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Non-invasive assessment of embryo reproductive potential: metabolomic profiling of biomarkers of oxidative metabolism (OM) during embryo development

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Objective: To determine if metabolomic profiling of OM biomarkers during embryonic development (D2 to D5) in-vitro, was associated with pregnancy in a single embryo transfer (SET) model

Materials and Methods: Embryos were cultured in individual drops of cleavage media (Cook) under oil. Embryo scoring and transfer was performed on Day 2 (N=75) while remaining embryos were transferred to new cleavage media (CCM-30, Vitrolife) and later selected for transfer at Day 3 (N=27) or Day 5 (N=49) based on morphological criteria. Only SET was performed. Spent media was collected and stored in liquid N₂. Pregnancy was determined by the presence of foetal cardiac activity (FCA) at (5-6) weeks.

Near infrared spectroscopy (NIR) measurements of blinded, randomized samples were conducted using an InGaAs spectrometer. Sample volume was 7 uL. Control media samples were used to compensate for any drift in signal. Sample properties were quantified from the resulting mean centered NIR spectra by determining the most parsimonious combination of variables in selected wavelength domains using a genetic algorithm optimization. Selected wavelength regions were weighted by a coefficients calculated by inverse least-squares regression. Each sample's pregnancy viability was estimated by a leave-one-out cross validation method. Notch box plots were used to plot the resulting viability indices, and t-tests were applied to determine significant differences.

Results: A 151 media specimens were analyzed. Embryos from each media specimen were considered viable if pregnancy ensued following SET as determined by positive FCA. NIR spectral analysis reproducibly defined 5 OM biomarker regions associated with changes in ROH, C=C, -SH, -CH and -NH functional groups that were used to distinguish between FCA+ vs FCA- samples based on bioinformatic analysis of the data. The FCA+ and FCA- groups were statistically different at 95% confidence level. Morphology scores did not correlate with neither pregnancy outcome nor metabolomic activity.

Conclusions: Unique metabolomic profiles obtained at each day of embryo development (D2 through D5) were significantly correlated with FCA+ or FCA- embryos in a SET study design not observed by morphological scoring alone. Further prospective randomized trials are planned with the aim of choosing between morphologically similar embryos and to distinguish their implantation potential.