Application of single cell (blastomere) gene expression profiles to the assessment of human embryos created by IVF

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Abstract

The need for technologies that can assay samples at the single cell level is becoming increasingly apparent. During development, it has been shown that many cells may look identical morphologically, but that the cells have already started down their path of differentiation and thus are showing discrete gene expression profiles. In order to fully characterize these stages of development, it is necessary to look at individual cells. In addition, with increasing interest in rare cell populations often only a very small number of cells, or even single cells, are available for study. One area in which analysis of single cells may be useful is in the assessment of embryo quality for in vitro fertilization (IVF). Using a modified version of the Global RT-PCR Amplification technique gene expression was analysed in single-cells (blastomeres) biopsied from 6-8 cell embryos. Information gathered using this technique may allow for the determination of factors affecting the ability of an embryo to implant after IVF. Advancing the assessment of embryo quality will allow for application of elective single embryo transfer in more cases, reducing the risks and complications of multiple pregnancies resulting from the current common practice of the transfer of several embryos in IVF.

Introduction

Many countries are moving towards single embryo transfers (SET) for in vitro fertilization (IVF) practices[1-2]. SET reduces the risk of multiple gestation and thus lowers the health risks to mother and child that are inherent with multiples. One major hurdle in the way of this practice, particularly for countries where the cost of IVF is not covered by the government, is that currently there is no reliable way to determine the viability of an embryo prior to implantation. Without reliable screening and considering the high costs associated with IVF rounds, it can be difficult to convince patients to choose SET despite the health benefits of single gestation and birth.

Current methods used to assess embryo quality mainly involve morphological assessment of the cleavage stage embryo. There is a range of morphological features examined and there are opposing views in the literature as to the importance of some of these features[3]. One main problem with morphological assessment is that it is subjective. There is a great deal of interest in developing more objective and reliable methods for assessing embryo viability prior to transfer. In the case of patients with a history of a specific gene abnormality Preimplantation Genetic Diagnosis (PGD) can be used to determine which, if any, embryos carry this mutation. This method is commonly used to screen for inherited diseases that are caused by a known gene defect[4]. Another similar procedure is Preimplantation Genetic Screening (PGS), which can be used to check for anueploidy in fertilized embryos[5]. The possible benefits of PGS are controversial as there are disparities in how the screening laboratories vary in their techniques and in how studies have been conducted to assess the risk/benefit of this procedure[5]. Analysis can be performed on various cells including; polar body biopsy, single-blastomere biopsy, some remove two blastomeres, or trophoectoderm cells[5]. There are concerns that removing cells from the embryo may have a detrimental effect on its development. There is a great deal of evidence that increasing embryo manipulation correlates with decreasing pregnancy rates[5]. For this reason there is a great push in the field towards finding more reliable and less-invasive methods to determine embryo viability.

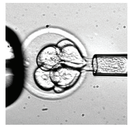
To work toward developing a reliable method for screening embryo viability we have begun by profiling the gene expression of single blastomeres from potentially viable embryos, obtained with consent from the Centre for Fertility and Reproductive Health at Mt. Sinai Hospital in Toronto, ON. Using a modified version of the Global RT-PCR Amplification technique[6], gene expression of 30 blastomeres was profiled using the Agilent 44k whole human genome arrays.

Methods

Embryos were labelled as “good” or “bad” based on morphological assessment considering both the number of blastomeres that survived the freeze/thaw cycle and the appearance of the embryo as a whole (amount of fragmentation and granulation). Blastomeres from a total of 12 embryos (7 to 10 cells after thawing) were removed by biopsy. Using a modified version of the Global RT-PCR Amplification technique[6], gene expression of 30 blastomeres was profiled using the Agilent 44k whole human genome arrays. Following amplification, fluorescently labelled material was hybridised to each Agilent 44k whole human genome array. Arrays were scanned on the Agilent G2565BA scanner and quantified using Feature Extraction version 9.5. Data was analysed using GeneSpring version 7.3 and R version 2.8.1.

