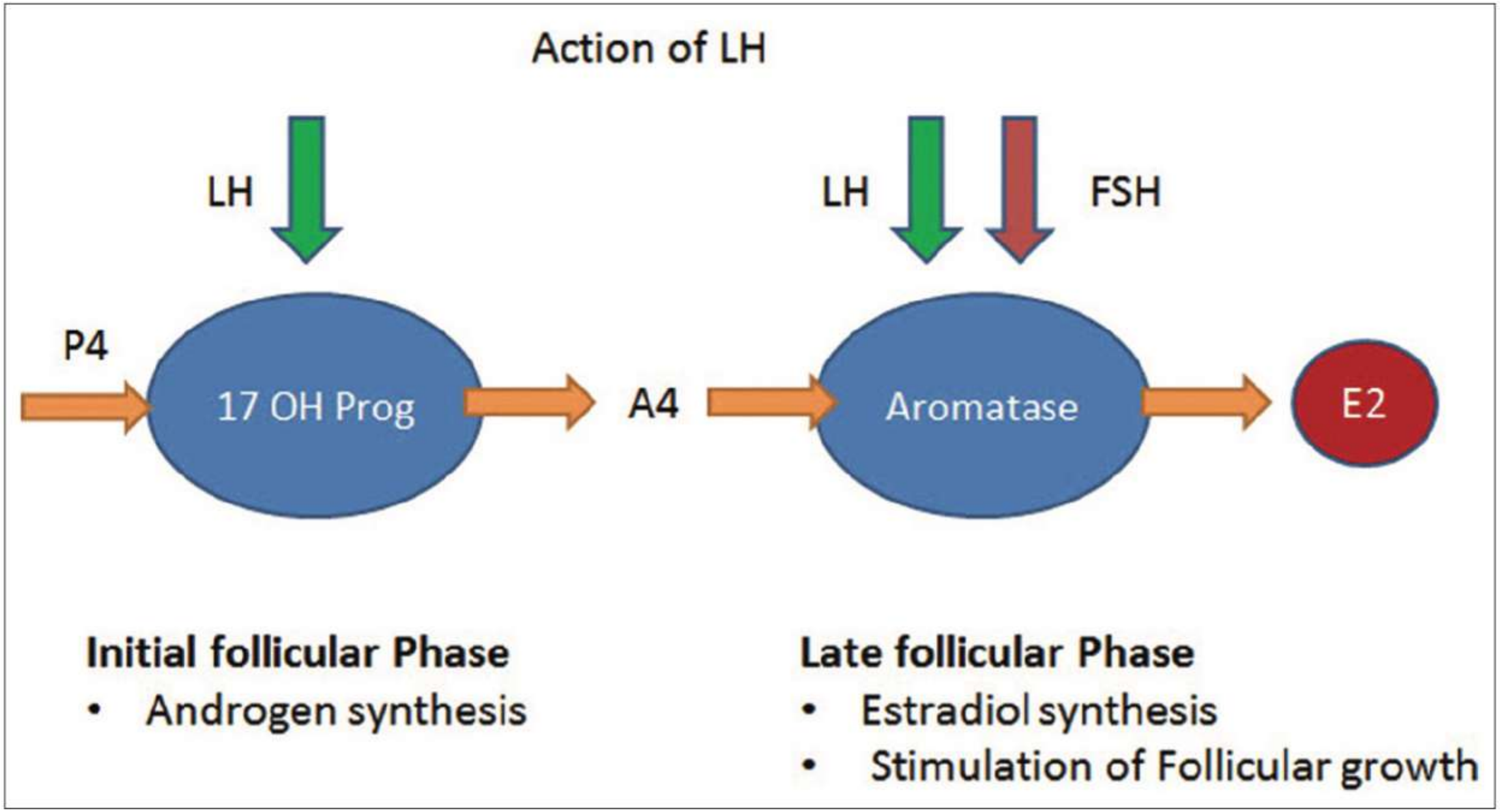
Two-cell Two-gonadotropin theory

…in a nutshells…



Ramaraju, *et al*., 2013: Role of LH in controlled ovarian hyper-stimulation

Two-cell Two-gonadotropin theory

…and more in detail…

The steroid hormones are produced from cholesterol through a series of reactions catalyzed by steroid cytochrome P450 (CYP) hydroxylases and hydroxysteroid dehydrogenases (HSD)

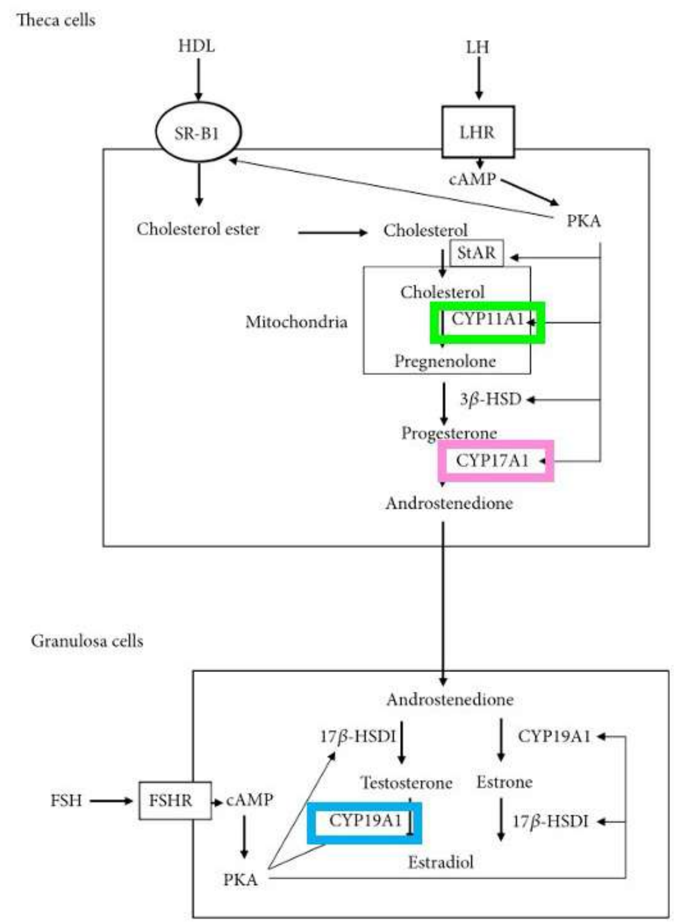


Figure 1

Schematic diagram of two-cell–two-gonadotropin theory in ovarian steroidogenesis. Theca cells autonomously produce androstenedione from cholesterol via the positive regulation of steroidogenic enzymes by LH/cAMP/PKA pathway. Granulosa cells convert androstenedione into estradiol. It is promoted by FSH/cAMP/PKA pathway.

Yazawa *et al*., 2019. Transcriptional Regulation of Ovarian Steroidogenic Genes

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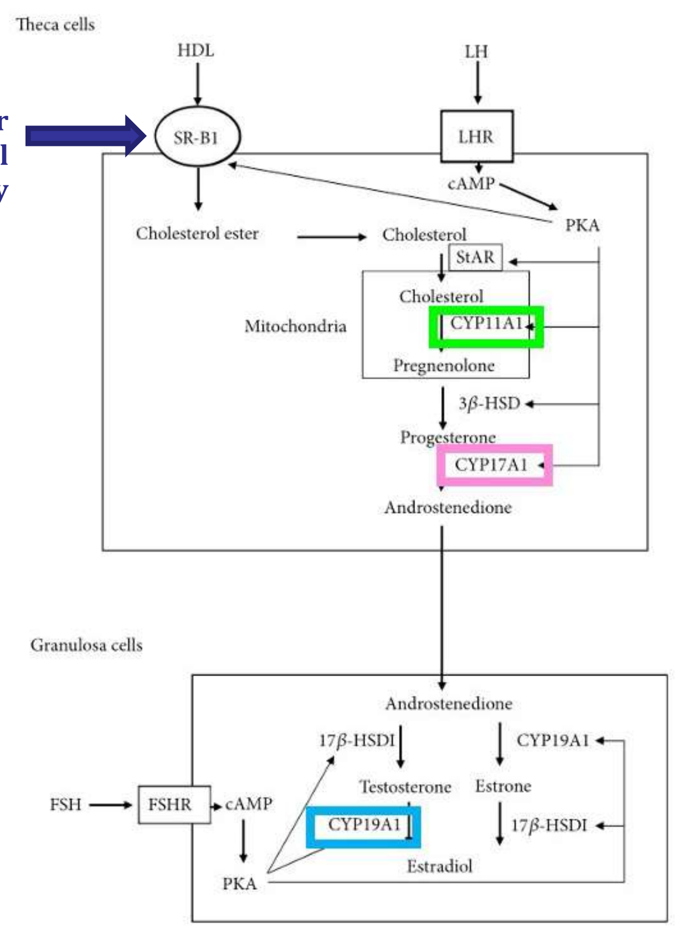
…and more in detail…

(1) The source of cholesterol for steroidogenesis depends on cholesterol ester uptake from plasma proteins by lipoprotein receptors, such us SR-B1



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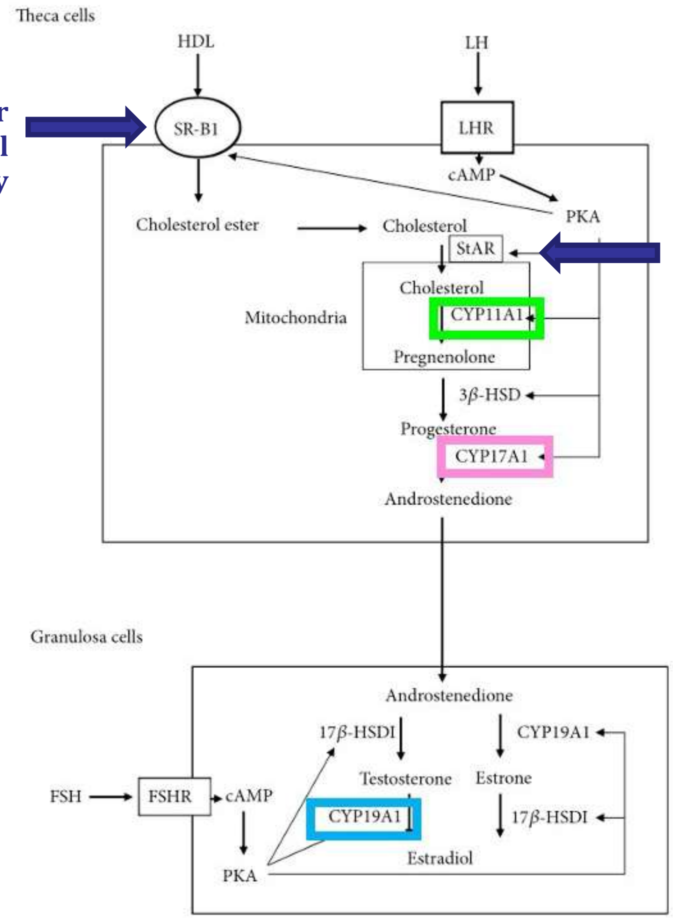

