Mitochondrial Contribution

Elpida Fragouli

IVI-RMA Global, Basic Research Laboratory, Oxford, UK

Nuffield Department of Women's and Reproductive Health, University of Oxford, Oxford, UK

Mitochondrial organelles are critical for the correct function of a cell. They are involved in the regulation of vital cellular processes, including apoptosis, amino acid synthesis, and the generation of energy in the form of ATP via the process of oxidative phosphorylation. For this reason, mitochondria are considered as the main power houses of a cell. Mitochondria are unique compared to other cellular organelles in that they contain one or more copies of their own genome, the mitochondrial DNA (mtDNA). The mtDNA is a circular double stranded molecule of 16.6 kb in size, encoding for genes that produce some of the electron transport chain subunits. This means that mtDNA genes have a direct role in cellular metabolism and the generation of energy.

Mammalian embryos inherit their mitochondria and mtDNA exclusively from a population found in the oocyte. Embryo mtDNA amounts remain stable during the first few days of preimplantation development. Significant mtDNA replication is initiated once the embryo has become a blastocyst, and it is first observed in the trophectoderm part, as this is a finally differentiated tissue which is destined to give rise to the placenta. Embryo development is a dynamic and energy demanding process. Preimplantation embryos require adequate energy levels in order to support their mitotic divisions. This means that correct mitochondrial function and mtDNA gene expression are critical during the first few days of life.

There has been an increasing interest on the quantities of mtDNA of blastocyst stage embryos in the past few years. Specifically, relationships between mtDNA quantity, female age, embryo ploidy, morphology and viability have been described in various different studies. Some of these investigations demonstrated that blastocyst stage embryos generated by reproductively younger women (aged between 21-22 years) contained significantly lower mtDNA amounts, compared to embryos generated by reproductively older women (aged between 42-48 years). It was also evident that aneuploid embryos had significantly more mtDNA, compared to euploid embryos. Additionally, mtDNA quantities were influenced by the morphology of blastocysts, with poorer quality embryos having higher mtDNA levels, compared to those of good morphology.

More interestingly, however, the assessment of mtDNA quantity in relation to embryo viability showed that blastocysts capable of implanting and leading to ongoing pregnancies and live births had significantly lower mtDNA quantities, compared to those which after transfer did not implant. In this context, mtDNA quantity thresholds or scores were established, above which

successful pregnancy after blastocyst transfer was rarely observed. Results obtained during a large blinded multicentre retrospective study showed that approximately 10% of all euploid blastocysts of good morphology, which would be considered for transfer, had mtDNA quantities above established thresholds, and were therefore of low implantation ability. It was also evident that the referring IVF clinic seemed to influence the mtDNA quantities of the embryos it generated. The mtDNA quantity implantation threshold's validity was assessed during a prospective blinded non-selection study which indicated that the negative predictive value of mtDNA quantification (failure of ongoing pregnancy per embryo with elevated mtDNA levels transferred) was 100%. Combination of the different datasets suggested that mtDNA quantification may enable the selection of euploid embryos with the highest implantation potential. This could in turn improve clinical outcomes after IVF with PGT-A by 5%-10%.

There were, however, some other studies that were unable to show a clear relationship between mtDNA quantities and blastocyst implantation potential. It should be noted that some of these studies were hampered by several technical issues, and assessed embryos generated in a single IVF centre. These differences among investigations have led to significant debate about the usefulness of mtDNA quantification as a new biomarker of embryo viability. The ability of mtDNA quantification to predict the embryo's implantation potential could definitively be determined only in a multicentre randomised control trial setting.

The biological mechanisms associated with the low implantation potential of blastocysts with elevated mtDNA quantities are not well understood. It is possible that embryos with elevated mtDNA levels are under some form of cellular stress and require additional energy as they attempt to resolve it. An increase in energy could be achieved by an increase of mitochondria (and mtDNA) replication, or a reduction of the mitophagy rate, allowing persistence of organelles that may have been eliminated. It is also possible that an increase in mitochondria (and mtDNA) represents a compensatory mechanism, employed when the existing mitochondria are not functioning properly. Studies assessing ATP production in blastocysts with unusually high mtDNA levels could provide an insight into the energy status of such embryos aiding, in this way, our understanding of this as yet unexplained phenomenon.