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

Jus St. John

Centre for Genetic Diseases

HUDSON
INSTITUTE OF MEDICAL RESEARCH

Declaration of competing interest

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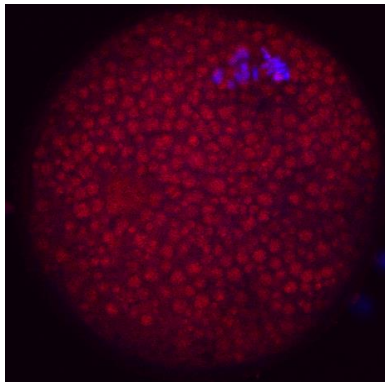
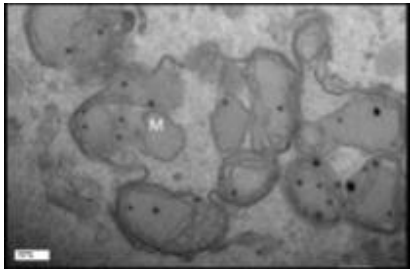
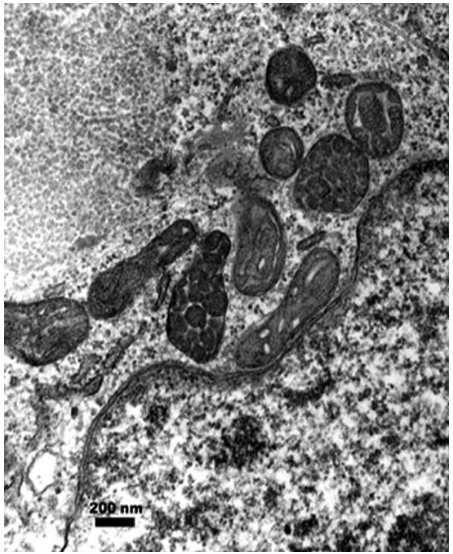
Mitochondria exist in very different shapes, numbers and locations in different cell types

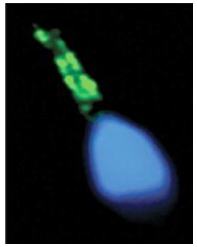


Oocyte

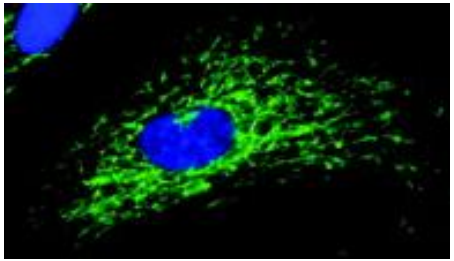




hES cells

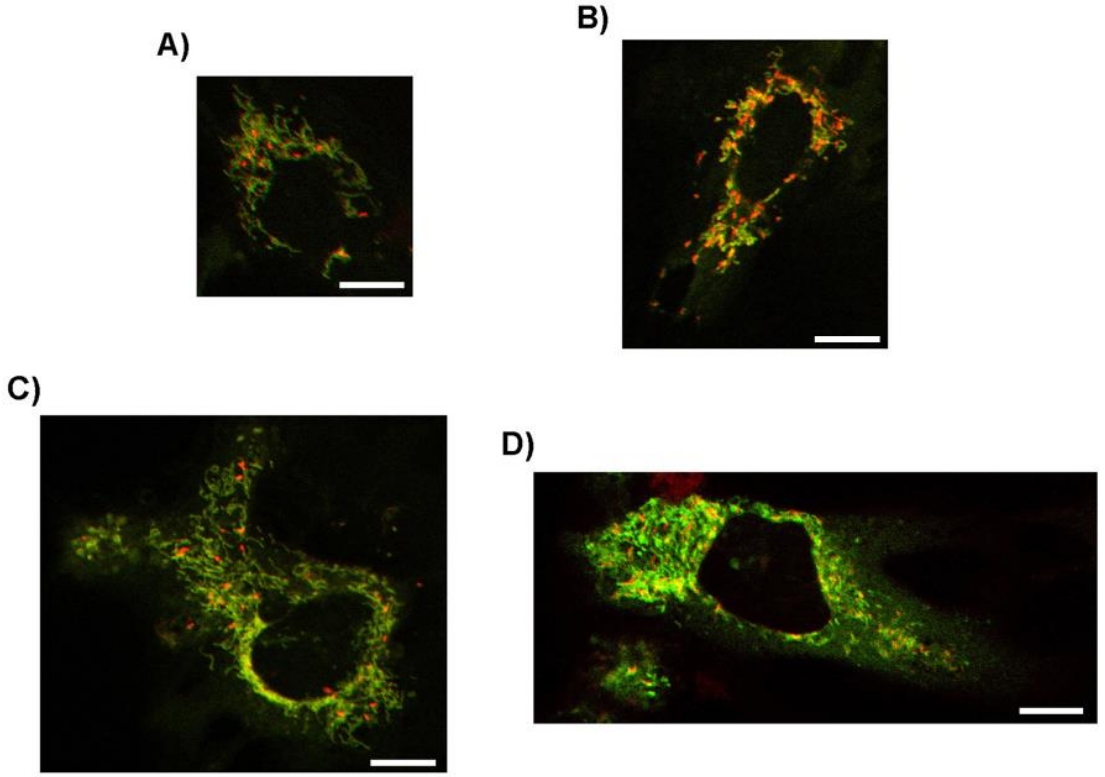


Sperm



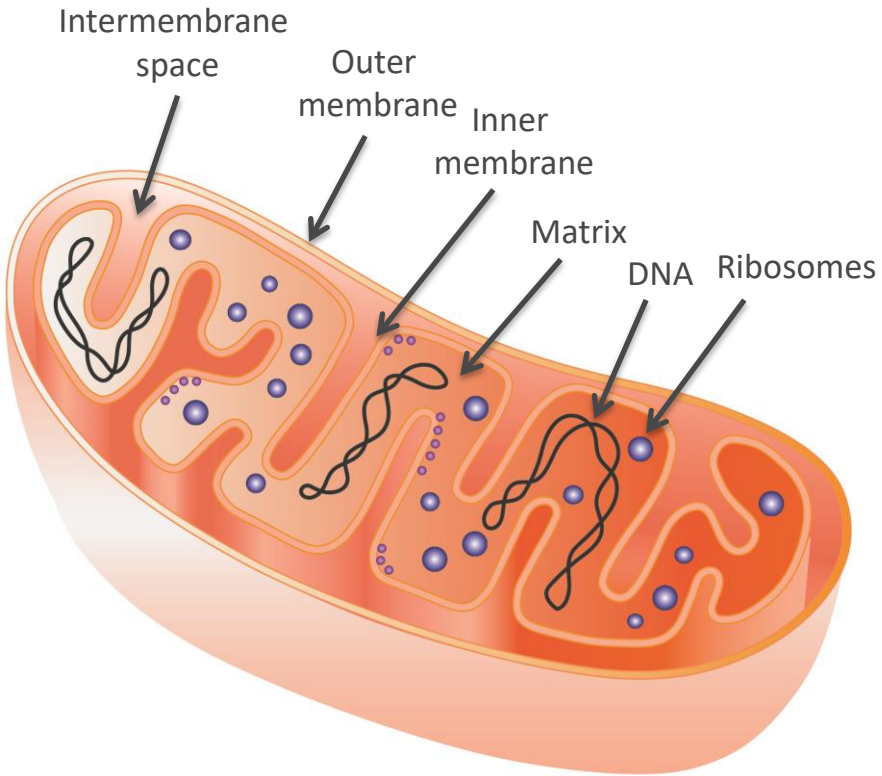
Skin cell

Mitochondrial colonisation in differentiating human ES cells



St. John et al. *Clon Stem Cell* 2005; **7:** 141-153

The mammalian mitochondrion



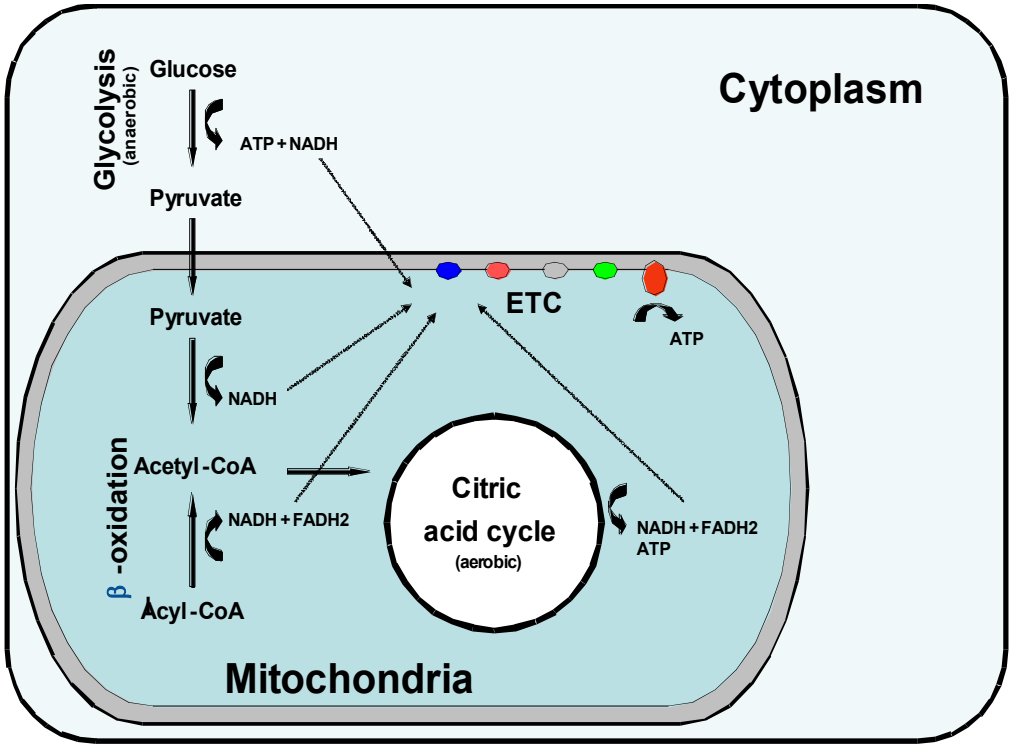
- 100 kcal/hour = 116W
- O_2 consumption 380 litres/day
- 65 kg ATP/day ($3M worth!)

