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Reproductive Medicine

Oogenesis and ovulation

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Two main functions of ovary

- Production of oocytes
- Production of steroid hormones

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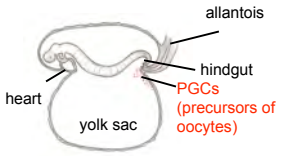
Origin of oocytes

- Primordial germ cells (oocyte precursors) first seen in endoderm of dorsal wall of yolk sac, at 3 weeks gestation
  - originate before gonadal differentiation
  - 15 - 20 µm diameter, with large nucleus



3-week human embryo

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Gonadal development

- First sign of gonadal development recognizable at 4 weeks
- Genital ridges form either side of central dorsal aorta on posterior wall of embryo (lower thoracic, upper lumbar region)
- 2 bilateral longitudinal prominences - genital ridges
  - mesenchyme
  - overlain by coelomic epithelium

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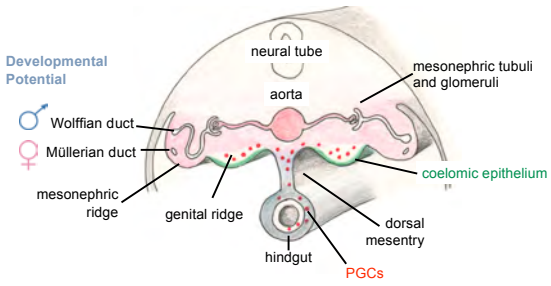
'Indifferent' gonad (4 - 5 weeks)



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PGCs migrate and proliferate

- PGCs migrate from yolk-sac endoderm
- Found in hindgut at 4 weeks
- Arrive at dorsal mesentery at 5 weeks
- Enter genital ridge at 5 - 6 weeks
- PGCs proliferate while migrating
- Migrate by amoeboid movements
  - demonstrated in vitro by timelapse photography
- May be directed by chemotactic substances from gonadal areas

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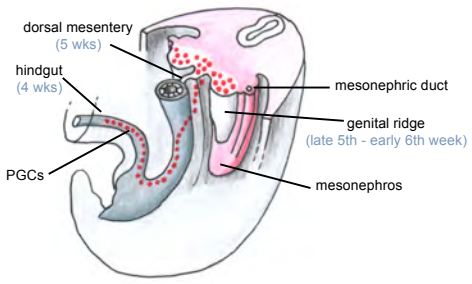
Primordial germ cell migration



Sex differentiation

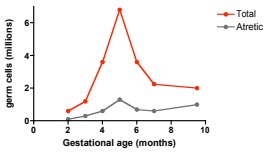
- Males have testosterone and anti-Müllerian hormone (AMH)

Males, presence of
- testosterone maintains Wolffian duct
- AMH in males leads to degeneration of Müllerian duct

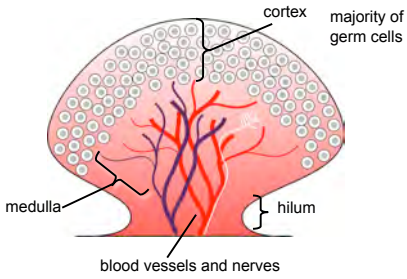
Females, absence of
- ~~testosterone~~ leads to Wolffian duct degeneration
- ~~AMH~~ leads to differentiation of the Müllerian duct into the oviduct, uterus and upper part of vagina.

PGC colonization of gonads

- After reaching genital ridge, PGCs undergo rapid mitosis
  - 5 week embryo: 700 - 1,300
  - 8 week embryo: 600,000
- Somatic tissue also undergoes hyperplasia - gonads increase in size
- PGCs occupy superficial areas of gonad
- Gonads still 'indifferent'



Gonadal differentiation



Formation of oogonia from PGCs

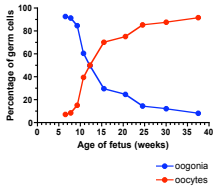
- Germ cell differentiation starts in cortical regions of ovary during 9th week
- PGCs become oogonia, which lose ability to migrate