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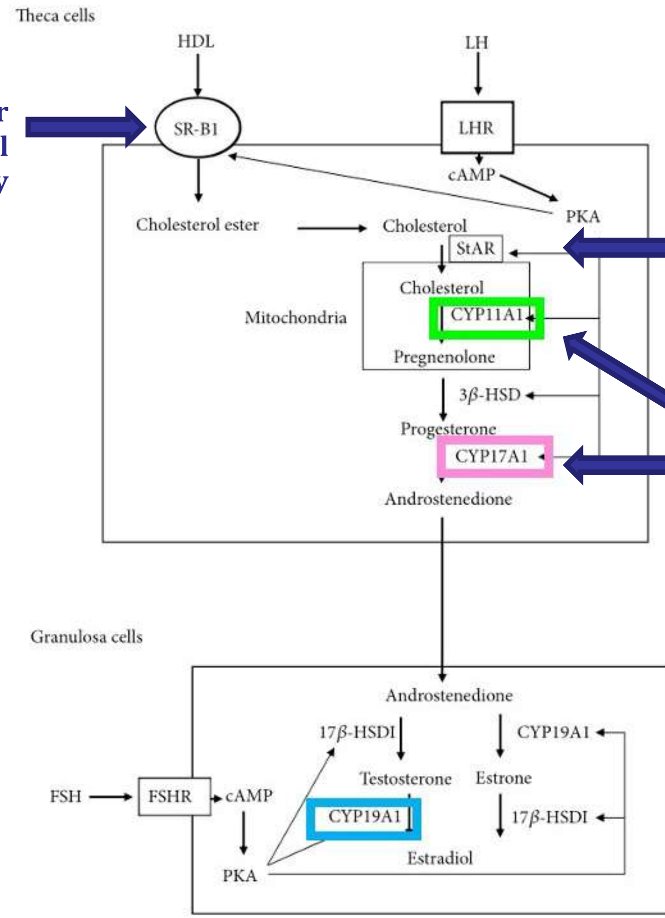

(2) Cholesterol transport from the outer to the inner mitochondria membrane by StAR represents a rate-limiting step of steroidogenesis

(3) Steroidogenesis begins with conversion of cholesterol into pregnenolone in mitochondria by CYP11A1
CYP17A1 catalyzes two sequential reactions (17α-hydroxylation and a 17,20-lyase reaction) leading to the formation of androstenedione from progesterone.

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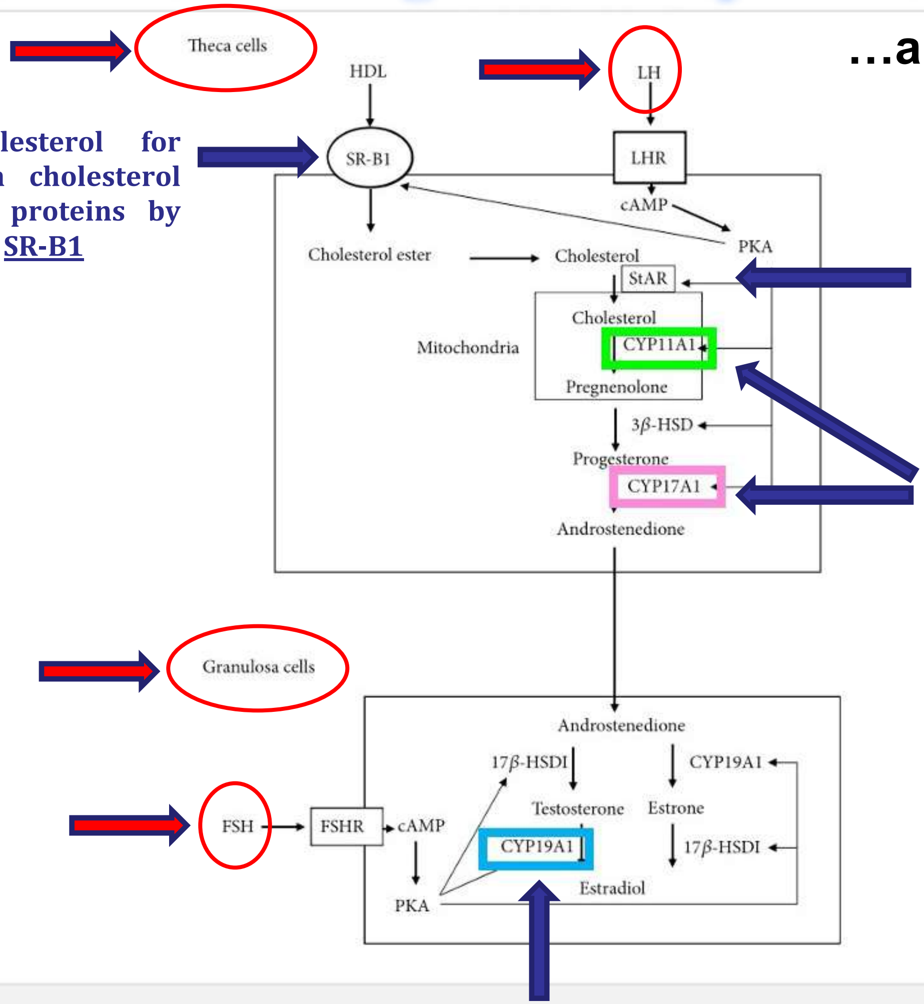

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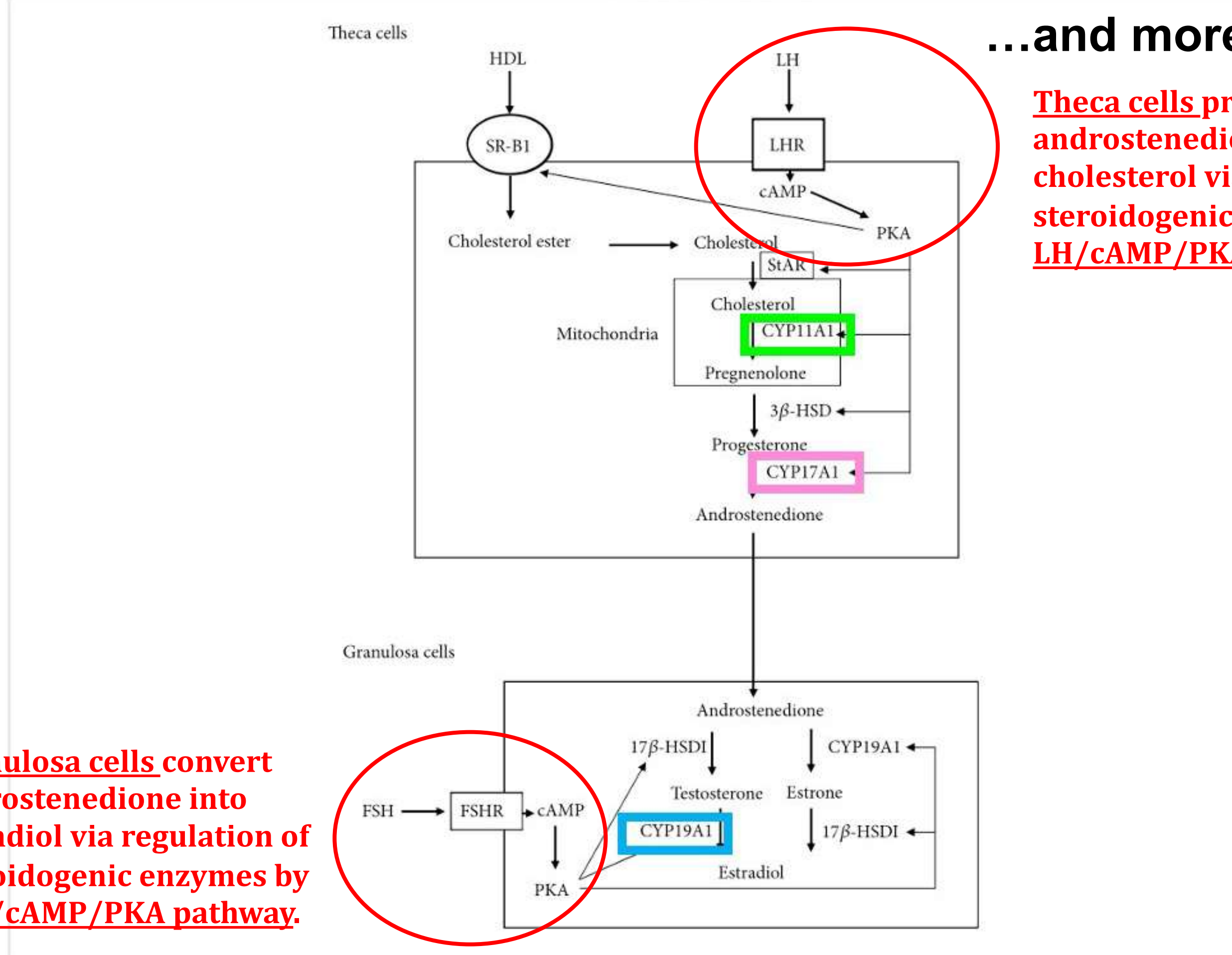
<u>Theca cells</u> produce androstenedione from cholesterol via regulation of steroidogenic enzymes by <u>LH/cAMP/PKA pathway</u>



<u>Granulosa cells</u> convert androstenedione into estradiol via regulation of steroidogenic enzymes by <u>FSH/cAMP/PKA pathway</u>.

