

EFFECT OF TIME AND SPERM CONCENTRATION ON REACTIVE OXYGEN SPECIES PRODUCTION BY HUMAN SPERMATOZOA

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Reactive oxygen species (ROS) have been implicated to play an important role in male infertility. ROS measurement by chemiluminescence assay using luminol as the probe is a simple and accurate method. The current protocols for ROS measurement are unclear about the optimum time period when the measurement is to be done following the specimen production. In addition, the optimum sperm concentration for the chemiluminescence assay is not defined. A concentration of 20×10^6 used generally for measuring ROS cannot be obtained in oligospermic patients. The purpose of our study was to examine the effect of time (age of the sample), and sperm concentration on ROS generation.

Semen specimens were obtained from 12 normal, healthy men according to the WHO criteria. Liquefied samples were split into 4 aliquots to assess the effect of time and sperm concentration on ROS production. Each aliquot was washed in PBS buffer and adjusted to a final concentration of 20×10^6 spermatozoa. ROS production was measured at 60, 100, 180 and 240 minutes after specimen collection. For this purpose, 10:L of 5mM luminol was added to 400:L of the sample and the luminescence measured for 15 min. using a luminometer (LB 953 Berthold, Wallac, Gaithersburg, MD). Effect of sperm concentration on ROS production was assessed by first washing the sample and then diluting the pellet into 8 different sperm concentrations ranging from 1 to 120×10^6 .

ROS levels decreased significantly over time; a dramatic decline in ROS production was seen in specimens over 60 min. in age ($P < 0.001$). ROS values on average decreased by 31% at 120 min. and 62% at 180 min. compared to 60 min. old specimen. A linear relationship was seen between the amount of ROS production and sperm concentration. Sperm concentration had no confounding effect on ROS levels when adjusted to 20 million/mL ($P = 0.60$).

ROS production decreases with specimen age. ROS levels can be measured accurately within 60 min. of specimen collection. Linear relationship between ROS production and sperm concentration allows measurement of ROS in specimens with greater than 1×10^6 spermatozoa/mL.

