Review

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Implications of mitochondrial function in embryonic development

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SUMMARY: Mitochondria are organelles that play a crucial role in various physiological processes. They are particularly important during embryonic development, as their proper function is required for essential processes such as fertilization, implantation, and embryonic growth. In addition to their well-known role in adenosine triphosphate (ATP) synthesis and energy production, mitochondria serve multiple other functions during embryonic development. These include the synthesis of important metabolites, involvement in cell signaling pathways, regulation of reactive oxygen species, and facilitation of interactions between organelles. The mitochondrial genome, known as mitochondrial DNA (mtDNA), also plays a unique role in embryonic development. Dysfunction in mitochondria can lead to failures in fertilization, suboptimal embryo development, post-implantation failures, and mitochondrial-related diseases in adults. Advances in sequencing technology and experimental techniques have greatly improved our understanding of mitochondrial function. This paper reviews the roles of mitochondrial functions in embryonic development and the influence of mitochondrial technologies and it highlights the potential impact of understanding mitochondria's unique genetic and functional characteristics on embryonic development and offspring health.

Keywords: mitochondria, metabolism, mtDNA, embryonic development

1. Introduction

Mitochondria are known to have a highly functional structure and are found in eukaryotic cells. They play crucial roles in various cellular processes including material metabolism, energy metabolism, and signal transduction (*1*). Human embryonic development is a complex and fascinating process that involves various stages and cellular activities. Throughout preimplantation development, embryo implantation, and subsequent post-embryonic development, the embryo undergoes a series of energetic cellular processes that heavily rely on adenosine triphosphate (ATP) for energy (*2*). ATP is the primary energy currency of cells, and its production is critical for various cellular functions, including growth, division, and differentiation. Therefore, the maintenance of mitochondrial function is crucial to successful embryogenesis. However, mitochondria also serve other important roles beyond energy production.

2. Mitochondrial mechanisms governing early embryonic development

2.1. Mitochondrial energy metabolism and embryonic development

There is a huge heterogeneity in the number of mitochondria in human cells, and a large number of mitochondria are known to be distributed in the cytoplasm of oocytes (*3*). After fertilization, fertilized oocytes adjust the mitochondrial density in different intracellular regions, but their exact functional significance remains unknown (*4,5*). Mitochondria have also been reported to be disproportionately distributed in the formed blastomeres of developing embryos (*6*). The hypothesis is that mitochondrial aggregation may contribute to the direct and rapid supply of energy to the nucleus (*7*).

Early embryonic mitochondria appear spherical (≤ 1 μm) under electron microscopy, featuring sparse cristae and dense matrices (*8*). They retain this immature form

until the blastocyst stage, where they begin to elongate and form mature cristae (*9*). Despite the high energy demands of cleavage and blastocyst formation (*10*), pre-implantation embryos rely heavily on pyruvate oxidation rather than glucose. This strategy is thought to limit mitochondrial reactive oxygen species (ROS) generation (*11-13*). Pyruvate enters mitochondria directly for the tricarboxylic acid (TCA) cycle — bypassing cytosolic glycolysis — so it is a more efficient fuel source here. Indeed, removing pyruvate blocks development, whereas removing glucose or lactate does not (*14,15*). In the blastocyst stage, increased oxidative respiration coincides with mitochondrial cristae expansion (*16*). The molecular transition from anaerobic to aerobic respiration is orchestrally regulated by the stabilization of hypoxia-inducible factor 1-alpha (HIF-1α), a master regulator of oxygen homeostasis constitutively expressed in cleavage-stage embryos. HIF-1α upregulates pyruvate dehydrogenase (PDH) kinase 1 (PDK1) to shunt pyruvate away from the TCA cycle (*17,18*). HIF-1α also influences mitochondrial morphology to support anaerobic metabolism by upregulating dynamin-related protein 1 (DRP1) expression, resulting in fragmented mitochondria with limited cristae formation, which is characteristic of anaerobic metabolism (*19,20*). Notably, cristae maturation is related to an ROS balance by increasing expression of antioxidant enzymes and preventing oxidative damage despite higher oxygen consumption (*21*).

2.2. Mitochondrial substance metabolism and embryonic development

Mitochondrial metabolism is at the core of the cellular metabolic network, where the TCA, oxidative phosphorylation (OXPHOS), fatty acid oxidation, nucleotide synthesis, and amino acid metabolism all occur (*22*). Alterations in these activities may mediate changes in the oocyte and early embryo epigenetic landscape that affect subsequent developmental capacity (*23*). There is a complex relationship between mitochondrial activity and calcium signaling (*24*). Calcium is an activator of OXPHOS by activating dehydrogenases, respiratory chains, and ATP synthases required for cycling. There are several contact zones between the endoplasmic reticulum (ER) and mitochondria, known as mitochondrial-associated ER membranes (MAMs), that serve as the key physical and functional platform for calcium signaling crosstalk during embryonic development (*25*). This mechanism is known to play a role in fertilization, where calcium released from the ER activates the mitochondria and induces calcium oscillations necessary for oocyte activation (*26*). MAMs are enriched with core regulatory proteins including mitofusin 1/2 (MFN1/2), inositol 1,4,5-trisphosphate receptors on the ER, and voltage-dependent anion channels on the mitochondrial outer membrane, which together form a high-efficiency calcium transfer channel (*27*). Moreover, phosphatidylinositol metabolites in MAMs, such as