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Two-cell Two-gonadotropin theory

…Key points to sum up…

1. The most accepted model of follicle development is the **Two-cell Two-gonadotropin theory.**

2. The ovary comprises of **two cellular components (Theca cells and Granulosa cells)**, which are stimulated by LH and FSH, leading to the production of ovarian steroid hormones.

3. These steroid hormones are produced from cholesterol through a series of reactions catalyzed by steroid cytochrome P450 (CYP) hydroxylases (**CYP11A1**, **CYP17A1**, **CYP19A1**). Specifically:

-**CYP11A1**: essential enzyme in the synthesis of all steroid hormones. In fact, steroidogenesis begins with conversion of cholesterol into pregnenolone in mitochondria by CYP11A1 enzymes. The enzyme can be found in theca cells
-**CYP17A1**: is an essential steroidogenic enzyme, which catalyzes two sequential reactions (known as 17α-hydroxylation and a 17,20-lyase reaction) leading to the formation of androstenedione from progesterone. The enzyme can be found in theca cells
-**CYP19A1**: also known as aromatase, is an enzymere responsible for a key step in the biosynthesis of estrogens. Aromatase enzyme is responsible for the »aromatization» (conversion) of androgens into estrogens. The enzyme can be found in granulosa cells.

4. **Theca cells** produce androstenedione from cholesterol via the positive regulation of steroidogenic enzymes by **LH/cAMP/PKA pathway**.
Granulosa cells, thanks to aromatase enzyme, convert androstenedione into estradiol. It is promoted by **FSH/cAMP/PKA pathway**.

TERTIARY or ANTRAL FOLLICLES: transition from antral to dominant follicle

FSH receptors are expressed exclusively in granulosa cells

LH receptors up to the stage of preovulatory follicles are expressed exclusively in theca cells, whereas in periovulatory follicles they start to be expressed also in granulosa cells (initiated by FSH and estrogens).

TERTIARY or ANTRAL FOLLICLES: transition from antral to dominant follicle

LH surge

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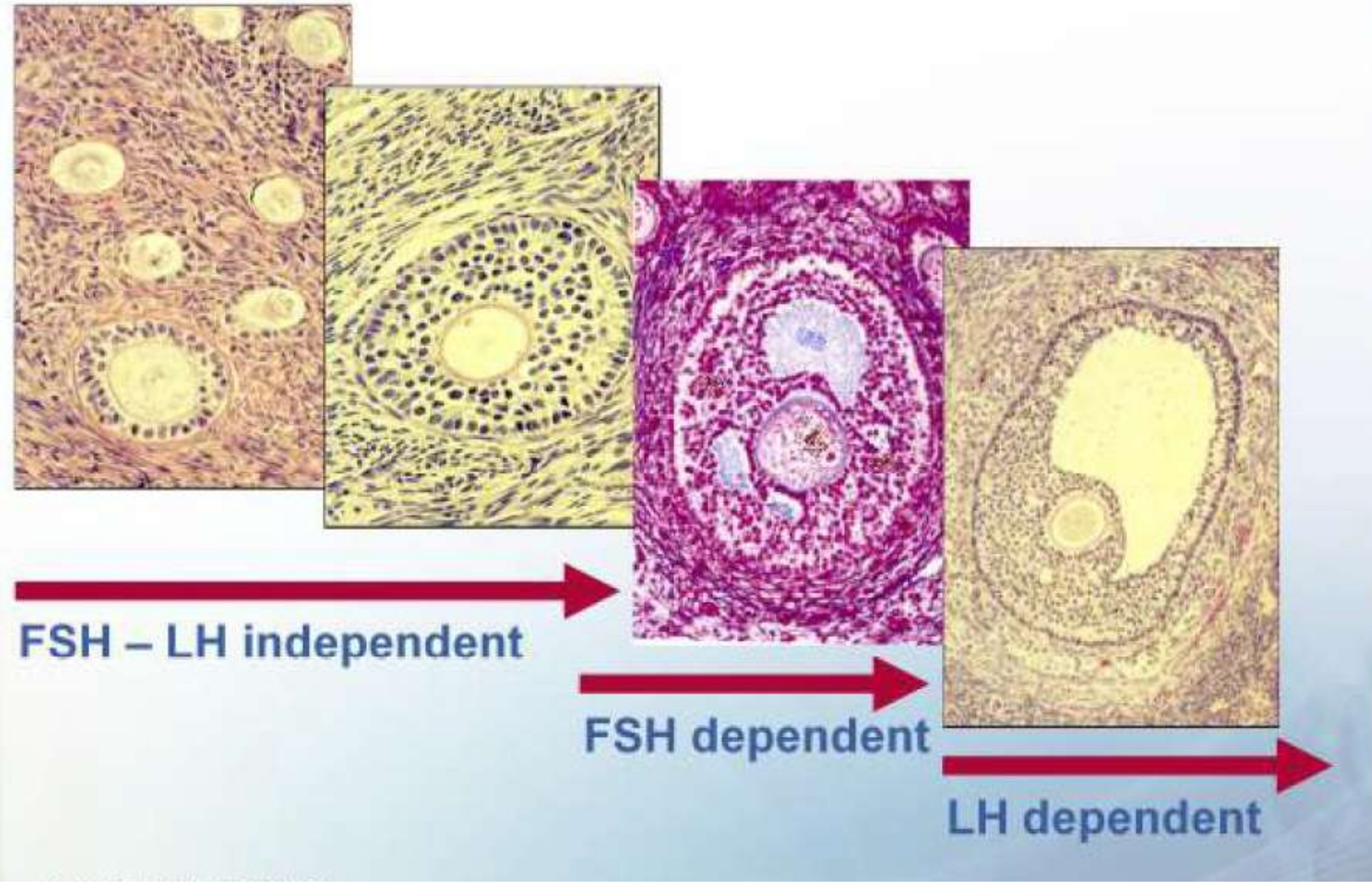
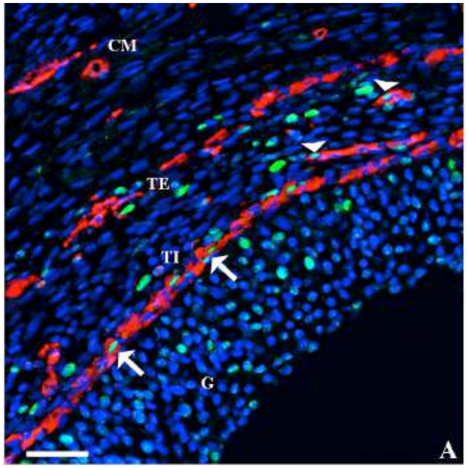

FSH – LH independent
FSH dependent
LH dependent

TERTIARY or ANTRAL FOLLICLES
PERIOVULATORY ANTRAL FOLLICLES
DOMINANT FOLLICLE

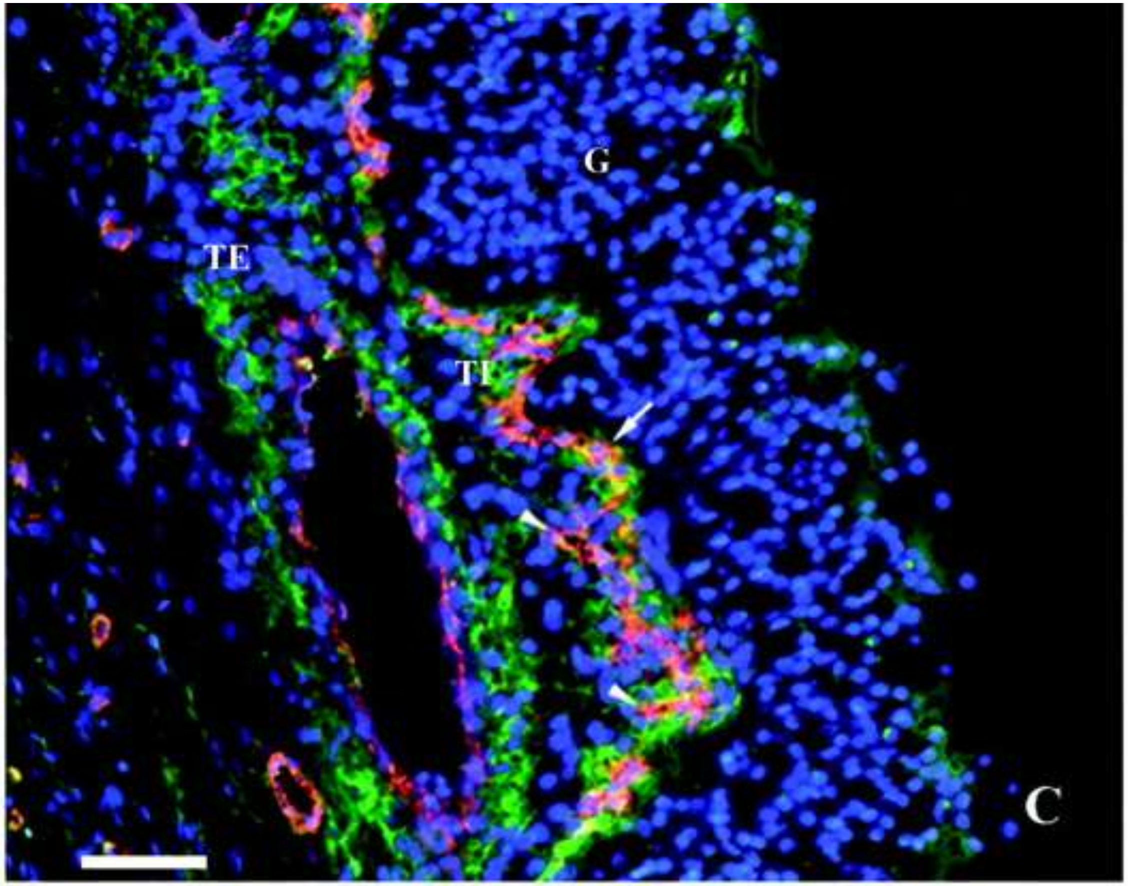
Species	Time of ovulation
Cattle	12 (10–15) hours after end of oestrus
Horse	24–48 hours before end of oestrus
Swine	38–48 hours after onset of oestrus
Sheep	18–20 hours after onset of oestrus
Goat	Near the end of oestrus
Dog	1–2 days after onset of oestrus
Cat	Induced ovulation

