

## REVIEW ARTICLE

# The role of mtDNA in oocyte quality and embryo development

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## Abstract

The mitochondrial genome resides in the mitochondria present in nearly all cell types. The porcine (*Sus scrofa*) mitochondrial genome is circa 16.7 kb in size and exists in the multimeric format in cells. Individual cell types have different numbers of mitochondrial DNA (mtDNA) copy number based on their requirements for ATP produced by oxidative phosphorylation. The oocyte has the largest number of mtDNA of any cell type. During oogenesis, the oocyte sets mtDNA copy number in order that sufficient copies are available to support subsequent developmental events. It also initiates a program of epigenetic patterning that regulates, for example, DNA methylation levels of the nuclear genome. Once fertilized, the nuclear and mitochondrial genomes establish synchrony to ensure that the embryo and fetus can complete each developmental milestone. However, altering the oocyte's mtDNA copy number by mitochondrial supplementation can affect the programming and gene expression profiles of the developing embryo and, in oocytes deficient of mtDNA, it appears to have a positive impact on the embryo development rates and gene expression profiles. Furthermore, mtDNA haplotypes, which define common maternal origins, appear to affect developmental outcomes and certain reproductive traits. Nevertheless, the manipulation of the mitochondrial content of an oocyte might have a developmental advantage.

## KEYWORDS

genomic balance, mitochondrial DNA, mitochondrial supplementation, nuclear transfer, oogenesis

## 1 | INTRODUCTION

In the last few years, the role played by the mitochondrial genome in fertilization outcome and embryo development has gained increasing interest. In this context, the pig (*Sus scrofa*) has largely been regarded as a model of human embryology and development (Humpherson et al., 2005) and as a model for conducting human preclinical testing and trials (Bode et al., 2010; Larsen & Rolin, 2004) for which it has

been deemed to be excellent in both respects (Perleberg et al., 2018). Nevertheless, the outcomes of these studies should be of significant value to pig scientists, veterinarians, and breeders who seek to maximize reproductive and other economic breeding traits to produce superior animals for the food chain and other breeding purposes. This review focuses on the role of the mitochondrial genome from the perspective of the oocyte and how it can influence fertilization outcome and development; and how its copy number is

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strictly regulated to ensure key developmental milestones are met. We further highlight how the nucleus and mitochondrial genome need to interact to ensure that development can progress.

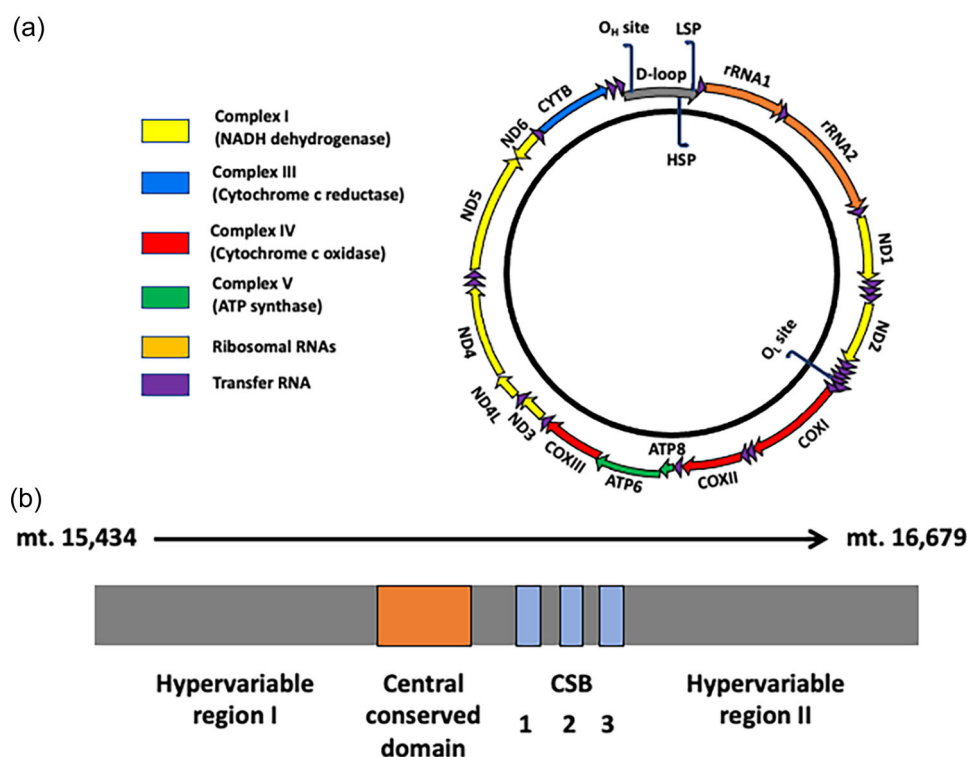
## 2 | MITOCHONDRIAL DNA (mtDNA)

