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Review:

Mitochondrial functions on oocytes and preimplantation embryos*

Li-ya WANG[1,2,3], Da-hui WANG[2], Xiang-yang ZOU[1], Chen-ming XU[†‡3]

([1]*Department of Biotechnology, Dalian Medical University, Dalian 116044, China*)

([2]*The Sperm Laboratory, Institute of Cell Biology and Genetics, College of Life Sciences, Zhejiang University, Hangzhou 310058, China*)

([3]*Department of Reproductive Endocrinology, Women's Hospital, School of Medicine, Zhejiang University, Hangzhou 310006, China*)

[†]E-mail: xuchenm@126.com

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Abstract: Oocyte quality has long been considered as a main limiting factor for in vitro fertilization (IVF). In the past decade, extensive observations demonstrated that the mitochondrion plays a vital role in the oocyte cytoplasm, for it can provide adenosine triphosphate (ATP) for fertilization and preimplantation embryo development and also act as stores of intracellular calcium and proapoptotic factors. During the oocyte maturation, mitochondria are characterized by distinct changes of their distribution pattern from being homogeneous to heterogeneous, which is correlated with the cumulus apoptosis. Oocyte quality decreases with the increasing maternal age. Recent studies have shown that low quality oocytes have some age-related dysfunctions, which include the decrease in mitochondrial membrane potential, increase of mitochondrial DNA (mtDNA) damages, chromosomal aneuploidies, the incidence of apoptosis, and changes in mitochondrial gene expression. All these dysfunctions may cause a high level of developmental retardation and arrest of preimplantation embryos. It has been suggested that these mitochondrial changes may arise from excessive reactive oxygen species (ROS) that is closely associated with the oxidative energy production or calcium overload, which may trigger permeability transition pore opening and subsequent apoptosis. Therefore, mitochondria can be seen as signs for oocyte quality evaluation, and it is possible that the oocyte quality can be improved by enhancing the physical function of mitochondria. Here we reviewed recent advances in mitochondrial functions on oocytes.

Key words: Mitochondria, Oocyte, Preimplantation embryo
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INTRODUCTION

Oocyte quality can be defined as its abilities to be fertilized, mature, and give rise to normal offspring (Duranthon and Renard, 2001). Good quality oocytes are exceedingly essential for fertilization, especially for successful in vitro maturation (IVM) and fertilization (IVF), which are two major assisted-reproductive technologies for female infertility. At present, the cellular mechanisms that impart oocyte quality have not been elucidated, although many researchers have studied the functions of mitochondria in oocytes (Table 1). Mitochondria are energy-supplying organelles, whose functional integrality is essential for cellular survival and development. Mitochondria in the oocyte can provide adenosine triphosphate (ATP) for fertilization and preimplantation embryo development (Torner *et al*., 2004) and they can act as stores of intracellular calcium (Ca) and proapoptotic factors as well.

Mitochondria have their own genetic materials, mitochondrial DNA (mtDNA) that is derived from maternal mtDNA exclusively, for the paternal mitochondria are not retained in the fertilized oocyte at the four-cell stage (Cummins *et al*., 1997). There are approximately 100000 to 200000 mtDNA copies in a mammalian oocyte (Reynier *et al*., 2001), which are divided among all daughter cells during the

‡ Corresponding author

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Table 1 Catalog of research progresses on mitochondrial function in oocytes and preimplantation embryos

Mitochondria	References[a]
Mitochondrial inheritance	Cummins *et al*., 1997; Cox and Spradling, 2003; 2006
Mitochondrial distribution	Wilding *et al*., 2001; Torner *et al*., 2004; Nishi *et al*., 2003
Mitochondrial movement	van Blerkom, 1991; Cox and Spradling, 2003; 2006
mtDNA content	Reynier *et al*., 2001; Spikings *et al*., 2007
mtDNA mutation	Yesodi *et al*., 2002; Hsieh *et al*., 2004
Mitochondrial polarity	van Blerkom *et al*., 2003; 2006;van Blerkom and Davis, 2006; 2007
Function in calcium regulation	Machaty *et al*., 1997; Krisher, 2004; Whitaker, 2008
Mitochondrial dysfunction	Tarín, 1996; Eichenlaub-Ritter, 1998; Yin *et al*., 1998; Ottolenghi *et al*., 2004; Thouas *et al*., 2004; Zhang *et al*., 2006
Mitochondria transfer	Pinkert *et al*., 1997; Malter and Cohen, 2002; Kong *et al*., 2004; Yi *et al*., 2007

