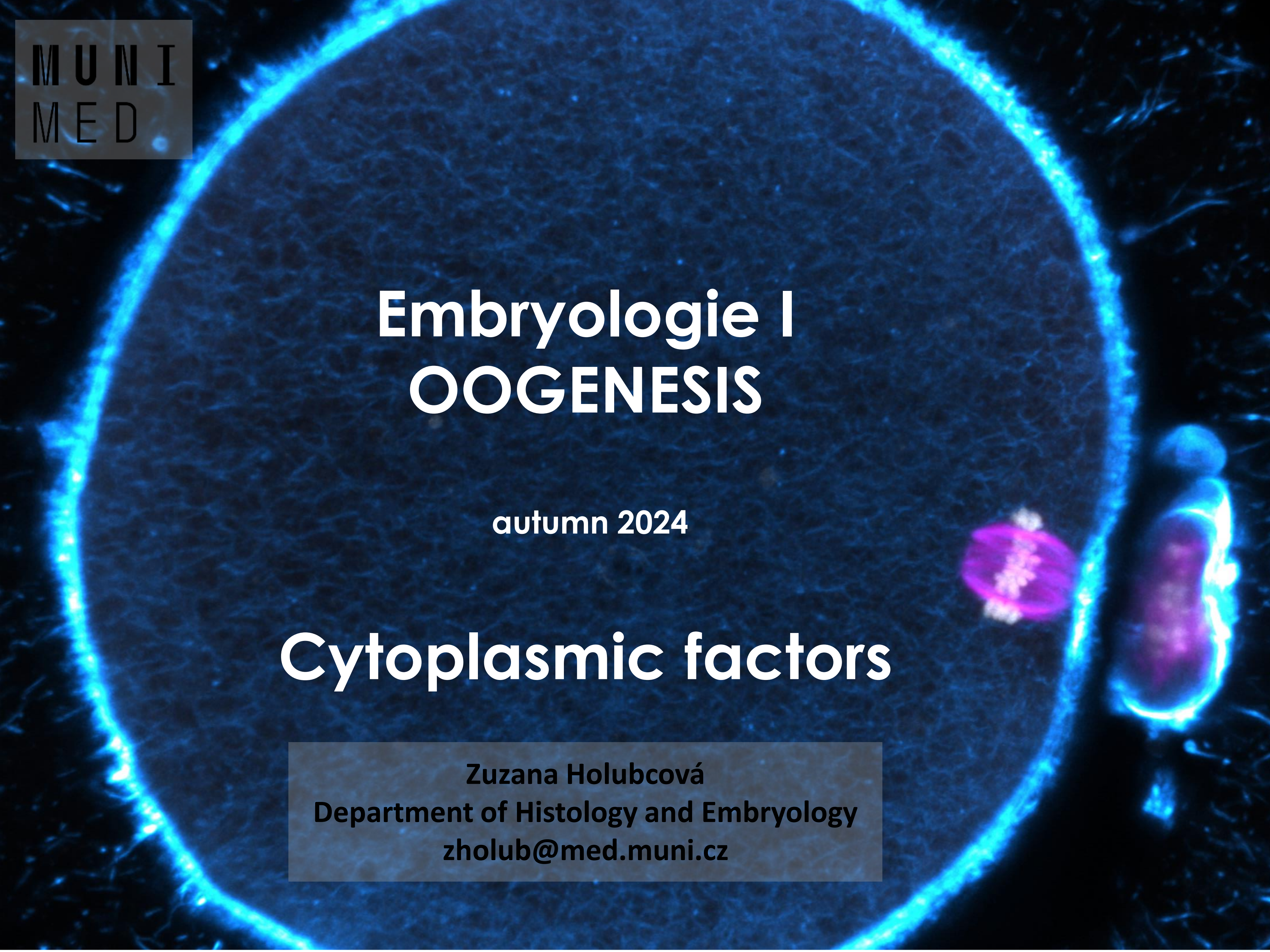

# Embryologie I
# OOGENESIS

autumn 2024

# Cytoplasmic factors


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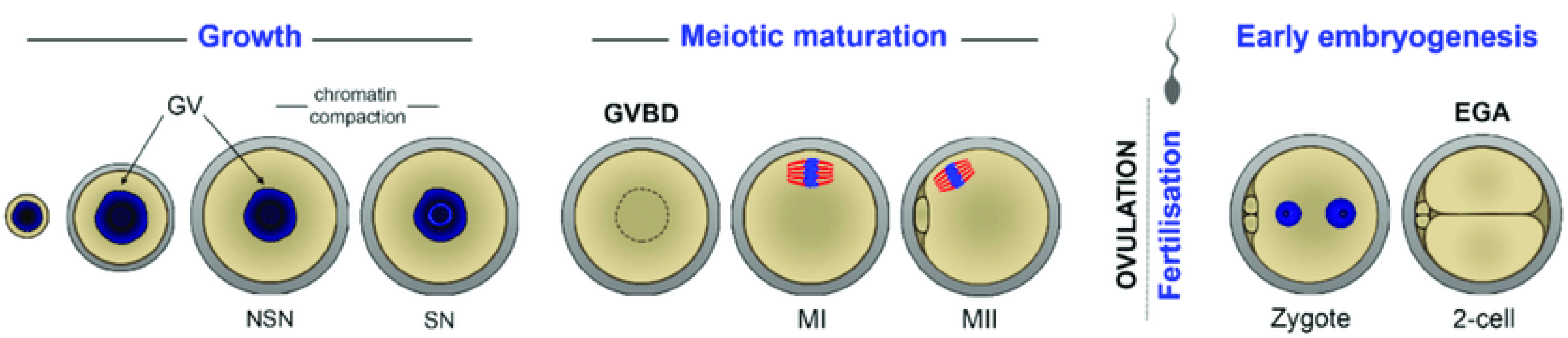
# Oogenesis

## Female gamete must acquire **functional competencies**

**1. Meiotic**
- capacity to reach the metaphase II arrest

**2. Activation**
- capacity to finish meiosis, block polyspermy, and form pronuclei at fertilisation

**3. Developmental**
- capacity to trigger and support embryonic development

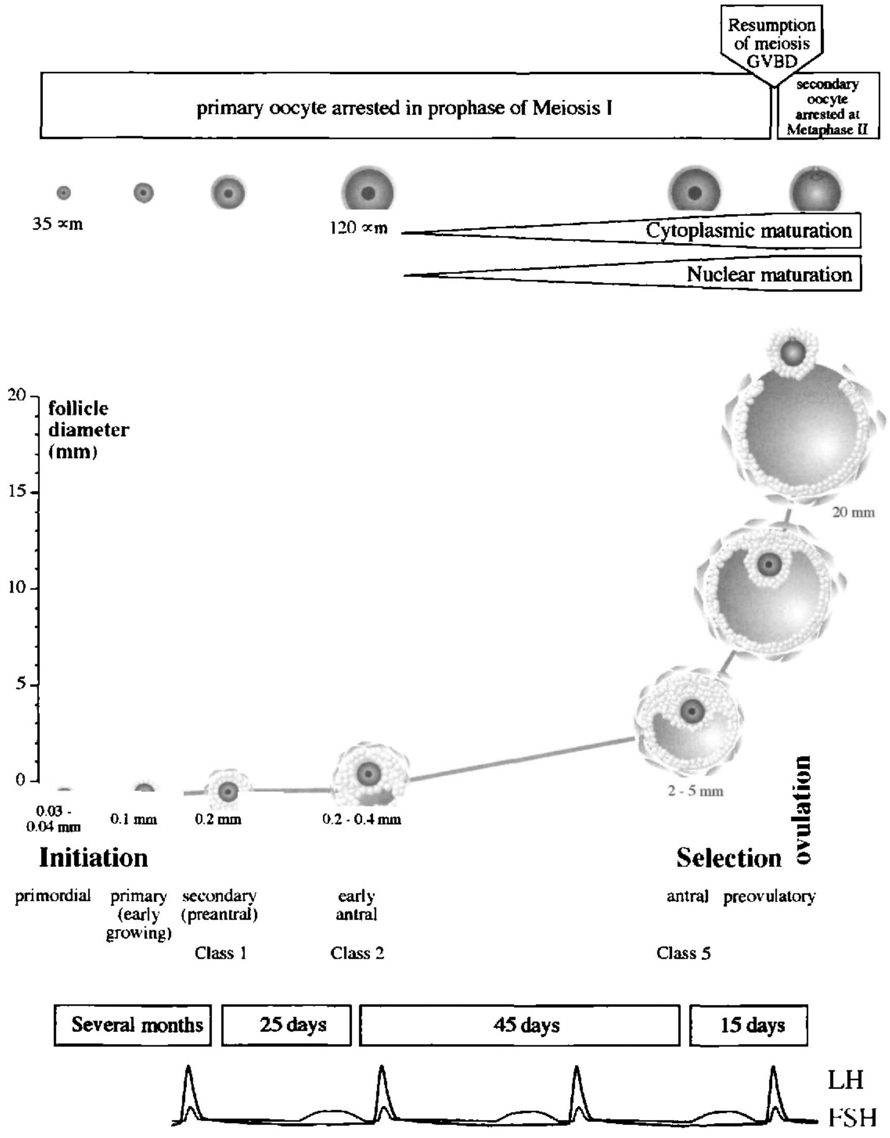


# Oogenesis

- **Dormant stage**
  - cellular quiescence (inactivity)

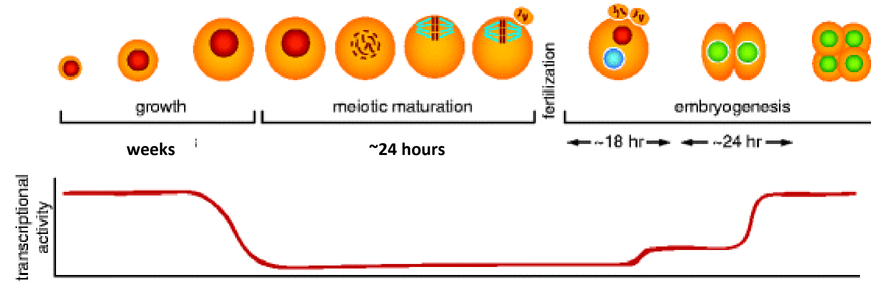
- **Growth stage**
  - synthesis of RNAs and proteins
  - intense accumulation of cellular material

- **Maturation stage**
  - **Nuclear maturation**
    - resumption of meiosis (release from prophase arrest)
    - chromosome segregation
    - MII arrest
    - capacity to complete meiosis II after activation
  - **Cytoplasmic maturation**
    - storage of maternal mRNA
    - modification organelles
    - global rearrangement of organelles




*Wright et al 2000*

# mRNA transcription and translation



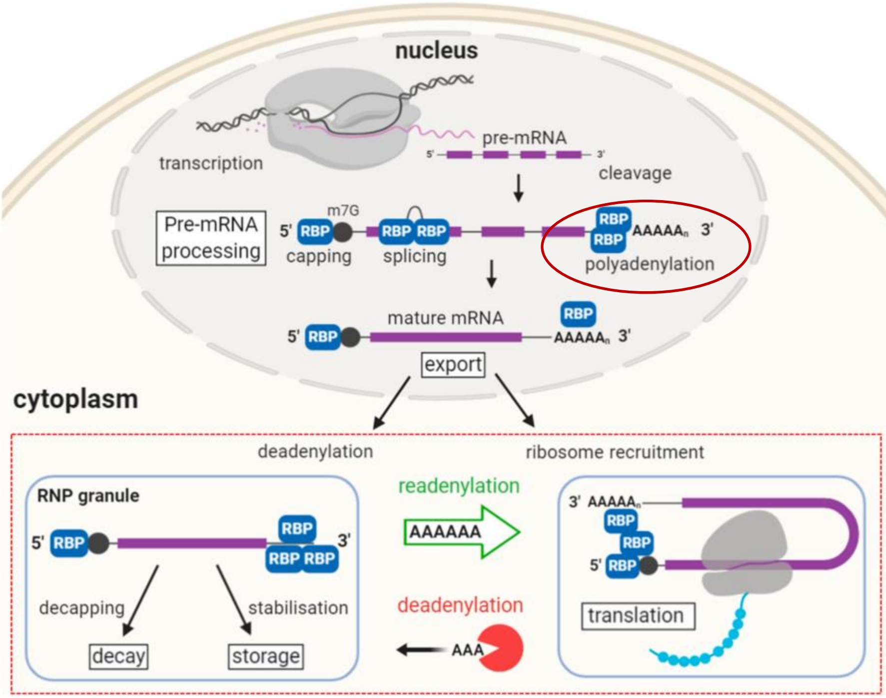

## ❑ Transcription

- executed by transcription factors
- regulated by epigenetic DNA modification
- active during growth phase
- silenced during oocyte maturation due to condensed chromatin state
- resumed only if fertization occurs = „embryonic genome activation - EGA“

## ❑ mRNA processing

- spatiotemporal control over activation, storage and degradation of transcripts
- translation is mediated by RNA-binding proteins (RBP) that recruit/deter actors of protein synthesis machinery

- polyadenylation of 3´end by Poly(A) Polymerase (PAP)
- long poly(A) tail favours efficient translation and protects mRNA from degradation
- deadenylation slows translation and initiates mRNA degradation

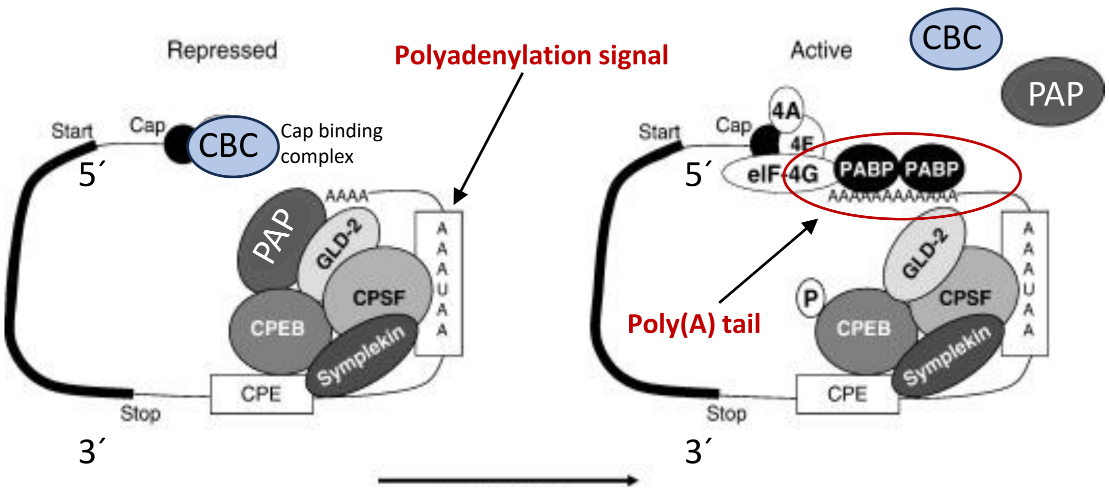


# mRNA transcription and translation

## ❑ mRNA processing

- the 5′ end of the nascent RNA molecule receives a 7-methylguanosine cap and bounds with nuclear cap-binding complex (CBC)

- molecular complex containing PAP binds the AAUAAA sequence (polyadenylation signal)




- PAP catalyses the addition of adenine bases to the 3′ terminus forming the 3′ poly-A tail of around 200 adenine nucleotides

- poly(A)-binding protein (PABP) binds to the poly(A) tail, protecting transcript from degradation and playing a major role in translation initiation

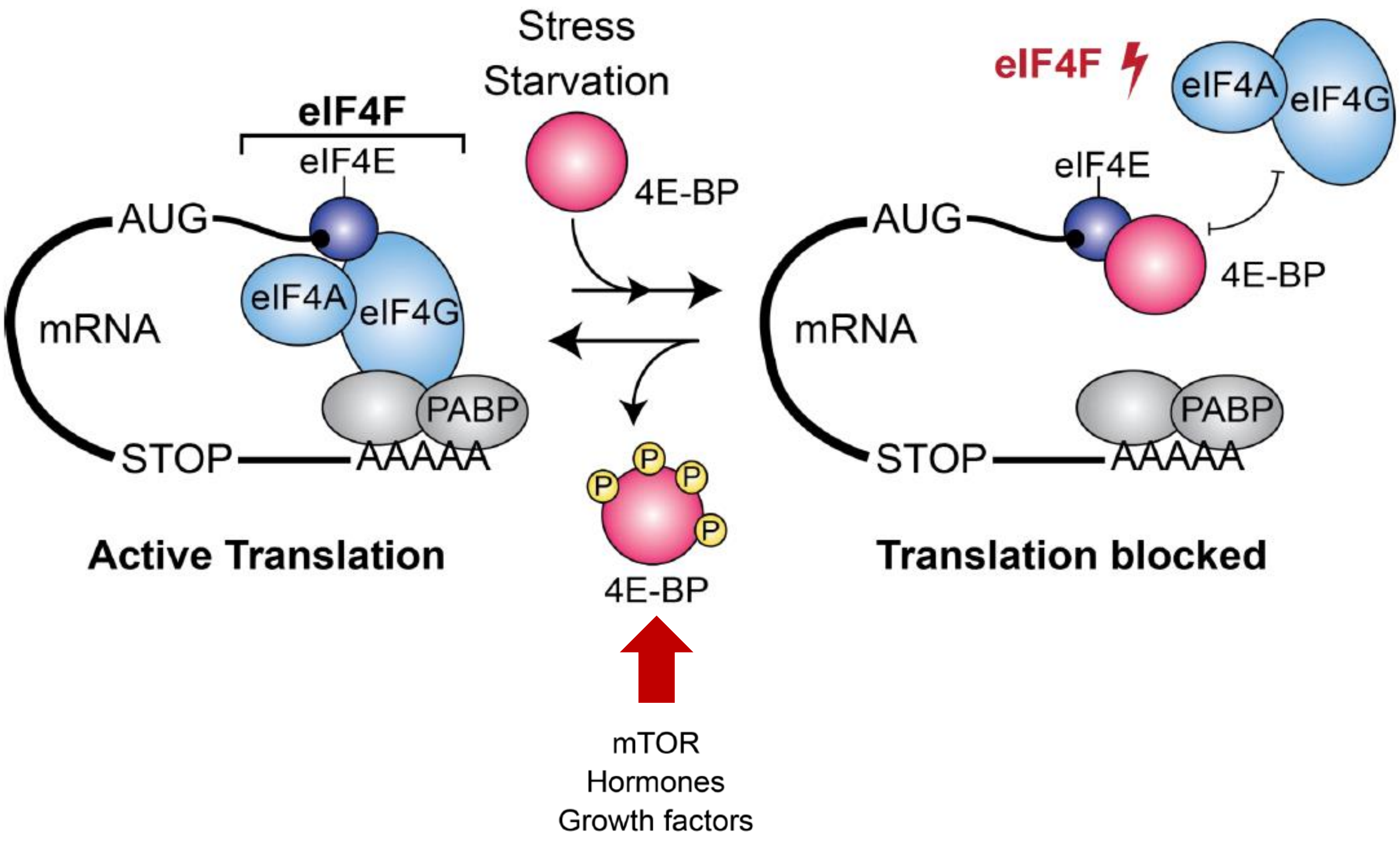
- the mature mRNA and its associated proteins, forming a ribonucleoprotein (RNP) complex, are exported from the nucleus to the cytoplasm

# mRNA transcription and translation

- **mRNA translation**

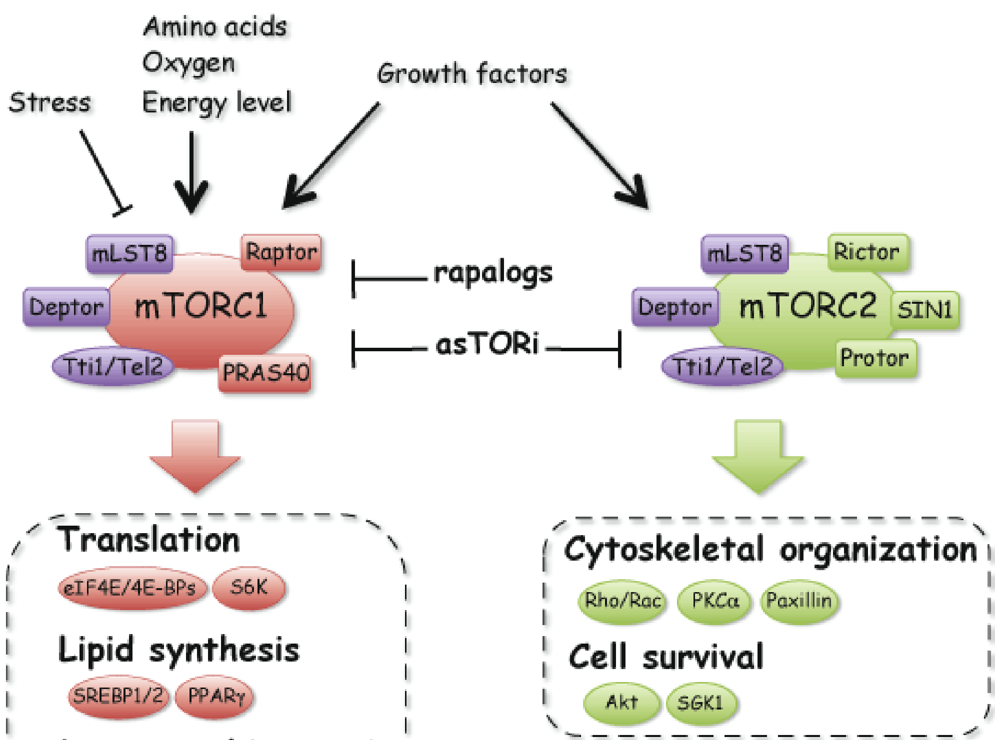
  - spatially and temporally controlled translation of these stored mRNAs

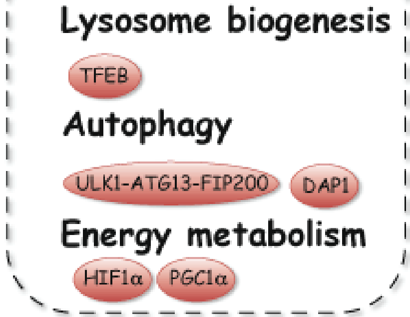
    (e.g. meiotic genes silencing in antral stage oocytes)



# mRNA transcription and translation

## ❑ mRNA translation

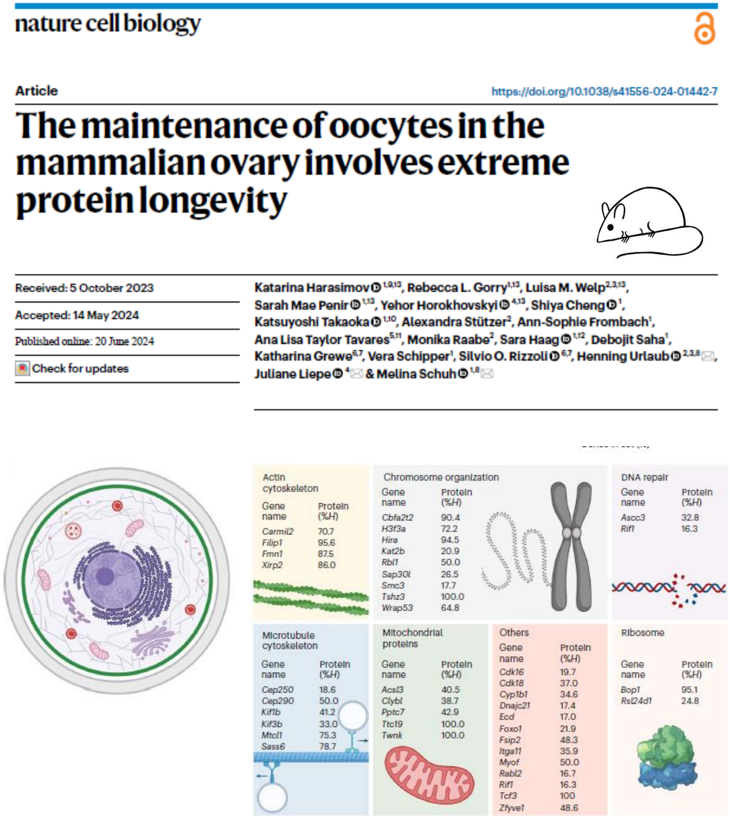



*Tsukumo et al 2014*

-translation driven by mTOR activity

mTOR = mammalian target of rapamycin
mTORC1/mTORC2 – complex 1/compex 2




nature cell biology

Article https://doi.org/10.1038/s41556-024-01442-7


**The maintenance of oocytes in the mammalian ovary involves extreme protein longevity**


Received: 5 October 2023

Accepted: 14 May 2024

Published online: 20 June 2024

Check for updates



Katarina Harasimov[1,9,13], Rebecca L. Gorry[1,13], Luisa M. Welp[2,3,13], Sarah Mae Penir[1,13], Yehor Horokhovskyi[4,13], Shiya Cheng[1], Katsuyoshi Takaoka[1,10], Alexandra Stützer[2], Ann-Sophie Frombach[1], Ana Lisa Taylor Tavares[5,11], Monika Raabe[2], Sara Haag[1,12], Debojit Saha[1], Katharina Grewe[6,7], Vera Schipper[1], Silvio O. Rizzoli[6,7], Henning Urlaub[2,3,8], Juliane Liepe[4] & Melina Schuh[1,8]


Actin cytoskeleton

| Gene name | Protein (%H) |
|---|---|
| Carmil2 | 70.7 |
| Filip1 | 95.6 |
| Fmn1 | 87.5 |
| Xirp2 | 86.0 |

Chromosome organization

| Gene name | Protein (%H) |
|---|---|
| Cbfa2t2 | 90.4 |
| H3f3a | 72.2 |
| Hira | 94.5 |
| Kat2b | 20.9 |
| Rbl1 | 50.0 |
| Sap30l | 26.5 |
| Smc3 | 17.7 |
| Tshz3 | 100.0 |
| Wrap53 | 64.8 |

DNA repair

| Gene name | Protein (%H) |
|---|---|
| Ascc3 | 32.8 |
| Rif1 | 16.3 |

Microtubule cytoskeleton

| Gene name | Protein (%H) |
|---|---|
| Cep250 | 18.6 |
| Cep290 | 50.0 |
| Kif1b | 41.2 |
| Kif3b | 33.0 |
| Mtcl1 | 75.3 |
| Sass6 | 78.7 |

Mitochondrial proteins

| Gene name | Protein (%H) |
|---|---|
| Acsl3 | 40.5 |
| Clybl | 38.7 |
| Pptc7 | 42.9 |
| Ttc19 | 100.0 |
| Twnk | 100.0 |

Others

| Gene name | Protein (%H) |
|---|---|
| Cdk16 | 19.7 |
| Cdk18 | 37.0 |
| Cyp1b1 | 34.6 |
| Dnajc21 | 17.4 |
| Ecd | 17.0 |
| Foxo1 | 21.9 |
| Fsip2 | 48.3 |
| Itga11 | 35.9 |
| Myof | 50.0 |
| Rabl2 | 16.7 |
| Rif1 | 16.3 |
| Tcf3 | 100 |
| Zfyve1 | 48.6 |

Ribosome

| Gene name | Protein (%H) |
|---|---|
| Bop1 | 95.1 |
| Rsl24d1 | 24.8 |

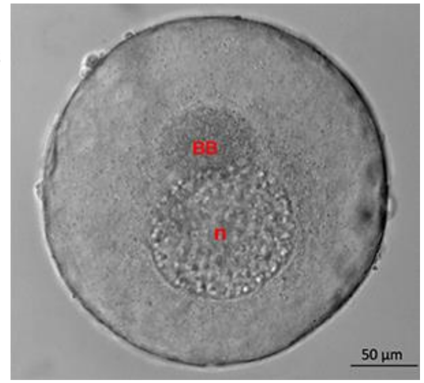
- mammalian oocytes and ovarian somatic cells contain large number of very longed-lived proteins
- role of chaperones and cellular antioxidants

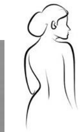
*Harasimov et al 2023*

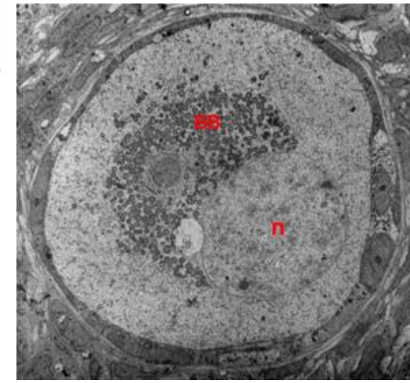
# Storage of mRNA

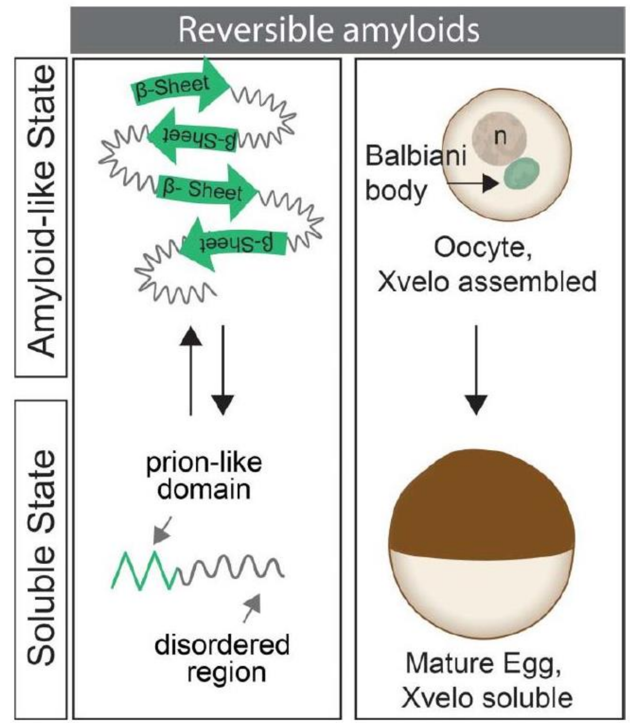
## ❖ Balbiani body

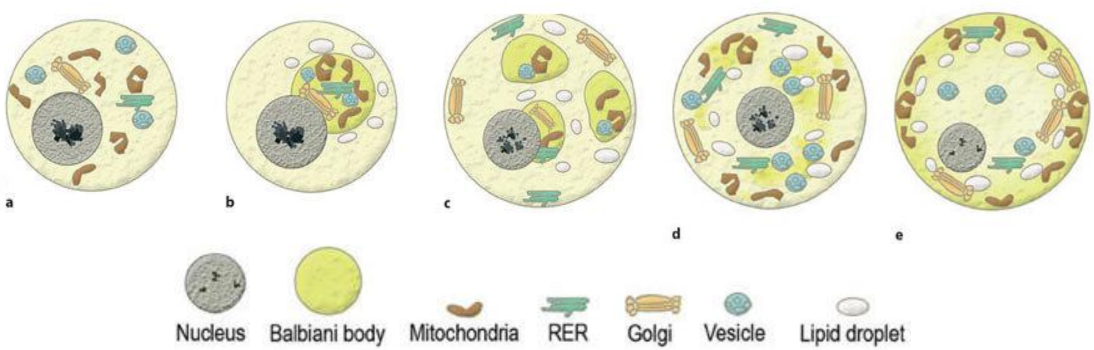
- transient membraneless organelle found in immature dormant oocytes of diverse species and typically dispersed during development
- adjacent to nucleus facing vegetal pole
- giant clump of RNA, proteins and organelles, embedded in a dense network of amyloid fibers
- storage of mRNA granules during prolonged development?






