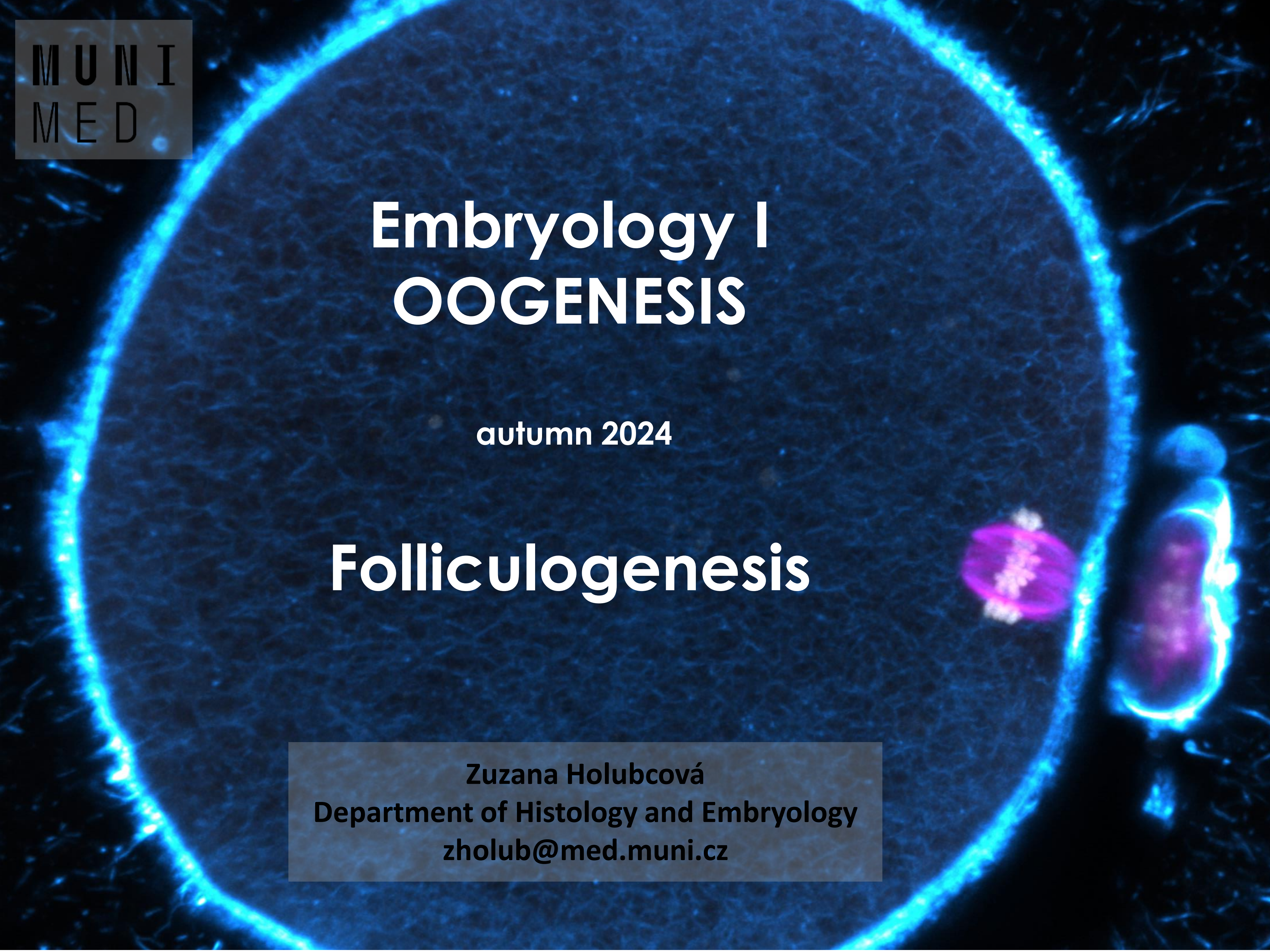

MUNI
MED

Embryology I
OOGENESIS

autumn 2024

Folliculogenesis

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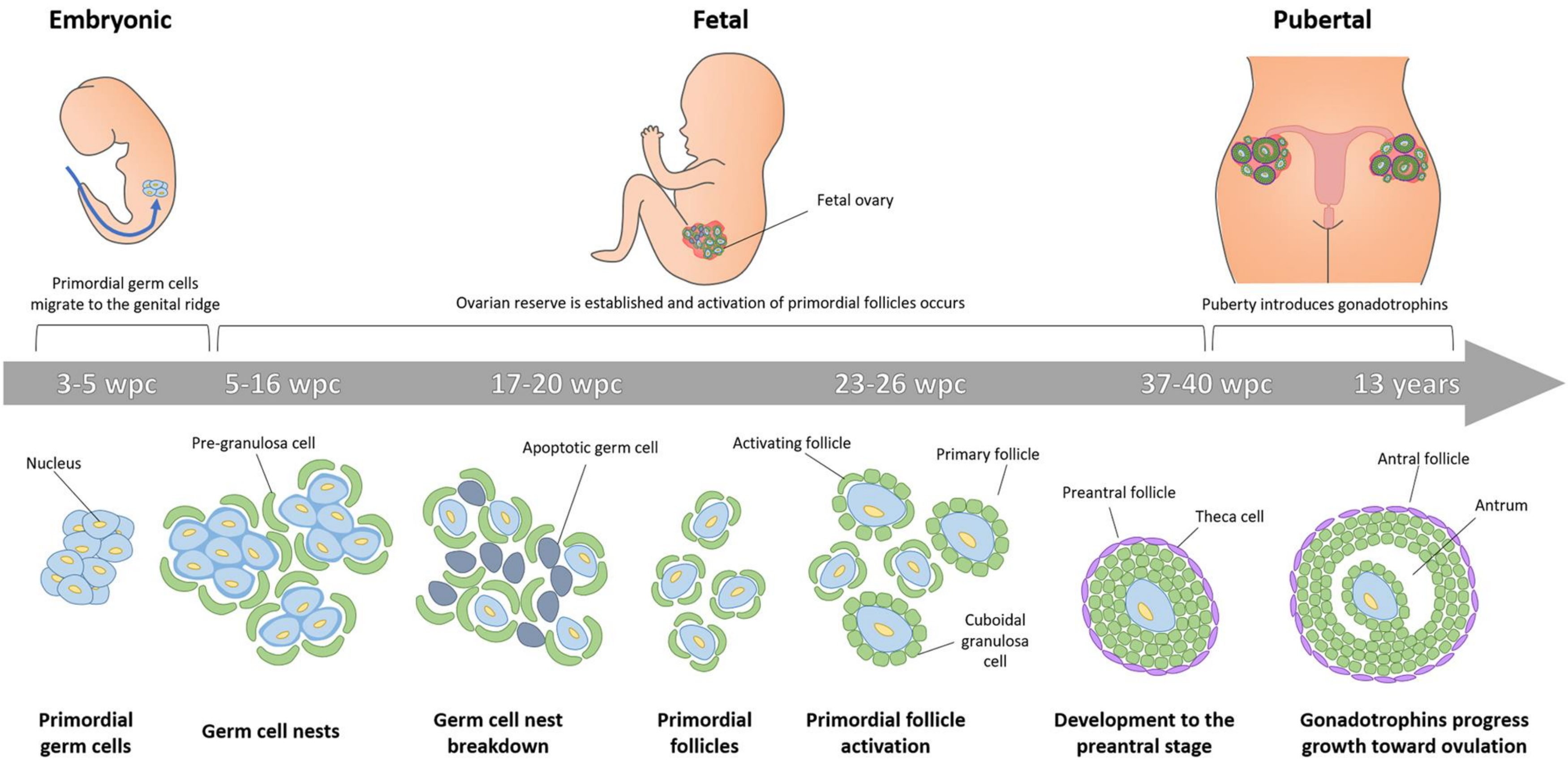
Folliculogenesis overview

- Follicle = female germ cell + somatic cells
  - symbiotic syncitium, functional unit of the ovary
- oogenesis (female gamete development) a folliculogenesis (follicle development) are interconnected processes
- endocrine, paracrine and autocrine regulation



Primordial follicles formation

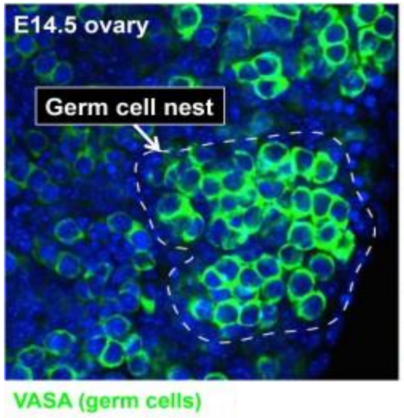
➢ Proliferation of oogonia

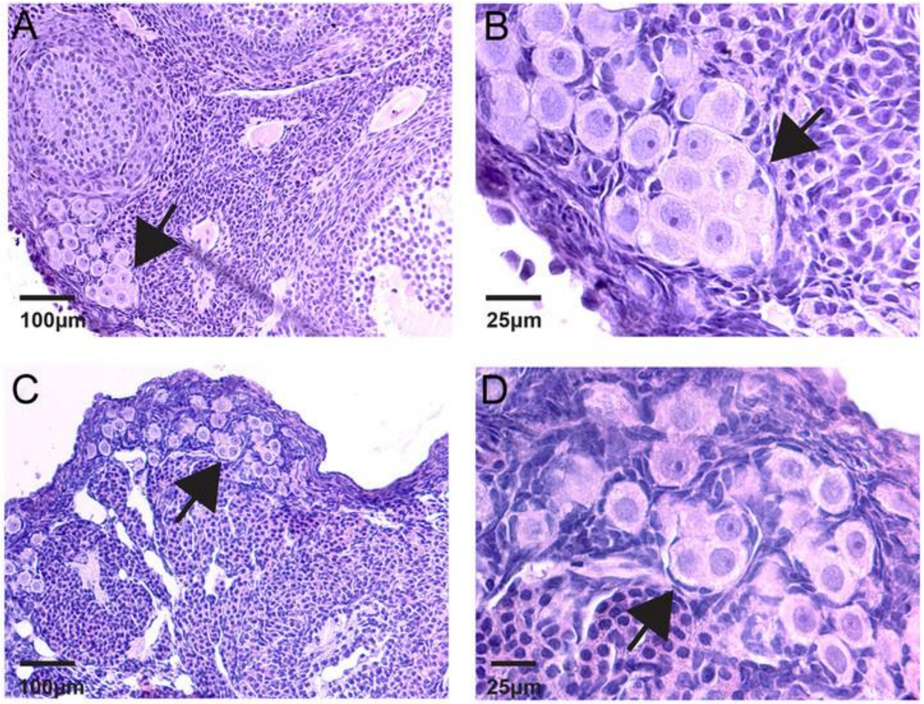
- first germ cells colonize primordial ovaries in 5. week of gestation (wg)/ 3. week post conception (pc) and divide completely producing fully separated oogonia

- second division wave is characterized by incomplete cytokinesis, daughter cells remain connected by intercellular bridges

syncitium

germ cell nest/cluster

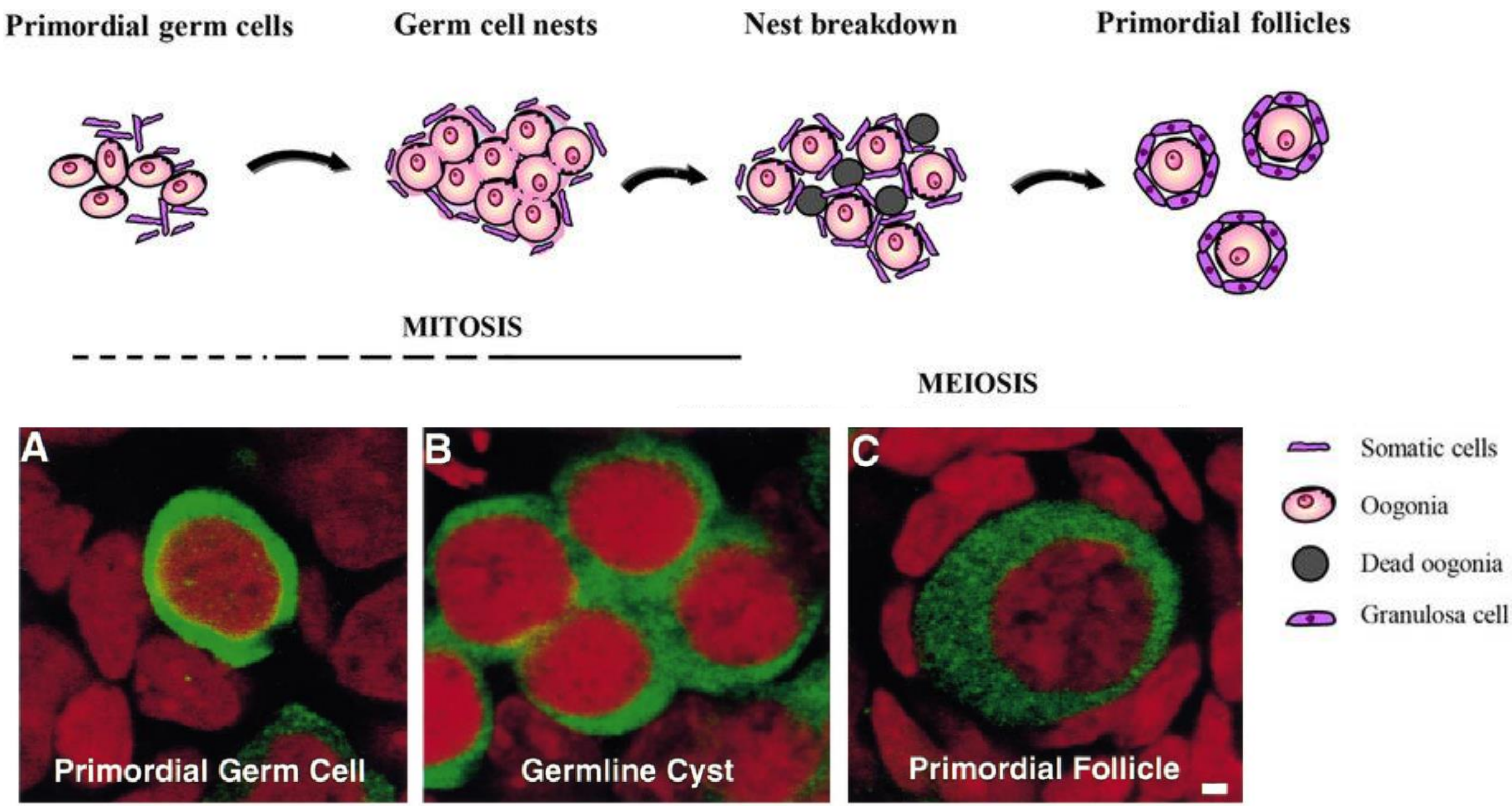




Primordial follicles formation

➢ Germ cell nest breakdown

- surrounding somatic cells invade syncitium a enclose individual oocytes which entered meiosis
- prenatally in humans (15-22. wg), perinatally in mouse



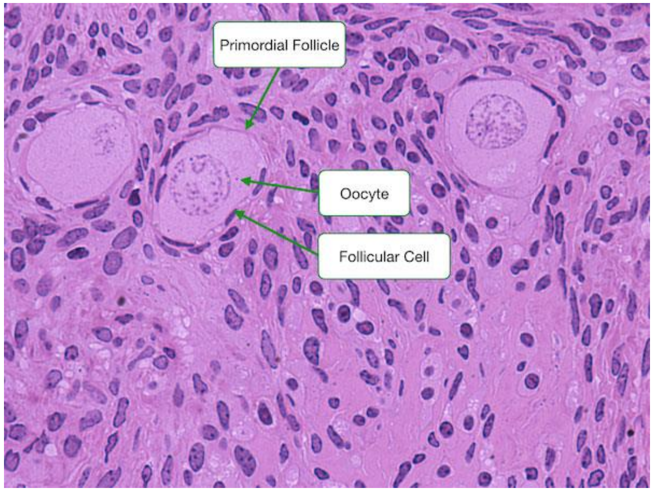
Primordial follicles formation

➢ **Germ cell nest break down**

⬇

primordial follicles (PF)

= diplotene non-growing oocyte surrounded by a single layer of flattened pre-granulosa cells

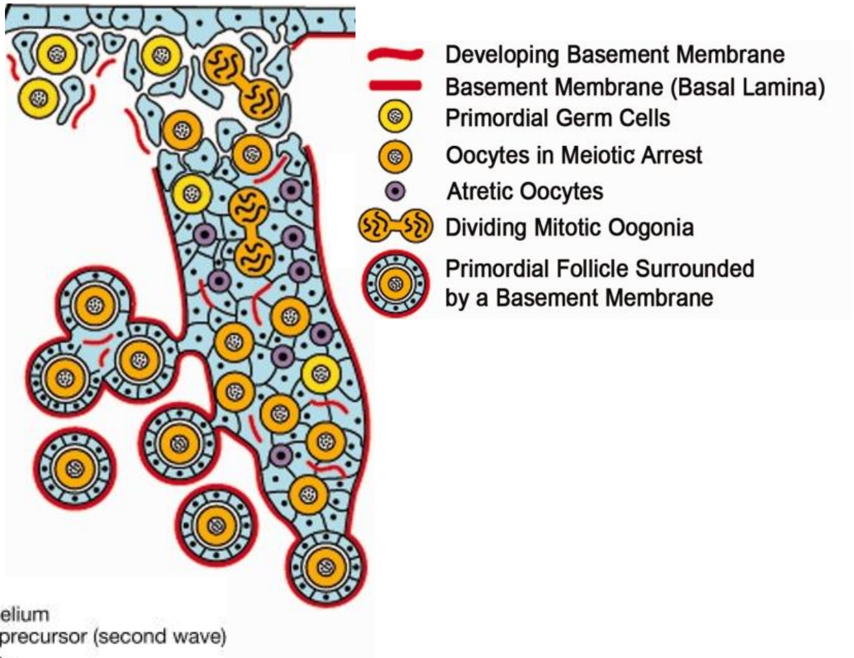


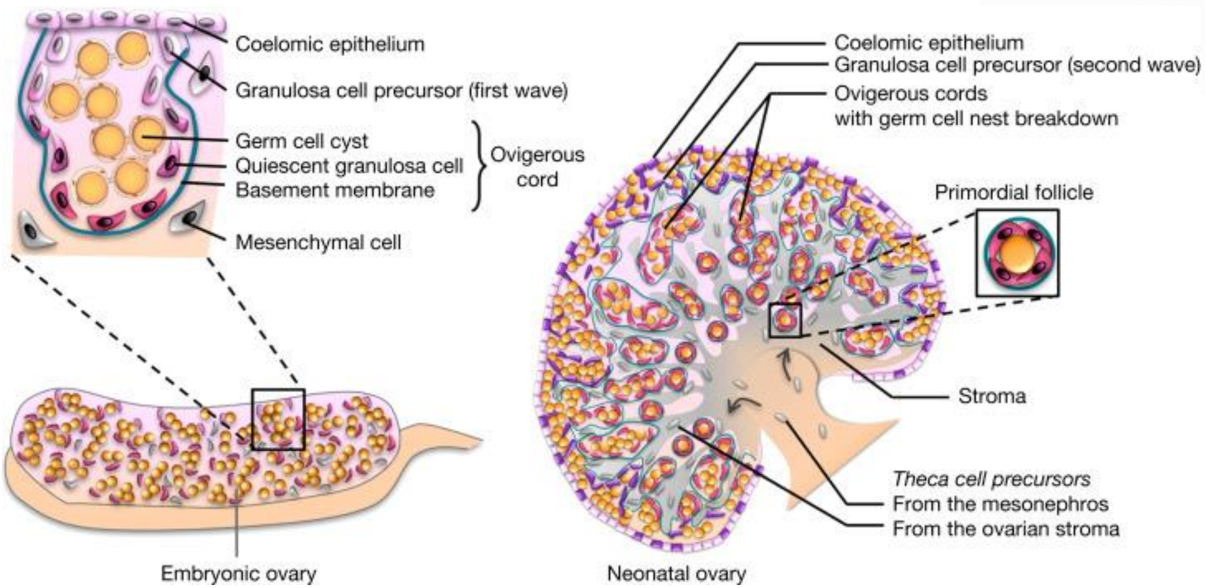
- primordial follicles represent extremely long-living symbiotic unit
  - somatic cell supply germ cells (oocytes) with nutritients and signalling factors
  - oocytes dictate follicular cell function

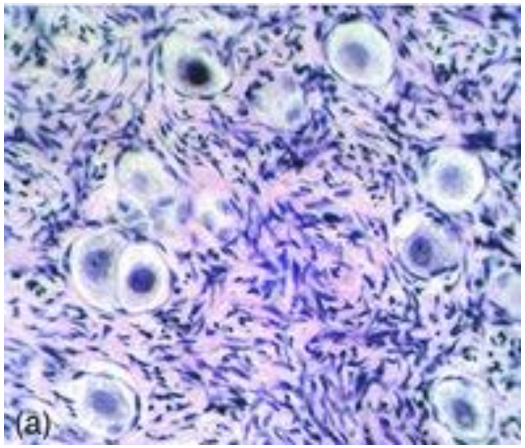
Primordial follicles formation

➤ **Basement membrane**

- thin sheet-like structure that surrounds and protects primodial follicle
- formed from extracellular matrix and mesnchymal cells of indiferent gonad
- components: colagen IV + laminin
  (+ fibronectin only later in antral stadium)

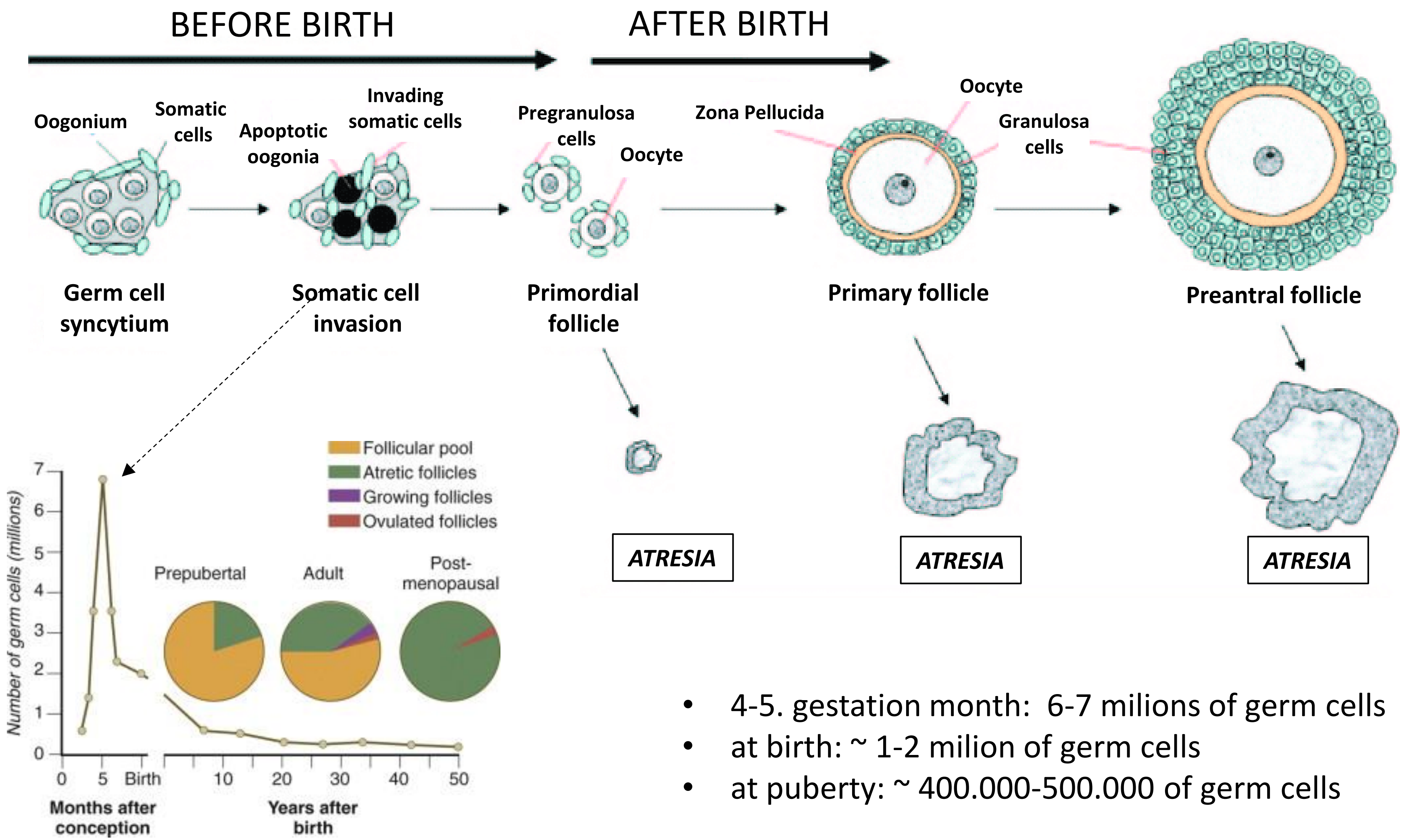






Atresia

- Selective resorption/degeneration of germ cells/follicles during oogenesis



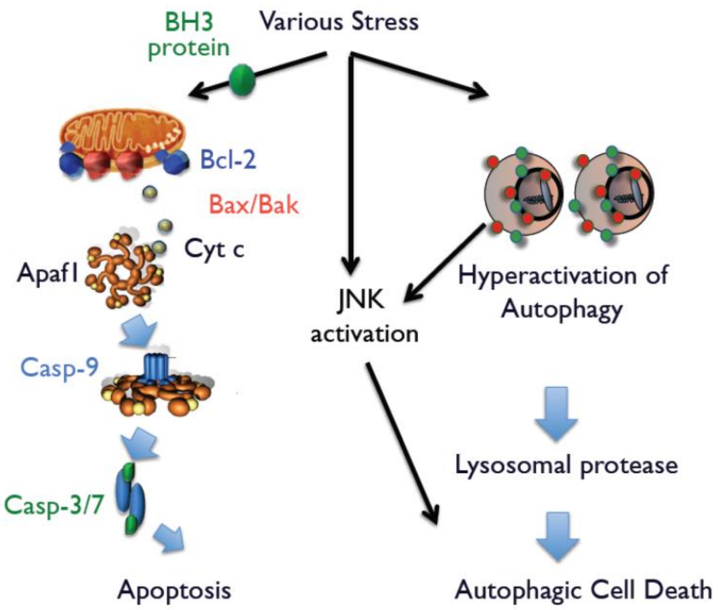
- 4-5. gestation month: 6-7 milions of germ cells
- at birth: ~ 1-2 milion of germ cells
- at puberty: ~ 400.000-500.000 of germ cells

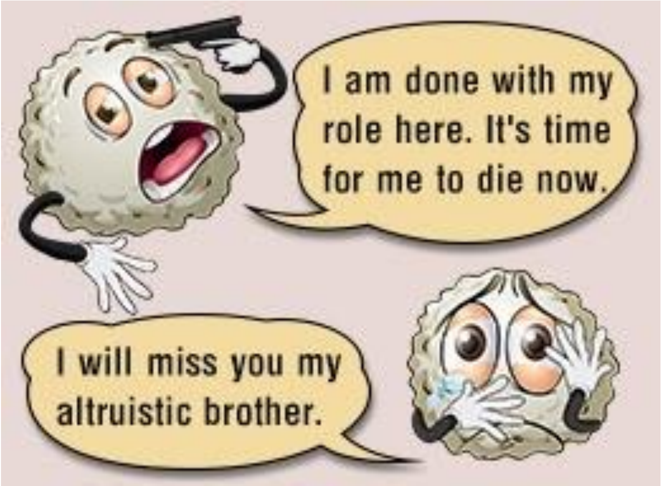
Atresia

- **Cell death mechanism?**
    - apoptosis – rarely observed (rapid progression)?
    - autophagy - increased lysosomal activation detected

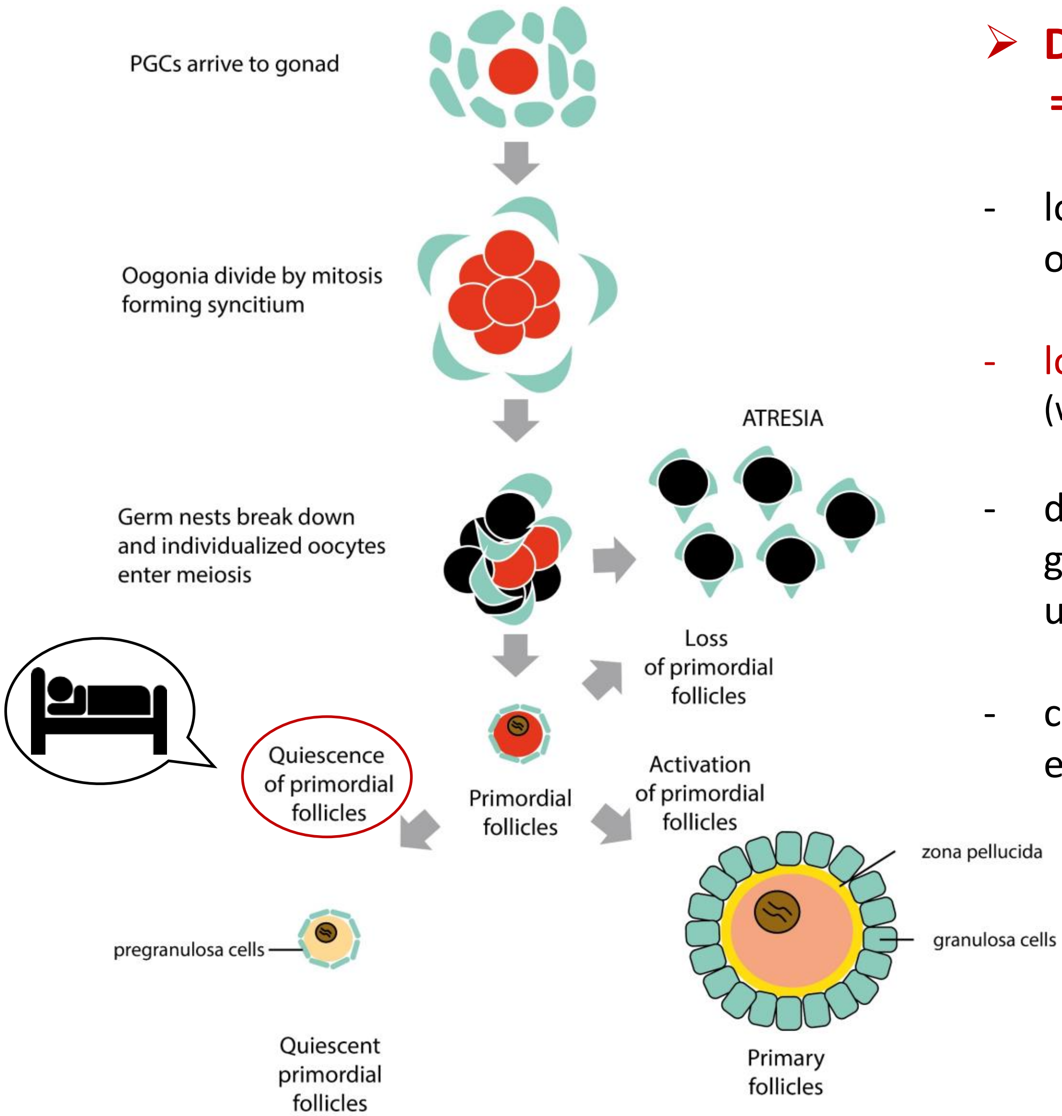
- **Purpose?**
    - Required for germ cell nest breakdown and individualization of primordial follicles?
    - Degenerated cells nurture survivors?
    - Quality control mechanism
      - elimination of defective germ cells

      (e.g. gene mutations, aneuploidy, non-functional mitochondria,...)





