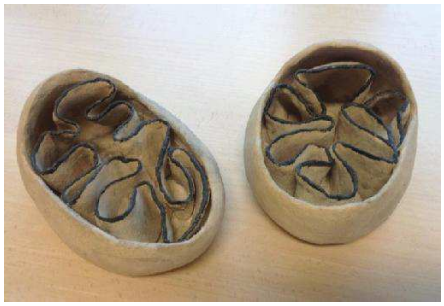
The (predictive) value of mitochondrial DNA



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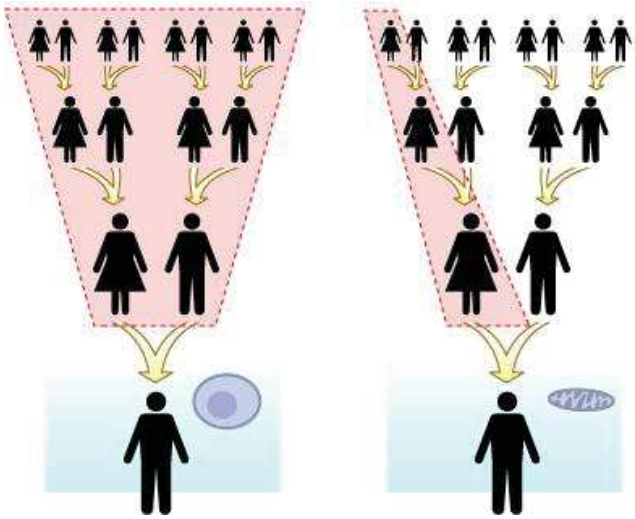
Mitochondrial DNA inherits maternally



- Asexual reproduction: no recombination events possible → mutations are irreversible
- Intense ROS production and no repair: mtDNA vulnerable to mutations
- **Accumulation of mutations:** **MULLER'S RATCHET**

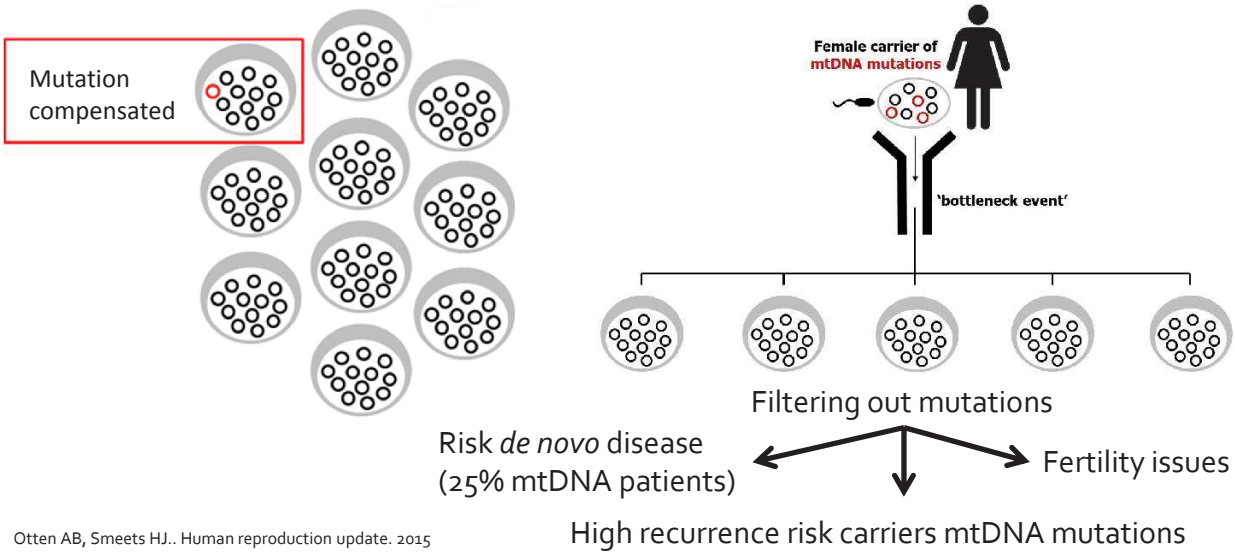
Avoiding accumulation of mtDNA mutations

All eukaryotes

- **Transfer of genes** from mitochondria to nucleus (reducing the risk)

Animal/Human specific

- Maintaining a **high mtDNA copy number** – compensates *de novo* mutations
- Mitochondrial DNA segregates through a **genetic bottleneck** during inheritance

