The role of mitochondria and mitochondrial DNA copy number in the human oocyte

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Declaration of competing interest

This work was primarily funded by:

OvaScience Inc., Waltham, MA, USA

National Health and Medical Research Council, Australia

Australian Pork Ltd, Australia

Research grants awarded to Jus St. John, Hudson Institute of Medical Research
Mitochondria exist in very different shapes, numbers and locations in different cell types.
Mitochondrial colonisation in differentiating human ES cells

The mammalian mitochondrion

- Intermembrane space
- Outer membrane
- Inner membrane
- Matrix
- DNA
- Ribosomes

- 100 kcal/hour = 116W
- O₂ consumption 380 litres/day
- 65 kg ATP/day ($3M worth!)

Rich Nature 2003; 421: 583
The mature mammalian mitochondrion

- Form highly structured networks - fuse later during development
- Act as Ca$^{2+}$ stores and regulators
- Initiate steroidogenesis
- Provide balanced free radical activity
- Regulators/mediators of apoptosis and necrosis
- Regulators of the epigenome (Fluxome)
- Vehicles for the transmission of mtDNA
Production of cellular energy

Glycolysis

Glucose → Pyruvate → ATP + NADH

Pyruvate

Acetyl-CoA

β-oxidation

Acyl-CoA

Citric acid cycle (aerobic)

NADH + FADH2

ETC

NADH + FADH2

ATP

Cytoplasm

Mitochondria

Pfeiffer et al. Science 2001; 292:504-7
Electron Transfer Chain

Complex I  II  III  IV  V
nDNA >18  4  8  10  10
mtDNA  7  0  1  3  2
The two genetic compartments in a cell

- Chromosomes: Maternally and paternally inherited
- Mitochondrial DNA: Maternally-only inherited

Nucleus (Chromosomal DNA)
Mitochondrial DNA
The mitochondrial genome

Human = 16,569 bp

Mouse = 16,295 bp

Pig = 16,613 bp
mtDNA haplotypes

- Confer an advantage or disadvantage to the individual

- Adaptation to warm and cold climates
  (Ruiz-Pesini et al. *Science* 2004;303:223-26)

- Growth and physical performance

- Longevity

- Predisposition to and against age-associated disorders
mtDNA haplotypes

- Milk quality in cows
- Sperm motility in men
  (Ruiz-Pesini et al. *Science* 2004;303:223-6)
- Fertility in cows
  (Sutarno et al. *Theriogenology* 2002;57:1603-10)
- Fertility in pigs
- Strongly influence cellular differentiation
  (Kelly et al. *Stem Cells* 2013: 31; 703-716)
- Outcomes related to cloning
  (Bowles et al. *Stem Cells* 2008; 26:775–782)
Mutation and deletion of the mitochondrial genome

- Series of pathogenic maternally inherited mtDNA mutations and deletions that lead to a number mtDNA diseases:
  - LHON; NARP; MERRF; Leigh Syndrome
  - 70 to 80% mutant load
  - 1:5000 to 1:10000
  - 1:200 women are carriers

- Poorly packaged
  - Susceptible to mutation and deletion
  - 1-2% heteroplasmy in all individual (Ye et al. *PNAS* 2014; 111: 10654–9)
  - Increase with age (Payne & Chinnery, *BBA* 2015; 1847 1347–53)

- Transgenerational transmission that is tightly regulated
  (Cagnone et al. *Genetics* 2016; 202: 931–944)
Distribution of mtDNA variant load in oocytes and early preimplantation embryos

Cagnone et al. Genetics 2016;202:931-944
Correlation of variant load with mtDNA copy number in offspring tissues

Cagnone et al. *Genetics* 2016;202:931-944
mtDNA copy number is strictly regulated during development
Mitochondrial transcription and replication

Factors associated with:
Mitochondrial biogenesis
Proliferation
Acetylation / deacetylation

Mitochondrial specific DNA polymerase $\gamma$ (*PolgA*)

- Sensor between nucleus and mitochondrial genome
- Intragenic CpG island in exon 2 of *PolgA*.
PolgA is DNA methylated in a tissue specific manner

Kelly et al. Nucleic Acids Res 2012:40; 10124–10138
Tissue specific mtDNA copy number

Kelly et al. *Nucleic Acids Res* 2012; **40**; 10124–10138
mtDNA copy number in human oocytes

Mean mtDNA copy number

** = p < 0.002
(t-test)

Santos et al. Fert Steril 2006; 85: 584-591
mtDNA copy number in unfertilised human oocytes

Santos et al. Fertil Steril 2006; 85: 584-91
Cytoplasmic transfer results in heteroplasmic offspring

