Embryologie I
OOGENESIS

autumn 2024

Reproductive aging

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Reproductive aging

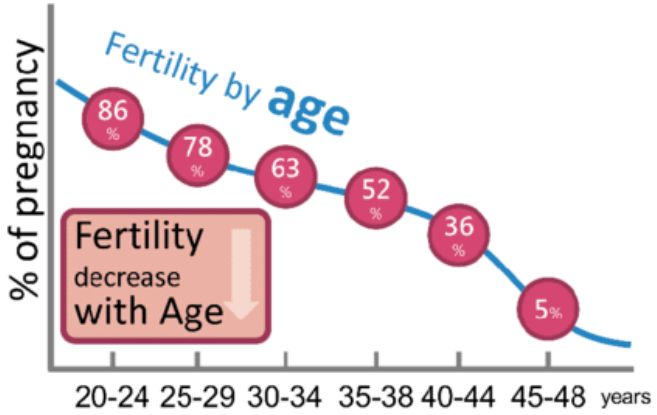
- **<u>gradual</u> age-related decline in the <u>quatity</u> and <u>quality</u> of oocytes in the ovaries**

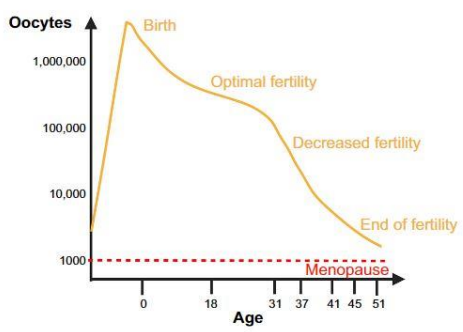
- ↓ chance of natural conception
- ↑ time to pregnancy
- ↑ pregnancy loss/miscarriages
- ↑ congenital defects

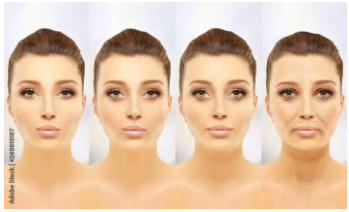
- ↓number of oocytes retrieved after COS
- ↓ fertility rate
- ↓ developmental rate
- ↑ implantation failure
- ↑ incidence of genetically abnormal embryos

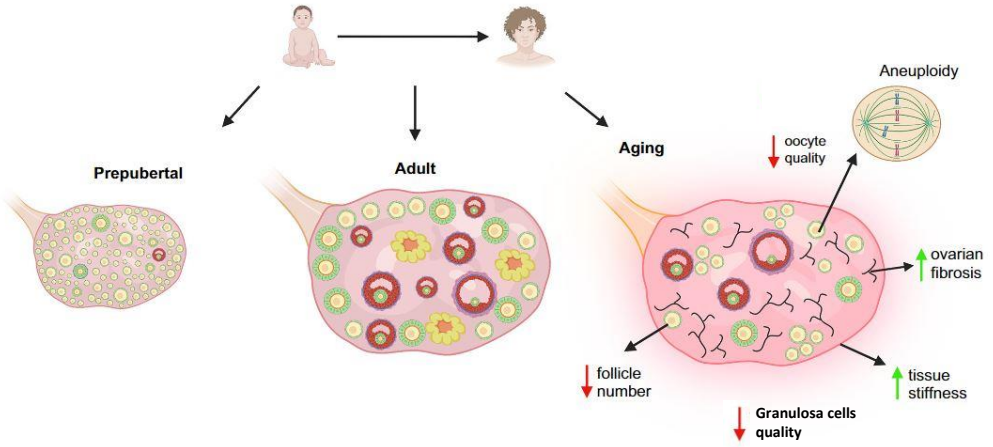
- **mutilifactorial process**
  - physiological factors
  - genetic factors
  - environmental and lifestyle factors

- outcomes of donor cycles indicate that reproductive aging **is related to ovarian not unterine factors**









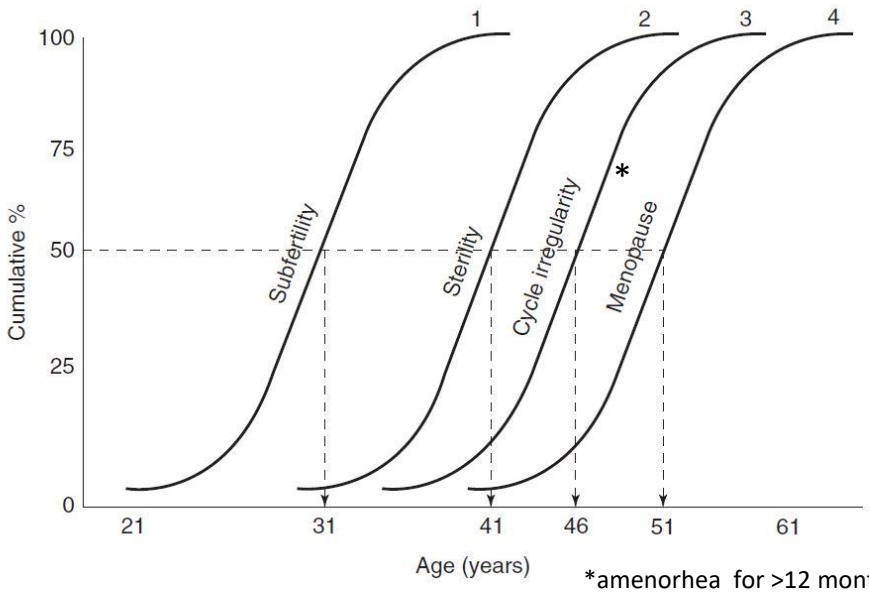
Reproductive aging

❖ Quantitative ovarian aging

- age-related exhaution of ovarian reserve
- continuous process

- markers:
  - Age (chronological vs. biological)
  - Menstrual cycle characteristics
  - FSH
  - Estradiol (E2)
  - LH/FSH ratio
  - Inhibin B
  - Antimüllerian hormone (AMH)
  - Basal antral follicule count (AFC)
  - Basal ovarian volume
  - Basal stromal blood flow

- age at menopause is highly heritable
- extreme malnutrition related to earlier menopause



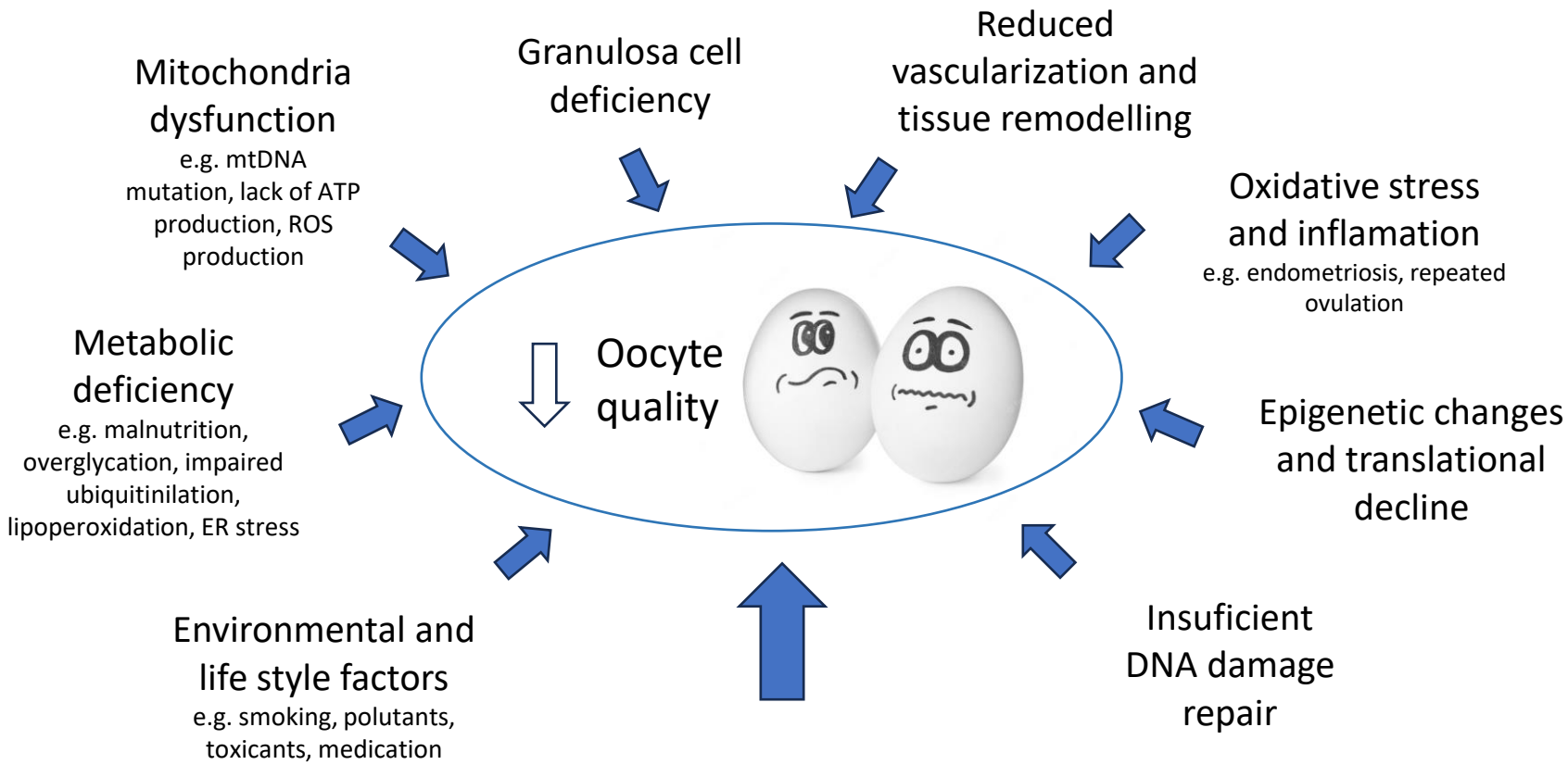
*amenorhea for >12 months

Ovarian reserve test	Favorable result	Unfavorable result
Age (years)	<35	≥35
FSH (IU/l)[1]	<10	≥10
d3 E_2 (pg/mL)	<75	≥75
d10 P_4[2]	≤0.9	≥1.1
AMH (pmol/L)[3]	15.7–48.5	<15.7
d3 inhibin-B (pg/mL)[4]	>45	≤45
AFC[5]	≥5	<5
Ovarian vascularity	Lower PI	Higher PI
Ovarian volume (cm^3)[6]	≥3	<3

Reproductive aging

❖ Qualititative ovarian aging

- diminishing of oocyte quality with advanced age

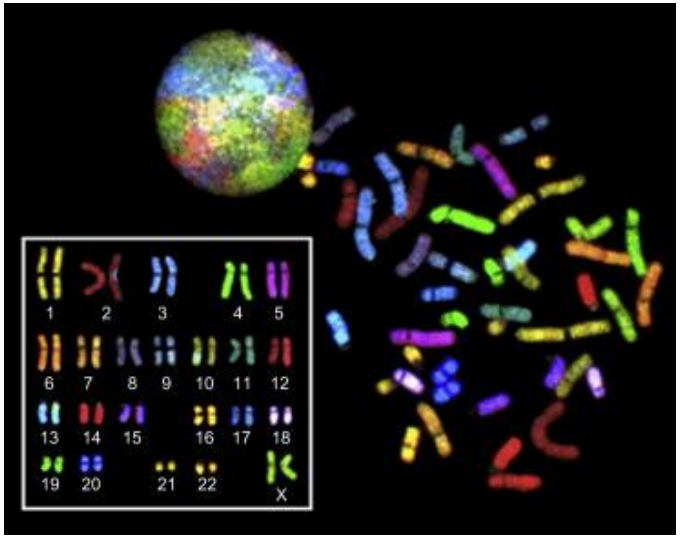


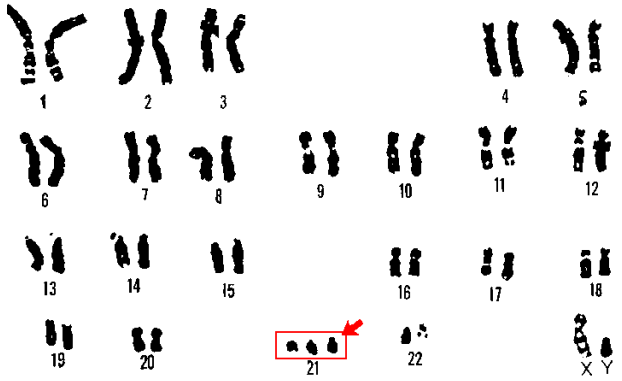
Aneuploidy

= presence of abnormal number of chromosomes
- chromome gain/loss resulting from unequal chromosome segregation