Onset of the LH surge to ovulation is species-specific

TERTIARY or ANTRAL FOLLICLES
PERIOVULATORY ANTRAL FOLLICLES
DOMINANT FOLLICLE

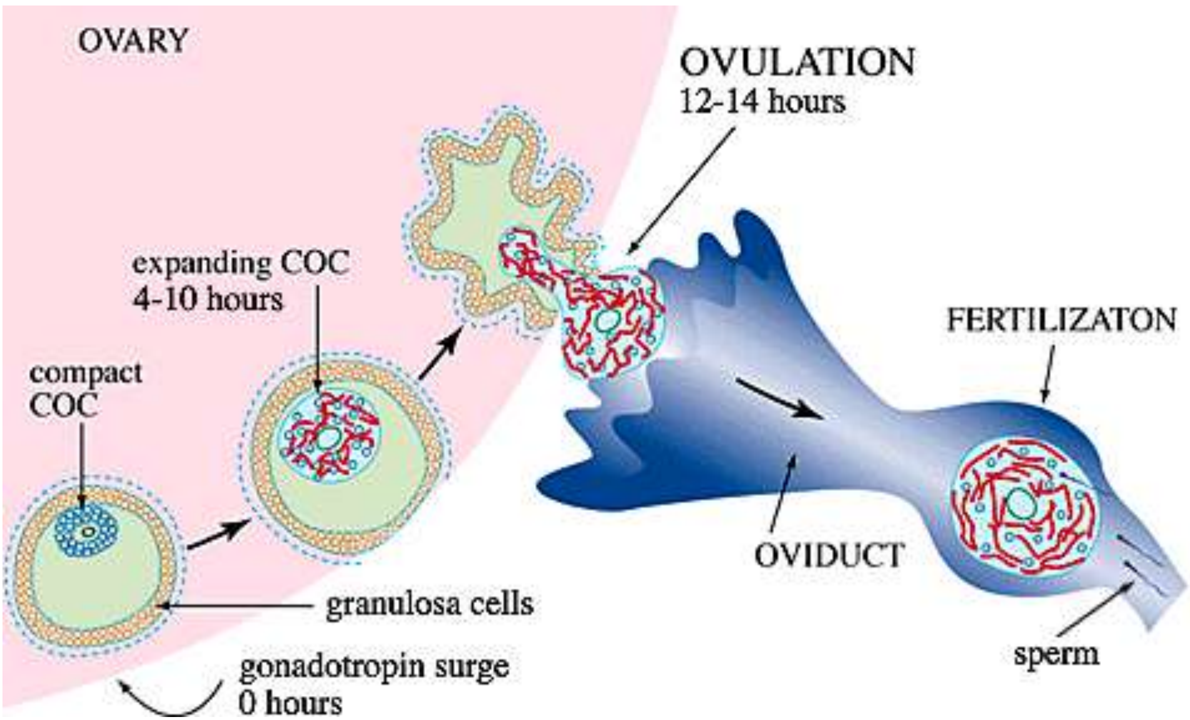


Follicular maturation



TERTIARY or ANTRAL FOLLICLES
PERIOVULATORY ANTRAL FOLLICLES
DOMINANT FOLLICLE

Cytoplamic oocyte maturation



At the time of hormonal stimulation, there is a **progressive accumulation of HA, a mucopolysaccaride, leading** to expansion of the cumulus oophorus matrix **expansion**. Shortly before ovulation the COC matrix reaches its maximal volume (20 times its initial state). It is a sort of **intercellular cement**. **Cytoplasmic oocyte maturation involves**:

- Disruption of the contact between the corona radiata cells and the oocyte membrane,
- Increase lipid content in oocyte cytoplasm,
- The cortical granules are aligned just below the oocyte membrane.

TERTIARY or ANTRAL FOLLICLES
PERIOVULATORY ANTRAL FOLLICLES
DOMINANT FOLLICLE

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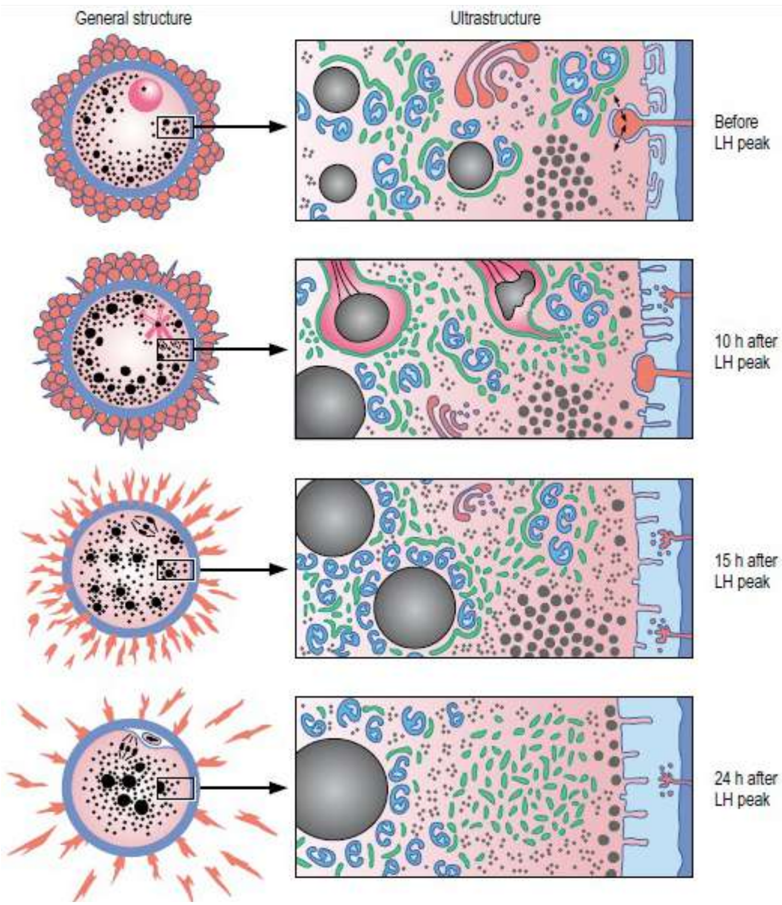


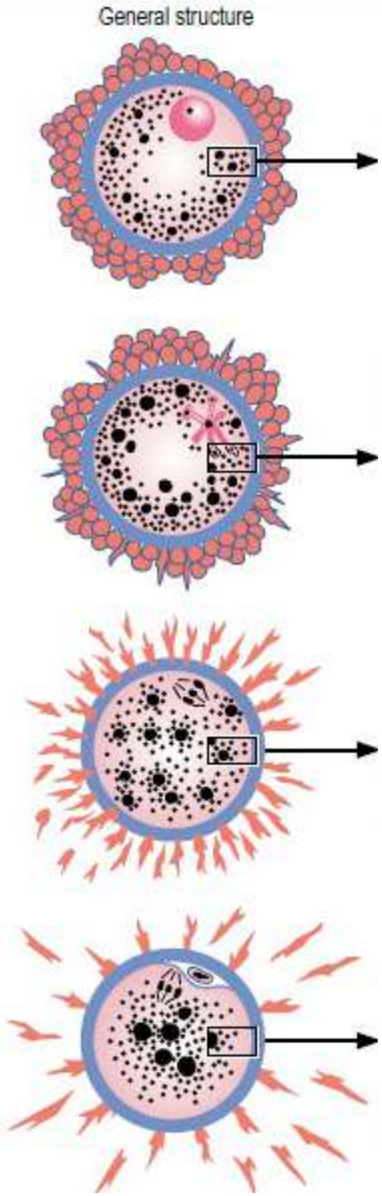
***Before** the LH peak*, the oocyte is characterized by a **peripherally located nucleus** (red) **and** a peripheral location of the **organelles**. At the ultrastructural level, the oocyte presents well developed smooth endoplasmic reticulum (SER; green), associated with lipid droplets (large black spheres) and mitochondria (blue), Golgi complexes (red), and clusters of cortical granules (small black spheres). The oocyte communicates through gap junctions with projections from the cumulus cells (arrows).

*10 h after the **LH peak***, the oocyte has resumed meiosis and the germinal vesicle, breaks down, and microtubules (black lines) appear adjacent to the condensing chromosomes (black in red nucleus). The perivitelline space between the oocyte and the zona pellucida develops, and in the oocyte the mitochondria tend to arrange around the lipid droplets and the Golgi complexes have decreased in size. The gap junctions between the oocyte and the cumulus cell projections are partially lost

***15 h after** the LH peak,* the oocyte has reached metaphase of the first meiotic division (metaphase I). The number and size of the lipid droplets have increased, mitochondria have assembled around the droplets, and these conglomerates have attained a more even distribution throughout the cytoplasm. Numerous ribosomes (black dots) have appeared, especially around the chromosomes, and the size of the Golgi complexes has decreased further. The gap junctions between the oocyte and the cumulus cell projections have been broken down

***24 h** after the LH peak*, the oocyte has reached the metaphase of the second meiotic division (metaphase II) and the first polar body has been extruded. The bulk of the cortical granules are distributed at solitary positions along the plasma membrane. The lipid droplets and mitochondria have attained a more central location in the cytoplasm leaving a rather organelle-free peripheral zone in which the most prominent features are large clusters of SER. Golgi complexes are practically absent. Ovulation occurs around 24 h after the peak of the LH surge.

