FREEZING EPIDIDYMAL SPERM FROM WHITE RHINOCEROS (*Ceratotherium simum*) TREATED WITH DIFFERENT CRYODILUENTS

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The aim of this study was to evaluate the motility of White rhinoceros caudal epididymal sperm using two cryodiluents, and included the sperm viability after cold storage. Sperm morphology and motility of different fertility level bulls were evaluated. Testes were collected from two hunted rhinoceros bulls in Tswalu Desert Reserve in Kalahari. Rhinos No.1 and No. 2 were nonbreeding and breeding bulls, respectively. Testes were collected 1 h after death, transported on ice (~8°C) and processing began 3 h thereafter. Sperm were frozen in 0.25 ml straws at 150 x 10^6sperm/ml. Straws were cooled to 4°C and equilibrated for 6 h, then placed in liquid N_2 vapor for 20 min and stored in liquid N_2. Sperm treatments were evaluated for progressive motility at pre-freeze and at post-thaw. Trial I included freezing epididymal sperm of rhino No.1, treated with either equine diluent (OP) or Triladyl® (Minitube), supplemented with 0, 5 or 10% fetal calf serum (FCS). Trial II was conducted using the epididymides from rhino No.2, epididymis A (time = 0 h only) and for epididymis B, extracting sperm at given intervals of 0, 6, 12 and 18 h, respectively, using Triladyl + 5% FCS. During the intervals, epididymis B was stored at 4°C. The last trial (Trial III) examined the morphological differences among sperm from rhinos No.1 and No.2. Sperm were fixed in gluteraldehyde and evaluated at 100X using light microscopy.

Table 1. Post-thaw motility assessment of White rhino sperm

	Trial I (Rhino No.1)						Trial II (Rhino No.2)				
Item	Equine diluent			Triladyl diluent			Epididymis A	Epididymis B			
Time (h)	-	-	-	-	-	-	0	0	6	12	18
% FCS added	0	5	10	0	5	10	5	5	5	5	5
% Motility:											
Pre-freeze	20	12	10	10	18	10	70	75	55	44	35
Post-thaw	12	5	5	1	5	6	43	48	42	38	30

Table 2. Morphology of caudal epididymal sperm of rhinos No.1 and No.2 (Trial III)

Morphological features	No.1 (%)	No.2 (%)	Morphological features	No.1 (%)	No.2 (%)
Proximal droplet	24.8	16.6	Distal midpiece reflex + droplet	1.9	4.1
Translocating droplet	10.5	9.7	Nuclear vacuole	9.5	2.7
Bent midpiece + droplet	23.8	25.0	Dag effect	8.6	4.1
Distal midpiece reflex	3.8	5.6	Miscellaneous	35.2	27.8

In this study, there was a marked difference in sperm motility between breeding and nonbreeding male rhinos. There was a noticeable difference between the epididymides of rhino No.1, with one epididymis being half the size of the other, which contained no sperm. There was no detectable difference in sperm morphology between the two bulls. There tended to be a difference between pre-freeze and post-thaw sperm motility in Trials I and II. The results at this stage suggest Triladyl + 5% FCS would be suitable for freezing the sperm of a White rhino breeding male.