[a]Studies involved in oocytes and embryos

developmental progress of embryos. Because there is no mtDNA replication until postimplantation, this renders oocytes more susceptible to any kind of mitochondrial dysfunction (Spikings *et al*., 2007). Recent studies have shown that mitochondrial dysfunctions, such as the structural, spatial and genetic abnormalities in the oocyte, may influence normal embryo development, so mitochondrial characteristics and other mitochondrion-related changes can serve as signs of oocyte quality.

MITOCHONDRIA AND OOCYTE QUALITY AND MATURATION

Oocyte maturation includes nuclear maturation and cytoplasmic maturation, and both of them are essential for the fertilization and embryo development (Eppig, 1996). The nuclear maturation was characterized by the release of the first polar body (Fulka *et al*., 1998), while the cytoplasmic maturation is difficult to evaluate. But presently, it is reported that mitochondria may be used to estimate cytoplasmic maturation because oocyte maturation in vitro is accompanied by the distribution changes of active mitochondria in addition to distinct cumulus morphological changes (Torner *et al*., 2004).

Mitochondrial distribution and oocyte maturation

Homogeneousness and heterogeneousness are two major mitochondrial distribution patterns in oocytes. Homogeneous distribution of mitochondria throughout the cytoplasm is more common at germinal vesicle stage (Nishi *et al*., 2003), while heterogeneous distribution is more commonly observed in the oocyte of metaphase I or II (Torner *et al*., 2004). The increased level of mitochondrial aggregation around the nucleus indicates oocyte maturation, during which mitochondrial distribution changed from being homogeneous to heterogeneous. More precisely, in the heterogeneous state, the mitochondrial distribution changed from granulated aggregation to clustered aggregation. It is also observed that morphologically poor quality embryos are only characterized by the homogenous distribution of mitochondria (Wilding *et al*., 2001). Inappropriate culture conditions may inhibit mitochondrial movement to the inner cytoplasm and thus affect its cytoplasmic maturation (Torner *et al*., 2004). It was reported that mitochondrial redistribution in different cellular areas is mediated by cytoskeleton, especially microtubule network (van Blerkom, 1991). The abnormal distribution of mitochondria is related to the inappropriate formation of the cytoplasmic microtubule network, which can lead to the retardation or arrest of oocyte development and exert negative effects on the embryogenesis due to the abnormal ATP distribution (Nagai *et al*., 2006), because high energy supply around nucleus is very important during embryonic development. Therefore, proper distribution of active mitochondria during oocyte maturation is necessary for further development.

Calcium signaling in mitochondria

Mitochondrial redistribution in the oocyte may result from the high demand for ATP and calcium during cytoplasmic maturation, since the main functions of mitochondria are ATP synthesis and calcium supply (Krisher, 2004). Mitochondria form aggregates with the smooth endoplasmic reticulum (SER),

and these aggregates are apparently involved in the energy production and deposition before fertilization (Torner *et al*., 2004). Mammalian oocyte maturation relies not only on a high level of ATP for nuclear envelope breakdown, but also on intracellular calcium, which is derived from the endoplasmic reticulum (ER) and mitochondria (Krisher, 2004).

Inositol 1,4,5-trisphosphate (IP3) and ryanodine receptors were found to localize on the surface of the ER, which are active from the germinal vesicle stage to oocyte maturation (Machaty *et al*., 1997). These two receptors enable redundant calcium to release from the intracellular stores. Ca^{2+}-induced Ca^{2+} release (CICR) is considered as a feedback process of IP3- and ryanodine-induced Ca^{2+} release (Chakraborti *et al*., 1999). Studies have shown that mitochondria have an enormous capacity to regulate Ca^{2+} and prevent cytoplasmic Ca^{2+} concentration from rising too high potently. When the Ca^{2+} in the matrix gets overloaded, mPT pore (mitochondrial permeability transition pore) will be formed for the Ca^{2+} release (Giacomello *et al*., 2007).

