# Effects of cryopreservation on mitochondrial function and sperm quality in fish

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The development of cryopreservation techniques has led to important changes in animal reproductive biotechnology. However, these techniques are associated with cellular and molecular damage, affecting the mitochondrial function and quality of spermatozoa; moreover studies in fish are limited. In this work, the effects of cryopreservation on ultrastructure, mitochondrial function and antioxidant defences in Atlantic salmon (Salmo salar) spermatozoa were assessed, along with intracellular calcium (Ca2+)i, mitochondrial DNA sequence and sperm function (motility and fertilization rate). Significant ultrastructure alterations of the middle piece and mitochondria were observed in cryopreserved spermatozoa as compared to controls. Oxygen consumption and ATP were also significantly different in cryopreserved spermatozoa, and in spermatozoa incubated with electron transport chain (ETC) uncouplers and inhibitors. Mitochondrial membrane potential, motility, fertilization rate and Ca2+ i in cryopreserved spermatozoa displayed significant reductions compared to fresh spermatozoa. Mitochondrial potential correlated significantly with motility and fertilization rate. A redox imbalance was observed in frozen spermatozoa due to increased lipid peroxidation and superoxide anion production as compared to fresh spermatozoa. Likewise, increased activity of glutathione peroxidase and total glutathione (GSH/GSSG), as well as reduced catalase activity, were observed in comparison with fresh spermatozoa. Our results contribute to a better understanding of cryodamage to mitochondrial functions in fish spermatozoa, and enabled us

to establish potential quality assessment indicators. The data suggest that cryopreservation induces a reduction in overall sperm quality and functionality through disruption of the mitochondrial ultrastructure and function, leading to energy depletion and increased oxidative stress. This knowledge may also lead to the identification of a potential biotechnological tool for improving reproductive efficiency in species of commercial and biological interest.