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BSc in Reproductive & Developmental Sciences

Oogenesis and meiosis: making a good egg

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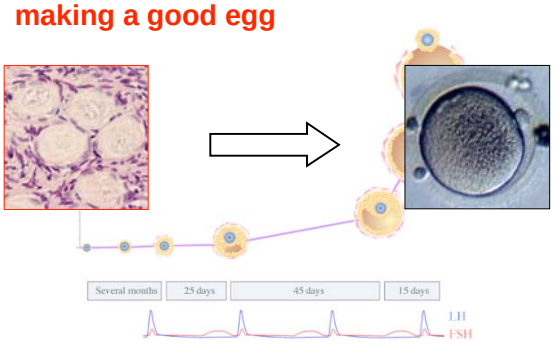
Institute of Reproductive and Developmental Biology

Oocyte maturation and meiosis: making a good egg



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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

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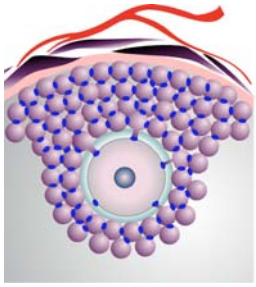
Extensive gap junction communication between granulosa cells (GCs) and oocyte



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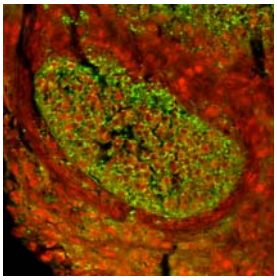
Gap junctions

- 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



Connexin 43

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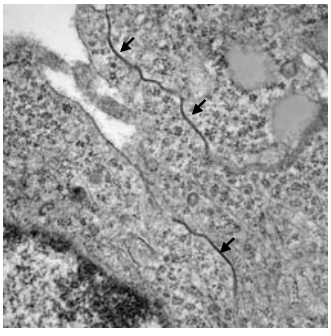
TEM of gap junctions between granulosa cells (mouse)



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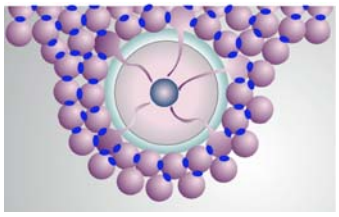
Trans-zonal processes



- can also penetrate deep into oocyte
- most abundant during oocyte growth

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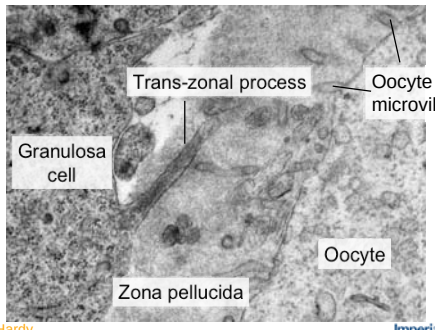
Mouse ovary (day 10)



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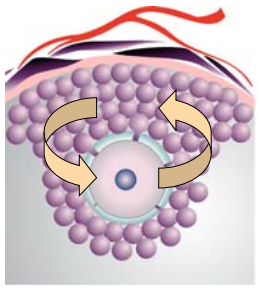
Paracrine communication between GCs and oocyte and vice versa



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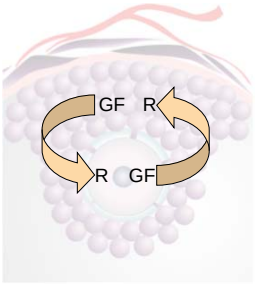
Paracrine communication between GCs and oocyte and vice versa

GF = growth factor
R = receptor



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

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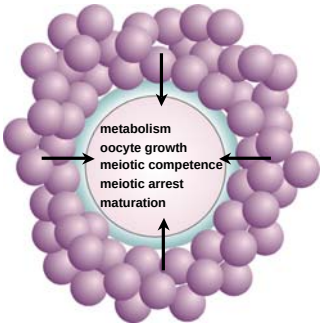
Granulosa cells essential for



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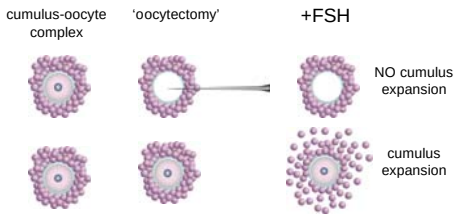
Oocyte essential for many processes



Conclusion: an oocyte-derived factor is essential for cumulus expansion.
Cumulus-expansion enabling factor (GDF-9?)