Mitochondria and ER are both important protectors involved in intracellular calcium homeostasis. Ca^{2+} enters mitochondria via the Ca^{2+} uniporter when Ca^{2+} concentration in the cytosol is over a certain threshold, and once the cytosolic Ca^{2+} has returned to its resting level, Ca^{2+} is pumped back into cytoplasm by the Na^{+}/Ca^{2+} exchanger (Bootman *et al*., 2001). These two channels together with the Na^{+}/H^{+} antiporter establish a transport cycle to mediate Ca^{2+} concentration in both cytosol and matrix. The cytosolic excessive Ca^{2+} is transported to the ER by sarco-endoplasmic reticulum Ca^{2+} ATPases (SERCAs) or removed from the cell through plasma membrane Ca^{2+}-ATPase isoforms (PMCAs) and Na^{+}/Ca^{2+} exchangers located in the plasma membrane to the extracellular medium. This cycle is quite crucial for intracellular Ca^{2+} homeostasis (Berridge *et al*., 2000; Smaili *et al*., 2000). Whitaker (2008) recently reviewed that calcium may perform an important function in embryonic cell-cycle transition and embryonic axis establishment.

Interaction between ooplasmic mitochondria and cumulus cells

Communication between the oocyte and its surrounding cumulus cells is also important for oocyte development potential (Krisher, 2004) and therefore for the success of IVM and IVF (Mermillod *et al*., 2008). The cumulus cells are responsible for nutrition supply for oocytes in the final phase of oocyte maturation (Gilchrist *et al*., 2008). Morphological characters of cumulus cells can be used to value oocyte quality and its maturation (Torner *et al*., 2004). Compact and complete cumulus and bright and homogeneous ooplasm are considered as a sign of high-quality immature oocytes, whereas cumulus with no more than three layers or dark and heterogeneous ooplasm indicates low-quality immature oocytes (Blondin and Sirard, 1995).

It has been suggested that mitochondrial polarity is a determinant of ATP generation and calcium homeostasis (van Blerkom and Davis, 2007; van Blerkom, 2008), and van Blerkom *et al*.(2008) recently found that mitochondrial membrane potential ($\Delta\varphi_m$) in the subplasmalemmal domain of oocytes could be mediated by cumulus-derived nitric oxide (NO) during oocyte maturation. NO is important for ovulation and it can de-energize mitochondria by depressing the generation of ATP in the subplasmalemmal domain of oocytes, which is required for ovulatory process. Previous studies have shown that suppression of NO synthesis can prevent ovulation (Bu *et al*., 2003) and the effect of NO on oocyte maturation is related to its inhibition on estradiol production (Voznesenskaya and Blashkiv, 2005).

As we know, during meiotic progression, oocyte maturation also couples with cumulus expansion or apoptosis and the number of cumulus cells attached to matured oocytes decreases with age (Qiao *et al*., 2008). Torner *et al*.(2004) found that mitochondrial redistribution and their oxidative activity are essential to the degree of cumulus cell apoptosis during oocyte maturation. Mitochondrial fine homogeneous distribution is correlated to low oxidative activity and low levels of cumulus cell apoptosis, while granulated homogeneous distribution is correlated to high oxidative activity and high levels of cumulus cell apoptosis. It is probable that oocytes transport proapoptotic molecules from the activated ooplasmic mitochondria to the surrounding cumulus. Paracrine factors and gap junctions participate in the communication between oocytes and cumulus cells (Andreuccetti *et al*., 1999; Albertini *et al*., 2001). Recent studies have proven that oocytes may regulate the functions

