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Comparison of mitochondrial DNA content in euploid and aneuploid embryos at cleavage stage and blastocyst stage with NGS

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Study question:

Is the mitochondrial DNA-content (mtDNA) and its dynamics in cleavage stage and blastocyst stage embryos different in euploid and aneuploid embryos when measured with NGS?

Summary answer:
mtDNA-content is comparable in euploid and aneuploid embryos at cleavage stage, however, it is significantly higher in aneuploid embryos at blastocyst state.

What is known already:
Mitochondria and their DNA (mtDNA) are lately increasingly investigated regarding their ability as a potential marker for embryo viability and implantation chances and an increased number of mtDNA-copies in the blastocyst seems to be associated with decreased implantation-rates. Replication of mtDNA is not initiated during the first 3 days of embryo development and because of cell division, mtDNA-content is expected to decline from cleavage to blastocyst stage biopsy. Increased mtDNA-numbers at blastocyst stage are found with advanced female age and in aneuploid embryos. However, so far, data on the mtDNA-content in euploid and aneuploid embryos at cleavage stage is rare.

Study design, size, duration:
Retrospective, blinded, observational study including 35 patients (118 embryos) which underwent preimplantation genetic testing (PGT-A) from August 2016 to January 2017. This study validated the chromosomal status of D5 embryos previously diagnosed on D3 and determined the mtDNA-content. Double biopsy (D3 and D5) was performed to all embryos that reached blastocyst stage on D5 and were not selected for transfer (including surplus euploid embryos that could not be vitrified according to the UAE law).

Participants/materials, setting, methods:
Infertile patients with normal karyotype undergoing preimplantation genetic screening with fresh oocytes and with ≥5 MII or more than 4 embryos to biopsy on D3. For embryo biopsies at D3, only embryos with ≥5 nucleated blastomeres and < 25 % fragmentation and for blastocyst biopsy, only hatching blastocyst were evaluated. Relative values of mitochondrial DNA were directly obtained from the software and were analyzed using IGenomix algorithm for day 3 and day 5 biopsies.

Main results and the role of chance:
After genetic screening at cleavage stage, there were 70 aneuploid and 48 euploid embryos. In the group of aneuploid embryos, mean mtDNA-content on D3 was 50.8 with a range from 31.7 to 99.4, a 95% confidence interval from 47.1 to 54.4 and a standard deviation (SD) of 15.3. In the group of euploid embryos, mean mtDNA on D3 was 51.1 with a range from 35.2 – 99.6, a 95% confidence interval from 47.3

to 54.8 and SD of 13.0. No significant correlation between mtDNA on D3 and the chromosomal status of the embryos was found (p = 0.628).

After blastocyst biopsy, the results of the genetic screening revealed 51 aneuploid and 62 euploid embryos. In the group of aneuploid embryos, mtDNA-content was 22.8 with a range of 12.2 – 48.4, a 95% confidence interval of 20.8 to 24.9 and SD of 7.3. In the euploid embryos, the results were 20.6 for the mean mtDNA-content with a range of 12.8 to 45.4, a 95% confidence interval of 19.0 – 22.2 and SD of 6.2. A significant difference in mtDNA on D5 between aneuploid and euploid embryos (p = 0.034) was found with Mann-Whitney test.

Limitations, reasons for caution:
It is a bivariate analysis (aneuploidies versus mtDNA-content), and not controlled by other covariates that could potentially bias the relationship we are studying. At blastocyst stage, 5 embryos with trophectoderm Mitoscore values above 1.000 were considered as “outliers” and therefore excluded from the comparison between euploid and aneuploid embryos.

Wider implications of the findings:
The fact that mtDNA-content is similar in euploid and aneuploid cleavage stage embryos, however significantly higher in aneuploid blastocysts supports the idea that mtDNA-content might have a direct impact on chromosome segregation during embryo development. It seems that embryos under energetic stress are forced to increase the biogenesis as compensation.

Trial registration number:
not applicable

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