Evaluation of post-thaw semen parameters for different extenders in white-tailed deer
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White-tailed deer farming in the United States relies heavily on the use of frozen semen for dissemination of valuable genetics, yet there are limited data available on the most suitable extenders for deer sperm cryopreservation. Previous studies suggest that the use of soybean-based extenders is superior to most egg-yolk based extenders. However, to date, the use of a synthetic liposome-derived extender in white-tailed deer has not been critically evaluated. The objective of the current study was to compare semen parameters of white-tailed deer semen cryopreserved using three different extenders: (1) soybean-based (AM, Andromed®); (2) liposome-based (OC, OptiXcell®); and (3) egg yolk-based (OR, Ovine Red®). Our hypothesis was that white-tailed deer semen cryopreserved with AM or OC extenders would present superior post-thaw sperm motility, increased viability and acrosome integrity, and reduced DNA fragmentation when compared to OR extender. White-tailed deer (n = 8, mean age of 1.6 ± 0.1 yr, range 1-2 yr) were anesthetized with tiletamine-zolazepam (0.4 mg/lb) and xylazine (1 mg/lb) intramuscularly. Semen was collected by electroejaculation, and the ejaculate from each buck was divided equally amongst the three extenders. Each aliquot was extended to a final concentration of 120 million sperm/mL, cooled to 5°C, and then incubated at 5°C for 3 to 4 hr, according to manufacturers’ recommendations. Semen was loaded into 0.5 mL straws and frozen manually by placing straws on a rack in liquid nitrogen vapor at a distance of 4 cm horizontally above the liquid nitrogen level for 10 min before submerging into the liquid nitrogen for final freezing and storage. Each semen straw was thawed in a 37°C water bath for 30s before post-thaw analysis. Percent of total sperm motility (TM) and progressive sperm motility (PM) were assessed for each sample using computer-automated semen analysis. Additionally, samples were stained with fluorescent probes for evaluation of sperm viability (SYBR-14/PI), acrosomal integrity (FITC-PNA/PI), and chromatin stability (acridine orange) using flow cytometry. Data were analyzed using a General Linear Models procedure for all analyses of variance in R. Data are expressed as mean ± SEM. Significance was set at p < 0.05. Total and progressive sperm motilities for AM (TM: 46 ± 8.8% and PM: 33 ± 7.7%), OC (TM: 56 ± 6.7% and PM: 40 ± 6.9%), and OR (TM: 51 ± 5.7% and PM: 28 ± 5.1) were not different (p ≥ 0.49). There were no differences in sperm viability (p ≥ 0.31), with the post-thaw population containing 78 ± 6.6%, 87 ± 3.5%, and 73 ± 8.0% viable sperm when cryopreserved with AM, OC, and OR extenders, respectively. There were no differences in acrosome integrity (p ≥ 0.18) with 90 ± 7.4% (AM), 80 ± 12% (OC), and 77 ± 3.6% (OR) of viable sperm having an intact acrosome. The DNA fragmentation index also did not differ (p = 0.68) and was 7.2 ± 2.5% (AM), 7.3 ± 2.4% (OC), and 9.8 ± 2.0% (OR), which is consistent with normal fertility in other mammalian species. These results suggest that non-egg yolk based extenders are equally effective as a traditional egg-yolk based extender for cryopreservation of white-tailed deer semen. Although we did not directly assess fertility in this study, the semen parameters herein evaluated appear to indicate that all three extenders are suitable for use in clinical practice.

Keywords: Cryopreservation, flow cytometry, sperm quality, white-tailed deer.