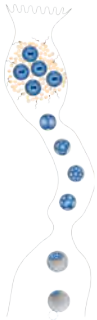
Imperial College London

Reproductive Medicine

Preimplantation Development

Kate Hardy
Institute of Reproductive and Developmental Biology

Preimplantation development (mouse)

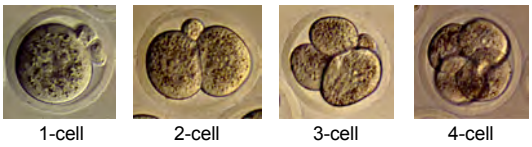


Embryo
- Unattached
- Moves from site of fertilization (ampulla) to site of implantation (uterus)
- Depends on maternal reproductive tract for nutrition
- Undergoes cell proliferation

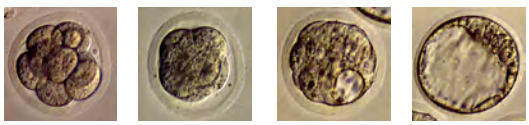
BUT
- No cell growth

Imperial College London

Preimplantation development (mouse)



1-cell 2-cell 3-cell 4-cell

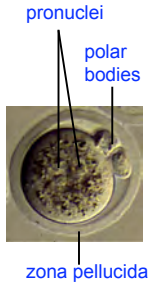


8-cell morula cavitating morula blastocyst

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

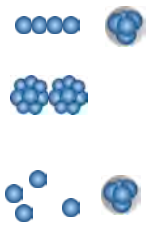
Functions of zona pellucida at fertilization

- 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

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

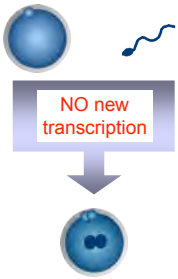
1 chromosome of each pair from mother
1 chromosome of each pair from father

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

2-cell embryo

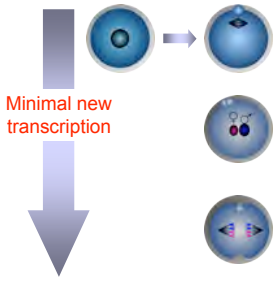
Parental → embryonic transition



highly differentiated gametes

totipotent embryonic cell

Early development relies on maternal transcripts



completion of meiosis

fertilization

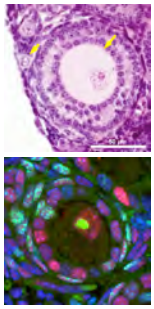
reprogramming of maternal and paternal genomes

first mitotic cleavage

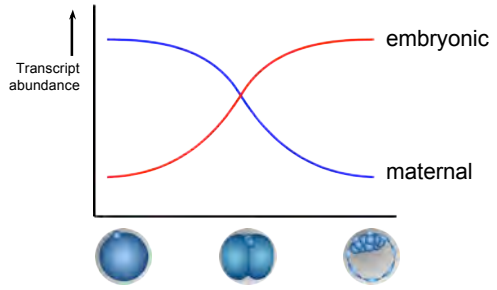
activation of embryonic genome

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



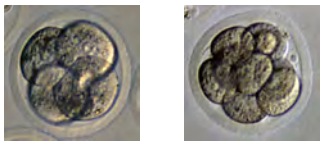
Embryonic genome activation



Cleavage

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

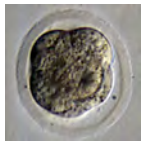
Cleavage



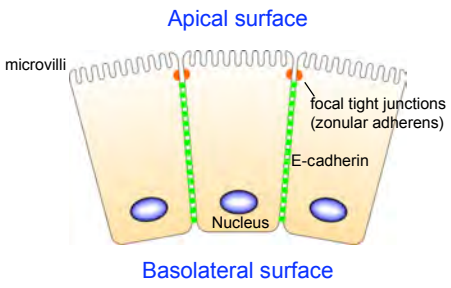
- cells spherical
- loosely attached
- totipotent

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

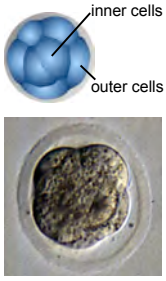


Polarization

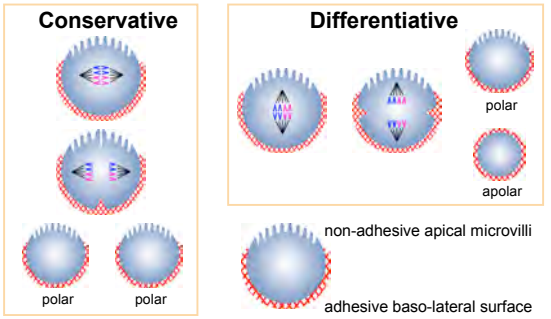


Morula: formation of 2 cell types

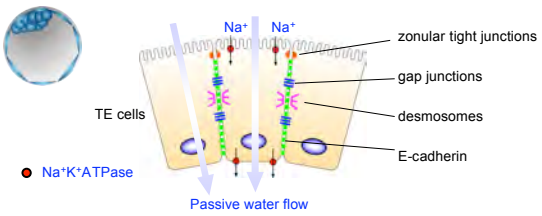
- Polarization of cells at compaction is critical for formation of 2 different cell types in blastocyst
  - outer polar trophectoderm (TE)
  - inner apolar inner cell mass (ICM)
- Differential adhesiveness maintains this pattern
- Morula formation marks first differentiation in embryogenesis