Rich *Nature* 2003; 421: 583

The mature mammalian mitochondrion

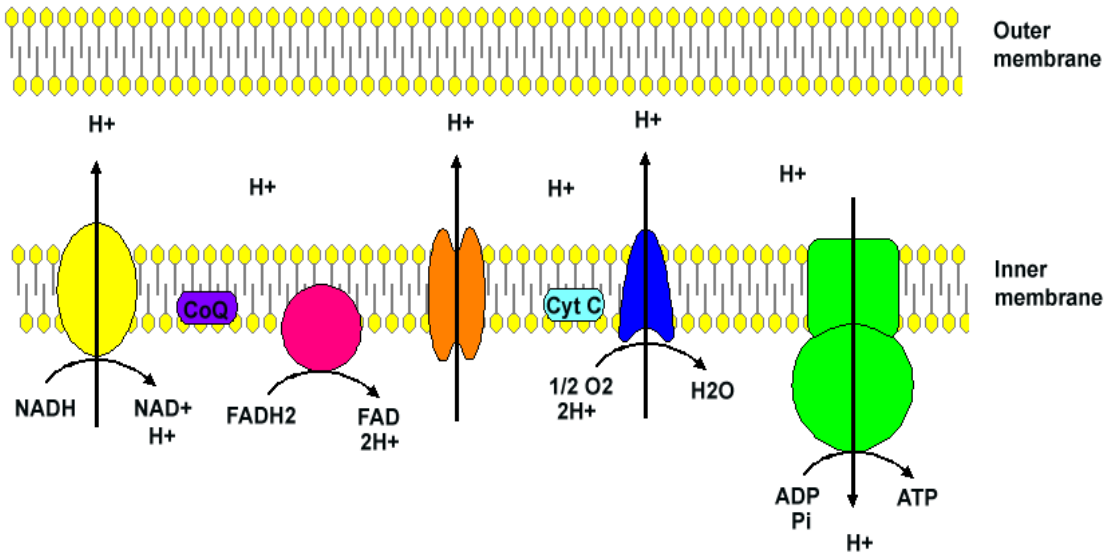
- Form highly structured networks - fuse later during development
- Act as Ca^{2+} stores and regulators
- Initiate steroidogenesis
- Provide balanced free radical activity
- Regulators/mediators of apoptosis and necrosis
- Regulators of the epigenome (Fluxome)
- Vehicles for the transmission of mtDNA

Production of cellular energy



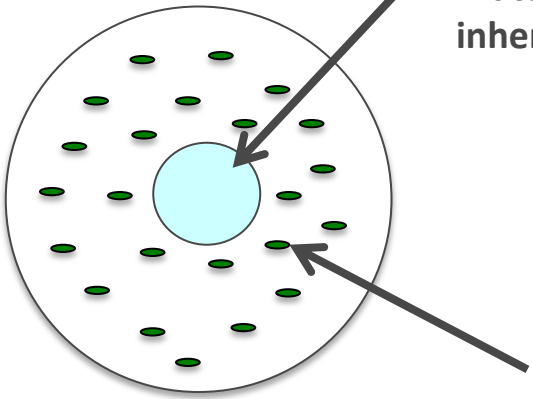
Pfeiffer et al. *Science* 2001; 292:504-7

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



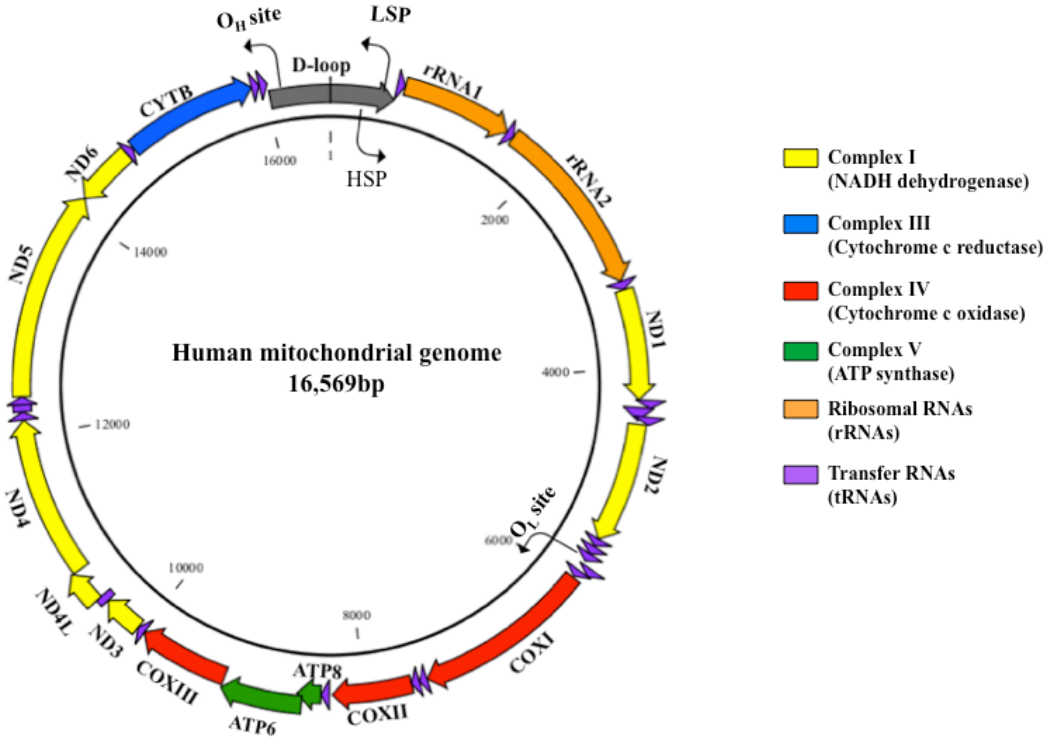
Chromosomes
Maternally and paternally inherited

Mitochondrial DNA
Maternally-only inherited

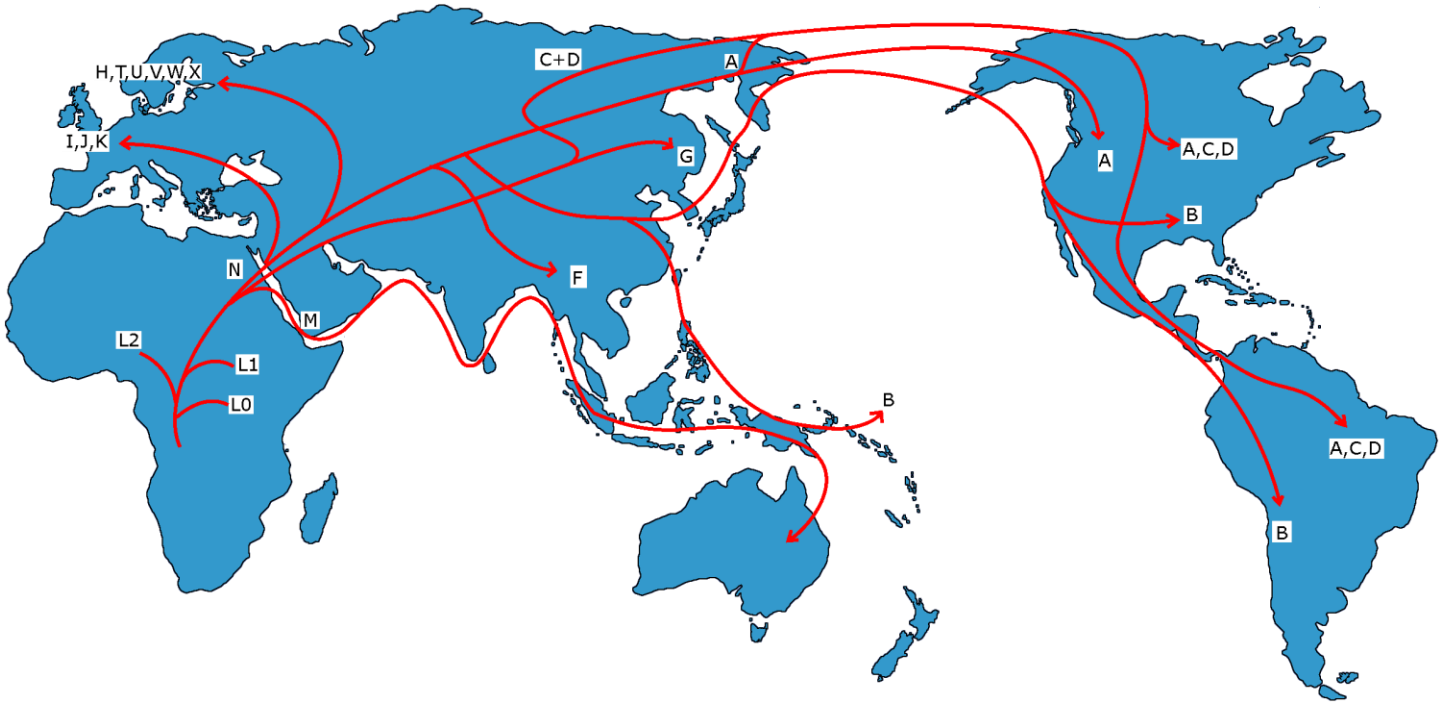
Nucleus (Chromosomal DNA)

Mitochondrial DNA

The mitochondrial genome



mtDNA haplotypes



St. John & Tsai, 2016: http://www.ivf-worldwide.com/vaoeh/chapters/the-role-of-mitochondria-and-mitochondrial-dna-in-fertilisation-and-development-outcome.html

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

  (Nagao et al. *Genes Genet Syst* 1998;73:21-27)

- Longevity

  (Tanaka et al. *Lancet* 1998;351:185-6)

- Predisposition to and against age-associated disorders
  - Cancer (Kaipparettu et al. *Ann N Y Acad Sci* 2010;1201:137-46)
  - Diabetes (Hwang et al. *PLoS One* 2011;6:e22116)

mtDNA haplotypes

- Milk quality in cows

  (Brown et al. *J Anim Sci* 1989;67:1926-32)

- 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

  (El Shourbagy et al. *Reproduction* 2006;131:233-45)

