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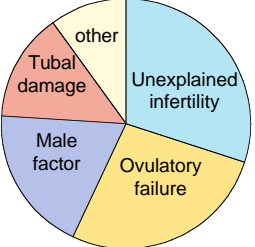
BSc Reproductive & Developmental Sciences

**Human IVF:
Developmental potential of
the human embryo**

Kate Hardy
Institute of Reproductive and Developmental Biology

Infertility

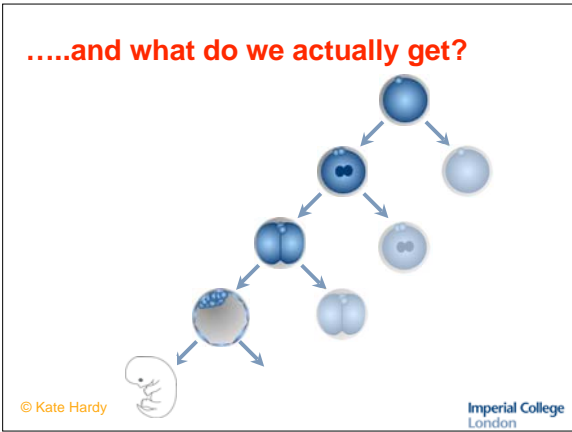
1 in 6 couples are infertile

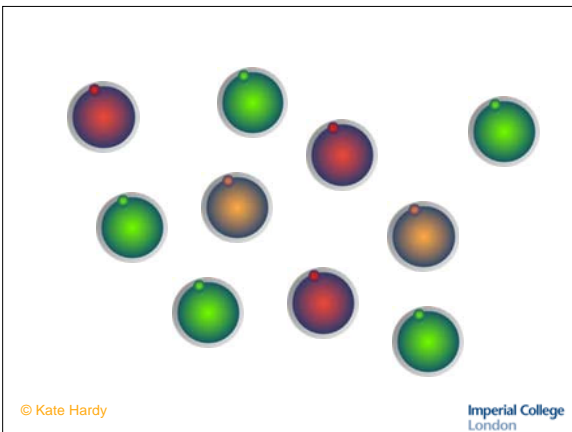


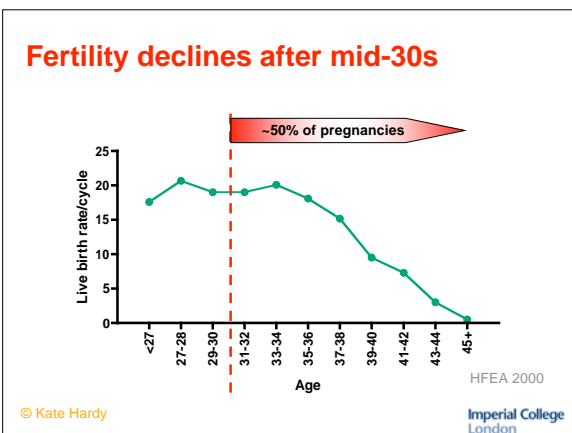
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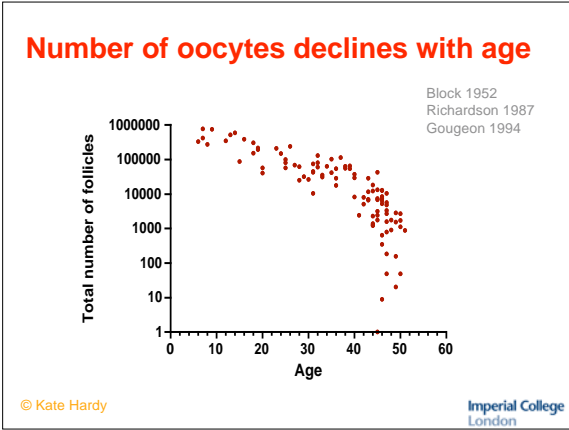
The ideal oocyte.....

