Mitochondrial DNA rearrangements in human oocytes and embryos

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Human mitochondrial DNA (mtDNA) rearrangements, including more than 150 deletions and insertions, accumulate with age and are responsible for certain neuromuscular diseases. Human oocytes, arrested for up to 50 years, may express certain mtDNA rearrangements possibly affecting function. Investigations have previously shown a single mtDNA rearrangement (dmtDNA4977) in human oocytes. Sequencing of other rearrangements and their correlation with maternal age have not been performed in human oocytes or embryos. Here we use a nested PCR strategy of long followed by short polymerase chain reaction (PCR) that amplifies two-thirds of the mitochondrial genome. mtDNA rearrangements were detected in 50.5% of the oocytes (n = 295) and 32.5% of the embryos (n = 197). This represents a significant difference in the percentage of mtDNA rearrangements between oocytes and embryos (P < 0.0001). Twenty-three novel mtDNA rearrangements with deletions, insertions and duplications were found. There was no significant age-related increase in the percentage of human oocytes or embryos that contained mtDNA rearrangements. Significant reductions in the number of oocytes containing mtDNA rearrangements occurred as oocyte development progressed from germinal vesicle to the mature metaphase II oocyte (P < 0.05). These findings are discussed as they relate to mitochondria, mtDNA, and ATP production in human oocytes and embryos.

Key words: DNA rearrangements/embryos/mitochondrial deletions/mitochondrial DNA/oocytes

Introduction

Mitochondria contain specific DNA (mtDNA) that is distinct from nuclear DNA. Human mtDNA is believed to be inherited exclusively from the mother, although this debate continues (Giles et al., 1980; Smith and Alcivar, 1993; Alkel-Simons and Cummins, 1996; Cummins, 1998). Most human cells contain ~1000 mitochondria, and each mitochondrion contains 1–10 copies of mtDNA (Giles et al., 1980). Human mtDNA is 16 569 base pairs (bp) of circular double-stranded DNA encoding 22 tRNA, two rRNA and 13 mitochondrial proteins (Anderson et al., 1981). Although mitochondria provide these proteins for the oxidative phosphorylation pathway, the majority are encoded by nuclear DNA and are imported into the mitochondria from the cytoplasm.

Mutations in mtDNA are responsible for certain neuromuscular syndromes including Kearns–Sayre syndrome (KSS), chronic progressive external ophthalmoplegia and Pearson’s syndrome (Moraes et al., 1989; Zeviani et al., 1989). More than 150 other types of mtDNA rearrangements have been described, including deletions, insertions and duplications (Wallace et al., 1993). Deleterious mtDNA rearrangements cause cellular energy deficiencies and result in clinical disorders affecting the brain, heart, skeletal muscle, kidney, bone marrow, and pancreatic cells seen in degenerative diseases. One of the most predominant mtDNA rearrangements is found in 50% of patients with KSS syndrome. This ‘common deletion’ is a 4977 bp mtDNA deletion (dmtDNA4977) that is located between nucleotides 8482 and 13460.

mtDNA rearrangements accumulate with age faster in non-dividing tissues such as muscle and brain (Cortopassi and Arnheim, 1990; Ikebe et al., 1990; Hattori et al., 1991; Corral-Debrinski et al., 1991; Kitagawa et al., 1993). When ageing tissues accumulate rearrangements, the percentage reaches a significant level and a reduction in oxidative phosphorylation efficiency may occur (Richter et al., 1988; Hattori et al., 1991). Arrested oocytes do not divide for periods of up to 50 years. It has been postulated that they may accumulate mtDNA rearrangements, although the relationship with reproductive senescence is ambiguous (Keefe et al., 1995; Brenner et al., 1998). The number of mitochondria increases 100-fold during oogenesis, but there is a decrease in the number of copies of mtDNA per mitochondrion (Giles et al., 1980; Hauswirth and Laipis, 1985; Chen et al., 1995). The lack of redundant copies of mtDNA may render the metaphase II (MII) oocyte sensitive to mutations. Affected oocytes may be unable to produce energy, because of a dysfunction of the oxidative phosphorylation system. Such alterations may alter fertility. Embryo arrest in mice is dependent on a specific threshold level of ATP in oocytes (Van Blerkom et al., 1995). The original 100 000 mitochondria in the mouse oocyte do not replicate, but are distributed between all cells of the blastocyst. The mitochondria must have produced and stored all the energy required for the resumption of meiosis II, fertilization, and development (Piko
and Taylor, 1987; Ebert et al., 1988; Meirelles and Smith, 1998). Mitochondrial replication and ATP accumulation during oogenesis appear to be crucial for further development.

