SNOW LEOPARD (Panthera uncia) SPERM LONGEVITY IN VITRO IS NOT INFLUENCED BY PROTEIN OR ENERGY SOURCE SUPPLEMENTS BUT IS AFFECTED BY BUFFER SOURCE

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The snow leopard (Panthera uncia) differs among felid species (even within the Panthera lineage) in that sperm motility longevity in vitro is enhanced by a simple (phosphate buffered saline; PBS) rather than a complex (Ham's F10) medium. In Ham's F10, snow leopard spermatozoa lose motility abruptly and, even using various simple media, there are distinct differences in the ability of snow leopard sperm to survive. This unique, adverse response indicates a species-specific sensitivity not observed in other felid taxa studied to date. To better understand the biology of these gametes and to facilitate assisted reproduction, our objective was to identify (1) the component(s) responsible for the detrimental effect on sperm survival in vitro and (2) the optimal medium for supporting sperm viability. Our approach involved systematically adding constituents and supplements found in other media to PBS to isolate the factor(s) influencing snow leopard sperm survival. Specifically, we examined the effects of (1) bovine serum albumin (BSA), fetal calf serum (FCS) and homologous snow leopard serum (SLS) at 2 concentrations, (2) 4 supplemental energy substrates and (3) $NaHCO_3$ buffer on snow leopard sperm motility over time. Electroejaculates were collected from 8 snow leopards maintained at 5 institutions (Brookfield Zoo, IL; Cheyenne Mountain Zoological Park, CO; Micke Grove Zoological Gardens, CA; San Francisco Zoological Gardens, CA; Woodland Park Zoological Gardens, WA). Aliquots (90 µl each) of raw semen from each ejaculate were transferred into Eppendorf tubes containing 90 µl of the appropriate warmed (37°C) medium. Treatments (Trt) were: 1) PBS + 5% FCS; 2) PBS + 5% SLS; 3) PBS + 20% FCS; 4) PBS + 20% SLS; 5) PBS + 0.4% BSA; 6) PBS; 7) PBS + 0.1 mM pyruvate; 8) PBS + 2.78 mM glucose; 9) PBS + 21.6 mM Na lactate; 10) PBS + 2.0 mM glutamine; and 11) PBS + 14.3 mM $NaHCO_3$. All except Trt 6 were supplemented with 0.1 mM pyruvate and Trt 8 to 10 contained 5% FCS. In all cases, sperm aliquots were centrifuged, supernatants discarded and sperm pellets resuspended in their respective medium at a concentration of 5×10^6 motile sperm/ml. Samples were protected from light at room temperature in air for 6 h, and sperm percent motility and forward progression (scale, 0 to 5; 5 = best) were evaluated at 0 h, 0.5 h and then every h for 6 h. Motility values were used to calculate a sperm motility index (SMI) profile for each treatment over time. Differences in longevity profiles were determined by ANOVA and treatment means compared using a least significant difference test. Good quality ejaculates were obtained from all snow leopards with mean (± SEM) volume = 2.9 ± 0.3 ml, SMI = 75.3 ± 1.3, percent morphologically normal spermatozoa = 55.5 ± 4.6 and total spermatozoa/ejaculate = $160 \pm 40.2 \times 10^6$. The SMI profile for Trt 1 (PBS + 5% FCS) was similar ($P \geq 0.05$) to that for Trt 2, 4 and 5, but higher ($P < 0.05$) than the profile for Trt 4 (PBS + 20% FCS). The SMI profiles for spermatozoa incubated in PBS containing no (Trt 6) or different energy substrates (Trt 7 to 10) did not differ ($P \geq 0.05$). Interestingly, compared to all other treatments, the SMI profile was lowest ($P < 0.05$) for spermatozoa incubated in PBS supplemented with $NaHCO_3$ (Trt 11). In summary, for maintaining snow leopard sperm in vitro, these results suggest that: 1) there is no benefit to using homologous serum (SLS) versus FCS or BSA; 2) increasing the concentration of FCS to 20% appears detrimental; 3) the presence of pyruvate, glucose, Na lactate and glutamine is innocuous; 4) a simple medium with no protein or energy substrate supplements is capable of supporting snow leopard sperm in vitro for up to 6 h; and 5) altering the buffer system by adding $NaHCO_3$ has a profound detrimental effect resulting in a rapid decrease in sperm motility and reduced longevity. In conclusion, snow leopard sperm appear particularly sensitive to pH changes, the buffering system in the culture medium or $NaHCO_3$ itself. This information will be useful for designing further studies to identify an optimal medium for maintaining snow leopard sperm in vitro. (Funded by the Philip Reed Foundation/NOAHS Center, NIH HD 23853, Ralston Purina Co./Conservation Endowment Fund of the American Zoo and Aquarium Association and the Smithsonian Institution Scholarly Studies Program.)