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Oocyte → granulosa cell signalling

- Oocyte essential for
  - follicle organization - follicles do not form in absence of oocytes
  - cumulus expansion after LH surge
  - granulosa cell differentiation (cumulus granulosa different from mural granulosa)
  - steroidogenesis
  - granulosa cell proliferation

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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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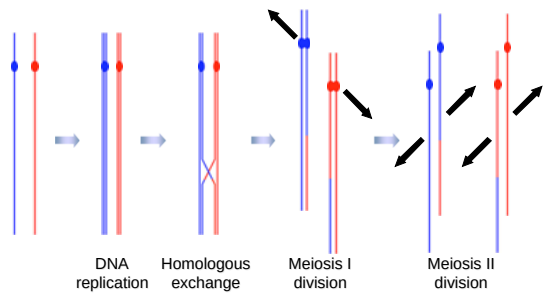
Landmarks of meiosis



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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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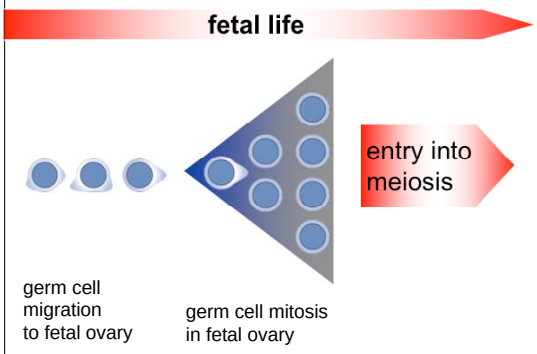

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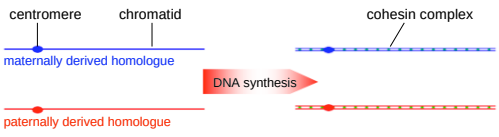
Entry into meiosis: leptotene

- 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
- intitiation of chromosome synapsis

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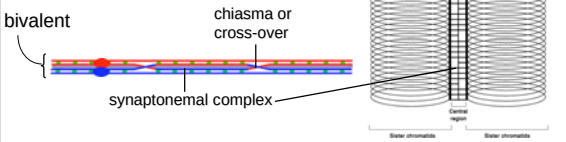
Entry into meiosis: pachytene

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



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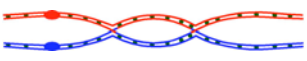
Entry into meiosis: diplotene

- 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



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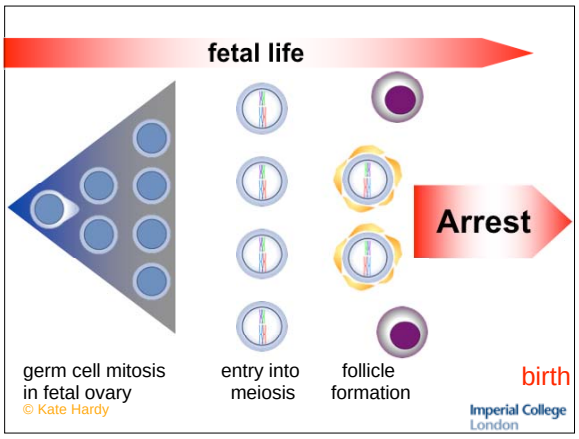

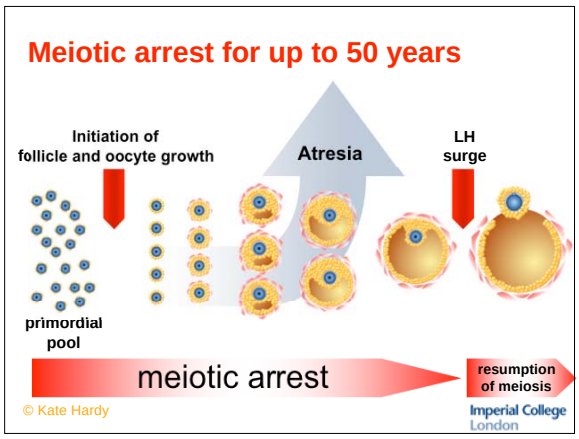

Maintenance of meiotic arrest

- Resumption of meiosis stimulated by
  - release of oocyte from follicle & culture in vitro
  - hormonal trigger (LH)
- Meiosis maintained by cAMP, probably supplied to oocyte from granulosa cell via gap junctions
  - membrane permeable cAMP analogues maintain meiotic arrest
  - Preventing normal decrease of cAMP (just before GVBD) with phosphodiesterase inhibitors maintains meiotic arrest