The maternally inherited mitochondrial genome (Giles et al., 1980), otherwise known as mtDNA, resides in each of the large numbers of mitochondria present within many cell types. It is a double-stranded circular genome that ranges from 16.2 kb (mice) to almost 16.7 kb (pigs) in size across mammalian species (Anderson et al., 1981; Bibb et al., 1981; Ursing & Arnason, 1998) (Figure 1a). It encodes 13 of the greater than 90 subunits of the electron transfer chain, which generates ATP through oxidative phosphorylation (OXPHOS), and is essential for cells with high energy requirements, such as neurons, and heart and skeletal muscle cells (Moyes et al., 1998). The remainder of the genes encoding the other subunits of the electron transfer chain and all other genes of the mitochondrion are encoded by the nuclear genome. mtDNA also encodes 2 ribosomal RNA (rRNA) subunits and 22 transfer RNAs (tRNAs) and has 1 noncoding region, the D-Loop (Figure 1b), which contains regulatory regions

that interact with the nuclear-encoded mtDNA-specific transcription and replication factors (Kucej & Butow, 2007). The D-Loop also contains two hypervariable regions that are used by molecular geneticists to map common maternal origins and migratory routes for many species (Wallace et al., 1999). Based on their maternal origins, individuals are grouped into mtDNA haplotypes that are characterized by similar mitochondrial genomes (Wallace et al., 1999).

## 3 | mtDNA REPLICATION

mtDNA replication is dependent on a number of nuclear-encoded mtDNA-specific transcription and replication factors that translocate and enter the mitochondrion, many of which possess mitochondrial-targeting tags (Kucej & Butow, 2007). The key transcriptional factors comprise the mitochondrial RNA polymerase (POLRMT) (Tiranti et al., 1997), and mitochondrial transcription factors A (TFAM) (Fisher & Clayton, 1988), B1 (TFB1M), and B2 (TFB2M) (Falkenberg et al., 2002). mtDNA transcription precedes mtDNA replication, which is essential as the transcript that is generated undergoes cleavage to provide the primer (Hillen et al., 2017), used by the mitochondrial-specific replicase, DNA polymerase gamma (POLG), to initiate and



**FIGURE 1** The porcine mitochondrial genome. (a) The porcine mitochondrial genome is 16.7 kb in size and encodes 13 of the subunits of the electron transfer chain, namely ND1 to 6 and ND4L, CYTB, COX I to III, and ATP6 and ATP8. It also encodes 2 rRNAs (12S and 16S rRNAs), and 22 tRNAs. It contains two noncoding regions. The smaller region is located two-thirds of the way around the genome and houses the origin of L-strand replication ( $O_L$ ). The major noncoding region is the D-Loop, which contains the regulatory regions, the H-strand promoter region (HSP), the L-strand promoter region (LSP), and the origin of H-strand replication ( $O_H$ ). (b) The D-Loop (mt. 15,434 to mt. 16,679) also contains two hypervariable regions I and II, the central conserved domain, and three conserved sequence boxes (CSB1-). rRNA, ribosomal RNA; tRNA, transfer RNA.

promote replication (Wernette & Kaguni, 1986). In mammals, the polymerase consists of two components, a catalytic subunit (encoded by *POLG*) and two accessory subunits (encoded by *POLG2*) which anchor the catalytic subunit to its template (Carrodeguas et al., 1999) (Figure 2). Replication is also dependent on the mtDNA-specific helicase, Twinkle (TWNK), and the mitochondrial single-stranded DNA binding protein (SSBP1) (Korhonen et al., 2003). To facilitate replication of this double-stranded molecule, the mtDNA-specific topoisomerase (encoded by *TOP1MT*) is responsible for cleaving and religating one of the strands (Zhang et al., 2007).

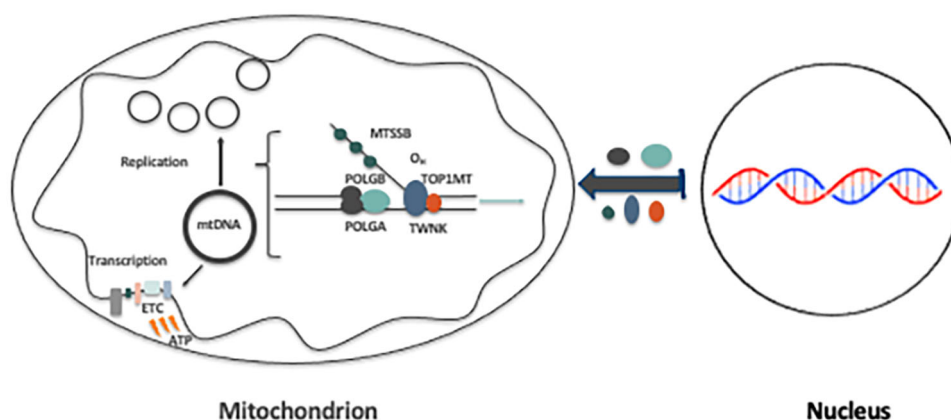
#### 4 | mtDNA COPY NUMBER

Each mitochondrion possesses between 2 and 10 copies of mtDNA which are tethered to nucleoid structures comprising the nuclear-encoded mtDNA transcription and replication machinery and anchoring proteins (Kucej & Butow, 2007). These copies of mtDNA originate from an individual's mother's primordial germ cells, the very first germ cells present just after gastrulation (Nguyen et al., 2019). This population ranges from approximately 200 copies in mouse (Cao et al., 2007; Cree et al., 2008; Wai et al., 2008) (Figure 3), possess approximately 1130 copies (Tsai et al., 2017). During oogenesis, this population increases exponentially resulting in the mature, fertilizable, metaphase II oocyte possessing between 180,000 and 500,000 copies of mtDNA (Figure 3) across a number of mammalian species including the pig (May-Panloup, Chretien, et al., 2005; May-Panloup, Vignon, et al., 2005; T. A. Santos et al., 2006).