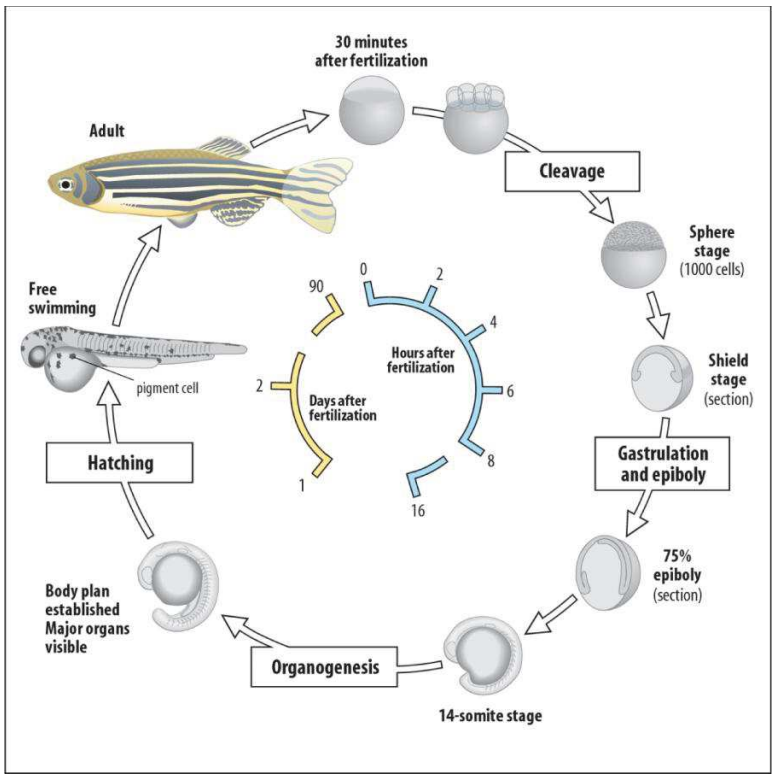


Otten AB, Smeets HJ.. Human reproduction update. 2015

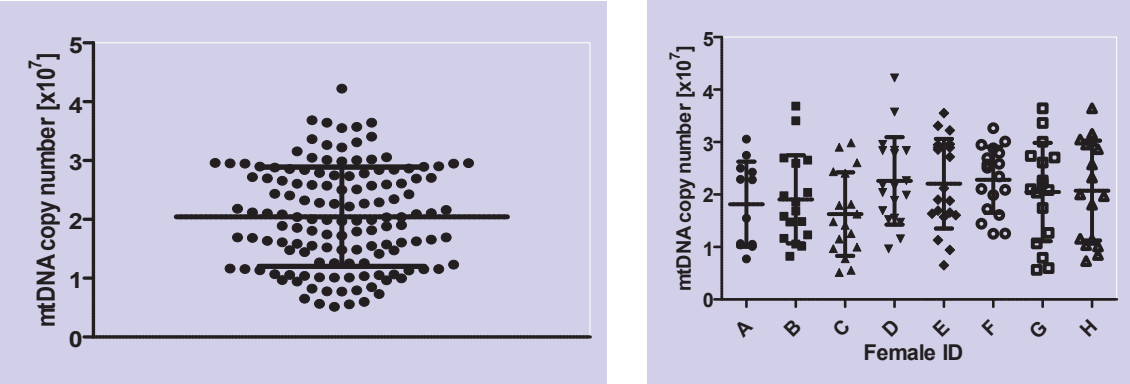
Zebrafish: model for mtDNA segregation

Zebrafish (*Danio rerio*)

- rely on many of the same organs as humans
- optical clarity during development (*in vivo* assays)
- rapid *ex utero* development
- high number of offspring (cheap in breeding and keeping)
- easy genetic manipulation
- highly suitable for large scale intervention studies



MtDNA copy number in mature zebrafish oocytes

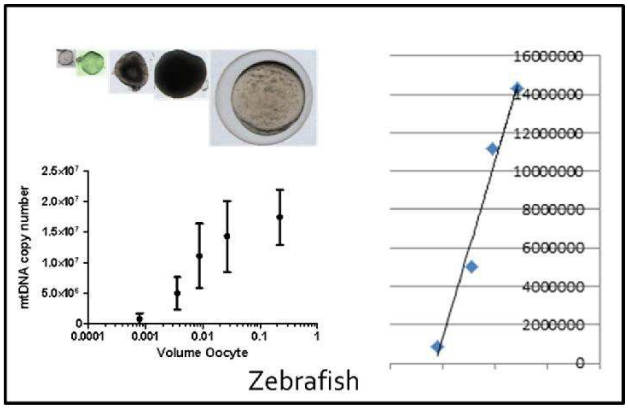


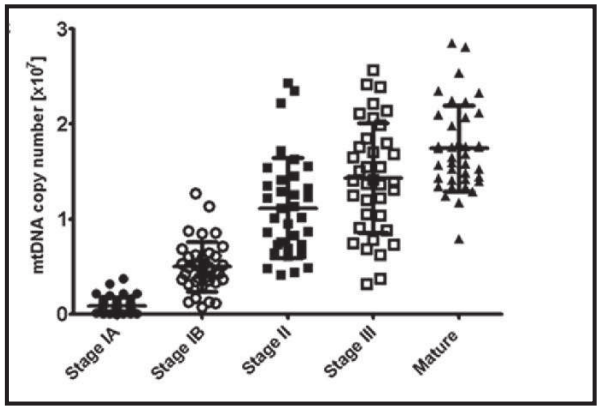
Otten et al. Cell Rep, accepted

- Q-PCR: critical (mtDNA copy number affected by isolation procedure/compounds)
- Selection against low mtDNA copy number zebrafish (<5 million copies)
- Low variation mean mtDNA copy among individual fish
- High intra-individual variation mtDNA copy number across oocytes individual fish