*Rodler and Sinowatz 2013*

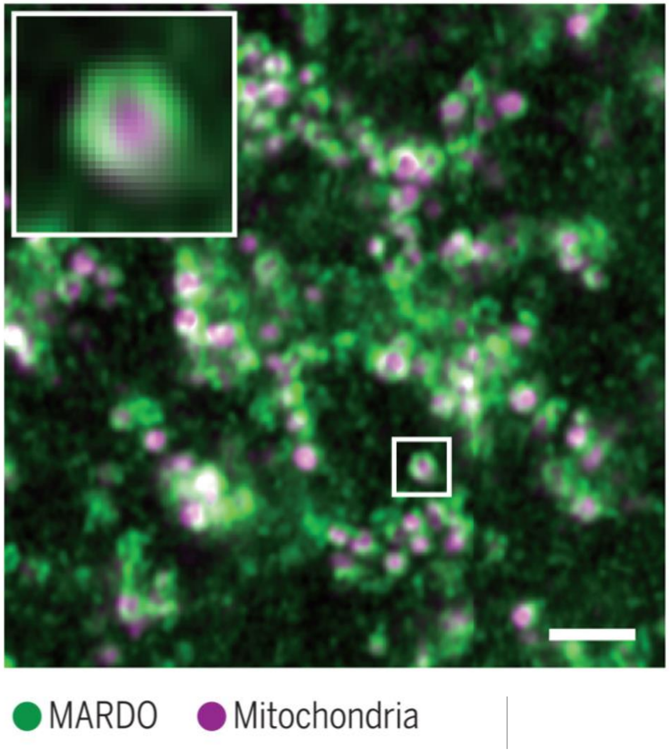
*Boke et al 2016*

# Storage of mRNA

## ❖ MARDO

= mitochondria-associated ribonucleoprotein domain

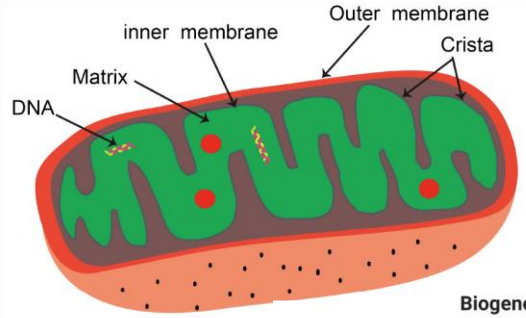
- membrane-less compartment with hydrogel-like properties located around Mts
- stores transiently transcriptionally repressed maternal mRNAs
- assembly **driven by RNA-binding** protein ZAR1 which clusters the Mts and protects the mRNAs against degradation
- dissolution in mature mammalian eggs ensures timely degradation of maternal mRNAs

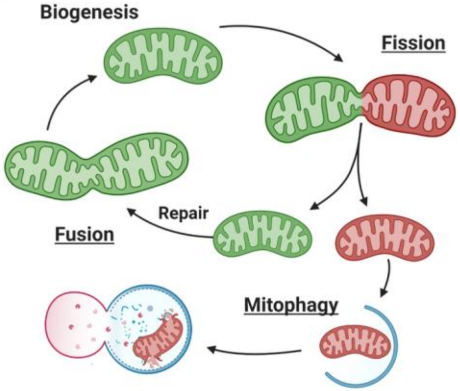



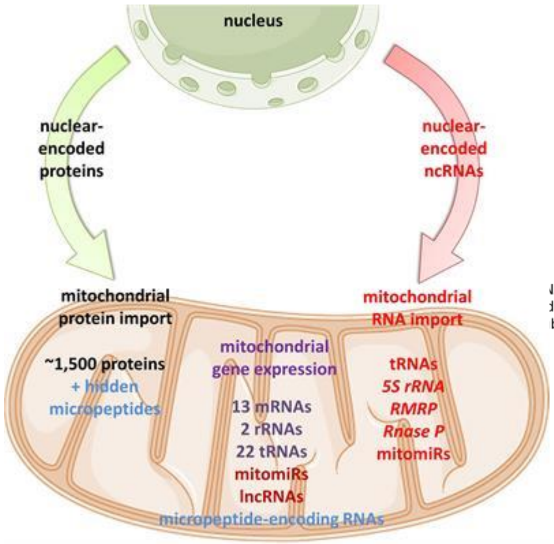

*Cheng et al, 2022*

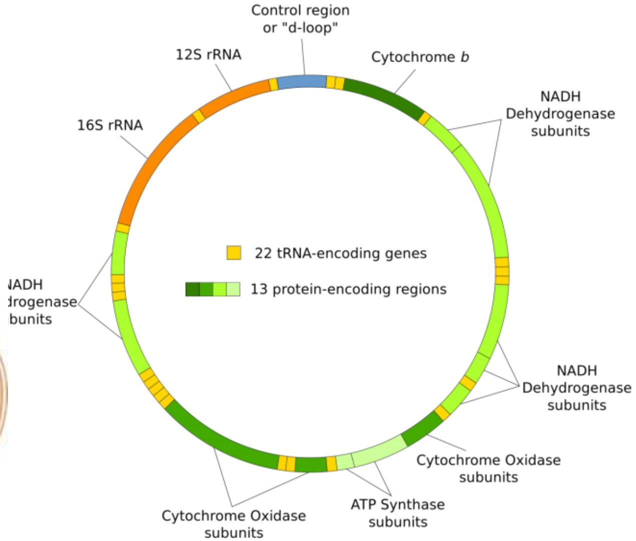
# Mitochondria

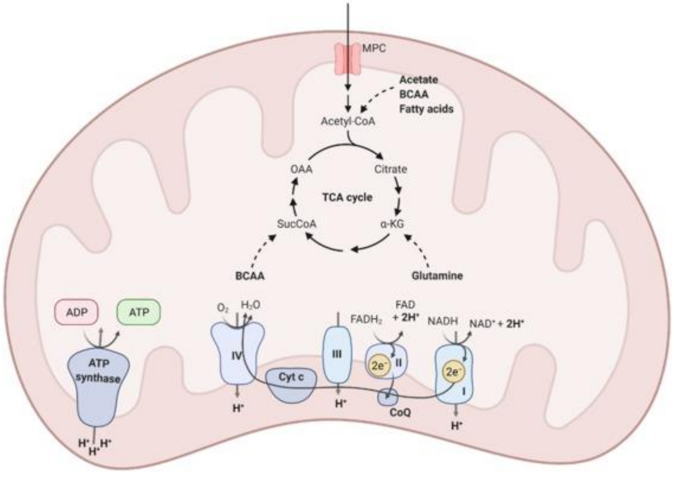
- semiautonomous double membrane- bound organelle producing energy
- production of 90% ATP necessary for cellular function via oxidative phosphorylation (OXPHOS)
- endosymbiotic origin
- circular mtDNA (16 569 bp encoding 37 genes: 13 polypeptides, 2rRNA, 22tRNAs)
- bioenergetic capacity optimized by fusion (enlargement) x fission (fragmentation)



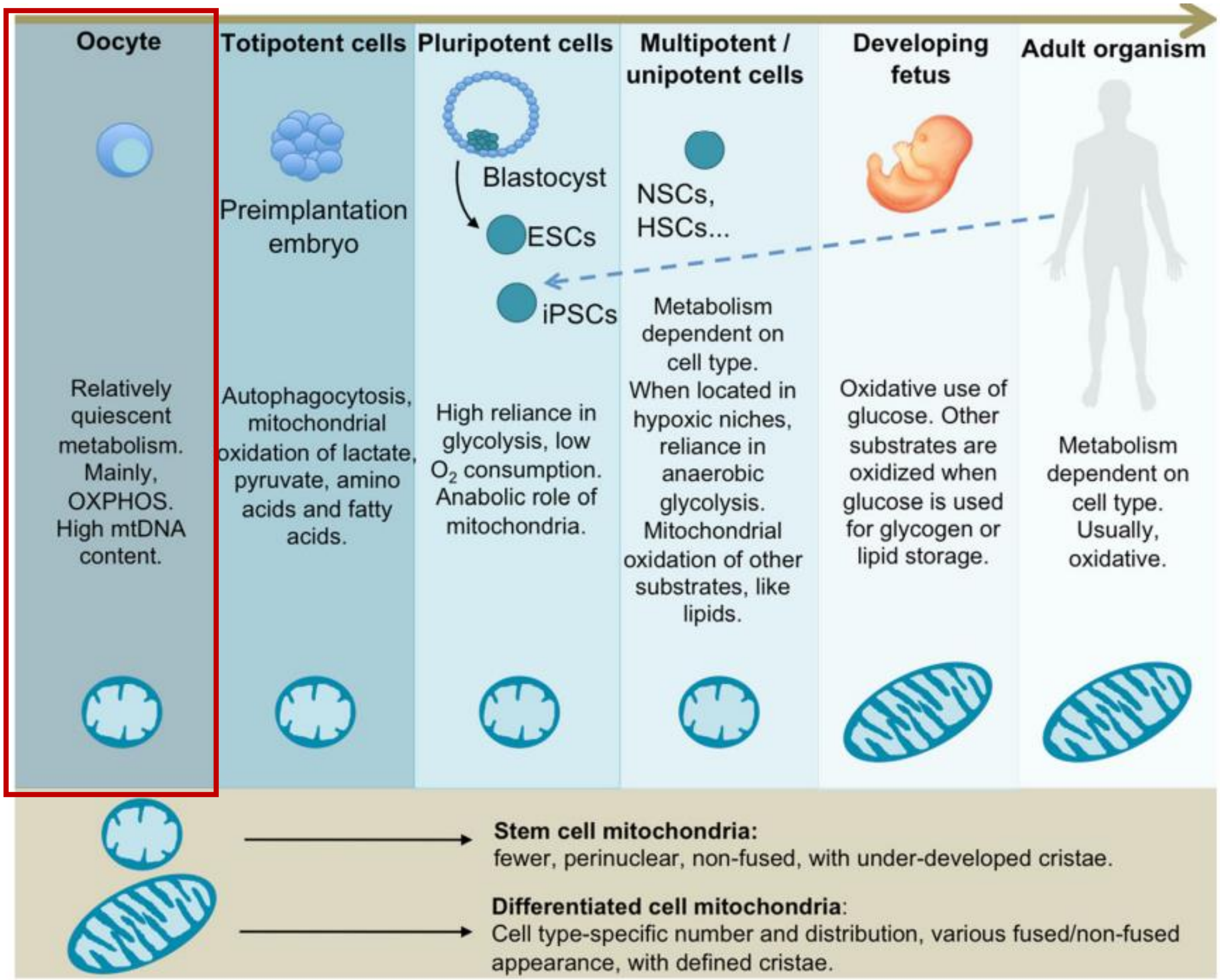



# Mitochondria

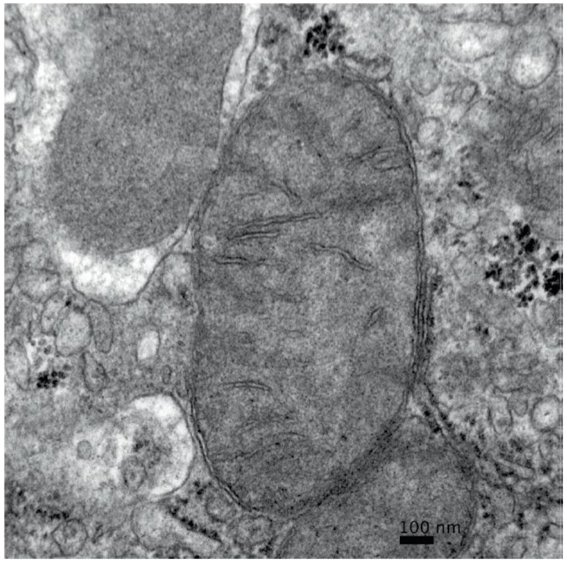
Atypical „primitive“ mitochondria

Somatic type mitochondria

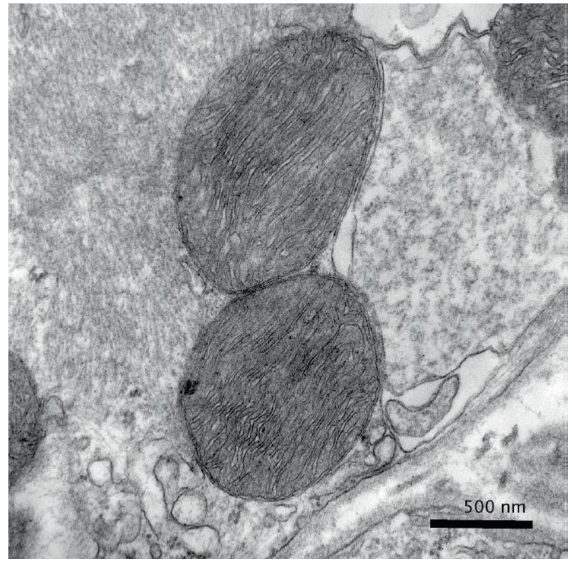



*Prigione et al 2015*

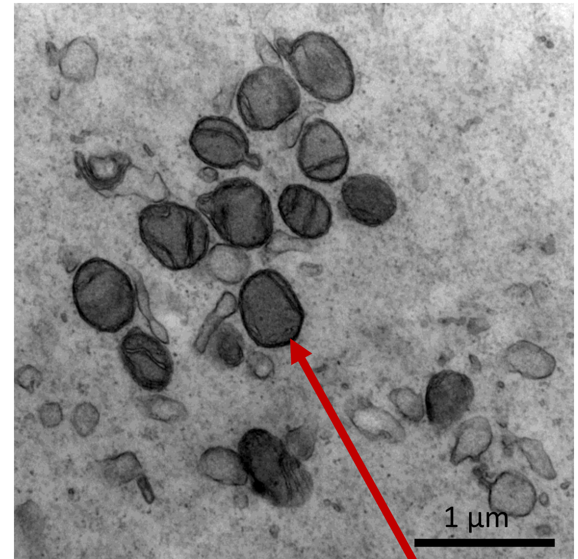
# Mitochondria



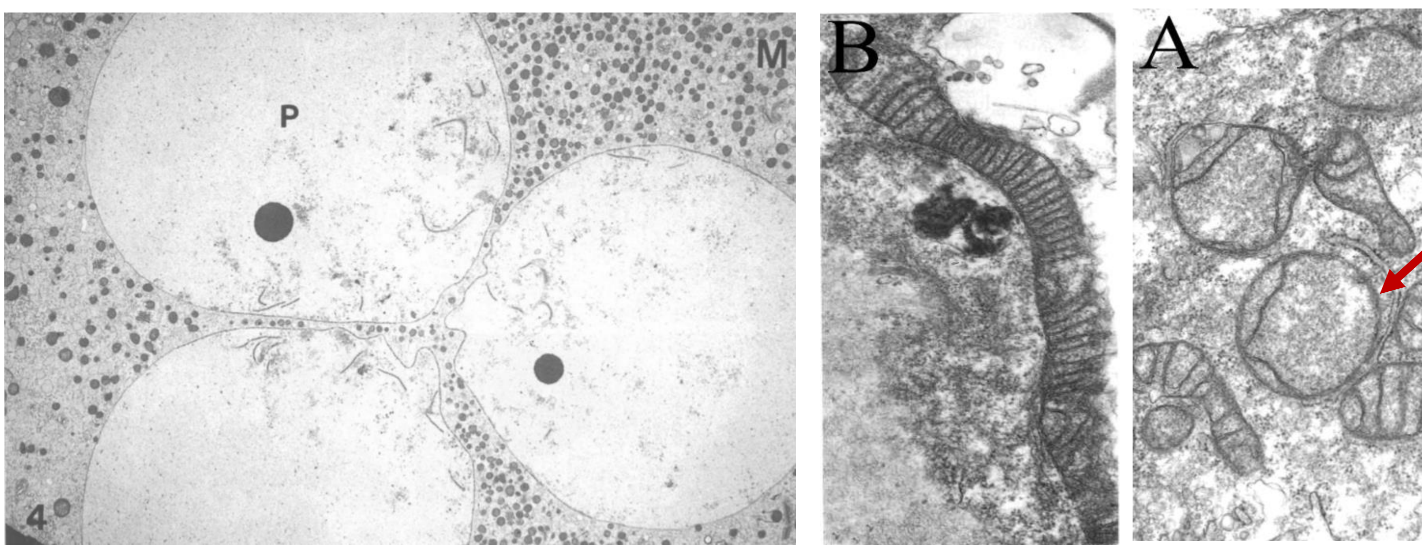

MOUSE LIVER




MOUSE HEART




HUMAN OOCYTE

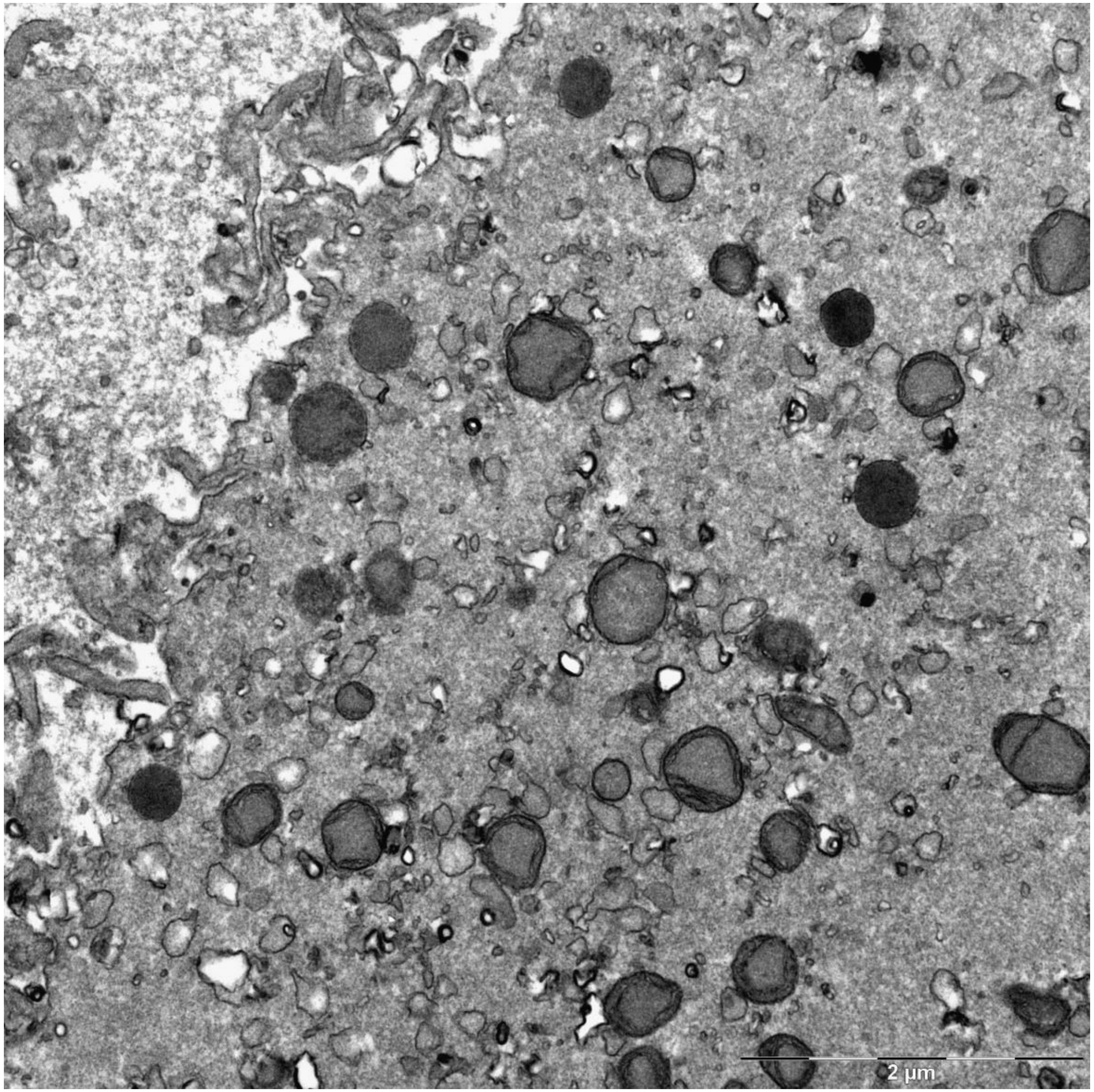


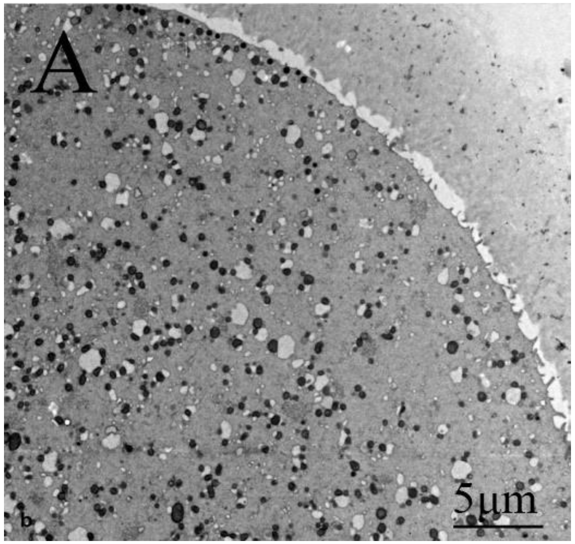

HUMAN EMBRYOS

Atypical „primitive“ mitochondria

- rounded/oval shape
- parallel/circular cristae

# Oocyte mitochondria

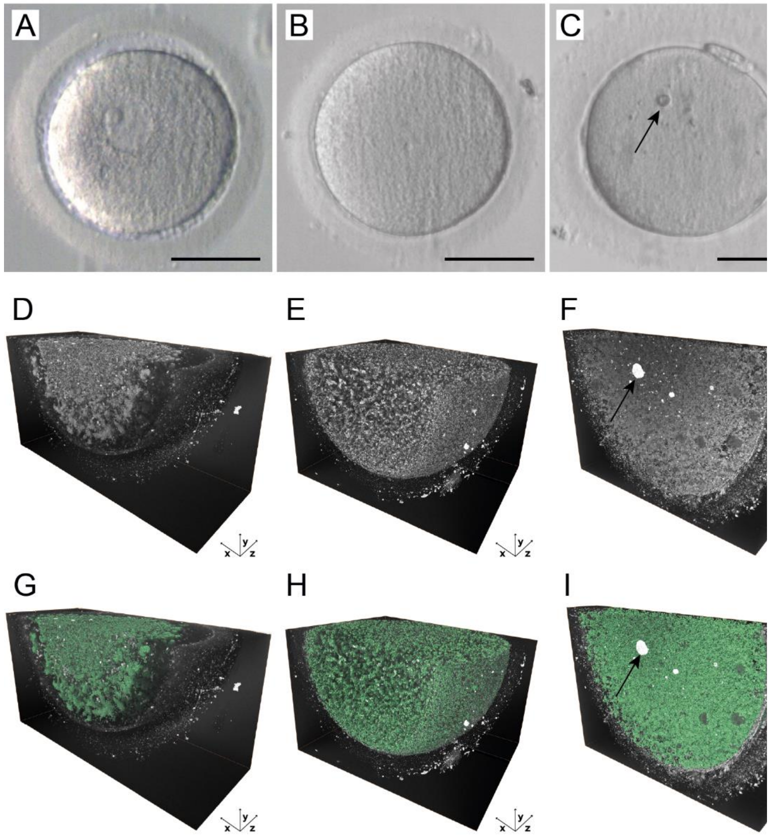


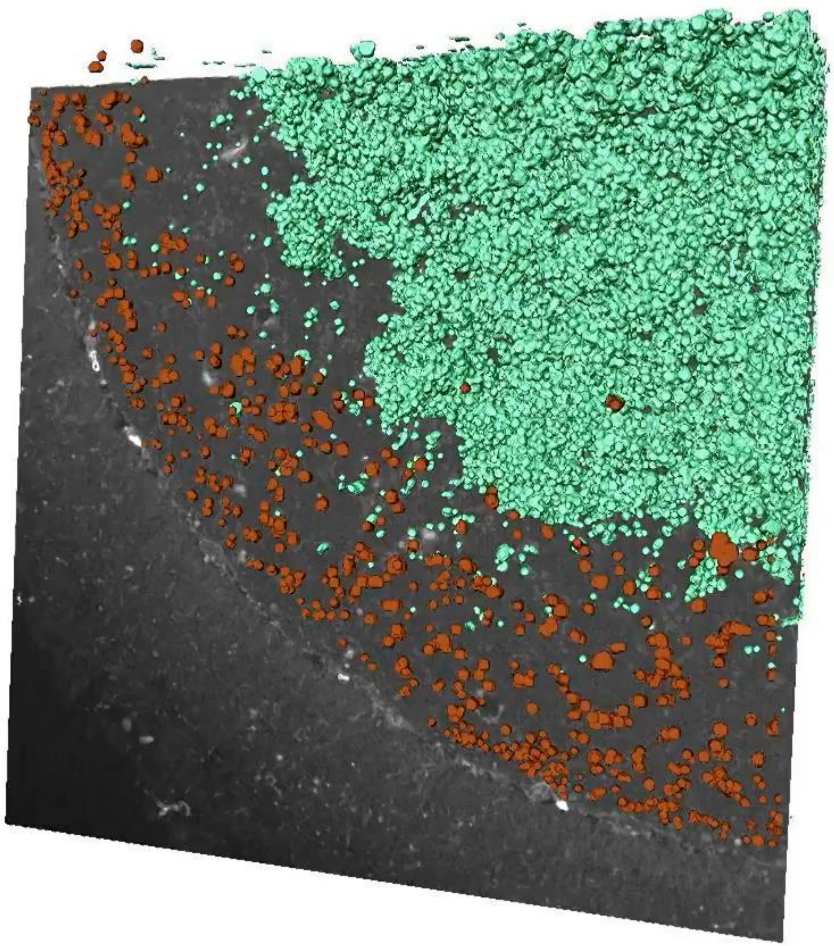




**Trebichalská, Z.** (2020). *Analýza ultrastrukturních znaků lidských oocytů. Brno, 2020. Diplomová práce. Masarykova univerzita, Přírodovědecká fakulta.*

# Oocyte mitochondria

Mitochondria are the most abundant oocyte organelle



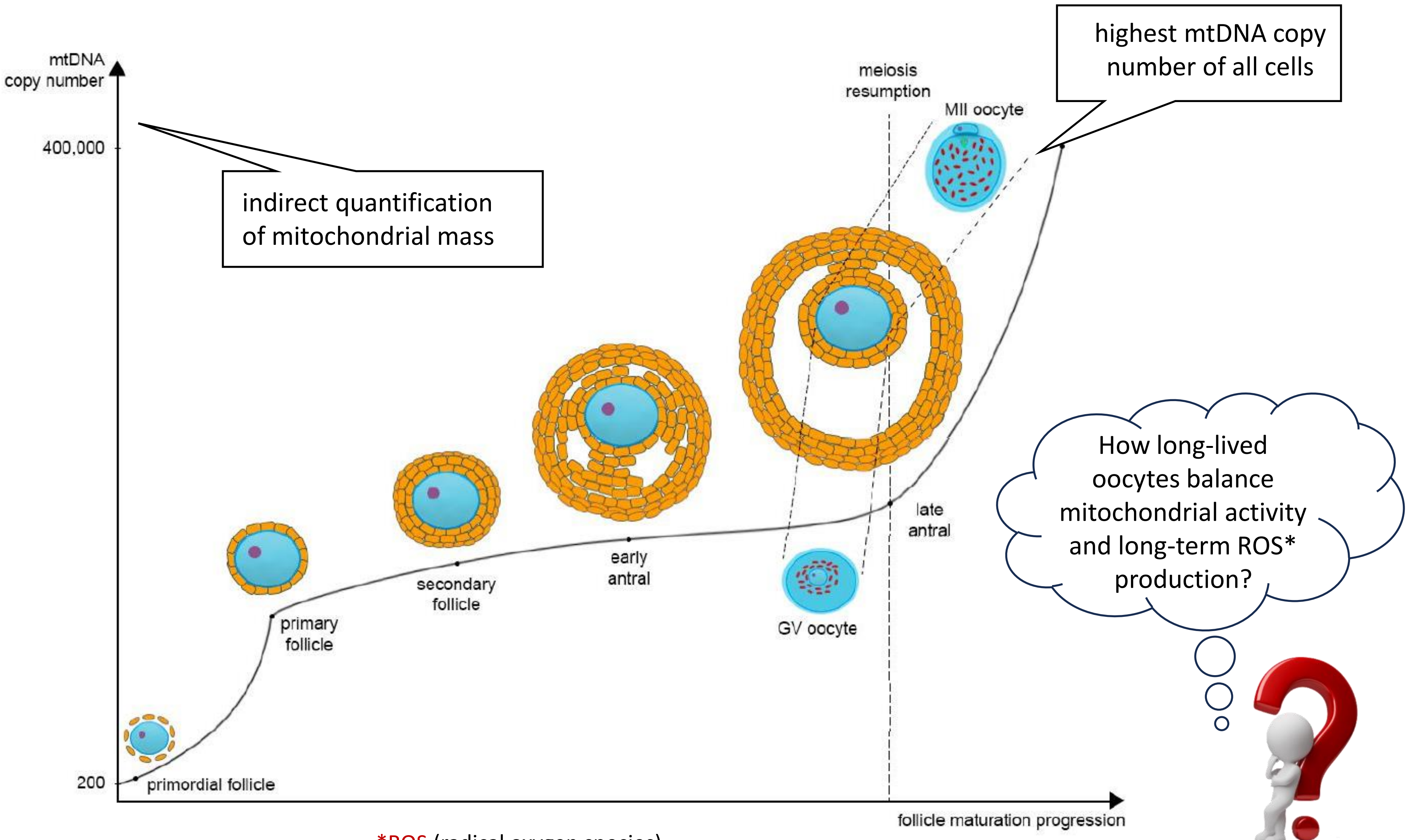



**GV oocyte**

Mitochondrion constitute 4-5 % of oocyte volume

*Trebichalska et al, 2021*

# Oocyte mitochondria




*ROS (radical oxygen species)

- by-products of mitochondrial activity
- cause of oxidative damage, mutagenesis and apoptosis

*Kirillova et al 2021*

# Oocyte mitochondria

- early oocyte avoid accumulation of ROS by a eliminating complex I of electron transport chain

- keeping complex I shut down (but rest of OXPHOS functional) during dormancy enables to avoid ROS built up

- functional complex I assembled in final stages of oogenesis

Article | Open Access | Published: 20 July 2022

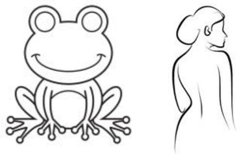
**Oocytes maintain ROS-free mitochondrial metabolism by suppressing complex I**

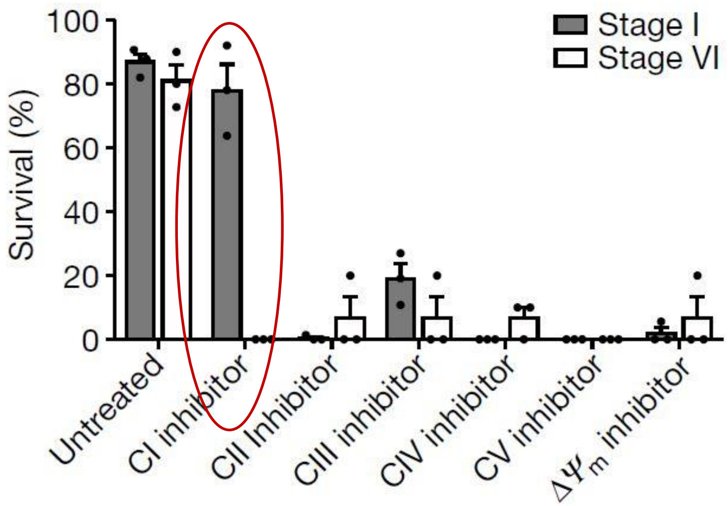
Aida Rodríguez-Nuevo, Ariadna Torres-Sanchez, Juan M. Duran, Cristian De Guirior, Maria Angeles Martínez-Zamora & Elvan Böke

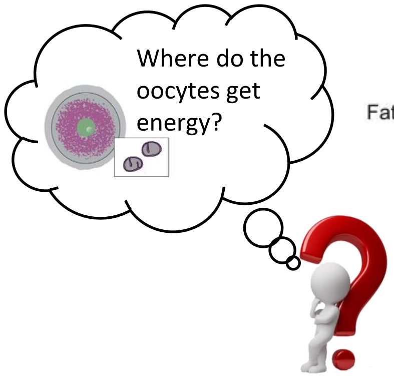
*Nature* **607**, 756–761 (2022) | Cite this article

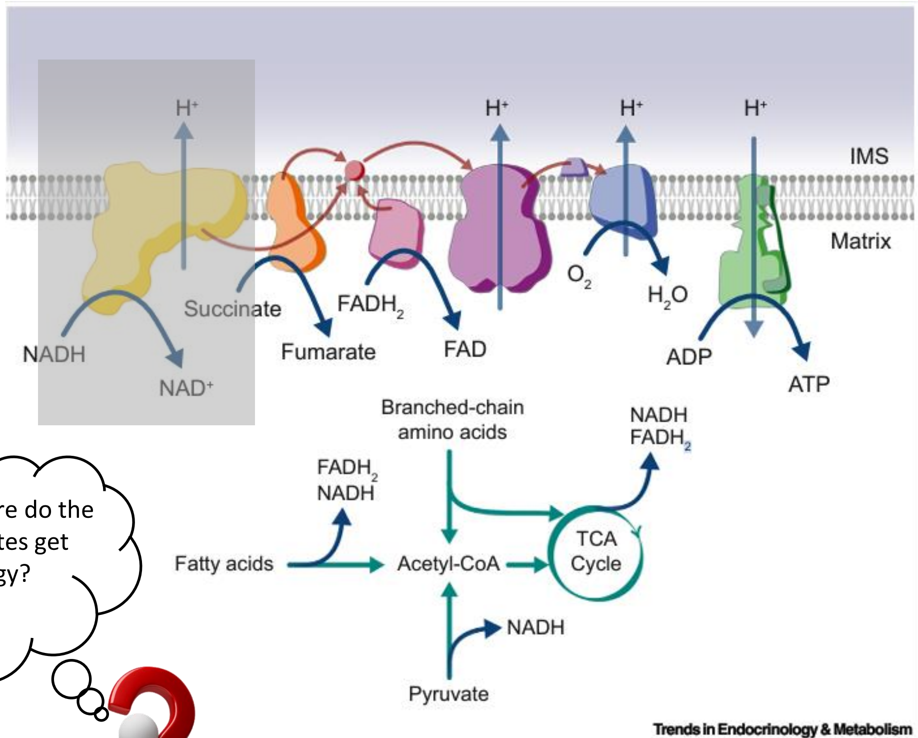


Elvan Böke





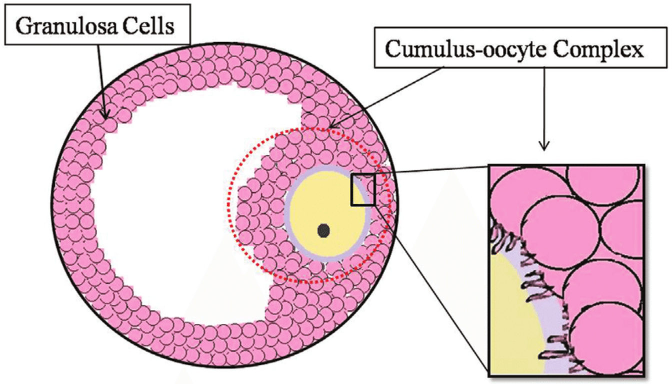

Rodriguez-Nuevo et al 2022

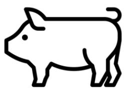
Bahety et al 2024

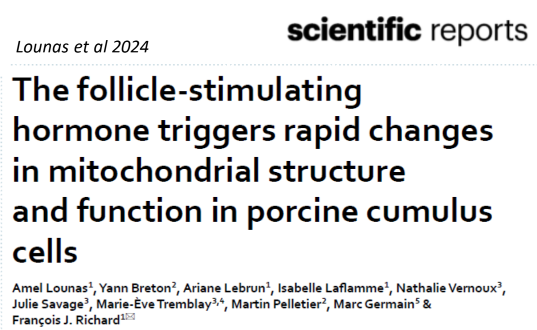
# Oocyte mitochondria

- the cumulus cells directly surrounding the oocyte supply nutrients and energy substrates such as pyruvate and lactate to the oocyte through gap junctions