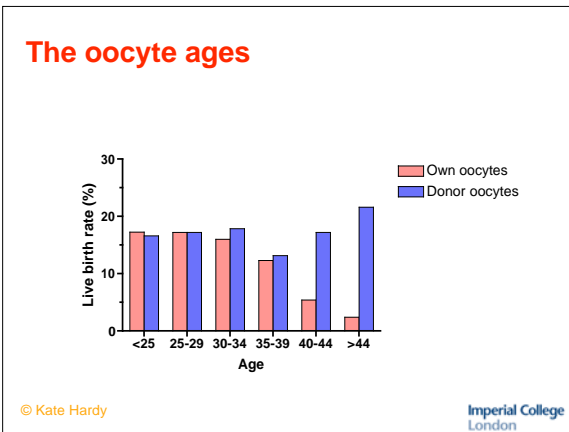


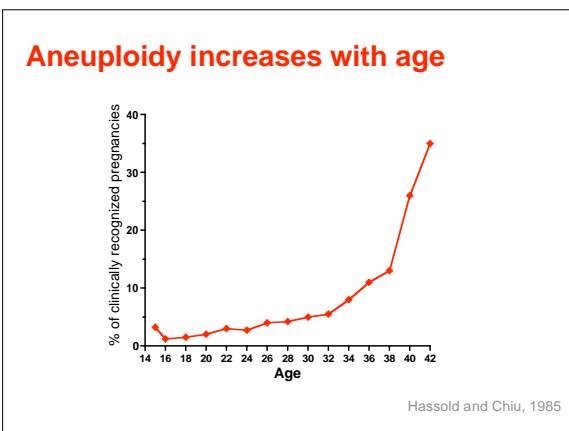












Impact of ART

Worldwide

- >1,000,000 babies born since 1978

USA 2001

- 40,687 babies born
- ~1% of all livebirths by ART

Europe 2001

- UK: 1.3% of all livebirths
- Denmark: 3.9%

www.cdc.gov/nccdphp/drfh/art.htm

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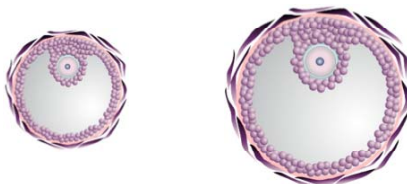
Production of multiple oocytes

- Downregulation of pituitary with GnRH agonist from Day 2 of cycle, for ~14 days
- Maturation of many follicles (~10) with FSH
- When 3 follicles are >17mm diameter, administer hCG (analogous to LH surge) for final maturation of oocytes
- 36 hours later, just before they would be ovulated, oocytes are retrieved from follicles

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Heterogeneous follicle sizes



↓ fertilization
↓ pregnancy
↓ E₂ and P

↑ fertilization
↑ pregnancy
↑ E₂ and P

Bergh et al, 1998; Teissier et al, 2000

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Gamete collection

Oocytes

- Oocytes collected using a needle inserted through vagina to follicles in the ovary, under ultrasound guidance
- Follicle aspirated by gentle suction
- Cumulus/oocyte complex identified and cultured in tube of culture medium

Sperm

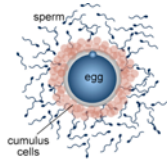
- Produced on morning of oocyte retrieval
- Swim-up into culture medium

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Insemination and embryo culture

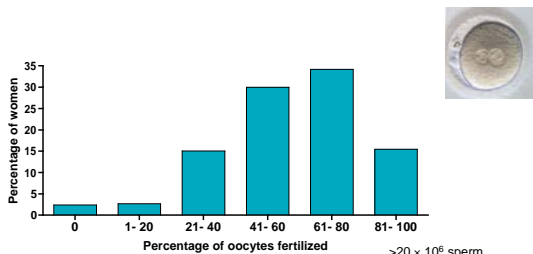
- Cumulus/oocyte complexes inseminated with sperm 40h after hCG
- Oocytes examined for fertilization next morning
- Fertilized oocytes cultured for further 24h (2- to 4-cell stage) or 48h (8-cell stage)



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Variability in ability to be fertilized



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>20 x 10⁶ sperm
>30% motility
tubal disease
IVF (n = 2694)
Hammersmith 1992-2001
Imperial College London

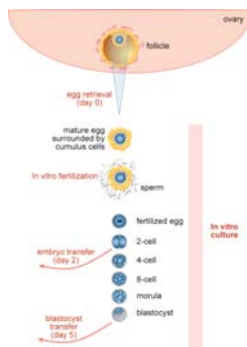
Embryo selection and transfer

- Embryos selected on basis of
 - morphology and
 - rate of development
- Embryos aspirated into fine plastic catheter. Catheter inserted into uterus through cervix, and embryos expelled into uterine cavity
- Implantation in ~a third of patients ~ 5 days later

