Journal of Assisted Reproduction and Genetics (2019) 36:1845–1846
https://doi.org/10.1007/s10815-019-01565-z

COMMENTARY

Mitochondrial DNA as a readout of embryo cellularity

Manuel Viotti [1]

Received: 9 August 2019 / Accepted: 13 August 2019 / Published online: 19 August 2019

When thinking about the human genome, it is easy to forget that, aside from the DNA found in the cell's nucleus, some of our genetic material is located in mitochondria. Mitochondrial DNA (mtDNA) is formatted in circular molecules containing genes necessary for mitochondrial function and therefore essential for the cell's survival. Each human cell contains a varying number of mitochondrial organelles and each mitochondrion can contain numerous copies of mtDNA. This copy number is not static and can change according to the energetic demand experienced by its host tissue.

The furor caused by reports correlating high mtDNA copy number with embryonic inviability was quickly met by other studies challenging those observations. After much discussion about appropriate technical and analytical methods of mtDNA quantitation and additional papers in both camps, it now appears that using mtDNA copy number as a biomarker for implantation might be valuable in some centers but not in others. Why?

Taking a step back, the original discovery concerning mtDNA copy number was that embryos classified as aneuploid with PGT-A generally contained higher levels of mtDNA than their euploid counterparts. This led to the postulation that general cell stress correlated with high mtDNA copy number and that stressed embryos increased their mtDNA content to compensate for an elevated energetic demand. Consequently, within the pool of euploid embryos, it was proposed that those with higher mtDNA levels might be experiencing cell stress, hence decreasing their viability. Fittingly, the initial reports noted that euploid embryos with very high mtDNA content did not implant.

A crucial, often overlooked point in the discussion is that under normal circumstances, mtDNA does not replicate between zygote and blastocyst stage. Replication of mtDNA commences specifically in the blastocyst trophectoderm, and only later in the ICM. During the first days of development, the initial set of mtDNA molecules present in the oocyte (the human cell with highest mtDNA copy number, whereas the sperm does not contribute to the mtDNA pool) becomes progressively diluted between dividing cells. It follows that an embryo with arrested or attenuated development, having undergone fewer cell divisions and containing fewer cells, should on average contain a higher mtDNA copy number per cell than a normally dividing embryo. The analysis of morphology and developmental progression is therefore key to the mtDNA discussion.

In this issue of *JARG*, Bayram and colleagues perform a correlative analysis of mtDNA copy number (which the authors call Mitoscore) and morphokinetics for cleavage stage embryos. The observations confirm that (1) the slower the development of the embryo, the higher the mtDNA content per cell, and (2) the more cellular the embryo, the lower the mtDNA copy number per cell. Importantly, at first glance and in general terms, it looks like aneuploid embryos have higher mtDNA copy number than euploid embryos. But when exquisitely normalizing for cellularity, the authors observe that this effect goes away. In other words, euploid and aneuploid embryos with an equal number of blastomeres contained the same mtDNA copy number per cell. The importance of this finding cannot be overemphasized for the discussion surrounding mtDNA quantitation. Bluntly stated, it means that aneuploidy alone does not increase mtDNA copy number per cell, as the hypothesized compensatory mechanism to cell stress would have predicted. Instead, it points to the fact that aneuploid embryos generally develop slower and have decreased cellularity, meaning mtDNA content is less diluted between existing cells. This is the reason aneuploid embryos have in general greater mtDNA copy number per cell than euploid embryos, on average and without controlling for cellularity.

What could this mean in regard to using mtDNA as a biomarker for viability? One could speculate that reports of embryos with very high mtDNA copy number always failing to

✉ Manuel Viotti
manuel@zouvesfoundation.org

[1] Zouves Foundation for Reproductive Medicine, 1241 E Hillsdale Blvd, Foster City, CA 94404, USA

implant come from laboratories more amenable to considering blastocysts with poor morphology. That is not a bad thing per se, as it increases the pool of possible embryos for transfer for a given patient, but at the possible cost of decreased implantation potential. Say a fertility center only considers embryos for transfer with good to excellent morphology and steady development. They will have a hard time finding embryos with very high mtDNA content per cell, obviating the need to quantify mtDNA clinically. On the other hand, a laboratory that is willing to consider euploid embryos with attenuated development and low cellularity will encounter some instances of very high mtDNA content. And indeed, in those cases, it might be a good idea to de-prioritize those embryos from transfer. The salient point is that mtDNA copy number is likely no more than a readout of mitogenic activity in early embryos, tightly linked to morphology and developmental kinetics.

Previous efforts analyzing mtDNA copy number in embryos have used more subjective evaluations of morphology both at the cleavage and blastocyst stages, which carries some obvious intrinsic error. Case in point, two additional papers in this issue of *JARG* by Lee et al. and De Munck et al. report opposing findings when assessing morphology by eye, one observing a correlation of mtDNA with ploidy and the other not seeing such a trend. In their study, Bayram and colleagues have meticulously counted each cell in the analyzed embryos. The authors of the manuscript are to be lauded for making this fundamental contribution to the discussion of mtDNA quantitation in embryos.