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BSc in Reproductive & Developmental Sciences

The human preimplantation embryo: Cell biology

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Institute of Reproductive and Developmental Biology

In vitro fertilization

Rock and Menke
Edwards et al., 1969

Steptoe and Edwards, 1978

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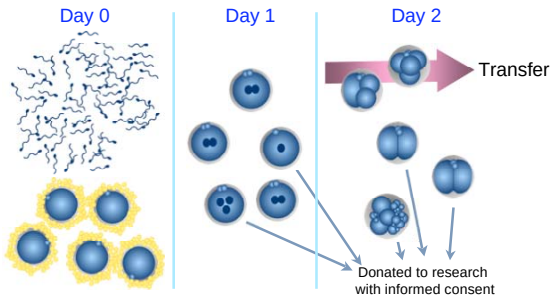
Source of embryos for research



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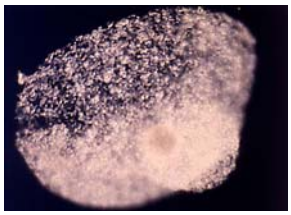
Oocyte and cumulus cells after retrieval



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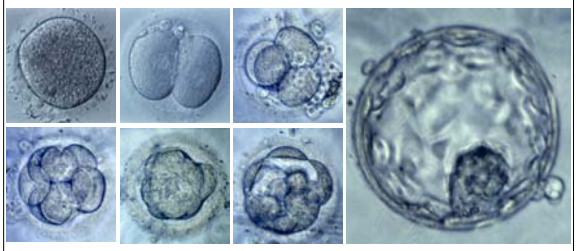
Human embryo development



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Hatching human blastocyst

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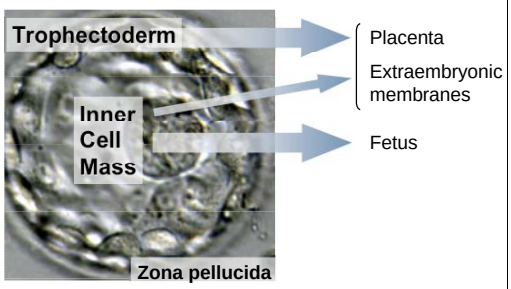
The human blastocyst



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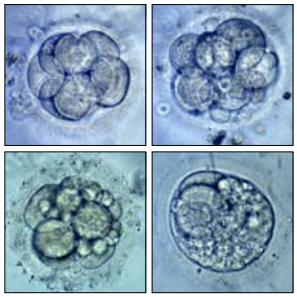
Fragmentation is common



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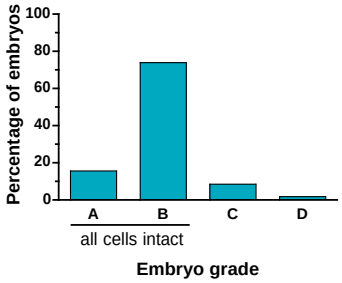
Embryos with a few fragments are the most common



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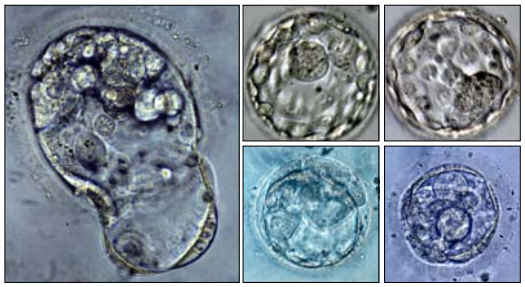
Blastocysts have fragments and excluded arrested cells



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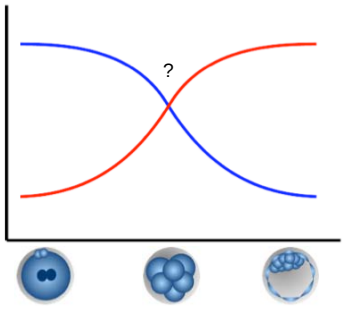
Transition from maternal to embryonic gene expression