TERTIARY or ANTRAL FOLLICLES
PERIOVULATORY ANTRAL FOLLICLES
DOMINANT FOLLICLE



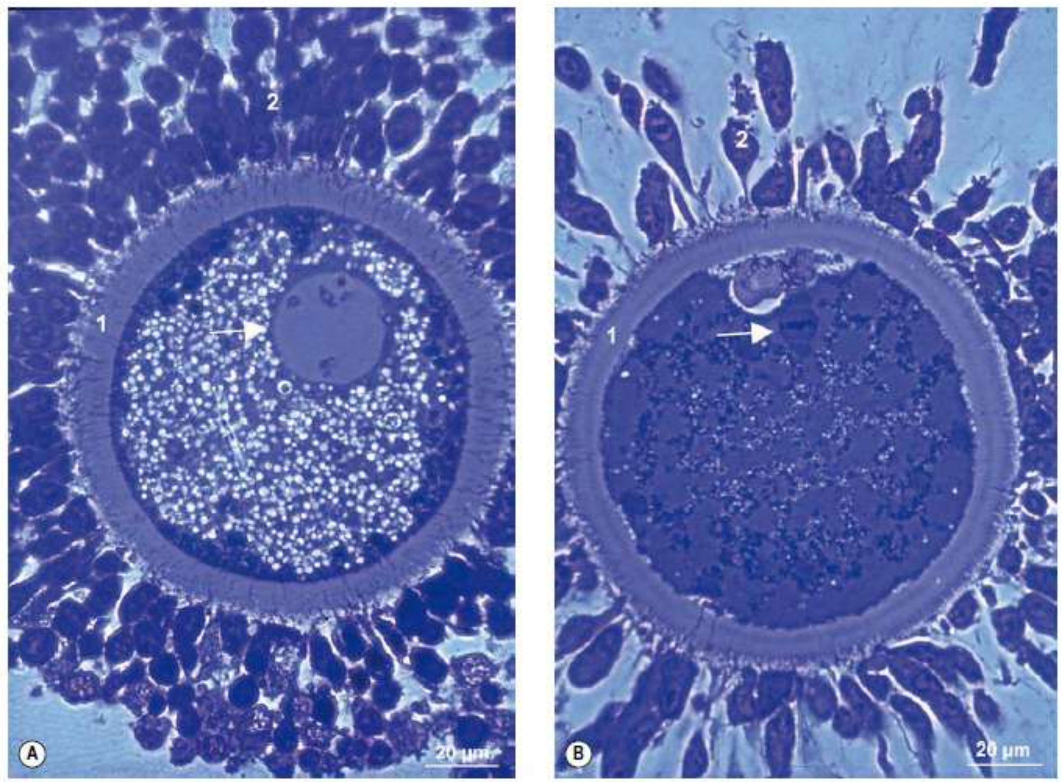
Germinal vesicle (GV)

Germinal vesicle break down (GVBD)

Metaphase I (MI)

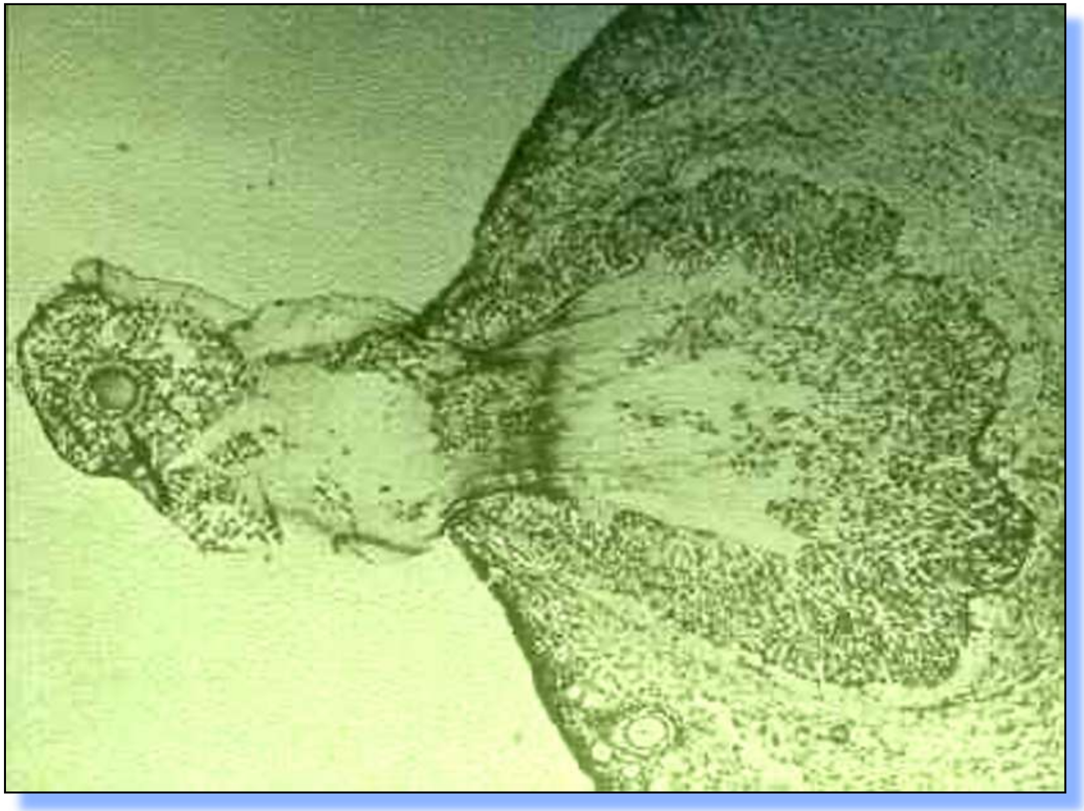
Metaphase II (MII)

