Abnormal Mitochondrial Structure in Human Unfertilized Oocytes and Arrested Embryos

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ABSTRACT: To clarify the relationship between mitochondria and embryo development, we collected human unfertilized oocytes, early embryos, and arrested embryos. Unfertilized oocytes and poor-quality embryos were collected, and the ultrastructure of mitochondria was determined by transmission electron micrography. Four criteria for determining the mitochondrial state were mitochondrial morphology, cristae shape, location, and number of mitochondria. In mature oocytes, mitochondria were rounded with arched cristae and a dense matrix and were distributed evenly in the ooplasm. In pronuclear zygotes, the size and shape of mitochondria were similar to those in mature oocytes; however, mitochondria appeared to migrate and concentrate around pronuclei. In this study, 67% of examined unfertilized oocytes had fewer mitochondria in the cytoplasm. A decreased number of mitochondria located near the nucleus was also demonstrated in 60% of arrested embryos. Fewer differentiated cristae were determined in all three arrested blastocyst stages of embryos. The relative expressions of oxidative phosphorylation genes in oocytes and embryos were also determined. These data imply that inadequate redistribution of mitochondria, unsuccessful mitochondrial differentiation, or decreased mitochondrial transcription may result in poor oocyte fertilization and compromised embryo development.

KEYWORDS: embryo; mitochondria; oocyte

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INTRODUCTION

Mature oocytes contain approximately 10^5 mitochondria, but these are structurally undifferentiated compared with those of later embryo stages.[1,2] Throughout oogenesis and early embryogenesis, mitochondria in germ cells differ in appearance from those of somatic cells. Mitochondria in female germ cells assume a unique spherical profile, and an elongated mitochondrial morphology can be observed after implantation.[3,4] There are significant differences in net ATP content between oocytes, and low concentrations of ATP are generated in oocytes and early embryos.[5] Mitochondrial function can affect the physiology of embryos in many ways. This organelle has been recognized as the "powerhouse" of the cell because of its role in oxidative metabolism. The electron transfer chain consists of four respiratory enzyme complexes arranged on the mitochondrial inner membrane.

In recent years, an increasing number of reports have shown that mtDNA mutations are associated with human aging and mitochondrial diseases.[6–8] Declining mitochondrial function in older women may contribute to declining fertility.[9,10] Male subfertility and sperm dysfunction are also associated with defective mitochondrial function.[11,12] The loss of mitochondrial activity in oocytes obtained from aging couples therefore may contribute to lower embryo development and pregnancy rates.[13] To determine the relationships of mitochondrial structure and location with the ability for embryo development, we compared the ultrastructure of mitochondria through oocytes to early embryos by electron microscopy.

MATERIALS AND METHODS

Human Oocytes and Embryo Collection

This study was approved by the institutional review board of Taipei Medical University Hospital. Unfertilized oocytes were donated to our laboratory for research from patients enrolled in an *in vitro* fertilization program. In addition, embryos that were abnormally arrested and tripronucleus zygotes unsuitable for embryonic replacement or cryopreservation were also donated and used for the following experiments. Fresh human oocytes were obtained after informed consent in cases in which the donation of these oocytes to the research program would have little effect on the outcome of an *in vitro* fertilization cycle.

Electron Microscopy

Human oocytes and early embryos were fixed for 2 h in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.2 M cacodylate buffer, washed in 0.1 M cacodylate buffer containing 0.2 M sucrose three times, and postfixed for 2 h in 1% osmium tetroxide. Dehydration was achieved by a graded series of 35, 50, 75, 95, and 100% ethanol, respectively. Samples then were infiltrated in a mixture of ethanol and spurr (Electron Microscopy Sciences, Fort Washington, PA) and were embedded in spurr. Ultrathin sections were cut on a Leica AG ultramicrotome, placed on 200-mesh copper grids, stained with uranyl acetate and lead citrate, and photographed on a Hitachi T-600 electron microscope.

