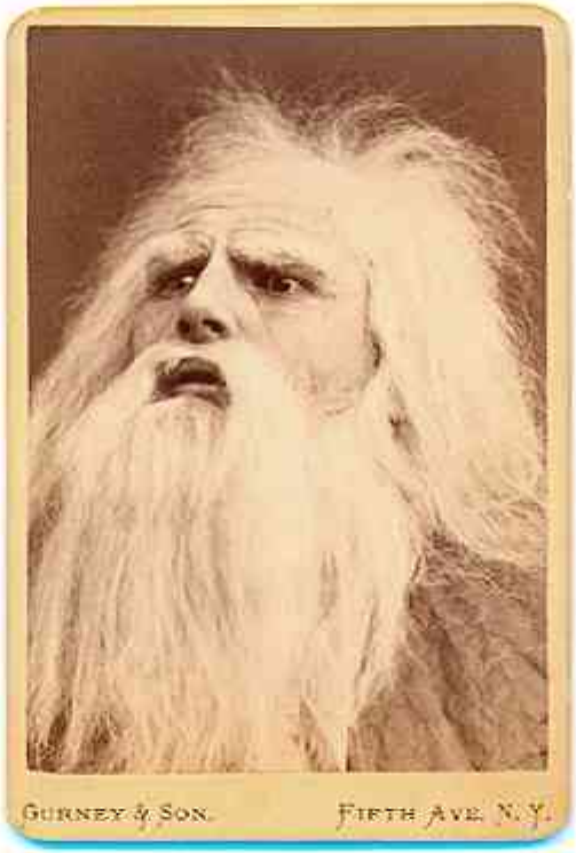
The ageing ovary - What changes ?

Varpu Jokimaa
TYKS Nkl
SGY 12.11.2009

Engagements 2007-09

- MD, PhD, Specialist in Obsterics and Gynaecology and Gynaecological Endocrinology

- **Main occupation**
  - Consultant in Dept Ob Gyn, Turku University Central Hospital
- **Secondary occupation**
  - Private practitioner
- **Other engagements**
  - Lecturer and participant in meetings and conferenses organised by Ferring, Merck-Serono and Schering-Plough



”Jokainen haluaa elää pitkään, mutta kukaan ei halua tulla vanhaksi ”

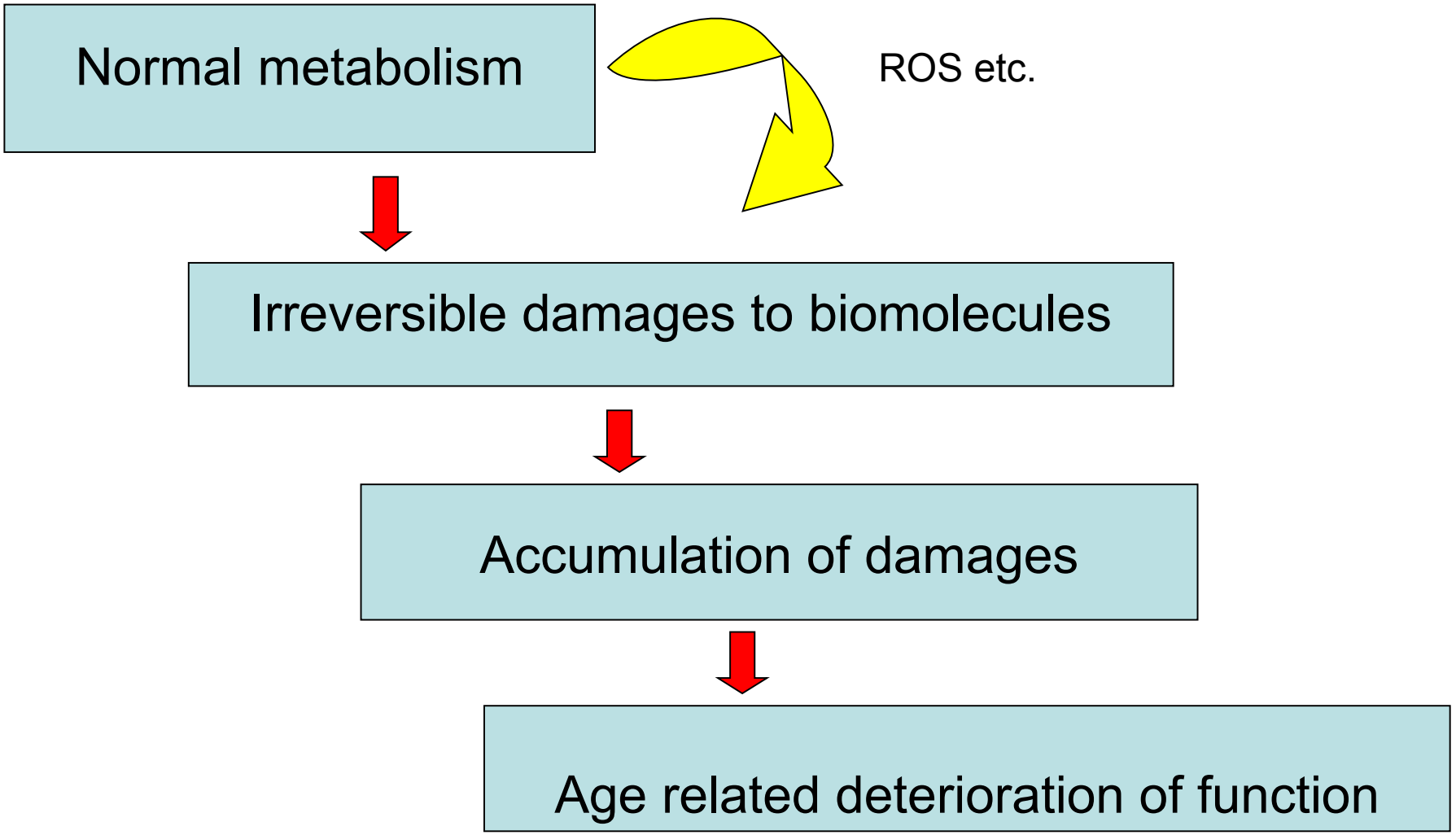
Jonathan Swift

The changes related to ageing ovary

- Anatomical
  - macroscopic
  - microscopic
- Functional
  - Hormonal
  - Fertility
  - Genetic risks in reproduction
- Secondary
  - Health concerns

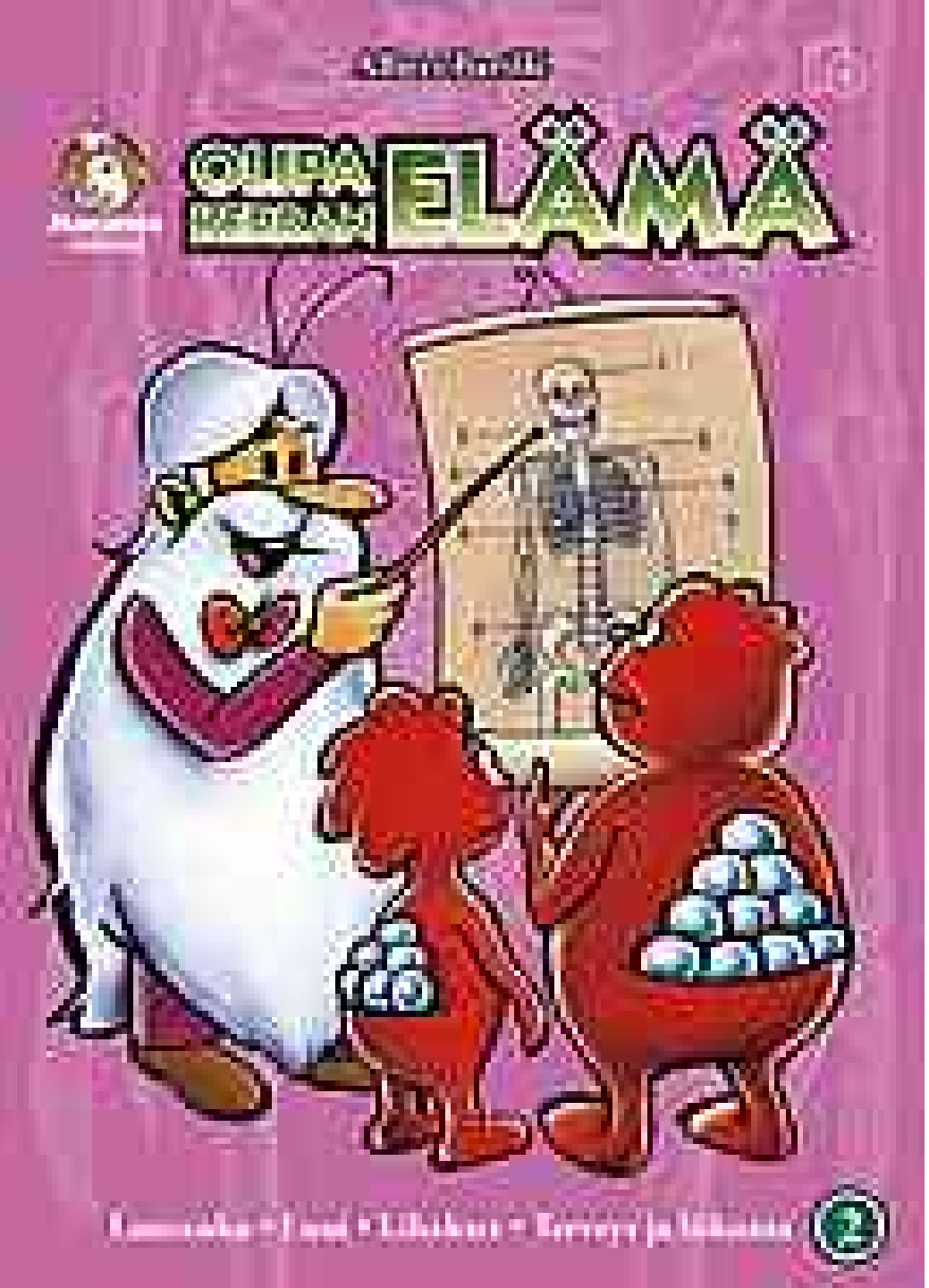

Oireet ja häiriöt ilman tyypillisiä tuntomerkkejä
Oireet ja häiriöt tyypillisillä tuntomerkeillä
vuotohäiriöt
vaihdevuosien vaivat
unihäiriöt,masentuneisuus
ihon oheneminen
luukato
virtsankarkailu
rintojen pieneneminen ja emättimen kuivuminen
painonnousu
korkea verenpaine
sydän- ja verisuonisairaudet
menopaussi
ika 40 45 50 55 60 65 70

Ageing


Normal metabolism
ROS etc.
Irreversible damages to biomolecules
Accumulation of damages
Age related deterioration of function

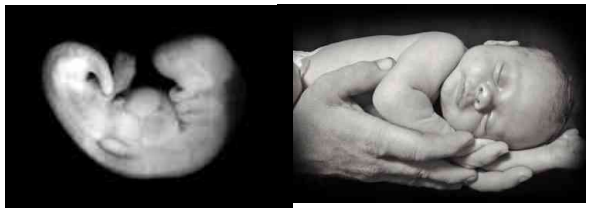

This is physiological and inevitable.