Rearranged mtDNA elimination has been postulated to occur by a mtDNA ‘bottleneck’, conserving normal mtDNA for offspring (Hauswirth and Laipis, 1982; Ashley et al., 1989; Howell et al., 1992; Jenuth et al., 1996). This theory states that a small number of mitochondrial genomes undergo replication (Jenuth et al., 1996). Such founder mtDNA replicate to give rise to almost all mtDNA in mature oocytes. If a rearranged mtDNA is randomly selected as one of the founder mtDNA, the resulting oocyte would contain an abnormally high percentage of rearranged mtDNA. An oocyte with replicated rearranged mtDNA would potentially fail to meet bioenergetic demands and arrest (Hauswirth and Laipis, 1982). The purpose of this study was to determine the types of mtDNA rearrangements in human oocytes and embryos, and whether they contribute to reproductive senescence.

Materials and methods

Human oocyte and embryo collection

The institutional review board (IRB) of Saint Barnabas approved research of compromised and discarded material. Exemption from review was granted by the Medical College of Virginia (MCV) IRB Committee Chairman. Oocytes and embryos were donated to research from consenting couples. Oocytes were considered to be discarded because they failed to develop into mature metaphase II oocytes or did not fertilize. Only compromised embryos that were arrested or abnormal and unsuitable for embryo replacement or cryopreservation were used for these experiments.

A limited number of oocytes isolated from ovaries of women undergoing oophorectomy were collected by the Department of Pathology at MCV. A small sample of the ovaries without pathological abnormalities was washed in phosphate-buffered saline (PBS). Oocytes were dissected from ovarian tissues by manual trituration. Individual ovarian oocytes were never incubated in vitro or exposed to spermatooza.

Oocytes and embryos were washed in acidified Tyrode’s solution (Sigma, St Louis, MO, USA) to remove the zona pellucida and all adhering sperm and cumulus cells. All samples were washed three times in PBS, added to 16 µl of sterile water, and frozen at –80°C. Single cell isolation was performed and verified by microscopy. All pipetting was performed with sterile barrier pipette tips.

Oocyte and embryo preparation

Oocytes and embryos were thawed at room temperature, and 1.7 µg proteinase K (ProK) (Sigma), 8 µl of sterile water and 10 µl of extra-long (el)PCR buffer containing 2 mM Mg²⁺ (elPCR kit reagent) were added. Reaction tubes were incubated at 37°C for 30 min, 30 min at 50°C, and at 95°C for 10 min.

PCR strategy

Two rounds of ‘nested’ PCR were performed with multiple sets of primers (Table I). The first round elPCR reaction (elPCR Kit, Life Technologies, Gaithersburg, MD, USA) amplified the entire larger arc (10.2 kilobases (kb)) of mtDNA between the primers (Figure 1). Five second round PCR reactions amplified regions within the first round elPCR product (Figures 2 and 3). The strategy of second round PCR was developed to amplify products only when mtDNA rearrangements were present. Minor mtDNA deletions and point mutations were not detectable using these techniques. All primers were designed using the ‘Oligo’ primer design program (National Biosciences, Inc., Baltimore, MD, USA) or were taken from existing literature and are shown in Table I.

First round elPCR protocol

First round elPCR (Figure 1) was performed using primers 5835 and 16065 on the samples after the ProK incubation step. Remaining elPCR reagents were added to produce 50 µl reactions containing 100 µmol/l of each dNTP, 25 pmol of each primer, and 1 µl elPolymerase. The reactions were carried out in a PTC-100 thermocycler (MJ Research Inc., Watertown, MA, USA) as follows: 94°C for 1 min; 35 cycles of 94°C for 30 s, 62°C for 30 s, and 68°C for 12 min; and a final 10 minute extension at 72°C. A 10 µl aliquot of the first product was run on a 0.8% agarose gel, stained with ethidium bromide, and photographed under ultraviolet (UV) illumination. Positive control verification of reagent quality and PCR function was carried out in each first round elPCR reaction.