- Oocytes deficient in mitochondria insufficient energy for fertilization/ embryogenesis
- Poor oocyte quality (aging) - deficiency in number of functional mitochondria.
- Functional mitochondria in oocytes play a key role in fertilization success

Linear relation between oocyte volume and mtDNA copy number in zebrafish





Species; mtDNA; size oocyte

- Salmon; ~3 billion; ~4.5mm
- Zebrafish; ~ 1 million; ~0,75mm
- Bovine, sheep, pigs; 0.3–1 million; ~0.15mm
- Human, mice, rats; 0.1-0.3 million; ~0,1mm

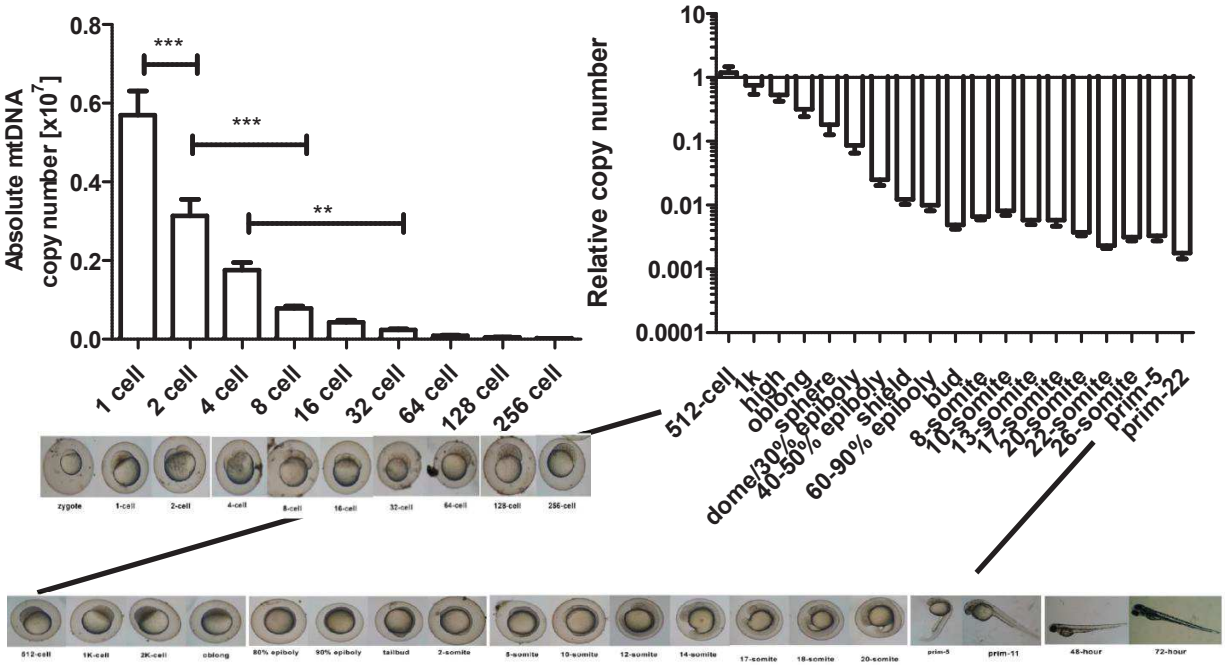
Copy number relates to pattern/speed implantation?

- absent, centric, eccentric/interstitial
- different energy requirements

Copy number correlates with size oocyte

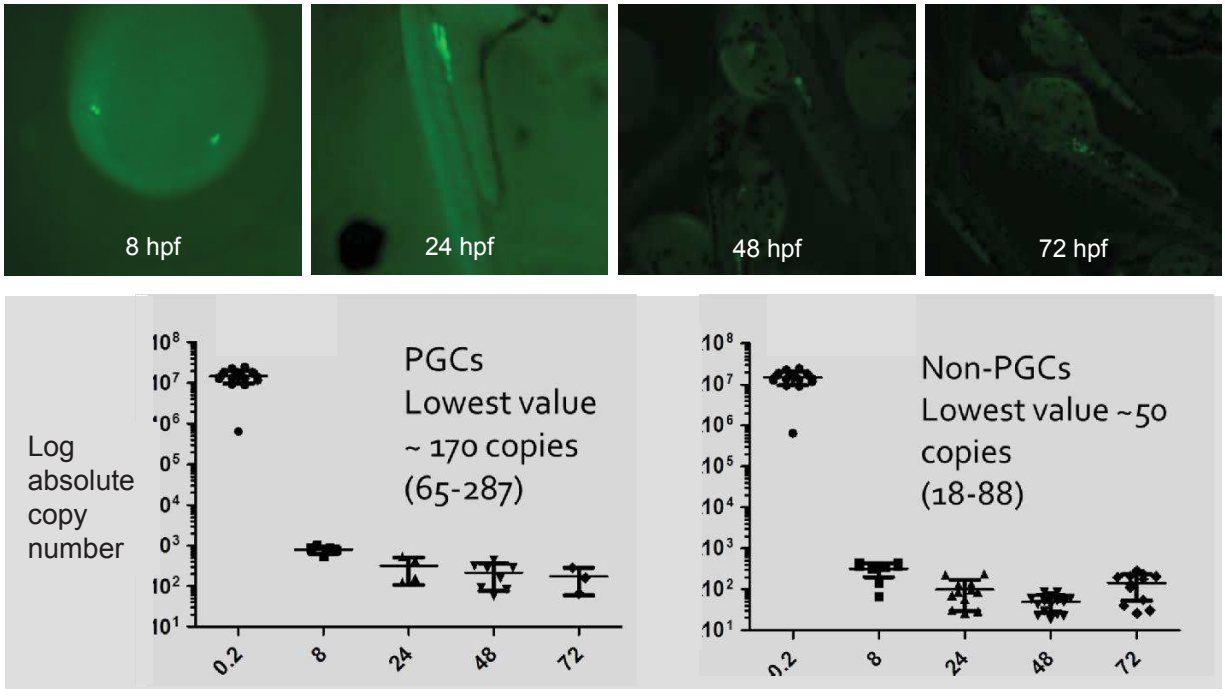
- mtDNA copy number per unit of volume seems equal across species

