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Assessment of oxidative stress levels in semen using spectroscopy-based metabolomic profiling: implications in male infertility

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Objective: Complex interactions between the pro-oxidants and antioxidants are crucial in the maintenance of intracellular homeostasis. An imbalance in these reactions results in oxidative stress (OS), which is known to affect the quality of spermatozoa, oocytes and embryos. Analytical methods that assess reactive oxygen species and OS rely primarily on biochemical methodologies that are cumbersome and labor intensive. In this pilot study we used a novel, non-invasive method of spectroscopy-based metabolomic profiling of biomarkers of OS to assess semen samples from 4 groups of patients and controls evaluated for their infertility.

Materials and Methods: Semen specimens were collected from 4 groups of patients: group I: varicocele (n = 70); group II: idiopathic male factor patients (n = 15); group III: vasectomy reversal (n = 9); group IV: female factor patients (n = 9). Control group consisted of 30 healthy donors of unknown fertility status. Seminal plasma was aliquoted and frozen at -80°C. Prior to analysis, 5µL of sample was diluted 1:3 in milliQ water and analyzed for specific biomarkers of OS at selected wavelengths by Near Infrared Spectroscopy (NIR). Each sample spectrum was subjected to statistical analysis using proprietary and non-proprietary chemometrics and bioinformatics (Molecular Biometrics, LLC, Chester, NJ). Total spectral analysis and bioinformatics analysis time is about one minute. The resulting data were first separated statistically into principle components and then represented as a self-organized twodimensional map showing the proximity of the obtained spectra relative to one another. Likewise, means for each of the groups were determined to examine where differences in the spectra were most significant.

Results: All five groups of semen specimens showed unique spectral "signatures" that were statistically different from each other, illustrating different levels of OS as indicated by unique changes in the ratios of -CH to ROH biomarker bonding. Likewise, significant differences in the -CH, -NH and -OH concentrations were observed. By contrast, spectra from Group I patients (varicocele) were broadly distributed among all groups and did not segregate as a separate population with uniquely identifiable biomarker characteristics.

Conclusion: Metabolomic profiling of semen, using NIR spectroscopy and proprietary chemometrics and bioinformatics, is a useful tool that may be used to identify different levels of OS. These are uniquely associated with normal spermatozoa compared to different forms of male factor infertility or sperm quality. The technology platform of metabolomic profiling may provide a rapid, non-invasive and cost-effective diagnostic method of analyzing semen for abnormalities related to ROS damage and oxidative stress.