Second round PCR protocols

Five separate second round PCR reactions (Figures 2 and 3) were performed using 1 µl of first round elPCR product as template. Second round primers were as follows: set 1, 7765 and 13962; set 2, 8300 and 15208; set 3, 5897 and 14905; set 4, MT1 and MT3; and set 5 MT2 and MT4. Each 50 µl PCR reaction including 100 µmol/l of each dNTP, 5 µl 10×PCR buffer II, 35 µl sterile water, 2 units of AmpliTaq DNA Polymerase, the specific primers (25 pmol of each), and MgCl₂ for reactions 1, 2 and 3 (3.5 mmol/l), and for reactions 4 and 5 (1.5 mmol/l). Second round PCR reactions were hot-started by adding the 1 µl of first round elPCR product at 80°C. The second round reactions were carried out in a PTC-100 thermocycler as follows: 94°C for 1 min; 30 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 3 min; and a final extension of 72°C for 10 min. A 10 µl aliquot of the reaction product was run on a 0.8% agarose gel, stained with ethidium bromide, and photographed under UV illumination.

DNA sequencing

Amplified products were sequenced to verify that they were from rearranged mtDNA. Some PCR bands (fragments) were purified following the protocols of the QIAquick PCR Purification Kit (Qiagen, Chatsworth, CA, USA). All purified fragments were sequenced by running sequencing reactions (ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit, with AmpliTaq DNA Polymerase, FS; Perkin-Elmer, Foster City, CA, USA), and were purified with Centri-Sep Columns (Princeton Separations Inc., Aldelphi, NJ, USA). Sequencing reactions were read on an ABI Prism Model 377 cycle sequencer (Perkin Elmer). Each sequence was visually and GCG database analysed using the Cambridge human mtDNA sequence (Anderson et al., 1981).

Statistical analysis of oocytes and embryos

Groups were compared by Fisher or χ²-analyses where appropriate. In order to eliminate age bias between the groups of patients, Mann-Whitney rank sum test (MWRST) was performed.

Results

Mitochondrial rearrangement analysis

First round elPCR reactions produced a 10.2 kb band that was a representative of the full length mtDNA (Figure 4). Samples that failed to amplify the 10.2 kb band of mtDNA, at a 17.5%
Table I. mtDNA primer sequences

<table>
<thead>
<tr>
<th>Primer set no.</th>
<th>Primer name</th>
<th>Strand</th>
<th>Sequence 5'-3'</th>
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<td>GAATCTAGATCGGAGCTGTTAAAAG</td>
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</tr>
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<td>H</td>
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<td>532</td>
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<td>TGTCTAGATCGGAGTTTGTGGCTCTAAGA</td>
<td>10230</td>
</tr>
<tr>
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<td>6197</td>
</tr>
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<td>10230</td>
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<td>L</td>
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<tr>
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<td>MT4</td>
<td>H</td>
<td>ACGGAACTCAGCAGGTTGTTGAGGTGACTC</td>
<td>6197</td>
</tr>
</tbody>
</table>

elPCR = extra-long polymerase chain reaction; L = lower; H = higher.

Figure 1. (A) Diagrammatic representation of the mitochondrial (mt)DNA genome showing the region that was amplified in the first round polymerase chain reaction with primers 5835 and 16065. The 10.2 kb region between the primers is represented by the line drawn between 5835 and 16065, and represents 2/3 of the mtDNA genome. (B) A 0.8% agarose gel showing first round reaction products. Lane 1: marker, with top band representing 12 kb. Lane 2: positive first round control reaction, showing the expected 20 kb band. Lane 3: negative first round control reaction (containing water), showing no bands as expected. Lane 4: first round reaction on a single human oocyte, showing the normal 10.2 kb band representative of full length amplification of undeleted mtDNA. Failure rate, were eliminated from the study because they contained no amplifiable mtDNA. A first round elPCR positive control reaction was used to amplify a 20 kb control fragment for verification of reagent quality and to monitor that the PCR machine was cycling correctly during each experiment (Figure 4). Negative control reactions (n = 65) included all elPCR reagents and produced no amplified mtDNA products (Figure 4). Only when first round elPCR experiments produced the positive control 20 kb fragment and reaction products were not detected in negative controls were the samples used. Control reactions using an isolated full-length mtDNA sequence were carried out for verification that the PCR reactions were not creating artefactual fragments (Figure 4).