	PGCs	Oogonia	Oocytes
Migration	✓	✗	✗
Proliferation	✓	✓	✗

Oogonia → oocytes

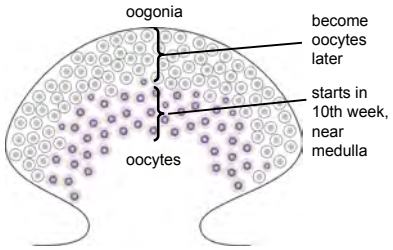
- After numerous mitotic divisions,
- oogonia differentiate into oocytes from 10 weeks onwards
- Most oogonia have become oocytes by 25 weeks
- Oogonia are capable of mitosis, oocytes are not

	PGCs	Oogonia	Oocytes
Migration	✓	✗	✗
Proliferation	✓	✓	✗



Kurilo, 1981

Oogonia → oocytes



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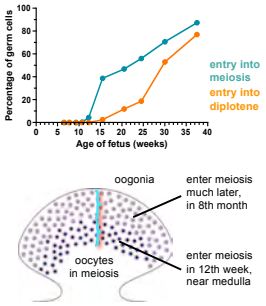
Entry into meiosis and then arrest

- Oocytes enter meiosis during the 12th - 13th week
- oocytes pass through
  - leptotene (DNA synthesis)
  - zygotene (pairing)
  - pachytene (SC formation)*
- and arrest in diplotene for between ~10 and 50 years



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Meiosis in the oocyte

- Produces single haploid gamete (secondary oocyte) from a diploid parental cell (primary oocyte)
- Chromosome number is reduced because there is one round of DNA replication followed by two rounds of chromosome segregation
  1. separation and segregation of homologous chromosomes to oocyte and first polar body
  2. separation and segregation of sister chromatids to oocyte and second polar body (cf mitosis)

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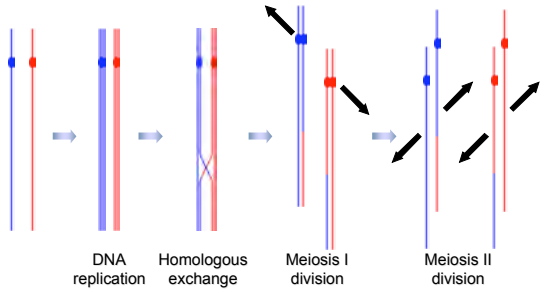
Landmarks of meiosis



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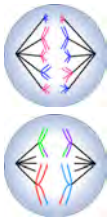
Functions of meiosis

- maintain chromosome number
  - consistency in offspring by producing haploid gametes
- introducing genetic variation
  - random assortment of parental chromosomes between the gametes
  - recombination of genetic material by 'crossing over' of chromosomal segments



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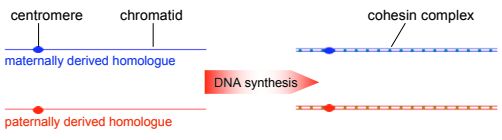
Entry into meiosis: leptotene

- DNA synthesis produces chromosomes with 2 chromatids
- This is the only DNA synthesis during meiosis
- Cohesins hold chromatids together



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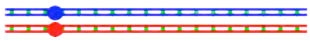
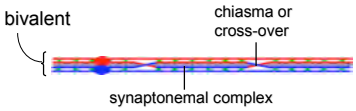
Entry into meiosis: zygotene

- distance pairing - homologues lie side by side
- initiation of chromosome synapsis



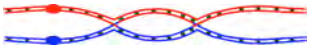
Entry into meiosis: pachytene

- two paired homologues synapse (become stuck together with synaptonemal complexes),
- undergo recombination, or crossing over, with formation of chiasmata
- form a 'bivalent'