Morula: formation of 2 cell types



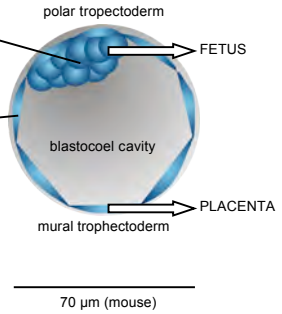
Cavitation and blastocyst formation



- 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"

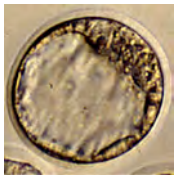
Blastocyst structure

- 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



Functions of trophectoderm

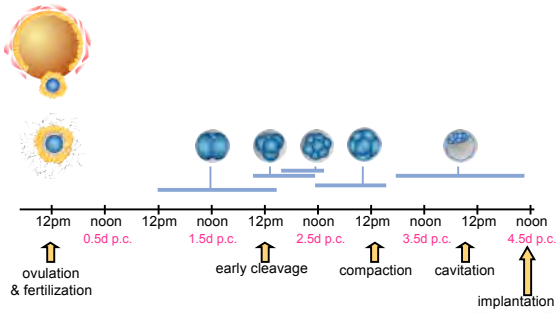
- pump fluid into cavity
- transport metabolites between maternal tissues and ICM
- initiate chemical and physical communication between fetus and mother
- provide a proliferative source of cells for placental TE



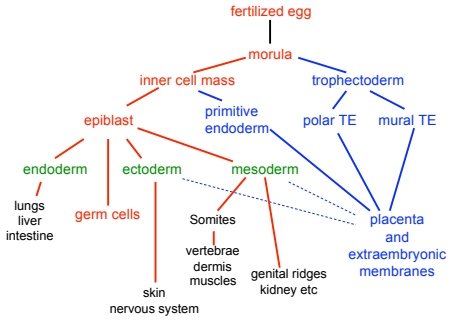
Blastocyst expansion and hatching

- Expansion
  - enlargement of blastocoel cavity, expansion of blastocyst
  - continued cell division, especially in TE
- Herniation and hatching
- Attachment and implantation

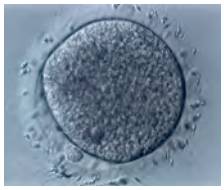
Timing of preimplantation development (mouse)



Lineage (mouse)



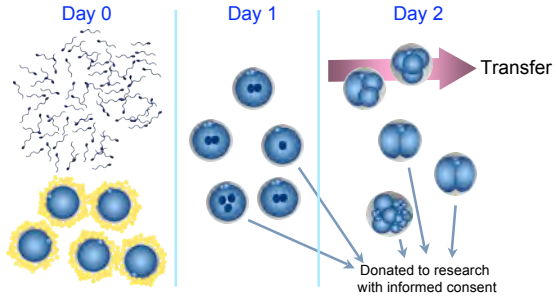
Human in vitro fertilization



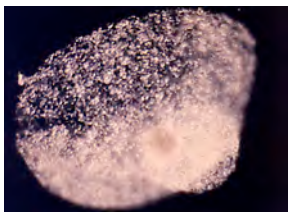
Rock and Menke
Edwards et al., 1969

Steptoe and Edwards, 1978

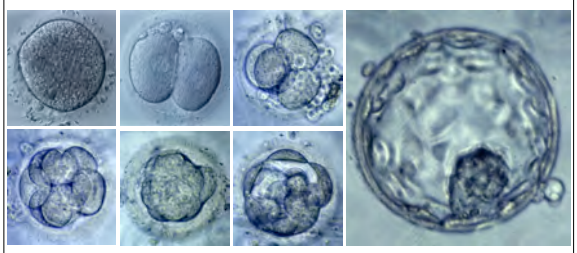
Source of embryos for research



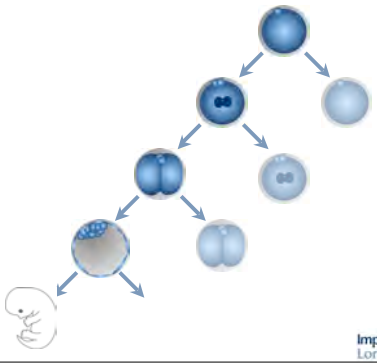
Oocyte and cumulus cells after retrieval