Activation of primordial follicles



- **Dormant follicles**
  =„quiescent"/„resting" PFs

- localised in the cortical region of mammalian ovary

- long survival due to local inhibitory signals (weeks, months, yeas, decades in different species)

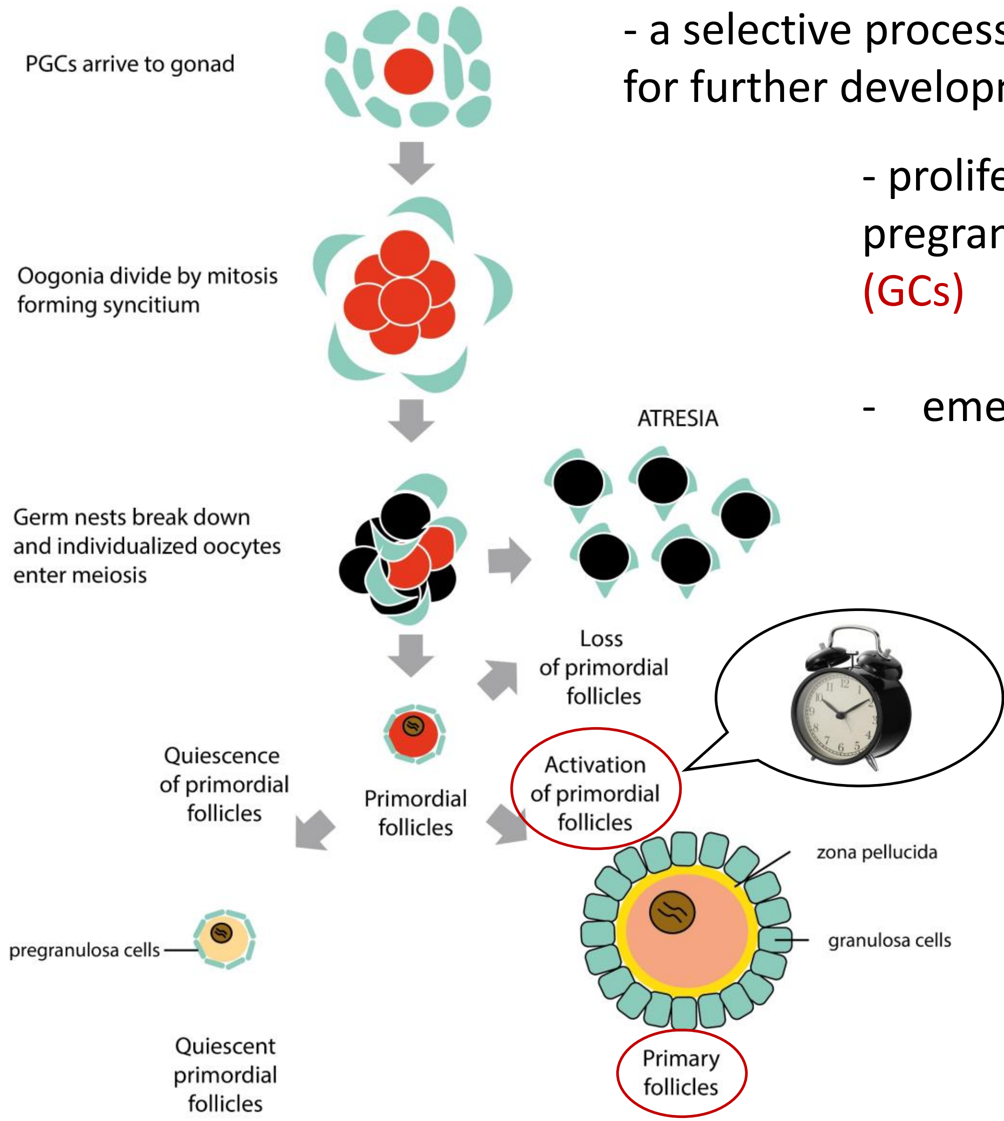
- dormant state sustained until recruited for growth by activating signal or receive signal to undergo atresia (>90% !)

- compact units, relatively resistant to environmental factors a cryopreservation

  - number of PFs defines fertility span

  - exhaustion of „ovarian reserve" (<1.000 primordial follicles) → menopauze

Activation of primordial follicles



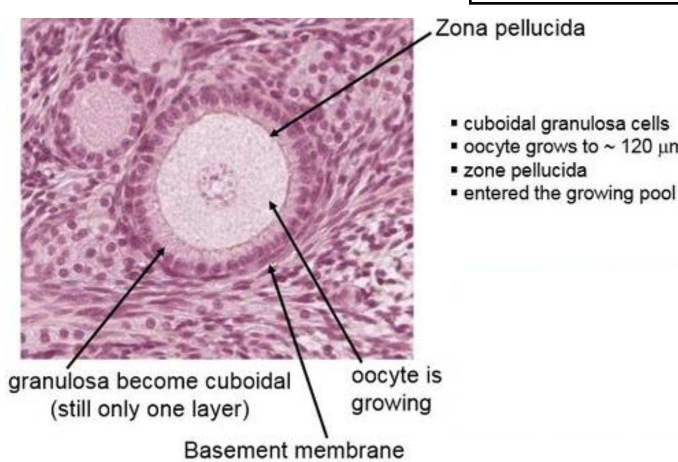
- a selective process by which a dormant PFs are selected for further development in preparation for fertilization

- proliferation and diferentiation of flat pregranulosa cells into cubic granulosa cells (GCs)

- emergence of zona pellucida

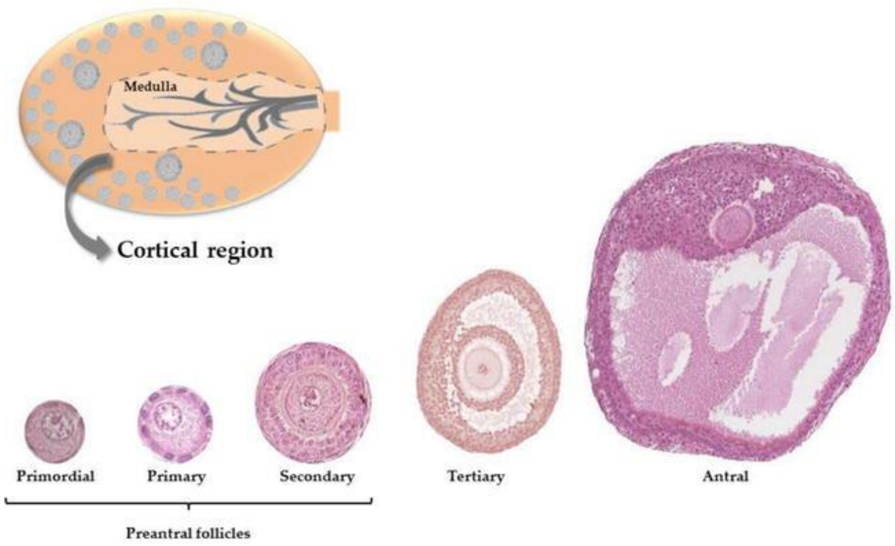
Primary follicle

= single layer of cubic granulosa cells enclosing diplotene oocyte with zona pellucida



- only little portion of PFs is activated (~1.000 per month)

Activation of primordial follicles

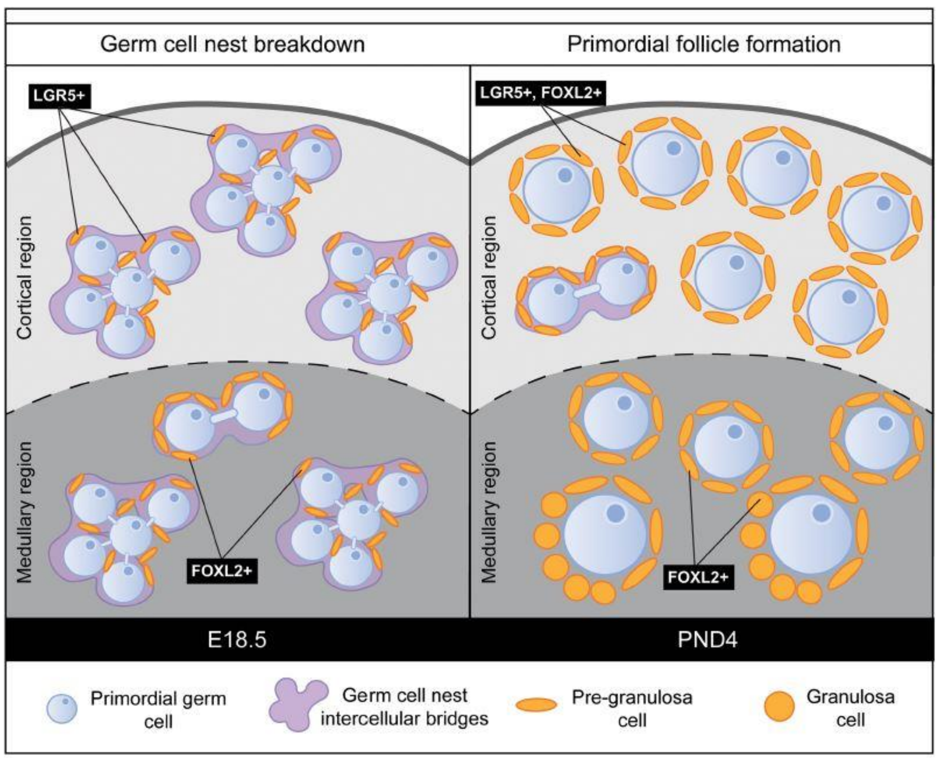


- Activation progresses form medulla to cortex

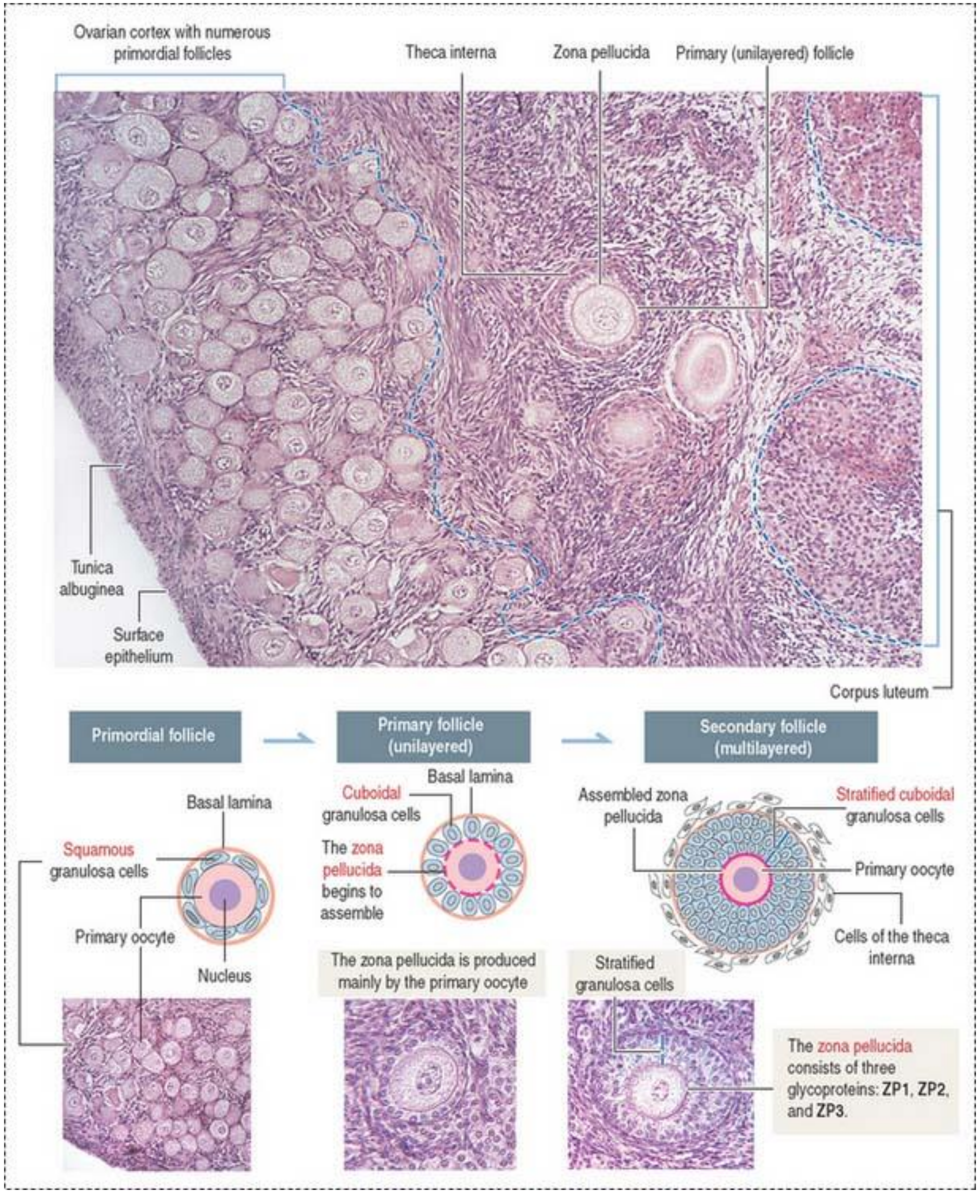
❑ 2 categories of PFs

- Medulla
  - Postnatal activation
  - Fast growth
  - Early specification of preganulosa cells

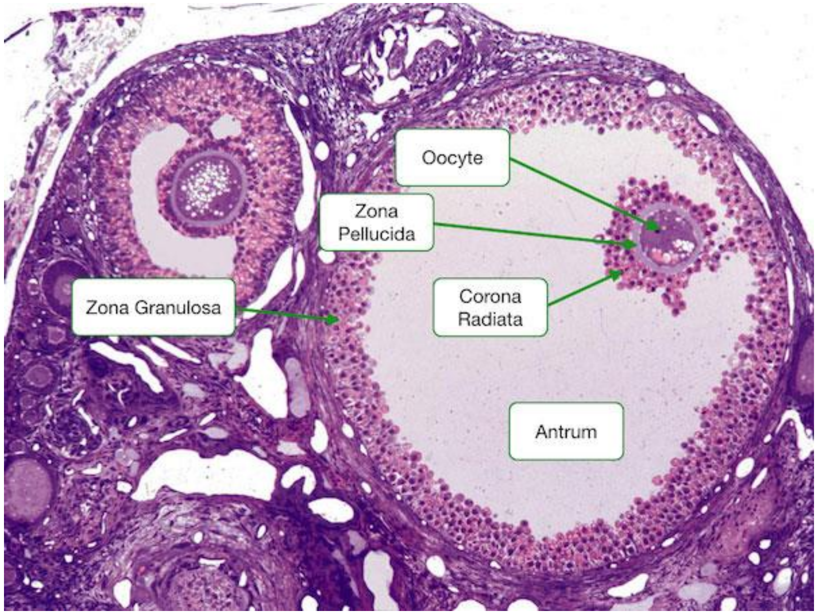
- Cortex
  - Dormant until puberty
  - Periodical activation
  - Late specification of pregranulosa cells

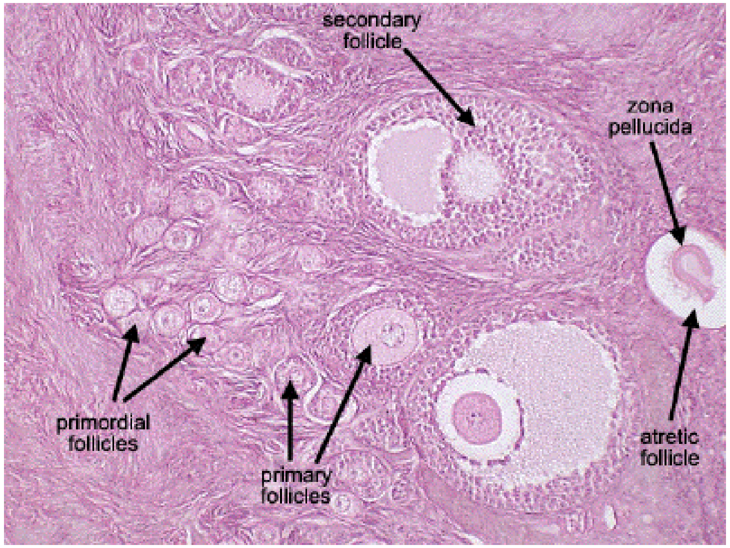


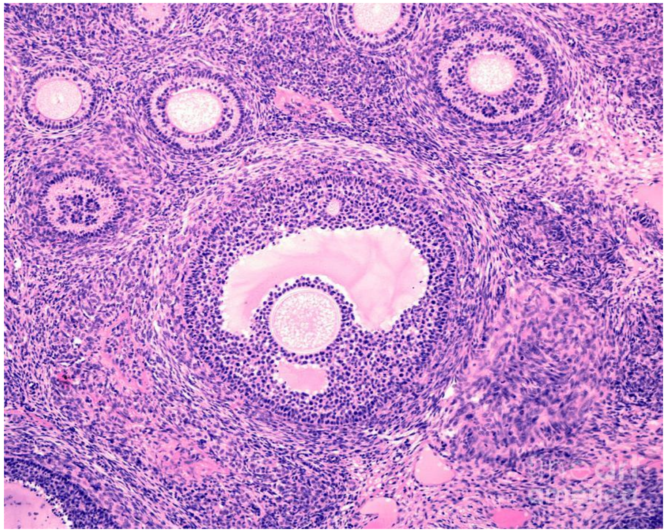
Follicular development



Follicular develoment

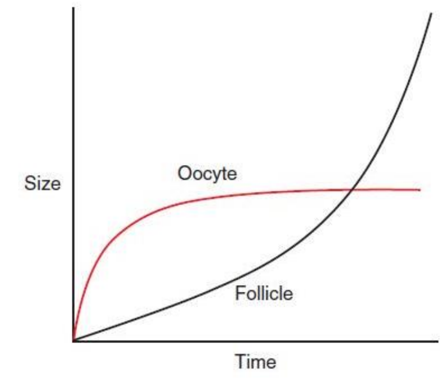


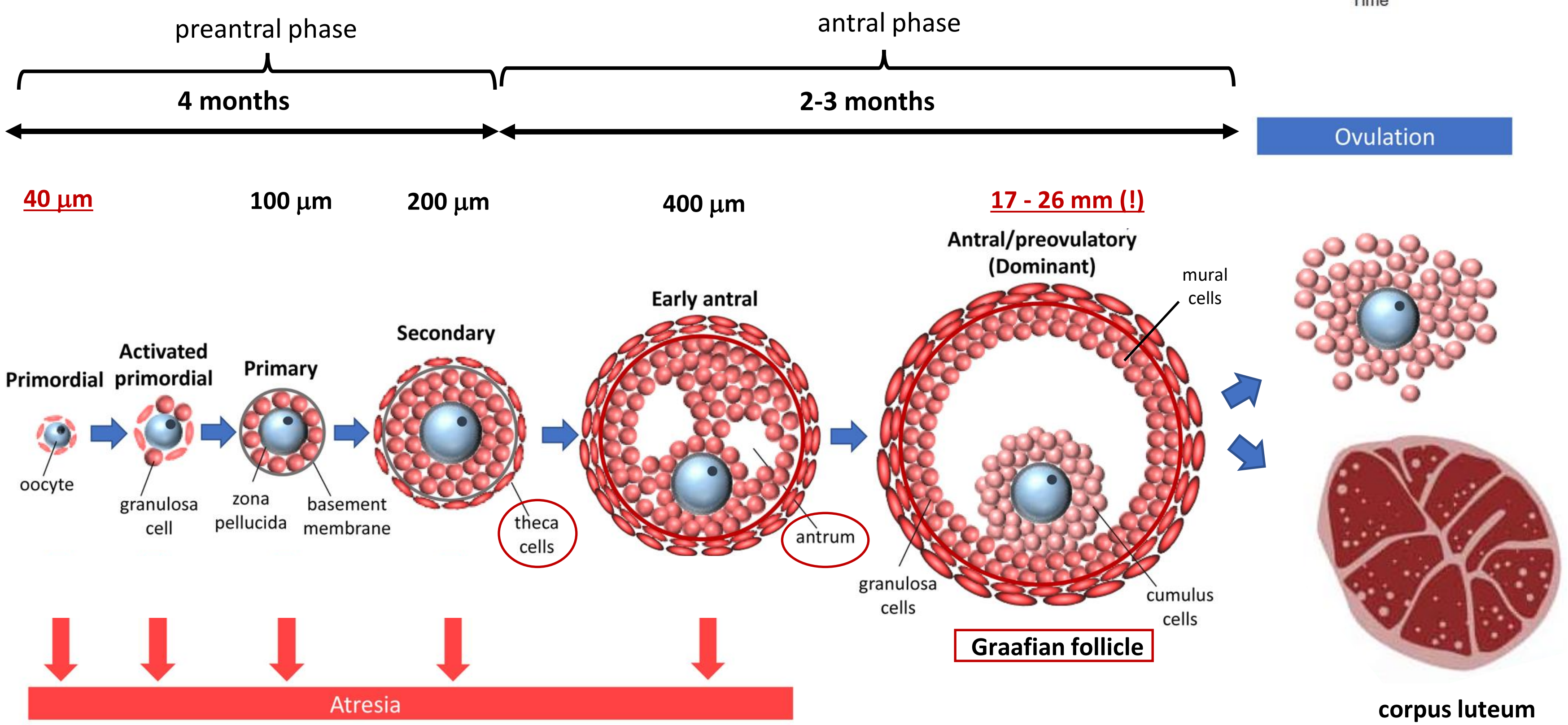




Follicle growth

- continual long-lasting irreverzible process of follicle enlargement
- disproportional growth of follicle and oocyte
- surrounding stroma cells align with basal membrane of secondary follicle an differentiate to **theca cells**
- weakening of cell contacts between granulosa cells produces multiple fluid-filled foci, they expand and coalesce giving rise to large central cavity called **antrum** with **follicular fluid**





Follicle growth

➤ Theca (lat. a case)

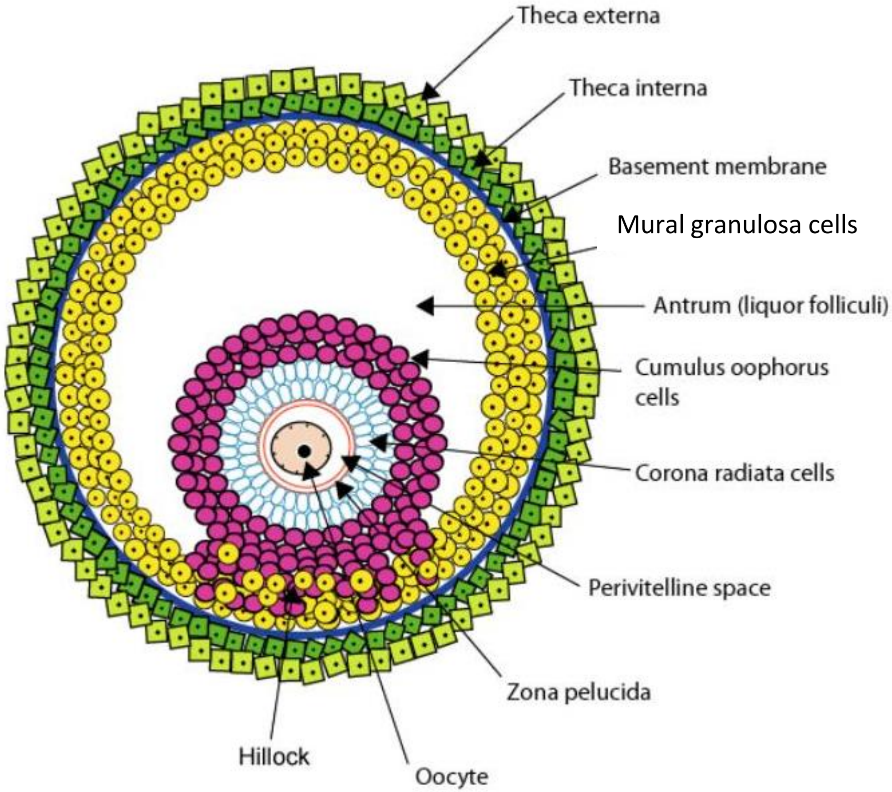
- vascularized somatic layer which provides nutrition, mechanical support and protection to the follicle, and plays the role in follicle ruture during ovulatory process
- recruited from surrounding stromal tissue by factors secrted by activated primary follicle

