Journal of Assisted Reproduction and Genetics, Vol. 21, No. 3, March 2004 (© 2004)

Opinion

Why Do Older Women Have Poor Implantation Rates? A Possible Role of the Mitochondria

Ana Karina Bartmann,[1,3] Gustavo Salata Romão,[1] Ester da Silveira Ramos,[1,2] and Rui Alberto Ferriani[1]

Submitted October 30, 2003; accepted February 27, 2004

Mitochondria are organelles responsible for oxidative phosphorylation, the main energy source for all eukaryotic cells. In oocytes and embryos, it seems that mitochondria provide sufficient energy for fecundation by supporting spindle formation during meiosis II, and for implantation. Since mitochondria are inherited from mother to child, it is important that oocyte mitochondria should be intact. Older women seem to have more mitochondrial DNA mutations, which can be responsible for poor implantation and aneuploidy, two conditions that occur more often in this group. In the present report we propose a new model to explain why older women have poor implantation rates.

KEY WORDS: Aneuploidy; mitochondria; older women; poor implantation.

Mitochondria are organelles which occupy a large volume of the cytoplasm in eukaryotic cells. They are responsible for oxidative phosphorylation, which transforms adenosine diphosphate to adenosine triphosphate (ATP), the main energy source for all eukaryotic cells. Mitochondria produce more than 90% of the ATP in mammals (1). The morphology and number of mitochondria vary among species. In humans, they represent 23% of the total cytoplasm of preimplantation embryos (2).

The mitochondria also play a role in genetic inheritance. They carry a small DNA strand organized circularly as a double helix (3) that is different from nuclear DNA. Mitochondrial DNA (mtDNA) is inherited from mother to child by a process that does not follow classic Mendelian laws (4). Morphological studies have shown that sperm-derived mitochondria are destroyed either immediately after penetration into the ooplasm or during early cleavage stages of embryonic development. Nevertheless, molecular genetic studies have confirmed that maternal mtDNA predominates in mammals (5).

Alleles of organelle genes segregate during mitosis as well as meiosis, so the descendants of a single cell by mitotic division are not necessarily genetically identical in terms of organelle genes. Alleles can be lost and mutant alleles can also be fixed (6).

The homogeneity of mtDNA (homoplasmy) within individuals and matrilineages is another important aspect that needs attention. Despite the large number of mitochondrial genomes in mammalian oocytes (200,000), heteroplasmy (more than one kind of mtDNA within the cell) in normal human populations has rarely been observed (7). The most accepted explanation for the widespread mtDNA homoplasmy is that of a genetic bottleneck effect enabling only a limited amount of mtDNA from a female to be transmitted and to colonize the progeny in the next generation (5,7,8)

Although a large amount of information is available on the effects of energy production on animal productivity and health, limited information is available

[1] Department of Obstetrics/Gynecology, Service of Human Reproduction, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil.
[2] Department of Genetics, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil.
[3] To whom correspondence should be addressed at A/C Dr. Ester S. Ramos, Departamento de Genética, Faculdade de Medicina de Ribeirão, Preto – USP, Avenue Bandeirantes, 3900, Ribeirão Preto, SP, CEP 14049-900, Brazil; e-mail: anabartmann@ig.com.br.

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on how mitochondria affect oocytes and embryos (5). Most cells in the body contain 10^3 to 10^4 copies of mitochondrial DNA (9).

Oogonia are assumed to contain an average of 200 mitochondria distributed sparingly, each carrying five copies of mtDNA. These mitochondria multiply exponentially during oogenesis, yielding 10^5 undifferentiated mitochondria in the mature oocyte, but they generate relatively low levels of ATP when compared to those observed at the morula and blastocyst stage (5). It may be possible that mitochondria multiply during oogenesis, but wait to produce ATP until the oocyte is fertilized. The embryo may need much more energy for implantation and development than the oocyte itself.

Mitochondria are usually excluded from meiotic and mitotic spindles but locate peripherally, apparently providing energy from centrosomal, cytoskeletal, and chromosomal activity during cell division. This may be the reason why many authors have postulated that the mitochondria have a determinant role in oocyte maturity and subsequent fertilization rate (9–11). The oxidative phosphorylation process is crucial for embryo development, playing a role in blastocyst differentiation, expansion, and hatching (12).

In spite of innovative techniques, maternal age remains a limiting factor in achieving a pregnancy. Only 5–30% of the embryos from women over 35 years of age implant after IVF. This group of women has also the highest incidence of trisomic deliveries, and therefore a link may exist between lower implantation, higher aneuploidy, and maternal age (13,14).

Why don't the embryos of older women implant as well as those of younger women? What is different in the former? Why do older women have more aneuploid babies? What is the relation between aneuploidy and poor implantation? In which way does aneuploidy arrest development? What occurs inside the oocyte or embryo? Is aneuploidy itself responsible for poor implantation?

