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RESEARCH ARTICLE

# Altered Levels of Mitochondrial DNA Are Associated with Female Age, Aneuploidy, and Provide an Independent Measure of Embryonic Implantation Potential

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## Abstract

Mitochondria play a vital role in embryo development. They are the principal site of energy production and have various other critical cellular functions. Despite the importance of this organelle, little is known about the extent of variation in mitochondrial DNA (mtDNA) between individual human embryos prior to implantation. This study investigated the biological and clinical relevance of the quantity of mtDNA in 379 embryos. These were examined via a combination of microarray comparative genomic hybridisation (aCGH), quantitative PCR and next generation sequencing (NGS), providing information on chromosomal status, amount of mtDNA, and presence of mutations in the mitochondrial genome. The quantity of mtDNA was significantly higher in embryos from older women (P=0.003). Additionally, mtDNA levels were elevated in aneuploid embryos, independent of age (P=0.025). Assessment of clinical outcomes after transfer of euploid embryos to the uterus revealed that blastocysts that successfully implanted tended to contain lower mtDNA quantities than those failing to implant (P=0.007). Importantly, an mtDNA quantity threshold was established, above which implantation was never observed. Subsequently, the predictive value of this threshold was confirmed in an independent blinded prospective study, indicating that abnormal mtDNA levels are present in 30% of non-implanting euploid embryos, but are not seen in embryos forming a viable pregnancy. NGS did not reveal any increase in mutation in blastocysts with elevated mtDNA levels. The results of this study suggest that increased mtDNA may be related to elevated metabolism and are associated with reduced viability, a possibility consistent with the 'quiet embryo' hypothesis. Importantly, the findings suggest a potential role for mitochondria in female reproductive aging and the genesis of aneuploidy. Of clinical significance, we propose that mtDNA content represents a novel biomarker with potential value for in vitro fertilisation (IVF) treatment, revealing chromosomally normal blastocysts incapable of producing a viable pregnancy.