Thawing straws of frozen stallion semen directly in a 37°C water bath versus inside a plastic bag within the water bath

Melissa J. Prell, a Patrick M. McCue, a Paula D. Moffett, a James K. Graham, b Ryan A. Ferris b
a Department of Clinical Sciences, b Department of Biomedical Sciences, Colorado State University, Fort Collins, CO

Introduction
The success of an artificial insemination program relies on proper semen handling techniques. Frozen semen shipments should be accompanied by thawing instructions aimed at optimizing post-thaw motility and, potentially, fertility. Standard thawing instructions must be altered when presented with a cracked or broken straw to prevent exposure of the spermatozoa to water. The goal of this study was to compare post-thaw motility between frozen semen straws immersed directly in a water bath versus frozen semen straws placed inside of a plastic bag and then immersed in a water bath.

Materials and methods
Ejaculates (n=9) were collected from healthy Quarter Horse and Arabian stallions using a Colorado model artificial vagina. The semen was centrifuged, diluted in freezing extender to a concentration of 200 million sperm/mL, loaded into 0.5 mL straws and cryopreserved. Treatment groups were: Group 1 – straws were thawed in a 37°C water bath for 30 seconds; Group 2 - straws were placed into a plastic bag and then immersed in a 37°C water bath for 30 seconds; Group 3 – straws were placed in a plastic bag and immersed in a 37°C water bath for 3 minutes. The latter time period was determined to be the time required for a straw within a plastic bag to reach 37°C as measured by a thermocouple. Sperm motility was evaluated using computer assisted sperm analysis (CASA; SpermVision®, MOFA Global, Verona, WI). Statistical analysis was performed using a one-way repeated measures ANOVA with post-hoc Tukey’s test. A significant difference was considered for a p value of <0.05. Data are presented as mean ± SD.

Results
There was no significant difference in post-thaw total sperm motility between the different thawing techniques. Post-thaw total sperm motility for Group 1 was 43.9 ± 17.2 %, Group 2 was 38.3 ± 15.6 % and Group 3 was 38.7 ± 17.1 %.

Discussion
These data confirm that there is no significant difference in post-thaw sperm motility if straws of frozen equine semen are thawed in a plastic bag immersed in a 37°C water bath versus the standard technique of direct immersion in a water bath. Consequently, the bag technique can be used if confronted with a clinical situation involving cracked or broken straws of frozen stallion semen.

Keywords: Stallion, frozen semen, cooled storage