Nuclear oocyte maturation

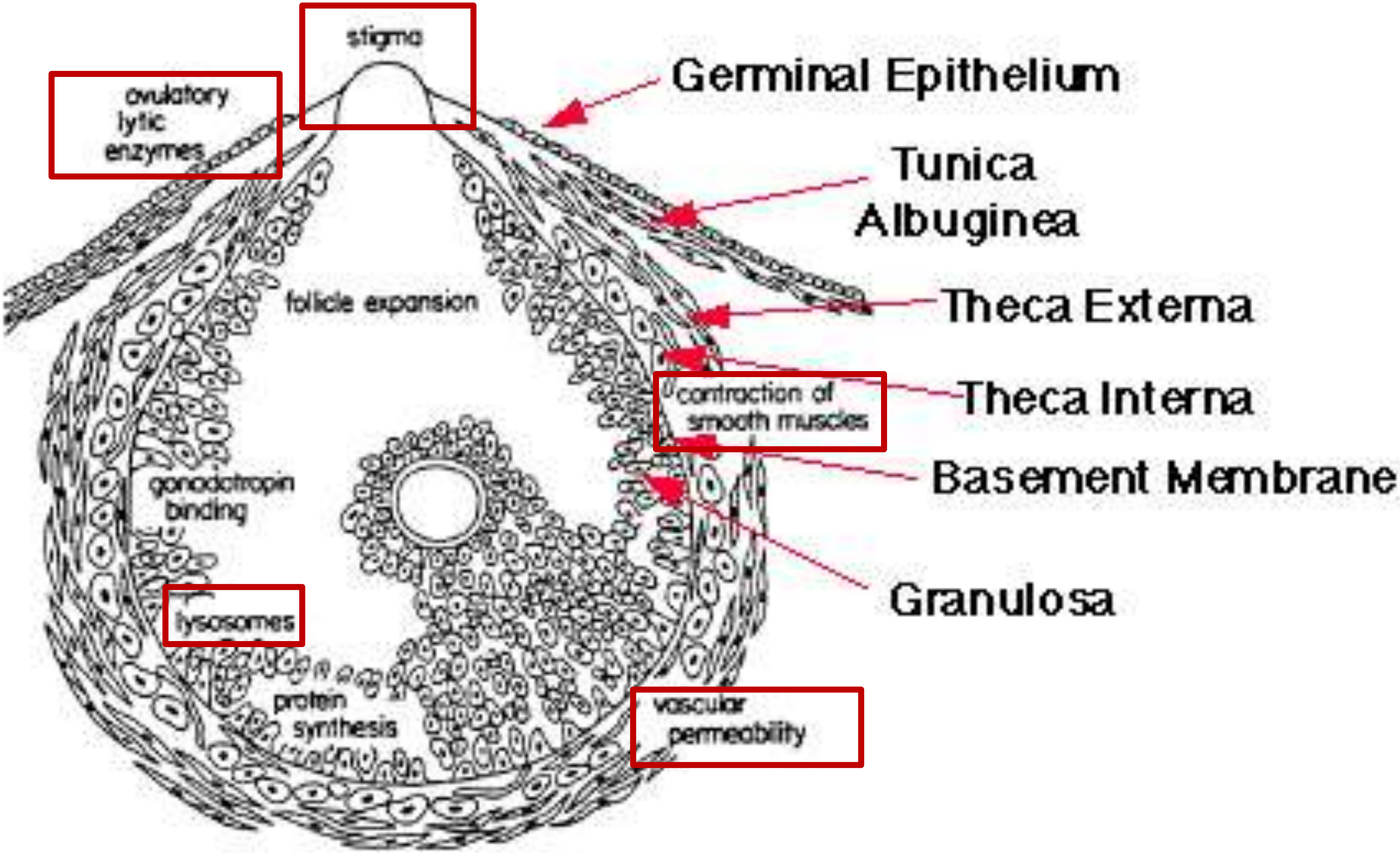


…in a nutshells…

OVULATION



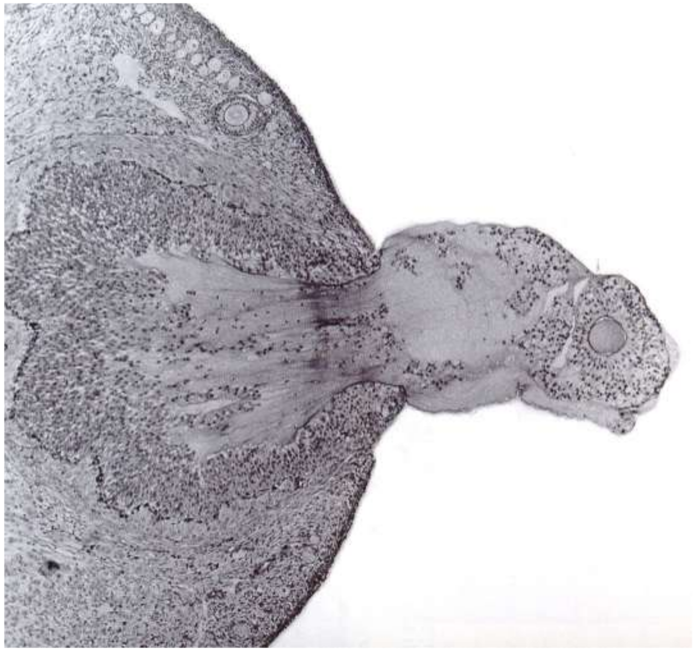
OVULATION

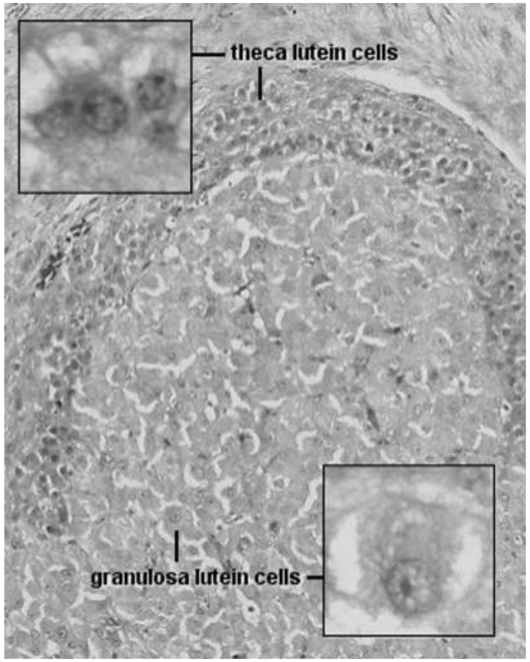

stigma
ovulatory
lytic
enzymes
Germinal Epithelium
Tunica
Albuginea
follicle expansion
Theca Externa
Theca Interna
contraction of
smooth muscles
Basement Membrane
gonadotropin
binding
Granulosa
lysosomes
protein
synthesis
vascular
permeability

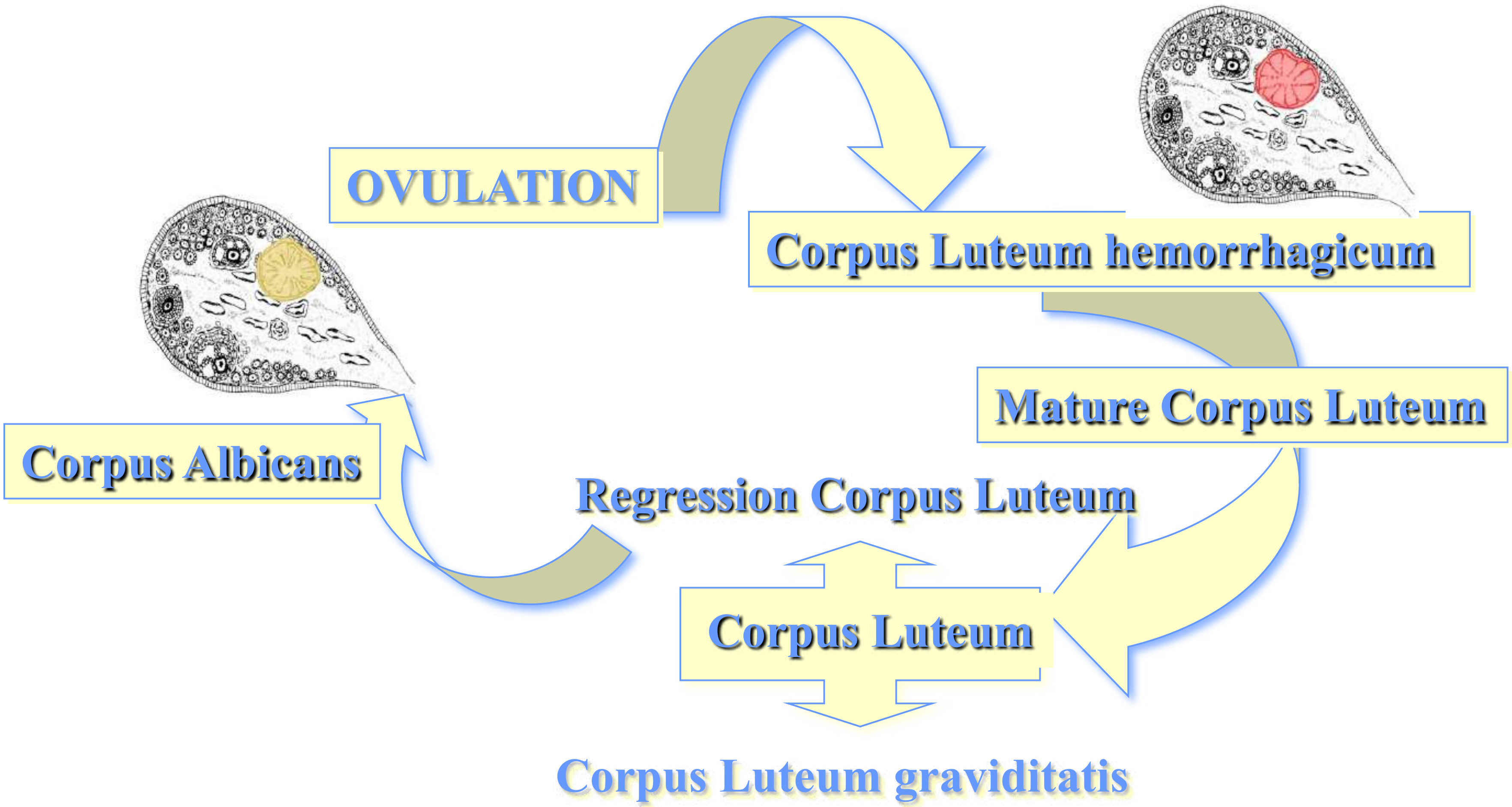
OVULATION

- An increase of intrafollicular pressure,
- Proteolytic enzyme activity on the follicular wall,
- Morphological changes in the stigma,
- Perifollicular ovarian smooth muscle contractions and
- Vascular modifications in the perifollicular vessels.

CORPUS LUTEUM

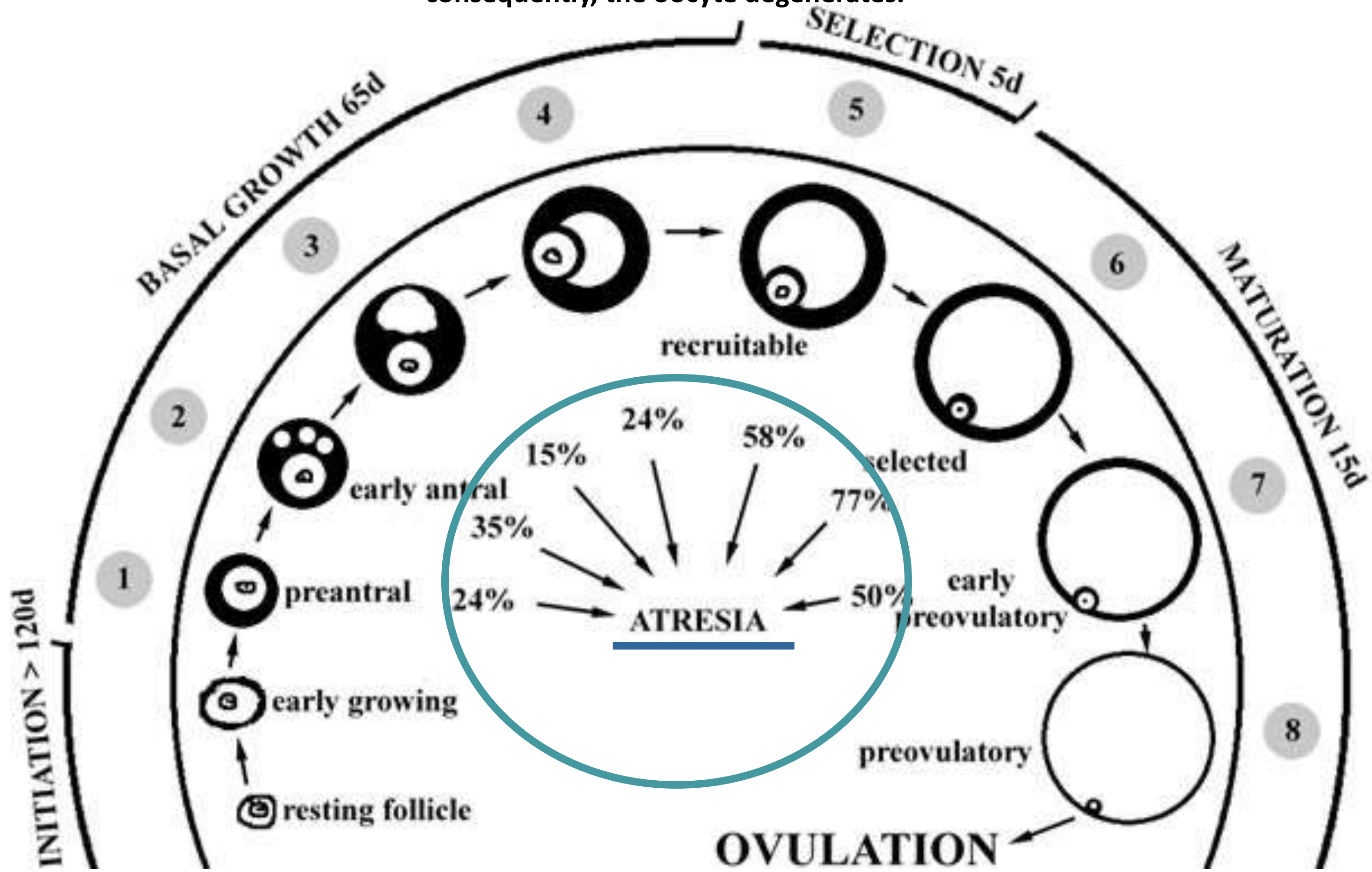





OVULATION
Corpus Luteum hemorrhagicum
Mature Corpus Luteum
Corpus Albicans
Regression Corpus Luteum
Corpus Luteum
Corpus Luteum graviditatis

ATRETIC FOLLICLES

Degeneration of follicle (atresia) can occur at any stage of development. The granulosa cells undergo apoptosis and consequently, the oocyte degenerates.



