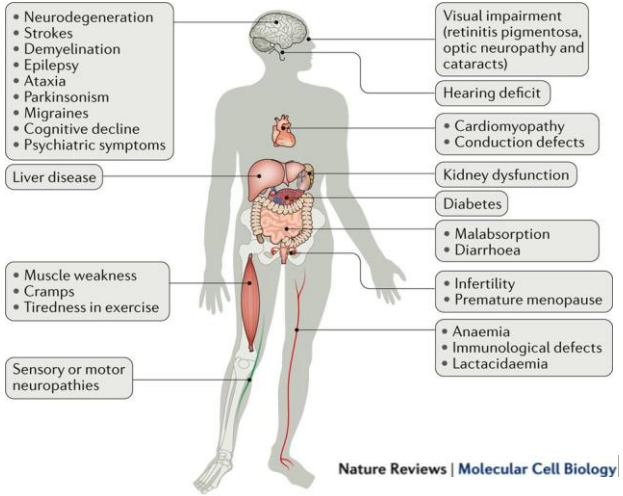
Exploring the feasibility of PGT for mitochondrial DNA mutation at late stages of preimplantation development

Paula RUBENS MD
Imagine Institute - Genetics of mitochondrial diseases
PGT Centers Necker-Béclère; Paris, France

PGDIS Leuven April 6-9, 2025

Introduction – mitochondrial disorders

Severe, multisystem, and uncurable



Genetic Origins:

nuclear DNA
(mendelian)
3/4 cases

mtDNA
(maternal)
1/4 cases

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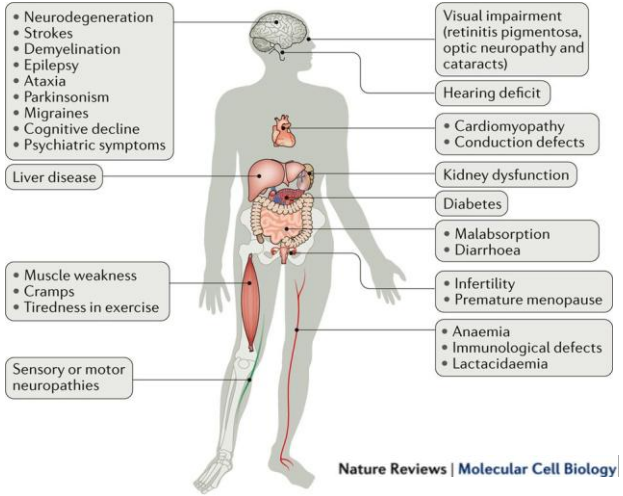

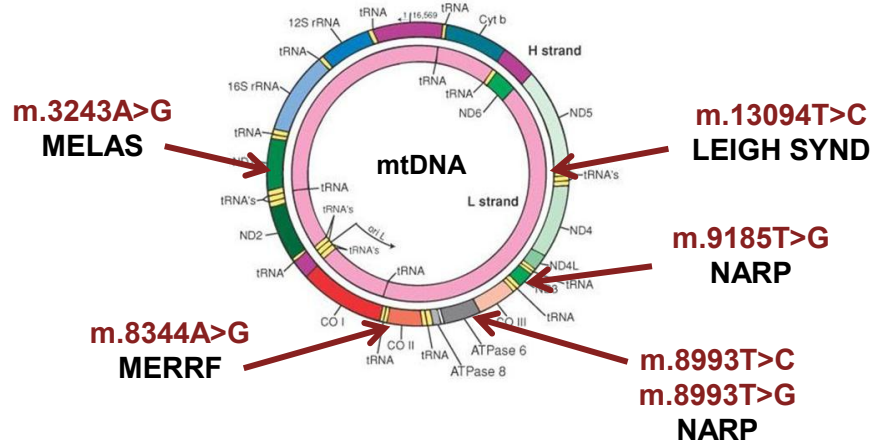

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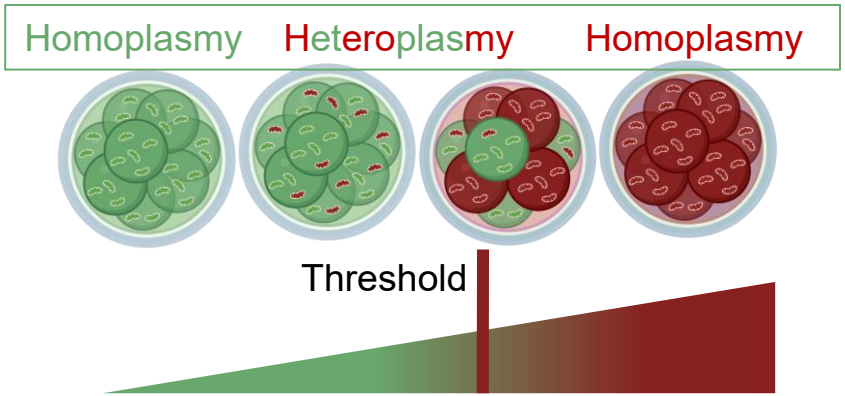
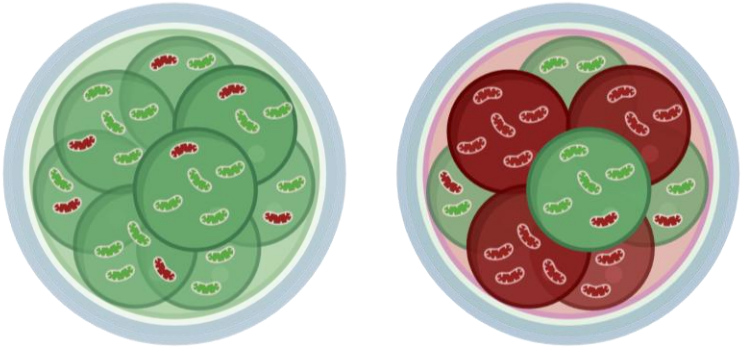