- pyruvate and lactate supplied by cumulus cells constitute energy source required for oocyte development



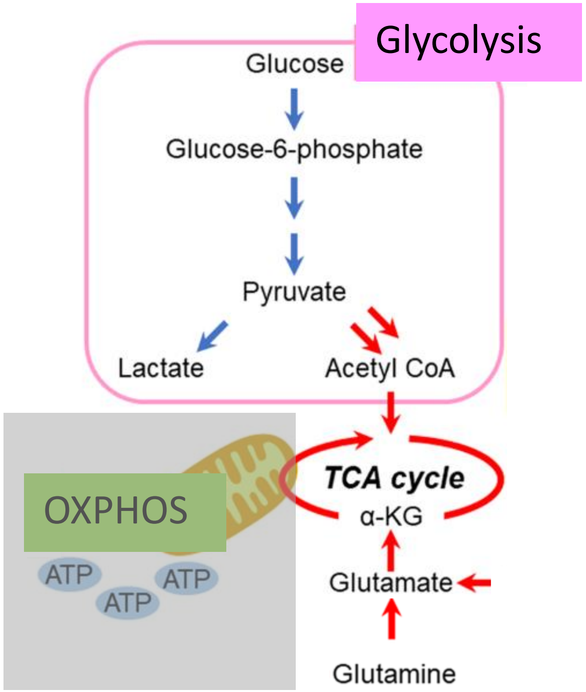



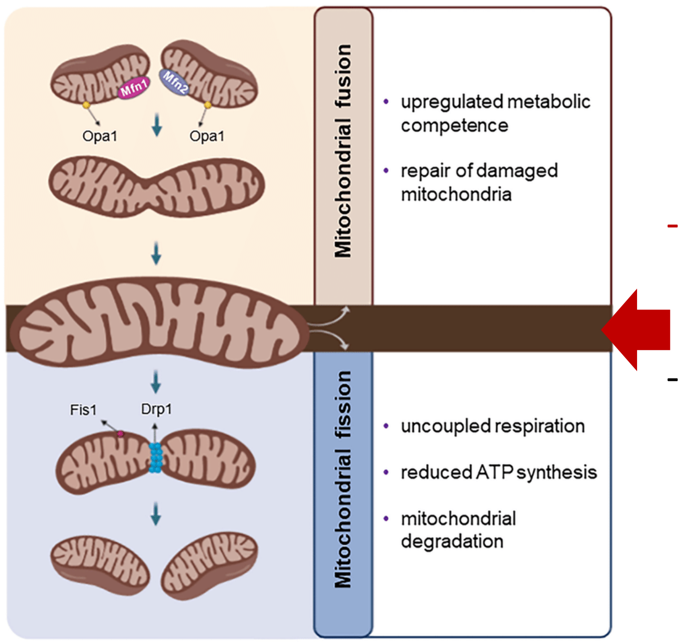

Lounas et al 2024

scientific reports

**The follicle-stimulating hormone triggers rapid changes in mitochondrial structure and function in porcine cumulus cells**

Amel Lounas[1], Yann Breton[2], Ariane Lebrun[1], Isabelle Laflamme[1], Nathalie Vernoux[3], Julie Savage[3], Marie-Eve Tremblay[3,4], Martin Pelletier[2], Marc Germain[5] & François J. Richard[1]



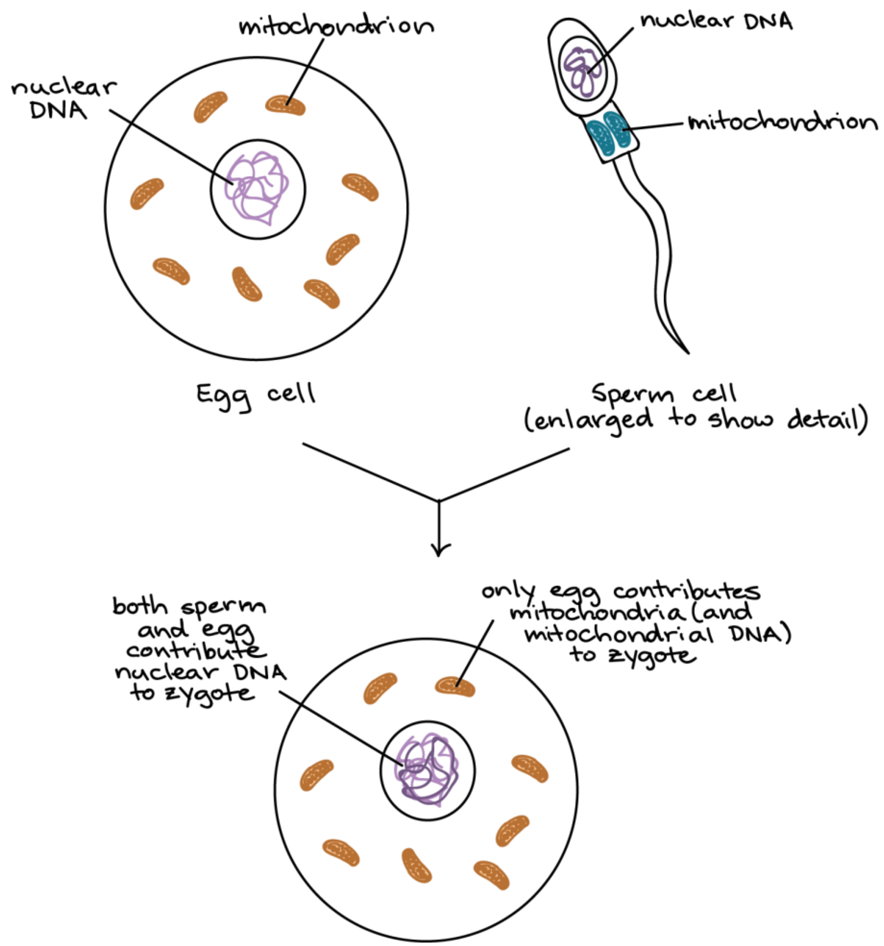



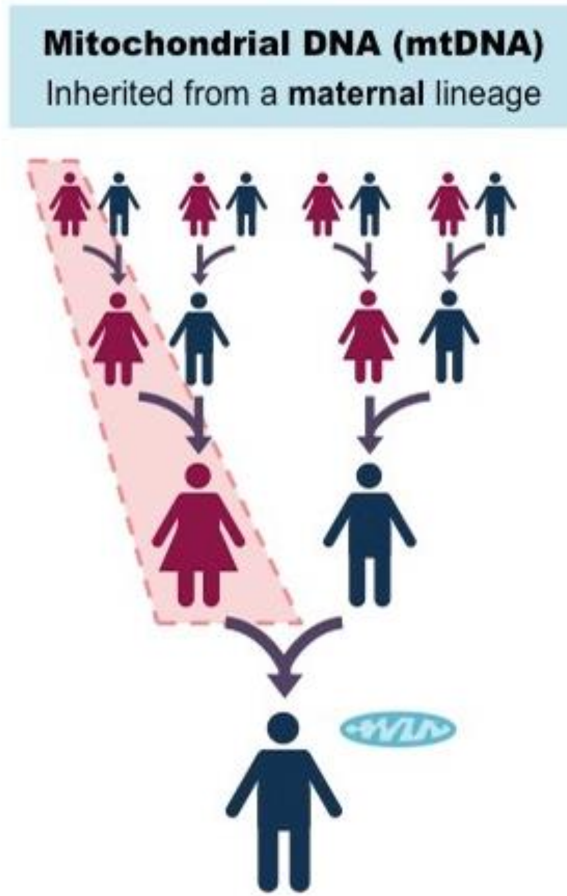

- FSH regulates mitochondrial structure and dynamics in cumulus cells
- the mitochondria elongation followed by fragmentation is accompanied by a decrease in mitochondrial activity and a switch to glycolysis

# Mitochondrial inheritance

→ ~ 150,000 mtDNA/oocyte

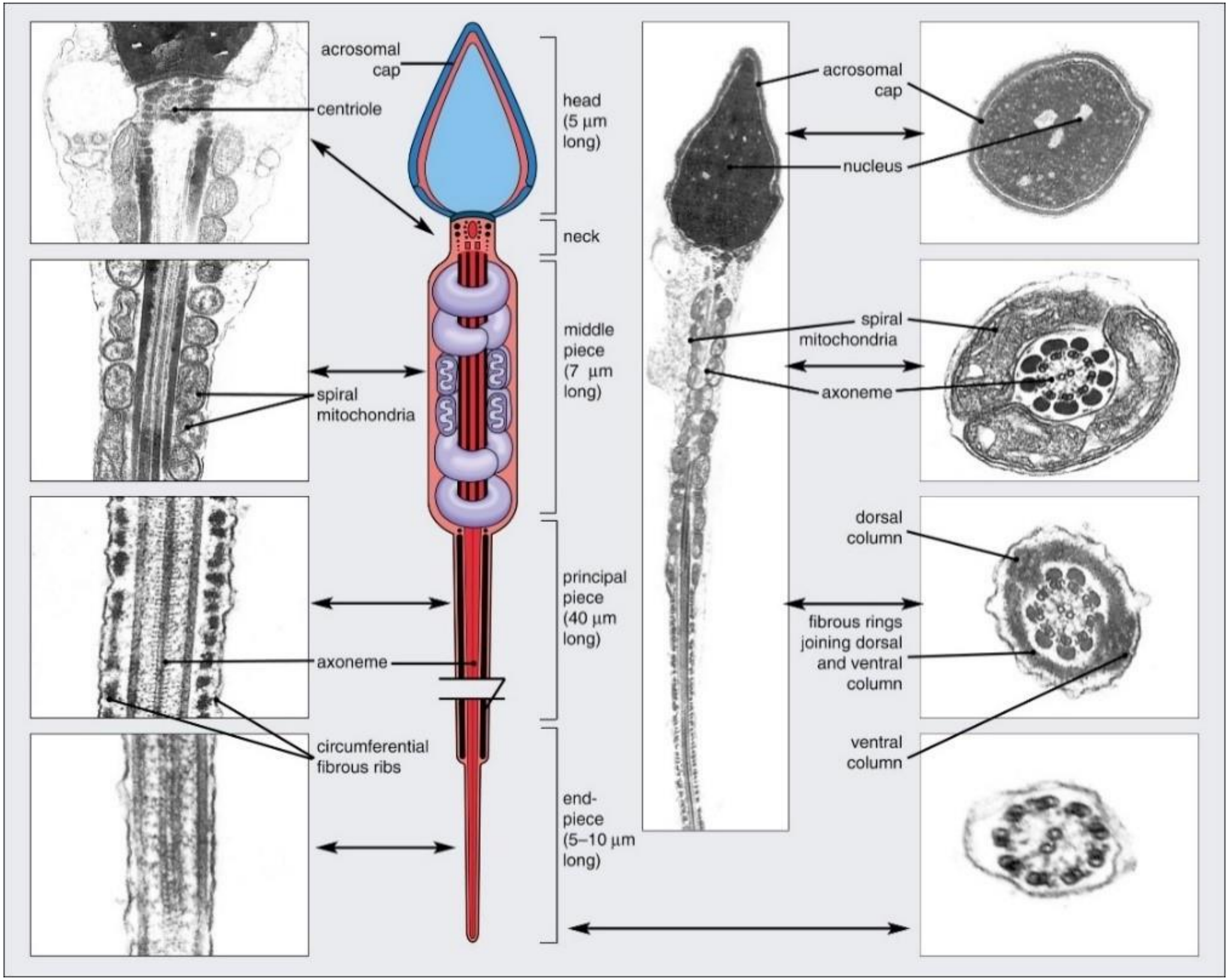
→ 3-15,7 mtDNA/sperm




Non-Mendelian
maternal inheritance

# Mitochondrial inheritance




- Sperm mtDNA quantity associated with semen quality

RESEARCH ARTICLE

Mitochondrial Biomarkers Reflect Semen Quality: Results from the MARCHS Study in Chongqing, China

Guowei Zhang[1], Zhi Wang[2], Xi Ling[2], Peng Zou[2], Huan Yang[2], Qing Chen[2], Niya Zhou[2], Lei Sun[2], Jianfang Gao[2], Ziyuan Zhou[1], Jia Cao[2*], Lin Ao[2*]

1 Department of Environmental Health, College of Preventive Medicine, Third Military Medical University, Chongqing, China, 2 Institute of Toxicology, College of Preventive Medicine, Third Military Medical University, Chongqing, China

**Sperm mitochondrial DNA measures and semen parameters among men undergoing fertility treatment**

Haotian Wu[#a], Alexandra M Huffman[#a], Brian W Whitcomb[b], Srinihaari Josyula[a], Suzanne Labrie[c], Ellen Tougias[c], Tayyab Rahil[c], Cynthia K Sites[c], and J Richard Pilsner[a]

[a]Department of Environmental Health Sciences, School of Public Health and Health Sciences, University of Massachusetts, 173A Goessmann, 686 North Pleasant Street, Amherst, MA 01003, USA.

***Carlson, B.*** *(2018). Human Embryology and Developmental Biology. Elsevier, 6th edition.*

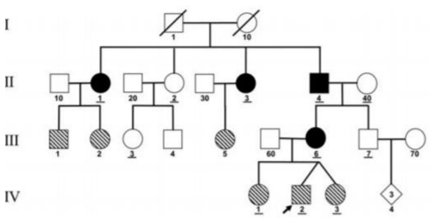
# Mitochondrial inheritance

**Biparental Inheritance of Mitochondrial DNA in Humans**

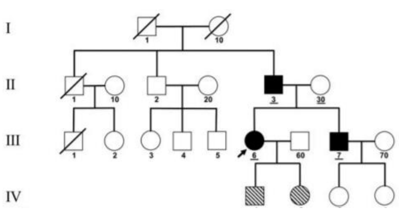
Shiyu Luo[a,b], C. Alexander Valencia[a,1], Jinglan Zhang[c], Ni-Chung Lee[d], Jesse Slone[a], Baoheng Gui[a,b], Xinjian Wang[a], Zhuo Li[a,2], Sarah Dell[a], Jenice Brown[a], Stella Maris Chen[c], Yin-Hsiu Chien[d], Wuh-Liang Hwu[d], Pi-Chuan Fan[e], Lee-Jun Wong[c], Paldeep S. Atwal[f,3], and Taosheng Huang[a,3,4]

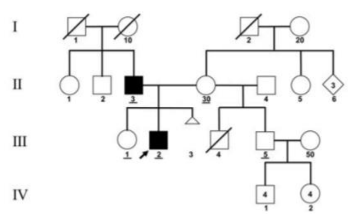
[a]Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229; [b]Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region, Nanning, 530003 Guangxi, China; [c]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030; [d]Department of Pediatrics and Medical Genetics, National Taiwan University Hospital, 100 Taipei, Taiwan; [e]Department of Pediatrics, National Taiwan University Hospital, 100 Taipei, Taiwan; and [f]Department of Clinical Genomics, Center for Individualized Medicine, Mayo Clinic Hospital, Jacksonville, FL 32224

Edited by Douglas C. Wallace, Children's Hospital of Philadelphia and University of Philadelphia, Philadelphia, PA, and approved October 29, 2018 (received for review June 26, 2018)

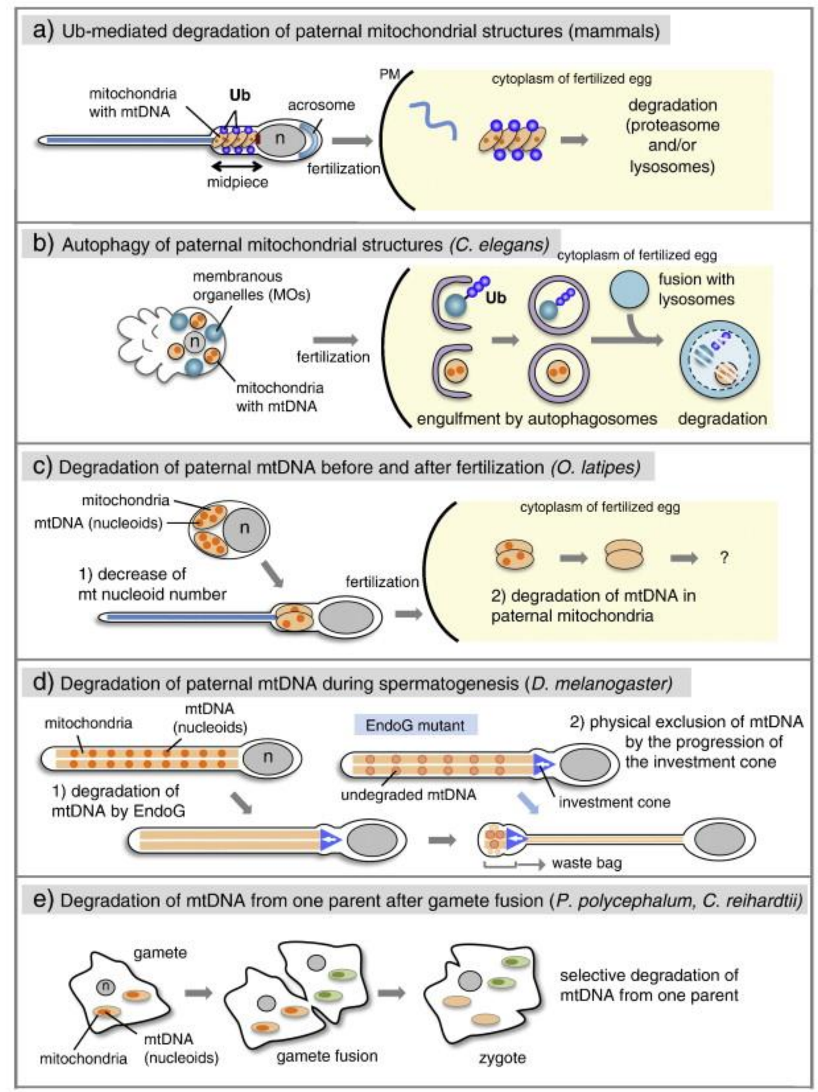







Failure to eliminate paternal mtDNA after fertilization?




Sato and Sato, 2013

# Mitochondrial diseases

- **mtDNA diseases**

  ← point mutation of mtDNA
  - maternally inherited

  - **MELAS** syndrome

    mitochondrial encephalopathy, lactic acidosis and stroke-like episodes

  - **LHON syndrome**

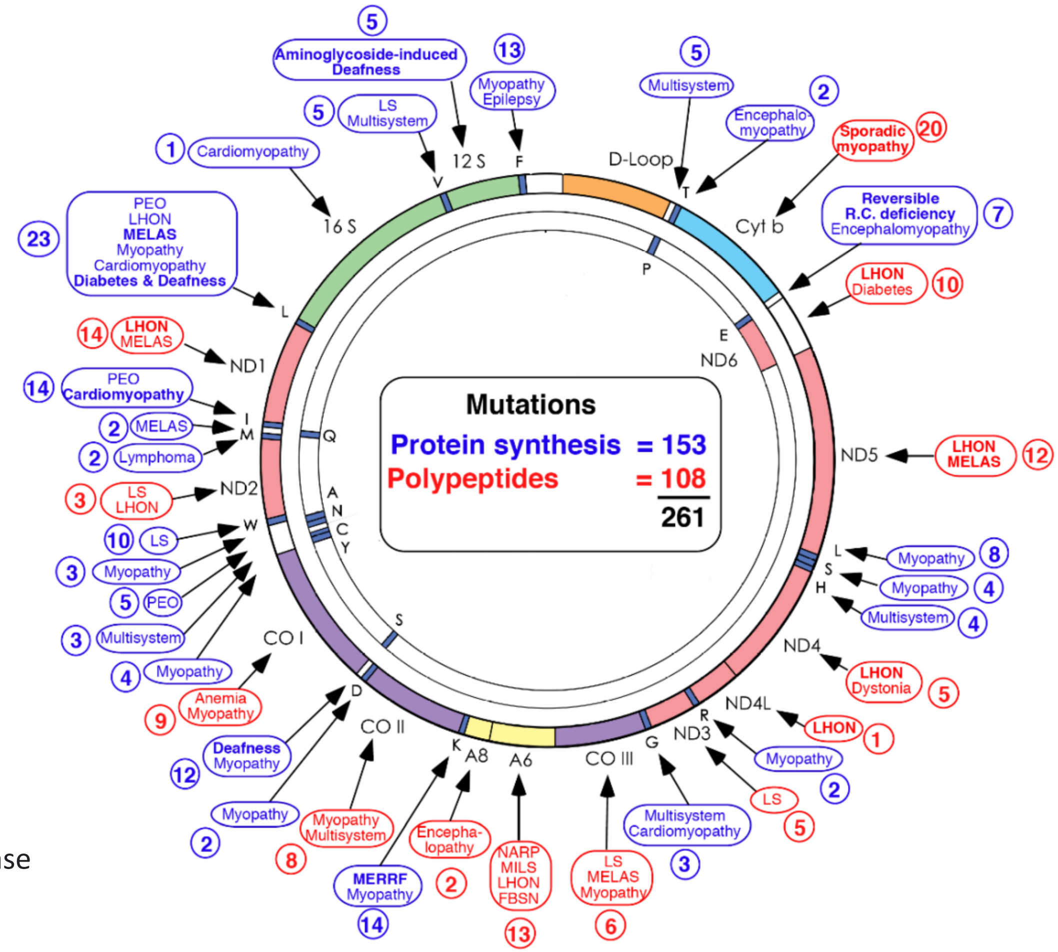
    Leber hereditary optic neuropathy

  - **Leigh syndrome (LS)**

    psychomotor regression

  - **MERRF disease**

    Myoclonic epilepsy and ragged-red fiber disease



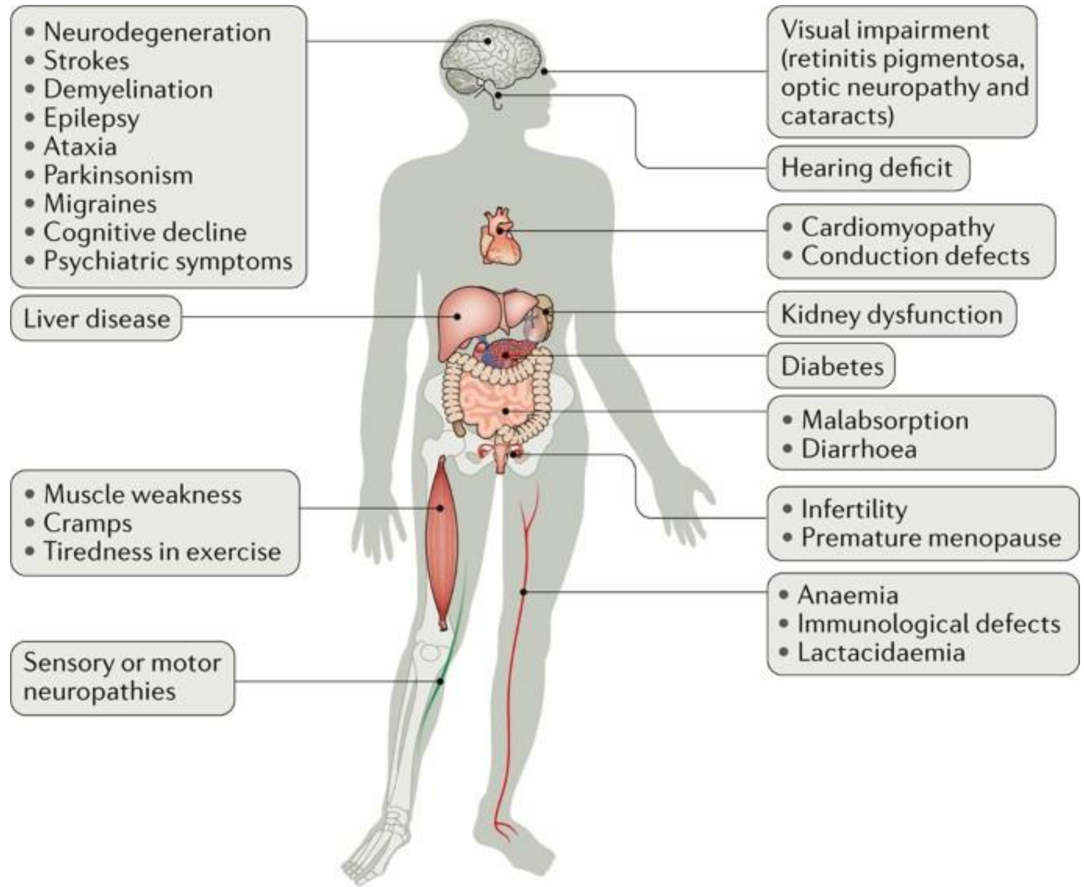

- **Mitochondrial diseases**

  ← mutation of nDNA encoding mitochondrial proteins
  - mendelian inheritance

  (+ mtDNA mutations)

*DiMauro, 2019.*

# Mitochondrial diseases

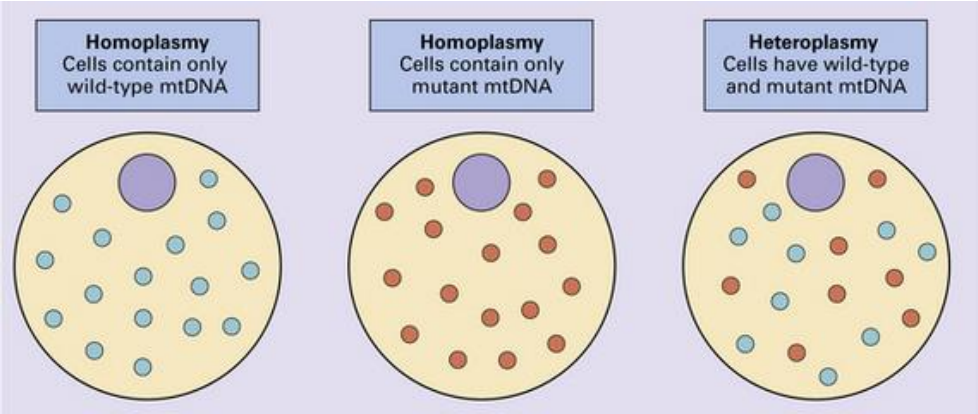



Nature Reviews | Molecular Cell Biology

- „mitochondrial cytopathy“
- multisystem syndrom
- clinically heterogenous
- rare but often progressive and severe

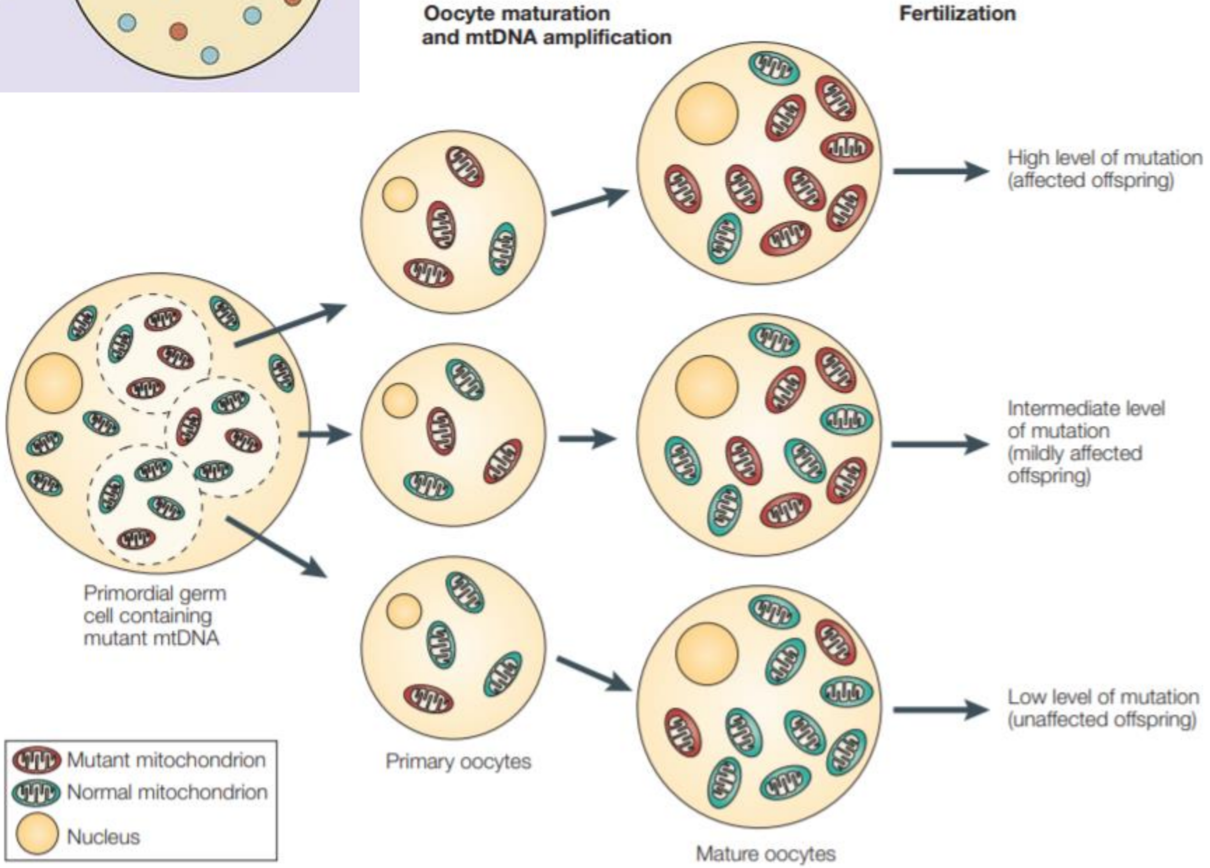
- deficient energy production
- embryo developmental arrest

# Mitochondrial diseases

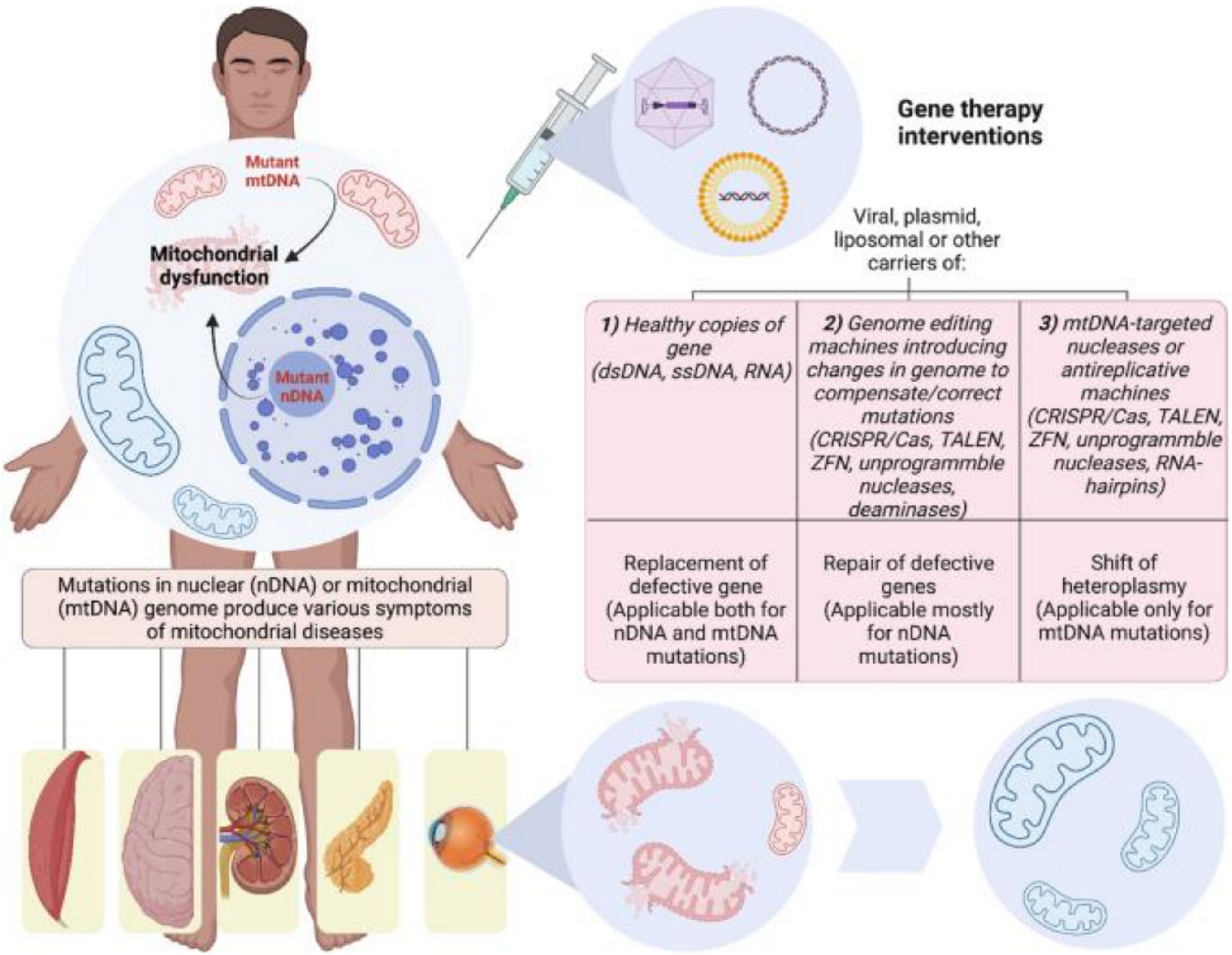



- clonal expansion of mitochondria with normal/defective mtDNA variants → degree of phenotype severity

