Exploring Oxylipid Changes in Human Sperm During Cryopreservation"

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Reactive oxygen species (ROS) have been associated with decreased sperm motility and a high number of defective sperm in the ejaculate. Sperm membranes contain an abundance of polyunsaturated fatty acids (PUFAs), making them especially susceptible to lipid peroxidation. ROS can activate phospholipases, leading to the cleavage and liberation of PUFAs from membrane phospholipids, which can then be oxidized via a combination of non-enzymatic and enzymatic reactions. Signaling by oxygenated derivatives of free PUFAs, also known as bioactive lipids, regulates intercellular communications and plays pivotal roles in signal transduction, inflammation, cell death, and disease. During sperm cryopreservation, there is a burst of ROS that impacts sperm membranes. The study of the oxylipidomic profile may reflect the functional status of sperm cells. This study aimed to detect and quantify changes in the levels of oxylipids during the cryopreservation of human semen samples.

To accomplish our objective, semen samples from normozoospermic sperm donors (n=15) were cryopreserved. Routine semen analysis and semen lipidomics were performed before cryopreservation and after thawing. Briefly, the fresh sample was divided into two aliquots. One aliquot was frozen with SpermFreeze™ (Fertipro, Belgium) according to the manufacturer's instructions. After 72 hours, samples were thawed at 37°C for 5 minutes. Lipids from fresh and cryopreserved-thawed samples were extracted using the hexane method followed by solid-phase extraction to obtain the free fatty acid fraction. Oxylipids present in those samples were analyzed by liquid chromatography–mass spectrometry (LC-MS/MS). T-tests were applied to compare both groups ($p< 0.05$). Enrichment pathway analysis was performed using MetaboAnalyst 6.0.

The oxylipidomic analysis showed differences in the lipid profile in post-thawed samples. In particular, the enrichment analysis exhibited an activation of the arachidonic acid pathway, with the following oxylipids levels statistically increased after thawing: 12-oxo-eicosatetraenoic acid (12-oxoETE), Prostaglandin E2 (PGE2),

and Isoprostanes F2α IV 5-iso PGF2). There was a decrease in the concentrations of 11-hydroxy-eicosatetraenoic acid (11-HETE), 15-hydroxy-eicosatetraenoic acid (15-HETE), and Prostaglandin F2α (PGF2 α), indicating an activation of pro-inflammatory pathways during cryopreservation. At the same time, the concentration of components of the eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) pathway increased (Resolvin D3 and (RvD3), Resolvin E1 (RvE1)) ($p < 0.05$). In addition, a small activation of the linoleic pathway was observed. The modifications of the oxylipid pattern did not correlate with the sperm parameters, as expected in a group of normozoospermic men.

We conclude that LC-MS/MS is a sensitive method to detect modifications of the lipid peroxidation status during sperm cryopreservation. A change in the lipidomic phenotype was observed in cryopreserved-thawed samples. Although a prevalence of pro-inflammatory lipids was detected in the cryopreserved-thawed group, resolvins were also detected. The latter are PUFA that may represent specialized pro-resolving mediators in tissue injuries, but their function is not known in sperm physiology. The study of biolipids (oxylipidomics) in sperm under different experimental conditions may be exploited to develop lipid-targeted therapies and strategies to protect cell membranes against oxidative attack.