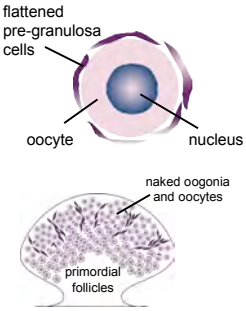
Entry into meiosis: diplotene

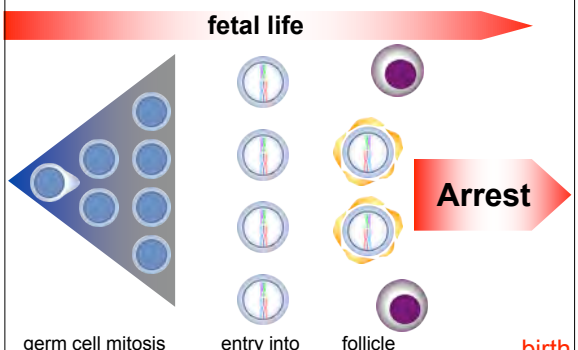
- synaptonemal complexes removed, so close apposition of homologues released,
- homologues held together only at crossovers (or chiasmata) through metaphase I, until anaphase I.
- oocyte arrested in this state of meiosis for up to 50 years



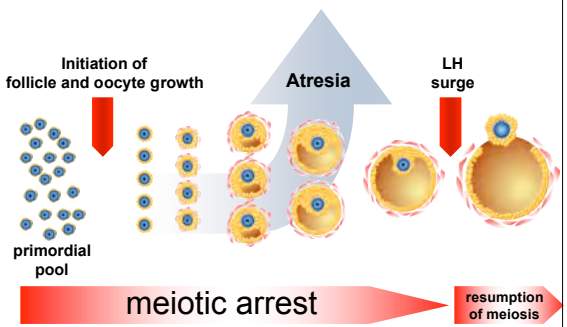
Oocytes in diplotene form follicles

- Oocyte reaches diplotene,
- becomes enveloped in pre-granulosa cells and forms a primordial follicle
- Follicle formation begins (near medulla) during the 15th - 16th week and continues early postnatally
- some oocytes do not form follicles and die (mainly in zygotene and pachytene)

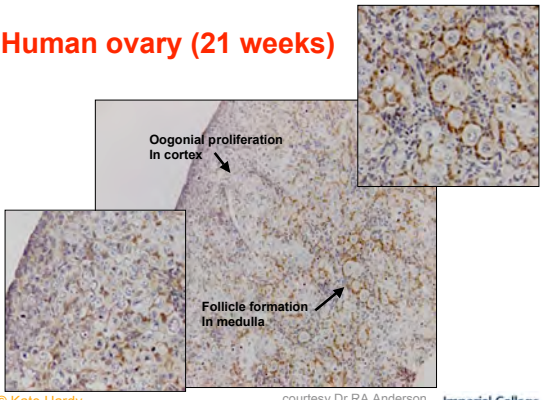




Meiotic arrest for up to 50 years



Human ovary (21 weeks)



courtesy Dr RA Anderson

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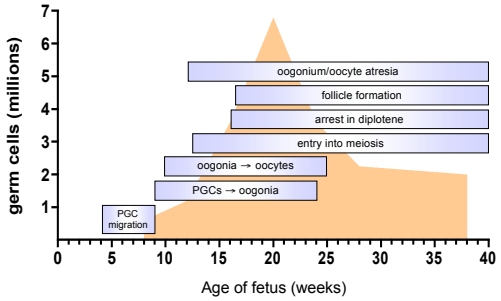
Asynchrony of developmental stages of female germ cells during gestation



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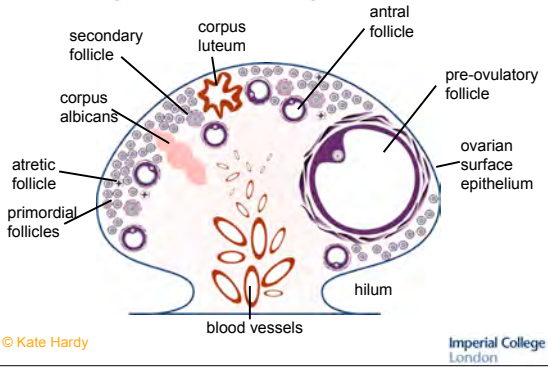
Anatomy of adult ovary



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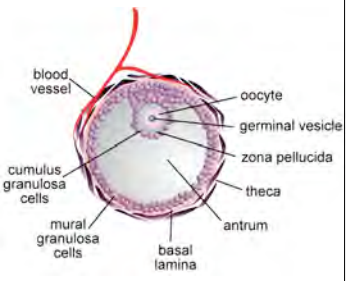
Function of a follicle

