Quality control of reactive oxygen species measurements
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Objectives: Elevated levels of reactive oxygen species (ROS) play an important role in the etiology of male infertility. Quality control of chemiluminescence assay used to measure ROS production is important in evaluating the changes not only within the assay but also among the various observers. The purpose of our study was to evaluate the reliability of ROS measurement performed by assessing the inter-observer, intra-observer, and intra-assay variability.

Design: Six healthy donors provided semen samples by masturbation after 2 to 3 days of sexual abstinence.

Materials and Methods: After liquefaction, semen specimens were processed for ROS measurement. Briefly, samples were centrifuged at 300g for 7 minutes and the seminal plasma was removed. Pellet was suspended in 3mL of PBS buffer and washed again at 300g for 7 min. The sperm concentration was adjusted to 20 X10^6 sperm/mL after centrifugation. ROS levels were measured by chemiluminescence method using luminol as the probe in a Berthold luminometer. Each sample was measured in duplicate by three different observers. In addition, each donor sample was measured 3 times over a 3-week period. After log transformation of the ROS results, variance components were calculated using random effects ANOVA to compute reliability (inter-class correlations).

Results: Average log ROS (ROS + 1) levels for donor sample’s was 0.218 with an inter-assay variability of 0.077 (SD). The inter-observer standard deviation was 0.002 for an inter-observer reliability of 97.5% (CV = 0.9%). The intra-observer (between replication) standard deviation was 0.001, for an inter-observer reliability of 98.7% (CV = 0.5%). The intra-assay standard deviation was 0.005 for an intra-assay reliability of 93.8% (CV = 2%). There was no significant inter-observer, intra-observer, inter- or intra-assay variation (P> 0.80).

Conclusions: Our results demonstrate that the chemiluminescence method for ROS measurement is both accurate and reliable. Seminal ROS levels can be measured easily in a clinical laboratory as a part of an infertility work-up. This project was supported by a grant from the Cleveland Clinic Foundation.