Imperial College London

BSc in Reproductive & Developmental Sciences

Cell Biology of Preimplantation Development

Kate Hardy
Institute of Reproductive and Developmental Biology

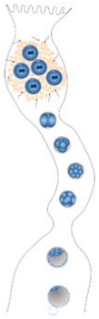
Preimplantation development (mouse)

Embryo

- Unattached
- Moves from site of fertilization (ampulla) to site of implantation (uterus)
- Depends on maternal reproductive tract for nutrition
- Undergoes cell proliferation

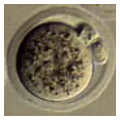
BUT

- No cell growth



Imperial College London

Preimplantation development

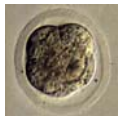

1-cell


2-cell

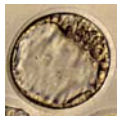

3-cell


4-cell


8-cell


morula

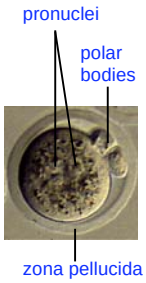

cavitating morula


blastocyst

Imperial College London

Zona pellucida

- Translucent glycoprotein coat, 7μm thick
  - 3 glycoproteins: ZP1, ZP2 and ZP3 (mouse)
  - synthesized by oocyte during oogenesis
- ZP3: Primary sperm receptor
  - binds sperm head
  - activates sperm – induces acrosome reaction
  - highly conserved – 60% homology between mouse & human
  - species specific (carbohydrate side-chains)
- ZP2: Secondary sperm receptor
  - binds acrosome reacted sperm



Imperial College London

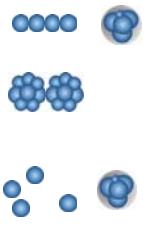
Functions of zona pellucida at fertilization

- Secreted by oocyte during early folliculogenesis
- Provides species specificity at fertilization
  - oligosaccharides on ZP3 provide specificity
  - sperm can fuse with zona-free oocytes from other species
- Induces sperm acrosome reaction
- Site of 'slow block' to polyspermy
  - cortical granule release → zona reaction
  - zona hardening
  - loss of sperm binding capacity

Imperial College London

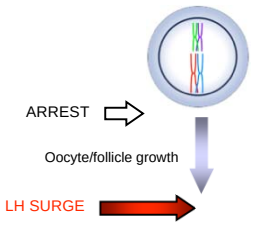
Protective functions of zona pellucida

- Maintains normal cleavage pattern
- Prevents sticking of embryos to one another or to oviduct
- Protects embryos moving down oviduct
  - Zona-free cleavage stage embryos disintegrate in oviduct
  - Protects against attack by leukocyte



Imperial College London

Oocyte: entry into meiosis and arrest

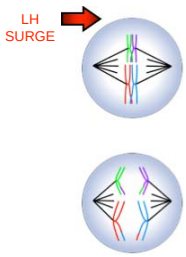


PROPHASE
- occurs during fetal life
- homologous chromosomes pair (1 from mother, 1 from father)
- chiasma form
- crossing over occurs
- oocyte arrested in primordial follicle for up to 50 years

RESUMPTION OF MEIOSIS
- only occurs after puberty

Imperial College London

Resumption of meiosis after LH surge

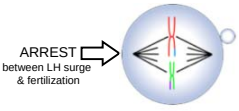


METAPHASE I
- nuclear membrane breaks down
- chromosomes align on equator, stabilized by chiasmata
- no centromere division
- no chromatid separation

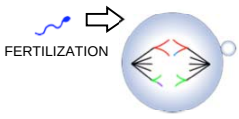
ANAPHASE/TELOPHASE
- 1 chromosome goes to one pole, other goes to other
- maternal and paternal chromosomes randomly migrate to either pole
- first polar body extruded

Imperial College London

Second meiotic division



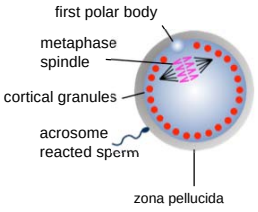
METAPHASE II
- oocyte ovulated while arrested at Metaphase II



ANAPHASE II/TELOPHASE
- only occurs if fertilization or activation occurs
- centromeres divide
- chromatids separate
- one set of chromatids expelled in second polar body