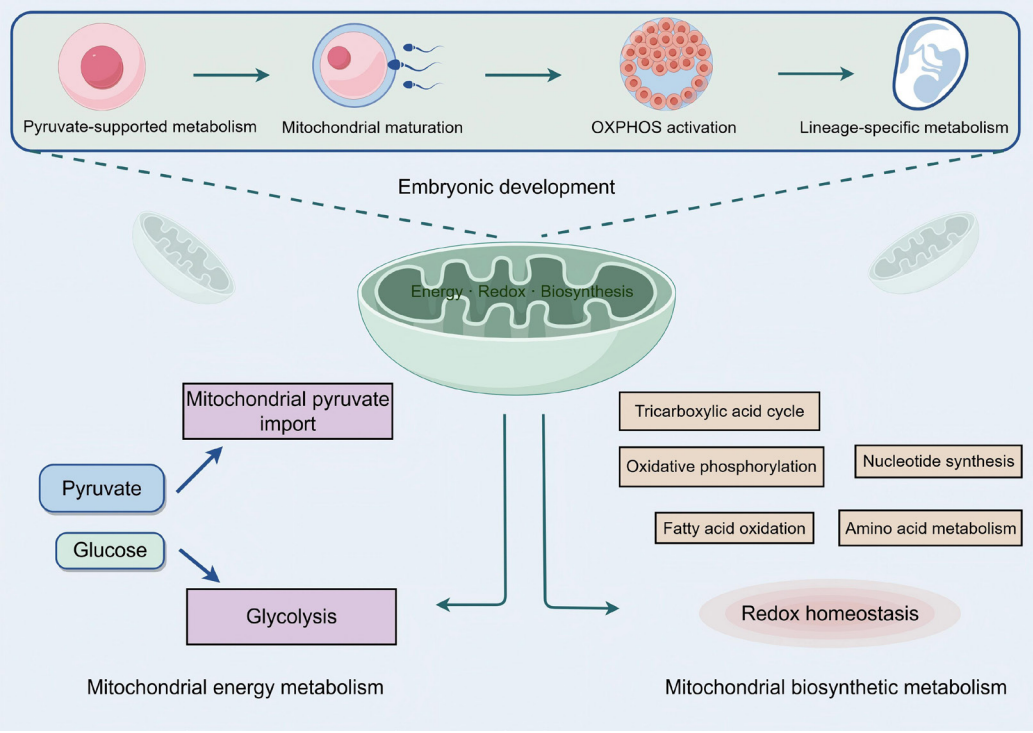

Figure 1. Metabolic transitions and mitochondrial maturation in early embryos. During early cleavage, metabolism relies primarily on pyruvate, while glucose utilization remains limited to the cytosol. As development progresses towards implantation, mitochondria undergo structural maturation and upregulate oxidative phosphorylation. Beyond ATP production, these organelles serve as hubs for the tricarboxylic acid cycle, fatty acid oxidation, and the synthesis of nucleotides and amino acids, thereby coordinating energy supply with redox homeostasis.

inositol 1,4,5-trisphosphate, can specifically bind to IP3Rs on the ER membrane to trigger calcium release, further amplifying calcium oscillation signals during fertilization (*28*). These findings collectively demonstrate that MAM-mediated precise regulation of calcium signaling is a prerequisite for normal oocyte-to-embryo transition.

Mitochondrial metabolites serve as critical substrates for epigenetic modifying enzymes and play pivotal regulatory roles in oocyte maturation and early embryonic development. α-Ketoglutarate functions as an essential cofactor for TET (ten-eleven translocation) family DNA demethylases and Jumonji C domain-containing histone demethylases, whereas acetyl-coenzyme A (acetyl-CoA) directly modulates histone acetylation levels as the obligate substrate for histone acetyltransferase. Notably, genetic ablation of *Drp1* results in pronounced reductions in both DNA methylation and H3K27me3 levels in oocytes, providing direct evidence for the indispensable role of mitochondrial functionality in the establishment of the maternal epigenome. The post-fertilization perinuclear redistribution of mitochondria surrounding the pronuclei may generate "localized metabolite microdomains," thereby establishing substrate concentration gradients that provide a spatially defined microenvironment for epigenetic modifying enzymes. This compartmentalized metabolic architecture is hypothesized to orchestrate the asymmetric epigenetic reprogramming of the paternal and maternal genomes during the earliest stages of embryogenesis (*29-31*).

In addition, there are interactions between the physical contacts of mitochondria and lipid droplets (LDs) (*32*). LDs staining of embryos from *in vitro* fertilization (IVF) and parthenogenetic activation (PA) sources in cattle and pigs, respectively, revealed possible dysregulation of lipid metabolism and mitochondrial dysfunction in PA embryos, suggesting significant differences in LD parameters between developmental stages and species studied (*33*). Research has demonstrated that LDs in mammalian eggs are utilized during embryonic diapause, revealing the functional role of LDs in embryonic development (*34*). A recent review highlighted the fact that LDs play essential roles in embryonic development across species by providing energy, supplying lipids for membrane formation, and protecting embryos against lipotoxicity, oxidative stress, and infection, with evidence showing that depletion of LDs in early embryos leads to developmental arrest and abnormalities (*35*).

