MEASUREMENT OF ROS LEVELS IN NEAT SEMEN FOR ASSESSMENT OF SEMINAL OXIDATIVE STRESS STATUS

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Introduction and Objective: Reactive oxygen species (ROS) are routinely measured in washed and neat semen samples for both diagnostic and research purposes. There are concerns that multiple centrifugation, resuspension, and vortexing steps involved in washing may increase ROS levels. Therefore, measurement of ROS levels in washed sperm may not accurately diagnose the presence of oxidative stress (OS). The aims of our study were: 1) to define the basal levels of ROS in normal healthy volunteers in neat semen samples, and 2) to compare ROS levels in the neat and washed semen samples. Methods: Healthy volunteers were selected on the basis of normal standard semen parameters according to WHO guidelines. Donors with leukocytospermia (>1X10^6 WBCs/mL seminal ejaculate) were not included in the study. ROS levels were measured directly in the neat semen samples (n = 34) following liquefaction while the seminal antioxidant protection was still present. These samples, were then centrifuged at 300 g for 7 min to remove the seminal plasma. The sperm pellet was washed with phosphate-buffered saline and resuspended to 1ml volume. ROS levels were determined in the washed sperm suspension by chemiluminescence assay using luminol (5mM) as the probe. Measurements were made using a Berthold luminometer (Autolumat LB 953, Wallac inc., Gaithersburg, MD) in the integrated mode for 15 minutes. The results were expressed as X 10^6 counted photons per minute (cpm) per 20 million sperm. Results: The median and interquartile values (25th and 75th percentiles) of ROS in neat and washed specimens were 0.029 (0.008, 0.13) and 0.112 (0.077, 0.407). The 95% confidence intervals for ROS levels in neat semen were 0.051 – 0.277. The levels of ROS were significantly lower in neat semen samples as compared to washed specimens (P = 0.002). Conclusions: Sperm washing and removal of the seminal plasma increases ROS levels. We recommend measurement of ROS in neat semen samples rather than in washed semen samples as it provides an accurate estimation of the oxidative stress (OS) status of an individual. We emphasize the need for minimal preparation of a semen specimen before use for ART procedure. The reference values of seminal oxidative stress may be used in diagnosing and treating infertile men with pathological levels of OS.

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