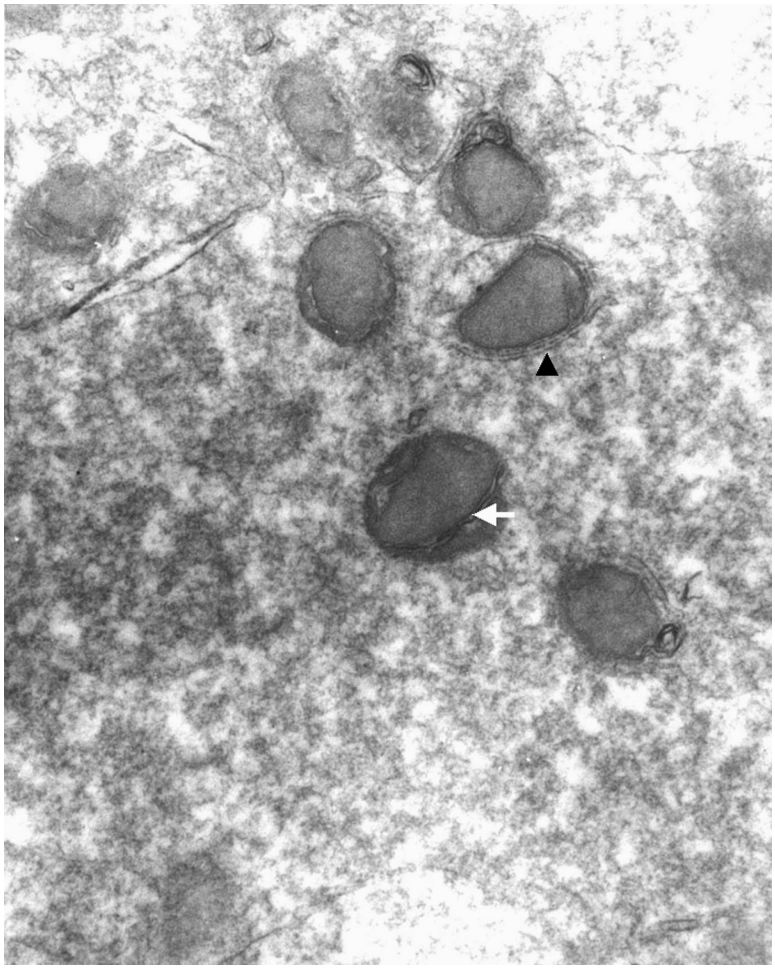

FIGURE 1. Ultrastructure of mitochondria in mature oocytes. The arched cristae were determined by electron microscopy. Mitochondrial cristae had an arched shape and were located in the mitochondria periphery (*arrow*). The smooth endoplasmic reticulum was also present together with mitochondria (*arrowhead*). Original magnification ×25,000.

RESULTS

To study the ultrastructure of mitochondria in human oocytes and early embryos, we examined normal mitochondrial structure and location by electron microphotography. In mature human oocytes, mitochondria are the prominent organelle. Mitochondria were rounded and possessed a dense matrix. Mitochondrial cristae had an arched shape and were located in the mitochondria periphery (FIG. 1). The smooth endoplasmic reticulum was also present together with mitochondria (FIG. 1). Some complexes existed in mature oocytes, which consisted of mitochondria aligned