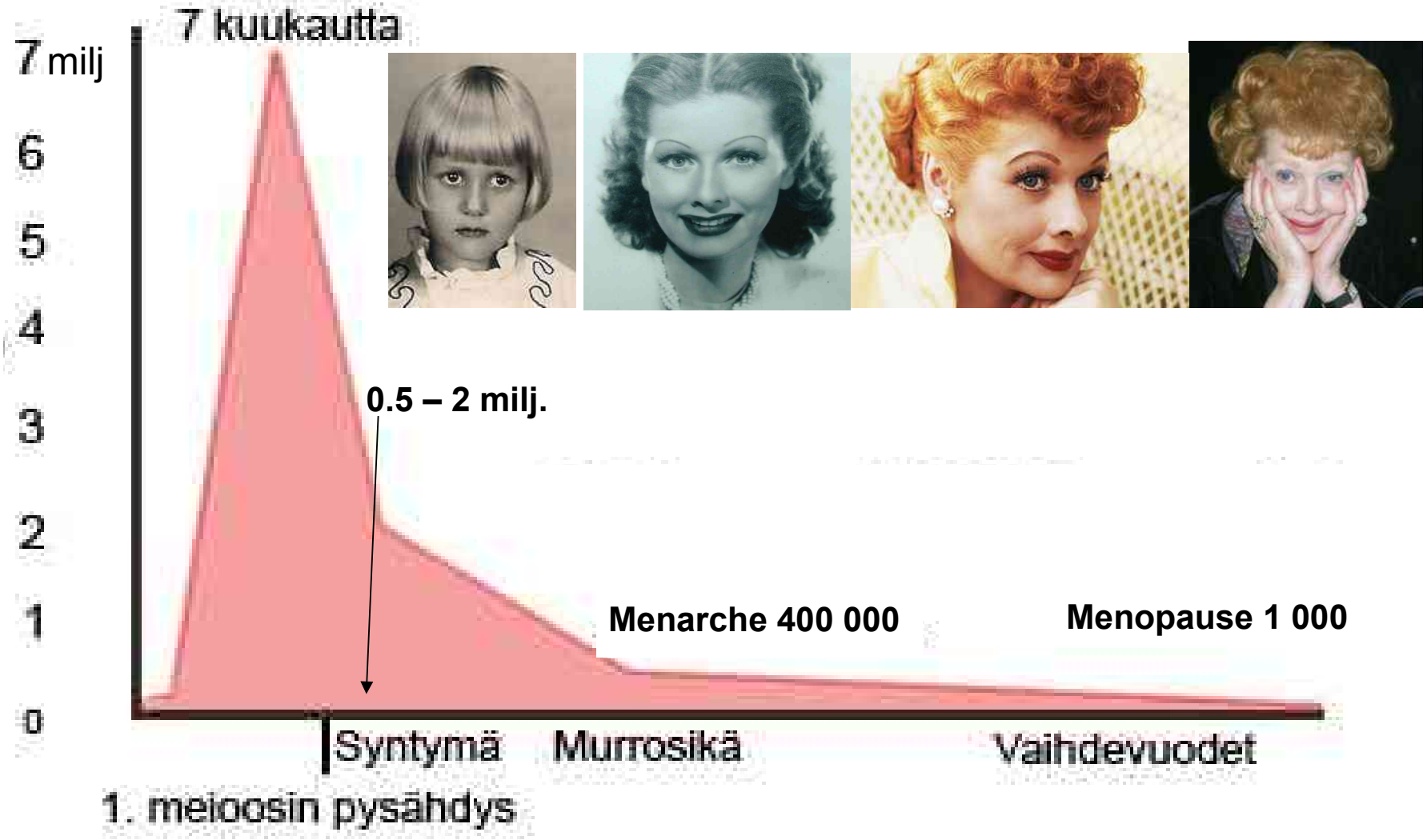

ataVegamin
W Your Way
HEALTH
VITAMEATAVEGAMIN
FOR HEALTH



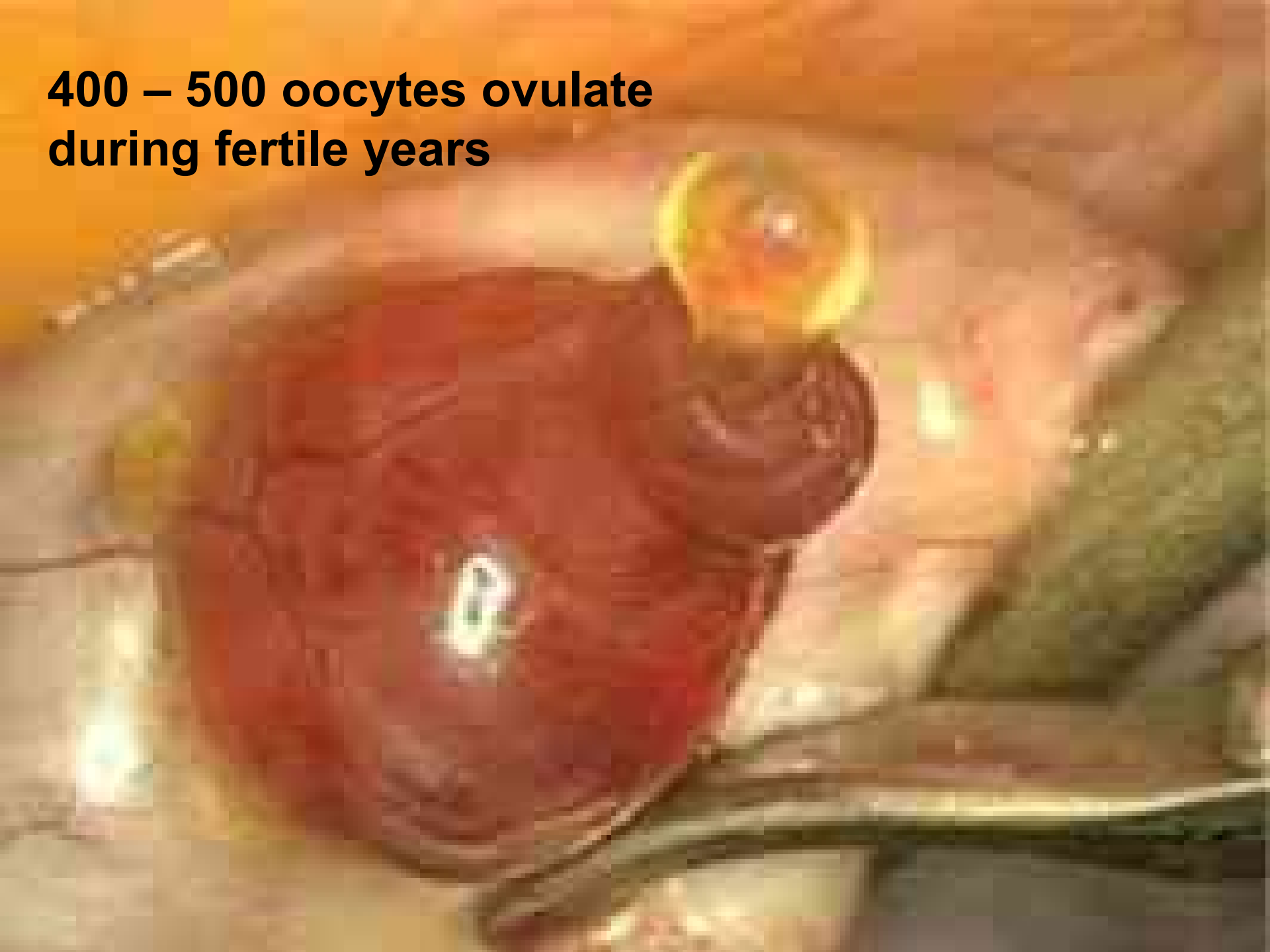
One has to know what is normal to understand abnormality.

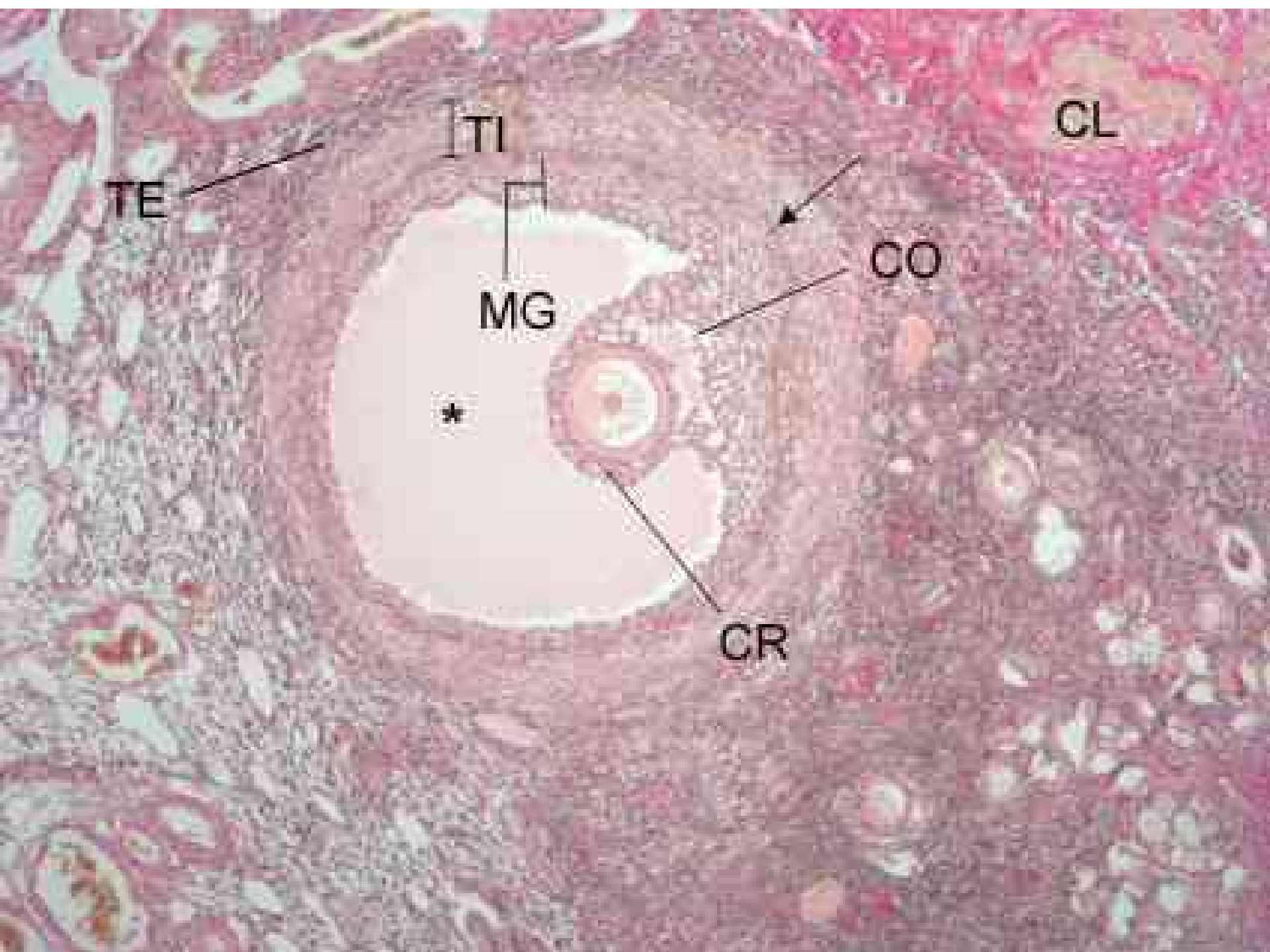
Someone somewhere




7 kuukautta
7 milj
6
5
4
3
2
1
0
0.5 – 2 milj.
Menarche 400 000
Menopause 1 000
Syntymä
Murrosikä
Vaihdevuodet
1. meioosin pysähdys

400 – 500 oocytes ovulate during fertile years




TE
TI
MG
CL
CO
*
CR

Aged ovary

The diminishing size of follicle pool is reflected into the menstrual cycle characteristics and fertility.

Also, there is evidence suggesting hypothalamic-pituitary insensitivity to E2

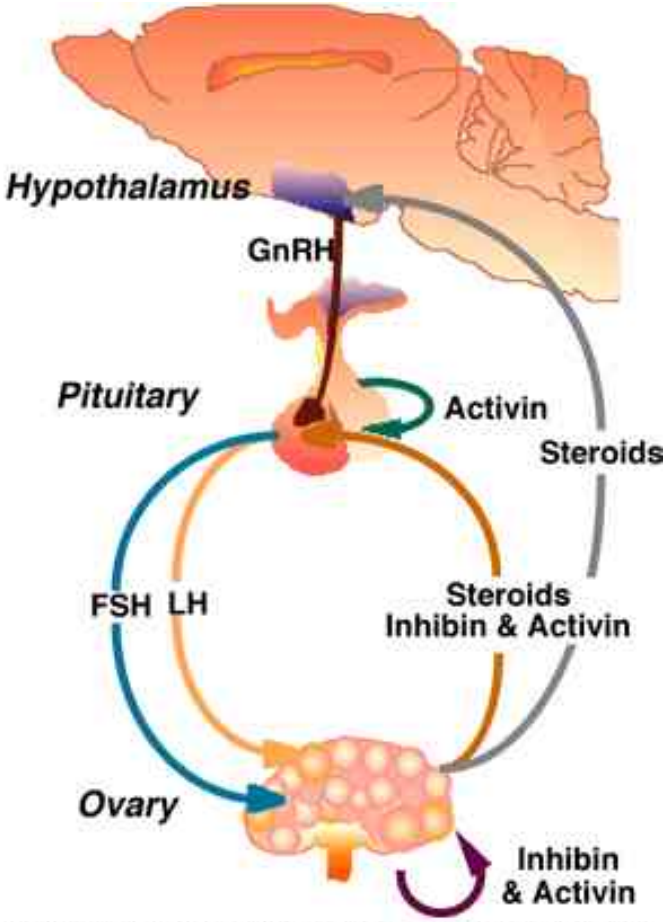
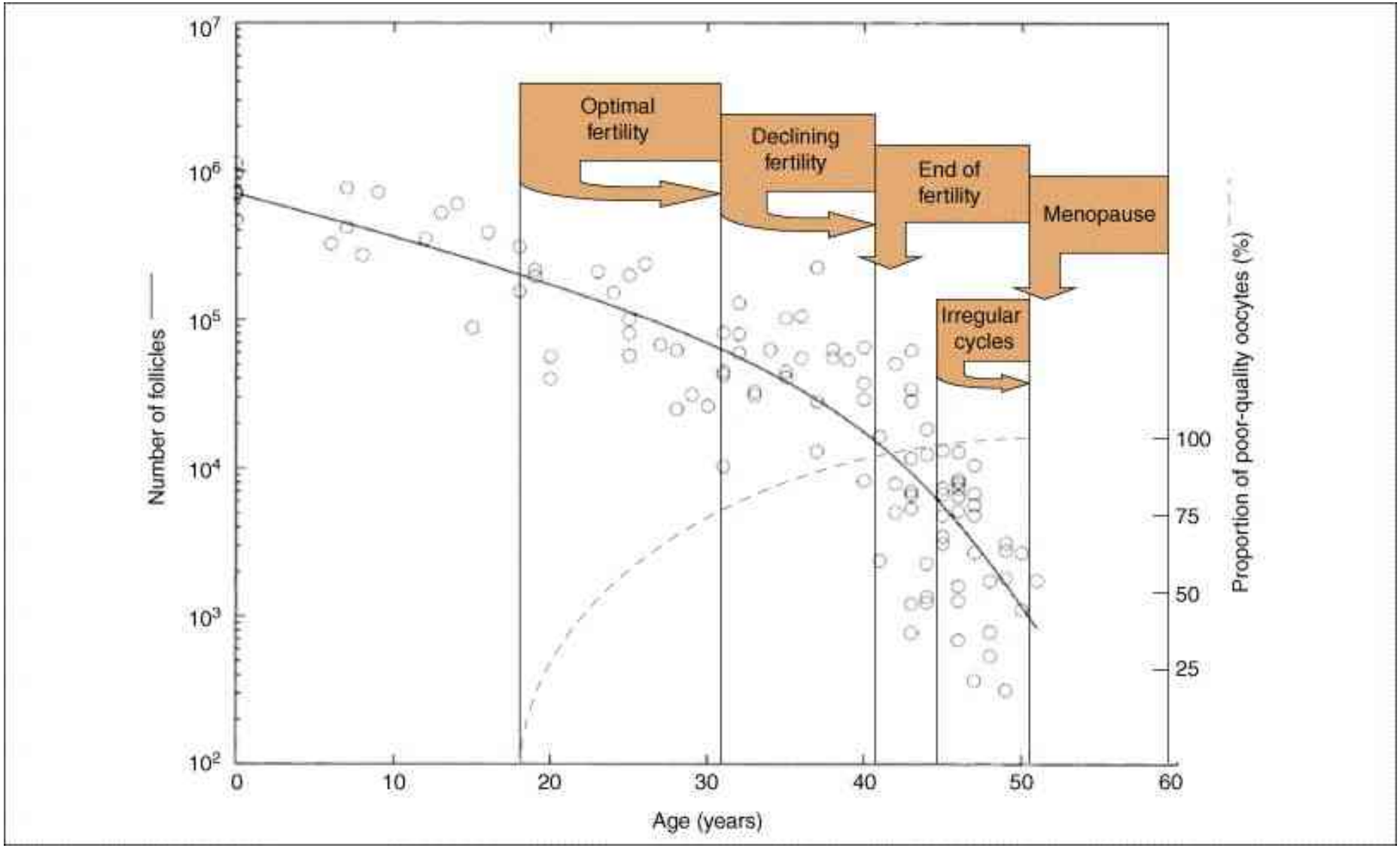
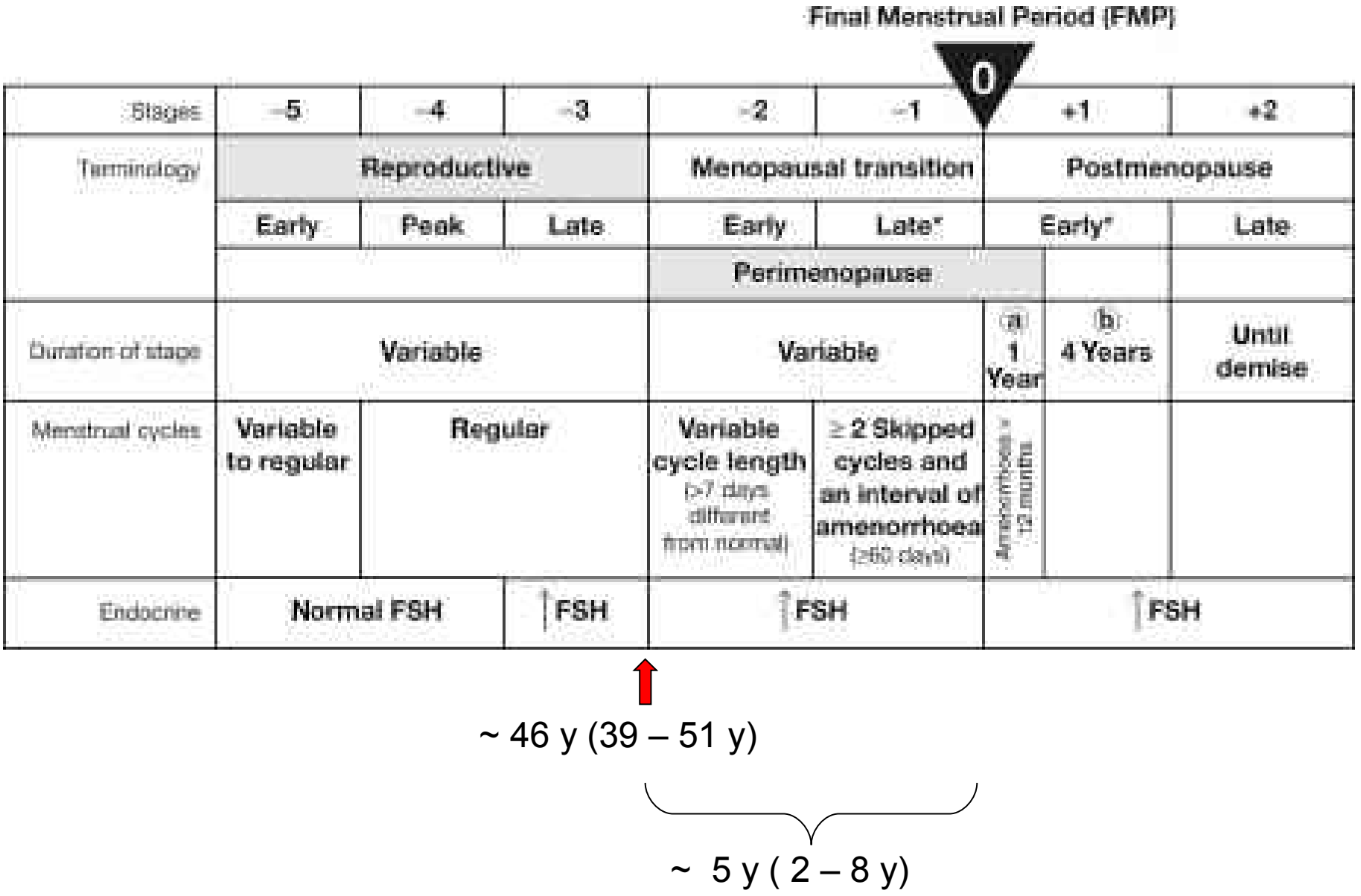


