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BSc in Reproductive & Developmental Sciences

Environmental regulation of preimplantation development

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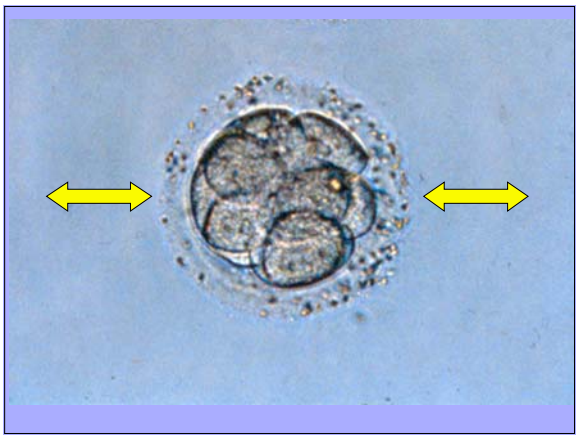
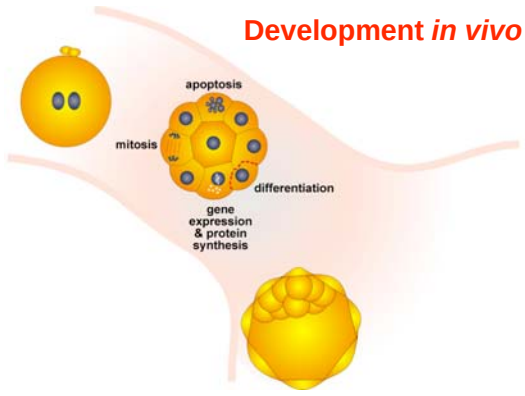
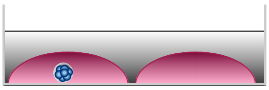


Development *in vivo*



Development *in vitro*



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Importance of embryo culture

- Culture of embryos and gametes is fundamental for
  - assisted reproductive technologies
  - research
- Successful culture, and further system optimization, requires understanding of embryo physiology
- Suboptimal culture has been associated with
  - altered gene expression & genomic imprinting (mice)
  - developmental defects (large offspring syndrome) in domestic species

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Assisted reproduction and environment

- IVF and embryo culture: gametes and embryos in artificial environment

Does this matter?

- Culture media contain chemicals at different concentrations to those found *in vivo*
- Media may contain chemicals not found *in vivo*
- Media may lack chemicals present *in vivo*

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Environment I

In vivo	*In vitro*
Dynamic	Static
Ciliated & secretory cells	No cells
Varies with cycle	Invariant
	Delayed development

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Environment II

	In vivo	*In vitro*
Salts	? $\uparrow K^+$ $\downarrow Ca^{2+}$	NaCl, $MgSO_4$, NaH_2PO_4, $CaCl_2$, $NaHCO_3$
Energy substrates	Pyr, Glc, Lac ↓plasma levels	Pyr, Glc = plasma levels
Amino Acids	Gln, Tau, Glu, Gly, Ala, Ser, Asp	—
Growth factors	IGF I, IGF II, LIF etc	—
Proteins	plasma proteins albumin, IgG	B/HSA or serum

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Components of culture media

- Water (very pure)
- Salts
  - Isotonic solution - requirements for cell survival
- Energy substrates
  - Glucose, lactate, pyruvate
- Protein source
  - Serum*, bovine serum albumin (BSA)*, human serum albumin (HSA) or a synthetic serum substitute
- Amino acids
- Growth factors

} Not essential but beneficial

* Not recommended for human use

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Evidence for suboptimal culture

Historically

- Embryos from several species blocked *in vitro*
  - outbred mouse strains at 2-cell
  - hamster embryos at 2-cell
  - pig embryos at 4-cell
  - coincided with time of embryonic genome activation

Recently

- Blocks overcome by modifying and modernizing culture media

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Optimization of culture media

- Recognizing changing environment *in vivo*
- Recognizing and meeting changing nutritional requirements
- Reducing inhibitory components e.g. glucose
- Identifying trophic factors e.g. growth factors
- Adding other components e.g. osmolytes, free-radical scavengers

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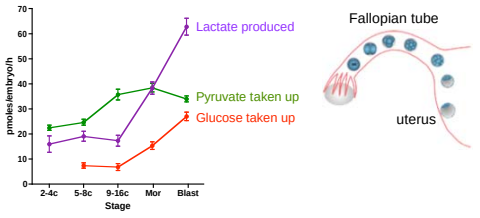
Changing requirements & environment



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Quantifying improvements in culture

In vitro

- Proportion developing to the blastocyst
- Rate of development
- Blastocyst cell number
  - Number of inner cell mass and trophectoderm cells
- Metabolism

