

**Posillico, J, *et al.* Selection of viable embryos and gametes by rapid, non-invasive metabolomic profiling of oxidative stress biomarkers. Chapter 20, Human Preimplantation Embryo Selection, Ed. Elder, K; Cohen, J. Informa Healthcare, 2007.**

Review:

In this text book chapter, the author reviews the issues to date with IVF, namely, generally low success rates, high treatment costs, high multiple birth rates, high pre-term deliveries, low birth weight babies and increased neonatal mortality. The author stipulates that many of these issues could be ameliorated if embryos of known viability and increased developmental potential could be identified prior to transfer, which would go a long way toward resolving the 'IVF Dilemma': how to increase pregnancy rates while reducing the number of embryos implanted, and, therefore multiple birth rates.

The author introduces 'metabolomics' as the study of cellular processes downstream to 'genomics' and 'proteomics'. As such, metabolomics is the study of global metabolite profiles in a cell, tissue or organism that leave behind a chemical 'fingerprint' of cellular processes and may be used as a small molecular biomarker of the functional phenotype of a cell; essentially a real-time characterization of the products of gene expression.

The author also reviews various analytical techniques used in metabolomics, including gas and high performance liquid chromatography, capillary electrophoresis, mass spectrometry, NMR spectroscopy and near-infrared and Raman spectroscopy.

The author also introduces rapid, non-invasive metabolomic-based embryo viability selection as capable of identifying viable gametes and embryos as part of IVF programs, effectively mitigating the 'IVF Dilemma'. The author concludes that there is significant potential to a rapid, non-invasive determination of embryo reproductive potential prior to transfer, which would lower multiple gestation rates while maintaining or increasing pregnancy outcomes.

Publisher Link: <http://catalogue.informahealthcare.com/pjbp/products/10001043400>

**Scott R., et al. Noninvasive metabolomic profiling of human embryo culture media using Raman spectroscopy predicts embryonic reproductive potential: a prospective blinded pilot study. *Fertility and Sterility*, 2008. *In Press*.**

Review: Investigators demonstrated a clear relationship between the reproductive potential of embryos and modification of their culture media as detected by metabolomic profiling using Raman spectroscopy. Embryo reproductive potential (viability indices) was demonstrated to predict embryo viability among a total of 41 samples taken from media surrounding 3- and 5-day embryos of 19. Viability indices for embryos that implanted were higher than for those that did not implant (0.875 vs. 0.56), yielding an aggregate diagnostic accuracy of 75%.

Abstract:

**Objective:** To determine if metabolomic profiling of embryonic development was associated with implantation rates in IVF.

**Design:** Prospective blinded. Setting: University-affiliated assisted reproductive technology program. Patient(s): Unselected assisted reproductive technology population. Intervention(s): None. Main Outcome Measure(s): Raman-based biospectroscopic metabolomic profiling of spent culture media and delivery rates.

**Results:** Forty-one spent media samples from 19 patients with known reproductive potential (0 or 100% delivery rates of each embryo that implanted) were evaluated. Raman-based metabolomic profiling was used to calculate a viability index for each sample. On day 3, the spent media of embryos with proven reproductive potential (n = 33) demonstrated higher viability indices (0.875 +/- 0.12) than those that failed to implant (0.56 +/- 0.09). Similar findings were present in spent media from embryos transferred on day 5 (n = 8) (-0.40 +/- -0.21 vs. -0.81 +/- -0.08). Receiver operating characteristic curve analyses were used to select thresholds with the greatest ability to discriminate outcomes. Overall diagnostic accuracy for predicting delivery or a failed implantation was 80.5%.

**Conclusions:** There is a clear relationship between the reproductive potential of human embryos and their modification of their culture media as detected by Raman biospectroscopy-based metabolomic profiling. This technology offers great potential for development as a tool to allow rapid noninvasive assessment of embryonic reproductive potential before transfer.

Link: <http://www.ncbi.nlm.nih.gov/pubmed/18281045>

**Seli, E., et al. Noninvasive metabolomic profiling of embryo culture media using Raman and near-infrared spectroscopy correlates with reproductive potential of embryos in women undergoing in vitro fertilization. *Fertility and Sterility* 2007; 88(5):1350-7.**

Review: This prospective study of 69 media samples surrounding embryos from 30 patients demonstrated sensitivities of 86% and 75% for correctly choosing viable embryos using Raman and NIR spectroscopy respectively, with specificities of 77% and 83%, respectively. Authors concluded that rapid, non-invasive metabolomic profiling of culture medium using Raman and NIR spectroscopy generated viability indices that correlate well with pregnancy outcomes.

Abstract:

**Objective:** To determine if metabolomic profiling of embryo culture media correlates with reproductive potential of individual embryos.

