109 Sperm cryopreservation in *Eulamprus quoyii* (Eastern water skink)

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Abstract

Australia has a rich diversity and high endemism (93%) of reptilian species, the largest family being *Scincidae* (252 species), yet there continues to be a paucity of reports for gamete cryopreservation in reptilians (Clulow and Clulow 2016 Reprod. Fertil. Dev. **28**, 1116-1132, DOI: 10.1071/RD15466). The goal of this study was to collect sperm from a locally abundant skink species (*Eulamprus quoyii*; Eastern water skink) to examine sperm sensitivity to cryopreservation. Wild-caught males ($n = 50$; snout-vent length = 103-126 mm) were held in seminatural conditions for the duration of the study. Semen was collected during the breeding season (September-October) using an adapted ventral-massage technique (Molinia *et al.* 2010 Herp. Cons. Biol. 5, 311-319; retrieved from http://www.herpconbio.org). Sperm metrics (volume, concentration, progressive and total motility, and membrane integrity) were assessed subjectively under light or fluorescence microscopy. Results were compared using ANOVA. Sperm volume (9.43 ± 5.18 µL) and concentration ($7.79 \times 10^8 \pm 5.32 \times 10^8$ sperm/mL) did not correlate with snout-vent length in adult males ($r^2 = 0.025$ and $r^2 = 0.15$, respectively). Due to small volumes, sperm samples (motility >80%) from 4 to 6 males were pooled before allocation across treatments. For all cryopreservation experiments, sperm samples (5-10 µL) were loaded into 0.2-mL French straws and frozen using a controlled-rate freezer (Cryobath; −6°C per minute), then plunged into LN. Sperm were thawed in a water bath at 35°C for 10 s. In year 1, pooled samples ($n = 3$) were maintained at room temperature (21 ± 1.5°C) either raw, or diluted in PBS, Tris-citrate glucose, TLHepes, or Ham's F-10 and assessed at 0, 1, 3, 16, 40 h. Tris-citrate-glucose-diluted sperm had significantly lower total motility from 3 h (36.7 ± 11.5%; $P < 0.05$), decreasing to 1% motility by 16 h. Up to 70% motility could be maintained for 16 h in all other treatment groups. In year 2, pooled samples ($n = 3$) were cooled to 4°C over a period of 2 h, then gradually diluted 1:1 with buffer to a final concentration of either 0.6, 1.35, or 2.7 M cryoprotectant (CPA; dimethyl sulfoxide, dimethyl acetamide, glycerol) in PBS or no CPA. Sperm diluted in 1.35 and 2.7 M dimethyl sulfoxide in PBS had significantly ($P < 0.05$) higher kinetic rating and proportion of live sperm than control or dimethyl acetamide treatments; 1.35 and 2.7 M glycerol were intermediate. In year 3, dilution and cryopreservation using 1.35 M CPA in complex diluents, Tris-yolk buffer (20.1 ± 2.6% live) and Beltsville poultry semen extender (29.7 ± 2.0% live), did not significantly improve sperm survival compared to PBS (26.4 ± 2.7% live); however, post-thaw progressive motility (5 ± 1.1%) was significantly ($P < 0.05$) higher with 1.35 M dimethyl sulfoxide Tris-yolk buffer than all other treatment groups. In conclusion, dimethyl sulfoxide yields promise for sperm cryopreservation in a skink, but further studies are required.