Figure 1: The Hypothalamic-Pituitary-Gonadal Axis

Broekmans ym., *Trends Endocr Met* 2007

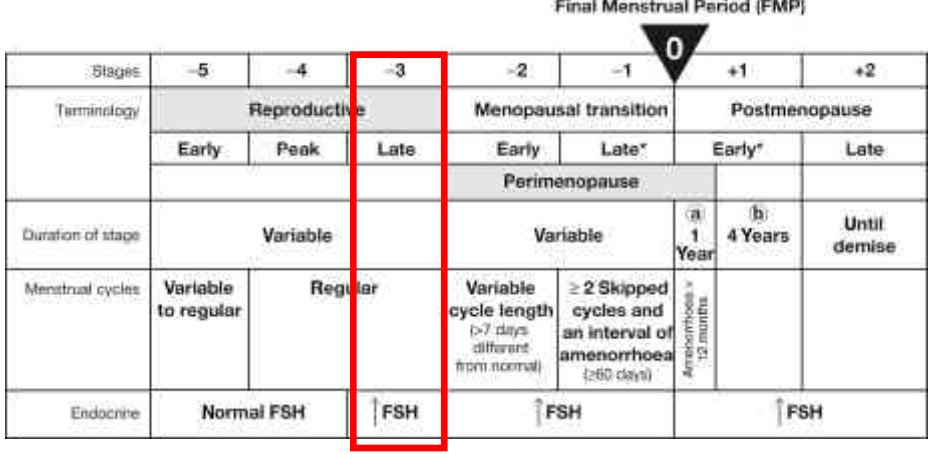


Stages of reproductive ageing (STRAW) stages

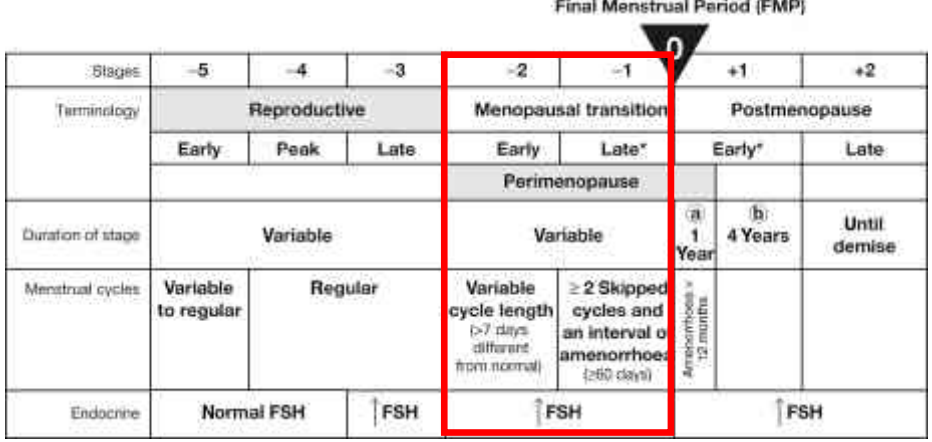


~ 46 y (39 – 51 y)

~ 5 y (2 – 8 y)



- In Late Reproductive Phase the cycle is shorter by 2 - 3 days but regular
  - Advancement rather than quicker follicle development
    - earlier and more profound rise in FSH-levels
  - Possibly FSH bioavaibility also ↑ during the luteal-follicular transition
  - Nadir at ~ 42 years



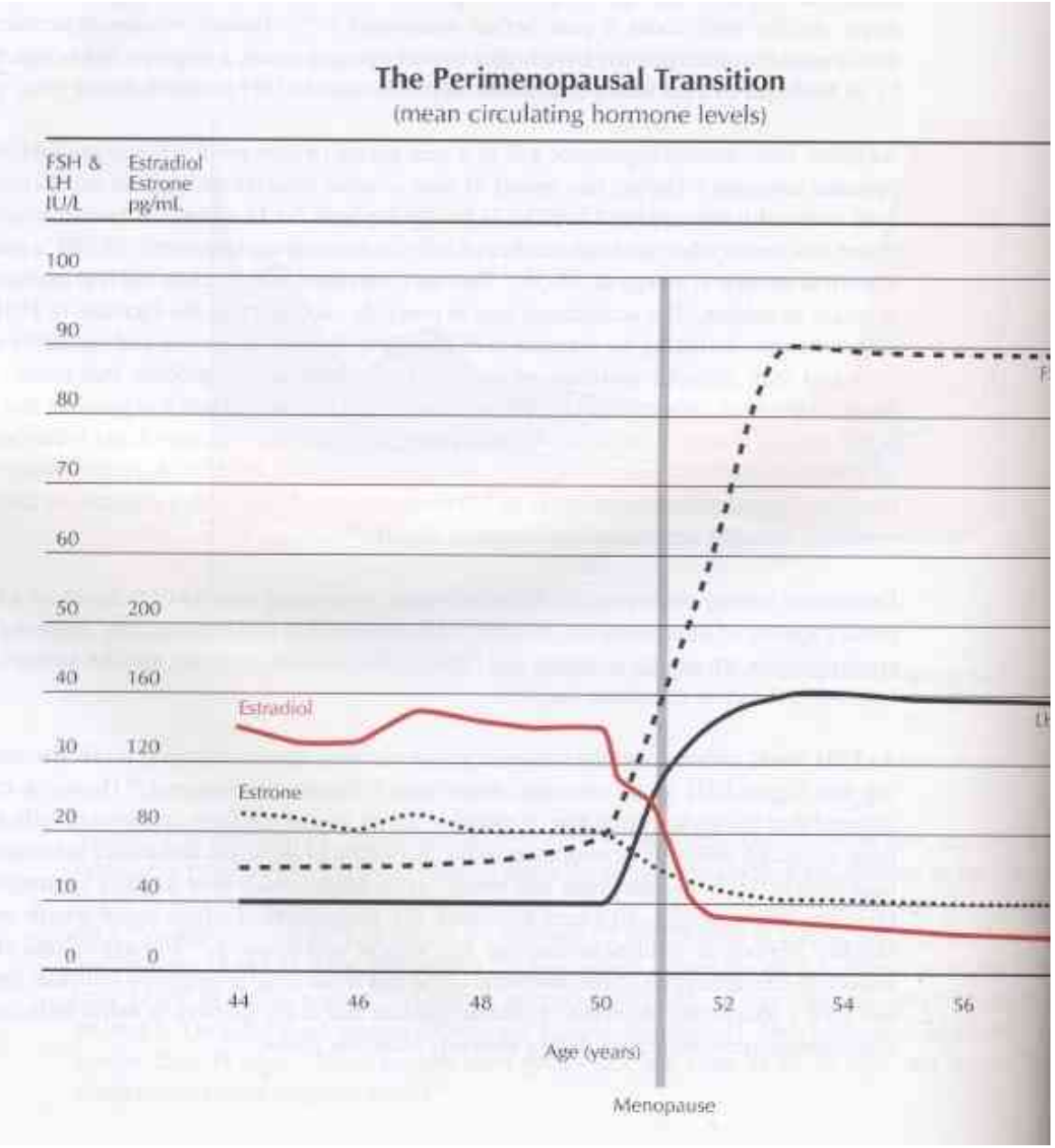
As the STRAW-stages ↑

- Cycles become progressively longer and irregular
- Anovulatory cycles ↑
  - SWAN-study daily hormone samples for 3 years
    - Age 47.3 → 49.3
    - Ovulatory cycles 80.8 % → 64.7 %
  - Both short & long cycles associate with anovulation
- The cycle length reflects the length of follicular phase
  (ESHRE Capri Workshop 2005)

- Irregular cycles and unpredictable hormone levels
- Dramatic swings in E2
- Individual variation in and between cycles (Hale et al, 2009)

- Hormone levels are unpredictable – although there is some logic
  - In ovulatory cycles Inh B ↓, FSH ↑, E2 ↑, P4 ↓

- E2 levels do not correlate with the cycle length



Atypical levels of E2 in ovulatory cycles

STRAW

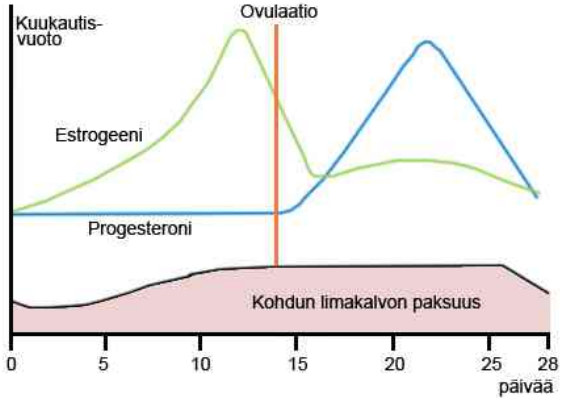
EMT	4/16	(31%)
LMT	7/13	(53%)

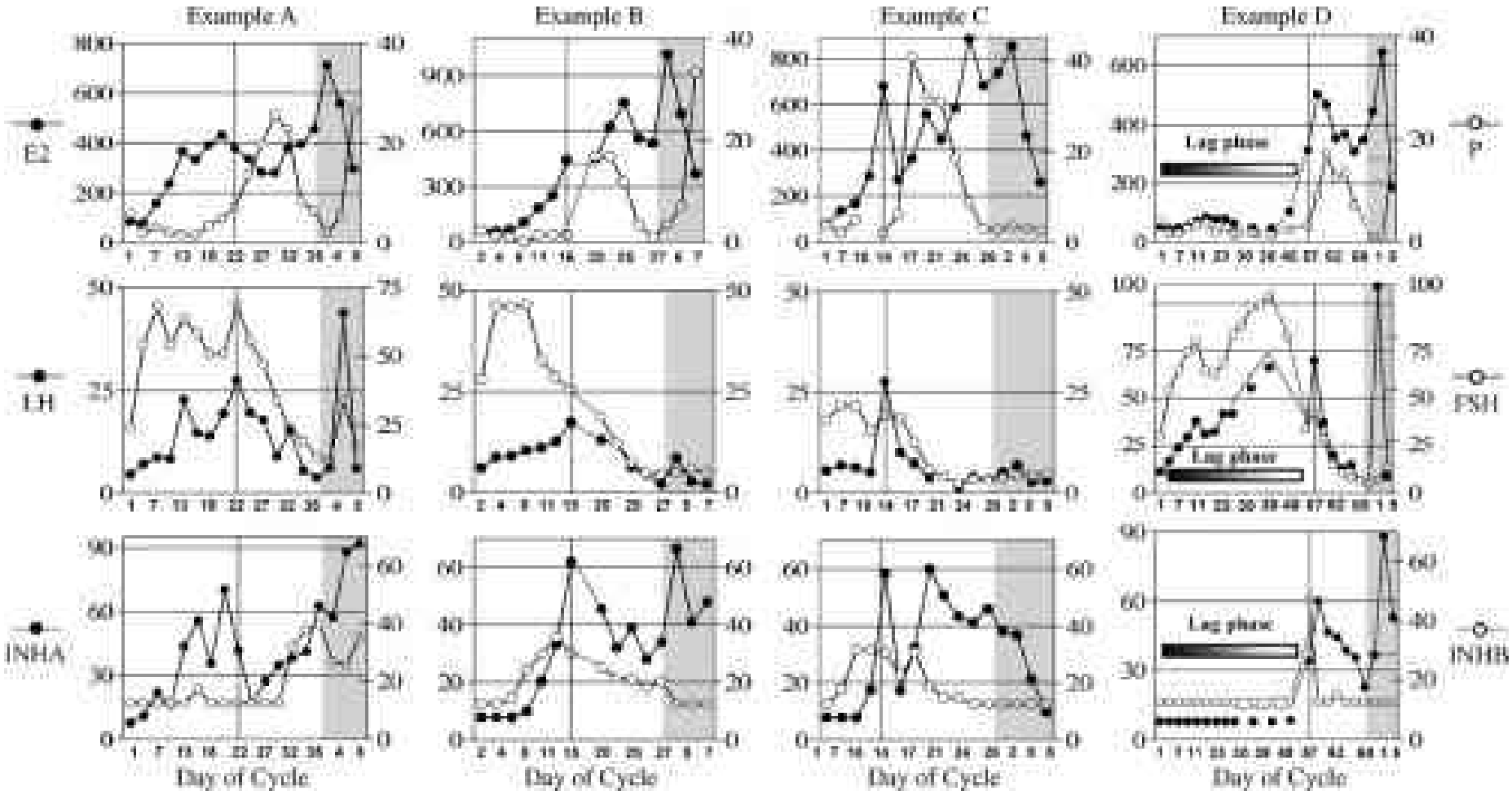
Luteal Out Of Phase event

LOOP

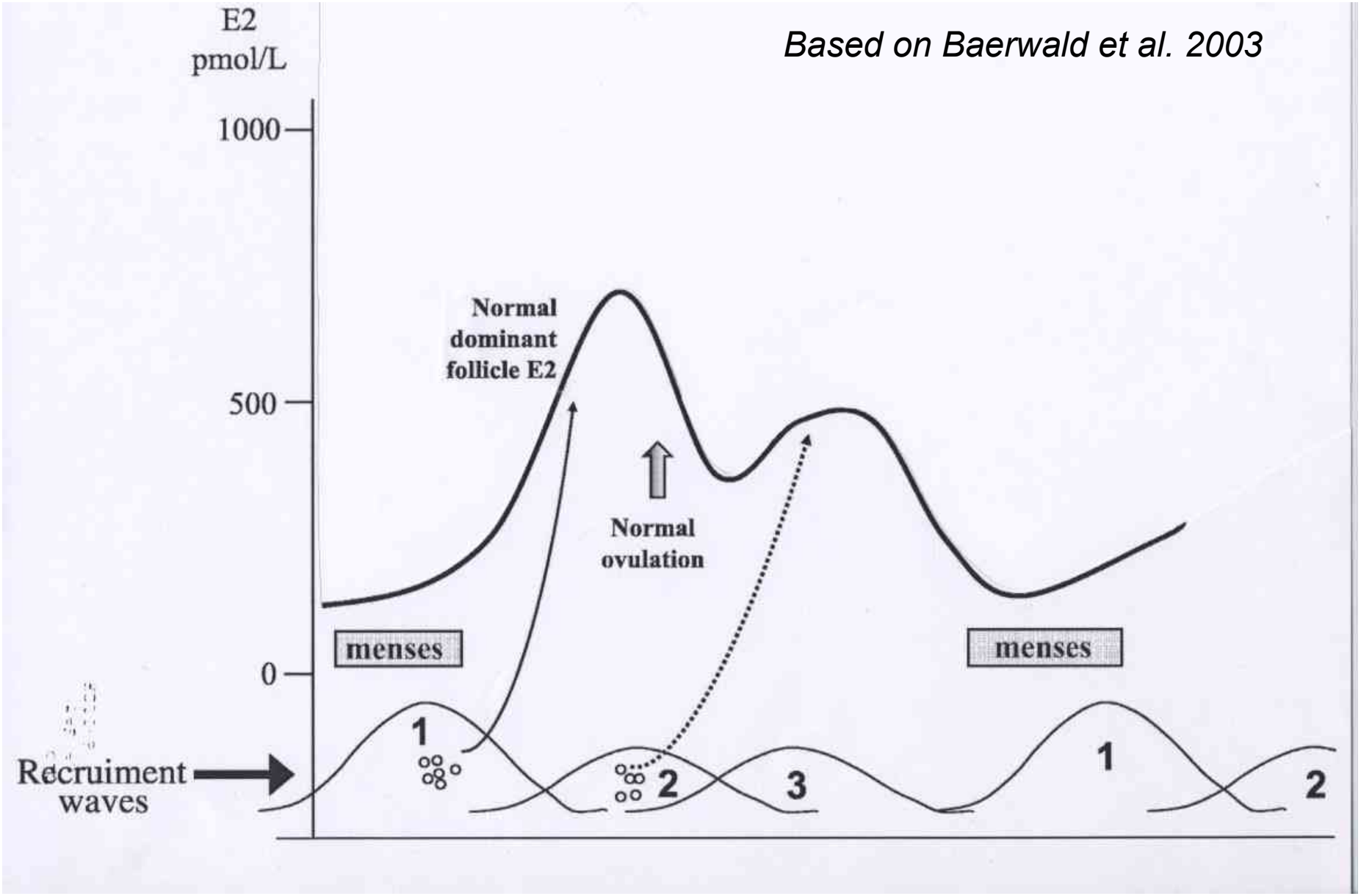
Rise in E2 levels resembling that in follicular phase in mid- and late luteal phases in ovulatory cycles

