Improved sperm freezing in the endangered African wild dog (*Lycaon pictus*) using a two-step dilution TRIS-egg yolk extender containing Equex STM.

F. Van den Berghe[1,2], M.C.J. Paris[1,2], Z. Sarnyai[1], M.B. Briggs[3], W.K. Farstad[4] & D.B.B.P. Paris[1]

[1]College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, QLD, Australia

[2]Institute for Breeding Rare and Endangered African Mammals, Edinburgh, UK

[3]African Predator Conservation Research Organisation, Las Vegas, NV, USA

[4]Department of Production Animal Clinical Sciences, Norwegian University of Life Sciences, Oslo, Norway

Introduction

Development of assisted breeding techniques can aid conservation & management of the endangered African wild dog (*Lycaon pictus*). Previous attempts to freeze sperm from this species have proven unsuccessful with sperm motility dropping to nearly 0% within 2 h of thawing. The aim of this study was to improve the freezing success of African wild dog sperm by testing two routinely used canine cryopreservation protocols.

Methods

(1) Sperm collected n=3 African wild dogs

(2) Analysis
- volume, colour, pH
- motility, viability, morphology
- sperm number
- acrosome status, DNA fragmentation

(3) Samples split	***Protocol 1***	***Protocol 2***
(4) Dilution	Tris-egg yolk extender	
	8% glycerol 20% egg yolk	3% glycerol 20% egg yolk
(5) Cooling	37°C to 4°C over 2.5 h	
(6) Dilution	none	Tris-egg yolk extender 7% glycerol 20% egg yolk 1% Equex STM
(7) Freezing	0.25 mL straws 10 min at 4 cm above LN_2, then immersed	
(8) Thawing	37°C water bath, 30 sec	
(9) Dilution	none	Tris-egg yolk extender
(10) Analysis	Incubated at 37°C motility *(5 min, 2h, 4h, 6h, 8h)* viability, morphology, acrosome *(5 min, 2h, 4h, 6h)* DNA fragmentation *(5 min; Fig.1)*	

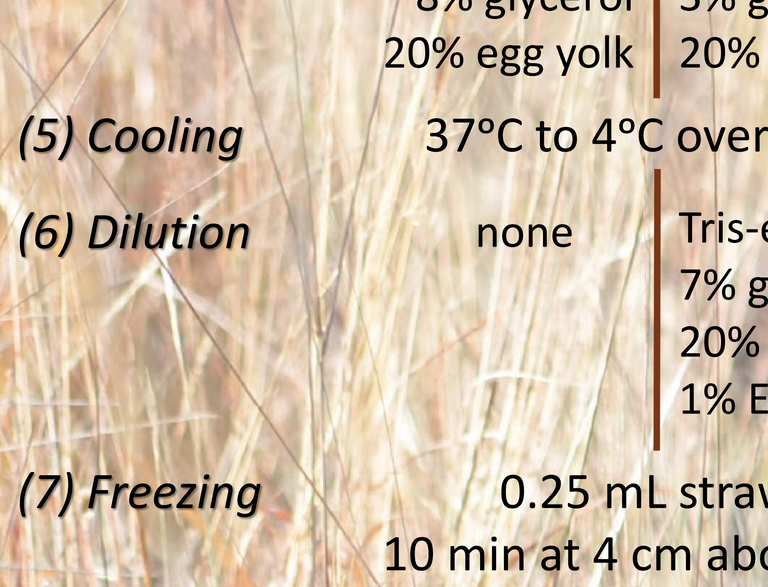

Figure 1. *DNA fragmented (green; FIT-C) & intact (blue; Hoechst 33342) African wild dog sperm heads evaluated by TUNEL.*

Results

Table 1. *Mean (± SEM) sperm quality before freezing & 5 min after thawing for the two different freezing protocols. Different letters indicate a significant difference between treatments (P ≤ 0.05). n = 3 males.*

