The strict regulation of the mitochondrial genome during development

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Declaration of competing interest

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Cellular Energy

- Fast replicating cells
- Embryonic cells
- Embryonic stem cells
- Tumour-initiating cells
- Blood cells

Glycolysis

- Glucose
- Pyruvate
- 4 ATP + NADH

β-oxidation

- Acyl-CoA
- NADH
- Acetyl-CoA
- NADH + FADH₂

Citric acid cycle

- ETC
- OXPHOS
- 32 ATP

Mitochondria

Cytoplasm

Heart

Muscle

Brain

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

<table>
<thead>
<tr>
<th>Complex</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>nDNA</td>
<td>&gt;18</td>
<td>4</td>
<td>8</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>mtDNA</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>2</td>
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</table>
The mitochondrial genome

Human mitochondrial genome
16,569bp

- Complex I (NADH dehydrogenase)
- Complex III (Cytochrome c reductase)
- Complex IV (Cytochrome c oxidase)
- Complex V (ATP synthase)
- Ribosomal RNAs (rRNAs)
- Transfer RNAs (tRNAs)
The two genetic compartments in a cell

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

- Nucleus (Chromosomal DNA)
- Mitochondrial DNA
Nucleo-mitochondrial interactions: not just a one way street

Require a degree of compatibility

- mtDNA haplotypes
  - Pigs
    - Tsai et al. *BMC Genetics* 2016; 17:67
  - Cattle
    - Srirattana et al. *BMC Genetics* 2017; 18:59

- mtDNA rearrangements

- mtDNA copy number
mtDNA copy number is strictly regulated during development.
Mitochondrial transcription and replication

Factors associated with:
- Mitochondrial biogenesis
- Proliferation
- Acetylation / deacetylation

Sun & St. John, Biochem J 2016; 473:2955-71
Mitochondrial specific DNA polymerase $\gamma$ (*PolgA*)

$N = \text{NotI}; \ Hp = \text{HpaII}; \ M = \text{McrBC}$

- 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; 10124–38
Tissue specific mtDNA copy number

PolgA expression is regulated by DNA methylation

Kelly et al. *Nucleic Acids Res* 2012: 40; 10124-38
mtDNA copy number in human oocytes (mtDNA deficiency)

** = p < 0.002 (t-test)

* = p < 0.02 (one-way analysis of variance)

Santos et al. Fert Steril 2006; 85: 584-91
How can mtDNA deficiency in oocytes be overcome?
Certain assisted reproductive technologies result in abnormal inheritance of mtDNA

One population of mtDNA

Maternal mtDNA only
In vitro fertilisation (IVF)
Intracytoplasmic sperm injection (ICSI)

Two populations of mtDNA

2 genetically diverse mtDNA populations (haplotypes)
Cytoplasmic transfer
Cloning (SCNT)
<table>
<thead>
<tr>
<th>Nucleotide position</th>
<th>Variant</th>
<th>Amino acid code</th>
<th>Amino acid</th>
</tr>
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<tr>
<td>16034</td>
<td>A→G</td>
<td>GGA→GGG</td>
<td>Gly→Gly</td>
</tr>
<tr>
<td>16181</td>
<td>T→C</td>
<td>ATT→ATC</td>
<td>Ile→Ile</td>
</tr>
<tr>
<td>16215</td>
<td>T→C</td>
<td>TTG→CTA</td>
<td>Leu→Leu</td>
</tr>
<tr>
<td>16217</td>
<td>G→A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16220</td>
<td>C→T</td>
<td>GCC→GCT</td>
<td>Ala→Ala</td>
</tr>
<tr>
<td>16224</td>
<td>G→A</td>
<td>GTA→ATA</td>
<td>Val→Met</td>
</tr>
<tr>
<td>16281</td>
<td>A→G</td>
<td>AGC→G GC</td>
<td>Ser→Gly</td>
</tr>
<tr>
<td>16293</td>
<td>C→G</td>
<td>CGA→GGA</td>
<td>Arg→Gly</td>
</tr>
</tbody>
</table>