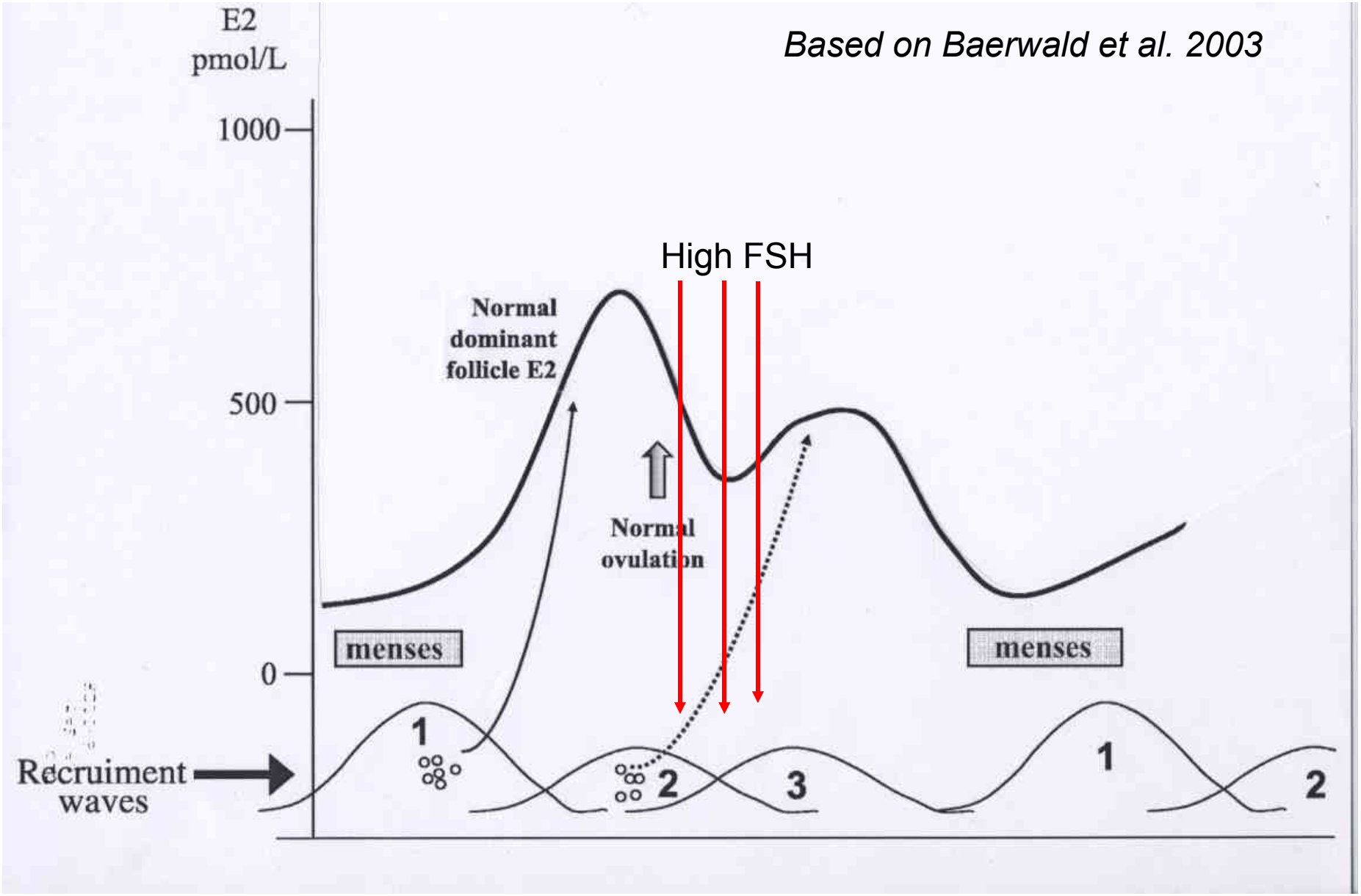
(Hale et al., 2009)

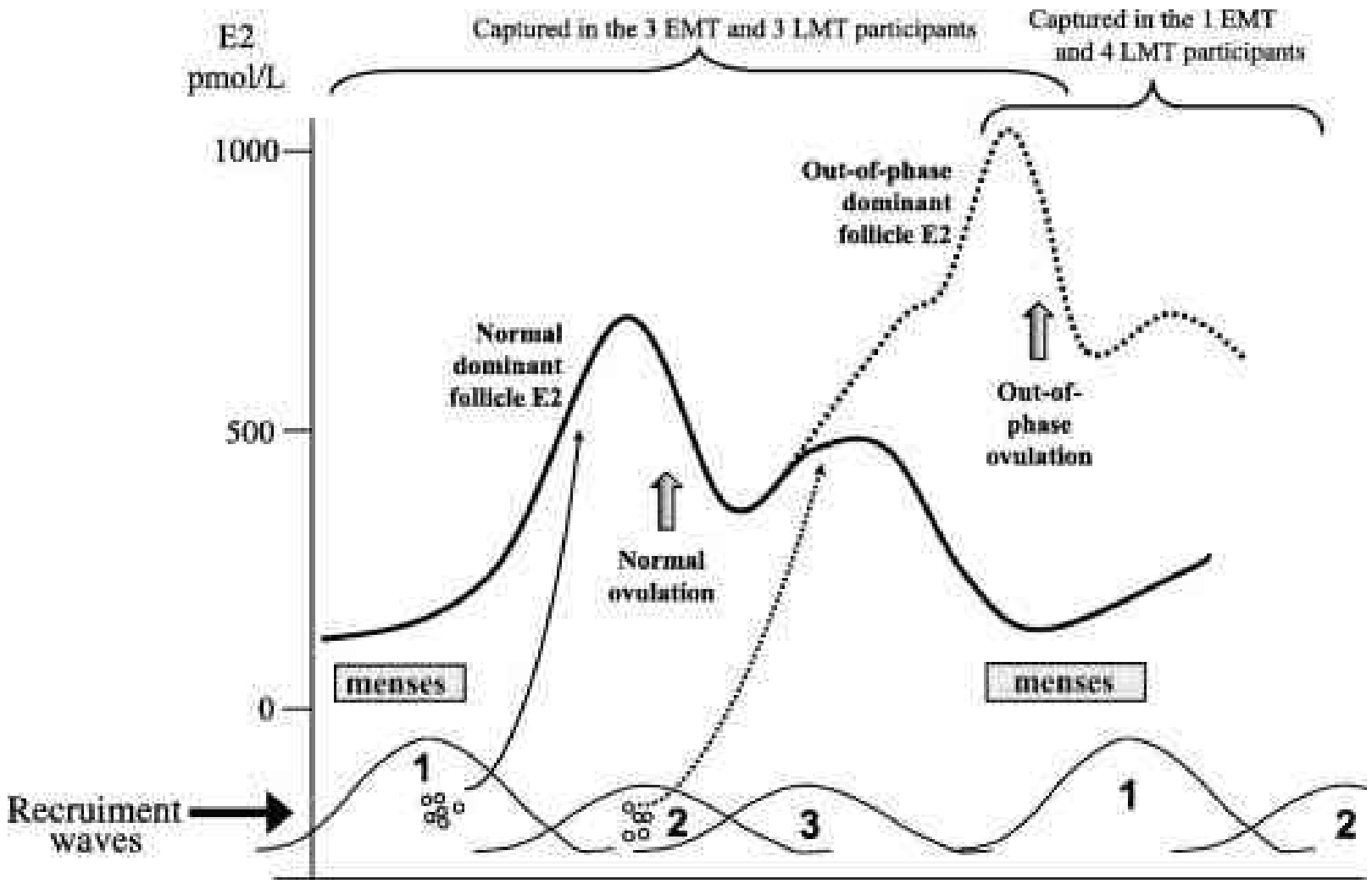

Kuukautis-
vuoto
Ovulaatio
Estrogeeni
Progesteroni
Kohdun limakalvon paksuus
0
5
10
15
20
25
28
päivää


Example A
Example B
Example C
Example D
E2
P
LH
FSH
INHA
INHB
Lag phase
Day of Cycle

Based on Baerwald et al. 2003




E2
pmol/L
1000
500
0
Based on Baerwald et al. 2003
High FSH
Normal
dominant
follicle E2
Normal
ovulation
menses
menses
Recruiment
waves
1
2
3
1
2


E2
pmol/L
Captured in the 3 EMT and 3 LMT participants
Captured in the 1 EMT
and 4 LMT participants
1000
500
0
Out-of-phase
dominant
follicle E2
Normal
dominant
follicle E2
Out-of-
phase
ovulation
Normal
ovulation
menses
menses
Recruiment
waves
1
2
3
1
2

Short menstrual cycle

- 5 – 20 % of the cycles during menopausal transition are short (14 – 21 days)
- Proposed causes
  - Early ovulation
  - Anovulation

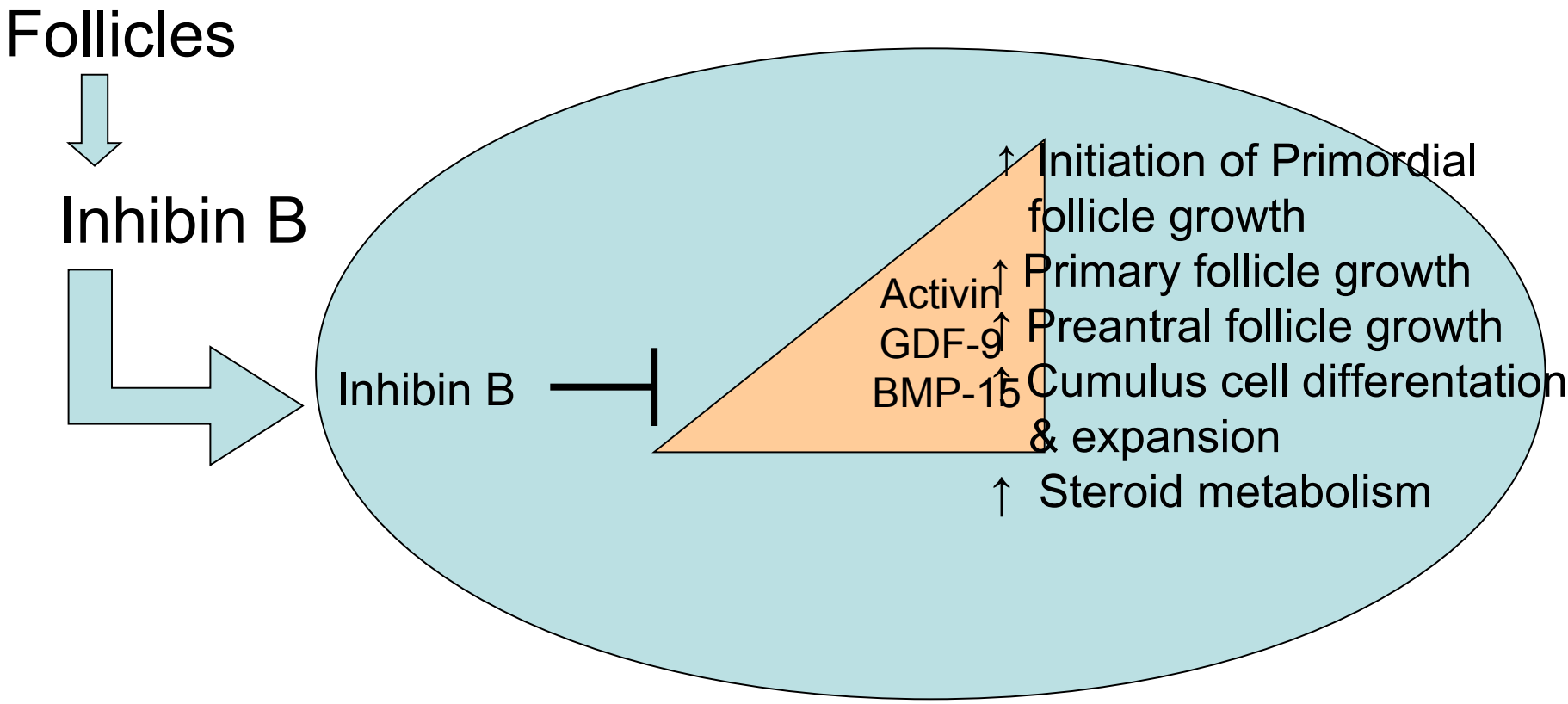
Anovulation

- Normal rise in E2 & LH with no ovulation – Ovarian cause ?
- Normal/high E2, no LH surge –

  Hypothalamic insensitivity ?
- Low E2, marginally elevated gonadotrophins –

  Relative hypothalamic insensitivity ?

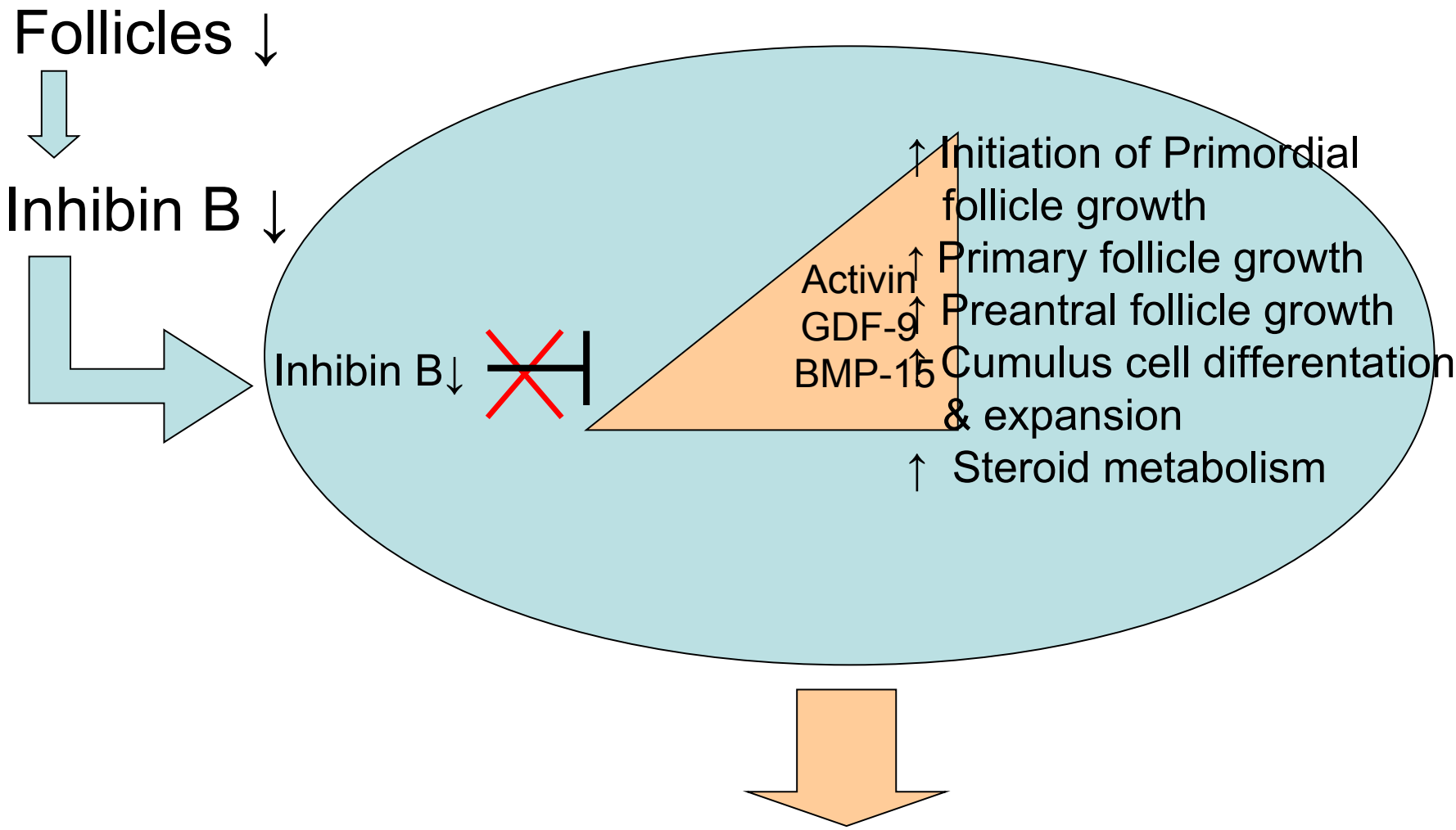
Weiss ym., 2004, Wu ym., 2005,
Skurnick ym. 2009



