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

The early embryo has high-energy requirements as it divides and differentiates before implantation. The energy needed to support the initial stages of preimplantation development is supplied by mitochondria. Just before fertilization, mammalian embryos inherit mitochondria (and its mtDNA) exclusively from the oocyte. Little is known about the extent of variation in mtDNA between individual embryos or about the biological and clinical impacts of such variation. Previous studies examining human mitochondria and mtDNA in relation to female reproductive aging have had variable results; most reported mtDNA levels are either unchanged or decreased with advancing age.

The aim of this study was to examine the relationship between human blastocyst mtDNA content, female patient age, embryo chromosome status, viability, and implantation potential. The biological and clinical relevance of the quantity of mtDNA was examined in 379 embryos (39 cleavage stage embryos and 340 blastocysts). A combination of microarray comparative genomic hybridization, quantitative polymerase chain reaction, and next-generation sequencing provided information on chromosomal status, amount of mtDNA, and presence of mutations in the mitochondrial genome.

A highly significant increase in embryo mtDNA quantity occurred with advancing female age ($P < 0.003$). Amounts of mtDNA were also significantly elevated in aneuploid embryos, independent of age ($P < 0.025$). After transfer of euploid embryos to the uterus, blastocysts failing to implant tended to contain higher mtDNA quantities than those successfully implanted ($P = 0.007$). An mtDNA quantity threshold was established, above which implantation never occurred. An independent blinded prospective study confirmed the predictive value of this threshold: mtDNA levels above the threshold were present in approximately 30% of nonimplanting euploid embryos, but were never seen in embryos that resulted in a viable pregnancy. Next-generation sequencing analysis demonstrated increased quantities of mtDNA in nonimplanting embryos compared with those shown to be viable. The above data show that the mtDNA content threshold is independent of morphology, age, and aneuploidy.

The investigators speculate that high levels of mtDNA in preimplantation embryos may be related to elevated metabolism in defective preimplantation embryos and are associated with reduced viability. Elevated mtDNA content in nonviable embryos may help explain why up to one third of implantation failures occur in chromosomally normal embryos. Measurement of mtDNA content may represent a novel biomarker for in vitro fertilization treatment by helping identify which chromosomally normal embryos are incapable of producing a viable pregnancy.

EDITORIAL COMMENT

(In the introduction to their study, the authors state that the early embryo has high-energy requirements as it divides and differentiates before implantation, and the energy needed to support the initial stages of preimplantation development is supplied by mitochondria. The first statement is correct, but the second is not. Mitochondrial oxidative phosphorylation is not the principal source of energy for early embryo development. Rather the adenosine salvage pathway provides the major source of ATP to the oocyte. Mitochondria in oocytes and cleavage stage embryos are remarkably quiescent. Transmission electron microscopy shows almost no cristae (morphological sign of active mitochondria), and measurement of oxygen consumption, the sine qua non of mitochondrial activity, is nearly absent in oocytes and embryos until the blastocyst stage of development. Experimental reduction of mtDNA in mice does not affect early embryo development, and women whose oocytes...
harbor severe mtDNA mutations are fertile. Presumably, the metabolically quiescent oocyte favors heritability of intact mtDNA, which otherwise would be mutated by reactive oxygen from OXPHOS.

Previous studies examining human mitochondria and mtDNA in relation to female reproductive aging reported decreased mtDNA copy number with advancing age. Fragouli et al examined the relationship of mtDNA content in blastocysts to age, aneuploidy, embryo viability, and implantation. They found increased mtDNA copy number with advancing female age, and in aneuploid embryos. Blastocysts failing to implant contained higher mtDNA copy number than those which implanted, with a threshold level of mtDNA, above which implantation never occurred.

The investigators speculate that high levels of mtDNA in preimplantation embryos may result from increased metabolic activity in dysfunctional embryos. Intriguingly, the increased mtDNA copy number was not associated with any change in the load mtDNA mutations in the embryos, so it’s not clear what factors might mediate such an altered metabolic state.

An alternative interpretation of these findings is that increased mtDNA copy number merely reflects the increased size of trophectoderm cells in nonviable embryos. Large trophectoderm is the most reliable marker of the nonviable embryo. Even subtle increases in cell radius would markedly increase cell volume because volume increases to the third power of radius. Because mitochondria are cytoplasmic organelles, mtDNA copy number would be expected to be increased in large cells. Future studies need to sort out whether increased mtDNA copy number in nonviable embryos results from their increased cell size, or from mitochondrial dysfunction.—DK)

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**Adult Human and Mouse Ovaries Lack DDX4-Expressing Functional Oogonial Stem Cells**

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**ABSTRACT**

The generally accepted viewpoint for more than 50 years has been that the number of oocytes is fixed in fetal or neonatal ovaries, and therefore, oocytes cannot renew themselves in postnatal or adult life. Over the past decade, however, the traditional viewpoint has been challenged by a number of investigators who have presented evidence that postnatal follicular renewal occurs in mammals, and that mitotically active oogonial stem cells (OSCs) exist in postnatal mouse ovaries.