Notably, mitochondrial fatty acid β-oxidation serves as a critical additional energy supply pathway for embryos transitioning to the blastocyst stage. As embryonic development progresses from the cleavage stage to the blastocyst, metabolic demands surge, and mitochondrial fatty acid β-oxidation is dynamically activated to complement pyruvate-dependent energy metabolism (*36,37*). This process involves a series of highly regulated steps: fatty acids stored in LDs are first activated to fatty acyl-CoA, then transported into the mitochondrial matrix *via* the carnitine palmitoyltransferase 1-mediated shuttle system, and subsequently degraded into acetyl-CoA. Acetyl-CoA then enters the tricarboxylic acid cycle to generate a large amount of ATP, meeting the high energy requirements for blastocyst expansion and lineage segregation (*38,39*). A recent study has confirmed that long-chain fatty acid β-oxidation is essential for preimplantation development. Inhibition of long-chain fatty acid β-oxidation results in downregulated expression of S phase-related genes and loss of H3K18ac modification. This finding provides evidence of the effect of fatty acid β-oxidation in metabolism-epigenetic crosstalk on early embryonic development (*40*).

A study found that the secretion of leptin increased in mothers with a high-fat diet by altering the mitochondrial function of mouse oocytes and fertilized eggs, activating nuclear PPAR-γ receptors, and stimulating the up-regulation of genes controlling fatty acid oxidation, resulting in abnormal development of fertilized eggs (*41*). As women age, lipid levels in the follicular fluid may disrupt the epigenetic landscape of the oocyte (*42*). Lipid-derived acetyl-CoA is more readily incorporated into histones than glucose-derived acetyl-CoA, suggesting plasticity in mitochondrial metabolism and a potential association with epigenetic remodeling (*43*). Culturing under atmospheric conditions (20% O_2) resulted in increased ROS levels in mouse embryos, abnormalities in mitochondrial morphology and function, and alterations in mitochondrial gene expression profiles compared to culturing under physiological conditions (5% O_2) (*44*). These studies suggest that the abnormal accumulation of ROS can seriously affect the normal development of embryos (*45*). Thus, maintaining mitochondrial metabolic function is vital for redox homeostasis and supports normal embryogenesis (Figure 2).

2.3. Mitochondrial dynamics in embryonic development

Mitochondria are the center of cell metabolism, and their size, density, and location are closely related to the status of cell metabolism (*46*). Mitochondria perform quality control through division and fusion to adjust their shape, length, and quantity. Mitochondrial dynamics allow for the exchange of lipid membrane and matrix contents, the repair of mitochondrial DNA (mtDNA) damage, and the regulation of mitochondrial biogenesis and apoptosis (*48*). In the context of reproductive biology, normal mitochondrial dynamics are indispensable for embryonic development, as evinced by gene-specific knockout models. Deficiencies in *Drp1*, mitochondrial fission factor (*Mff*), or *Mfn1/2* consistently result in developmental arrest, reduced embryo size, and impaired differentiation (*49*). The knockout of *Drp1* in mice