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IVF, culture and embryo transfer

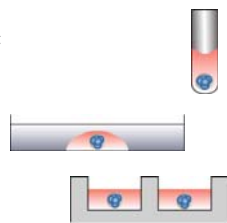


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Embryo culture

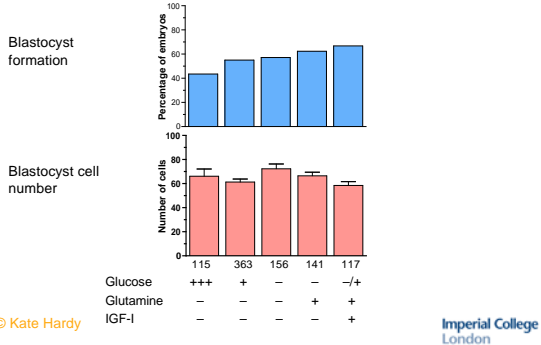
- Embryos cultured in culture medium at 37°C in 5%CO₂, 5% O₂, 90% N₂
- Water
- Salts
 - Isotonic solution - requirements for cell survival
- Energy substrates
 - Glucose, lactate, pyruvate
- Protein source
 - Serum, BSA, HSA
- Amino acids & Growth factors (Not essential)



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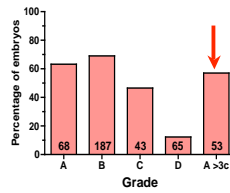
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Blastocyst development

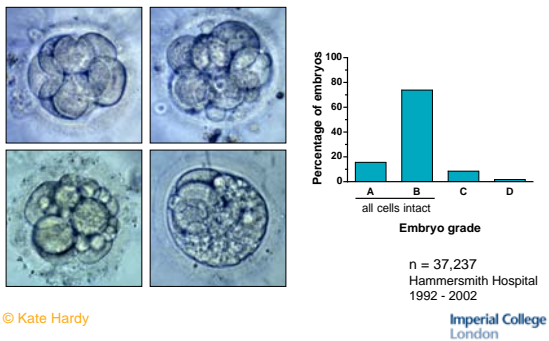


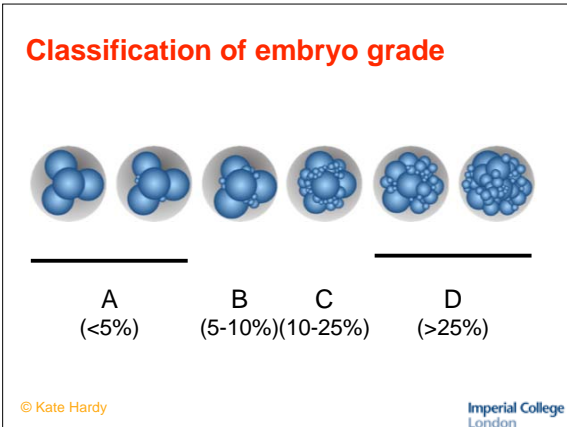
Embryo selection

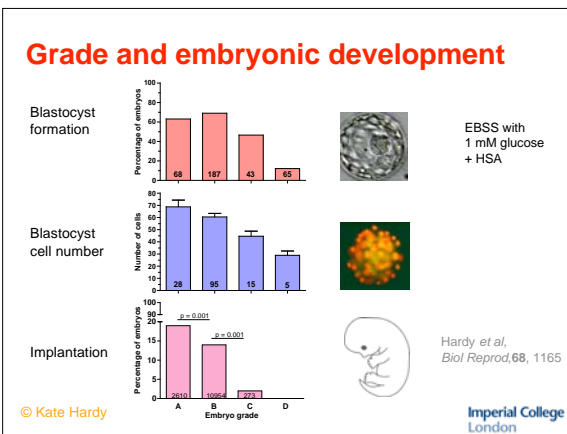
- selection techniques
 - simple
 - accurate
 - quick
 - safe
- embryo scoring
 - morphology
 - rate of development
- non-invasive biochemical tests

