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Metabolomic assessment of Day 2 (D2) embryos based on pregnancy outcome after single embryo transfer (SET)

**O. Kato, S. Teramoto, H. Morita, L. Botros, P. Roos, D. Burns.
Kato Ladies Clinic, Tokyo, Japan; Molecular Biometrics, LLC, Chester, New Jersey, USA
and McGill University, Montreal, Quebec, Canada**

OBJECTIVE: To assess the status of metabolic activity and viability of Day 2 embryos selected for SET using non-invasive metabolomic profiling of biomarkers of oxidative metabolism (OM).

DESIGN: Prospective, blinded study.

Material and Methods: A "mild" ovarian stimulation protocol was employed using 50 mg clomiphene citrate daily combined with 50 IU hMG on day 8 until GnRHa administration prior to OPU. Fertilized zygotes were cultured individually in 20µl of cleavage medium (SAGE) with 12mg/ml SPS. Discarded media samples were collected on Day 2, frozen and stored in liquid N₂. Media controls were also obtained for each embryo media specimen collected.

Near Infrared spectroscopy (NIR) measurements of blinded, randomized samples were conducted using an InGaAs spectrometer; sample volume was 7µl. 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 statistical significance.

Results: A total of 159 D2 media specimens plus an equal number of controls were analyzed. NIR spectral analysis reproducibly defined 5 to 6 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- using proprietary bioinformatics. The FCA+ and FCA- groups were statistically different at $p < .05$. The morphology scores of embryos between FCA+ and FCA- groups were not statistically different indicating no correlation with prediction of reproductive potential or metabolomic activity.

Conclusions: A number of studies have implicated oxidative metabolism as an important factor effecting embryo development and viability. It is generally agreed that Day 2 embryos are at a crucial stage of growth and development involving the activation of new metabolic pathways. Unlike metabolomic data obtained by NIR, morphological criteria were not correlated with embryo competency. Variations in drugs and dosages used in different stimulation protocols may affect metabolomic activity and quality in developing (D2) embryos (data on file). Metabolomic profiling appears to be a reliable indicator of metabolic activity and embryo viability as early as D2, whereas morphology was not predictive of either parameter.

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