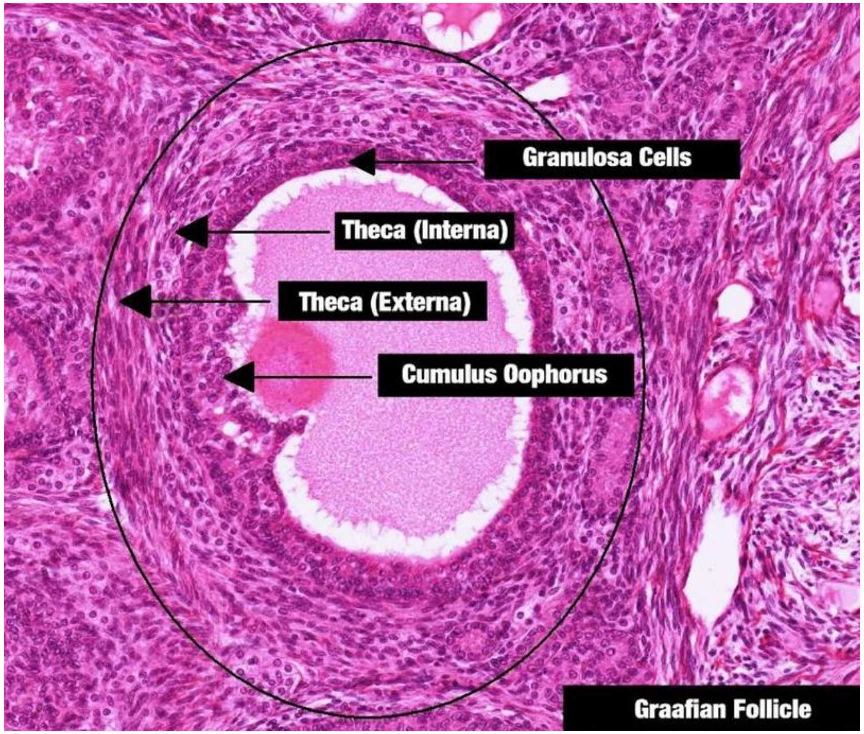
❑ Theca externa

- external part made up of connective tissue
- smooth muscle-like cells and fibroblast-like cells producing ECM

❑ Theca interna

- inner layer in contact with basal membrane
- endocrine cells responsible for synthesizing androgens

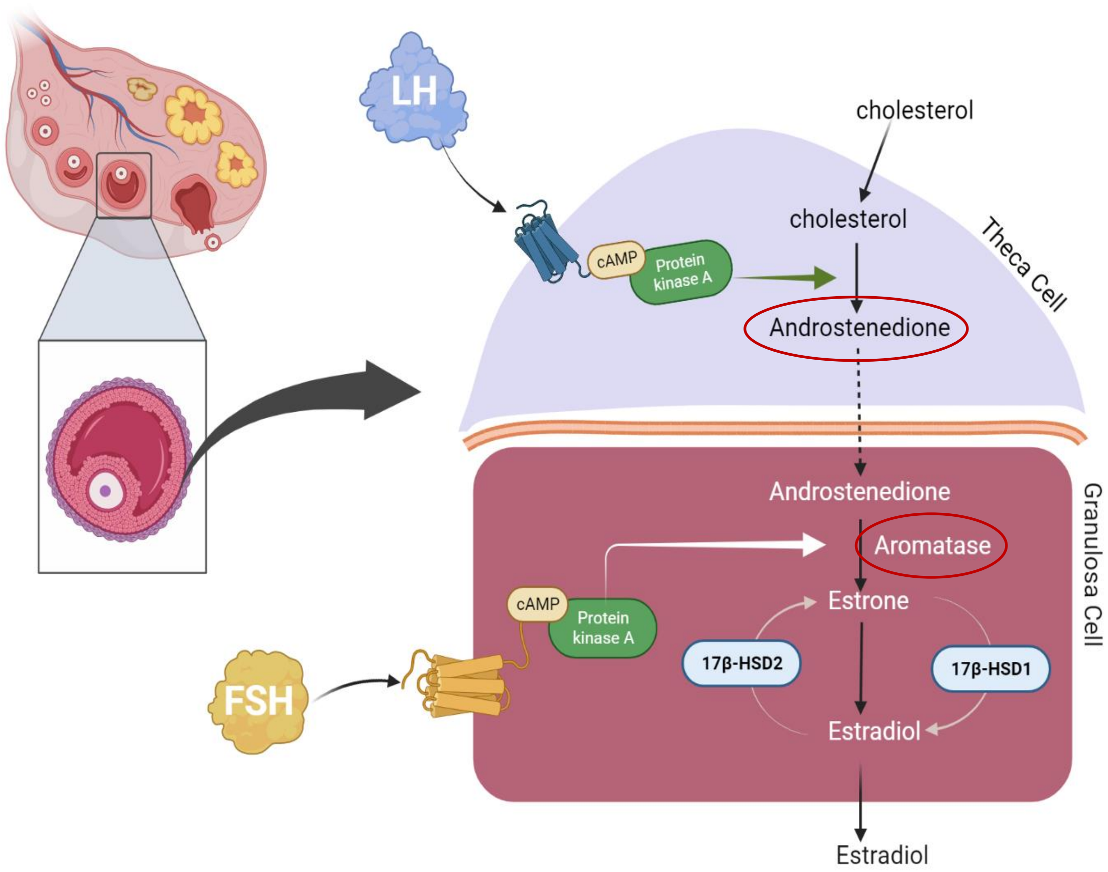




Follicle growth

„2 hormones – 2 cells concept"

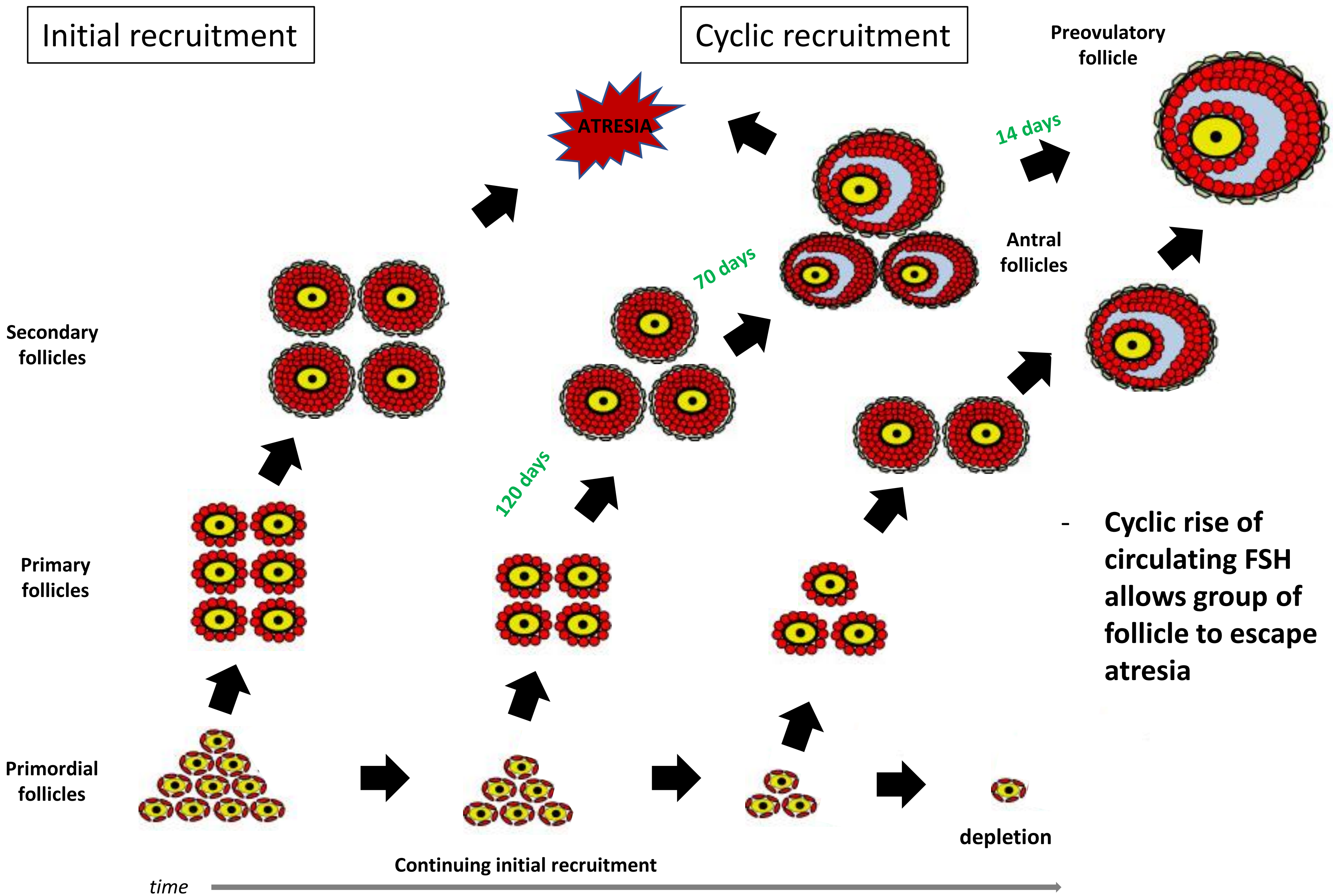
Amstrong 1979



- under control of LH, thecal cells synthesize androgens

- stimulation of FSH receptors in granulosa cells leads to activation of **aromatase**, which converts androgens to estrogens

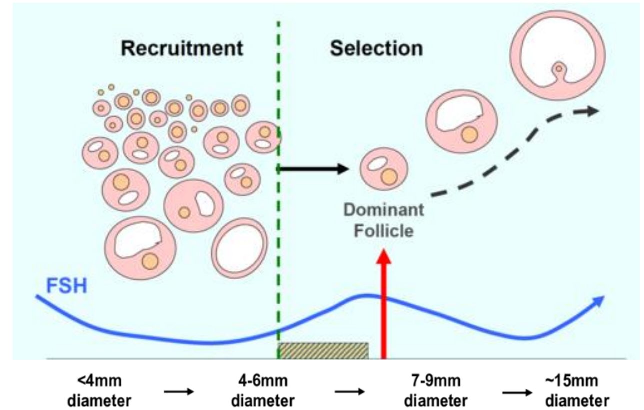
Follicle selection

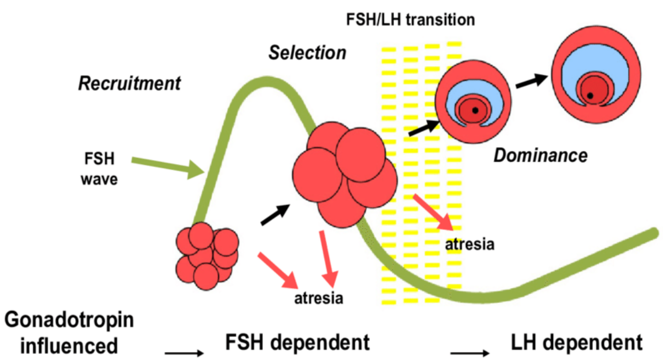


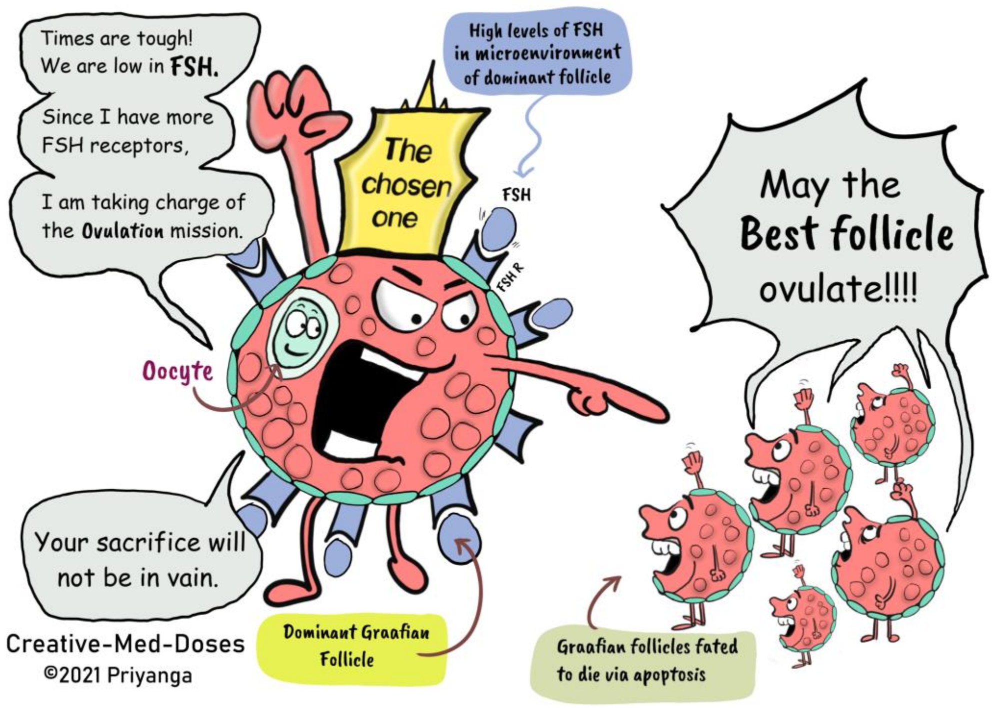
- Cyclic rise of circulating FSH allows group of follicle to escape atresia

Follicle selection

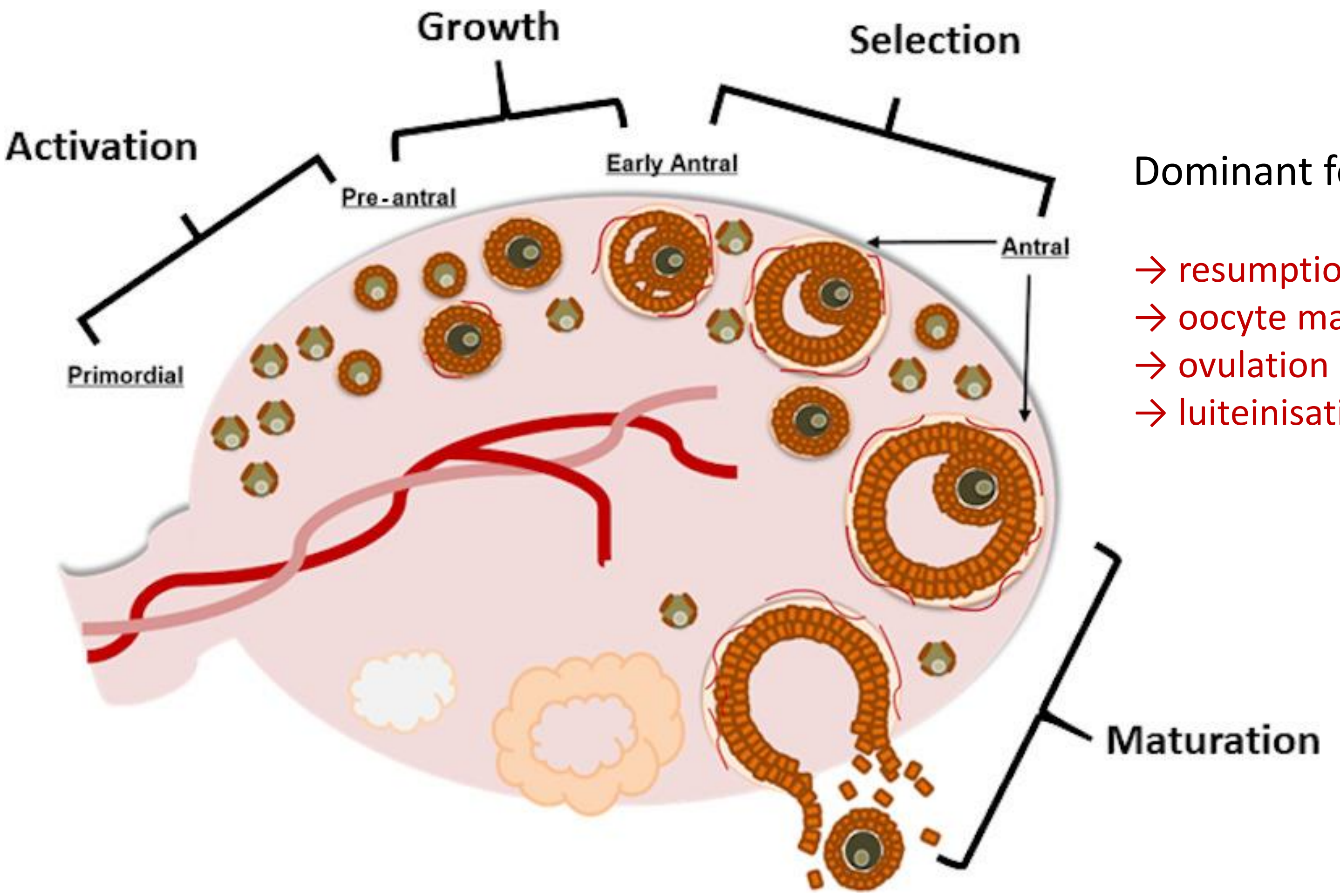
- dominant follicle selection based of abundance of FSH receptors (FSHR) at granulosa cells
- rollicle with the highest number of FSHR gows faster
- dominant follicle secrete estrogens and inhibin → lower pituitary FSH release → the growth of other follicles slowed → apoptosis







Follicle selection



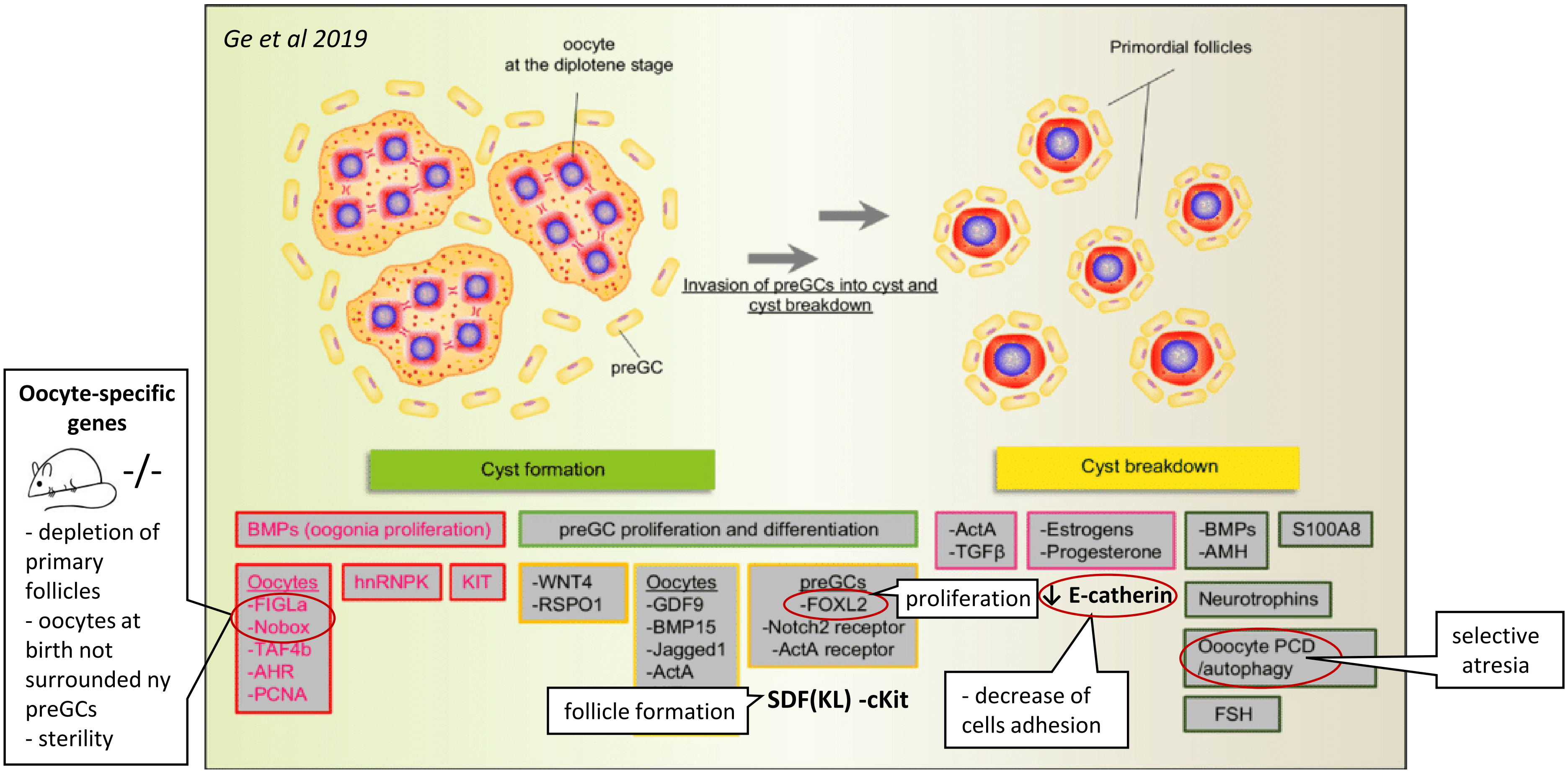
Dominant follicle faces LH peak

→ resumption of meiosis
→ oocyte maturation
→ ovulation
→ luiteinisation of GCs

Regulation of folliculogenesis

➢ Regulation of primordial follicles formation

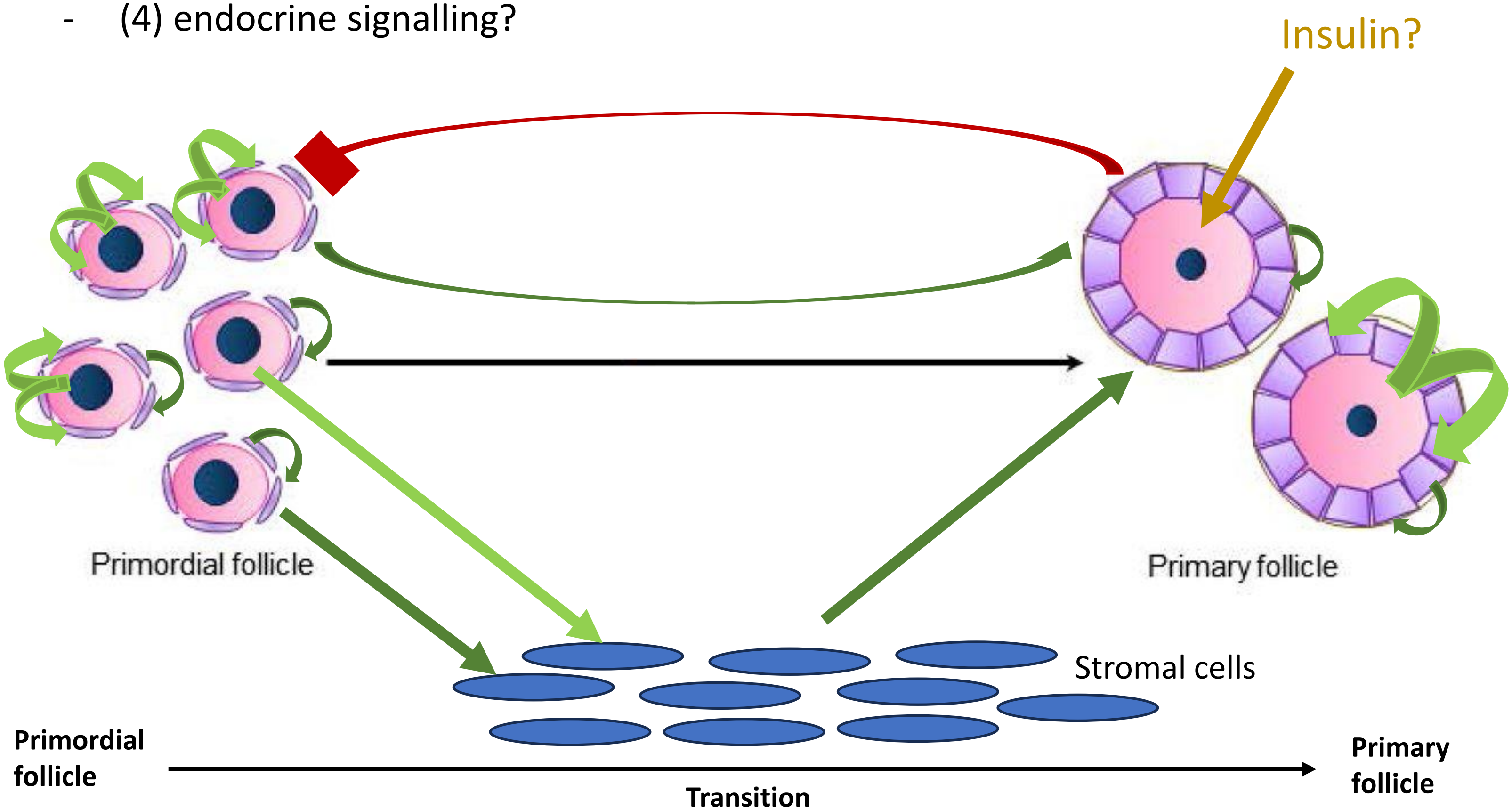
- coordinated expression of genes in both somatic and germ cells
- interplay of secreted factors



Regulation of folliculogenesis

➢ Regulation of primordial follicles activation

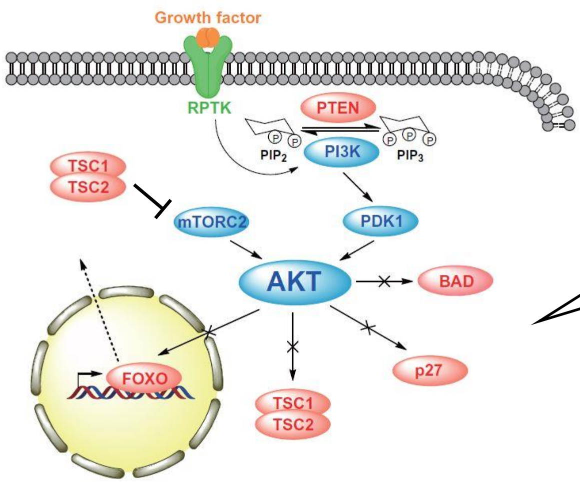
- not completely understood
- ratio of local activation and inhibition signals
  - (1) oocyte-secreted factors
  - (2) granulosa cells-secreted factors
  - (3) paracrine inhibition signals from other growing follicles
  - (4) endocrine signalling?



