DEVELOPMENT OF AN ULTRA-SENSITIVE PROTEOMIC METHODOLOGY FOR THE ASSESSMENT OF EMBRYO VIABILITY

M. Poli[1,2], A. Ori[1,3]
[1]*Clinical Research, Reproomics, UK*
[2]*Nuffield Dept. of Obstetrics and Gynaecology, University of Oxford, UK*
[3]*Structural and Computational Biology Unit, European Molecular Biology Laboratory, Germany*

Despite its wide application, Assisted Reproduction Technologies (ART) remains a relatively inefficient procedure, with less than a third of treatments resulting in a healthy pregnancy. In the last decade, clinics have focused on the safety of ART treatments, minimizing the likelihood of multiple gestations and their associated risks (i.e. premature birth, miscarriage). To achieve this result, single embryo transfer policy has been increasingly adopted by many clinics around the world. In this scenario, it is critical to prioritize for transfer the embryo with the highest implantation potential. However, current methods for embryo assessment present limitations. Morphological assessment is easy to implement but offers limited sensitivity in terms of defining embryo's potential to implant. Preimplantation genetic screening (PGS) has been widely adopted to identify aneuploid embryos and exclude them from transfer. Despite its robust technical validation, PGS is not yet perceived as the ideal methodology for embryo assessment due to its invasiveness. Additionally, only half of the euploid embryos transferred to patients end up implanting in the uterus, suggesting that there may be other levels of regulation beyond chromosomal status. It has been suggested that expression patterns of proteins secreted by the embryo may correlate with its ability to implant. So far, however, the development of embryo assessment strategies based on the measurement of proteins has been hampered by the complexity of the media the embryos are cultured in, and the low concentration of the secreted proteins.
In this study, we describe the use of a procedure that allows the safe retrieval of embryo's inner fluid, that we named Blastocentesis. In clinical *in vitro* fertilization practice, the blastocoelic cavity is commonly emptied prior to embryo cryopreservation procedures, to allow higher post-thaw survival rates. The fluid filling this space is generally discarded. Using a tailored mass spectrometry strategy, we analyzed 4 batches of 20 blastocoel fluids and generated a catalogue of 288 proteins present in the human blastocoel. We used microarray and PCR-based gene expression analyses on 19 whole blastocysts to validate the embryonic origin of 182 of these proteins. Further, using targeted proteomics, we demonstrated the feasibility of measuring the abundance of 9 proteins in single blastocoel fluid from single embryos. This allowed us to measure the protein expression profile of 14 individual embryos and correlate it to their chromosomal status. Our data show that embryo chromosomal status can be inferred from the abundance of key protein markers in the blastocoelic fluid. Finally, we assessed the safety of blastocentesis procedure on embryo's viability. The minimally invasive procedure resulted in extremely high survival rates in both embryos allowed to re-expand in culture and embryos that underwent vitrification/warming procedures. The blastocentesis procedure and the validated catalogue of embryonic proteins provided by this study represent a unique resource of novel target molecules for basic and clinical research in human embryology. Additionally, this study provides the biological and technological ground for the development of proteomic-based embryo assessment strategies aimed at improving clinical outcomes of ART treatments.