

Oxidative Stress and Male Infertility: What is New in the Laboratory?

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Mammalian spermatozoa generate a variety of reactive oxygen species (ROS), which are thought to play a role during sperm capacitation, acrosome reaction, and oocyte fusion. However, oxidative stress (OS) occurs if the generation of ROS overwhelms the body's limited antioxidant defenses. Infertility is the main complication associated with high levels of OS in the seminal fluid. Patients with idiopathic infertility present with significantly higher seminal ROS levels than fertile men. It is evident that OS is an independent measure of sperm quality.

The process by which spermatozoa produce ROS is not fully understood. Immature spermatozoa contain excessive cytoplasm containing enzymes such as NADPH oxidase, which may be responsible for ROS production. Recently, we prospectively evaluated its role in ROS generation.

Semen was collected from healthy donors and from patients undergoing infertility evaluation. The specimens were assessed for sperm concentration, motility, morphology, and WBCs. Density gradient separated spermatozoa were used to assess ROS levels. Lucigenin and luminol were used as probes before and after incubation with NADPH.

When lucigenin was used, basal levels of ROS ($X 10^6$ cpm / $20 X 10^6$ sperm) were significantly higher in patients with male infertility than in the healthy donors [0.73 (0.5, 5.5) vs. 0.20 (0.0, 0.5); $P = 0.03$]. Compared with basal levels, $O_2^{\cdot -}$ generation was significantly higher after incubation with NADPH (5 mM and 10 mM) in the whole study population ($P < 0.001$; $P < 0.001$). Levels of ROS pre- and post-NADPH incubation are summarized in the table. The overall increase in ROS generation was detected with both 5 mM and 10 mM

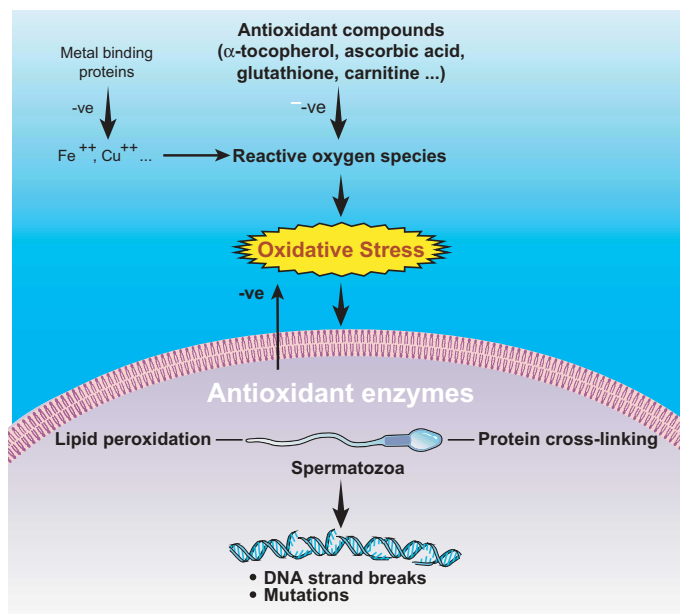
Effect of Exogenous NADPH on Levels of Lucigenin Detectable Superoxide

	Subjects (n = 17)	P ^a
NADPH (5mM)	-13.70 (-35.09, -6.45)	< 0.001
NADPH (10mM)	-18.61 (-52.80, -8.99)	< 0.001

Results are expressed as median (25th and 75th percentile)

ROS levels expressed as $X 10^6$ counted photons per minute (cpm) / $20 X 10^6$ sperm and represent the within-subject differences between post-incubation and pre-incubation values

^aWilcoxon sign rank test (comparing the values in absence and in presence of NADPH); $P < 0.025$ was considered significant.



Mechanisms of oxidative stress-induced damage to human spermatozoa.

of NADPH but only when lucigenin was used as a probe. $O_2^{\cdot -}$ generation was negatively correlated with sperm concentration ($r = -0.75$, 95% CI: 0.38, 1), motility ($r = -0.69$, 95% CI: 0.28, 1), and % normal morphology ($r = -0.78$, 95% CI: 0.36, 1).

Chemiluminescent signals were higher in the infertile patient population than in healthy donors, suggesting poor quality spermatozoa from patients with male infertility have deranged redox metabolic activity and a greater ability to produce $O_2^{\cdot -}$. Our findings support that NADPH, which is present in the residual sperm cytoplasm of the mid-

piece, plays an important role in $O_2^{\cdot -}$ production and can result in the production of increased levels of such toxic oxygen radicals (illustration). Because the overall detection was possible using lucigenin only, which specifically detects $O_2^{\cdot -}$ it appears that the impact of NADPH on the levels of free radical generation by the spermatozoa is solely due to $O_2^{\cdot -}$.

In conclusion, it appears that NADPH in human spermatozoa mediates ROS production, specifically the superoxide anion. Thus, when measuring levels of OS in semen from infertile patients, the superoxide anion should be assessed.