Background – PGT for mtDNA mutations

Prerequisites for **PGT-mt** is mutant load stability :

- among embryonic tissues/cells
- during preimplantation development



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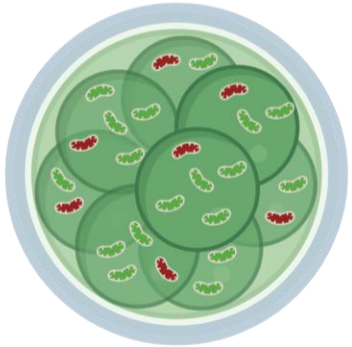
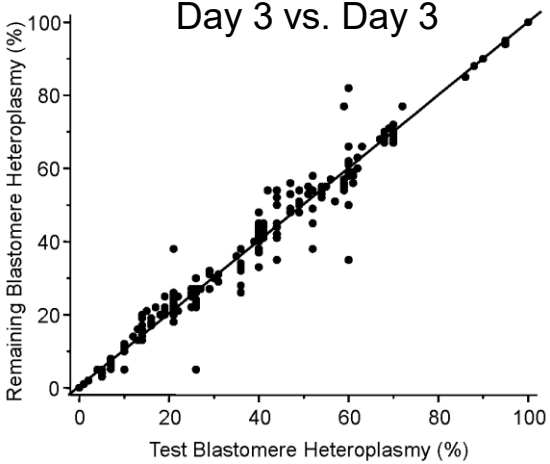


mtDNA heteroplasmy in human blastomeres

Day 3 vs. Day 3



(*Steffann et al., Cell Reports 2014*)

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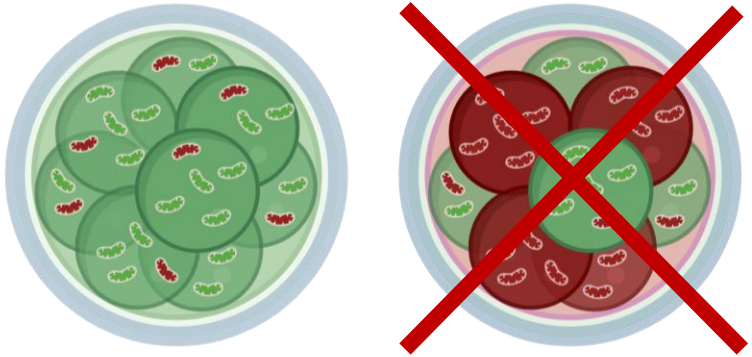

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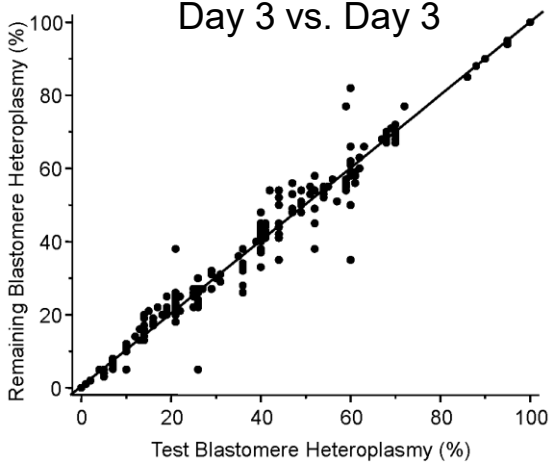

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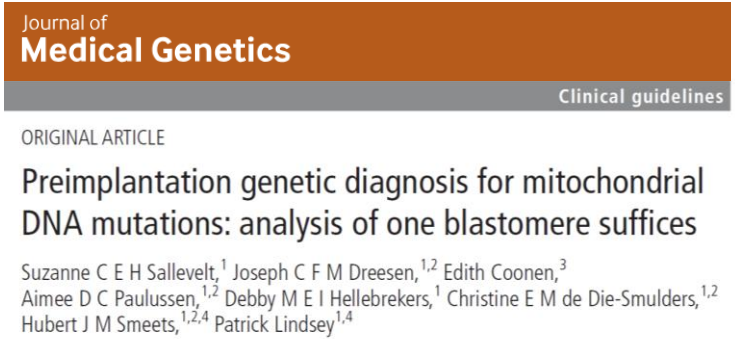


→Stability of heteroplasmy between blastomeres at Day 3

PGT Timing - a tendency to delay

PGT – nuclear DNA

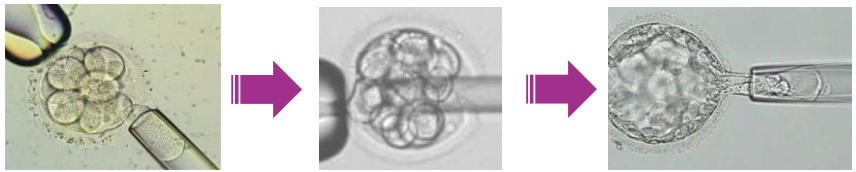
→Impaired clinical outcomes with cleavage (Day 3) biopsy

Scott et al., Fert Steril 2013

→Improved clinical outcomes with morula (Day 4) or blastocyst (Day 5/6) biopsy

Scott et al., Fert Steril 2013
Orvieto et al., PLoS One 2014
Zakharova et al., PLoS One 2014

Day 3 Day 4 Day 5/6



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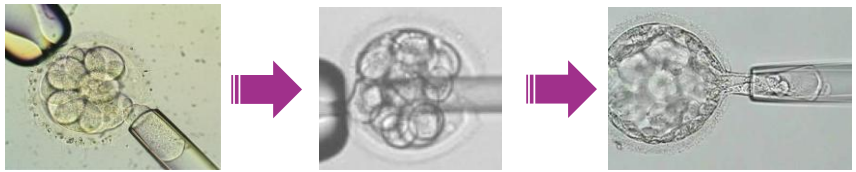
PGT - mitochondrial DNA