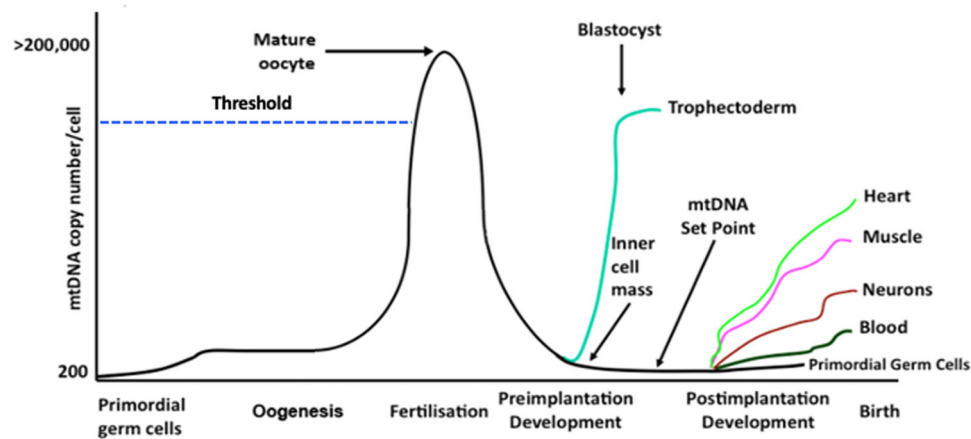
The population present in the metaphase II oocyte is considered to be an investment in subsequent developmental events (St John, 2016). Indeed, the metaphase II oocyte possesses significantly more copies of mtDNA than any other cell type and, when compared with the nuclear genome at this stage of development, is an equal partner in terms of genomic or DNA contribution on a per nucleotide basis (St John, 2019). This investment is necessary as, apart from a minor

mtDNA replication event that takes place between fertilization and the 2-cell stage (McConnell & Petrie, 2004), there are significant reductions in copy number per cell between the 4- and 16-cell stages, as evidenced in pig embryo development (Spikings et al., 2007) as each newly formed blastomere divides and, thus, possesses fewer copies of mtDNA (El Shourbagy et al., 2006) (Figure 3). These decreases are matched by low levels of expression of the nuclear-encoded mtDNA-replication factors which, again from studies in the pig, demonstrates the strict regulation of this process (Spikings et al., 2007). Furthermore, there appears to be an active process of extrusion of mtDNA into the embryo's neighboring environment (Hammond et al., 2017; Stigliani et al., 2014). As argued by others, the developing embryo, thus, begins to increasingly rely on aerobic glycolysis for energy production (Krisher & Prather, 2012), a combination of low levels of oxygen consumption and glycolysis, and a process that is also utilized by tumor cells and first proposed as the Warburg Hypothesis (Warburg, 1956). In the pig, this is coupled with an amino acid metabolism at the blastocyst stage that is similar to the human embryo (Humpherson et al., 2005).

At the blastocyst stage, the final stage of preimplantation development, mtDNA replication is initiated but this is restricted to the trophectoderm (Houghton, 2006; Spikings et al., 2007) (Figure 3), which gives rise to the placenta. This most likely supports the metabolic requirements of these cells to both provide the inner cell mass with its required nutrients, and to promote implantation, and once the blastocyst implants, it is able to feed on the metabolites produced by its neighboring environment to promote OXPHOS and provide nutrients throughout fetal development. Unlike nuclear DNA, the mitochondrial genome is replicated multiple times per cell cycle. This process does not take place uniformly in all mitochondria but rather in a limited number and is likely focal (Chatre & Ricchetti, 2013), which may account for the disparity in the assessment of mtDNA copy number in trophoblast biopsies and its relevance to aneuploidy and implantation rates (Fragouli et al., 2015; Treff et al., 2017).



**FIGURE 2** The mitochondrial replication machinery mtDNA replication is dependent on nuclear-encoded mtDNA-specific transcription and replication factors that translocate to the mitochondrion. Firstly, mtDNA transcription is initiated, which is a pre-requisite for replication to proceed. The key factors involved in mtDNA replication are POLGA, POLGB, TWNK, TOP1MT, and MTSSB. Each is specific to mtDNA replication only. ETC, electron transfer chain; mtDNA, mitochondrial DNA.



**FIGURE 3** The strict regulation of mtDNA copy number during development. During oogenesis, mtDNA copy number exponentially increases to reach a peak at fertilization. Failure of the maturing oocyte to increase mtDNA copy number above the threshold (dotted blue line) can result in fertilization failure or early embryo arrest. mtDNA copy number per cell progressively decreases during preimplantation development until the blastocyst stage when replication is initiated in the trophoblast only. The inner cell mass cells continue to reduce mtDNA copy number and the “mtDNA set point” is established, which is essential for mature cells to acquire the required numbers of mtDNA copy as their precursor cells undergo differentiation into their mature, fully functional forms. mtDNA, mitochondrial DNA.

