

($P = 0.002$). Mean ($\pm$ 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

DOI: 10.1016/j.theriogenology.2007.05.015

FREEZING RATE EFFECTS RHESUS MONKEY SPERM SURVIVAL

S. Sumigama, M. McCarthy, S. Meyers

Department of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

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 BWB 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 $100 \times 10^6 \text{ mL}^{-1}$ 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 $50 \times 10^6 \text{ mL}^{-1}$ 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 $46.7 \pm 2.6\%$ for all three treatments. Post-thaw progressive motility and viability were comparable among the standard and fast freeze curves ($45.9 \pm 6.64\%$ and $49.8 \pm 6.2\%$; $50.9 \pm 1.55\%$ and $63.2 \pm 1.57\%$ respectively). However, sperm cryopreserved using the slow freeze curve exhibited low progressive motility ($33 \pm 5.6\%$, $P < 0.001$) and low viability ($50.9 \pm 1.55\%$, $P < 0.001$) 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

DOI: 10.1016/j.theriogenology.2007.05.016

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

L.J. Minter, C.R.F. Pinto, M. Davis, D.M. Kozink

Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA

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 FreezinTM—"LE", Animal Reproduction Systems, Chino, CA, USA) and two types of packaging

(0.5 mL plastic straws and 1.0 mL cryotube vials) on post-thaw motility, viability and changes in morphology of domestic cat epididymal spermatozoa. Epididymides were harvested from testes of 40 male feral cats after routine castration. Sperm samples were collected by macerating the cauda epididymides in 1.0 mL of Kenney's type semen extender (EZ, Animal Reproduction Systems) with a scalpel blade to release spermatozoa. Immediately after collection, sperm samples were evaluated for motility, viability and changes in morphology (eosin-nigrosin) and divided into four aliquots. Each aliquot was centrifuged ($700 \times g$ for 8 min), supernatant was removed and the resulting pellet was randomly assigned to one of the four treatment combinations arranged in a 2×2 factorial design (two cryoprotective extenders and two packaging types). After dilution and equilibration, both straws and vials were cooled by forced LN₂ vapor in programmable freezer (Micro Digitcool ZH 300 & UE 300 cycle freezer with 900 HP programmer 3T software CE (IMV Technologies, L'Aigle, France) until frozen and then transferred to liquid nitrogen storage dewar for at least 1 week before being subjected to post-thaw semen analyses. A two-way ANOVA was used to evaluate the effects of the two factors and the interaction on sperm motility, survival and morphology. Mean ($\pm$ S.E.M.) percentages of motile and viable spermatozoa, immediately after collection, were $62.8 \pm 12.7\%$ and $84.1 \pm 5.1\%$, respectively. The mean ($\pm$ S.E.M.) percentage of morphologically normal spermatozoa after collection was $61.2 \pm 10.4\%$. After thawing, findings supported that cryopreservation of domestic cat epididymal spermatozoa frozen using the LE cryoprotective media in cryotube vials resulted in a more vigorous post-thaw motility ($27.5 \pm 2.5\%$, $P < 0.05$), a higher level of viability ($77.9 \pm 1.1\%$, $P < 0.05$) and the greatest percentage of morphologically normal spermatozoa ($42.5 \pm 1.9\%$, $P < 0.0001$). There was, however, a marked decrease in motility, viability and appearance of normal morphology after freezing and thawing. Therefore, a more in-depth study addressing each stage of cryopreservation is needed to gain further understanding of where and when damage occurs.

Keywords: Feline; Epididymal spermatozoa; Cryopreservation; Cryoprotective media; Semen packaging

DOI: 10.1016/j.theriogenology.2007.05.017

EVALUATION OF THREE DIFFERENT TECHNIQUES TO RECOVER CANINE EMBRYOS USING A POST-MORTEM MODEL

J.E. Claudia, B.B. Omar

Facultad de Medicina Veterinaria y de Zootecnia, Universidad Nacional de Colombia, Bogotá, Colombia

Reproductive technologies such as embryo transfer in canids may become a very important tool in conservation efforts. The dog is a good model for implementing such studies and could lead to important advances in these technologies that could be applied to wild animals such as foxes and other endangered wild canids. The present study compared three different recovery techniques, using simulation models in which denuded oocytes were introduced in recently recovered uteri. Ten denuded oocytes were deposited in the ovarian tip of each uterine horn using a tomcat catheter. Twenty replicates were made for each protocol. Technique one (T1) consisted of a puncture made close to the ovarian tip of the uterine horn with a 20-gauge needle connected to a 20 mL syringe loaded with 20 mL of PBS (flush medium); in the base of each horn a 2.5 cm incision was made to place a tomcat catheter, from which the fluid was recovered into a Petri dish. For technique two (T2), a puncture in the ovarian tip was made (as in T1), but the oocytes were recovered with an 8-French catheter that was passed through the cervix and the fluid recovered in a Petri dish. In technique three (T3), an 8-French catheter was inserted through the cervix and was used for infusing and recovering collection media. Each horn was flushed independently (20 mL of PBS was used for each horn). The recovery per horn was 7.3 ± 2.4 (73%), 8.5 ± 1.9 (85%) and 5.8 ± 2.2 (58%) for T1, T2 and T3, respectively. The first two techniques were not different ($P > 0.5$) and were selected to conduct an in vivo trial with six bitches whose embryos were collected 5 days after a diestrous smear. The average of embryos flushed using either technique was 6 ± 2 .

The findings of the study suggested that canine embryos could be retrieved using a less invasive technique by passing an 8-French catheter through the cervix, thereby reducing the damage to the uterus and the possible generation of adhesions.

Keywords: Embryos; Oocytes; Embryo transfer; Recovery; Canine

DOI: 10.1016/j.theriogenology.2007.05.018