of cumulus itself instead of being controlled by cumulus (Gilchrist *et al*., 2008). Hussein *et al*.(2005) demonstrated that soluble paracrine signals from the oocyte may contribute to the levels of apoptosis in cumulus cells rather than gap junctions, because destroying the gap junctions between cumulus cells and oocytes has effects on apoptosis in all cumulus cell layers instead of the closest layer to the oocyte. Growth-differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) are two key oocyte-secreted factors (OSFs), which are expressed in the ooplasm and function in cumulus cells. These two factors can activate the signaling pathways in cumulus cells to mediate the development of their neighboring oocytes. It is obvious that GDP9 and BMP15 play vital roles in oocyte maturation and quality determination (Gilchrist *et al*., 2008). Sharov *et al*.(2008) have detected the expression of GDP9 and BMP15 decreased in aged oocytes, which can be also used for oocyte quality evaluation.

MITOCHONDRIA AND OOCYTE AGING

Aging is the accumulation of function loss in diverse organs and tissues. Natural human fertility decreases with the maternal age, which may reduce the success rate of IVF. The success rate of IVF pregnancy in women older than 35 years old is markedly lower than that in younger ones (Hsieh *et al*., 2004). Previous investigations suggested that poor oocyte quality was responsible for the age-related decline in female fertility competence (Wu *et al*., 2000). For the aged oocytes, mitochondrial pathophysiology occurs in the ooplasm, which includes an increased density of mitochondrial matrix, increased mtDNA damage, and higher frequency of ruptured mitochondrial membranes (Ottolenghi *et al*., 2004), while $\Delta\varphi_m$, ATP synthesis and metabolic reactions in the electron transport chain decrease with age (Sharov *et al*., 2008). All these changes may link to the female infertility.

MtDNA and oocyte aging

Human mtDNA strictly derives from maternal inheritance (Kaneda *et al*., 1995; Cummins *et al*., 1997). The number of mtDNA copies varies in oocytes, and lower copy numbers may be caused by inadequate cytoplasmic maturation or mitochondrial biogenesis (Reynier *et al*., 2001). The mtDNA content in oocytes with normal fertilization capability is higher than that in abnormal oocytes (Reynier *et al*., 2001), and aging has been reported to exert a negative influence on the process of mitochondriogenesis (López-Lluch *et al*., 2008).

Over 150 mtDNA mutations have been identified (Yesodi *et al*., 2002), among which the 4977-bp deletion named as $\Delta mtDNA^{4977}$, is the most common one. The $\Delta mtDNA^{4977}$ accumulation with aging suggests that it should be a marker of aging (Meißner *et al*., 1997). $\Delta mtDNA^{4977}$ results from the removal of structural genes including ATPases 6, ATPase 8, cytochrome oxidase III, and NADH-CoQ oxidoreductase subunits 3, 4L, 4 and 5 (Wang and Lü, 2009). The accumulated mtDNA mutations induce insufficient amounts of active mitochondria and a decline of oxidative phosphorylation efficiency, all of which may contribute to poor oocyte fertilization (Hsieh *et al*., 2004). In addition, mtDNA mutation in aged cells may generate a specific peptide for triggering cytochrome-c release (Dubec *et al*., 2008). At present, a study showed another mtDNA deletion, $\Delta mtDNA^{5286}$, that was a non-age-related marker for impaired oocyte quality in IVF (Yesodi *et al*., 2002).

The production of reactive oxygen species (ROS) is the main reason for oxidative damages of DNA. 8-oxodeoxyguanosine (8-oxo-dG) is the most common base modification in mutagenic damage, which is usually used as a biomarker of oxidative stress (Haghdoost *et al*., 2005). Previous observations suggested that oxidative damage and a lack of DNA repair mechanism may contribute to mtDNA mutation accumulation. Anson *et al*.(1998) demonstrated that there was a DNA repair pathway in the mitochondrion by detecting lesion ratio between nuclear DNA and mtDNA. A common sense is that the level of 8-oxo-dG is much higher in mtDNA than in nuclear DNA, if there is no DNA repair capability in the mitochondrion. But the result that the ratio was a constant suggested the existence of DNA repair capability. Vermulst *et al*.(2008) also proved the existence of a homology-directed double-stranded break (DSB) repair mechanism in mammalian mitochondria. Kujoth *et al*.(2005) found that accumulation of mtDNA mutations increases in the incidence of apoptosis with aging instead of responding to

oxidative stress. It was also found that mtDNA mutation was not correlated with increased ROS production (Trifunovic *et al*., 2005). Therefore, it may be a phenotypic expression, which differs from mtDNA mutation accumulation that causes cellular aging (Vermulst *et al*., 2008).