Chand, Harrison and Shelling

Human Reproduction Update

Advance Access Sep 2009


Follicles ↓
Inhibin B ↓
Inhibin B↓
Activin
GDF-9
BMP-15
↑ Initiation of Primordial follicle growth
↑ Primary follicle growth
↑ Preantral follicle growth
↑ Cumulus cell differentation & expansion
↑ Steroid metabolism
- Poor quality of primordial follicle pool
- Abnormal folliculogenesis
- Follicle depletion ↑

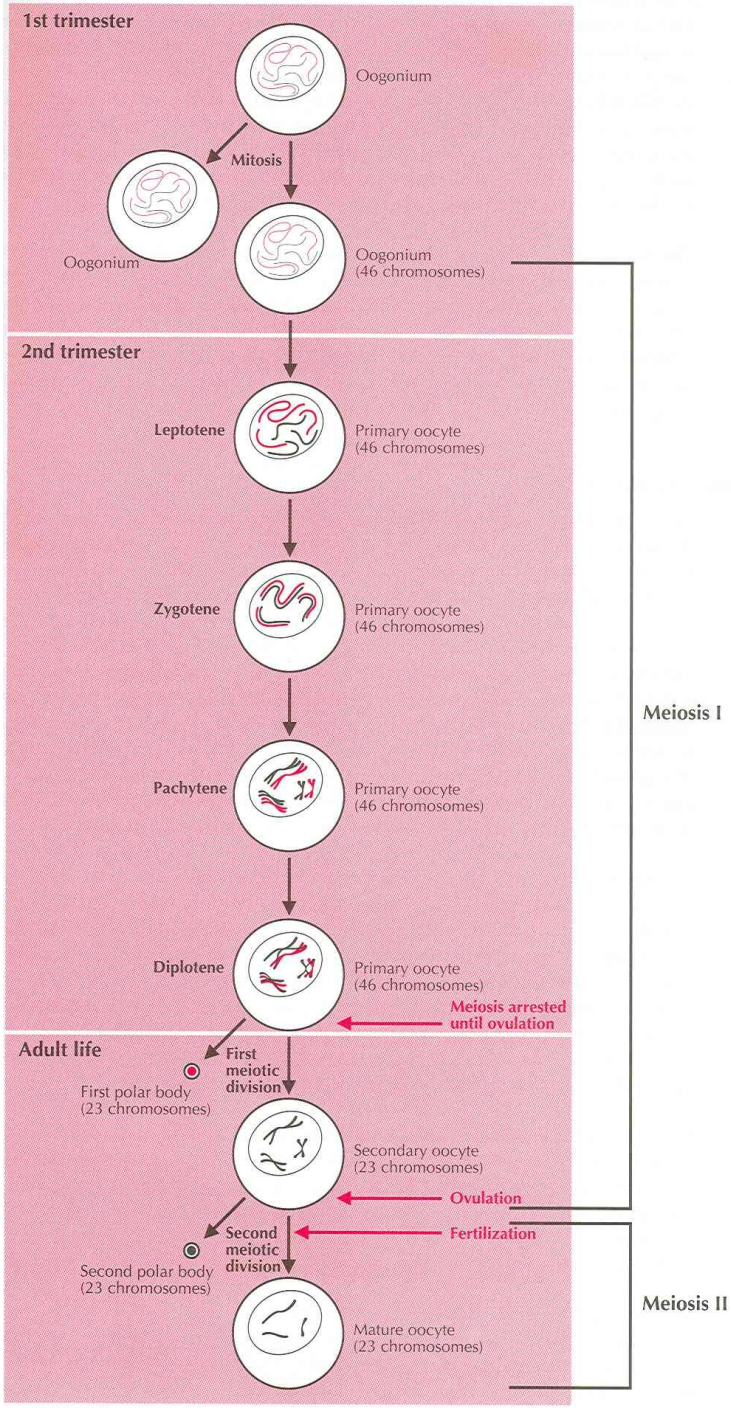
Functional changes associated with oocyte aging

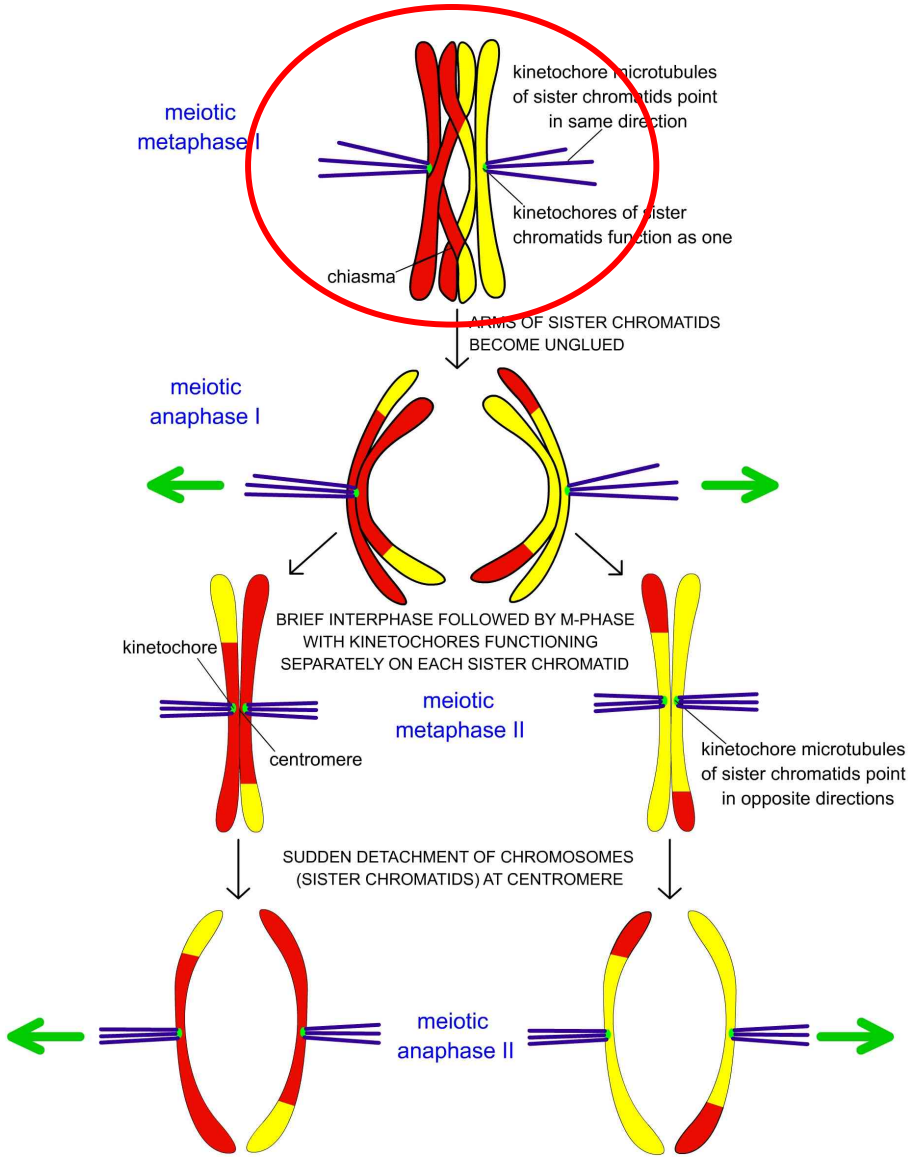
1. Decreased fertilization rates
   - Partial exocytosis of cortical granules (CG), structural alteration and hardening of the zona pellucida
   - Quicker postovulatory ageing and shorter lifespan
2. Aneuploid fertilization
   - chromosomal anomalies, polyspermy, digyny
3. Increased susceptibility to activating stimuli
   - Parthenogenesis
   - A decrease in maturation promoting factor (MPF) and mitogen-activated protein kinase (MAPK) activity
   - Onset of anaphase II
4. Apoptosis ↑
5. Epigenetic changes
6. Abnormal and/or retarded development of embryos

'An egg cell before fertilisation is on pause. At the moment of fertilisation, when a sperm fuses with the egg, the egg bursts into life.

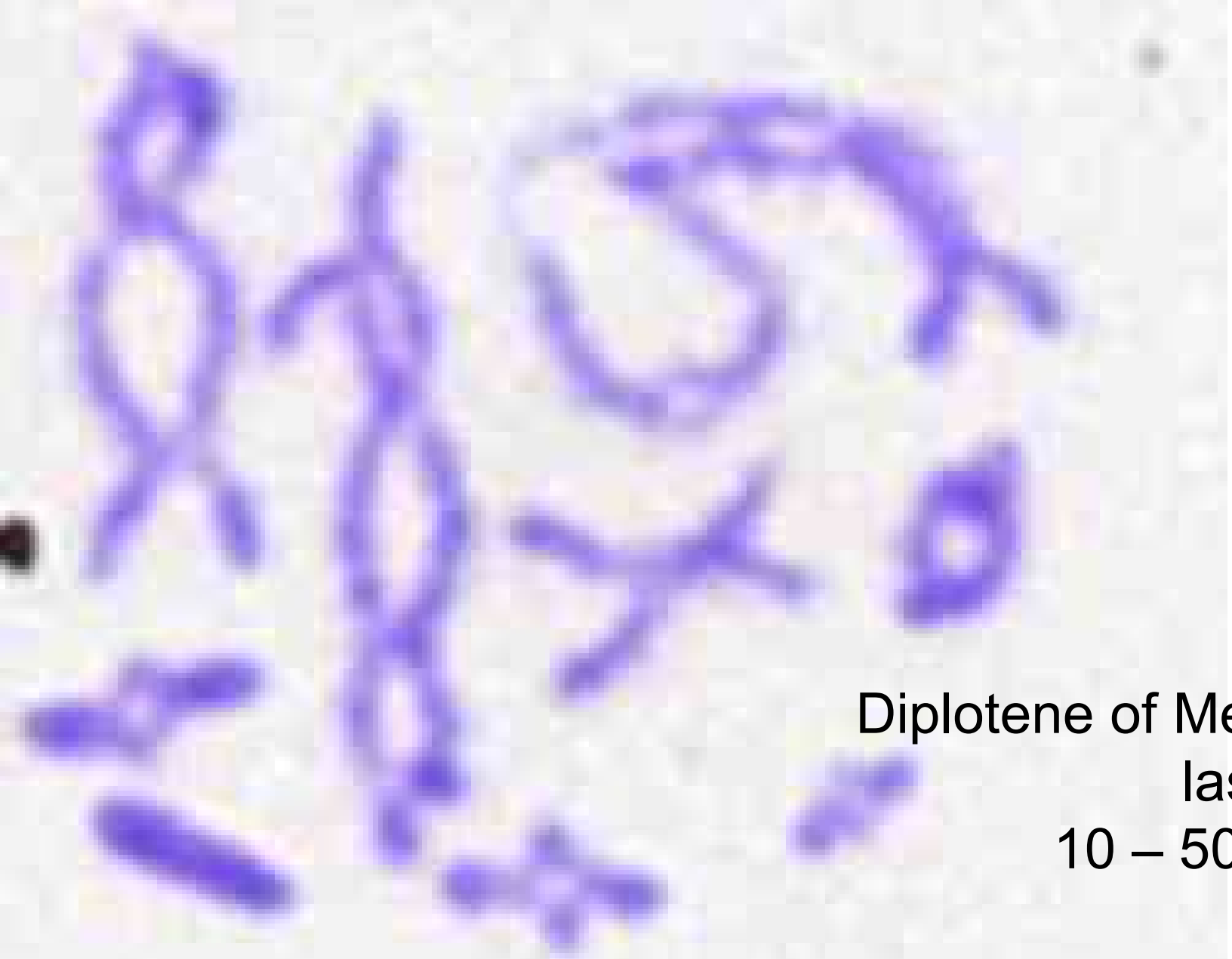
It's like a Prince waking Sleeping Beauty.'

Dr John Parrington
University of Oxford.

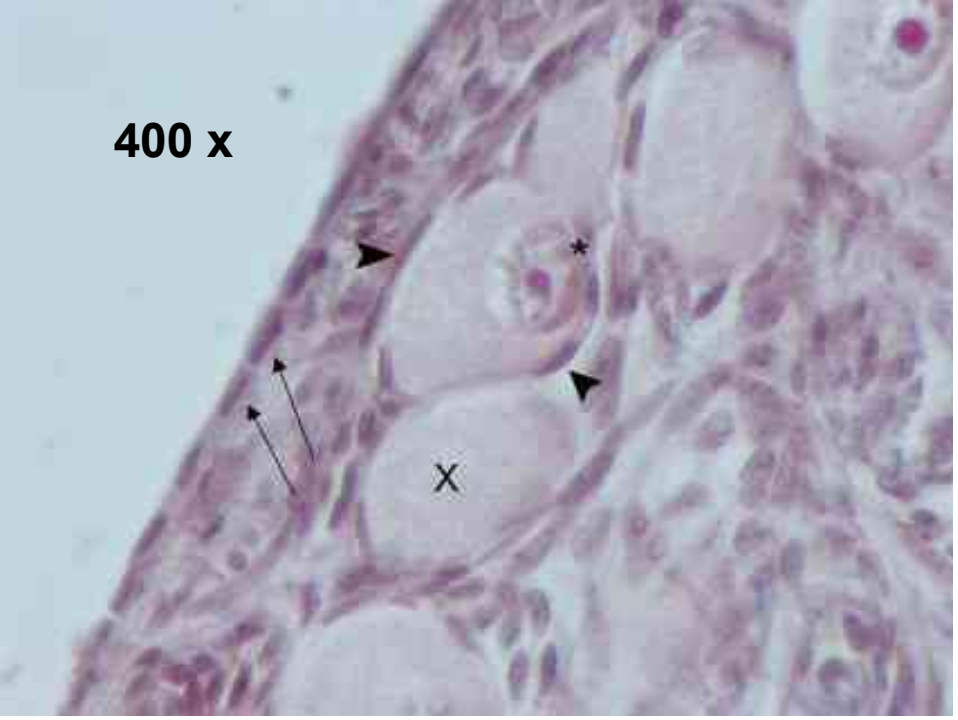


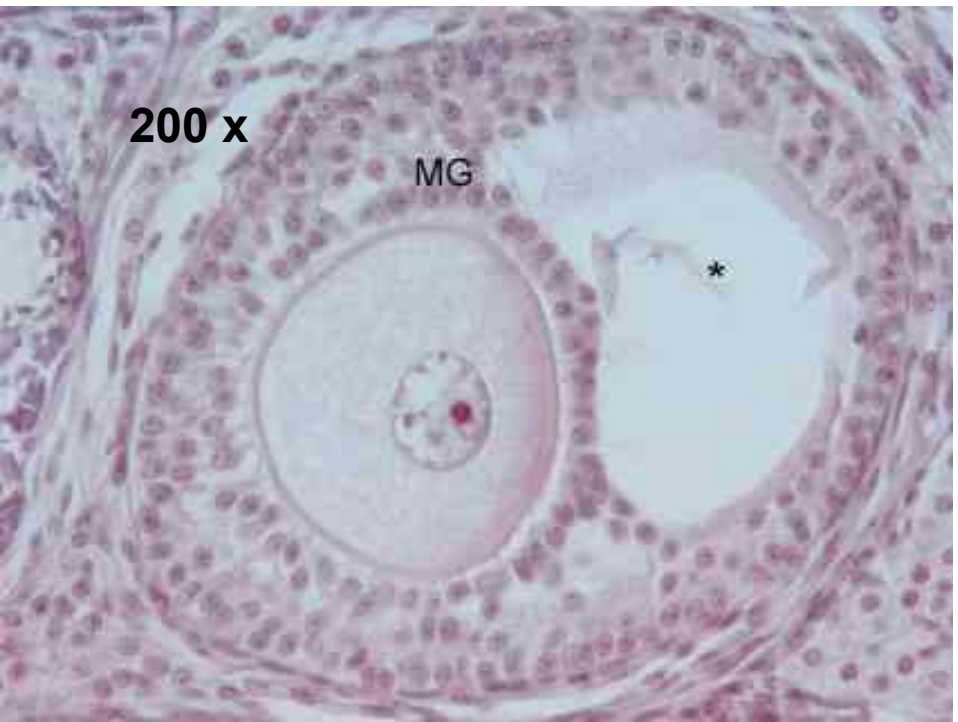


http://www.johnkyrk.com/meiosis.html



Diplotene of Meiosis I
lasts for
10 – 50 years





Primordial follicle recruited

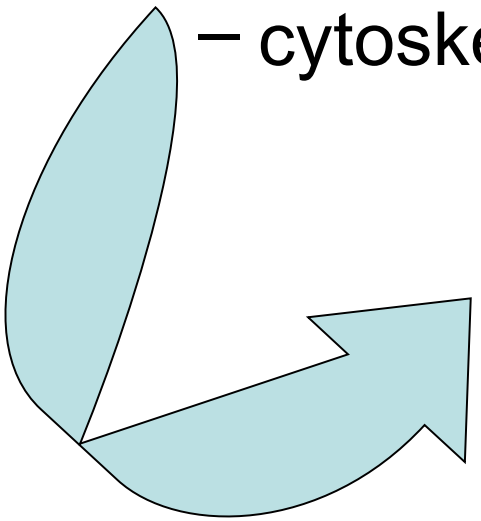
- production of RNAs and proteins ↑
- mtDNAcopies ↑↑↑
- the size of oocyte ↑

