Effect of osmolality dilution on motility of frozen thawed equine spermatozoa
T.M. Collop, E.J. Hand, J.L. Loy, S.T. Norman
Charles Sturt University, Wagga Wagga, NSW, Australia

The longevity of osmotically challenged frozen/thawed spermatozoa is postulated to be inhibited in vivo. This study was conducted to evaluate the gradual reduction of thawed semen osmolality on motility over time. The null hypothesis of this study was that a progressive reduction of osmolality of post-thaw spermatozoa would not influence the longevity of the spermatozoa.

Semen was collected from three stallions, housed within 10 km of Wagga Wagga, NSW, and transported fresh to Charles Sturt University, Veterinary Clinical Centre, for analysis and cryopreservation. Semen was extended with EquiPro™ (MiniTüb, Tiefenbach, Germany) and centrifuged prior to the addition of freezing media (5 % glycerol) and processed utilizing standard laboratory protocols, with a controlled freezing mechanism (Freeze Control®, CryoLogic, Mulgrave, VIC, Australia). Straws were thawed in a water bath at 37 °C for 30 sec. semen from the Control straw was placed in a pre-warmed 37 °C microcentrifuge tube. Semen osmolality was determined utilizing a Fiske 210 Micro-Osmometer (Fiske Associates, Norwood, MA) and progressive individual motility was visually analyzed as a percentage of spermatozoa. Test dilutions were based on EquiPro™ containing varying amounts of analytical grade glycerol (Chem-Supply, Gillman, SA, Australia). Dilution one, total volume of 1 mL, contained 2.5 % glycerol and had a target osmolality of 730 mOsm/kg. Dilution two, total volume of 1 mL, contained 1.25 % glycerol and had a target osmolality of 530 mOsm/kg. Dilutions three and four contained EquiPro™ only (315 mOsm/kg). However, dilution three equalled 1 mL and dilution four equalled 3 mL. Thawed semen, 0.5 mL, was placed in dilution step one, pre-warmed to 37 °C, and allowed to equilibrate at 37 °C for 5 min, prior to being analyzed for osmolality and motility. The subsequent dilutions were added to the total volume of the previous dilution following the same protocol. Once the dilution steps were complete, both Control and Test semen were placed at 22 °C for the remainder of the study and analyzed for motility every hour for 7 hrs and then at 24, 27, 30, 36, and 48 hrs post-thaw.

Results (Figure 1) show that serial dilution of frozen/thawed spermatozoa to a more isosmolar level significantly increases the motility of spermatozoa for the period between 4 and 30 hrs post-thaw in comparison to the Control sample. Further studies could investigate if this thawing technique leads to improved fertility after the insemination of frozen-thawed equine semen.

![Progressive individual motility of sperm over time](image)

**Figure 1 –** Plot of motility means for each assessment time. Solid plot-points indicate significant differences between treatments at that assessment time.
D = Dilution (Test) sample
ND = Control sample

**Keywords:** Osmolality, spermatozoa, motility, cryopreservation, longevity