Second round PCR reactions for primer sets 1 and 2 showed no bands unless a rearrangement was present between the primers. Second round PCR reactions for primer set 3 showed a 400 bp band when mtDNA was present (sequence verified as a mispriming fragment) and showed no other bands unless a rearrangement was present between the primers. Second round PCR reactions for primer set 4 showed no bands unless rearrangements were present and a 349 bp band when dmtDNA4977 was present (sequence verified). Finally, second round PCR reactions for primer set 5 (positive control for mtDNA) showed a 532 bp band when mtDNA was present (sequence verified).

Samples that contained the 532 bp band from second round primer set 5, but contained no other bands with second round primers, were assumed to contain mtDNA without mtDNA rearrangements. Samples that contained the 532 bp band from second round primer set 5, in addition to bands smaller than the full length between any of the second round primers, were assumed to contain mtDNA with characteristic mtDNA rearrangements.

Figure 2. Diagrammatic representation of the first round polymerase chain reaction (PCR) product between 5835 and 16065 is at the top of this figure. Five second round PCR reactions are represented by lines between the base pairs of the primers used; second round reaction (1)7765–13962, second round reaction (2)8300–15208, second round reaction (3)5897–14905, second round reaction (4)8491–13528, and second round reaction (5)13200–13707. Second round PCR reaction products were only produced if a rearrangement existed between the primers being used, except for positive control, because the PCR reaction conditions used did not allow for >5 kb products to be produced.

Second round PCR reactions for primer set 1 and 2 showed no bands unless a rearrangement was present between the primers. Second round PCR reactions for primer set 3 showed a 400 bp band when mtDNA was present (sequence verified as a mispriming fragment) and showed no other bands unless a rearrangement was present between the primers. Second round PCR reactions for primer set 4 showed no bands unless rearrangements were present and a 349 bp band when dmtDNA4977 was present (sequence verified). Finally, second round PCR reactions for primer set 5 (positive control for mtDNA) showed a 532 bp band when mtDNA was present (sequence verified).

Samples that contained the 532 bp band from second round primer set 5, but contained no other bands with second round primers, were assumed to contain mtDNA without mtDNA rearrangements. Samples that contained the 532 bp band from second round primer set 5, in addition to bands smaller than the full length between any of the second round primers, were assumed to contain mtDNA with characteristic mtDNA rearrangements.
Figure 3. A 0.8% agarose gel showing second round reaction products. Lane 1: second round reaction product with primer set 1 from a single oocyte, showing multiple bands (1.7, 2.0, 2.2 and 2.9 kb) characteristic of rearrangements. Lane 2: second round reaction product with primer set 1 from a single oocyte, showing two bands characteristic of rearrangements (1.3 and 2.3 kb). [Sequencing the 1.3 kb band confirmed a 7855 bp deletion (deletion 2 in Table II).] Lane 3: second round reaction product with primer set 2 from a single oocyte, showing multiple bands characteristic of rearrangements (0.7 and 1.5 kb). Sequencing reactions on the 0.7 kb band confirmed a 5463 bp deletion (deletion 3 in Table II). Lane 4: second round reaction products with primer set 2 from a single oocyte, showing multiple bands characteristic of rearrangements (0.8 and 1.4 kb). [Sequencing the 0.8 kb band showed a 6088 bp deletion (deletion 4 in Table II).] Lane 5: second round reaction product with primer set 3 from a single oocyte, showing a single band characteristic of a rearrangement (1.3 kb). [Sequencing the 1.3 kb band showed a 7903 bp deletion (deletion 5 in Table II).] Lane 6: second round reaction product with primer set 3 from a single oocyte, showing a single band representing the positive control fragment (0.5 kb). Sequencing the 0.5 kb band verified it as the amplified mtDNA control fragment.