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LH surge causes

- shift in steroid production by GCs from E_2 to P
- 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

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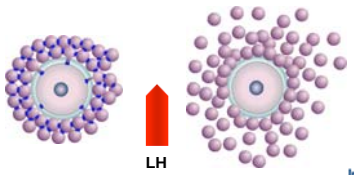
The LH surge downregulates gap junction communication and the cumulus expands

- Oocytes resume meiosis spontaneously when removed from follicle
- cAMP maintains meiotic arrest
- ↓ cAMP → resumption of meiosis
  - hypoxanthine and adenosine may also be involved



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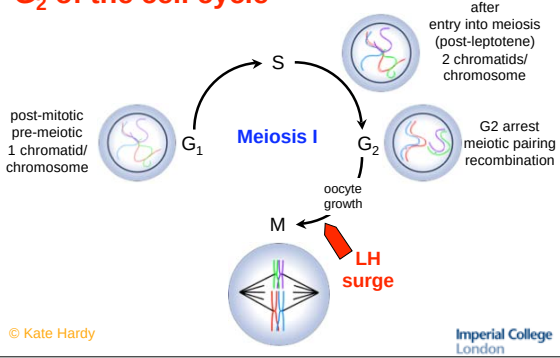
Oocytes arrested in prophase I are in G_2 of the cell cycle



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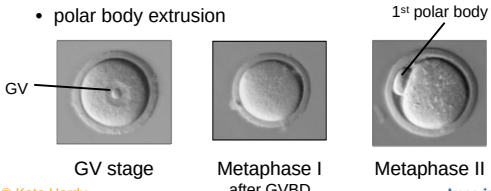
LH surge stimulates

- Resumption of meiosis

manifest by morphological changes known as

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



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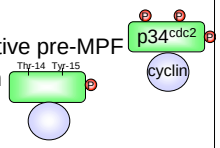
Nuclear maturation

- Initiation of nuclear maturation requires active maturation promoting factor (MPF)
- MPF is universal regulator of G2 - M transition
- MPF is a dimer of
  - cyclin B
  - $p34^{cdc2}$
- Oocytes have stockpile of inactive pre-MPF
- activated by dephosphorylation



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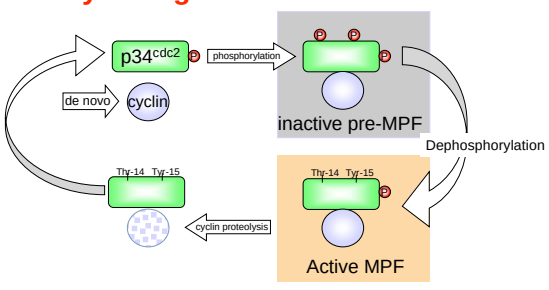
Cell cycle regulation



Anaphase

$G_2 \rightarrow$ metaphase

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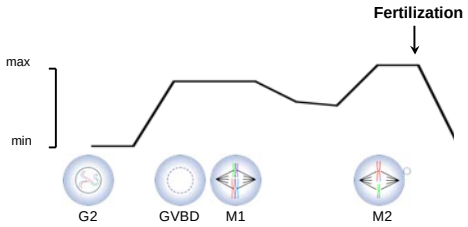
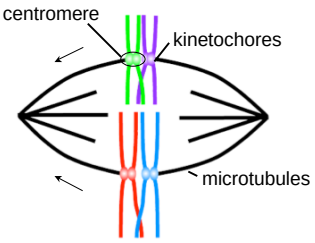
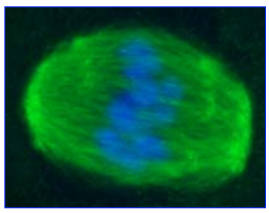
MPF levels



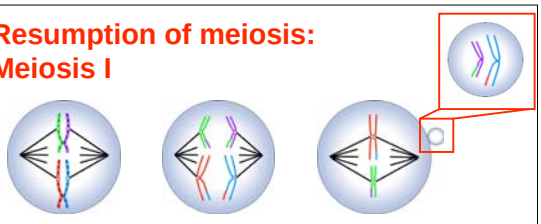
Meiosis resumes with formation of metaphase I spindle



