

# Embryology I

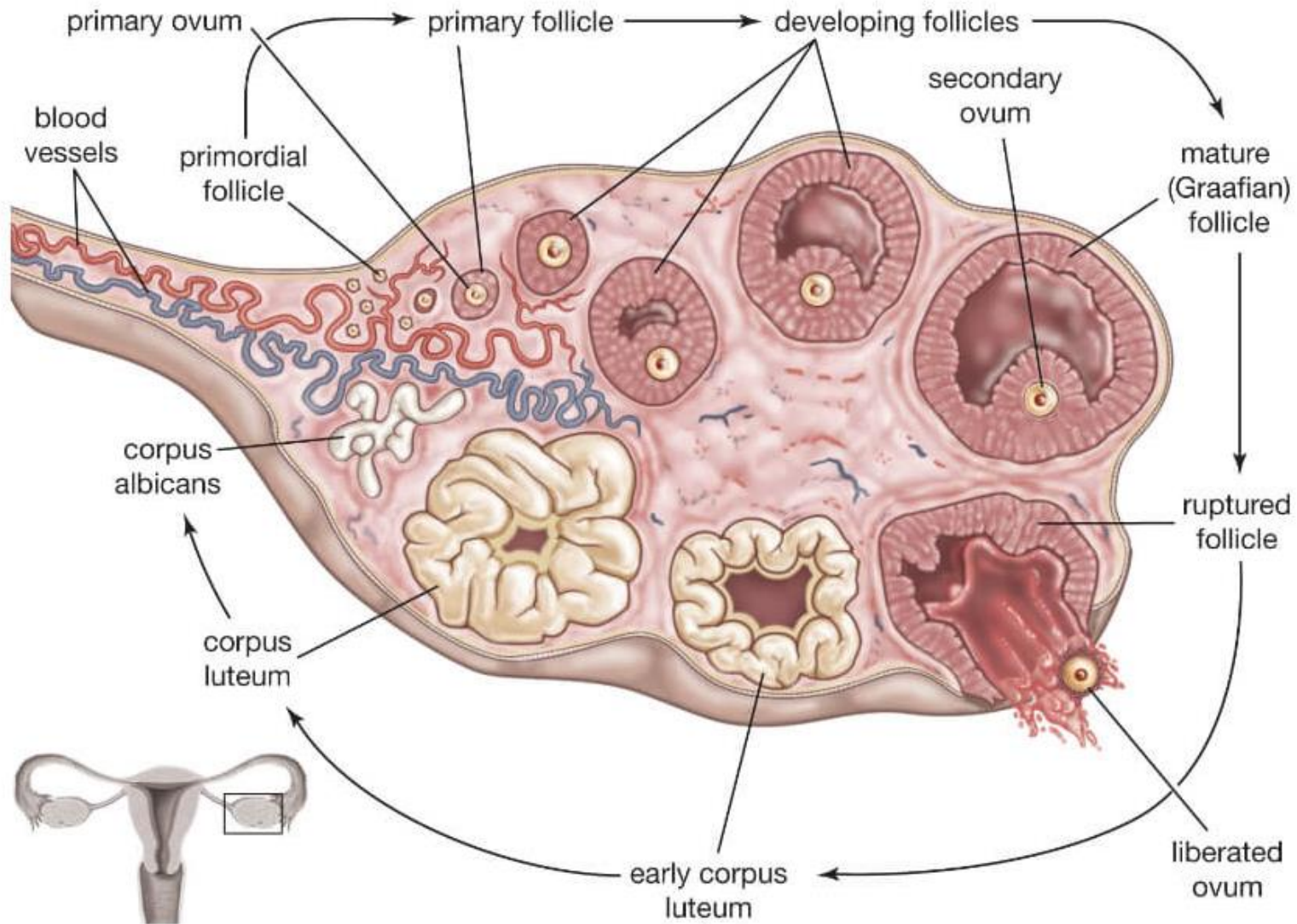
# OOGENESIS

autumn 2024

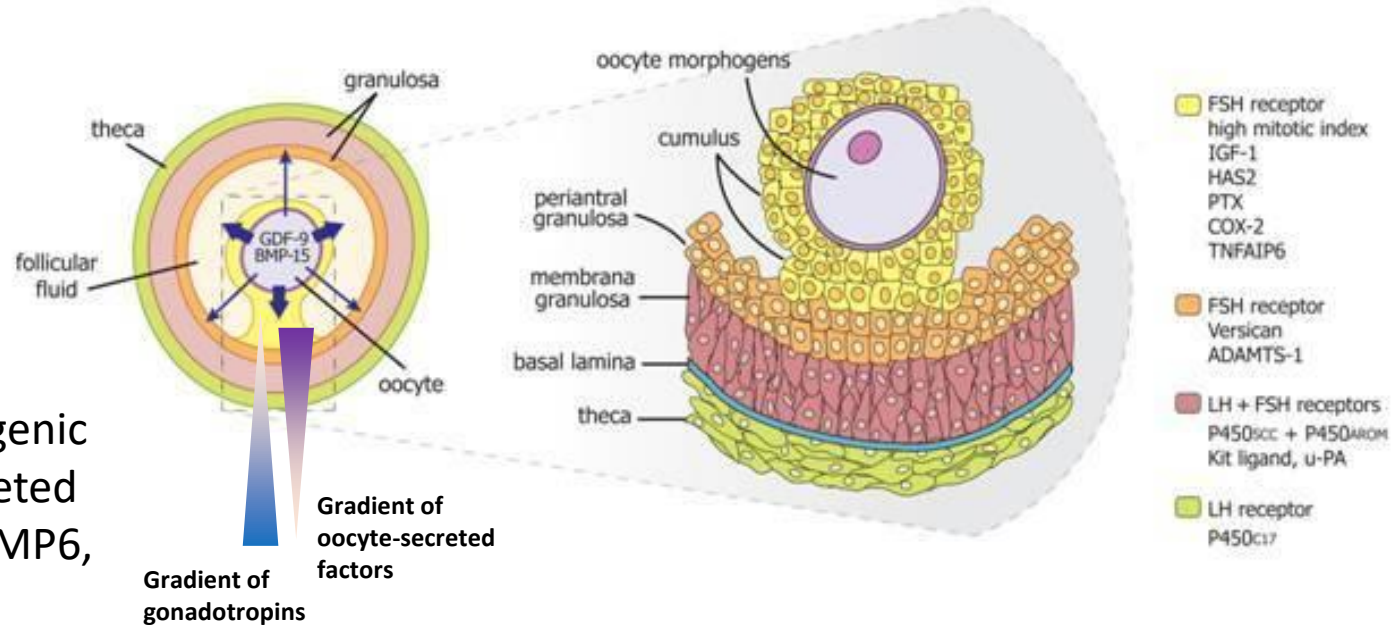
## Ovulation and luteinization

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# Ovarian cycle



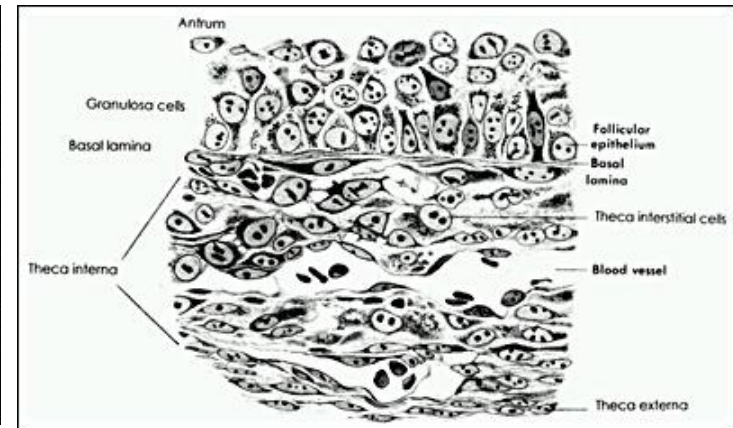
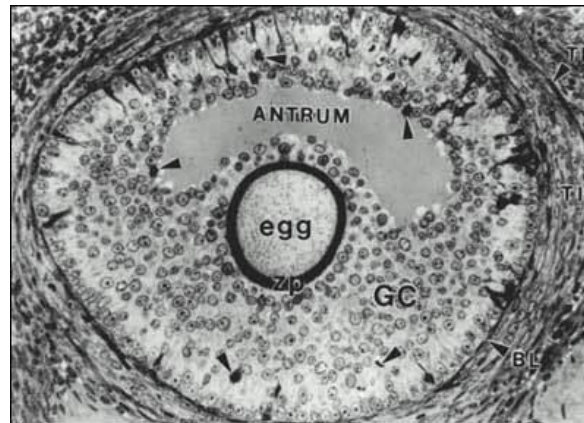
# Preovulatory follicle



COC microenvironment maintained by morphogenic gradient of oocyte-secreted factors (**GDF9**, **BM15**, BMP6, BMP8,...)



- prevention of apoptosis
- expansion of COCs
- prevention of premature luteinisation

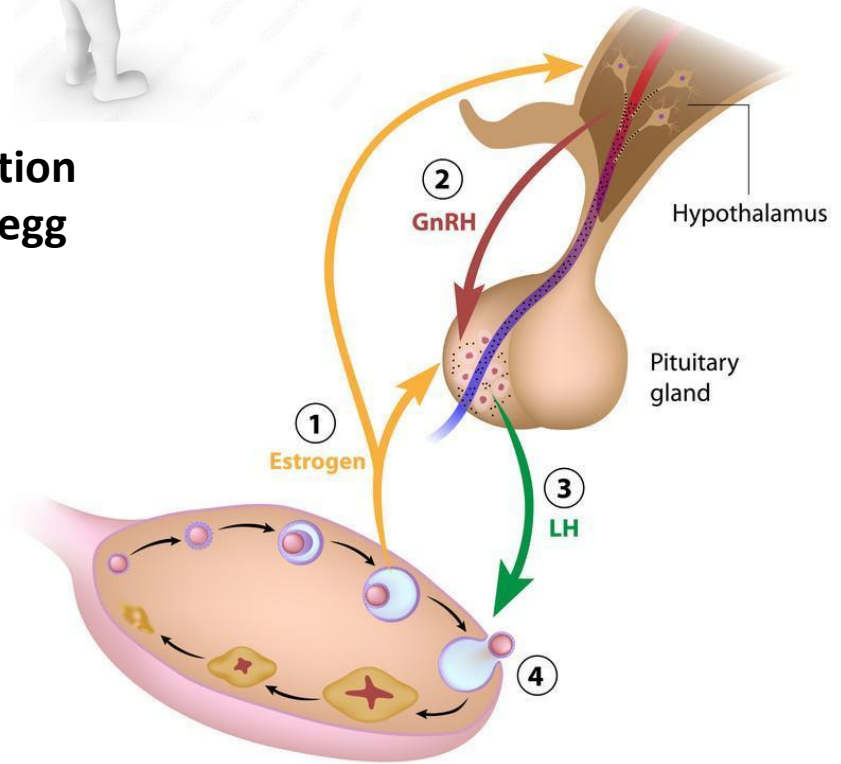
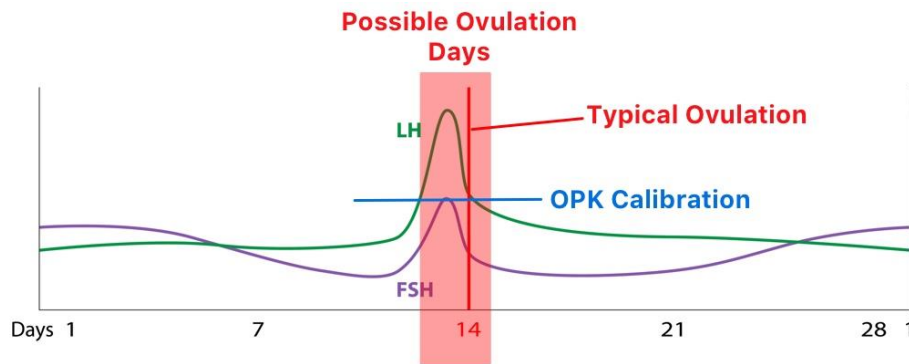


→ supplement to IVM culture?

# Ovulation induction

## ❖ LH surge

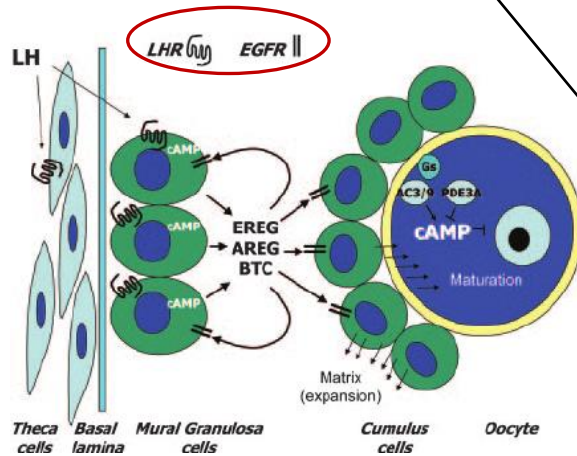
- begins ~36h before ovulation
- initiates molecular signaling leading to
  - (1) COC expansion
  - (2) meiosis resumption and oocyte maturation
  - (3) rupture of Graafian follicle a release of egg
  - (4) luteinization of emptied follicle



# Ovulation induction

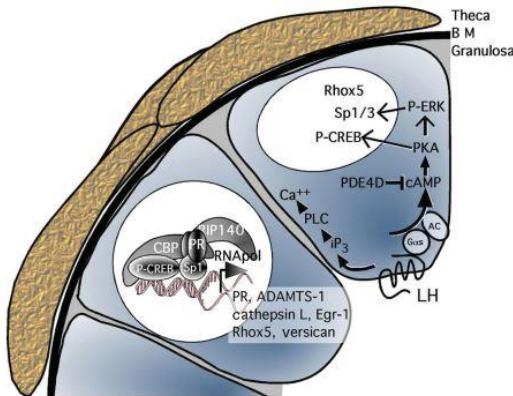
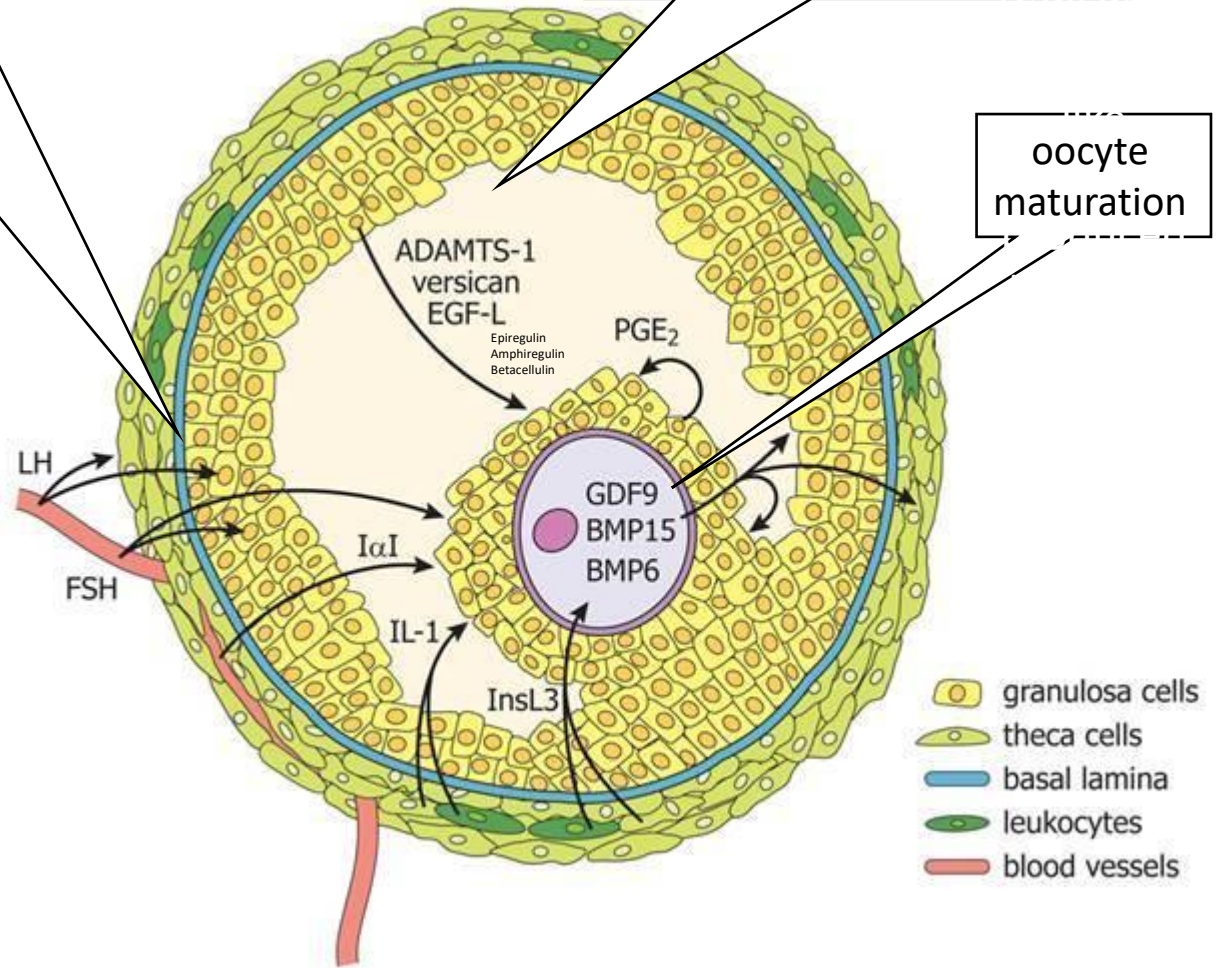
## LH surge