- Produce steroid hormones
- Create a specialized environment to nourish and mature a healthy oocyte



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Ovary contains 'pool' of arrested oocytes, enclosed in follicles

- Female born with complete supply of oocytes
- **All** oocytes are arrested in prophase of Meiosis I, except the ovulating oocyte
- Most follicles in cortex are arrested at primordial and primary stages
- Some follicles towards medulla are at later stages
- Throughout reproductive life, a small proportion of follicles escape arrest and start growing

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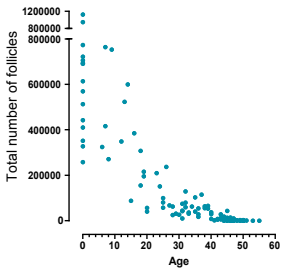
Declining number of oocytes

- Exponential decline in oocyte number
- Great variability in oocyte endowment at birth (~600,000)
- Only 10,000 oocytes remain in 20s
- 60-fold decline in first 20 years!

Decline:

- entry into growth phase
- atresia (follicle death)



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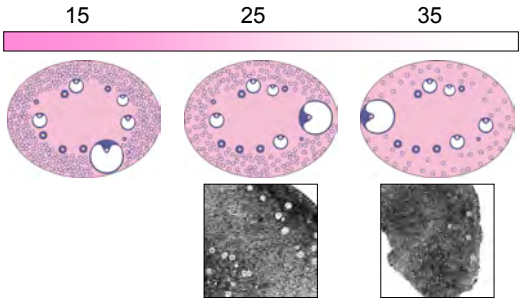
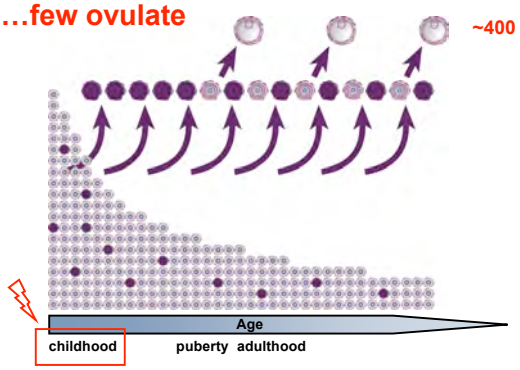
Primordial pool declines with age



Follicles initiate growth all the time …few ovulate



What regulates initiation of growth?

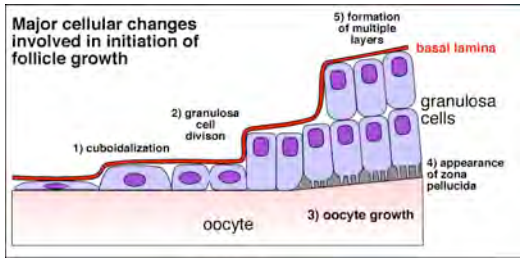
Regulation

- Not known
- Release of inhibitory signal?
- Stimulatory signal?

Growth

- Granulosa cells become cuboidal and start dividing
- Oocyte starts to grow

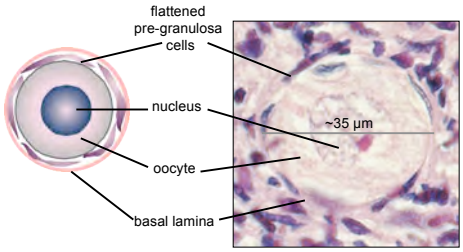
Cellular changes during initiation



Oocyte growth

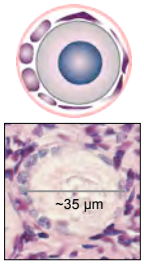
- Human oocyte grows in diameter from 35 µm to 120 µm
- 40-fold increase in volume
- Oocytes start growing at primary follicle stage
- Oocyte growth complete around the time of antrum formation
- Accumulating organelles and stored molecules needed for fertilization & preimplantation development
  - stable RNA
  - proteins