Regulation of folliculogenesis

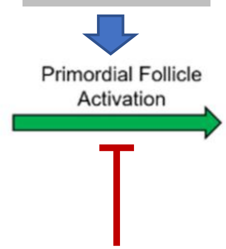
➢ Regulation of primordial follicles activation

❖ PI3K signalling



- growth factors stimulate receptor tyrosine kinase which phophorylates-activates PI3K
- PIP3 formed from PIP2 binds and activates PDK1
- PDK1 phosphorylates Akt
- Akt inactivates transcription factor Fox (translocation from nucleus)

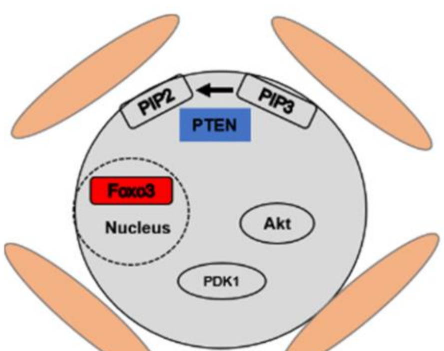
PIP3
Akt
PDK1
mTORC



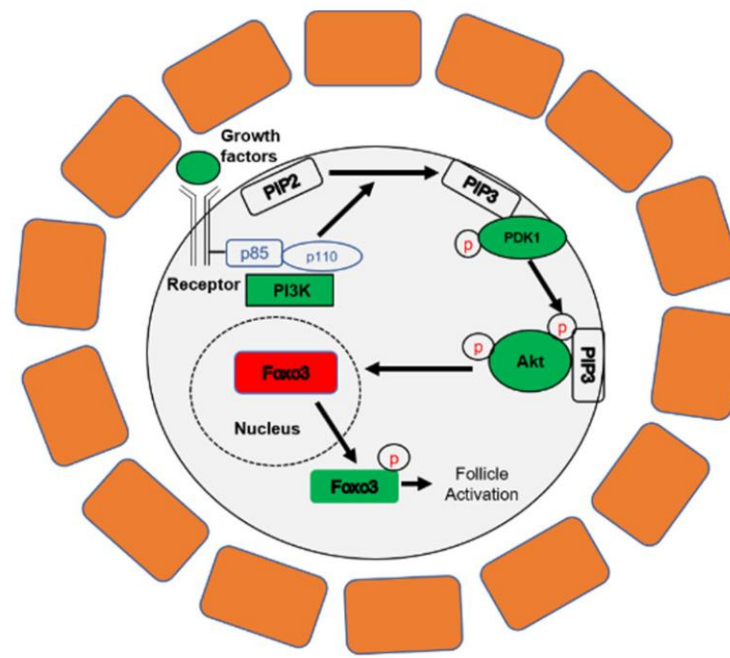
PTEN
FOXO3
P27
TSC1/2

Pten-/-
Foxo3-/-
Pdk1-/-

- premature activation
- depletion of primordial follicles
- premature ovarian failure (inferility)



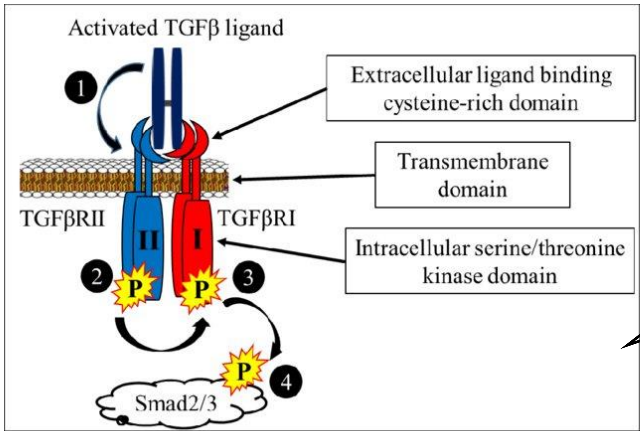
A dormant follicle

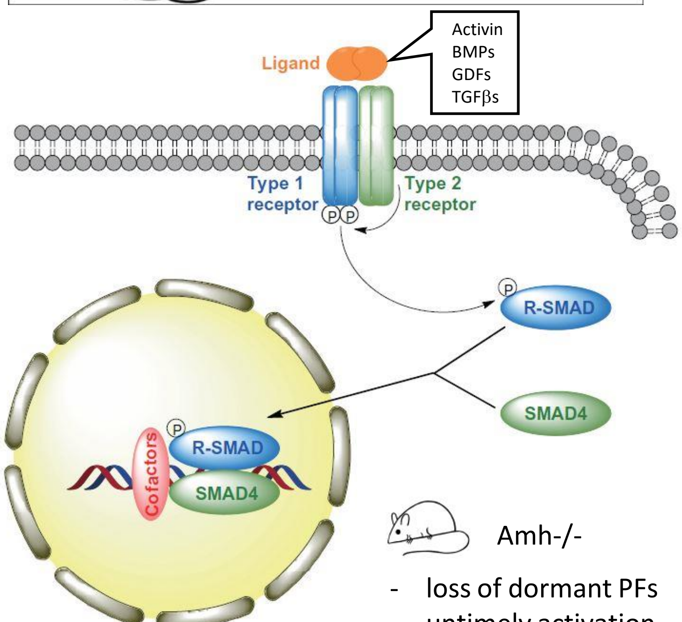


An activated follicle

Regulation of folliculogenesis

➢ Regulation of primordial follicles activation





Amh-/-

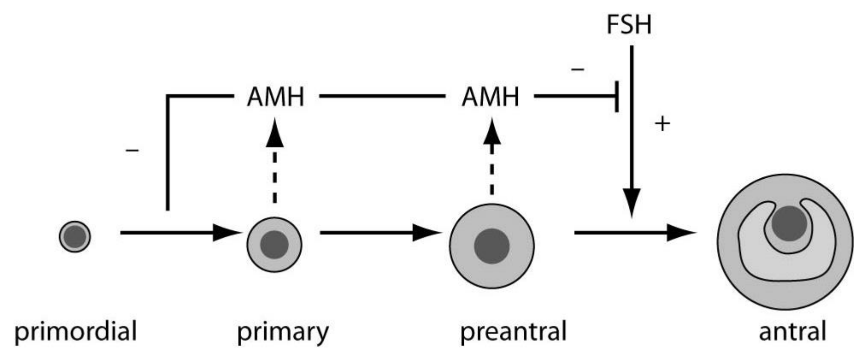
- loss of dormant PFs
- untimely activation

❖ TGFβ signalling

- binding of hetero-/homo-dimeric ligand on outer domain of receptor kinase I and II
- formation of heterotetrameric complex
- receptor´s subunit II phophorylates subunit I which activates SMAD pathway regulating transcription

▪ AMH – Antimüllerian hormone

- produced by GCs of preantral follicles
- negative regulator of folliculogenesis
  - prevents activation and growth of PFs
  - prevents proliferation and diferentiation of GCs
  - inhibits aromatase
  - suppresses FSH-stimulated growth of antral follicles



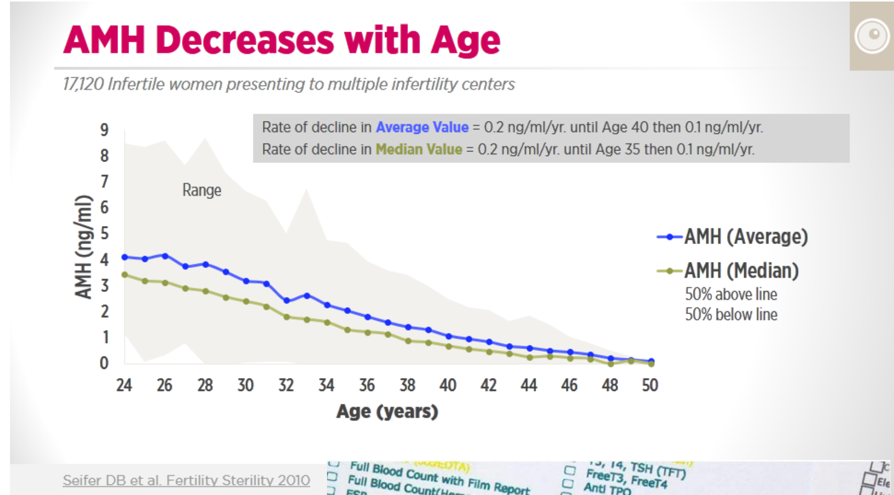
Regulation of folliculogenesis

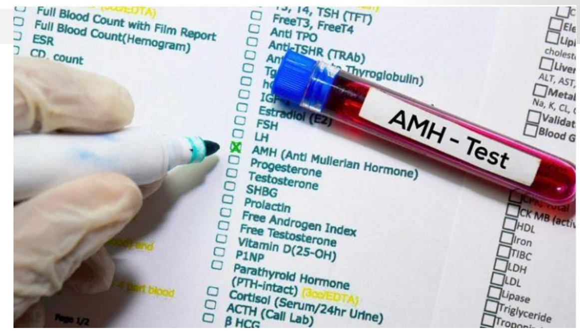
- **AMH as a clinical marker**

- indicates presence of dormant PFs cohort during reproductive lifespan
- the level decreases with reproductive aging in correlation with the exhausion of ovarian reserve

⇩

- circulating level used a **predictor of response to hormonal stimulation** in ART
- extremely high levels imply risk of PCOS and OHSS
- inappropriately advertised as a marker of female fertility (!)
- contraception potential?



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Durable contraception in the female domestic cat using viral-vectored delivery of a feline anti-Müllerian hormone transgene

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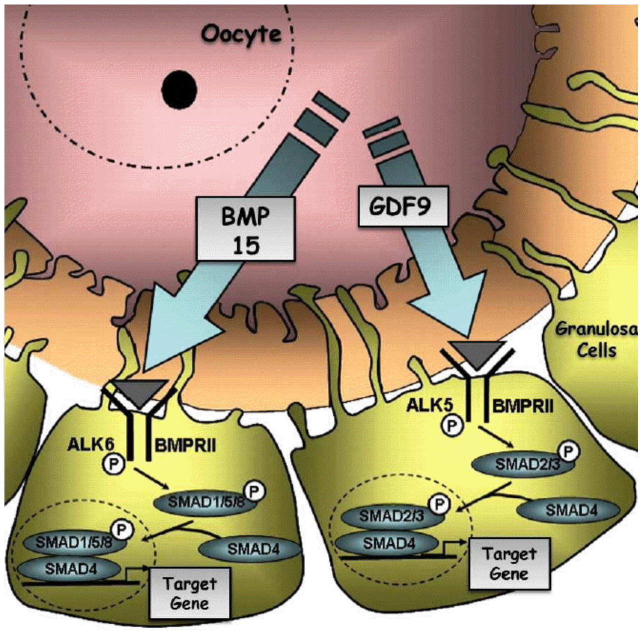
Check for updates

Lindsey M. Vansandt[1], Marie-Charlotte Meinsohn[2], Philippe Godin[2], Nicholas Nagykery[2], Natalie Sicher[2], Motohiro Kano[2], Aki Kashiwagi[2], Maeva Chauvin[2], Hatice D. Saatcioglu[2], Julie L. Barnes[1], Amy G. Miller[1], Amy K. Thompson[1], Helen L. Bateman[1], Elizabeth M. Donelan[1], Raquel González[1], Jackie Newsom[1], Guangping Gao[3], Patricia K. Donahoe[2], Dan Wang[3], William F. Swanson[1] & David Pépin[2]

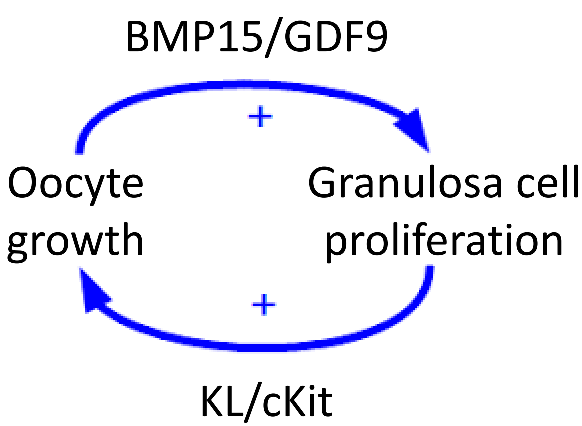
Regulation of folliculogenesis

➢ Regulation of primordial follicles activation and growth

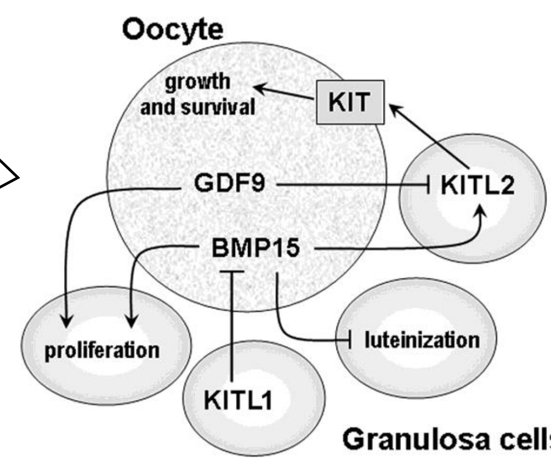


- **GDF9 (Growth differentiation factor 9)**
- **BMP15 (Bone morphogenic protein 15)**

- oocyte-secreted factors from TGFβ family of growth factors
- synergic activity (formation of homo-/hetero-dimers)
- major positive regulators of GCs growth, proliferation and diferentiation
- inhibition of apoptosis, stimulation of secretion of paracrine growth factors in GCs
- regulatory feedback loop promoting follicule growth



- Oocyte-secreted factors stimulate GCs proliferation and secretion of KL (Kit ligand/SDF)
- KL activates oocyte´s c-Kit kinase promoting oocyte´s growth



Regulation of folliculogenesis

➢ Regulation of primordial follicles activation and growth

- Gdf9-/-
  - development arrested in primary follicle stage
  - infertility
- Bmp15-/-
  - decreased ovulation frequency
  - subfertility
- Gdf9+/-Bmp15-/-
  - low numebr of growing follicles
  - infertility
- BM15 overexpression – depletion of follciles

- Bmp15-/- i Gdf9-/-
  - infertility
- Bmp15+/- i Gdf9+/-
- mutation fo BMP15 receptor
- immunisation against BMP15

(High fecundity)

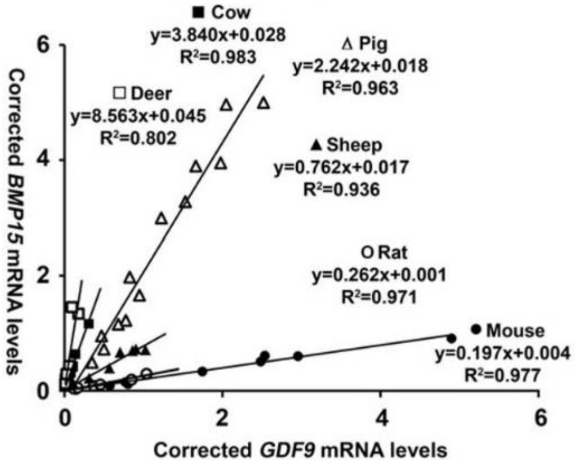
- mutace GDF9/BMP15 spojené s předčasným ovariálním selhání a neplodností
- GDF9 mutace spojené s familiárním výskytem dyzigotických dvojčat
- Abnormlání exprese BM15/GDF9 u PCOS
- Polymorfismus BMP15 u OHSS

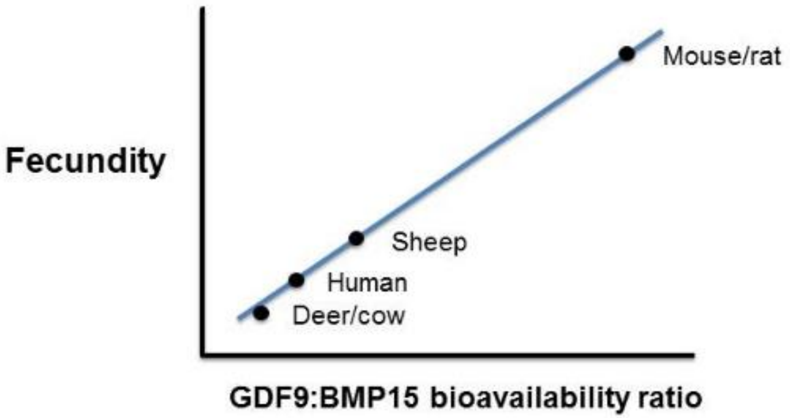
Differences between mono- a poly-ovulatory species?

Regulation of folliculogenesis

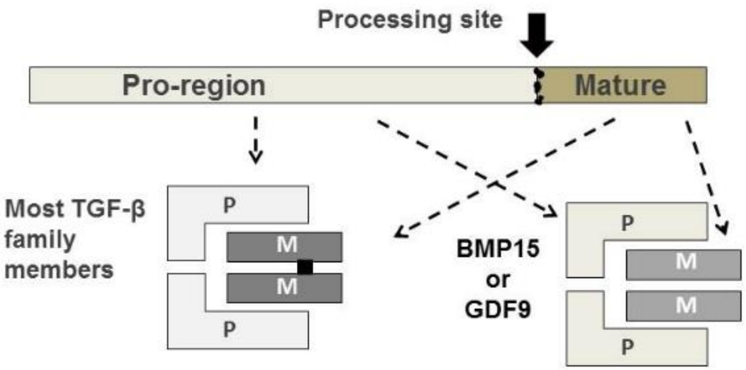
➢ Regulation of primordial follicles activation and growth

Robert Gilchrist





Poly-ovulatory species have higher GDF9:BMP15 ratio than mono-ovulatory species



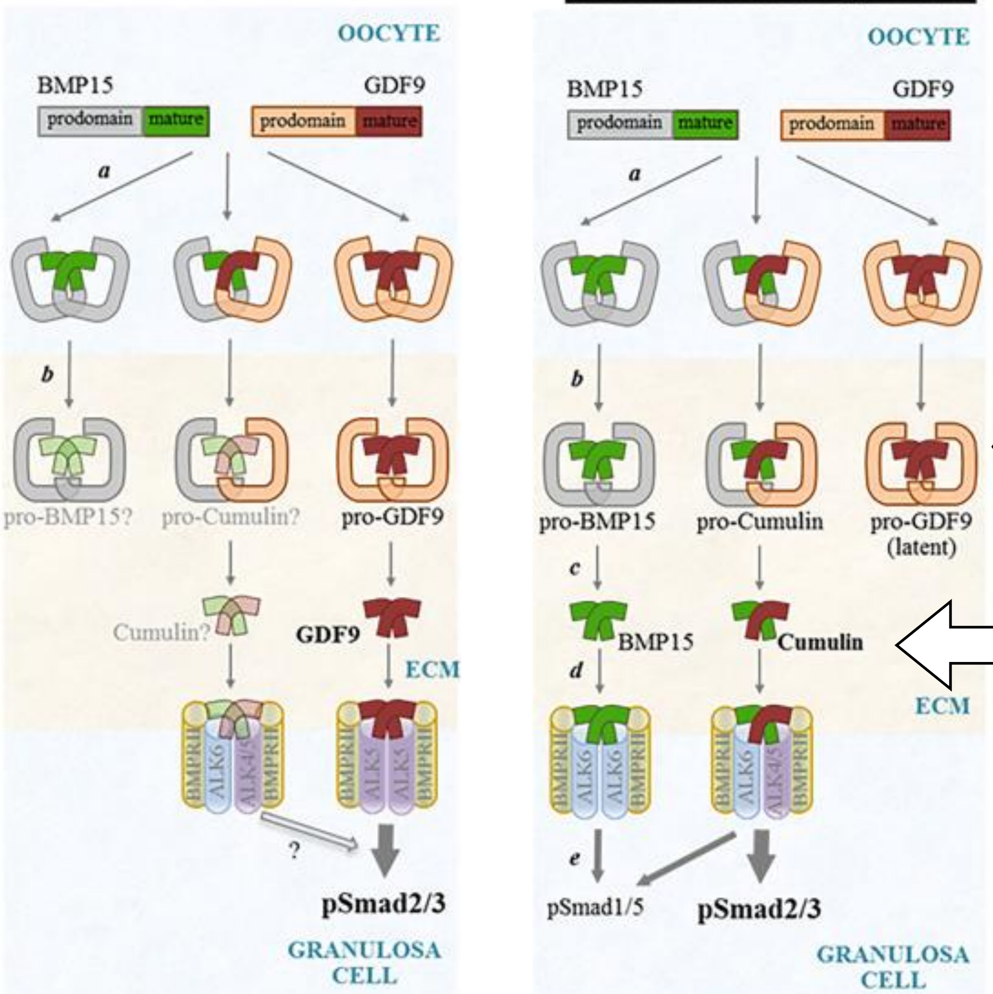
Non-covalent homo/heterodimer

Regulation of folliculogenesis

- **Regulation of primordial follicles activation and growth**

Robert Gilchrist

Poly-ovular species | **Mono-ovular species**



Single point mutation causes latency of human GDF9

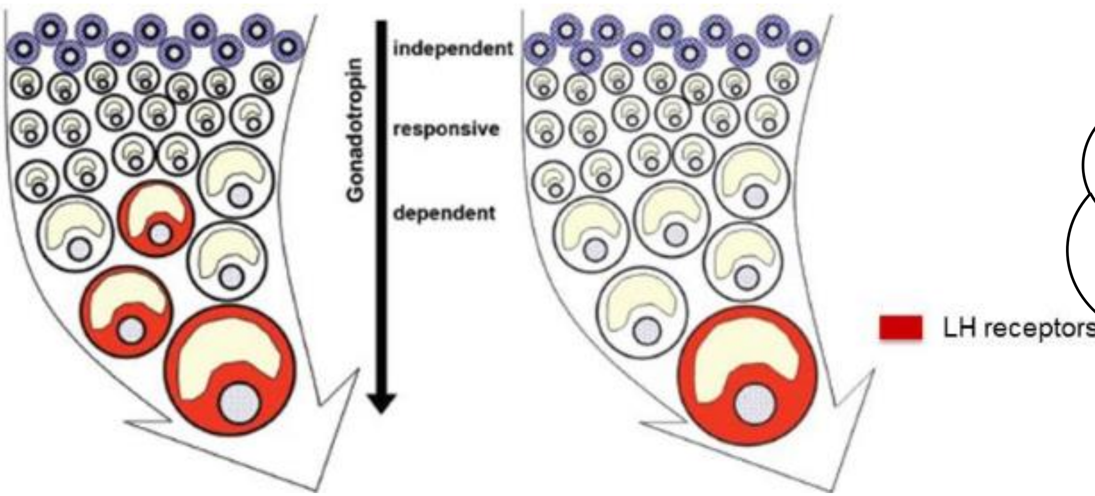
Supplementation of IVM media with recombinant **cumulin**

Regulation of folliculogenesis

➢ **Regulation of primordial follicles activation and growth**

Availability of BMP15 and altered ratio of GDF9:BMP15 heterodimer changes the timing of LH receptor expression

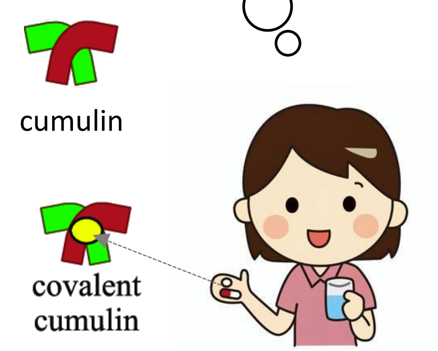
Robert Gilchrist



Reduced level of BMP15

Normal level of BMP15

Development of low-molecular drug promoting short-term BMP15+GDF9 pairing would allow natural selection of multiple best quality oocyte from the cohort for IVF purpose...



- Smaller and more follicles acquire LH receptors
- More follicles „see“ the LH surge and are selected for ovulation

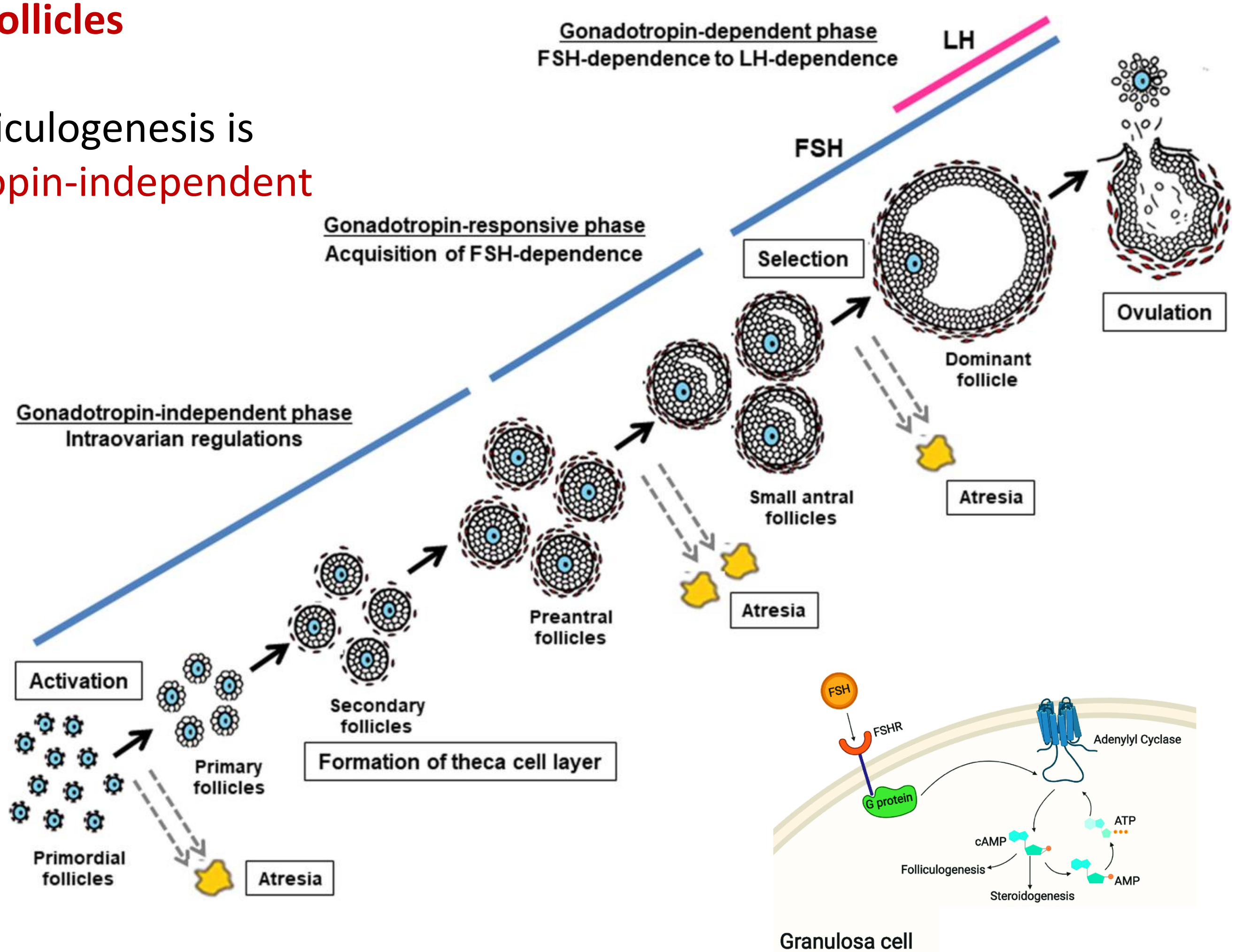
Increased fertility

Regulation of folliculogenesis

➢ Role of gonadotropins

- **stimulation of antral follicles**

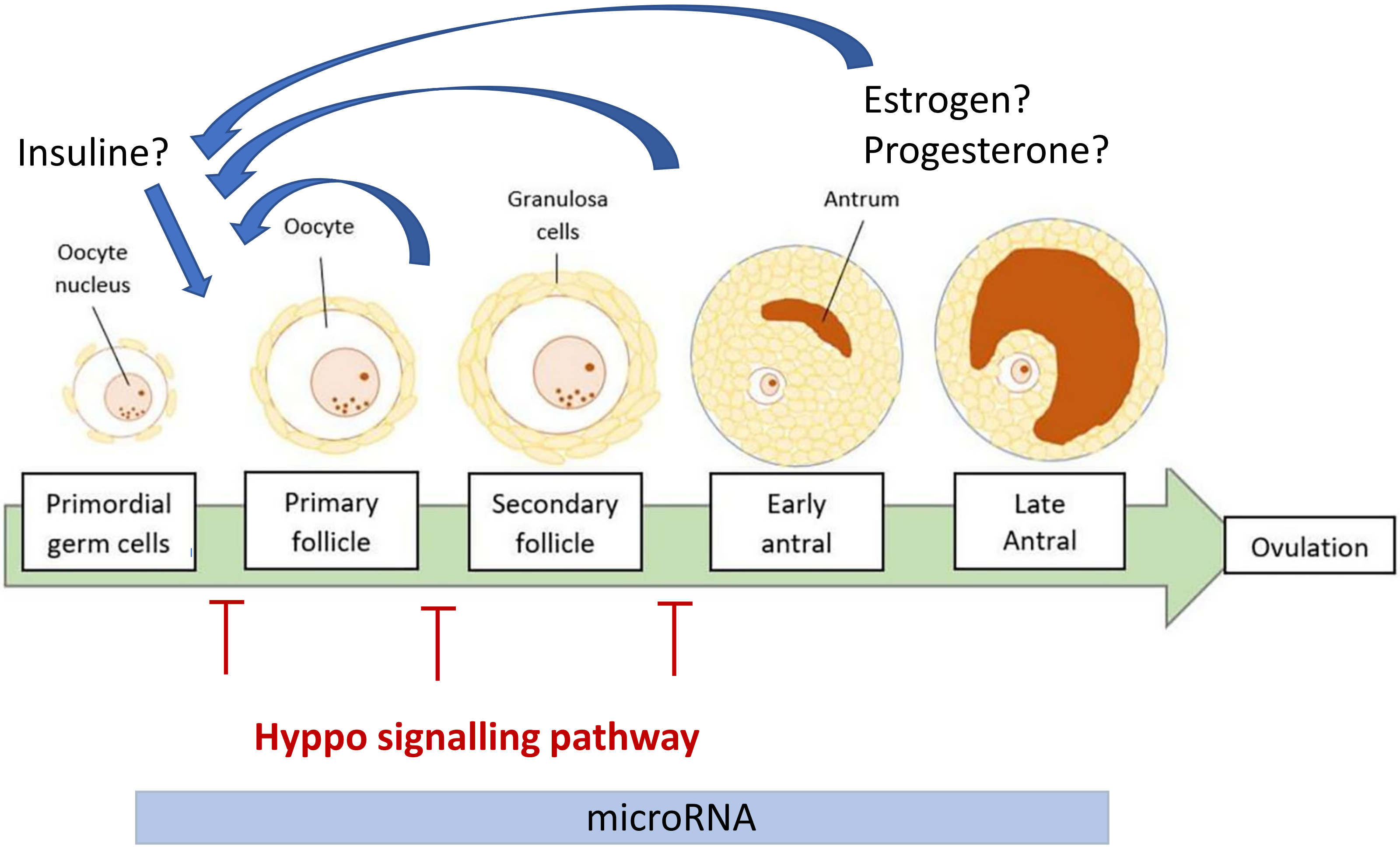
- preantral phase of folliculogenesis is regarded as gonadotropin-independent