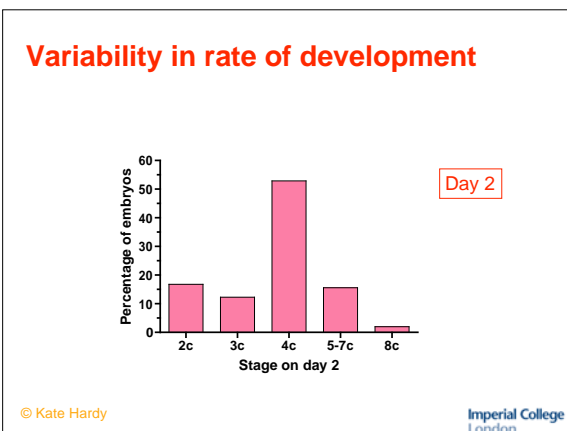


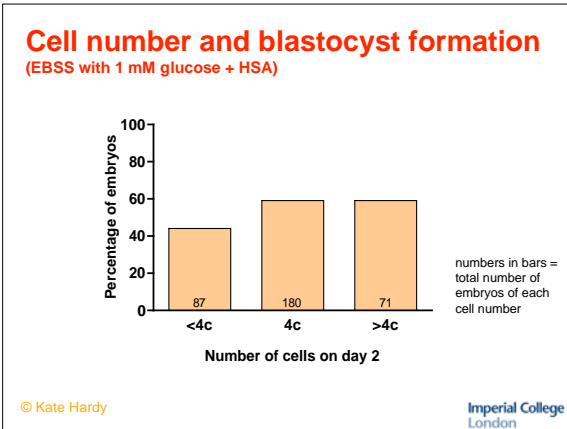
Morphology and fragmentation

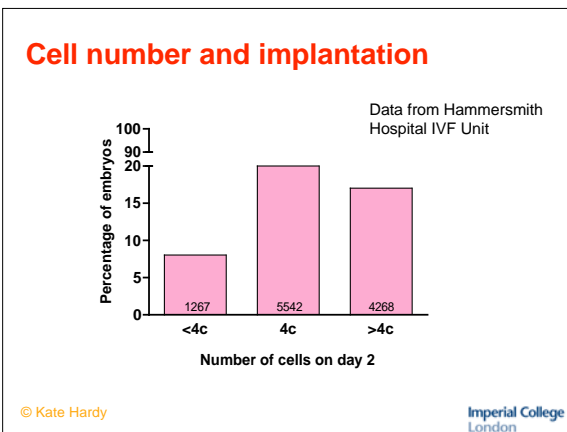


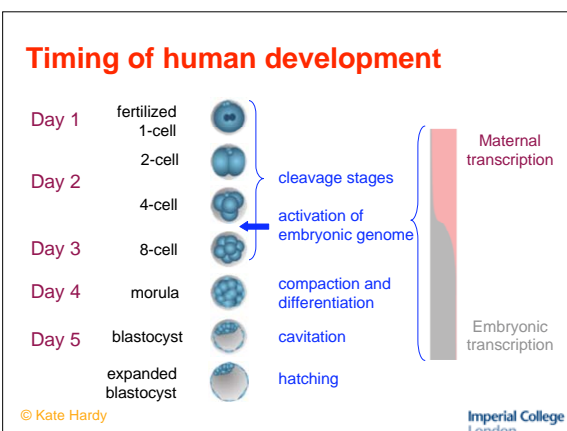


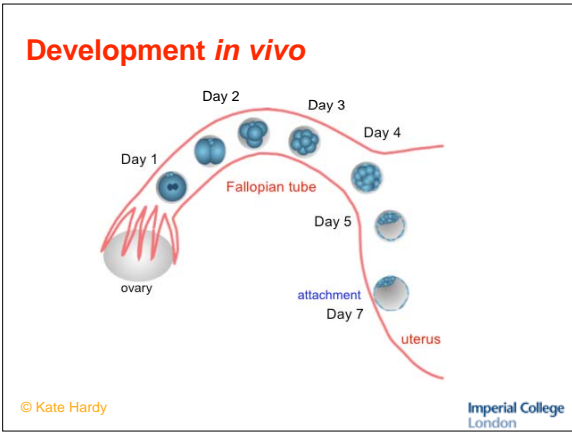


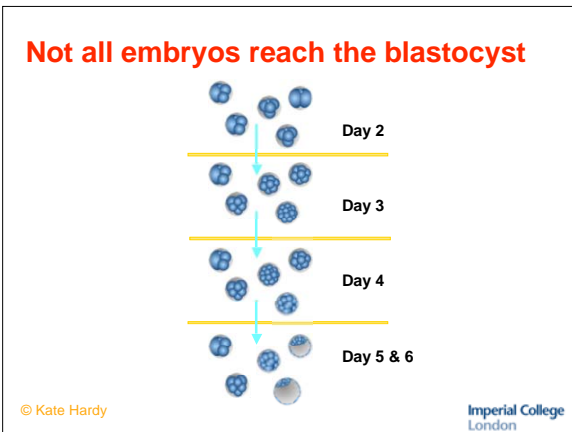


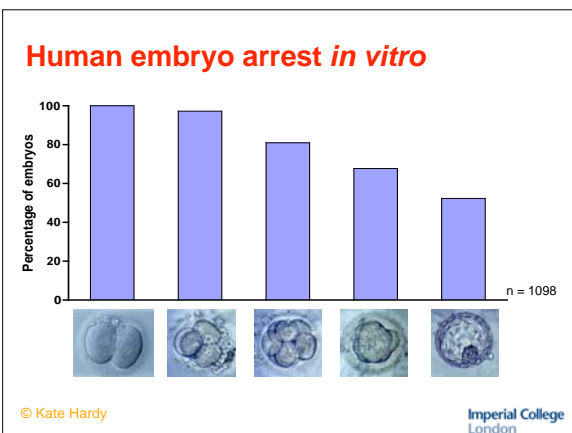




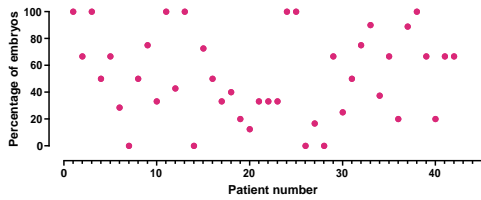








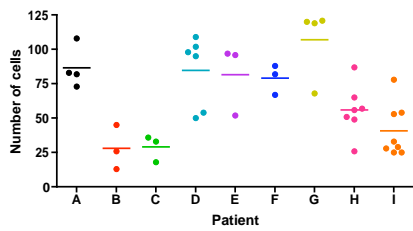
Variability and blastocyst formation



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'spare' embryos
EBSS + 1mM glucose + HSA
>3 embryos
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Variability and blastocyst cell number



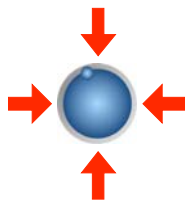
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'spare' embryos
EBSS + 1mM glucose + HSA
>3 blastocysts
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Why do embryos arrest?

Environment

- embryo culture?
- follicle environment?



Oocyte

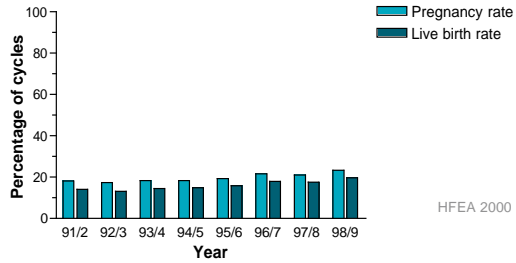
- 'quality'?
- chromosomal abnormalities?



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Low pregnancy and live birth rates following IVF

