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

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## 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 integrity

*(3) Samples split* — ***Protocol 1*** | ***Protocol 2***

*(4) Dilution* — Tris-egg yolk extender

| Protocol 1 | Protocol 2 |
|---|---|
| 8% glycerol<br>20% egg yolk | 3% glycerol<br>20% egg yolk |

*(5) Cooling* — 37°C to 4°C over 2.5 h

*(6) Dilution*

| Protocol 1 | Protocol 2 |
|---|---|
| none | Tris-egg yolk extender<br>7% glycerol<br>20% egg yolk<br>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*

| Protocol 1 | Protocol 2 |
|---|---|
| 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 integrity *(5 min; Fig.1)*

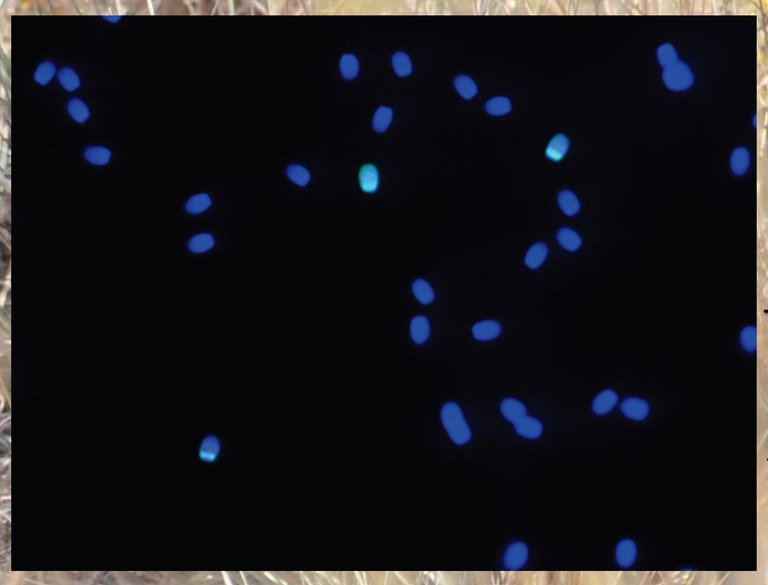
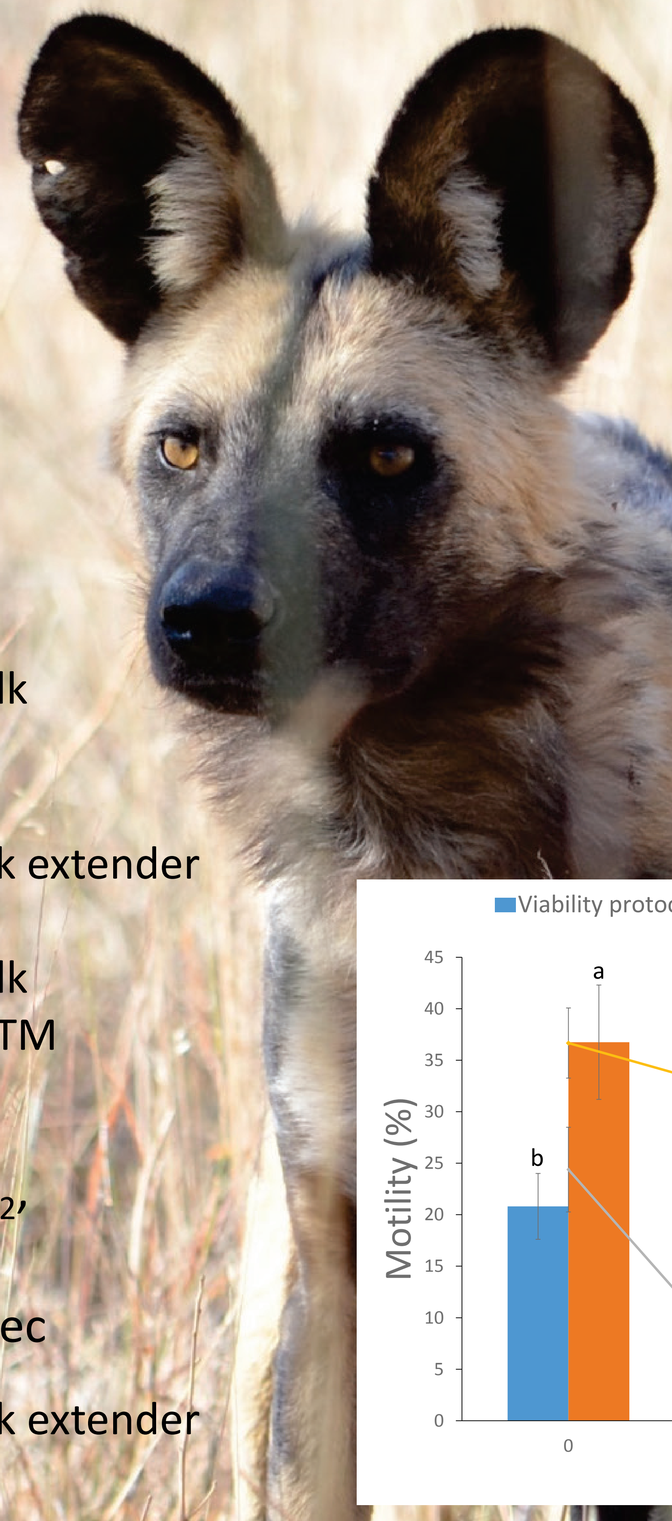