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

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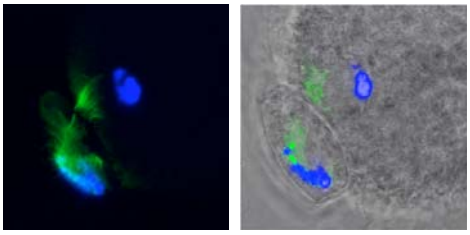
Extrusion of the 1st polar body



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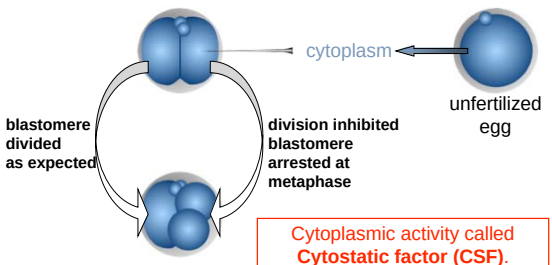
Maintenance of metaphase II arrest



Cytoplasmic activity called **Cytostatic factor (CSF)**.
Thought to be Mos protein kinase

Frog (*Rana pipiens*)

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c-*mos* and metaphase II arrest

- Maturing and mature mouse oocytes have Mos proteins (as well as CSF-like activity)
- Oocytes from c-*mos* knockout mice progress through metaphase II, and do not arrest as +/+ normally do.
- CSF/Mos thought to stabilize MPF. High levels of MPF are preserved, so cannot progress to anaphase (where MPF normally falls)

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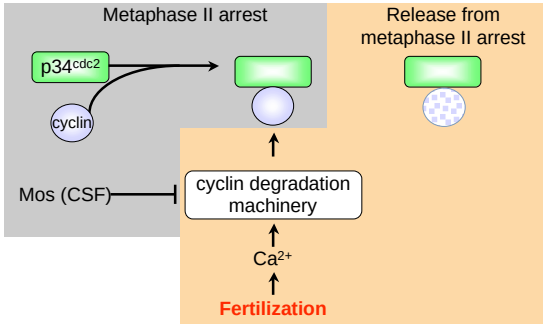
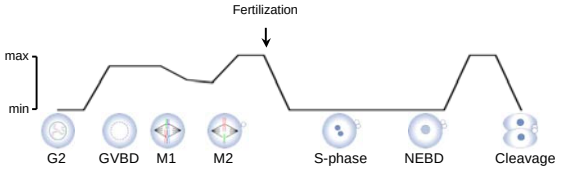
Fertilization causes release from metaphase II arrest



MPF levels



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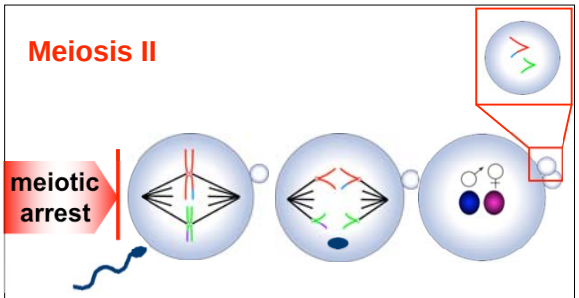
Meiosis II



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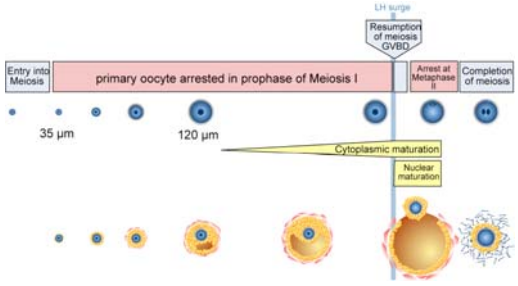
Meiosis and oogenesis



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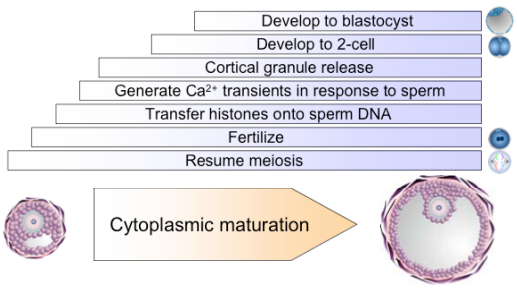
Stepwise acquisition of developmental competence



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