LH stimulate thecal and mural GCs



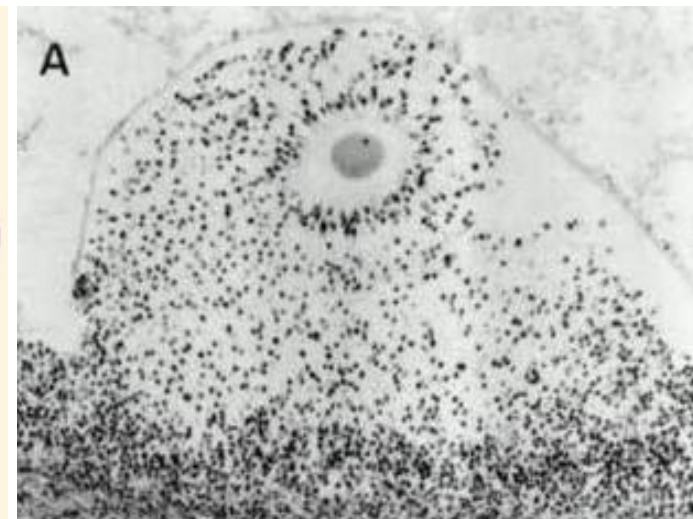
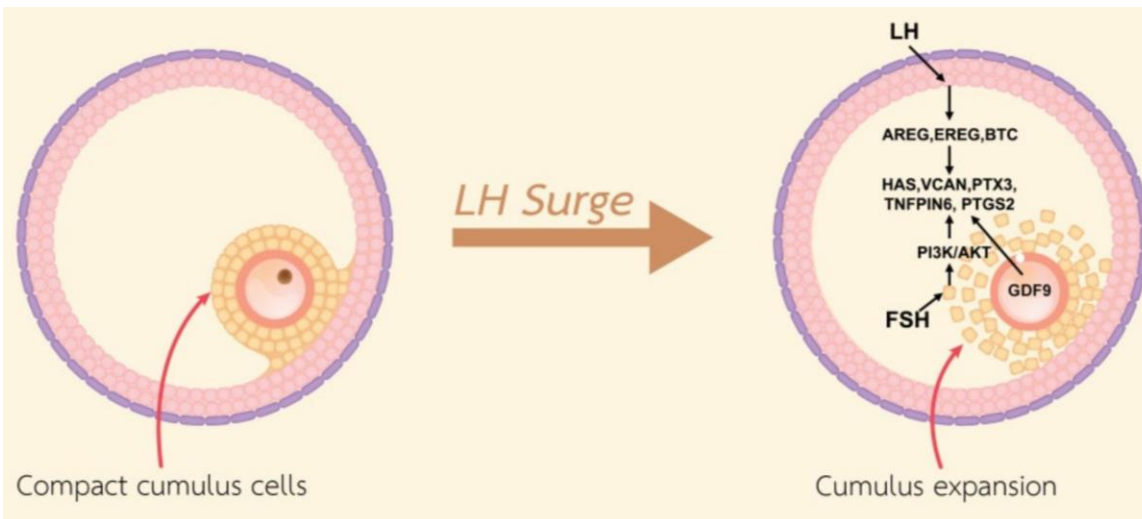
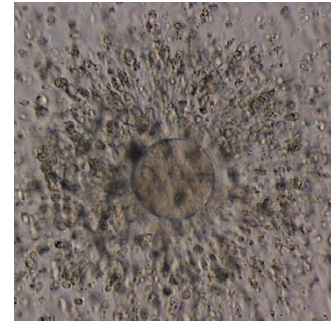
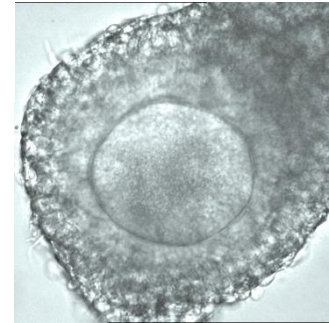
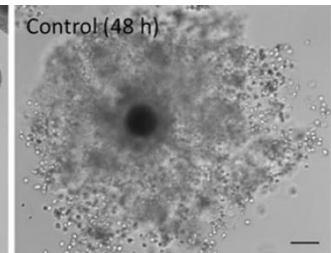
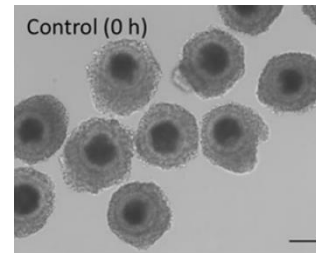
signal transduced by EGF-like peptides produced by mural GCS

oocyte maturation



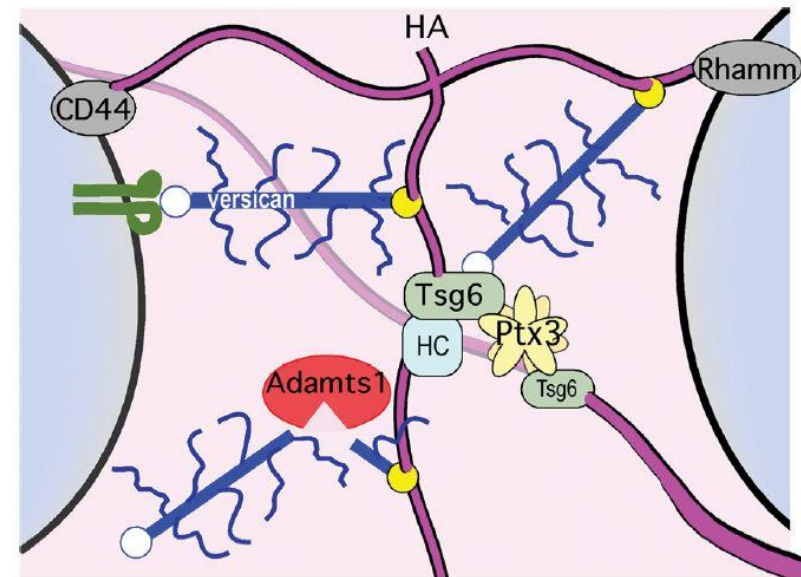
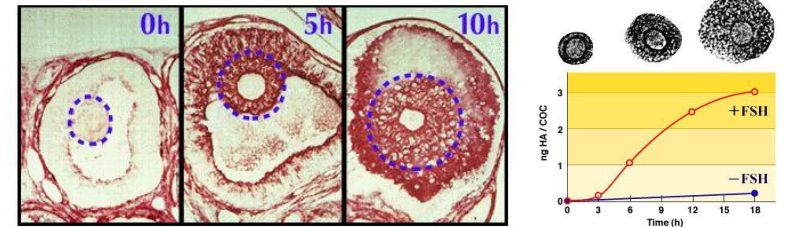
# Cumulus expansion

- LH/hCG - induced morphostructural changes of cumulus cells surrounding the oocyte - **cumulus oocyte complex (COC)**
- **enlargement, loosening and mucification** of cumulus

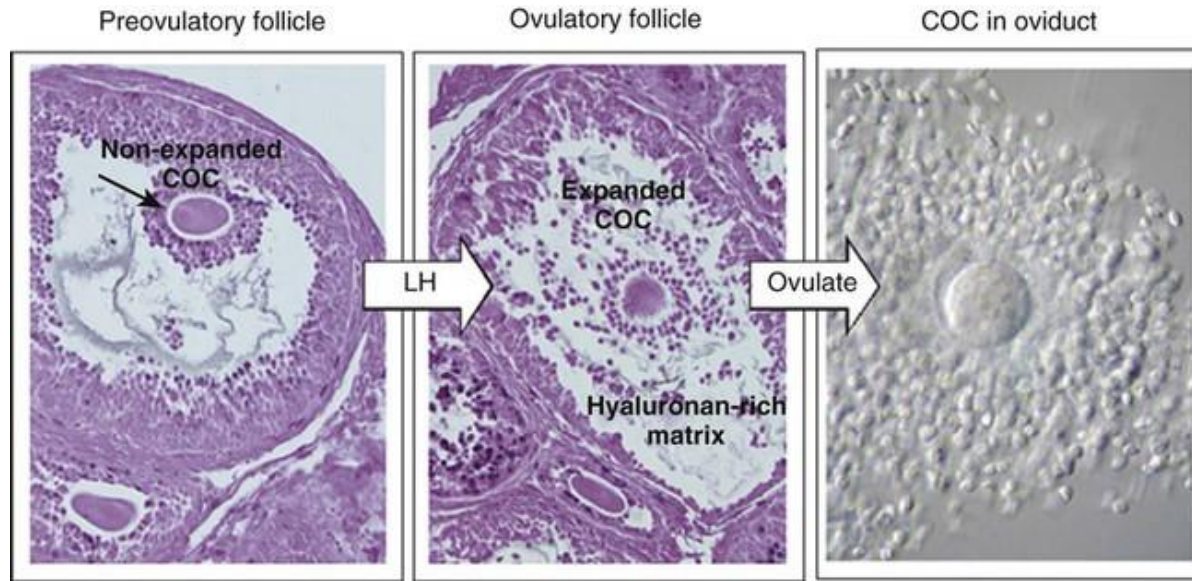


# Cumulus expansion

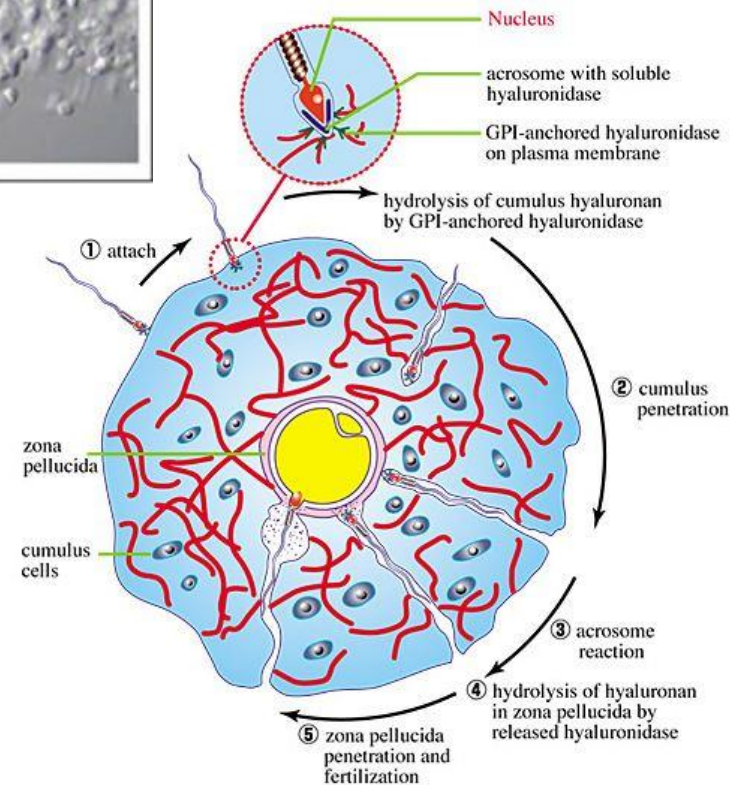
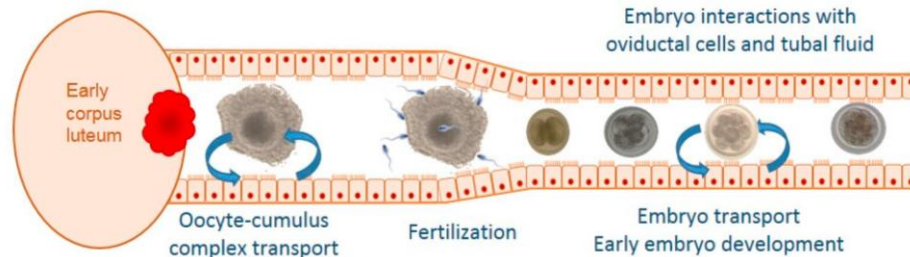
- cumulus ECM composed of strands of **hyaluronic acid (HA)** and crosslinking proteins, glycoproteins and proteoglycans (TSG-6, versican,  $\alpha 1$  HC, Ptx3) required for cumulus retention and stability
- LH surge triggers expression of HA synthase and Glc uptake
- synthesis and deposit of mucoelastic matrix in extracellular space leads to cumulus cells distancing with Gap junction maintained
- further distancing leads to retraction of TZP → oocyte meiotic maturation
- **↑HA → ECM water attraction → ↑ follicular fluid pressure**



# Cumulus expansion



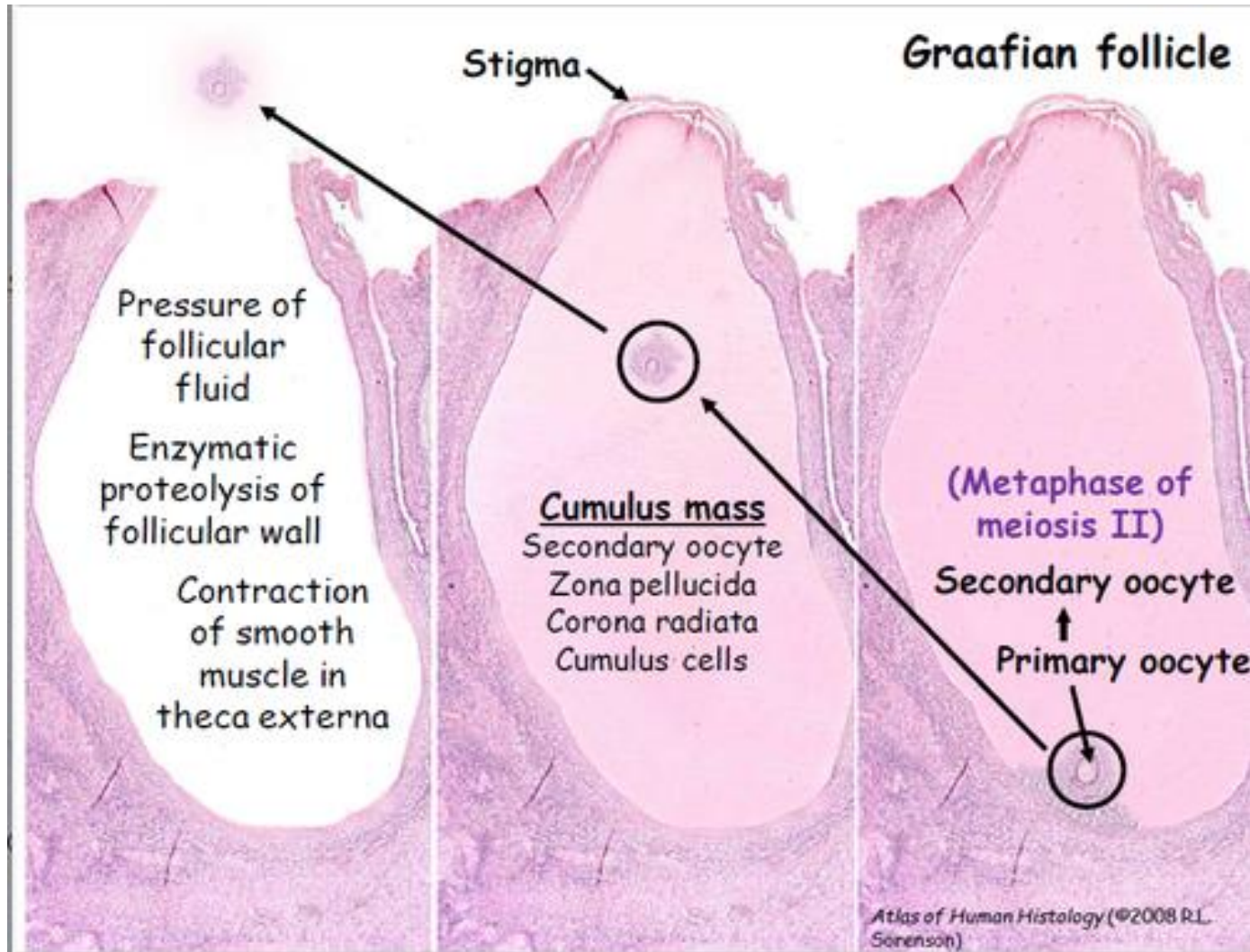
- Cumulus cells play role in
- (1) deposition of molecular factors
- (2) ovulation
- (3) fertilization
- (4) embryo passage through oviduct



