Enhanced techniques to "power" embryonic mitochondria research

Characterizing the reproductive competence of individual human embryos remains one of the most significant research goals in contemporary reproductive endocrinology. Recent advances in extended culture, aneuploidy screening, and the assessment of embryonic-endometrial synchrony have all produced meaningful improvements in implantation rates and clinical outcomes. Unfortunately, a substantial number of embryos which appear optimal at the time of selection fail to implant and progress to delivery. Clearly, other significant factors are contributing to reproductive senescence and the limits of embryonic competence.

An intriguing topic for investigations into senescence involves mitochondria. Changes in the number, structure, and function of mitochondria are central to aging in virtually all tissues. Given that, it seems almost intuitive that changes in mitochondria would play a role in reproductive senescence, particularly in the human oocyte.

The article by Victor and colleagues (1) in this month's *Fertility and Sterility* advances our understanding of the changes in mitochondrial DNA copy number relative to specific markers of reproductive senescence including maternal age, aneuploidy rates, embryo development, and ultimately implantation rates.

The role of mitochondria in limiting the efficiency of human reproduction has been an active area of investigation for more than two decades. Keefe et al. (2) described an increasing prevalence of microdeletions in the hypervariable region of mitochondrial DNA in older women compared to their younger counterparts. Early efforts to quantitate mitochondrial DNA copy number by Barritt et al. (3) found very high copy numbers (hundreds of thousands of copies), a high degree of variability even amongst the oocytes from a single woman, and no correlation with age.

More recently, methodologies to assess mitochondrial DNA copy number have been refined. The relative quantity of mitochondrial DNA may be contrasted to one or more loci of nuclear DNA to allow calculation of the mitochondrial DNA copy number per cell. Some investigators found that elevated copy number is associated with extremely poor clinical outcomes (4). While neither study evaluated the clinical utility directly, the authors seemed to speculate that mitochondrial DNA copy number might be used to assign a prognosis to individual embryos and to aid in selection of which embryos should be transferred.

Victor et al. (1) had the keen insight that there is significant variability in the nuclear DNA based on aneuploidy and sex chromosomal differences. They developed a statistical model which corrects for these variables allowing a more accurate determination of the average mitochondrial DNA copy number per cell in a given biopsy. The authors use multiple methodologies including those which are dependent on whole genome amplification as well as more targeted screening with quantitative polymerase chain reaction. The authors then go on to compare the adjusted copy number and found that there was no correlation with aneuploidy status, increasing maternal age, blastulation rates, and implantation rates. Significantly, the results were equivalent with all three strategies.

This study is an important contribution to our field for several reasons. It speaks to the importance of thoughtful analysis and careful validation before speculating on clinical utility. Does the study imply that mitochondrial functions are unimportant in reproductive senescence? It does not. It simply suggests that changes in structure and function may be more significant than copy number. Alterations in mitophagy, the process wherein cells remove dysfunctional mitochondria from the cytoplasm may impair function have been studies in animals. Failures to complete this critical function normallyhave been associated with limit implantation rates and increase loss risk in a murine model (5).

An interesting consideration in the clinical setting is the issue of the purpose of mitochondrial DNA copy number screening? If the primary goal to accurately and precisely determine the number of copies present per cell so that insight might be gained into the processes which limit human reproductive efficiency, then the methodology here is the most rigorously validated to date and will empower many future studies.

Alternatively, what if the purpose is solely as a marker to prognosticate implantation rates? It may not provide insight into the physiology of the embryo, but might still prognosticate outcome. While the latter possibility seems far-fetched, those who may continue to advocate the use of mitochondrial DNA density as a marker of embryo viability would be encouraged to do a rigorously designed randomized clinical trial to demonstrate any value.

These are very exciting times. Rigorous methods, new tools, and increasingly sophisticated bioinformatics provide myriad opportunities to study and possibly improve the efficiency of human reproduction. A great deal of work remains to be done. This study is an important step forward in our understanding the fundamental biology of human reproduction and its limits.

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