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## JOURNAL ARTICLE

# Snow leopard (*Panthera uncia*) spermatozoa are sensitive to alkaline pH, but motility in vitro is not influenced by protein or energy supplements

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To better understand the biology of snow leopard spermatozoa and to facilitate developing assisted reproduction, a series of studies was conducted to: 1) identify the component(s) of complex culture media responsible for the detrimental effect on sperm survival in vitro, 2) optimize medium for supporting sperm viability, and 3) evaluate sperm capacitation in vitro. Constituents of complex media were added systematically to phosphate-buffered saline (PBS) to isolate the factor(s) influencing snow leopard sperm motility in vitro. Sperm capacitation was also assessed following incubation in PBS with bovine serum albumin (BSA), fetal calf serum (FCS), or heparin. For maintaining sperm motility, there was no benefit ( $P > \text{or} = 0.05$ ) to supplementing PBS with low (5%) or high (20%) concentrations of snow leopard serum (SLS) versus FCS or BSA. Likewise, adding supplemental energy substrates (pyruvate, glucose, lactate, or glutamine) did not enhance or hinder ( $P > \text{or} = 0.05$ ) sperm motility. However, motility rapidly decreased ( $P < 0.05$ ) with the addition of  $\text{NaHCO}_3$  to PBS or Ham's F10 nutrient mixture. Surprisingly, Ham's F10 with no buffering component or with both  $\text{NaHCO}_3$  and N-Z-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) maintained sperm motility at levels similar ( $P > \text{or} = 0.05$ ) to PBS. Although sperm motility in all treatments decreased with time, there was a strong inverse relationship ( $P < 0.01$ ;  $r = 0.90$ ) between motility and sample pH at 6 hours. Spermatozoa incubated in PBS containing FCS, BSA, or heparin did not undergo the acrosome reaction when exposed to calcium ionophore. In summary, alkaline pH has a profound detrimental effect on snow leopard sperm motility, and capacitation does not occur under conditions that normally promote this event in other felid species. These results clearly demonstrate a high degree of interspecific variation among felids in fundamental sperm function, and they provide evidence for the necessity of basic research when developing assisted reproduction in little-studied nondomestic species.

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