Effects of seminal plasma on frozen-thawed stallion epididymal semen plasma membrane integrity
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Frozen-thawed epididymal sperm of stallions has variable post-thaw motility. Epididymal sperm are collected postmortem or postcastration and therefore are not exposed to the effects of seminal plasma as is ejaculated semen. The objective of this study was to evaluate the effects of seminal plasma plus extender versus extender or seminal plasma alone on epididymal spermatozoa plasma membrane integrity using the hypoosmotic swelling test (HOST). Our hypothesis was that epididymal sperm exposed to seminal plasma would demonstrate better post-thaw membrane integrity as assessed by HOST than epididymal sperm frozen without exposure to seminal plasma. Epididymides were dissected from the testes of mature stallions during the breeding season within one hour of routine castration or euthanasia. Seminal plasma was obtained from a stallion with high fertility and high freezing ability and was centrifuged and stored at -20°C until use. In the first experiment (n=6 stallions) epididymal flushing with a commercial extender (INRA 96, IMV Technologies France, L’Aigle, France) plus 20% seminal plasma (EXSP) was compared to flushing with extender alone (EX). In the second experiment (n=5 stallions), epididymal flushing with extender plus 20% seminal plasma (EXSP) was compared to flushing with seminal plasma alone (SP). Total volume of flushing medium was 20 mL. Semen was extended after 10 min incubation at room temperature to approximately 100 x 10^6 spermatozoa per mL. The extended semen was then centrifuged at 700g for 15 min and the semen pellet suspended to 400 x 10^6 spermatozoa per mL in a commercial freezing extender (E-Z Freezin-LE, Animal Reproduction Systems, Chino, CA), loaded in 0.5 mL straws and frozen 2 cm over liquid nitrogen vapor for 20 min. Frozen straws were thawed in a 37°C water bath for 30 sec then incubated at 37°C for 10 min. The HOST was performed using 100 µL of semen added to 1 mL of a 100 mOsm sucrose solution. The percentage of spermatozoa with intact membranes for each treatment was compared using a paired-t test within each experiment after 10 min and 60 min incubation. The results of the first experiment demonstrated that the percentage (± SEM) of spermatozoa with intact (swollen) membranes after epididymal flushing with EXSP was lower than those flushed with EX (53.7 ± 3.4 vs. 58.0 ± 2.7%, p=0.05) at 10 min but a difference was not seen at 60 min (43.7 ± 2.7 vs. 45.7 ± 0.8%, p=0.51). In the second experiment, the percentage of spermatozoa with intact membranes after epididymal flushing with EXSP was not significantly different from those flushed with SP at 10 min (66.8 ± 6.8 vs. 55.6 ± 8.5%, p=0.06) or at 60 min (55.6 ± 8.1 vs. 54.8 ± 8.7%, p=0.9). In conclusion, the addition of seminal plasma to the epididymal flushing extender does not seem to improve post-thaw semen quality of recovered stallion epididymal sperm and in one experiment was slightly detrimental after short incubation. However, additional studies are needed to evaluate if the addition of seminal plasma before processing of epididymal sperm for freezing would improve their fertilizing ability.

Keywords: Hypoosmotic swelling test, cryopreservation, equine, spermatozoa