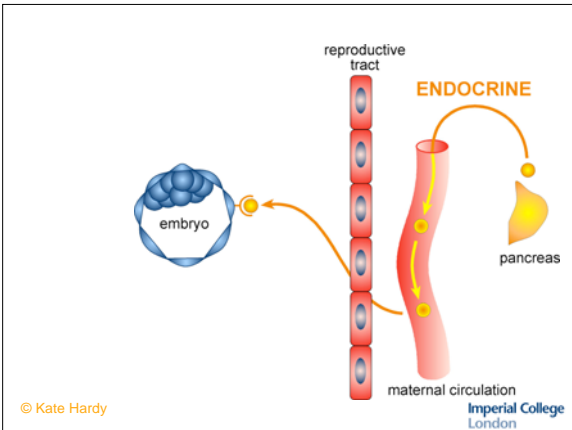


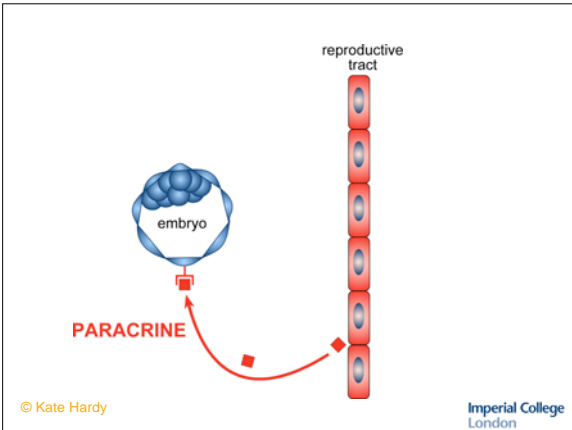
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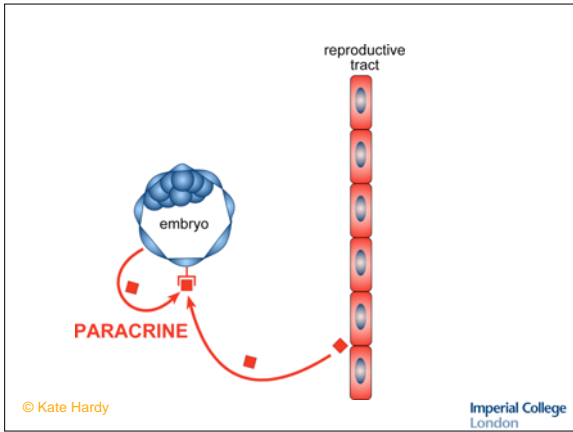
BSc in Reproductive & Developmental Sciences

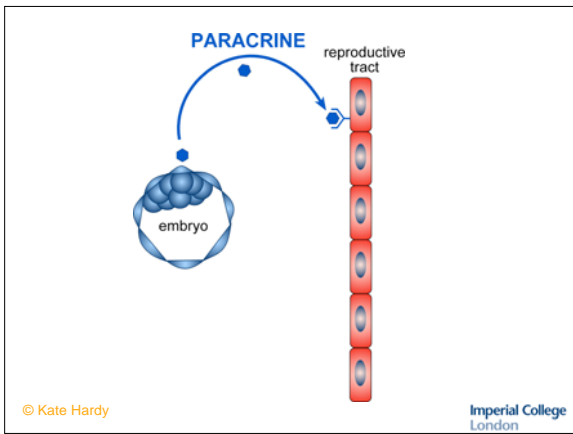
**Growth factors and
preimplantation development**

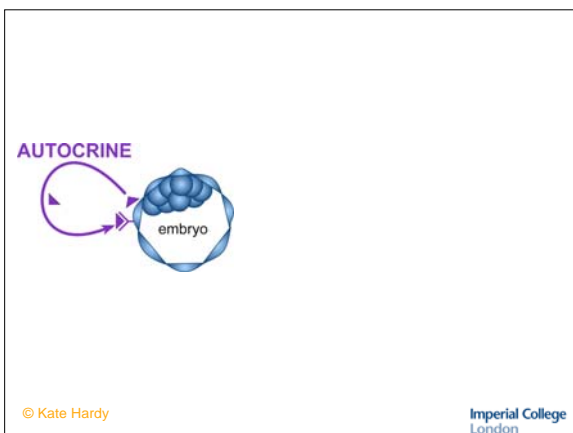
Kate Hardy
Institute of Reproductive and Developmental Biology

