

## ESHRE 2008 Abstract:

Non-invasive metabolomic profiling of Day 5 spent culture media aids in the prediction of blastocyst viability

T. Hardarson<sup>1</sup>, L. Rogberg<sup>1</sup>, A. Ahlström<sup>1</sup>, M. Wikland<sup>1</sup>, L. Botros<sup>2</sup>, P. Roos<sup>2</sup>, D. Sakkas<sup>2</sup>, T. Hillensjö<sup>1</sup>, D. Burns<sup>2</sup>.

<sup>1</sup>Fertility Center Scandinavia, Carlanderska Hospital, Göteborg, Sweden.

<sup>2</sup>Molecular Biometrics, LLC, New Jersey, U.S.A..

**Introduction.** The commercial availability of sequential culture media systems has led to the routine use of blastocyst culture in many IVF clinics. It has proved as an excellent tool in particular for single embryo transfers (SET). The type of blastocyst obtained is however of critical importance. As with the scoring of embryos during the cleavage stages, time and morphology play an important part in selecting the best blastocyst. In addition to morphology it has long been known that the intrinsic metabolism of a particular embryo can also provide strong clues to whether a particular embryo will be viable. Recently, the development of a rapid non-invasive screening technology using near infrared (NIR) spectroscopy of spent culture media has allowed us to establish a metabolic profile for individual embryos. In this study we examined whether the individual metabolic profile of a single Day 5 blastocyst aids in predicting its viability.

**Materials and methods:** Thirty one patients (mean age  $\pm$ SD: 34.4  $\pm$ 4.1) received SET of day 5 blastocysts. Embryos were cultured in individual 20  $\mu$ l drops of cleavage media (Cook) under oil and transferred to new 40  $\mu$ l drops of cleavage media (CCM-30, Vitrolife) on day 2. Embryos were then selected for SET on Day 5 (N=31) based on morphological criteria. Spent media was collected and stored in liquid N<sub>2</sub>. Pregnancy was determined by the presence of foetal cardiac activity (FCA) at (6-7) weeks. Media samples (7  $\mu$ L) were transferred to a 3mm path-length cell, and NIR measurements of blinded, randomized samples were conducted using an InGaAs spectrometer. Control media samples were used to compensate for any instrumental drift over time. Sample properties were quantified from resulting mean-centred NIR absorbance spectra by determining a parsimonious linear combination of spectral regions that discriminated between pregnant and not-pregnant patients using a genetic algorithm optimization. Selected spectral regions were weighted by a coefficients calculated by inverse least-squares regression. Each sample's pregnancy viability was quantified using the linear combination of selected spectral regions and their weighted coefficients through 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 between test groups.

**Results:** Of 31 day 5 blastocyst 15 (48.4%) ongoing pregnancies were established as detected by Foetal Cardiac Activity. When the Viability Index was examined, a significant difference (P=0.0015) was evident between the Viability Index of media samples that established a viable pregnancy (0.675  $\pm$  0.35) compared to those that failed to implant (0.279  $\pm$  0.33). The mean age of the two groups was not significantly different (pregnant: (34.7  $\pm$  4.0) versus non-pregnant: (33.9  $\pm$  4.3)). Furthermore, using a cut-off value of 0.52, generated by ROC curve analysis, the accuracy of predicting implantation using the Viability Index > 0.52 was 12/15 (80.0%) whereas 13/16 (81.3%) blastocysts scoring below 0.52 failed to establish a pregnancy.

**Conclusions:** Although blastocyst culture has gone a long way in aiding the selection of viable embryos our study has shown that a unique metabolomic profile exists that further establishes viability of an individual embryo. A prospective randomized trial is planned with the aim of seeing if the viability score is an independant predictor of pregnancy.