MtDNA replication is quiescent in the inner cell mass, which contains undifferentiated, pluripotent cells (Spikings et al., 2007) that progress through gastrulation, differentiate, and give rise to the fetus (Figure 3). The persistent dilution of mtDNA copy number before gastrulation establishes the “mtDNA set point,” which is the population of mtDNA, that is, the founder population of mtDNA, that contributes to the offspring’s somatic cells (Facucho-Oliveira et al., 2007; Kelly et al., 2013). Therefore, when cells initiate specialization during organogenesis, they use this founder population of mtDNA as the template for replication. Replication then proceeds in a cell-specific manner to provide individual cell types with sufficient copies of mtDNA to support their requirements for OXPHOS-derived ATP. This, thus, enables mature cells to meet the energy requirements associated with their specialized functions (St John et al., 2010) (Figure 3). For example, human skeletal muscle and cardiac cells will acquire approximately 6800 and 3650 copies per cell, respectively (Miller et al., 2003). Similar studies in mini pigs show wide-ranging differences in mtDNA copy number among tissues (G. Cagnone et al., 2016). Nevertheless, with tissue-specific studies, it must also be remembered that the cell types within tissues would have varying numbers of mtDNA copy based on their specific requirements for OXPHOS-derived ATP. Interestingly, the regulation of mtDNA copy number in a cell-specific manner is best exemplified by the differences between mature human sperm (purified populations) that have fertilization potential and their mtDNA copy number limited to approximately 10 copies per cell (Amaral et al., 2007) and mature oocytes (stripped of their cumulus cells) which possess more than 200,000 copies. Ultimately, mtDNA copy number in oocytes and resulting embryos is critical for embryo development and resulting fetal development.

## 5 | THE MITOCHONDRIAL GENETIC BOTTLENECK

At gastrulation, some pluripotent cells give rise to primordial germ cells which are populated with copies of mtDNA that are transmitted to the next generation through the metaphase II oocyte. It has been proposed that these copies pass through a filtering or purifying process, described as the mitochondrial genetic bottleneck (Marchington et al., 1998) that would normally weed out mutant copies of the mitochondrial genome to ensure that the recycling of this maternally inherited genome passes the least deleterious effects to the next generation of offspring. However, when the mutant load is high in these cells, mtDNA disease can ensue (McFarland et al., 2007). Indeed, mtDNA disease is well-documented in humans and is likely to have existed in other species but has been eradicated due to breeding selection programs where only the fittest or commercially most beneficial lineages survive. Nevertheless, the bottleneck appears to exist in livestock species. Early studies in cattle reported a large drift of mtDNA genotypes across generations of maternally related cows (Laipis et al., 1988; Olivo et al., 1983).

In the pig, naturally occurring mtDNA variants (somatic mutations and deletions), some of which might be deleterious, can persist at high levels in oocytes and preimplantation embryos, and be transmitted from one generation to the next through the female germline (G. Cagnone et al., 2016). To this extent, we identified four naturally occurring mtDNA variants (single base pair deletions) in a mini pig population. Each of the four variants indicated different levels in oocytes and embryos but their levels were suppressed in somatic tissues, especially those with a high requirement for OXPHOS-derived ATP. Consequently, the levels did not surpass

the threshold associated with the phenotypic onset of mtDNA disease (Russell & Turnbull, 2014; Wei & Chinnery, 2020). For example, the onset of Leber's hereditary optic neuropathy requires over 60% of the mtDNA molecules to be mutated in the affected tissue (Chinnery et al., 2001) while 85% mutant mtDNA load is necessary for the onset of Myoclonic epilepsy with ragged red fibers syndrome (Boulet et al., 1992). Interestingly, there were gender-specific differences for heart and liver tissues for one variant and generational differences for several tissues for the same variant (G. Cagnone et al., 2016). Indeed, the consensus opinion is that mtDNA point mutations associated with mammalian aging are insufficient to cause a phenotypic response (Moore et al., 2020). For example, in hair, the exponential increase in mutation with age ranges from 0 to  $1.436 \pm 0.2086\%$  of total mtDNA content (Zheng et al., 2012), which, in the context of mtDNA copy number per cell suggests little if no impact on health and well-being. However, there is evidence from mouse models generated through ooplasmic transfer, where cytoplasm, which includes mitochondria and mtDNA, is transferred from one oocyte into a recipient, suggesting that there are differences in levels of heteroplasmy (the mixing of two genotypes) between generations (Burgstaller et al., 2018) and that the divergence of heteroplasmic molecules in germ and somatic lineages take place early in development (Johnston et al., 2015).

Nevertheless, analysis of the breeding lines of commercial pigs in Australia showed that there was no correlation between the total number of mtDNA variants harbored by each of the mtDNA haplotypes investigated and developmental competence, maturation to metaphase II, fertilization rates, blastocyst rates, and litter size (Tsai et al., 2016). However, when individual variants were assessed at a presence of greater than 25%, there was a negative correlation with oocyte developmental competence; and more specifically with the number of variants present at greater than 25% in the *Cytochrome B* gene. Furthermore, the level of a variant at position 16,383 in one of the regulatory regions, conserved sequence box II (Figure 1b), correlated positively with mtDNA copy number for developmentally competent oocytes. This particular variant is within the site of interaction for the Mitochondrial Transcription Elongation Factor (Jiang et al., 2019). Termination at this site results in the production of a primer that enables mtDNA replication to proceed.

