($P = 0.002$). Mean (±S.E.M.) RLU concentrations for 1:1-diluted and post-centrifugation 1:1-diluted ejaculates were 112.8 ± 30.5 and 143.5 ± 39.9 ($P = 0.89$). The effect of diluent within non-centrifuged and centrifuged semen samples had P values of 0.06 and 0.001, indicating that semen diluents rapidly scavenged ROS. This reinforces the argument for adding skim milk-based diluent to canine semen immediately following collection as a standard processing technique to reduce iatrogenic increases of ROS concentrations and their potential harmful effects on semen quality.

Keywords: Chemiluminescence; Reactive oxygen species; Canine; Semen; Luminometer

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FREEZING RATE EFFECTS RHESUS MONKEY SPERM SURVIVAL

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The rate at which intracellular ice crystal formation occurs and, hence, induces damage to spermatozoa during cryopreservation is highly dependent on temperature, freezing rate, and cryopreservation medium. The objective of this study was to determine the optimal rate at which monkey sperm can be cooled for maximum post-thaw sperm survival. Semen samples were obtained by penile electroejaculation from unanesthetized male rhesus macaques (*Macaca mulatta*; $n = 4$) under chair restraint. Semen samples were then diluted in 4 mL BWW medium containing BSA (0.5 mg/mL) and gently rocked for 5 min. The coagulum was removed and samples were maintained at room temperature for 10 min. The supernatant was removed and transferred into a separate tube for determination of initial motility, sperm concentration and subsequent processing for cryopreservation. Semen samples were centrifuged at $300 \times g$ for 5 min and sperm pellets were resuspended at a final concentration of 100×10^6 mL^{-1} in cryopreservation extender (TEST yolk with 3% glycerol). Extended semen was loaded into 0.5 mL polyvinylchloride straws and sealed with polyvinyl chloride sealing powder. Nine straws were loaded per monkey (three per freeze curve). Sperm motility was evaluated before and after cryopreservation by computer-assisted semen analysis utilizing HTM CEROS and post-thaw viability was determined using a BD FACScan flow cytometer. Straws were frozen with a programmable freezer (Planar) according to each of the following three separate two-step freeze curves: fast curve, cooled from 20 to 8 °C, −0.2 °C/min and rapidly lowered to −110 °C at −34 °C/min; slow curve, cooled from 20 to 8 °C, −0.2 °C/min and slowly lowered to −110 °C at −5 °C/min; standard curve, cooled from 20 to 8 °C, −0.2 °C/min and lowered to −110 °C at −17 °C/min. Once sample temperature reached −110 °C, straws were plunged into liquid nitrogen (−196 °C) and transferred to liquid nitrogen storage tanks until evaluated. Straws were thawed for 30 s at 37 °C and resuspended to 50×10^6 mL^{-1} in TEST yolk for analysis of motility and viability. Sperm viability was determined with a dual DNA staining technique using Sybr 14 (100 μM) and propidium iodide (5 μM; Molecular Probes; Eugene, OR, USA). Pre-freeze progressive motility was 46.7 ± 2.6% for all three treatments. Post-thaw progressive motility and viability were comparable among the standard and fast freeze curves (45.9 ± 6.64% and 49.8 ± 6.2%; 50.9 ± 1.55% and 63.2 ± 1.57% respectively). However, sperm cryopreserved using the slow freeze curve exhibited low progressive motility (33 ± 5.6%, $P < 0.001$) and low viability (50.9 ± 1.55%, $P < 0.001$) when compared with the standard and fast freeze curves. In conclusion, both a fast and standard freeze curve produced sperm of similar post-thaw quality; however, a slow freeze curve was detrimental and resulted in poor quality sperm.

Keywords: Primate sperm; Rhesus macaque; Cryopreservation

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INFLUENCE OF EXTENDER AND PACKAGING ON POST-THAW SURVIVAL OF EPIDIDYMAL CAT SPERMATOZOA

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Cryopreservation of germplasm, in combination with assisted reproductive techniques such as AI, will play a critical role in sustaining the future of the earth's threatened animal biodiversity. The objective of this study was to examine the effect of two types of cryoprotective media (Test Yolk Buffer, TYB, Irvine Scientific, Santa Ana, CA, USA) and Lactose-EDTA, E-Z Freezin™—"LE", Animal Reproduction Systems, Chino, CA, USA) and two types of packaging