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A NOVEL, NON-INVASIVE METHOD TO ASSESS HUMAN EMBRYO VIABILITY USING METABOLOMIC PROFILING OF OXIDATIVE STRESS BIOMARKERS IN EMBRYO CULTURE MEDIA.

Metabolomics Study Group in ART*

OBJECTIVE: 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. We describe herein, a novel technology platform to assess OS based on 1) biospectroscopy, or the application of different forms of spectral analysis to identify, quantify and validate proteomic and molecular biomarkers and 2) metabolomics, or 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. The technology is used to quantify a sample's molecular biomarker makeup and convert the data into a novel "metabolomic profile" or "fingerprint". Each profile is analyzed using chemometrics and bioinformatics that correlate the data to a clinical condition or outcome. The goal of this work was to establish a rapid, non-invasive methodology to assess embryo viability during each stage of development in-vitro by examining the OS biomarkers present in the embryo culture media.
MATERIALS AND METHODS: Discarded culture media (N=228) were collected from single embryo culture microdrops at the time of transfer from D3 and D5 embryos under informed Consent. All samples were immediately frozen and stored at -80°C. Individual spectra were obtained from one 106L sample using three different forms of biospectroscopy: Nuclear Magnetic Resonance (NMR), Raman and Near Infrared (NIR). Specific OS biomarkers of ROH, CH, OH, and NH groups were identified at specific wavelengths yielding unique metabolomic profiles which were then quantified using proprietary bioinoformatics (Molecular Biometrics, LLC, Chester, NJ). The spectra obtained from each instrument were separately analyzed using a wavelength selective genetic algorithm (GA) to determine regions predictive of pregnancy outcome as determined by a logistic regression of the light attenuation from the wavelength included. To avoid random correlations, a leave-one out cross-validation was used. Total analysis time was ~1 min per sample. The resulting metabolomics data were correlated to pregnancy outcomes on an embryo-by-embryo basis.
RESULTS: Unique metabolomic profiles reflective of OS were reliably produced from discarded culture media of embryos that resulted in a pregnancy compared to those that did not. These observations were consistent by all three methods of spectroscopy and were significant 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). Assay sensitivity and specificity was >80%.
CONCLUSION: OS pathways are a crucial intracellular homeostatic mechanism in both health and disease and are implicated in infertility. Our findings suggest that OS can be assessed rapidly and non-invasively in embryos using spectroscopy-based metabolomic profiling. This method may prove useful for the selection of viable embryos within a cohort for IVF.
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