MtDNA bottleneck in zebrafish embryos



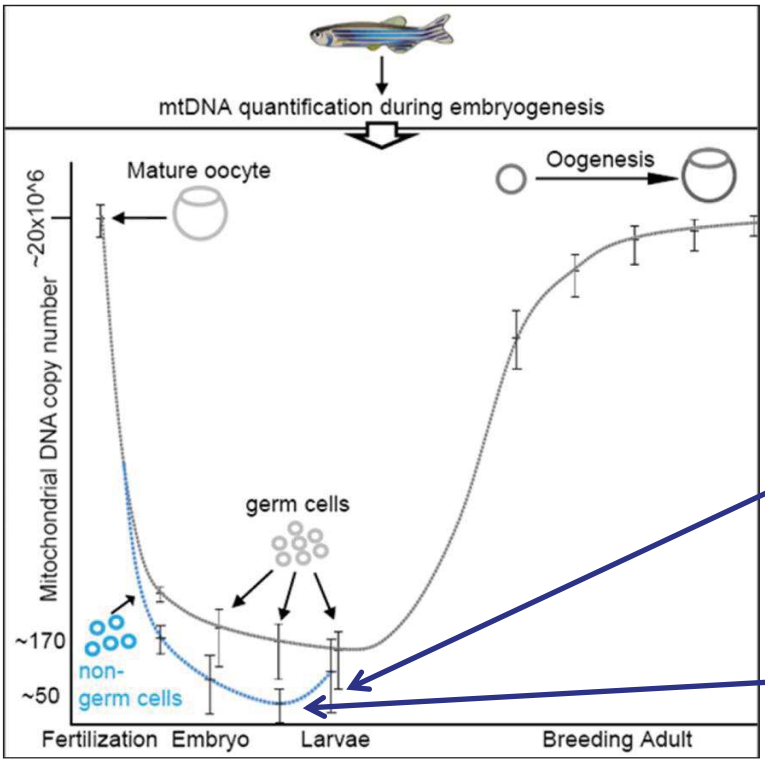
No mtDNA replication until epiboly and early somitogenesis
Epiboly phase has similarities with implantation

Isolation of PGCs/non-PGCs from zebrafish embryos with FACS-sorting (*nanos3*)



High variation in all stages of development

Regulation mtDNA copy number during development



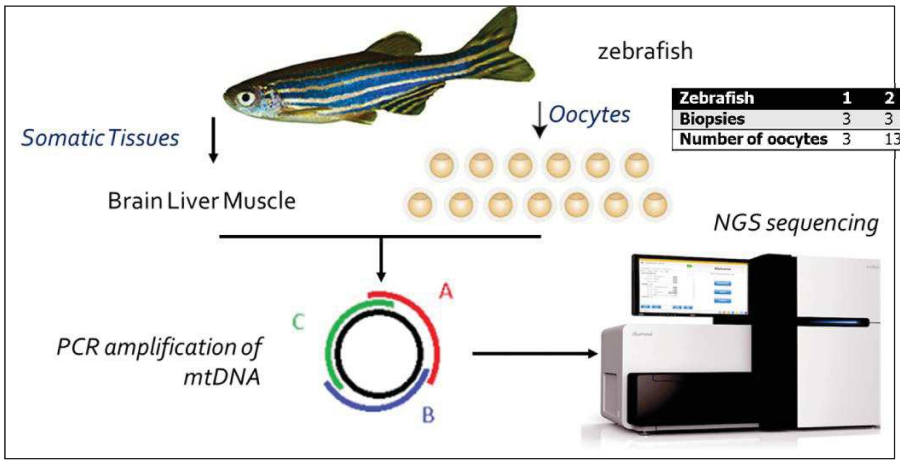
PGC bottleneck sizes fairly constant across species

