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Albumin modification in culture media as a surrogate marker that is predictive of embryo viability.

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Introduction: Clinical assisted reproductive techniques (ART) provide reproductive opportunities for the infertile. A pivotal aspect of the ART procedure is *in-vitro* embryo culture and implantation. However, rates of embryo implantation remain low despite considerable effort to improve success rates through new embryo selection techniques, enhanced culturing methods and improved culture media formulation. During the culture period the embryo undergoes rapid growth and transformation, using the media as both nutrient resource and waste reservoir. During this period, the developing embryo establishes dynamic biochemical equilibrium with the media. Thus, the molecular species that remain in the media at the end of the culture period may be useful biomarkers of the metabolic pathways involved in embryo development. Pathways of oxidative metabolism (OM) are known to influence both gamete and embryo function and are necessary for the development of competent, viable embryos that have the potential to implant. Conversely, abnormal perturbations of the OM are known to alter embryo viability. We previously reported the identification of biomarker functional groups using non-invasive spectroscopic profiling of the media. To further elucidate the key molecular species of the embryonic metabolome responsible for signaling viable versus non-viable embryos, we analyzed embryo culture media using sensitive capillary electrophoresis and sophisticated chemometric techniques. This report details our preliminary findings and identifies key molecular constituents in embryo culture media that may be useful as predictors of embryo health.

Materials and Methods: Standard IVF protocols utilizing single embryo transfer were performed at the VUmc in Amsterdam, The Netherlands. 59 samples of normally discarded D-3 culture media were collected at the time of embryo transfer and frozen at -80°C until analyzed. All embryos were cultured individually in 20 μl micro drops. Samples, diluted with three volumes of distilled water, were injected and separated by capillary electrophoresis using a 50 cm effective length fused silica capillary at 330 V/cm. The separation media was 75 mM sodium borate pH 9.25 buffer with 5 mM sodium dodecyl sulfate. Absorbance data, collected at 195 nm, was normalized to height of albumin's major peak and aligned using correlation optimized time warping. Four wavelets were obtained from each electropherogram using the Haar transform and used as the basis set for a genetic algorithm (GA) that selected the wavelets according to a Bayesian statistical classification process. Leave-one-out cross validation was used to validate the developed model.

Results: The Bayesian classification model was able to achieve a high level of accuracy in distinguishing between viable embryos that implanted and non-viable embryos that did not implant. With this data set, the model correctly predicted 38/41 implanted embryos and 16/18 non-implanted giving rise to a sensitivity of 92% and specificity of 89%. The wavelets selected by the Bayesian model corresponded to an early eluting fraction of human serum albumin (HSA) and two unidentified fractions eluting before albumin.

Conclusion: These data suggest that during development in culture, molecular species, or biomarkers, generated by the embryo are selectively modifying the HSA. Specific modifications of HSA can be detected and quantified by capillary electrophoresis and characterized mathematically by proprietary chemometrics and bioinformatics (Molecular Biometrics, Chester, New Jersey, USA). These findings are consistent with previous reports showing that non-invasive metabolomic profiling of the culture media could be used to determine embryo viability and reproductive potential. It is possible that an imbalance in OM in the embryo leads to oxidative stress that ultimately compromises embryo health. The deleterious changes in OM can be detected by selected modifications to HSA. These methods should prove useful as diagnostic tools for the assessment of embryo viability in IVF.