Sequencing reactions were performed and verified that fragments produced in these experiments were mtDNA rearrangements. Sequencing reactions identified 23 previously unpublished mtDNA rearrangements from 21 of the samples analysed (Table II). The rearrangements included 23 deletions, two insertions, and one duplication. One of the rearrangements had a deletion and an insertion. Another rearrangement had a deletion, insertion, and duplication.

Rearrangements were compared according to insemination type. Oocytes never exposed to spermatozoa (n = 41) had mtDNA rearrangements 56.1% of the time, oocytes that had undergone intracytoplasmic sperm injection (ICSI) (n = 80) had mtDNA rearrangements 36.3% of the time, and oocytes that had undergone conventional insemination (n = 133) had mtDNA rearrangements 46.6% of the time. \( \chi^2 \)-analyses showed no significant differences between these three groups. Similar results were found when embryos from ICSI and in-vitro fertilization (IVF) were compared. Oocytes isolated from ovarian tissues that were never exposed to spermatozoa or incubated in vitro had mtDNA rearrangements in 6/7 samples (85.7%), whereas oocytes removed from antral follicles after human chorionic gonadotrophin (HCG), but never exposed to spermatozoa, had mtDNA rearrangements in 23/41 samples (56.1%).

mtDNA rearrangements and dmtDNA\(^{4977} \) were observed in 64/197 (32.5%) and 42/197 (21.3%) of embryos respectively (Table III). Multiple rearranged fragments were seen in 28/197 (14.2%) of the embryos.

mtDNA rearrangements and age

Population age variation was analysed by MWRST to verify that there was no statistically significant age difference in the sampled populations of oocytes and embryos. The mean ± SEM of the maternal ages of 235 oocytes analysed was 33.0 ± 0.3 years, and showed no significant difference from the 196 embryos analyzed (33.2 ± 0.3 years).

The donor age of MII oocytes (n = 184) with mtDNA rearrangements (30.5 ± 0.6 years; mean ± SEM) was significantly different (MWRST; \( P < 0.0001 \)) from the donor age of MII oocytes without mtDNA rearrangements (33.4 ± 0.3 years). The presence of mtDNA rearrangements in oocytes from donors aged <38 years was 84/192 (43.8%). The presence of mtDNA rearrangements in oocytes from donors aged ≥38 years was 14/40 (35.0%). \( \chi^2 \)-Analysis revealed no age-related change in the presence of mtDNA rearrangements in human oocytes. The donor age of MII oocytes (n = 182) with dmtDNA\(^{4977} \) (31.1 ± 0.8 years) was not significantly different from the donor age of MII oocytes without dmtDNA\(^{4977} \) (32.8 ± 0.3 years).
MtDNA rearrangements in human oocytes and embryos

Table II. Mitochondrial DNA Rearrangements in human oocytes

<table>
<thead>
<tr>
<th>Deletion no.</th>
<th>Size of deletion (base pairs)</th>
<th>Type and size of repeat</th>
<th>Repeat location</th>
<th>Stage of development</th>
<th>Type of insemination</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>5989</td>
<td>Direct, 7/10</td>
<td>7943–52/13931–38</td>
<td>MII</td>
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<td>7855</td>
<td>Direct, 7/8</td>
<td>6422–28/14275–82</td>
<td>MII</td>
<td>IVF</td>
</tr>
<tr>
<td>3</td>
<td>5463</td>
<td>Direct, 5/6</td>
<td>8143–47/13602–07</td>
<td>MII</td>
<td>ICSI</td>
</tr>
<tr>
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<td>6088</td>
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<td>8793–99/14880–85</td>
<td>MII</td>
<td>ICSI</td>
</tr>
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<td>No spermatozoa</td>
</tr>
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<td>6979–80/14881–82</td>
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<td>ICSI</td>
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<td>5367–73/13844–50</td>
<td>MI</td>
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<td>IVF</td>
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MII = metaphase II oocyte with one polar body; MI = metaphase I oocyte with no polar body; GV = oocyte containing a germinal vesicle; IVF = insemination with 150,000–500,000 spermatozoa; ICSI = intracytoplasmic sperm injection.

