Relevance of JC-1 for mitochondrial activity measurement in trout rainbow spermatozoa

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Cryopreservation allows the preservation of genetic heritage and the regeneration of lines particularly in fish farming. However, upon thawing, the spermatozoa suffer several subcellular damages that can have consequences on their fertilizing ability. Mitigation of such damages requires the development of methods to study the quality of cryopreserved spermatozoa. The present work will focus on the mitochondria, whose electron transport chain drives proton transfer from the matrix to the intermembrane space. The created proton gradient will ultimately fuel ATP production via proton reentry in the matrix. Functional mitochondria therefore possess an electrochemical potential whose intensity can be assessed using lipophilic and cationic fluorochromes such as JC-1. In healthy cells with active mitochondria, JC-1 specifically accumulates in the negatively charged mitochondrial matrix to form red fluorescent aggregates (em = 590 nm). In altered mitochondria, inner membrane potential decreases, the interior of the mitochondria is therefore less negative and JC-1 accumulation in the matrix is lessened. JC-1 thus remains in a monomeric form that emits green fluorescence (em = 529 nm). This dual fluorescence is used to express the mitochondrial activity of the cells, where a high red/green fluorescence ratio indicates cells with active mitochondria.
JC-1 is available as a kit and the labeling and assay conditions were optimized for mammalian cultured cells. In this work, we are questioning the conditions of the JC-1 probe use in order to accurately estimate the quality of rainbow trout sperm mitochondria. We carried out various cell labelling tests with JC-1, namely probe concentration, incubation temperature and duration. The fluorescent profiles of the spermatozoa were assessed by flow cytometry and controlled on a fluorescent microscope. We observed that JC-1 concentrations above 0.5 µM induced spermatozoa aggregation, thereby impairing measurement reliability. On the contrary, incubation temperature and duration (20-37 °C and 10-30 min) had little effect on the spermatozoa labeling pattern and we could obtain homogenous cell populations with intense red labelling and stable red/green ratio. The next step was to assess how changes in mitochondrial quality would be conveyed through changes in JC-1 labeling pattern. Various conditions were tested including sperm cold storage (7 days), cryopreservation, and chemical decoupling using the oxidative phosphorylation uncoupler CCCP. The obtained patterns were confusing, either in flow cytometry or microscopy, and the canonical red/green ration did not always faithfully report the changes in cell quality. We discussed that the unique mitochondria behavior in trout spermatozoa may yield a specific response to JC-1 labeling.

Funding: French CRB Anim project ANR-11-INBS-0003