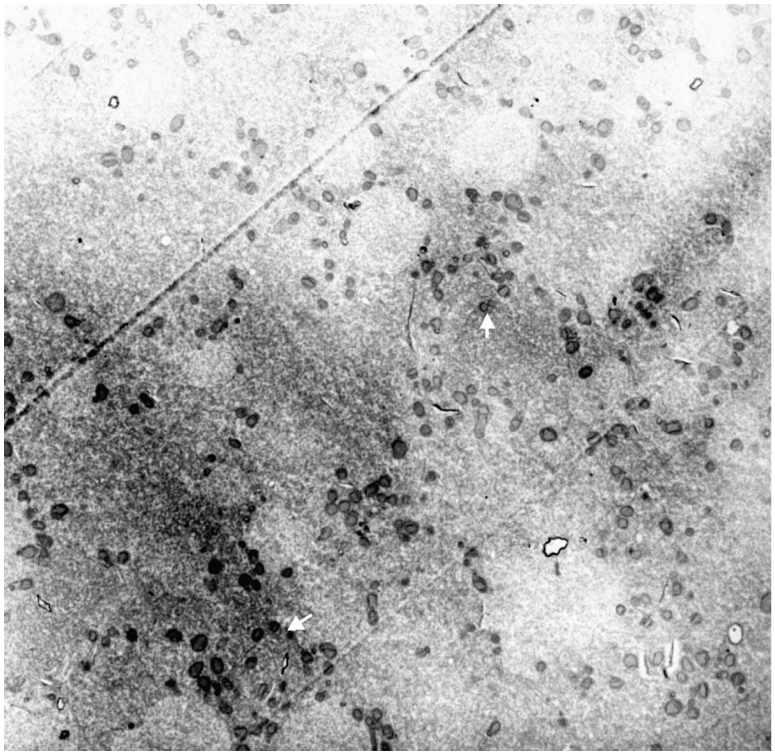

FIGURE 2. Multivesicular complexes distributed in mature oocytes. Mitochondria are arranged around vesicules and form multiple complexes scattered evenly throughout the ooplasm. Multivesicular complexes are indicated (*arrows*). Original magnification ×3,000.

around a vesicule. Multivesicular complexes were randomly distributed in mature oocytes (FIG. 2). Pronuclear zygotes had a similar size and shape compared with mature oocytes. The multivesicular complexes were also still observed at this stage. The mitochondria migrated and were concentrated around the pronuclei. In the eight-cell stage of embryos, mitochondria that were more elongated were seen together with rounded elements, and the cristae were more differentiated (FIG. 3). Some of the mitochondria began to form transverse cristae in the blastocysts (FIG. 4). A decrease in the number of mitochondria was also observed.

To determine whether the number and differentiation patterns of mitochondria affect the ability of oocyte fertilization and embryo development, we also collected 12 unfertilized oocytes and 15 arrested embryos to study their mitochondrial structure and location. There were multiple vacuoles and fewer mitochondria in unfertilized oocytes compared with functional mature oocytes. Eight unfertilized oocytes with less than 100 mitochondria were examined. Significantly decreased numbers of mitochondria located near the nucleus were observed in arrested embryos. Nine of 15 arrested embryos were characterized by this phenomenon. Peripheral arched cristae that were insufficiently differentiated to transverse cristae also were determined in

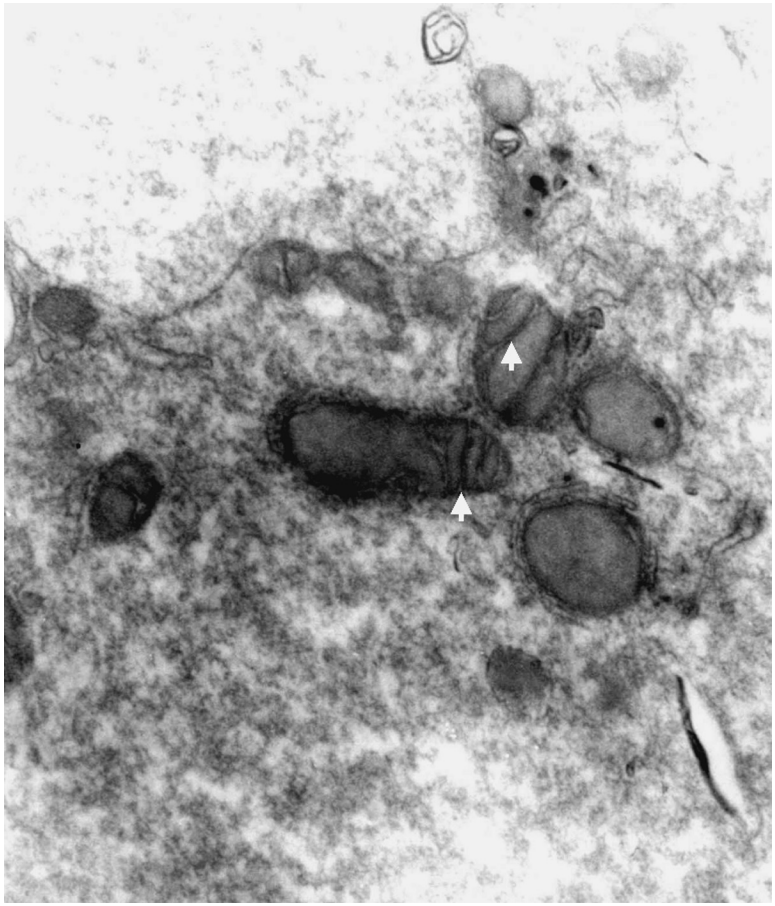

FIGURE 3. Electron microscopic examination of the eight-cell stage of an embryo. There are both round and elongated mitochondria in the embryo. Mitochondria are differentiated with few transverse cristae (*arrows*). Original magnification ×25,000.