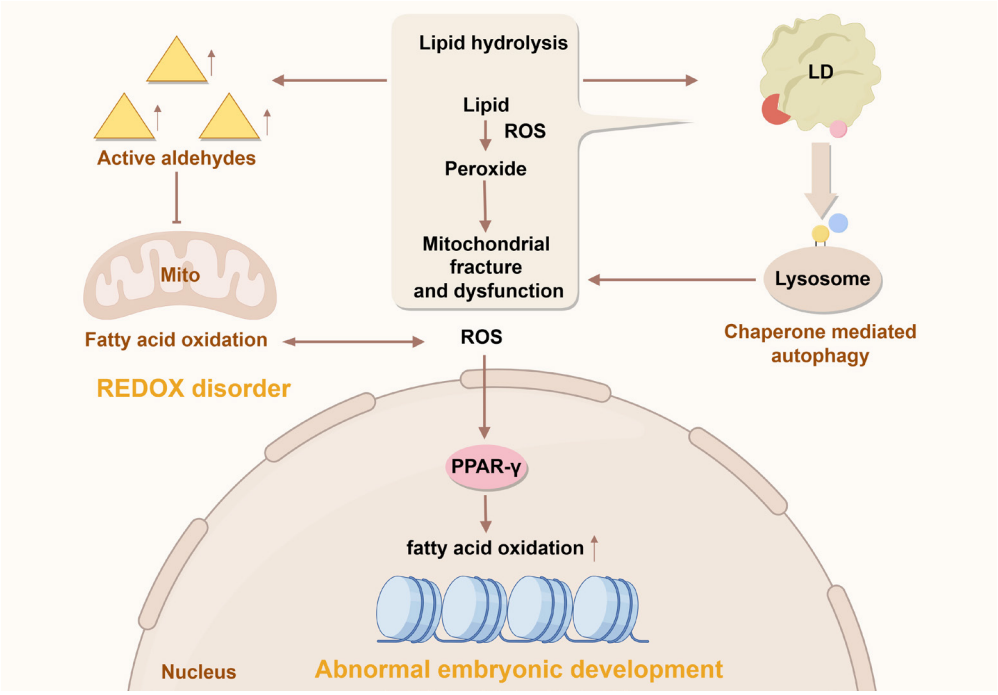

Figure 2. Redox imbalance drives lipid oxidation and mitochondrial dysfunction in embryonic cells. Elevated ROS levels trigger lipid peroxidation, generating active aldehydes and disrupting lipid homeostasis. Lipid droplets (LDs) undergo hydrolysis *via* lysosomal pathways, including chaperone-mediated autophagy, in response to stress. Under these oxidative conditions, mitochondria exhibit structural remodeling and altered fatty acid oxidation profiles. Mechanistically, ROS signaling activates nuclear PPAR-γ and downstream transcriptional programs regulating fatty acid metabolism. Cumulatively, these redox-induced metabolic and organelle defects predispose embryos to developmental abnormalities.

resulted in death of the embryo at E11.5, accompanied by a reduction in volume, which was associated with damage to placental giant cells (*30*). Disruption of mitochondrial fission by inhibiting DRP1 recruitment to the mitochondria leads to G2/M growth arrest in cells undergoing reprogramming and affects the early phase in reprogramming, suggesting that mitochondrial fission affects pluripotential reprogramming (*50,51*). A study suggested that MFNs play an important role in germ cell formation and embryonic development, including spermatogenesis, oocyte maturation, and embryonic development (*52*). MFNs are necessary for embryonic development and are involved by regulating mitochondrial fusion and homeostasis, thereby maintaining normal levels of ATP and a normal mitochondrial membrane potential (MMP) during embryonic development (*53*). During early embryonic development, low levels of MFN1 expression lead to the lethal fragmentation of the early embryo by destroying mitochondrial MMP and OXPHOS components, ultimately resulting in reduced embryo survival (*54*). Normal expression of MFN2 maintains blastocyst formation, while reduced expression of MFN2 leads to mitochondrial dysfunction and induces apoptosis through BCL2/BAX and $Ca2^{+}$, ultimately reducing blastocyst formation (*55*). These genes related to mitochondrial function are associated with various modes of cell death, and their roles in fertilization and embryonic development warrant further examination.

In addition to mitochondrial dynamics-associated genes, cell competition has emerged as a novel quality control mechanism capable of eliminating cells with compromised mitochondrial function during early embryonic development. Studies have demonstrated that approximately 35% of epiblast cells are eliminated prior to gastrulation in murine embryos, with single-cell transcriptomic analyses revealing that these eliminated cells exhibit pronounced signatures of mitochondrial dysfunction. This "purifying selection" mechanism ensures that cells harboring high-load mtDNA mutations or exhibiting suboptimal mitochondrial performance are culled through apoptotic pathways, thereby optimizing the overall mitochondrial fitness of the embryo prior to gastrulation. Notably, even non-pathogenic mtDNA sequence variants may be sufficient to trigger cell competition, suggesting that the behavior of mtDNA heteroplasmy may manifest along a continuum ranging from stochastic genetic drift to stringent selective pressure, contingent upon nuclear-cytoplasmic interactions and metabolic determinants (*56,57*).

Human embryos also rely on mitochondrial fission-fusion dynamics to regulate mtDNA heteroplasmy. Mutated mitochondria often exhibit reduced MMP and impaired OXPHOS, which are recognized as "damage signals". Mitochondrial fission prevents the spread of harmful mutations to the entire mitochondrial population and marks the mutated mitochondria for subsequent selective clearance *via* mitochondrial autophagy (*58*).

Mitochondrial fission-fusion imbalance impairs mtDNA quality control, leading to the accumulation of mutated mitochondria, which in turn disrupts NADH/NAD^+ redox homeostasis and reduces oocyte and embryo quality. Conversely, enhancing mitochondrial fusion *via* MFN activation or inhibiting USP30 to promote mitochondrial autophagy can improve the developmental competence of embryos with high mtDNA mutation loads (*59*). The crucial role of mtDNA mutation loads during embryonic development is described in further detail below. By screening out high-load mtDNA mutations and optimizing ATP production, mitochondrial dynamics ensure the metabolic homeostasis and genomic stability necessary for successful fertilization and embryogenesis.

2.4. mtDNA and embryonic development

In humans, like most mammals, mitochondria and mtDNA are entirely passed down maternally. Sperm contain very few mitochondria, which are destroyed after fertilization, while in oocytes, the mitochondrial reservoir is fully expanded, making an oocyte the cell with the most mitochondria in the organism (*60,61*). In human oocytes, the average number of copies of mtDNA is estimated to be about 250,000 (*62*). While most of the genes associated with mitochondrial biological activity are encoded by the nuclear genome, mtDNA encodes 13 proteins involved in the respiratory chain, as well as 22 transfer RNAs and two ribosomal RNAs (*63*). The mtDNA copy number grows exponentially during ovulation and peaks during fertilization (*64*). Failure of mature oocytes to increase the mtDNA copy number above the threshold may result in fertilization failure or early embryo arrest (*65*). During pre-implantation development, the number of mtDNA copies per cell gradually decreases. mtDNA is at a low level after fertilization and comparatively increases during embryo implantation (*66*). During the blastocyst stage, the final stage of pre-implantation development, mtDNA replication begins, but this is limited to the trophectoderm, where mtDNA replication is quiescent in the inner cell mass (*67*).