- Strongly influence cellular differentiation

  (Kelly et al. Stem Cells 2013: 31; 703-716)

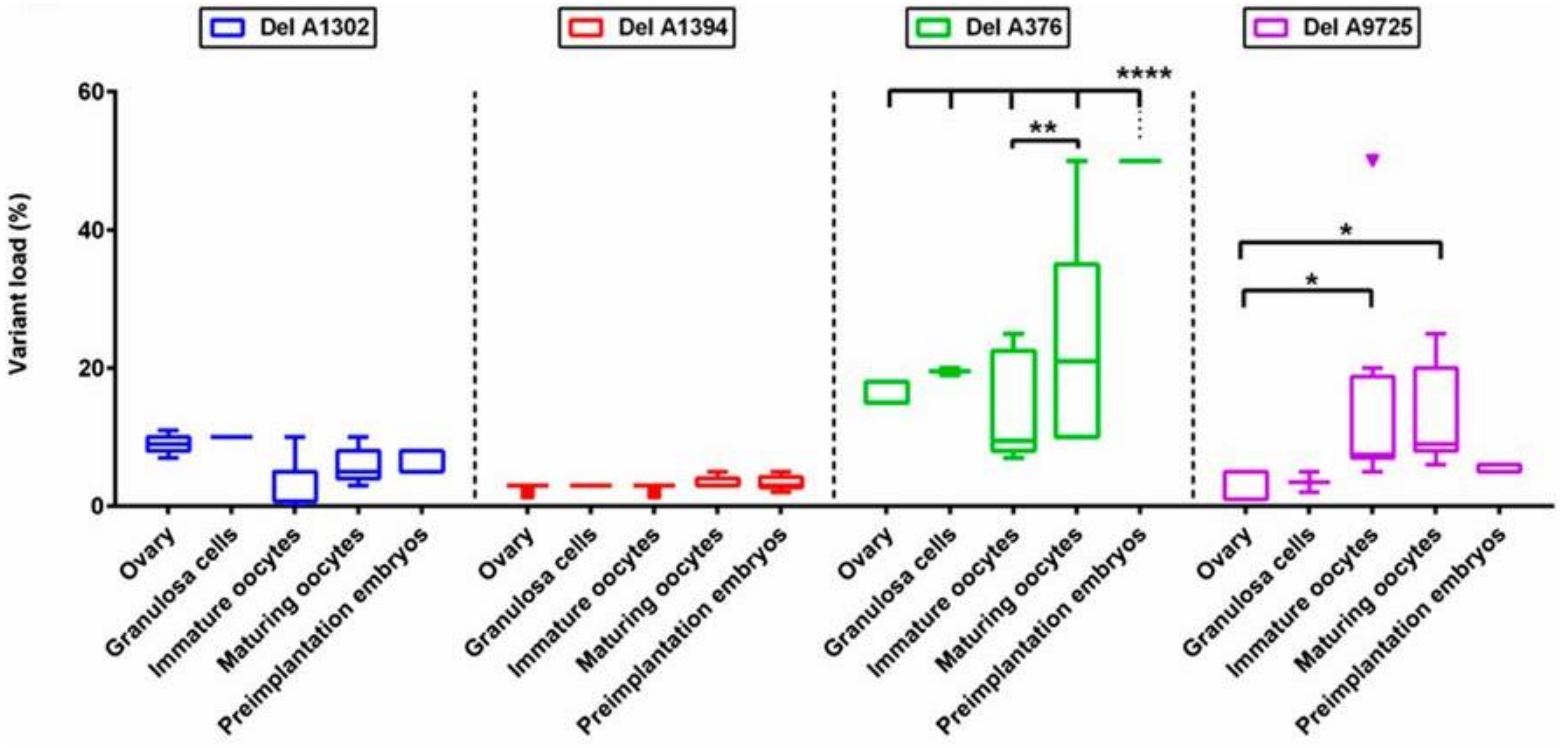
- Outcomes related to cloning

  (Bowles et al. *Stem Cells* 2008; 26:775–782)

Mutation and deletion of the mitochondrial genome

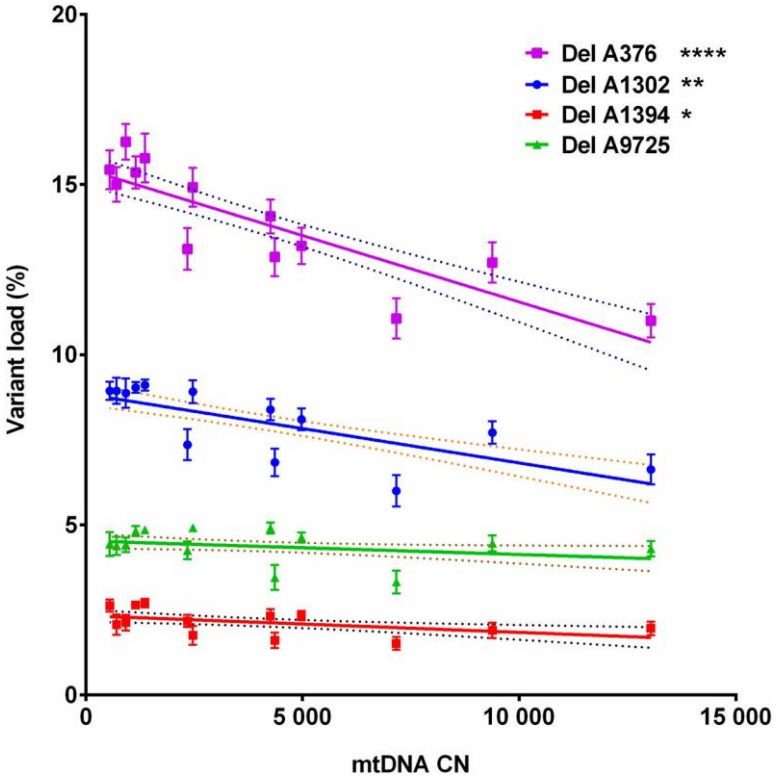
- Series of pathogenic maternally inherited mtDNA mutations and deletions that lead to a number mtDNA diseases:
  - LHON; NARP; MERRF; Leigh Syndrome
  - 70 to 80% mutant load
  - 1:5000 to 1:10000
  - 1:200 women are carriers
- Poorly packaged
  - Susceptible to mutation and deletion
  - 1-2% heteroplasmy 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



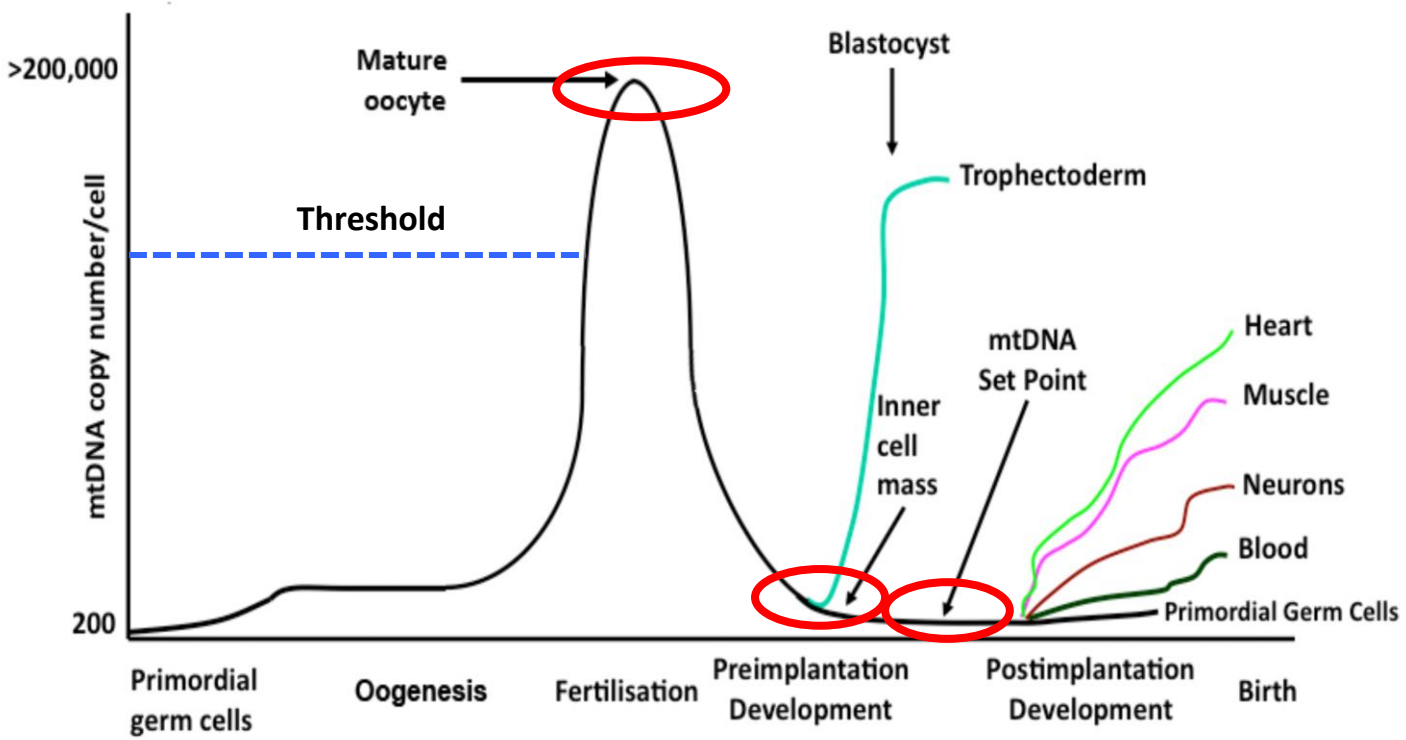
Cagnone et al. *Genetics* 2016;202:931-944