In vivo

- Implantation after transfer

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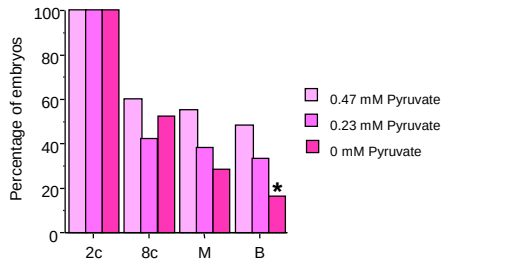
Pyruvate & blastocyst formation: human



Conaghan et al, 1993, *J. Reprod. Fertil.* **99**, 87

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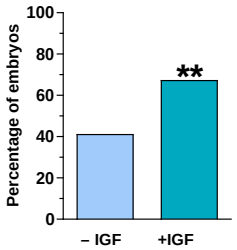
IGF-I and human blastocyst formation



** $p < 0.05$

Lighten et al., 1998
Hum. Reprod. **13**, 3144

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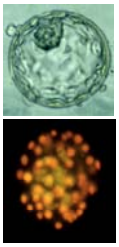
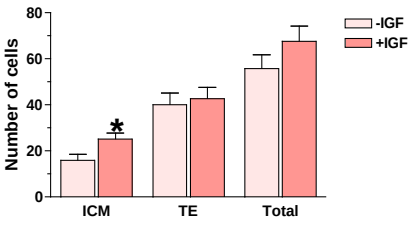
Environment and allocation to ICM





Lighten et al., 1998
Hum. Reprod. **13**, 3144

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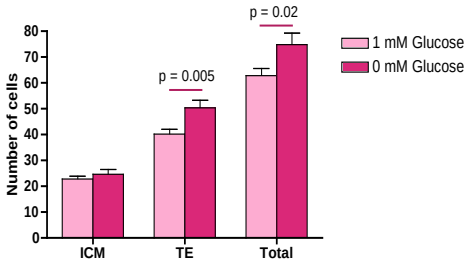
Environment and allocation to TE



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Consequences?

- What are the consequences of disturbing number of cells in two lineages?
- Effects on
  - implantation?
  - embryo/fetal growth?

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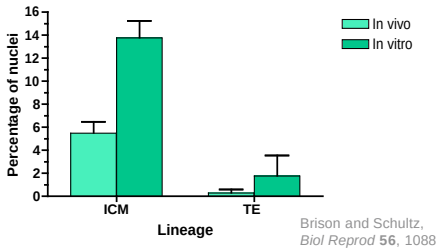
Environment (in vivo vs in vitro) and apoptosis (mouse)



Brison and Schultz, *Biol Reprod* **56**, 1088

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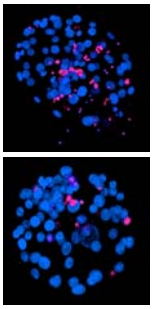
Environment and apoptosis (human)

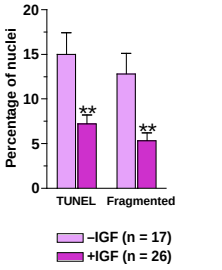
– IGF

82 nuclei
14.6% TUNEL

+ IGF

79 nuclei
6.3% TUNEL





Spanos *et al*., 2000 *Biol. Reprod*. **63**, 1413

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Environment and metabolism

Culture

- decreases protein synthesis in rabbit embryos
- increases protein degradation in rabbit embryos
- increases glycolysis (conversion of glucose to lactate) (mouse and cow) -
  - stress response? - embryos with high glycolysis do not implant as well
- increases glycogen content 3-fold (mouse)
- increases reactive oxygen species (ROS) (mouse)
- serum in culture medium causes lipid accumulation in blastomeres (visible by TEM)

Leese, 2002, BioEssays 24, 845

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Environment and metabolism

- Amino acids in culture medium improve postimplantation development

However

- Amino acids in culture medium can degrade to ammonium
- Toxic for embryos - leads to anencephaly in fetuses (mouse)

Lane and Gardner, 1994, J. Reprod. Fertil. 102, 305

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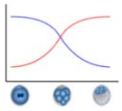
Environment & embryo development

- Failure to activate embryonic genome



- Gene expression
  - mouse — Ho et al, 1995 Mol Reprod Dev, 41, 232
  - cow — Niemann et al, 2000 Theriogenology, 53, 21
- Imprinting
  - mouse — Doherty et al, 2000 Biol. Reprod. 62 15
  - sheep — Young et al, 1999 Theriogenology, 53, 627

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Environment and gene expression in cow embryos *in vivo* and *in vitro*

Gene	In vivo	In vitro
Connexin 43	+	–
bovine LIF	–	+
LIF receptor β	–	+
Hsp 70.1	+	↑
Glut-1	+	↓

Niemann et al, 2000 *Theriogenology*, **53**, 21

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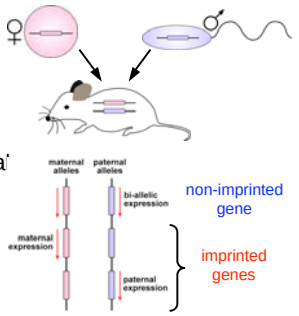
Genomic imprinting