The effects of third party mtDNA on gene expression profiles – a bovine model

- REMOVAL of Maternal Chromosomes from egg
- INTRODUCTION of somatic cell chromosomes into egg
- FORMATION OF BLASTOCYST

Lloyd et al. *Genetics* 2006; 72: 2515–27
Bowles et al. *Genetics* 2007; 176: 1511–26
Srirattana & St. John, *G3* 2017; 7:2065-80
Differentially expressed genes in bovine SCNT blastocysts generated from mtDNA\(^0\) and mtDNA\(^+\) cells

- **Most affected networks**
  - DNA Replication and Repair, Nucleic Acid Metabolism
  - Cell Cycle, Cell Death and Survival

- **Most affected canonical pathways**
  - PPAR Signaling
  - CDK5 Signaling

35 DEGs

Srirattana & St. John, *Genes, Genomes, Genetics* 2017; 7:2065-80
BCB selects fertilisable and non-fertilisable oocytes

mtDNA copy in maturing oocytes

Higher mtDNA copy numbers in developmentally competent oocytes

Comparison of MII oocytes (t-test)

Cagnone, Tsai et al. Sci Rep 2016; 6:23229
Differential mitochondrial clustering between BCB$^+$ and BCB$^-$ oocytes
Mitochondrial clustering in BCB⁺ and BCB⁻ oocytes

Cagnone, Tsai et al. Sci Rep 2016; 6:23229
Depletion of mtDNA during IVM

Red = MitoTracker Red; Blue = DAPI; ddC = 2',3'-dideoxycytidine

Minimum threshold of mtDNA copy number required for fertilisation

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

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

Mitochondrial supplementation

BCB^+ and BCB^-

Blood, Heart, Muscle, Neurons, Primordial Germ Cells, BCB^+ cells, BCB^- cells
Supplementation with genetically identical mitochondria (mICSI) to overcome threshold

~ 800 copies of mtDNA

Mitochondria isolated from sister oocytes
## Supplementation

<table>
<thead>
<tr>
<th>MitoTracker Green</th>
<th>TMRM</th>
<th>MitoTracker Deep Red</th>
<th>Merge</th>
<th>Brightfield</th>
<th>Zoom</th>
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<tbody>
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<td>1 hrs</td>
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<td>24 hrs</td>
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Improvement in fertilisation and development of BCB⁻ embryos following mICSI

<table>
<thead>
<tr>
<th></th>
<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>BCB⁺</td>
<td>IVF</td>
<td>764*</td>
<td>58.4</td>
<td>23.7</td>
<td>20.6 ± 13.9</td>
</tr>
<tr>
<td></td>
<td>ICSI</td>
<td>255</td>
<td>77.7</td>
<td>34.9</td>
<td>33 ± 15.3</td>
</tr>
<tr>
<td></td>
<td>mICSI</td>
<td>98</td>
<td>62.2</td>
<td>31.6</td>
<td>31.5 ± 13.8</td>
</tr>
<tr>
<td>BCB⁻</td>
<td>IVF</td>
<td>507*</td>
<td>38.1</td>
<td>10.6</td>
<td><strong>7.6 ± 5.8</strong></td>
</tr>
<tr>
<td></td>
<td>ICSI</td>
<td>136</td>
<td>59.9</td>
<td>22</td>
<td>23.9 ± 11.0² ³</td>
</tr>
<tr>
<td></td>
<td>mICSI</td>
<td>139</td>
<td>40.4</td>
<td>27.8</td>
<td><strong>31.5 ± 15.6</strong></td>
</tr>
</tbody>
</table>

Cagnone, Tsai et al. Sci Rep 2016; 6:23229
Improvement in development of BCB⁻ embryos following mICSI as evidenced by increased cell number

Cagnone, Tsai et al. Sci Rep 2016; 2016; 6:23229
Early rescue supports blastocyst development in BCB− embryos

Cagnone, Tsai et al. Sci Rep 2016; 2016; 6:23229
Global genome microarray: mICSI BCB⁻ blastocysts cluster more closely with ICSI BCB⁺ blastocysts than with ICSI BCB⁻ blastocysts

