AN ACCURATE AND RELIABLE METHOD FOR THE DIAGNOSIS OF SEMINAL OXIDATIVE STRESS IN INFERTILE MEN
Ramadan A. Saleh, Navid Esfandiari, Saad S. Al-Dujaily, Rakesh K. Sharma, Anthony J. Thomas, Ashok Agarwal,
The Cleveland Clinic Foundation

Objective:
There is a lack of consensus in the literature regarding the cut-off values of seminal oxidative stress (OS) despite a strong correlation between OS and male infertility. Since OS in semen is the result of an imbalance between reactive oxygen species (ROS) production and antioxidant strategies, we hypothesized that levels of ROS in neat semen of men undergoing fertility evaluation may reflect OS status in vivo. The objectives of this study were to 1) establish a cut-off value of seminal OS by measurement of ROS levels directly in neat semen in a group of normal healthy donors (controls) and comparing the results with a population of infertile men, and 2) evaluate the characteristics of laboratory variability of ROS measurement in neat semen.

Design:
Prospective study in a male infertility clinic.

Materials/Methods:
Semen samples were obtained from normal donors (n = 9) and from infertile men (n = 34) after 2-3 days of sexual abstinence. Donors were selected on the basis of normal semen parameters and negative Endtz (\(\leq 1 \times 10^6\) WBCs/mL) as per World Health Organization guidelines. Levels of ROS were determined in neat semen, within one hour of sample collection, by a chemiluminescence assay using luminol as a probe. Results were expressed as \(10^4\) counted photons per minute (cpm)/20 \(10^6\) sperm/mL. ROS measurement in neat semen from normal donors were tested for intra-assay (running the same sample in duplicate or triplicate), inter-assay (same sample observed on 3 successive weeks by the same observer), inter-observer (multiple observers with the same sample) variability.

Results:
Seminal ROS levels [median (25%, 75% inter-quartile ranges)] in donors [0.3 (0.2, 0.9) \(10^4\) cpm] were significantly lower than in patients [5.8 (1.0, 81.0) \(10^4\) cpm, \(P = 0.0037\)]. The cut-off value for seminal OS was 1.5 \(10^4\) cpm based on maximum levels of ROS in neat semen of normal donors. Sixty-eight percent (23/34) of patients were classified as OS-positive based on ROS levels >1.5 \(10^4\), while 32% (11/34) were classified as OS-negative (\(\leq 1.5 \times 10^4\)). The inter-donor variability [standard deviation (SD)] was 0.001. The intra-assay SD was 0.0004 with an intra-assay reliability of 99% [coefficient of variation (CV) = 17%]. The inter-assay SD was 0.0004 with an inter-assay reliability of 96% (CV = 18%). The inter-observer SD was 0.0006 with an inter-observer reliability of 88% (CV = 30%).

Conclusions:
Our results indicate that measurement of ROS in neat semen is easy, accurate and reliable method for in vivo assessment of OS status. Based on the newly established cut-off values of seminal OS, men undergoing fertility evaluation can be classified as OS-positive or negative irrespective of their clinical diagnosis or semen characteristics. This test for OS can be incorporated into routine laboratory practice as an independent tool, which may help in the assessment of the fertility status of the male partner.

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