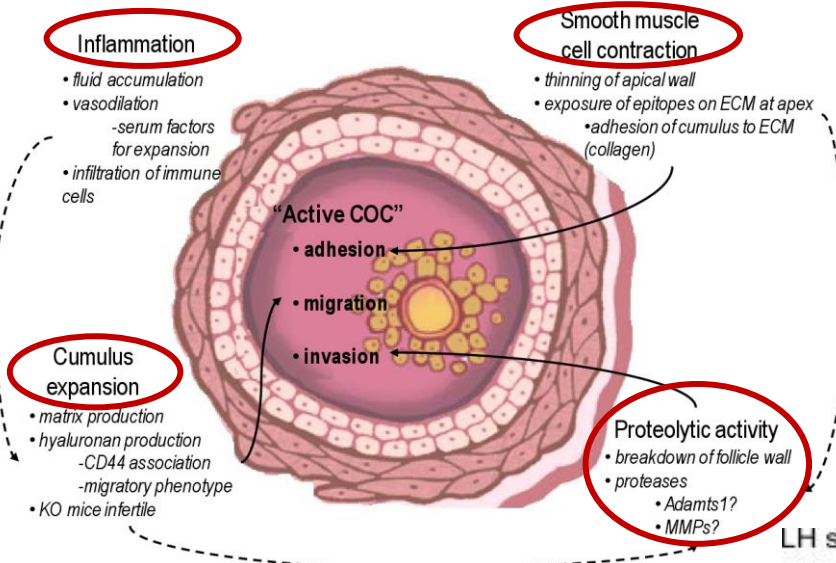


# Cumulus expansion

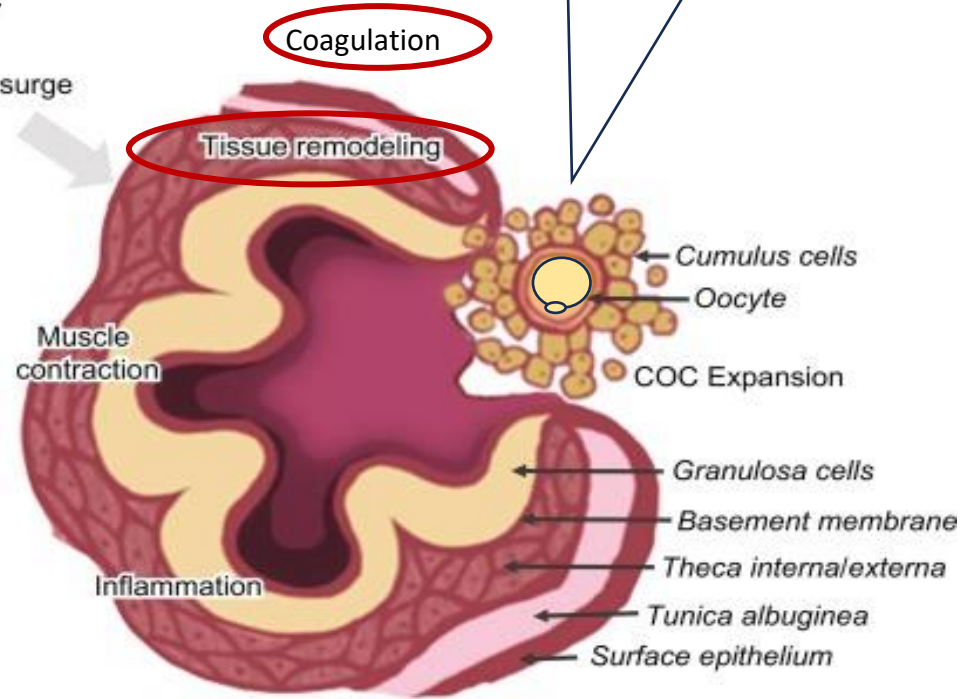
## COC detachment from follicular wall



# Ovulation

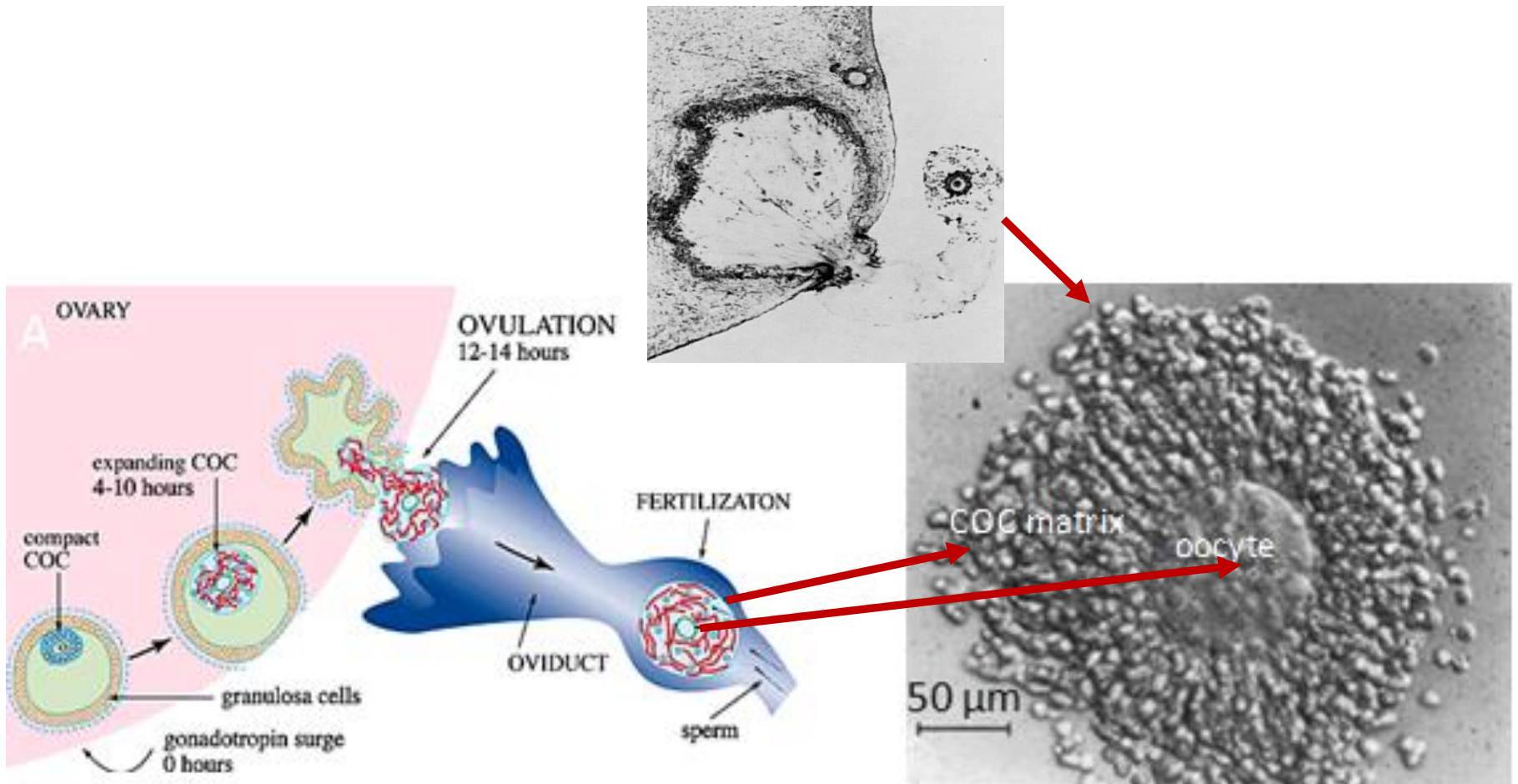


Acquisition of viscoelastic properties facilitates COCs release through follicle rupture

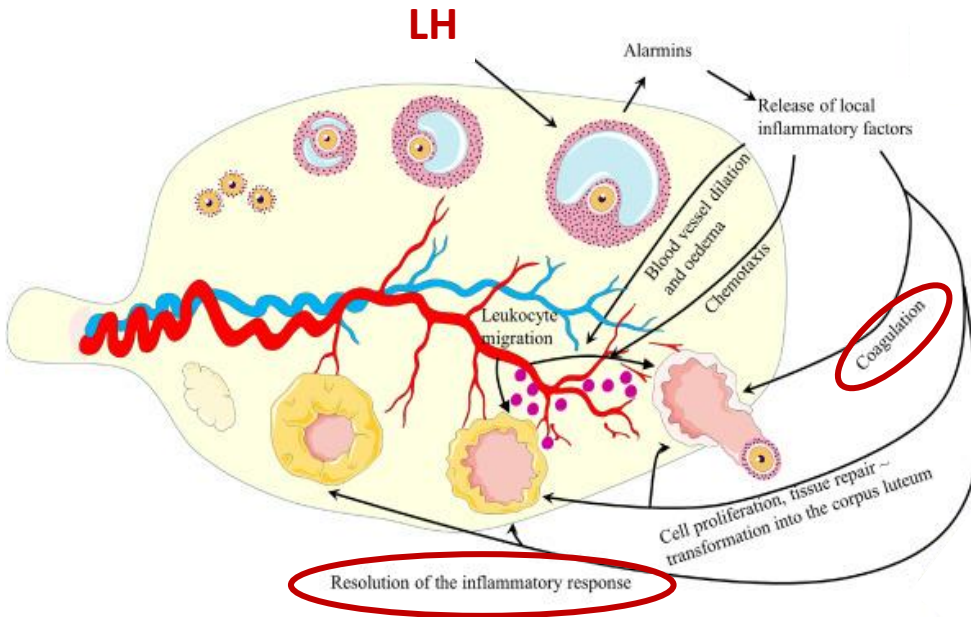


# Ovulation

- rupture of Graffian follicle and expulsion of COC containing **secondary oocyte (MII oocyte, egg)** and **granulosa cells of corona radiata**



# Ovulation



## ❖ Vascular changes

- vasodilatation
- perforation of capillars
- increased permeability



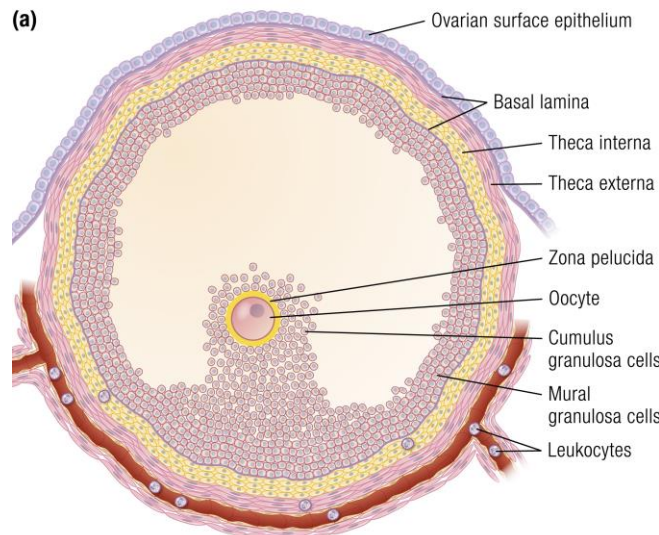
local edema

## ❖ Chemotaxis

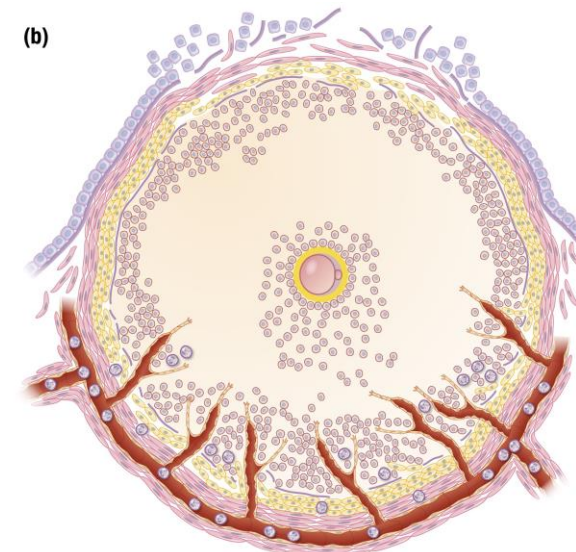


release of proinflammatory and antiinflammatory factors

Prior LH surge



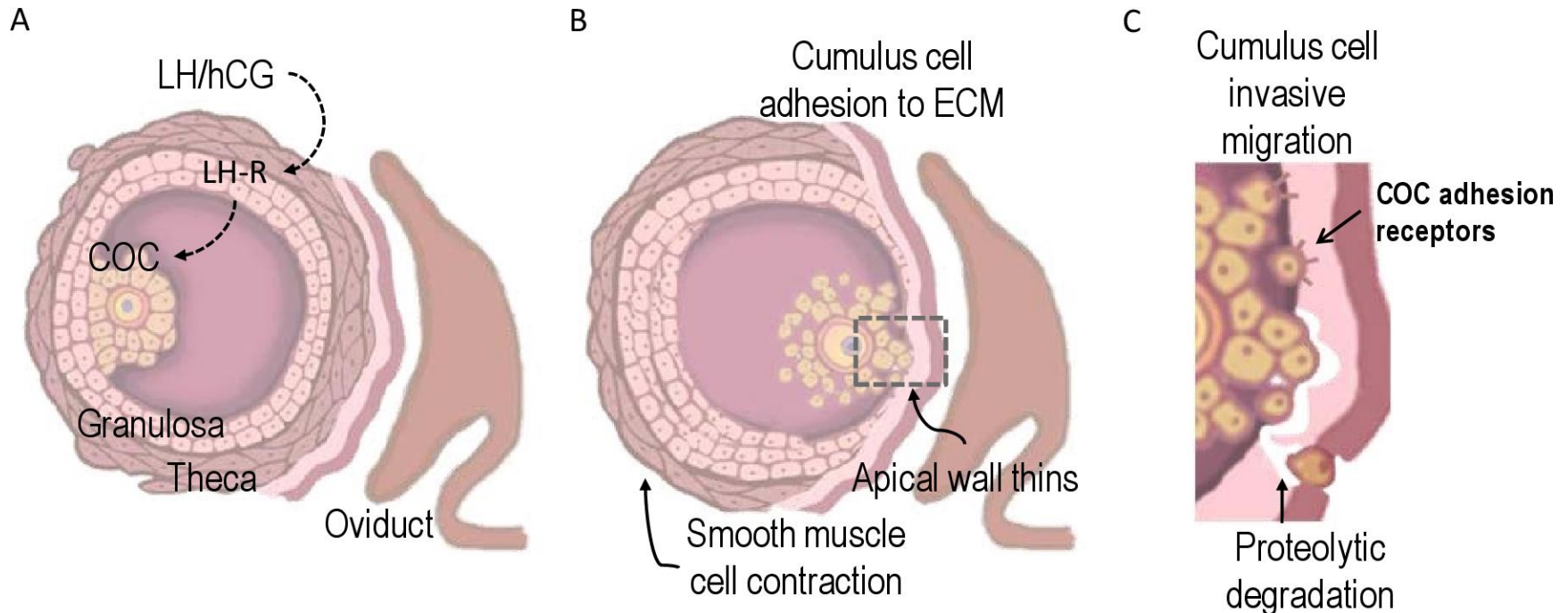
After LH surge



# Ovulation

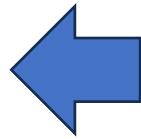
## ❖ Follicle rupture

- HA activates CD44 receptor on cumulus cells → migratory and invasive phenotype
- COC separation from follicular wall and adhesion to apex epithelium
- proteolytic degradation of tunica albuginea
- role of lysosomal **matrix metalloproteinases and ADAMTS-1**
- just before ovulation apical region becomes avascular (ischemia)



# Ovulation

## ❖ Follicle rupture



## ■ Prostaglandins

### PGE2

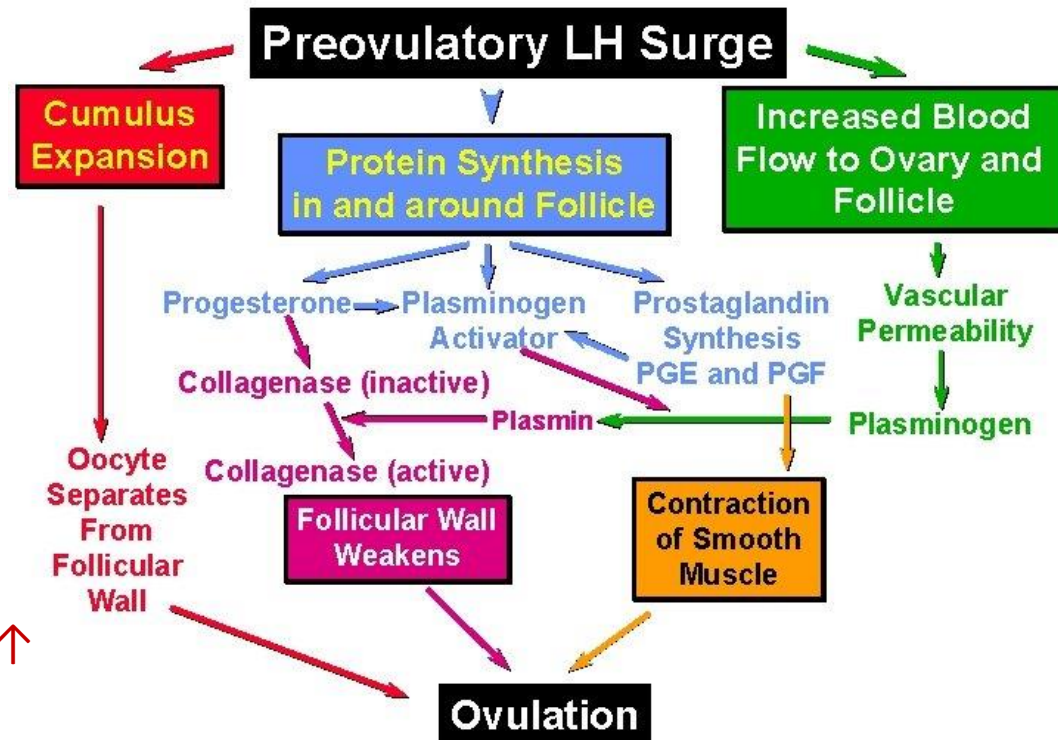
→ stimulates **plasminogen activator** → plasminogen converted to plasmin → activation of collagenase → **dissolution of connective tissue**

### PGF2a

→ induces **rupture of lysosomes**  
→ follicle and ovarian **contractions** ↑

## ■ Progesterone

→ stimulates production of collagenase by theca cells → **dissolution of connective tissue**  
→ displace cortisol from its binding protein → ↑ cortisol mitigates inflammatory reaction



# Ovulation

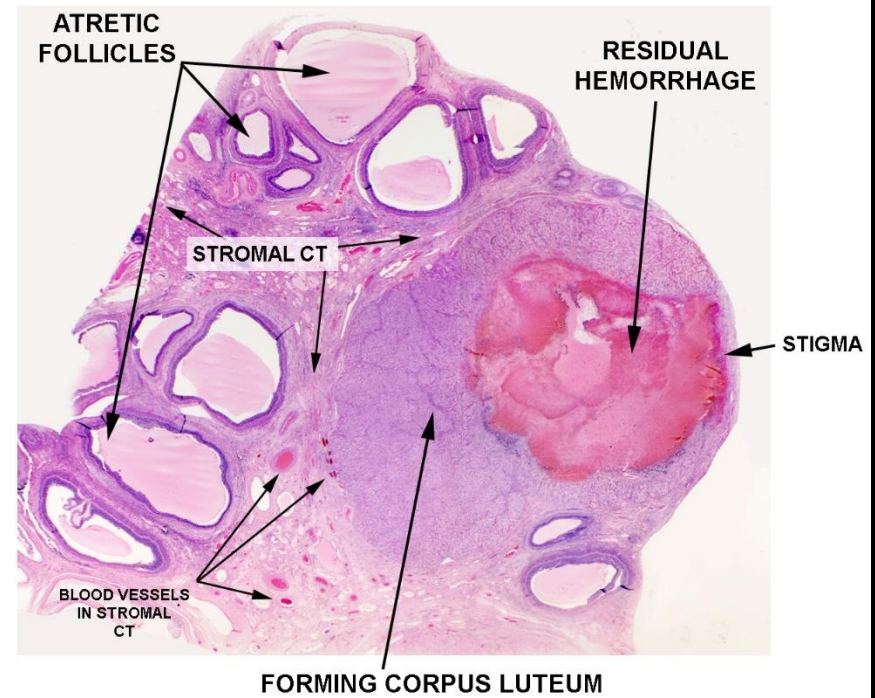
**What Ovulation Looks Like**



**Color Removed to make it  
less "shocking"**

# Ovulation

- **stigma** (*macula pellucida*) = avascular spot left on the ovarian surface





# Ovulation

Transgenic mouse  
Myr-Tomato  
H2B-GFP



nature cell biology



Technical Report

<https://doi.org/10.1038/s41556-024-01524-6>

## Ex vivo imaging reveals the spatiotemporal control of ovulation

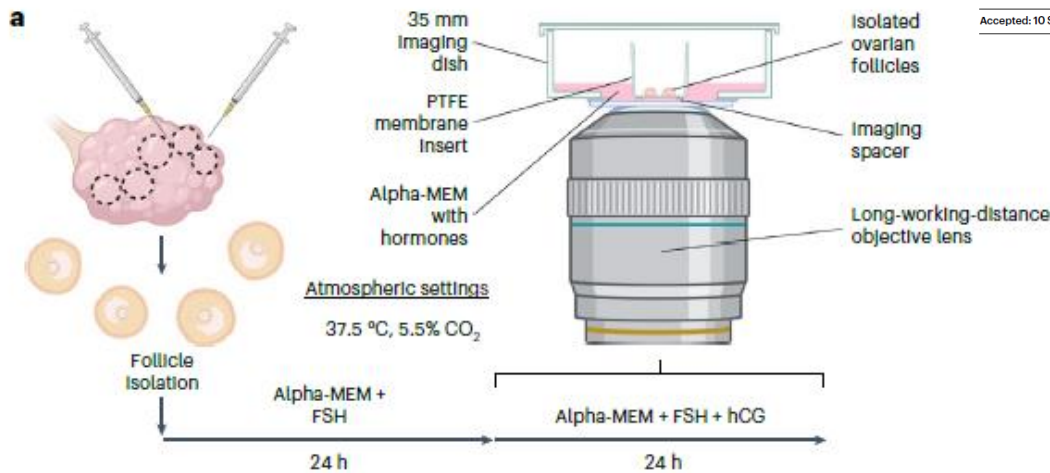
Received: 29 April 2024

Christopher Thomas<sup>1,2,5</sup>, Tabea Lilian Marx<sup>1,3,5</sup>, Sarah Mae Penir<sup>1</sup> & Melina Schuh<sup>1,4,5</sup>