Correlation of variant load with mtDNA copy number in offspring tissues

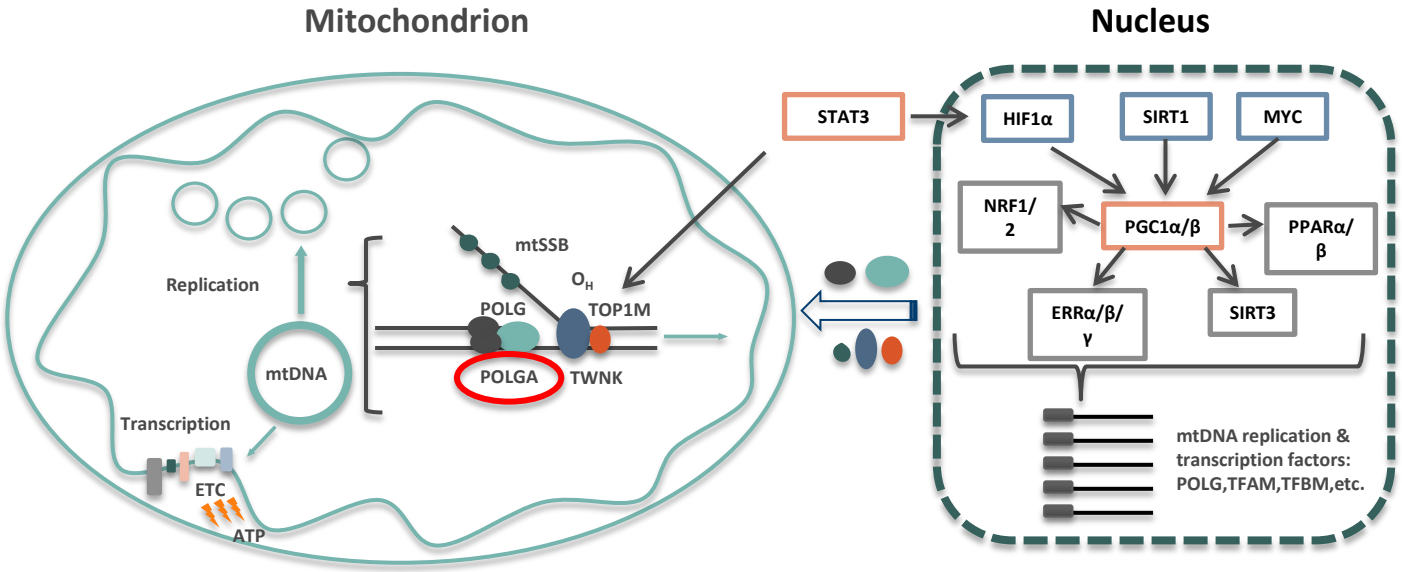


Cagnone et al. *Genetics* 2016;202:931-944

mtDNA copy number is strictly regulated during development



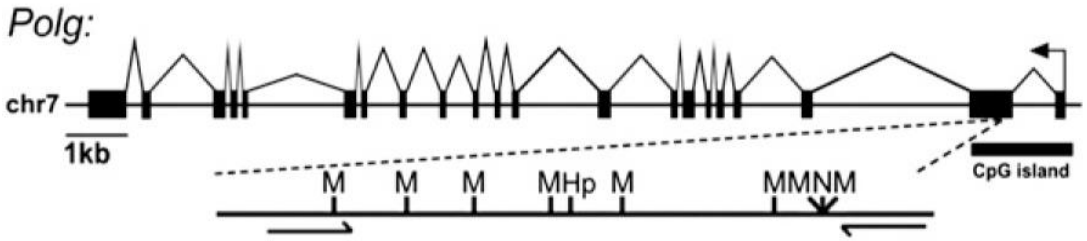
Mitochondrial transcription and replication



Factors associated with:
Mitochondrial biogenesis
Proliferation
Acetylation / deacetylation

Sun & St. John, *Biochem J* 2016; 473:2955-71

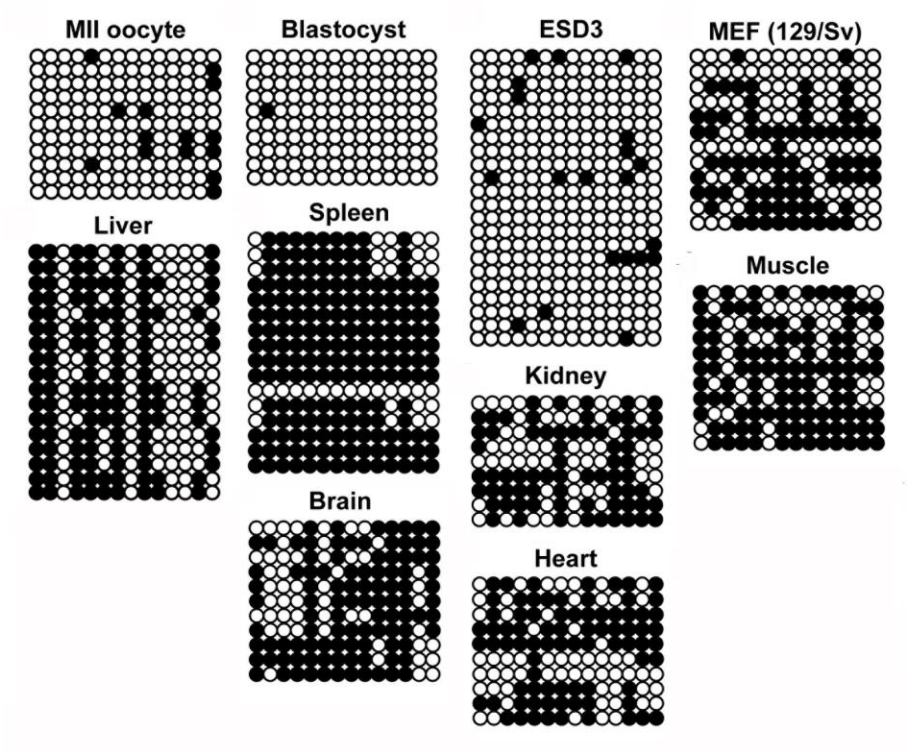
Mitochondrial specific DNA polymerase γ (*PolgA*)



N = NotI; Hp = HpaII; M = McrBC

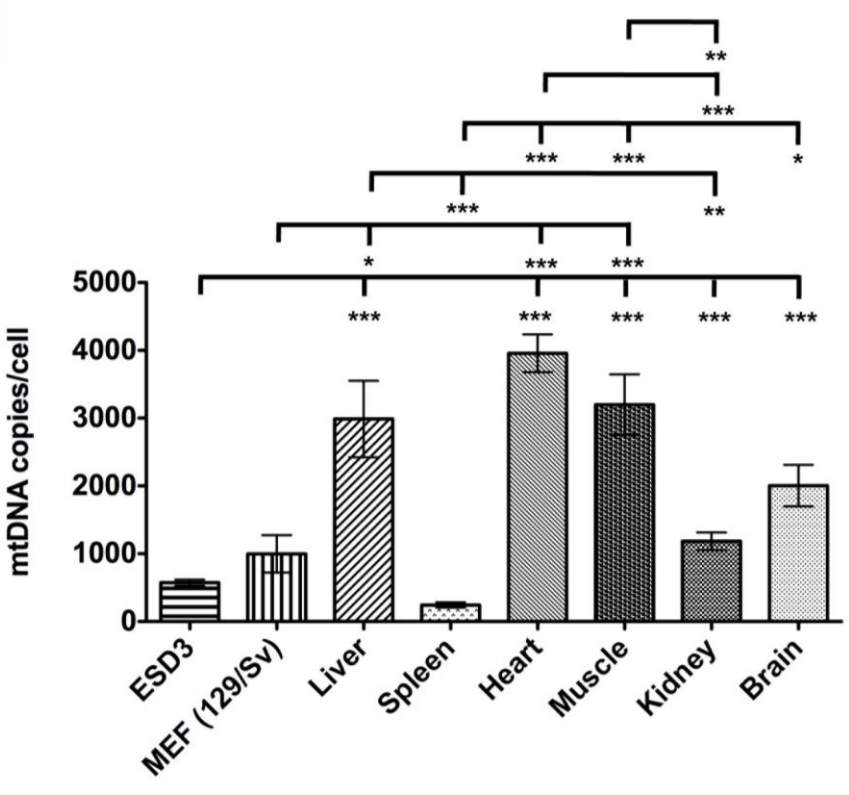
- Sensor between nucleus and mitochondrial genome
- Intragenic CpG island in exon 2 of *PolgA.*

PolgA is DNA methylated in a tissue specific manner



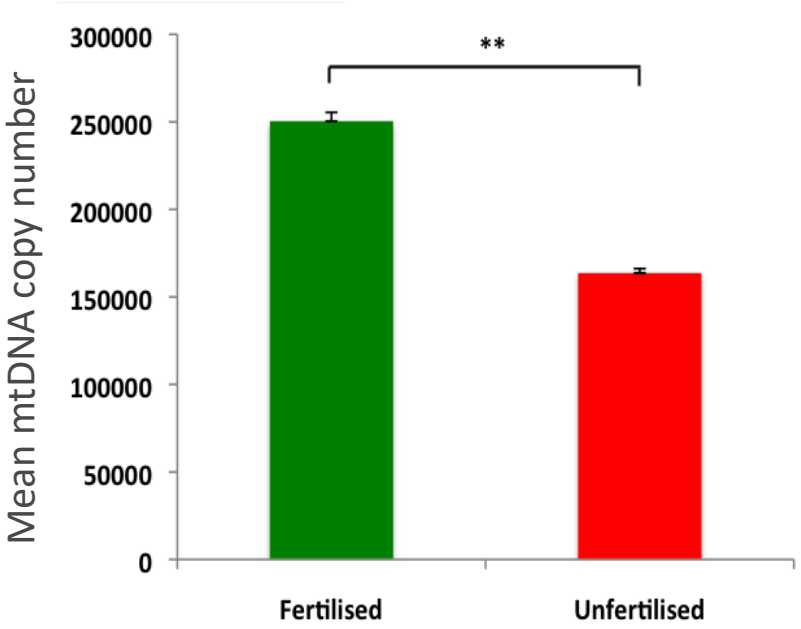
Kelly et al. *Nucleic Acids Res* 2012:**40**; 10124–10138

