10. CRYOPATHOLOGY OF MACROPOD SPERMATOZOA

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Cryopreservation of kangaroo spermatozoa has proven a significant challenge (1). This study documents kangaroo sperm pathology at an ultrastructural level associated with glycerol cytotoxicity and cryoinjury in an attempt to better understand the causes of poor post-thaw sperm survival in this species. Spermatozoa were dissected from the cauda epididymides of eastern grey kangaroos (n = 2) and recovered into a Tris citrate buffer (pH 7.2). Sperm preparations were then exposed to one of three temperature treatments in buffer containing a final glycerol (G) concentration of either 0 or 20%. Treatment 1 involved storing sperm at 35°C for 10 mins. In treatment 2, sperm were initially cooled from 35°C to 4°C at 10°C / min before a 10 min exposure to 0 or 20% G. Sperm in treatment 3 were frozen–thawed according to a standard macropod sperm cryopreservation protocol (1). Glycerol in the freeze–thaw treatment was added after the sperm had cooled to 4°C. All sperm treatments were then subsequently fixed and processed for standard transmission electron microscopy. Sperm injury was described in detail and quantified based on ultrastructural damage and disruption of the axoneme (A), mitochondria (M), plasma membrane (P) and the presence of distinctive periaxonemal spaces along the sperm tail (S). The incidence of each pathology was determined after evaluating approximately 100 spermatozoa. Means for each pathology were compared separately using balanced ANOVAs and least significance difference (P = 0.05) post hoc tests. Results indicate the cytotoxic nature of 20% glycerol at 35°C on kangaroo sperm ultrastructure; an effect that was not as detrimental when sperm were cooled to 4°C and exposed to glycerol. While glycerol was clearly detrimental to sperm at 35°C, it did provide some cryoprotective value during the freeze–thaw procedure, reducing the incidence of mitochondrial, axonemal and periaxonemal pathology.

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