Non-cancerous somatic cells	<1%
Sperm	1-4%
Human MII oocyte	**~20%**
Mouse MII oocyte	<5%

fetal losses	50%
still births	4%
live births	0.3%

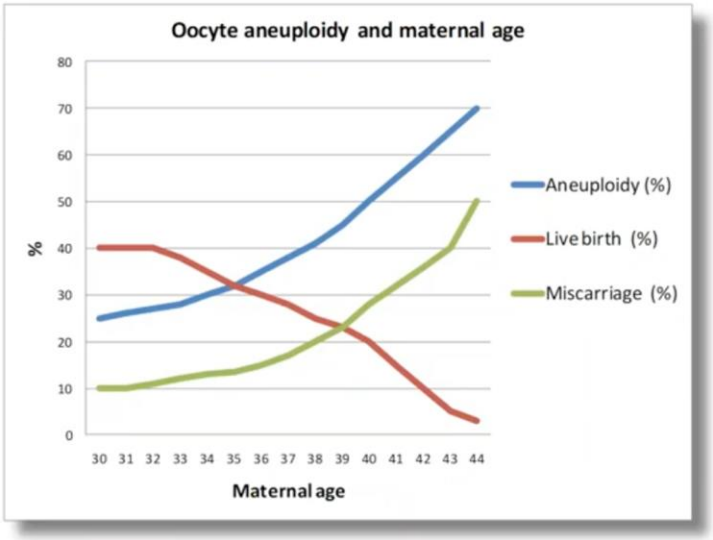




Egg aneuploidy

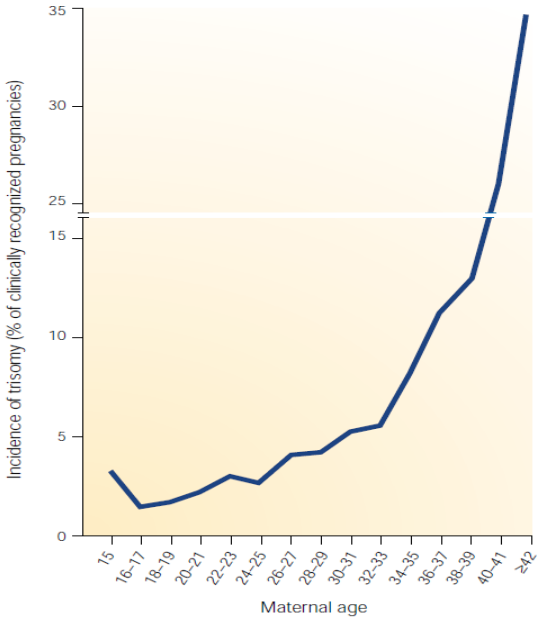
- incidence of oocyte aneuploidy increases with age

in women in their early 30s	~ 10-25%
in women above 40 years	~ 50-90%



= maternal age effect

↑ trisomic pregnancies



Hassold and Hunt 2001

Errors in maternal meiosis

Prevalence of the common aneuploidies in newborns

Aneuploidy	Prevalence
Trisomy 21 (Down syndrome)	1 : 700
Trisomy 18 (Edwards syndrome)	1 : 7.000
Trisomy 11 (Patau syndrome)	1 : 20.000
47, X (Turner syndrome)	1 : 2.5000
47, XXX ("super female")	1 : 1.200 females
47, XXY (Klinefelterův syndrome)	1 : 900 males
47, XYY ("supermale")	1 : 1000 males

Segregation errors in female meiosis

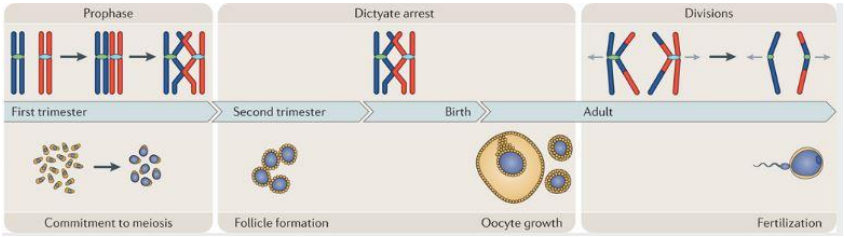
- **recombination defects predisposes oocytes to chromosome segregation errors**
  - recombination failure
  - inadequate number and position of crossovers

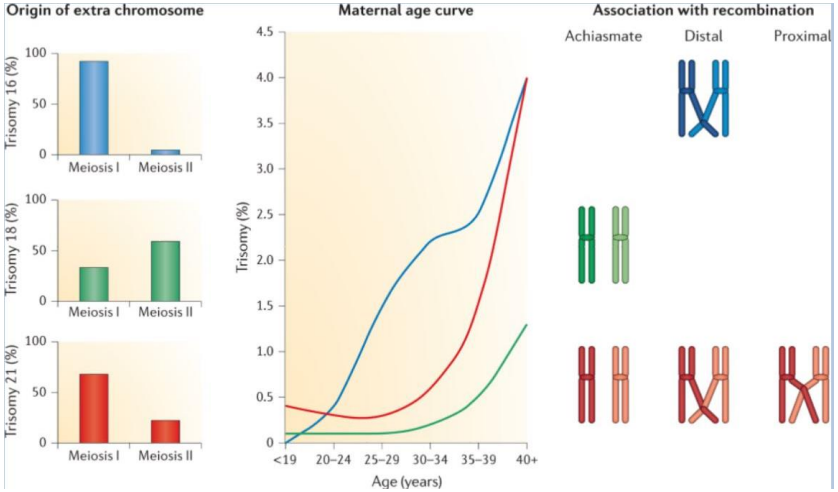
⇩

PREMATURE or NO
separation of homologous chromosomes

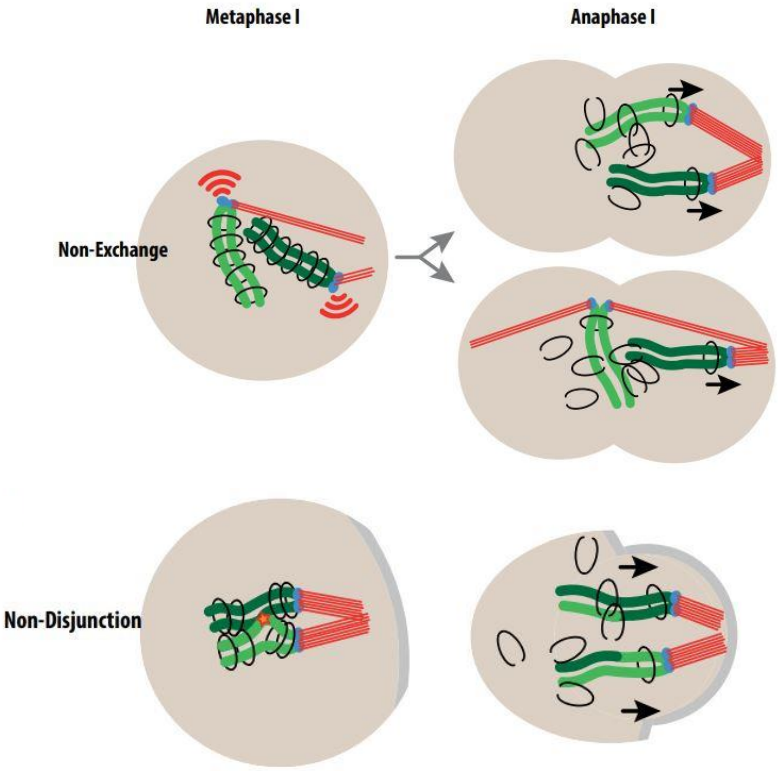
⇩

random segregation of univalents





Nagaoka et al 2012



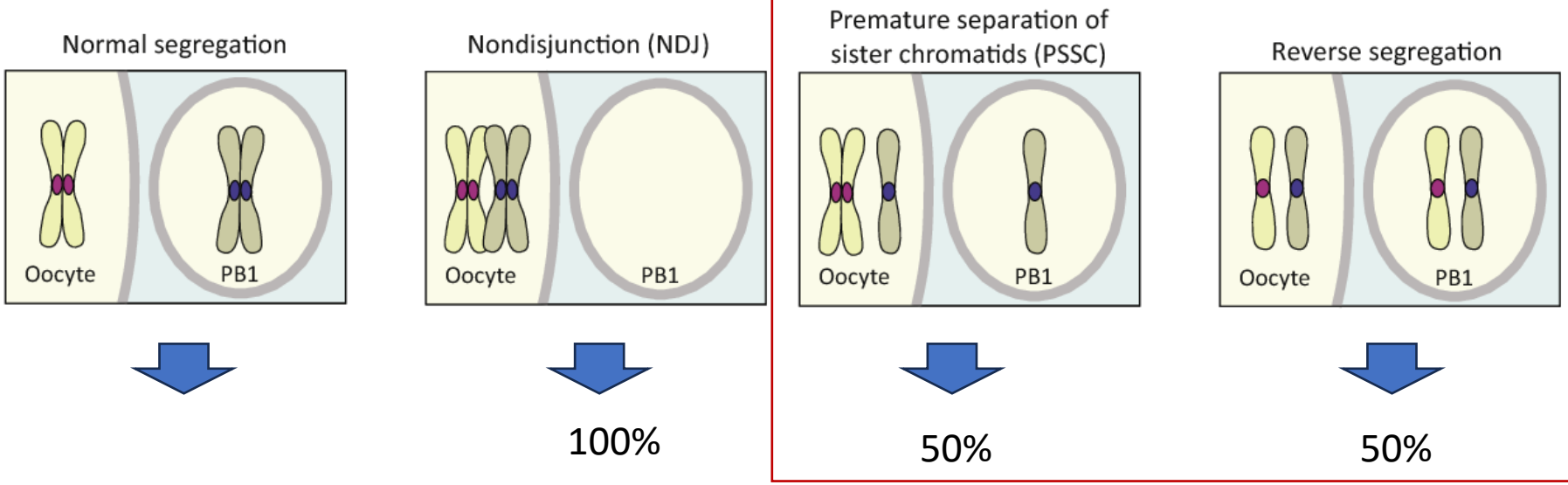
Lane and Kauppi 2019

Segregation errors in female meiosis

Most chromosome segregation errors occurr in meiosis I

Some MI erros may be balanced in meiosis II

Meiosis I



Euploid embryo

Embryonic aneuploidy

Webster and Schuh 2017

Origin of human egg aneuploidy

Gruhn et al, Science 2019

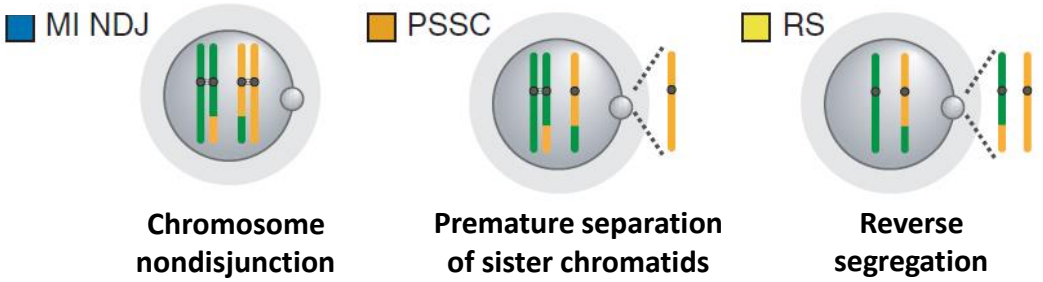
RESEARCH

REPRODUCTIVE BIOLOGY

Chromosome errors in human eggs shape natural fertility over reproductive life span

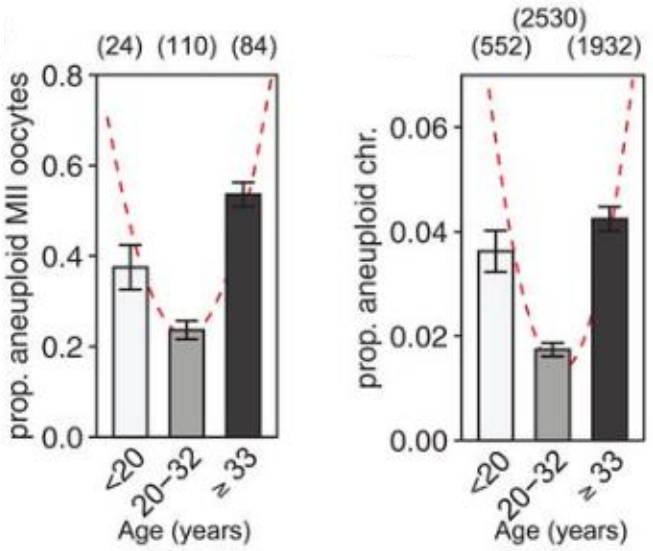
Jennifer R. Gruhn[1]*, Agata P. Zielinska[2]*, Vallari Shukla[1]*, Robert Blanshard[3,4]*, Antonio Capalbo[5], Danilo Cimadomo[6], Dmitry Nikiforov[7,8], Andrew Chi-Ho Chan[1], Louise J. Newnham[3], Ivan Vogel[1], Catello Scarica[9], Marta Krapchev[10], Deborah Taylor[11], Stine Gry Kristensen[7], Junping Cheng[7], Erik Ernst[12], Anne-Mette Bay Bjørn[12], Lotte Berdiin Colmorn[13], Martyn Blayney[14], Kay Elder[14], Joanna Liss[10,15], Geraldine Hartshorne[11], Marie Louise Grøndahl[16], Laura Rienzi[6], Filippo Ubaldi[6], Rajiv McCoy[17], Krzysztof Lukaszuk[10,18,19], Claus Yding Andersen[7], Melina Schuh[2], Eva R. Hoffmann[1]†

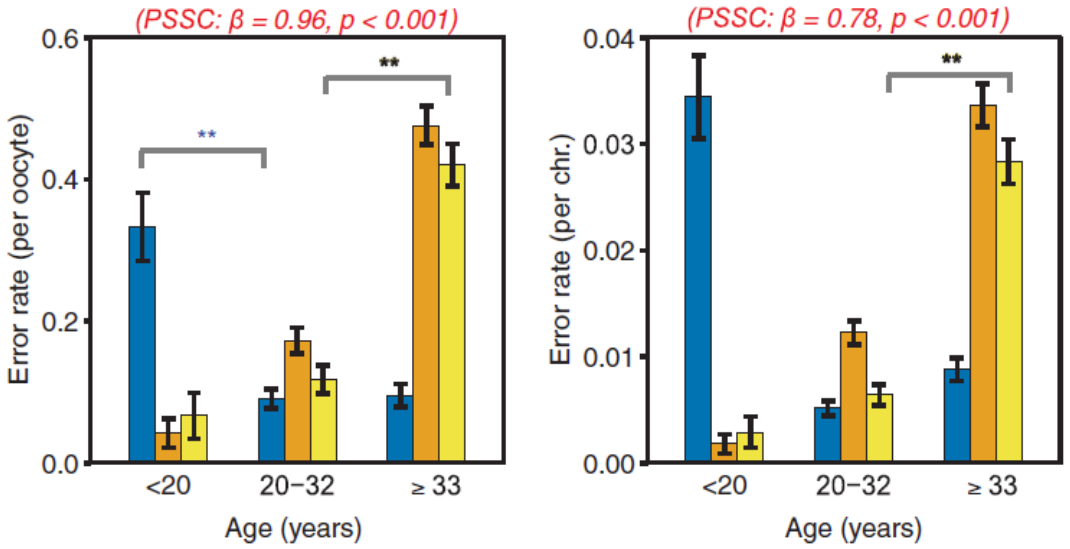
Meiosis errors and aging



- **Young age: nondisjunction of homologous chromosomes**
- **Advanced age: premature separation of sister chromosomes**