- ~173 copies humans
- 30-35 copies humans
- 65-287 copies zebrafish
- 65-163 copies cows
- 80-88 salmons
- 87-395 crickets

Risk de novo mutations reaching functional significance at lowest copy number

Somatic mtDNA replication starts at early somatogenesis

mtDNA bottleneck and *de novo* disease risk

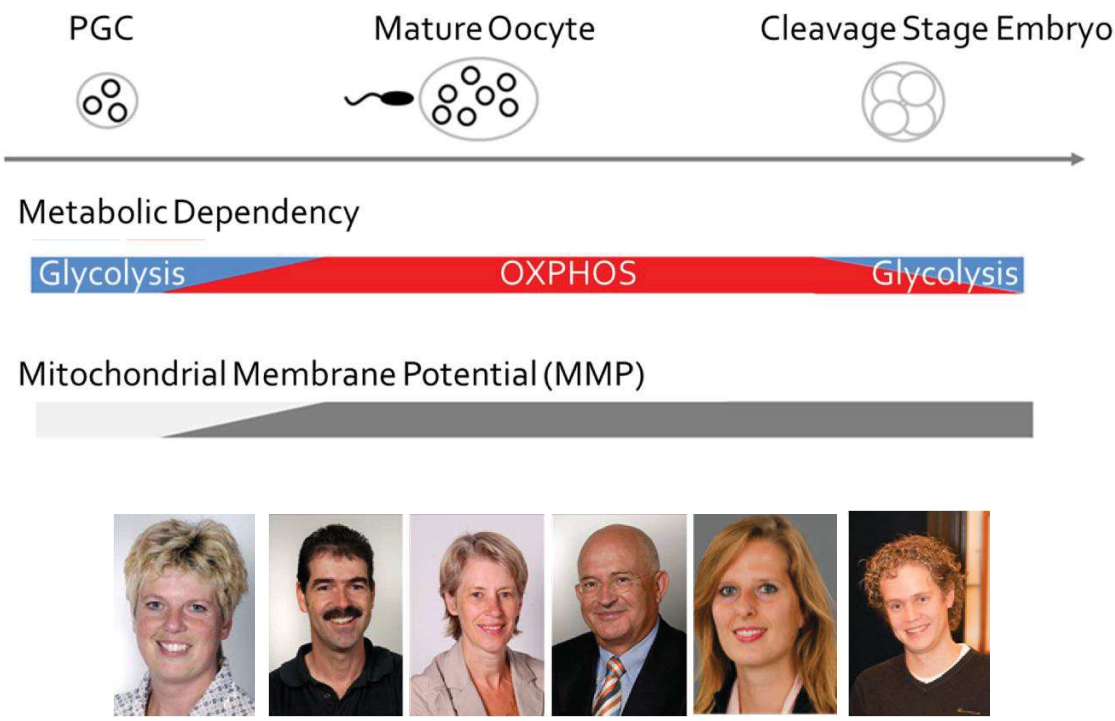


Zebrafish	1	2	3	4	5	6	7	8
Biopsies	3	3	3	3	3	3	3	3
Number of oocytes	3	13	15	14	15	14	15	14

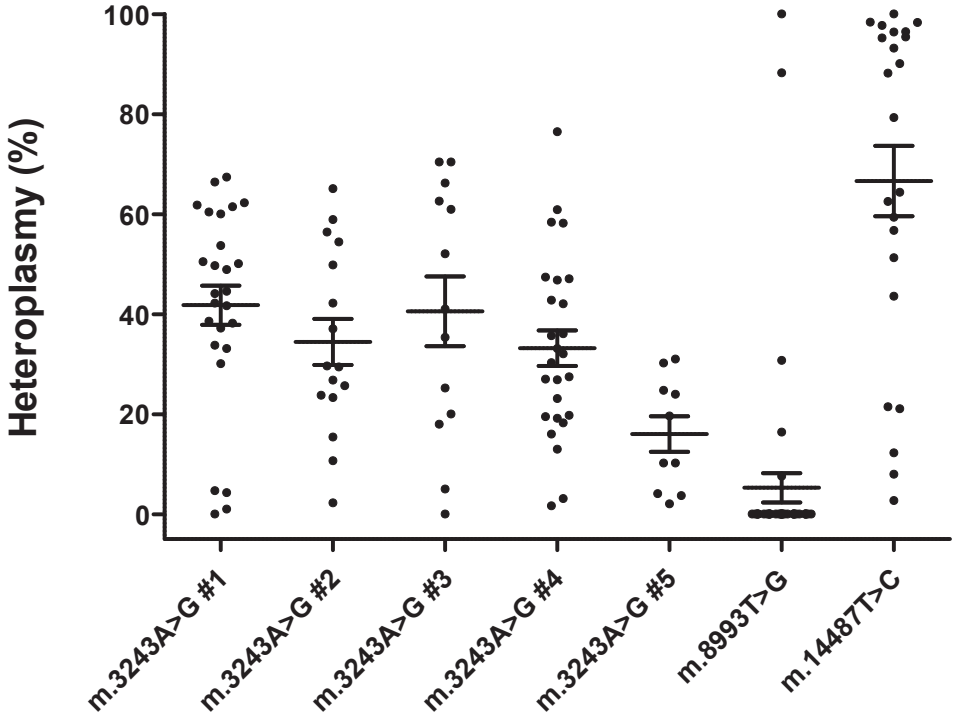
- De novo mutation: present in oocytes not in mother (technical detection limit: 1.5%)
- Only 19 oocytes (18%) possessed a de novo mutation of 1.5-9.0%
- Calculated bottleneck size based on percentage de novo mutation: 11 to 67
- Calculated bottleneck size all oocytes:43-353 - 20% below 65 (1.5% detection threshold)

