Characterization of reproductive parameters from Pennsylvania bull elk (*Cervus canadensis*)

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Pennsylvania (PA) elk (*Cervus canadensis*) are a population of free-ranging cervids that live in north central PA, and were initially reintroduced in 1913 as a measure to recover the elk population that was extirpated by uncontrolled hunting. Currently, the herd size of PA elk approaches ~ 1,400 individuals. During the 2019 calving season, a reduction in the number of calves was noticed. Gross anatomic inspection from the uteruses and ovaries harvested from selected cows revealed evidence suggestive of embryonic death whereas conventional sperm parameters (e.g. morphology, viability) analyzed from selected bulls were largely normal. This study was conducted to characterize certain sperm quality parameters from bulls (n = 24) harvested during the 2020 hunting season, to determine potential causes for reduced fertility. Testes and epididymides were harvested from hunted animals, immediately cooled at 5 °C and shipped from PA to Texas within 24 hours. Upon arrival, each testis and epididymis were isolated and weighed. Tissue wedges were obtained from testes and fixed in Davidson's fixative. Each caudal epididymis was flushed with 5 ml of INRA-96® extender and total sperm numbers (TSN; 10^9), total and progressive motility (TMOT, PMOT; %), curvilinear velocity (VCL; µm/s), plasma membrane/acrosomal intactness (VAI; %), DNA integrity ($COMP_{\alpha\text{-}t}$; %), and sperm morphology (Normal; %) were determined by CASA, flow cytometry and DIC microscopy, respectively. Overall, the sperm quality was consistent with parameters from other species (e.g. stallion, bull; Table). The % (mean ± SD) of morphologically normal sperm was low due to the incidence of proximal, distal droplets (13 ± 11, 43 ± 21; respectively), and bent midpieces (14 ± 11). Surprisingly, the % $COMP_{\alpha\text{-}t}$ was high (mean ± SD 45 ± 17; range: 3 - 71). Spearman's correlation analysis revealed no linear relationship ($p > 0.05$ [positive or negative]) among testes and epididymides sizes, sperm morphology, or $COMP_{\alpha\text{-}t}$ values. At this point, it can be speculated that the observed values of $COMP_{\alpha\text{-}t}$ (susceptibility of DNA to denaturation) in the wild PA elk population may partially explain the reduced fertility recently observed. Further studies are warranted to confirm this hypothesis and to determine a potential cause for such increased values of sperm DNA damage in the absence of other sperm quality abnormalities.

Keywords: Pennsylvania elk, *Cervus canadensis*, sperm quality, fertility, DNA integrity

Table. Sperm quality parameters from 24 PA elks obtained after epididymal harvest

Parameter	Testis	Epid.	TSN	TMOT	PMOT	VCL	VAI	$COMP_{\alpha\text{-}t}$	Nortmal
(Mean ± SD)	83 ±21	21 ± 5	0.6 ± 1	55 ± 20	34 ± 15	162 ± 51	87 ± 6	45 ± 17	34 ± 14

Expression and abundance of prostaglandins in healthy and fibrotic mare endometrium

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Inflammation and fibrosis of the endometrium is a major cause of infertility in the mare. A better understanding of the mechanisms of degenerative diseases of the endometrium can help to improve diagnosis and clinical outcomes in the mare. Prostaglandins have a wide range of roles including in tissue remodeling, inflammation, and fibrosis in the endometrium. We hypothesized that expression of prostaglandin-related genes in the endometrium and abundance of prostaglandins in low-volume uterine lavage is different in healthy mares compared to mares diagnosed with fibrosed endometrium. Our objective was to discern any changes in prostaglandin abundance and gene expression among healthy and fibrosed endometrium in order to better understand the mechanisms of endometrial inflammation and fibrosis. A total of 27 estrous mares were enrolled in this study. A uterine lavage using 250 ml of 0.9% NaCl solution was performed with a sterile bivona catheter, and the concentration of prostaglandins in the fluid was measured using enzyme-linked immunosorbent assays (ELISAs) for PGE_2 and $PGF_{2\alpha}$. An endometrial biopsy was collected and sectioned in half. One half of the endometrial biopsy was fixed in 10% formalin, sectioned, stained with hematoxylin & eosin and graded according to the Kenney-Doig system by a board-certified pathologist; the second half was snap frozen in liquid nitrogen and stored at -80 °C until analysis. Mares were assigned to either the healthy group (n = 15) if the endometrial biopsy score was I or IIA, or to the fibrosed endometrium group (n = 12) if the endometrium biopsy score was IIB or III. Total RNA was extracted from endometrial biopsies and real-time PCR was performed to evaluate the relative abundance of the following genes: PTGES, PTGS2, PTGFR, PTGER2, PTGER4, CBR1, and SLCO2A1. Mean threshold cycle (Cq) was determined and then normalized to the reference gene (GAPDH) (ΔCT). Statistical analyses were performed with JMP® software using $p < 0.05$. Data were

analyzed for normality and rank transformed when necessary. Student's *t*-test was used to compare differences between healthy and fibrosed endometrium groups. Healthy endometrium had higher expression of PTGES ($p < 0.03$) and SLCO2A1 ($p < 0.05$) compared to fibrosed ones. There was no difference ($p > 0.05$) in abundance of PGE_2 or $PGF_{2\alpha}$ in low-volume lavage between the 2 groups. In endometrial fibrosis, degenerative inflammatory conditions can increase cytokine production and disrupt normal cellular function that may impact pathways including prostaglandin synthesis and prostaglandin uptake/transport. SLCO2A1 is involved in the transport of PGE2 and $PGF_{2\alpha}$ that are present during pregnancy and luteolysis. A disruption of prostaglandin E-synthase could similarly affect prostaglandin concentrations, altering the PGE/PGF ratio and resulting in a deficient environment for development and maintenance of conceptus and corpus luteum.

Keywords: Endometrial biopsy, fibrosis, equine, prostaglandin