Gonadotrophin responsive antral follicle

In old oocytes

Changes in gene expression related to

- Mitochondrial function, energy metabolism
- Oxidative stress, stress responses
- Control of cell cycle and transcription
- cytoskeleton

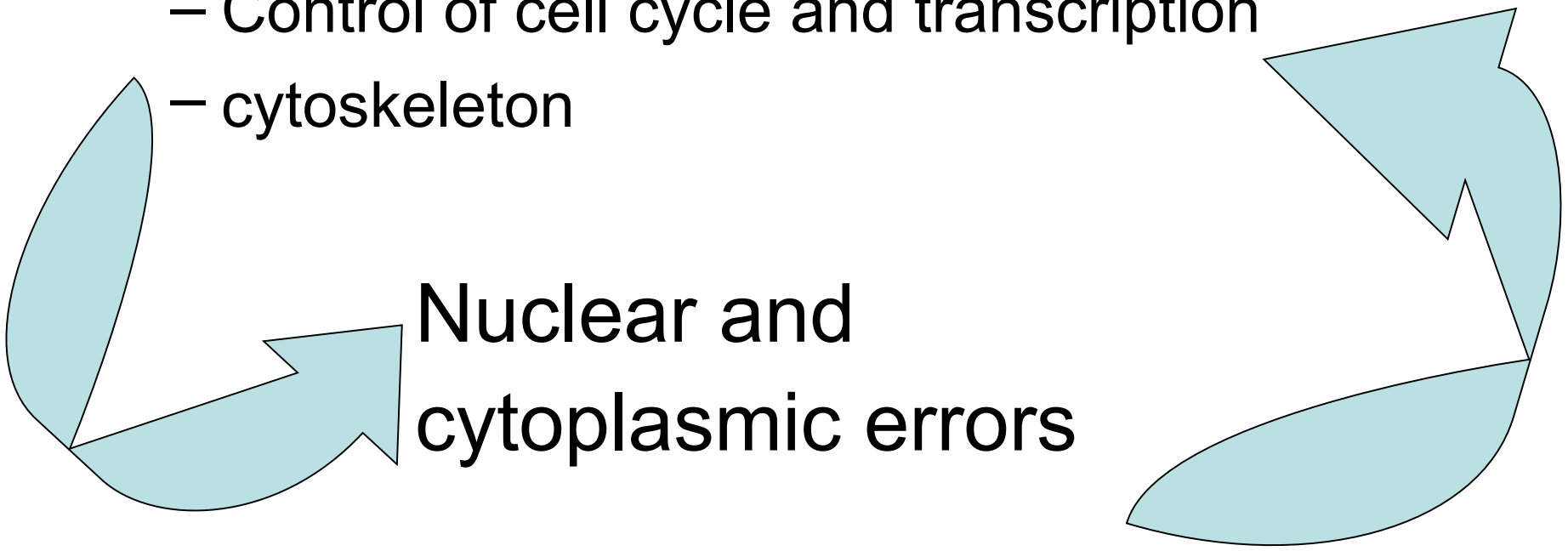


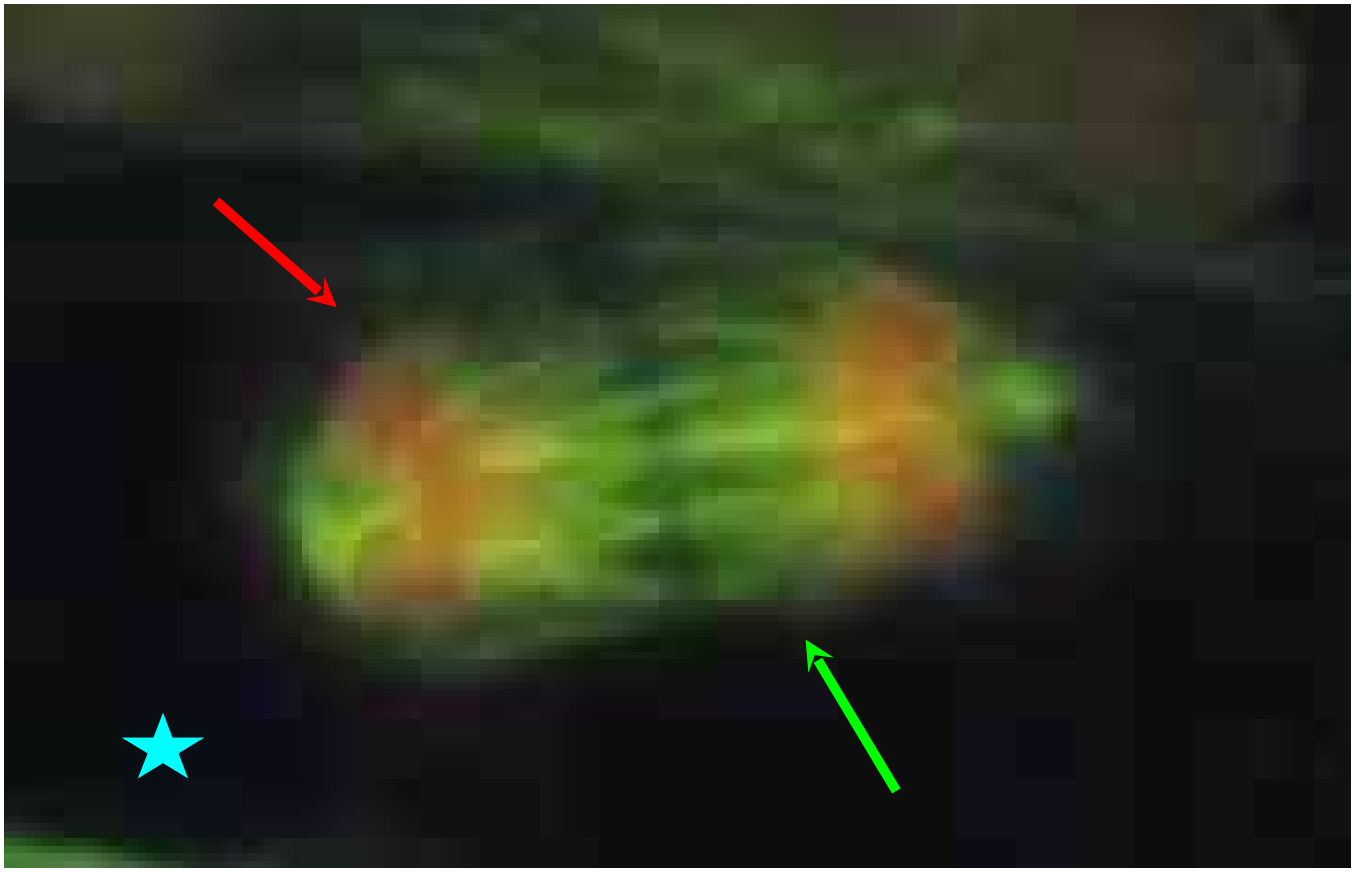
Nuclear and cytoplasmic errors

In old oocytes

Changes in gene expression related to

- Mitochondrial function, energy metabolism
- Oxidative stress, stress responses
- Control of cell cycle and transcription
- cytoskeleton





What a cell touches has a major role in determing what a cell does.

This principle yields also intracellularly.

More rapid postovulatory ageing

→ shorter postovulatory lifespan

→ shorter time to be fertilised

- Activities of cell cycle kinases (MPF, MAPK) decrease more rapidly →
  - More problems in Meiosis II; spontaneous activation; initiation of apoptosis

Tatone *et al*. *Biol Rep*, 2006

Cytoplasmic changes in ageing oocytes

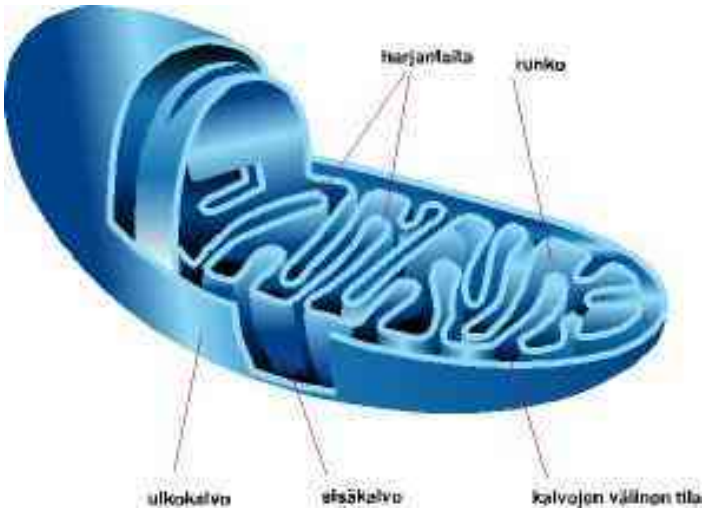
- Increased vacualisation of cytoplasm
- High density of matrix
- Enlargement of Golgi apparatus ja endoplasmic reticulum

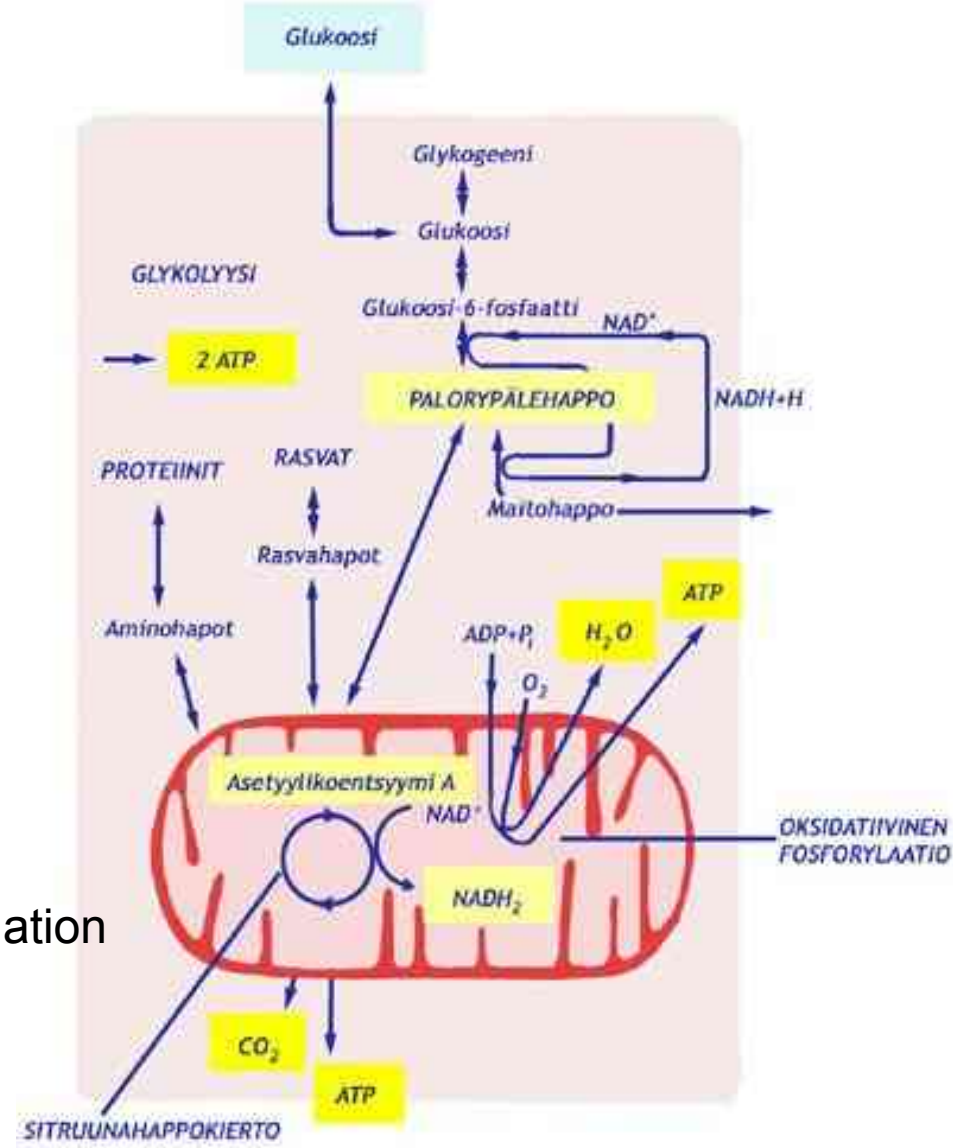
- Changes in mitochondria
  - also in the granulosa cells

The changes related to ageing
differ from those related to atresia.