During gastrula formation, some pluripotent cells produce primordial germ cells, which contain copies of mitochondrial DNA that are passed to the next generation *via* metaphase II oocytes (*68*). These copies undergo a filtration or purification process that typically removes mutated copies of the mitochondrial genome to ensure that the maternal transmission of only certain genomes passes on minimal harmful effects to the next generation, also known as the mitochondrial genetic bottleneck (*69*). However, when the mutant load is high in these cells, mtDNA diseases occur (*70*). mtDNA copy number is generally considered to be an indicator of the number of mitochondria and is associated with oocyte fertilization potential (*71*). However, this is not consistent with the metabolic requirements of the blastocyst embryo, and this view is still a subject of debate (*72*). This controversy may be reconciled through the "compensatory biogenesis" hypothesis: when embryos are subjected to metabolic stress, they upregulate mitochondrial biogenesis to sustain ATP provision, consequently resulting in elevated mtDNA copy numbers. Accordingly, a heightened mtDNA copy number may reflect an overcompensatory response to adverse conditions rather than superior developmental competence. Supporting this notion, studies conducted in IVF patients with favorable prognoses have failed to demonstrate a significant correlation between the cumulus cell mtDNA copy number and implantation success rates, with no significant differences in mtDNA content observed between implanted and non-implanted embryos. Moreover, the mtDNA/gDNA ratio exhibits a negative correlation with patient age, underscoring the imperative of establishing "tissue-specific" and "age-dependent" threshold values rather than relying upon a singular universal numerical criterion (*73-75*). The advent of single-cell multi-omics technologies has afforded unprecedented opportunities to monitor how mtDNA mutations alter fateful decisions within specific cell lineages in real time. Integrating single-cell transcriptomic data with chromatin accessibility profiles enables the construction of regulatory networks governing early embryonic development and the identification of pivotal regulatory determinants orchestrating the differentiation of the inner cell mass and trophectoderm. Recent studies involving single-cell transcriptomic analyses have delineated mtDNA mutation-specific and lineage-specific compensatory mechanisms; these compensatory pathways are governed by transcription factors that promote organellar resilience and sustain mitochondrial functionality throughout critical developmental windows (*76,77*). Preimplantation embryo development is characterized by extensive reprogramming of the epigenetic landscape to support and regulate events in specific stages, including embryo genome activation and lineage norms (*78*). Perturbations of the nutritional environment around oocytes and preimplantation embryos may regulate the long-term health and viability of offspring by influencing metabolic changes in the programmed epigenome. In mouse and human oocytes, highly polarized mitochondria cluster around the oocyte cortex, and mitochondria with a low membrane potential are evenly distributed throughout the cytoplasm (*79*). After fertilization, mitochondria translocate and cluster around the two pronuclei to facilitate synthesis and ensure a uniform distribution of organelles between the resulting blastomeres, and mitochondria may be associated with mtDNA functioning (*80*). Mitochondrial repositioning occurs at the same time as differential regulation of the paternal and maternal epigenomes, so clustering may enable crosstalk between the nuclear and mitochondrial genomes and the establishment of local metabolite

domains to regulate epigenetic modifiers (*81*).

In order for cells to function effectively in different stages of development and maturation, the nuclear and mitochondrial genomes need to change in sync (*82*). During critical stages of development, cells strike a balance between their two genomes in order to be able to move on to the next stage of development (*83*). This process is mediated by the constant exchange of regulatory information between the nuclear and mitochondrial genomes (*84*). This ensures that the cell gets enough copies of mtDNA in any given stage so that the cell can use as much or as little OXPHOS-derived ATP as possible to perform certain functions (*85*). At the same time, nuclear genomes contribute to a genomic balance by altering epigenetic changes such as levels of DNA methylation that control gene expression (*86*). The mitochondrial genome contributes to a genomic balance through the mtDNA copy number, which affects cell metabolism, and metabolism is also influenced by the cell's mtDNA haplotype (*87*). These lineage-specific remodeling events are summarized in Figure 3.

2.5. Mitochondrial autophagy plays an important role in early embryonic development

The quality of mitochondria in the oocyte is widely recognized to determines the quality of the oocyte and, as a consequence, the developing embryo (*88*). The autophagy system targets damaged mitochondria and transports them to lysosomes for degradation (*89*). This catabolic process, called mitochondrial autophagy, helps maintain mitochondrial quality control in a variety of cell types (*90*). Autophagy occurs after conception and throughout embryogenesis. In addition to providing survival mechanisms in times of nutrient deficiency, autophagy also eliminates organelles and protein aggregates at specific points in time during development (*91*). Survival after a specific developmental stage also requires several autophagy genes (*92*).

Proteins of maternal origin that are present in oocytes after fertilization are widely believed to promote autophagy (*93*). After oocyte-specific ATG5 knockout, mice were fertilized with ATG5-deficient sperm; the embryos failed to survive and development stalled in the 4-cell to 8-cell stage (*94*). There is a lack of complete understanding of why embryos with defective autophagy die in the 4-to-8-cell stage, but protein synthesis decreases significantly. The autophagy-related gene Beclin1 plays an important role in early embryogenesis in mice (*95*). Homozygous mutations in Beclin1 led to early embryo death between days E7.5 and E8.5, and severe developmental delays were present in animals on day E7.5. Beclin1 may be involved in promoting amniotic duct closure and amniotic fold development (*96*).

