THE EFFECT OF TIME ON REACTIVE OXYGEN SPECIES (ROS) LEVEL IN HUMAN SEMEN. M Shekarriz, AJ Thomas and A Agarwal, Andrology Laboratory, Department of Urology, Cleveland Clinic Foundation, Cleveland, OH 44195,

Objectives: Recent methods of ROS assessment in human semen lack a standardized protocol. Although, generally accepted standards exist for routine semen analysis, it is so far unclear, how the time interval between semen collection and measurement of ROS affects its level in semen. The purpose of this study was to investigate the correlation between the level of ROS and the time interval between collection and analysis of specimens.

Design: Semen specimens from 20 men who were consulting for infertility evaluation were used for routine semen analysis and ROS measurement during a 24 hours time interval after semen collection.

Materials and Methods: The white blood cell (WBCs) were quantitated in semen specimens by Endtz test. Significant leukocytospermia (Endtz positive) was defined as greater than 1X10⁶ WBCs/mL. The sperm concentration was adjusted to 20X10⁶/mL in all Endtz negative specimens. The formation of ROS in the whole semen was measured by the chemiluminescence method using a Berthold luminometer. The ROS formation was regarded as positive when a value was >10X10³ counted photons per minutes (CPM). ROS measurement and semen analysis were performed after one hour, three hours, five hours and 24 hours respectively. Specimens were preserved in incubator at 37°C during the experiment.

Results: There was a significant decrease in ROS formation as well as sperm motility with time. The mean ROS level was: 343.4X10⁴ CPM (one hour), 133.5X10⁴ CPM (three hours, p<0.004), 66.0X10⁴ CPM (five hours, p<0.001) and 22.2X10⁴ CPM (24 hours, p<0.001) respectively. Fourteen specimens were initially positive for ROS formation. Four out of 14 specimens were also positive for Endtz test (>1X10⁶ WBC/mL). The number of positive specimens after three, five and 24 hours was: eight, six and two respectively.

Conclusions: The results of this study indicate the importance of time as a variable for assessment of ROS levels in human semen. The decrease in sperm motility with the time may explain the decrease in ROS level. A significant decrease in ROS with the time was also seen in Endtz-positive specimens. This may be due to a decline in WBCs activity. In summary, in order to allow comparison of results from different studies, ROS measurement using chemiluminescence method should be performed within an hour after semen collection.