MS Thesis
Department of Environmental Science and Policy
College of Science
George Mason University

Candidate: Church Humphreys
Defense Date and Time: Friday, June 12, 2020 at 10:30 am
Defense Location: Webex (E-mail Stephanie Lister, slister1@gmu.edu, to request link)
Title: Inhibition of Equine Sperm Motility using Sodium Tetraphenyl Borate and Reactivation with Caffeine - Potential to Improve Post-thaw Recovery

Thesis Director: Dr. Alonso Aguirre
Committee Members: Dr. Thomas Wood and Dr. Budhan Pukazhenthi

ABSTRACT

Frozen semen provides several advantages for preservation of endangered equids and domestic horse relative to fresh or chilled semen. However, cryopreservation results in extensive loss of sperm motility and viability arising from freeze-thaw induced membrane damage, osmotic stress, and oxidative stress leading to suboptimal fertility outcomes. The main objectives of this study were to:1) assess the ability of sodium tetraphenyl borate (TPB) to inhibit sperm motility in equids, 2) assess the ability of caffeine to reverse the inhibitory effects of TPB, and 3) evaluate if the inhibition of sperm motility prior to cryopreservation improves post-thaw sperm motility. Spermatozoa from domestic horse (*Equus ferus caballus)*, Przewalski's horse (*E. f. przewalskii),* and Persian onager (*E. f. hemionus)* were exposed to varying concentrations of TPB. Sperm total motility, progressive motility, and several motility characteristics (curvilinear motility, average path velocity, linearity, and straightness) were assessed using Computer Assisted Sperm Analysis (CASA) over 2 h of *in vitro* incubation. After 2 h of incubation, TPB was washed and sperm were resuspended in fresh medium containing 10 mM caffeine. Motility parameters described above were analyzed using CASA for 2 h of *in vitro* incubation. In a separate experiment, sperm suspensions were pre-treated with 0 mM, 150 mM or 300 mM TPB and cryopreserved using standard protocols. Following thawing, sperm samples were assessed

for motility parameters (as described above), sperm viability and acrosomal integrity. Exposure to TPB (150 mM, 300 mM and 500 mM) resulted in a sharp ($P < 0.05$) decline in total sperm motility and progressive motility within 30 min of *in vitro* incubation. Caffeine (10 mM) failed to restore sperm motility in TPB treated samples. Pre-treatment with TPB prior to cryopreservation failed to improve post-thaw sperm motility, progressive motility, viability or CASA parameters. However, there was no change in acrosomal integrity before or after cryopreservation. Overall, although TPB was successful in inhibiting sperm motility in all three species of equids, contrary to earlier reports in human sperm, caffeine was unable to restore sperm motility in TPB treated samples. Furthermore, pre-treatment with TPB failed to improve post-thaw motility in equid spermatozoa. Further research is warranted to evaluate alternate cellular pathways that regulate reversible inhibition of equid spermatozoa and their effects on post-thaw sperm survival.