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Review

MITOCHONDRIA IN MAMMALIAN OOCYTES AND EARLY EMBRYOS. A REVIEW ON MORPHOLOGICAL AND FUNCTIONAL STUDIES

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ABSTRACT

Mitochondria are the most abundant organelles in mammalian oocytes and early embryos. The central role of mitochondria in the establishment of developmental competence of oocytes and early embryos come out from basic research in experimental models and clinical studies, including those from Assisted Reproductive Technologies (ARTs) such as *in vitro* fertilization and embryo culture. We here review major concerns about mitochondrial bioenergetic function and morphology as well as their involvement in oocyte and early embryo development.

1. Introduction

Mitochondria are the most copious organelles in the mammalian oocytes and early preimplantation embryos. They are involved in the energy synthesis, generation of reactive oxygen species (ROS) and regulation of the apoptotic processes (1). The mature oocyte contains more mitochondria and mitochondrial DNA (mtDNA) than other cell types. Numerous studies considerate mitochondria as key factors of reproductive competence (2, 3) and as crucial determinants of physiological oogenesis and preimplantation embryogenesis (4).

In humans, the female reproductive potential slowly declines from the age of 32 to 37, to be then followed by a rapid downfall (5, 6). Almost the half of meiotically mature oocytes obtained by IVF from 35 years-old women are aneuploid and this rate gradually increase with aging (7, 8). The mechanisms underlying this age-related oocyte and embryo quality decrease are not fully unraveled but one of the main targets of senescence are mitochondria, with alterations in their function and ultrastructure (9, 10). Many studies tried to define the role of the mitochondria during oocyte maturation and early preimplantation embryonic development by correlating ATP content, meiotic and mitotic spindle organization and chromosomal segregation (8, 9, 11, 12).

We here reviewed the role of mitochondria as main determinants of the oocyte and embryonic developmental competences as well as their clinical implication.

2. Mitochondria in oocytes and early preimplantation embryos: bioenergetics and development

Oocytes are subjected to a complex and energy-consuming remodeling before ovulation and fertilization, to sustain the post-fertilization processes (13). Mitochondria are the primary source of ATP in the oocyte; their number is relevant for the bioenergetic ability of the future preimplantation embryo to normally develop after fertilization (14, 15). Since they are maternally inherited, the complement, i.e. the total number of mitochondria/mature oocyte at the fertilization stage, is fixed (9). Due to a temporarily suspension of their replication until the post implantation stage, the total number of mitochondria remains constant during the preimplantation development (16). This clearly means that the complement of mitochondria at fertilization is responsible for the energy supply during the subsequent early post-fertilization phases of the embryonic development, as confirmed by better developmental rates in human oocytes and embryos with higher ATP content (1, 2, 17).

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The oocyte may remain in a close anoxic environment for part of its life. Its energy demands may be minimal and supplied by the ATP produced through the oxidative phosphorylation within the electron transport chain (ETC). The main substrate used by the oocytes is the exogenous pyruvate, produced by cumulus or granulosa cells, whose cytoplasmic extensions deeply penetrate into the ooplasm and directly communicate with the oolemma via gap junctions (18, 19). Other amino acids and intermediate metabolites are also potentially involved (20) but the glycolysis in the oocytes is limited by low phosphofructokinase expression (21). In early preimplantation embryo, the embryonic metabolism switches during the development, changing from a predominantly glycolytic pathway ending with the production of pyruvate, to a full aerobic respiration that include Krebs cycle and oxidative phosphorylation (3). Simultaneously to this metabolic transition, a morphological change is clearly identified by an increase in the mitochondrial cristae, the presence of a denser matrix, and an elongated or branched appearance. Abnormalities in the mitochondria ultrastructure are linked to functional alterations leading to embryo degeneration or death (9).

3. Mitochondria ultrastructure evaluation in oocyte and early embryo

Morphological changes affect the mitochondria and associated organelles in the mammalian female germ cell during oogenesis, maturation and fertilization (22). Transmission electron microscopy (TEM) is the gold standard to reveal and analyze ultrastructural differences in germ and somatic cells (23-33). In the primordial germ cell (PGC), rounded mitochondria with a pale matrix and small vesicular cristae are located close to the nucleus. In the early stages of mammalian oogenesis, aggregates of mitochondria are clustered around the *nuage*. In oocytes at the early prophase stage, mitochondria growth and align along the outer surface of the nuclear membrane. In this phase, they show a denser matrix and visible lamellar cristae (22). Oocytes of primordial and primary follicles have round, or irregular mitochondria clustered near the nucleus, with the typical arched cristae. During the follicular growth, the numerical density of the mitochondria gradually increases to spread in all the ooplasm (34). Microtubules regulate the perinuclear accumulation and the following cytoplasmic dispersion of mitochondria. Cumulus cells are essential for the mitochondrial maturation during the oogenesis. They are responsible for the ATP production necessary to the oocyte energy supply in the latest phases of oocyte maturation (35). Morphological characteristics of cumulus cells can be indirectly used to evaluate oocyte quality and its maturation. During the ovulation, mitochondria are the most represented organelles in the ooplasm (22, 25). They form clear aggregates with either the smooth endoplasmic reticulum (SER) and the vesicles. These so-called “mitochondria-SER aggregates” (M-SER) and “mitochondria-vesicle complexes” (MV) seems to be involved in the production of substances or membranes necessary for the subsequent fertilization and early embryogenesis. After fertilization, significant changes occur in mitochondria size and shape. In the pronuclear zygote, they are distributed around the pronuclei. During the first embryonic cleavage division, round or oval mitochondria with a dense matrix and few arched cristae are gradually replaced by elongated ones, with a less dense matrix and numerous transverse cristae (3, 36). A progressive reduction in size and number of M-SER aggregates and MV complexes was observed in the early embryos (36).