Some authors believe that a senescence process within the oocyte may cause further malsegregation of the chromosomes (15). Others believe that there may be a preferential recruitment of normal oocytes early in reproductive life, so that more abnormal (aneuploid) oocytes than normal ones would be available for later recruitment (16–19).

In mammals, including humans, the mature ovulated oocyte remains arrested in second meiotic metaphase, with its condensed chromosomes lying on the equator of the spindle (20). Chromosome segregation appears to be controlled by the meiotic spindle, but spindle components are largely supplied by the ooplasm. Therefore, it has been suggested that dysfunctional cytoplasmic factors are responsible for structural abnormalities of this structure, which lead to eventual chromosomal malsegregation and poor implantation (21,22).

Ooplasmic factors are so important, that it was proposed that oocytes from women with poor implantation rates or poor embryo cleavage should receive a transfer of ooplasm isolated from oocytes of known developmental capacity (23). This approach has been successful in achieving a pregnancy, because the injection of fresh ooplasm appears to restore cytoplasmic deficiencies (22,23). This technique has led to the birth of nearly 30 babies worldwide (24).

In fact, it seems that the ooplasmic factors that improve implantation rates could be the mitochondria. This possibility has been confirmed by the experiments of Hsieh (25) who described the association between rearrangements of mtDNA, altered oxidative phosphorylation, and poor fertilization rates. It seems that mitochondrial dysfunction contributes to oocyte senescence, as observed in other tissues such as muscle and brain (11).

Wilding (26) has confirmed by confocal measurements that the mitochondrial activity is reduced in older women, concluding that the inherent mitochondrial activity can determine the potential of the oocyte to form a top quality embryo. Lee (9) has also hypothesized that the amount of mtDNA deletion may have an effect on oocyte maturity with subsequent fertilization. Mitochondrial DNA point mutations are known to accumulate in an age-dependent fashion in somatic tissues (10). Smith (5) has suggested that developmental arrest and abnormalities during preimplantation development may result from inadequate capacity to generate ATP at levels sufficient to support normal chromosomal segregation or normal biosynthetic activities within blastomeres.

The expression of oxidative phosphorylation genes reflects the need for this process as energy generator at early stages of development, so that the mitochondrial activity of human embryos is related to the rate of development (26). It may be possible that a mechanism of natural selection may operate during preimplantation development, eliminating abnormal embryos (27). Since the mitochondria initiate apoptosis by opening an inner membrane channel, which causes a collapse of the membrane potential and release of death-promoting factors (5), this natural selection can be mediated by them.

As the major site of oxidation reactions in the cell, mitochondria are also the most important source of reactive oxygen superoxides. These are very toxic byproducts of respiration implicated in mtDNA mutations and in the aging process. Normally superoxides are eliminated by transformation of $O_{2\,to}$ H_2O_2 by mitochondrial Mn++ superoxide dismutase, which is later converted to water by glutathione peroxidase (5).

Mitochondria also generate energy for spindle formation. Meiotic spindles are composed of microtubules and are crucial for normal chromosome alignment and separation of maternal chromosomes during meiosis I and meiosis II.

Disruption of the meiotic spindle results in abnormal chromosome alignment in embryos and may contribute to aneuploidy (28,29). Battaglia (21) found that the proportion of oocytes with abnormal meiotic spindles was significantly higher in older women than in younger women. Changes in spindle structure may reflect cytoplasmic dysfunction or other damage to the oocytes, like temperature stress (28). The microtubule system of the human oocyte is highly susceptible to disruption by cryoprotectants and temperature fluctuations (30).

The presence of a meiotic spindle predicts higher fertilization and embryo development rates, so it serves as a marker for oocyte quality, reproductive senescence, cytoplasmic maturation and/or pH, and/or temperature stress during handling (28,29).

Are the mitochondria, in terms, responsible for the process of fertilization? Possibly yes, first because they provide the energy necessary for oocyte maturation and fertilization and for embryo implantation, and second because without the energy produced by the mitochondria it would be impossible for the cell to divide.

The mitochondria also seem to be responsible for apoptosis, which performs a selection between "good" and "bad" oocytes before maturation.

We suggest that in women with poor implantation rates the first pathological step is a mutation in mtDNA. Once they have an error in their DNA, mitochondria cannot "work" normally. This abnormal mitochondrial activity reduces the production of ATP in the cell, which in turn reduces the implantation rates by limiting cell division. As we know, the implantation process needs much energy and would therefore be compromised by reduced ATP production.

Also the meiotic spindles, that are crucial for normal chromosomes segregation, may not be formed normally because of the poor ATP levels and altered spindles may result in aneuploid embryos, which implant poorly.

Finally, abnormal or reduced mitochondrial activity may cause an augmentation of reactive oxygen superoxides within the cell, resulting in oocyte damage that can also reduce implantation rates (Fig. 1).