BUT

some data indicate that in prenatral stage FSH effect is transmitted via local factors and acts in synergy with paracrine factors produced by ovarian tissue

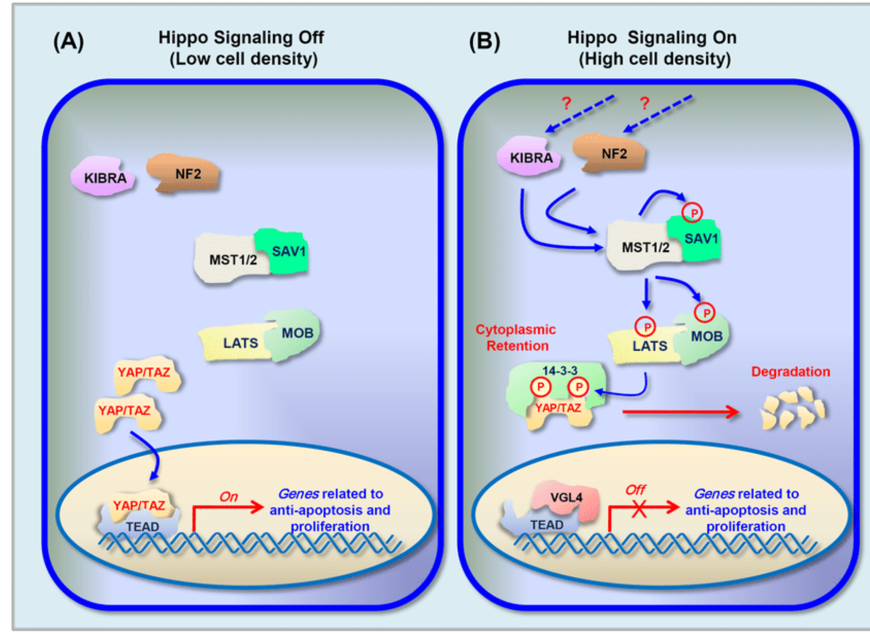
Regulation of folliculogenesis

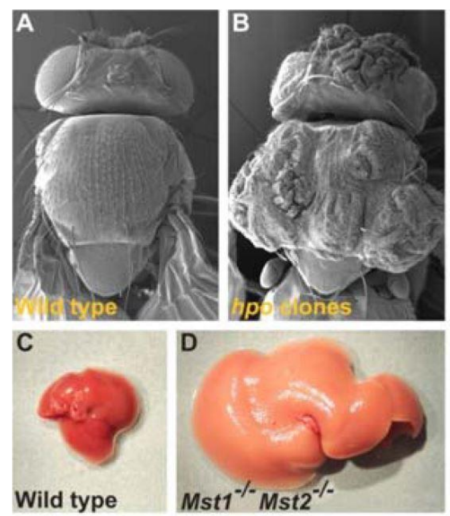


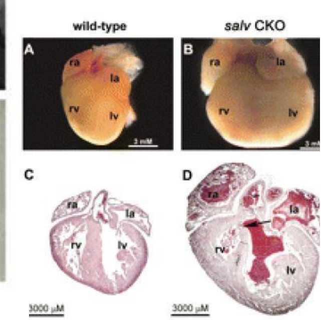
Regulation of folliculogenesis

❖ Hyppo signalling

- Tumor supresor signalling pathway regulating organ size
- limits proliferation and induced apoptosis during organogenesis
- dysregulation leads to tissue overgrowth
- Hpg gene (MST in mammals) named after mutant Drosophyla phenotype which head resembles a hippo
- **normal follicle growth requires suppression of Hyppo pathway**



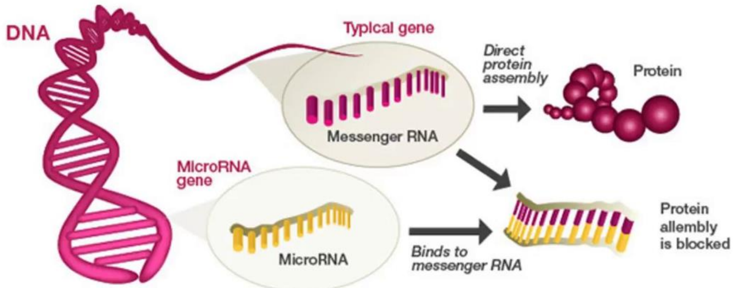


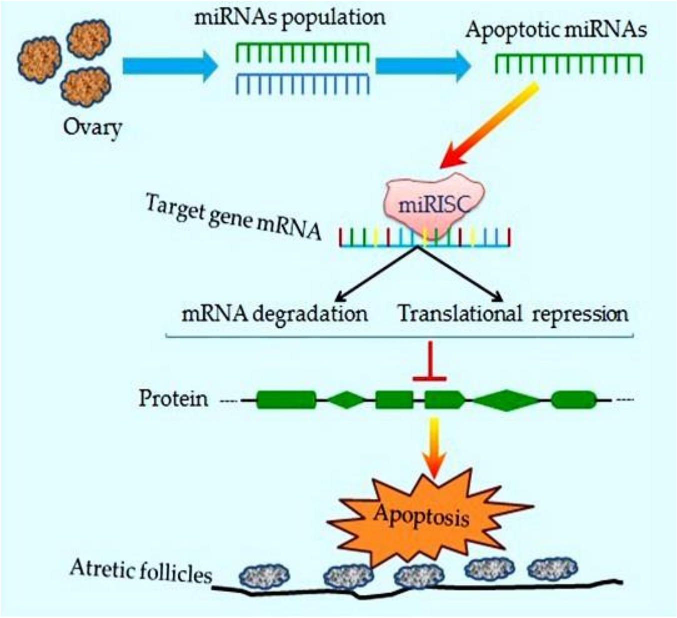


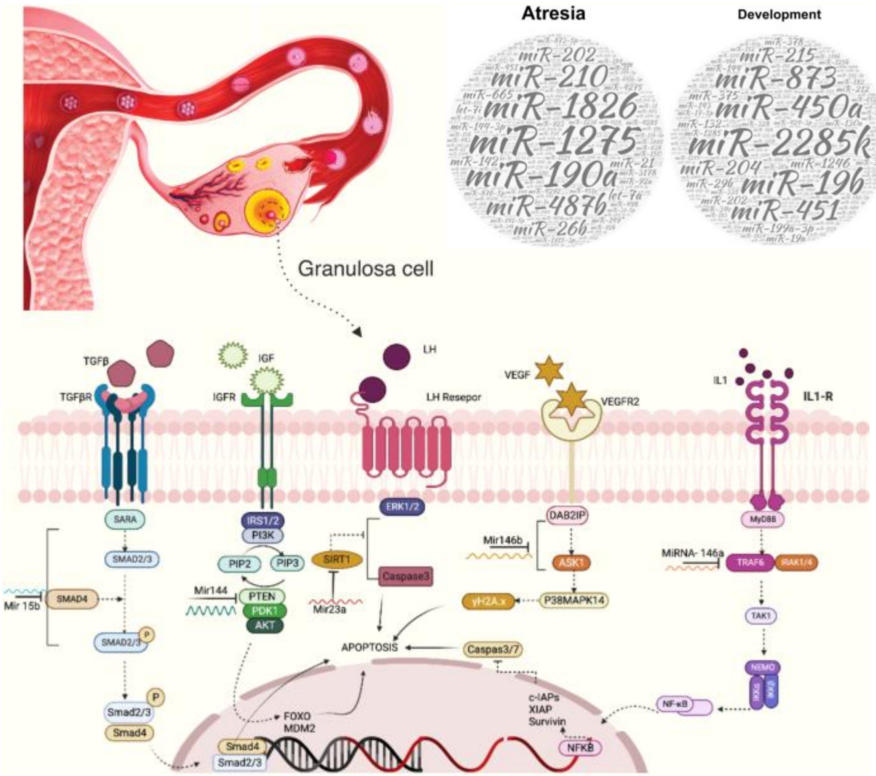
Regulation of folliculogenesis

❖ microRNA

- small non-coding RNAs involved in regulation of gene expression in ovarian cells
- influence development of gonads, folliculogenesis, ovulation, steroidogenesis, luteinisation and apoptosis
- stage-specific oocyte/GCs miRNA profiles
- pathophysiological miRNA profiles







Reviewed in *Nouri et al., Cell Commucation and signaling, 2022*

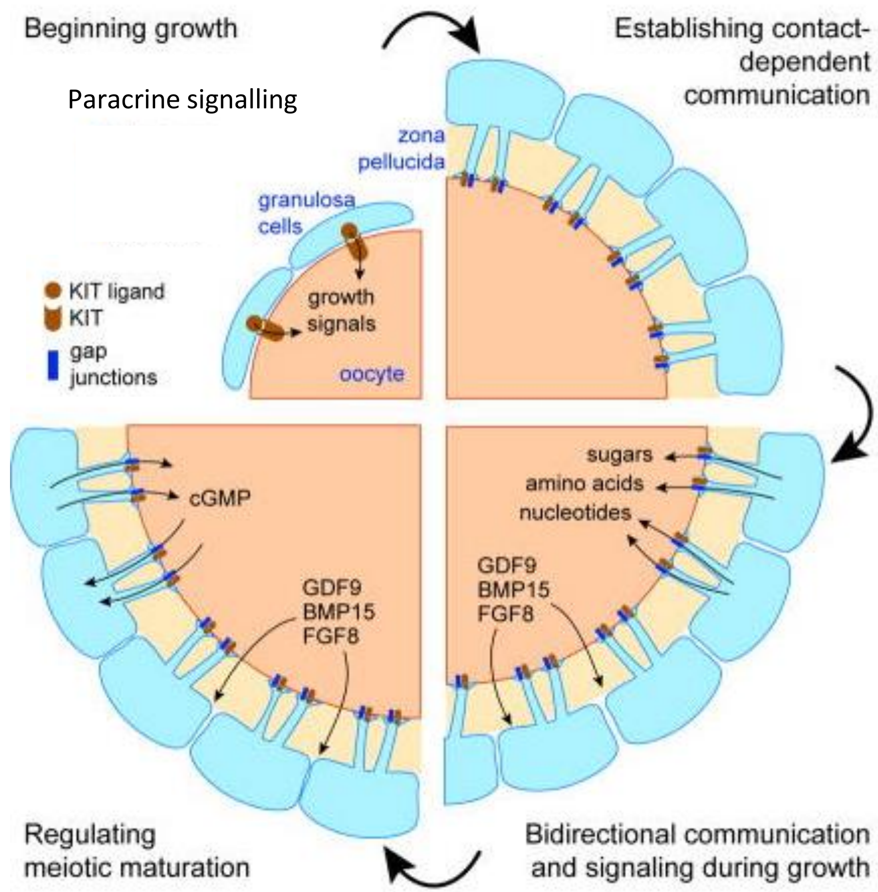
Oocyte and granulosa cells interaction

Bidirectional communication between oocyte a follicular cells

- coordination of
  (1) oocyte growth and maturation
  (2) granulosa cells proliferation and differentiation
  (3) steroidogenesis
  (4) metabolism
  (5) apoptosis

Communication via:

(A) Paracrine signals (growth factors, microRNA)
(B) Extracellular vesicles
(C) Transzonal projections



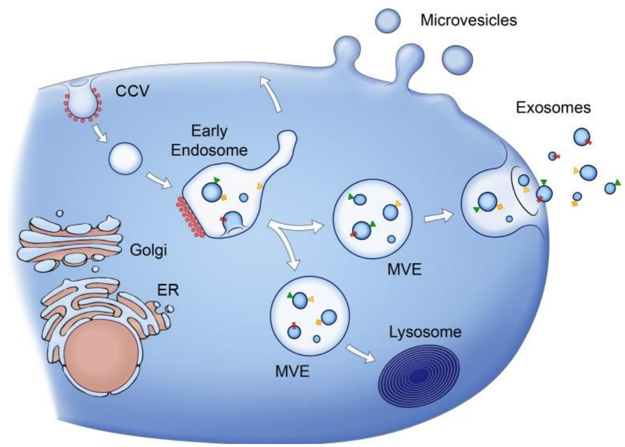
Oocyte and granulosa cells interaction

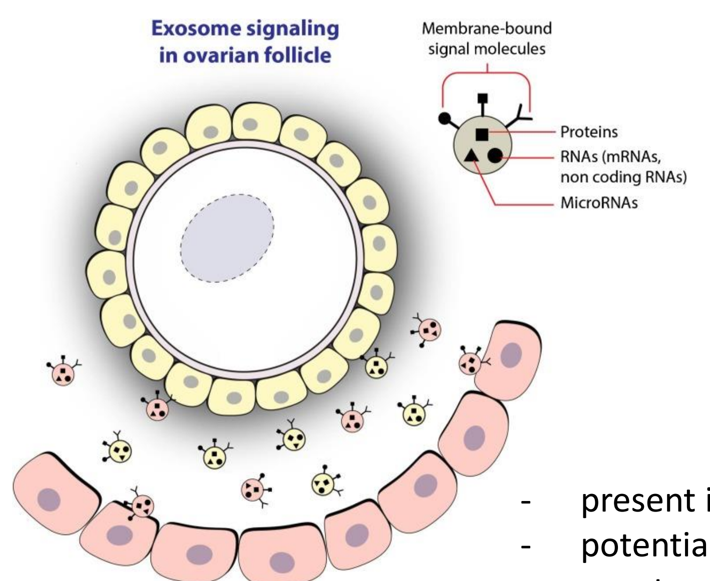
❖ Extracelular vesicles

= mebrane vesicles

- exosomes (40-100 nm)
  - ← fusion of endosomes with plasma membrane
- microvesicles (>100nm)
  - ← released from cell membrane

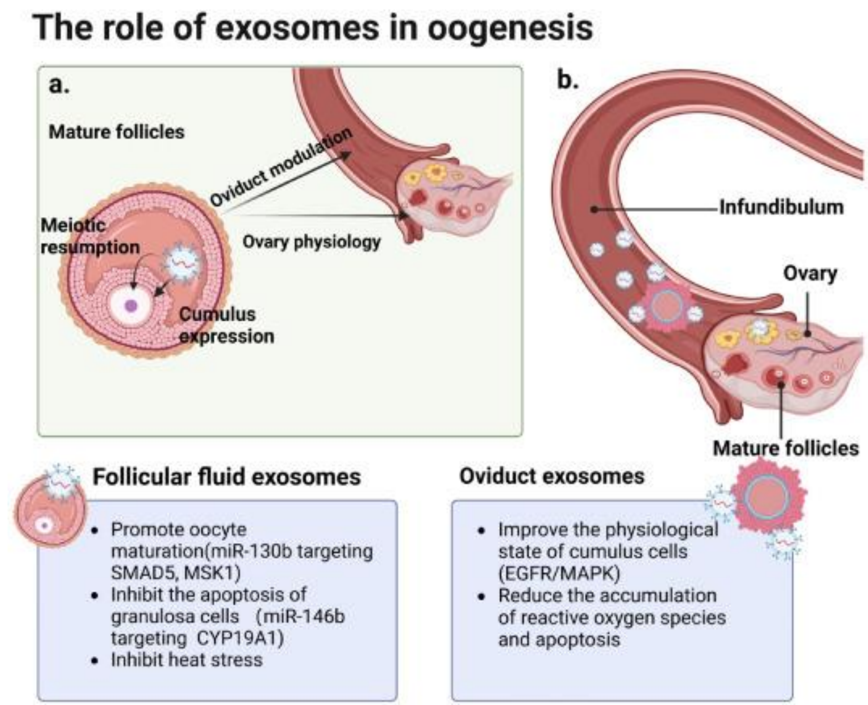
- transfer of membrane and cytosolic proteins, lipids, nc-RNAs, microRNA,..





- present in follicular fluid
- potential biomarkers of oocyte quality?

Pietro et al., JARG 2016



Chen et al, 2023

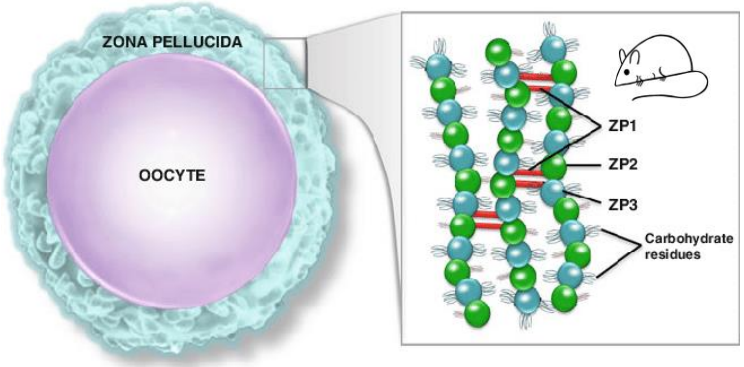
Oocyte and granulosa cells interaction

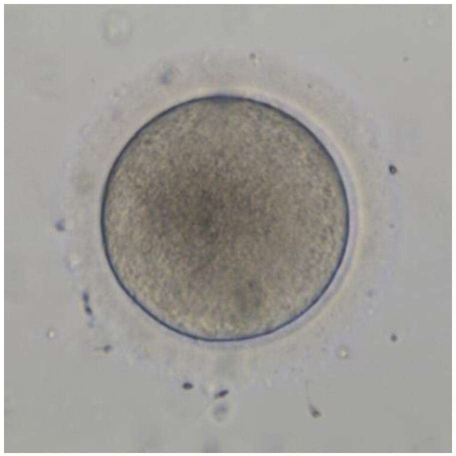
❖ Zona pellucida (ZP)

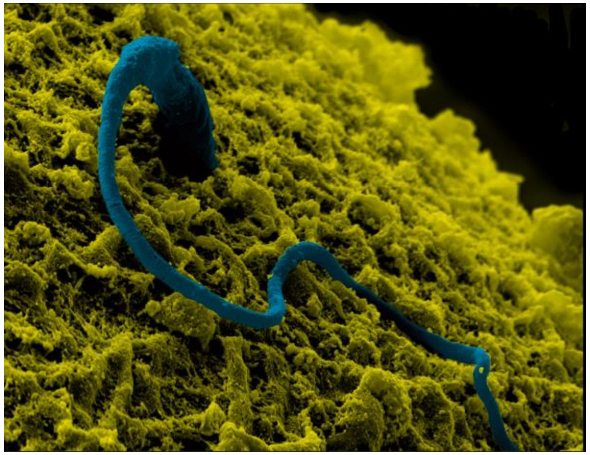
- thick extracellular coat surrounding mammalian oocytes
- composed of 4 glycoproteins ZP1-4*
    - ZP-2 and ZP 3 form long chains
    - ZP-1 and ZP-4 (its paralog) crosslinks the chains

- sialylation and sulfation → acidic character

- FUNCTION:
    - protection of oocyte (and early embryo)
    - storage of biactive molecules
    - receptivity and sperm attachment during fertilization
    - prevention of polyspermy





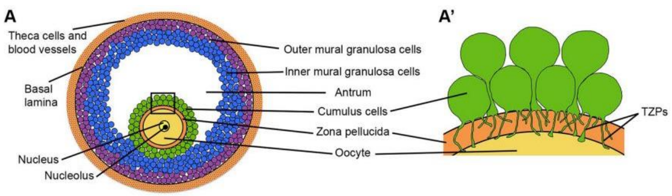


*u myší jen ZP1-3

Oocyte and granulosa cells interaction

❖ Transzonal projections (TZP)

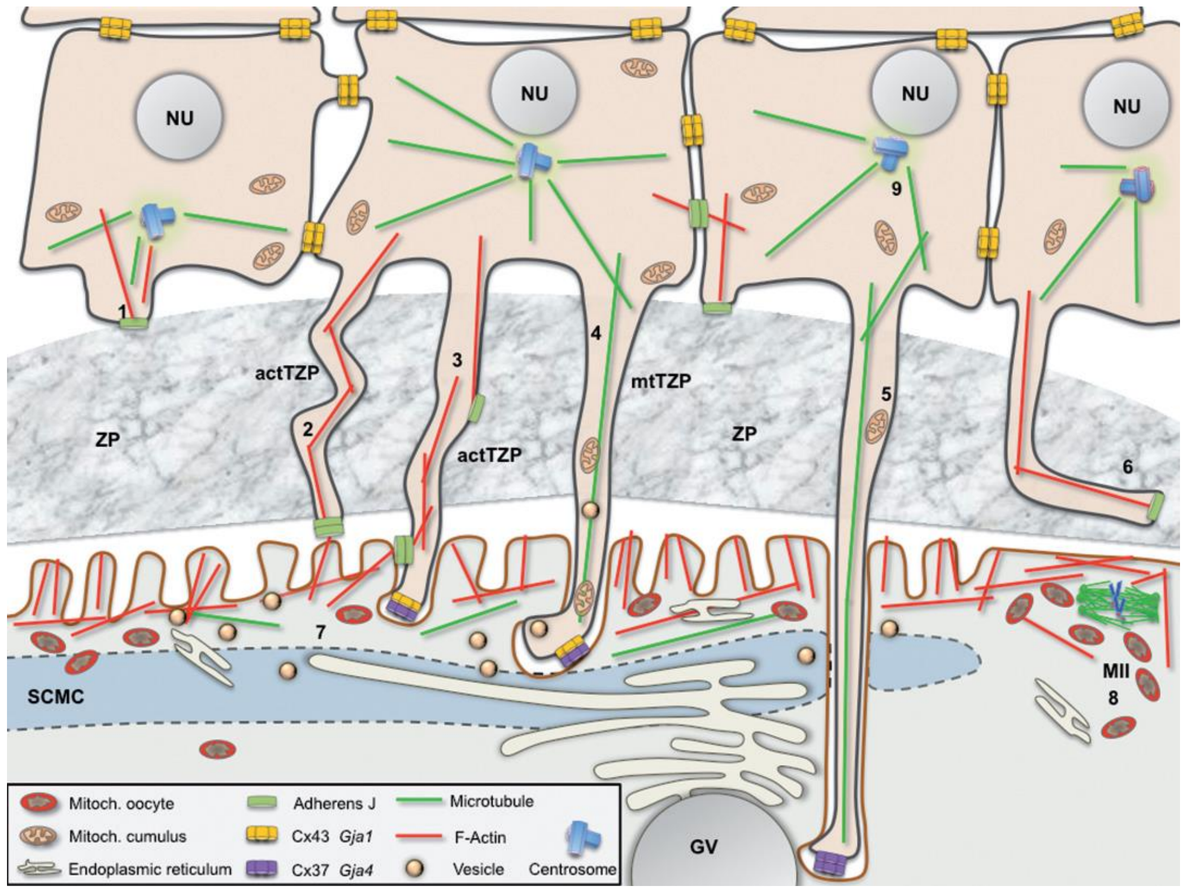
- projections of cumulus GCs enabling their communiation with oocyte through ZP



- FUNCTION:

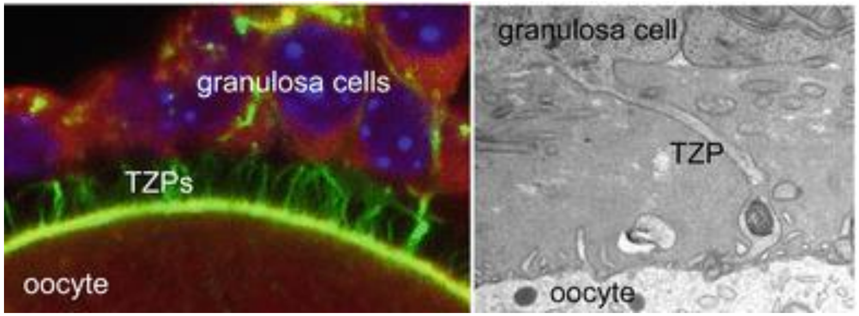
(1) mechanical contact
(2) intercellulra signalling
(3) supply of nutrients

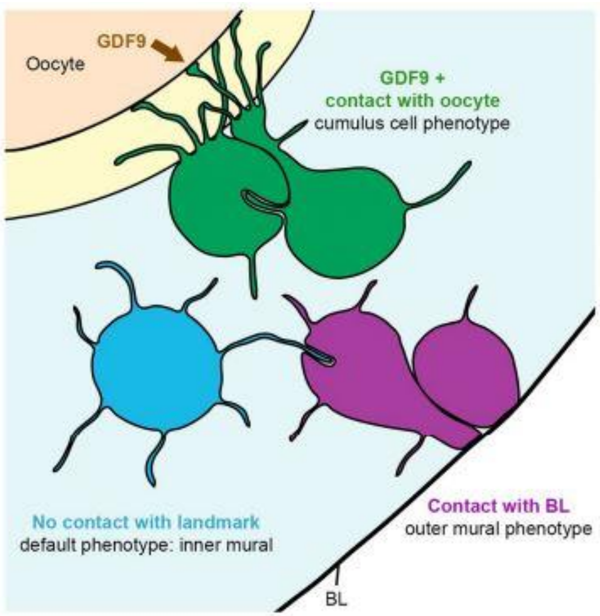
- presence of cytoskeletal structures (actin/acetylated microtubulin) stabilizes TZP and facilitates intercellular transport of molecules

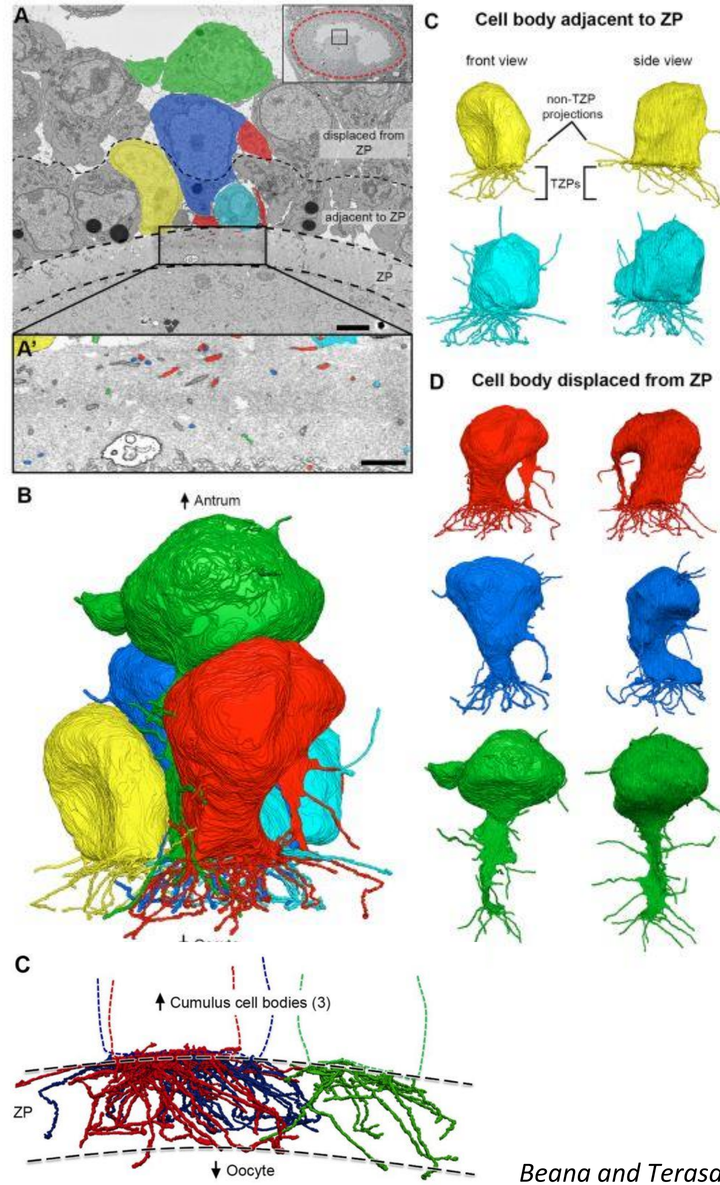


Oocyte and granulosa cells interaction

❖ Transzonal projections (TZP)