- heteroplasmy can drastically change during development



# Treatment mitochondrial diseases




*Soldatov et al 2022.*

# Mitochondrial dysfunction therapies

## ❖ Mitochondrial replacement techniques (MRT)

**= micromanupulation procedures designed to <u>prevent</u> maternal transmission of mtDNA diseases**

- GERMINAL VESICLE TRANSFER
- MEIOTIC SPINDLE TRANSFER
- PRONUCLEAR TRANSFER
- POLAR BODY TRANSFER

Total cytoplasm transfer → **Avoidance of mitochondrial diseases**

## ❖ Egg rejuvenation

**= micromanupulation procedures designed <u>to boost egg fitness and fertilization potential</u>**

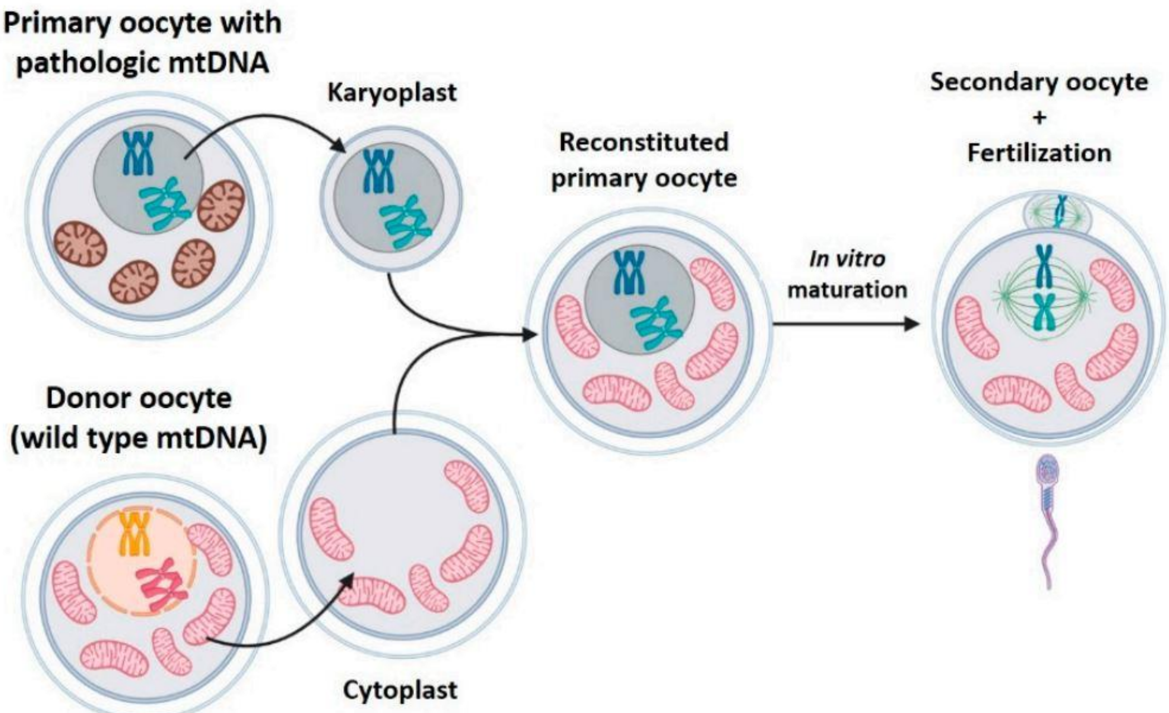
- CYTOPLASMIC TRANSFER
- AUTOLOGOUS GERMLINE MITOCHONDRIAL TRANSFER (AUGMENT TREATMENT)

Partial cytoplasm supplementation → **Energizing egg with healthy mitochondria**

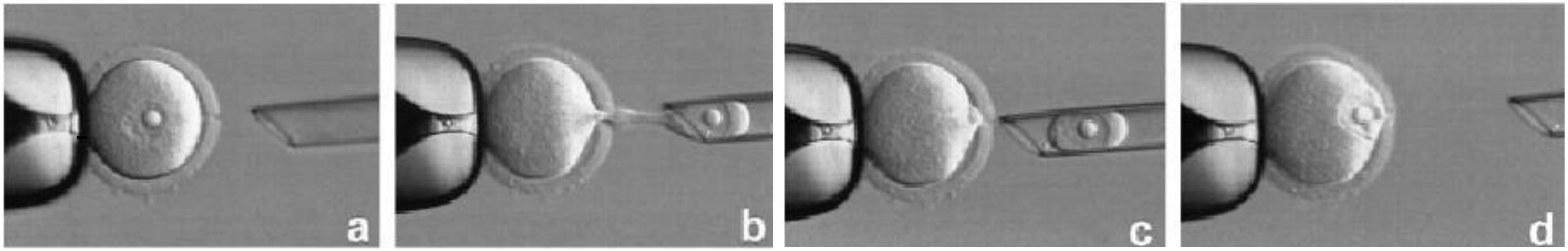
# Mitochondrial replacement techniques (MRT)

## Germinal vesicle transfer

- transplanting the GV from a patient's immature oocyte to an enucleated oocyte derived from a healthy donor
- fusion of karyoplast and cytoplast achieved by electroporation or HVJ-E (Sendai virus extract)
- only in animal models
- low efficiency



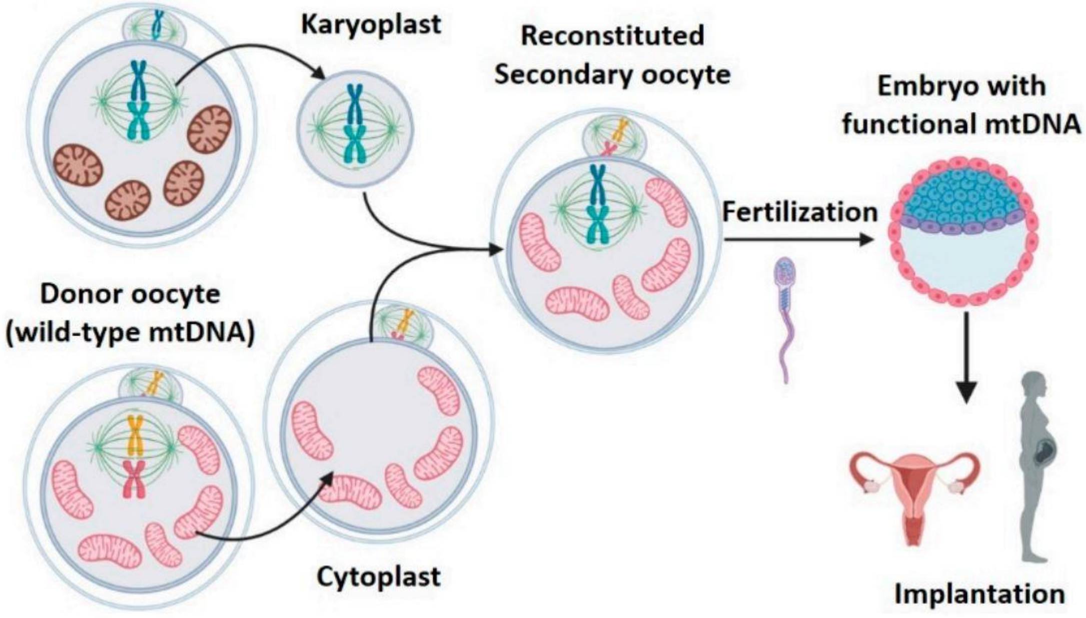

*Sendra et al 2021*



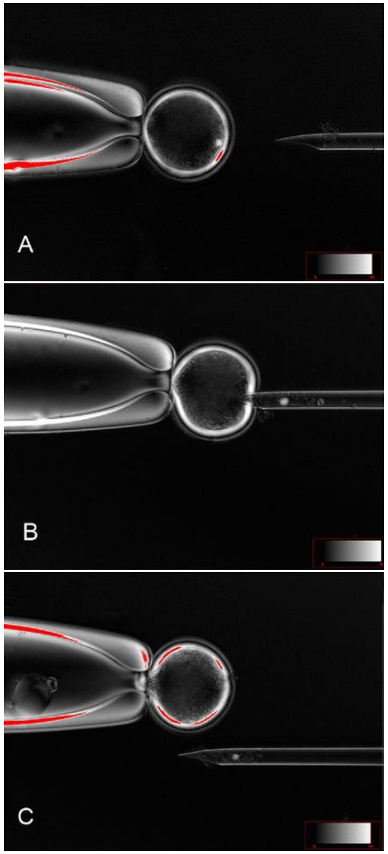
# Mitochondrial replacement techniques (MRT)

## ❑ Meiotic spindle transfer

- transferring the meiotic spindle of a patient's metaphase II oocyte to a healthy enucleated donor oocyte



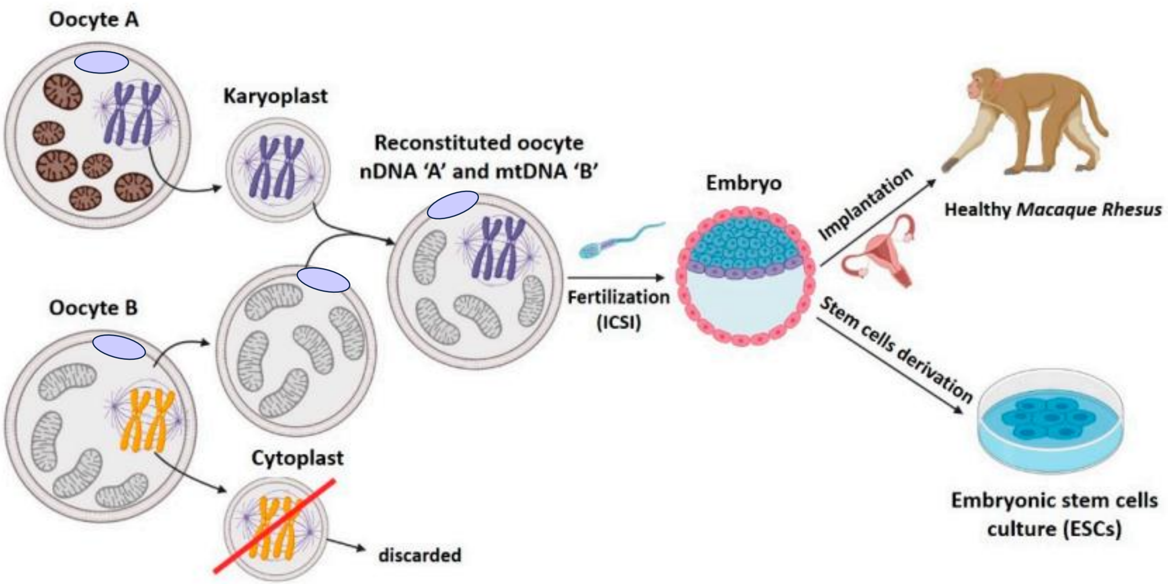

- spindle visualized by PLM




*Sendra et al 2021*

# Mitochondrial replacement techniques (MRT)

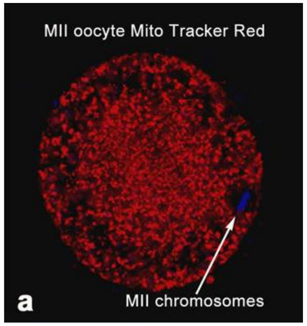
## ❑ Meiotic spindle transfer

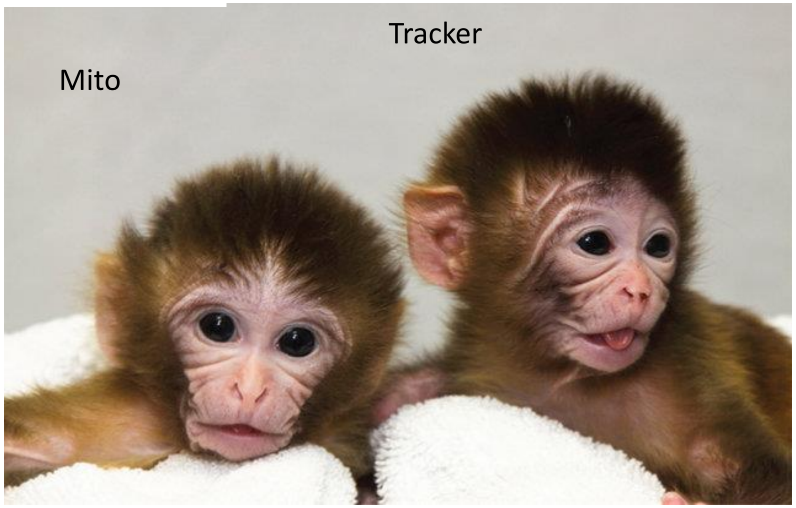



*Sendra et al 2021*

Shoukhrat Mitalipov

- successfully used in primates
- low level of mtDNA carry over
- 3-year postnatal follow-up




*Tachibana et al., 2009*

# Mitochondrial replacement techniques (MRT)

## ❑ Meiotic spindle transfer

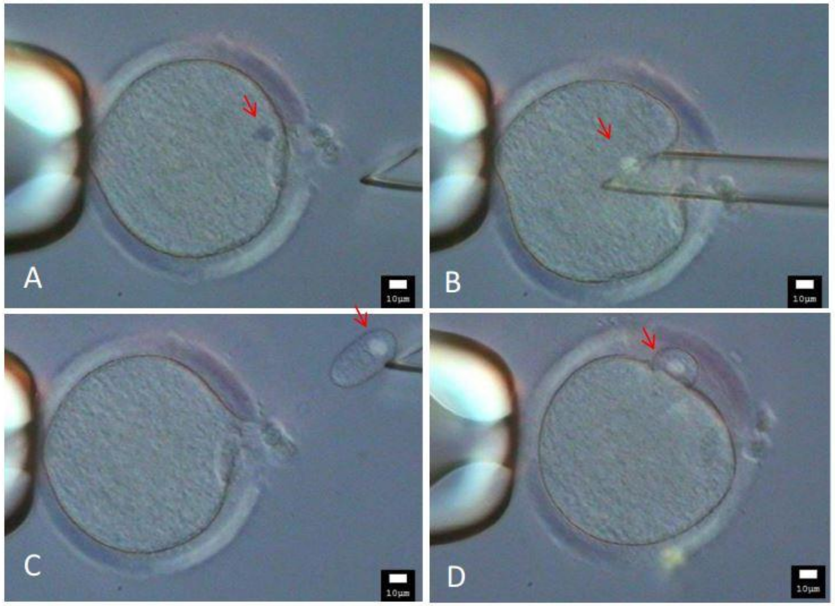
- 1st baby (Mexico, 2016)

**Live birth derived from oocyte spindle transfer to prevent mitochondrial disease**

CrossMark

John Zhang [a,b,*], Hui Liu [b], Shiyu Luo [c], Zhuo Lu [b], Alejandro Chávez-Badiola [a], Zitao Liu [b], Mingxue Yang [b], Zaher Merhi [d], Sherman J Silber [e], Santiago Munné [f], Michalis Konstantinidis [f], Dagan Wells [f], Jian J Tang [g], Taosheng Huang [c,*]

[a] New Hope Fertility Center, Punto Sao Paulo, Lobby Corporativo, Américas 1545 Providencia, Guadalajara, Mexico
[b] New Hope Fertility Center, 4 Columbus Circle, New York, NY 10019, USA
[c] Division of Human Genetics, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229, USA
[d] Department of Obstetrics and Gynecology, Division of Reproductive Biology, NYU School of Medicine, 180 Varick Street, New York, NY 10014, USA
[e] Infertility Center of St Louis, St Luke's Hospital, St Louis, MO 63017, USA
[f] Reprogenetics, 3 Regent Street, Livingston, NJ 07078, USA
[g] Department of Obstetrics and Gynecology, The Mount Sinai Hospital, E 101st Street, New York, NY 10029, USA






**Exclusive: World's first baby born with new "3 parent" technique**

HEALTH 27 September 2016, updated 27 September 2016

- prevention of Leigh syndrome transmission

# Mitochondrial replacement techniques (MRT)

- **Pronuclear transfer**

  - the pronuclei of a patient's oocyte is isolated in a karyoplast and transferred into a donor oocyte without its pronucleus

*Craven et al., Nature 2010*

LETTER

doi:10.1038/nature18303

**Towards clinical application of pronuclear transfer to prevent mitochondrial DNA disease**

Louise A. Hyslop[1,2], Paul Blakeley[3], Lyndsey Craven[4], Jessica Richardson[1], Norah M. E. Fogarty[3], Elpida Fragouli[5], Mahdi Lamb[1], Sissy E. Wamaitha[3], Nilendran Prathalingam[1,2], Qi Zhang[1]†, Hannah O'Keefe[1], Yuko Takeda[1], Lucia Arizzi[1,2], Samer Alfarawati[5], Helen A. Tuppen[4], Laura Irving[1]†, Dimitrios Kalleas[1]†, Meenakshi Choudhary[2], Dagan Wells[6], Alison P. Murdoch[2], Douglass M. Turnbull[4], Kathy K. Niakan[3] & Mary Herbert[1,2]

*Hyslop et al. Nature, 2016.*

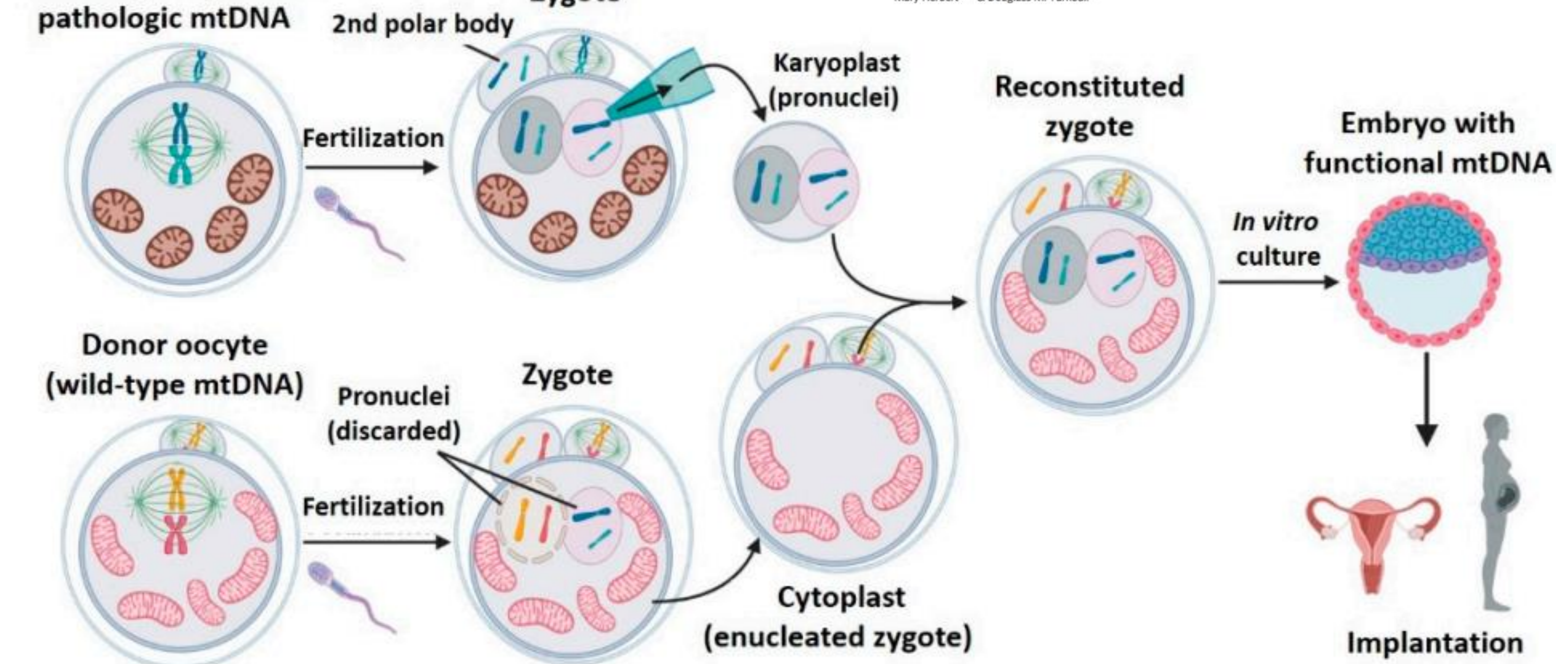
nature Vol 465|6 May 2010|doi:10.1038/nature08958

LETTERS

**Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease**

Lyndsey Craven[1], Helen A. Tuppen[1], Gareth D. Greggains[3,4], Stephen J. Harbottle[3], Julie L. Murphy[1], Lynsey M. Cree[1], Alison P. Murdoch[3,5], Patrick F. Chinnery[1], Robert W. Taylor[1], Robert N. Lightowlers[1], Mary Herbert[3,4,5] & Douglass M. Turnbull[1,2,5]

Mery Herbert



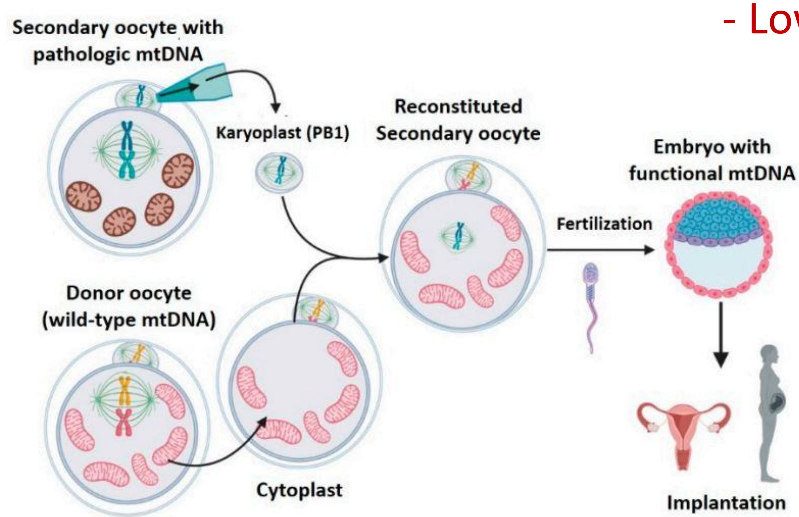

- time-dependent effciency
- early (8h post ICSI) better than late PNs transfer (16-20h post ICSI)

# Mitochondrial replacement techniques (MRT)

## ❑ Polar body transfer

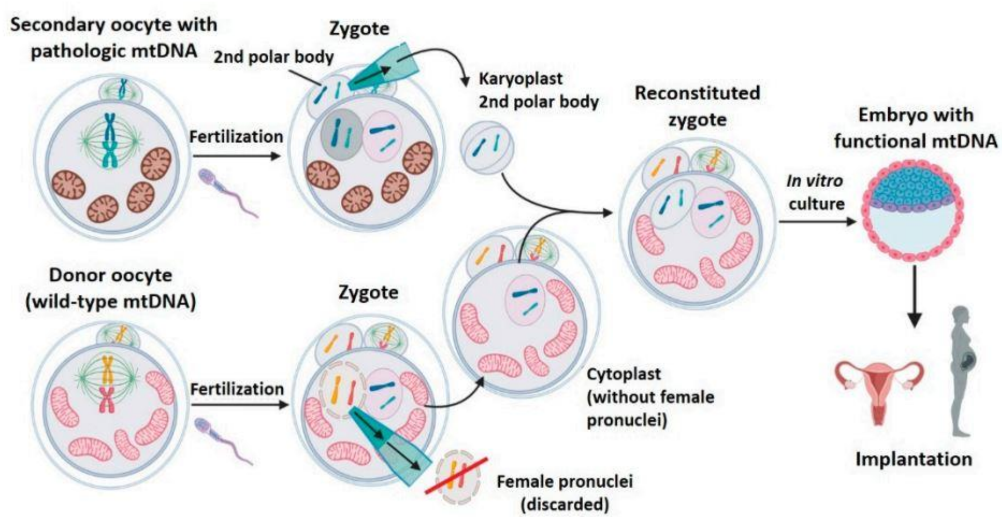
- Low Mt carry-over!

PB1 transfer




Transfer of the 1st PB from a patient's MII to a donor MII oocyte without meiosis spindle

PB2 transfer




Transfer of the PB2 from a patient's zygote to a donor zygote whose female pronuclei has been previously removed

*Sendra et al 2021*

# Mitochondrial replacement techniques (MRT)

- **Clinical applications**

Human Fertilisation & Embryology Authority

Home | Contact us

I am… | Treatments | Donation | Choose a clinic | About us

## Mitochondrial donation treatment

Mitochondrial donation treatment can be used by people with severe mitochondrial disease to avoid passing the condition onto their children. This page introduces you to what the treatment involves and how you can apply to have it.

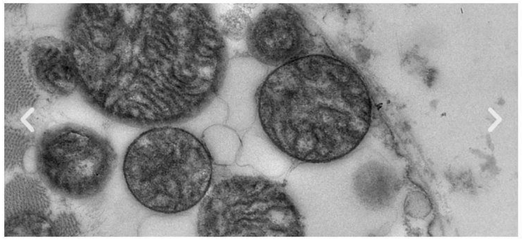
wellcome centre mitochondrial research | Newcastle University

Home | Patient And Public | Research | Clinical | Clinical Trials | Team Members | News | Contact

Clinical Research | Mitochondrial Research Bank | Mitochondrial Gene Expression | Preventing Mitochondrial Disease Transmission | Clinical Mitochondrial Disease | Mitochondria in Common Disease | Young Scientists | Career Support

### Research

The Wellcome Centre for Mitochondrial Research is built on our long standing clinical and scientific expertise in mitochondrial disease. We are totally committed to providing the best of care for our patients and this means that we must understand more about the mechanisms underlying mitochondrial disease. If we are to develop new strategies for preventing or treating mitochondrial disease, it is fundamentally dependent upon very high quality research.



## Newcastle awarded world's first mitochondrial licence

**Published on: 16 March 2017**

Newcastle has been granted the first licence to offer a fertility treatment to mothers affected by mitochondrial disease.

The Human Fertilisation and Embryology Authority (HEFA) has granted a treatment licence to the Newcastle Fertility Centre, part of the Newcastle Hospitals NHS Foundation Trust. This allows a variation to the current clinical licence so that pro-nuclear transfer can be offered to reduce the risk of mothers transferring mitochondrial disease.

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## Fourth baby born in 14 months using the maternal spindle transfer method as part of pilot trial conducted by the Institute of Life and Embryotools scientific team

Within 14 months from the birth of the first baby, threemore babies have been born using the maternal spindle transfer method, **as part of the pilot trial** conducted by the scientific team of the Institute of Life and Embryotools in Greece.

The fourth baby was born at 10:40 am on June 20, 2020, at IASO Hospital, to a Greek mother with a long history of multiple IVF failures. Both the mother and the infant are in very good health.

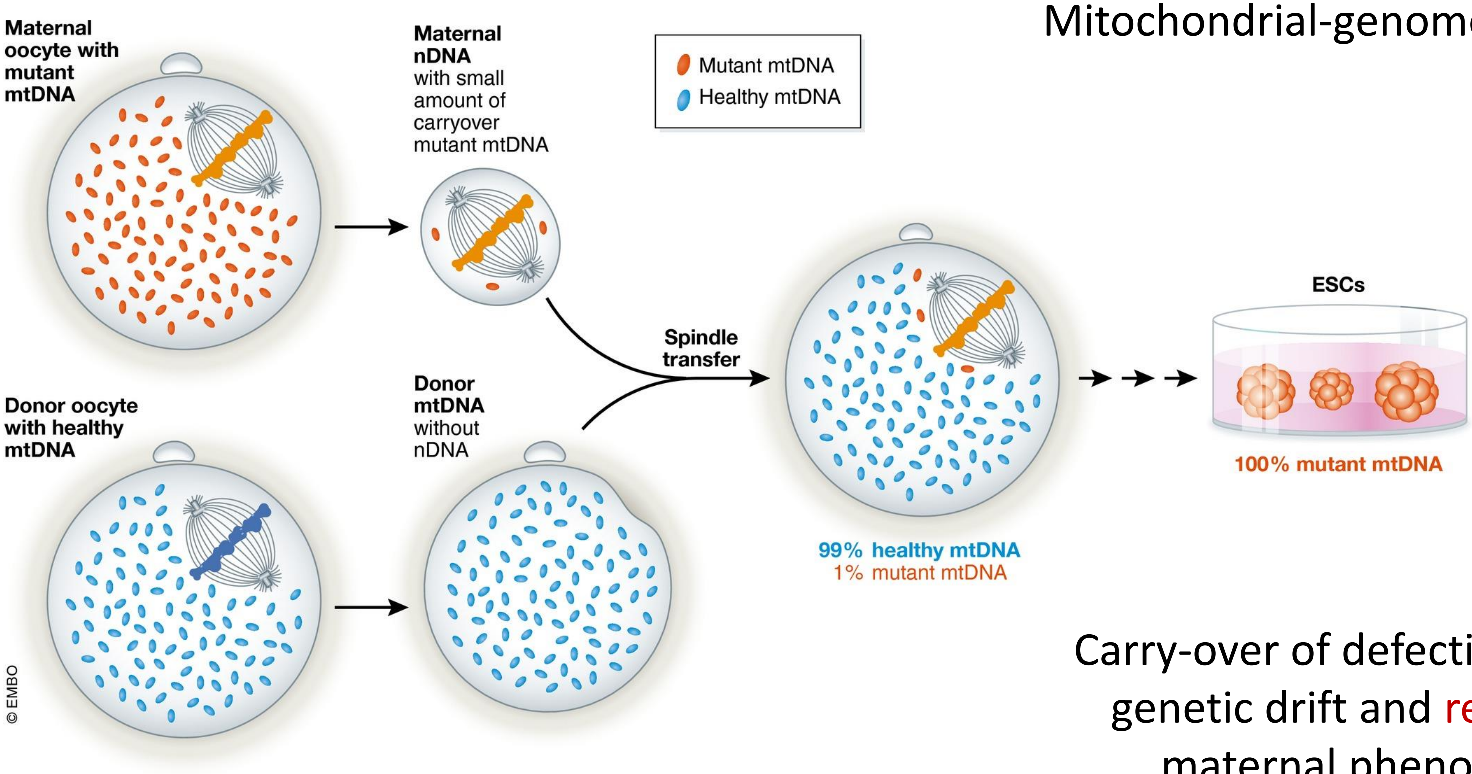
## V Británii se narodily první děti s DNA od tří lidí

10. 5. 2023, 16:18 – Londýn
Filip Šára, Novinky, ČTK

V Británii se narodily první děti vytvořené experimentální technikou kombinující genetickou informaci tří dárců, která má zabránit tomu, aby zdědily vzácné genetické choroby. Ve středu to potvrdil britský úřad pro lidskou fertilizaci a embryologii (HFEA), podle nějž jde o méně než pět dětí, ale další podrobnosti neposkytl, aby ochránil identitu rodin.

# Mitochondrial replacement techniques (MRT)

- **Risk of MRT?**




Mitochondrial-genome mismatch ?!

Carry-over of defective mtDNA, genetic drift and reversal of maternal phenotype?!

LETTER

doi:10.1038/nature20592

**Mitochondrial replacement in human oocytes carrying pathogenic mitochondrial DNA mutations**

Eunju Kang[1,2]†, Jun Wu[3], Nuria Marti Gutierrez[1,2], Amy Koski[1,2], Rebecca Tippner-Hedges[1,2], Karen Agaronyan[4], Aida Platero-Luengo[3], Paloma Martinez-Redondo[3], Hong Ma[1,2], Yeonmi Lee[1,2]†, Tomonari Hayama[1,2], Crystal Van Dyken[1,2], Xinjian Wang[5], Shiyu Luo[5], Riffat Ahmed[1,2], Ying Li[1,2], Dongmei Ji[1,6], Refik Kayali[7], Cengiz Cinnioglu[7], Susan Olson[8], Jeffrey Jensen[9], David Battaglia[9], David Lee[9], Diana Wu[9], Taosheng Huang[5], Don P. Wolf[1,2], Dmitry Temiakov[4], Juan Carlos Izpisua Belmonte[3], Paula Amato[9] & Shoukhrat Mitalipov[1,2,9,10,11]

MATTERS ARISING

https://doi.org/10.1038/s41586-019-1623-3

**Reversion after replacement of mitochondrial DNA**

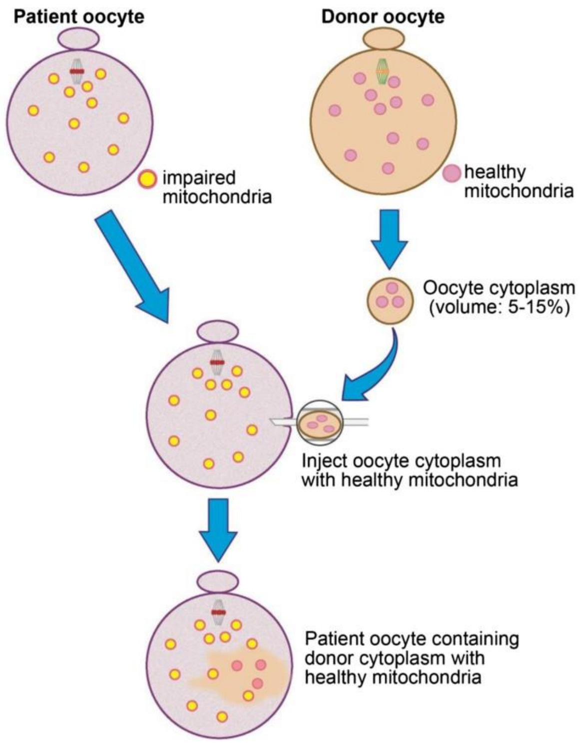
Gavin Hudson[1]*, Yuko Takeda[1] & Mary Herbert[1,2]*

ARISING FROM: E. Kang et al. *Nature* https://doi.org/10.1038/nature20592 (2016).