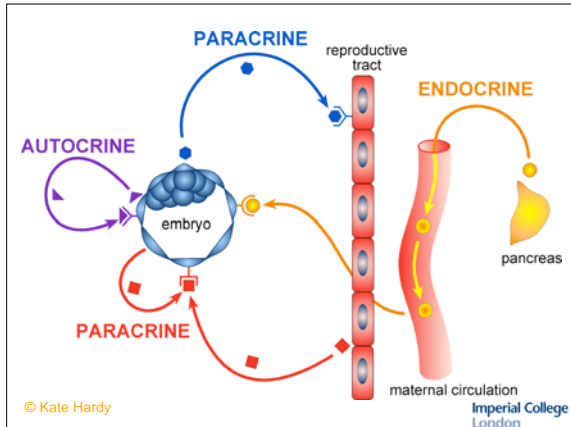












Growth factors and preimplantation development

- Not essential, but important

Evidence:

- Slower development *in vitro*
- Poor development of single embryos *in vitro*
- Improved development in groups/small drops
- Expression of growth factor receptors
- Expression of growth factor ligands by embryo and tract
- Improved development with co-culture
- Improved development with exogenous growth factors

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Assessment of growth factor effects

In vitro

- Blastocyst formation and rate of development
- Cell number and allocation to ICM and TE
- Apoptosis
- Metabolism
- Gene expression and imprinting

In vivo

- Implantation and fetal development
 - following exposure *in vitro* preimplantation
 - introduced *in vivo* to uterus

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Culture volume



Mouse (Lane and Gardner, 1992)

- Comparison of single embryo development between 20 µl and 80µl

Smaller volume:

- no difference in blastocyst formation
- ↑ blastocyst cell number
- ↑ implantation

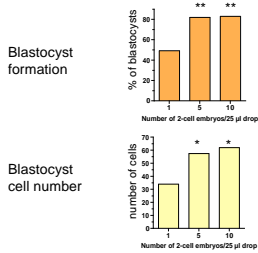
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Culture density



Mouse (Paria & Day, 1990)



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Hypothesis

- Preimplantation embryos produce factors which stimulate development and enhance viability

Factor is

- diluted in large volumes



- concentrated with many embryos



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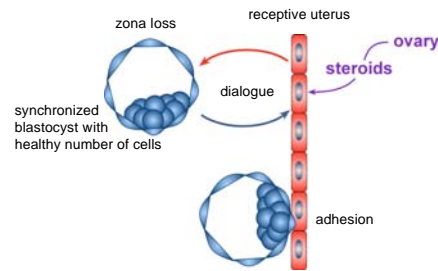
Mouse knockout studies

EGF-R	implantation failure	Threadgill et al 1995
CSF-1	↓ blastocyst cell number	Pollard 1997
GM-CSF	↓ blastocyst cell number	Robertson 2001
TGF-α	↑ blastocyst apoptosis	Brison & Schultz 1998
LIF	implantation failure	Stewart et al 1992

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Requirements for successful implantation



EGF family act as local mediators of steroid hormone actions

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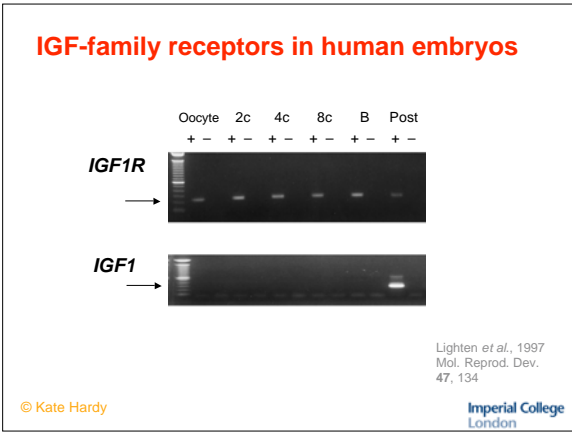
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Insulin-like growth factor I: Story of 2 PhD projects

- IGF Family**
- Ligands
 - Insulin
 - IGF-I
 - IGF-II
 - Receptors
 - Insulin receptor
 - IGF-IR
 - IGF-IIR
 - IRR
 - IGF Binding proteins
- IGF-I
 - similar to proinsulin
 - secreted by many cells
 - ↑ glucose uptake
 - ↑ amino acid uptake

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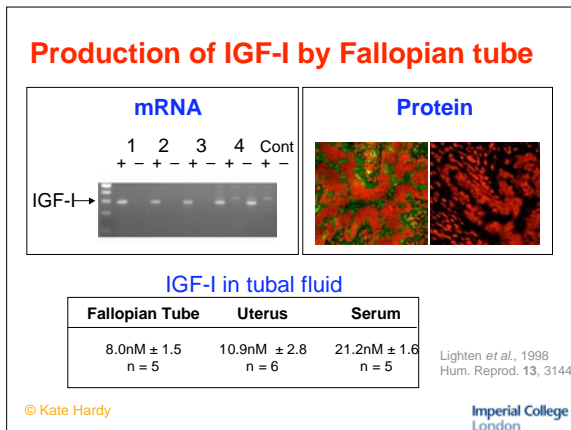