Primordial follicle

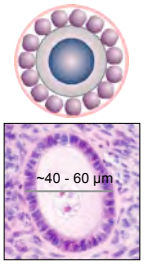


Granulosa cells become cuboidal

Transitional follicle



Primary follicle

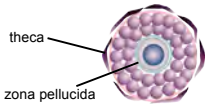


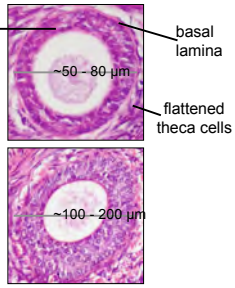
single layer of cuboidal granulosa cells

oocyte starts to grow

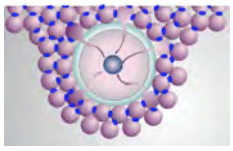
Granulosa cells form multiple layers

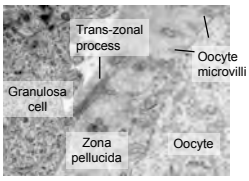
- multiple layers of granulosa cells
- theca cells start to assemble
- zona pellucida around oocyte produced





Trans-zonal processes





- close and extensive direct contact between oocyte and granulosa cells
- can also penetrate deep into oocyte
- most abundant during oocyte growth

Control of follicle growth

Intraovarian growth regulatory factors (important early)

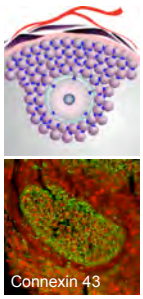
- IGF-I
- Steroids
- TGFβ superfamily members
  - Growth differentiation factor-9 (GDF-9)
  - Bone Morphogenetic Proteins (BMPs)
  - Anti Müllerian hormone (AMH)
- Wnt/frizzled family

Endocrine factors (essential during antral development)

- FSH
- LH

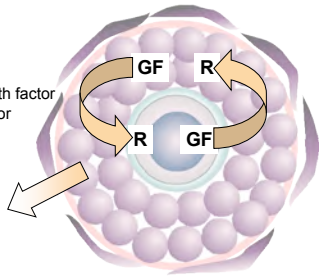
Extensive gap junction communication between granulosa cells (GCs) and oocyte

- Allow passage of small molecules such as cAMP
- Coordinates cellular events throughout follicle (eg luteinization, atresia)
- Granulosa cells can nourish oocyte
  - pyruvate
  - metabolic precursors
    - amino acids
    - nucleotides



Paracrine growth factor signalling within and between follicles

GF = growth factor
R = receptor



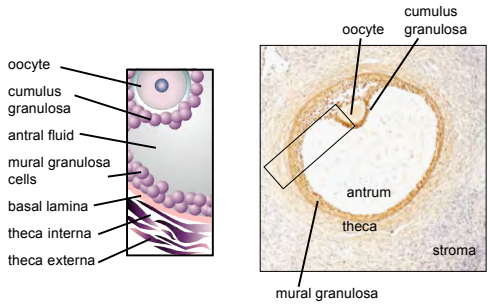
e.g.
- Kit Ligand
- BMPs (Bone morphogenetic proteins)
- AMH (Anti-Müllerian hormone)
- TGFβs (Transforming growth factor βs)

Antrum formation

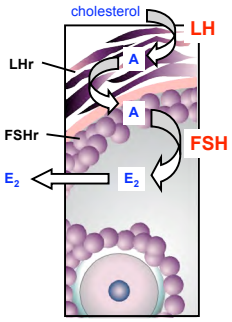
Antrum:

- Fluid-filled cavity
- Forms when follicle is ~200μm in diameter
- Occurs when ~2000 granulosa cells in all species
- Function
  - prevent necrosis in centre of follicle?