Mitochondrial polarity, ATP content, and oocyte aging

The embryo development is strongly correlated with the activity of mitochondria in oocytes, which is characterized by $\Delta\varphi_m$ (Wilding *et al*., 2001). High-polarized mitochondria are commonly seen in the pericortical cytoplasm of oocytes and early embryos, which may be associated with their functions in metabolic regulation including ATP production and calcium homostasis (van Blerkom *et al*., 2003). Intracellular high-polarized mitochondria can reflect the embryo ability to reactivate development in the diapausing state (van Blerkom and Davis, 2006). The high-polarized mitochondrial domain in an oocyte may also have an effect on sperm penetration and cortical granule exocytosis (van Blerkom and Davis, 2007). Low-polarized mitochondria are associated with abnormal embryos (Wilding *et al*., 2001). $\Delta\varphi_m$ may be involved in material transport and energy transition, so a decrease of $\Delta\varphi_m$ may lead to mitochondrial dysfunction. Studies on embryo fragmentation showed that large number of fragmentation resulted in the residual cell without any high-polarized mitochondria in subplasmalemmal domain, while studies on 2-cell embryos with unequal blastomeres showed that the daughter cells without high-polarized mitochondria in subplasmalemmal domain stopped dividing (van Blerkom and Davis, 2006). van Blerkom *et al*.(2006) demonstrated that $\Delta\varphi_m$ was related to intercellular contact and communication instead of morphological difference and cell specificity. In addition, studies have shown that maternal age had an effect on oocyte mitochondrial activity. The age-related decline of $\Delta\varphi_m$ was observed in oocytes and preimplantation embryos (Wilding *et al*., 2001). Cyclosporine A (CsA) is an inhibitor of mPT pore opening, and it can restore $\Delta\varphi_m$ decease caused in aged cells (Rottenberg and Shaolong, 1997), which suggests that the opening of mPT pore may have an effect on $\Delta\varphi_m$. Fan *et al*.(1998) showed that the expression of adenine nucleotide translocase (ANT), an important component in mPT pore, decreased in aged cells, which may be a reason for $\Delta\varphi_m$ decease. $\Delta\varphi_m$ decline is a signal of mitochondrial membrane permeability transition, and continuous membrane permeability transition represents the abolished proton gradient across the inner mitochondrial membrane, which blocks ATP synthesis. Reduced ATP level may limit the energy supply for the oocytes or preimplantation embryos (Thouas *et al*., 2004) and high ATP content may support high $\Delta\varphi_m$ effectively (van Blerkom *et al*., 2006). However, embryos are contained in the hypoxic uterine lumen, which is not beneficial for ATP synthesis (Lonergan *et al*., 2007). So high ATP level can also limit embryo development.

Mitochondria and apoptosis in oocytes

Previous IVM studies showed that apoptotic rate of immature oocytes from old patients is significantly higher than that from young ones, which suggested that age-related apoptosis in poor-quality oocyte might be one of the reasons for the female infertility (Wu *et al*., 2000). Excessive ROS produced in aged cells has been reported to provoke the opening of mPT pores and subsequently the release of cytochrome-c, which activates caspase cascade pathway that can induce cellular degradation (Thouas *et al*., 2004).

The mPT pore is a nonselective and high-conductance channel. It is responsible for the mitochondrial membrane permeability transition, which is a critical control point in apoptosis. Induction of the mPT pore can lead to mitochondrial swelling and cell death. It is believed that mPT pore opening is a consequence of apoptosis, not the reason for it (Kinnally and Antonsson, 2007). Excessive ROS generation, Ca^{2+} overload, or the expression of Bcl-2 family proteins can lead to the formation of the mPT pore, followed the release of cytochrome-c and the activation of caspase cascade.