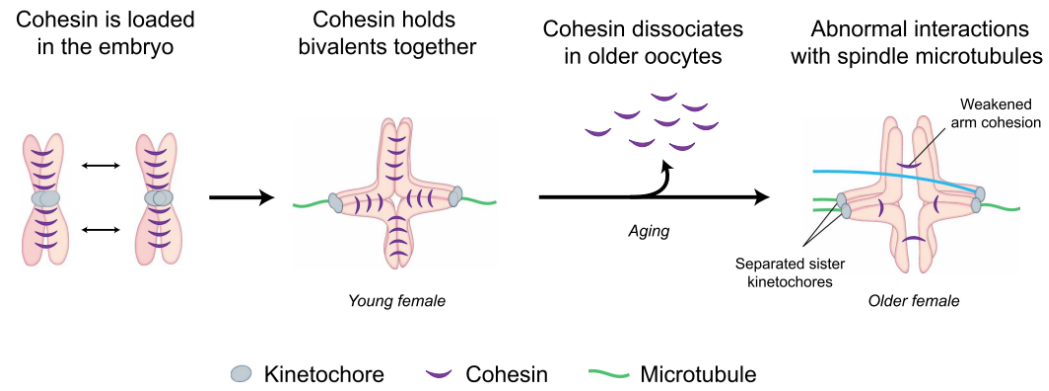
Egg aneuploidy

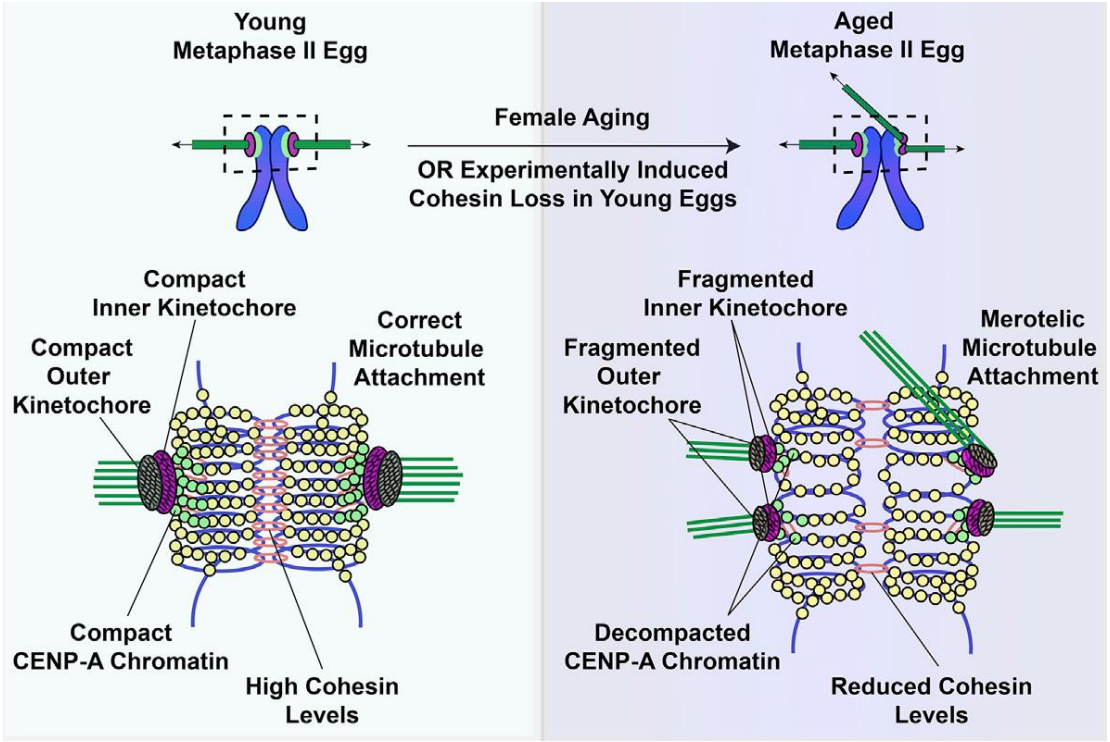




Cohesion deterioration

- **age-dependent loss of cohesins from chromosome arms**





eLIFE elifesciences.org

Zielinska et al 2015.

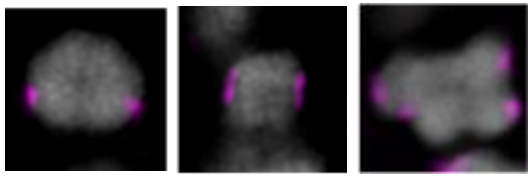
Sister kinetochore splitting and precocious disintegration of bivalents could explain the maternal age effect

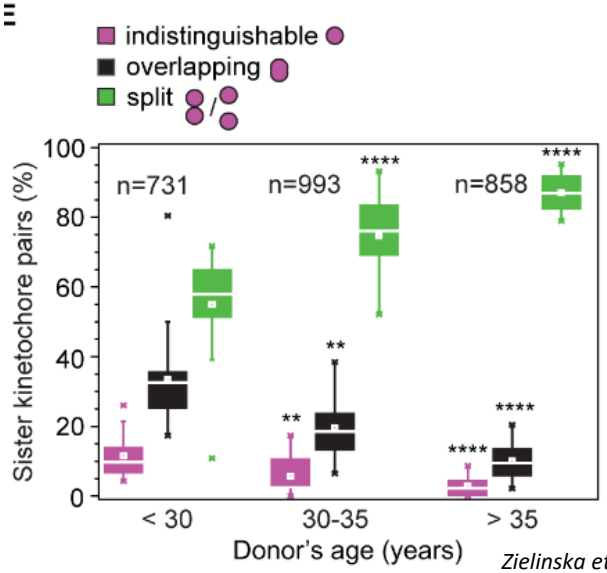
Agata P Zielinska[1], Zuzana Holubcova[1], Martyn Blayney[2], Kay Elder[2], Melina Schuh[1,3]*

[1]Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom; [2]Bourn Hall Clinic, Cambridge, United Kingdom; [3]Max Planck Institute for Biophysical Chemistry, Goettingen, Germany

Melina Schuh

- **precocious splitting of sister kinetochores during meiosis I**



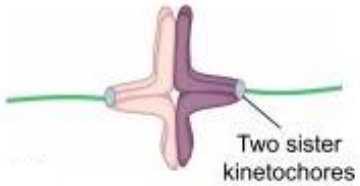


Zielinska et al 2019.

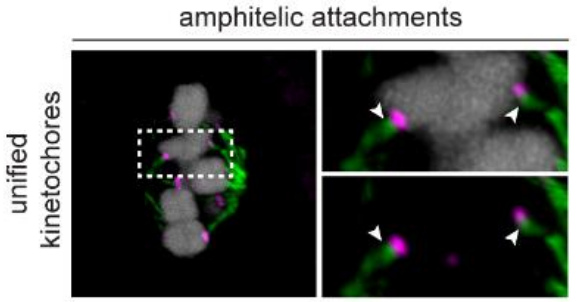
Cohesion deterioration

Meiosis I

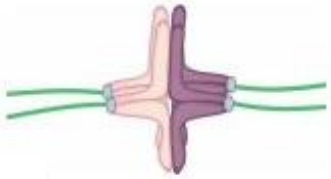
Correct alignment in meiosis I



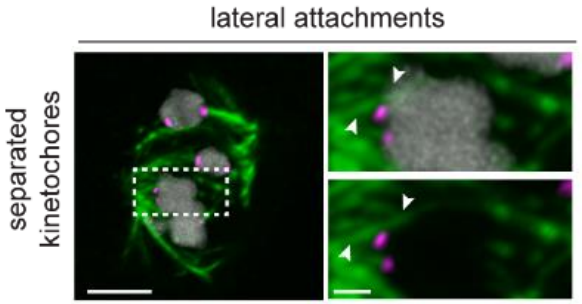
- In meiosis I, sister kinetochores of a single chromosome should act as one functional unit.
- In this way, sister kinetochores face the same spindle pole and whole chromosomes can segregate in anaphase I.



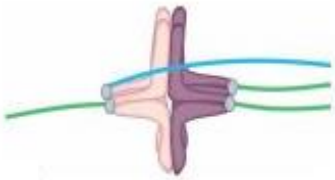
Sister kinetochore separation



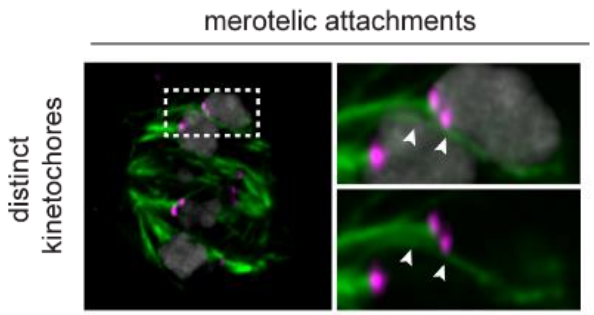
- Sister kinetochores separate as women become older.
- Separated sister kinetochores interact with microtubules independently.



Merotelic attachment



- Separation of kinetochores increases the probability of merotelic attachments.
- Merotelic attachments increase with maternal age.



Thomas et al 2021.

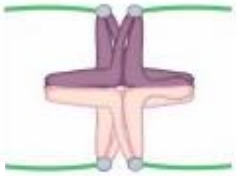
Zielinska et al 2015.

Separation of sister kinetochores in meiosis I promotes abnormal kinetochore-microtubule attachments

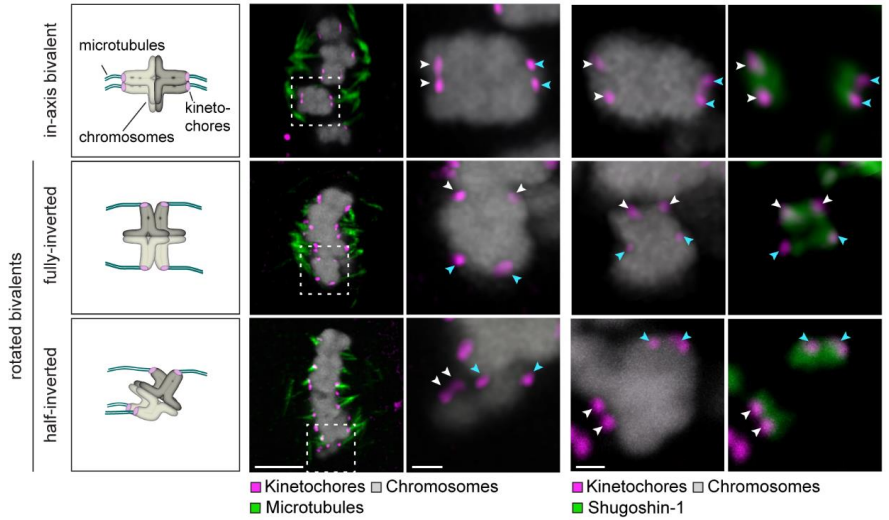
Cohesion deterioration

Meiosis I

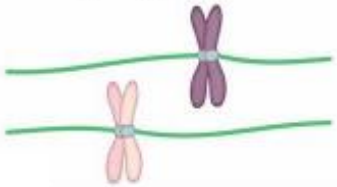
Bivalent rotation



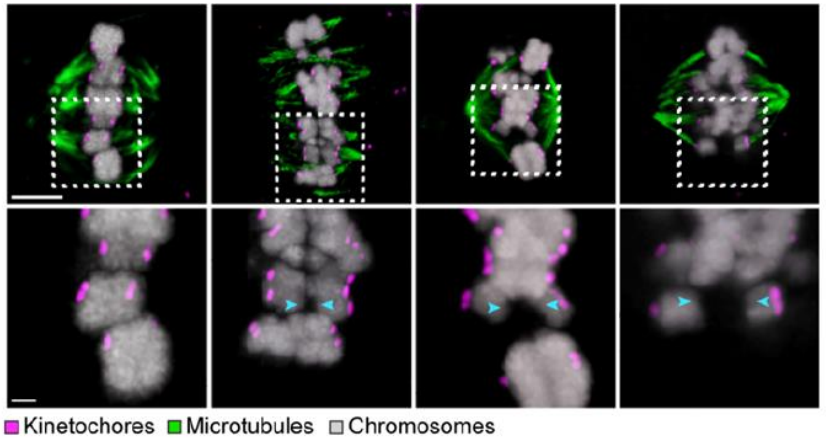
- Separation of sister kinetochores allows bivalents to rotate on the spindle.
- Here, sister kinetochores orient like in mitosis.
- This could result in the reverse segregation pattern of chromosome segregation.



Bivalent splitting



- Bivalents may prematurely separate into univalents prior to anaphase I.
- These univalents can align on the spindle and could give rise to PSSC and reverse segregation.



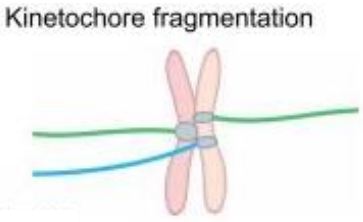
Thomas et al 2021.

Zielinska et al 2015.