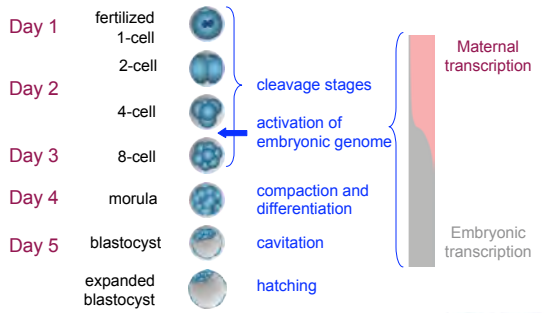
Human embryo development



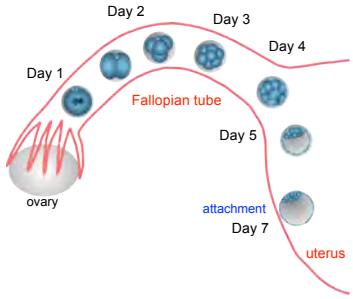
Human Preimplantation Development



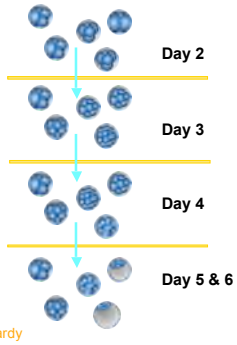
Human Development



Development *in vivo*

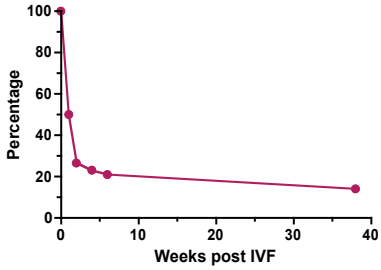


Not all embryos reach the blastocyst



Only ~50% reach the blastocyst stage

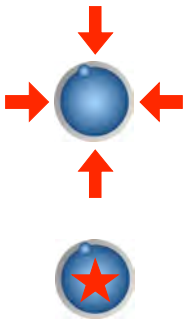
Embryonic loss



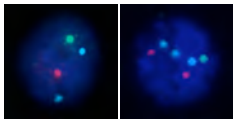
Hardy, 1993
Dawson *et al.*, 1995
HFEA, 1999

Cause of poor development?

- Environment
  - embryo culture?
  - follicle environment?
- Oocyte
  - 'quality'?
  - chromosomal abnormalities?

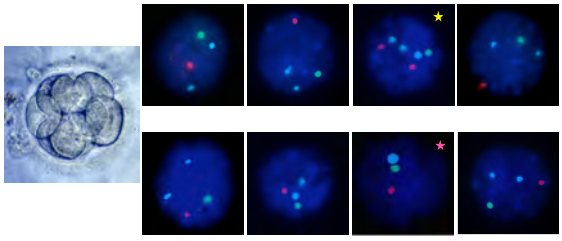


Chromosomal & nuclear anomalies in human preimplantation embryos



Chromosomal anomalies
- ~20% have gross anomalies
- ~40% mosaic, with 1 or more abnormal cells

Mosaic 8-cell human embryo (d3)

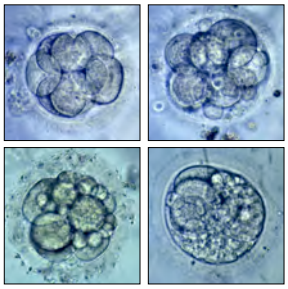


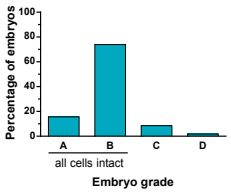
X Y 18 18 ★X Y 18 18 18

★X Y 18

Imperial College London

Morphology and fragmentation

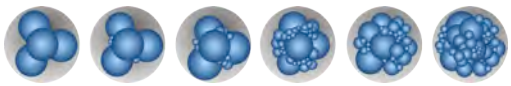




n = 37,237
Hammersmith Hospital
1992 - 2002

Imperial College London

Classification of embryo grade



A (<5%) B (5-10%) C (10-25%) D (>25%)

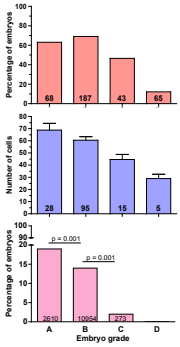
Imperial College London

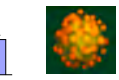
Grade and embryonic development

Blastocyst formation

Blastocyst cell number

Implantation





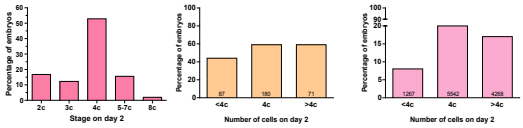
EBSS with 1 mM glucose + HSA

Hardy *et al*, *Biol Reprod*,**68**, 1165

Imperial College London

Rate of development and developmental potential

Cell number on day 2 | Blastocyst formation | Implantation



Data from Hammersmith Hospital IVF Unit

numbers in bars = total number of embryos of each cell number

Imperial College London

Summary

- Preimplantation Development
  - Cleavage
  - Compaction
  - Cavitation
  - Implantation
- Human Preimplantation Development
  - 50% arrest
  - Fragmentation common
  - Aneuploidy common
  - Poor developmental potential

Imperial College London