Several proteins have been thought to form the mPT pore, which include ANT, voltage-dependent anion channel (VDAC), and cyclophilin-D (Crompton, 1999). ANT is located in the inner mitochondrial membrane and can exchange adenosine diphosphate (ADP) for ATP between the mitochondrial matrix and the cytosol. Active ANT is a dimer that can alternate between two conformations: ANT-c (cytosol) and ANT-m (matrix) (Vyssokikh and Brdiczka, 2003).

When induced by atracyloside or pyridoxal phosphate, the ADP/ATP-binding site of ANT would face the cytosol in a way that it can interact with VDAC in the outer membrane. In this state, the mPT pore is opened up. But when induced by bongkrekate, the ADP/ATP-binding site faces the matrix without interaction with VDAC, and the mPT pore is inhibited (Crompton, 1999).

VDAC is a highly conserved protein in the outer mitochondrial membrane and belongs to the porin family that can form aqueous channels, which allow for the passive diffusion of metabolites less than 5000 kDa (Vieira *et al*., 2000). VDAC is also a dimer, which can also change between VDAC-t (tensed) and VDAC-r (relaxed) (Vyssokikh and Brdiczka, 2003). The VDAC-t can interact with the ANT-c, while the VDAC-r is not bound to ANT, only existing as a monomer. On the surface of VDAC, there are some specific binding sites for proteins including hexokinase, glycerol kinase, and Bax. Studies have shown that hexokinase and Bax can compete for the same binding site (Pastorino *et al*., 2002). When this binding site is occupied by hexokinase, the permeability transition caused by ANT-c will be prevented potently (Beutner *et al*., 1998). In the mitochondrion, there is an octamer of creatine kinase that can inhibit the interaction between VDAC and ANT, for it binds to VDAC from the inner surface of the outer membrane, and the octamer reduces the affinity of VDAC for both Bcl-2 and Bax (Vyssokikh and Brdiczka, 2003). So the octamer has been suggested to be the mitochondrial target of the pro-apoptotic proteins, Bax and Bcl-2, which would allow VDAC to form a cytochrome-c release channel.

Cyp D belongs to the cyclophilin family. It possesses peptidyl-prolyl *cis*/*trans* isomerases (PPIase) activity, which is crucial for protein folding (Galat and Metcalfe, 1995). Cyp D is localized in the mitochondrial matrix normally. When activated, its PPIase activation may induce a conformational change of ANT and then result in the opening of the mPT pore. PPIase is inhibited by CsA, which is another member of the cyclophilin family (Tsujimoto and Shimizu, 2007). Ca^{2+} and adenine nucleotides are the regulators of the affinity between Cyp D and CsA. Ca^{2+} can reduce the binding affinity while the adenine nucleotides promote it (Crompton, 1999). In addition to the CsA-sensitive and Ca^{2+}-dependent mPT pore, there exists a CsA-insensitive mPT pore (He and Lemasters, 2002), although its mechanism is totally unknown.

Mitochondria are the major sources of ROS, which can do some oxidative damage to themselves (Takahashi *et al*., 2004). It has been recently suggested that mPT pore opening is the result of co-actions of Ca^{2+} overload and ROS generation (Mather and Hagai Rottenberg, 2000), and Ca^{2+} overload can also enhance ROS generation in mitochondria (Grijalba *et al*., 1999). However, the mPT pore opening does not destine cellular apoptosis. In a low conductance state, the opening is reversible, and the excitable state may contribute to the generation of Ca^{2+} waves. The mPT pore becomes irreversibly open under a high conductance state with the accumulated Ca^{2+} in the mitochondria exceeding a certain threshold-concentration, and these irreversible opening results in the collapse of transmembrane potential and the release of cytochrome-c, which initiates apoptosis (Ichas *et al*., 1997).

