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NMR, Raman and Near Infrared Spectroscopy Determination of embryo Culture Predicts Implantation and Pregnancy Outcomes in Human IVF

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INTRODUCTION: The identification of viable gametes for IVF and the subsequent selection of competent embryos for transfer are crucial procedures in the ART laboratory. 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. We describe herein, a novel technology platform to assess OS based on: (1) *biospectroscopy*, a method of spectral analysis that is used to identify, quantify and validate molecular biomarkers; and (2) metabolomics, a form of systems biology that examines and integrates the dynamic interplay of small molecule biomarkers at the cellular level. The technology quantifies a sample's biomarker makeup and then converts that data into a unique "metabolomic profile" or "fingerprint". Each profile is analyzed using targeted bioinformatics that correlates the data to a clinical condition or outcome.
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 profiles were obtained from 10µ samples using three different forms of biospectroscopy: Nuclear Magnetic Resonance, Raman and Near Infrared. Specific OS biomarkers of ROH, SH, C=C, CH, OH, and NH groups were identified yielding unique metabolomic profiles which were then quantified using a wavelength selective genetic algorithm, proprietary bioinoformatics (Molecular Biometrics, LLC, Chester, NJ), and leave-one out cross-validation used in conjunction with logistical regression. Total analysis time was ~1 min per sample. The resulting metabolomics data were correlated to pregnancy outcomes.
RESULTS: Unique metabolomic profiles of OS biomarkers 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 biospectroscopy. Significant differences were noted in D3 and D5 embryos that produced a pregnancy vs those that did not ($p=.003$ vs $p=.009$, respectively). Profiles of D3 embryos were significantly different from profiles 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 consistently >85%.
CONCLUSIONS: This analysis demonstrates a clear relationship between the reproductive potential of human embryos, as determined by implantation and pregnancy outcomes, and the inventory of biomarkers of OS released in the culture media. Likewise, there appear to be unique metabolomic signatures in FF that are predictive of oocyte viability; and in seminal plasma of infertile vs normal men. Spectroscopy-based metabolomics, coupled with targeted bioinformatics, is a sensitive method for mining the metabolome of embryos and gametes. Metabolomic profiling may serve as a useful tool for rapid, non-invasive gamete and embryo assessment and selection in IVF that can be used in concert with, or in place of, the current practice of embryo morphology grading.

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