Each reproductive cycle a pool of follicles will grow (folliculogenesis), but only one (mono-ovulatory species) or few (poli-ovulatory species) will ovulate; most of them will undergo to atresia

STAGES OF ATRESIA

Healthy (non-atretic) follicle

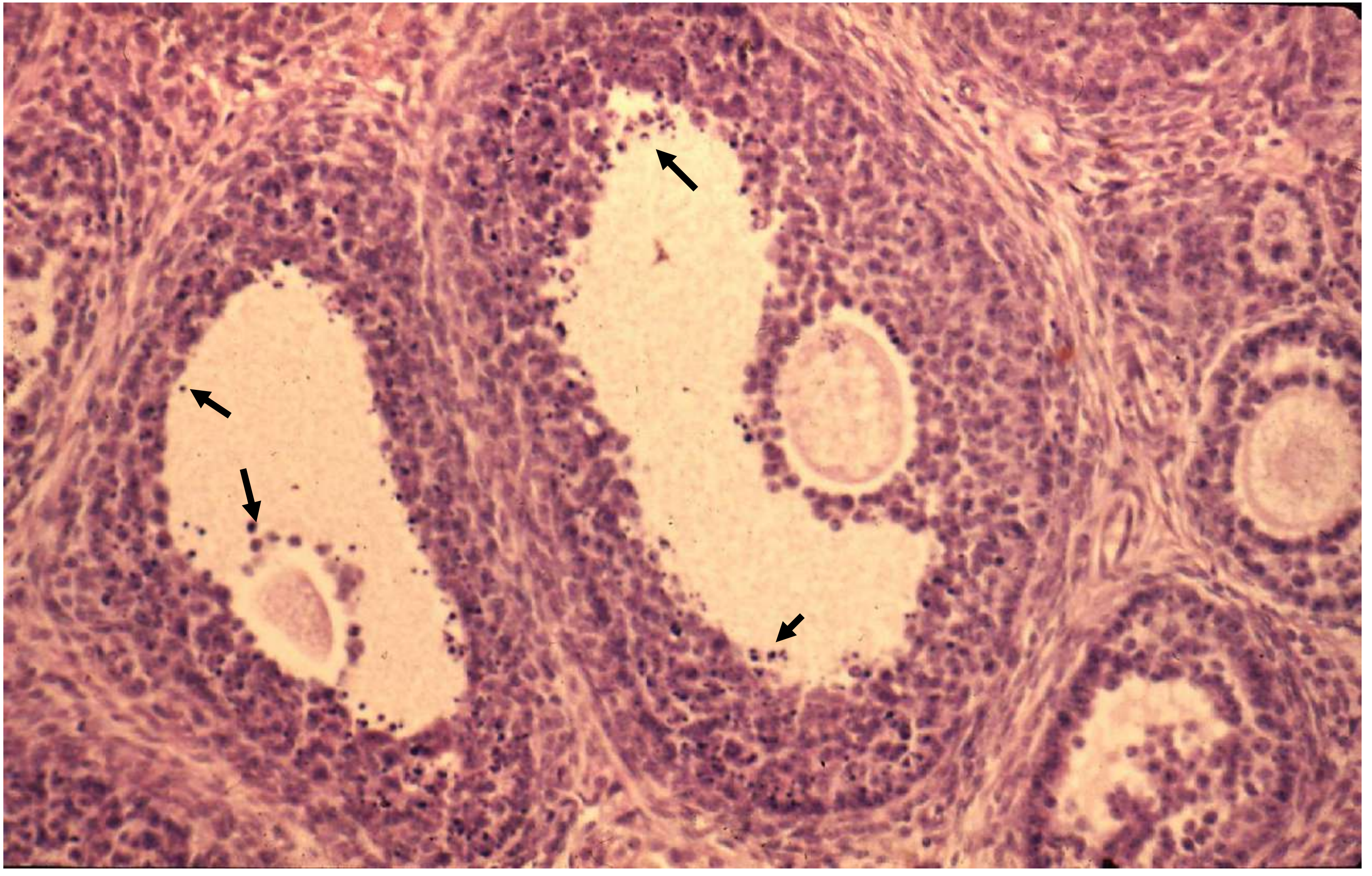
- many dividing granulosa cells (high mitotic index)
- No/very low % pyknotic granulosa cells
- follicle fluid “clean” without cell debris
- oocyte in the resting stage of prophase MI
- theca extensively vascularized

Atresia

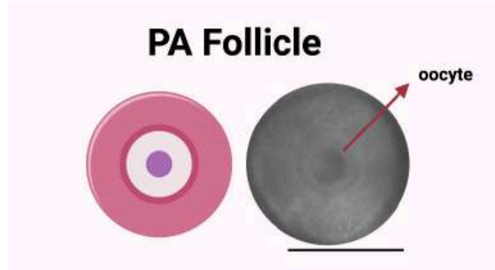
- no dividing granulosa cells
- 10-30% pyknotic granulosa cells
- detachment of granulosa cells from basement membrane
- follicle fluid contains many cell debris or atretic bodies

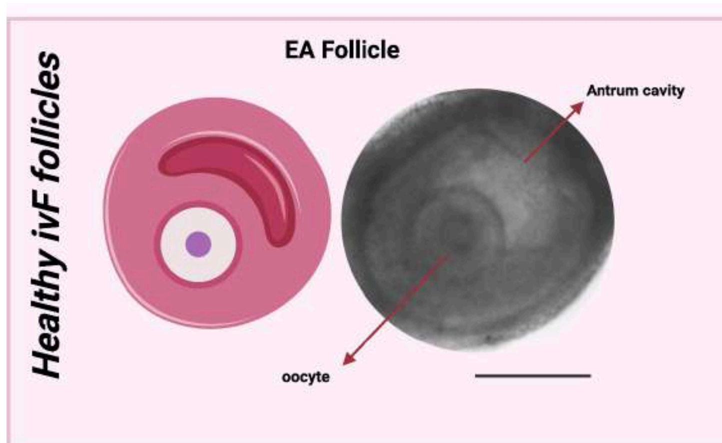
- reduction in number of granulosa cells
- oocyte fragmentation
- connective tissue invasion into follicle fluid
- follicle starts to collapse

