior to freezing maintained higher total and progressive motility of equine sperm after cryopreservation. In addition, this procedure can easily be incorporated into routine processing of stallion sperm for cryopreservation.

Keywords: Cholesterol; Semen; Equine; Cryopreservation; Motility

EFFECT OF CENTRIFUGATION ON EQUINE SEMEN QUALITY OVER TIME DURING COOL-STORAGE INCUBATION

C.L. Fogal¹, D.M. Kozink¹, C.R. Pinto¹, R.H. Douglas²

¹*Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606, USA;* ²*BET LABS, 1501 Bull Lea rd, Suite 102, Lexington, KY 40511, USA*

The objective of this study was to determine the effect of centrifugation over time on equine semen quality during cool-storage incubation. We hypothesized that the removal of seminal plasma would result in higher semen quality over time than non-centrifuged semen samples.

Twenty ejaculates were collected from eight stallions of known fertility. Immediately after collection, concentration, motility, membrane integrity (HOST; swelling test), and viability (eosin–nigrosin) were determined. Samples from each ejaculate were extended with one of five commercially available semen extenders (four skim-milk based and one egg-yolk based) to a final concentration of 50×10^6 mL⁻¹. An aliquot of these samples were centrifuged at $400 \times g$ for 10 min and resuspended to 50×10^6 mL⁻¹ with its respective extender. All samples were stored at 5 °C and analyzed at 24 h and 48 h. Data were statistically analyzed utilizing a repeated measurements design with a 2×5 treatment structure (SAS Institute Inc., Cary, NC). Results, summarized in Table 1, were expressed as