→ Morula (Day 4) biopsy
No data

→ Day 5/6 trophectoderm (TE) biopsy
Insufficient data / Uncertainties

Day 3 Day 4 Day 5/6



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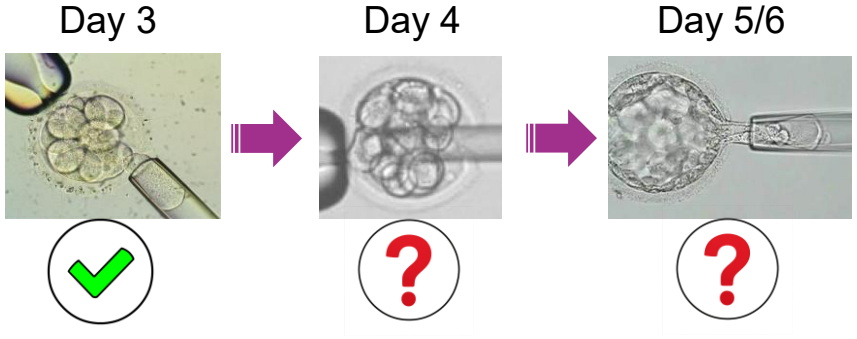


PGT - mitochondrial DNA

→ Morula (Day 4) biopsy
No data

→ Day 5/6 trophectoderm (TE) biopsy
Insufficient data / Uncertainties

Case report: m.3243A>G
Trophectoderm: 12% mutant load

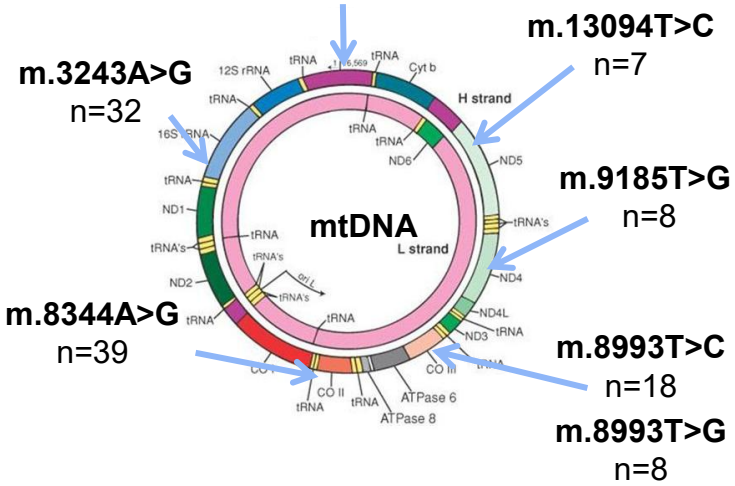
	Bucal	Blood	Urine
Birth	15%		
1.5 months		47	52
18 months		46	42

Clinical presentation (?)

Treff et al., Fertil Steril 2012
Mitalipov et al., Cell Reports 2014

Materials and Methods

Hypervariable Region 2 (HV2)
n=28



28 “control” and 112 “mito” embryos

Materials and Methods

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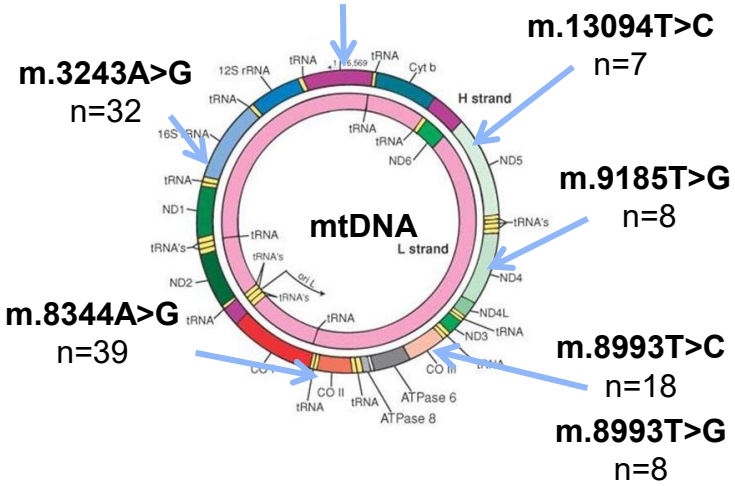


28 “control” and 112 “mito” embryos

48 embryos sampled at two time points

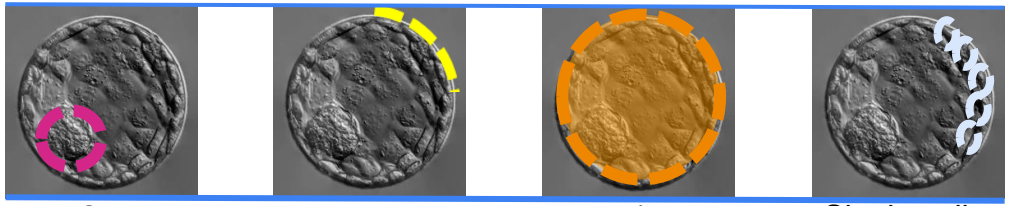
Day 3

Blastomere

Day 4

Blastomere

Day 5/6



Inner Cell Mass (ICM)

Trophectoderm (TE)