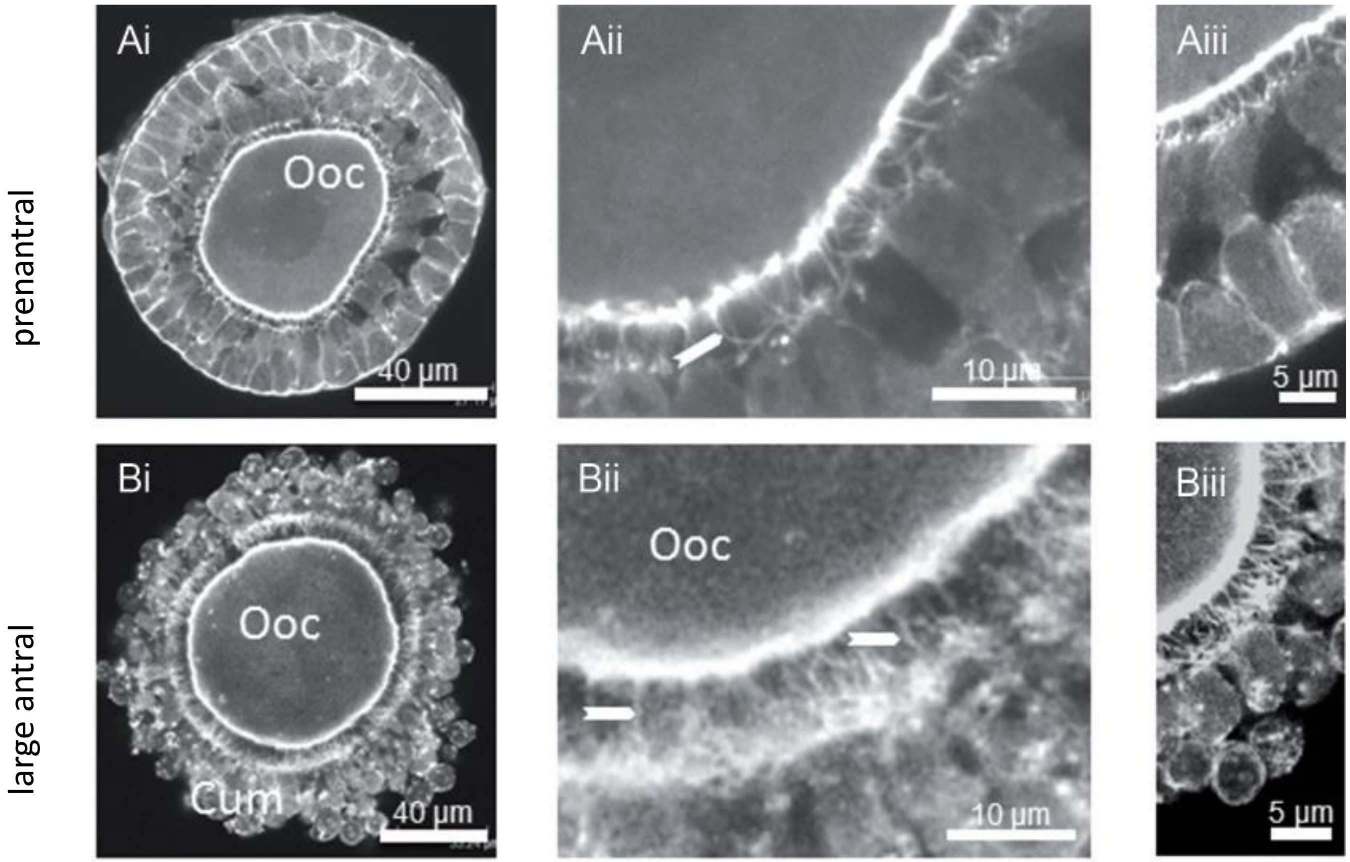




Beana and Terasaki 2018

Oocyte and granulosa cells interaction

❖ Transzonal projections (TZP)

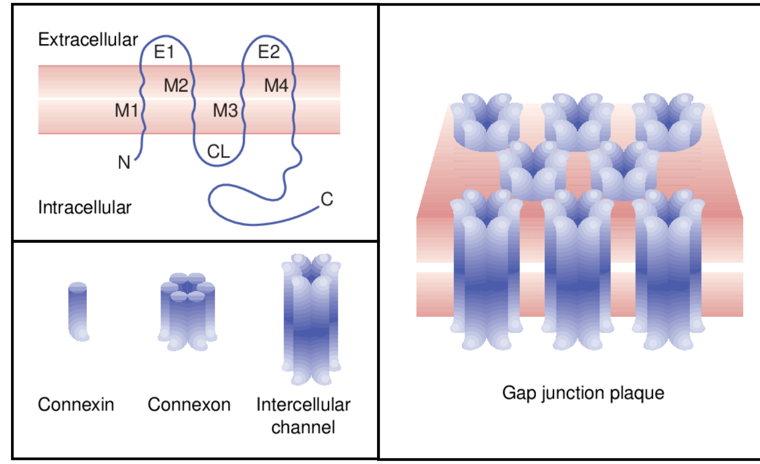


- prominent during preantral stage (oocyte growth)
- retracted in late antral stage
- maintain meiotic arrest
- mechanical removal of cGCs → spontaneous resumption of meiosis in denuded oocytes

Oocyte and granulosa cells interaction

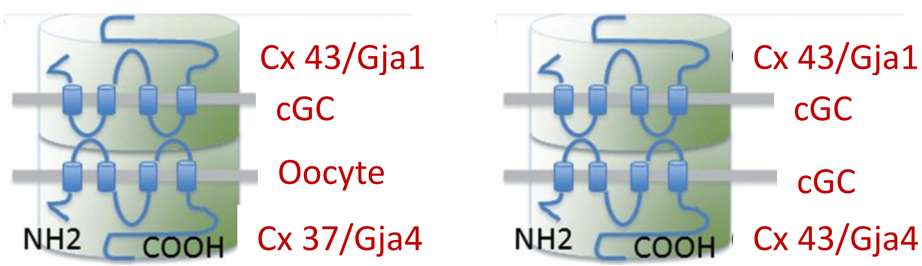
❑ Gap junctions

- transmembrane channels
- made of two „connexomes“ (hemichannels) located on both apposed membranes
- each connexom composed of six connexins with 4 transmembrane domains

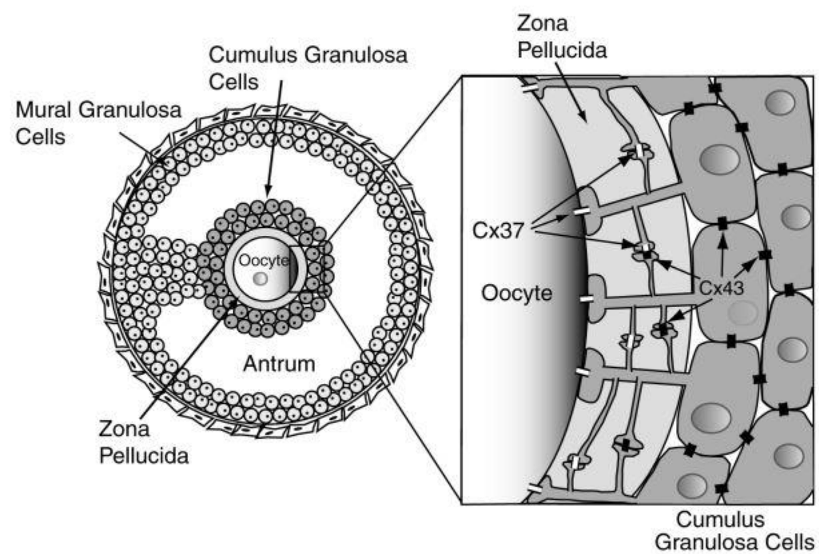


- connection:

(A) between oocyte and GC **(B) between two GCs**



functional syncitium



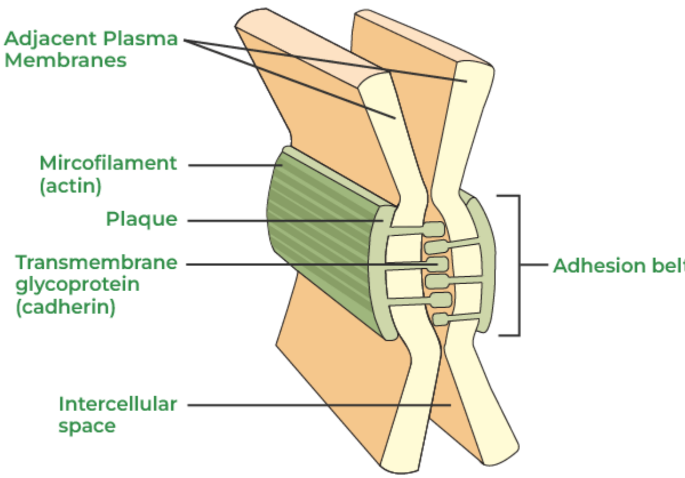
Oocyte and granulosa cells interaction

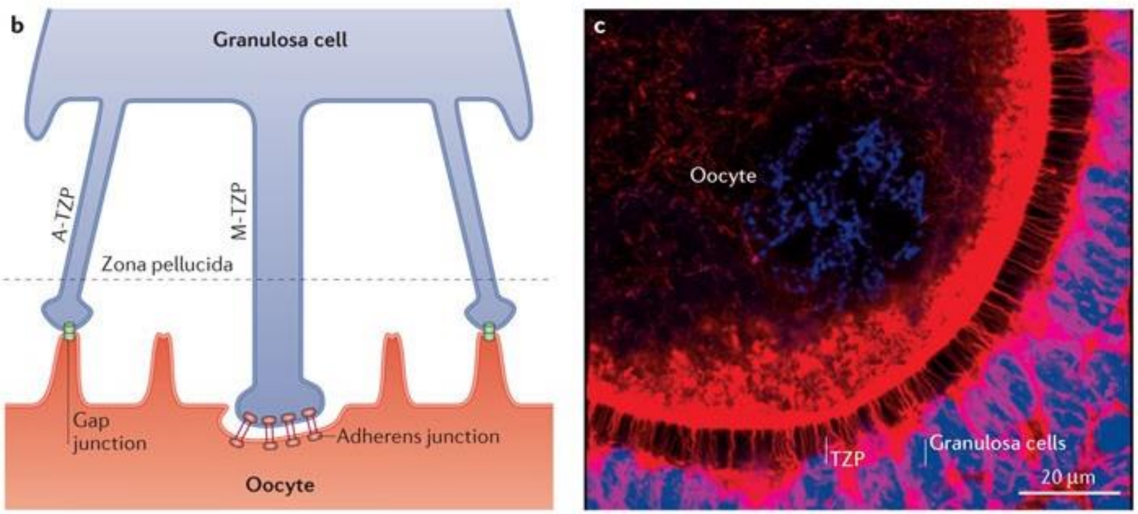
❑ Adherent junctions

- bridge neighboring plasma membranes via transmembrane glycoprotein cadherin

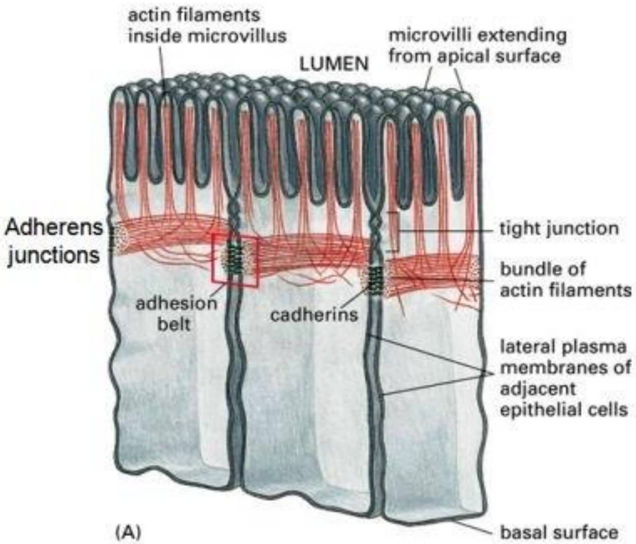
- cytoplasmic face linked to actin

- E-cadherin and N-cadherin involved in physical contact of GCs with oocyte´s ZP





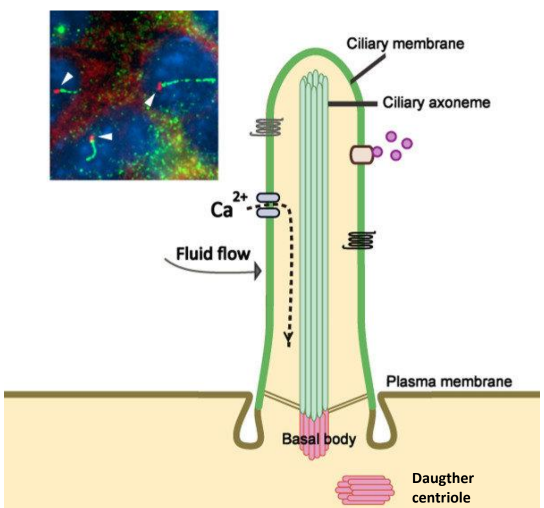
Nature Reviews | Molecular Cell Biology

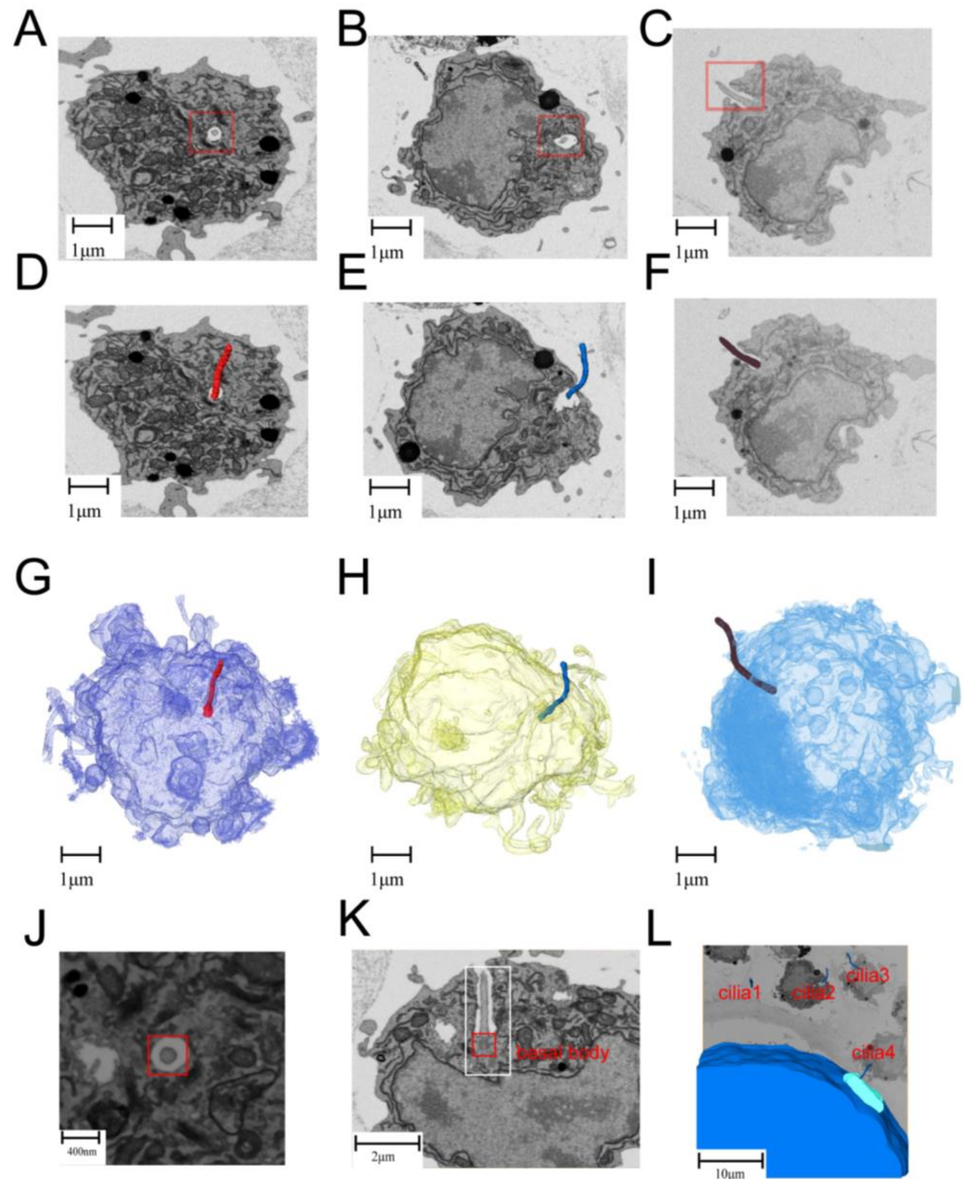


Oocyte and granulosa cells interaction

❑ Cilia of granulosa cells

- primary cilia (9+0 pairs of microtubules)
- communication between oocyte and GCs
- senzoring cumulus microenvironment



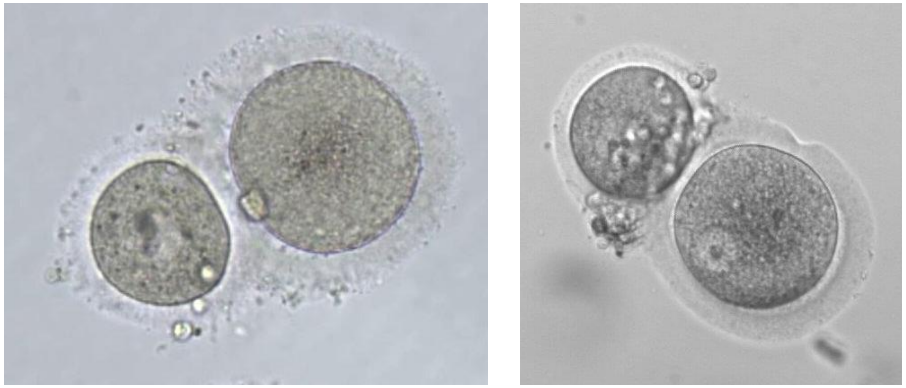


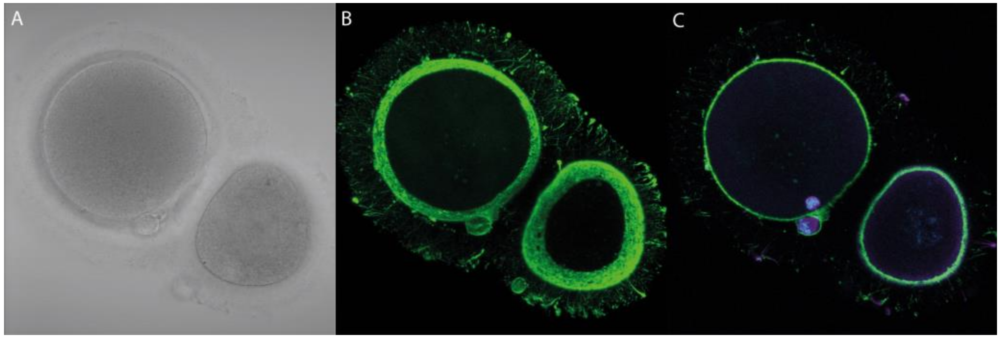
Shu et al, 2023

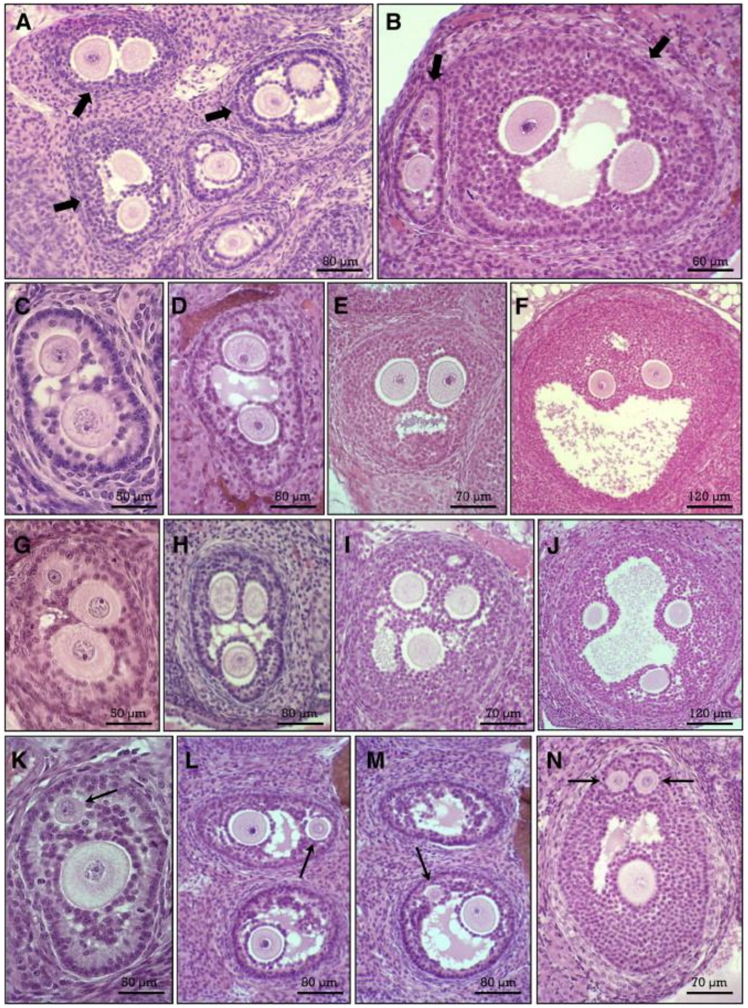
Folliculogenesis disorders

➢ MOF – multiple oocyte follicle syndrome

- presence of multiple preovulatory follicles within one follicle
- failure of follicle individualisation
- two oocytes of different maturation stage enclosed by one ZP commonly observed in cohorts of oocytes retrieved for IVF





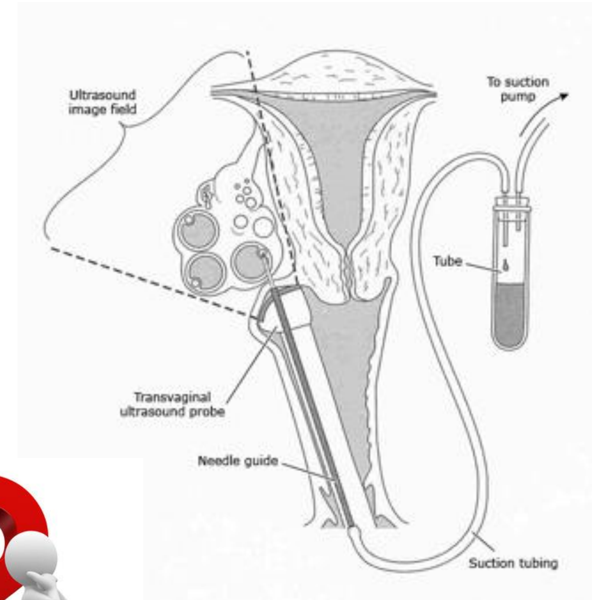


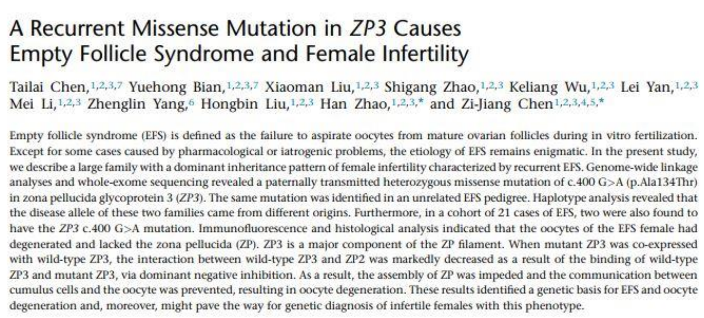
Gaytan et al 2014.

Folliculogenesis disorders

➢ EFS – empty follicle syndrome

- no oocytes obtained from preovulatory follicles during IVF procedure despite normal hormonal response and UZK monitoring
- usually technical problem during COCs aspiration and/or hCG trigger administration
- genuine absence of oocyte very rare ((„genuine EFS“ - 0.0016%)
- Possible cause?
  - COC stick to follicle wall due to insufficient LH/hCG trigger which causes COC expansion and detachment
  - Oocyte degeneration
    - genetic predisposition for proapoptotic genes expression in GCs
    - defect ZP leading to impaired GCs-oocyte communication



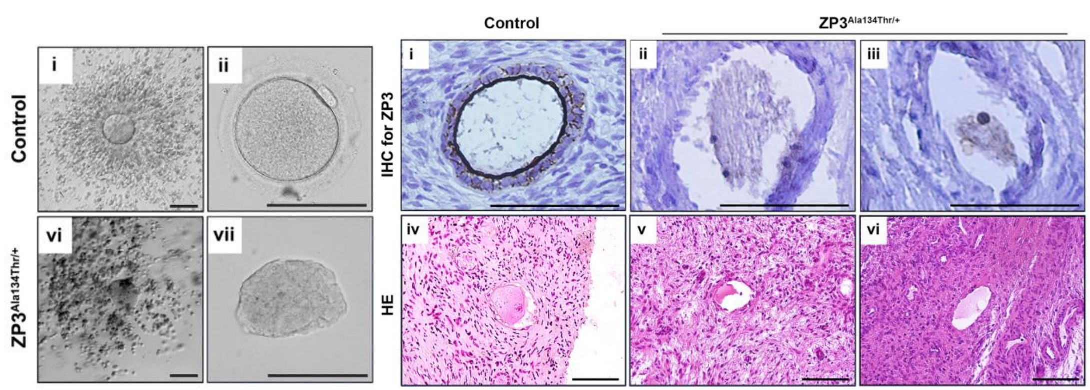


A Recurrent Missense Mutation in *ZP3* Causes Empty Follicle Syndrome and Female Infertility

Tailai Chen,[1,2,3,7] Yuehong Bian,[1,2,3,7] Xiaoman Liu,[1,2,3] Shigang Zhao,[1,2,3] Keliang Wu,[1,2,3] Lei Yan,[1,2,3] Mei Li,[1,2,3] Zhenglin Yang,[6] Hongbin Liu,[1,2,3] Han Zhao,[1,2,3,*] and Zi-Jiang Chen[1,2,3,4,5,*]

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.



