DEFINING REACTIVE OXYGEN SPECIES (ROS) LEVELS IN MATUR AND IMMATURE SPERMATOZOA IN HEALTHY VOLUNTEERS

SSR Allamaneni, KP Nallella, TM Said, RK Sharma, AJ Thomas, Jr, A. Agarwal, The Cleveland Clinic Foundation, Cleveland, OH

The production of reactive oxygen species (ROS) by spermatozoa is a normal physiological process. Excessive generation of ROS by abnormal and immature spermatozoa is harmful to the fertilizing potential. Although ROS levels can be measured directly in the whole ejaculate or after simple wash and re-suspension, there is a wide-spread concern that leukocytes present in the semen may generate additional ROS and induce damage to the sperm cell. The majority of sperm preparation methods used for various assisted reproduction procedures use mature spermatozoa free from leukocyte contamination. Our objective was to establish the basal levels of ROS in mature and immature spermatozoa in a group of normal healthy men after double density gradient technique. Thirty-two healthy donors were selected on the basis of normal standard semen parameters according to WHO guidelines. Men with leukocytospermia (>1X10⁶ WBCs/mL seminal ejaculate) were excluded. Samples were processed using the double-density gradient technique (47% and 90%). ROS levels were determined in both mature and immature fractions by chemiluminescence assay using luminol. The results were expressed as X 10⁶ counted photons per minute (cpm) per 20 million sperm. ROS levels were significantly higher in the immature fraction compared to mature spermatozoa: median (25th and 75th percentiles) 0.066 (0.015, 0.156) versus 0.021 (0.004, 0.075) (P = 0.01). In conclusion, we have defined the normal levels of ROS in mature and immature spermatozoal fractions in normal healthy men. These reference values may be used to determine pathological levels of ROS in patients examined for infertility and may be relevant in selecting semen samples for ART procedures.