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Quantitative and qualitative changes of mitochondria in human embryos

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Abstract

Study question: What roles do mitochondria play in the development of human embryos?

Summary answer: The mitochondrial respiratory function of human embryos developed along with embryonic growth although the copy numbers of mitochondrial DNA (mtDNA) decreased transiently before blastulation.

What is known already: Although the rate of oxygen consumption (OCR) in mice and cattle changes during preimplantation embryogenesis, the number of mtDNA copy remains unchanged in mice but changes in cattle and pigs. However, dynamic aspects of mitochondrial functions and mtDNA copy numbers in human embryos during preimplantation development remains obscure.

Study design, size, duration: Sixteen oocytes and 100 embryos were used to assess mtDNA copy numbers and OCR. Three oocytes and 12 embryos were used to determine cytochrome c oxidase activity. All specimens were obtained between July 2004 and November 2014, and donated from couples after they had given informed consent.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Mature oocytes and developed embryos to 2, 3-4, and 5-8 cell, morula and blastocyst stages after ICSI were used to assess their OCR in the presence or absence of mitotoxins. The mtDNA copy number was determined using the samples after analysis of OCR. The relationships between developmental stages and OCR, and developmental stages and mtDNA copy number were analyzed. Furthermore, cytochrome c oxidase activity was determined in oocytes, 4, 8, morula and blastocyst stages.

Main results and the role of chance: Mitochondrial OCR (mtOCR) was calculated by subtracting the value obtained in the presence of cyanide from those obtained without any mitochondrial toxins. No difference in mtOCR was found from oocyte to 8 cell stages. However, mtOCR increased rapidly at 9-14 cell and later stages ($P < 0.01$) compared with those until 8 cell stage. Furthermore, the mtOCR at blastocyst stage were significantly higher than those until 14 cell stage ($P < 0.01$). The number of mtDNA copy per specimen in embryos decreased transiently ($P < 0.01$) at 2 cell, 9-14 cell and morula stages compared with oocytes. The number of mtDNA copy significantly increased ($P < 0.01$) in expanded blastocyst stage compared with those in earlier stages. The numbers of mtDNA copy per cell in embryos decreased gradually ($P < 0.01$) from oocytes toward morula and blastocyst stages. Taken together, OCRs increased toward the morula stage ahead of an increase of mtDNA at the time of blastulation. The undifferentiated state of inner cell mass appears to be associated with a low OCR. On the other hand, the aerobic metabolism of mitochondria in trophectoderm cells increased.

Limitations, reasons for caution: All samples except for oocytes were used after vitrification

and warming following the guidelines of the Japan Society of Obstetrics and Gynecology. To avoid contamination with mtDNA from spermatozoa attached to the zona pellucida, all embryos were obtained by using intra-cytoplasmic sperm injection.

Wider implications of the findings: Modifying the energy sources required for mitochondrial functions would provide an environment with less stressful conditions for the culture of embryos. The present work showing changes in mitochondrial structure, function, and mtDNA copy numbers during preimplantation development would be useful in optimizing culture media for the development of human embryos.

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Key words: mitochondrial DNA /mitochondrial function / oxygen consumption

Trial registration number: This study was approved by the IRB of IVF Namba Clinic and the Japan Society of Obstetrics and Gynecology (Registry numbers 135 and 138).