Is it still necessary to conduct research on human embryos, including the creation of embryos for research purposes?

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Centre for Reproductive Medicine
Reproduction and Immunology

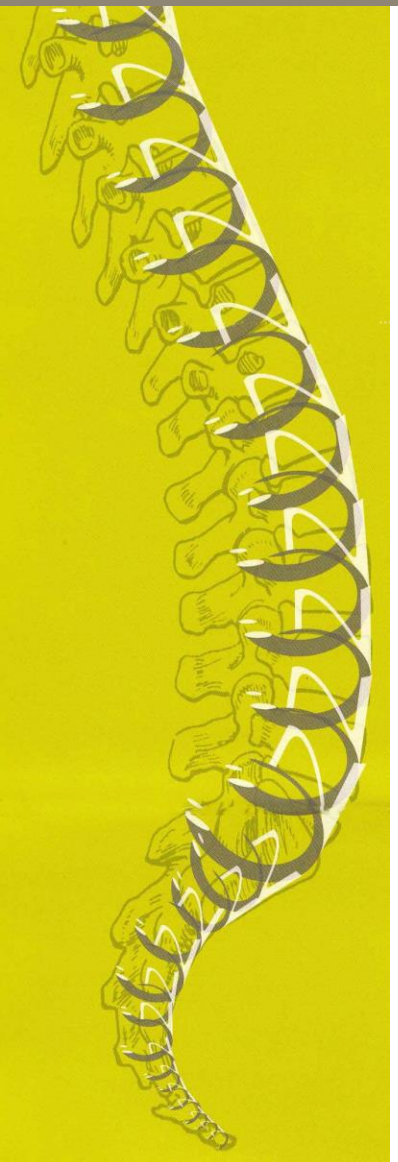
REIM

Symposium FCE
25 November, 2016

Conflict of interest

Nothing to declare

Outline



- Plea for research on human embryos
  - → Spare human embryos
  - → Examples
  - → Compare with research on mouse embryos
  - → Belgium - UZ Brussel

- Need to create human embryos for research
  - → Belgium - UZ Brussel
  - → Examples

Revolution in ART procedures

- IVF (Steptoe and Edwards, 1978)
  → Female infertility

- ICSI (Palermo et al. 1992)
  → Male infertility
  → Invasive

- Embryo biopsy for genetic testing (Handyside et al. 1993)

- *In vitro* culture of human embryos
  → Available for research

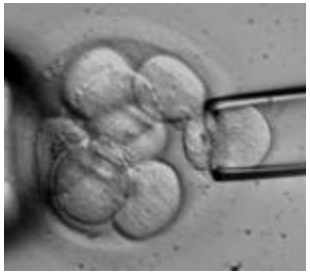

Daily Mail

WORLD EXCLUSIVE

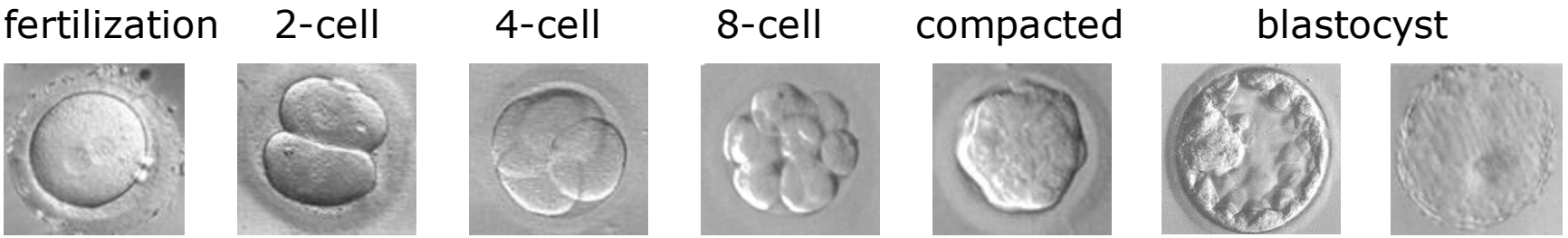
And here she is...

THE LOVELY LOUISE

LOUISE BROWN, bright-eyed at 10 hours old: The test-tube baby in hospital yesterday



ART children

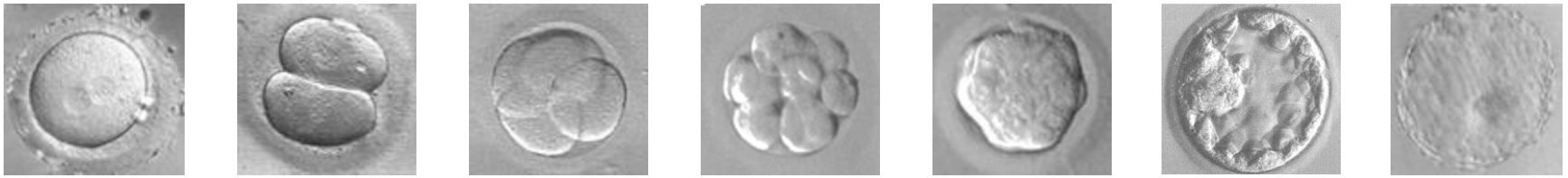


- New ART procedures are introduced without appropriate testing

Ca2+ ionophore for poor fertilization	IVM
Extended embryo culture Culture media supplemented with growth factors Ca2+ ionophore for poor embryo development	Mitochondrial transfer
Oocyte and embryo vitrification	Genome editing

