Semen collected from stallions can be extended and used for immediate insemination, extended and stored at 5 to 8°C for 24 to 48 hours prior to insemination, or cryopreserved and stored indefinitely in liquid nitrogen. Breeding mares with cryopreserved semen requires specialized equipment for storage and thawing of semen and more intensive mare management. However, not all equine breeding programs are equipped or able to utilize frozen semen. Consequently, the objective of this study was to evaluate the longevity of frozen stallion semen once it has been thawed, extended and maintained at 5°C for up to 48 hours. Eight ejaculates from five healthy Quarter Horse stallions were utilized. Semen was collected and two aliquots were cooled in INRA96 (IMV Technologies, Maple Grove, MN) at a concentration of 50 million sperm/mL as non-cryopreserved controls. The remainder of the sample was frozen in CryoMax LE® (Animal Reproduction Systems, Chino, CA) extender at a concentration of 200 million/mL. Treatment groups were: Group 1 - straws thawed in a 37°C water bath for 30 seconds; Group 2 - straws were thawed in a 37°C water bath for 30 seconds, followed by centrifugation at 400 x g for 10 minutes; Group 3 - straws were thawed in a 37°C water bath for 12 seconds (time required to reach a temperature of 5°C). Semen samples were subsequently diluted to a concentration of 50 million/mL in INRA96 and cooled to 5°C. Sperm motility was evaluated at 24 and 48 hours using computer assisted sperm analysis (CASA; SpermVision®, MOFA Global, Verona, WI). Statistical analysis was performed using a one-way ANOVA, with p-value set at <0.05. Data is presented as mean ± SD. Total sperm motility for the non-frozen control semen at 24 and 48 hours of cooled-storage (77.1 ± 6.9 and 75.4 ± 9.2 %, respectively), was higher than all frozen-thawed treatment groups (p<0.05). There was no difference (p>0.05) in total motility at 24 or 48 hours of cooled-storage post-thaw between Group 1 (50.3 ± 9.6 and 51.6 ± 12.8 %), Group 2 (48.6 ± 10.1 and 47.0 ± 13.9 %), and Group 3 (32.8 ± 6.6 and 41.5 ± 6.5 %). In summary, frozen stallion sperm can be thawed, extended and cooled to 5°C for up to 48 hours and still maintain acceptable (i.e. > 30 %) total motility. Potentially, frozen semen could be thawed, extended and shipped by overnight courier to another location to inseminate into a mare. A breeding trial is clearly warranted to evaluate the fertility of frozen-thawed stallion semen after cooled-storage.

Keywords: Stallion, semen, frozen, cooled-storage