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Near Infrared Spectroscopy as a tool to predict embryo viability: a novel, non-invasive method for embryo selection.

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Introduction: One of the most important complications of IVF treatment is the high multiple pregnancy rate which leads to a higher incidence of medical, perinatal and neonatal complications and hence to higher health care costs. Single embryo transfer (SET) is an effective way to minimize the risks of multiple pregnancies. Because only one embryo is transferred, the selection of the embryo with an optimum implantation potential is of great importance. Currently, embryo selection is mainly based on morphological criteria using light microscope analysis. Because of its limited predictive value for ongoing pregnancy, new selection tools are being sought-after. Recently, new parameters to predict embryo viability like non-invasive metabolomic profiling of embryos have been studied. Pathways of oxidative metabolism (OM) influence gamete and embryo function and are necessary for the development of viable embryos with implantation potential. Conversely, perturbations of OM that result in oxidative stress have been shown to be deleterious to embryo health. Previous studies have shown that non-invasive metabolomic profiling can predict the developmental ability of embryos and lead to pregnancy and live birth following transfer. In this study, we investigated if metabolomic profiling of biomarkers of OM by Near Infrared (NIR) Spectroscopy correlates with ongoing pregnancy when the transferred embryos were selected by conventional selection criteria.

Material and Methods: From July—November 2006, embryos of 250 patients scheduled for IVF or ICSI treatment with a SET were included. Embryos were cultured individually in 25 µl pre-equilibrated medium drops and alongside, embryo-free media drops were incubated as controls. Transfer was either on day 2 or day 3. Embryos were selected for transfer by routine stringent morphological criteria. After transfer, the medium drop in which the transferred embryo was cultured and a control medium drop were immediately frozen (-196°C). Samples were shipped and stored at -80°C until analysed by NIR. Pregnancy was detected by serum HCG 12 days post embryo transfer. Ongoing pregnancy was defined by the observation of a positive heart beat at 12 weeks of amenorrhoea. Individual metabolomic profiles were obtained from 7 µl media samples using NIR spectroscopy. OM biomarkers of ROH, -SH, C=C, -CH, -OH, and -NH groups were identified yielding unique profiles which were then quantified using a wavelength selective genetic algorithm, proprietary bioinformatics and leave-one out cross-validation were used in conjunction with logistical regression. A Bayesian statistical classification model was used to assign relative “embryo viability scores” to embryos that correlated to pregnancy outcomes. Total analysis time was <1 min per sample. The resulting metabolomics data were compared to strict morphological assessment and pregnancy outcomes.

Results: NIR spectral analysis of discarded culture media samples collected from 250 SET cycles produced unique metabolomic profiles of OM. The Bayesian mathematical model produced relative embryo viability scores that correlated to an embryo's reproductive potential as determined by pregnancy outcomes. Statistical analysis performed on the metabolomic profiles established a viability score between the pregnancy and non-pregnancy group that was statistically significant (95% confidence interval, P<0.001). The sensitivity and specificity of the methodology was 87% and 93%, respectively. Metabolomic profiles from HCG positive patients that failed to sustain an ongoing pregnancy, showed a significantly lower mean profile score compared to those values obtained from profiles observed in ongoing pregnancies. Greater than 98% of all embryos transferred were classified as Grade A embryos by strict morphological criteria but only 29% implanted and resulted in an ongoing pregnancy.

Conclusion: Metabolomic profiles of OM biomarkers present in discarded embryo culture media were able to distinguish between viable embryos that produced an ongoing pregnancy versus their non-viable counterparts. Metabolomic profiles seem to provide a strong addition to the selection of a viable embryo and may serve as a useful methodology for rapid, non-invasive embryo selection.