In all, there is no clear-cut evidence to suggest that the presence of naturally occurring variants in oocytes is indicative of oocyte quality or the potential of any given oocyte to give rise to offspring. However, it is worth noting that mutations to *POLG* can result in a host of deleterious mtDNA mutations in oocytes, as demonstrated in the *Polg* mutator mouse model (Ma et al., 2019). Nevertheless, these should be seen in the context of the mutations associated with the nuclear-encoded mtDNA replication factors that can give rise to mtDNA disease (Fekete et al., 2019; Rahman & Copeland, 2019; Remtulla et al., 2019). Likewise, it is important to note that high mutant loading of pathogenic variants can lead to spontaneous abortions even at the late stages of gestation (Monnot et al., 2011). Indeed, the persistence of mtDNA in humans might be explained by the high numbers of carriers of mtDNA

rearrangements (Elliott et al., 2008) and efficient medical practice to support affected individuals; and the likely elimination in livestock species since affected animals would have been selected against, based on their lack of commercial viability.

## 6 | ALTERING mtDNA COPY NUMBER IN THE OOCYTE

Autologous mitochondrial supplementation primarily arose from the refinement of another assisted reproductive technology, namely cytoplasmic transfer (Cohen et al., 1997), due to the necessity to determine if the cytoplasmic factors introduced into the oocyte that enhanced development were specifically mitochondrial or not. A study employing a pig model used purified populations of mitochondria isolated from mature oocytes and introduced these into oocytes deficient in mtDNA (El Shourbagy et al., 2006). The initial findings showed that fertilization rates were enhanced to match those of oocytes possessing sufficient copies of mtDNA. These experiments highlight the clinical data that showed a relationship between passing a putative threshold (>150,000 copies; Figure 3) for mtDNA copy number and fertilization outcome and subsequent development (May-Panloup, Chretien, et al., 2005; May-Panloup, Vignon, et al., 2005; T. A. Santos et al., 2006). Further work has shown that supplementation of mtDNA deficient oocytes not only results in improved fertilization outcomes, but also enhanced blastocyst rates (G. L. M. Cagnone et al., 2016). A similar outcome has been observed when adding the growth factor neuregulin 1 to the oocyte *in vitro* maturation media (Mao et al., 2012), as was also the case with supplementation of the glycoside mogroside V to the maturation media (Nie et al., 2020). Both resulted in increased mtDNA copy number in mature oocytes and improved embryo developmental competence. There were also significant changes in gene expression profiles of the nuclear genome (approximately 190 genes) at the blastocyst stage of development to the extent that the supplemented populations exhibited gene expression profiles more similar to those of blastocysts from oocytes that had appropriate levels of mtDNA (G. L. M. Cagnone et al., 2016). Moreover, these changes appeared to occur throughout preimplantation development (Tsai & St John, 2018) and arose from differences in gene expression for metaphase II oocytes deficient in mtDNA compared to those with sufficient levels (Tsai et al., 2018).

The outcomes of these studies open the debate as to whether the supplementation process is a cellular, metabolic or genomic event. This has arisen in the context of the use of mitochondria isolated from oogonial stem cells or egg precursor cells (White et al., 2012). The proponents of the approach argue that the additional mitochondria are added to fuel development (Woods et al., 2018), that is, they are adding extra units of energy. However, the process only introduces approximately 780 copies of mtDNA (G. L. M. Cagnone et al., 2016). Normally mtDNA deficient oocytes possess approximately 50,000 copies of mtDNA. If each mitochondrion possesses two copies of mtDNA per mitochondrion (Wai et al., 2008),



increasing the mitochondrial number by 390 mitochondria is likely to have very little impact. The more likely outcome is that the addition of extra copies of mtDNA from sister oocytes transformed the minor replication event between fertilization and the 2-cell stage into a major replication event (G. L. M. Cagnone et al., 2016). Indeed, this replication event increases mtDNA copy number by 4.4-fold in mtDNA-deficient oocytes and ensures sufficient mtDNA is present at the blastocyst stage to support subsequent developmental events. A similar outcome was also observed at the blastocyst stage in the aforementioned studies using neuregulin 1 and mogroside V (Mao et al., 2012; Nie et al., 2020); and through a protease inhibitor MG132 and resveratrol on in vitro matured oocytes (Sato et al., 2014). Furthermore, the DNA methylation profiles in the CpG island of POLG are altered with increases in expression for this gene during this short window of mtDNA replication (Tsai et al., 2018). These events take place before the major round of embryonic genome activation in the pig at the 4-cell stage (K. Lee et al., 2014). In addition, knockdown of POLG2 leads to reduced maturation and blastocyst formation in pig oocytes along with reduced mtDNA copy number (S. K. Lee et al., 2015).

Taken together, these events, along with changes in nuclear gene expression, suggest that these are genomic rather than metabolic events, although certain gene networks associated within metabolism are affected (G. L. M. Cagnone et al., 2016). Indeed, the use of a model that employs nonselected pig metaphase II oocytes (i.e., does not discriminate between mtDNA deficient and mtDNA-normal oocytes) shows that mitochondrial supplementation alone induces significant changes in DNA methylation and nuclear gene expression by the blastocyst stage (Okada et al., 2022). Consequently, it appears that mitochondrial supplementation triggers a resetting of the genomic balance that was established between the two genomes during oocyte maturation (St John, 2019). Interestingly, though, it does not appear to affect genes associated with imprinting (Okada et al., 2022).

