Excessive ROS formation in human semen has been associated with impaired sperm function and fertility potential in some men. The presence of ROS in semen specimens from normal, fertile men emphasizes the importance of defining a normal range of ROS formation. The purpose of this study was to establish a normal range of ROS generation and to investigate the effect of sperm concentration on the level of ROS. ROS was determined in a population of 15 healthy donors. The sperm concentration was adjusted to 20X10^6/mL in all 15 donor specimens. ROS was measured by chemiluminescence method using luminol in a Berthold luminometer and was regarded as positive when the value was ≥ 10X10^4 cpm (counted photons per minute). All 15 donor specimens were negative for ROS formation. The range of ROS formation in 15 donors was 0.0-5.5X10^4 cpm with a mean of 1.1X10^4 cpm. In addition, ROS formation was evaluated in 14 patients at three different sperm concentrations (60, 30 and 15X10^6/mL). Eight patients were positive for ROS formation. The level of ROS in these specimens showed a positive correlation with the sperm concentration. ROS levels were significantly lower at a sperm concentration of 15X10^6/mL as compared to 30 or 60X10^6/mL (p=0.03). Our results indicate that a ROS formation of 0.0-5.5X10^4 at a sperm concentration of 20X10^6/mL may be considered as normal for healthy donor semen. The positive correlation of ROS formation with the sperm concentration at the time of measurement emphasizes the importance of concentration adjustment prior to analysis. ROS levels will change significantly at different sperm concentrations from the same specimen. ROS measurements using chemiluminescence method should be performed at a standard sperm concentration in order to allow comparison of results from different studies.