# Egg rejuvenation

## ❑ Cytoplasmic transfer

- microinjection of patient´s eggs with healthy mitochondria from young egg donor



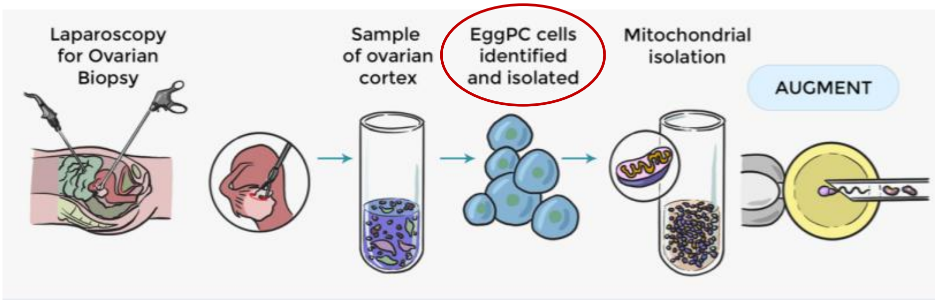

*Reznichenko, et al 2016*

CONTROVERSIAL

## ❑ Autologous germline mitochondrial transfer (Augment treatment/OvaScience)

- microinjection of patient-matched mitochondria isolate from putative ovarian stem cells


*Woods and Tilly 2015*

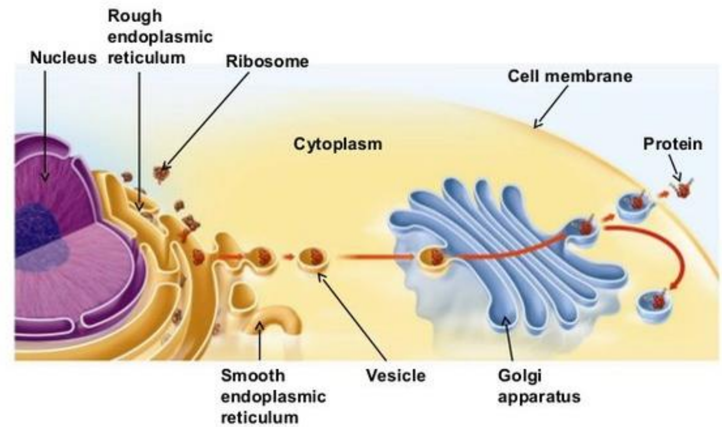



*Cozzolino, et al 2019*

mitigation of ovarian aging

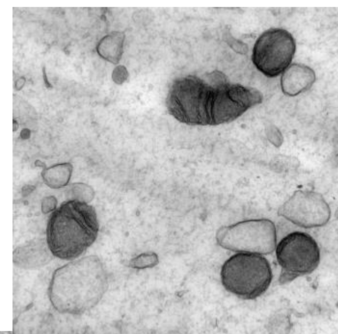
CONTROVERSIAL

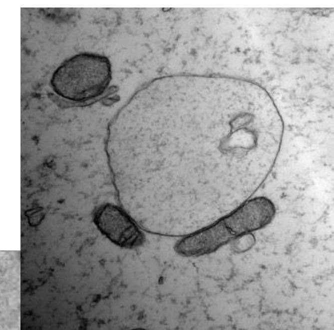
# Oocyte cytoplasmic organelles

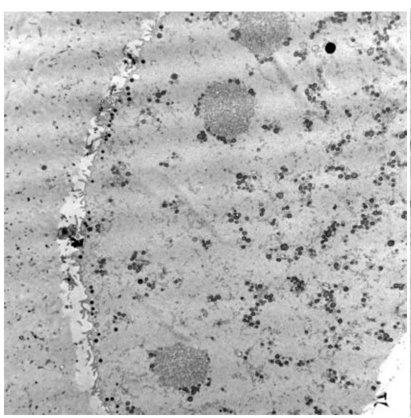


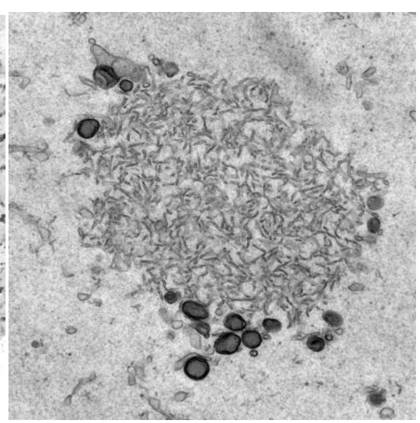

## ❖ Endoplasmic reticulum (ER)

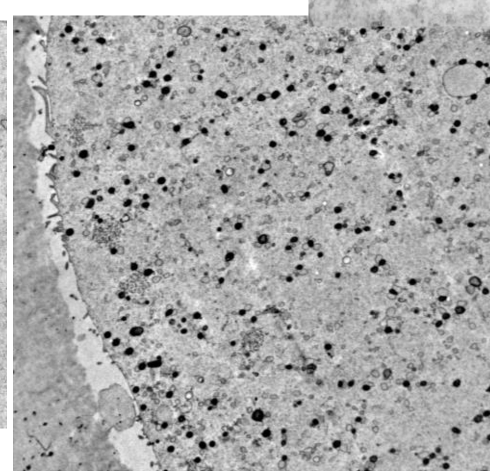
- smoth ER (sER) - ribosomes not detected
- major storage of $Ca^{2+}$, mediates $Ca^{2+}$ signalling
- perinucler location in GV oocytes, relocation to cortex after GVBD
- sER types
  - (1) vesicular (cisternea)
  - (2) tubular (dense arrays and large clusters in the cortex area)
- gradual association with mitochondria during maturation → coordination of $Ca^{2+}$ homeostasis and ATP production

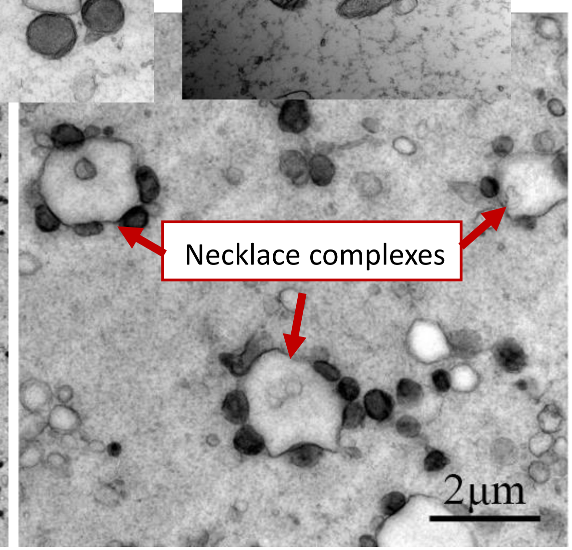








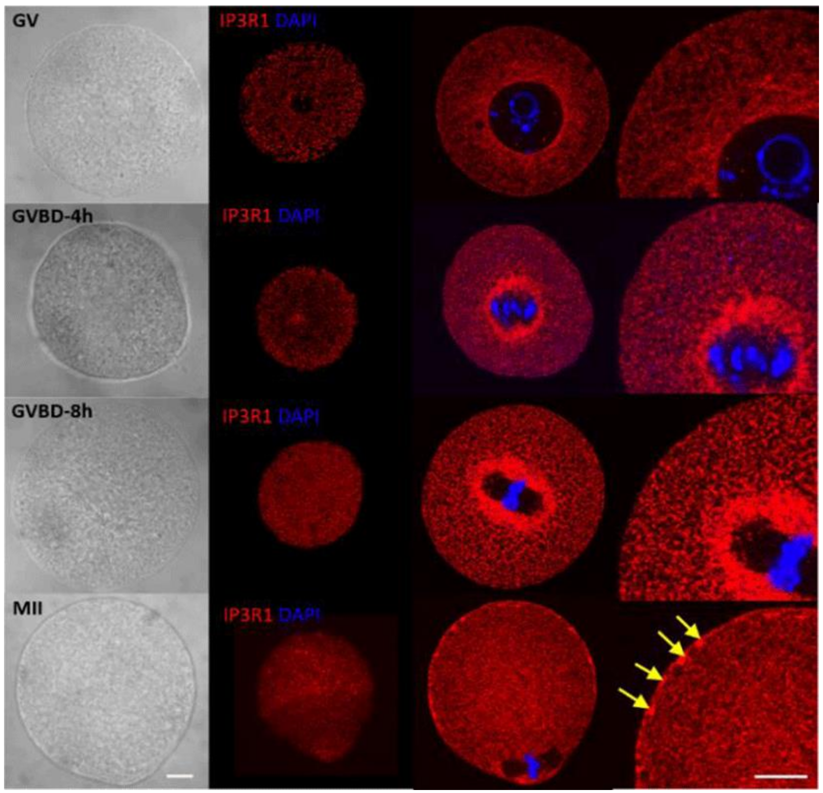


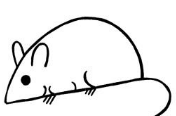



*Trebichalska et al, 2021*

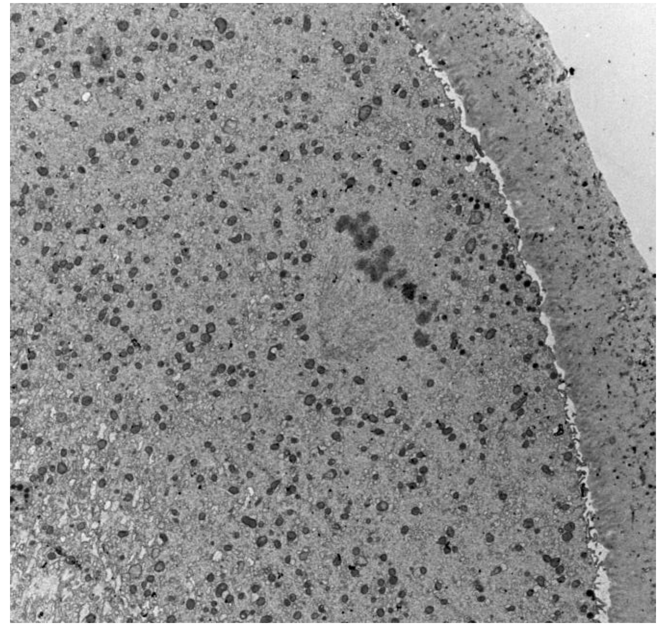
# Oocyte cytoplasmic organelles

## ❖ Endoplasmic reticulum (ER)



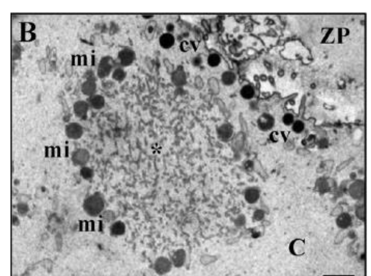



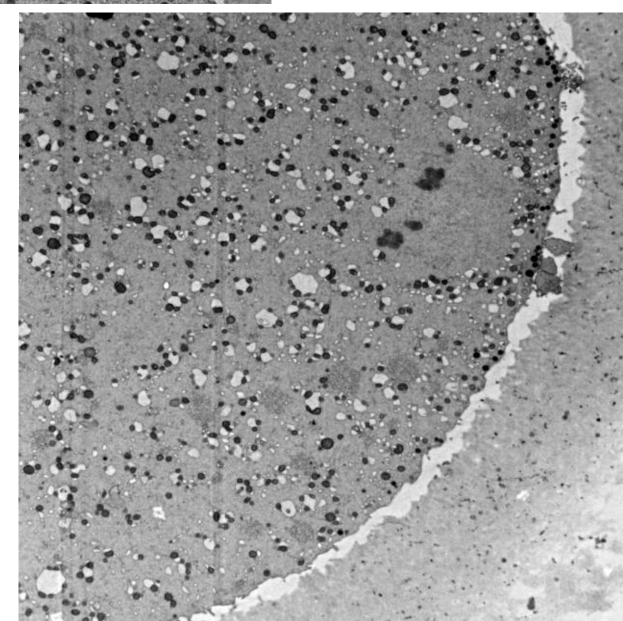
- ER clusters around MI spindle
- cortical ER clusters in MII



- no ER clusters around MI spindle

- cortical ER clusters in MII

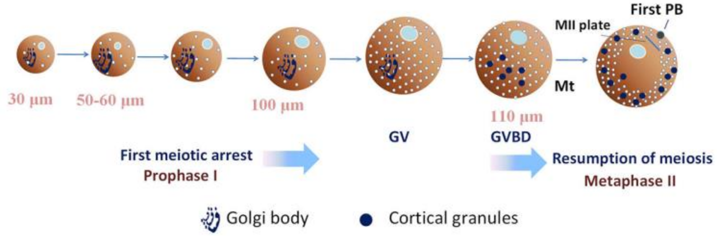


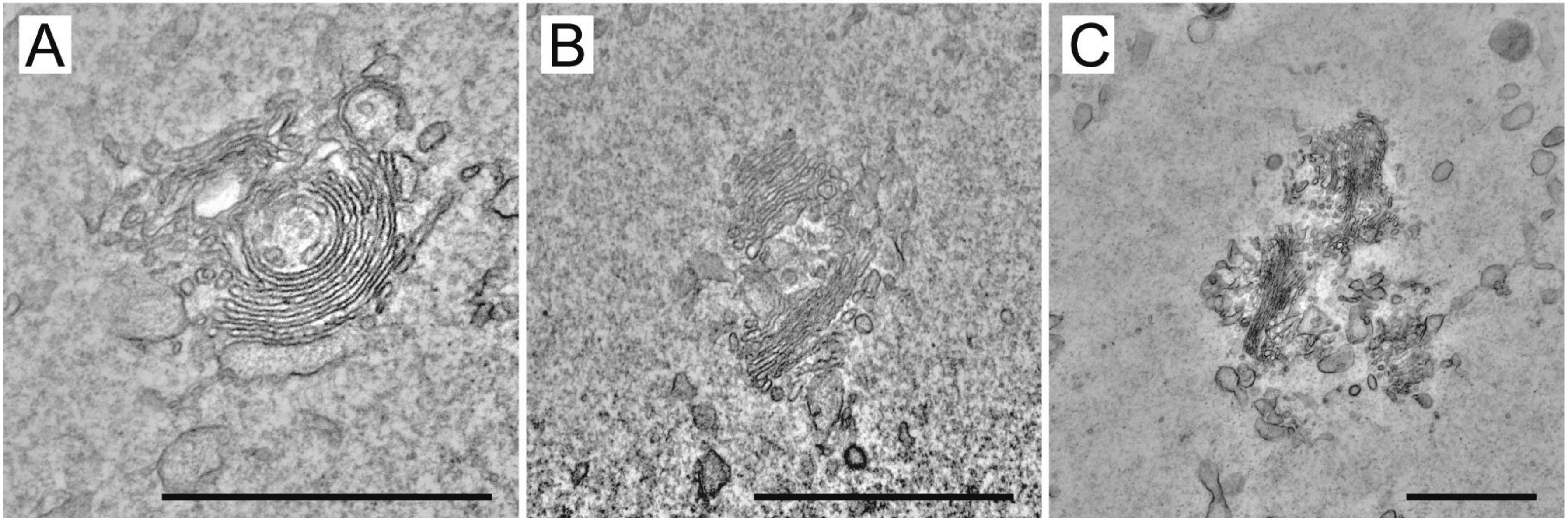



# Oocyte cytoplasmic organelles

## ❖ Golgi apparatus (GA)

- modifying, sorting, packing of macromolecules for intracellular trafficking and cell secretion
- perinucler location in GV oocytes, fragmentation at GVBD
- GA fragmentation generates vesicles taht are relocated to the cortex in actin dependent manner



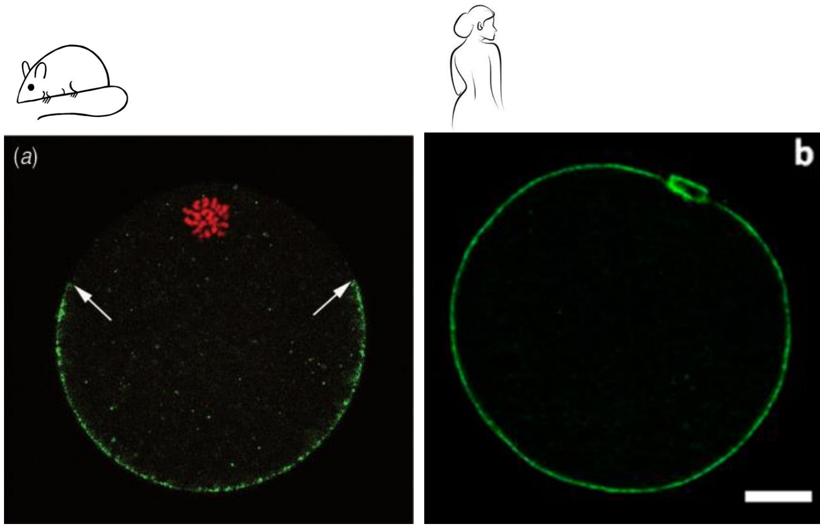



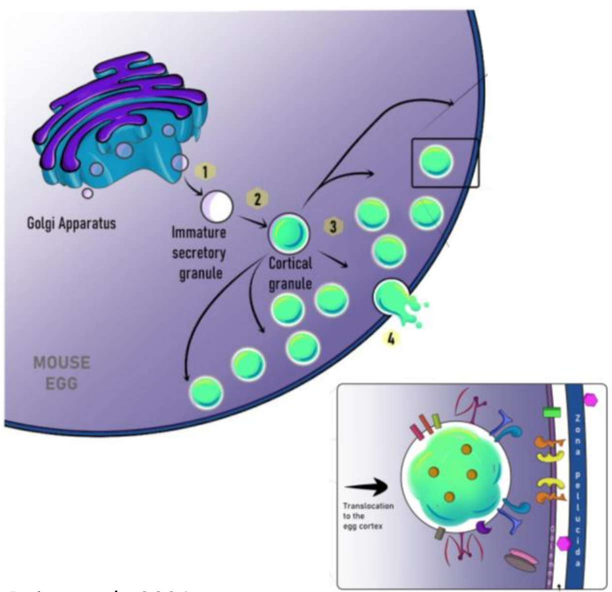

*Trebichalska et al, 2021*

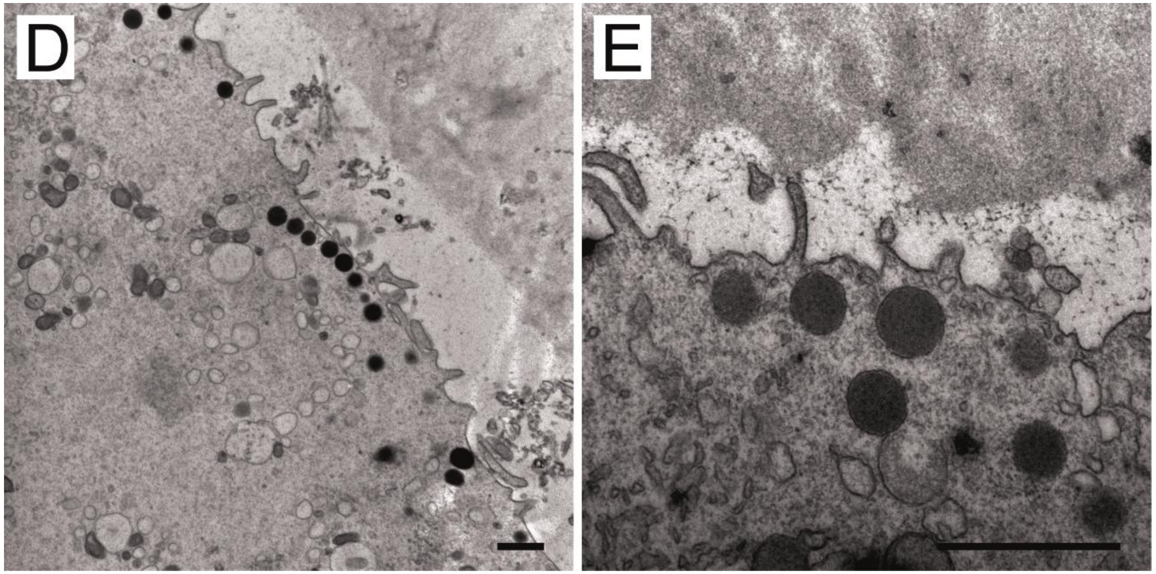
# Oocyte cytoplasmic organelles

## ❖ Cortical granules (CG)

- oocyte-specific secretory vesicles derived from GA
- located in suboolema cortex of mature oocytes
- critical role in fertilization and prevention of polyspemy
- acquire peripheral and cortical position during oocyte maturation
- in mouse, CGs-free zone in spindle proximity



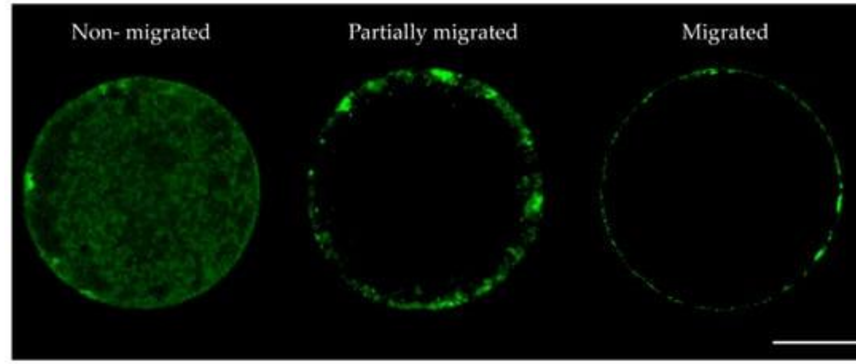

Rojas et al., 2021

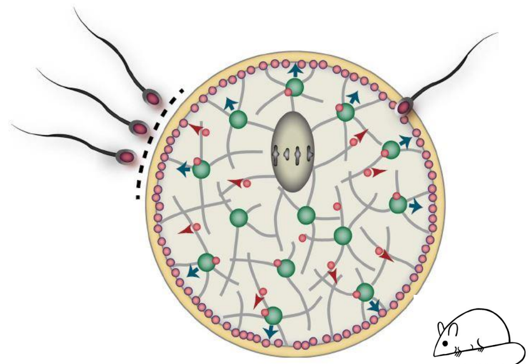
Trebichalska et al, 2021

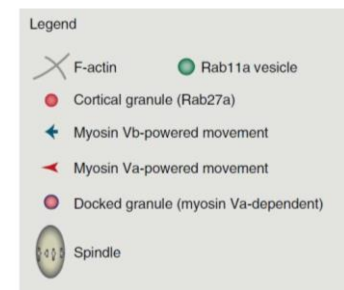
# Oocyte cytoplasmic organelles

❖ **Cortical granules (CG)**

- translocated to the cortex during oocyte maturation
- myosin-dependent movement along actin filaments, hitchhiking on specific vesicles
- anchoring to the cortex is dependent on subcortical maternal complex (SCMC, MATER)
- clearance of cortical actin prior CGs exocytosis

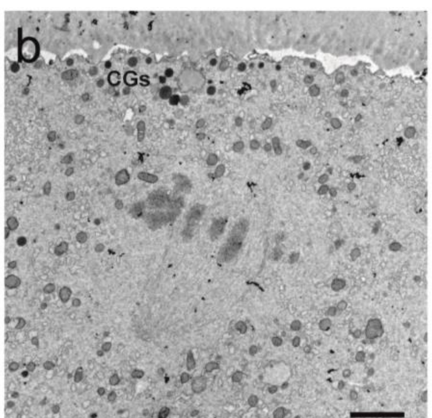


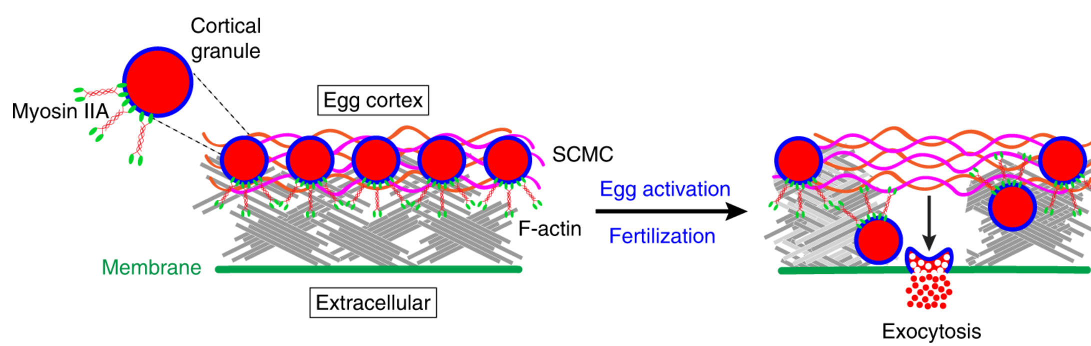






*Cheeseman et al, 2016*

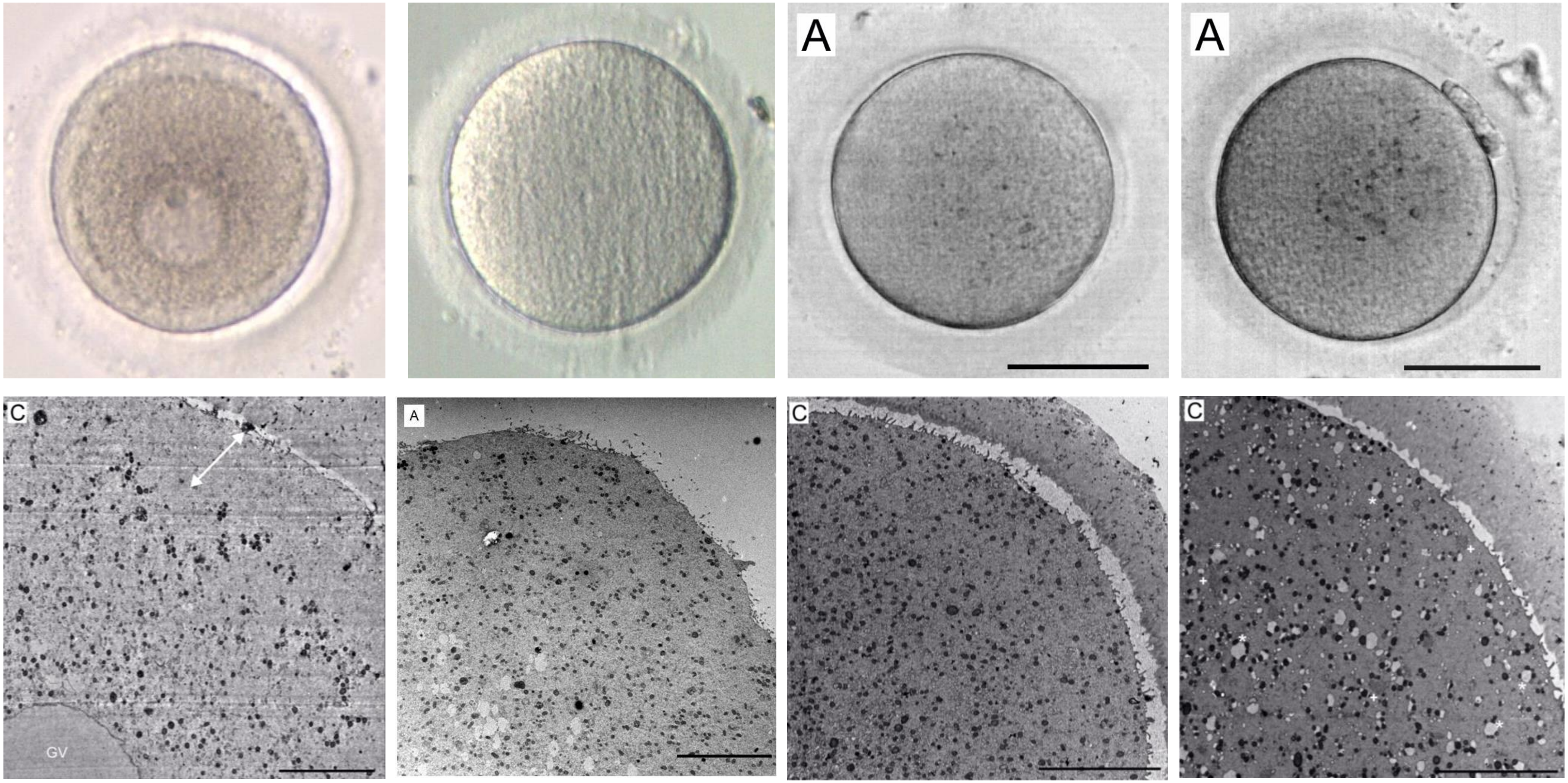
- no CGs depletion close to spindle pole




*Vogt et al, 2019*

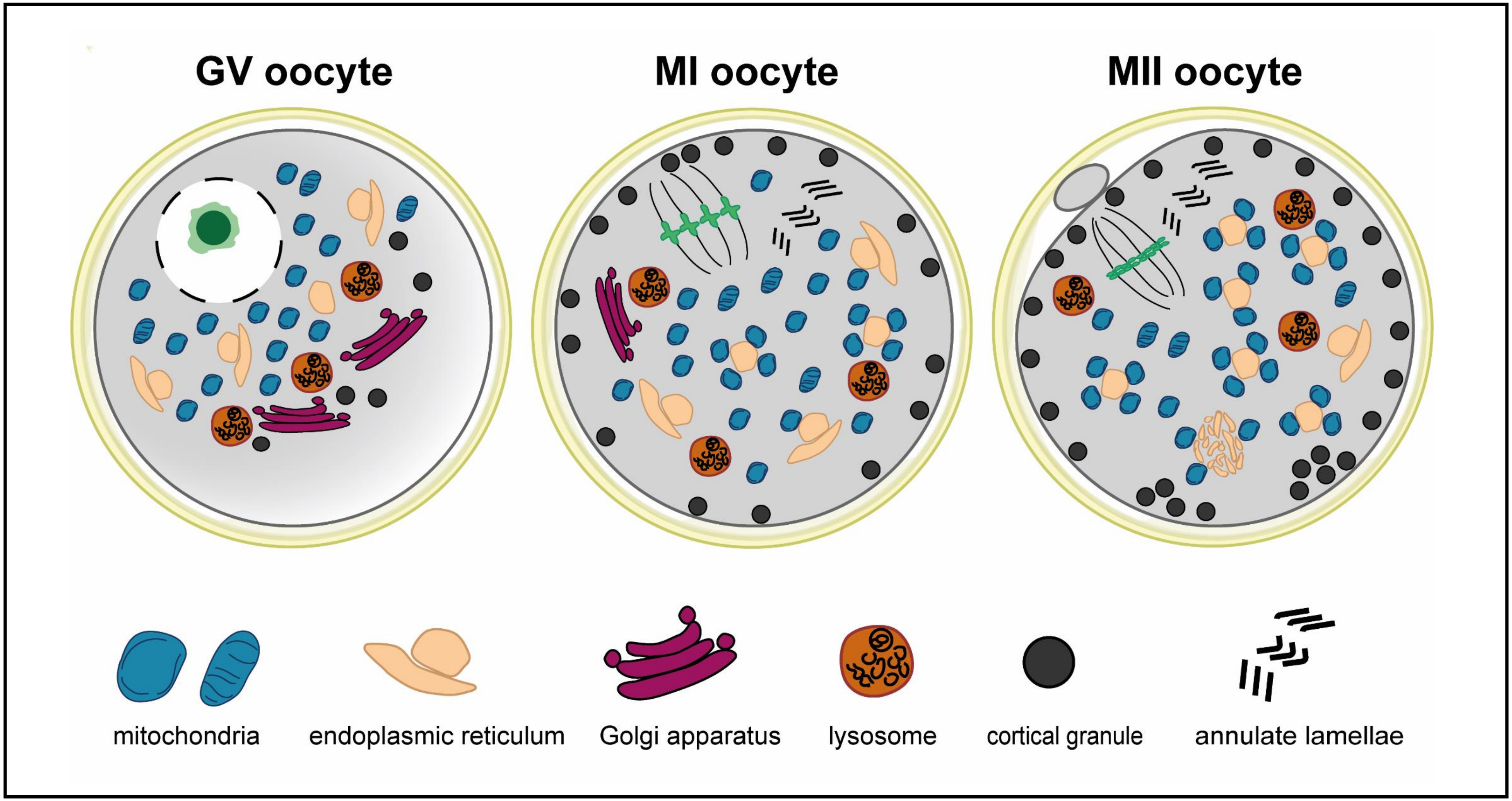
# Organelle rearrangement during maturation




- cortical area populated by organelle after NEBD
- SER-Mt association and SER agreggation
- GA fragmentation and CGs relocation to cortex

*Trebichalska et al, 2021*

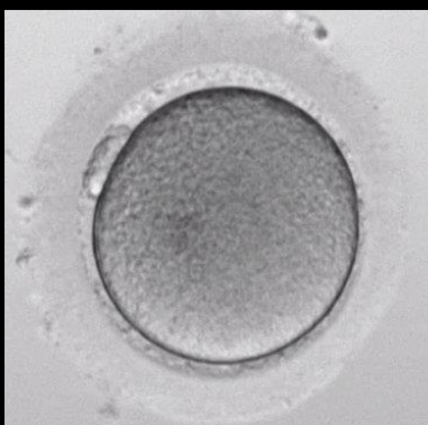
# Organelle rearrangement during maturation




- cortical area populated by organelle after NEBD
- SER-Mt association and SER agreggation
- GA fragmentation and CGs relocation to cortex