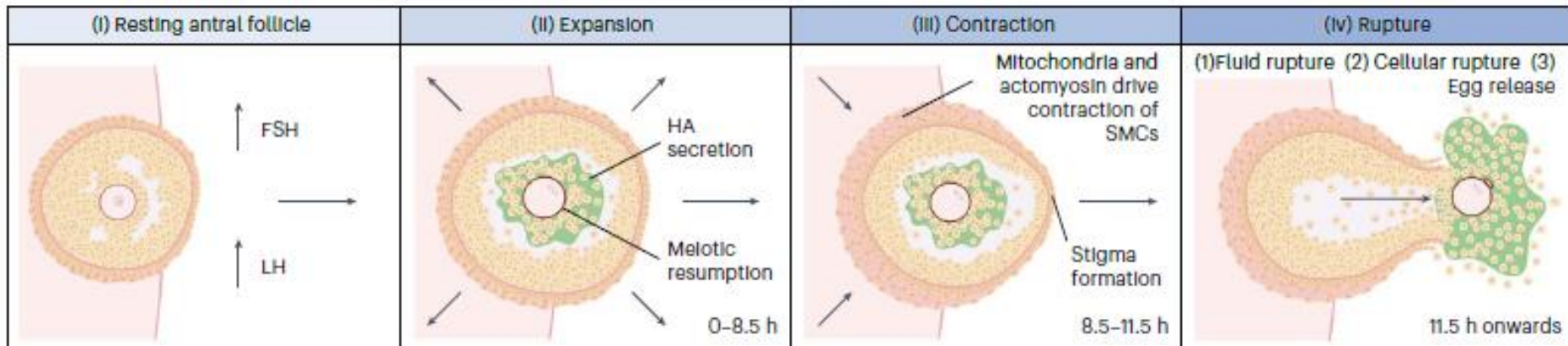
Accepted: 10 September 2024



Melina Schuh



- ex vivo imaging of ovulation in isolated mouse follicles



# Ovulation

Transgenic mouse  
Myr-Tomato  
H2B-GFP



nature cell biology



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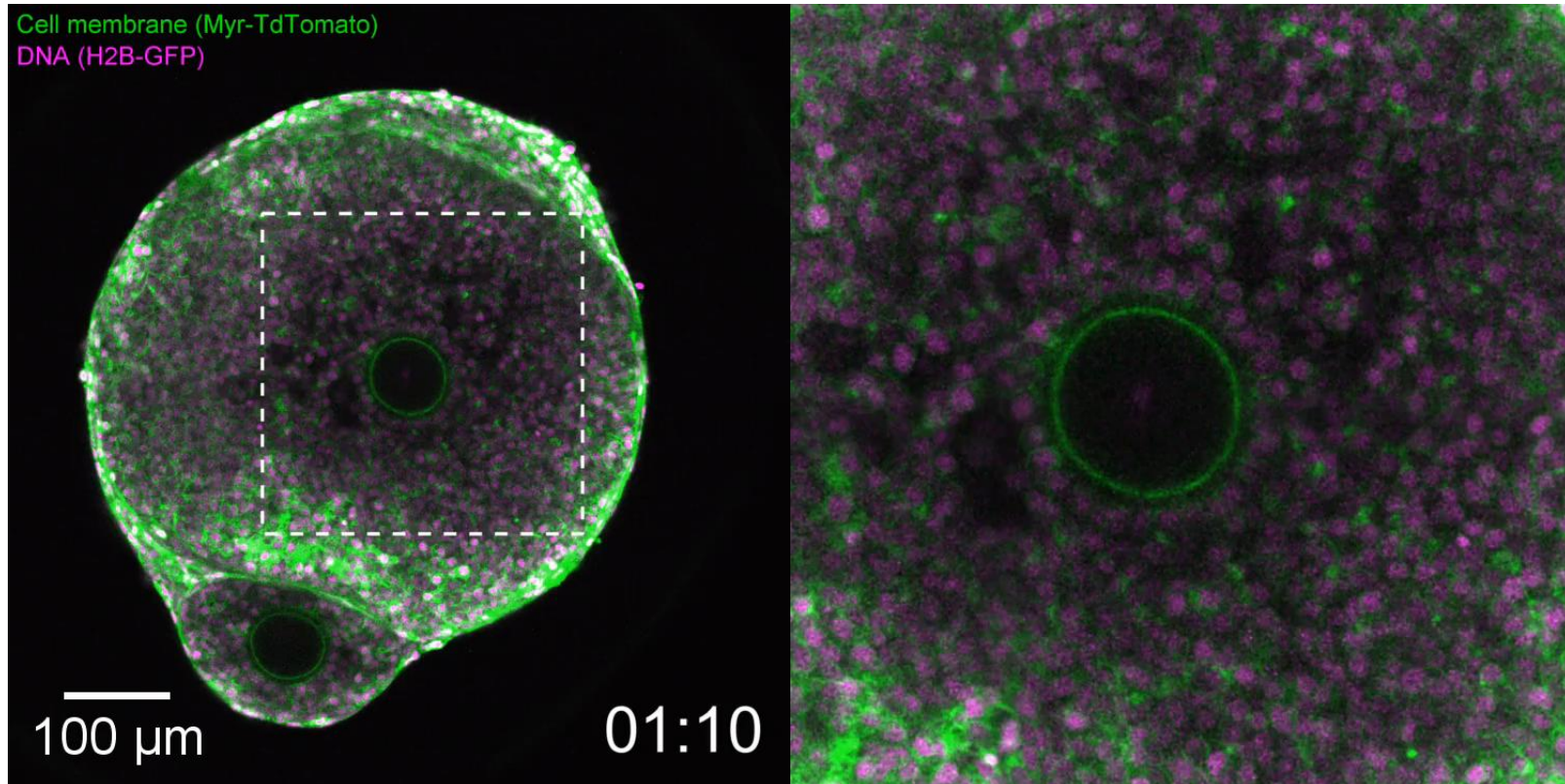
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nature cell biology



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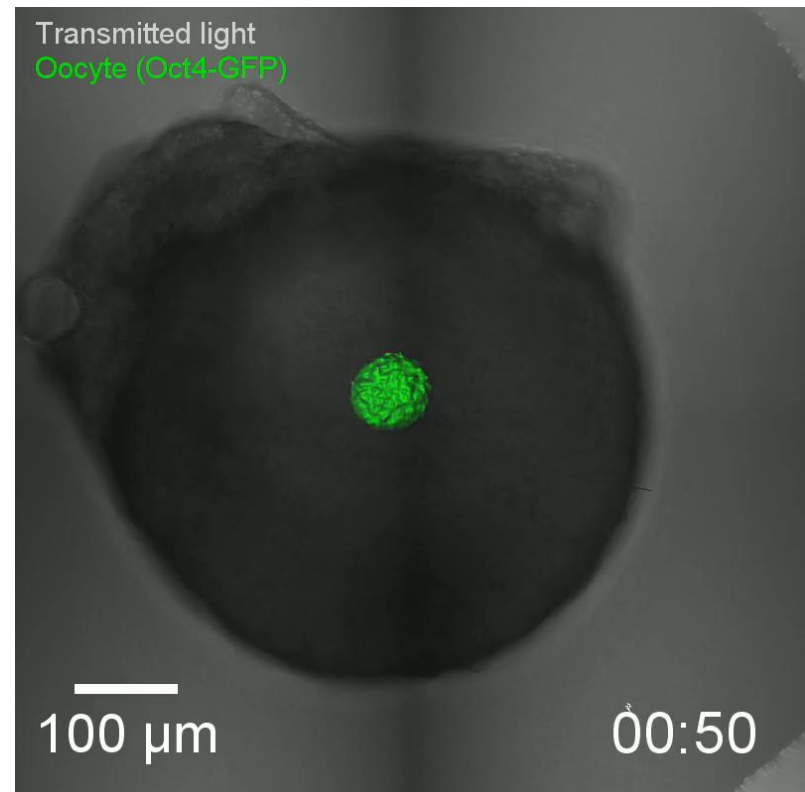
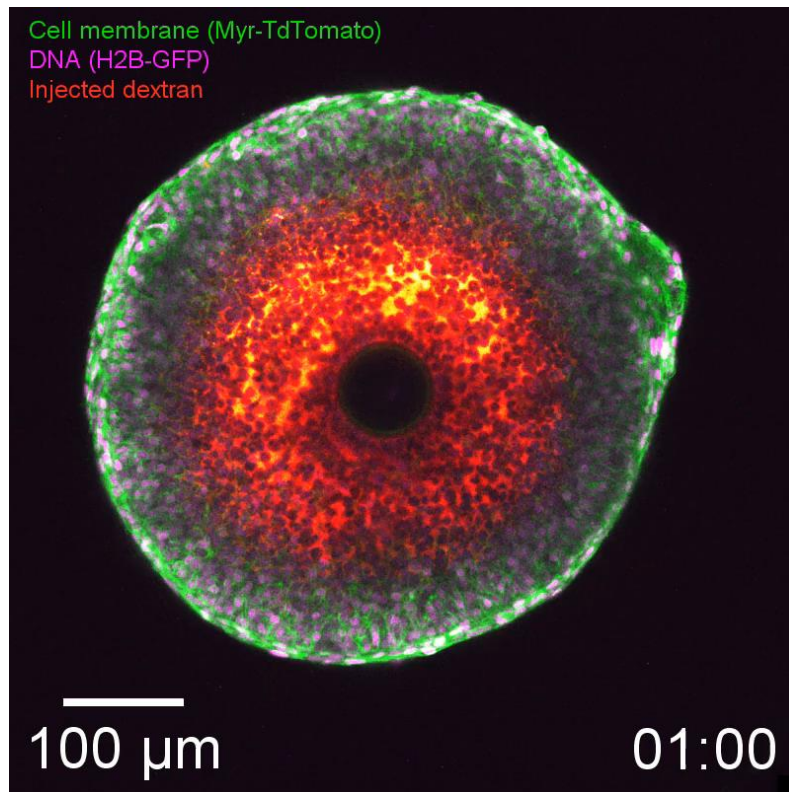
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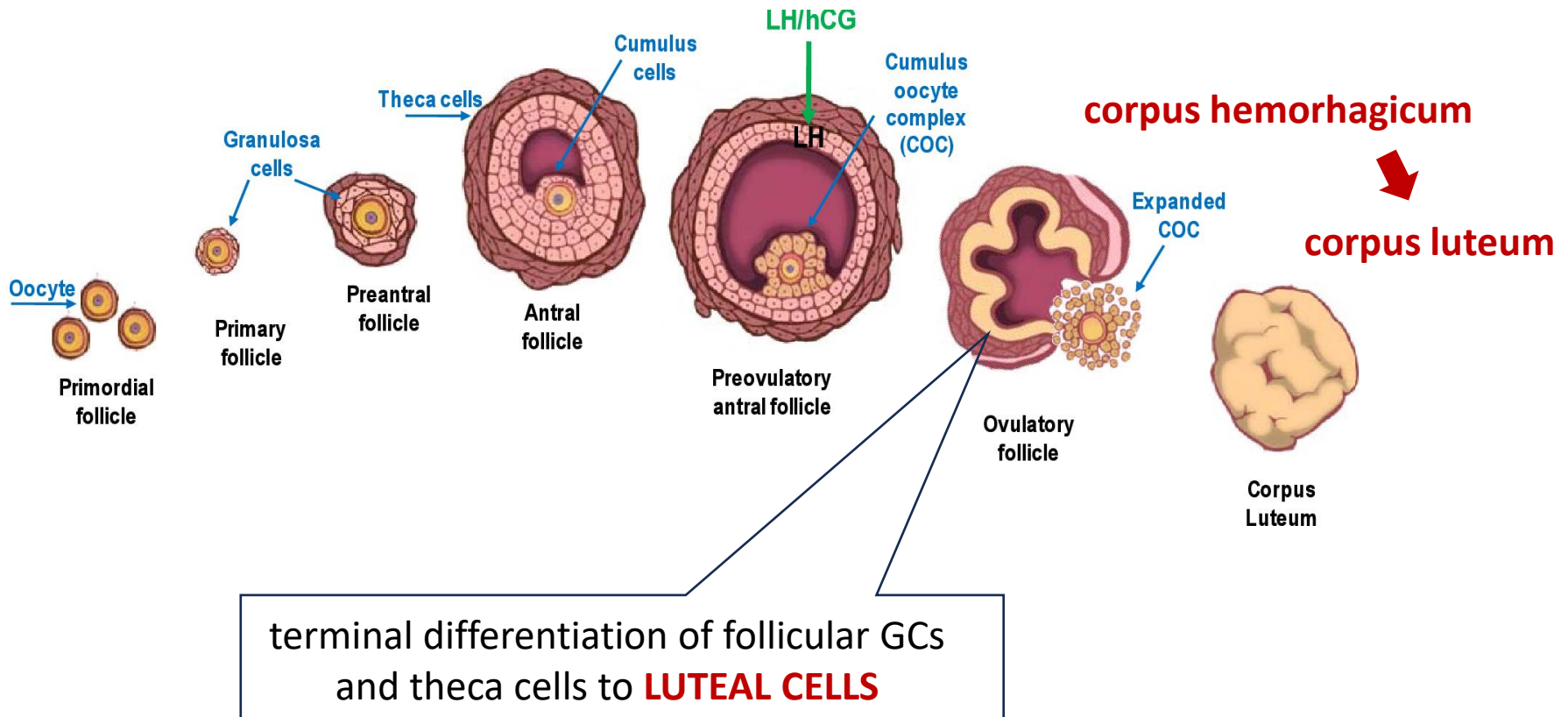
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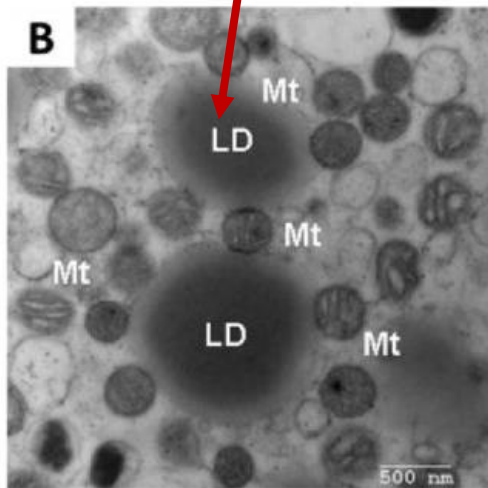
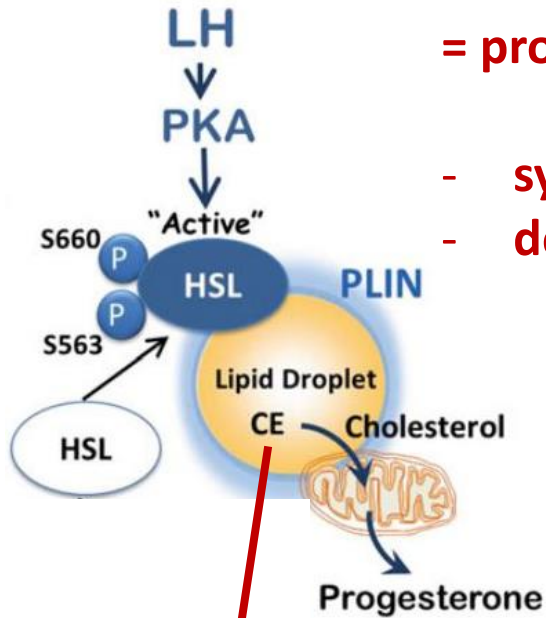
# Luteinisation