In summary, it seems that the mitochondrion is not only an important organelle for reproduction—we dare say that the mitochondrion is THE important organelle, which not only contributes to reproduction, but also regulates the energy necessary for it and selects oocytes for recruitment. May we say that the mitochondria are responsible for natural selection?

Since the first ooplasm transplantation was performed, we have thought about its clinical repercussions. Barritt (24) demonstrated that children born from this procedure have had a great heteroplasmy, expressing both maternal and donor mtDNA.

Many diseases derived from mtDNA mutations are currently recognized. Schon (31) has reviewed diseases associated with defects in mtDNA. The most prominent disorders associated with mtDNA mutations include Kerns–Sayre syndrome (a fatal multisystemic disorder), progressive external ophthalmoplegia, MELAS syndrome (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes), MERRF (myoclonus, epilepsy, with ragged-red fibers), deafness, cardiomyopathies, nonsyndromic hearing loss (31,32), β-cell dysfunction, and decreased insulin insensitivity (33).

There is a great diversity of phenotypes associated with mtDNA mutations. According to Schon (31),

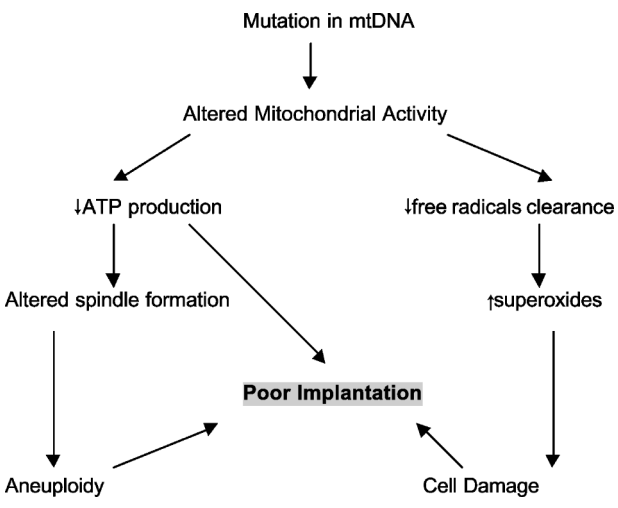

Fig. 1. Model proposed to explain a possible role of mitochondria in women with poor implantation rates.

"a particular clinical phenotype is, in essence, the product of the interplay between the level of heteroplasmy and the distribution of mutant genomes, both in space and in time."

If our model is true, it will be necessary to determine mitochondria "well-being." This may be achieved by determining mitochondrial ATP or free radical production. McGinnis (2) has measured the energy competence of mitochondria by using a specific medium with Mitotracker Red^{TM}. It has been demonstrated that gross morphology alone could only predict, at best, 23% of the embryos surviving continued culture, and when combined with the number of cleavage divisions, this figure improves only slightly to 34% (34).

Another field for future research will be the interaction between mitochondria and nucleus. It seems that this interaction plays an important role in oocyte development and maturation (35). Of the 70 or so proteins used in the process of oxidative phosphorylation, only 13 polypeptides are encoded by mtDNA, whereas nuclear genes encode all other oxidative phosphorylation proteins and all the mtDNA regulatory factors.

Therefore, mitochondrial biogenesis depends on the coordinated expression of the nuclear and mitochondrial genetic systems (5).

Many aspects about mitochondria physiology remain obscure. For example, are maternal mitochondria the only factors responsible for implantation? It is known that the centrosome of the embryo in most species is biparentally inherited, with the sperm contributing the centriole and the egg contributing the microtubule-organizing center (36), which means that also the sperm may play a role in embryo development.

And what about the poor implantation rates achieved after ICSI? Why almost 35% of all ICSI embryos do not develop? ICSI embryos have a lower developmental potential as measured by blastocyst formation (37). This could be explained by the fact that ICSI is able to "save" spermatozoa with genetic errors. We may also hypothesize that ICSI embryos have poor implantation rates because during sperm injection the meiotic spindles of the oocyte could be affected. Until now, the effects of ICSI on chromosomes, especially with respect to the 30–40% of oocytes that fail to fertilize, are unknown (28,29).

We presume that it is too early to start experiments with ooplasm transplantation in humans. At the present time, there are no clinical data available about children born after this procedure. We suggest that these children should be followed closely for early detection of eventual mitochondrial diseases. We also suggest that women who are candidates for ooplasm donation should be evaluated in terms of mitochondrial diseases in their families.

It is too soon to determine if mitochondria have, in fact, all the mentioned functions in human reproduction as we presume in our model. Further experiments are necessary to confirm our speculations.

Finally, since we do not know exactly what kind of role the interaction between nucleus and mitochondria plays, it would be advisable not to start human cloning in humans until we do.

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