*Trebichalska et al, 2021*

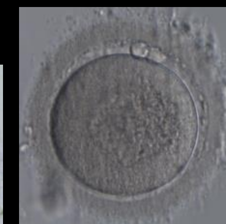
# Human oocyte dysmorphism



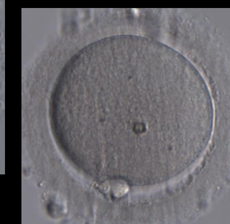
Normal oocyte

Vacuoles

sER disc



Granularity



Refractile bodies

Big polar body

Abnormal perivitelline space

Abnormal shape

Abnormal zona pellucida

Cellular fragments

For more examples see:
aAtlas of Clinical Embryology (MUNI) https://is.muni.cz/do/med/el/ake/index.html
Atlas of human embryology (ESHRE) https://atlas.eshre.eu/

# Cytoplasmic abnormalities

## ❖ Vacuoles

- membrane-bound, translucent bodies with 3D appearance
- persist after fertilization







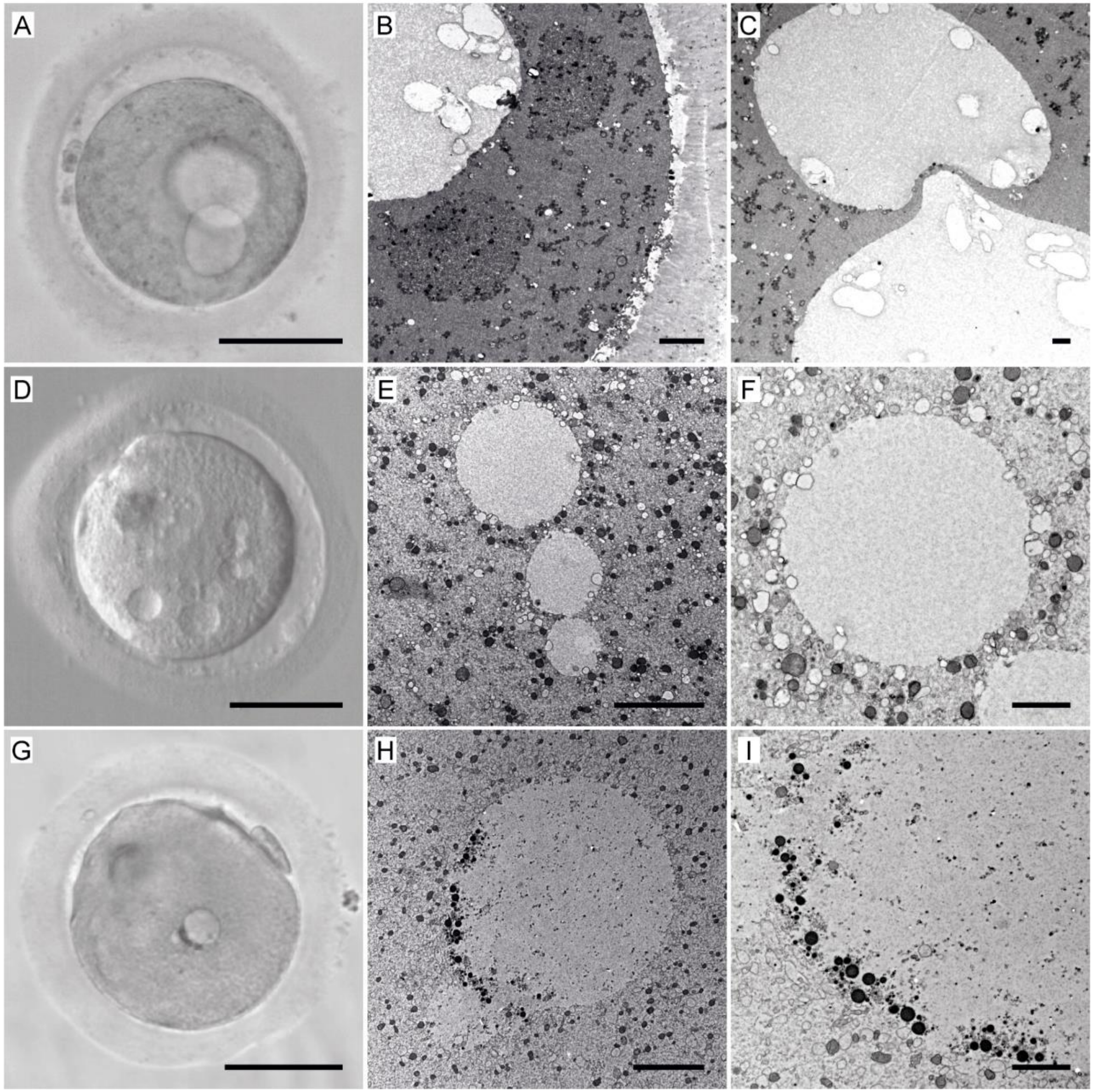
cortical vacuolisation common shortly after thawing

# Cytoplasmic abnormalities

❖ **Vacuoles**

- membrane- bound, fluid-filled structures
- some tend to merge
- may contain granular material

granular vacuole ➡

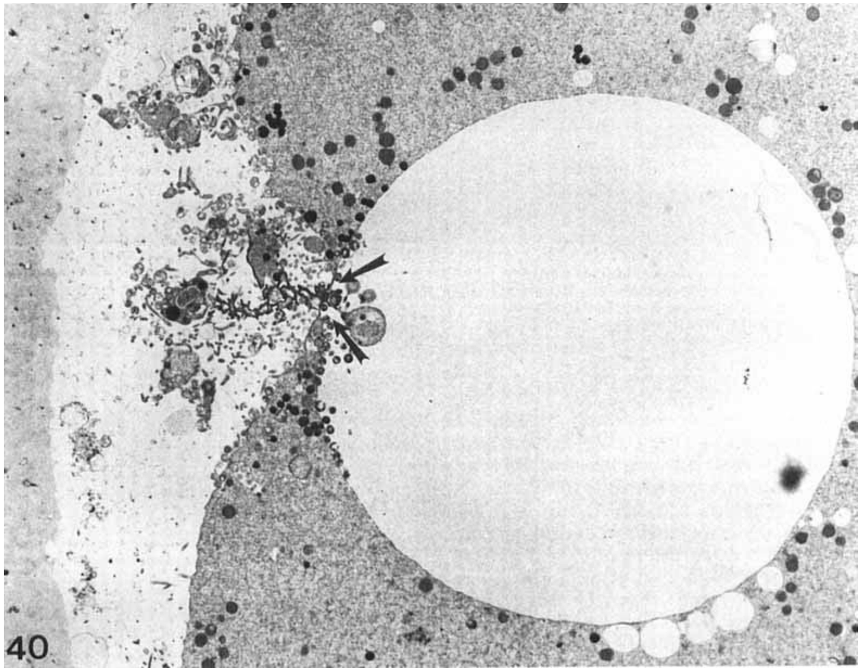



*Tatíčková et al 2023*

# Cytoplasmic abnormalities

❖ **Vacuoles**

- endocytic origin
- vacuolisation caused by instability of cell cortex?
- contain follicular fluid
- persist after fertilization




*Van Blerkom 1990*

# Cytoplasmic abnormalities

❖ **sER disc**

- a smooth plate-like structure
- rare feature
- dissolutes after fertilization



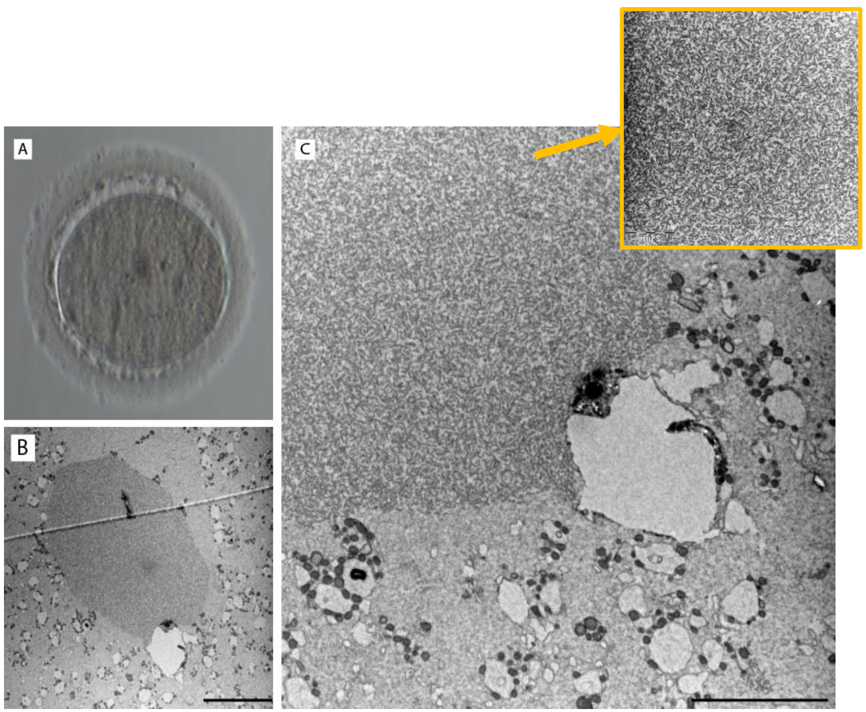




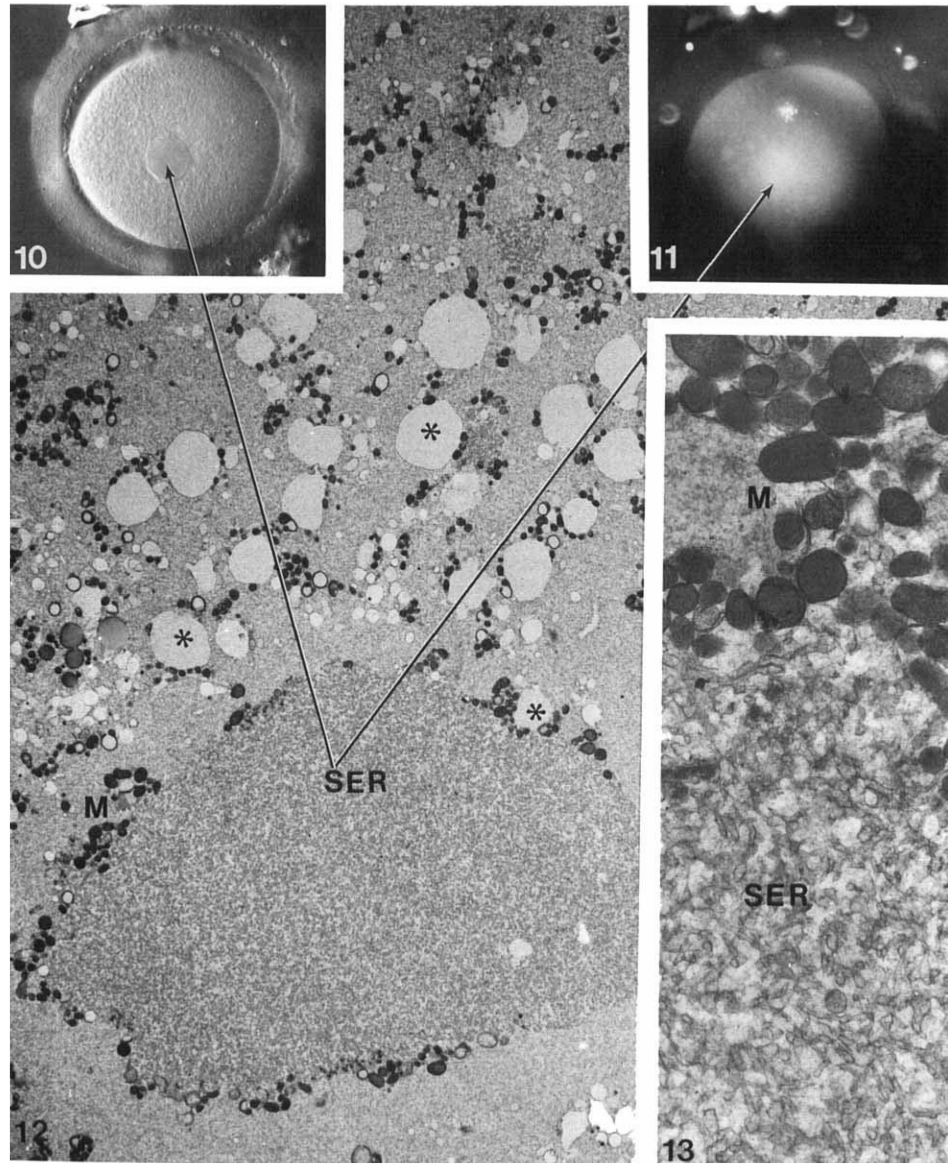

# Cytoplasmic abnormalities

## ❖ sER disc

- enormous aggregate of tubular sER
- decreased fertilisation capacity reported
- not recommended to used for ICSI, nevertheless healthy children born




*Tatíčková et al 2023*

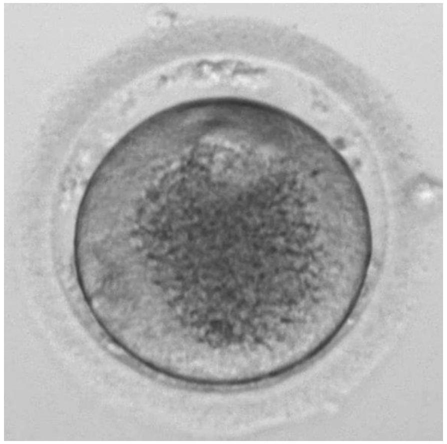



*Van Blerkom 1990*

# Cytoplasmic abnormalities

❖ **Cytoplasmic granularity**
- irregular texture of cytoplasm
- crater-like appearence
- disappears after fertilization

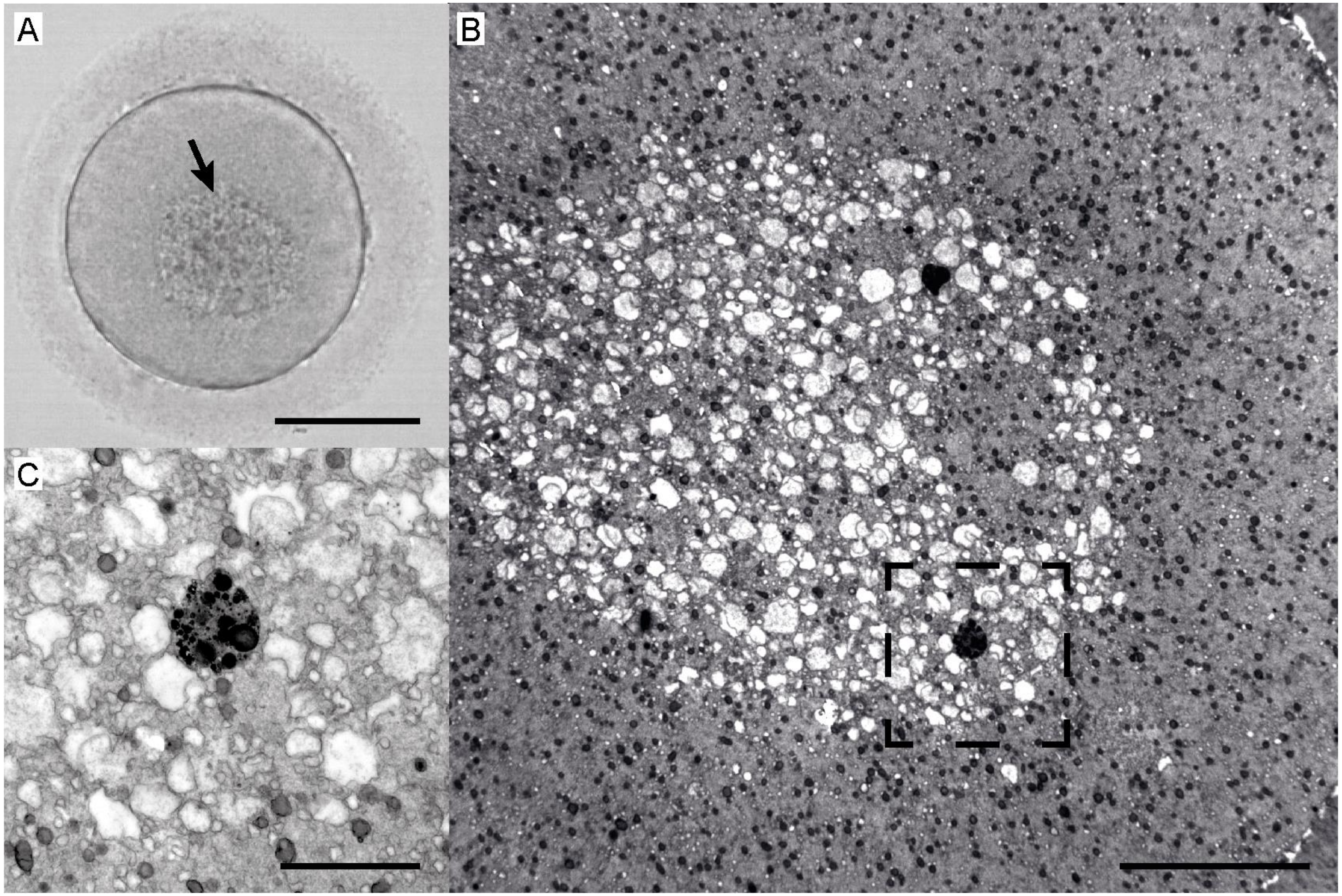



# Cytoplasmic abnormalities

❖ **Cytoplasmic granularity**

- excessive aggregation of organelles
- dysfunction of actin cytoskeleton?




*Tatíčkoví et al 2023*

# Cytoplasmic abnormalities

❖ **Refractile bodies**

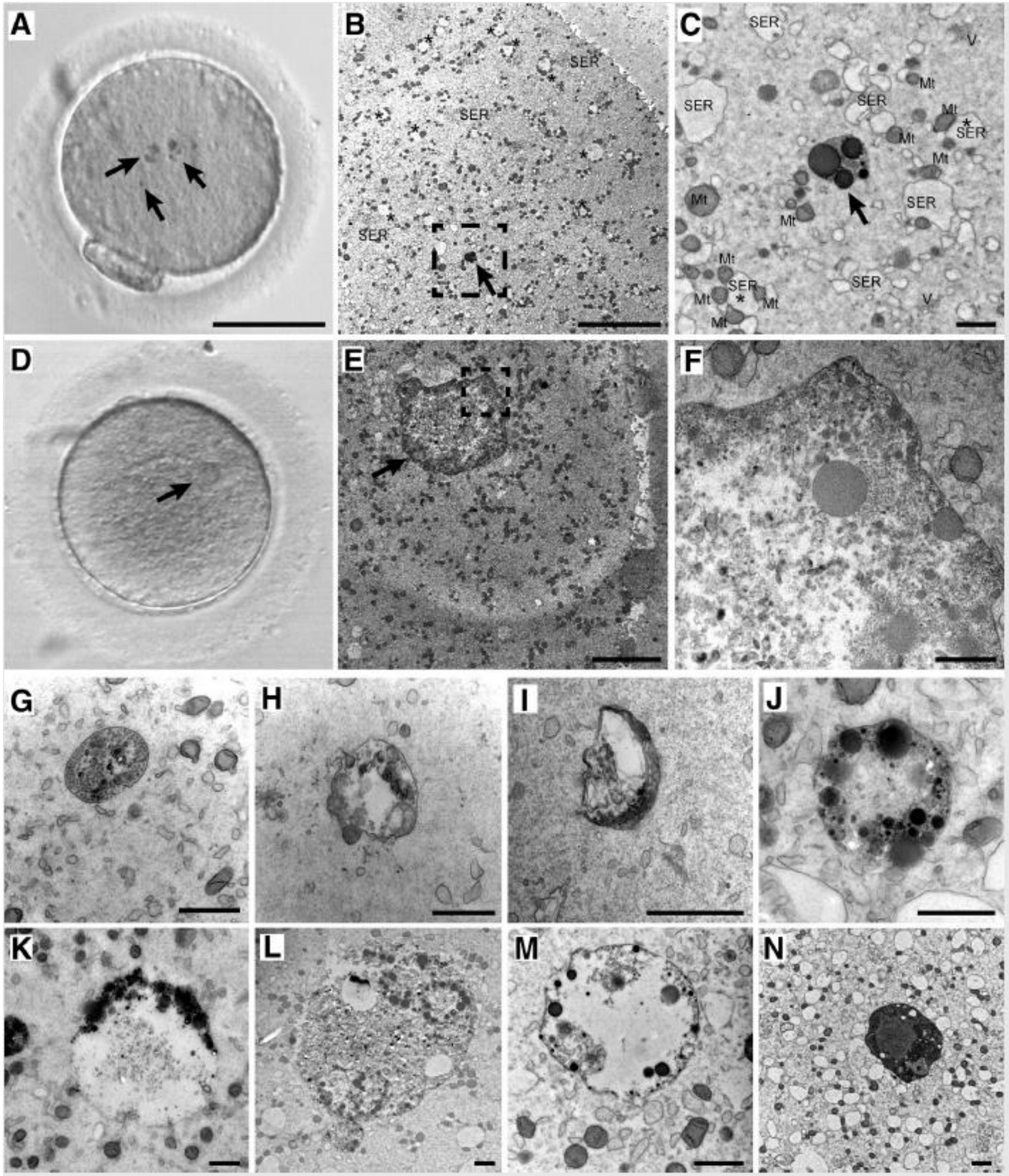
- various-sized dark inclusions in the cytoplasm
- incidence increases with reproductive aging
- persist after fertilization

*„Bull eye- inclusion*“*

# Cytoplasmic abnormalities

❖ **Refractile bodies**

Heterogenous clumps corresponding to tertiary lysosomes

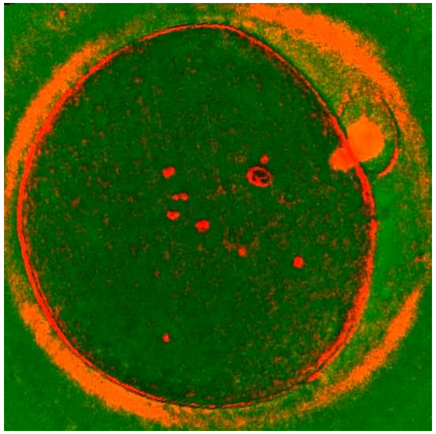



**Composed of**

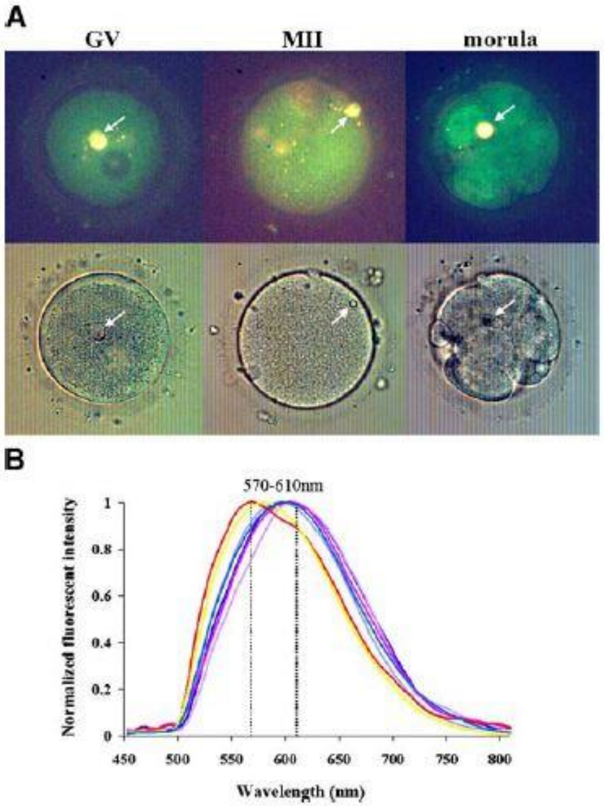
- electron-dense granules
- fibrilar material
- amorphous substance
- membrane remnants
- lipid droplets

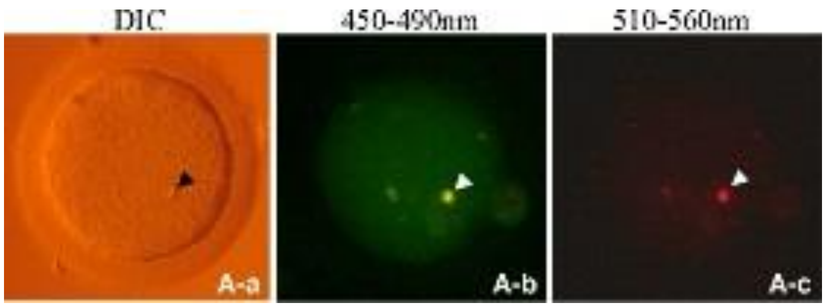
*Tatíčková et al 2023*

# Cytoplasmic abnormalities



- **Refractile bodies**
  - birefringent specs (PLM signal)
  - exhibit autofluorescence characteristic for lipochrome lipofuscin („lipofuscin bodies“) – an insoluble pigment which accumulates in aged terminally differentiated cells




- incorporate biological “garbage” and/or sequester xenobiotics?

← impaired protein and/or lipid metabolism?
← oxidative stress?
← reduced intralysozomal degradation?

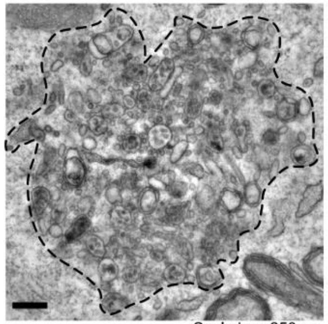
*Otsuki et al 2017*

# Cytoplasmic abnormalities

❖ **Refractile bodies**

## ~ ELVA

= EndoLysosomal Vesicular Assemblies



Scale bar: 250nm.

- non-membrane-bound compartments composed of endolysosomes, autophagosomes, and proteasomes held together by a liquid-like protein matrix
- in immature mouse oocytes, ELVAs sequester aggregated proteins and degrade them upon oocyte maturation
- ELVAs degradative activity increases upon oocyte maturation promoting healthy embryogenesis
- retention of protein aggregates in the embryo leads to early embryonic arrest

- Strategy to deep-clean toxic substances and damaged protein and promote logitivity´

*Zaffagnini et al 2024*

Cell
CellPress OPEN ACCESS

Article

**Mouse oocytes sequester aggregated proteins in degradative super-organelles**

Gabriele Zaffagnini,[1] Shiya Cheng,[2] Marion C. Salzer,[1] Barbara Pernaute,[1,6] Juan Manuel Duran,[1] Manuel Irimia,[1,3,4] Melina Schuh,[2,5] and Elvan Böke[1,3,7,*]
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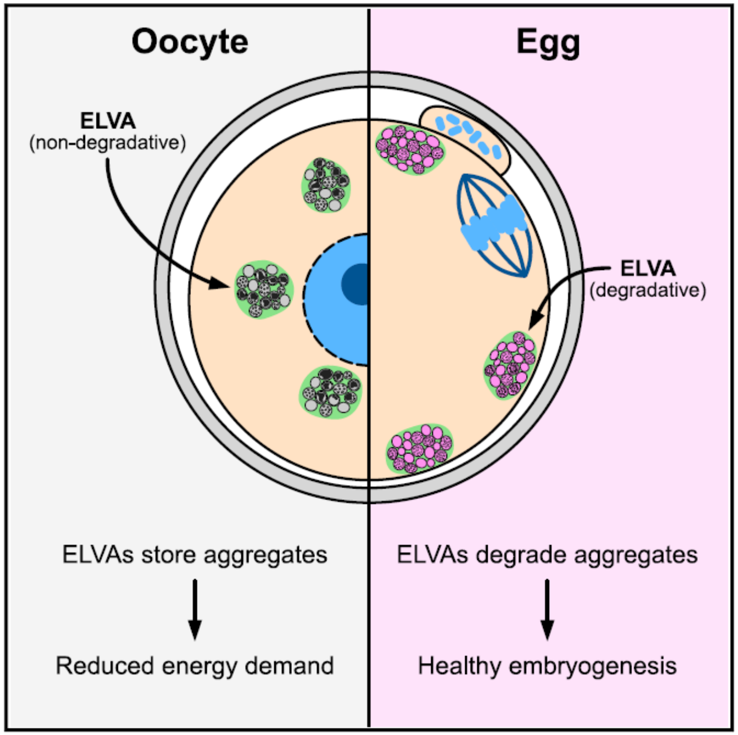
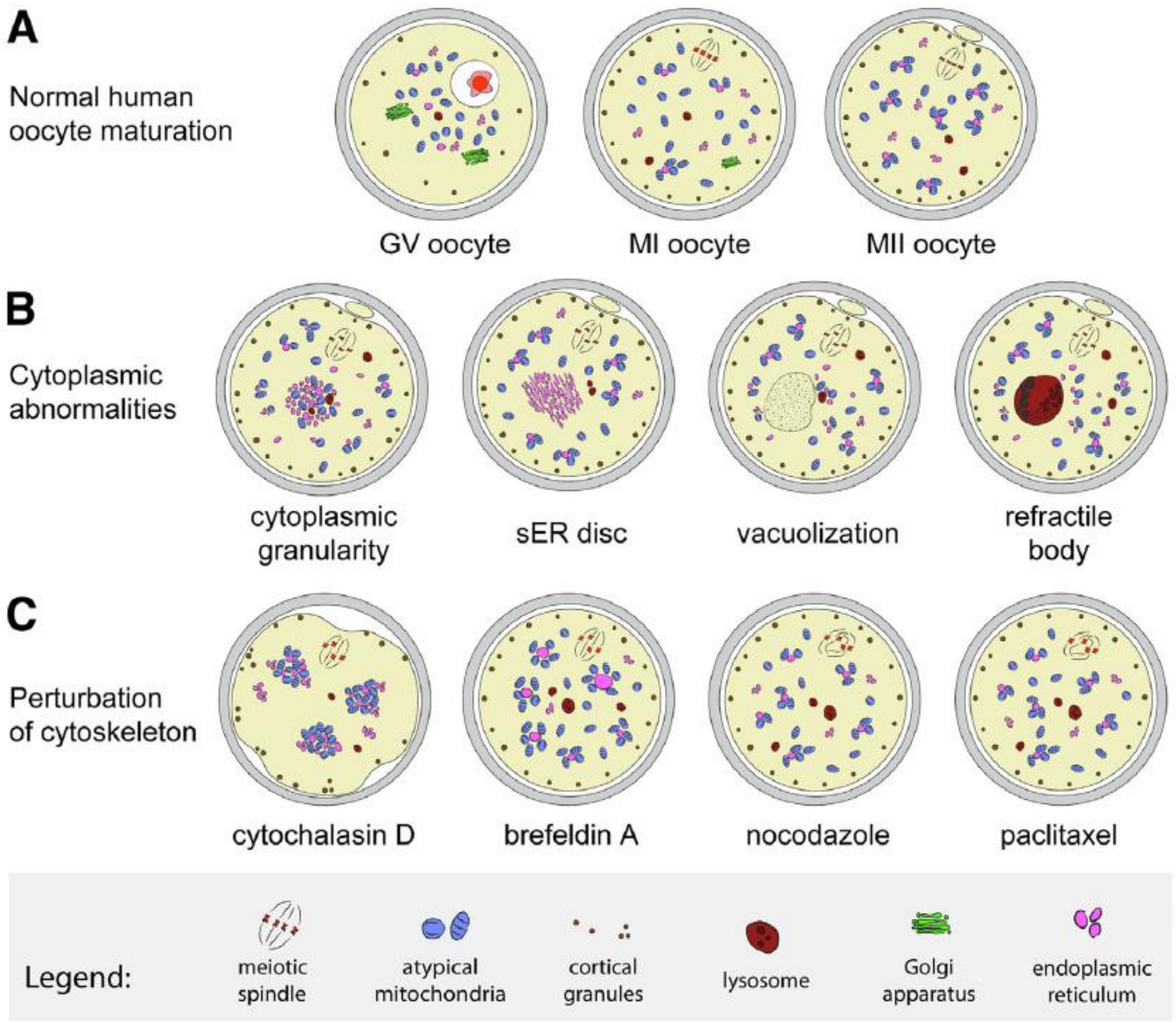
https://doi.org/10.1016/j.cell.2024.01.031