It has been suggested that mPT pore opening is a protective mechanism against mitochondrial Ca^{2+} overload (Crompton, 1999). Buchholz *et al*.(2007) reviewed the influences of aging-caused functional changes of mitochondria and ER on Ca^{2+} regulation. These changes contribute to Ca^{2+} overload and apoptosis. However, the mechanism of Ca^{2+} overload is not well understood. Additionally, the Ca^{2+} threshold for mPT pore decrease is accompanied by an irreversible increase of oxidative stress (Ott *et al*., 2007).

Mitochondria and meiosis in oocytes

It is possible that embryos with normal appearance may contain genetic lesions. These abnormalities may affect the viability of postimplantation embryos or result in abnormal fetuses. Munne *et al*.(1995) indicated that aged female has a much higher proportion of embryos with normal appearance but abnormal genes, especially chromosomal aneuploidies, compared with younger ones. As we know, motor proteins and protein kinases are necessary for spindle assembly and chromosome alignment (Eichenlaub-Ritter, 1998). Chromosomal aneuploidies often occur during the first meiotic division (Robertson, 1998) and aberrant activity of motor proteins and kinetochores-related kinases may contribute to these

chromosomal aneuploidies in aged female (Yin *et al*., 1998). Polo-like kinases (Plks) are key cytokinesis-related protein kinases in oocytes during meiotic maturation for spindle formation and chromosome separation (Pahlavan *et al*., 2000), and their activities are correlated with protein phosphorylation, which depends on the endogenous substrates and ATP as phosphate donor (Golsteyn *et al*., 1994).

Mitochondrial functional integrity is essential for ATP provision. Yin *et al*.(1998) cultured mouse oocytes with diazepam (DZ), a drug that binds with the benzodiazepine receptors on the outer mitochondrial membrane. This binding may limit ATP synthesis, affect calcium regulation, and disrupt the distribution of mitochondria, which can lead to the retardation of mouse oocyte maturation and chromosome aneuploidies. The similar conditions between the maternal age-related aneuploidy and drug-exposure aneuploidy suggest that mitochondrial dysfunction affects spindle formation and the segregation of chromosomes during the maturation of aged oocyte (Eichenlaub-Ritter, 1998). Oxidative stress can also influence the oocyte spindle assembly (Tarín, 1996), because oxidative stress may trigger mPT pore opening and decrease ATP content in oocytes (Zhang *et al*., 2006). It is therefore not surprising that injection of cytoplasm may improve embryo quality and support the embryo development (Cohen *et al*., 1998).

IMPROVEMENT OF OOCYTE QUALITY BY MANIPULATION OF MITOCHONDRIA

Many treatments are available for improving embryo quality and increasing success rate of IVF targeting at mitochondria, including mitochondrial transfer and various drugs. Accumulation of mtDNA mutation with aging mainly contributes to mitochondrial dysfunction, and the growing number of abnormal mitochondria impairs oocyte fertilization and subsequent embryo development. Pinkert *et al*.(1997) primarily microinjected mitochondria isolated from *Mus spretus* liver samples into fertilized ovary, and made mitochondrial genome applied for experimental and therapeutic purposes. Yi *et al*.(2007) have provided direct scientific evidence on the improvement of embryo development after mitochondrial transfer. Microinjecting a certain level of mitochondria into oocytes may compensate for the insufficiency of active mitochondria and raise the morula formation rate. However, allo-mitochondrial transfer may lead to potential risks such as mitochondrial heteroplasmy or mitochondrial genetic diseases (Malter and Cohen, 2002). Self-mitochondrial transfer can prevent these risks effectively, and the transfer of self-granulosa cell mitochondria was also proved to enhance the pregnancy rate by improving embryo quality during preimplantation development (Kong *et al*., 2004). Further research on mitochondria has also concentrated on the design and development of drugs. The rationale of targeting drugs to mitochondria is based on possible mitochondrial impairments during cellular metabolism, such as oxidative damage, apoptotic inducement, or uncoupling agent (Armstrong, 2007), which can help to overcome or reduce disadvantageous factors to mitochondria.

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