Reactive oxygen species levels are independent of sperm concentration, motility, and abstinence in a normal, healthy, proven fertile man: a longitudinal study

In a longitudinal study over a period of 21 months, we demonstrated that seminal reactive oxygen species (ROS) levels are independent of sperm concentration, motility, and abstinence duration within a healthy sperm donor, although some variations were observed in ROS levels. We suggest that fluctuation in seminal ROS values may be related to physiologic or transient changes in spermatogenesis. (Fertil Steril® 2010;94:1541–3. ©2010 by American Society for Reproductive Medicine.)

Reactive oxygen species (ROS) generated by spermatozoa play an important role in normal physiologic processes, such as sperm capacitation, acrosome reaction, oocyte fusion, and stabilization of the mitochondrial capsule in the midpiece (1, 2). ROS produced by spermatozoa and leukocytes are scavenged by various antioxidants in the seminal plasma. Uncontrolled production of ROS that exceeds the antioxidant capacity of the seminal plasma leads to oxidative stress (OS), which is harmful to spermatozoa (1). In addition, ROS is an independent marker of male factor infertility. ROS measurement by chemiluminescence method is a highly sensitive and accurate method, with low interobserver and intraobserver variability (2–4).

Levels of ROS have been correlated with semen parameters; in all of these studies, a single ejaculate from each subject was examined at a single time point (2, 3). However, longitudinal studies describing the association of ROS levels with semen parameters within a fertile man are lacking. Our aim was to conduct a longitudinal study and monitor within individual variations in ROS levels and correlate this with semen parameters.

This study was approved by the Cleveland Clinic Institutional Review Board. A single normal healthy proven fertile man provided 36 semen samples over a period of 21 months (from October 2006 to June 2008) with an abstinence of 1–10 days.

Following complete liquefaction at 37°C for 20 minutes, 5 µL of each specimen was loaded on a 20-µL Microcell chamber (Conception Technologies, San Diego, CA) and analyzed for sperm concentration and motility according to World Health Organization guidelines (5). Seminal smears were stained with Diff-Quick (Baxter Healthcare, McGraw Park, IL), and sperm morphology was assessed using WHO criteria (5).

The presence of white blood cells (WBCs) in the specimen was detected by the Endtz test (2). Briefly, 20 µL of the liquefied specimen was placed in a 0.5-mL sample cup, and 20 µL Tyrode buffer and 40 µL benzidine solution were added. The mixture was vortexed and allowed to sit at room temperature for 5 minutes. Peroxidase-positive WBCs, which stain dark brown, were counted in all 100 squares of the grid in a Makler chamber under a ×10 brightfield microscope. Results were recorded as ×10⁶ wbc/mL of semen.

Seminal ejaculates that had not undergone any additional processing (neat samples) were used for ROS measurement by chemiluminescence assay using luminol (5-amino-2,3-dihydro-1,4-phthalazinedione; Sigma Chemical Co., St. Louis, MO). A 100-nmOL/L stock solution of luminol was prepared in dimethyl sulfoxide (DMSO). For the analysis, 10 µL of the working solution (5 mmol/L) was added to 400 µL of neat sperm sample. Chemiluminescence was measured for 15 minutes using a Berthold luminometer (LB 953; Berthold, Bad-Wildbad, Germany). Results were expressed as ×10⁶ counted photons per minute (cpm)/20 × 10⁶/mL sperm (p=.01) (4). ROS levels >0.0185 were considered to be positive based on our lab cutoff value. Data analysis and
correlation studies were done by JMP software version 7.0 for windows. A P value of < .05 was considered to be significant.

Sperm concentration (mean ± SD) was 106.3 ± 36.3 × 10^6/mL and motility 71.6 ± 7.96%. ROS levels ranged from 0.00 to 0.06 × 10^6 cpm/20 × 10^6 sperm. The mean ROS level was 0.0122 ± 0.0137 U (95% CI 0.0169 U). Coefficient of variance for sperm concentration, motility, and ROS were 30%, 10%, and 110%, respectively. Correlation analysis demonstrated no significant correlation between ROS levels and sperm parameters. The fluctuations in sperm motility, concentration, and ROS are shown in Figure 1. A complete semen analysis, including the presence of round cells, WBCs, and sperm morphology, was evaluated only during the first visit, and it was within normal range according to the WHO criteria.

Semen analysis is one of the important clinical laboratory tests to evaluate male infertility, and intra-individual variations in the semen parameters have been reported (6). Reports from our center have shown that measurement of ROS level should be included in routine semen analysis (2, 7, 8). Previously, we have reported that ROS cutoff value of 0.0185 × 10^6 cpm/20 × 10^6 sperm is highly predictive of infertility (2). However, monitoring ROS level in a fertile man and studying correlation between ROS level and sperm parameters are extremely important before oxidative stress profile can be included to evaluate infertile men.

In the present study over a period of 21 months, we did not find any significant (positive or negative) correlation of ROS levels with sperm concentration and motility over a period of time within one individual. No significant correlation of motility and concentration with ROS suggests that ROS is an independent marker of fertility in healthy fertile donor.

In the present study on a single healthy man, incidence of ROS-positive seminal ejaculates was 13.8% (5 out of 36 samples). Despite positive ROS (5 samples), all (36) samples tested by Endtz test were negative (meaning an absence of any detectable peroxide-positive leukocytes in the specimens) (3). In all of these instances, sperm motility and concentration were not correlated with increased levels of ROS. Previously, we reported that sperm motility and concentration are inversely correlated with ROS levels in infertile men. On the other hand, earlier studies have linked ROS in various physiologic processes of spermatozoa (i.e., sperm capacitation, acrosome reaction, oocyte fusion, and stabilization of the mitochondrial capsule in the midpiece). Therefore, we suggest that levels of ROS may rarely fluctuate within a fertile man but do not affect sperm concentration and motility. This may be possible due to the presence of adequate antioxidant defense mechanisms in the present healthy individual. These fluctuations in ROS might also be due to physiologic process. Earlier reports have also suggested that there might be seasonal variation in ROS level in semen (9–11). Therefore, we suggest that fluctuation in ROS value may be physiologic or due to transient subclinical infection, transient abnormalities in spermatogenesis, such as retention of cytoplasm, or periodic presence of abnormal spermatozoa in semen.

In conclusion, we demonstrated that within a normal healthy individual, although variations in the ROS levels are seen over an extended period, ROS levels are independent of concentration, motility, and abstinence period. The results show seminal ROS variation in a healthy individual of proven fertility that is different from the sperm concentration or sperm motility. This variation may have physiologic, seasonal, or lifestyle-related causes. However, we measured morphology and leukocyte counts in only the first specimen from this fertile donor. We did not measure morphology and leukocyte counts in subsequent specimens. Therefore, further studies on many fertile donors over a long period of time are necessary to explore this phenomenon.

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REFERENCES