		5 min Post-thaw	
Sperm Quality Parameter	**Pre-freeze**	**Protocol 1**	**Protocol 2**
Total Motility (%)	78.9 ± 2.6[a]	24.4 ± 5.0[b]	36.7 ± 4.2[b]
Normal Morphology (%)	76.3 ± 5.9[a]	35.0 ± 9.5[b]	39.1 ± 12.0[ab]
Viability (%)	92.0 ± 0.6[a]	37.0 ± 5.7[b]	65.3 ± 9.9[a]
Acrosome Integrity (%)	92.0 ± 2.3[a]	22.8 ± 8.3[b]	69.3 ± 8.8[a]
DNA Fragmentation (%)	0.3 ± 0.3	0.6 ± 0.2	0.8 ± 0.2

- Sperm motility was significantly lower for both protocols immediately after thawing (Table 1), but remained significantly higher for Protocol 2 from 2 h after thawing (Fig. 2), and persisted for up to 8 h.
- Sperm frozen with Protocol 2 also had significantly higher viability & acrosome integrity after thawing (Table 1, Fig. 2).
- DNA fragmentation & normal morphology did not differ between protocols.

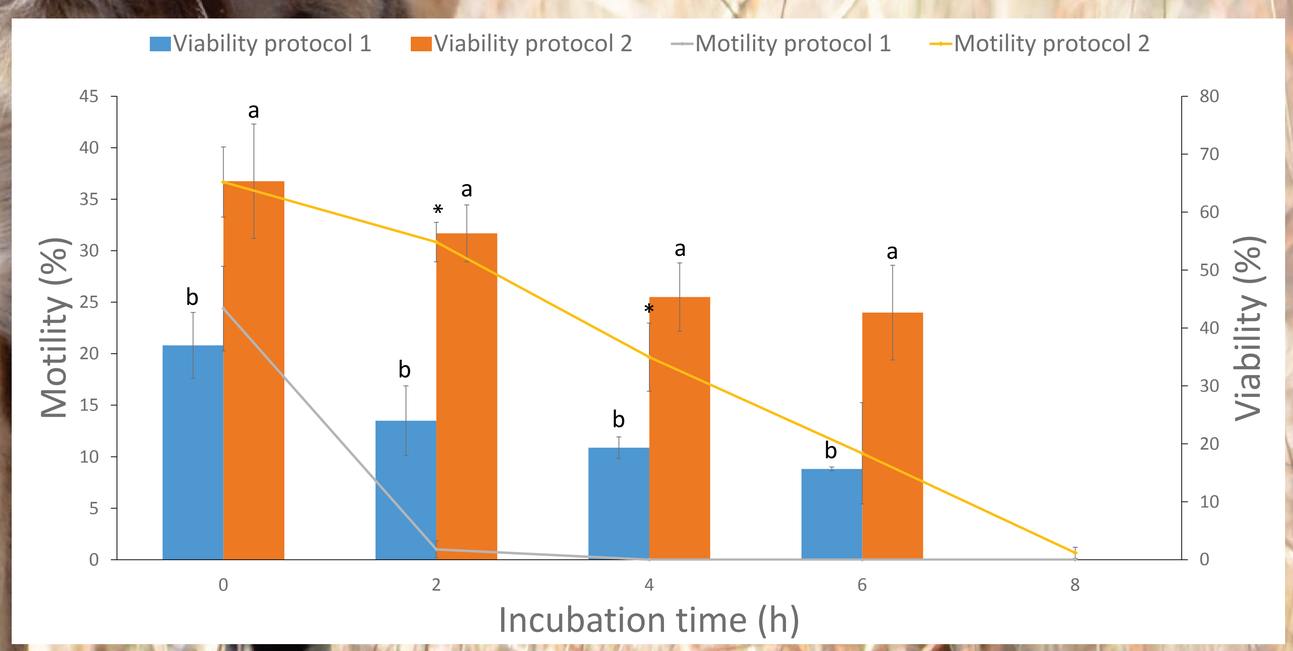

Figure 2. *Mean (± SEM) post-thaw motility & viability of sperm at different times after incubation at 37°C. Different letters (viability) or * (motility) indicate a significant difference between treatments (P ≤ 0.05). n = 3 males.*

Conclusion

Our results demonstrate that using a two-step dilution with TRIS-egg yolk extender containing Equex STM yields greatly improved post-thaw quality & longevity in African wild dog sperm; making it suitable for use in artificial insemination.

Acknowledgements

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