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**Non-Invasive Metabolomic Profiling of Human Embryo Culture Media
Correlates With Pregnancy Outcome. Initial Results of the Metabolomics
Study Group for ART**

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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. In this study, 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. Design: A prospective multi-center study was conducted in 2 academic, and one private ART centers. Materials and Methods: Embryo culture medium of 108 embryos from ART cycles using fresh donor or nondonor oocytes were evaluated. Media (G1.3; VitroLife, Sweden) were individually collected after embryo transfer on day 3, and analyzed using both Raman, and Near Infrared Spectroscopy (NIR). 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 correlations, a leave-one out cross-validation was used. Sensitivity and specificity of predicting pregnancy (defined as presence of fetal cardiac activity) were calculated. 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, four 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 80% and a sensitivity of 95%. Analysis of the NIR required two wavelength regions, and provided a specificity of 83% and a sensitivity of 73%. 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 3 different centers, and achieved high sensitivity and specificity. Confirmation of these initial observations is planned through a

larger prospective study involving additional ART centers. Metabolomic profiling may serve as a useful methodology for rapid, non-invasive embryo assessment and selection. 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.