IGF-family receptors & ligands in human embryos

	unfert	2c	4c	8c	Blast
Receptor					
IGF-IR	+	+	+	+	+
Ligand					
IGF-I	-	-	-	-	-

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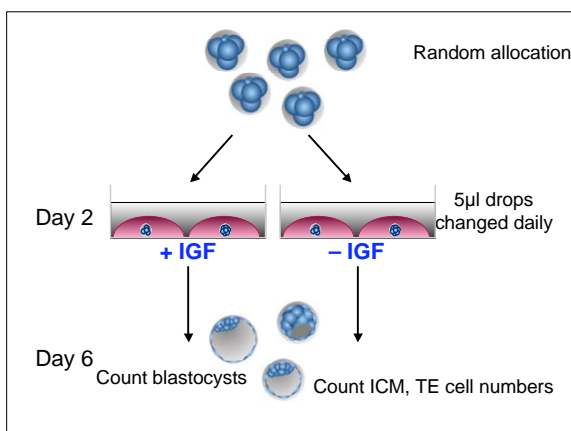


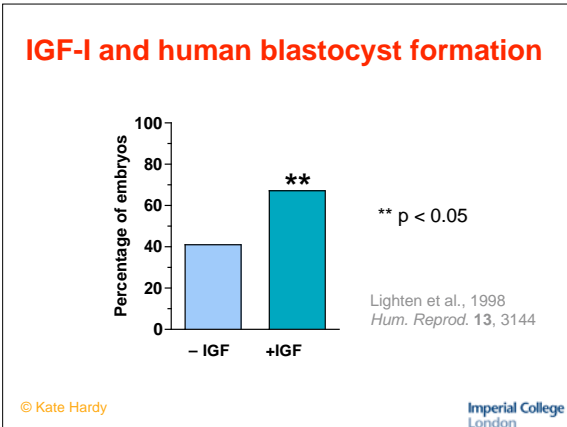
Summary

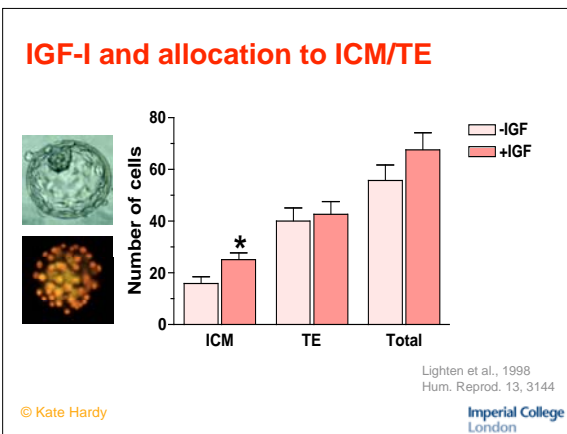
- Human preimplantation embryos express mRNA for IGF-I receptors
- IGF-I receptor protein present on blastocyst
- Fallopian tube produces IGF-I

Is human preimplantation development regulated by IGF-I?

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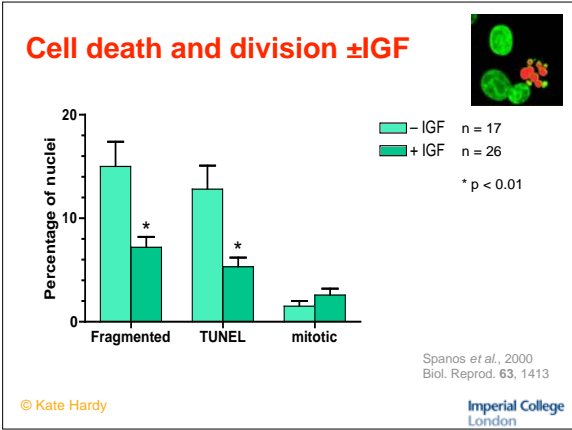