deBruin 2004

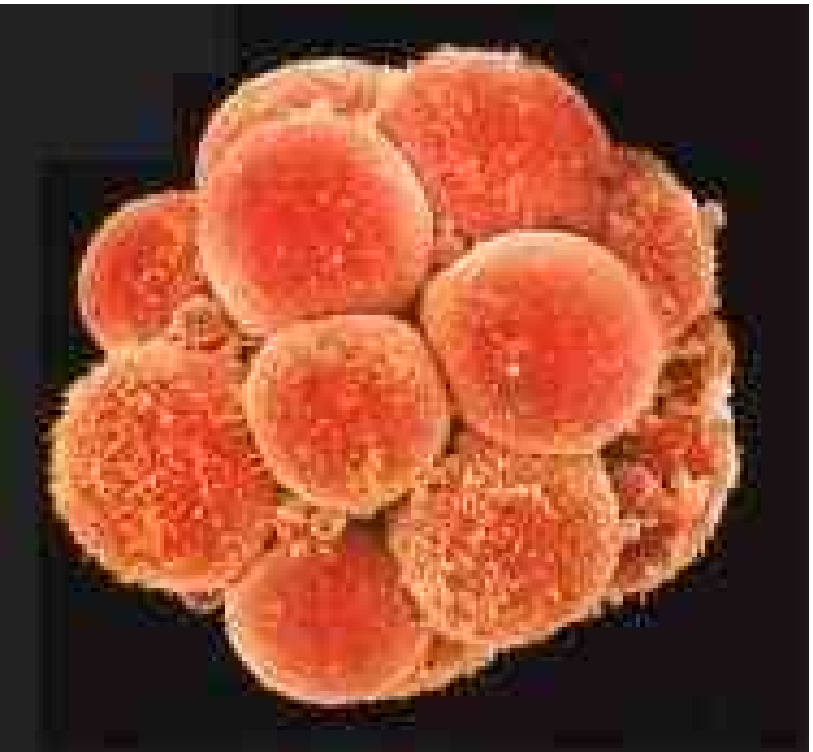
Mitochondria, the power stations

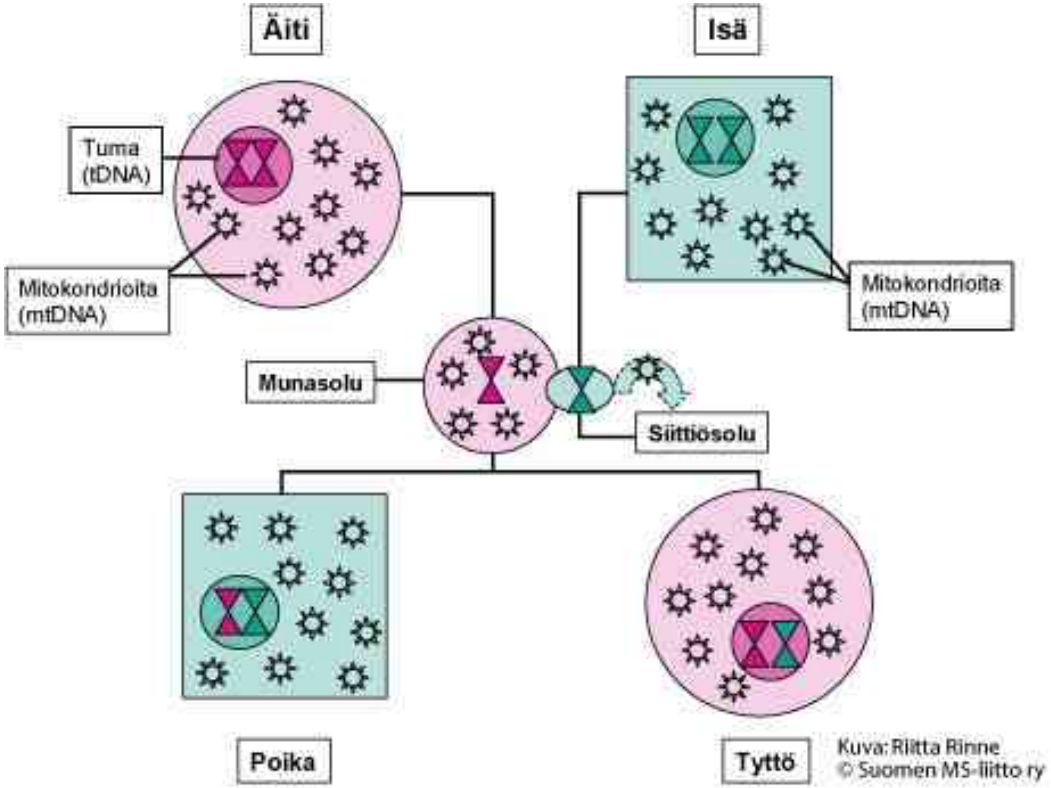




- ATP production via oxidative phosphorylation
- Participation in apoptosis

All mitochondria derive from the oocyte



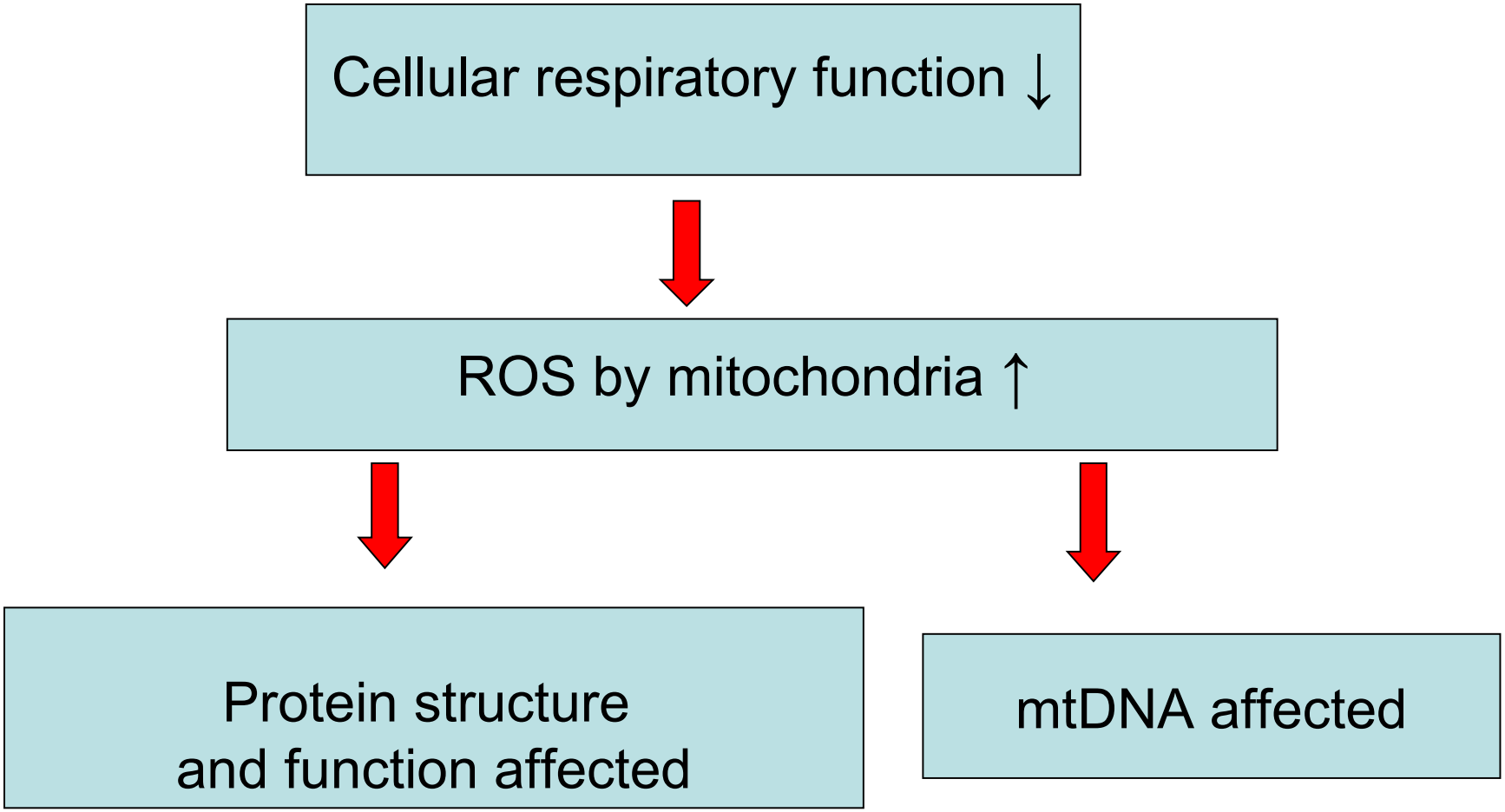


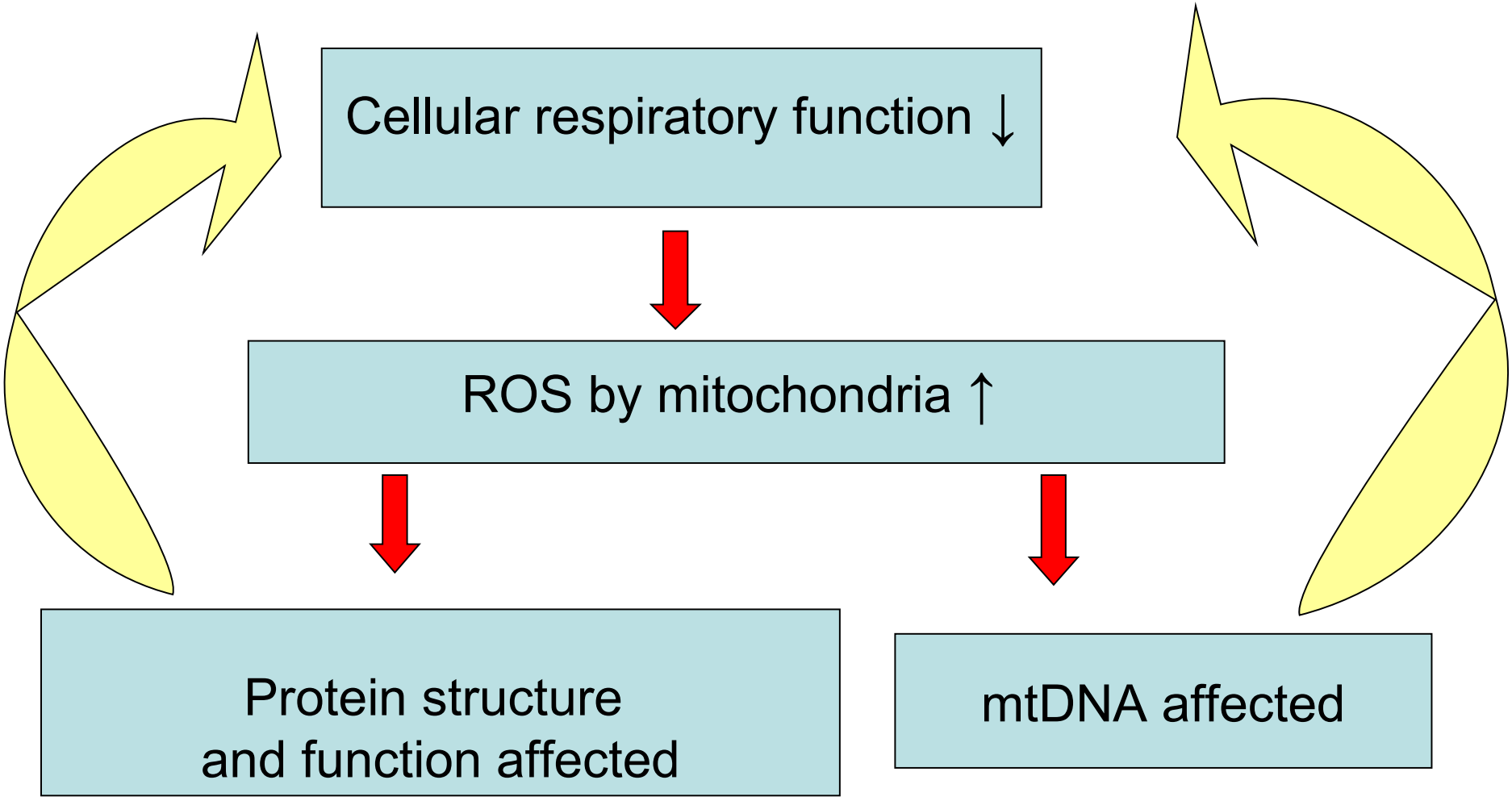
The genome of the embryo is activated in 8 cell stage

In aged oocytes

- ATP ↓
- Altered mitochondria
  - Morphologically, genetically and functionally
    - mtDNA point mutations, deletions and rearrangements ↑
    - Electric charge of the inner membrane ↓

Muller-Hocker et al., 1996
Attone et al., 2008

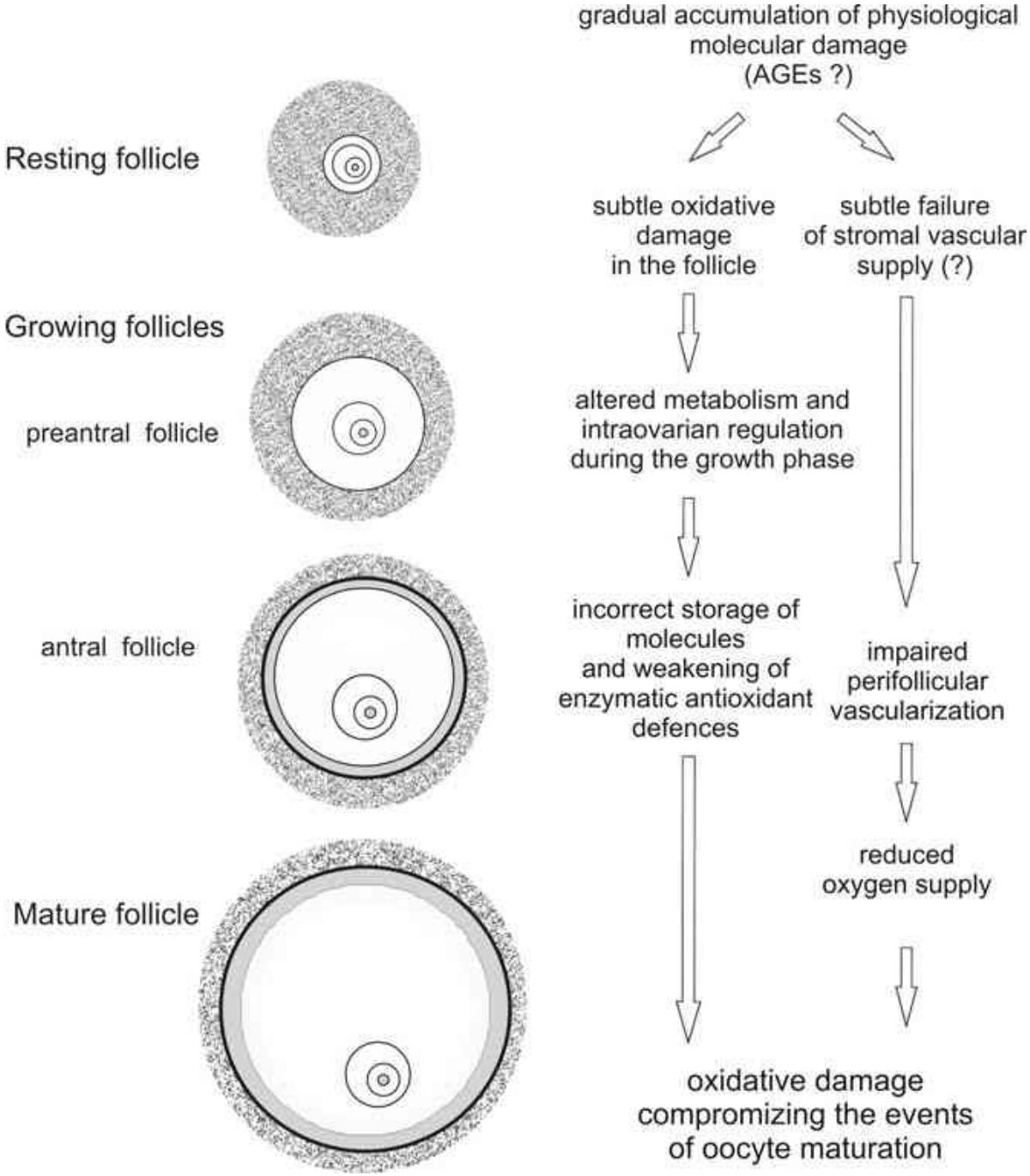

Cellular respiratory function ↓
ROS by mitochondria ↑
Protein structure
and function affected
mtDNA affected


Cellular respiratory function ↓
ROS by mitochondria ↑
Protein structure
and function affected
mtDNA affected

Animal and human studies:

Primordial and periovulatory oocytes suffer from

1. age-related oxidative stress
2. an impairment of antioxidant enzymatic defences