In rodents, developmental programmed cell death is involved in embryonic development and usually occurs early in embryogenesis. The solid mass of ectodermal cells undergoes programmed cell death and forms a preamniotic cavity (*97*). Mouse ATG5−/− embryos feature a defect in apoptotic-corpse engulfment in the retina and lungs (*98*). Autophagy-dependent ATP production promotes PS-mediated apoptotic cell clearance in some developmental programmed cell death

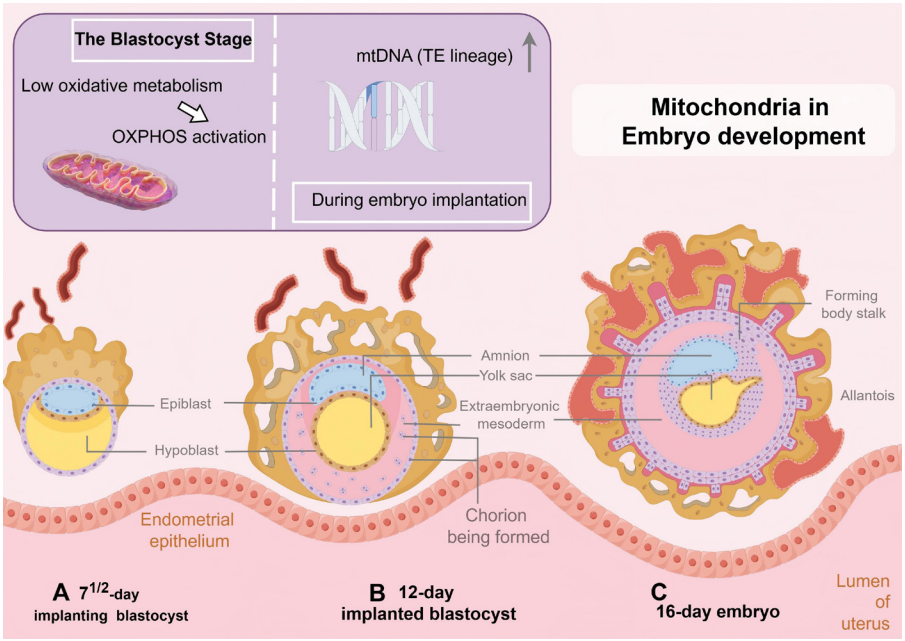

Figure 3. Mitochondrial dynamics during human implantation and early development. As the blastocyst implants, trophectoderm (TE) cells switch to a high-OXPHOS state with amplified mtDNA levels, supporting the metabolic demands of tissue invasion. In parallel, the spatial reorganization of mitochondria around pronuclei points to a direct role in nuclear reprogramming. These metabolic shifts provide the bioenergetic foundation required for embryonic genome activation and the epigenetic remodeling characteristic of early human embryogenesis.

contexts, but not all. The exact roles of mitochondrial autophagy and key genes in human embryonic development still need to be further studied and better understood.

2.6. Use of new techniques to study mitochondrial and embryonic development

A comprehensive analysis of age-related changes in gene expression profiles of mouse oocytes in the germinal vesicle (GV) stage was performed using single-cell RNA sequencing (scRNA-seq) (*99*). A study found that mitochondrial dysfunction, ER stress, and decreased antioxidant capacity may be involved in the process of oocyte senescence (*100*). In particular, downregulation of the mitochondrial coding subunit of the respiratory chain complex may play a key role in the relevant mechanisms. Mitochondria are not only organelles necessary for cell development but also play an important role in cell competition, eliminating unqualified cells during development. One study performed scRNA-seq on eliminated mouse epiblast cells and found that the eliminated cells not only displayed cellular competition characteristics but also impaired mitochondrial function (*101*). This finding suggests that differences in mitochondrial activity are key determinants of cellular competitiveness during early mammalian embryonic development.

Mitochondrial genome sequencing was used to study mtDNA heterogeneity, quantify single nucleotide variants and large structural variants, track heteroplasmy dynamics, and analyze the genetic linkage between variants at the individual mtDNA molecule level in single oocytes and human blastoids (*102*). A study observed the haplotype-resolved mitochondrial genomes from single human oocytes and single blastoids, revealing the linkage of rare heteroplasmic mutations and tracking heteroplasmy dynamics in the blastoid model of human early development (*103*). Several studies have showed that mtDNA content in cumulus cells does not predict development to a blastocyst or implantation (*104,105*). Another study has developed a new method of mtDNA sequencing, a cost-effective mtDNA targeted-sequencing protocol called single-cell sequencing that targets amplification of multiplex probes; this technique could be used to more economically ascertain the functional significance of mtDNA mutations in various stages of embryonic development (*106*). As these new technologies continue to be explored, they have continued to reveal the function of mitochondria and the role of mtDNA.

