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ROS generation by arachidonic acid in cat frozen semen

Freitas-Dell’Aqua, CP\(^A\); Ackermann, C L\(^A\); Costa, TA\(^A\); Papa, FO\(^A\); Lopes MD\(^A\)

\(^A\)Department of Animal Reproduction and Veterinary Radiology, FMVZ, UNESP, Botucatu – Brazil.
cpaulafreitas@fmvz.unesp.br

The measurement of ROS generation by frozen-thawed semen is an important investigative tool in the diagnosis of semen quality. Another important aspect is the type, origin and main role of the different ROS that can be formed during sperm metabolism. Thus, the aim of this work was to determine the sensitivity of different ROS detection tests and to compare their ability in determining ROS generation in different cellular compartments. In this study, we utilized arachidonic acid (AA) which has been shown to be an activator of mitochondrial ROS in human spermatozoa (1) and had no impact on any aspect of sperm motility and sperm vitality (2). Six pool (n= 3 animals) containing 3 samples from the same cat each pool. And one pool containing 1 sample from each cat and split in three sample, each one was exposed to 0, 25 or 50µM AA at 37°C for 15min to induce cellular ROS generation. At the end of the incubation period, the cells were washed by centrifugation (300g/5min, 2 times) The samples were split in 4 tubes and each tube was stained with: A:MitoSox Red (MSR, M36008) to assess superoxide (O\(_2^\cdot\)) production in mitochondrial matrix; B:DHE (D23107; Dihydroethidium) to detect O\(_2^\cdot\) intracellular generation; C:CM-H2DCFDA (C6827; Chloromethyl derivative of H\(_2\)DCFDA) has been used to detect the generation of hydrogen peroxide (H\(_2\)O\(_2\)) and peroxynitrite (ONOO\(^{-}\)); and D:C11Bodipy (D3861) for lipid peroxidation. The results were evaluated by GraphPadInstat 5.0 software (San Diego, CA, USA) and expressed as mean and standard error. Data were subjected to the Kolmogorov-Smirnov test to check for normality. For the averages obtained with normal distribution, one-way ANOVA follow by Tukey was used. For results without normal distribution the nonparametric Kruskal-Wallis test followed by Dunn test was applied. The generation of ROS was indicated by a highly significant and dose-dependent stimulation for DHE (77.7±1.2 vs 84.11±1.5 vs 85.3±2.3); C11Bodipy (41.6±10.0 vs 75.7±7.9 vs 84.0±5.7); and CM-H2DCFDA (10.1±2.4 vs 30.0±12.0 vs 32.6±11.0). A less significant but dose-dependent increase in the MSR (73.23±1.5 vs 74.8±1.8 vs 79.7±1.5). These results showed that the ROS signals generated in the presence of AA was significant and dose-dependent in all tests; DHE, C11Bodipy and CM-H2DCFDA were more responsive to AA than MSR. This may happened due to large amounts of ROS produced during the cryopreservation process, and mitochondria are especially sensitive to changes induced by sperm cryopreservation (3), this way, the sensitivity of MSR test may have been diminish since the control sample had high ROS generation.

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