Atretic follicles

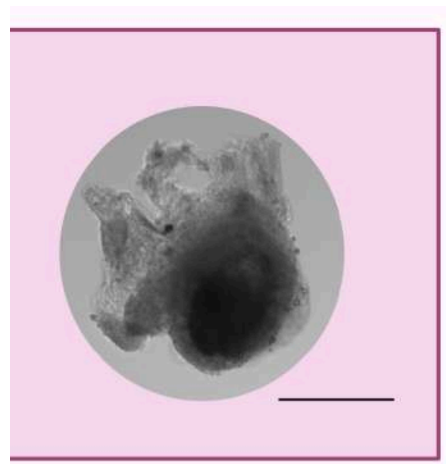


Detachment and apoptosis of granulosa cells

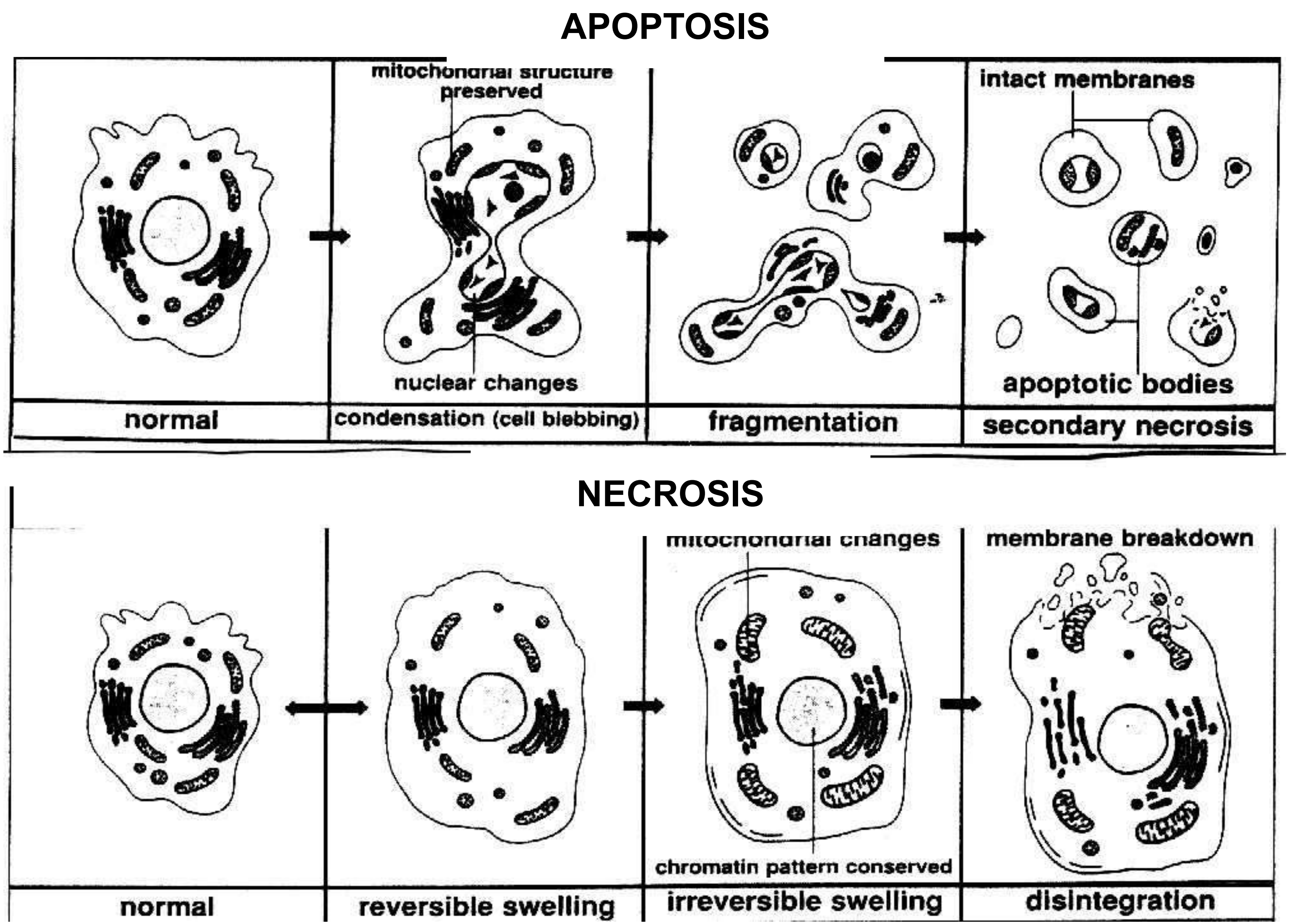




ATRETIC FOLLICLE



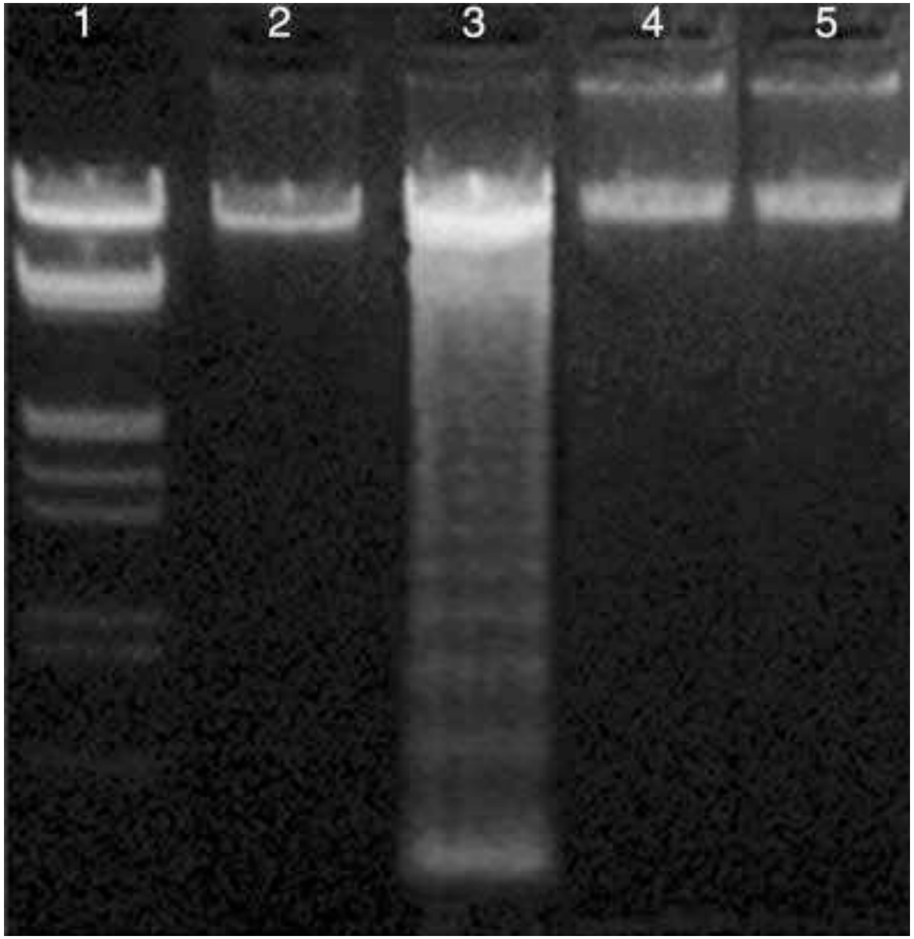
Apoptosis-programmed cell death



APOPTOSIS	NECROSIS
AFFECTS SCATTERED INDIVIDUAL CELLS	AFFECTS TRACTS OF CONTINGUOUS CELLS
CHROMATIN AND CYTOPLASMIC CONDENSATION CELL SHRINKAGE	CELL SWELLING AND RUPTURE OF PLASMA MEMBRANE
MAY REQUIRE mRNA AND PROTEIN SYNTHESIS	NOT DEPENDENT UPON NEW mRNA OR PROTEIN SYNTHESIS
NORMAL ATP LEVEL	DECREASED ATP LEVEL
NO INFLAMMATION	ELICITS INFLAMATORY RESPONSE
ENDONUCLEASE ACTIVATION AND DNA CLEAVAGE (ladder pattern)	ACTIVATION OF NONSPECIFIC Dnases (smearing)

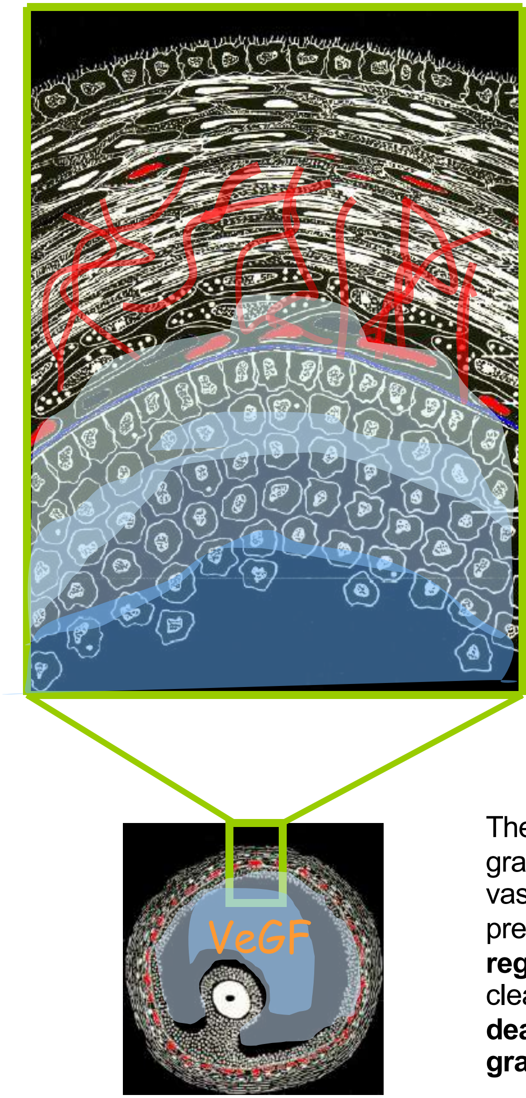
DNA FRAGMENTATION (ladder pattern)

Fig. 1. Analysis of extracted DNA from granulosa and theca layers of 4–5 mm antral and atresic follicles by 2% agarose gel electrophoresis with ethidium bromide staining. Lane 1: control (λ Eco/Hind); lane 2: healthy antral granulosa; lane 3: atretic granulosa; lane 4: healthy antral theca; lane 5: atretic theca.

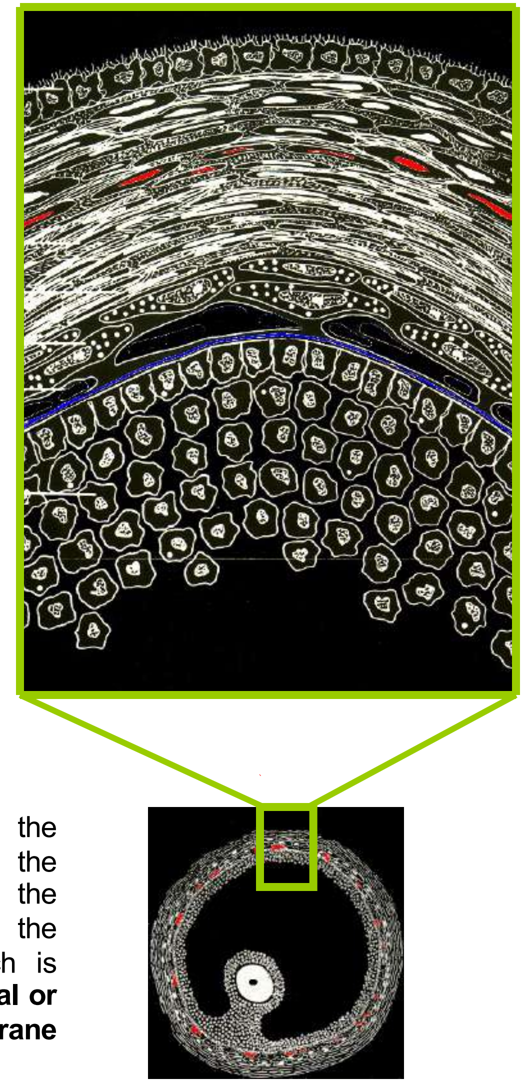


The **hallmark** of apoptosis is the presence of a typical **DNA fragmentation** that is identified by the appearance of a ladder pattern on gel electrophoresis. This gel indicates that the **cells involved in apoptosis** are for the most part **granulosa cells**.

Antral Follicle



Atretic Follicle



The apoptotic process starts within the granulosa layer, as a consequence of the vascular changes that accompany the precocious signs of atresia that involve the **regression of the inner network** which is clearly of primary importance to the **survival or death** of the adjacent avascular **membrane granulosa**

APOPTOSIS IN FOLLICULAR GRANULOSA CELLS

Bcl-2 family

Anti-apoptotic

Bcl-2

Bcl-X_L

Bcl-W

Mcl-1

Pro-apoptotic

Bax

Bcl-X_S

Bak

Bad

Caspase family

Caspase-9 (initiators)

Caspase-3 (effectors)

Death receptors

Fas-FasL

(TNF/NGF receptor family)

Caspase3 - TUNEL

Intense Caspase-3 immunoreactivity was principally observed in the granulosa cells of early atretic follicles