- De novo mutations occur at random (non-pathogenic and pathogenic)
- If pathogenic mutations exceed expression threshold → de novo disease
- Replication errors Polymerase Gamma (replicates mtDNA) most likely cause
- Due to the high intra-individual variation every female seems at risk

mtDNA bottleneck and fertility



Mutation load distribution in PGD oocytes, zygotes and blastomeres

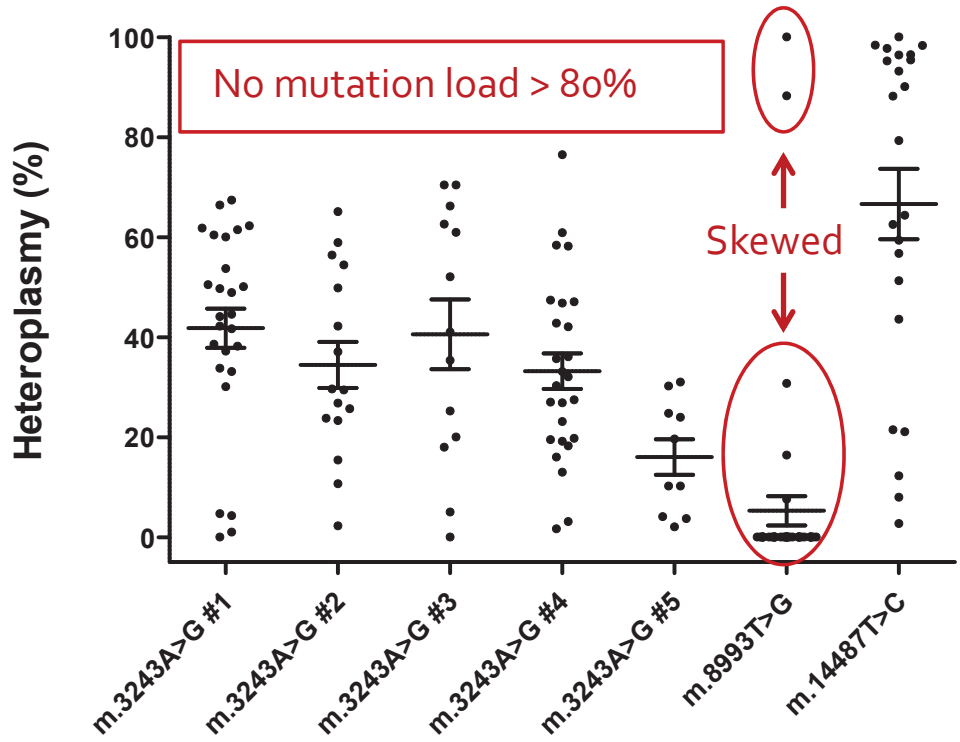


Bottleneck sizes for m.3243A>G, m.8993T>G and m.14487T>C mutation carriers

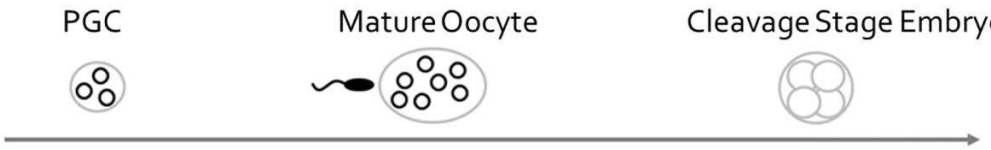
Carrier	n	p_o, heteroplasmy in samples (Average ± SEM)	Effective bottleneck size (N_{eff}) (value [95% CI])
m.3242A>G #1	26	0.42 ± 0.04	83 [50-159]
m.3242A>G #2	16	0.34 ± 0.05	94 [50-233]
m.3242A>G #3	13	0.41 ± 0.07	49 [24-117]
m.3242A>G #4	26	0.33 ± 0.04	92 [55-173]
m.3242A>G #5	10	0.16 ± 0.04	152 [69-473]
m.8993T>G	46	0.05 ± 0.03	10 [4-57]
m.14487T>C	23	0.67 ± 0.07	21 [13-38]