## 7 | mtDNA REPLICATION AND DNA DEMETHYLATION PROFILES DURING OOGENESIS AND IN THE EARLY EMBRYO

In the context of oogenesis, it appears that the regulation of the synergy between the two genomes is a key to the developmental outcome. During the proliferation and migration of primordial germ cells, DNA methylation is globally erased (Smallwood & Kelsey, 2012). Large-scale de novo DNA methylation takes place during oogenesis coupled with the significant increases in mtDNA copy number. Following fertilization, there is differential genome-wide DNA demethylation that takes place in the newly formed embryo based on paternal and maternal contributions (Smallwood & Kelsey, 2012; Stewart et al., 2016). Indeed, early development is marked by significant changes to DNA methylation, other epigenetic regulators and gene expression (von Meyenn et al., 2016). For example, during preimplantation development, the TET family of enzymes ensures the

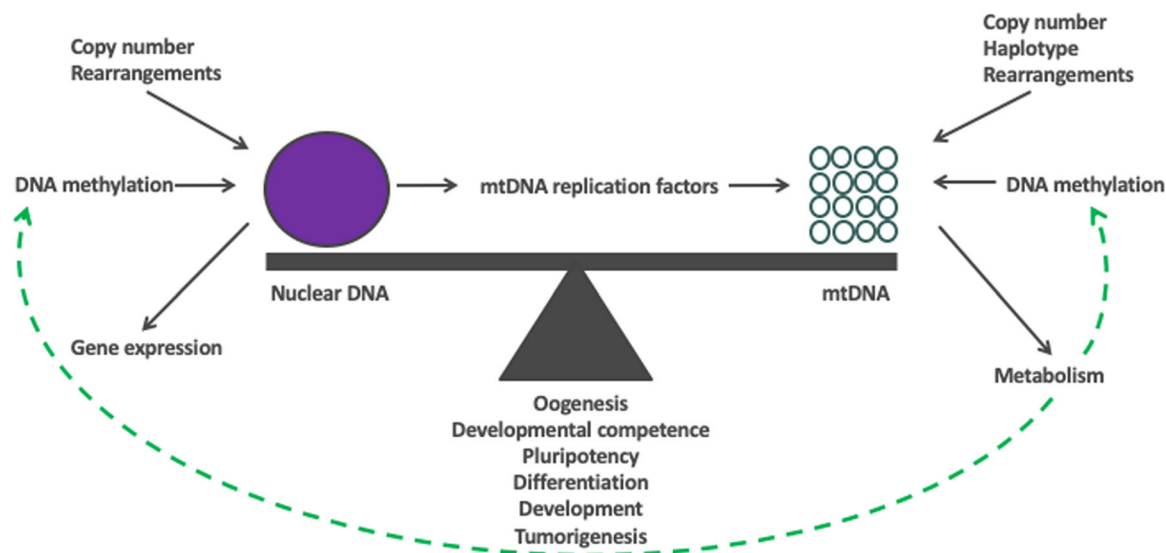
erasure of parental DNA methylation patterns up to the blastocyst stage and the de novo methyltransferases, that is, DNMT3a and DNMT3b, mediate de novo DNA methylation during preimplantation and postimplantation development. At the same time, DNMT1 maintains the predestined profiles (Guo et al., 2014; F. Santos et al., 2002).

The synchronous changes between the nuclear and mitochondrial genomes are key to the concept of cells achieving "genomic balance" (Figure 4) in order that cells function effectively at different stages of development and when mature (St John, 2019). Perturbing genomic balance could result in disease, as shown in models of tumorigenesis with cells being unable to mediate differentiation which requires replication of mtDNA copy to support advanced cellular function (Dickinson et al., 2013). However, genomic balance can be reset through DNA methylation agents such as 5-Azacytidine and Vitamin C, which can prevent tumorigenesis and allow cells to undergo differentiation with expected increases in mtDNA copy number (Dickinson et al., 2013; Sun & John, 2018; Sun et al., 2018). Likewise, failure of oocytes to acquire sufficient copies of mtDNA leads to fertilization failure or developmental arrest during preimplantation development (May-Panloup, Chretien, et al., 2005; May-Panloup, Vignon, et al., 2005; T. A. Santos et al., 2006) though this can be countered through mitochondrial supplementation (G. L. M. Cagnone et al., 2016). Interestingly, the addition of homocysteine, an intermediate in the one-carbon metabolism that donates methyl groups for methylation processes involved in epigenetic gene regulation, results in reduced mtDNA copy number and affected the quality of porcine oocytes which then recovered through the addition of 5-Azacytidine (Jia et al., 2019).