- During normal development, a small subset of genes (imprinted genes), are only expressed from one allele, the maternal or paternal one

genomic imprinting: parent-of-origin-dependent differences in gene expression



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Environment and genomic imprinting

Gametogenesis and early development is a critical period for:

- erasure
- acquisition
- maintenance

} of genomic imprints

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Environment and genomic imprinting

Mouse

- H19 - imprinted gene
  Doherty 2000
  - *in vivo* mouse embryos: no paternal expression
  - *in vitro* culture in Whitten's medium: biallelic expression
  - *in vitro* culture in KSOM: only 10% paternal expression

Sheep

- IGF2R - imprinted gene
  Young 2001
  - IGF-2 R expression reduced in LOS syndrome
  - Loss of methylation

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Culture and fetal weight

- Culture decreases fetal weight
  Khosla 2001
- Mouse embryos cultured in the presence of serum are
  - less able to implant
  - 20% smaller on day 14
  - decreased expression of the imprinted *Igf2** and *H19* genes
  - increased expression of the imprinted *Grb10* gene
  - Decreased *Igf2* expression probably results in decreased growth

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Environment and postimplantation development

In other species, culture medium affects:

- Implantation rates
- Fetal development
- Embryo loss
- Fetal abnormalities

Gardner and Sakkas, 1993, Hum Reprod. 8 288
Lane and Gardner, 1994, J. Reprod. Fertil. 102, 305

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Large Offspring Syndrome

(Sheep embryos cultured with human serum)

Preimplantation	Fetal/Postnatal
Early cavitation	↑ fetal death
↓ compaction	↑ fetal heart/liver weights
↑ fragmentation	↑ gestation length
↑ lipid accumulation	↑ birthweight
↓ glucose oxidation	↑ neonatal death
	↑ limb abnormalities
	↑ postnatal growth

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Fetal programming

↓ birthweight –↑ heart disease Barker, 1993 BMJ

Preimplantation programming?

Rat studies
↓ maternal nutrition (low protein diet) during preimplantation stages
leads to
↓ ICM/TE cell numbers in blastocysts
↓ birth weight
↑ compensatory postnatal growth & disproportionate organ growth
↑ blood pressure Kwong et al, 2000 Development 127, 4195

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Effects of low birthweight

- Susceptibility to adult-onset disease
  - growth
  - cardiovascular
  - metabolic (eg Diabetes)
  - neurological disorders associated with stress
  - autism
- Different behaviour of adult mice derived from embryos cultured in different media

Ecker et al (2004) PNAS 101, 1595

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Alcohol

- Causes fetal abnormalities (fetal alcohol syndrome) and decreases fetal survival
- Mouse oocytes exposed to alcohol have elevated incidence of aneuploidy (action on spindle?)
- reduced fertilization rates
- reduced embryo development
- increased fragmentation

Cebral et al, 1999 Alcohol Alcoholism 34, 551

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Smoking

- Aberrant meiotic maturation in human oocytes - increased proportion of diploid and triploid oocytes
  - (should be haploid - failure to separate homologues or chromatids?)
- Increased aneuploidy in sperm
- Decreased fragmentation in embryos developed from follicles with high cotinine concentrations - inhibiting apoptosis?

Zenzes, 2000 Hum Reprod Update 6, 122

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Diabetes

- Increased risk of
  - First trimester spontaneous abortion
  - Major fetal malformations
- Hyperglycaemia major factor
  - Increases apoptosis in rodent preimplantation embryos
  - Decreases ICM size (diabetic rat)
  - Disturbs gene expression (diabetic mouse)

Moley, 2001 Trends Endocrinol Metab 12, 78
Pampfer et al, 1990, Diabetes, 39, 471

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Toxins

Pesticides

- Organochlorines: reduced oocyte maturation, embryo transport, implantation

Microtubule poisons (chemotherapy)

- increased aneuploidy

Solvents

- Methylnitrosourea and preimplantation embryos: cleft palate, exencephaly, malformed vertebrae

Walker et al, 2000 Hum Reprod Update 6, 564

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ART and environment

- gonadotrophins and oocyte maturation
- anaesthetic?
- temperature fluctuations? (spindle)
- composition of culture medium
- plastic culture dishes?
- embryo transfer catheters?
- water?
- copper tubing for gas?
- rubber gloves?

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Environment modulates:

- Blastocyst formation and rate of development
- Cell number and allocation to ICM and TE
- Apoptosis
- ?Cell cycle defects (nuclear & chromosomal abnormalities)
- Metabolism
- Gene Expression
- Genomic imprinting
- Implantation and fetal development

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Effect of culture on…….

culture ⟶ Fetal health? ⟶ Postnatal health?

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