

## Metabolic modification of culture medium by human embryos; correlations with embryonic characteristics and outcome in single embryo transfers

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**Introduction:** The accuracy with which the embryo with the highest implantation potential can be selected from a cohort generated in a cycle of IVF treatment is of crucial importance in determining the success of single embryo transfer (SET). Observation of a number of developmental markers ranging from early cleavage on day 1, through cleavage rate and morphology on day 2/3 to blastocyst formation on day 5/6, assists as a basis for rational selection but can often fail to distinguish sufficiently between candidate embryos for transfer. A number of markers of embryo function, such as measurement of uptake of carbohydrates or turnover of amino acids, have been shown to provide additional insight into embryo viability but, while they fulfil the essential criterion of being non-invasive, they are difficult to apply in routine practice. In the present study, we report the measurement of the modification of the chemical composition of the surrounding medium by individual embryos using a rapid Near Infra-red (NIR) spectroscopy approach. In addition we report relationships between an algorithm generated "viability index", traditional predictive markers of embryo viability and actual viability as assessed by fetal development.

**Materials and methods:** Fertilised oocytes from IVF and ICSI cycles involving SET (n=144) were transferred from Quinn's Advantage Fertilisation medium to individual 50µl drops of Quinn's Advantage Cleavage medium under oil 18 hours post insemination (hpi) and incubated for a further 24 hours prior to selection for transfer. A parallel 50µl control drop with no embryo was incubated over the same period. Medium samples from transferred embryos and parallel controls were collected at the end of this culture period and stored at -80°C until analysed by NIR spectroscopy. Metabolomic profiles, based on measuring concentrations of key functional groups (e.g. -SH, C=C, -CH, -OH, and -NH groups) and adjusted for parallel controls, were generated for individual transferred embryos. A wavelength specific genetic algorithm and inverse least-squares regression (Molecular Biometrics, USA) were used to determine a relative embryo viability index (VI) based on differences between transferred embryos which did or did not result in implantation (fetal heart detection) [Seli et al., *Fertil Steril*, 88, 1350-1357, 2007].

**Results:** Embryos which resulted in implantation (n=43) had a significantly higher mean VI than those (n=101) which did not (0.3349 vs 0.2809; p=0.0103 by one sided t-test). The mean VI of embryos transferred at the 2 cell stage was 0.2353 compared to 0.2959 for those with 4 cells at transfer and 4 cell embryos which underwent early cleavage by 23-24 hpi had a higher mean VI than those which were still at the 1 cell stage (0.3277 vs 0.2674). Furthermore, using a cutoff value of 0.2884, generated by ROC curve analysis, the accuracy of predicting implantation using the VI was 59.0% compared to 38.2% when using morphological markers alone. In addition, 37% of top quality embryos with a VI >0.2884 implanted compared to 24% of those with a VI <0.2884.

**Conclusions:** Cleavage stage IVF embryos which are destined to implant following transfer alter their culture environment differently from those which subsequently fail to implant and these differences are quantifiable using a rapid NIR spectroscopy method. A Viability Index based on these differences has a higher predictive accuracy than traditional morphological markers of viability. Metabolomic profiling by NIR may be a useful adjunct to currently available methods used to select embryos for transfer.