Antral follicle

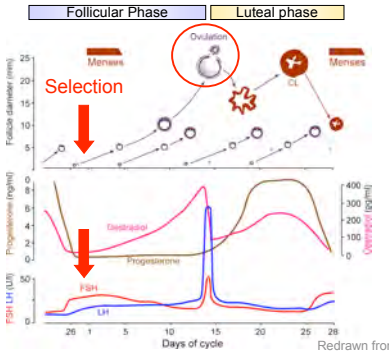


Steroidogenic function of antral follicle



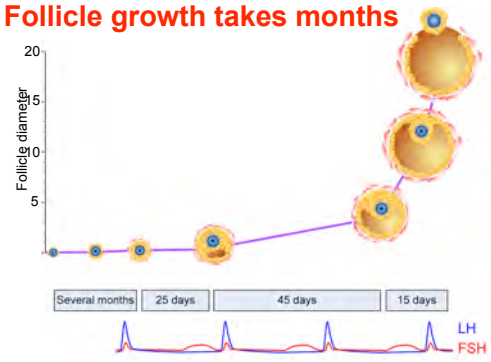
- Theca cells express LH receptors
- With LH stimulation, theca cells produce androgen
- Androgen enters granulosa cells
- Granulosa cells express FSH receptors.
- Under FSH stimulation, androgen converted to oestradiol

The ovulatory cycle

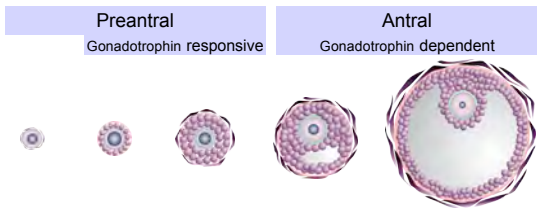


Redrawn from Baird 1981

Follicle growth takes months



Human follicle development

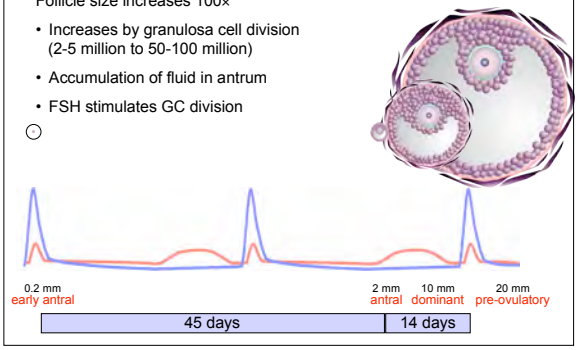


40 μm	50 μm	>70 μm	0.2 mm	20 mm
primordial	primary	secondary	early antral	Graafian (pre-ovulatory)

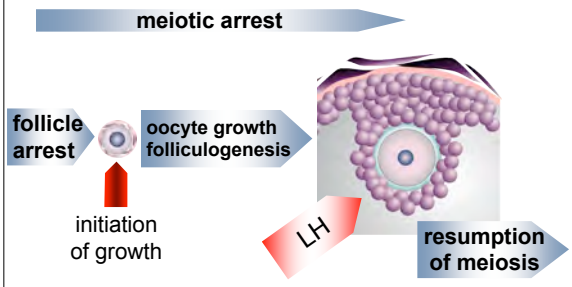
Antral follicle growth

Follicle size increases 100×

- Increases by granulosa cell division (2-5 million to 50-100 million)
- Accumulation of fluid in antrum
- FSH stimulates GC division



Remember! Oocyte has been arrested throughout folliculogenesis, until…



Maintenance of meiotic arrest

- Resumption of meiosis stimulated by
  - hormonal trigger (LH)
  - release of oocyte from follicle & culture *in vitro*
- Meiosis maintained by cAMP, probably supplied to oocyte from granulosa cell via gap junctions

LH surge causes

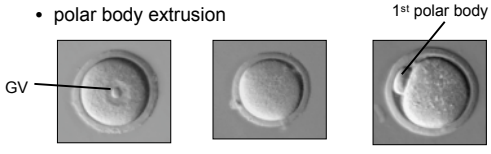
- withdrawal of GC processes from oocyte
- ↓gap junction communication
- ↓cAMP within oocyte
- resumption of meiosis
- production of hyaluronic acid → mucification and expansion of the cumulus GCs
- ovulation of oocyte
  - LH surge stimulates expression of
    - prostaglandins
    - proteolytic enzymes
- luteinization of follicle
  - shift in steroid production by GCs from E_2 to P

LH surge stimulates

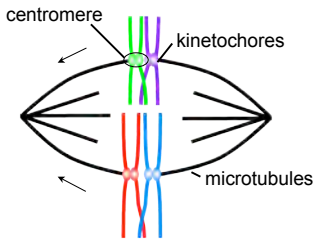
- Resumption of meiosis

manifest by morphological changes known as

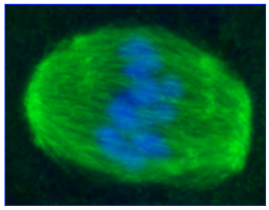
- Nuclear maturation
  - germinal vesicle breakdown (GVBD)
  - polar body extrusion



