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

(3) Samples split

(4) Dilution

(5) Cooling

(6) Dilution

(7) Freezing

(8) Thawing

(9) Dilution

(10) Analysis

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.

<table>
<thead>
<tr>
<th>Sperm Quality Parameter</th>
<th>Pre-freeze</th>
<th>Post-thaw 5 min</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Protocol 1</td>
<td>Protocol 2</td>
</tr>
<tr>
<td>Total Motility (%)</td>
<td>78.9 ± 2.6a</td>
<td>24.4 ± 5.0b</td>
</tr>
<tr>
<td>Normal Morphology (%)</td>
<td>76.3 ± 5.9a</td>
<td>35.0 ± 9.5b</td>
</tr>
<tr>
<td>Viability (%)</td>
<td>92.0 ± 0.6a</td>
<td>37.0 ± 5.7a</td>
</tr>
<tr>
<td>Acrosome Integrity (%)</td>
<td>92.0 ± 2.3a</td>
<td>22.8 ± 8.3a</td>
</tr>
<tr>
<td>DNA Fragmentation (%)</td>
<td>0.3 ± 0.3</td>
<td>0.6 ± 0.2</td>
</tr>
</tbody>
</table>

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

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.

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