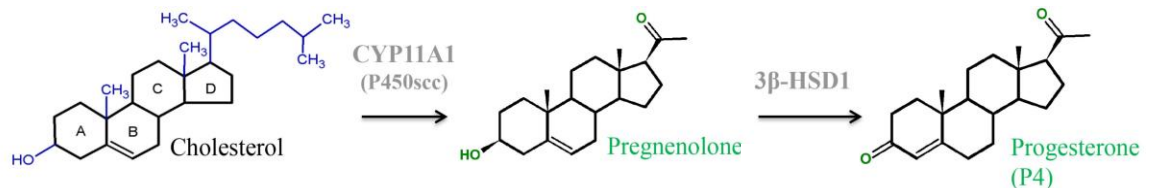
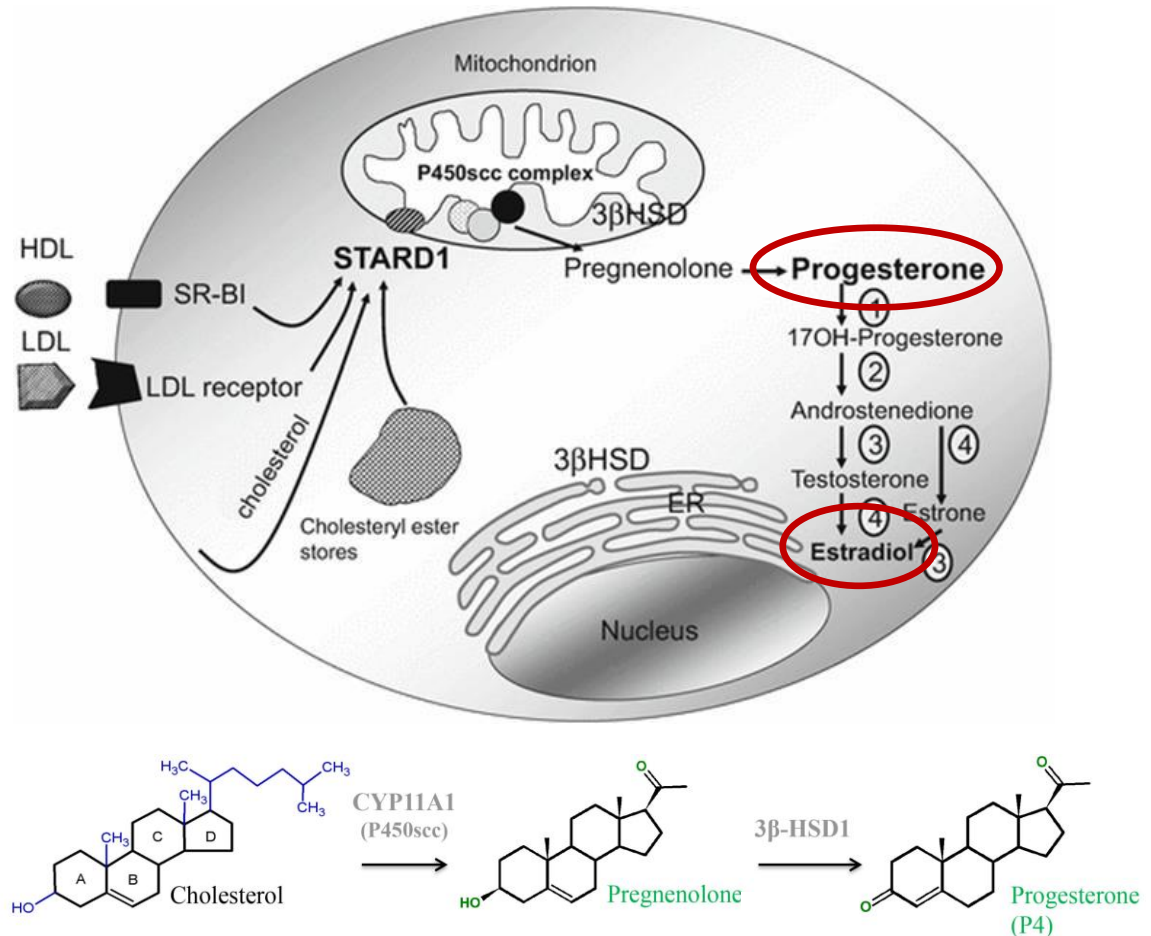
# Luteinisation

= proces of lipid accumulation

- synthesis of progesterone (P4) in response to LH
- dependent of bioavailability of cholesterol

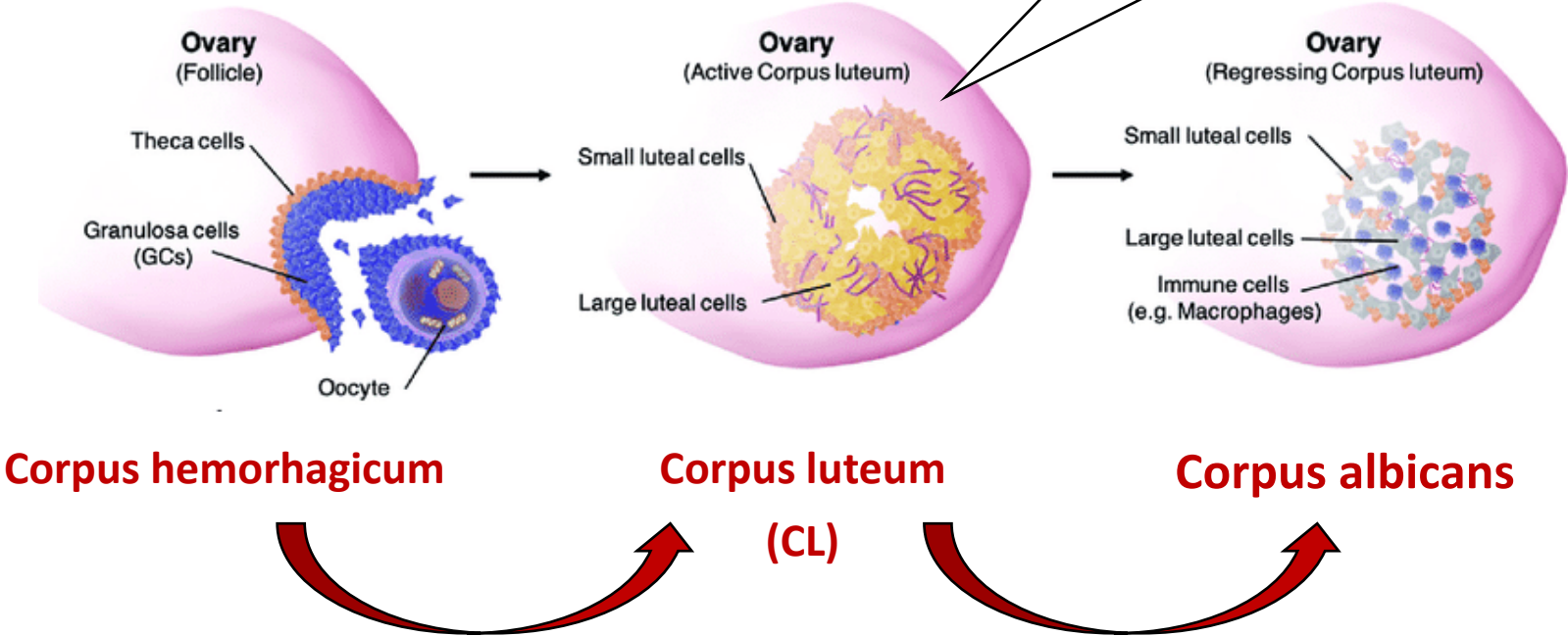
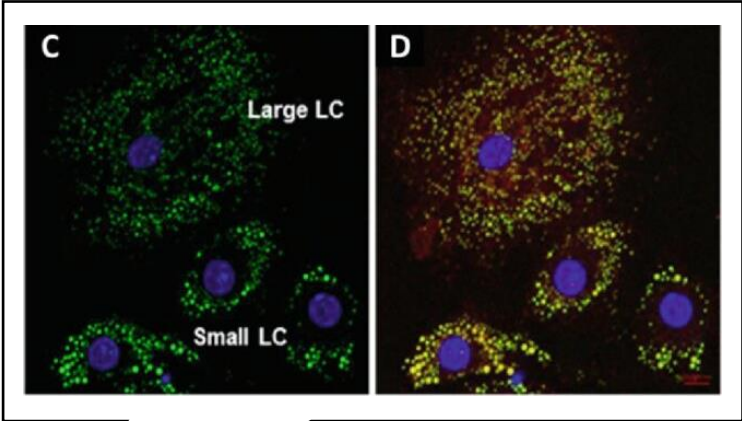


Talbott and Davis 2017

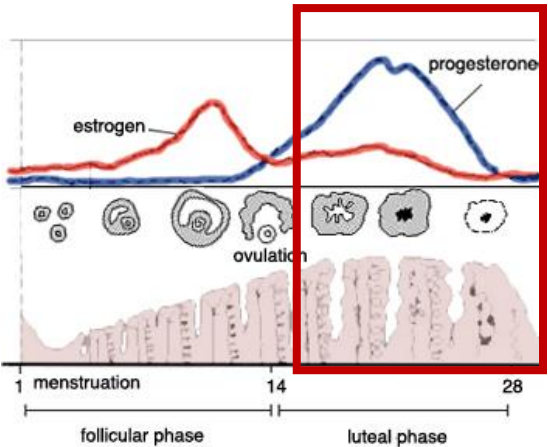
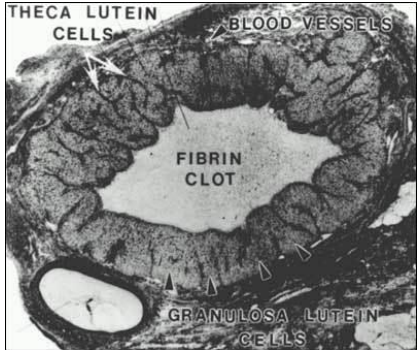
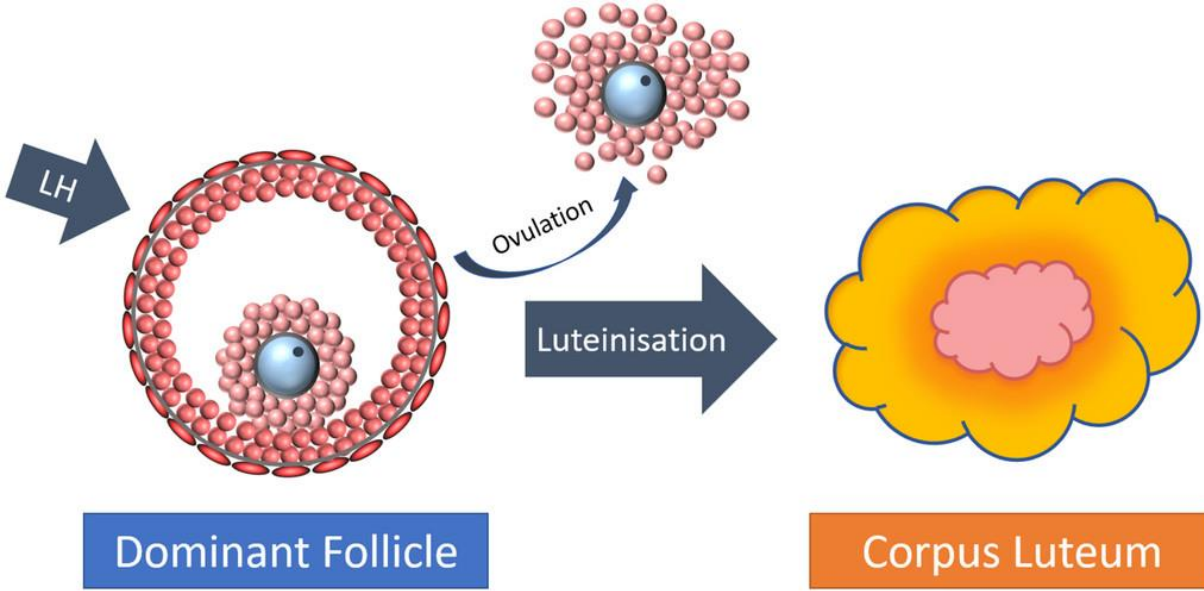


# Corpus luteum

- vascularization of GCs layer
- fibroblast proliferation
- basement membrane break down
- GCS grow and become vacuolized
- **GCS acquire LH receptor and start steroidogenesis**
- cell cycle arrest (p27, p21, cycD)
- proliferation of sER and structural modification of mitochondria



# Corpus luteum

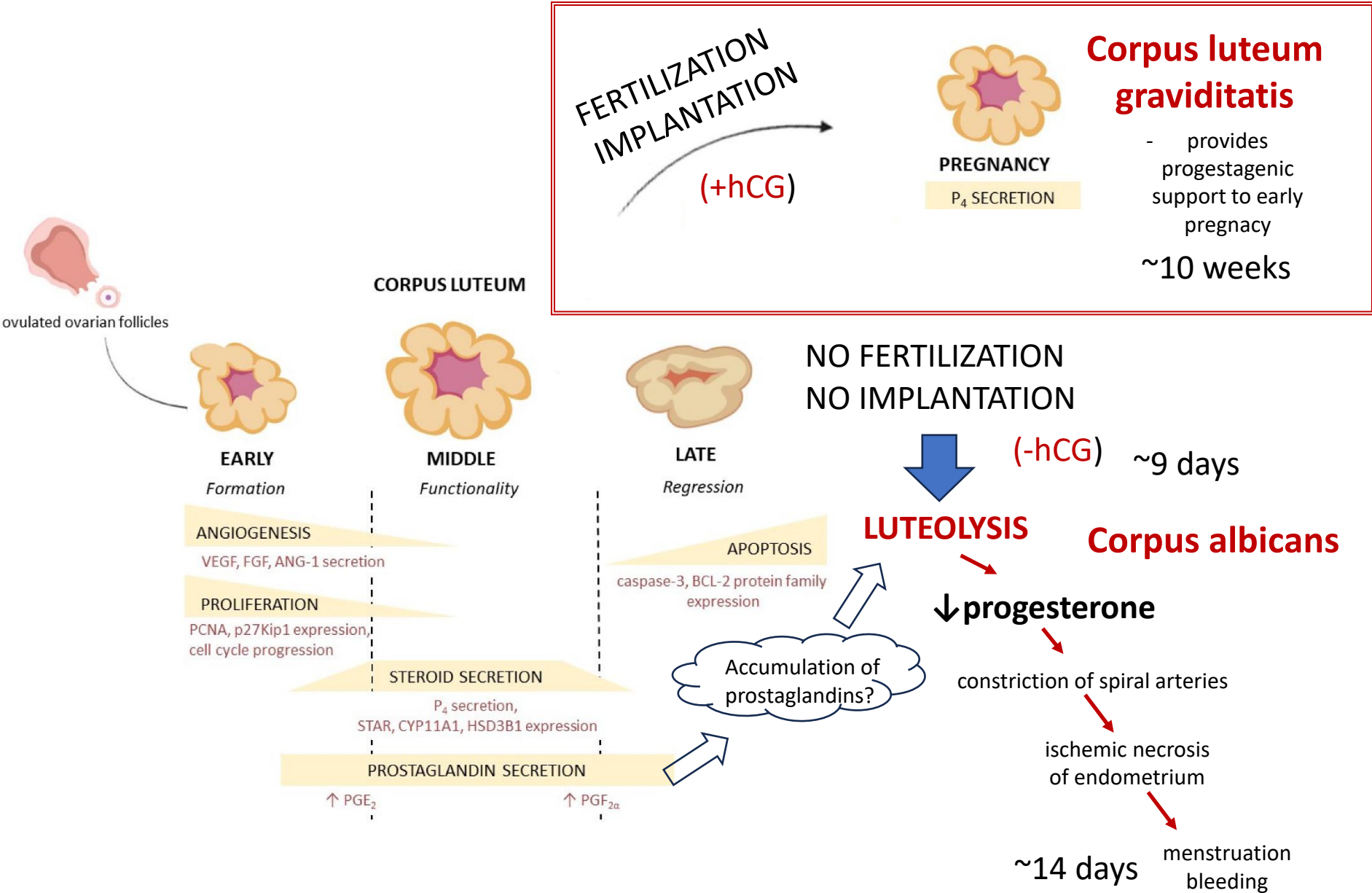


- cyst-like „yellowish“ structure 2-5cm
- temporary endocrine organ
- arises from ovulatory follicle
- secretion of steroid hormones
  - **PROGESTERON (and estradiol)\***
- feed-back loop inhibition of GnRH, FSH and LH

Hyperplasia of endometrium ensuring suitable environment for implantation

\* + relaxin, inhibin

# Corpus luteum



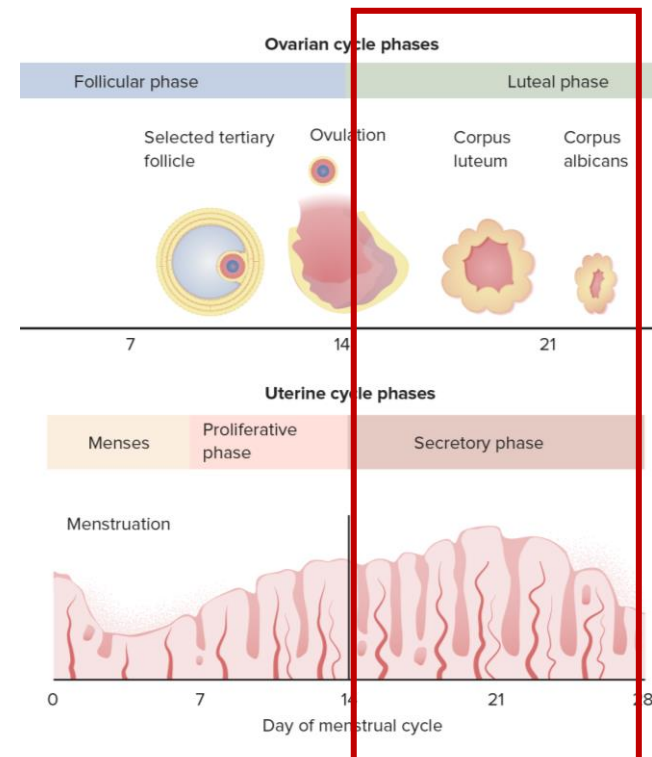
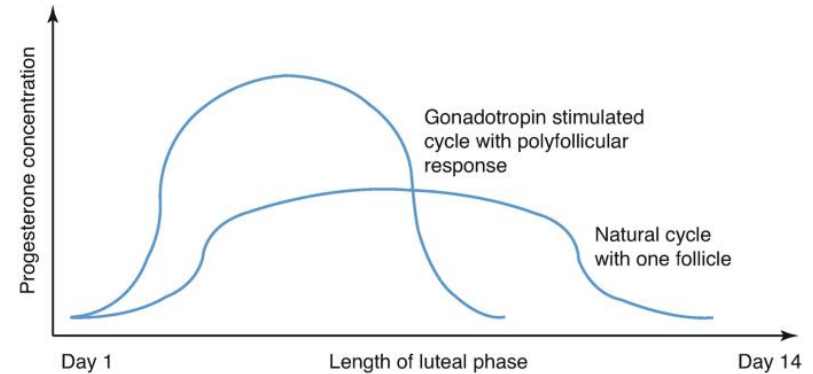


# Corpus luteum dysfunction

- normal P4 production by CL peaks 4 days after ovulation and drops after next 9 days
- Supraphysiological level of steroids (FSH) and prevention of LH release during ovarian stimulation for IVF cause GnRH suppression and may cause **corpus luteum dysfunction**