Imperial College London

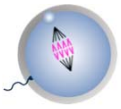
Ovulation and fertilization



- Ovulated oocyte arrested at the second metaphase stage of meiosis
- Still surrounded by cumulus cells

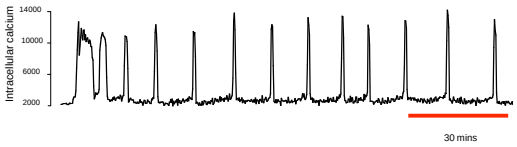
Imperial College London

Fertilization



Sperm fusion

- Activates calcium wave & spikes
- Induces cortical granule release, 'hardens' zona, to prevent polyspermy
- Metaphase spindle rotates
- Meiosis resumes



Imperial College London

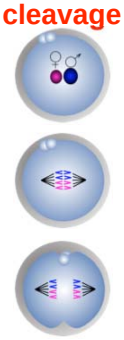
Fertilization (continued)

Pronucleus formation

- Meiotic division completes
- Second polar body extruded
- Sperm nucleus decondenses & male pronucleus forms
- Female pronucleus forms

Imperial College London

First cleavage



Diploid zygote
- pronuclei do not fuse
- chromosomes duplicate DNA
- pronuclei migrate together

First mitotic metaphase
- pronuclear membranes break down
- first mitotic spindle forms
- maternal and paternal chromosomes align on single metaphase plate

First anaphase
- chromatids separate and move apart
- cleavage furrow forms

1 chromosome of each pair from mother
1 chromosome of each pair from father

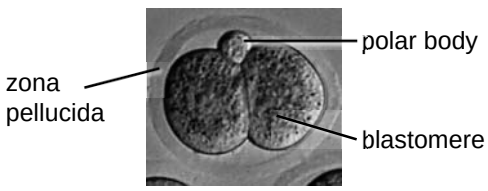
Imperial College London

Timing of first cell cycle (mouse)

• Extrusion of second polar body	2 – 5 h
• Formation of male pronucleus	4 – 7 h
• Formation of female pronucleus	6 – 9 h
• DNA replication	11 – 18 h
• Cleavage to 2-cell	17 – 20 h

Imperial College London

2-cell embryo



Imperial College London

Meanwhile – what is happening to gene expression?

TRANSCRIPTION

TRANSLATION
occurs on ribosomes (rRNA & proteins)
Amino acids added on to growing polypeptide chain by tRNA

DNA
↓
mRNA
↓
Protein

Imperial College London

Parental → embryonic transition

highly differentiated gametes

totipotent embryonic cell

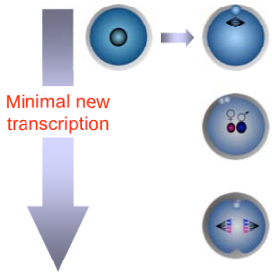
Imperial College London

In absence of transcription

- Molecular changes, necessary to accomplish transition from differentiated gamete to undifferentiated embryonic cell, rely on:
  - stability and differential translation of maternal mRNAs
  - stability of proteins
  - post-translational processing

Imperial College London

Early development relies on maternal transcripts



completion of meiosis

fertilization

reprogramming of maternal and paternal genomes

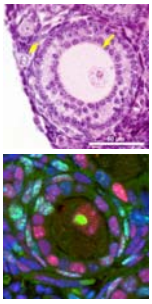
first mitotic cleavage

activation of embryonic genome

Imperial College London

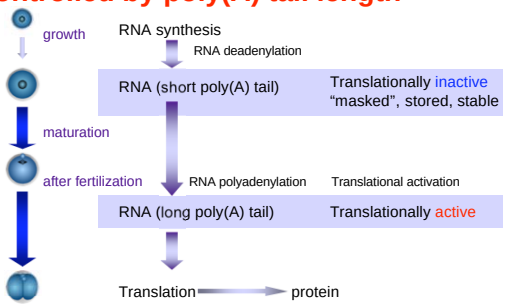
Maternal transcripts accumulated during ocyte growth and maturation

