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OXIDATIVE STRESS IN VIABLE SPERMATOZOA: DETECTION IN NATIVE SEMEN SAMPLES

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Abstract

Oxidative stress is involved in many known causes of impaired sperm function. It is of great importance to have methods able to detect oxidative stress in native semen samples to be used in male infertility work-up. In this study, we challenged three probes for sperm ROS generation: CM-H2DCFDA, CellROX Green and MitoSOX Red. Each probe was coupled by flow cytometry to a suitable stain to exclude non-sperm elements and focus viable spermatozoa, the clinically most relevant sperm fraction. We found that the three probes have different cell localization: in the midpiece (CM-H2DCFDA) and in the head of spermatozoa (CellROX Green and MitoSOX Red). In addition, H_2O_2 treatment highly increased MitoSOX Red fluorescence (14.18±4.6 vs 31.13±9.74, %, $p<0.01$), but not, or only slightly, the labelling with CM-H2DCFDA and CellROX Green, respectively. Menadione treatment highly increased CellROX Green (1.98±0.28 vs 7.71±4.53, mean fluorescence intensity, $p<0.05$) and MitoSOX Red fluorescence (20.38±10.31 vs 65.34±32.34, %, $p<0.05$), but not that one of CM-H2DCFDA. Finally, only MitoSOX Red resulted able to detect spontaneous sperm ROS generation during a short-term in vitro incubation. We also found that MitoSOX labelling is strictly associated to sperm DNA fragmentation, as assessed in sorted spermatozoa by Comet and SCD Assay. In conclusion, the three probes showed different localization and specificity in human spermatozoa. MitoSOX Red appears a probe able to detect both spontaneous and H_2O_2- and menadione-induced ROS production. Labelling with this probe also identifies a viable sperm fraction susceptible to sDF, a clinically important semen parameter.

Key words: Native semen, Flow cytometry, Oxidative stress, DNA fragmentation.