

**2nd International Conference on the Cryopreservation of the Human Oocyte
Bologna, Italy
October 5-7, 2006**

Metabolomic Biomarkers of Oxidative Stress in ART: A Rapid, Non-invasive Methodology to Assess and Select Viable Gametes and Embryos From Their Non-viable Cohorts.

Metabolomics Study Group in ART

OBJECTIVE: It is well known that oxidative stress (OS) affects the quality of gametes as well as early embryo development and implantation, and thus affects pregnancy. Reactive oxygen Species (ROS) have been shown to influence sperm, oocytes and embryos and to modulate their interaction in their respective microenvironments. We describe herein, a novel technology platform to assess OS that is based on the confluence of two scientific disciplines: (1) *biospectroscopy*, the different forms of spectral analysis in human biology that are used to identify, quantify and validate molecular biomarkers; and (2) metabolomics, the science that systematically examines and integrates the dynamic interplay of small molecule biomarkers at the cellular level. The technology is used to quantify a sample's molecular biomarker makeup and converts that data into a novel "metabolomic profile" or "fingerprint". Each profile is analyzed using bioinformatics that correlates the data to a clinical condition or outcome. The goal of this work is to establish a rapid, non-invasive and cost-effective point-of-care methodology to select viable gametes and embryos from their non-viable cohorts in IVF.

MATERIALS AND METHODS: A total of 433 discarded specimens were blinded and analyzed (228 embryo media; 72 follicular fluid (FF); 133 seminal plasma). Media was collected from single embryo culture at the time of transfer (D3 and D5). Individual spectra were obtained from 10 μ samples using three different forms of biospectroscopy: Nuclear Magnetic Resonance, Raman and Near Infrared. Specific OS biomarkers of ROH, CH, OH, and NH groups were identified yielding unique metabolomic profiles which were then quantified using a wavelength selective genetic algorithm, proprietary bioinformatics (Molecular Biometrics, LLC, Chester, NJ), and leave-one out cross-validation methods used in conjunction with logistical regression. The resulting metabolomics data were correlated to pregnancy outcomes (embryo-by-embryo) or by diagnosis in the case of semen. Total analysis time was ~1 min per sample.

RESULTS: Unique metabolomic OS profiles were reliably produced from discarded culture media, FF and seminal plasma that correlated with pregnancy vs non-pregnancy outcomes. These observations were consistent by all three methods of spectroscopy. In the case of embryo media, significant differences were noted for D3 and D5 embryos ($p=.003$ vs $p=.009$, respectively). Spectra from D3 embryos were significantly different from the spectra of D5 embryos ($p=.0001$). Statistical correlations were also observed between the OS profiles of FF and semen and their respective pregnancy outcomes. Assay sensitivity and specificity was >80%.

CONCLUSIONS: This is the first study to establish a link between metabolomic profiles of OS and treatment outcomes (pregnancy) in ART. This analysis demonstrates a clear relationship between the reproductive potential of human embryos and their modification of the culture media in which they developed. There also appear to be unique metabolomic signatures in FF that may be predictive of oocyte viability (and by inference, embryo quality) and pregnancy. OS profiles of seminal plasma from infertile men were distinct from normal men. Metabolomic profiling may serve as a useful methodology for rapid, non-invasive gamete and embryo assessment and selection. This in turn may make it possible to decrease the number of embryos transferred, decrease the multiple birth rate, and possibly also improve the pregnancy rates. Confirmation of these initial observations is planned through larger prospective studies.

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