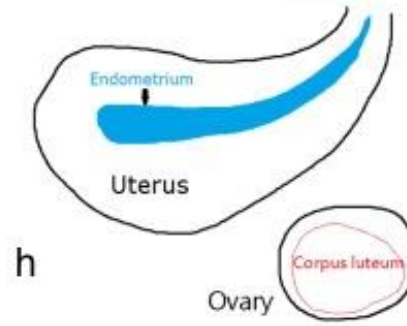
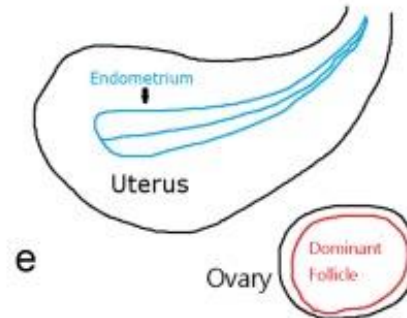
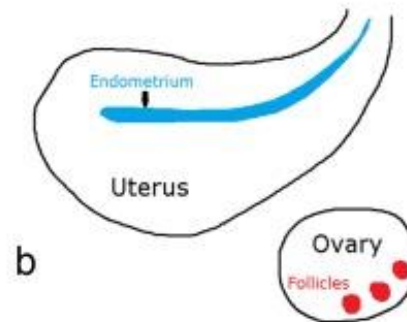
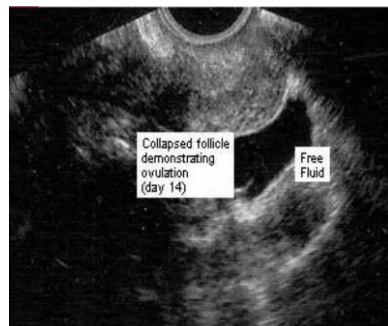
## ❖ Luteal support

- administration of **progesterone** and/or **hCG** to complement CL secretion
- encouraging the uterine lining to support implantation



# Monitoring ovulation

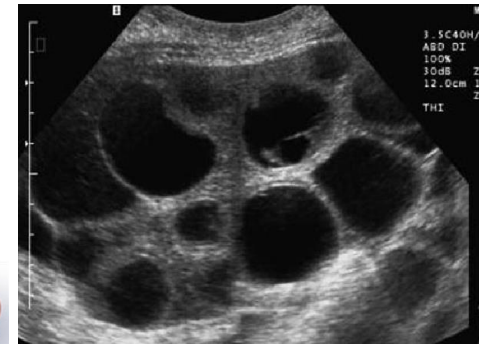
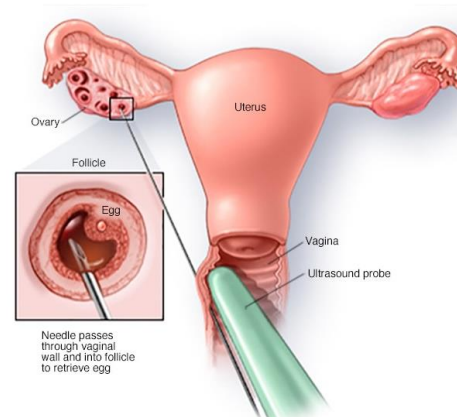
## - Transvaginal Sonographic Folliculometry



→ sexual intercourse, IUI timing

# Oocyte retrieval in ART

- from preovulatory follicles
- in central anesthesia
- ultrasound guidance
- transvaginally
- each follicle invaded by a hollow needle and COCs are aspirated
- performed by accredited IVF specialist (MD)



<https://www.youtube.com/watch?v=0h3LaaL97e0>

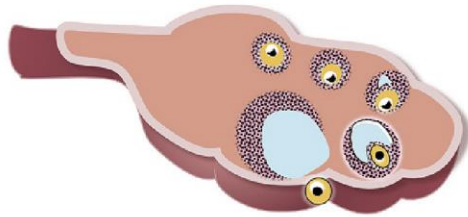
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# Oocyte retrieval in ART

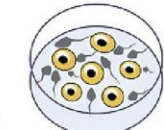
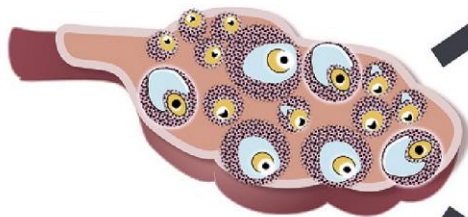
## REGULAR MENSTRUAL CYCLE

*Spontaneous pregnancy*



## ASSISTED REPRODUCTION

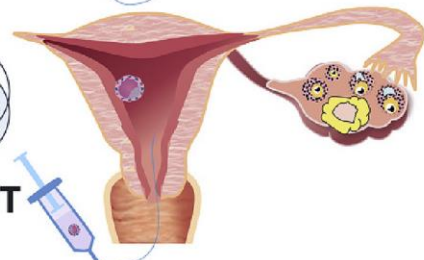
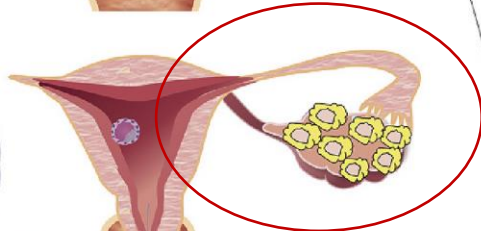
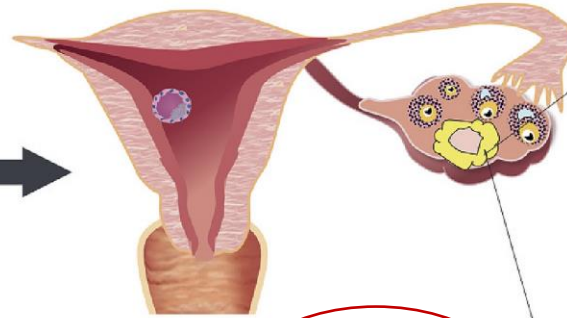
*Controlled ovarian hyperstimulation*



Fresh ET

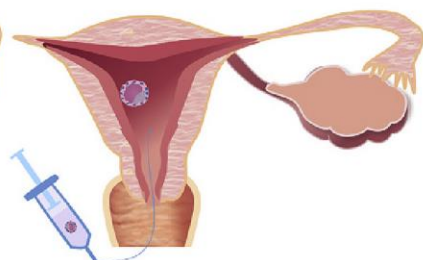


Frozen ET



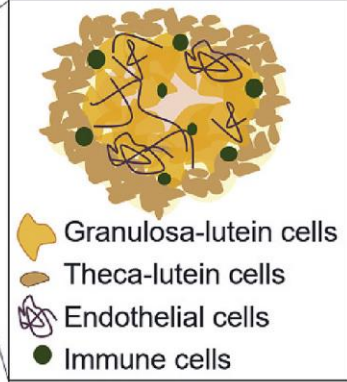
Natural cycle

OR



Programmed cycle

### CORPUS LUTEUM

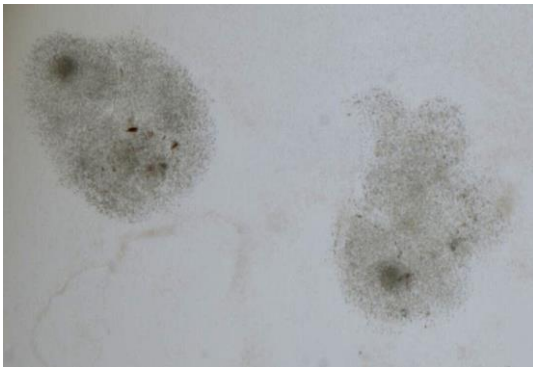
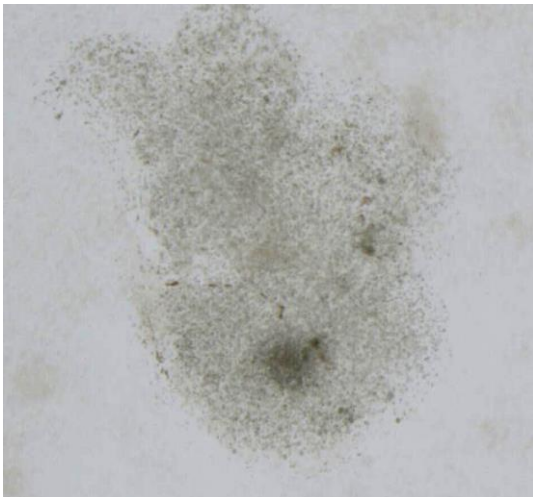


# Oocyte retrieval in ART

## ❖ Putative markers of oocyte quality

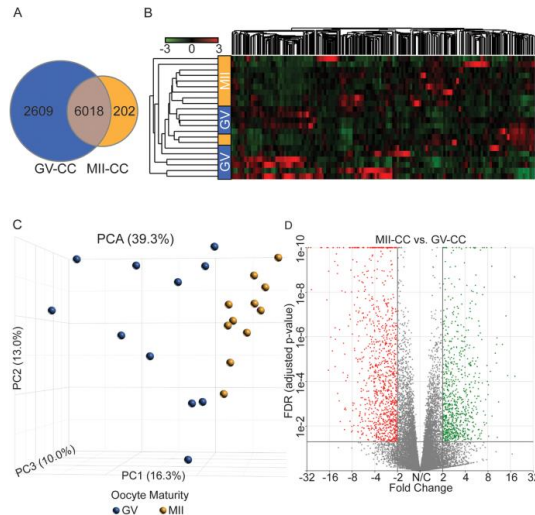
### ■ cumulus cell phenotype

- number of layers, compactness, color, blood clot presence
- reaction to enzymatic treatment



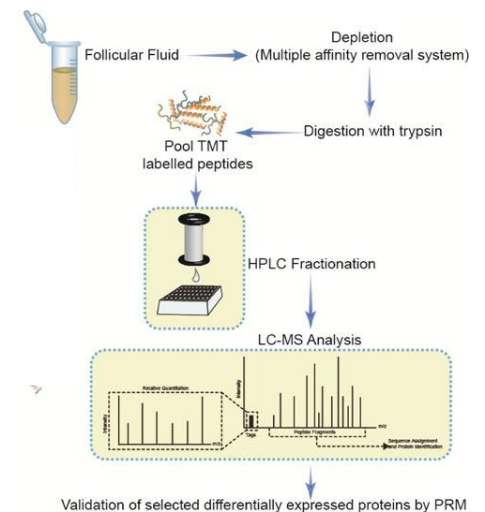
### ■ cumulus cells gene expression

- cell growth/survival/apoptosis, steroidogenesis, intercellular signalling, lipid metabolism, ECM formation, vesicle trafficking, inflammatory factors,..

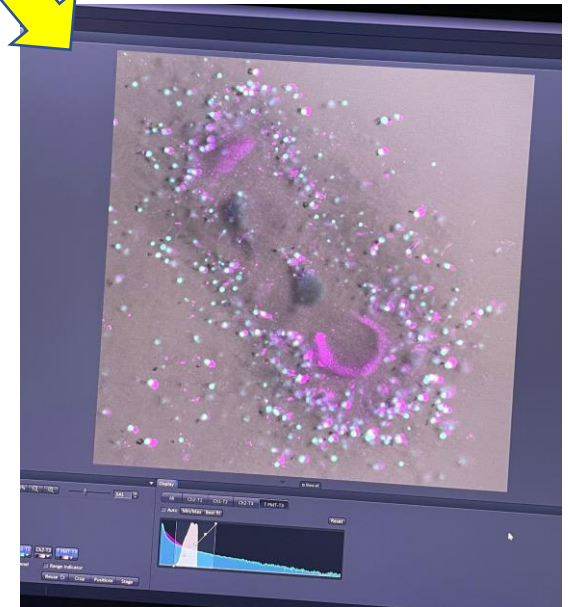
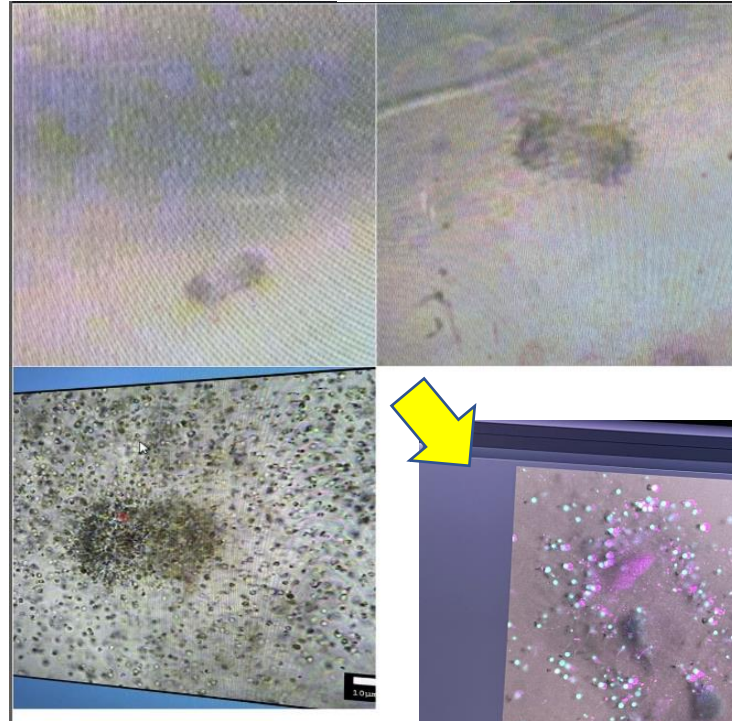
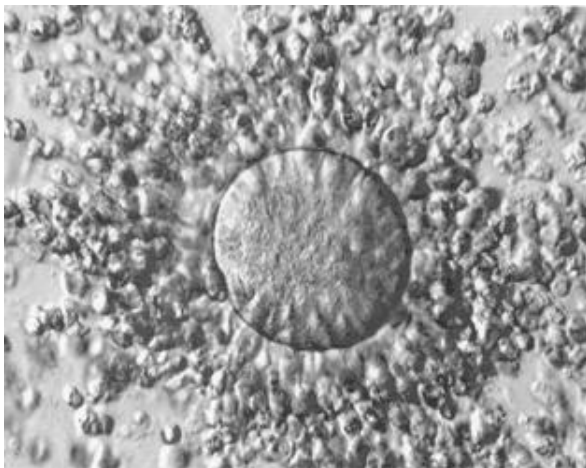
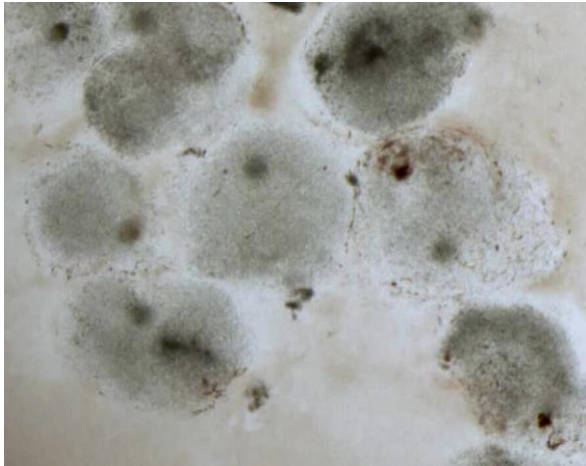


### ■ follicular fluid content

- biochemical/proteomic profile, presence of specific cytokines, ox-red potential,..

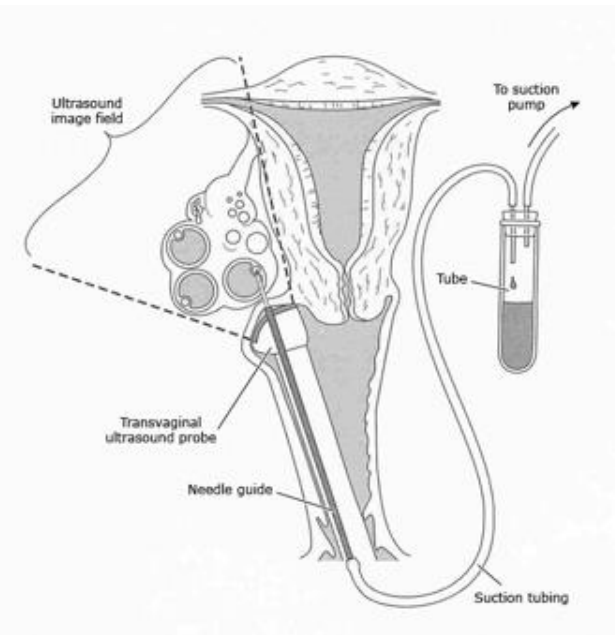


# Oocyte retrieval in ART



# Empty follicle syndrome (EFS)

- no oocytes obtained from preovulatory follicles during IVF procedure despite normal hormonal response and ultrasound monitoring
- usually technical problem during COCs aspiration and/or hCG trigger administration
- genuine absence of oocyte very rare („genuine EFS“ - 0.0016%)



## Possible cause?

A) COC stick to follicle wall due to insufficient LH/hCG trigger which causes COC expansion and detachment

B) Oocyte degeneration within follicle

- genetic predisposition for proapoptotic genes expression in GCs
- defect ZP leading to impaired GCs-oocyte communication

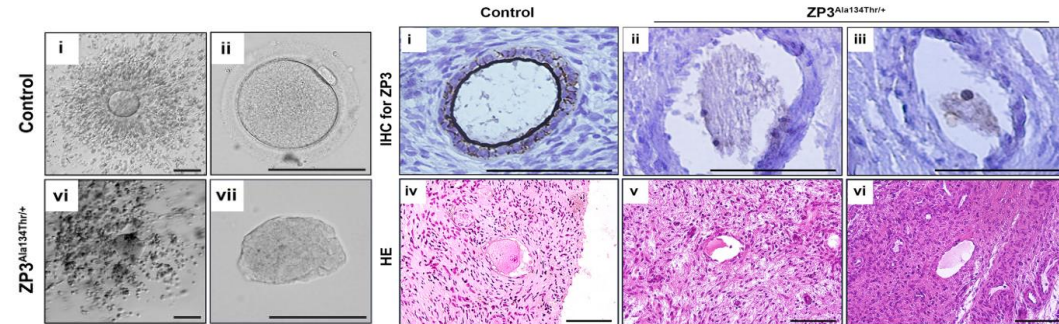


## A Recurrent Missense Mutation in *ZP3* Causes Empty Follicle Syndrome and Female Infertility

Tailai Chen,<sup>1,2,3,7</sup> Yuehong Bian,<sup>1,2,3,7</sup> Xiaoman Liu,<sup>1,2,3</sup> Shigang Zhao,<sup>1,2,3</sup> Keliang Wu,<sup>1,2,3</sup> Lei Yan,<sup>1,2,3</sup> Mei Li,<sup>1,2,3</sup> Zhenglin Yang,<sup>6</sup> Hongbin Liu,<sup>1,2,3</sup> Han Zhao,<sup>1,2,3,\*</sup> and Zi-jiang Chen<sup>1,2,3,4,5,\*</sup>

Empty follicle syndrome (EFS) is defined as the failure to aspirate oocytes from mature ovarian follicles during in vitro fertilization. Except for some cases caused by pharmacological or iatrogenic problems, the etiology of EFS remains enigmatic. In the present study, we describe a large family with a dominant inheritance pattern of female infertility characterized by recurrent EFS. Genome-wide linkage analyses and whole-exome sequencing revealed a paternally transmitted heterozygous missense mutation of c.400 G>A (p.Ala134Thr) in zona pellucida glycoprotein 3 (*ZP3*). The same mutation was identified in an unrelated EFS pedigree. Haplotype analysis revealed that the disease allele of these two families came from different origins. Furthermore, in a cohort of 21 cases of EFS, two were also found to have the *ZP3* c.400 G>A mutation. Immunofluorescence and histological analysis indicated that the oocytes of the EFS female had degenerated and lacked the zona pellucida (ZP). *ZP3* is a major component of the ZP filament. When mutant *ZP3* was co-expressed with wild-type *ZP3*, the interaction between wild-type *ZP3* and *ZP2* was markedly decreased as a result of the binding of wild-type *ZP3* and mutant *ZP3*, via dominant negative inhibition. As a result, the assembly of ZP was impeded and the communication between cumulus cells and the oocyte was prevented, resulting in oocyte degeneration. These results identified a genetic basis for EFS and oocyte degeneration and, moreover, might pave the way for genetic diagnosis of infertile females with this phenotype.