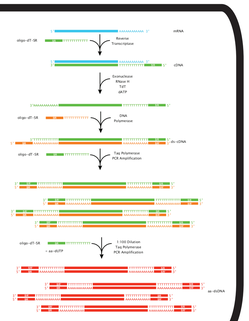
Extract

Single blastomeres are removed from a developing embryo for analysis



Label

RNA is extracted from the blastomeres and amplified using a modification of the Global RT-PCR Amplification technique[6]. This material is further labelled with dyes which will act as an indicator to the relative quantities of individual mRNA transcripts.

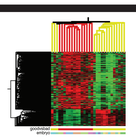


Hybridise

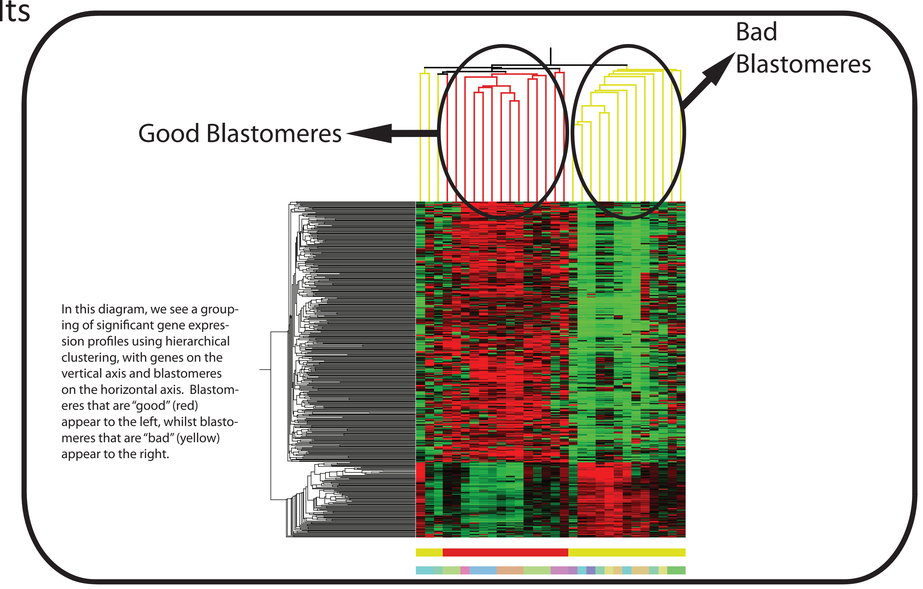
The labelled material is hybridised to slides with probes for each gene in the human genome and then read with a laser scanner.

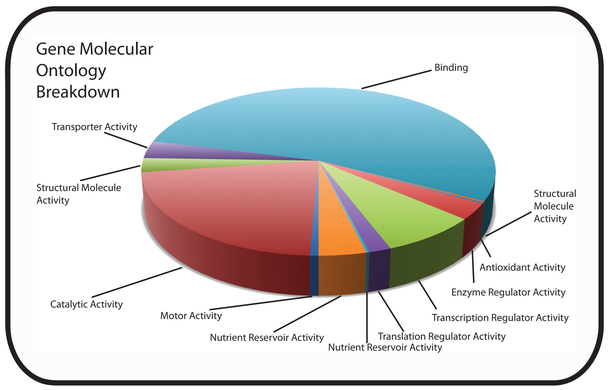
Analyse

The resulting expression data is then normalised, filtered, and statistically analysed for genes which delineate the boundaries of groups.



Results





Conclusions

- Reliable gene expression profiles can be obtained using RNA amplification from single cells
- In the majority of cases a single blastomere from an embryo portrays a very similar gene expression pattern to other blastomeres from the same embryo
- A putative gene expression signature to define “good” vs. “bad” embryos based on single blastomere analysis has been identified
- This gene expression signature will be mined to find proteins detectable in the spent media from embryo culture which may allow for the development of a noninvasive screen to determine embryo viability

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