Anomalous attachment of separated sister kinetochores may cause bivalent rotation, twisting and splitting in meiosis I

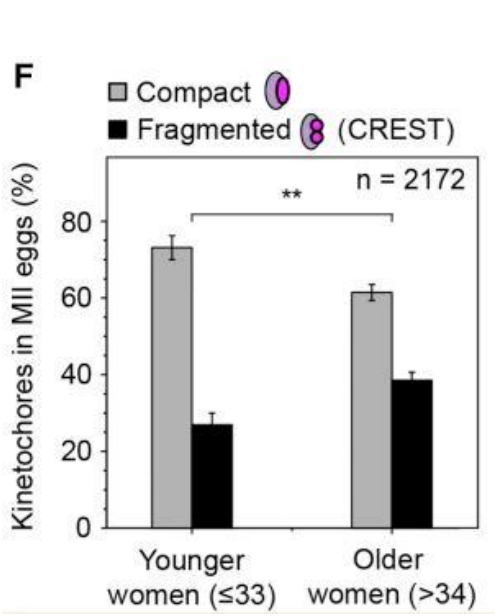
Cohesion deterioration

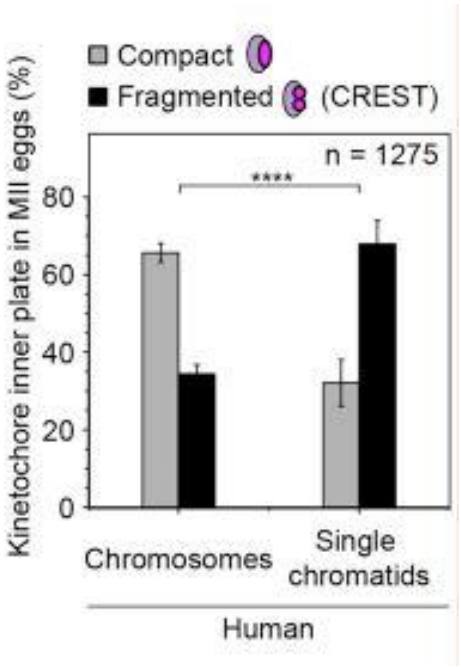
Meiosis II

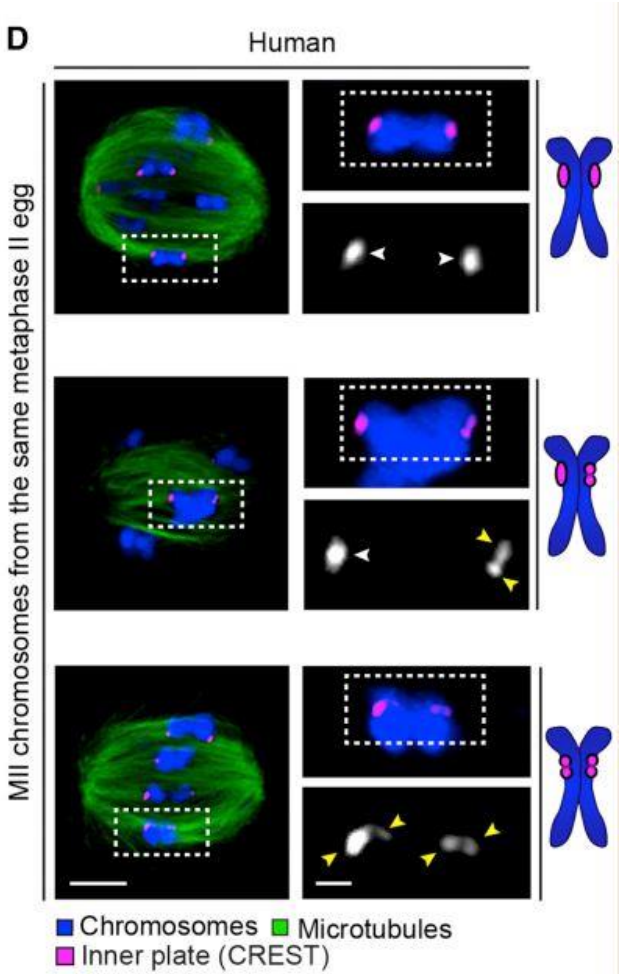


- Kinetochores fragment into multiple lobes in oocytes.
- Fragmented kinetochores frequently form incorrect attachments with spindle microtubules in meiosis II.

Thomas et al 2021.



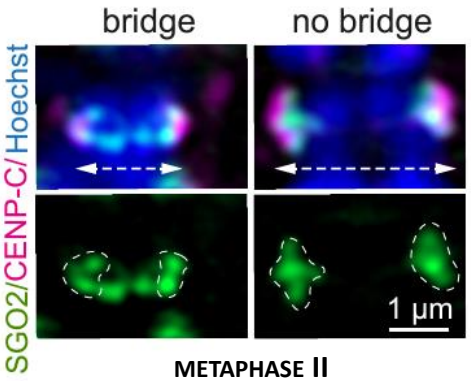




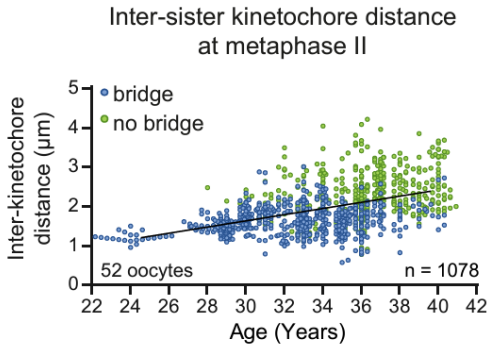
Zielinska et al 2019.

Loss of cohesion induces kinetochore fragmentation in aged MII eggs.

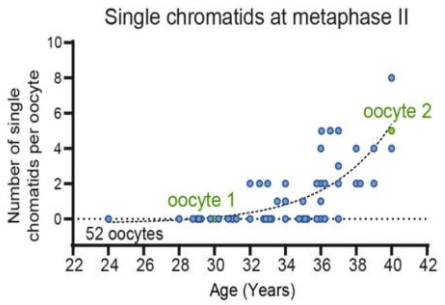
Cohesion deterioration

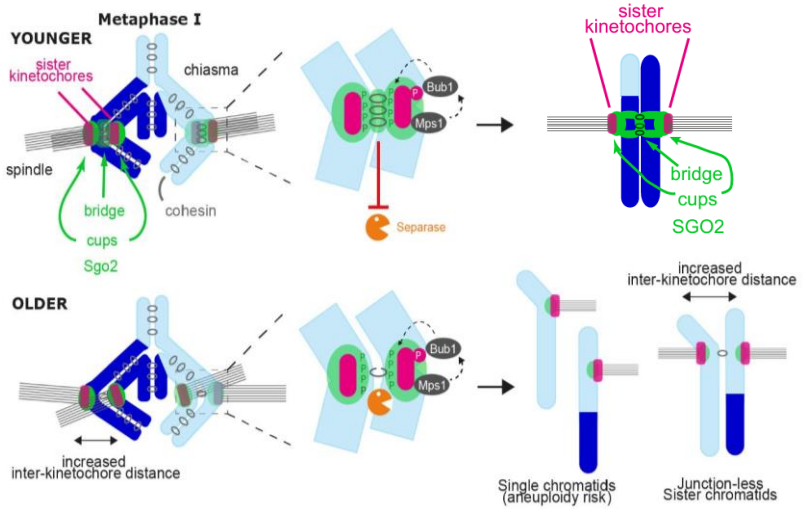


METAPHASE II



- **reduced Sgo2 location** at the pericentromeric bridge is associated with increased inter-sister kinetochore distance and incidence of single chromatids in MII oocytes





Current Biology *Mihalas et al. 2024*

CellPress OPEN ACCESS

Article

Age-dependent loss of cohesion protection in human oocytes

Bettina P. Mihalas,[1] Gerard H. Pieper,[1] Mansour Aboelenain,[1,2] Lucy Munro,[1] Vlastimil Srsen,[3] Cerys E. Currie,[4] David A. Kelly,[1] Geraldine M. Hartshorne,[4,5] Evelyn E. Telfer,[3,6] Andrew D. McAinsh,[4] Richard A. Anderson,[6] and Adele L. Marston[1,7,8,*]

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[3]Institute of Cell Biology, School of Biological Sciences, University of Edinburgh, Edinburgh EH8 9XD, UK
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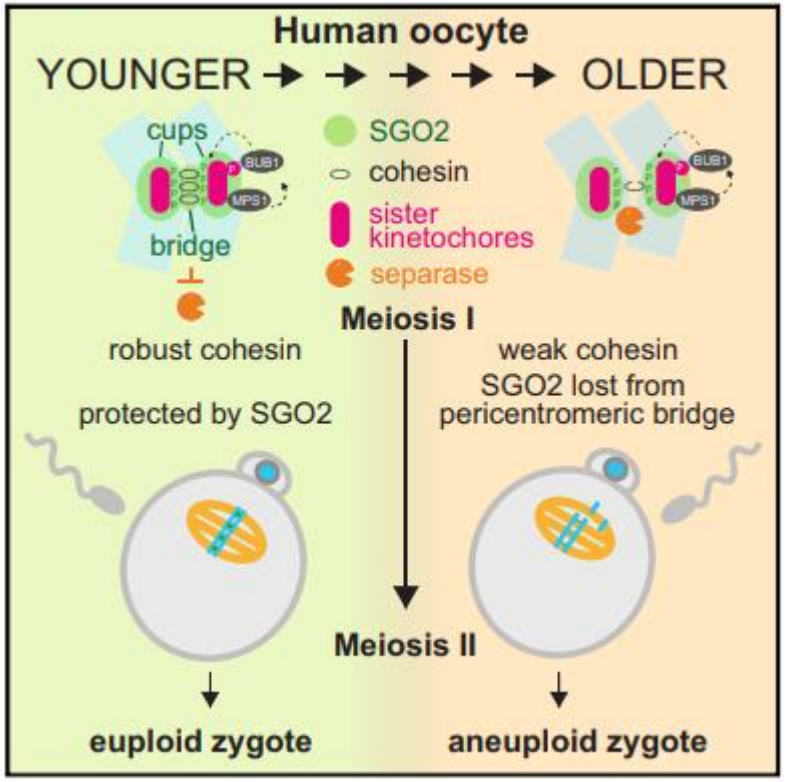
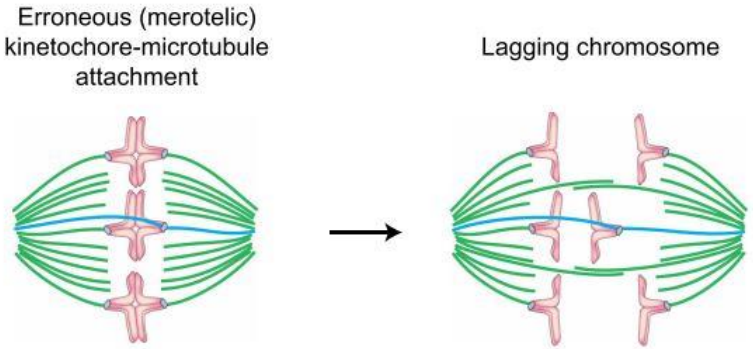
[5]University Hospitals Coventry and Warwickshire NHS Trust, Coventry CV2 2DX, UK
[6]Medical Research Council Centre for Reproductive Health, University of Edinburgh, Edinburgh EH16 4TJ, UK
[7]X (formerly Twitter): @Marston_lab
[8]Lead contact
*Correspondence: adele.marston@ed.ac.uk
https://doi.org/10.1016/j.cub.2023.11.061

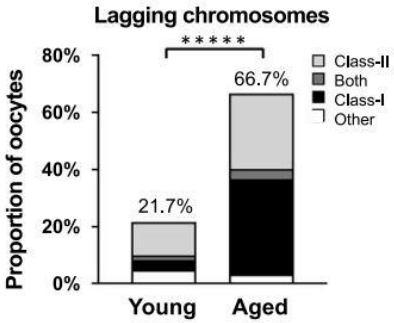


Chromosome lagging

- merotelic attachments promote chromosome lagging



- distinct velocity types of chromosome laggards with different risk to result in aneuploidy



- controlled prolongation of meiosis I specifically lessens class-I lagging to prevent aneuploidy

Developmental Cell
CellPress
Short Article

Distinct classes of lagging chromosome underpin age-related oocyte aneuploidy in mouse

Aleksandar I. Mihajlović,[1] Jenna Haverfield,[1] and Greg FitzHarris[1,2,3,*]
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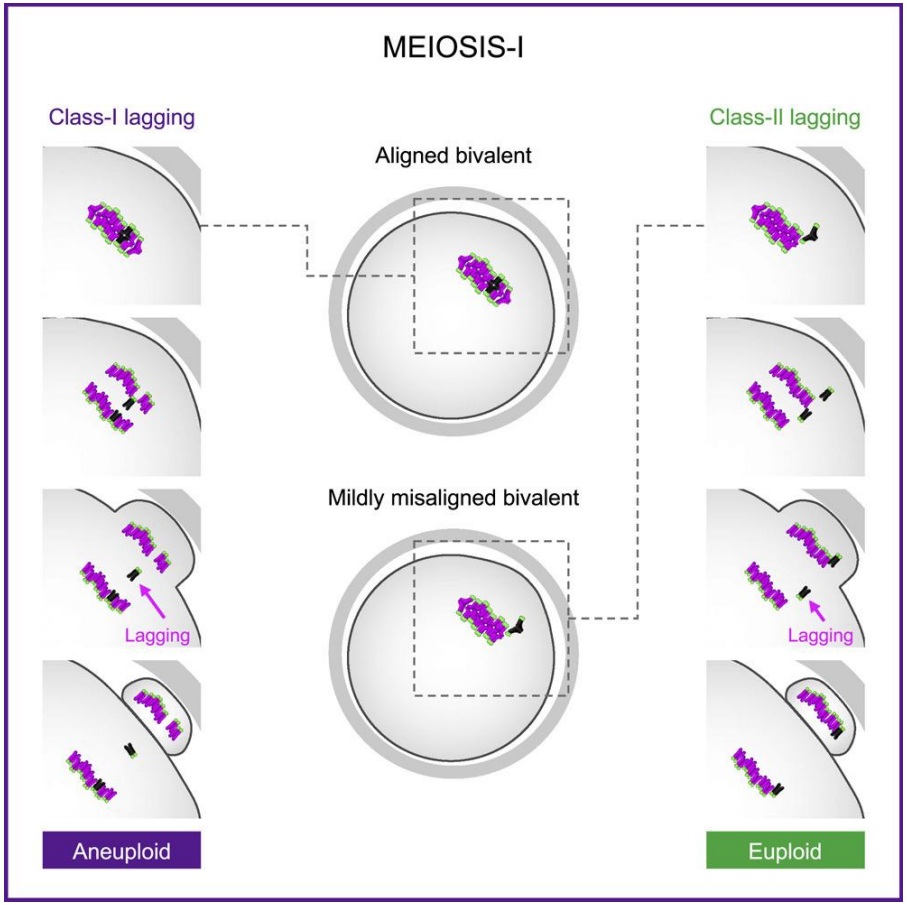
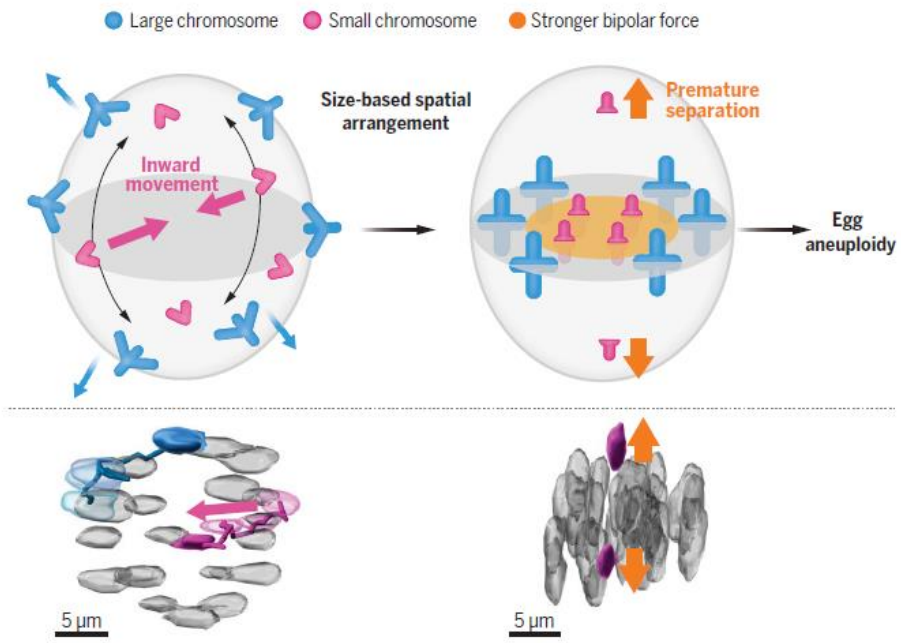
Greg FitzHarris

