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Utility of the Nitroblue Tetrazolium Reduction Test for Assessment of Reactive Oxygen Species Production by Seminal Leukocytes and Spermatozoa

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The purpose of this study was to evaluate the ability of spermatozoa and leukocytes in semen to produce reactive oxygen species (ROS) by using nitroblue tetrazolium (NBT) staining and to examine the association between NBT staining and levels of ROS as measured by chemiluminescence. Twenty-one infertility patients (leukocytospermia; n = 8; nonleukocytospermia, n = 13) and 9 healthy donors were included. Standard semen analysis and density gradient centrifugation were performed to test NBT staining, ROS, and total antioxidant capacity. A ROS-total antioxidant capacity (ROS-TAC) score was calculated by using principal component analysis. In the leukocytospermic group, after separation on a density gradient, the percentage of NBT-positive staining was significantly higher in sperm suspensions contaminated with leukocytes (median [25th, 75th percentiles]; 70% [61%, 79%]) compared to the nonleukocytospermic group (14.5% [9%, 25.5%]; P = .03) and donors (7% [3%, 11%]; P = .02), respectively. A strong positive correlation was seen between levels of ROS in whole ejaculates and NBT-positive staining in leukocytes (r = 0.59; P < .0006) and in leukocyte fractions (r = 0.72; P < .0001) after density gradient separation. Similarly, ROS was positively correlated with excessive cytoplasmic retention in spermatozoa from whole ejaculates and abnormal spermatozoa after separation on density gradients (r = 0.72; P < .0001). The ROS-TAC score was inversely correlated with NBT staining in leukocytes in whole ejaculates (r = -0.960, P < .0007) and in both leukocyte fractions (r = -0.39; P < .04) and spermatozoa with cytoplasmic retention (r = -0.38; P < .04). Our results indicate that the NBT reduction test can be used to assess the contribution of seminal leukocytes and defective spermatozoa towards ROS generation in semen. Levels of ROS assessed by chemiluminescence assay are strongly correlated with the results of NBT staining.

Key words: Male infertility, oxidative stress

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