- Mouse oocytes grow from 15 - 80 µm diameter
- Human oocytes grow 35 - 120 µm diameter
- Fully grown mouse egg contains 25µg protein and 0.3 - 0.55 ng RNA
- Growing oocytes contain large nucleoli and synthesize large amounts of RNA



Imperial College London

Translation of maternal mRNA controlled by poly(A) tail length



Imperial College London

Formation of pronuclei

Male

- Breakdown of nonpermeable sperm nuclear envelope
- Reformation of permeable pronucleus nuclear membrane
- Decondensation
  - removal of protamines
  - replacement with histones

Female

- Completion of meiosis
- Formation of female pronucleus
- Pronuclei remain separate until first mitosis

Imperial College London

Nuclear changes during cleavage

- Histone composition continues to change
- Acetylation of histones changes, facilitating transcription
- Embryo generally acquires transcriptionally repressive chromatin state: for tightly regulated gene regulation
- Specific genes can then be transcribed in a regulated manner (enhancers, transcription factors etc)

Imperial College London

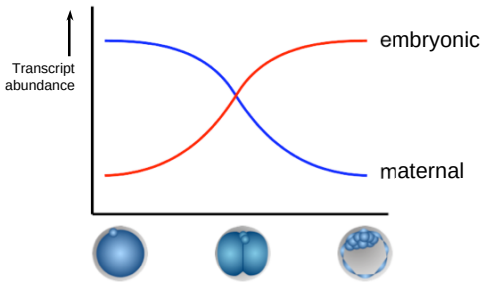
Activation of the embryonic genome

Transition from maternal control to embryonic genome control marked by:

- Degradation of maternal mRNA
- Activation of transcription of embryonic genome, with transcription of new mRNAs not seen in oocyte including gene products which influence:
  - metabolism,
  - rate of cell proliferation,
  - embryo cell number &
  - differentiation.

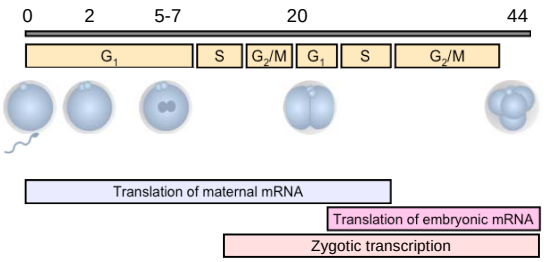
Imperial College London

Embryonic genome activation



Imperial College London

First 2 cell cycles (hours) - mouse



Imperial College London

Cleavage

- asynchronous cell division
- no growth
- at each division, cell volume halves

Imperial College London

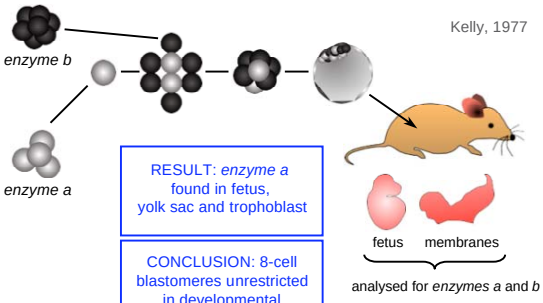
Cleavage


- cells spherical
- loosely attached
- totipotent

Imperial College London

Totipotency during early cleavage

Kelly, 1977



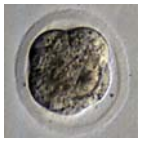
RESULT: *enzyme a* found in fetus, yolk sac and trophoblast

CONCLUSION: 8-cell blastomeres unrestricted in developmental potential

Imperial College London

Compaction and morula formation

- Occurs at 8-cell stage (mouse), 16-cell stage (human)
- Mediated by E-cadherin (adhesion glycoprotein)
- Blastomeres:
  - flatten and maximize intercellular contacts
  - become more adhesive
  - become polar - embryo develops radial axis

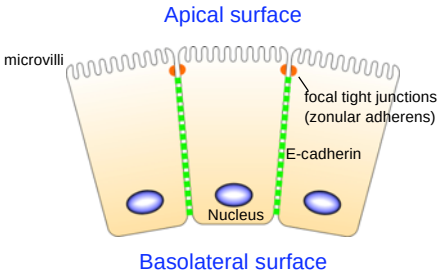


Imperial College London

Morula (mulberry)