Cagnone, Tsai et al. Sci Rep 2016; 6:23229
Genes differentially expressed between ICSI BCB⁻ and ICSI BCB⁺ blastocysts

- Most affected networks
  - Cellular assembly and organisation
  - Cellular morphology
  - Amino acid metabolism

- Canonical pathways
  - PPAR signaling

- Upstream regulators
  - CREB1, ERB2 and BMP2
  - Troglitazone pathway

378 DEGs

Cagnone, Tsai et al. Sci Rep 2016; 6:23229
Genes differentially expressed between mICSI BCB and ICSI BCB blastocysts

- Most affected networks
  - Cellular movement
  - Cellular development
  - Cellular morphology

- Canonical pathways
  - PPAR signaling and CREB1

- Upstream regulators
  - NFKB, ILS and HRAS
  - Resveratrol pathway

192 DEGs

Summary

Blastocyst Offspring

mtDNA copy number
Resetting of embryonic genome

BCB⁻

Blastocyst Offspring

Resetting of embryonic genome

BCB⁺
mtDNA haplotypes

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
- Organism longevity
- Predisposition to various age-associated disorders
mtDNA haplotypes

- Milk quality in cows

- Sperm motility in men (Haplotype T)
  (Ruiz-Pesini et al. *Science* 2004; 303:223-6)

- Fertility in cows
  (Sutarno et al. *Theriogenology* 2002; 57:1603-10)

- Fertility in pigs

- Outcomes related to cloning
  (Bowles et al. *Stem Cells* 2008; 26:775–782)
Five haplotypes identified in Australian domestic pigs

216 sows from commercial breeders (multiple breeds)
152 randomly selected abattoir gilts or sows

Tsai et al. BMC Genetics 2016; 17(1):67
Developmental efficiencies amongst porcine mtDNA haplotypes

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>BCB⁺:BCB⁻ / Offspring</th>
<th>Maturation rate / Offspring</th>
<th>Fertilisation rate / Offspring</th>
<th>Blastocyst rate / Offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.2728</td>
<td>0.0725</td>
<td>0.0753</td>
<td>0.0187</td>
</tr>
<tr>
<td>B</td>
<td>0.2779</td>
<td>0.0681</td>
<td>0.0745</td>
<td>0.0162</td>
</tr>
<tr>
<td>C</td>
<td>0.3304</td>
<td><strong>0.0536</strong></td>
<td><strong>0.0575</strong></td>
<td>0.0136</td>
</tr>
<tr>
<td>D</td>
<td><strong>0.1906</strong></td>
<td>0.0605</td>
<td>0.0677</td>
<td><strong>0.0116</strong></td>
</tr>
<tr>
<td>E</td>
<td>0.2037</td>
<td>0.0614</td>
<td>0.0684</td>
<td>0.0157</td>
</tr>
</tbody>
</table>

The relationship between mtDNA haplotype and litter size

* P < 0.05; ** P < 0.01

n = 94 sows with 3 to 10 parities

Tsai et al. BMC Genetics. 2016; 17(1):67
Inner cell mass to adult cell

**Pluripotency**

- OCT4, SOX2,
- NANOG, ESSRB,
- REX1, KLF4,
- DPPA5, PRAMEL7,
- NDP521L

- PGCs
- Oocytes
- Sperm

- Endoderm
- Mesoderm
- Ectoderm

- Alveolar
- Cardiac Muscle
- Nerve
Model of mtDNA divergence

CC9\textsuperscript{mus} ESC → mtDNA depleted donor cell + Enucleated Recipient cell → mtDNA divergent ESC line

Lee et al. Cell Death Discovery 2017; 3:17062
mtDNA haplotype, pluripotency and DNA methylation

Kelly et al. Stem Cells 2013; 31:703-716
Differentiation potential (Day 21)

Kelly et al. Stem Cells 2013; 31:703-716
Lee et al. Cell Death Discovery 2017; 3:17062
mtDNA haplotypes

Hypermethylation

Hypomethylation

Lee et al. *Cell Death Discovery* 2017; 3:17062
Acknowledgements

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