Rest/Whole

Single cells of TE

Materials and Methods

Hypervariable Region 2 (HV2)
n=28

m.13094T>C
n=7

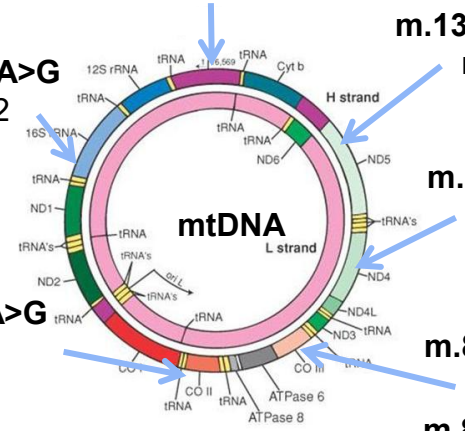
m.3243A>G
n=32

m.9185T>G
n=8

m.8344A>G
n=39

m.8993T>C
n=18

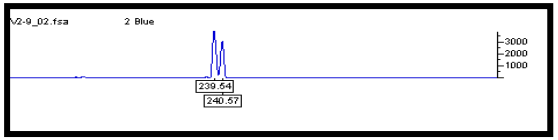
m.8993T>G
n=8



28 “control” and 112 “mito” embryos

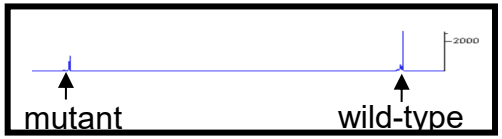
Semi-quantitative fluorescent PCR

for HV2
134 “control” samples



Lee et al., Electrophoresis 2004

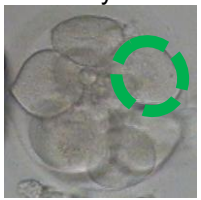
+ enzymatic digestion
285 “mito” samples



Gigarel et al., MGM 2005

48 embryos sampled at two time points

Day 3

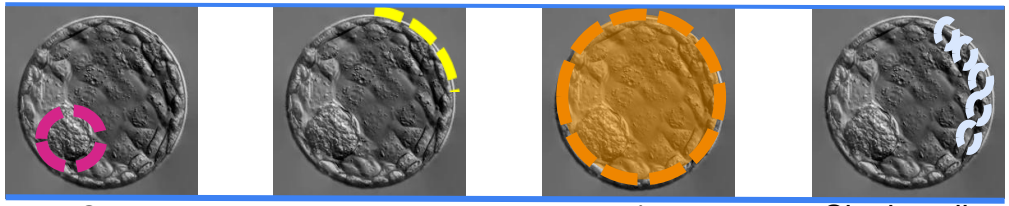


Blastomere

Day 4

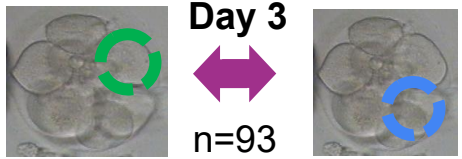
Blastomere

Day 5/6



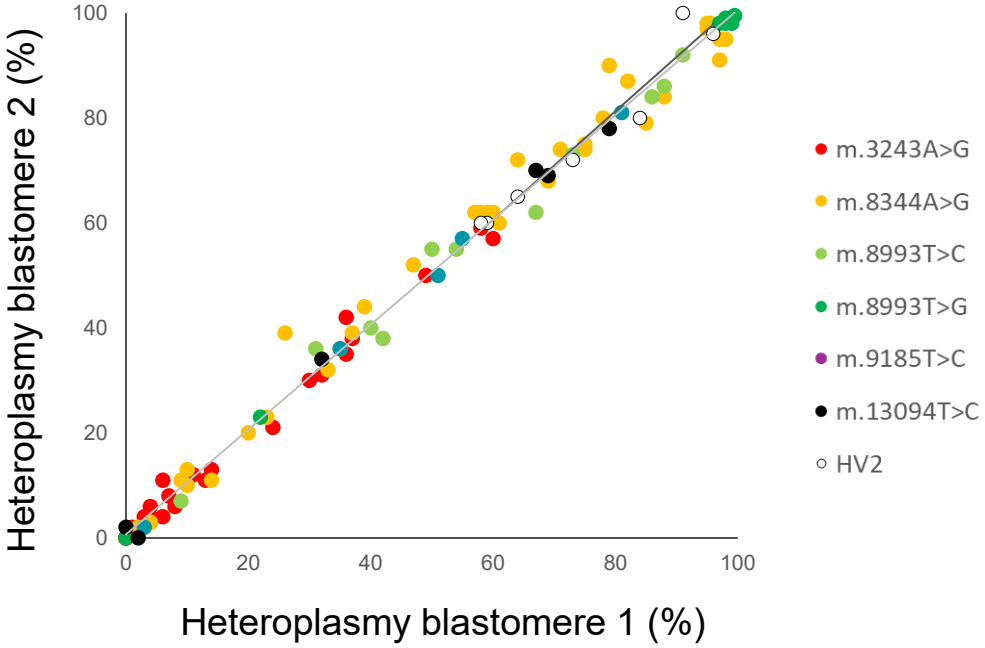
Inner Cell Mass (ICM) | Trophectoderm (TE) | Rest/Whole | Single cells of TE

Results: Day 3 vs Day 3 (cleavage stage)



Day 3

n=93



→Stability of heteroplasmy between blastomeres at Day 3

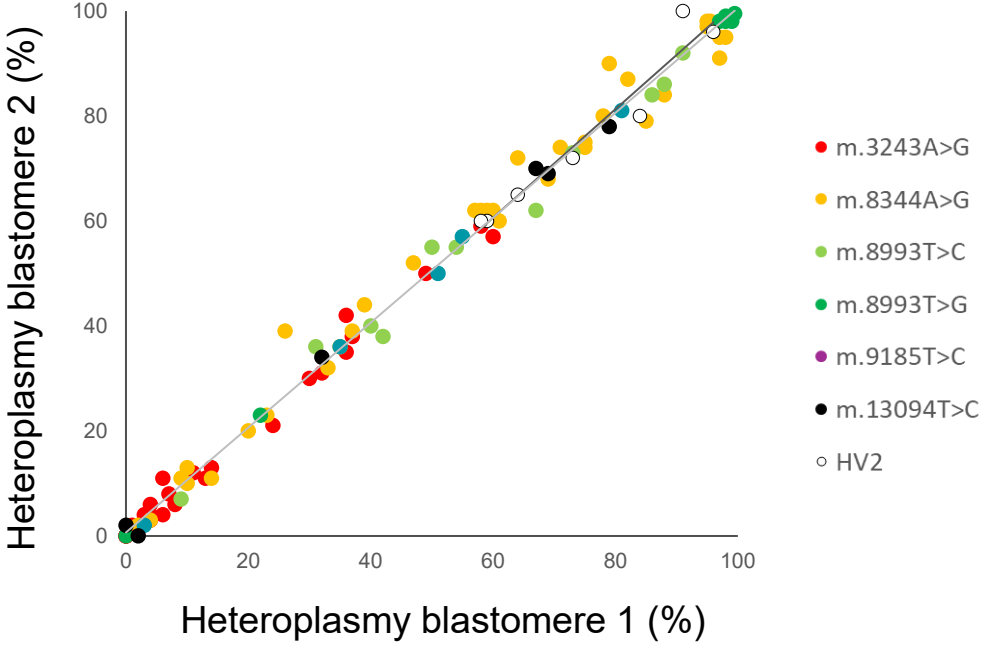
Results: Day 3 vs Day 3 (cleavage stage) and Day 4 vs Day 4 (morula)

