performed to confirm pregnancy (linear array transducer, Tringa®, Pie Medical; Maastricht, Holland), and on Days 35–38, pregnant queens ($n = 6$) were injected subcutaneously with 10 mg/kg aglepristone (Alizin®, Virbac, Germany) on two consecutive days. Blood samples were taken before the administration of aglepristone (Day 0), during treatment (Days 1 and 2), the day after treatment (Day 3), the day of abortion, and the two following days; to measure progesterone (P_4) by RIA (Coat-A-Count, Diagnostic Product Corporation, Los Angeles, CA, USA). The general condition, vaginal cytology and presence and character of vaginal discharge were recorded daily. The condition of the uterus was assessed by ultrasonography the day before and the days after the administration of aglepristone until confirmation of complete fetal expulsion. Progesterone concentrations were analyzed by least squares means analysis of variance using the GLM procedure of SAS® (Statistical Analysis System, Cary, NC, USA). Termination of pregnancy was achieved in all queens; the mean interval between administration of aglepristone and fetal expulsion was 5 ± 2 days. Progesterone plasma concentration increased after aglepristone injection (15.0 ng/mL versus 28.0 ± 2.5 ng/mL; $P > 0.004$). Mean progesterone concentration started to increase 28 h after aglepristone injection and peaked 77 h after drug administration. On the day of abortion, mean serum P_4 concentration was 19.5 ± 3.9 ng/mL. There were no significant differences in P_4 plasma concentration between Day 0 and the two consecutive days after the abortion (15.0 ± 4.0 ng/mL versus 18.4 ± 4.9 ng/mL). Hemorrhagic vaginal discharge was observed in all queens during the abortion and during the following week. The mean interval between the abortion day and the beginning of the next estrus was 24.6 ± 6.6 days. Few negligible side effects were observed; two queens had a slight decrease in feed intake and three had increased vocalization. In conclusion, the use of aglepristone in queens at Days 35–38 of pregnancy induced abortion 3–7 days later without side effects.

Keywords: Queen; Abortion; Aglepristone; Pregnancy termination; Progesterone receptor antagonist

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DETECTION OF REACTIVE OXYGEN SPECIES IN CANINE SEMEN

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The presence of reactive oxygen species (ROS) have been documented in canine semen. In humans, centrifugation of semen has resulted in increased iatrogenic ROS concentrations during laboratorial processing. The purpose of this study was to determine the concentrations of ROS, in raw and diluted canine ejaculates prior to and post-centrifugation. The study was designed to test the hypothesis that centrifugation would increase the concentration of ROS in semen. Ejaculates ($n = 21$) were collected via digital manipulation from sexually mature dogs ($n = 6$). Raw semen was immediately evaluated for motility, viability, morphology, membrane integrity (hypo-osmotic swelling test, HOST) and presence of leukocytes with Hema 3® (Fisher Diagnostics, Middletown, VA, USA). Sperm concentration and motility were determined by computer-assisted sperm analyzer (CASA, IVOS; Hamilton-Thorne). Viability and morphology were determined under light microscopy at 1000× magnification using eosin-nigrosin stain. Membrane integrity was evaluated with a 100 mM sucrose solution (HOST) and visualized under phase contrast microscopy at 400×. Concentrations of ROS were determined using a luminometer (automatic measuring system for chemiluminescence; Lumat LB 9507, Berthold Technology Oak Ridge, TN, USA). Each ejaculate was divided into two fractions, raw and diluted 1:1 with a skim milk-based diluent (Har-vet, Spring Valley, WI, USA). Following initial analysis, each fraction was then centrifuged at $700 \times g$ for 10 min (Eppendorf Centrifuge 5702, Eppendorf North America; Westbury, NY, USA) and reassessed for ROS. For each chemiluminescence analysis, 20 μL of luminol (Sigma Aldrich, St. Louis, MO, USA) were added to 500 μL of semen and placed in the luminometer. Eleven, relative light unit (RLU) measurements, taken over the course of 5 min every 30 s, were converted into RLU/10^6 spermatozoa for each sample and statistical analyses. Data were analyzed using a two-way ANOVA with repeated measures to evaluate the effects of diluent and centrifugation on seminal concentrations of ROS, and expressed as means ± S.E.M. Concentrations of RLU in raw and post-centrifugation raw ejaculates were 975.0 ± 292.9 and 1804.2 ± 579.7, respectively

($P = 0.002$). 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