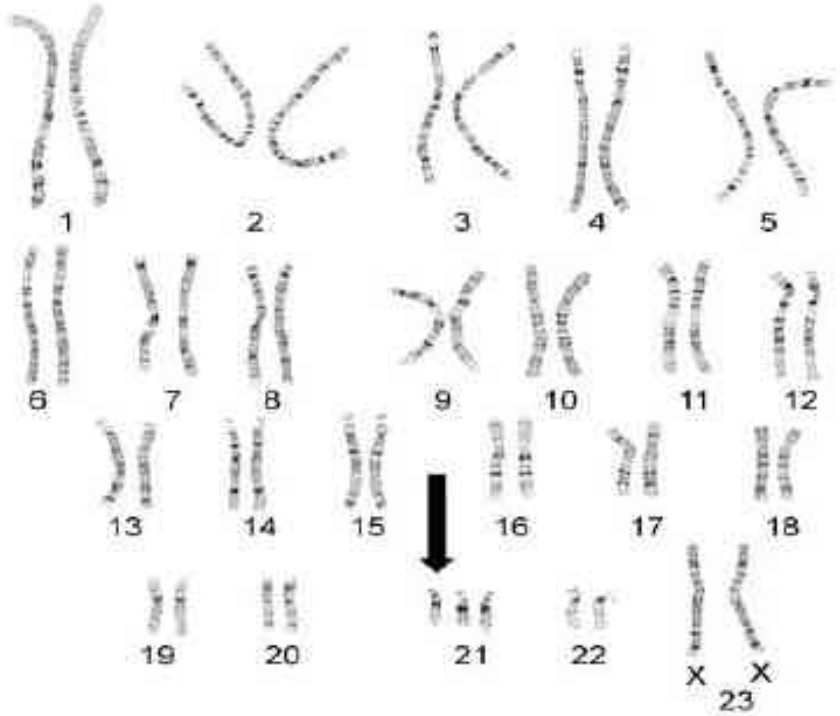


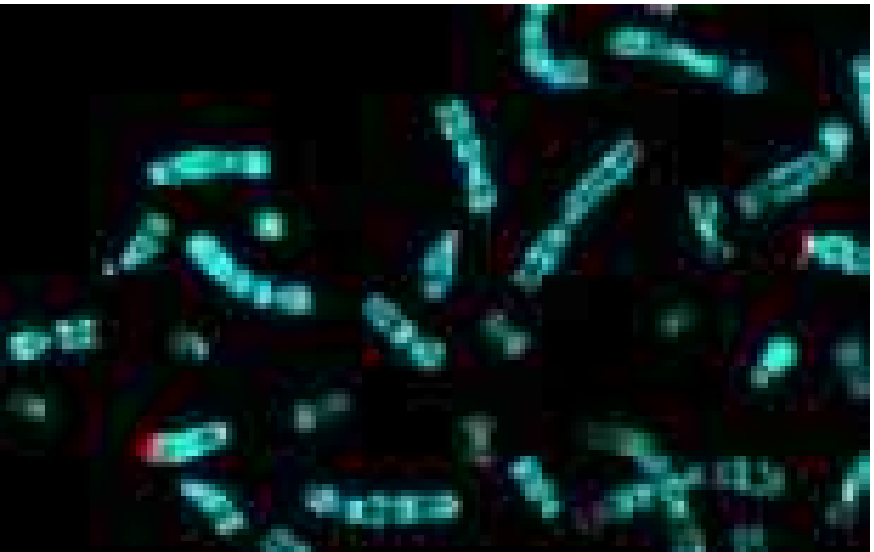
Tatone et al.,

Hum Rep UpDate 2008

Oxidative stress increases the risk for meiotic errors

- mtDNA affected$\rightarrow$ ATP $\downarrow$ $\rightarrow$ replicatory capacity $\downarrow$
- Telomere shortening ?





Risk for aneuploidy ↑ with age

- At the age of 38 about 1%
- At 45 years about 5 %.

Several mechanisms proposed

1. Hormonal imbalances
2. Ageing of the somatic cells in the follicles
3. Impaired perifollicular microcirculation

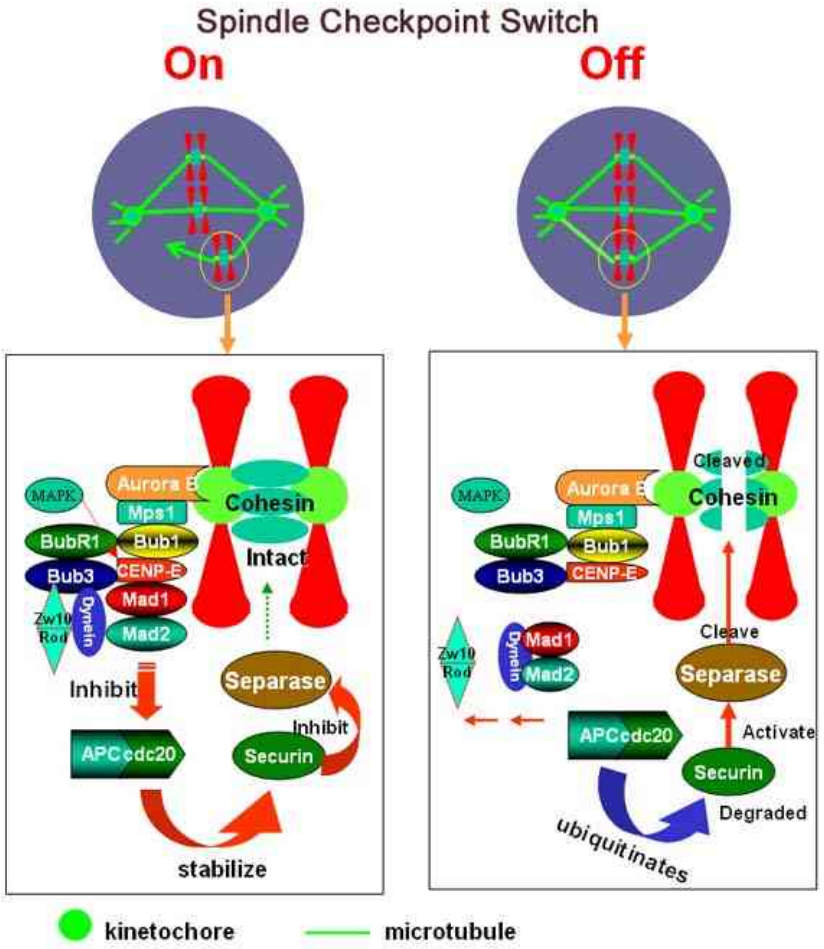
Aneuploidia

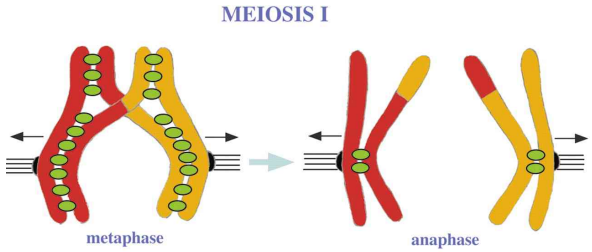
Compromised meiotic clock

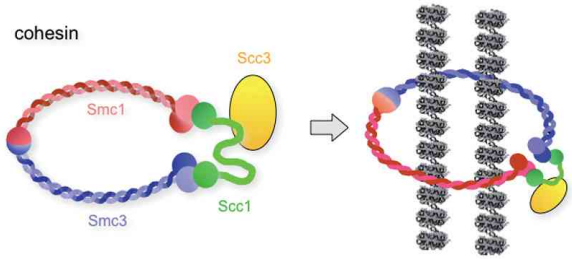
- Acceleration of the meiotic events
  - transition to I anaphase and to metaphasis II
- Precocious chromosome segregation / separation of sister chromatides
- *Failure in the coordination of nuclear and cytoplasmic meiotic events*
  - Altered folliculogenesis

Age-related aneuploidies result mainly from an aberrant meiosis I

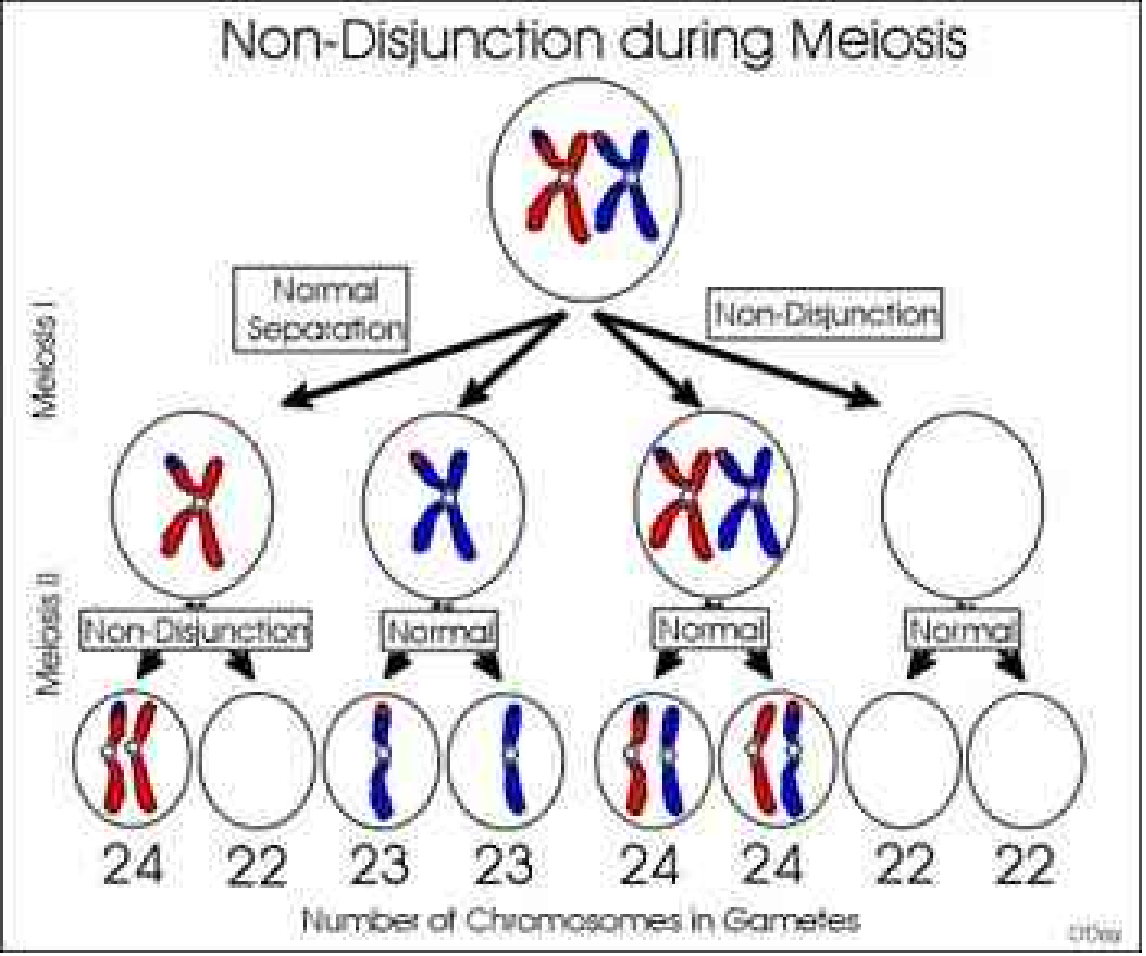
- Incorrect storage of molecules involved in cell cycle control
- Changes in spindle checkpoint proteins
- Changes in cohesion proteins

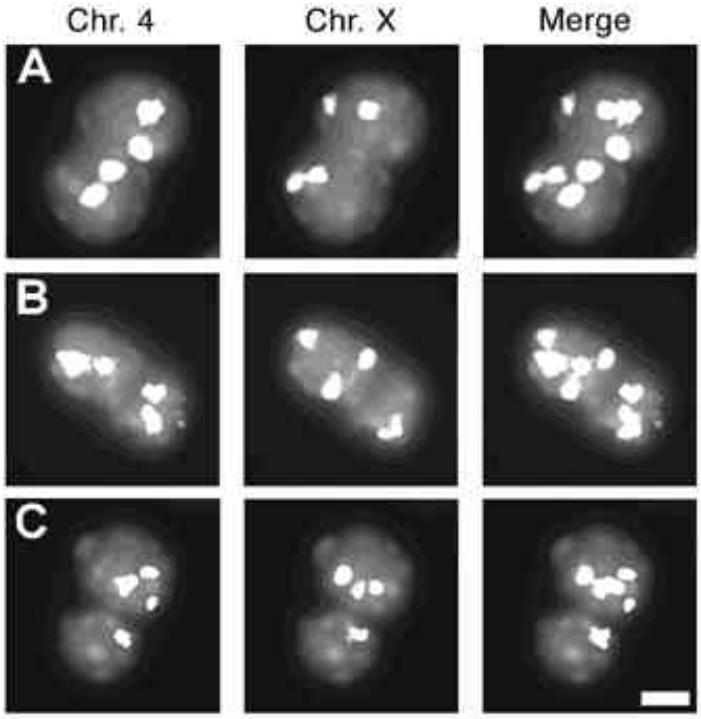






Rec8-containing meiotic cohesin


Non-Disjunction during Meiosis
Meiosis I
Meiosis II
Normal Separation
Non-Disjunction
Non-Disjunction
Normal
Normal
Normal
24
22
23
23
24
24
22
22
Number of Chromosomes in Gametes


Chr. 4
Chr. X
Merge
A
B
C

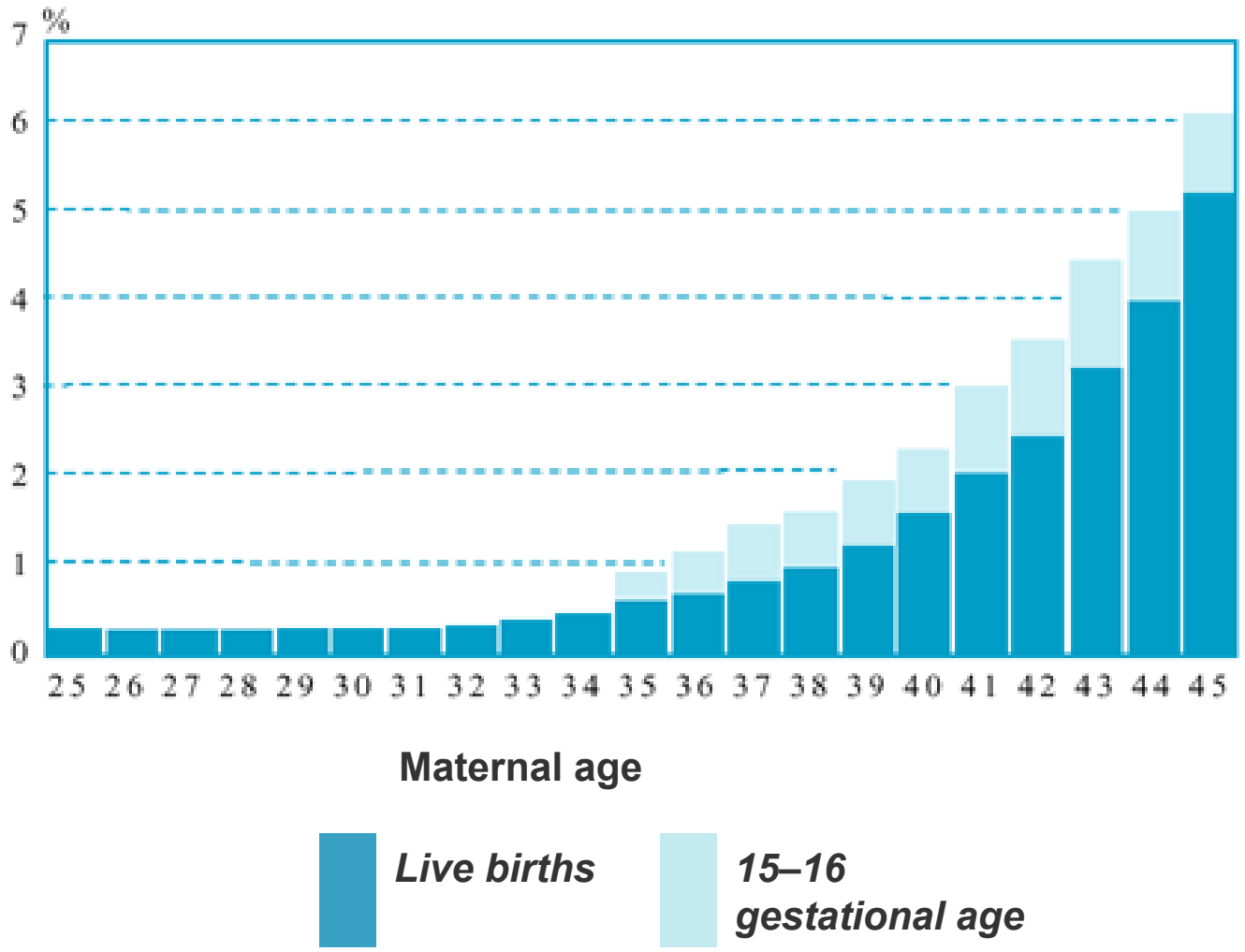


Fig 1. Risk for Down sdr in relation to maternal age

The changes related to ageing ovary

- Anatomical
  - macroscopic
  - microscopic
- Functional
  - Hormonal
  - Fertility
  - Genetic risks in reproduction
- Secondary
  - Health concerns

Time for discussion
and questions...