

## Assessing Oxidative Stress Levels in Semen Using Spectroscopy-Based Metabolomic Profiling: Implications in Male Infertility

Ashok Agarwal, Ph.D., H.C.L.D.

Free radicals such as ROS exert their effect at the molecular level in all cell types and play a role in both physiological and pathological functions. 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). Biomarkers of OS have been found in the male and female reproductive tracts and are known to affect the quality of gametes, early embryo development and implantation, which, in turn, affects pregnancy. Spermatozoa used for insemination in assisted reproduction techniques (ART) are likely to be exposed to ROS, which can cause extensive DNA damage. Thus, OS has been implicated in the etiology of different forms of male factor infertility.

We evaluated a novel technology platform (Molecular Biometrics, LLC, Chester, N.J.) to assess OS based on the confluence of two scientific disciplines: 1) biospectroscopy, or the applications of different forms of spectral analysis to identify, quantify and validate small proteomic and molecular biomarkers; and 2) metabolomics, the science that examines and integrates the dynamic interplay between the inventory of small molecule biomarkers at the cellular level that characterize complex biological processes and functions. Biospectroscopy is used to quantify a sample's molecular biomarker makeup by producing novel spectra, which appear as highly unique "metabolomic profiles" or "fingerprints." Each profile is analyzed using proprietary chemometrics and bioinformatics that correlate the data to a clinical condition or outcome. We examined the potential utility of this technology to explore the possible role of OS in the pathophysiology of male factor infertility.

Seminal plasma was collected with informed consent in four groups of patients: varicocele (N=70); idiopathic male infertility (N=15); vasectomy reversal (N=9); female factor infertility (N=9); and healthy donors (N=30). The specimens were individually analyzed using nuclear magnetic resonance, Raman, and near-infrared spectroscopy. The

individual spectra obtained from each sample were separately analyzed using a wavelength selective genetic algorithm. Four spectral regions associated with the OS biomarkers were identified for each patient group. Results were further evaluated by a logistic regression of the light attenuation from the wavelength regions analyzed. Compiled results from the leave-one-out cross-validation of the logistic regression from all three spectroscopic measurements resulted in a specificity and sensitivity of >80%. In addition, two-dimensional self-organized maps (SOM) were constructed based on the input data to locate natural clustering or grouping patterns of the patient data. The total analysis time per sample was ~1 minute and required 10 $\mu$ L of SP.

Unique metabolomic profiles describing differences in the OS biomarkers—concentrations of -CH, -NH and -OH—were observed. When each metabolomic profile was quantified using a direct exponential curve resolution algorithm and coupled with logistic regression analysis, the ratio of the -CH to ROH content—which is reflective of OS—also was different between the groups. The female factor infertility, healthy donor and vasectomy reversal patient groups were well-defined within the self-organized map. The profiles of the varicocele patients were more randomly distributed and did not segregate as a separate population with uniquely identifiable biomarker characteristics. The idiopathic patients were defined as two regions in the SOM.

These results suggest that high-speed, non-invasive metabolomic profiling of seminal plasma using biospectroscopy, along with proprietary bioinformatics, can be used to identify different levels of OS in seminal plasma. The ability to quantify differences in the metabolomic profiles observed in different groups of male patients should prove useful as a diagnostic tool to evaluate semen quality and function. Additional studies are planned to further elucidate the role of OS in normal semen function vs. male factor infertility and to determine if metabolomic profiling of biomarkers of OS can be developed as a routine method for assessing sperm function in ART, as well as for other diagnostic applications in the urologic examination of male patients.