## 24 The pros and cons of urethral catheterization for semen collection in rhinos

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Electroejaculation (EEJ) has been the chosen method for semen collection in rhinos, but urethral catheterization (UC) is becoming increasingly popular since first trialed successfully in a white rhino over a decade ago. Yet, the efficacy of UC remains unreported. This study compares the characteristics of semen collected via UC with those previously published for EEJ. The pros and cons of the collection methods are described as these are important considerations for researchers and animal care staff alike. Semen was collected opportunistically via UC from black (n = 1), white (n = 2), and greater one-horned (n = 1) rhinos on seven occasions. In several cases, more than one UC was performed during the collection. The anaesthesia protocol included medetomidine, an α2-adrenergic agonist known to stimulate the release of semen into the urethra. The UC samples (n = 13) were characterised and found to be comparable to EEJ samples (n = 68 from black (n = 4), white (n = 26), and GOH (n = 10) rhinos; <u>Table 1</u>) for all characteristics except volume and total number of sperm recovered, both of which trended lower for UC samples. Regardless, the amount of semen obtained via UC often was sufficient for sperm banking and some would have been sufficient for an AI attempt (4 out of 13 samples ≥500 × 10<sup>6</sup> total motile sperm). Levels of alkaline phosphatase (ALP) activity support the notion that sperm recovered with UC are from emission (seminal ALP < 100 U/L is indicative of pre-ejaculate fluid, whereas >1000 U/L is indicative of testicular contribution). As previously reported for EEJ samples, the ALP activity in UC samples was strongly correlated with the total number of sperm recovered (Pearson R = 0.79, P = 0.004). Although urine contamination has been noted in some EEI fractions, all UC-derived samples appear to contain urine (as measured by creatinine; ≥21 µg of creatinine/mL). Only 3 of the 13 samples had concentrations below 100 µg/mL, which is concerning as urine can compromise sperm quality and cryosurvival. However, EEJ often yields highly viscous samples, posing challenges to sperm recovery and processing. In UC samples, issues with viscosity are rare due to limited seminal fluid. Both EEJ and UC can be conducted opportunistically during scheduled procedures (e.g. dental work, physical exams). However, EEJ often stimulates body movements, particularly hindleg extension, so it is typically performed after other procedures and prolongs the immobilization period. Also, EEJ necessitates specialised equipment and multiple trained personnel. In contrast, UC can be performed concurrently with most procedures, involves simple equipment, and can be completed in less than 15 min. This study is part of the American Institute of Rhinoceros Science (AIRS) funded in part by the Institute of Museum and Library Services.

Table 1. Semen characteristics

Item	Urethral catheterization			Electroejaculation <u>1</u>	
	Minimum	Maximum	Median	Minimum	Maximum
Age (yr)	21.9	33.5	(33.2)	6.0	42
Volume (mL)	0.8	4.0	(2.5)	0.5	338
Concentration (×10 <sup>6</sup> /mL)	16	2671	(250)	0.2	1683
Total sperm (×10 <sup>9</sup> )	0.012	10.7	(0.625)	0	66.4
Motility (%)	0	93	(69)	0	90
Total motile sperm (×10 <sup>9</sup> )	0	9.9	(0.29)		
рН	7.3	8.8	(8.2)	7.3	9.5
Osmolality (mOsm/kg)	263	539	(343)	262	410
ALP activity (U/L)	327	24 412	(2808)	<5	11 780
Creatinine (µg/mL)	30	2381	(410)		



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