Tissue specific mtDNA copy number

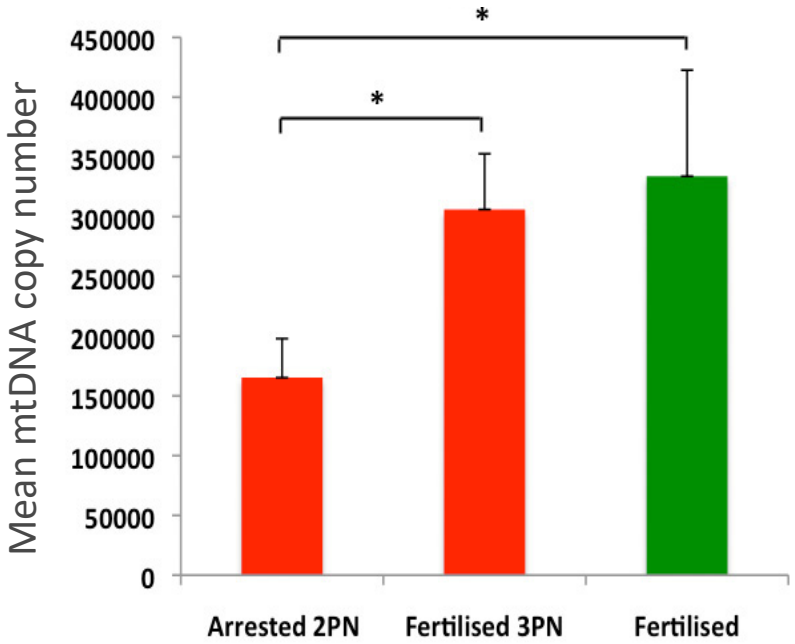


Kelly et al. *Nucleic Acids Res* 2012; **40:**; 10124–10138

mtDNA copy number in human oocytes



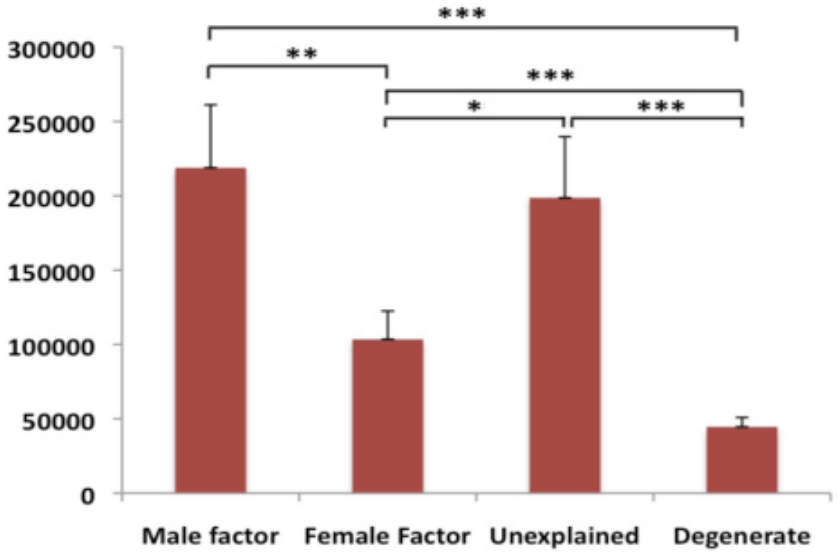
** = p < 0.002
(t-test)



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

Santos et al. *Fert Steril* 2006; **85:** 584-591

mtDNA copy number in unfertilised human oocytes



*** = P < 0.05**
**** = P < 0.02**
***** = P < 0.001**

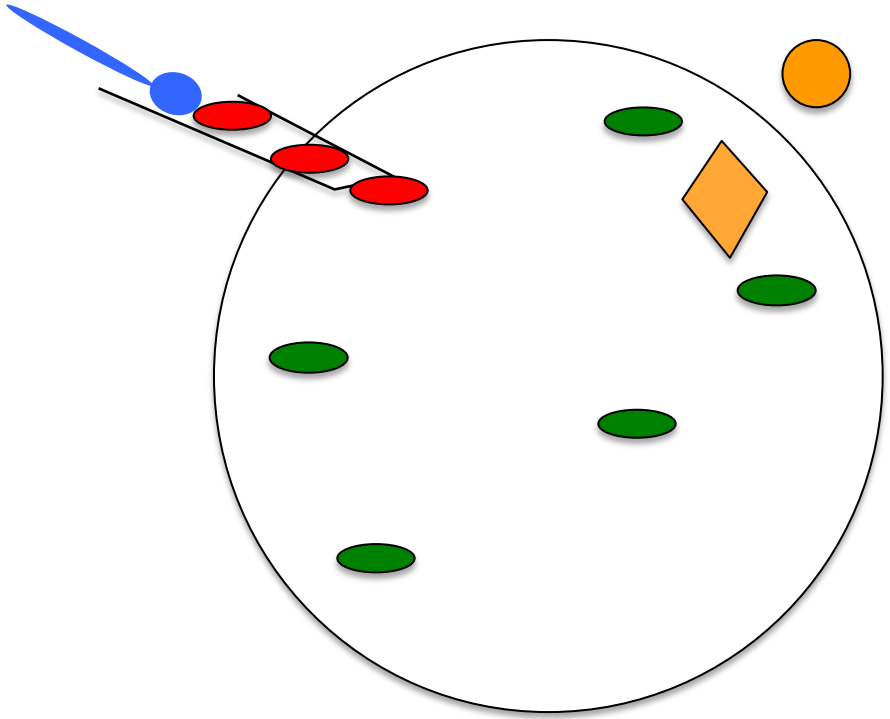
Santos et al. *Fertil Steril* 2006; **85:** 584-91

Cytoplasmic transfer results in heteroplasmic offspring

Proposed to enhance embryonic developmental outcome

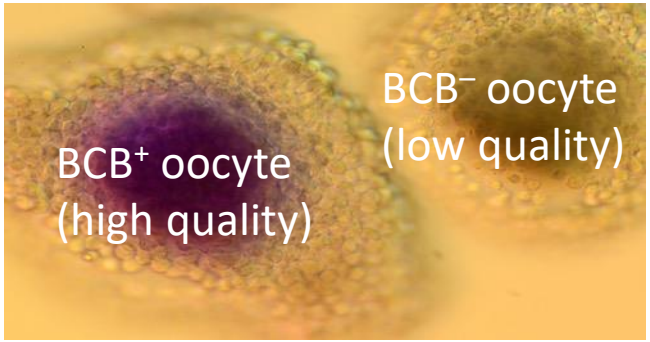
Resulted in heteroplasmic offspring **3-parents**

Developmental disorders reported in human and mouse



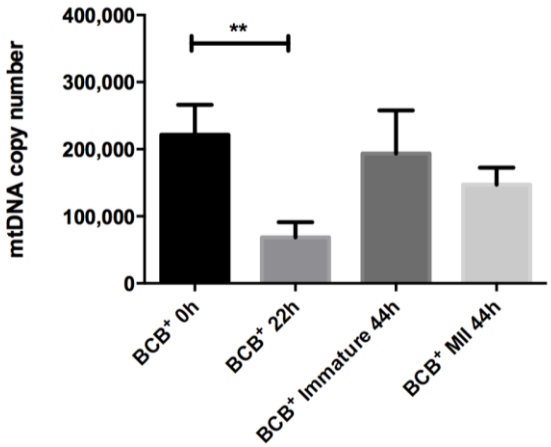
Cohen et al. *Lancet* 1997; **350:** 186-7
Brenner et al. Fertil Steril 2000; **74:** 573-8
Barritt et al. *Reprod Biomed Online* 2001; **3:** 47-48
Acton et al. *Biol Reprod* 2007; **77:** 569-76

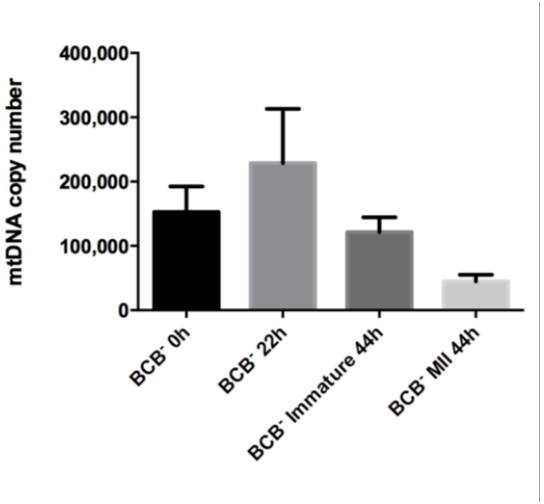
BCB identifies fertilisable and non-fertilisable oocytes

