

Seasonality affects semen cryopreservation of white-tailed deer bucks (*Odocoileus virginianus*) throughout rut

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White-tailed deer farming is a growing industry in the United States, with breeding operations contributing significantly to the industry's economic impact. Artificial insemination with frozen semen allows for selection and dissemination of valuable genetics, yet surprisingly little information is available regarding the best time throughout rut to perform semen cryopreservation. The objective of this study was to compare the efficacy of semen cryopreservation of white-tailed deer bucks collected early in the breeding season (September), at peak rut (December), and late season (March). We hypothesized that semen freezing ability would be enhanced at peak rut. Mature bucks ($n = 7-11$) were anesthetized with tiletamine-zolazepam (0.88 mg/kg) and xylazine (2.2 mg/kg) administered intramuscularly via projector. The penis was manually exteriorized, and semen was collected by electroejaculation (Pulsator IV, Lane Manufacturing, Inc). An aliquot of the ejaculate was diluted 1:60 in warm (37°C) Optixcell extender (IMV Technologies) and placed into a pre-warmed 20 μ M chamber slides (Vitrolife, Microcell Counting Chambers) for assessment of motility via computer-automated sperm analysis (Spermvision II, MiniTube of America). The remainder of each sample was extended with Optixcell to a final concentration of 120 million sperm/mL. Ejaculates were cooled to 5°C over 1 h, loaded into 0.5-mL straws, and incubated for 3 to 5 h. Straws were frozen manually in liquid nitrogen vapors at 4 cm above the liquid nitrogen level for 15 m before submerging them for final freezing and storage. Each semen straw was thawed in a 37°C water bath for 30 s for post-thaw analyses. Overall and progressive sperm motilities were evaluated in each sample using computer-automated semen analysis. Additionally, samples were stained with fluorescent probes and subjected to flow cytometry for characterization of sperm viability (SYBR-14/PI), acrosome integrity (FITC-PNA/PI), and chromatin stability (acridine orange). Data were analyzed using a Kruskal-Wallis chi-squared test with a Dunn posthoc test (non-parametric) or ANOVA with repeated measures and Tukey post-hoc test (parametric) in R Version 3.2.2 (<https://www.r-project.org/>). Pre-freeze overall and progressive sperm motilities were lowest in March and highest in December ($p \leq 0.04$). However, post-thaw overall and progressive motilities were lowest in September and highest in December ($p = 0.01$). The DNA Fragmentation Index was lowest in December ($p < 0.01$), but did not differ between March and September ($p = 0.23$). The percentage of live sperm cells was highest in December ($p = 0.03$), and the percentage of intact acrosomes per live sperm cell was highest in September ($p = 0.02$). This study confirms that bucks have superior semen quality when in peak rut (December). Though semen collected early or late in rut may present acceptable motility, DNA stability appears to be impaired, which could adversely affect fertility rates. Therefore, we suggest that semen cryopreservation be performed closer to peak rut to optimize post-thaw semen quality and ensure successful artificial insemination outcomes.

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