

Commentary

Mitochondrial DNA Copy Number, a Marker of Viability for Oocytes¹

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Finding a biological marker of viability for oocytes and sperm has been a challenge for reproductive biologists. Such a marker could be used to choose gametes that have a higher chance of producing healthy descendants. This is especially important in humans and would benefit couples facing reproductive problems. The enormous amount of mitochondrial DNA (mtDNA) in oocytes and the fact the mtDNA is not replicated before implantation have led to speculation that the number of mtDNA copies in oocytes could be used as a marker of viability [1–4].

Mitochondrial DNA is present inside the mitochondrion and codes for proteins that are essential for cellular energy production [4]. Multiple copies of mtDNA in the same mitochondrion encode the proteins necessary for mitochondrial function, with the number of copies being directly proportional to the amount of ATP synthesized [4, 5]. Mitochondrial DNA from sperm does not contribute to the new embryo; all the mtDNA arises from the oocyte [4]. Hence, mtDNA copy number in oocytes could be a key determinant of development. Although some evidence supports this hypothesis (e.g., low mtDNA copy number in human oocytes has been linked to infertility [1–3]), little is known about how mtDNA copy number affects oocyte viability. In this issue, Wai et al. [6] report that the mtDNA content in mouse oocytes is involved in gamete viability, and a minimum number of copies is required by oocytes/embryos to support postimplantation development.

To study the importance of mtDNA copy number in oocytes and sperm, Wai et al. [6] partially depleted these cells of mtDNA using Cre recombinase-mediated excision of *Tfam*, which is an important component of nucleoids [4]. The heterozygous knockout *Tfam* mice obtained by expressing Cre recombinase in the germline reduced the mtDNA copy number by approximately 60% in oocytes and sperm. However, the investigators observed no effect on their reproductive performance when the heterozygous knockout *Tfam* mice were mated with wild-type animals. This suggests that mouse oocytes and sperm contain more mtDNA copies than are needed to support development to term. One might guess the large number of

mtDNA copies in oocytes is necessary to provide ATP during fertilization and preimplantation [1–4], but the rate of mitochondrial respiration during early development is actually very low [5]. Moreover, embryonic development does not require mtDNA-encoded proteins up to the blastocyst stage in mice [7]. Replication of mtDNA does not appear to be essential for mouse development up to the egg cylinder stage [8], further confirming that mitochondrial function is not crucial for preimplantation in this species.

If, however, the mtDNA content in oocytes can be reduced by more than half with no effect on development, why do oocytes contain so many mtDNA copies? Previous reports have shown that the homozygous knockout of genes essential to the maintenance, replication, and expression of mtDNA in the mouse leads to developmental arrest between Embryonic Day (E) 8.5 and E10.5 [9–11]. Hence, re-establishment of mtDNA replication around E6 [8] must be essential to development. Otherwise, dilution of nonreplicating mtDNA molecules among daughter cells would result in mtDNA depletion and developmental arrest. Thus, one would expect to find a threshold mtDNA copy number in oocytes below which development after E8.5 to E10.5 is not possible.

Wai et al. [6] investigated this hypothesis using a gene promoter that allowed Cre recombinase expression to excise *Tfam* while mtDNA is intensively replicating during oocyte growth. As a result, the heterozygous females that were produced carried oocytes with mtDNA content depleted by approximately 90%; the majority of these oocytes were infertile. Although these oocytes were not able to develop to term, they developed into blastocysts with no obvious effect on rates of development. This confirms that mtDNA copy number in oocytes is critical for preimplantation in the mouse.

To further clarify whether female infertility was caused by the reduced levels of mtDNA in oocytes, blastocysts with low and with high mtDNA copy number were transferred into pseudopregnant females. Blastocysts were obtained by fertilization of *Tfam* heterozygous knockout oocytes (from females made heterozygous during early germline development) and from which a single cell had been biopsied at the 8-cell stage to determine the mtDNA content. Wai et al. [6] used 40 000–50 000 copies as a cutoff point, assuming that the lower limit of mtDNA copy number found in normal mouse oocytes defines the threshold number of copies necessary for development. In agreement with this, those authors found that development to either E10.5 or E12.5 failed when the number of mtDNA copies in 8-cell embryos was less than 50 000.