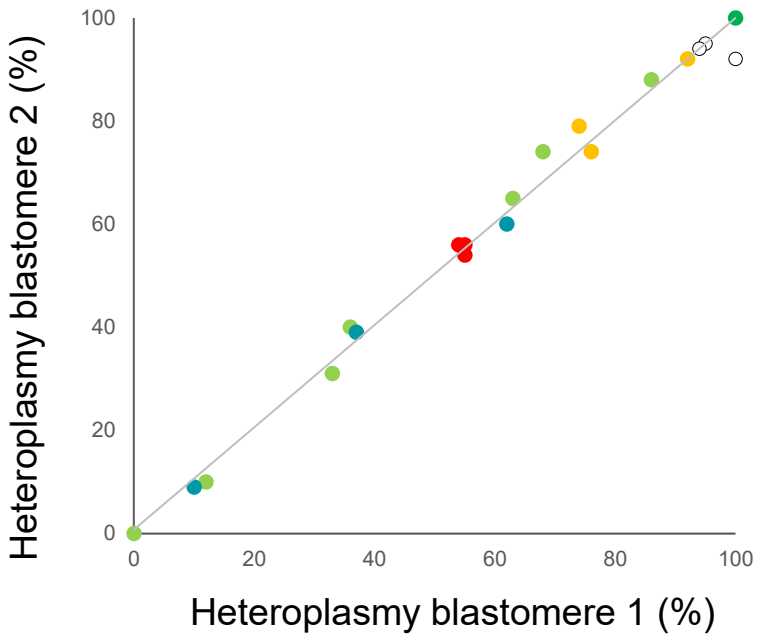
Day 3

n=93

Day 4

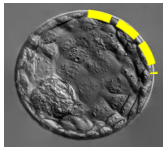
n=15



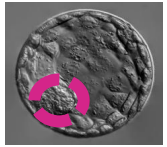


→Stability of heteroplasmy between blastomeres at Day 3 and between blastomeres at Day 4

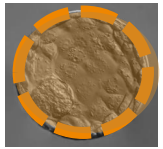
Results: Day 5/6 - Trophectoderm vs ICM / Rest



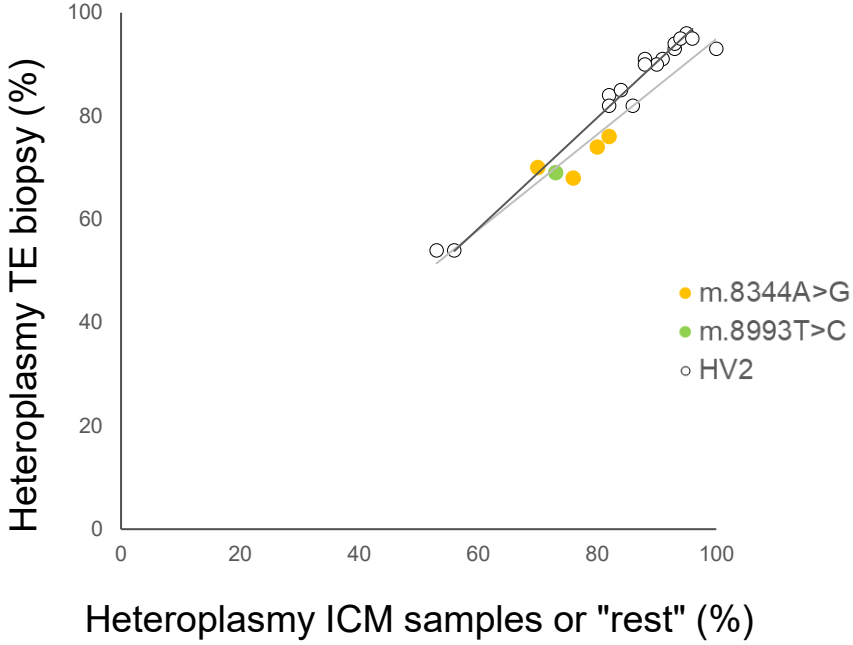
Trophectoderm
n=29



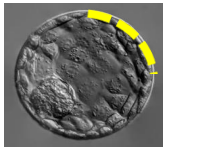
ICM
n=7



Rest
n=21



Results: Day 5/6 - Trophectoderm vs ICM / Rest



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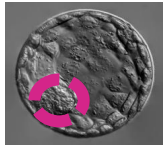

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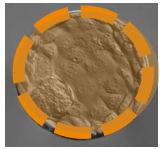