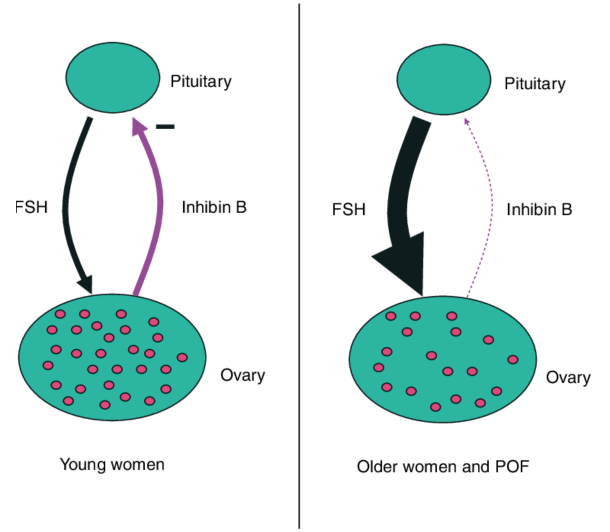
Folliculogenesis disorders

➢ Premature ovarian insuficiency (POI)

= premature ovarian failure - POF

→ premature depletion of ovarian reserve (<40 years), early menopause and infertility

- 1% women
- amenorrhea, hypoestogenism, low AMH, elevated FSH



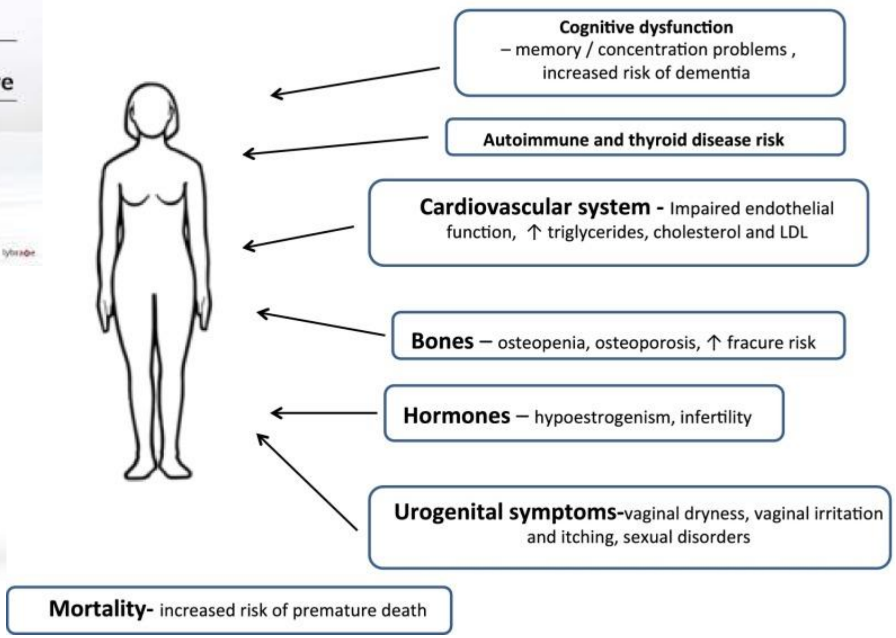
- poor prognosis, gonadotropin stimulation inefficient, usually necessary to become a recipient of donor egg

Cause?

- genetic mutations (e.g. Figla, Nobox, Sohlh)

→ failure of oocyte individualisation and apoptosis of oogonia non-surrounded by follicular cells

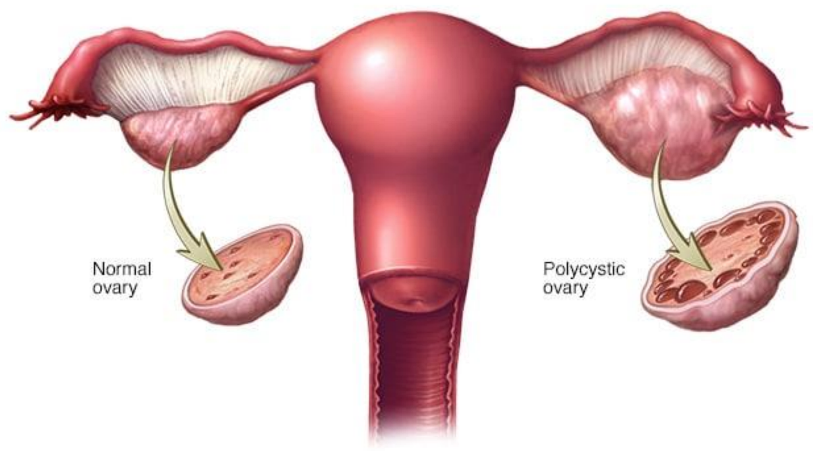
→ deregulated activation of primordial follicles and depletion of ovarian reserve

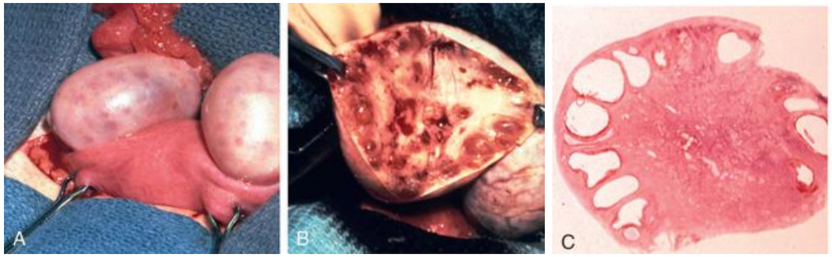


Folliculogenesis disorders

➢ Polycystic ovary syndrome (PCOS)

- enlarged ovaries with thickened sclerotic capsules (ultrasound visible cysts)
- high number of small antral follicles (2-9 mm), but no preovulatory
- oligo-/a-menorrhea, dysmenorrhea, anovulation, subfertility
- **↓FSH, ↑LH, ↑ androgens (hirsutismu, acne), extremely high AMH**
- inzuline resistance, obesity, hypertensis
- 5-10 % women in reproductive age (12-45 years)
- Difficult hormonal stimulation, risk of OHSS

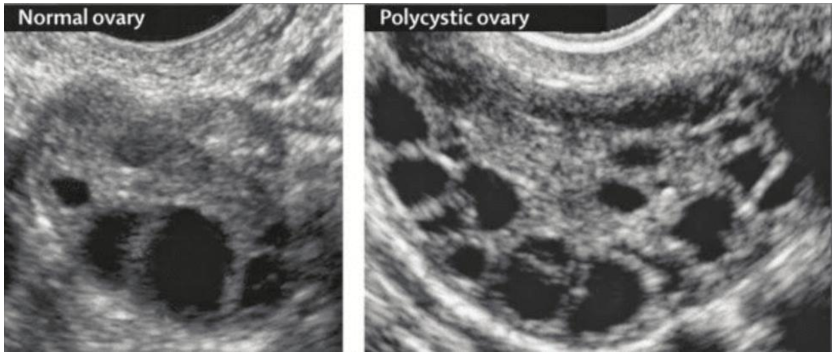




Cause?

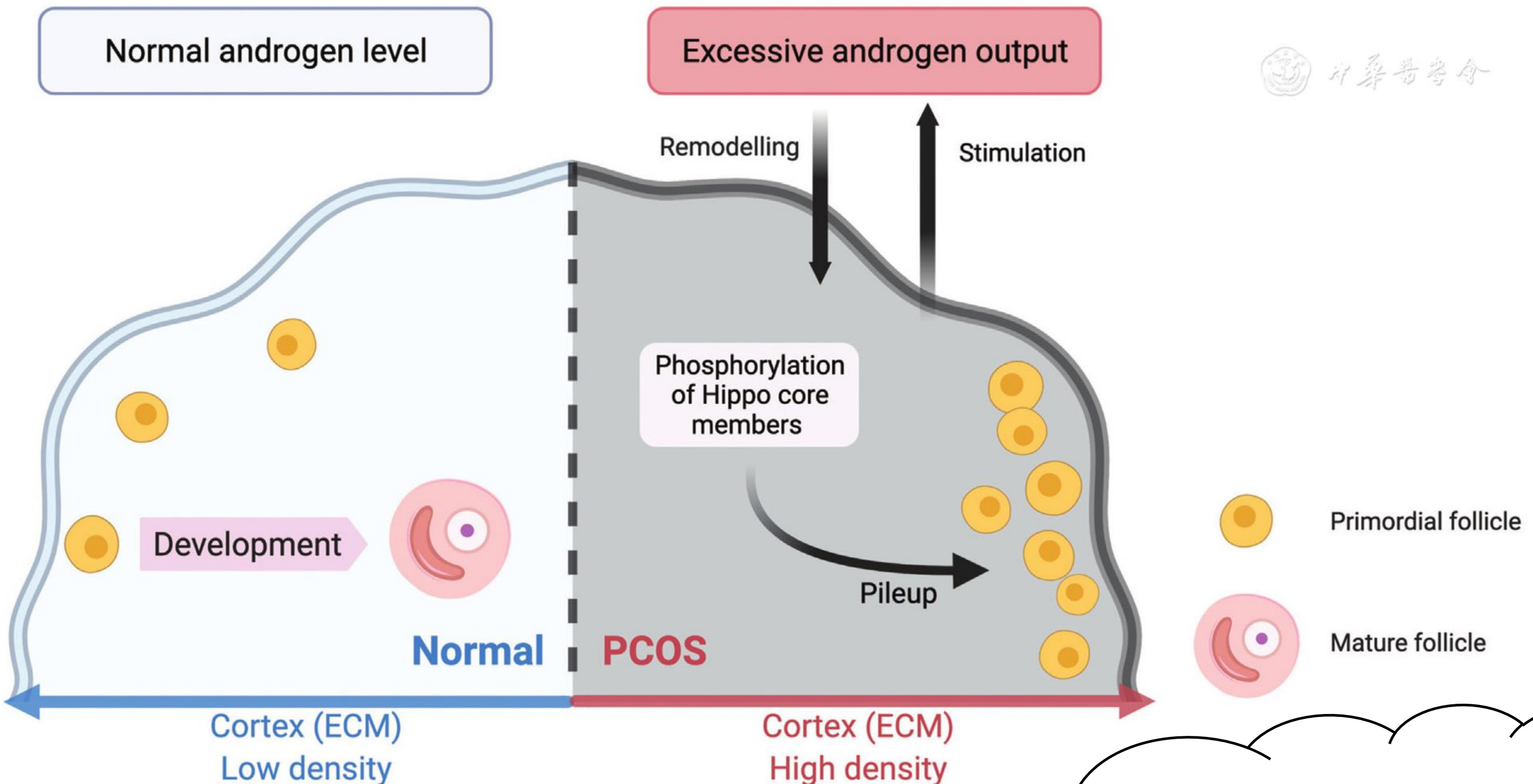
- defect of growth and/or selection of dominant follicle
- genetic predisposition
- altered miRNA profile

Lean vs. obese PCOS



Folliculogenesis disorders

➢ Polycystic ovary syndrome (PCOS)

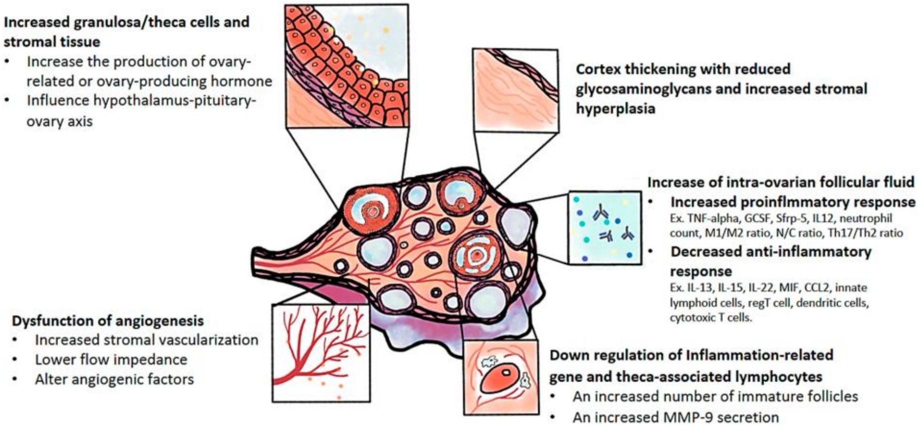


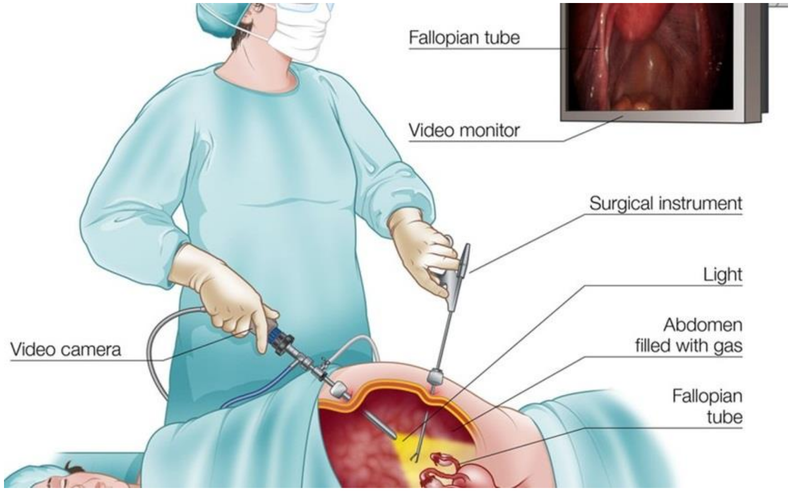
Possible to „unleash“ development of small follicles?

Therapeutical strategies to improve folliculogenesis

❖ Ovarian drilling

- historical empiric technique for fertility treatment of PCOS patients
- mechanical disruption of ovariant tissue integrity
- surgical multiperforation or laparoskopic diathermy
- risk of adhesions, vaskular changes, bleeding, tissue damage





Therapeutical strategies to improve folliculogenesis

❖ In vitro activation (IVA) of primordial follicles

- **experimental intervention** technique

(1) Mechanical fragmentation of surgically removed ovarian tissue
(2) Cryopreservation of ovarian strips
(3) In vitro treatment with activating substances
(4) Autologous transplantation of ovarian tissue

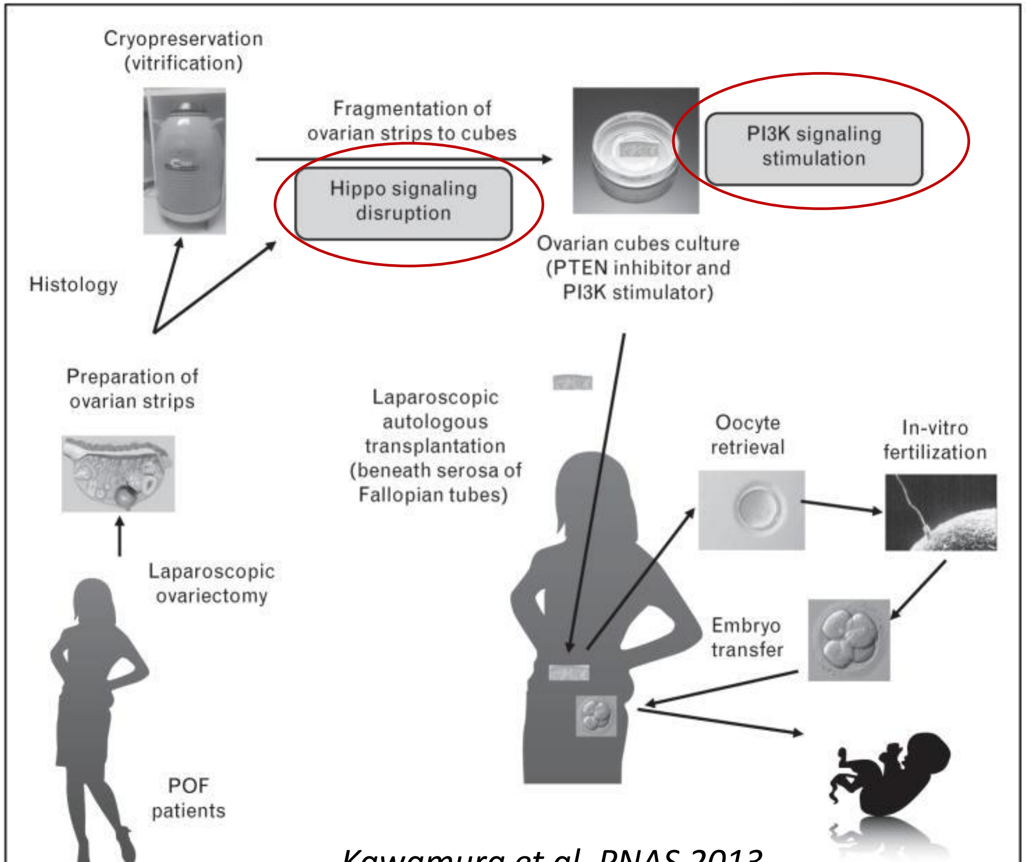
Mature oocytes for IVF

- method of last choice for POI patients

Kazuhiro Kawamura

Hippo signaling disruption and Akt stimulation of ovarian follicles for infertility treatment

Kazuhiro Kawamura[a,b,1,2], Yuan Cheng[c,1], Nao Suzuki[a], Masashi Deguchi[c], Yorino Sato[a,c], Seido Takae[a,c], Chi-hong Ho[c], Nanami Kawamura[b,d], Midori Tamura[a], Shu Hashimoto[e], Yodo Sugishita[a], Yoshiharu Morimoto[e], Yoshihiko Hosoi[f], Nobuhito Yoshioka[a], Bunpei Ishizuka[d,2], and Aaron J. Hsueh[c,2]



Kawamura et al. PNAS 2013

❖ In vitro activation (IVA) of primordial follicles

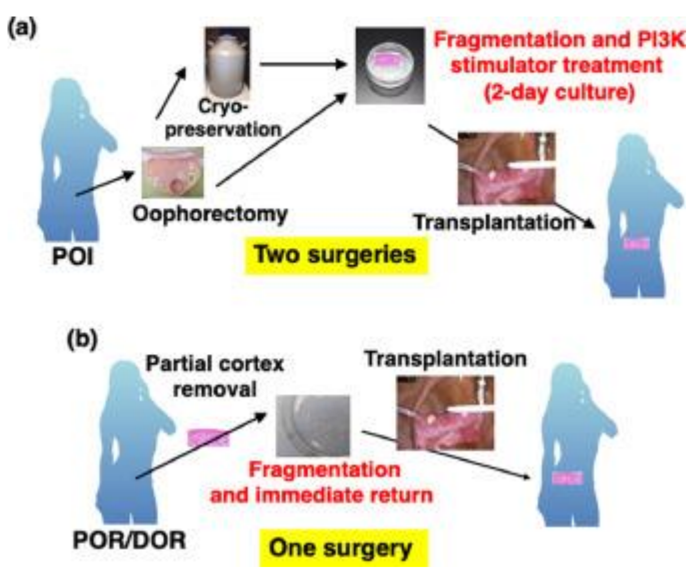
- „drug-free“ IVA

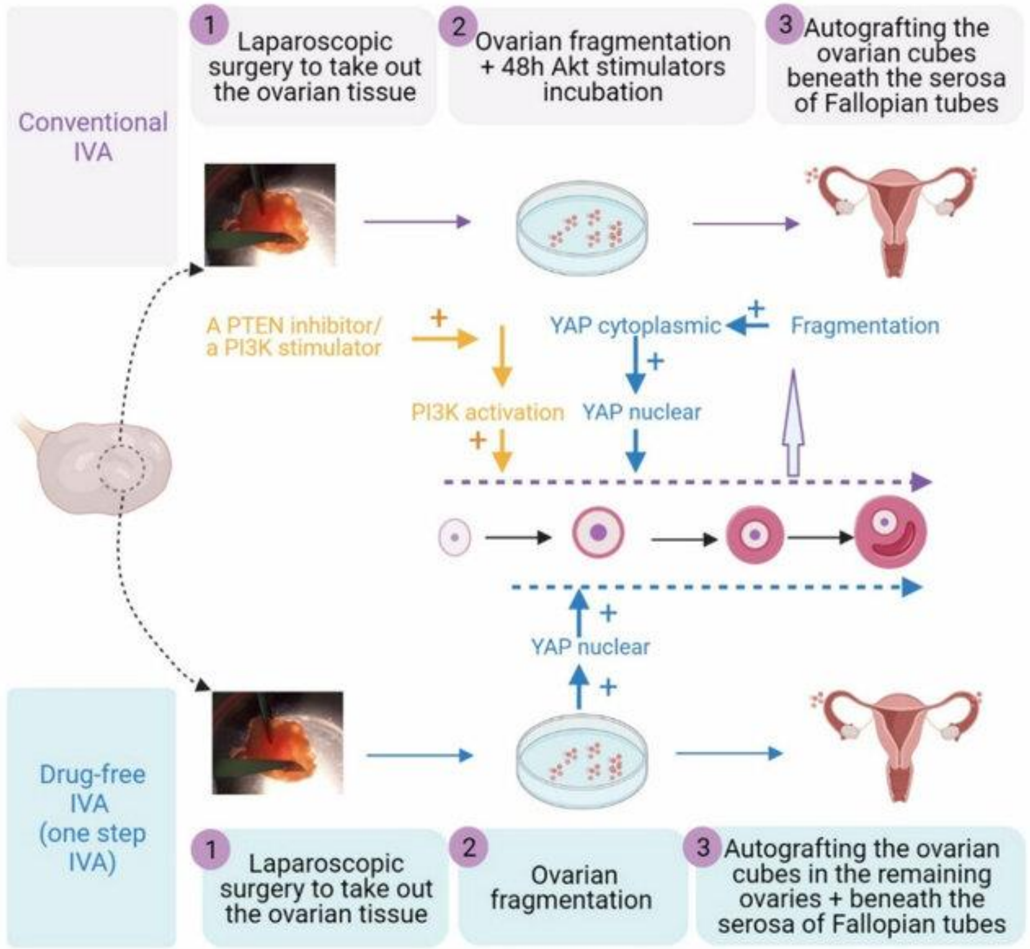
Drug-free in-vitro activation of follicles for infertility treatment in poor ovarian response patients with decreased ovarian reserve

BIOGRAPHY
Dr Kazuhiro Kawamura is Director of the Advanced Reproductive Medicine Research Center at the International University Health and Welfare (IUHW) School of Medicine. He is also a Professor of Obstetrics and Gynecology of IUHW School of Medicine. He received his medical and philosophy degrees from the Akita University School of Medicine.

Kazuhiro Kawamura[1,*], Bunpei Ishizuka[2], Aaron J.W. Hsueh[3]

KEY MESSAGE
Drug-free in-vitro activation (IVA) as infertility treatment for poor ovarian response patients with decreasing ovarian reserve could allow the retrieval of more oocytes to achieve higher pregnancy rates. The new approach omits the 2-day incubation of ovarian tissues with drugs used in the original IVA and requires only one surgery.



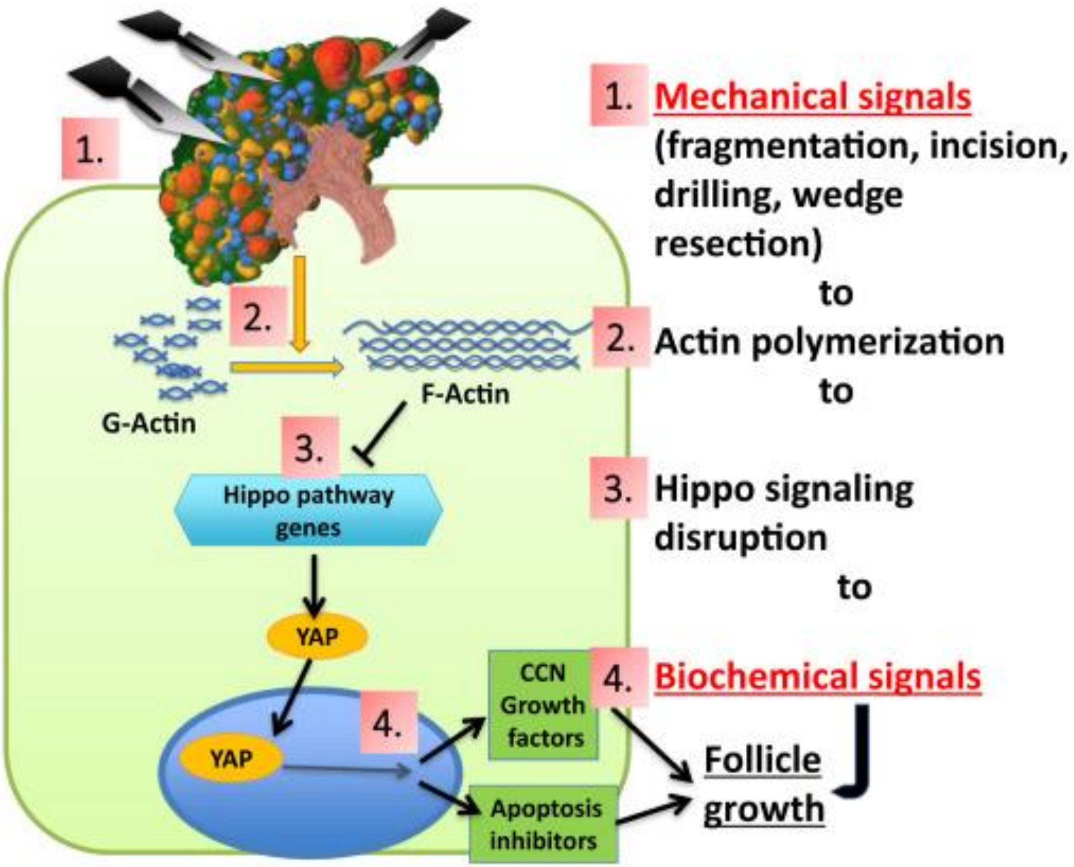


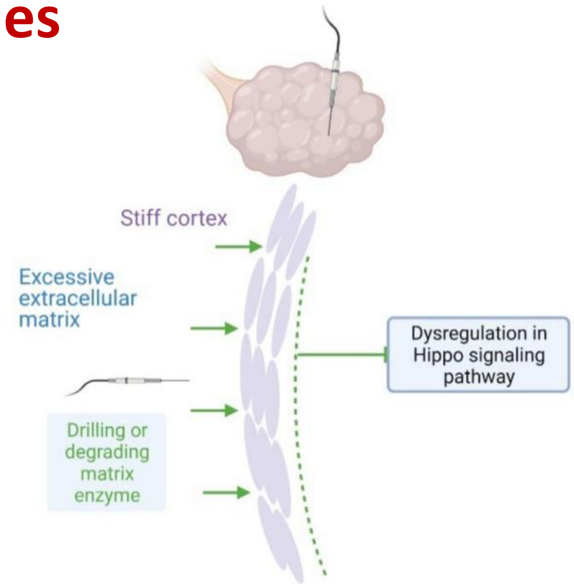
Vo KCT, Kawamura K. Int J Mol Sci. 2021

Kawamura K. Et al, RMBO. 2020

Therapeutical strategies to improve folliculogenesis

❖ In vitro activation (IVA) of primordial follicles



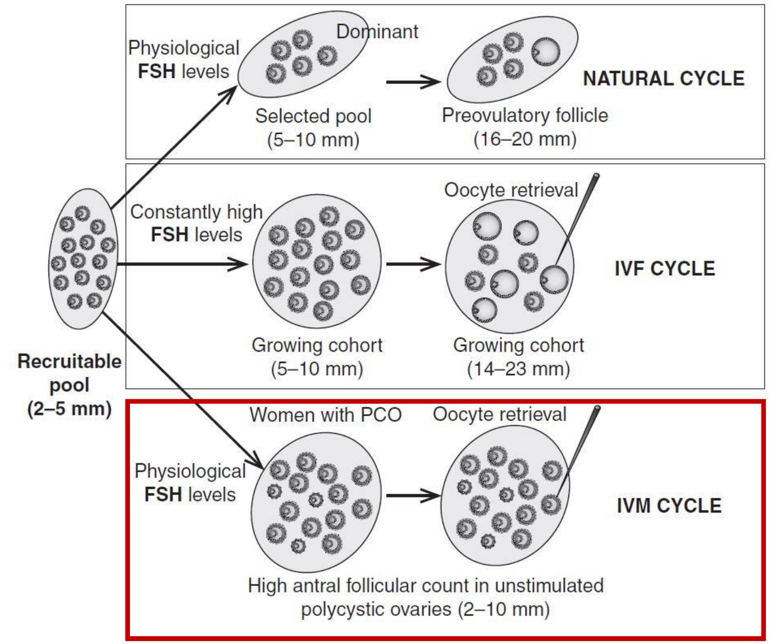


- overactivation of Hyppo signalling pathway blocks growth of follicles in PCOS patients
- tissue irritation induces reparation and actin polymerization which inactivates Hyppo signalling