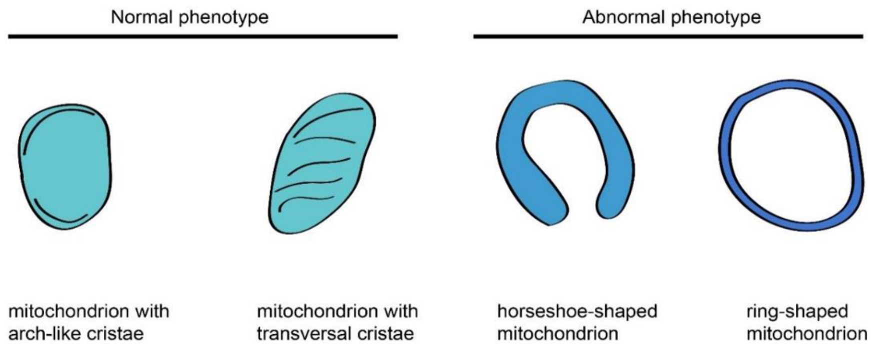
# Cytoplasmic abnormalities

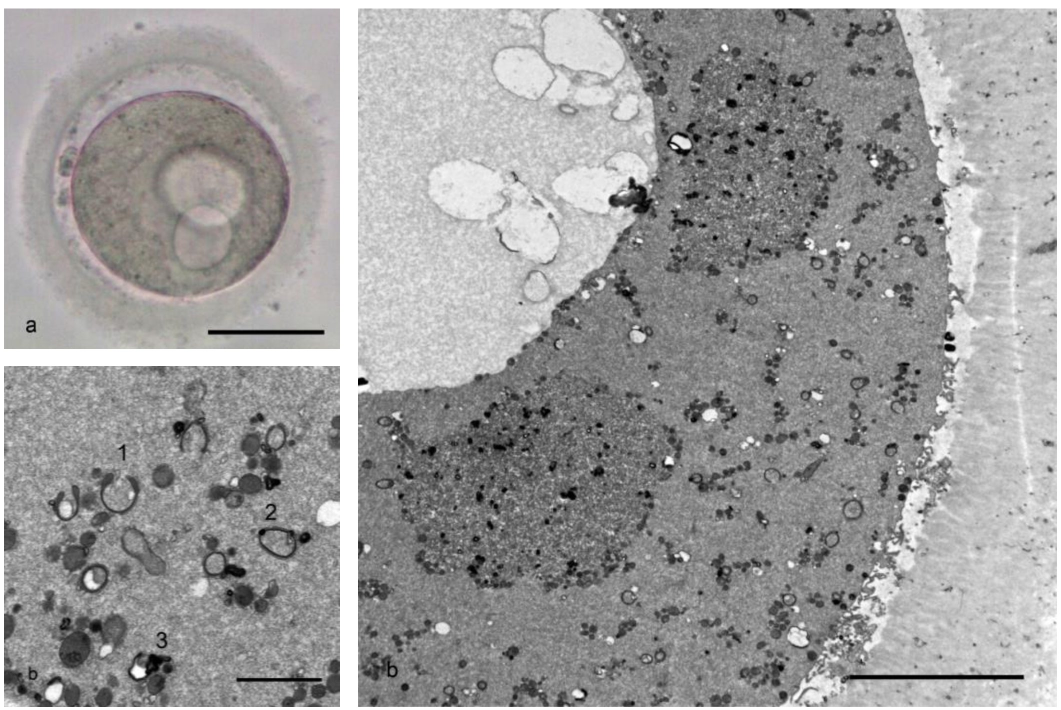



*Tatíčková et al 2023*

# Cytoplasmic abnormalities

## ❖ Patological mitochondria




*Trebichalska Z, Diploma thesis, Faculty of Science, Masaryk University 2020*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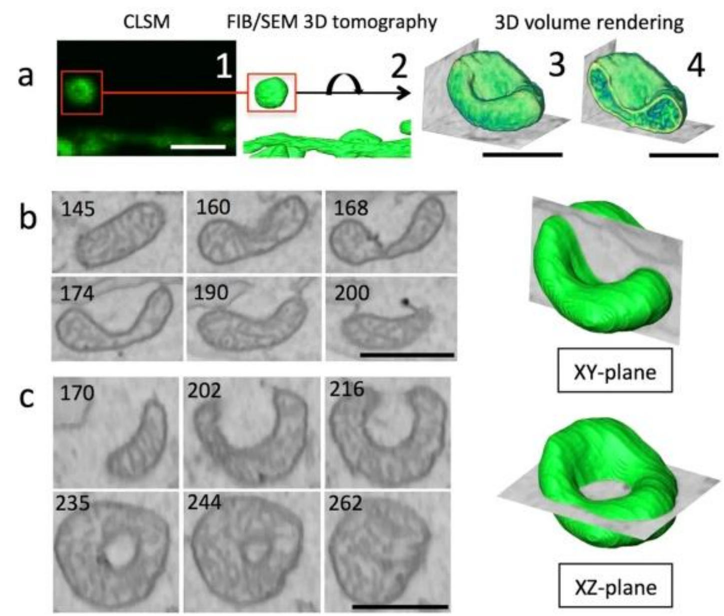
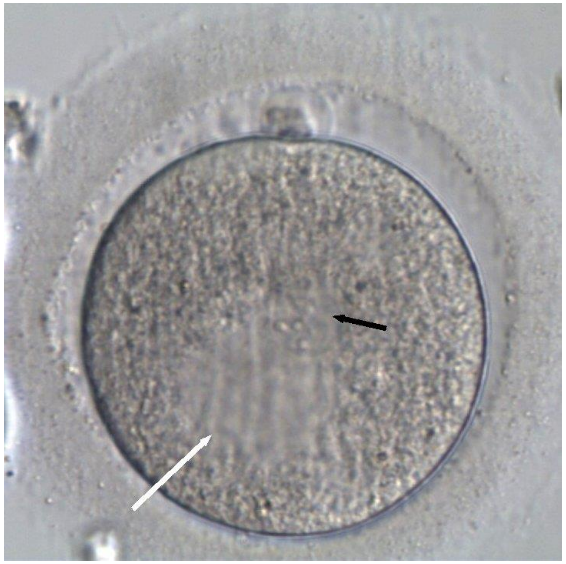
**Uncoupled mitochondria quickly shorten along their long axis to form indented spheroids, instead of rings, in a fission-independent manner**


Yoshihiro Miyazono[1,2], Shingo Hirashima[1], Naotada Ishihara[3], Jingo Kusukawa[2], Kei-ichiro Nakamura[1] & Keisuke Ohta[1,4]


- loss of ΔΨm triggers collapse of mitochondrial structure



# Cytoplasmic abnormalities



# Oocyte morphology and IVF outcome

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journal homepage: www.elsevier.com/locate/ejogrb

Relationship between oocyte abnormal morphology and intracytoplasmic sperm injection outcomes: a meta-analysis

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- meta-analysis of 14 studies

**Table 2**
Influence of oocyte morphological abnormalities on fertilisation rate and embryo quality.

| Morphological abnormality | Fertilisation rate | | Embryo quality | |
|---|---|---|---|---|
| | OR (CI) | Studies (n) | OR (CI) | Studies (n) |
| Fragmented IPB | 1.00 (0.77–1.29) | 7 | 0.74 (0.50–1.10) | 8 |
| Large IPB | 0.29 (0.09–0.90) | 4 | 0.42 (0.16–1.11) | 5 |
| Rough IPB surface | 0.98 (0.84–1.15) | 4 | 0.81 (0.47–1.40) | 5 |
| Large PVS | 0.86 (0.74–0.99) | 4 | 0.99 (0.58–1.70) | 4 |
| PVS granularity | 1.03 (0.85–1.26) | 2 | 1.15 (0.72–1.84) | 3 |
| Colour of the ZP | 0.94 (0.77–1.13) | 3 | 1.17 (0.93–1.47) | 2 |
| Shape of the oocyte | 0.99 (0.83–1.17) | 4 | 1.02 (0.83–1.25) | 3 |
| Cytoplasmic colour | 0.92 (0.76–1.10) | 3 | 0.64 (0.25–1.62) | 3 |
| Cytoplasmic granularity | 0.89 (0.69–1.15) | 2 | 1.15 (0.56–2.36) | 3 |
| Refractile bodies | 0.66 (0.51–0.84) | 3 | 1.06 (0.74–1.51) | 2 |
| Vacuoles | 0.59 (0.42–0.83) | 3 | * | * |

OR, odds ratio; CI, confidence interval; IPB, first polar body; PVS, perivitelline space; ZP, zona pellucida; *, not evaluated.

Egg fertilization capacity significantly reduced by:
(1) large PB1
(2) large PVS
(3) refractile bodies
(4) vacuoles

**Figure 2.1 Effect of large IPB on fertilization rate**

| Study or Subgroup | Experimental Events | Experimental Total | Control Events | Control Total | Weight | Odds Ratio M-H, Random, 95% CI | Year |
|---|---|---|---|---|---|---|---|
| Ebner et al. | 9 | 18 | 155 | 174 | 22.5% | 0.12 [0.04, 0.35] | 2000 |
| De Santis et al. | 15 | 25 | 202 | 340 | 24.1% | 1.02 [0.45, 2.35] | 2005 |
| Fancsovits et al. | 31 | 62 | 468 | 714 | 26.2% | 0.53 [0.31, 0.89] | 2006 |
| Navarro et al. | 75 | 363 | 967 | 1366 | 27.2% | 0.11 [0.08, 0.14] | 2009 |
| **Total (95% CI)** | | **468** | | **2594** | **100.0%** | **0.29 [0.09, 0.90]** | |
| Total events | 130 | | 1792 | | | | |

Heterogeneity: Tau² = 1.22; Chi² = 46.71, df = 3 (P < 0.00001); I² = 94%
Test for overall effect: Z = 2.13 (P = 0.03)

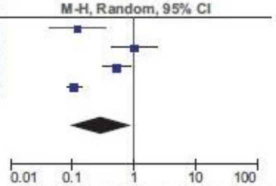



**Figure 2.2 Effect of large PVS on fertilization rate**

| Study or Subgroup | Experimental Events | Experimental Total | Control Events | Control Total | Weight | Odds Ratio M-H, Fixed, 95% CI | Year |
|---|---|---|---|---|---|---|---|
| De Sutter et al. | 39 | 66 | 135 | 194 | 7.2% | 0.63 [0.35, 1.13] | 1996 |
| Xia | 70 | 96 | 107 | 126 | 6.4% | 0.48 [0.25, 0.93] | 1997 |
| Balaban et al. | 620 | 864 | 1589 | 2180 | 65.4% | 0.95 [0.79, 1.13] | 1998 |
| Ten et al. | 108 | 176 | 975 | 1446 | 21.0% | 0.77 [0.56, 1.06] | 2007 |
| **Total (95% CI)** | | **1202** | | **3946** | **100.0%** | **0.86 [0.74, 0.99]** | |
| Total events | 837 | | 2806 | | | | |

Heterogeneity: Chi² = 5.68, df = 3 (P = 0.13); I² = 47%
Test for overall effect: Z = 2.12 (P = 0.03)

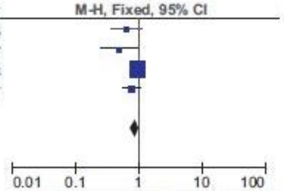



**Figure 2.3 Effect of refractile bodies on fertilization rate**

| Study or Subgroup | Experimental Events | Experimental Total | Control Events | Control Total | Weight | Odds Ratio M-H, Fixed, 95% CI | Year |
|---|---|---|---|---|---|---|---|
| De Sutter et al. | 57 | 98 | 135 | 194 | 26.2% | 0.61 [0.37, 1.01] | 1996 |
| Balaban et al. | 113 | 167 | 1589 | 2180 | 50.5% | 0.78 [0.56, 1.09] | 1998 |
| Ebner et al. | 58 | 84 | 249 | 300 | 23.3% | 0.46 [0.26, 0.79] | 2000 |
| **Total (95% CI)** | | **349** | | **2674** | **100.0%** | **0.66 [0.51, 0.84]** | |
| Total events | 228 | | 1973 | | | | |

Heterogeneity: Chi² = 2.72, df = 2 (P = 0.26); I² = 27%
Test for overall effect: Z = 3.29 (P = 0.001)

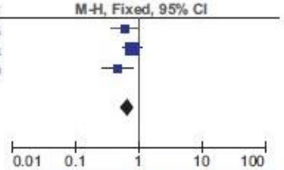
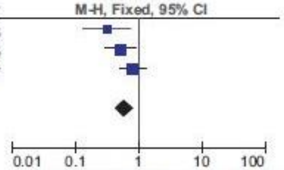



**Figure 2.4 Effect of vacuoles on fertilization rate**

| Study or Subgroup | Experimental Events | Experimental Total | Control Events | Control Total | Weight | Odds Ratio M-H, Fixed, 95% CI | Year |
|---|---|---|---|---|---|---|---|
| De Sutter et al. | 10 | 24 | 135 | 194 | 21.5% | 0.31 [0.13, 0.74] | 1996 |
| Ebner et al., | 23 | 47 | 752 | 1151 | 37.3% | 0.51 [0.28, 0.91] | 2005 |
| Ten et al. | 42 | 68 | 1040 | 1554 | 41.3% | 0.80 [0.48, 1.32] | 2007 |
| **Total (95% CI)** | | **139** | | **2899** | **100.0%** | **0.59 [0.42, 0.83]** | |
| Total events | 75 | | 1927 | | | | |

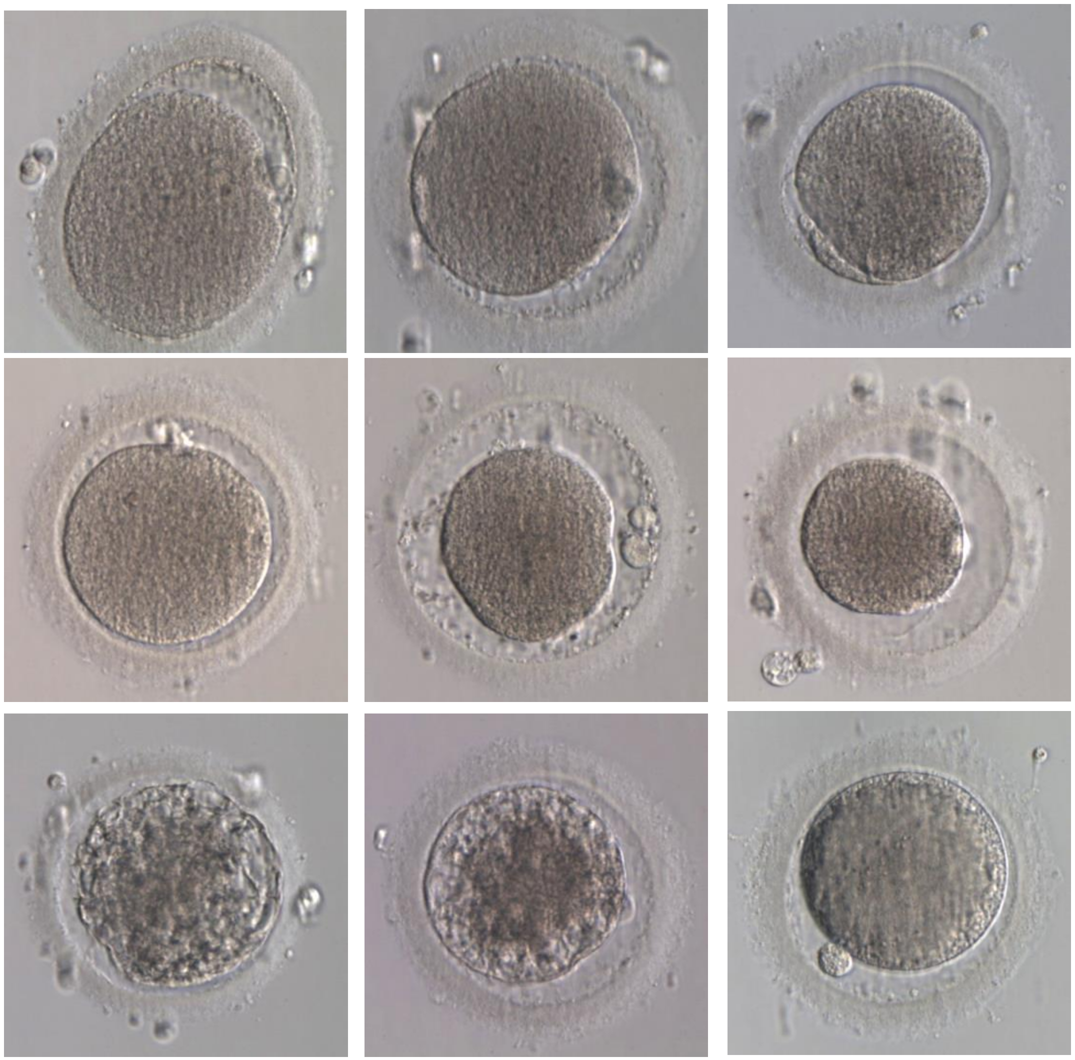
Heterogeneity: Chi² = 3.72, df = 2 (P = 0.16); I² = 46%
Test for overall effect: Z = 3.05 (P = 0.002)




*Setti et al 2011*

# Oocyte degeneration

- dark, granular cytoplasm



videos

bursting

shrinking

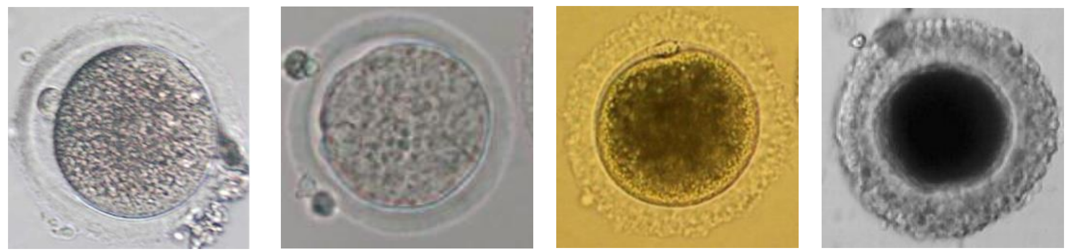
vacuolization

# Lipid droplets

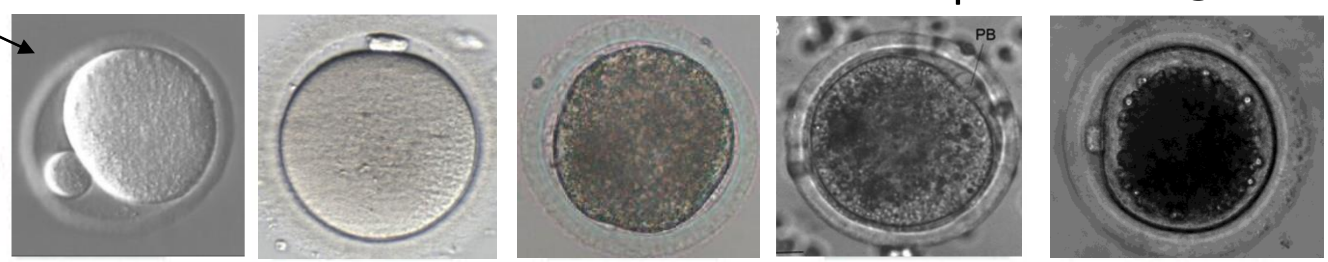
- dark, lipid storing inclusions
- composed of neutral lipids (triacylglycerides) and cholesterol esters
- interspecies variability in amount of lipid droplets

- the oocytes with high lipid content droplets appear darker

Rabbit | Rat | Cat | Dog



strain-dependent

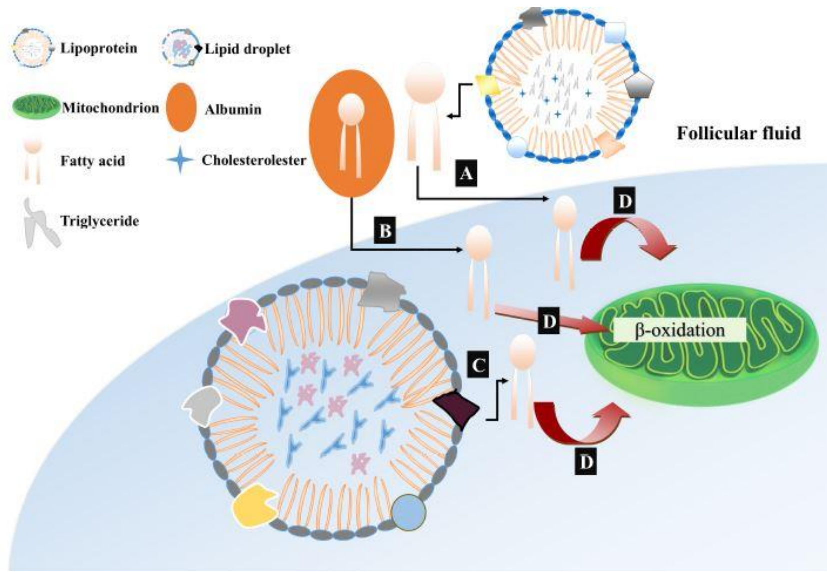


| | Mouse | Human | Cow | Sheep | Pig |
|---|---|---|---|---|---|
| *Lipid droplet accumulation* | | | | | |
| *Intraoocyte lipid content* | * | | ** | **/*** | *** |
| *Fatty acid oxidation dependency* | * | | ** | | *** |

*Dalbies-Tran et al 2020*

# Oocyte lipids

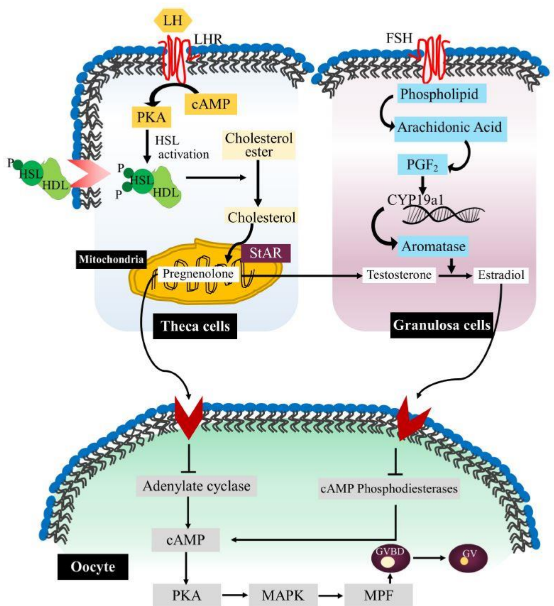
- lipid droplets
- phospholipids
- free fatty acids
    - saturated (palmitic C16, stearic C18 acid)
    - monounsaturated (oleic acid)
    - polyunsaturated (linoleic, arachidonic acid)

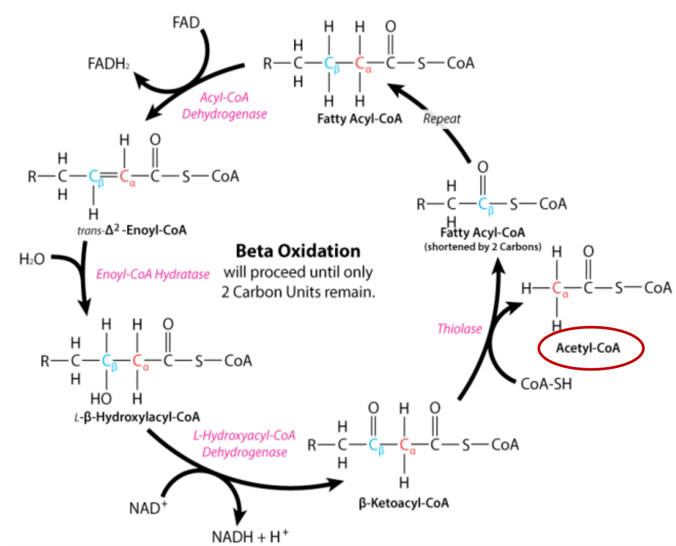



❖ **ROLES**

- ➢ energy production via mitochondrial β-oxidation and TCA (tricarboxylic acid) cycle
- ➢ precursors of steroid homones
- ➢ cellular signalling
- ➢ membrane components

- balanced amount of saturated and unsaturated fatty in oocyte´s microenvironment is critical for cytoplasmic maturation



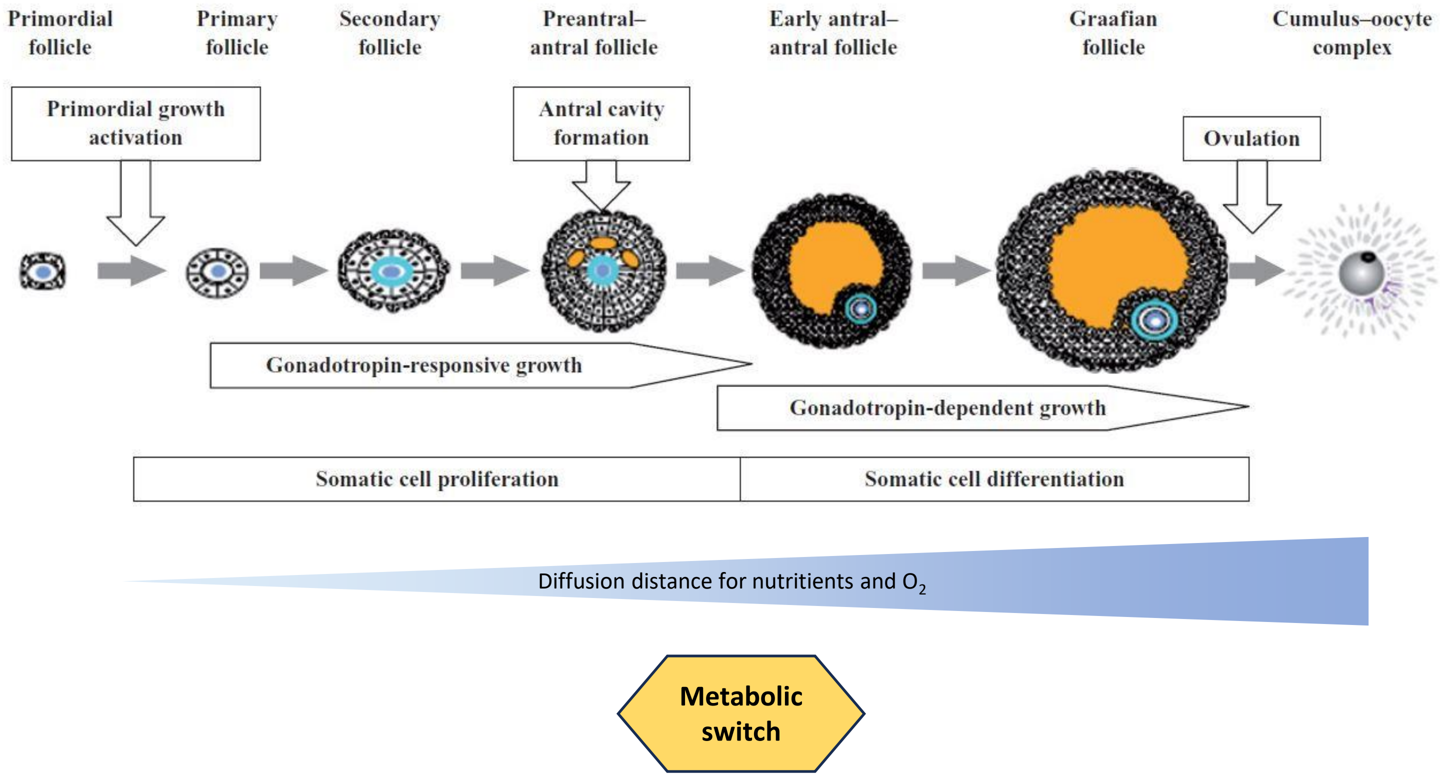




*Khan et al 2021*

# Oocyte metabolism

- metabolic cooperativity between oocytes and follicular cells
- metabolic coupling through gap-junctions
- different nutritional needs of these cell types – “nutrient partitioning“
- change of energy source during growth and differentiation
- influenced by endocrine environment and oxygen availabilty

Helen Picton



# Oocyte metabolism

## ❖ Glucose (Glc)

- uptake by GCs affected by FSH, LH, and insulin
- glycolysis in GCs or transfer to oocytes via gap junctions (oocytes have only low glycolytic capacity)
- metabolized to Pyr and Lac
- Glc consumption in COC increase during follicle growth and peaks before ovulation

## ❖ Pyruvate (Pyr)

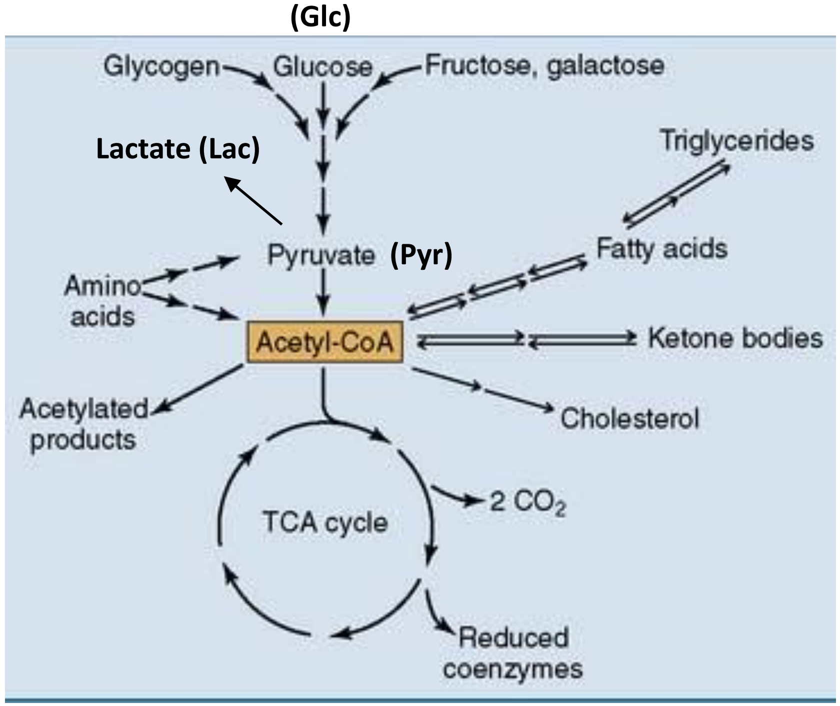
- preferred energy source for mammalian oocytes
- supplemented by GCs
- oxidation through TCA and OXPHOS
- acts as free ROS scavanger and buffer

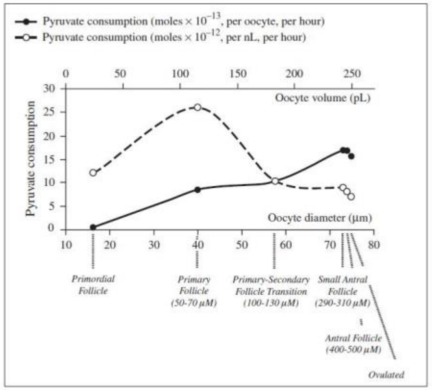
## ❖ Amino acids

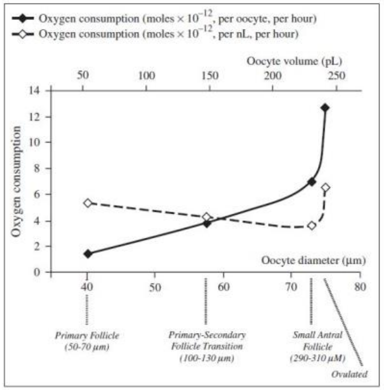
- transferred to oocyte from cumulus cells
- energy substrates
- heavy metal chelatators
- pH regulation
- elimination of amonia

## ❖ Cholesterol

- supplemenated by cumulus cells under oocyte´s influence (i.e. BMP15, GDP9)
- transfer through raft structures
- oocytes promote steroidogenesis and suppress luiteinisation in cumulus cells



# Oocyte metabolism

Impact of maternal diet on egg quality?

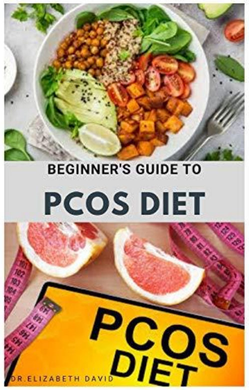
## ❖ Malnutrition

- undernutrition
  - ← decresed caloric intake (starving, anorexia, bulimia)
  - ← increased nutritional requirement/loss (athletes, disease)
  - ← impaired ability to absorb or utilize nutritiones (intolerance)


- overnutrition
  - Overweight BMI=25-29.9
  - Obese BMI ≥ 30

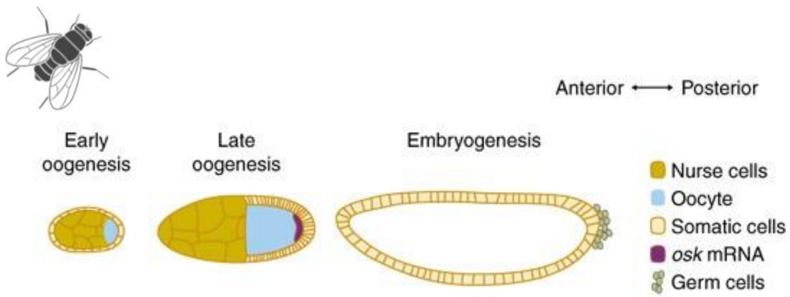
  } > 30% world population!

- conflicting data from human and animal studies
- overnutrition seems to be more detrimental than undernutrition
  - → lipotoxicity, ER stress, mitochondria alteration, absence of microvilli, ROS production, maturation arrest, inflamation, apoptosis (of GSc)...
- high-fat and high-protein diet impair oocyte developmental competence
- drastic weight loss before IVF treatment?