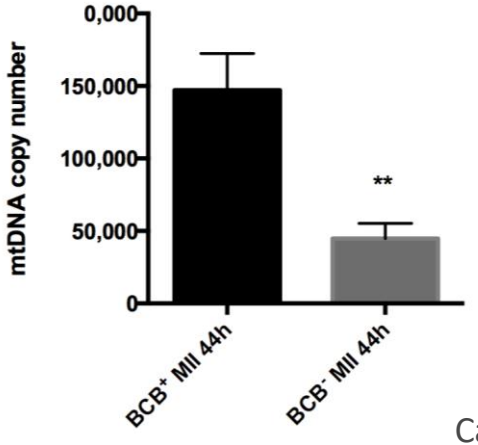


El Shourbagy et al. *Reproduction* 2006; **131:** 233-45

mtDNA copy in maturing and MII oocytes

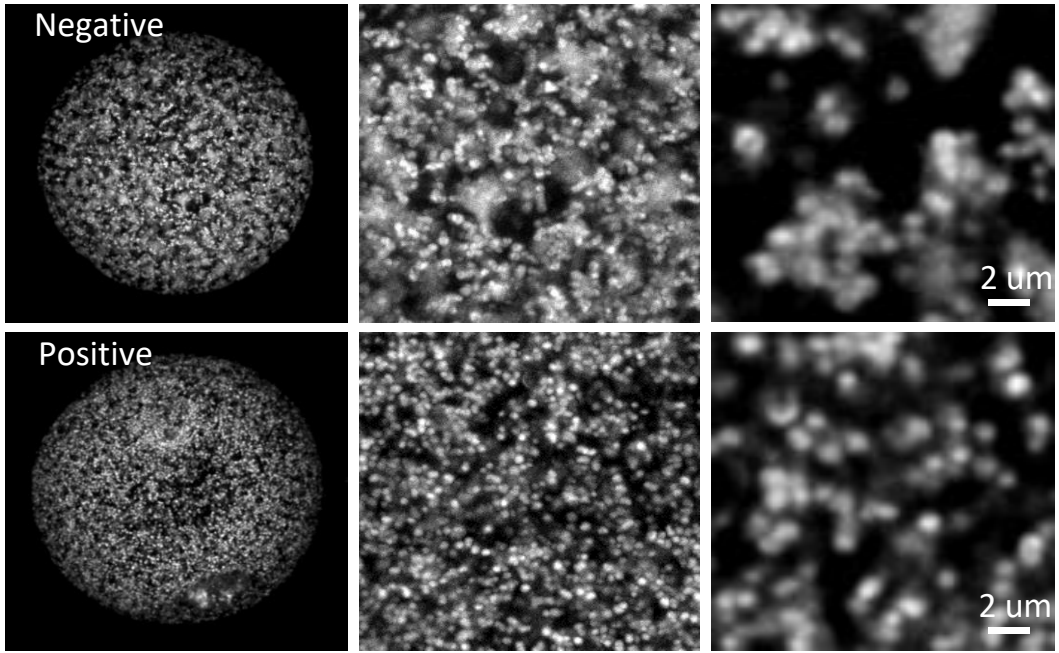






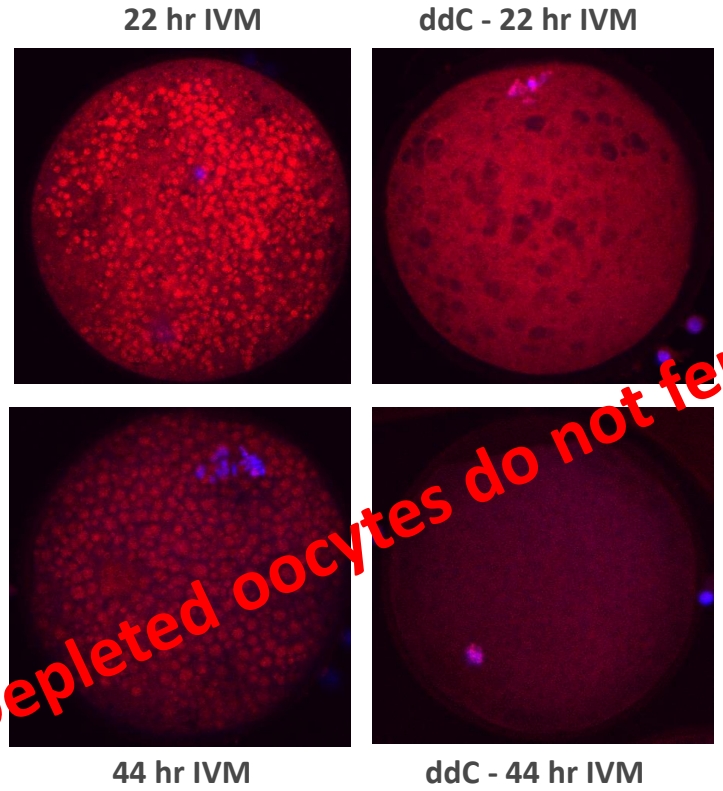
Cagnone et al. *Sci Rep* 2016; 2016; **6:** 23229

Differential mitochondrial clustering between BCB^{+} and BCB^{-} oocytes



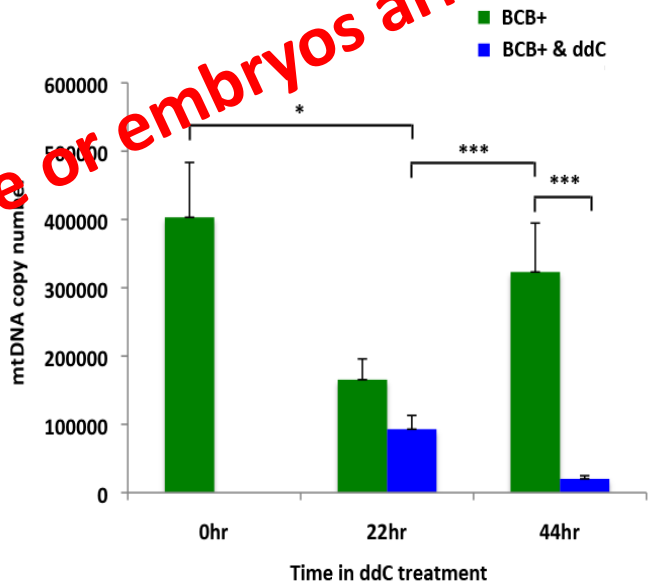
Cagnone et al. *Sci Rep* 2016; 2016; **6:** 23229

Depletion of mtDNA during IVM



Red: MitoTracker Red; Blue: DAPI

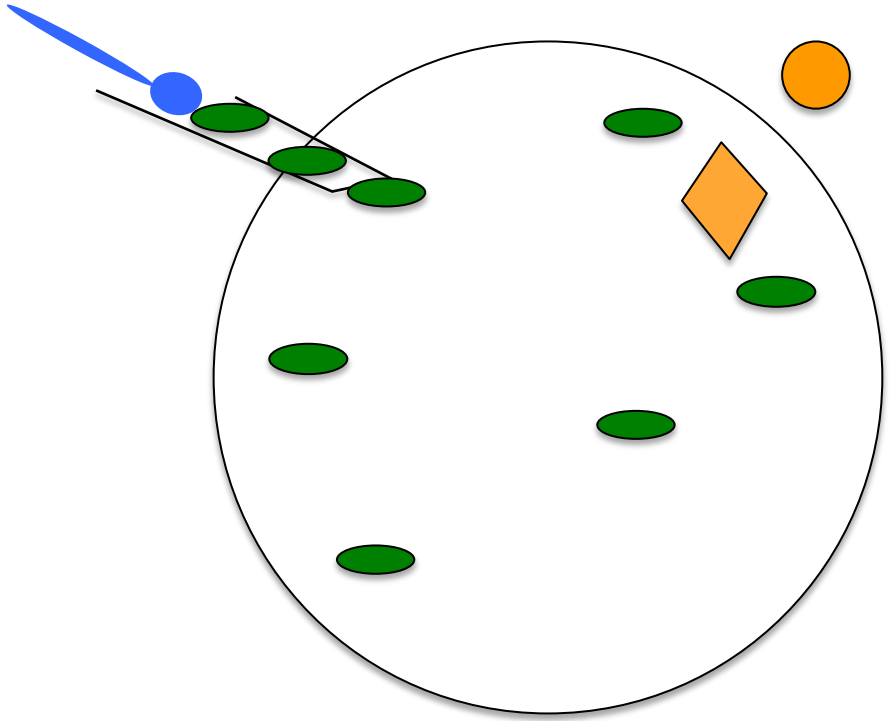
Depleted oocytes do not fertilise or embryos arrest



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

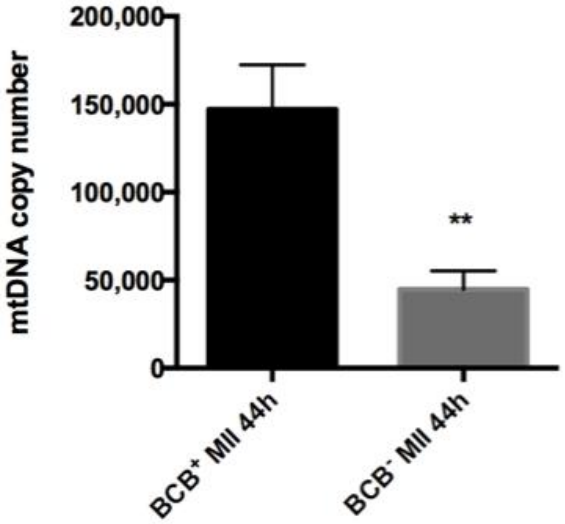
Autologous mitochondrial supplementation

Mitochondria isolated from BCB^{+} sister oocytes



El Shourbagy et al. *Reproduction* 2006; **131:** 233-45

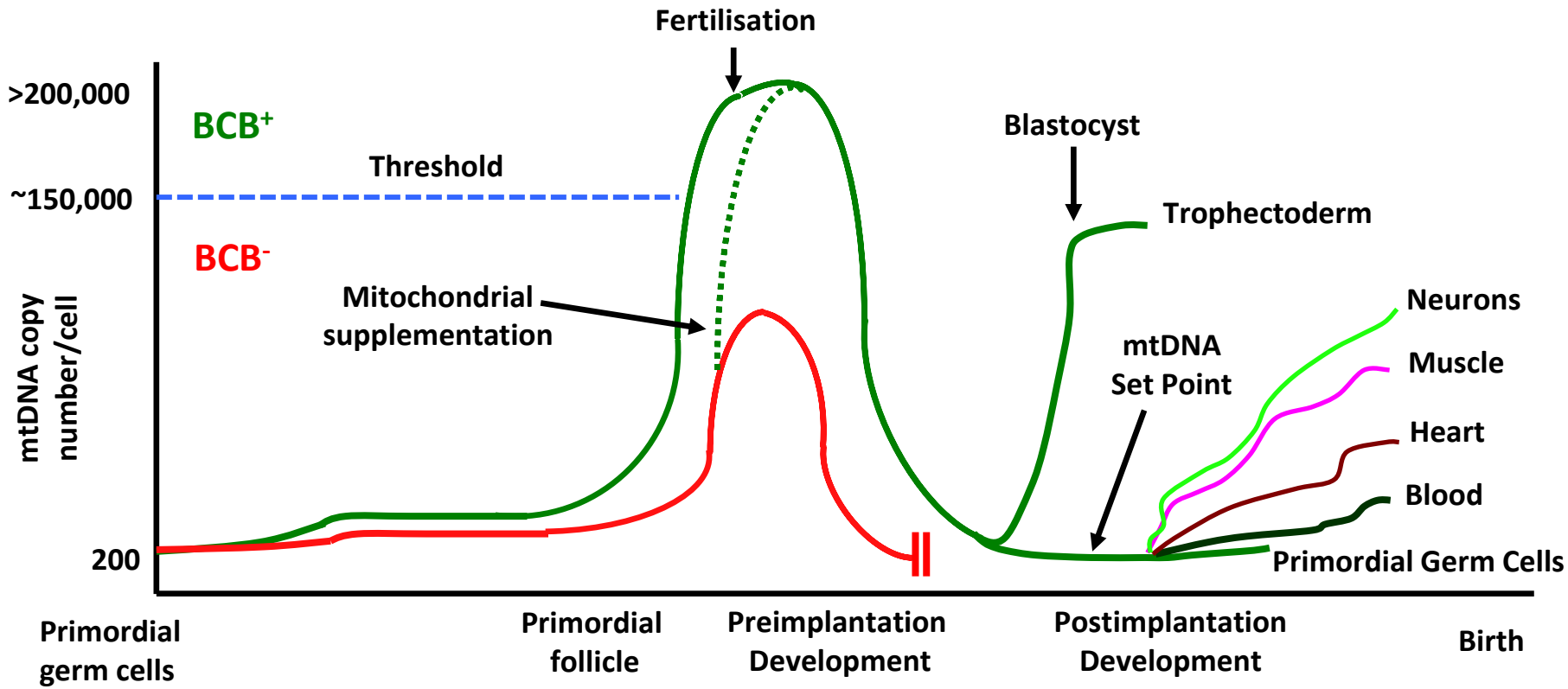
Higher mtDNA copy numbers in developmentally competent oocytes