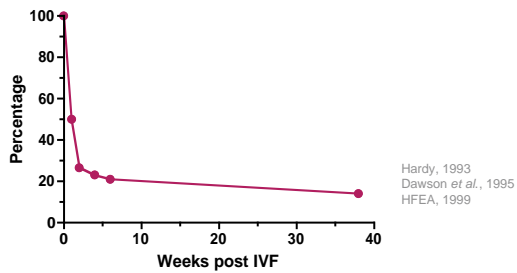


HFEA 2000

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Embryonic loss

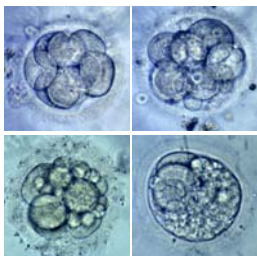


Hardy, 1993
Dawson et al., 1995
HFEA, 1999

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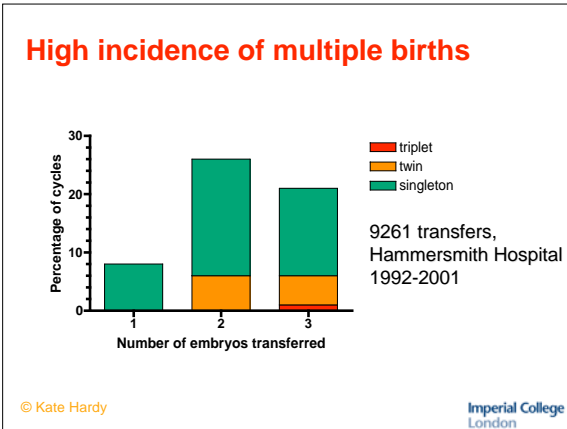
Human embryo development

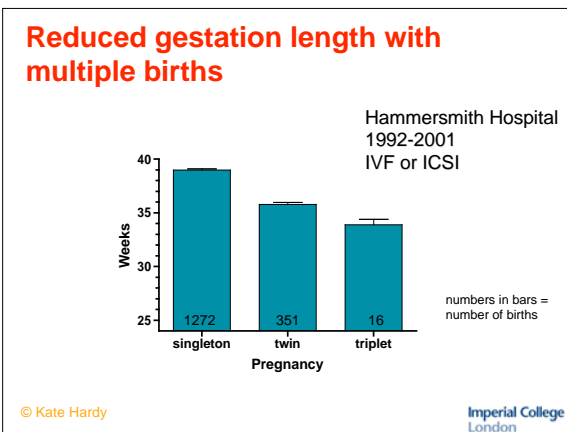


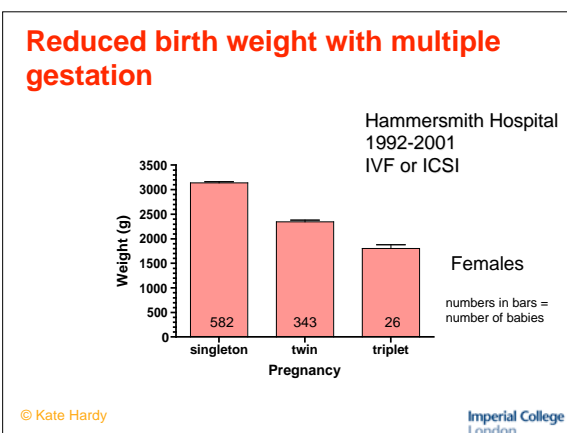
- Low implantation, pregnancy and live birth rates
- 50% of embryos arrest *in vitro*
- Fragmentation
- Chromosomal and nuclear abnormalities

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Improving success: superovulation

- Ovulation induction *alone* may increase
 - Prematurity
 - Low birthweight Olivennes 1993
- Superovulation decreases embryo viability
 - hamster McKiernan and Bavister 1998
 - mouse Van der Auwera & D'Hooghe 2001
- Superovulation increases aneuploidy?

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Blastocyst transfer as a strategy for improving success

Advantages

- Select non-arrested embryos
- Synchrony
- ↓ multiple pregnancies
- ↑ pregnancy rates

Disadvantages

- Prolonged culture?
- Risk of no blastocysts -no transfer
- ↑ Monozygotic twins?
- ↑ Males

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Prolonged culture and embryo development

- Blastocyst formation and cell number
- Metabolism
- Apoptosis Spanos et al 2000
- Gene expression Ho et al, 1995
Niemann & Wrenzycki, 2000
- Genomic imprinting Doherty et al, 2000
- Adult health Kwong et al, 2000

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Factors affecting human embryo development

- Culture medium (i.e. environment)
- Embryo morphology
 - development to blastocyst, blastocyst cell number and implantation declines with increasing fragmentation
- Rate of development
 - slower developing embryos less viable
- Chromosomal abnormalities
 - 35% of miscarriages are aneuploid
 - no monosomies detected in clinical pregnancies, except XO

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Abnormalities following IVF and ICSI

Increased incidence of

- Poor perinatal outcome
Helmerhorst, 2004
- Prematurity
Doyle et al. 1992
- Low birth weight babies
Schieve et al. 2002
- Neurological problems
Stromberg et al. 2002
- Congenital abnormalities
Hansen et al. 2002
- Imprinting defects
Cox et al., 2002

However, women who have assisted conception are older, have fewer previous pregnancies and different socio-economic status. They are also subfertile

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