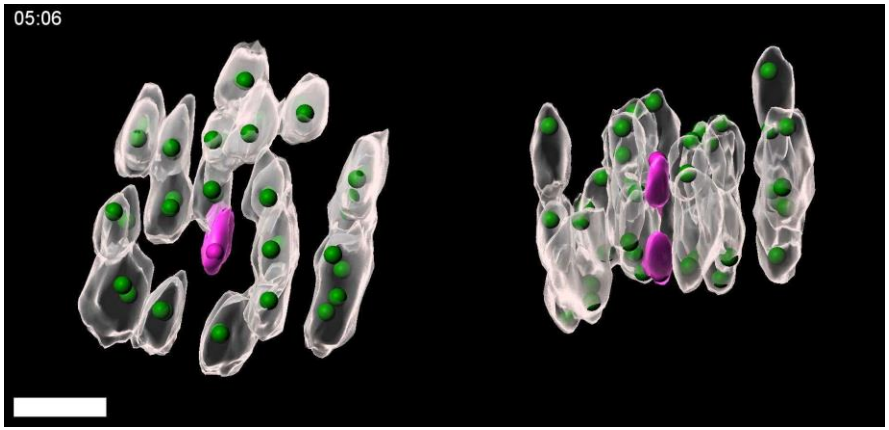


Mihajlovic et al 2021

Chromosome lagging

- flourescent probes for invidual chromosomes
- 3D tracking of meiosis I in live mouse oocytes





Science AAAS

Takenouchi et. 2024

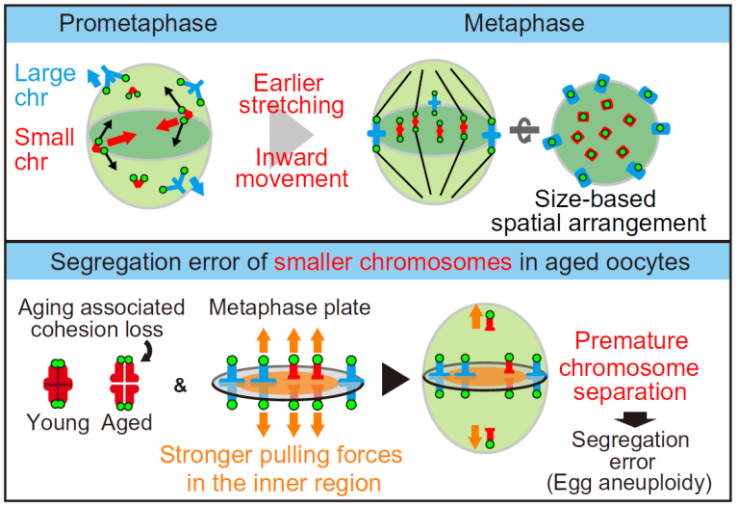
Live chromosome identifying and tracking reveals size-based spatial pathway of meiotic errors in oocytes

Osamu Takenouchi*, Yogo Sakakibara†, Tomoya S. Kitajima*

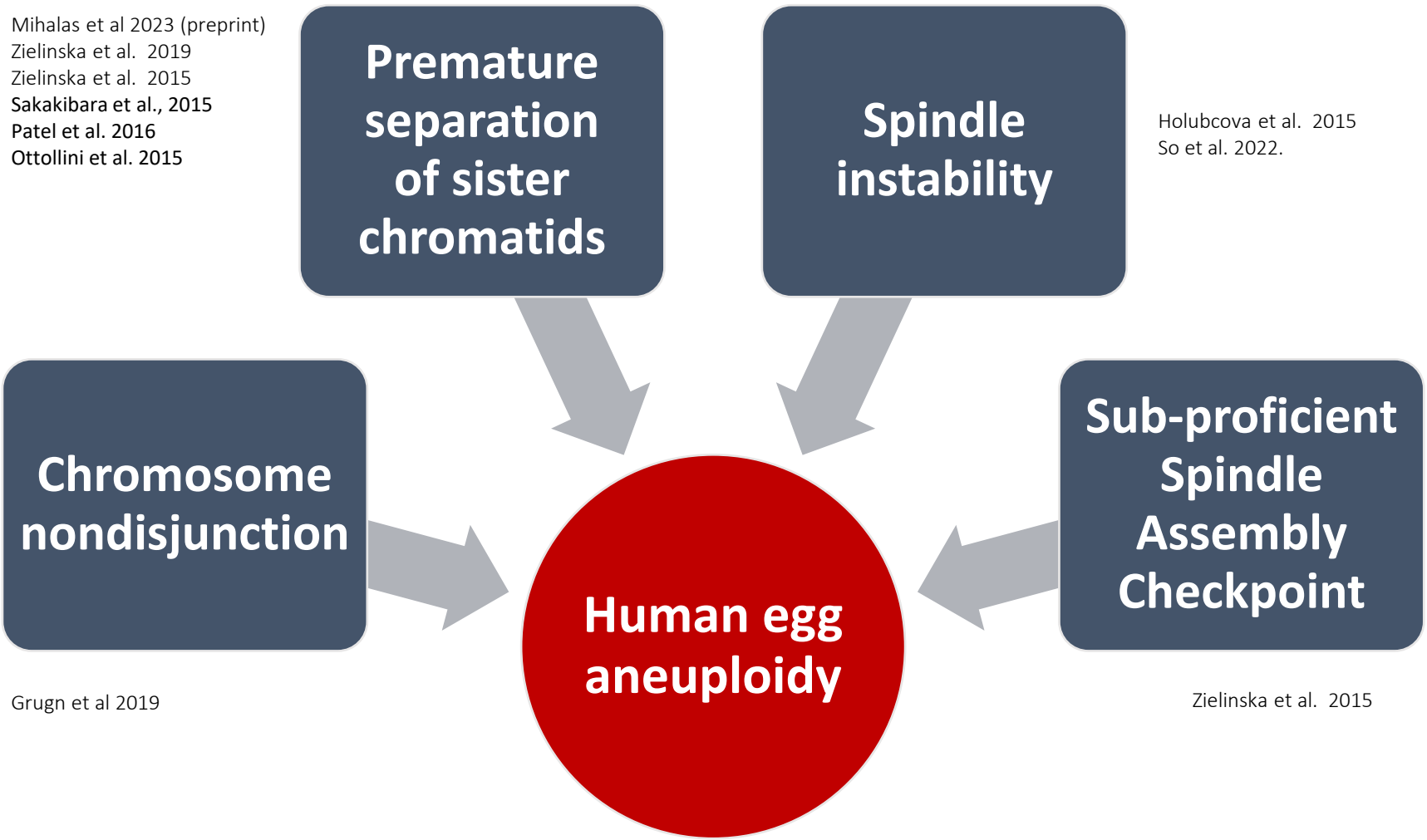
Tomoya Kitajima

- smaller chromosomes actively moved to the center of metaphase plate
- in the inner region, the chromosomes are pulled by the stronger bipolar MT forces, which facilitates premature chromosome separation

  → ↑risk of chromosome missegregation in aged oocytes with weakend cohesins



Origin of human egg aneuploidy



Mihalas et al 2023 (preprint)
Zielinska et al. 2019
Zielinska et al. 2015
Sakakibara et al., 2015
Patel et al. 2016
Ottollini et al. 2015

Holubcova et al. 2015
So et al. 2022.

Grugn et al 2019

Zielinska et al. 2015

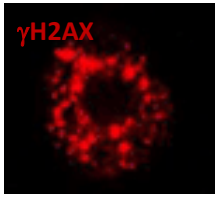
DNA damage response

meiotic recombination

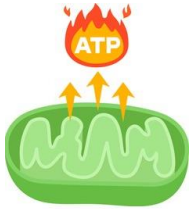
induced DNA damage

ROS
UV
Chemical toxicants

DSB



DNA repair



tolerance

genetic mutations

developmental arrest
misscarriage
birth defects

apoptosis

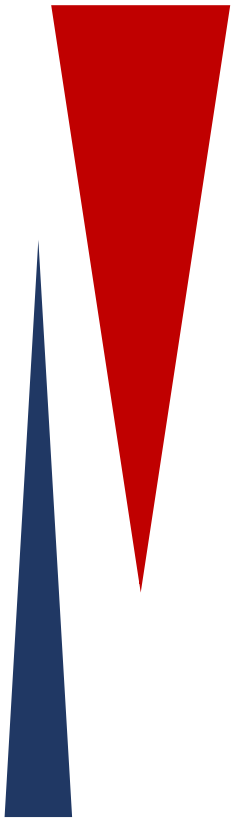
loss of ovarian reserve

reduced reproductive lifespan

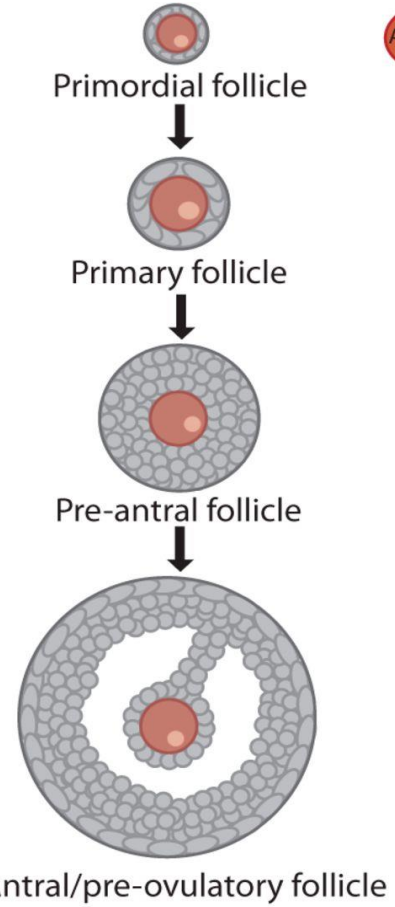
Low tolerance for DNA damage
Stringent quality control

DNA damage response

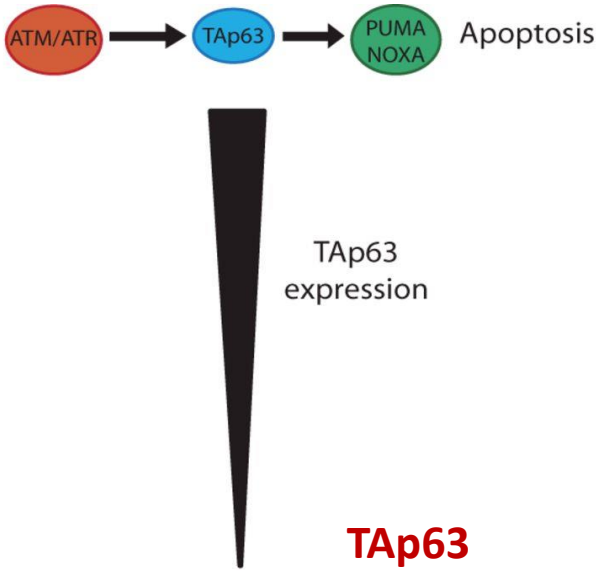
Sensitiveness to DNA damage



DNA repair



DNA damage survey



TAp63

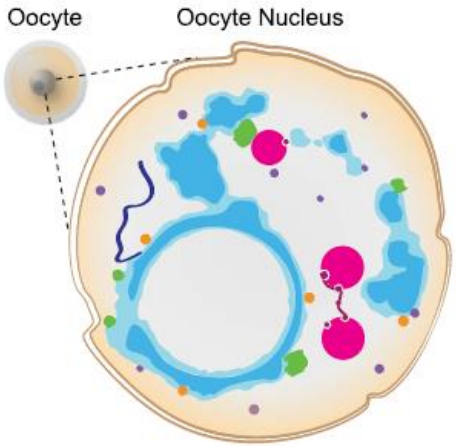
- isoform of p63
- homologue of p53
- **proapoptotic factor**

Role of p53 and p73?