Human:
4- to 8-cell

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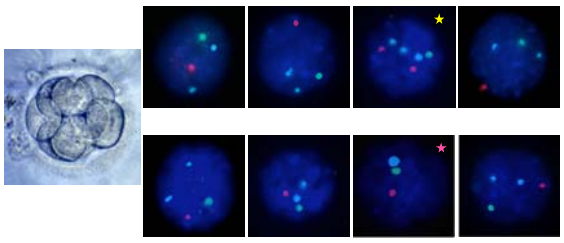
Aneuploidy is VERY common

Mosaic 8-cell human embryo (d3)



X Y 18 18

☆X Y 18 18 18

☆X Y 18

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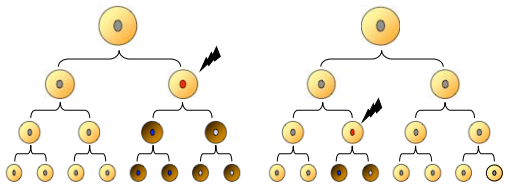
Mosaic embryos



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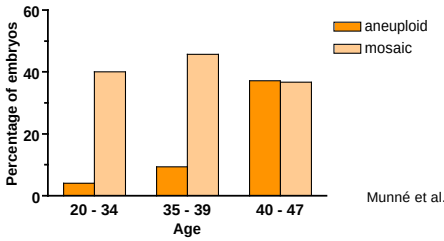
Aneuploidy in embryos increases with age, mosaicism is constant



Munné et al., 1995

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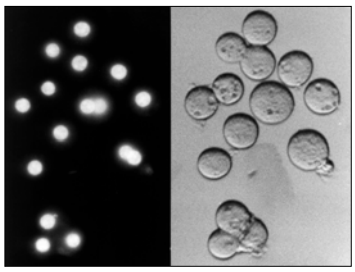
Nuclear abnormalities are common



Hardy *et al.*, 1993 JRF. **98**, 549

60 % day 4 embryos have binucleate, anucleate or multinucleate cells

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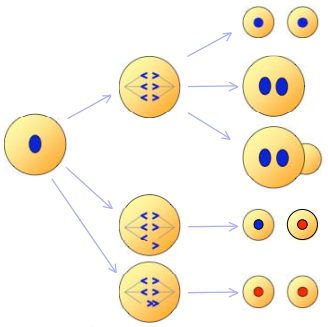
Cell cycle defects



Normal mitosis

Failure of cytokinesis

Asymmetric cytokinesis

Anaphase lag

Non disjunction

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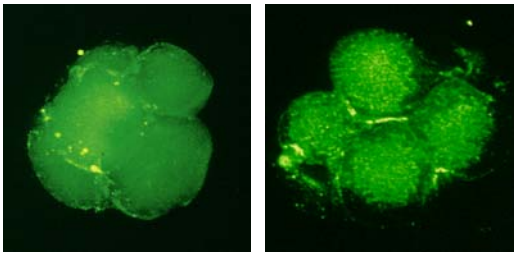
Gap junctions (Cx43)

Hardy et al., 1996



4-cell

8-cell

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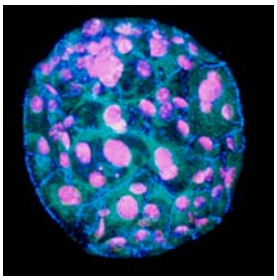
Gap Junctions (Cx43)



nuclei pink
Cx43 blue

Hardy et al., 1996

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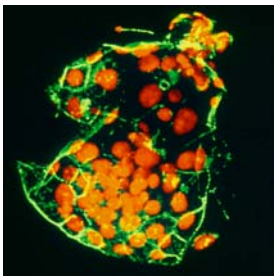
Desmosomes



nuclei orange

Desmosomes green

Hardy et al., 1996

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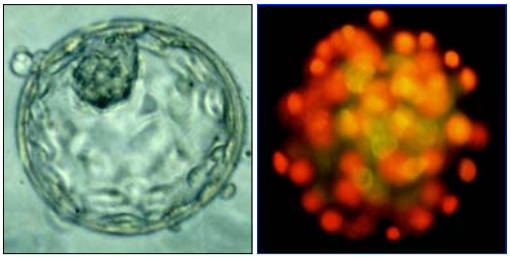
Cell number ICM/TE