Table III. Mitochondrial (mt)DNA rearrangements in human oocytes and embryos

<table>
<thead>
<tr>
<th>No. analysed</th>
<th>mtDNA rearrangements (%)</th>
<th>dmtDNA&lt;sup&gt;4977&lt;/sup&gt; (%)</th>
<th>Multiple mtDNA rearrangements (%)</th>
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<tr>
<td>Oocytes</td>
<td>295</td>
<td>50.5</td>
<td>34.2</td>
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<tr>
<td>Embryos</td>
<td>197</td>
<td>32.5</td>
<td>21.3</td>
</tr>
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</table>

Significantly different by χ²-analysis: P = 0.0001; P = 0.0028; P < 0.0001.

dmtDNA<sup>4977</sup> = 4977 basepair mtDNA deletion.

The donor age of embryos (n = 196) with mtDNA rearrangements (33.2 ± 0.5 years) was not significantly different from the donor age of embryos without mtDNA rearrangements (33.2 ± 0.4 years). The presence of mtDNA rearrangements in embryos from donors aged <38 years was 48/155 (31.0%). The presence of mtDNA rearrangements in embryos from donors aged ≥38 years was 16/42 (38.1%). χ²-analysis revealed no age-related difference. The donor age of embryos (n = 196) with dmtDNA<sup>4977</sup> (32.5 ± 0.6 years) was not significantly different from the donor age of embryos without dmtDNA<sup>4977</sup> (33.4 ± 0.4 years).

Mitochondrial rearrangements during oocyte development

When the oocytes were grouped by meiotic stage [ovarian, germinal vesicle (GV), and MI arrested at prophase of meiosis I, compared with MII oocytes arrested at metaphase of meiosis II] there was a significant difference by Fisher exact test (P = 0.048), with 32/53 and 107/230 respectively containing mtDNA rearrangements. The presence of mtDNA rearrangements in the ovarian and GV oocytes grouped together was 65.0% (26/40), and this was significantly different (Fisher exact test; P = 0.022) from the presence of mtDNA rearrangements in the MI and MII oocytes grouped together, 46.5% (113/243). The presence of mtDNA rearrangements in oocytes isolated from ovarian tissues was 85.7% (67/); in GV oocytes collected from IVF was 60.6% (20/33); in MI oocytes collected from IVF was 46.2% (6/13); and in MII oocytes collected from IVF was 46.5% (107/230).

Mitochondrial rearrangements and embryo quality

Statistical analyses found no significant differences based on mtDNA rearrangements for percentage of fragmentation, number of cells, or the presence of multinucleated cells. There was a significant difference based on mtDNA rearrangements between embryos considered sub-optimal without distinct morphological characteristics and embryos considered slow-developing (P < 0.05), with the slow embryos having fewer mtDNA rearrangements (13/53, 24.5%) than the normally dividing embryos (16/31, 51.6%).

Discussion

Approximately half of human oocytes and one-third of embryos contain single mtDNA rearrangements. Multiple mtDNA
rearrangements occurred in fewer samples, but again, at least twice as often in oocytes than in embryos. Over 20 new rearrangements were detected in this study. Although the differences between oocytes and embryos confound the idea of a fertilization ‘bottleneck’, one has to be careful in drawing conclusions, since the material selected here may contain an inherent bias common in human embryo research: apparently normal MII oocytes and normal embryos were not included due to their obvious need in the clinical process. Moreover, a study of bottleneck mechanisms and timing is dependent on the determination of quantifiable mtDNA mutations in single cells, rather than the presence of any mutation. The latter is important since the human oocyte contains an abundance of mitochondrial genomes and the presence of just one mutation may be insignificant. Nevertheless, trends observed in this work confirm the likelihood of a selection mechanism aimed at removing cells containing any mutated mtDNA during or after fertilization.