Imperial College London

Polarization



Imperial College London

E-cadherin (uvomorulin)

- Involved in establishment and maintenance of epithelial morphology
- Expressed in egg (cytoplasm) and embryo (cell surface)
- Redistributed to cell contact points at 8-cell stage
- Responsible for Ca^{2+}-dependent compaction
  - 8-cell morulae decompact in Ca^{2+}-free medium
  - antibodies to E-cadherin prevent compaction
- Maybe mediated by protein phosphorylation (8c)
- Mice lacking E-cadherin
  - compact (residual maternal transcripts) **but**
  - fail to form trophectoderm or a blastocoel cavity

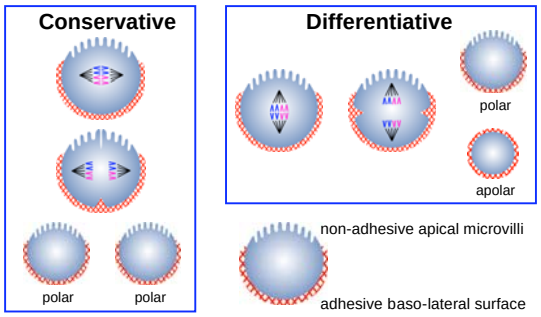
Imperial College London

Morula: formation of 2 cell types

- Polarization of cells at compaction is critical for formation of 2 different cell types in blastocyst
  - outer polar trophectoderm (TE)
  - inner apolar inner cell mass (ICM)
- Differential adhesiveness maintains this pattern
- Morula formation marks first differentiation in embryogenesis

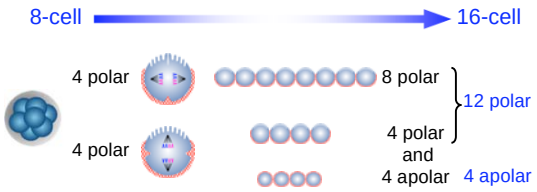
Imperial College London

Morula: formation of 2 cell types



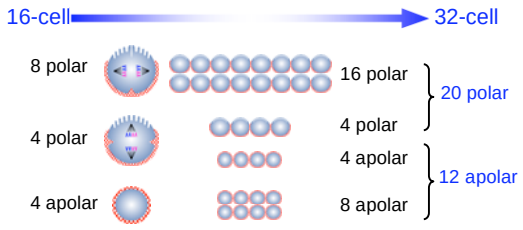
Imperial College London

Allocation to TE and ICM



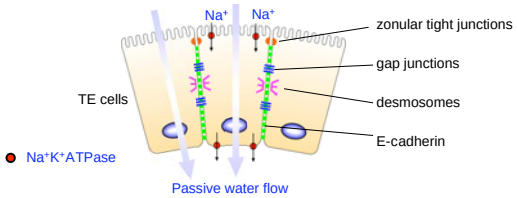
Imperial College London

Allocation to TE and ICM



Imperial College London

Cavitation and blastocyst formation



- $Na^+K^+ATPase$: pumps Na^+ into cavity, drawing in water
- Tight junctions: form a watertight "belt" around each TE cell
- Gap junctions: intercellular communication
- Desmosomes: structural "bolts"

Imperial College London

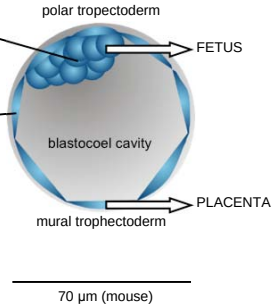
Cavitation and blastocyst formation

- Formation of fluid filled cavity (function unknown)
  - Occurs at completion of fifth cleavage division (mouse: 16- to 32-cell stage)
- Cell number at cavitation varies with species
  - mouse 32-cell
  - human 32-cell
  - rabbit 128-cell
  - pig 16-cell
  - sheep 64-cell
- Independent of
  - cell number (half and double embryos cavitate at same time)
  - number of cell divisions
  - DNA replication

Imperial College London

Blastocyst structure

- Inner cell mass (ICM)
  - apolar
  - uniformly adhesive cells
  - stem cell morphology
  - pluripotent
- Trophectoderm (TE)
  - polar cells, epithelial morphology
  - extensive intercellular junctions
  - differentiated - specialized for fluid transport and implantation