- **stage-dependent vulnerability to DNA damage**

DNA damage response

DNA repair proteins are organized into distinct repair compartments in GV oocyte nucleus



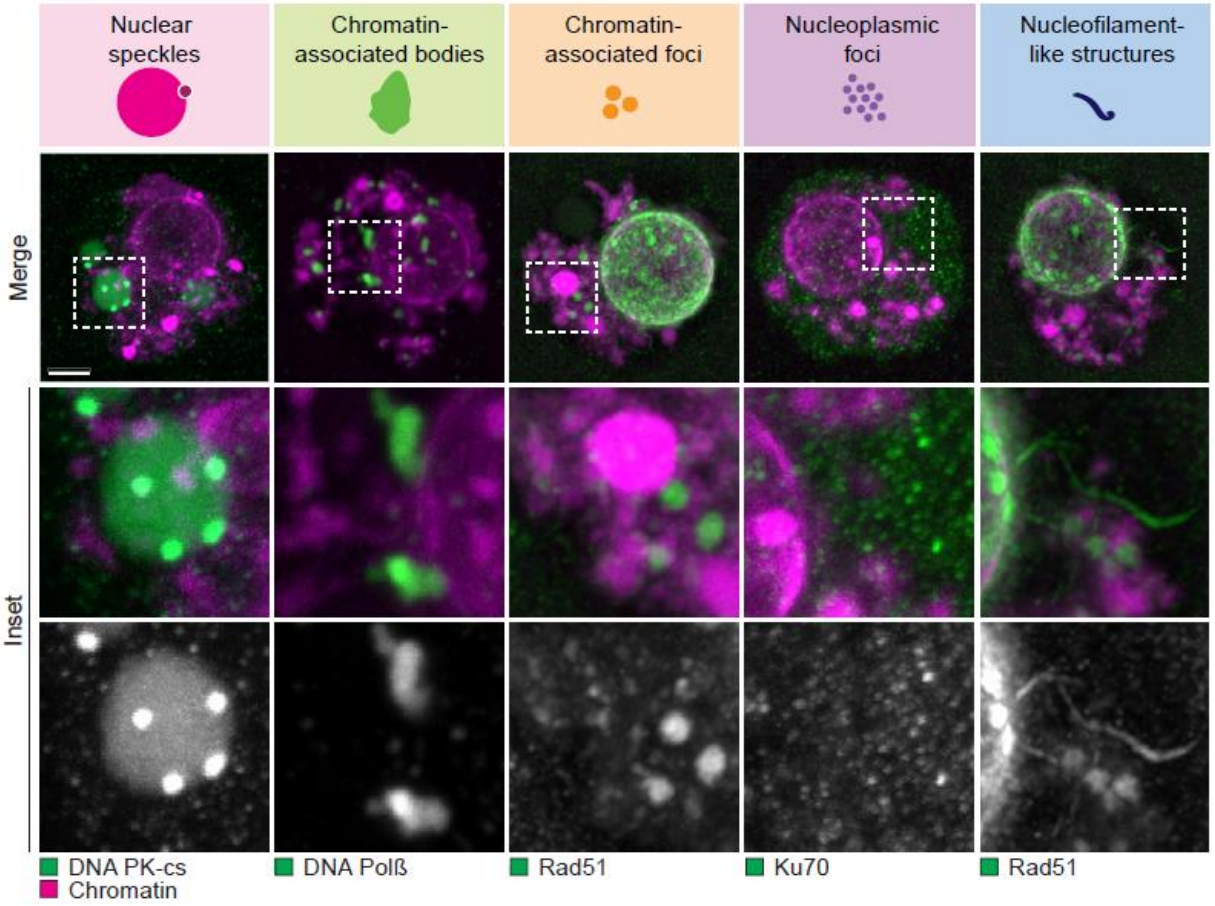
Nuclear Speckles
BRCA1
BRCA2
XRCC4
LIG4
TOP2A
DNA PK-cs

Chromatin-associated bodies
FANCI
HP1a
DNA Polß
Smc3

Nucleoplasmic foci
Ku70
Mlh1
BRCA1
BRCA2

Chromatin-associated foci
Rad51
γH2AX

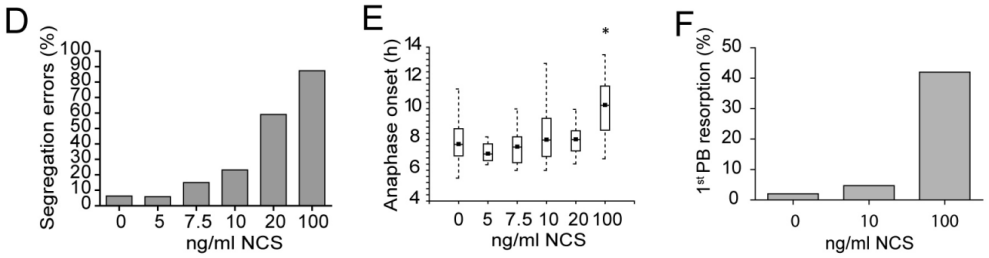
Nucleofilaments
Rad51
WRN

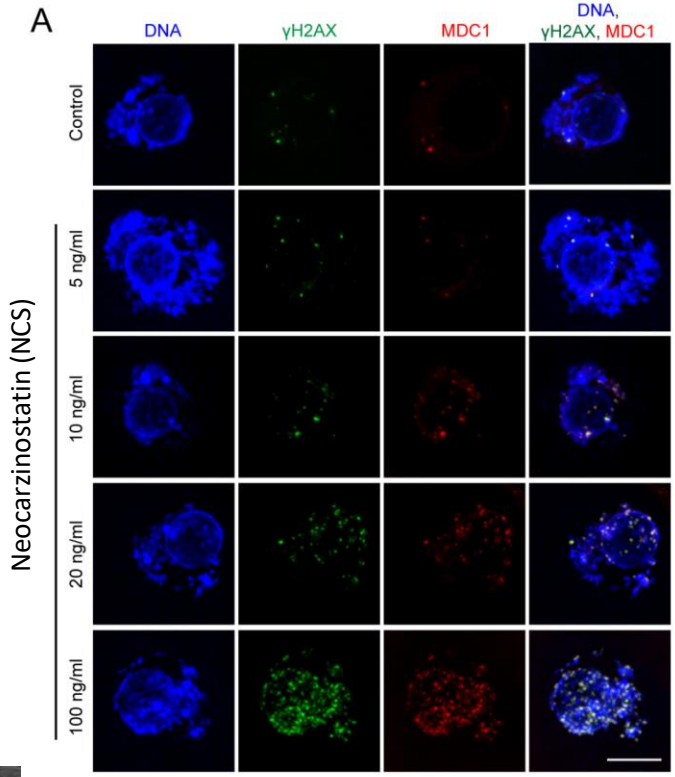


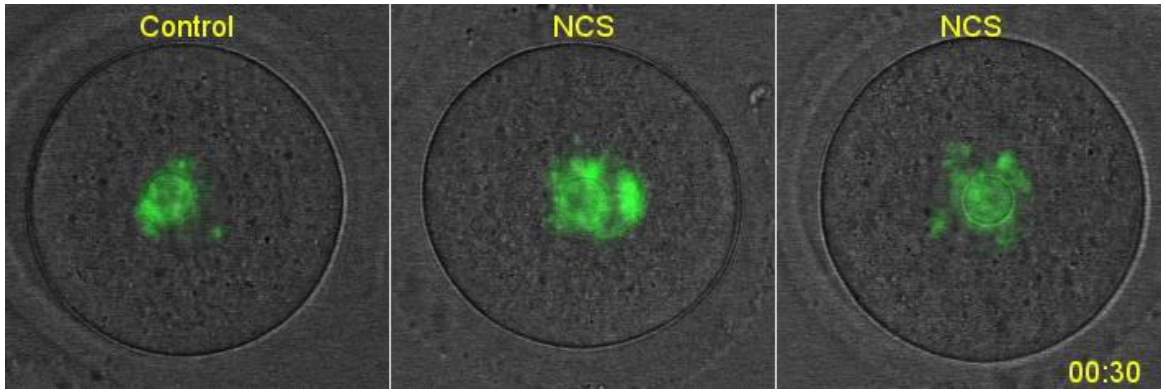
Sharma et al 2024

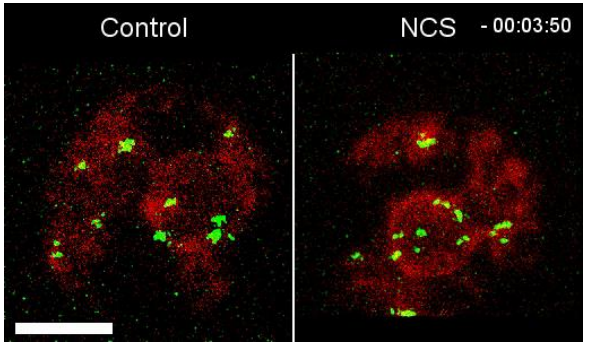
DNA damage response

- detection of DSBs by prophase I oocyte
- increased DSBs during meiotic maturation result in segregation errors due to chromosome fragmentation
- no cell cycle arrest or significant delay in anaphase onset





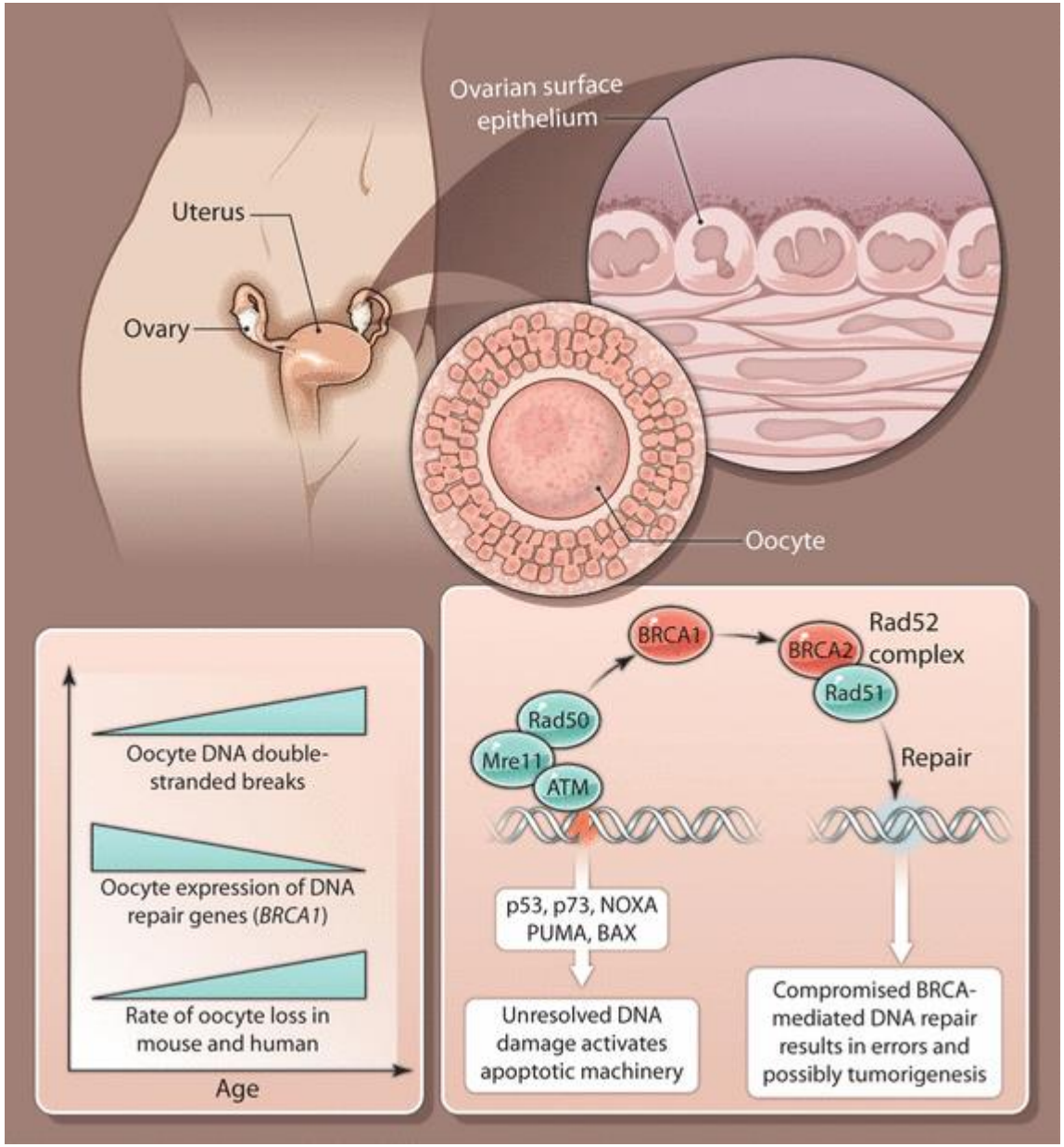




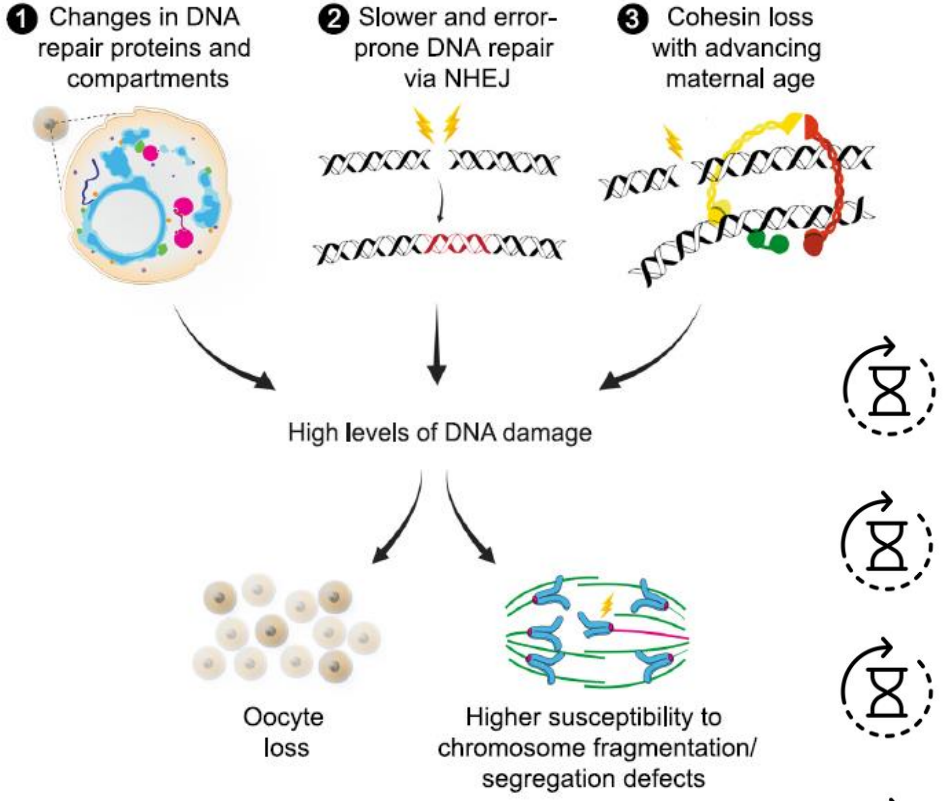
Mayer et al 2016

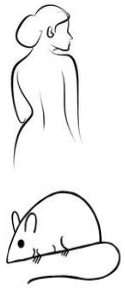
DNA damage response during aging

- age-related deterioration of DNA damage sensing and repairing machinery



DNA damage response during aging





Current Biology
CellPress OPEN ACCESS
Article

Changes in DNA repair compartments and cohesin loss promote DNA damage accumulation in aged oocytes