Bottleneck sizes calculated on the assumption of genetic drift only

Mutation load distribution in PGD oocytes, zygotes and blastomeres

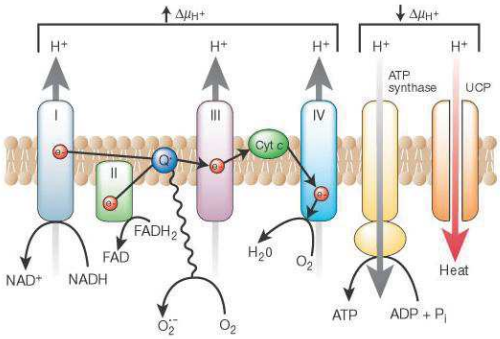


Selection on OXPHOS function in oogenesis



Metabolic Dependency

Mitochondrial Membrane Potential (MMP)



Most mutations:
- Reduced OXPHOS function
- Reduced MMP

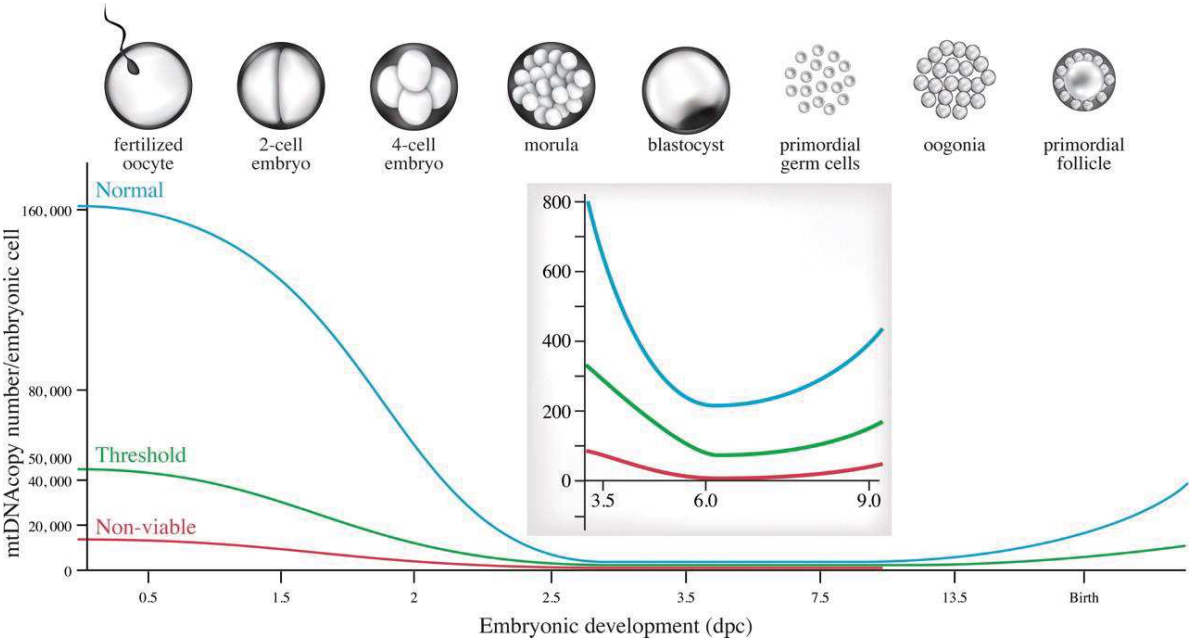
But:
- Differences between mutations exist
- Mutation loads are involved as well

Bottleneck, genetic drift and selection define mtDNA mutation distribution in oocytes

Segregational mechanism	**m.3243A>G**	**m.8993T>G**	**m.14487T>C**
Genetic drift	+	+	+
Selection OXPHOS - ATP production - OXPHOS assembly - Membrane potential	>80% drops to 0% >90% lost (CI) Strongly reduced	At 100%: activity 20-30% CV affected Increased	No major effect CI affected No effect
	Random, but no mutation loads >80% (negative selection)	Random, but positively selected for high mutation loads	Random, no selection

- Only 20-30% of OXPHOS capacity required for embryogenesis
- Normally 200,000 copies mtDNA → 50,000 copies will be enough (fully functional)
- Reserve capacity reflected in mtDNA copy number
- Patients develop severe disease during life (less reserve capacity)

Critical threshold of mtDNA copy number during mouse embryogenesis



Timothy Wai et al. Biol Reprod 2010;83:52-62

Selection for ooplasmic and blastomere volume

Group	No. of oocytes	Mean volume ± SE	
Pregnancy	44	625,019±7,448[a]	(511,026–723,604)
Pregnancy (excluded miscarriage)	35	63,911±8,255[b]	(537,143–723,604)
Miscarriage	9	598,217±14,787	(511,026–659,671)
Non-pregnancy	630	609,456±2,122[c]	(443,232–767,134)
Non-pregnancy (excluded frozen embryos that do not thaw)	549	608,569±2,246[d]	(443,232–767,134)

[a,d] $p<0.05$, [b,c] $p<0.05$, [b,d] $p\leq0.01$, μm^3

- Higher fecundity is associated with an increased number of mtDNA copies in the embryo.
- A significant positive correlation exists between blastomere volume and the number of mtDNA copies.
- Low-invasive quantification of ooplasmic and blastomere volume is a novel predictor for successful clinical outcome in selecting embryos to be transferred.