## 8 | mtDNA HAPLOTYPES

Over millions of years, different maternal lineages have evolved and, based on their mtDNA sequences, they cluster into groupings known as mtDNA haplotypes (Ruiz-Pesini et al., 2004). Consequently, a single mtDNA haplotype is defined by groups of mtDNA sequences from individuals that cluster together and define a group's common maternal origins. mtDNA haplotypes can confer advantages and disadvantages to the individual (Gerber et al., 2001; Innocenti et al., 2011). They are thought to predispose individuals to disease (Blanco et al., 2018; Fuku et al., 2007; Koo et al., 2019; Liou et al., 2016; Marom et al., 2017; Shen et al., 2022); influence tolerance to warm and cold climates (Ruiz-Pesini et al., 2004; Wallace et al., 2003) and high altitude (Ji et al., 2012); sperm motility (Ruiz-Pesini et al., 2000); the size of the ovarian reserve (May-Panloup et al., 2014); and overall reproductive capacity and fertility (Sutarno et al., 2002; Tsai et al., 2016). They also influence growth and physical performance (Nagao et al., 1998), milk quality (Brown et al., 1989), and key economic breeding values (EBVs) associated with animal production (St John & Tsai, 2018).

In the context of reproductive capacity, Australian commercial pigs belong to one of five mtDNA haplotypes (A–E) indicative of their



**FIGURE 4** Genomic balance. At key stages in development, a cell strikes a balance between its two genomes in order that it can progress to the next stage of development. This process is mediated by the constant exchange of regulatory information between the nucleus and the mitochondrial genome. This ensures that the cell at any given stage acquires sufficient copies of mtDNA in order that the cell can undertake its specialized function using as much or as little ATP derived through OXPHOS. At the same time, the nuclear genome contributes to genomic balance through epigenetic changes by altering, for example, the levels of DNA methylation, that control gene expression. Other factors include DNA rearrangements, such as mutations and deletions, and copy number variants that will have an impact on phenotype. The mitochondrial genome will contribute through the levels of mtDNA copy number available that will influence the means of cellular metabolism available to the cell, which, is also influenced by the cell's mtDNA haplotype. This results in metabolic factors being released that regulate DNA methylation and other epigenetic modifiers, which act on both the nuclear and mitochondrial genomes. mtDNA, mitochondrial DNA; OXPHOS, oxidative phosphorylation.

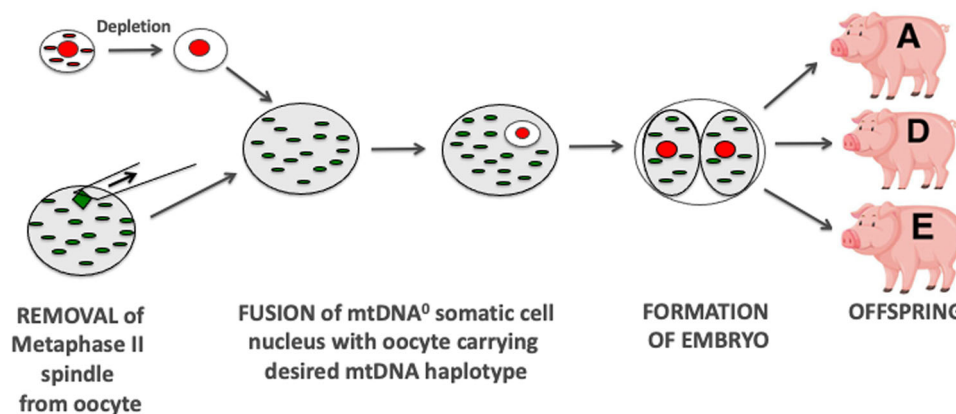
origins arising from one of five female founders (St John & Tsai, 2018; Tsai et al., 2016). Three of these founders originated from Asia while two were of European origin. Between the five mtDNA haplotypes, there are approximately 200 single nucleotide variants, which affect both protein coding genes and noncoding regions. These genomic variations result in differences in litter size based on mtDNA haplotypes. Females from haplotypes A and B were predisposed to producing fewer piglets per litter than C, D, and E, while females from haplotypes C ( $p < 0.05$ ), D ( $p < 0.01$ ), and E ( $p < 0.05$ ) were more likely to produce at least three litters of  $\geq 15$  piglets per parity than A (Tsai et al., 2016). Furthermore, there were differences in developmental efficiencies among the mtDNA haplotypes. By determining the efficiency of each developmental stage relative to the number of piglets born, haplotype C was the most efficient at converting mature and fertilized oocytes into offspring, and haplotype D was the most efficient at blastocyst conversion. However, haplotypes A and B were the least efficient at converting mature and fertilized oocytes and blastocysts into live offspring. In addition, given the relationship between mtDNA copy number and the ability of an oocyte to mature and develop once fertilized (Spikings et al., 2007), developmentally competent oocytes from each mtDNA haplotype exhibited differences in mtDNA copy number (Tsai et al., 2016). Haplotype D had the highest copy number and haplotype B had the lowest. Similarly, others have seen differences between female pigs having either high or low levels of mtDNA (K. Lee et al., 2014), which suggests a haplotype effect. In all, these outcomes indicate that each haplotype has a different

reproductive strategy that impacts on litter size across generations, and is, thus, a heritable trait. In addition, other EBVs such as fat density, muscle depth, fat to leanness ratios, lifetime daily gain, and teat quality were influenced by mtDNA haplotype. There were also gender-specific effects on teat quality (St John & Tsai, 2018).