- Comparison of MII oocytes (T-Test)

Cagnone et al. *Sci Rep* 2016; 2016; **6:** 23229

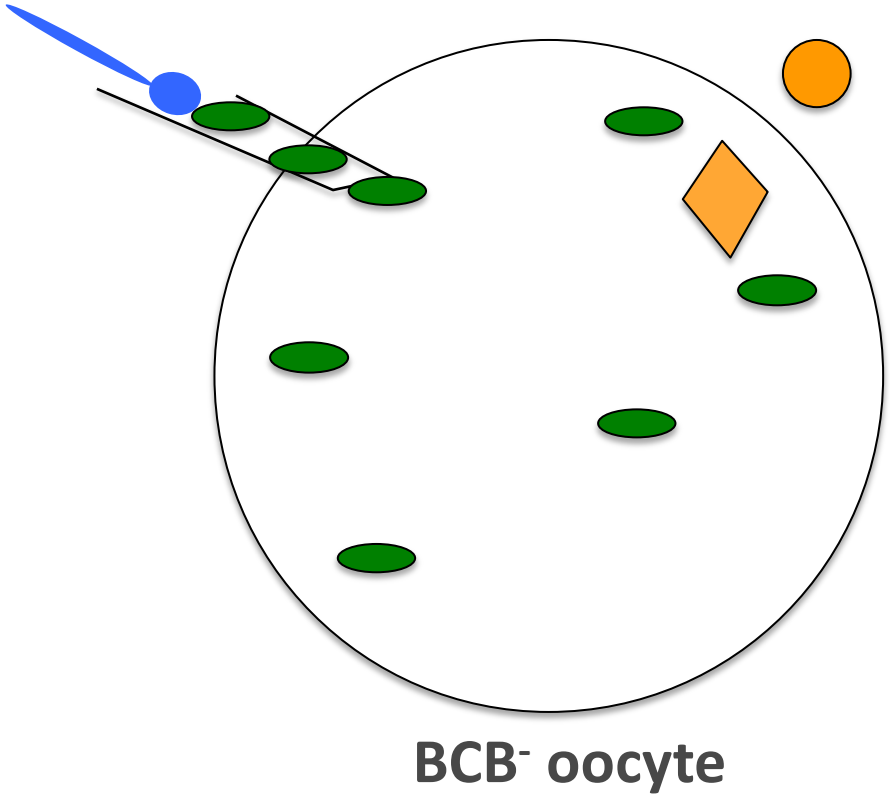
Minimum threshold of mtDNA copy number required for fertilisation



Supplementation with genetically identical mitochondria (mICSI) to overcome threshold

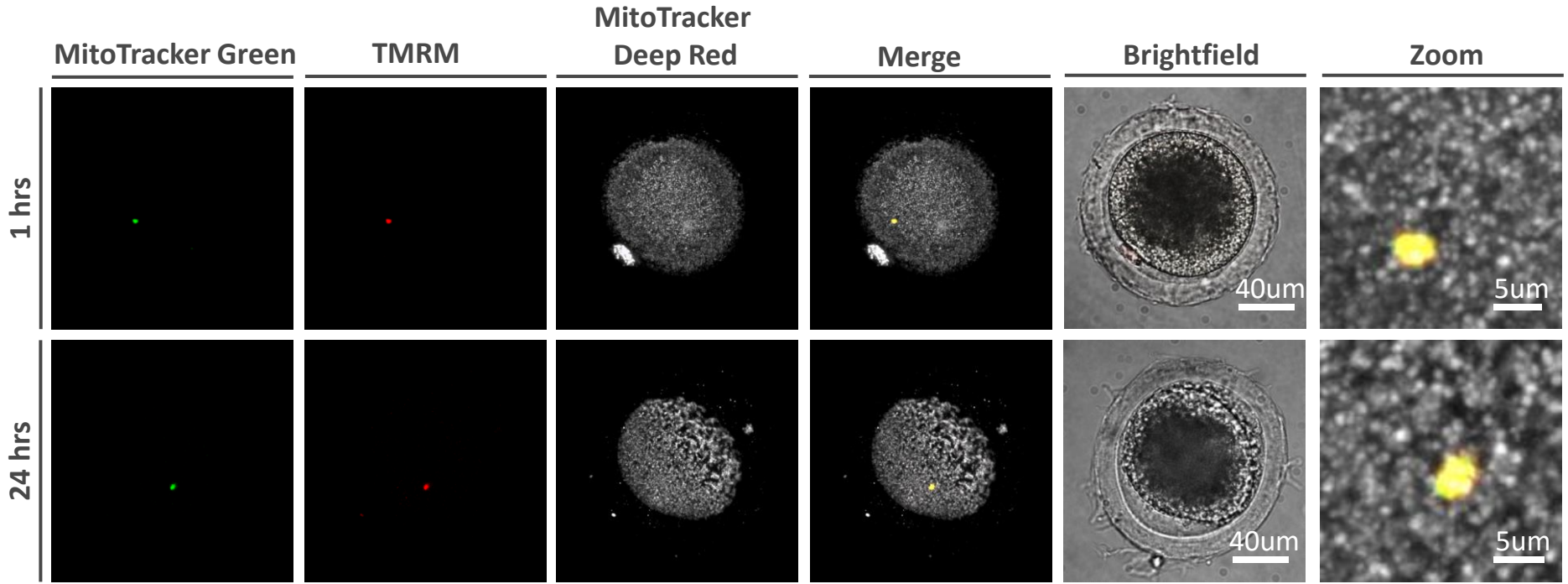
~ 800 copies of mtDNA

Mitochondria isolated from BCB^{+} oocytes



BCB^{-} oocyte

Supplementation



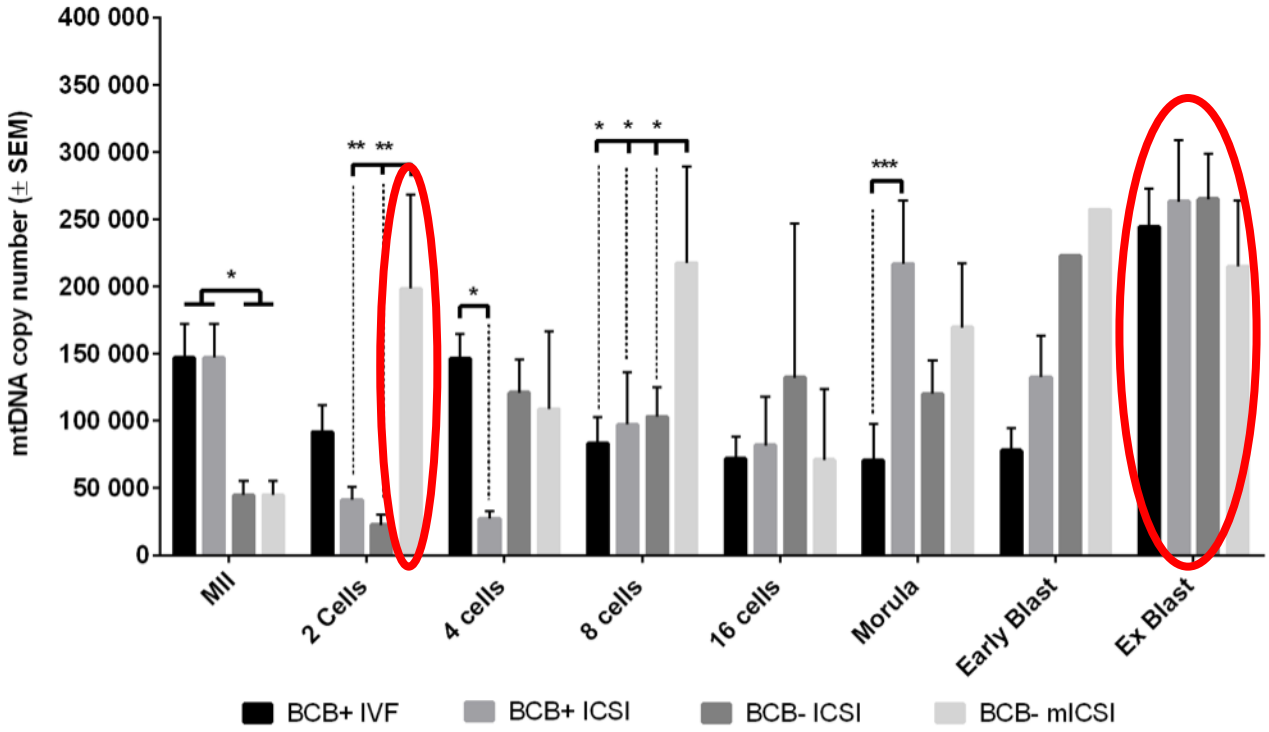
Cagnone et al. *Sci Rep* 2016; 2016; **6:** 23229