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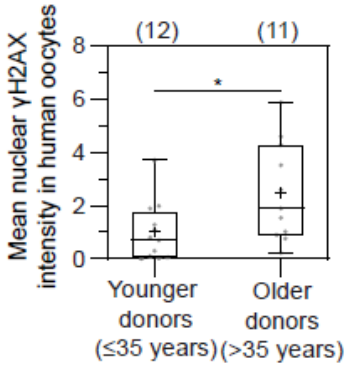
https://doi.org/10.1016/j.cub.2024.09.040

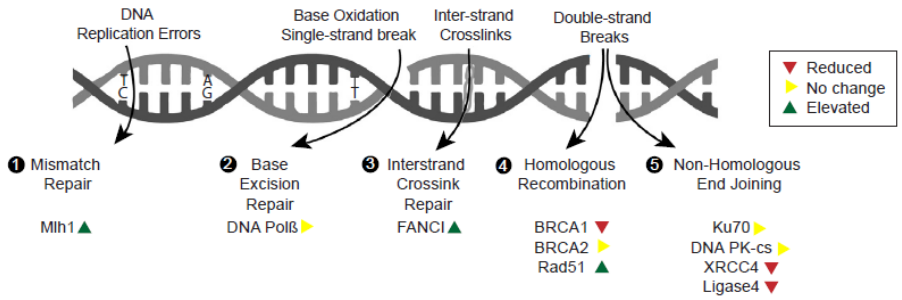
Aged oocytes accumulate DNA damage and take longer than young oocytes to repair DNA damage

DNA repair proteins are organized into distinct nuclear DNA repair compartments

Aged oocytes show changes in DNA repair machinery, favoring error-prone NHEJ repair pathway

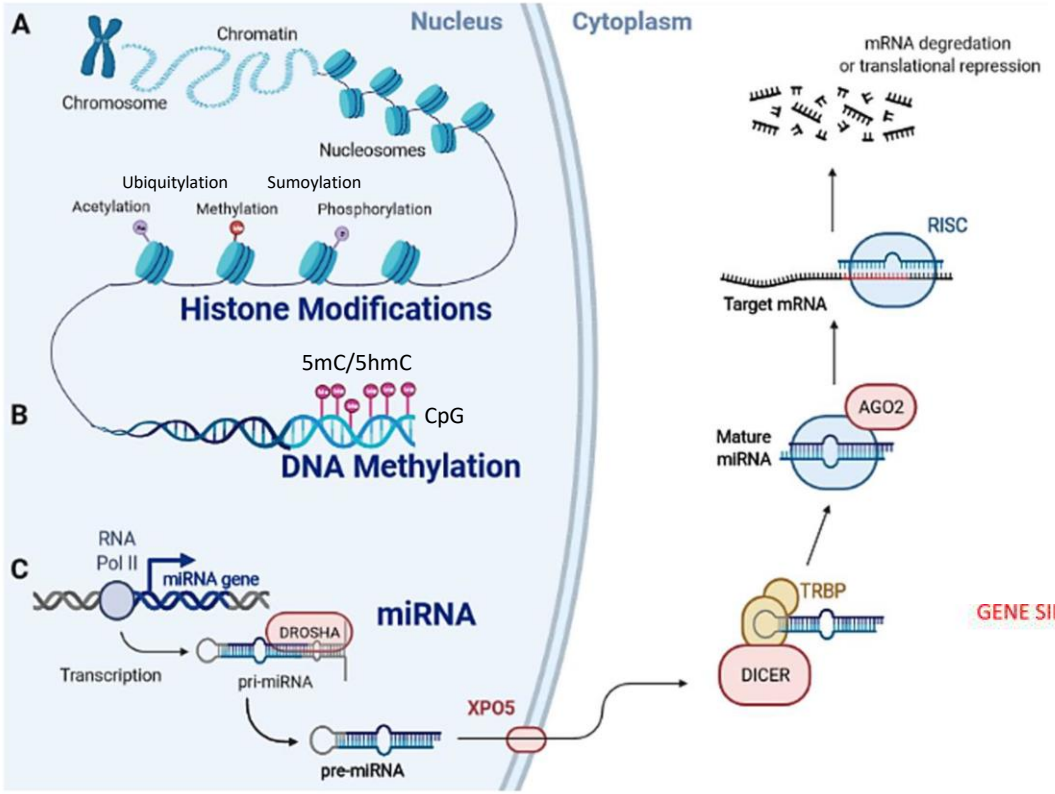
Age-related cohesin loss results in reduced DNA damage repair efficiency



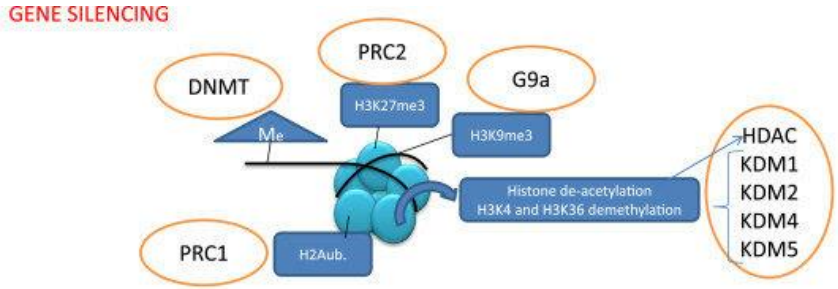


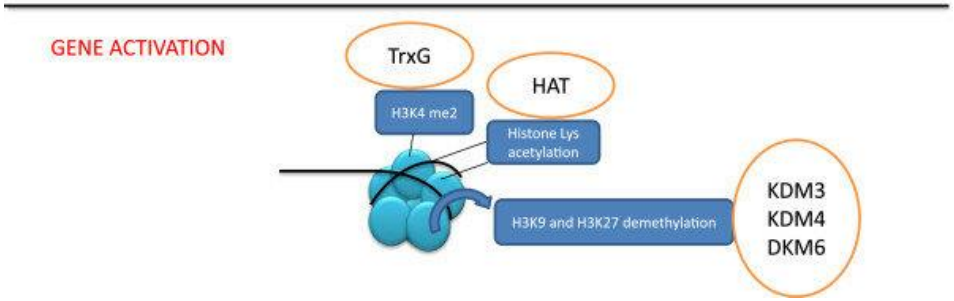
Reduced capacity for DNA repair in aged oocytes

Epigenetic regulation



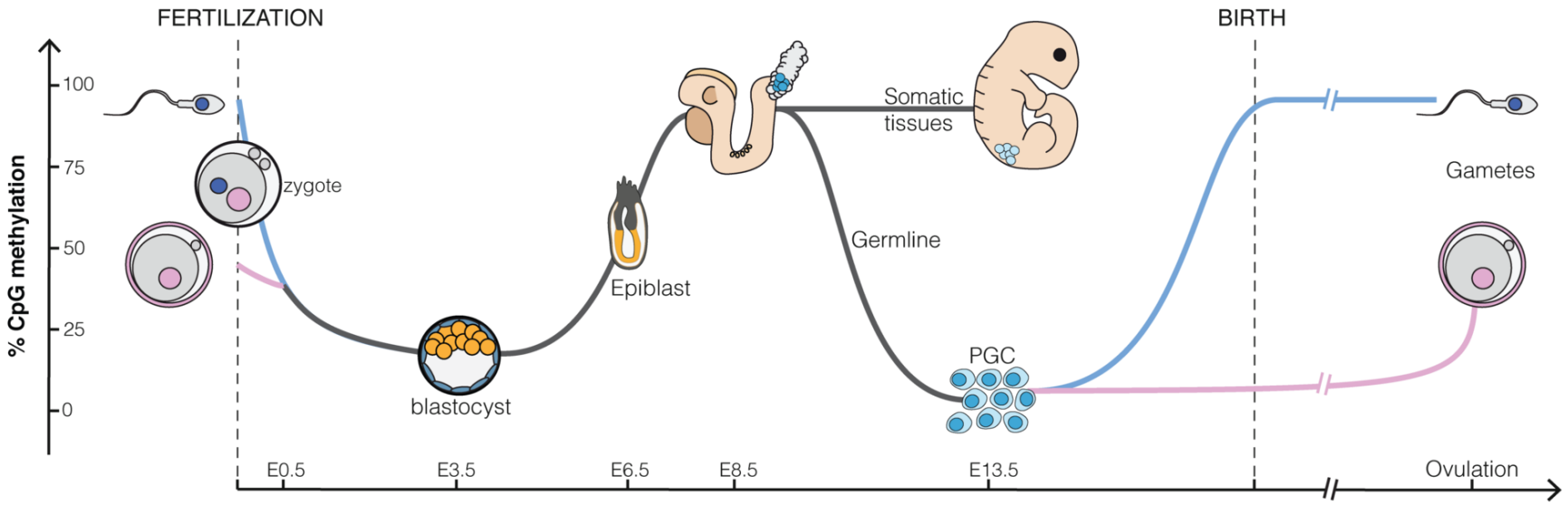
- regulation of gene expression without DNA sequence modifiation
- affect DNA accessibility for transcription factors
- gene trascription switched „ON“ and „OFF“ depending on cell needs





- explains complexity of multicellular organism from a single genetic blueprint
- enables cellular plasticity/genomic integrity during development and in response to environmental factors

Epigenetic reprograming during development



fertilization

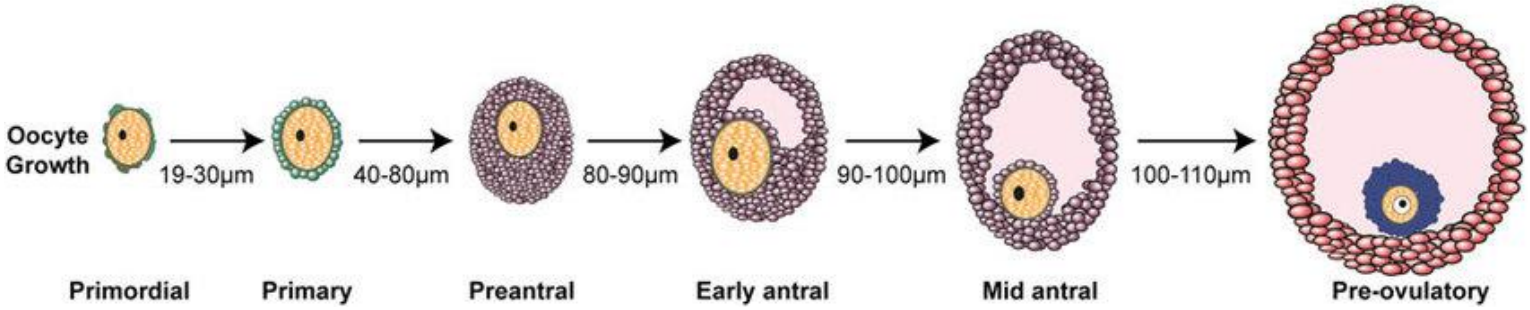
PGCs

Global erasure of epigenetic marks

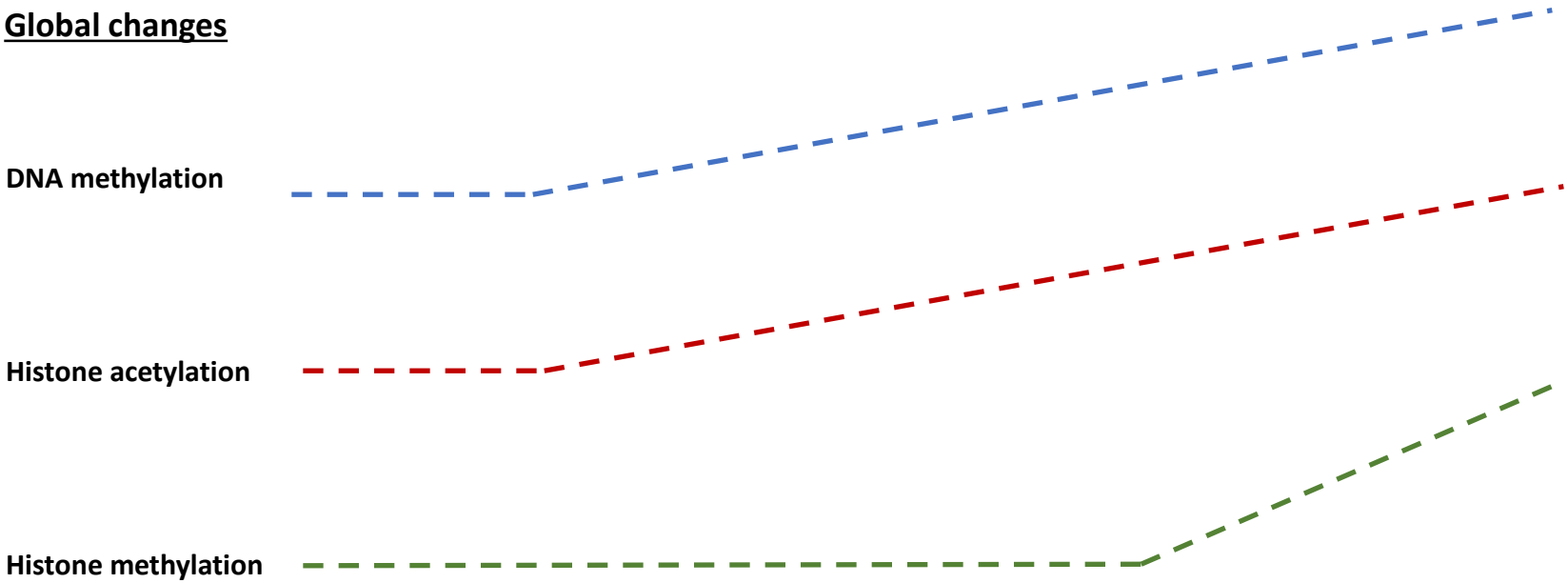
Transgenerational epigenetic inheritance??

