Association of Clinical Embryologists Annual Conference
Manchester, England
January 3, 2008

Non-Invasive Metabolomic Profiling of Human Embryo Culture Media Correlates with Pregnancy Outcome

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Objective: More than 100.000 assisted reproductive technology (ART) cycles are started yearly in the U.S. Partly due to our inability to select the best embryo(s) to be transferred, a mean number of 3.1 embryos are transferred in ART cycles using fresh non-donor oocytes. This leads to a 34.3% overall pregnancy rate, 29.0% of which result in multiple-infant live births. Similarly, a mean number of 2.5 embryos are transferred in ART cycles using fresh donor oocytes, achieving a 50.4% pregnancy rate, 44.7% of which result in multiple-infant live births (SART 2002). These statistics have remained essentially unchanged for several years and reflect a need for improvement over our current embryo selection methodology that is based on cleavage rates and morphology. We hypothesized that, embryos that result in pregnancy may be different in their metabolomic profile compared to embryos that do not and that the difference may be detected by the rapid, non-invasive evaluation of the embryo culture media using targeted spectroscopic analysis and bioinformatics.

Materials and Methods: Prospective studies were conducted in one academic, and three private ART centers. Embryo culture medium of transferred embryos from ART cycles using fresh donor or nondonor oocytes were evaluated. Media (n=619) were individually collected after embryo transfer, and analyzed using Raman, and/or Near Infrared Spectroscopy (NIR). Initially 69 samples were tested. In order to develop a model, 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 correlation, a leave-one out cross-validation was used. Sensitivity and specificity of predicting pregnancy (defined as presence of fetal cardiac activity) were calculated. Once spectral models developed were tested using embryo culture medium samples from different centers that perform multiple (n=4l) or single (n=509) embryo transfers.

Results: Spectral profile describing differences in –CH, -NH and -OH concentrations showed distinct differences between culture media of embryos that resulted in pregnancy and those that did not. The ratio of the -CH to ROH content in the media that is reflective of oxidative stress was also different between the two groups. Using GA with Raman, three spectral regions associated with these molecular species were identified as predictive of pregnancy outcome. Compiled outcomes from the leave-one-out cross-validation of the logistic regression using the Raman measurements resulted in a specificity of *86%* and a sensitivity of *76.5%.* Analysis of the NIR required four wavelength regions, and provided a specificity of 75% and a sensitivity of 83.3%. These findings were confirmed in studies using embryo culture media samples collected from centers that use multiple embryo transfer and single embryo transfer.

Conclusion: Our findings suggest that a detectable difference exists in the metabolomic profiles found in culture media obtained from embryos that cause pregnancy compared to those that do

not. The reported metabolomic parameters were established using two different forms of spectroscopic analysis, with samples from different centers, and achieved high sensitivity and specificity. Metabolomic profiling may serve as a useful methodology for rapid, non-invasive embryo assessment and selection. Consequently, metabolomic evaluation may make it possible to decrease the number of embryos transferred, lead to a decrease in multiple-infant live birth rates, and possibly also improve the pregnancy rates.