Prediction of embryo viability by morphology and metabolomíc profiling

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This thesis is based on the following studies, referred to in the text by their roman numerals.

I. Ahlström, A., Wikland, M., Rogberg, L., Siques Barnett, J., Tucker, M. and Hardarson, T. Cross-validation and predictive value of near-infrared spectroscopy algorithms for day 5 blastocyct transfer. *RBMOnline 2011; 22:477-484*.

II. Hardarson, T., Ahlström, A., Rogberg, L., Hillensjö, T., Westlander, G., Sakkas, D., and Wikland, M. Non-invasive metabolomic profiling of Day 2 and Day 5 embryo culture medium: A prospective randomized trial.. *Human Reproduction 2011; 27:89-96.*

III. Ahlström, A., Westin, C., Reismer, E., Wikland, M., and Hardarson, T. Trophectoderm morphology: an important parameter for predicting live birth after single blastocyst transfer. *Human Reproduction 2011; 26:3289-96*.

IV. Ahlström, A., Westin, C., Wikland, M. and Hardarson, T. Prediction of live birth in frozen-thawed single blastocyst transfer cycles by pre-freeze and post-thaw blastocyst morphology. *submitted manuscript*.

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Prediction of embryo viability by morphology and metabolomics profiling

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Abstract

The ultimate challenge for all *in vitro* fertilisation (IVF) clinics is to develop the ability to select for transfer the best single embryo first, from the patient's cohort of embryos, thereby maximising the chance of pregnancy while the incidence of multiple pregnancies is kept to a minimum and fewer transfer cycles are required. This ambition has driven extensive research and development into methods that can be used to predict embryo viability. The aims of this thesis were to investigate two non-invasive methods; one new method of metabolomic profiling using Near Infrared (NIR) spectroscopy to analyse spent embryo culture media and the most routine method of morphological grading at the blastocyst stage.

In our initial study we investigated metabolimic profiling by NIR spectroscopy and demonstrated that there were distinct differences between NIR spectral profiles of spent embryo culture media of implanting embryos and non-implanting embryos on day 5 of development. These differences were successfully used in a predictive model to calculate viability scores that were positively correlated ($R^2 = 0.82$, $P = 0.03$) to implantation rates. In addition, viability scores were not related to morphology indicating that this method could be used as an adjunct to current morphological selection criteria. We also showed, by a method of cross-validation, that a predictive algorithm was accurate even when used at different clinics using different blastocyst culture media. These findings, in addition to other published studies, suggest that selection of embryos with high NIR viability scores could potentially improve implantation rates.

Unfortunately, when the application of this technology was tested in a prospective randomized controlled trial (RCT) for selection of embryos on day 2 and day 5 for transfer, its use in adjunct to morphology did not significantly improve the ongoing pregnancy rate when compared to morphology alone (34.8% versus 35.6%, $P = 0.97$). As such, NIR spectroscopy, in its current form, did not improve selection of the most viable embryo for transfer. These results demonstrate the importance of performing RCT's before committing to the clinical application of any new technology or treatment.

We also investigated the independent predictive strength of morphological parameters used to predict blastocyst viability in both fresh and frozen-thawed cycles.

We found through our retrospective studies looking at blastocyst morphology and prediction of live birth found that trophectoderm (TE) morphology was the most important predictor after fresh single blastocyst transfer cycles and one of the most important predictors after frozen thawed transfer cycles. Expansion grade was found to be the other most important predictor of live birth after frozen-thawed transfer cycles. The inner cell mass (ICM) in both studies was not shown to be one of the most significant predictors of live birth. We have shown, for the first time, the predictive strength of TE grade over ICM for selecting the best blastocyst for embryo replacement. It may be that, even though ICM is important, a strong TE layer is essential at this stage of embryo development, allowing successful hatching and implantation. Furthermore, we found that for thawed blastocysts degree of re-expansion was the most important post thaw morphological predictor of live birth.

In conclusion, we have been able to show that morphology is a strong predictor of embryo viability and by understanding the predictive strength of each parameter being used in a grading system, we can better use these parameters when making our decisions. Furthermore, there is still a need for alternative methods to predict embryo viability, but these new methods should be validated in properly conducted studies before clinical implementation, as shown by the conflicting results in our two studies when testing the NIR technology platform.