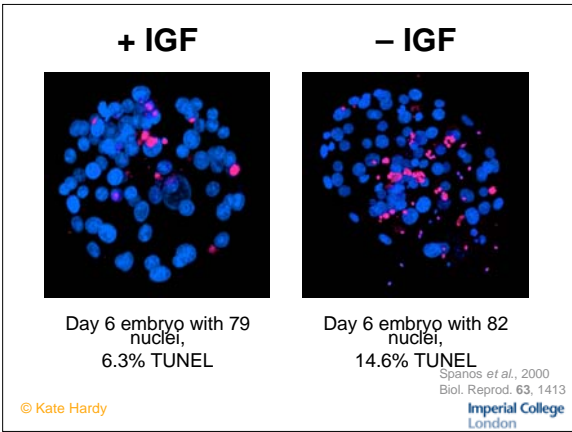
IGF-I and human development

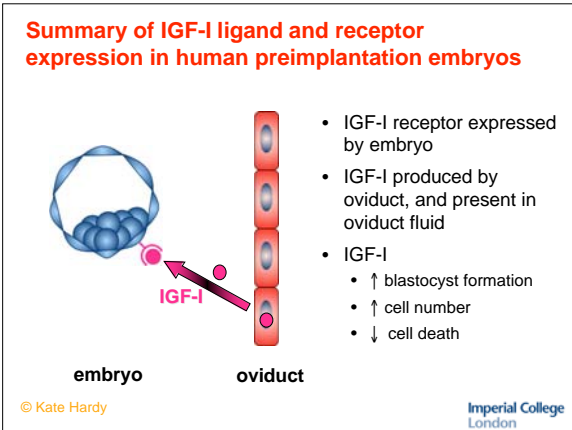
- Human preimplantation embryos express IGF-I receptors Lighten et al., 1997
- Fallopian tube produces IGF-I Lighten et al., 1998
- Exogenous IGF-I
 - ↑ blastocyst formation
 - ↑ cell number Lighten et al., 1998

Is IGF-I mitogenic and/or anti-apoptotic?

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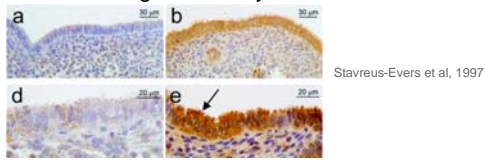






HB-EGF (Heparin binding-epidermal growth factor)

- Binds to EGF-R and ErbB4
- HB-EGF expressed in uterine epithelium
 - mouse: 6-7 h before implantation, at site of apposition Das et al, 1994
 - human: levels highest on day 18-19 Yoo et al, 1997



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HB-EGF

- Two biologically active forms
- transmembrane form
 - adhesion
- mature soluble form
 - stimulates cell proliferation, migration and cell motility

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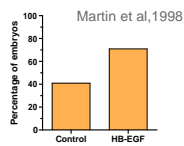
HB-EGF and embryo development

Human embryo development

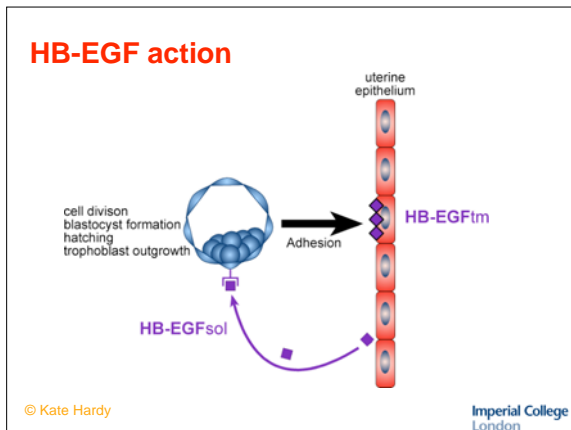
- Increases blastocyst formation and hatching

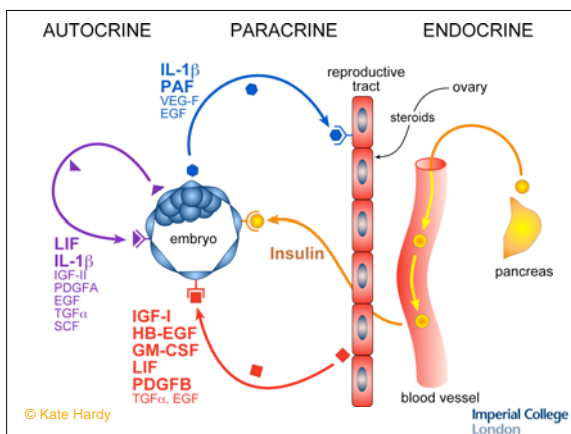
Mouse embryo development

- Stimulates blastocyst cell number, hatching & outgrowth Das et al 1994
- Transmembrane form promotes blastocyst adhesion
- ErbB4 expressed on TE cells Raab et al, 1996



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Conclusions

- human embryos and reproductive tract express a variety of growth factor receptors and ligands
- some are involved in regulating cell division and apoptosis (e.g. Granulocyte-macrophage colony-stimulating factor, IGF-1)
- others involved in blastocyst development and hatching (eg Transforming growth factor- α , HB-EGF) and implantation itself (HB-EGF, Leukaemia inhibitory factor)