This progressive, stage-specific ultrastructural changes of mitochondria during the preimplantation embryogenesis is physiological and with clinical significance. As a consequence, after fertilization, embryos are characterized by a finite number of organelles and this progenitor pool of non-replicating mitochondria is segregated between daughter cells at each division (9). The modifications in the mitochondrial fine structure, during the early development, are related to the progressive increase of the levels of respiratory activity. This would be expected to be compensatory respect to diminished mitochondrial number/cell in maintaining sufficient ATP levels to meet the increasing energy demands of the developing blastomeres during cleavage (15). These morphological changes result in a high oxidative metabolism and ATP production, influencing the oocyte quality and contributing to a good embryonic development (37-39).

Mitochondria distribution is another essential factor during oocyte maturation and embryo development. Differently to the differentiated cells, the progenitor population of mitochondria in the fully-grown oocyte is generally uniformly distributed within the cytoplasm; subsequently, mitochondria are subjected to stage-specific modification in their spatial organization. TEM analysis of mouse and human oocytes show mitochondria in direct contact with the SER cisternae; moreover, small clusters of mitochondria surround compact assemblages of SER elements in the pericortical and subplasmalemmal cytoplasm (9, 40). This different mitochondrial distribution, mediated by calcium fluxes between these two organellar systems, suggest an up-regulation of mitochondrial ATP production (41). This peculiar spatial organization also indicates a specific spatial functional heterogeneity for oocyte mitochondria, which may be an important regulatory factor in the early embryonic development (8, 41). Mitochondrial ultrastructure, distribution and number could be considered a sign of oocyte and embryo viability. Consequently, ultrastructural alterations could be a possible cause of mitochondrial dysfunction.

4. The role of mitochondria in the Assisted Reproductive Technologies (ARTs)

The regulation of mitochondrial activity is one of the focus in ARTs, to clarify if the premature arrest of oocyte maturation or early embryogenesis can be associated with mitochondrial alterations and, therefore, to insufficient ATP supply (38).

Several studies demonstrated that alterations in number, morphology and functionality of mitochondria, could be the cause of different developmental alteration during ARTs, such as: premature arrest of preovulatory maturation, altered organization of meiotic and mitotic spindles leading to chromosomal aneuploidy at MII, fertilization failure and arrested blastomeres division during the preimplantation phase (38, 42-44).

A healthy mitochondrial status of oocytes and embryos is also associated with the oxygen concentration used during *in vitro* maturation (IVM) or *in vitro* culture, that can interfere with the developmental competences (28, 32).

The developmental competence of oocytes matured under low oxygen concentration (5%) was, in fact, higher than in oocytes cultured under

atmospheric oxygen tension (20%) (45). Embryos cultured under a physiological O_2 concentration (5%) showed better developmental performance and higher embryo production rates than those cultured under atmospheric O_2 conditions (20%). Improved oocyte competence and embryo developmental rate seems to be linked to a lower reactive oxygen species (ROS) production under physiological oxygen conditions (46).

5. Conclusions and future perspectives

In ARTs, positive outcomes are strictly based on the proper selection of high-quality oocytes and embryos. Regarding the need to improve the embryo's performance ability during the early developmental stages, mitochondrial preservation can ensure the success of ART programs. The developmental viability, in both oocytes and embryos, is strictly connected to mitochondrial activity, function, morphology and distribution (12, 42). The lethal levels of fragmentation are one of the most remarkable developmental dysfunction originating from mitochondria, that can be clinically treated by invasive methods (9). Recently, bioactive molecules are used to ameliorate mitochondrial function, due to their ability to provide protection against oxidative damage and to increase the overall efficiency of ATP production (1).

The findings here discussed evidence the importance of further studies to improve activity and ultrastructural preservation of mitochondria during ART protocols, by optimizing culture conditions before the embryo transfer, especially for infertility connected to the advanced age (46-50).

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