TE orange
ICM green

Hardy et al., 1989

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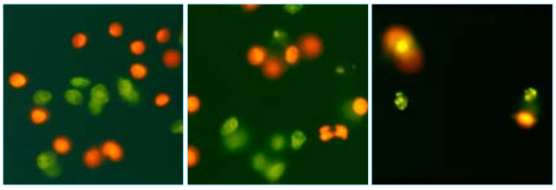
Analysis



TE orange
ICM green

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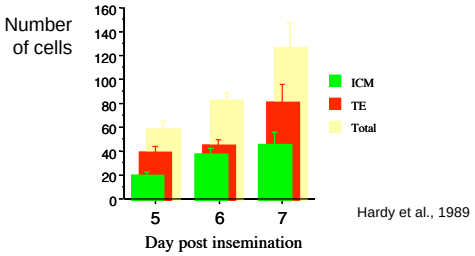
Cell numbers in human blastocysts



Hardy et al., 1989

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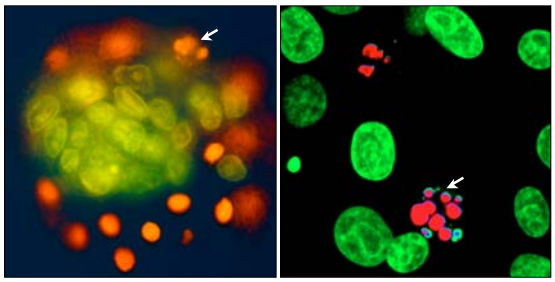
Fragmenting nuclei and DNA



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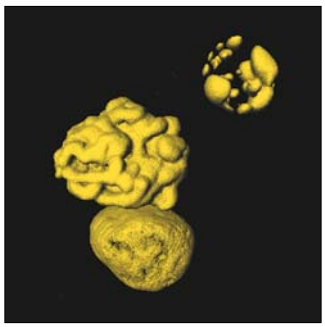
Condensed DNA



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Natural embryo loss and cell death

Buster et al 1985

- Uterine lavage of embryo donors inseminated with father's sperm (*in vivo* fertilization)
- 25 embryos recovered on day 5 - 7 post-LH peak
- 5 blastocysts (25%)
- 1 morula
- 9 at 8- to 32-cell
- 6 < 8-cell

Am J Obstet Gynecol, 153, 211

Hertig et al, 1954

- Embryos found at hysterectomy

4 normal

- 2-cell, 12-cell & 2 blastocysts

4 abnormal

- 5-cell Irregular cells & fragmented nuclei
- 8-cell 2 necrotic cells with pyknotic nuclei, 3 cells with multiple nuclei
- 9-cell fragments & multiple nuclei
- 12-cell multiple nuclei

Contrib Embryol, 35, 199

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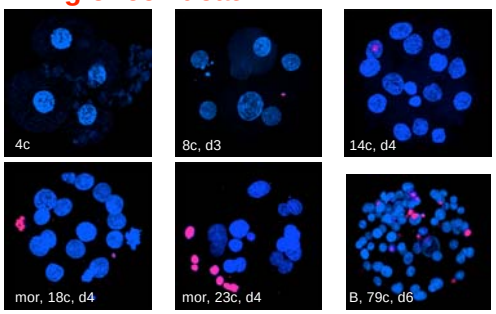
Timing of cell death