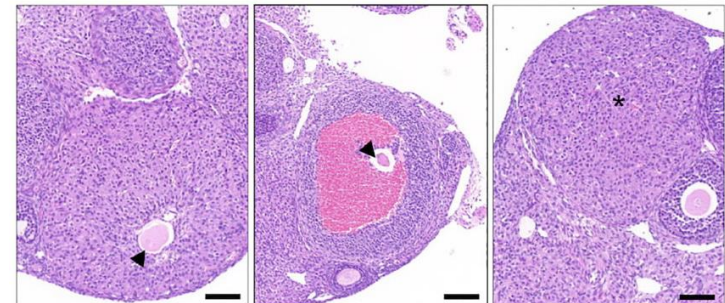
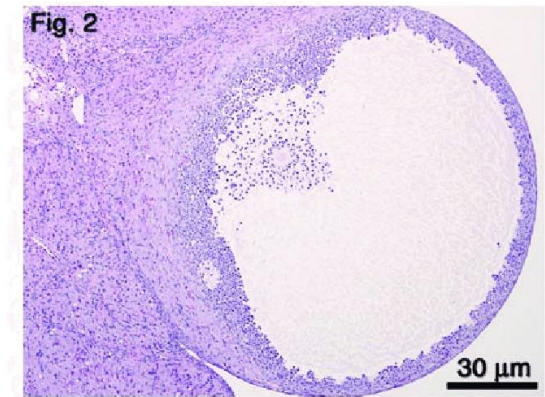
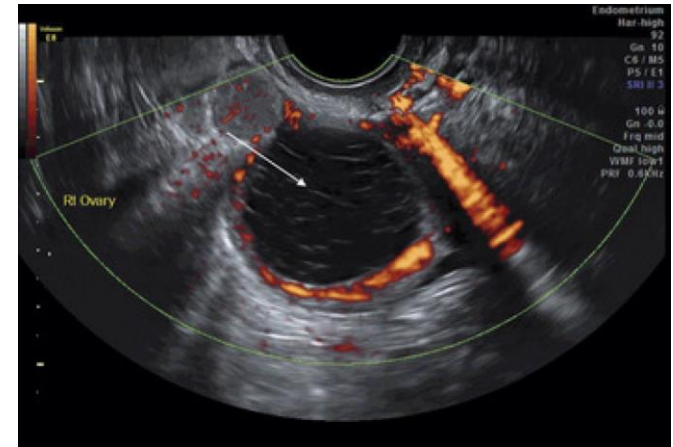
Chen et al., 2017.



# Anovulation

## ❖ Luteinized unruptured follicle (LUF)

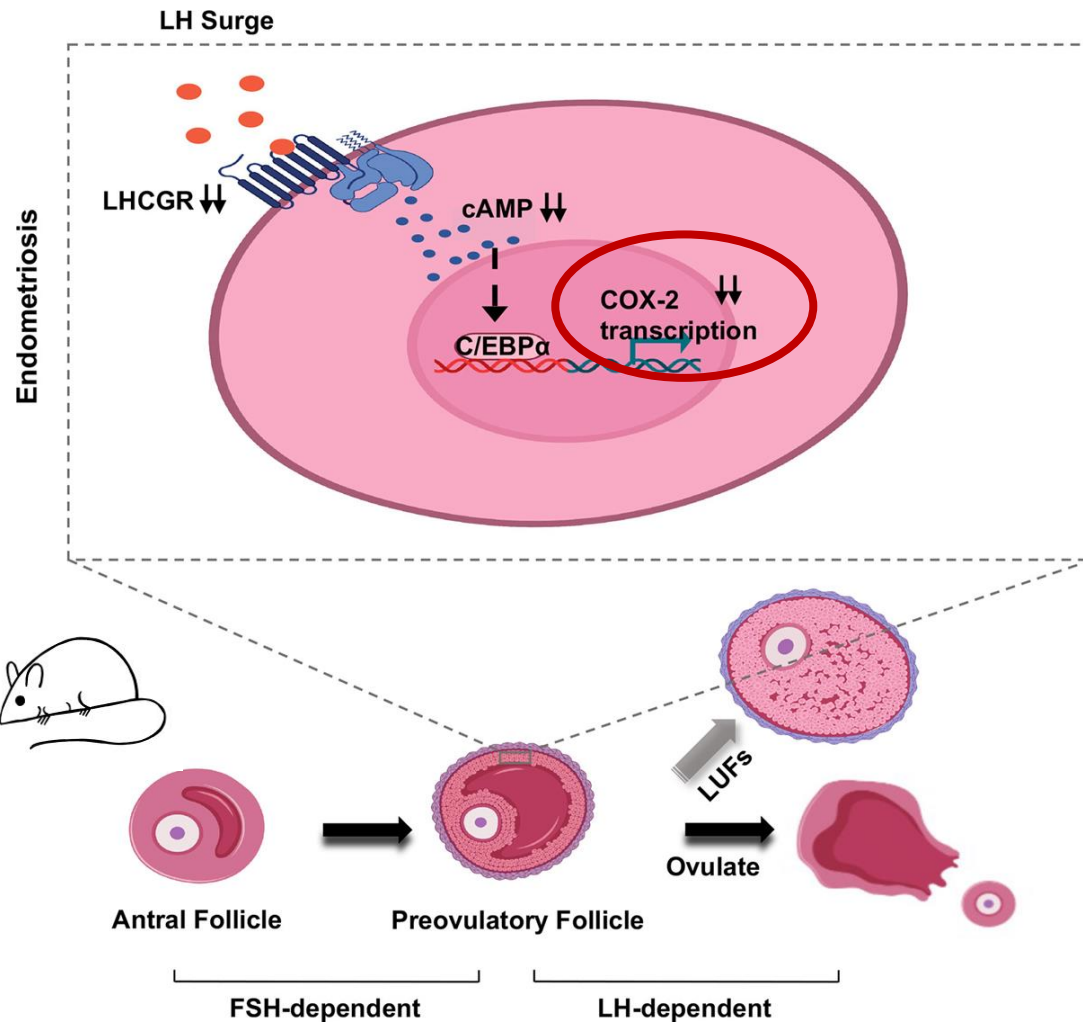
- failure of Graaffian follicle to rupture after LH peak
- egg trapped inside the persistent follicle with thick wall
- altered endocrine profile - low FSH and LH, high progesteron
- altered folliculogenesis dynamics - earlier follicular selection, faster and longer growth
- progressive loss of cystic appearance and luteinization without ovulation
- thick echogenic endometrium
- mature/premature LUFs
- high recurrency, sub-/in-fertility
- associated with endometriosis (73%), pelvis inflammatory disease and excessive used of NSAIDs (!)





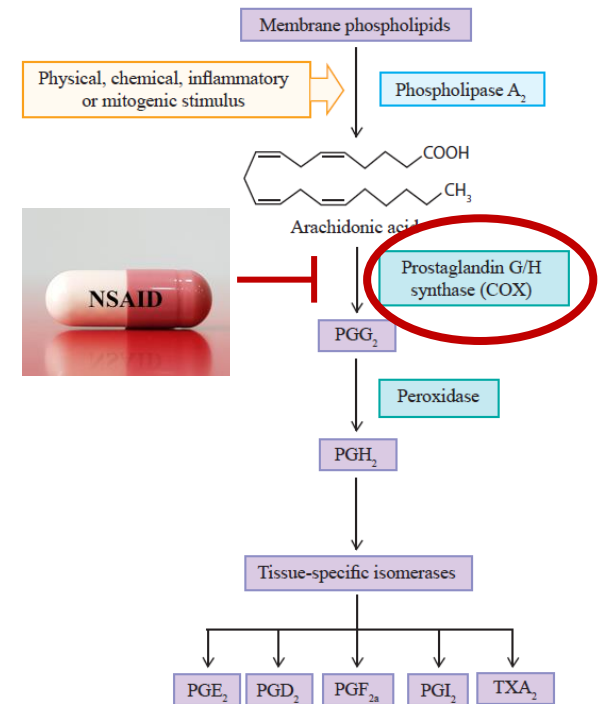
# Anovulation

## ❖ Luteinized unruptured follicle



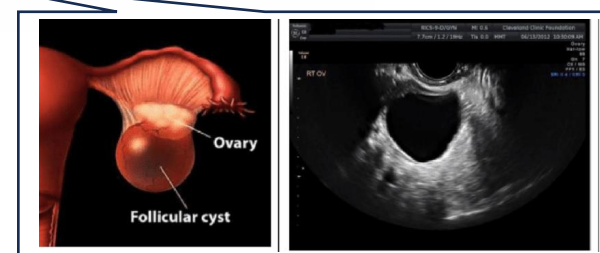
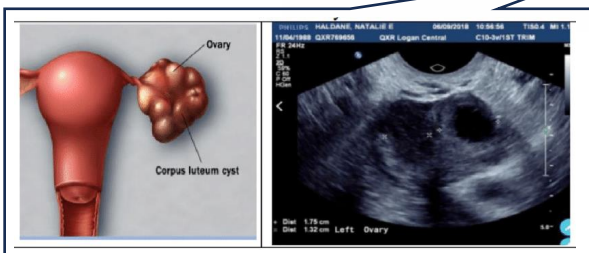
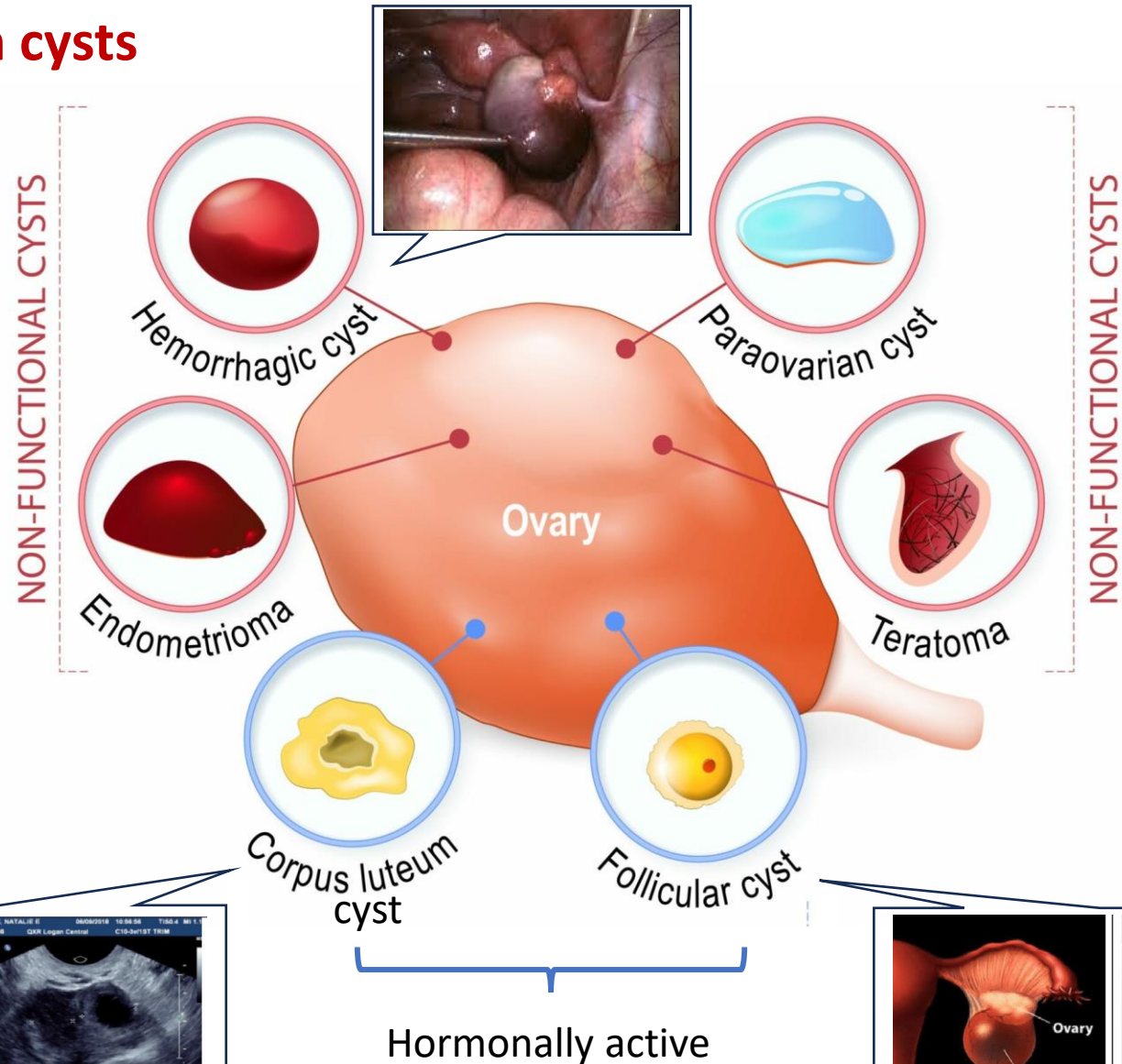
## - role of COX2

- production of prostaglandins required for follicular maturation and rupture



# Anovulation

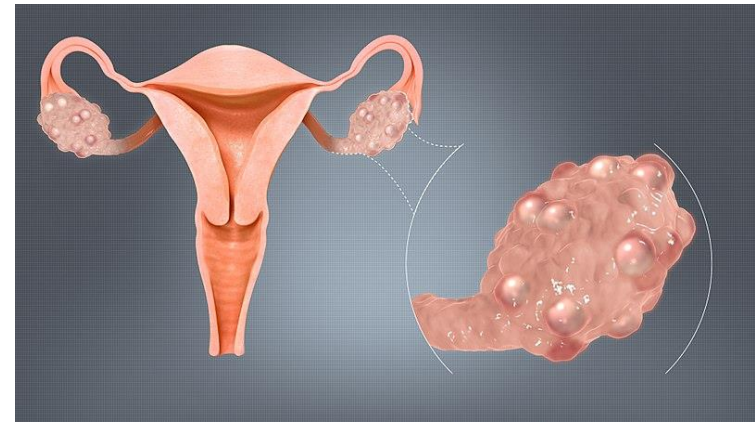
## ❖ Ovarian cysts



# Anovulation

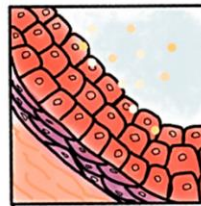
## ❖ Polycystic ovary syndrom

- 2 out of 3 Rotterdam criteria:
  - follicle arrest in antral stage, multiple growth ceasing follicles (and ovarian cysts)
  - reduced ovulation rates, oligo-/a-menorhea
  - hyperandrogenism



### Increased granulosa/theca cells and stromal tissue

- Increase the production of ovary-related or ovary-producing hormone
- Influence hypothalamus-pituitary-ovary axis

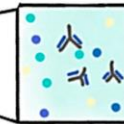


### Cortex thickening with reduced glycosaminoglycans and increased stromal hyperplasia



### Increase of intra-ovarian follicular fluid

- **Increased proinflammatory response**  
Ex. TNF-alpha, GCSF, Sfrp-5, IL12, neutrophil count, M1/M2 ratio, N/C ratio, Th17/Th2 ratio
- **Decreased anti-inflammatory response**  
Ex. IL-13, IL-15, IL-22, MIF, CCL2, innate lymphoid cells, regT cell, dendritic cells, cytotoxic T cells.