all three arrested blastocysts examined. These data indicate that a reduced number of mitochondria may affect the fertilization potential of oocytes. Arrested embryos may occur because of a lack of redistribution of mitochondria or successful mitochondrial differentiation.

DISCUSSION

In our studies, mitochondria present peripherally arched to transverse cristae from mature oocytes to the blastocyst stage. The dynamic nature of cristae may be caused by proteins, which mediate electron transport and oxidative phosphorylation, being bound to the inner mitochondrial membrane. The varied crista structures repre-

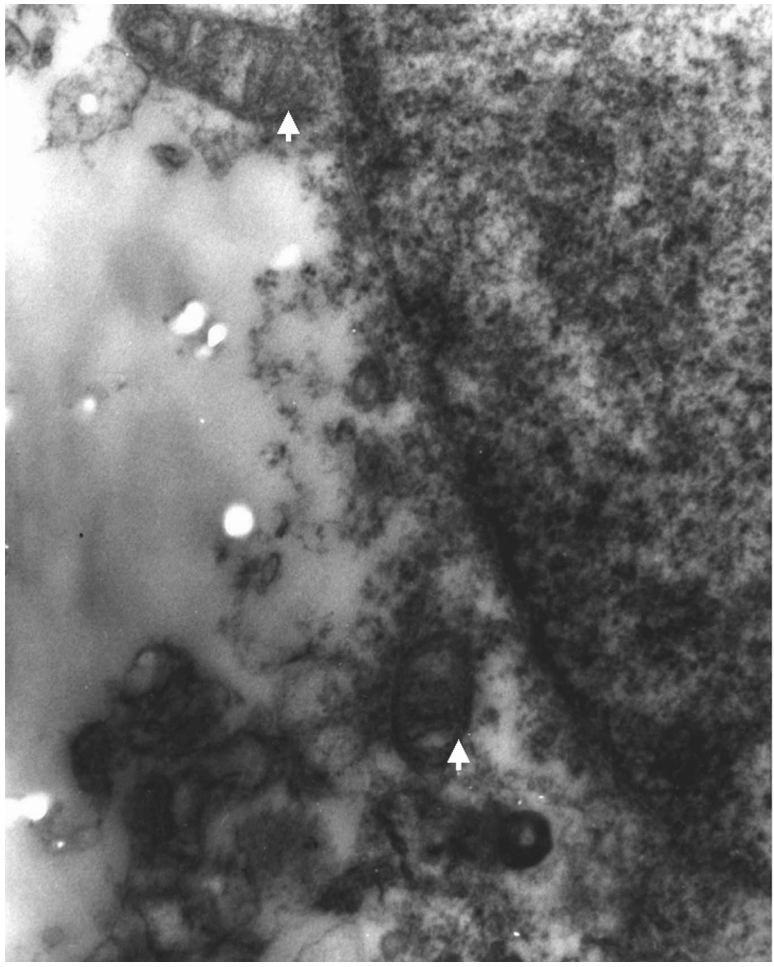

FIGURE 4. Electron microscopic examination of a blastocyst. Some of the mitochondria are differentiated with fully transverse cristae (*arrows*). Original magnification ×25,000.