Rest
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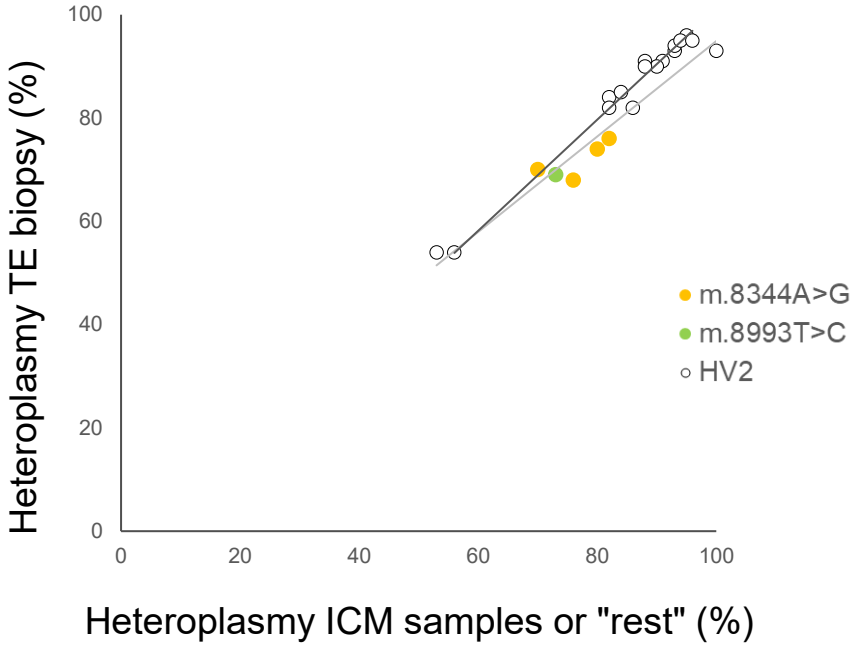
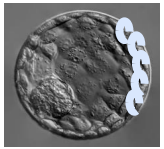
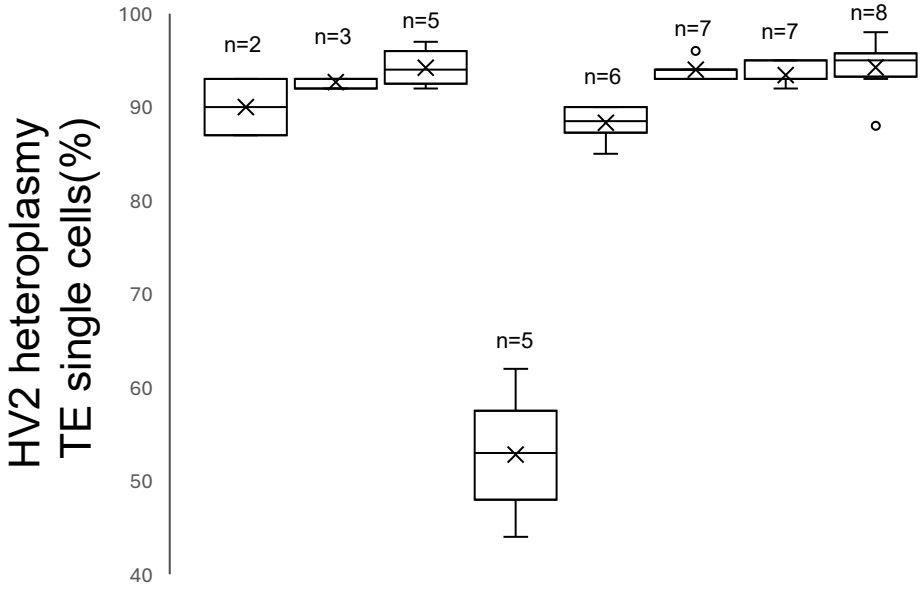


→Stability of heteroplasmy between different tissues of the blastocyst
→TE biopsy accurately represents the embryo

Results: Single Cells of Trophectoderm



Single cells of TE
8 control embryos
n=43



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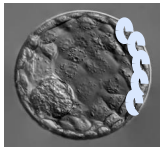

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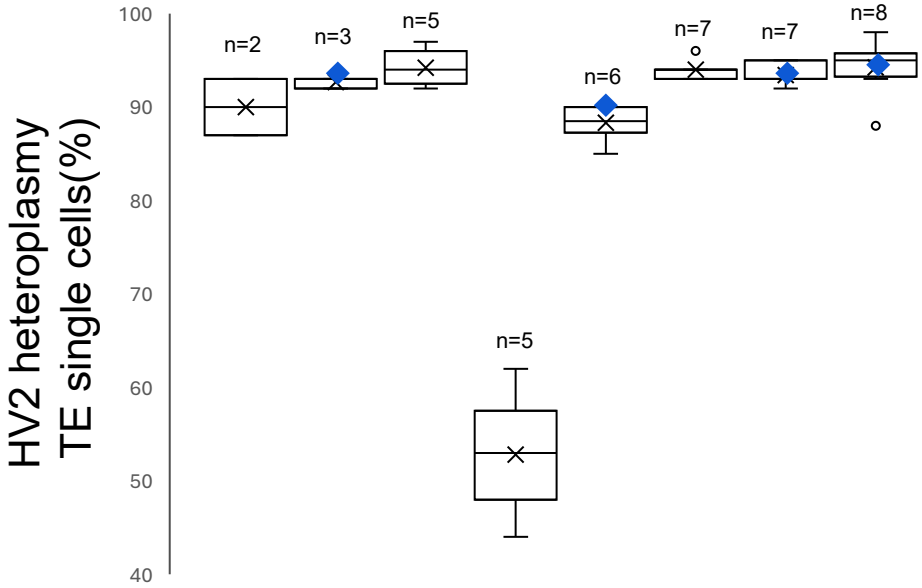
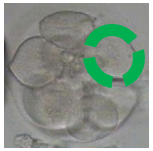
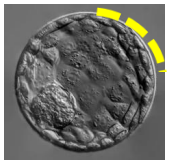