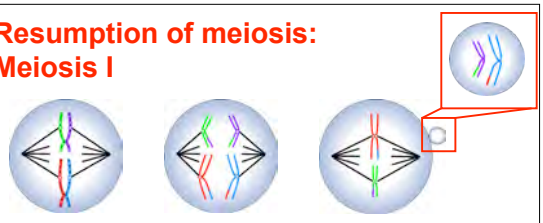
Meiosis resumes with formation of metaphase I spindle



Metaphase I spindle: separation of homologous chromosomes



Resumption of meiosis: Meiosis I



Metaphase I

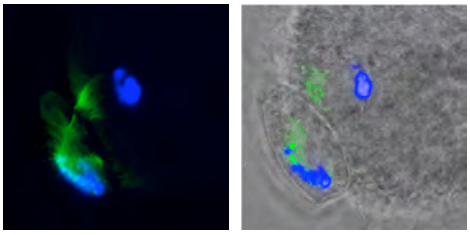
Anaphase I
- Resolution of chiasmata (cross-overs),
- Separation of homologues

Metaphase II

Ovulation

meiotic arrest

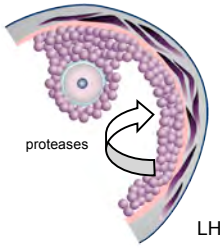
Extrusion of the 1st polar body



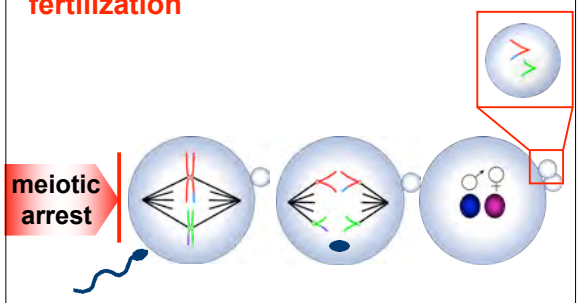
Ovulation

- Rupture of ovarian surface to allow release of oocyte
  - activation of collagenase and proteases
- Reorganization and remodelling of follicle → corpus luteum
  - recruitment and invasion of leukocytes and macrophages
  - vascularization of follicle

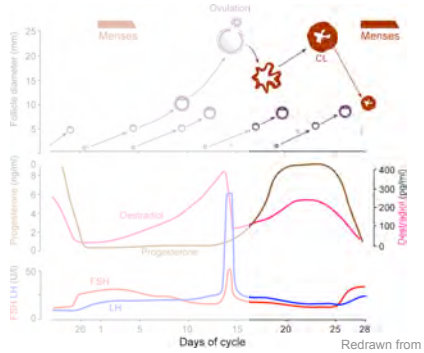
Follicle rupture



Meiosis II only resumed at fertilization



The luteal phase

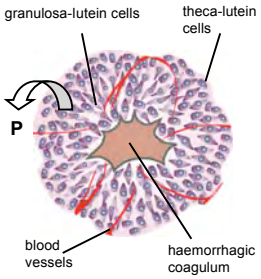


Redrawn from Baird 1981

Corpus luteum formation

After ovulation

- Granulosa and theca cells luteinize
- blood vessels invade granulosa-lutein cells
- increased vasculature supplies exogenous cholesterol for progesterone production



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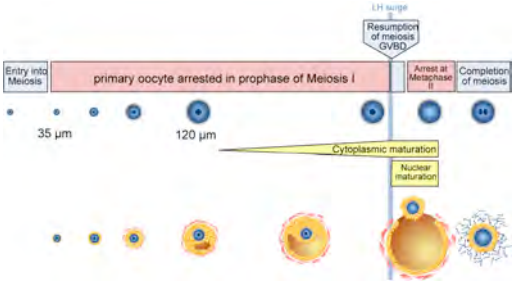
Meiosis and oogenesis



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