Hardy, Spanos et al., *PNAS* **98,** 1655, 2001

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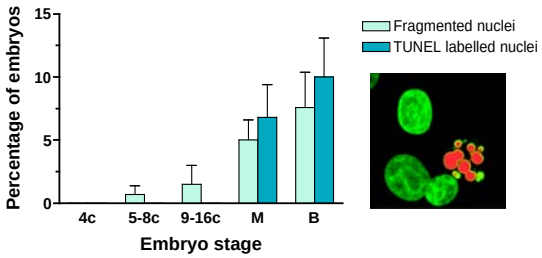
Timing of cell death



Hardy, Spanos et al., *PNAS* **98,** 1655, 2001

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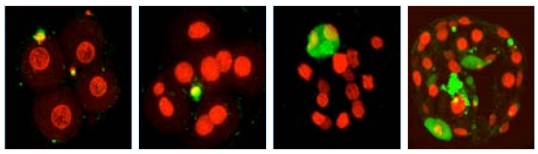
Active caspase expression

4-cell 8-cell morula blastocyst



Spanos et al., *Reproduction*, **124**, 353 (2002)

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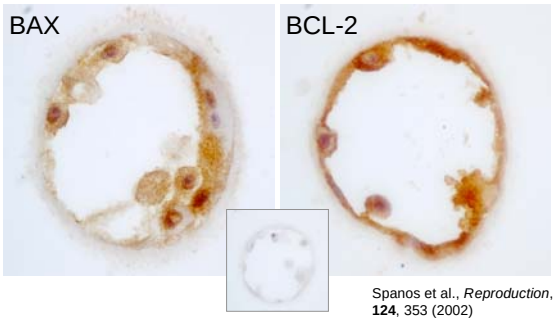
BAX and BCL-2 expression

BAX BCL-2



Spanos et al., *Reproduction*, **124**, 353 (2002)

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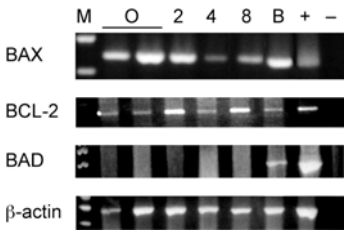
BAX and BCL-2 expression



Spanos et al., *Reproduction*, **124**, 353 (2002)

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Possible causes & roles of cell death

Possible causes

- Suboptimal culture
- Lack of survival factors

Roles

- Elimination of a particular lineage of cells?
- Removal of chromosomal and nuclear abnormalities?
- Maintenance of rapidly dividing undifferentiated stem cell population?

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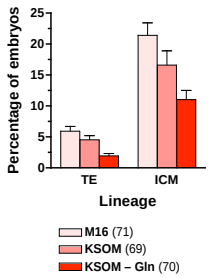
Suboptimal culture: mouse embryos



- Cell death not an *in vitro* artefact
- Cell death found in *in vivo* mouse blastocysts
- Suboptimal culture ↑ levels of cell death

Devreker and Hardy, 1997

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Survival factors: evidence for effect on preimplantation cell death

- Certain growth factors important for cell survival
- ↑ levels of cell death in
  - singly cultured embryos
  - diabetic rat embryos
- ↓ levels of cell death in
  - embryos cultured in groups
  - embryos cultured in TGF-α, IGF-1, insulin etc

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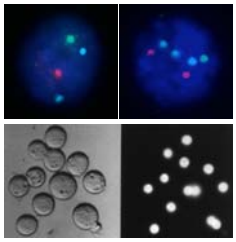
Chromosomal & nuclear anomalies in human preimplantation embryos



Chromosomal anomalies

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

Nuclear abnormalities

- ~60% of day 4 embryos have 1 or more binucleate, anucleate or multinucleate cells

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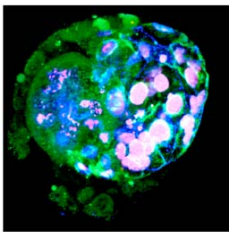
The human preimplantation embryo

Variability:

- Between embryos
  - developmental potential
  - morphology
  - metabolism
  - chromosomal/nuclear normality
- Within embryos
  - mosaic embryos



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