→Mostly stable heteroplasmy among single cells of trophectoderm

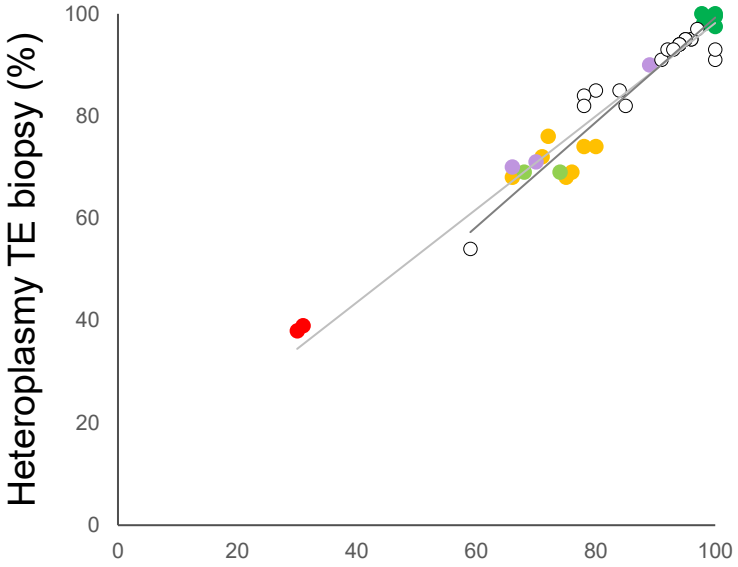
Results: Different time points - Cleavage/Morula vs. Blastocyst

Day 3/4

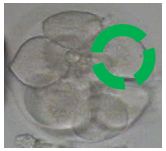


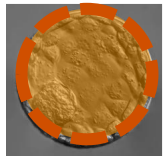


TE
n=25

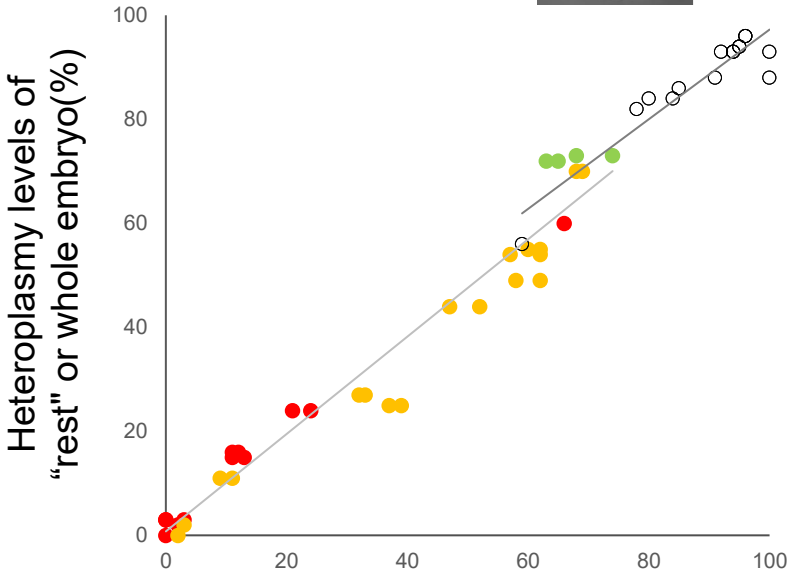


Day 3/4



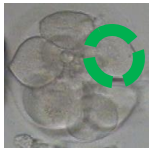


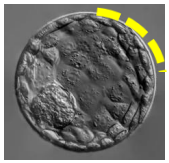
Rest
Whole
n=29



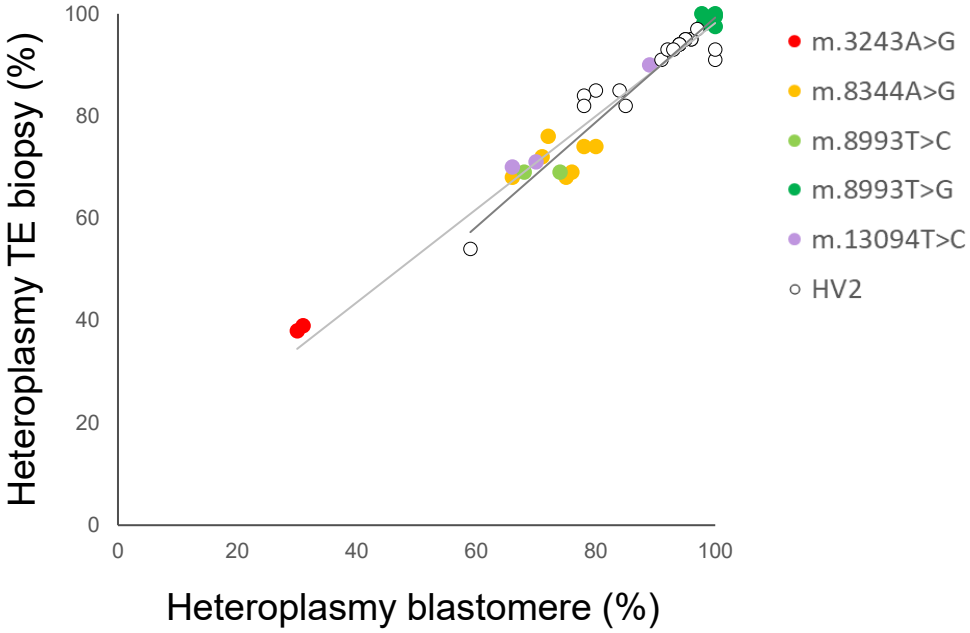
Results: Different time points - Cleavage/Morula vs. Blastocyst

Day 3/4





TE
n=25



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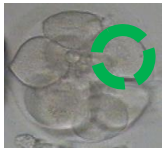