## 9 | NUCLEAR TRANSFER AND mtDNA TRANSMISSION

Nuclear transfer primarily involves the transfer of a nucleus, often within a cell, that is required to be propagated into an enucleated oocyte and the reconstructed oocyte is activated (Campbell, 1999). The reconstructed oocyte will then progress to an embryo and divide in much the same manner as a fertilized oocyte. This concept originated from the work of Gurdon and colleagues who were the first to use nuclear transfer to produce frogs with a somatic cell (Gurdon et al., 1958), and, thus, gave rise to the term somatic cell nuclear transfer (SCNT) (Figure 5). This was followed by the transfer of cultured embryonic-derived (Campbell et al., 1996) and somatic cells to produce the first mammalian offspring, namely Dolly the Sheep (Wilmut et al., 1997); and blastomeres to produce rhesus macaque monkeys (Meng et al., 1997), cattle (Bondioli et al., 1990; Prather et al., 1987), and pigs (Prather et al., 1989).

A key problem is that the mtDNA present in the somatic or embryonic cell accompanies the nucleus (sometimes referred to as



**FIGURE 5** Utilization of SCNT to promote pig breeding lines. The somatic cell is depleted of its mtDNA and introduced into an oocyte containing mtDNA from a specific mtDNA haplotype (e.g., A, D, or E). Once activated, it can develop into an embryo and give rise to offspring. This allows for embryos and offspring to be studied to determine if mtDNA haplotypes influence phenotypic traits with the aim of generating enhanced lines of breeding pigs. mtDNA, mitochondrial DNA; SCNT, somatic cell nuclear transfer.

“mtDNA carryover”) and, once fused to the enucleated oocyte, it is incorporated into the reconstructed oocyte and can be potentially transmitted to the offspring (Steinborn et al., 1998). The levels of transmission of accompanying mtDNA are random. In large animal models of SCNT, namely sheep, pigs, and cattle, the offspring can inherit from 0% to 59% of their total mtDNA content from the somatic cell (Burgstaller et al., 2007; Takeda et al., 2003, 2006). Where there is a mixing of two diverse mtDNA genotypes (heteroplasmy), there is the potential for the offspring to inherit two mtDNA genomes, namely from the donor cell and recipient oocyte and, thus, from two individuals. If they are of the same or very similar mtDNA haplotypes, the issue of compatibility is not necessarily significant. However, if the genotypes are more distant then compatibility between the haplotypes is an issue (St John et al., 2005; St John et al., 2010). This arises, as within a species, there is variability in the sequences from different mtDNA haplotypes for the encoding genes. This would result in the amino acids contributing to each of the 13 mtDNA-encoded subunits of the electron transfer chain having different configurations, as shown in a study of pig serial nuclear transfer (St John et al., 2005). Consequently, when two mitochondrial genomes are present, electron transfer chains can be assembled that have different efficiencies for generating ATP through OXPHOS as demonstrated in a pig model of SCNT where discordant interaction between the nucleus and mtDNA affected OXPHOS gene expression (Park et al., 2015); and mouse cybrid (cells derived from the fusion of an mtDNA depleted cell with an enucleated cell) (McKenzie et al., 2003) and pig cybrid (Yu et al., 2015) models.

The question that needs to be addressed is whether mtDNA carryover can be completely avoided. In SCNT, it is possible to deplete the somatic cell's mtDNA using an mtDNA depletion agent to produce embryos (Lloyd et al., 2006; Srirattana & John, 2017) and live offspring that inherit their mtDNA from the oocyte only (J. H. Lee et al., 2010), as in the case with natural conception (Figure 5). Consequently, it is possible to generate animals with a mixture of

traits present in the nuclear genome and match these with desired traits associated with the mitochondrial genome that would give enhanced breeding lines. In the pig, this might constitute an animal with desired meat quality that would be transmitted through the nuclear genome and matching with an mtDNA haplotype associated with enhanced oocyte quality and litter size (Figure 5). In countries where the import of new pig genetics through breeding lines is not allowed, for example, Australia, this offers an opportunity to increase and diversify breeding lines and expand pig genetics by alerting genomic combinations that might require large numbers of breeding rounds to achieve.

## 10 | CONCLUSION

In the context of understanding the role of the mitochondrial genome in the oocyte and embryo, the pig has proven to be an invaluable model. It has shown how mtDNA copy number is strictly regulated in the oocyte and developing embryo and the importance of oocyte mtDNA copy number and variants to the developmental outcome. Furthermore, it highlights how mtDNA haplotypes influence the developmental outcome and how the association of mtDNA haplotypes and traits could be exploited to produce animals with enhanced phenotypes. Nonetheless, the knowledge gained from the pig oocyte and embryo with regard to mtDNA variants and genetics is not only relevant in terms of generating human preclinical models. It has specific relevance to pig reproductive capacity. In many respects, the outcomes are waiting for the pig industry to use them to enhance pig production, capacity, and quality.

## AUTHOR CONTRIBUTIONS

**Justin C. St John:** Conceptualization; funding acquisition; writing—original draft; formal analysis; writing—review & editing. **Takashi Okada:** Writing—review & editing; writing—original draft; investigation; formal analysis. **Eryk Andreas:** Writing—original draft;



writing—review & editing; investigation; formal analysis. **Alexander Penn:** Investigation; writing—original draft; writing—review & editing; formal analysis.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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