**Design:** Prospective study. **Setting:** An academic and a private assisted reproduction program; a university research center. **Patient(s):** Women undergoing assisted reproduction treatment. **Intervention(s):** Sixty-nine spent media samples from 30 patients with known outcome (0 or 100% sustained implantation rates) were individually collected after embryo transfer on day 3 and were evaluated using Raman and/or near-infrared spectroscopy. The spectra obtained from each instrument were separately analyzed using a wavelength selective genetic algorithm to determine regions predictive of pregnancy outcome. Viability indices reflective of reproductive potential were calculated for each sample. To avoid random correlations, a leave-one-out cross-validation was used. Sensitivity and specificity of predicting viability (described as implantation and delivery) were calculated. **Main Outcome Measure(s):** Metabolomic profile of culture media and embryo viability.

**Results:** Viability indices calculated by Raman or near-infrared spectroscopy were higher for embryos that implanted and resulted in a delivery, compared with those that failed to implant. Raman spectroscopy predicted viability of individual embryos with a sensitivity of 86% and a specificity of 76.5%; near-infrared provided a sensitivity of 75% and a specificity of 83.3%.

**Conclusion:** Rapid, noninvasive metabolomic profiling of human embryo culture media using Raman or near infrared spectroscopy combined with bioinformatics correlates with pregnancy outcome.

Link: <http://www.ncbi.nlm.nih.gov/pubmed/17923129>



**Bromer J.G., et al. Assessment of embryo viability in assisted reproductive technology: shortcomings of current approaches and the emerging role of metabolomics. Curr Opin Obstet Gynecol 2008; 20:234-241.**

Review:

In this review article, the authors discuss various modes of embryo viability assessment employed in assisted reproduction technologies (ART) and the possible role of metabolomics. The authors note that as the number of ART cycles increase globally, there is increasing emphasis on developing quick and non-invasive strategies to determine the best embryo for transfer. The authors suggest that metabolomic profiling using media culture may be a useful adjunct to the findings of morphology and cleavage rate examination.

Abstract:

**Purpose:** The present article describes the current status of embryo assessment in assisted reproductive technologies and discusses two important issues that derive, at least in part, from our inability to adequately assess the reproductive potential of individual embryos: low implantation rates and high multiple pregnancy rates. After an overview of studies evaluating embryo metabolism as a predictor of embryo viability, use of metabolomics and additional emerging approaches for rapid, noninvasive, embryo assessment are discussed.

**Recent findings:** Morphology and cleavage rate remain the mainstay of embryo assessment. A number of technologies are, however, under investigation. These include the assessment of glucose, lactate, pyruvate, and amino acid metabolism, proteomic profiling, evaluation of oxygen consumption, and most recently, examination of the metabolome.

**Summary:** As the number of assisted reproductive technology cycles increases worldwide, improvements in the ability to quickly and noninvasively determine the best embryos for transfer remain a critical goal for reproductive medicine. Recent studies suggest that metabolomic profiling of embryo culture media may provide a useful adjunct to the current embryo assessment strategies based on morphology and cleavage rate.

Link: <http://www.ncbi.nlm.nih.gov/pubmed/18460937>

**Vergouw, C.G. *et al.* Metabolomic profiling by near-infrared spectroscopy as a tool to assess embryo viability: a novel, non-invasive method for embryo selection. *Human Reproduction* 2008; 23:1499-1504 .**

Review: Pilot study of NIR-based metabolomic assessment of embryo viability of 333 patients confirmed the results of Seli, *et al.* The overall accuracy of metabolomic profiling (53.6%) exceeded by a significant margin that of standard morphological selection (38.5%), suggesting that metabolomics profiling using NIR is able to distinguish between viable and non-viable embryos, and may be a valuable adjunct to morphological assessment.

Abstract:

**Background:** The morphology of an embryo has a limited predictive value for assessing viability and ongoing pregnancy, therefore new selection tools are needed to maintain success rates with single-embryo transfer (SET). In this study, we investigated if metabolomic profiling of biomarkers of embryo culture medium by near-infrared (NIR) spectroscopy has a correlation with ongoing pregnancy in SET.

**Methods:** A total of 333 patients scheduled for in vitro fertilization (IVF) with SET were included in the study. Embryos were selected for transfer by morphological criteria on Days 2 and 3 of in vitro culture, and left over culture media samples were analyzed by NIR spectroscopy.

**Results:** The NIR spectral analysis produced unique metabolomic profiles that correlated to an embryo's reproductive potential. Resulting relative viability scores between positive and negative pregnancy outcomes were statistically significant ( $P < 0.03$ ). A logistic regression of factors correlated to pregnancy outcomes showed that maternal age, percent fragmentation and relative viability scores all demonstrated a relationship. The extent of the correlation was determined by accuracy computation, where the accuracy of assessing viable embryos on Day 3 by metabolomic profiling was 53.6% and the accuracy of the morphological selection was 38.5%. In addition, the positive predictive value of metabolomic profiling was 0.365 and the negative predictive value was 0.830.

**Conclusions:** NIR metabolomic profiling of spent embryo culture media was able to distinguish viable embryos from non-viable embryos for reproduction.

Link: <http://www.ncbi.nlm.nih.gov/pubmed/18424459>