Mitochondrial replacement therapy (MRT) prevents the transmission of mtDNA-linked diseases by transferring nuclear DNA into enucleated donor oocytes—using pronuclear, spindle, or polar body transfer techniques (*107*). However, there are significant hurdles to its clinical use. Technologically, the carryover of even trace amounts of maternal mutant mtDNA poses a risk of "reversion," where mutant haplotypes proliferate and eventually overtake donor mtDNA, negating the therapy's benefit. Clinical trial data from children born following spindle transfer revealed a carryover of maternal mtDNA of a mere 0.8% in the blastocyst stage in one child; this subsequently increased to 30-60% at birth, demonstrating pronounced mtDNA reversion. Corroborating these findings, studies utilizing non-human primate models have similarly documented that in certain MRT-derived individuals, the proportion of maternal mtDNA in specific tissues increased from initial levels of <3% to levels as high as 17%. These observations indicate that trace quantities of carryover pathogenic mtDNA may confer a replicative advantage during development and undergo re-amplification. Studies indicate that this low-level heteroplasmy can undergo genetic drift or selective amplification during early embryonic development and after birth, potentially leading to the "reversal" of disease-causing mtDNA variants to clinically significant levels in tissues of offspring (*23*). Biological safety is also a major open question, particularly with regard to mito-nuclear compatibility. While recent single-cell data from spindle-transferred embryos have indicated seemingly normal development, these snapshots cannot rule out subtler disruptions arising from the mismatch between evolutionary co-adapted nuclear and mitochondrial genomes. Such mismatches could theoretically compromise metabolic fine-tuning or epigenetic stability in the long run. Beyond these biological risks, MRT sits at the center of heated ethical debates over germline modification and "three-parent" offspring. Therefore, despite its promise, MRT requires strict, long-term follow-up to validate its safety and efficacy before widespread adoption.

Non-invasive metabolic profiling represents another promising technological avenue. The analysis of metabolic fingerprints derived from spent embryo culture media has enabled the assessment of developmental competence and mitochondrial functional status without compromising embryo integrity. Nevertheless, precisely deducing mitochondrial homeostasis from culture medium metabolomics signatures is still technically challenging, owing to constraints in detection sensitivity and the inherent complexity of metabolite provenance. Future endeavors necessitate the integration of microfluidic platforms with high-throughput targeted metabolomics technologies to develop automated, non-invasive systems for the comprehensive evaluation of embryonic mitochondrial function (*108-110*).

3. Implications for embryo quality, developmental competence, and reproductive health

Mitochondria are now recognized as multifunctional organelles that extend far beyond ATP production. They are central to the dynamic regulation of

cellular metabolism, organelle interactions, signal transduction, and quality control systems. The stability of mitochondrial function plays an important role in all aspects of embryonic development.

Embryonic development is a key susceptibility window in the DOHaD framework, with mitochondrial dysfunction increasingly recognized as a link between early environmental stress and adult disease. Environmental toxicants like perfluorooctanoic acid (PFOA), for example, disrupt this bioenergetic balance by inducing oxidative stress and calcium dysregulation, which in turn leads to spindle defects and arrested development (*111*) Genetic factors are equally critical; mutations in maternal complex proteins such as OOEP and NLRP5 have been linked to mitochondrial insufficiency and early embryonic arrest (*112*). Importantly, these early defects may leave a lasting imprint. Studies in human iPSCs have found that mtDNA mutations can permanently shift metabolic profiles and alter differentiation trajectories (*113*). This suggests that early mitochondrial impairment could produce a form of "metabolic memory," potentially priming the individual for metabolic disorders later in life.

Research on mitochondria and embryonic development is expanding and promising. Mitochondria participate in the synthesis of several key metabolites such as amino acids, fatty acids, and nucleotides. These metabolites are necessary for the growth and development of embryos. Moreover, mitochondria are involved in the metabolism of glucose and fatty acids, which are important energy sources during embryogenesis. Dysfunctional mitochondria can lead to an insufficient supply of these metabolites, compromising embryonic development. Mitochondria also involved in calcium signaling, which regulates important cellular processes like cell division, gene expression, and apoptosis. Mitochondria can take up and release calcium ions, thereby influencing the activity of various proteins and enzymes. In addition, disruptions in mitochondrial reactive ROS homeostasis can have detrimental effects on embryonic development. Mitochondria interact with the ER, Golgi apparatus, and peroxisomes, facilitating processes like lipid metabolism, calcium signaling, and autophagy. Dysfunctional mitochondria can disrupt these interactions, leading to impaired embryonic development. The mitochondrial genome, mtDNA, also plays a unique role in embryonic development. Mutations in mtDNA can impair oxidative phosphorylation and ATP production, leading to mitochondrial diseases. These diseases can be transmitted from the mother to the offspring and have a significant impact on embryonic development and offspring health (Figure 4).

Advances in single-cell multi-omics now enable non-invasive assessment of mitochondrial function and mtDNA integrity in preimplantation embryos. Conventional methods of evaluating mitochondrial function in embryos often require invasive sampling, which compromises embryonic integrity and limits clinical applicability. Recent advances in single-cell multiomics technologies have revolutionized mitochondrial screening by enabling accurate, non-destructive assessment of mitochondrial status (*114*).