These findings [6] support the hypothesis that a minimum number of mtDNA copies in oocytes/embryos is necessary to allow the progression of postimplantation development (Fig.

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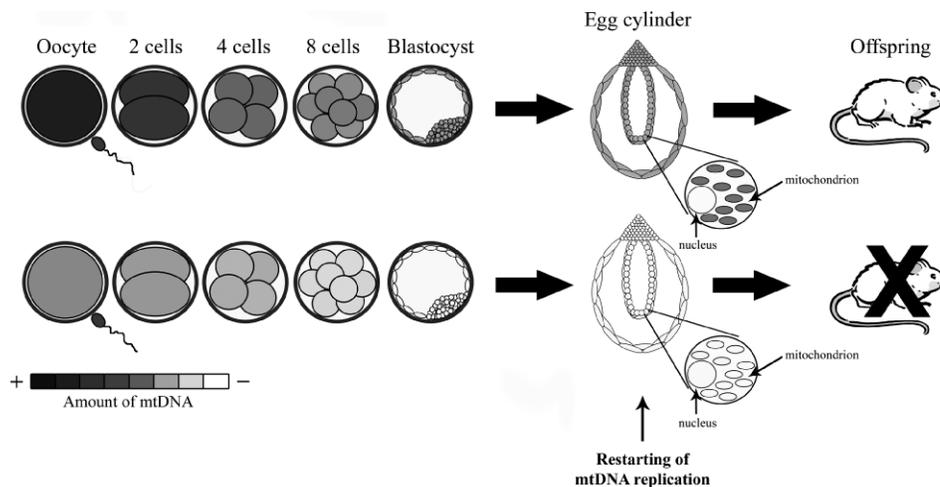


FIG. 1. Model of the effect of mtDNA copy number on developmental progress in mouse oocytes/embryos. This illustrates the postfertilization development of oocytes with mtDNA copy number above (**top**) and below (**bottom**) the threshold (40 000–50 000). After fertilization, embryo cleavage leads to mtDNA segregation among daughter cells, because mtDNA replication is downregulated up to E6 (egg cylinder stage). Thus, the number of mtDNA molecules per cell decreases after each cell cycle, leading to postimplantation developmental arrest if the starting copy number is below the threshold. Developmental arrest is thought to be caused by dilution of mtDNA copies below a minimum number necessary to support the cellular energy requirement. Therefore, mtDNA copy number can be used to select mouse oocytes/embryos with higher chances of development to term. Nevertheless, because the stage at which mtDNA replication starts again has been shown to differ among species, it remains to be proven whether mtDNA copy number can be used for the same purpose in other species.

1). Although mitochondrial function appears to be dispensable during preimplantation [5, 7], it is essential in subsequent stages of development [6, 9–11]. Thus, the oocyte needs enough mtDNA copies to provide a minimum number of molecules for each daughter cell before restarting of mtDNA replication around E6 [8]. These findings likely are of fundamental importance for human and livestock fertility, in which mtDNA copy number could be used as a viability marker to select oocytes/embryos with higher chances of development. Because preimplantation genetic diagnosis has been used in humans to select unaffected embryos for several disorders [12], quantification of mtDNA could be easily adapted to select embryos with higher levels of mtDNA. However, other selection criteria (e.g., embryo morphology) should be regarded as equally or even more important until studies have been carried out in species other than mice. In cattle, depletion of mtDNA content in zygotes can be replenished up to the blastocyst stage by a compensative replication [13], which minimizes the importance of mtDNA copy number in oocytes. The data reported by Wai et al. [6] also highlight the importance of mtDNA segregation among daughter cells, which could contribute to selection of wild-type mtDNAs to populate the germline. Dilution of mtDNA molecules to only a few copies per cell may enhance the effect of mtDNA mutations (normally mixed with wild-type molecules) on cell phenotype, and this might enhance selection of mtDNA molecules at the cellular level [14].

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