Improvement in fertilisation and development of BCB^{-} embryos following mICSI

	Insemination	Total oocyte*/ MII number	% Fertilisation (total)	% Blastocyst/ Fert (total)	% Blastocyst/ Fert (±S.D)
BCB^{+}	IVF	764*	58.4	23.7	20.6 ± 13.9
	ICSI	255	77.7	34.9	33 ± 15.3
	mICSI	98	62.2	31.6	31.5 ± 13.8
BCB^{-}	IVF	507*	38.1	10.6	7.6 ± 5.8^{a}
	ICSI	136	59.9	22	23.9 ± 11.0$^{a.b}$
	mICSI	139	40.4	27.8	31.5 ± 15.6^{b}

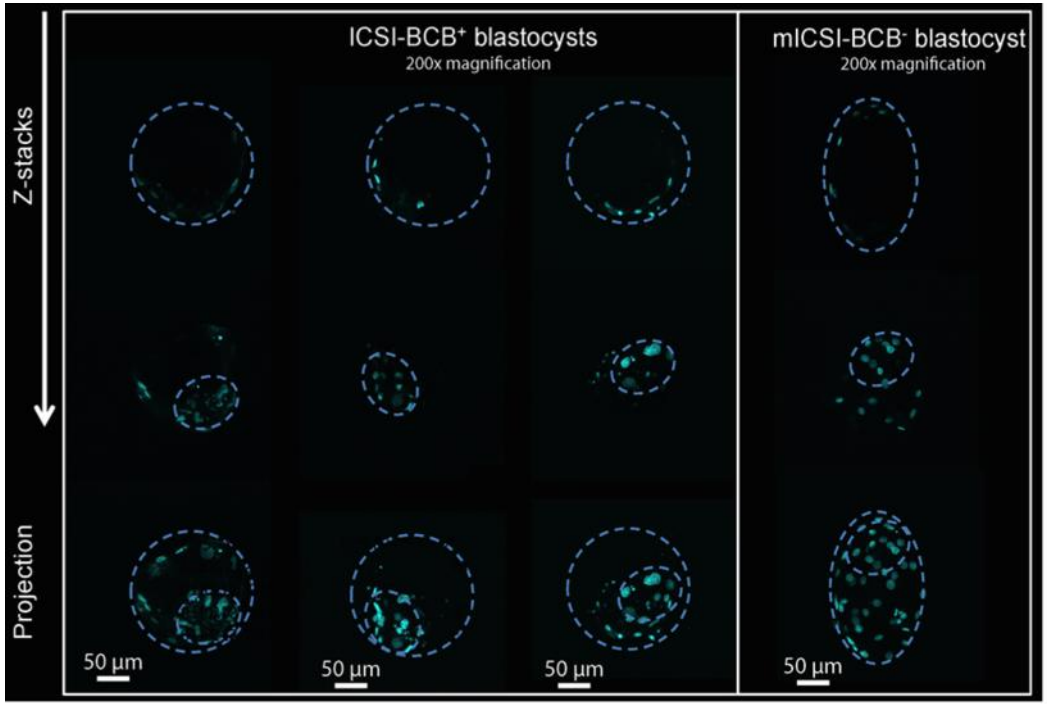
Cagnone et al. *Sci Rep* 2016; 2016; **6:** 23229

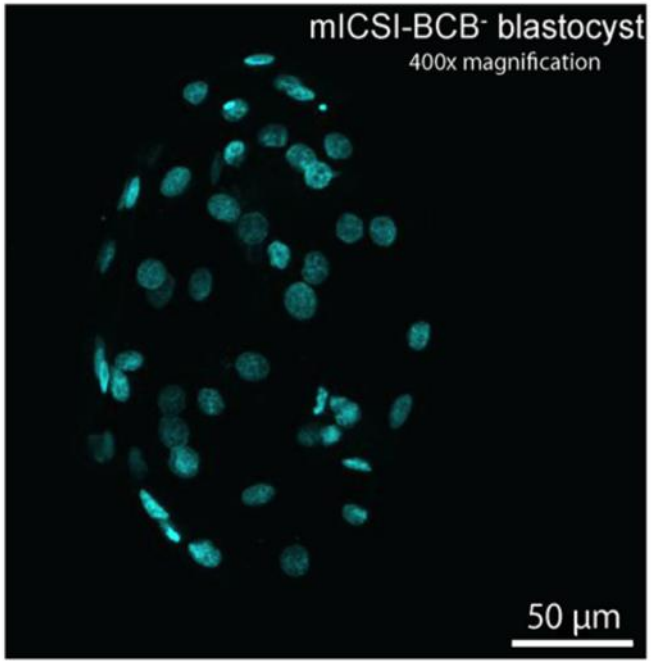
Early rescue supports blastocyst development in BCB^{-} embryos



Cagnone et al. *Sci Rep* 2016; 2016; **6:** 23229

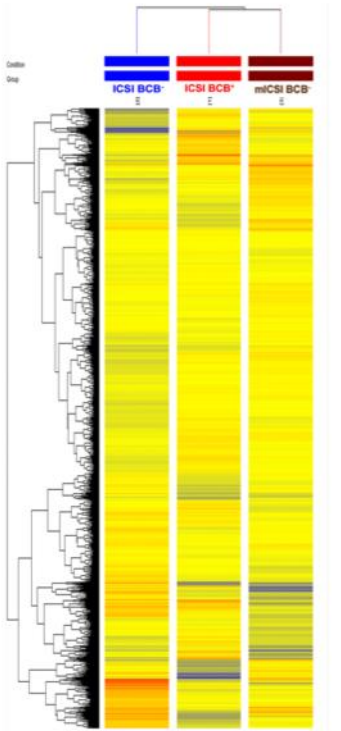
Improvement in development of BCB^{-} embryos following mICSI as evidenced by increased cell number





Cagnone et al. *Sci Rep* 2016; 2016; **6:** 23229

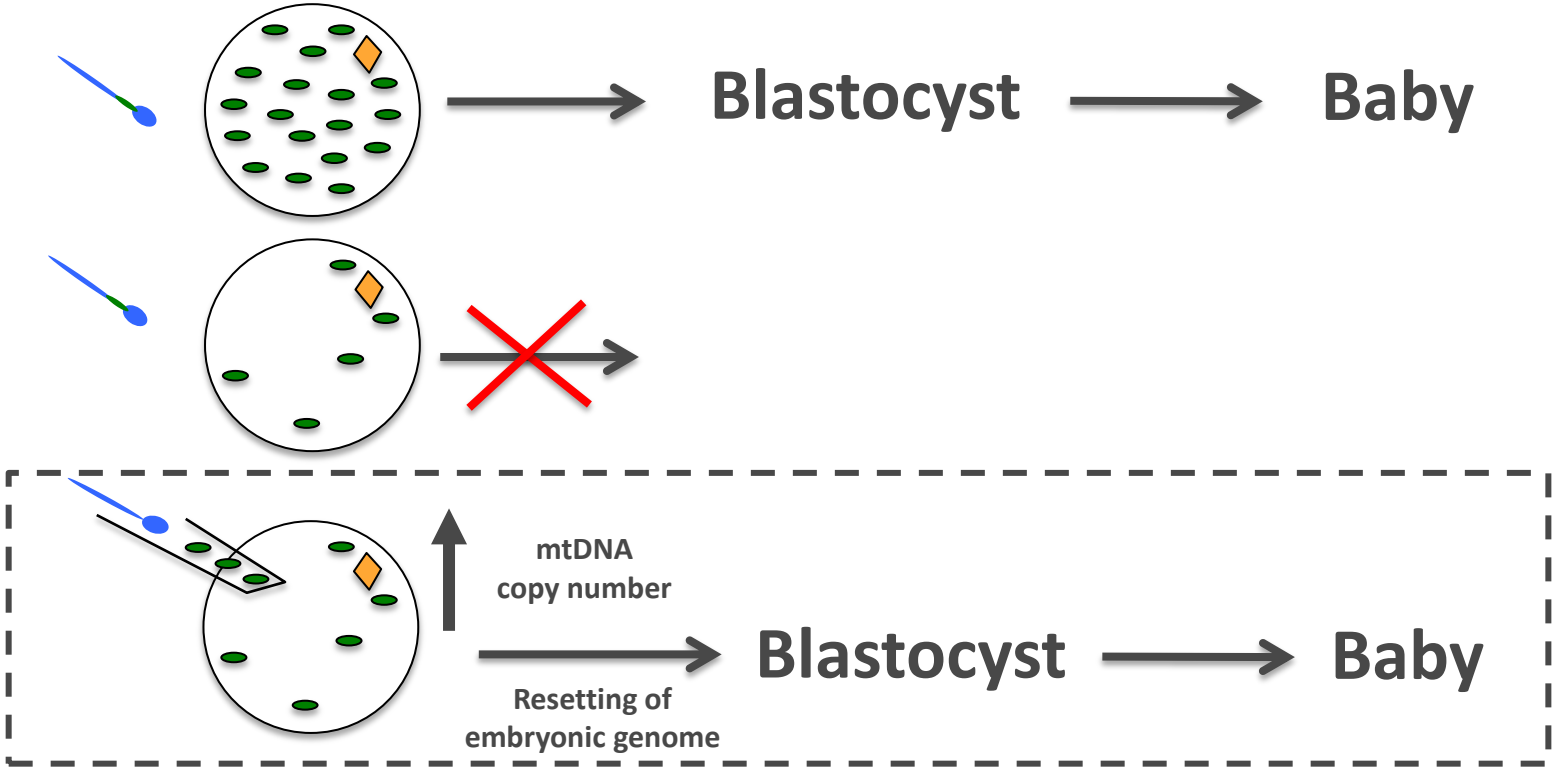
Genes differentially expressed between mICSI BCB$^-$ and ICSI BCB$^-$ blastocysts



- Most affected networks:
  - Cellular movement
  - Cellular development
  - Cellular movement
- Canonical pathways
  - PPAR signaling and CREB1
- Upstream regulators
  - Cell proliferation

Cagnone et al. *Sci Rep* 2016; 2016; **6:** 23229

Conclusion


Blastocyst
Baby
mtDNA
copy number
Resetting of
embryonic genome
Blastocyst
Baby

Acknowledgements

Centre for Genetic Diseases

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