Murakoshi et al. J Assist Reprod Genet (2013) 30:1367–1375

Mitochondrial copy number and preimplantation development

Pig data:

- Deficient pig oocytes supplemented with **autologous populations of mitochondrial isolate** (~800 copies mtDNA) at fertilization (minimum amount pig ~120,000 copies)
- Increase mtDNA copy number 2cell-stage (4.4 fold versus 1/2 -1/4 IVF/ICSI) and blastocyst (4.8 fold versus 1.7-1.8 fold)
- Brief replication event between fertilization and 2-cell stage
- Increased development to blastocyst and **promoted mitochondrial DNA replication** prior to embryonic genome activation
- Blastocysts exhibited transcriptome profiles developmentally competent oocytes.

Cagnone, G. L. M. et al. 2016.. Sci. Rep. 6, 23229

Human data:

- Normally mtDNA replication starts after blastocyst formation, earlier start in embryos of older woman as compensatory mechanism
- But increase in mtDNA copy number in blastocysts associated with loss of viability
- The 'quiet' hypothesis: early embryonic metabolism works at a quiet pace
- Insufficient metabolic support induces an adaptive response through increased gene expression that compromises embryonic development

Fragouli E et al. (2015) PLoS Genet 11(6): e1005241

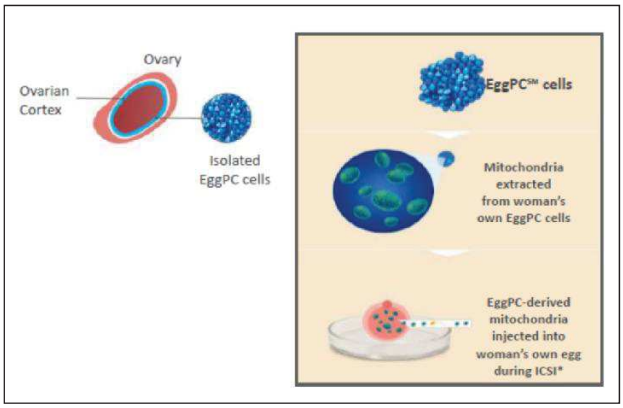
Journal of Fertilization : In Vitro, IVF-Worldwide, Reproductive Medicine, Genetics & Stem Cell Biology

OVA SCIENCE TRANSFORMING FERTILITY

Research Article

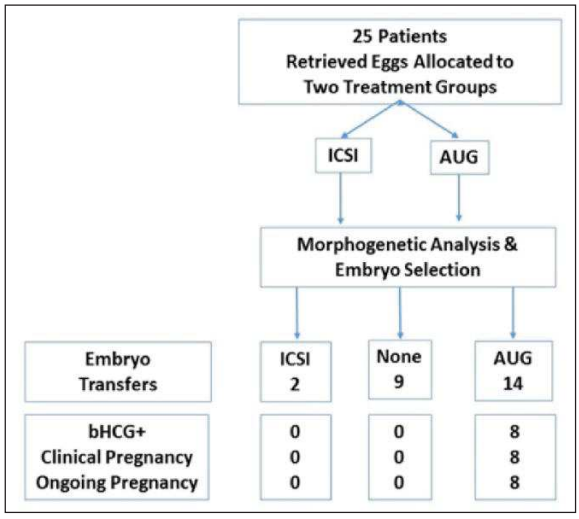
The AUGMENT℠ Treatment: Physician Reported Outcomes of the Initial Global Patient Experience

Michael H Fakih[1*], Mohamad El Shmoury[1], Julia Szeptycki[2], Dennis B dela Cruz[2], Caroline Lux[2], Suleman Verjee[3], Colleen M Burgess[4], Gabriel M Cohn[4] and Robert F Casper[2*]



- Number of egg precursor cells not provided
- Number of mitochondria not provided
- Proprietary information
- 1-2 pl injected

Increased pregnancy rates with the AUGMENT treatment



Embryo Transfers	ICSI 2	None 9	AUG 14
bHCG+	0	0	8
Clinical Pregnancy	0	0	8
Ongoing Pregnancy	0	0	8

Conclusions

1. Animal models provide insight in (common) normal and abnormal processes concerning the role of mitochondria in oogenesis and development
2. Sufficient functional mitochondria/mtDNA are required for oogenesis and embryonic development (estimated threshold ~50,000 copies of mtDNA or 25% of OXPHOS capacity)
3. Selection occurs at the level of OXPHOS function and MMP
4. The mtDNA bottleneck is evolutionary well-conserved (30-300 copies mtDNA)
5. Profound individual variation in mtDNA copy number and bottleneck levels is common in oocytes , PGCs and somatic cells
6. Low bottleneck sizes in PGCs are at risk of acquiring *de novo* mtDNA mutations and develop *de novo* mtDNA disease (25% of all mtDNA patients)
7. In zebrafish every female has such a risk, likely the same in other species
8. Risk of *de novo* disease reduced by limited fertility oocytes with low copy numbers
9. Reducing mtDNA copy number during embryogenesis in zebrafish cause a stress response and developmental problems

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