Evaluation of commercial semen extenders and crystalloids for short term cooled extension of epididymal spermatozoa in squamates and chelonians

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Our working knowledge of assisted reproduction in male reptiles is limited to our ability to collect semen from less than 0.2% reptile species. To date, cooled storage using egg yolk based semen extender has had some short term benefits for corn snakes (*Elaphe guttata*), green iguanas (*Iguana iguana*), and leopard tortoises (*Stigmochelys pardalis*).[1-3] Our objective was to evaluate commercial semen extenders and crystalloid solutions utility to provide short-term sperm storage in squamates and chelonians. The hypothesis was that commercial semen extenders maintain motility of squamate and chelonian sperm, specifically the green anole (*Anolis carolinensis*), the diamondback water snake *(Nerodia fasciata),* and the red eared slider (*Trachemys scripta elegans*). Epididymal sperm from *A. carolinensis* (n = 6), *N. fasciata* (n = 6)*,* and *T. scripta elegans* (n = 6) were collected post mortem. *A. carolinensis* and *N. fasciata* samples were aliquoted into 6 commercial extender treatments (Ham's F 10 without albumin, TEST yolk buffer, INRA 96, sperm washing media, Andro pro chill LT and Hank's balanced salt solution) and 3 crystalloid treatments (0.9% sodium chloride injection, phosphate buffered saline, and Lactated Ringer's solution), whereas *T. scripta elegans* samples were aliquoted into 6 extender treatments (Ham's F 10 without albumin, TEST yolk buffer, INRA 96, sperm washing media, electrolyte free media, and Hank's balanced salt solution). Samples were stored at 4°C for 72 and 96 hours respectively. Motility analysis was performed using a Computer Assisted Sperm Analysis system at time 0, 12, 24, 48, and 72 hours for *A. carolinensis* and *N. fasciata* and at 0, 6, 12, 24, 48, 72, and 96 hours for *T. scripta elegans*. Linear mixed models were used for statistical analyses. Epididymal sperm motility in *A. carolinensis* declined over time for each treatment over the first 24 hours ($p < 0.001$), with no significant difference in motility between commercial semen extenders and crystalloid solutions. Epididymal sperm motility in *N. fasciata* remained high in commercial semen extenders compared to crystalloid solutions ($p < 0.001$) for more than 72 hours. Sperm wash media, F 10, and INRA 96 maintained highest sperm motility for *N. fasciata*. Epididymal sperm motility in *T. scripta elegans* remained high over 96 hours in the sperm wash media, Test yolk buffer, and INRA 96 ($p < 0.001$). However, motility declined over time in all treatments except INRA 96 ($p < 0.001$). We concluded that INRA 96, Sperm wash media, or F 10 were the best choices for handling and cool storing reptile sperm.

Keywords: Semen extender, reptiles, *Trachemys scripta elegans*, *Anolis carolinensis*, *Nerodia fasciata*

References

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