### Dysfunction of angiogenesis

- Increased stromal vascularization
- Lower flow impedance
- Alter angiogenic factors



### Down regulation of Inflammation-related gene and theca-associated lymphocytes

- An increased number of immature follicles
- An increased MMP-9 secretion



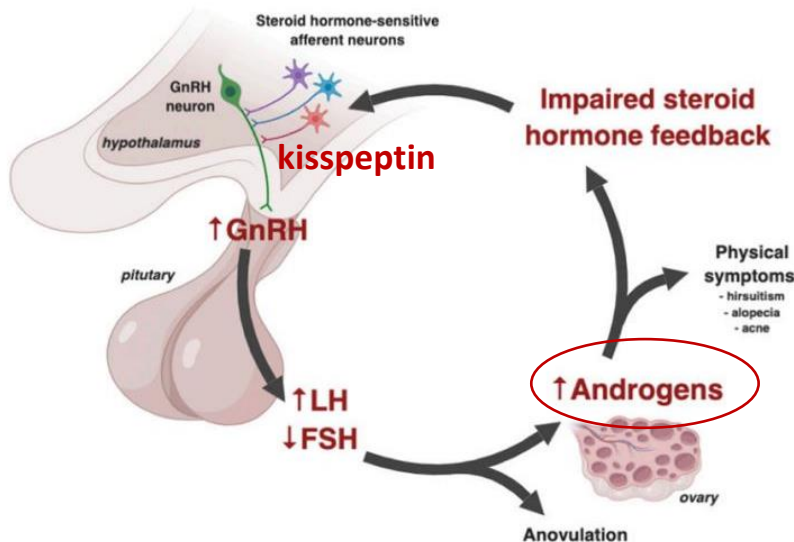
# Anovulation

## ❖ Polycystic ovary syndrom

- Role of androstenedione in follicular cyst formation

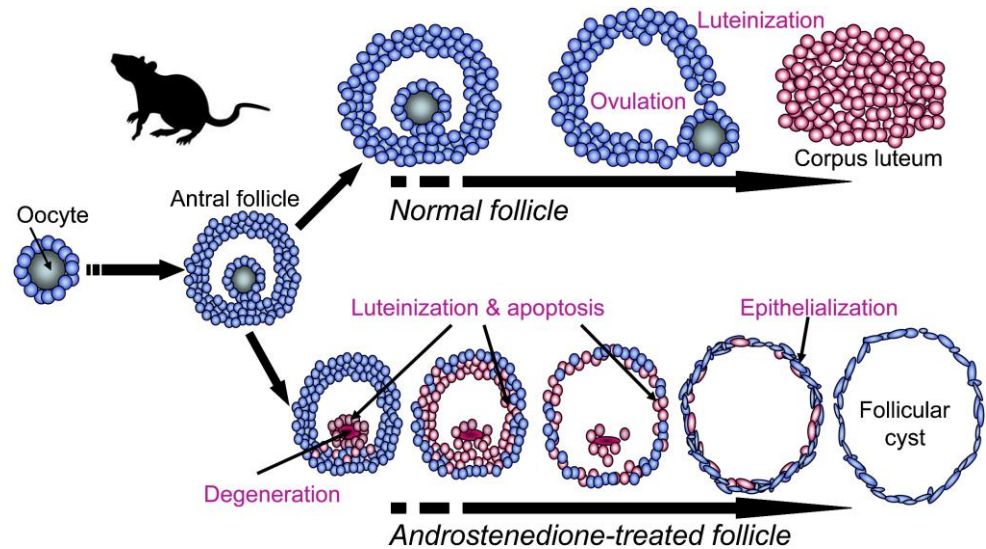


- oocyte degeneration
- increased GC apoptosis
- premature luteinisation of GCS



Ruddenklau and Campbell 2019

Okutsu et al 2008

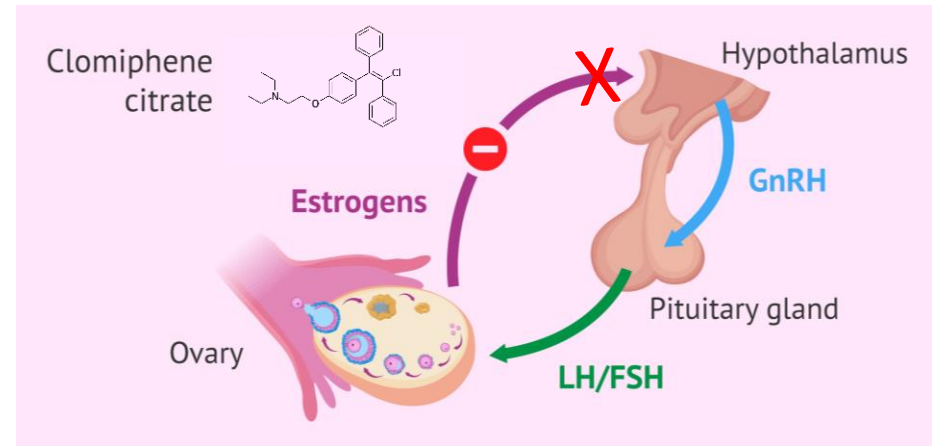


- Role of neuroendocrine impairment ?
  - hyperactivity of GnRH neurones?
  - role of kisspeptin?

# Pharmacological induction of ovulation

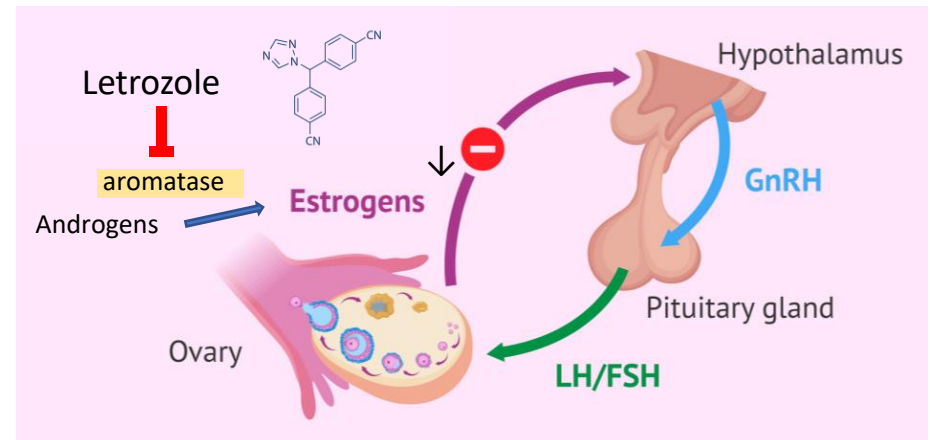
## ❖ Clomiphene (p.o.)

- non-steroid selective modulator of estrogen receptors in hypothalamus
- induce release of GnRH ( $\uparrow$ FSH, LH)
- long clearance, negative impact on endometrial lining
- used in an-/oligo-ovulation disorders (e.g. PCOS)
- RISK OF MULTIPLE PREGNANCY!



## ❖ Letrozole (p.o.)

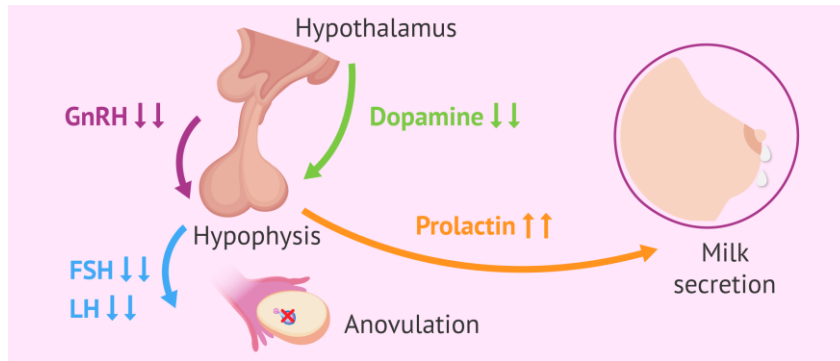
- aromatase inhibitor (blocks estrogen synthesis)
- negative loop increases GnRH production and thus FSH a LH levels
- anticancer drug (breast cancer), gynecomastia treatment
- „off-label“ use for induction of ovulation
- lower impact on endometrium, more expensive



# Pharmacological induction of ovulation

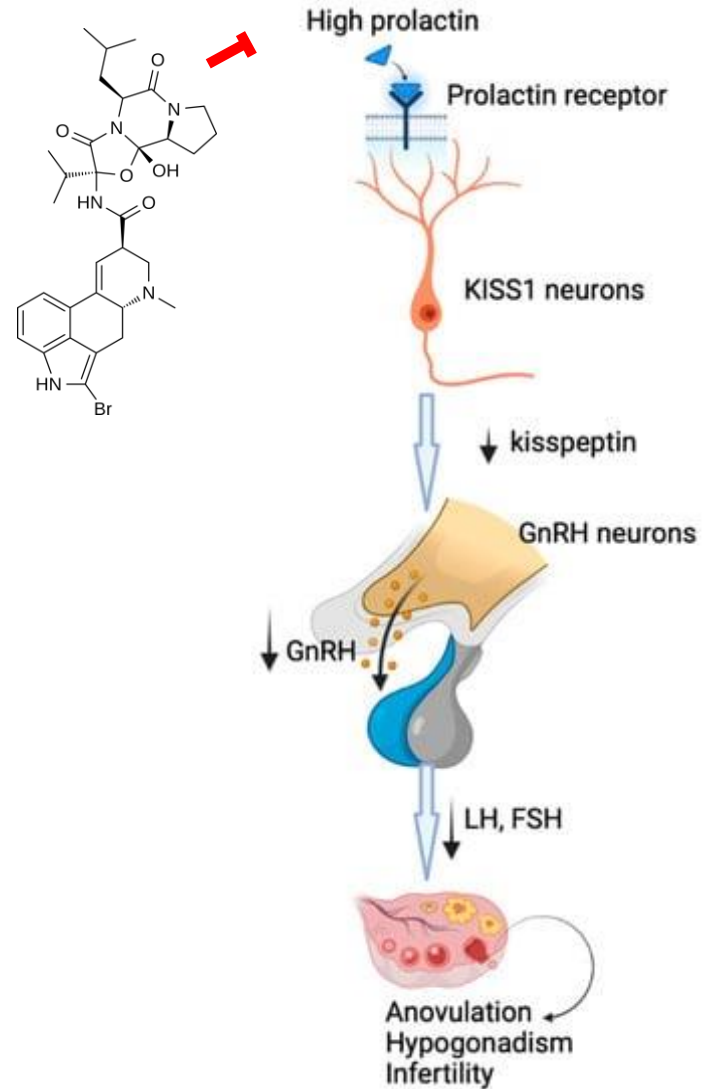
## ❖ Bromocriptine (p.o.)

- ergoline derivate and dopamin agonist
- reduces production of **prolactin** by pituitary gland
- treatment of **anovulation caused by hyperprolactinemia**



### ↑Prolactin

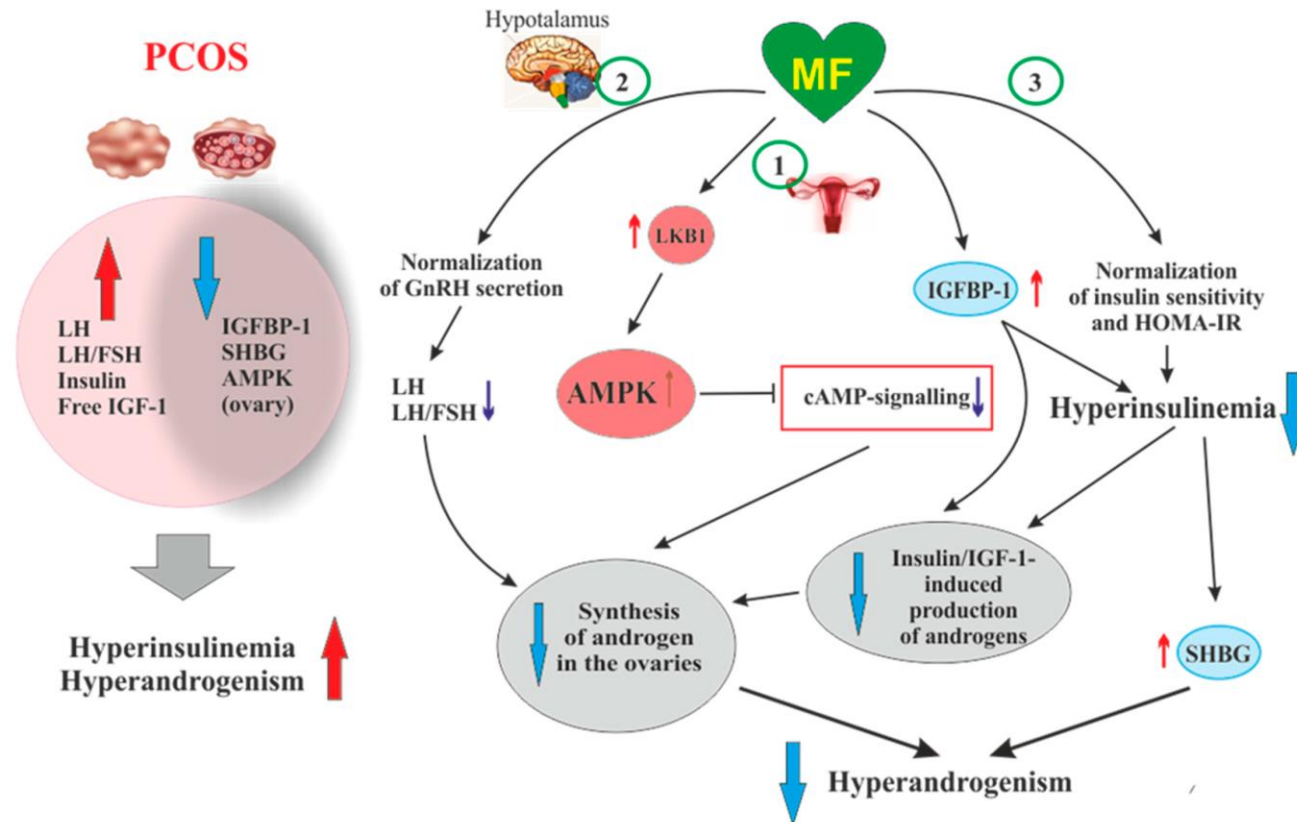
- decreased GnRH production
- granulosa cell dysfunction
- inhibition of corpus luteum function
- endometrium dysfunction



# Pharmacological induction of ovulation

## ❖ Metformin (p.o.)

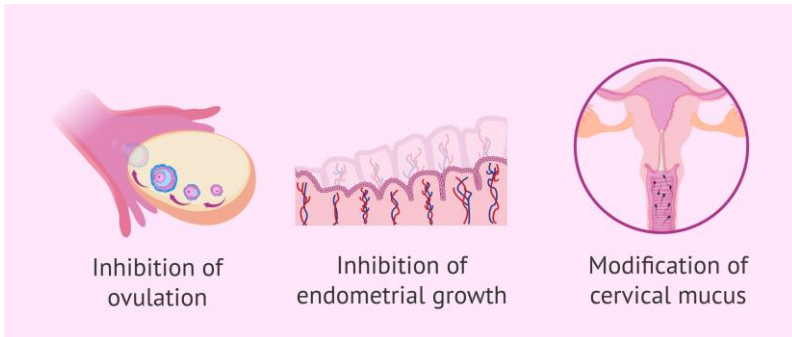
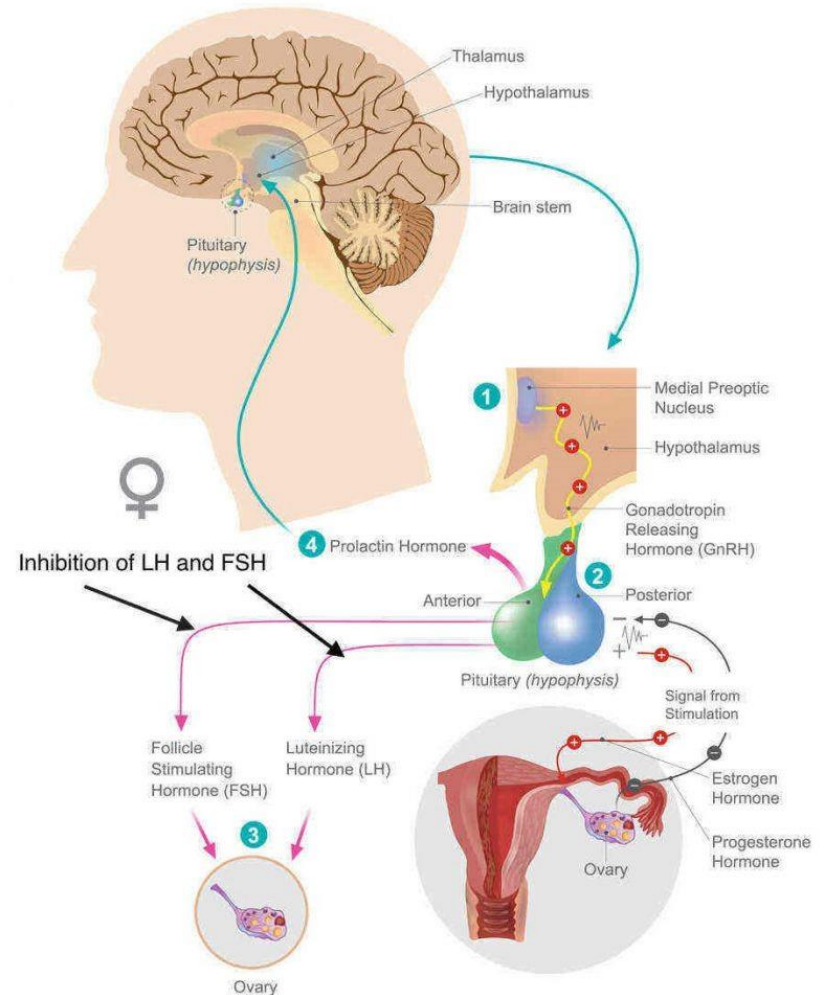
- antidiabetic drug (DM 2)
- stimulation of ovulation in insulin-resistant PCOS
- empirical treatment
- gastrointestinal side effects



# Pharmacological inhibition of ovulation

## ❖ Combined contraceptive pills (p.o.)

- **estrogen + progesterone**
- feedback **inhibition of FSH and LH secretion** from pituitary gland
- **inhibition of ovulation**, suppression of endometrial growth, thickening of cervical mucus
- resumption of fertility after discontinuation
- relief from dysmenorhea, prevention of endometriosis recurrence
- ↓ risk of endometrial cancer
- ↑ risk of breast cancer





# Pharmacological inhibition of ovulation

Can suppression of ovulation have a protective effect against ovarian aging by preventing tissue damage?



Original Article

## Administration of Oral Contraceptives Could Alleviate Age-Related Fertility Decline Possibly by Preventing Ovarian Damage in a Mouse Model

Wataru Isono, MD, PhD<sup>1,2</sup>, Osamu Wada-Hiraike, MD, PhD<sup>1</sup>, Yumiko Kawamura, PhD<sup>2</sup>, Tomoyuki Fujii, MD, PhD<sup>1</sup>, Yutaka Osuga, MD, PhD<sup>1</sup>, and Hiroki Kurihara, MD, PhD<sup>2</sup>

Reproductive Sciences  
1-11  
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DOI: 10.1177/1933719117746758  
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SAGE



Mouse model of ovulation suppression  
- daily administration of OC 2-12m

### - OC-treated mice

- no significant difference if follicle number compared to control
- higher number of oocytes after stimulation
- more living fetuses after spontaneous mating
- reduced amount of brownish foamy fibrous tissues

- tissue damage caused by ovulation cycle might contribute to age-related fertility decline