***Figure 1.*** *DNA fragmented (green-FIT-C) and intact (blue-Hoechst 33342) African wild dog sperm heads evaluated by TUNEL.*



## 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.*

| | | Post-thaw 5 min | |
|---|---|---|---|
| **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.

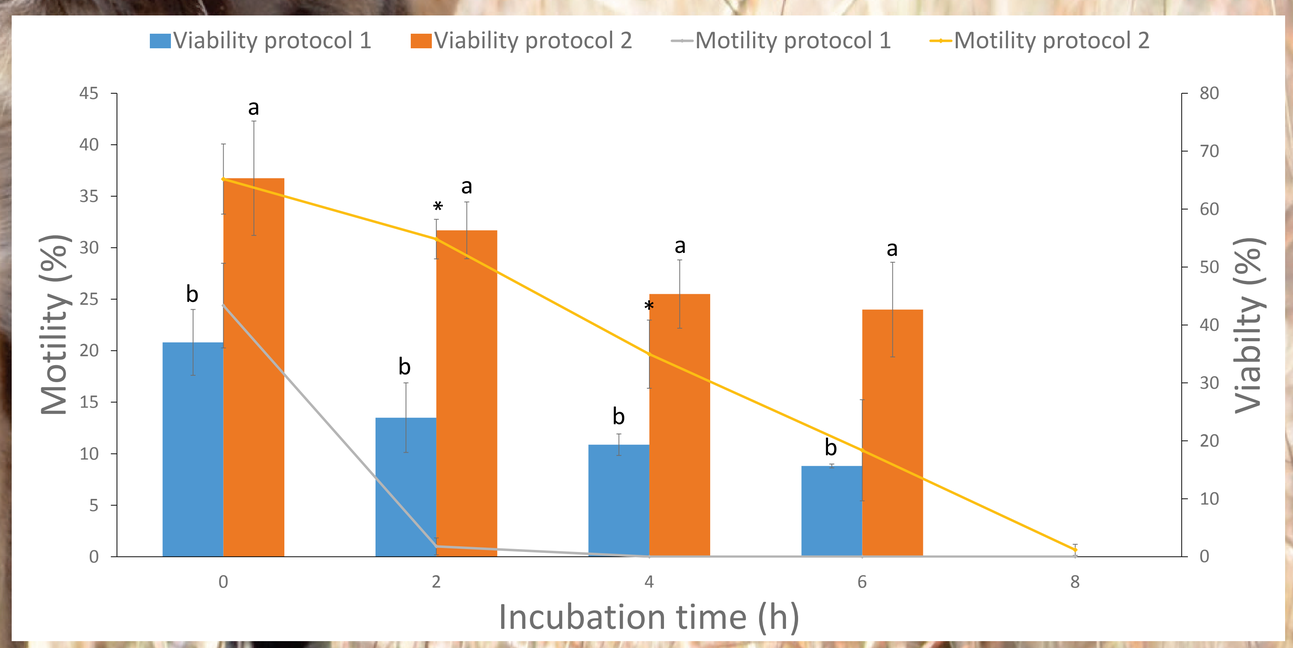



***Figure 2.*** *Mean (± SEM) post-thaw motility & viability of sperm at different times after incubation at 37°C. Different letters (viability) or * (motility) indicate a significant difference between treatments (P ≤ 0.05). n = 3 males.*

## 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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