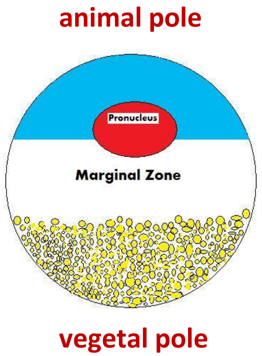


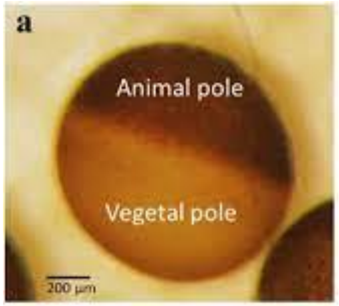

# Oocyte compartmentalization

- polarization of ooplasmic determinants and embryonic prepatterning in lower species

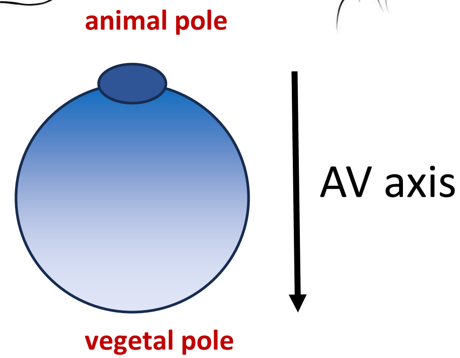



- marked asymmetric deposition of pigments and yolk in Xenopus eggs

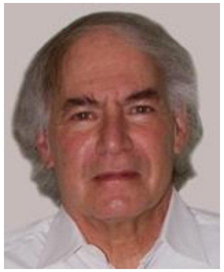



- mammalian oocyte compartmentalization?
    - actin polarization (thickening of actin = actin cap)
    - hyperpolarized mitochondria
    - lipid rafts
    - localization of maternal factors




# Oocyte compartmentalization

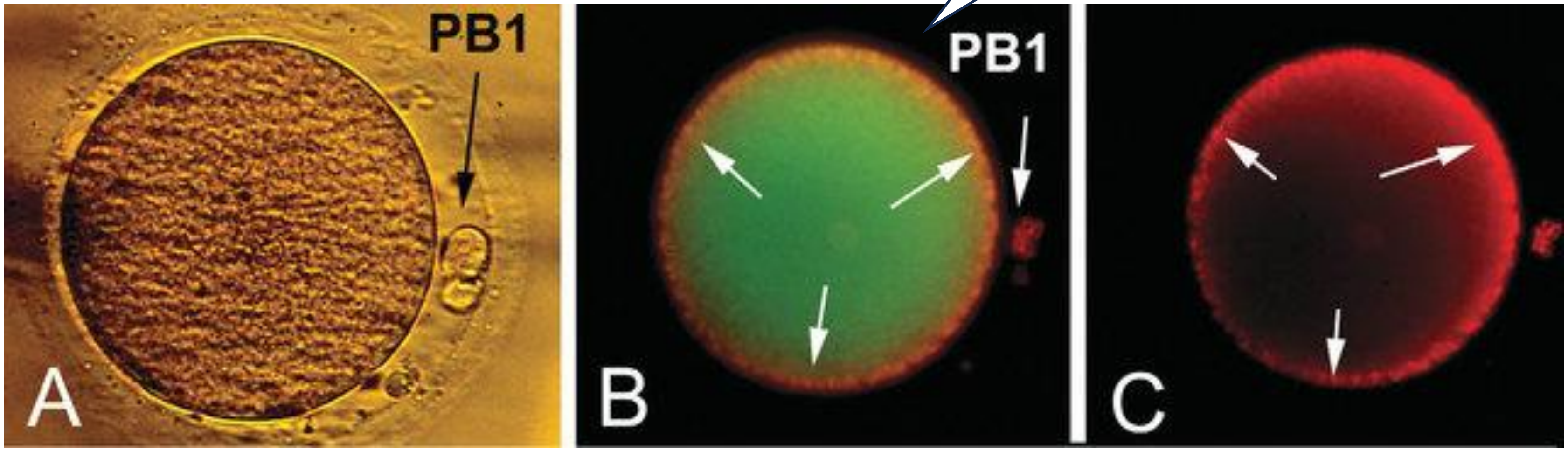


*Johnathan van Blerkom*

## ❖ Hyperpolarized mitochondria in subplasmalemal region

- 5-10 µm zone beneath oolema of mouse oocytes was found to be enriched for mitochondria with higher membrane potential (ΔΨm)*
- detected by mitochondria-specific potentiometric fluorescent stain JC-1
- asymmetric distribution mitochondria of higher functional activity at the periphery?
- proximity to 02 source?
- lower ATP consumption at the periphery?

- low potential – green monomer
- high potential → multimerization (J-aggregates) orange-red signal



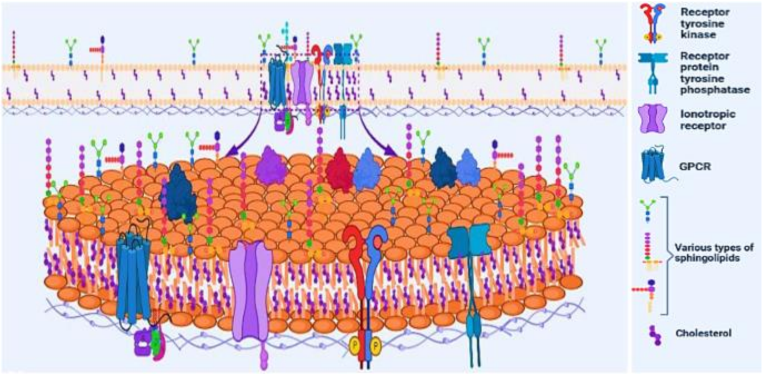

* difference across inner and outer Mt membrane (-mV)

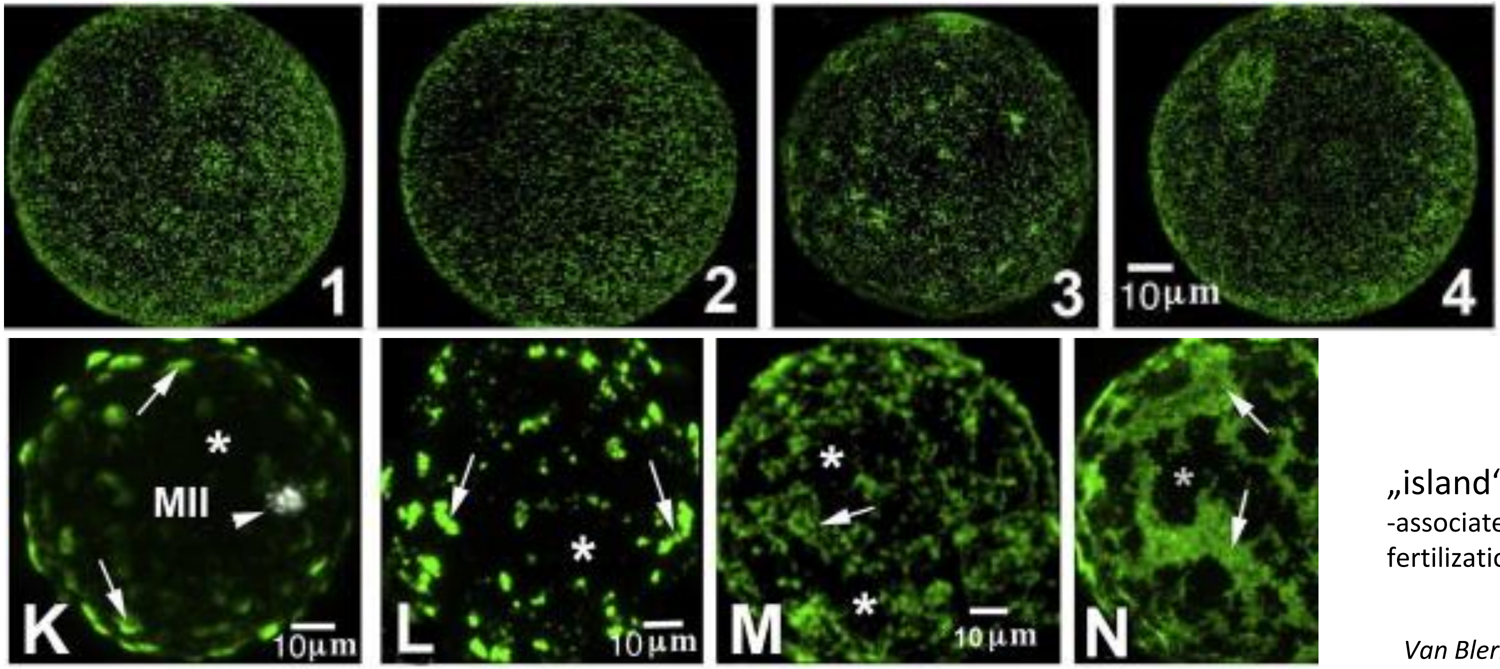
*Van Blerkom et al 2008*

# Oocyte compartmentalization

## ❖ Lipid rafts

- submicron sized oolemal microdomains which serve as concentrating platforms for membrane activities
- composed of cholesterol, sphingolipids and protein receptors; enriched for ganglioside GM-1
- organisation influenced by ΔΨm of suboolemmal Mt
- disruption of lipid rafts might explain fertilization failures



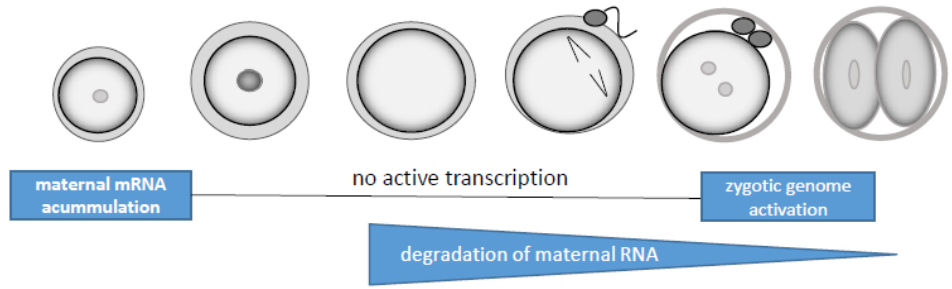

„finely punctate" pattern

„island" pattern
-associated with fertilization failure

*Van Blerkom and Caltrider 2013*

# Oocyte compartmentalization

❖ **Localization of maternal factors**



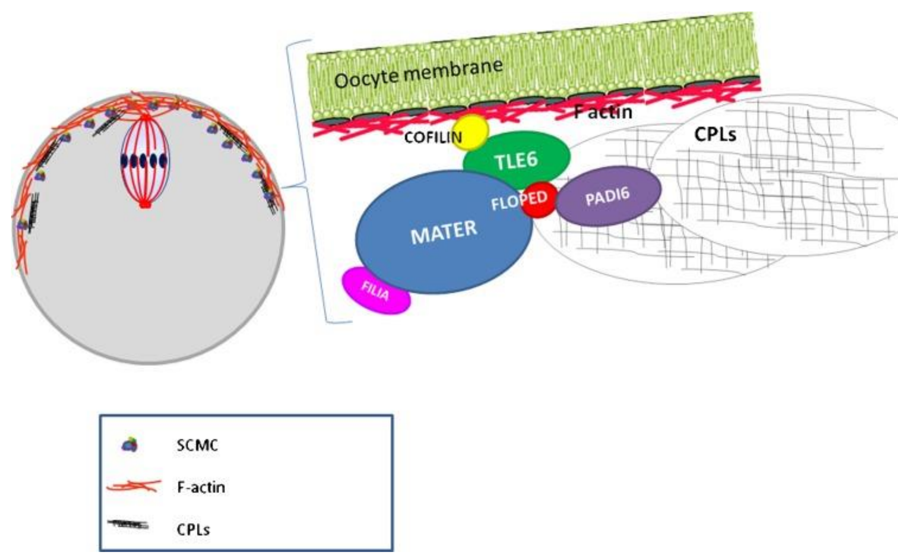

- **Maternal effect genes (MEG)**

  - transcribed during oogenesis but function during fertilization and preimplantation development (e.g., molecular remodelling of paternal genome, degradation of maternal mRNA and proteins, completion of EGA,...)

  - mutations typically manifest as idiopathic infertility and cleavage arrest in IVF and imprinting disorders

SubCortical Maternal Complex (SCMC)

Developmentally lethal -/- mutations



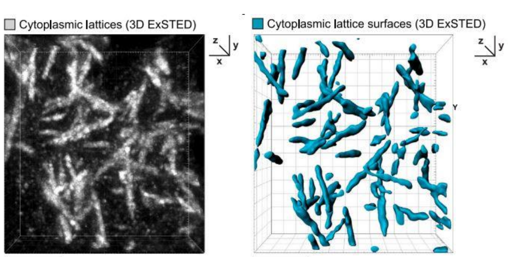
# Maternal factors

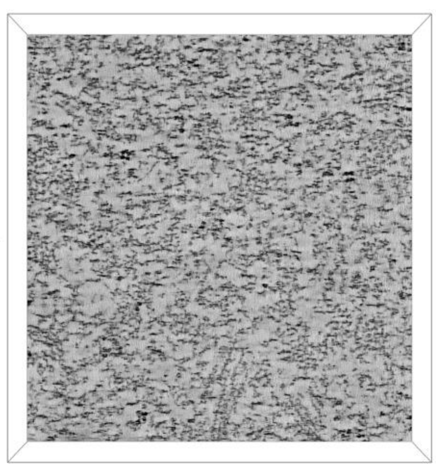
- maternal factors are stored at cytoplasmic lattices
- fibers made of short twisted filaments filaments with high surface area
- accumulate maternal proteins that are critical for postfertilization epigenetic reprogramming and early embryonic development
- separation of maternal factors from cytosol prevents their premature activity and degradation

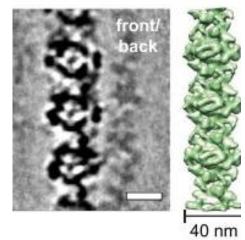
- PADI6 and SCMC (subcortical maternal complex) essential for cytoplasmic lattices formation

- embryos from PADI6 and SCMC -/- females (mouse/human!) arrest early in development

- discovered using super resolution and cryoelectron microscopy tomography






*Jentoft et al. 2023*


Cell
CellPress
OPEN ACCESS
Article
Mammalian oocytes store proteins for the early embryo on cytoplasmic lattices

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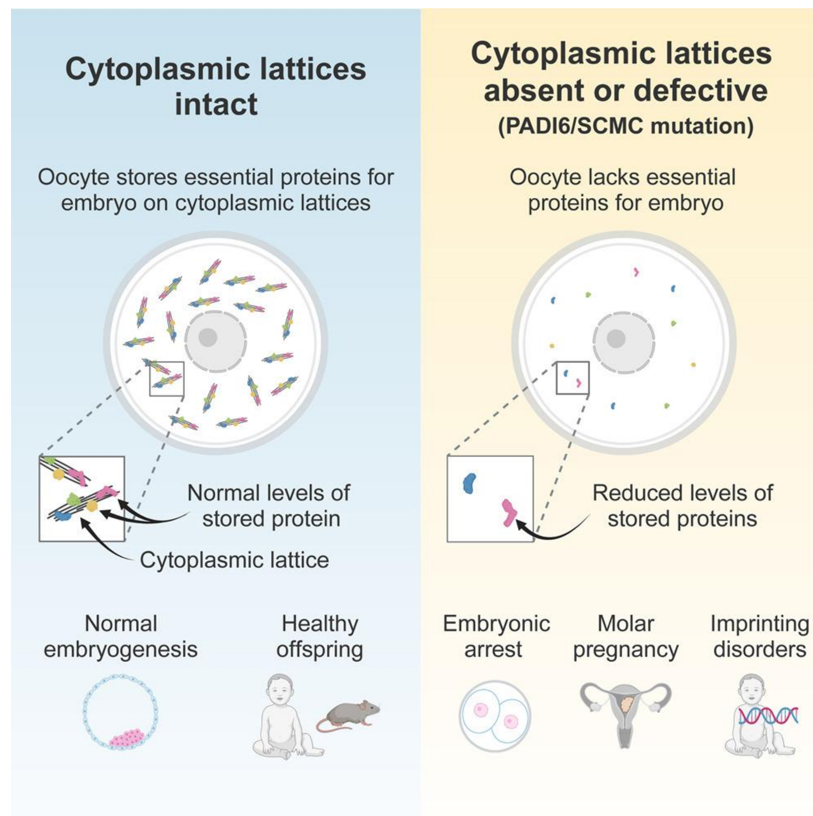
https://doi.org/10.1016/j.cell.2023.10.003

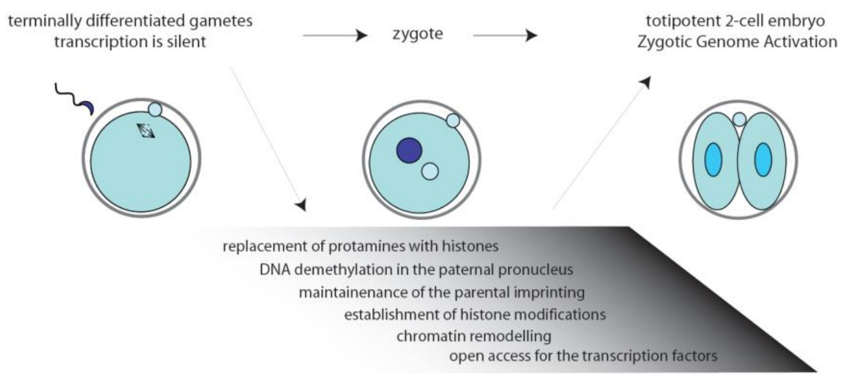


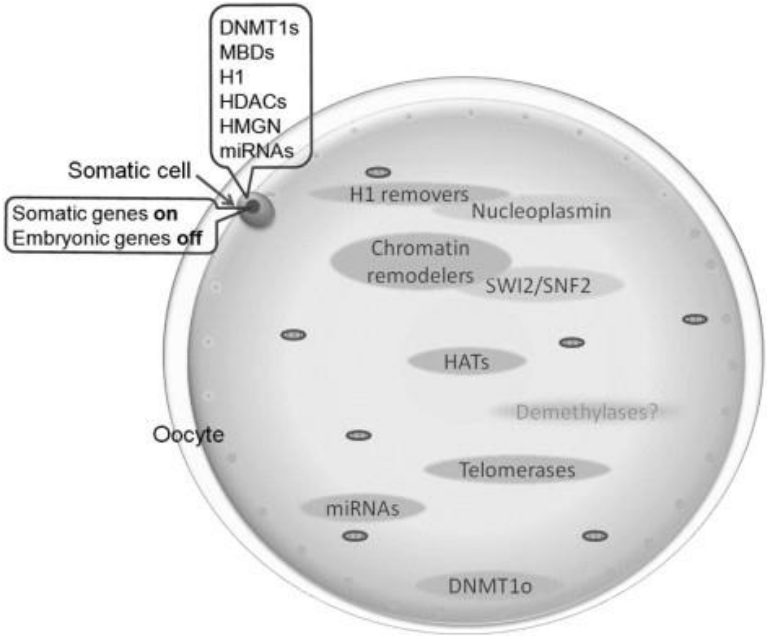
# Maternal factors

- capable to orchestrate chromatin remodelling and reprogramming

(A) sperm-delivered genome during fertilization

(B) somatic nucleus after somatic cell nuclear transfer (SCNT)



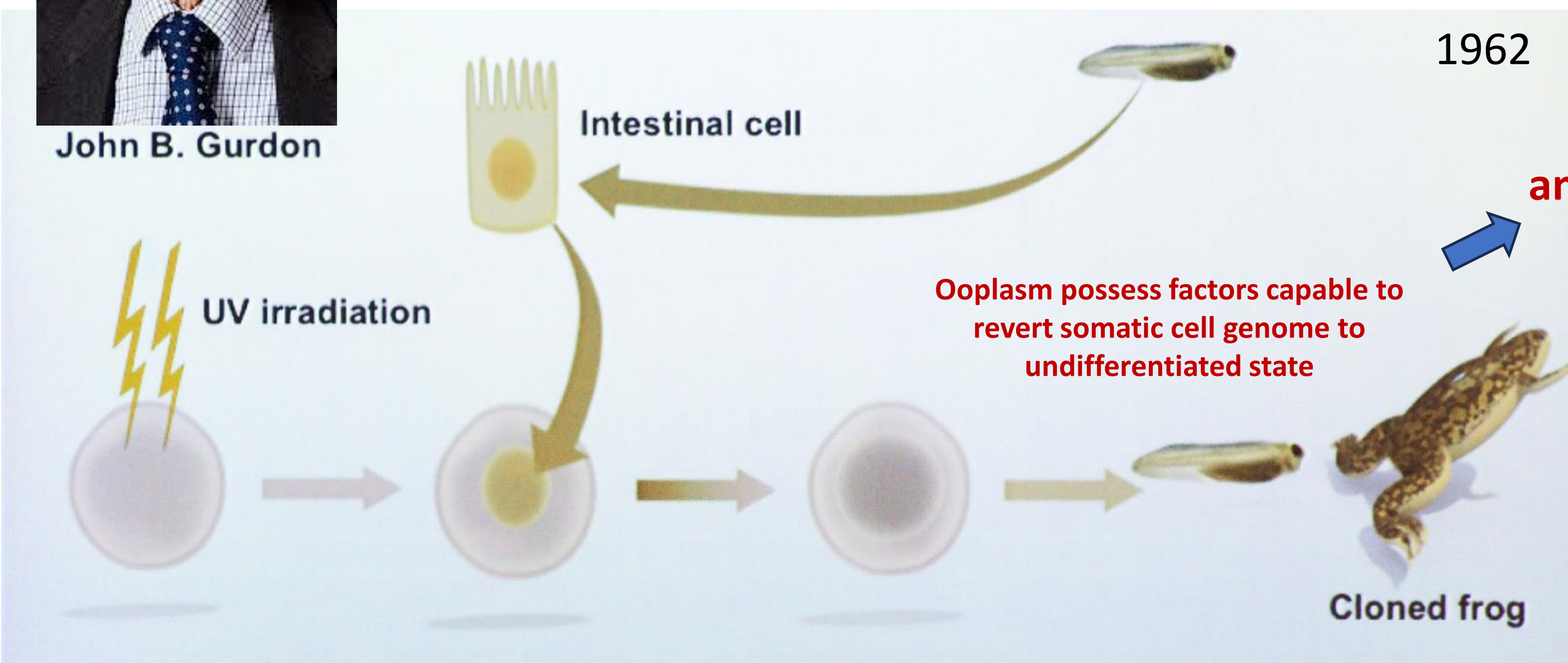
# Somatic cell nuclear transfer (SCNT)

Shinya Yamanaka  Ian Wilmut  John Gurdon

*„for the discovery that mature cells can be reprogrammed to become pluripotent"*

2012


John B. Gurdon



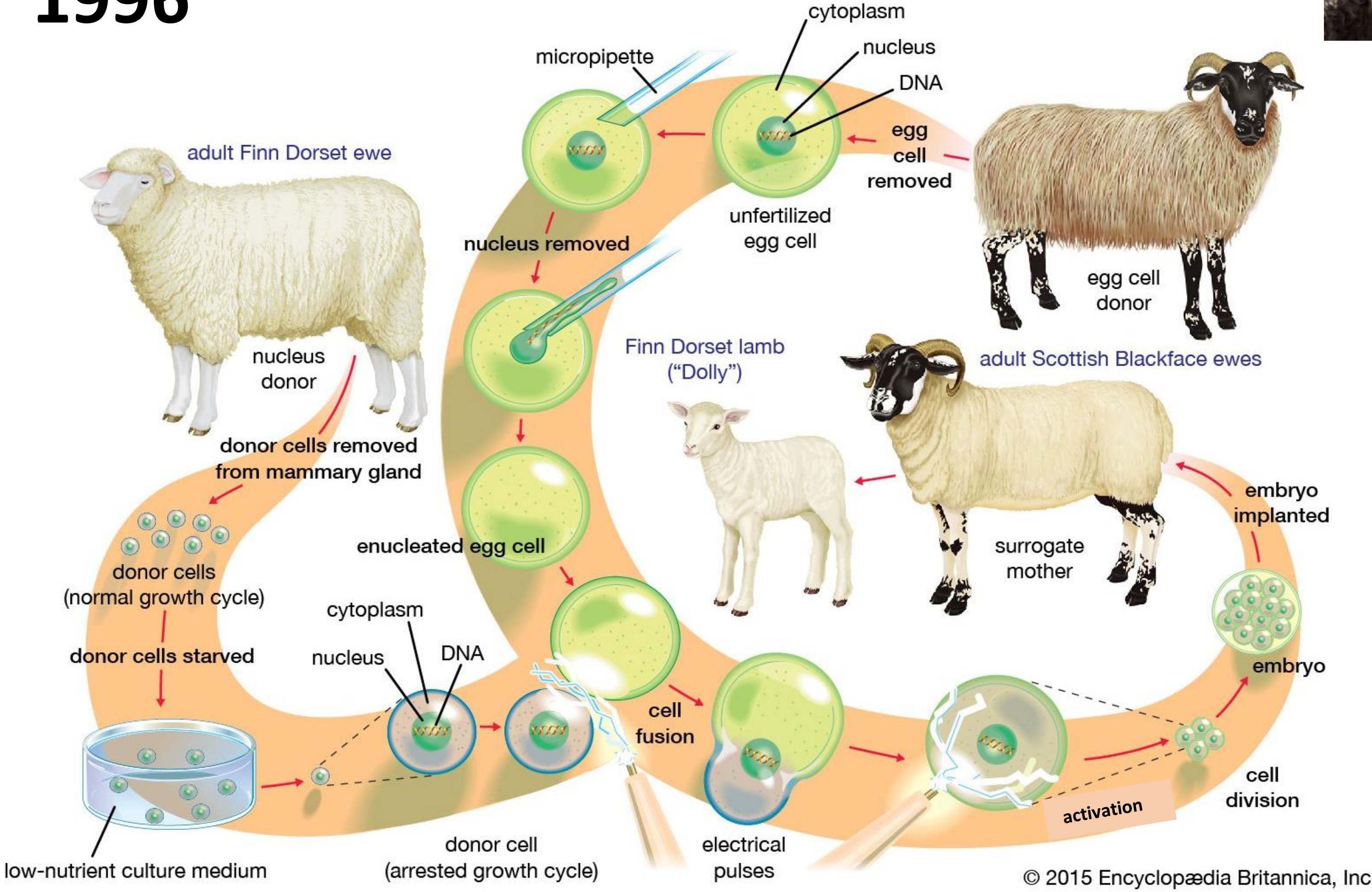

Ooplasm possess factors capable to revert somatic cell genome to undifferentiated state

animal cloning

# Somatic cell nuclear transfer (SCNT)

## Dolly: the cloned sheep

## 1996




Dolly Ian Wilmut

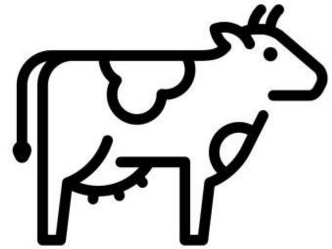

- low efficiency
- epigenetic alterations

- cell cycle stage mismatch between cytoplast (MII oocytes) and cell nucleus (G0/G1) ??

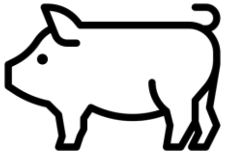
# Somatic cell nuclear transfer (SCNT)

## SCNT in other species

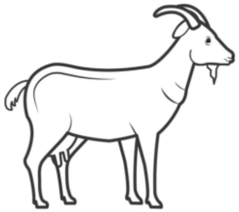
*(Wakayama et al. 1998)*



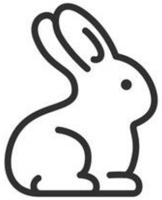
*(Kato et al. 1998)*



*(Polejava et al. 2000)*



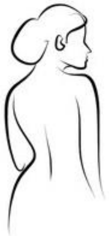
*(Baguisi et al. 1999)*



*(Chesné et al. 2002)*

*(Lee et al. 2005)*

?



The New York Times

***The 5 Clones in Argentina's Election***

Javier Milei, a far-right libertarian, might soon be Argentina's next president. He credits his cloned "four-legged children."

The American newspaper **The New York Times** , which defined Javier Milei as "the candidate of the extreme right", dedicated a curious article to those whom the libertarian calls **"his four-legged children"** .

Milei's five mastiffs **were cloned from her previous dog named Conan** in laboratories located in Texas, United States.

# Somatic cell nuclear transfer (SCNT)

## Cloned macaque monkeys

# 2018

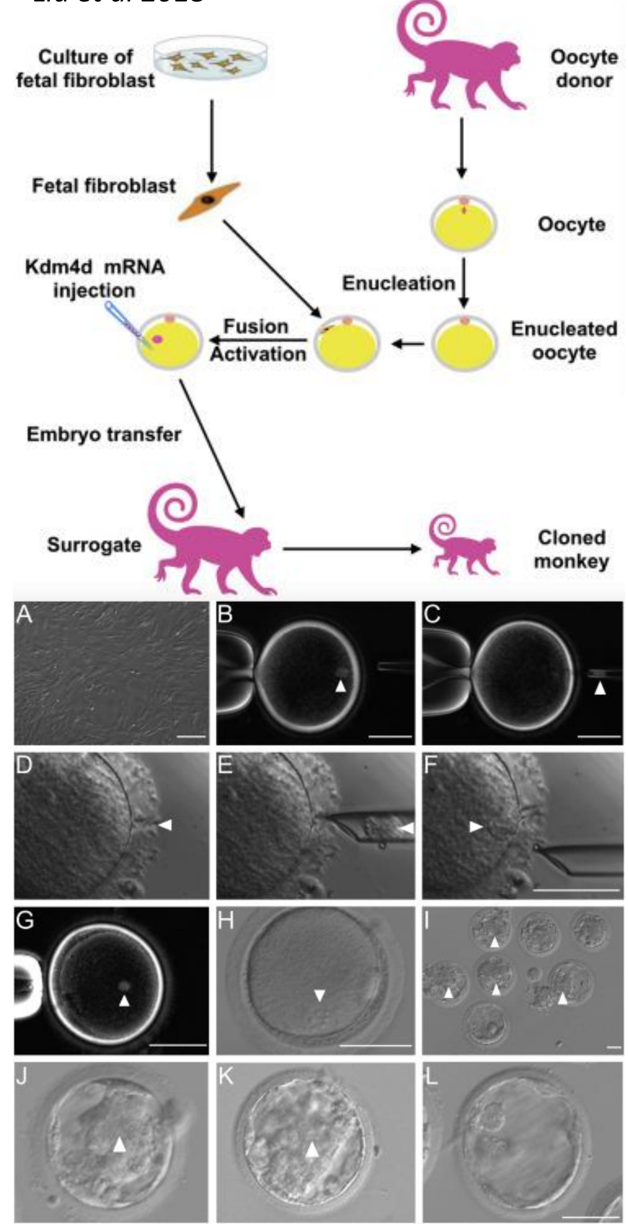
- genetically uniform non-human primates
- live but short-lived


- fetal fibroblast nucleus fused with enucleated MII oocyte using HVJ-E virus

- artificial activation with ionomycin and protein synthesis inhibition with 6-dimethylaminopurin (I/D)

- **epigenetic modification**
  (injection of H3K9me3 demethylase **Kdm4d** mRNA and treatment with histone deacetylase inhibitor **trichostatin A** at 1cell stage)

*Liu et al 2018*



# Somatic cell nuclear transfer (SCNT)

nature communications

Article https://doi.org/10.1038/s41467-023-43985-7

**Reprogramming mechanism dissection and trophoblast replacement application in monkey somatic cell nuclear transfer**

Received: 16 March 2023

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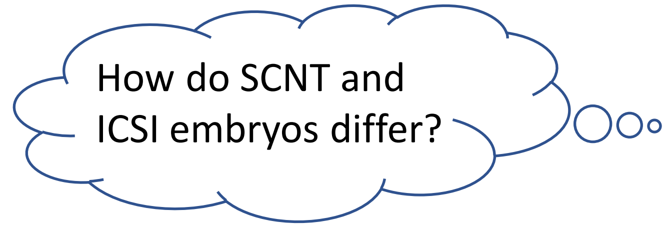
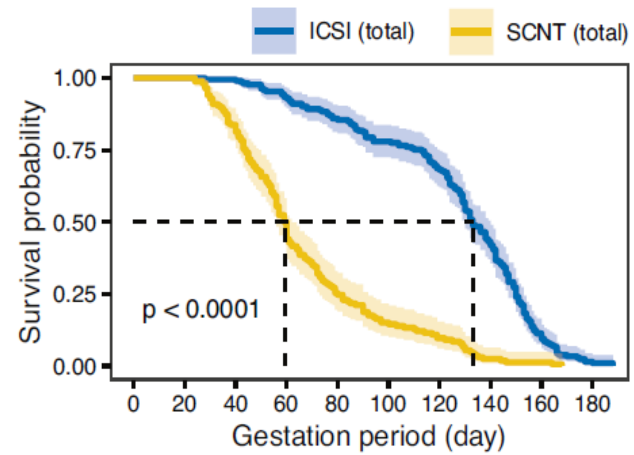
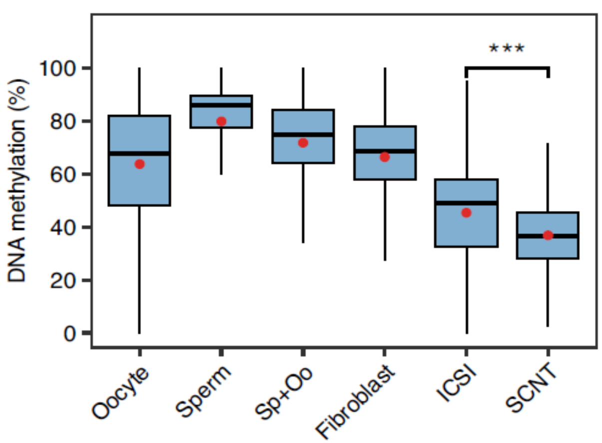
Zhaodi Liao[1,2,3,5], Jixiang Zhang[3,4,5], Shiyu Sun[1,2,3,5], Yuzhuo Li[1,2], Yuting Xu[1,2], Chunyang Li[1,2], Jing Cao[1,2], Yanhong Nie[1,2], Zhuoyue Niu[3,4], Jingwen Liu[3,4], Falong Lu[3,4,6], Zhen Liu[1,2,6] & Qiang Sun[1,2,6]

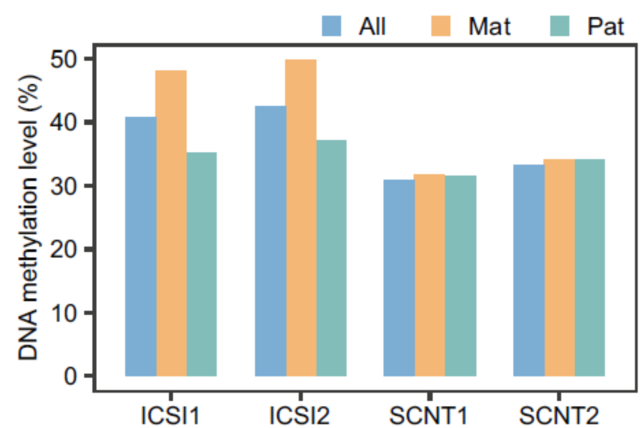



- SCNT embryos show hyperplasia and calcification of placenta

- SCNT embryos have ↓DNA methylation and loss of maternal gene imprinting



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- trophoblast replacement method
  → healthy adult male monkey
  **= succesful cloning of primates**

CLONING >

### The birth of Retro, a cloned monkey that presents the possibility of cloning humans

A group of Chinese researchers has perfected the technique for producing identical macaques but says that cloning people would be 'completely unacceptable'