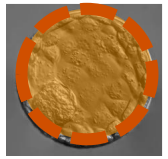

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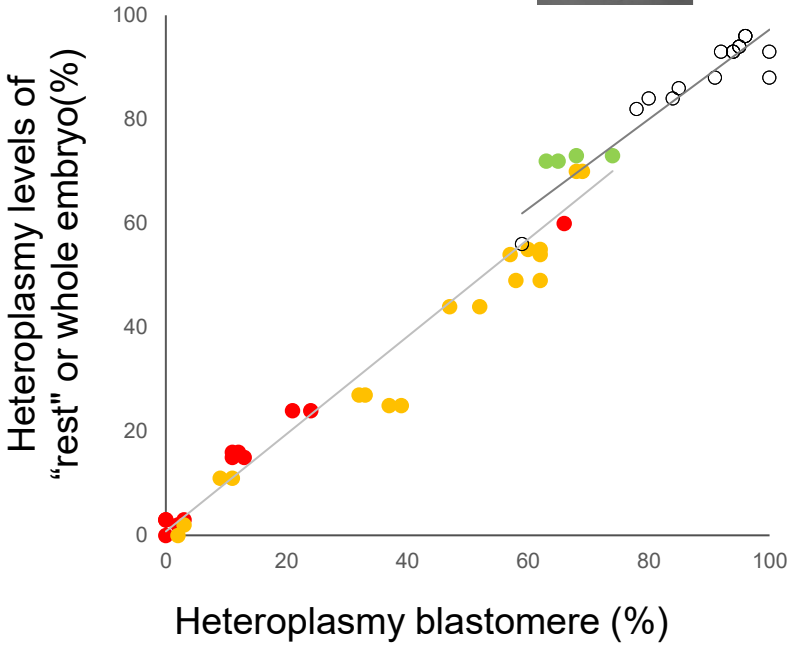


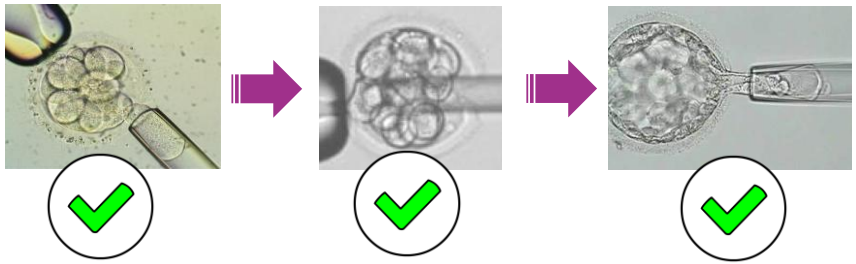
→Stability of heteroplasmy between different time points in preimplantation development

Conclusions

Prerequisites for **PGT-mt** is mutant load stability :

- among embryonic tissues/cells
- during preimplantation development

Day 3 Day 4 Day 5/6



<u>OUR RESULTS SUPPORT</u>:

Morula biopsy for PGT-mt
Trophectoderm biopsy for PGT-mt

Conclusions

Prerequisites for **PGT-mt** is mutant load stability :

- among embryonic tissues/cells
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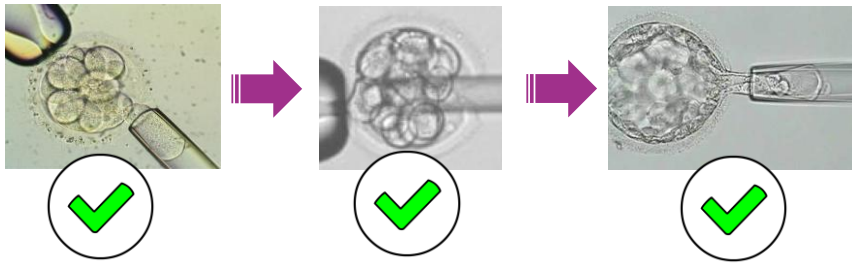
Day 3 Day 4 Day 5/6



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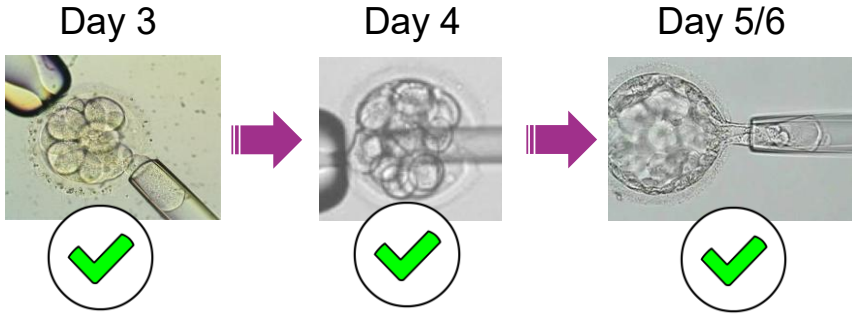

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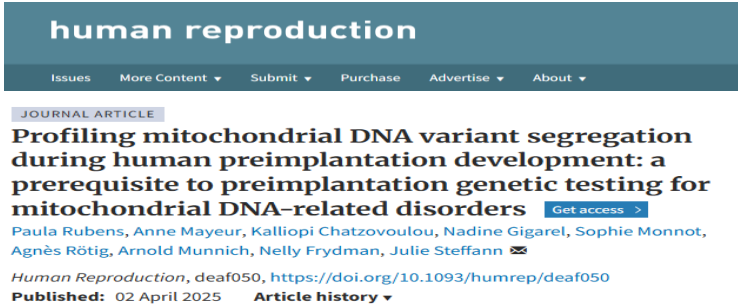
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Profiling mitochondrial DNA variant segregation during human preimplantation development: a prerequisite to preimplantation genetic testing for mitochondrial DNA–related disorders Get access

Paula Rubens, Anne Mayeur, Kalliopi Chatzovoulou, Nadine Gigarel, Sophie Monnot, Agnès Rötig, Arnold Munnich, Nelly Frydman, Julie Steffann

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Necker ENFANTS MALADES HÔPITAL UNIVERSITAIRE

Molecular Genetics Unit

Hôpital Antoine-Béclère AP-HP

Reproductive Biology Unit

Julie Steffann
Agnès Rötig
Anne Mayeur
Arnold Munnich
Kalliopi Chatzovoulou
Nadine Gigarel
Nelly Frydman
Sophie Monnot
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