(In)complete erasure?

Oocyte epigenomic profile



Global changes



- Epigenetic marks established during oogenesis
- Specific heterochromatin profile
- Different histone variants and epigenic modifiers

Translational decline

- mRNA polyadenylation, translation and protein levels are decreased in old mouse
- oocytes premature CDK1 activation, and accelerated reentry into meiosis
- dysfunction in the oocyte translation program associated with the decline in oocyte quality during aging in mouse

nature communications

Article

https://doi.org/10.1038/s41467-023-35994-3

CPEB1-dependent disruption of the mRNA translation program in oocytes during maternal aging

Received: 12 April 2022

Accepted: 11 January 2023

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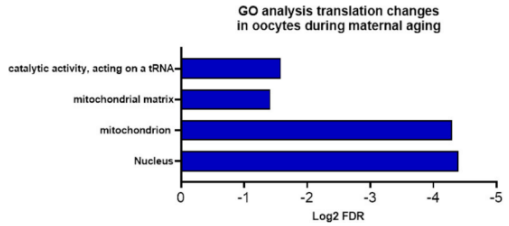
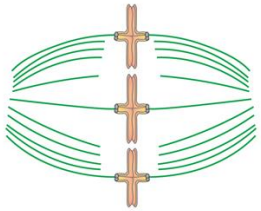


Time from primordial follicle activation to oocyte meiotic maturation

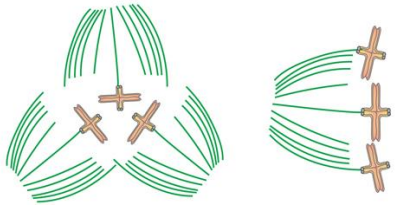
Young ovary → **Oocyte ribosome and mRNA "health" is maintained**

Bipolar Spindles



Aged ovary → **Slower follicle development leads to aging of mRNAs and ribosomes**

Multipolar/Unipolar Spindles



- reduced translation in late-stage oocytes during ovarian aging might contribute to meiotic defects

Danielson et al 2024

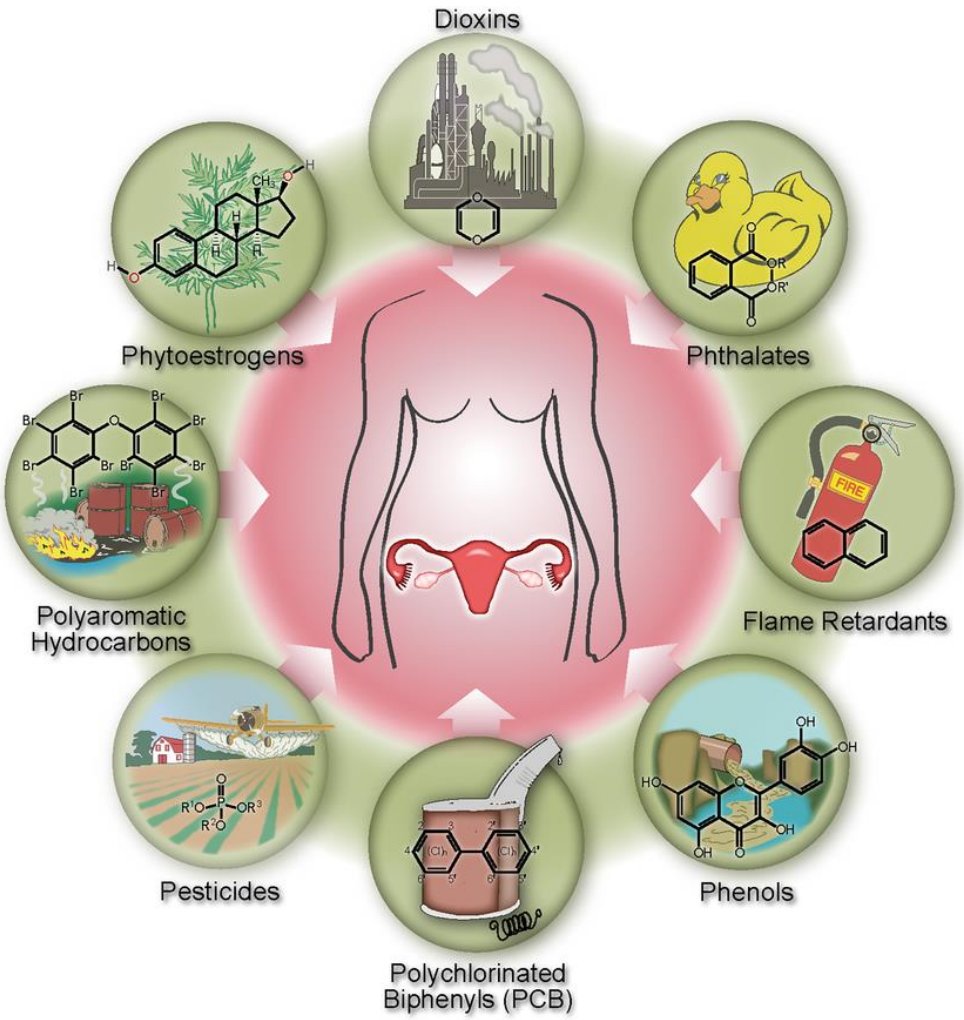
Environmental factors

- Radiation
- Air pollution
- PFAS
- Endocrine disruptors
- Plastics
- Heavy metals

etc....

100.000 human-made chemicals released to environment

70.000 **unknown** effects

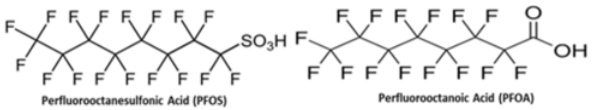


Environmental factors

❖ PFAS

= **p**er- and polyfluoroalkyl substances

- synthetic chemicals containg carbone-fluorine bonds
- low biodegradability („forever chemicals“)

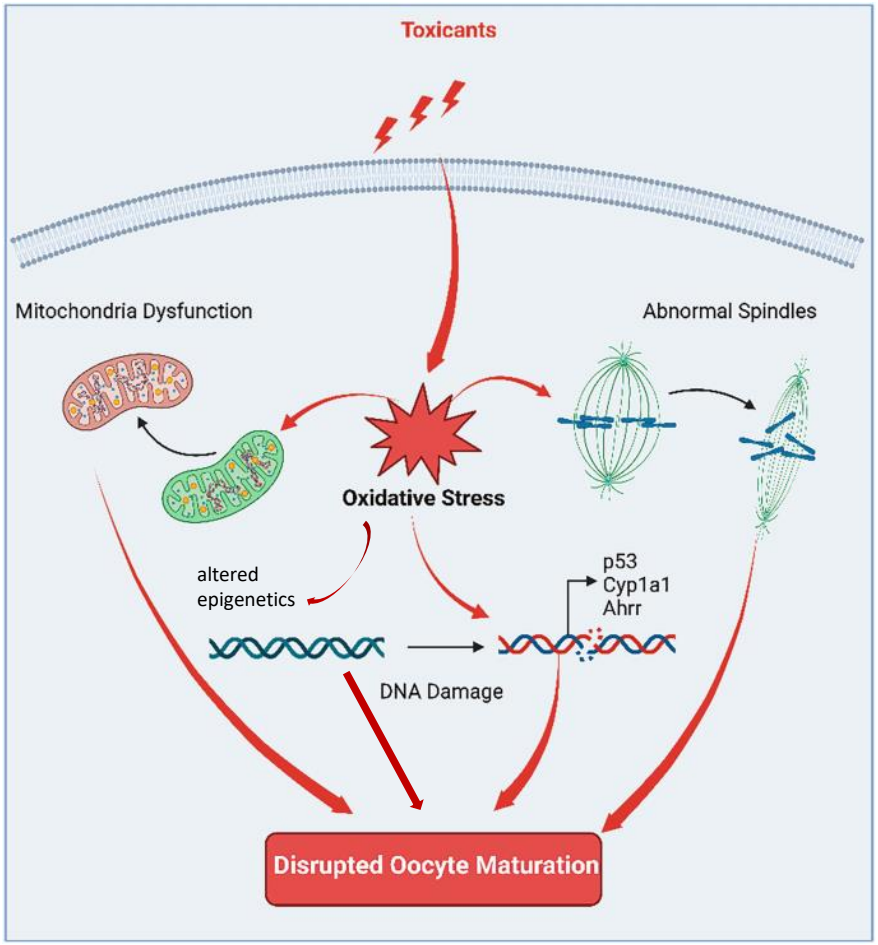


❖ Plastics

- **Microplastics**
  - particles **5 mm - 1 µm**
- **Nanoplastics**
  - particles < **1 µm**

← degradation of plastics

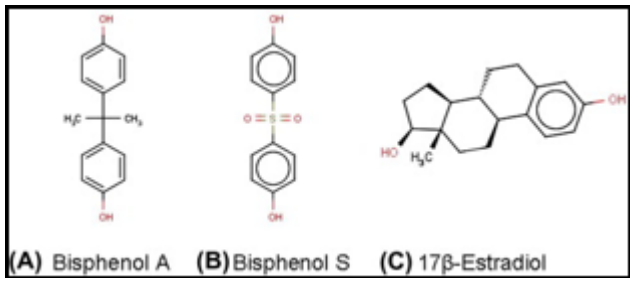
- found in human blood, milk, urine, placenta, meconium, follicular fluid, altered microbiome,...
- difficult identification, classification and quantification
- unknown effect on environment and human health



Environmental factors

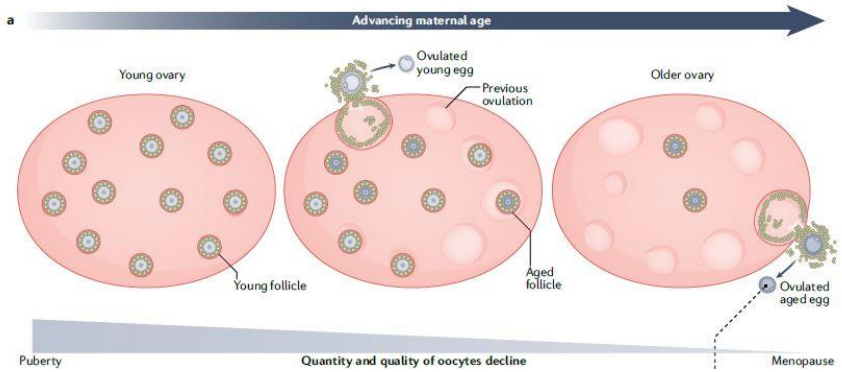
❖Endocrine disruptors

= natural or man-made chemicals that may mimic or interfere with synthesis, secretion, transport, bidning, action or elimination of hormones in the human or animal body



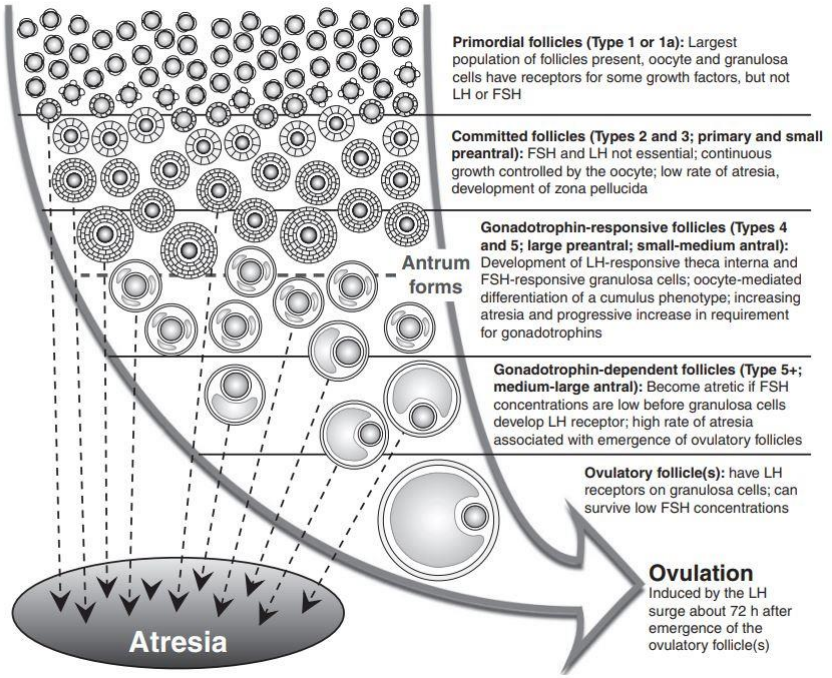
Oocyte quality hierarchy

- limited oocyte pool hypothesis
  (Warburton 1989)



- poor quality oocyte would not become dominant in young female ovary because of abundance of better quality oocytes

- in older women with small ovarian reserve a defective oocyte would be more likely ovulated



Scaramuzzi et al 2011

Human egg misery?

Evolutionary advantage of female **sub**fertility?

Preference of quality over quantity?