Proposed to enhance embryonic developmental outcome

Resulted in heteroplasmic offspring **3-parents**

Developmental disorders reported in human and mouse

Cohen et al. *Lancet* 1997; **350**: 186-7
*Brenner et al. Fertil Steril* 2000; **74**: 573-8
Barritt et al. *Reprod Biomed Online* 2001; **3**: 47-48
Acton et al. *Biol Reprod* 2007; **77**: 569-76
BCB identifies fertilisable and non-fertilisable oocytes

El Shourbagy et al. *Reproduction* 2006; **131**: 233-45
mtDNA copy in maturing and MII oocytes

Differential mitochondrial clustering between BCB$^+$ and BCB$^-$ oocytes

Depletion of mtDNA during IVM

Spikings et al. Biol Reprod 2007; 76:327-335

Red: MitoTracker Red; Blue: DAPI

Depleted oocytes do not fertilise or embryos arrest
Autologous mitochondrial supplementation

Mitochondria isolated from BCB⁺ sister oocytes

El Shourbagy et al. *Reproduction* 2006; **131**: 233-45
Higher mtDNA copy numbers in developmentally competent oocytes

- Comparison of MII oocytes (T-Test)

Minimum threshold of mtDNA copy number required for fertilisation

- Primordial germ cells
- Primordial follicle
- Fertilisation
- Preimplantation Development
- Blastocyst
- Trophectoderm
- Postimplantation Development
- Birth

mtDNA copy number/cell:
- >200,000
- 150,000
- 200

Mitochondrial supplementation

BCB⁺

BCB⁻

Threshold

mtDNA Set Point

Primordial Germ Cells

BCB⁺:
- Blood
- Heart
- Muscle

BCB⁻:
- Neurons
- Blood
- Primordial Germ Cells

Mitochondrial supplementation

Blood

Heart

Muscle

Neurons

Primordial Germ Cells
Supplementation with genetically identical mitochondria (mICSI) to overcome threshold

~ 800 copies of mtDNA

Mitochondria isolated from BCB⁺ oocytes

BCB⁻ oocyte
Supplementation

MitoTracker Green | TMRM | MitoTracker Deep Red | Merge | Brightfield | Zoom
---|---|---|---|---|---
1 hrs

## Improvement in fertilisation and development of BCB⁻ embryos following mICSI

<table>
<thead>
<tr>
<th>Insemination</th>
<th>Total oocyte*/MII number</th>
<th>% Fertilisation (total)</th>
<th>% Blastocyst/Fert (total)</th>
<th>% Blastocyst/Fert (±S.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BCB⁺</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVF</td>
<td>764⁺</td>
<td>58.4</td>
<td>23.7</td>
<td>20.6 ± 13.9</td>
</tr>
<tr>
<td>ICSI</td>
<td>255</td>
<td>77.7</td>
<td>34.9</td>
<td>*33 ± 15.3</td>
</tr>
<tr>
<td>mICSI</td>
<td>98</td>
<td>62.2</td>
<td>31.6</td>
<td>*31.5 ± 13.8</td>
</tr>
<tr>
<td><strong>BCB⁻</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVF</td>
<td>507⁺</td>
<td>38.1</td>
<td>10.6</td>
<td>7.6 ± 5.8</td>
</tr>
<tr>
<td>ICSI</td>
<td>136</td>
<td>59.9</td>
<td>22</td>
<td>23.9 ± 11.0(^ab)</td>
</tr>
<tr>
<td>mICSI</td>
<td>139</td>
<td>40.4</td>
<td>27.8</td>
<td>31.5 ± 15.6(^b)</td>
</tr>
</tbody>
</table>

Early rescue supports blastocyst development in BCB⁻ embryos

Improvement in development of BCB⁻ embryos following mICSI as evidenced by increased cell number

Genes differentially expressed between mICSI BCB- and ICSI BCB- blastocysts

- Most affected networks:
  - Cellular movement
  - Cellular development
  - Cellular movement

- Canonical pathways
  - PPAR signaling and CREB1

- Upstream regulators
  - Cell proliferation

Conclusion

Blastocyst → Baby

mtDNA copy number

Resetting of embryonic genome

Blastocyst → Baby
Acknowledgements

Centre for Genetic Diseases

Gael Cagnone
Te-Sha Tsai
Yogeshwar Makanji
Pam Matthews
Mat McKenzie
Shahy El Shourbagy
Emma Spikings

Harvard, USA

David Sinclair
Michael Bonkowski

UNSW
Ashley Wong
Lindsay Wu

Monash University
Kirstin Elgass

MHTP
Jodee Gould