70 µm (mouse)

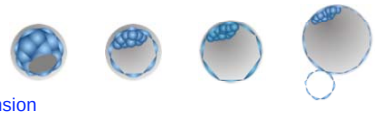
Imperial College London

Functions of trophectoderm

- pump fluid into cavity
- transport metabolites between maternal tissues and ICM
- initiate chemical and physical communication between fetus and mother
- provide a proliferative source of cells for placental TE

Imperial College London

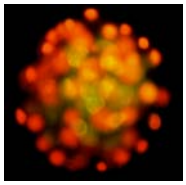
Blastocyst expansion and hatching



- Expansion
  - enlargement of blastocoel cavity, expansion of blastocyst
  - continued cell division, especially in TE
  - cell number in ICM remains at ~15 - 20, until implantation
  - cell death, especially in ICM
  - ICM differentiates
- Herniation and hatching
- Attachment and implantation

Imperial College London

Cell number in the mouse blastocyst



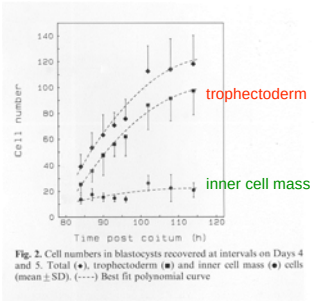
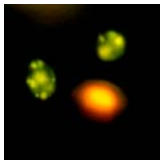


Fig. 2. Cell numbers in blastocysts recovered at intervals on Days 4 and 5. Total (•), trophectoderm (■) and inner cell mass (●) cells (mean ± SD). (----) Best fit polynomial curve

Imperial College London

Apoptosis in the mouse blastocyst

- Apoptosis occurs in ICM
- Role unknown
  - Remove cells with potential to form TE?
  - Remove defective cells?
  - Maintain ICM size?
- Signalling pathways unknown
  - BCL-2 family members and caspase genes present

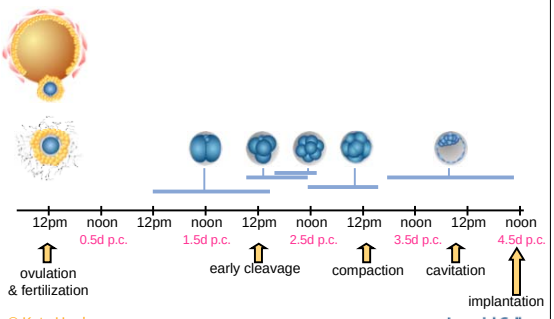


Imperial College London

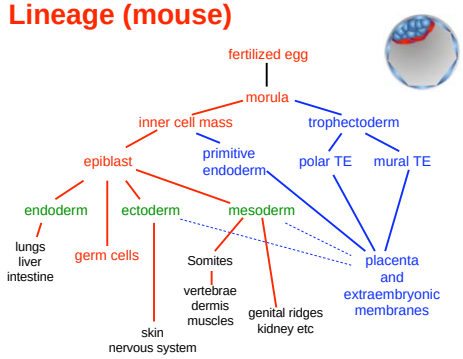
Hatching blastocyst

Imperial College London

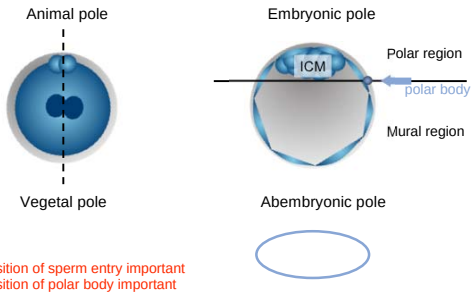
Timing of preimplantation development (mouse)



Lineage (mouse)



Polarization (embryo)



Position of sperm entry important
Position of polar body important

Comparative preimplantation development

- All mammalian species similar overall
- Differences in
  - Size (small)
  - Time in oviduct
  - Length of cell cycles
  - Timing of switch-on of embryonic genome
  - Timing of compaction/cavitation
  - Cell number at compaction/cavitation
  - Timing of implantation
  - Cell number at implantation

Imperial College London