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A mathematical correction method to accurately determine mitochondrial DNA levels in human embryos

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Introduction: In accordance with the quiet embryo hypothesis (Leese 2002), the level of mitochondrial DNA (mtDNA) has been proposed as a biomarker for implantation potential amongst euploid embryos, and overall differences in mtDNA amounts have been shown between embryos grouped by ploidy and age (Fragouli et al. 2015; Diez-Juan et al. 2015). We developed a mathematical formula resulting in an accurate calculation of mitochondrial levels as established by next generation sequencing (NGS) or quantitative polymerase chain reaction (qPCR).

Materials & Methods: We tested mtDNA content of 833 embryos derived from 181 patients by NGS, and 150 embryos derived from 96 patients by qPCR. For each embryo, the level of mtDNA was determined from a trophectoderm biopsy by whole genome amplification followed by NGS and/or qPCR. The value was subjected to mathematical analysis tailored to the genomic DNA composition of said embryo.

Results: On average our quantitation method changed the conventionally determined mtDNA level via NGS by 1.35% +/-1.58%, with changes ranging up to 17.42%, and via qPCR by 1.33% +/-8.08%, with changes ranging up to 50.00%. Our data also shows a proof of principle how the correction factor can considerably impact the P value when calculating the significance of mean changes between embryo groups substratified by ploidy, age, or implantation potential.

Conclusions: We recommend the implementation of our correction factor to all laboratories evaluating mtDNA levels in their embryos by NGS or qPCR.

References:

Leese H.J. (2002) Quiet please, do not disturb: a hypothesis of embryo metabolism and viability. Bioessays 24: 845–849.

Fragouli E., Spath K., Alfarawati S., Kaper F., Craig A., Michel C.-E., Kokocinski F., Cohen J., Munne S., Wells D. (2015) Altered Levels of Mitochondrial DNA Are Associated with Female Age, Aneuploidy, and Provide an Independent Measure of Embryonic Implantation Potential. *PLoS Genetics* 11(6): e1005241.

Diez-Juan A., Rubio C., Marin C., Martinez S., Al-Asmar N., Riboldi M., Díaz-Gimeno P., Valbuena D., Simón C. (2015) Mitochondrial DNA content as a viability score in human euploid embryos: less is better. *Fertility and Sterility*, Volume 104, Issue 3, Pages 534-541.e1