sent mitochondria that progress from the arrested to the active state with embryo development. The relationship between energy production and cristae area has been shown in other studies. Proportional increases in respiratory chain enzymes and cristae surface areas have been observed.[14] The high energy demand of cells is met by an increase in the surface area of cristae.[15] Cristae differentiation may provide an efficient energy power supply for embryo development. Mitochondrial cristae change from a tubulovesicular pattern to a sparse, lamellar configuration in primordial germ cells during differentiation into oogonia.[16] Throughout oogenesis to early embryogenesis, despite cristae changes, mitochondria are also differentiated into various

shapes to fit the energy requirements of different developmental stages. Mitochondria vary considerably in size and structure depending on their source and metabolic state. Mitochondria in mature oocytes assume a unique spherical profile. The arrested state of round mitochondria in ovulatory oocytes was also reported by other groups.[4] Postfertilization changes in mitochondria are characterized by a gradual transition from round or oval mitochondria with a dense matrix and few arched cristae to forms that are more elongated, possessing a lighter matrix and more numerous cristae oriented transverse to the long axis of the mitochondria.[16] Increased mitochondrial metabolism appears to coincide with a decrease in density of the mitochondrial matrix and an increase in the number of cristae.

In mature oocytes, mitochondria are the prominent organelles and are evenly distributed in the cytoplasm. After fertilization, mitochondria become concentrated in the center of the oocyte, around the developing pronuclei (FIG. 3). The mitochondria are persistently located around the nucleus from fertilization to the early developmental stage. Pronuclear formation and fusion presumably require energy. Mitochondria were reported to move close to the nucleus along microtubules to satisfy this energy requirement.[17,18] The observation that mitochondrial DNA replication in somatic cells is preferentially located close to the nucleus,[19] with human pachytene oocytes giving the appearance of a necklace of mitochondria around the nucleus,[20,21] implies that mitochondria migrate close to the nucleus when replication is required in both germ cells and somatic cells. In immature oocytes, mitochondrial aggregation is granular and clumped. Maturation of oocytes to metaphase I or II leads to the appearance of evenly distributed mitochondria.[13] Mitochondria evenly distributed in the cytoplasm are translocated to the perinucleus area as embryos develop.

There is a decrease in the number of mitochondria in normal blastocysts compared with mature oocytes. This may result from the original mitochondria segregating into the blastomeres without biogenesis of mitochondria from fertilization to the blastocyst stage. With oocyte maturity at ovulation, mitochondrial amplification[22] and mtDNA replication cease.[23] The gap between oogenesis and resumption of new mtDNA synthesis means that mitochondria are diluted and partitioned into multiplying daughter blastomeres. At ovulation, each oocyte contains around 10^5 mitochondria.[5] The mtDNA does not replicate until gastrulation in diverse species.[22–24] In arrested embryos, we also observed that fewer mitochondria existed in the cytoplasm. There were not enough mitochondria to supply energy for embryo development because of less-functional mitochondria or defective mitochondria in aging oocytes.

The average expression proportions of the eight studied genes were 4.4, 5.8, and 12.9 in unfertilized oocytes, arrested embryos, and tripronucleus zygotes, respectively. Higher expression levels in tripronucleus zygotes compared with unfertilized oocytes and arrested embryos were determined.[5] In this study, the arrested embryos collected at the two- to four-cell stage and tripronucleus zygotes collected at around the eight-cell stage had normal growth rates. In previous studies, Piko and Taylor reported that mouse mtDNA does not replicate during preimplantation development but is transcribed actively from the two-cell stage.[26] There is an approximately 30-fold increase during cleavage through the blastocyst stage.[22] Embryos with normal growth rates are assumed to have more than two times the expression level compared with unfertilized oocytes. However, there were no significant differences in expression levels between unfertilized oocytes and arrested embryos. Reduced mitochon-

drial transcription may affect the development of embryos. There was a three-fold greater expression level in 3PN compared with unfertilized oocytes. Mitochondrial RNA expression does not seem to be modified in embryos developing with abnormal tripronucleus. The expression of the ATPase 6 gene in unfertilized oocytes decreases compared with that in early cleavage–stage embryos.[27] We previously determined multiple deletions of mtDNA in unfertilized oocytes and arrested embryos, as well as significant increases in the proportion of deleted mtDNA in unfertilized oocytes.[28] It is probable that there is a minimum requirement for ATP content for normal embryo development including chromosomal segregation, normal mitosis, and physiological events. Fully differentiated mitochondria, successful translocation, an optimal amount of mitochondria, and sufficient transcripts may be the minimum requirements for embryo development. Our study results provide some criteria for selecting adequately developed oocytes.

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