In a previous study (Brenner et al., 1998), the frequency of dmtDNA4977 deletion using a nested primer strategy was 32.8% in oocytes and 8% in embryos. Using a long PCR–short PCR nested primer strategy, the frequency of dmtDNA4977 was found to be 47% oocytes and 20% in embryos (Brenner et al., 1998). Using the same nested PCR approach, a similar frequency of mtDNA deletions was found in oocytes as reported by Chen (n = 104, 49%) and Keefe (n = 50, 43%).

So far, more than 150 somatic types of mtDNA rearrange-
ments have been described, including deletions, insertions and duplications. The long PCR technique allows the rapid analysis of a large portion of the mitochondrial genome and may provide new information on the mtDNA instability in human gametes. The finding of novel mtDNA rearrangements in human oocytes and embryos presents some interesting biological questions. It is likely that a larger percentage of oocytes may have been found to contain mtDNA rearrangements if the entire mtDNA could have been analysed. However, one previous investigation using a long PCR followed by Southern blot hybridization, which only detects high copy number mtDNA rearrangements, showed that only 40% of oocytes contained mtDNA rearrangements (Reynier et al., 1998). If a limited number of mtDNA contain rearrangements in single oocytes, there would most likely be no adverse effect on the amount of ATP being produced. The presence of any rearranged mtDNA may not be meaningful, especially if one assumes that rearranged mtDNA are not replicated. In fact, this may be a possible mechanism for elimination of the damaged mitochondria from the oocyte. If, however, a larger percentage of mtDNA contain rearrangements there may be adverse effects on ATP production, and a higher chance of rearranged genomes being selected for replication during clonal expansion.

Accumulation of mtDNA rearrangements in human tissues appears to be age-related (Holt et al., 1988; Linnane et al., 1989; Trounce et al., 1989; Cortopassi and Arnheim, 1990; Ikebe et al., 1990; Harding, 1991; Hattori et al., 1991; Wallace, 1992; Kitagawa et al., 1993; Brown and Wallace, 1994; Cummins et al., 1994). The hypothesis that an age-related accumulation of mtDNA rearrangements would be found in human oocytes could not be supported here. This is in contrast to another study (Keefe, 1995) which showed an association between patient age and mtDNA mutation; however, the sample size was small, and only one mutation was analysed. The fact that there is not an age-related decline in respect to mtDNA rearrangements does not necessarily mean that there may not be a decrease in the function of the respiratory chain enzymes over time (Muller-Hocker, 1996). Studies are underway to determine the ATP content of human oocytes in association with their developmental competence.

A number of issues require further investigation. The possibility of localization of mtDNA rearrangements in oocytes and embryos needs to be determined. Furthermore, as mentioned above, these experiments did not quantify the amount of rearranged mtDNA. Therefore, the total amount of all types of rearranged mtDNA in human oocytes and embryos is unknown. Presently there is no known technique that can quantify multiple mtDNA mutations by single cell PCR. It is quite possible that only a small number of the 100 000 mitochondria in human oocytes and embryos are damaged and that these mitochondria are removed by apoptosis or simply cease to multiply.

The bottleneck is postulated to occur in a limited number of mtDNA that are randomly selected for clonal expansion (replication) during oocyte development (Jenuth et al., 1996). The results presented here support the notion that there is a decrease in the presence of mtDNA rearrangements in human oocytes during growth and after germinal vesicle breakdown.
The incidence of mitochondrial myopathy in newborns is a fraction of the incidence of mtDNA rearrangements seen in oocytes and embryos, suggesting that there is either no link between the two phenomena, or rearrangements are actively eliminated during development (Harding, 1993). Elimination may occur through apoptosis or some other mechanism. Elimination of rearranged mtDNA may be the result of a mechanism that requires an energy threshold for continued oocyte development. In such a model, an oocyte containing normal mtDNA will be able to produce enough ATP (>2 pmol; Van Blerkom et al., 1995) to sustain development. If fertilized, the resulting embryo will use the reserve of ATP until later stages when oxidative phosphorylation energy production resumes (Van Blerkom, 1980). The findings suggest that mitochondrial function and the subsequent production of ATP may play an important role in the selection process of maturing oocytes. Further studies of mitochondrial function in human oocytes and embryos need to be undertaken before any conclusions can be drawn about the effects of mtDNA rearrangements.

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References


MtDNA rearrangements in human oocytes and embryos


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