Therapeutical strategies to improve folliculogenesis

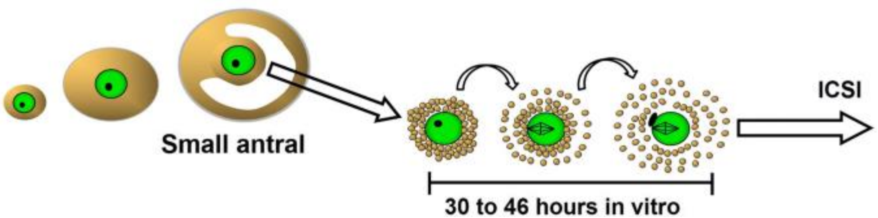
❖ In vitro maturation (of follicles)

- clinical strategy involving collection of **immature oocytes surrounded by follicular cells** from **small or mid-sized follicles (2-10 mm)** and their in vitro culture obtain fertilizable eggs



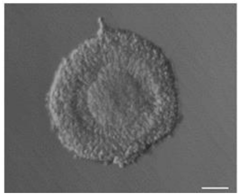
- method of choice
  - in patients with risk/history of OHSS (e.g. PCOS)
  - oncological patients (fertility preservation)
  - in „poor-responders“

- follicle in vitro culture for 24-48 hours in maturation media suplementaed with gonadotropins (FSH, LH/hCG)



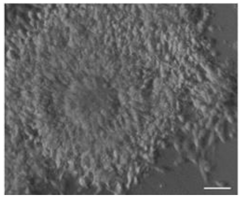
- mature (MII) oocytes fertilized by ICSI (IVF not recommended du to ZP hardening)

- relatively low efficiency



serum/HSA
growth factors*

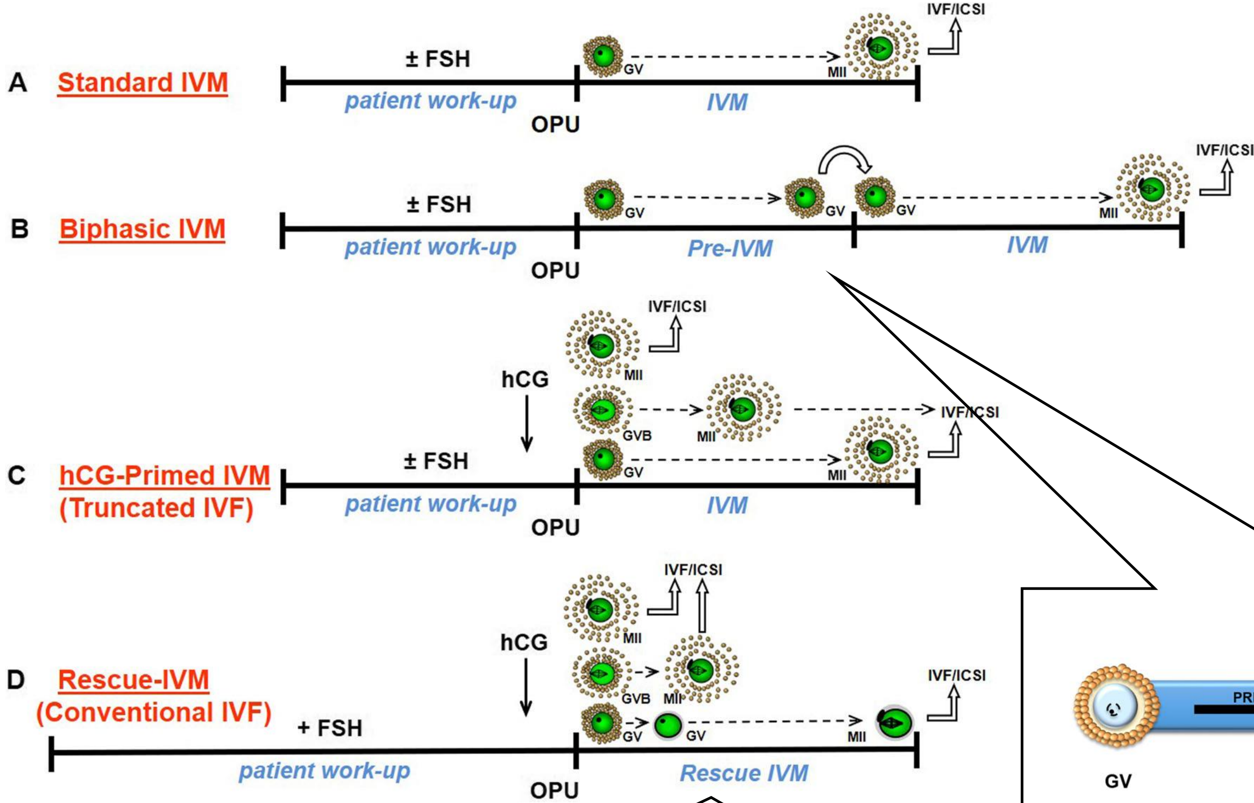
Hormones**
nutritients
antioxidants



* např. GDF9, BMP15, KL, cumulin,...

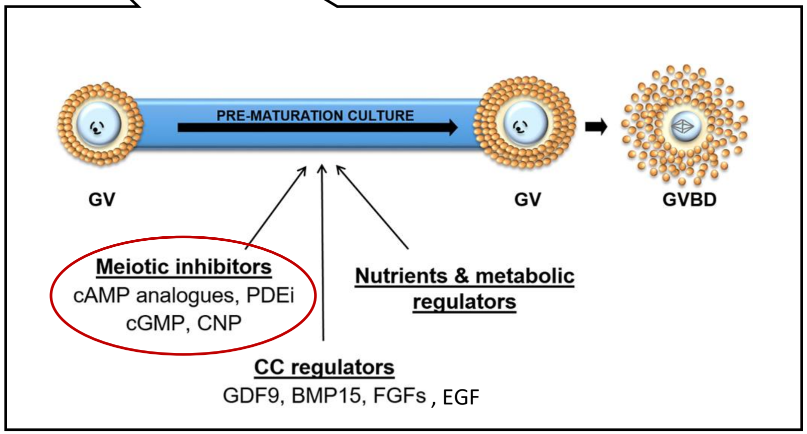
Therapeutical strategies to improve folliculogenesis

❖ In vitro maturation (IVM)



➡ MII ~ 50%

➡ MII ~70%



„Rescue“ *in vitro* maturation

hCG „priming“ *in vivo* – oocyte maturation *in vitro*

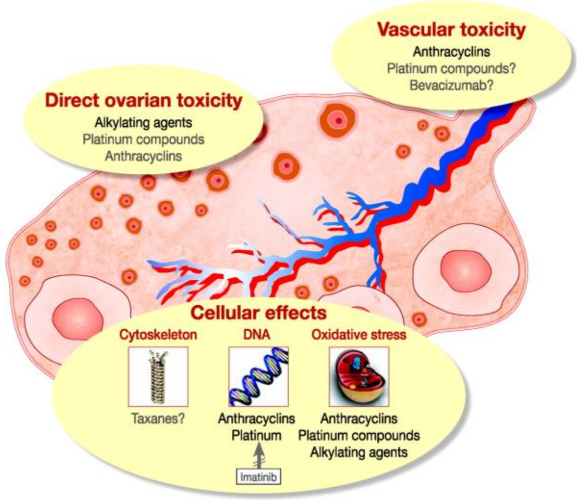
- Poor/delayed response to stimulation, maturation incomplete in 36hours after hCG

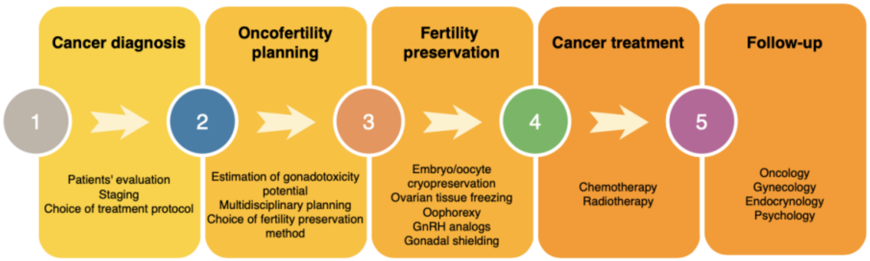
Therapeutical strategies to improve folliculogenesis

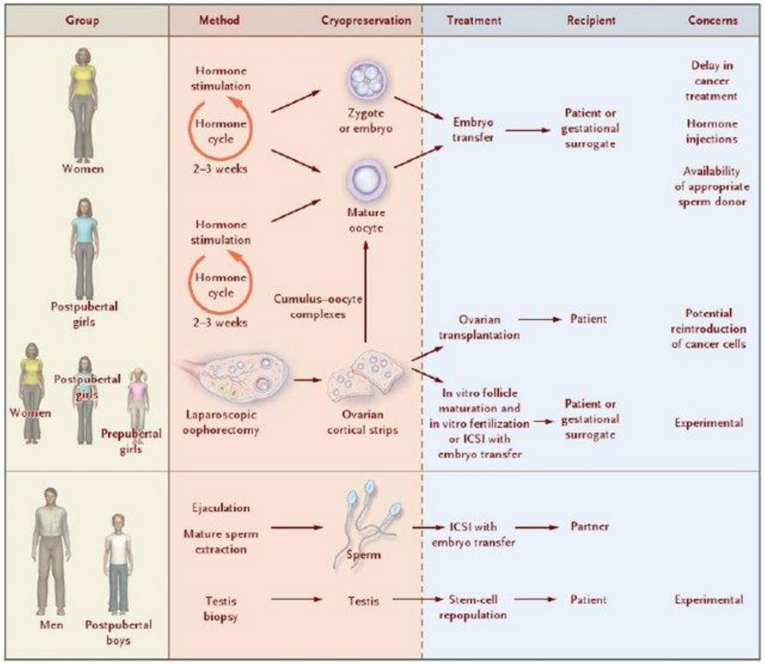
❖ Fertility preservation

- in cancer patients (including children!) undergoing radiotherapy and/or gonadotoxic treatment

- cryopreservation of ovarian tissue containing primordial follicles followed by autologous **transplantation or in vitro culture**





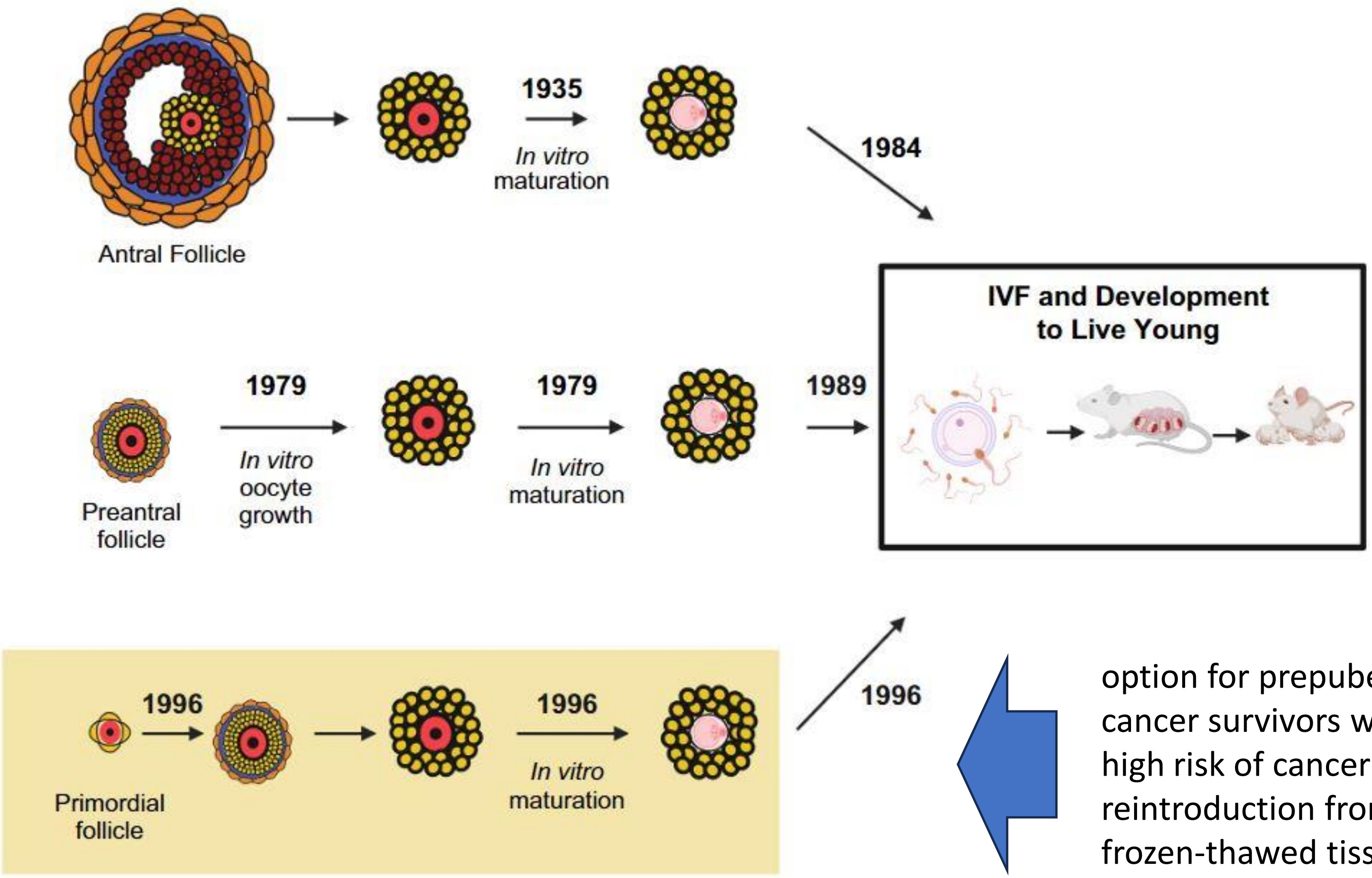


- hormonal replacement therapy?

- reimplantation of thawed tissue graft might constitute risk of reintroducing malignant cells and cancer remission!

Therapeutical strategies to improve folliculogenesis

❖ Egg development in vitro



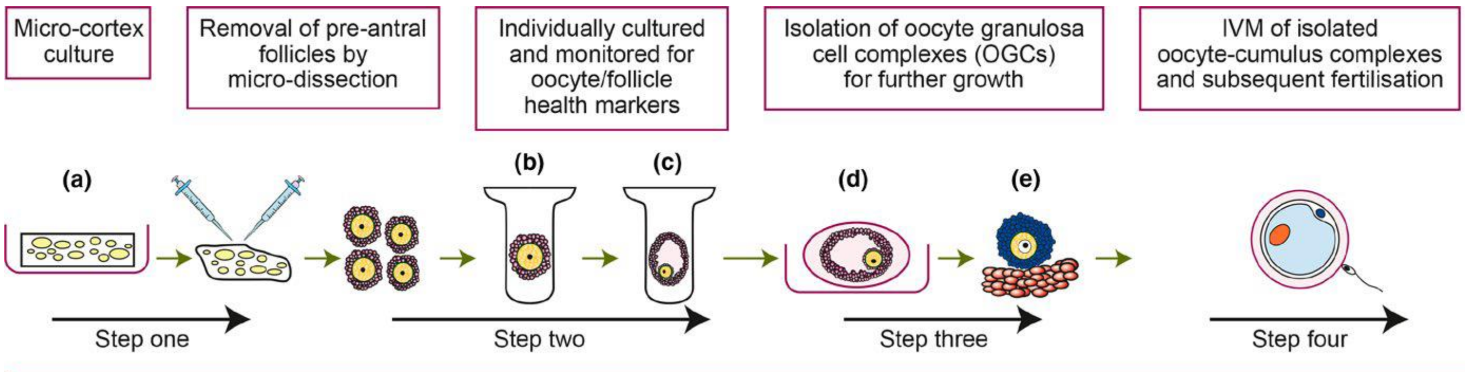
option for prepubertal cancer survivors with high risk of cancer cell reintroduction from frozen-thawed tissue

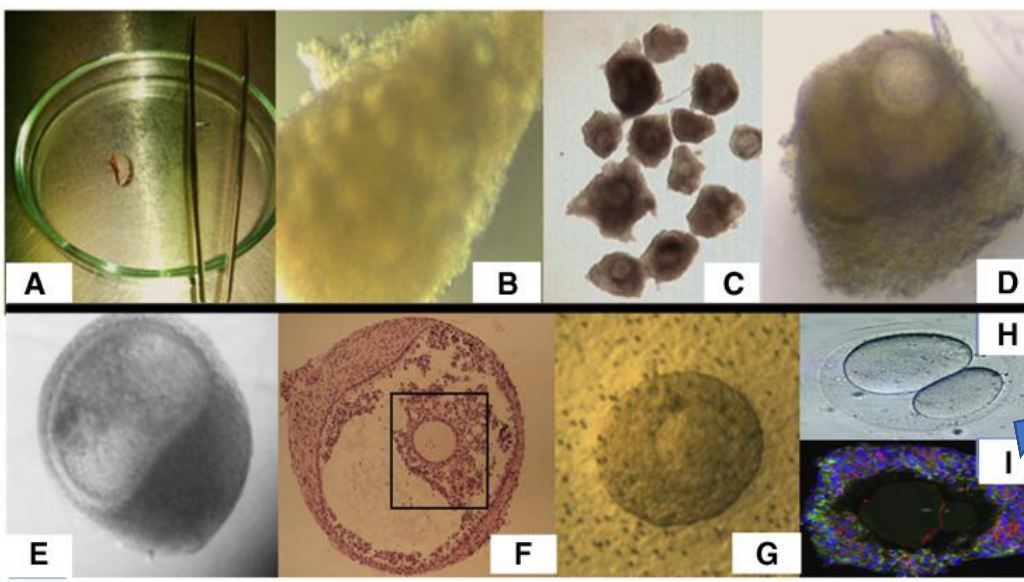
Telfer et al 2023

Therapeutical strategies to improve folliculogenesis

❖ In vitro foliculogenesis


Evelyn Telfer





Optimalisation of in vitro folliculogenesis

(A) isolation of follicles from stroma

(B) cultivation in media containing grow factors, low-molecular inhibitors, hormones, nutrients, antioxidants,...

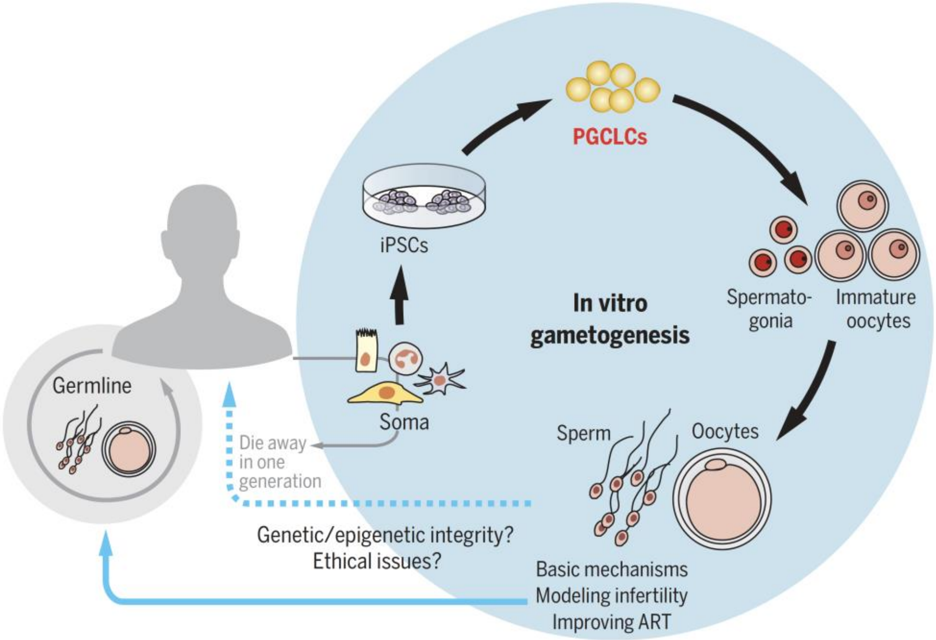
(C) supporting 3D biomatrix, non-adheren culture conditions

Suboptimal quality of in vitro grown oocytes

Future prospects

❖ In vitro gametogenesis (IVG)

- **experimental** technique of making female/male gametes outside of body
- full recapitulation of gametogenesis in vitro
- use of reprogrammed somatic/embryonic cells
- co-culture of in vitro-produced gametes and gonadal soma-derived cells
- chance of having biological child for cancer survivals
- possibility to rescue endangered and/or revive extinct animal species
- efficiency and safety?
- ethical and legal aspects



Startup aims to make lab-grown human eggs, transforming options for creating families

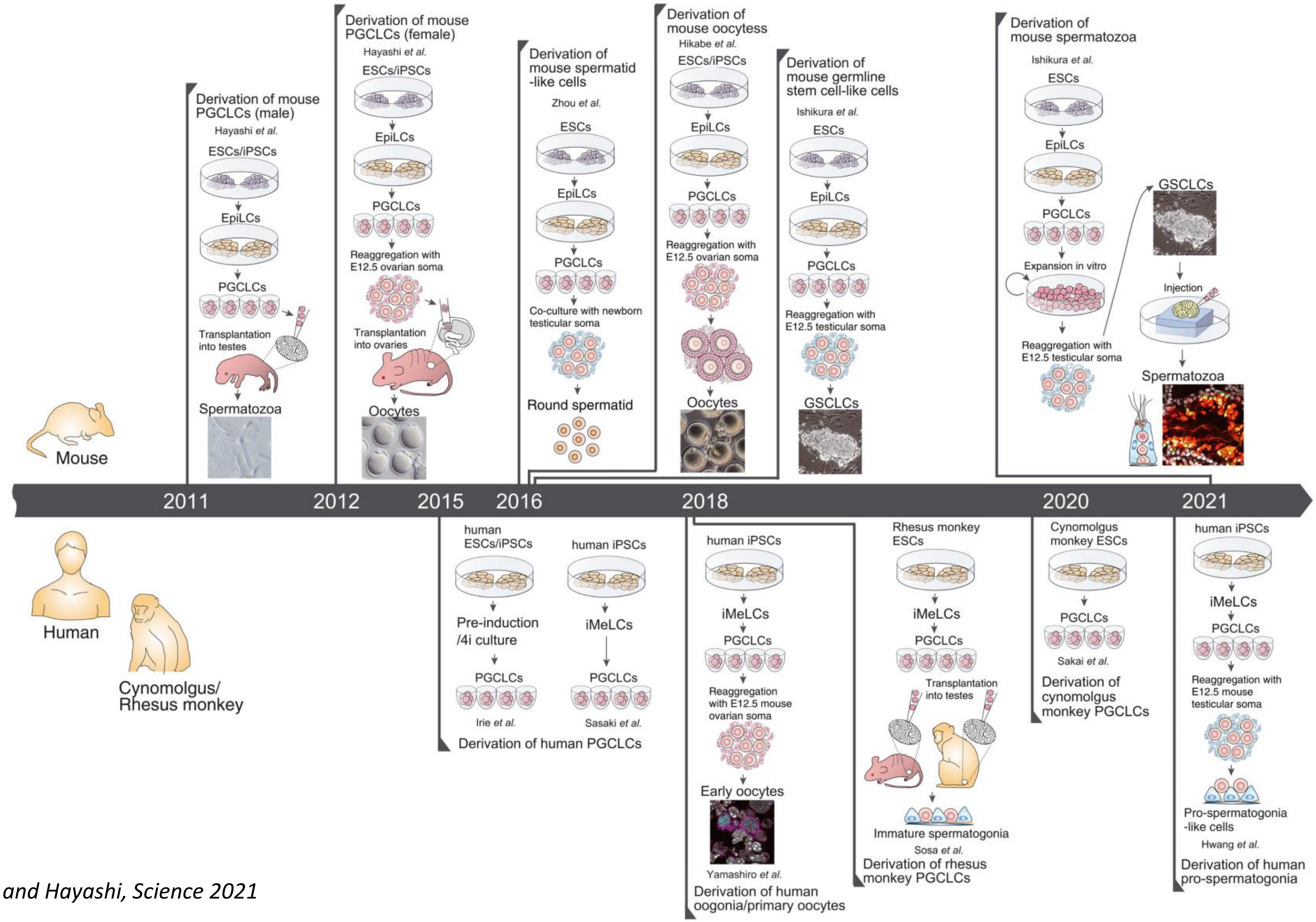
July 15, 2023 · 6:01 AM ET

Rob Stein

Conception's chief scientific officer, Pablo Hurtado, examines very early primordial germ cells under a microscope in a company lab in Berkeley, California.

Future prospects

❖ In vitro gametogenesis (IVG)

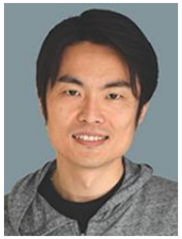


Saitou and Hayashi, Science 2021

Future prospects

❖ In vitro gametogenesis (IVG)

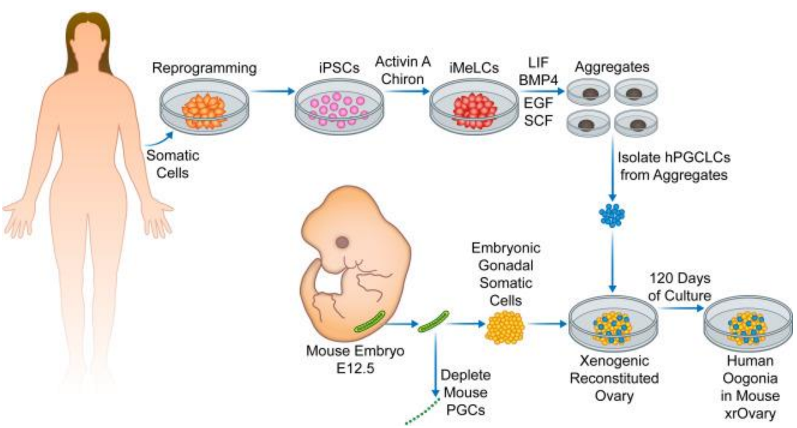
Mitinori Saitou



Kotaro Sasaki

Generation of human oogonia from induced pluripotent stem cells in vitro

Chika Yamashiro[1,2], Kotaro Sasaki[1,2], Yukihiro Yabuta[1,2], Yoji Kojima[1,2,3,4], Tomonori Nakamura[1,2], Ikuhiro Okamoto[1,2], Shihori Yokobayashi[1,2,4], Yusuke Murase[1,2], Yukiko Ishikura[1,2], Kenjiro Shirane[5,6], Hiroyuki Sasaki[5,6], Takuya Yamamoto[3,4,7], Mitinori Saitou[1,2,3,4]*



Yamashiro et al.,Science 362, 356–360 (2018)

nature COMMUNICATIONS

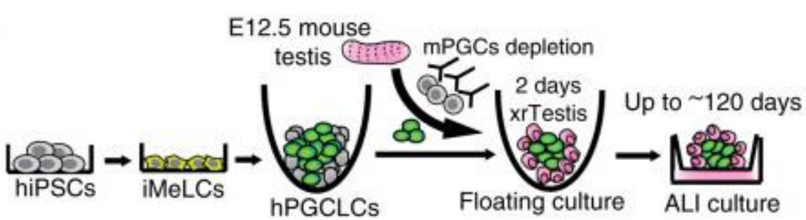
ARTICLE

Check for updates

https://doi.org/10.1038/s41467-020-19350-3 OPEN

Reconstitution of prospermatogonial specification in vitro from human induced pluripotent stem cells

Young Sun Hwang[1,5], Shinnosuke Suzuki[2,5], Yasunari Seita[1,3,5], Jumpei Ito[4], Yuka Sakata[1], Hirofumi Aso[4], Kei Sato[4], Brian P. Hermann[2] & Kotaro Sasaki[1✉]



Hwang et al., Nature Communication, 2167–2179, 2021

Future prospects

- **In vitro gametogenesis (IVG)**