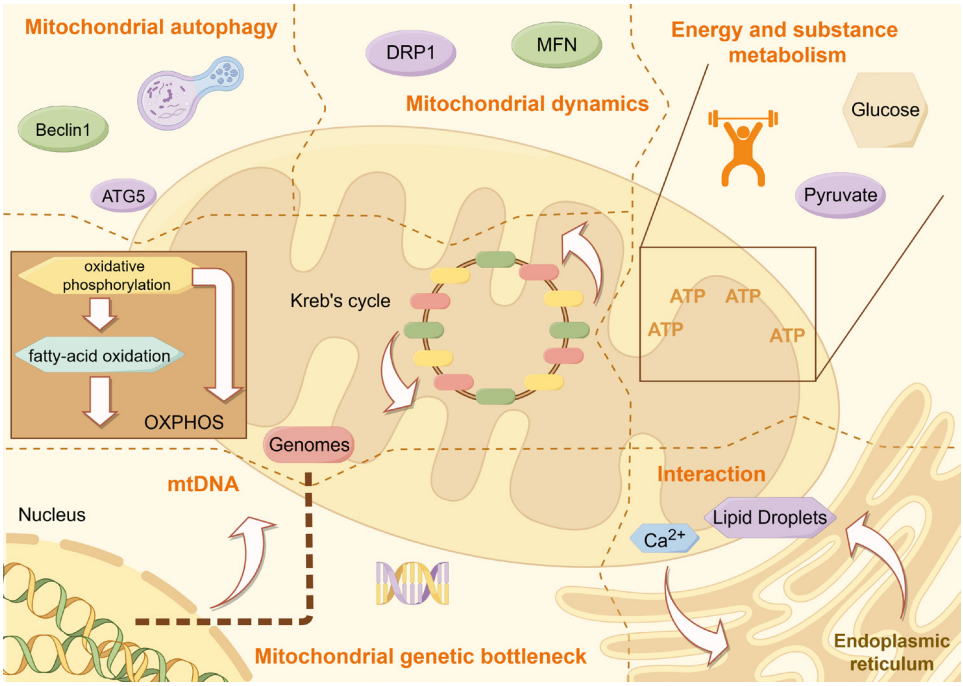

Figure 4. The role of mitochondria in embryonic cells. Mitochondria serve as central bioenergetic hubs, generating ATP *via* oxidative phosphorylation and fatty acid oxidation fueled by glucose and pyruvate metabolism. Organelle plasticity and quality control are governed by DRP1-mediated fission, MFN-dependent fusion, and ATG5/Beclin1-driven mitophagy. Beyond bioenergetics, mitochondria engage in extensive crosstalk with the endoplasmic reticulum and lipid droplets to coordinate Ca^{2+} signaling and lipid homeostasis. Moreover, the regulation of maternally inherited mtDNA involves a strict genetic bottleneck, ensuring the maintenance of mitochondrial genetic fidelity during embryogenesis.

Mounting evidence implicates mitochondrial metabolites as critical modulators of the epigenetic landscape in early embryos. Micro magnetic resonance spectroscopy (micro MRS) represents a breakthrough non-invasive technique for single-cell scale metabolic profiling of oocytes and preimplantation embryos (*109*). This method enables quantitative analysis of mitochondrial metabolites without compromising cellular integrity, generating metabolic fingerprints that correlate with oocyte maturity and embryonic developmental potential (*109*). Mitochondria are multifunctional regulators of embryonic development, integrating energy production, metabolic signaling, and epigenetic remodeling to ensure developmental success. MRT holds great promise for preventing mtDNA disorders and improving assisted reproductive technology (ART) outcomes, with ongoing technical refinements addressing key limitations such as residual mutations and mito-nuclear incompatibility. Future advances will likely focus on personalized MRT approaches, integration of non-invasive mitochondrial screening into routine ART workflows, and verification of long-term safety, ultimately expanding reproductive options for patients with a mitochondrial dysfunction and inherited mitochondrial diseases.

4. Future Perspectives

Despite substantial progress, the field still lacks a rigorous quantitative framework for defining "mitochondrial quality," with current assessments relying heavily on descriptive proxies such as the mtDNA copy number. A key unresolved question is how discrete mitochondrial features combine at the organelle level to influence developmental competence. In this context, recent evidence that oocytes actively suppress mitochondrial Complex I activity to limit ROS production challenges the prevailing assumption that elevated respiratory activity is inherently indicative of higher mitochondrial quality (*79*). These findings suggest that mitochondrial function must be evaluated in a developmental and context-dependent manner rather than through static measures of activity alone.

Current conceptual models also lack sufficient resolution in terms of lineage. The mechanisms governing mitochondrial fission, fusion, and mitophagy — and how these processes are differentially regulated between the inner cell mass and the trophectoderm — have yet to be fully understood. The increasing availability of single-cell multi-omics data has further exposed this limitation, raising the unresolved question of whether mitochondrial heterogeneity reflects stochastic variation or instead represents a regulated form of metabolic plasticity required for lineage commitment (*77,115*).

Finally, these uncertainties carry important implications for clinical translation. MRT shows therapeutic promise, but its broader implementation will require rigorous evaluation of potential mito-nuclear incompatibilities as well as the long-term stability of epigenetic states across generations (*116,117*). Addressing these challenges will require a shift from predominantly correlative studies towards mechanistic interventions that directly probe the functional limits of mitochondrial plasticity during early development.

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