Abstract Details

Session title: Embryology
Session type: Poster viewing
Presentation number: P-265

Abstract title:

Total mitochondrial DNA (mtDNA) content decreases along embryo development, insights of mtDNA turnover in human preimplantation development.

Biography

Senior Embryologist

M.F. Insua[1], A. Díez-Juan[2], A. Mercader[3], J. Bellver[4], L. Escrich[1], A. Pellicer[5], M.J. De los Santos[6].
[1]IVI Valencia, IVF laboratory, Valencia, Spain.
[2]Igenomix, PCR Laboratory, Paterna, Spain.
[3]IVI Valencia, PGT Laboratory, Valencia, Spain.
[4]IVI Valencia, Reproductive Medicine, Valencia, Spain.
[5]IVI Roma, Reproductive Medicine, Roma, Italy.
[6]IVI Valencia, IVF Laboratory Director, Valencia, Spain.

Study question:

To assess the mtDNA content along the human embryo development.

Summary answer:

This study demonstrates that not only the mtDNA content per cell decreases but also the total mtDNA copy number experiences a significant reduction before implantation.

What is known already:

It has been shown that mtDNA has a half-life in culture of 2-4 days. In animals, mtDNA copy number has been revealed to change depending on the species. Hashimoto et al., (2017) observed that total and per cell mtDNA copy number decreased along human embryo development, however there was an increase of total mtDNA copy number at the blastocysts stage. mtDNA content along embryo development has not been widely studied in humans but this is an important topic to address in order to better understand the mitochondrial dynamics in preimplantation embryos under in vitro culture conditions.

Study design, size, duration:

An observational prospective study was performed with a total of 89 samples. 36 fresh unfertilized oocytes, 21 vitrified day 3 embryos and 32 vitrified aneuploid blastocysts were warmed and immediately collected in PCR tubes with 2,5 µl of PBS. Finally, a Q-PCR was performed to determine total and per cell mt DNA copy number.

Participants/materials, setting, methods:

Q-PCR was performed with SurePlex DNA Amplification System (Illumina) using specific primers for the ATP8 and β-Actin genes to assess the total and per cell mtDNA copy number. Data was statistically analysed by ANOVA test with Scheffé multiple comparison for categorical variables and linear regression

for numerical variables.

Main results and the role of chance:

Fresh oocytes have significantly more total and per cell mtDNA copy number (Log_2 ATP8= 20.32, Log_2 ATP8/β-Actin=7.49) than embryos on day 3 of development (Log_2 ATP8= 18.26, Log_2 ATP8/β-Actin=5.46; $P<0.05$). At the same time, day 3 embryos have more total and per cell mtDNA copy number than blastocysts (Log_2 ATP8= 16.39, Log_2 ATP8/β-Actin=3.15; $P<0.05$). Interestingly, mtDNA content increased significantly ($P<0.05$) with age in oocytes (18-34 years) and embryos on day 3 of development (25-37 years) but not statistically significant ($P>0.05$) in blastocysts (24-44 years). Once fertilization takes place, mtDNA content in embryos on day 3 of development decreases along cleavage achieving minimal levels at blastocyst stage. No differences on mtDNA content were found when grouping blastocysts by quality or by day of development ($P>0.05$). This study shows that mtDNA content diminishes along embryo development and no mitochondrial biogenesis takes place at blastocyst stage. Also we have found a relation with the patient age in oocytes and embryos on day 3 of development within the age ranges of the study.

Limitations, reasons for caution:

All the analyzed blastocyst were aneuploid, so we need to check whether euploid human blastocysts will have similar behavior. Moreover, some of the embryos included were vitrified however, since they were tubed immediately after warming, the impact should be minimal.

Wider implications of the findings:

According to previous studies in animals, during preimplantation development human embryos seem to experience a significant decrease on mtDNA content that support the embryo quiet hypothesis. Therefore any situation resulting in mtDNA content increase should be understood as a stress condition. This findings could be used to improve culture conditions.

Trial registration number:
Not applicable

Keywords:
mtDNA
Q-PCR
Embryo
development