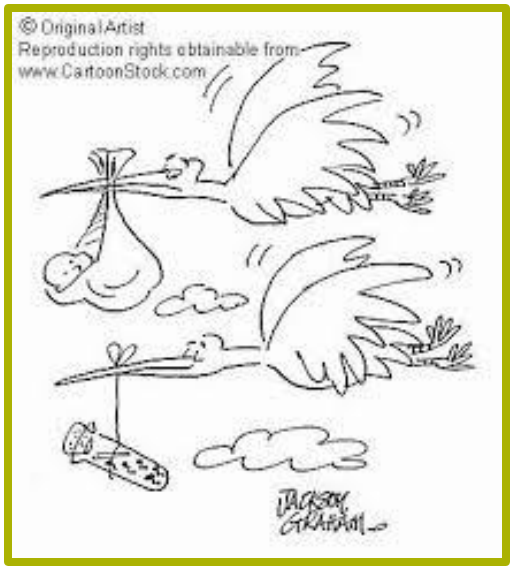
ART children

fertilization 2-cell 4-cell 8-cell compacted blastocyst



- New ART procedures are introduced without appropriate testing

- Developmental origin of disease
  - → Metabolic disorders
    - Diabetes
    - Obesitas
  - → Cardiac diseases
  - → Imprinting disorders



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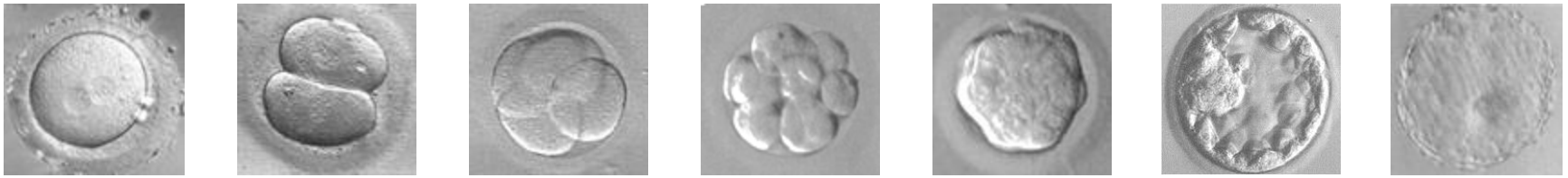

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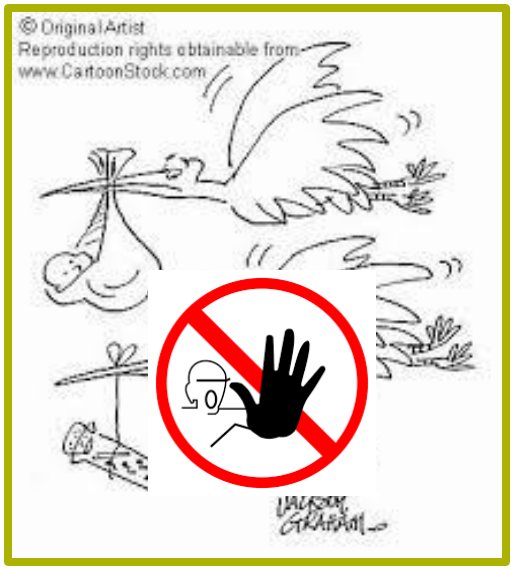

Research on the efficacy and safety of ART procedures

- Hypothesis
- Preclinical research in animal models
  - → Small animals (rodents)
  - → Large animals (cows and pigs)
- Preclinical research with human gametes and embryos donated to research
- Prospective clinical trials in IVF centres
  - → Small scale single centre
  - → Large RCT multi centre
- Assess clinical and cost effectiveness
- Longterm children follow up

Harper et al. 2012; Brison et al. 2013

Research on human embryos

- Reproductive medicine
  - → Efficacy and safety of ART techniques
  - → Infertility treatment

- Basic knowledge
  - → Reproductive biology
    - Fertilization
    - Preimplantation development
    - Implantation
  - → Stem cell biology
    - Model early embryogenesis
    - Transplantation therapy
    - Infertility treatment: germ cell differentiation
    - Cancer

Research on human embryos

- Hypothesis
- Basic research in animal models
  - → Small animals (rodents)
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- Basic research with human gametes and embryos donated to research
- Basic research translated to the IVF clinic
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Research in animal models

- Human population is outbred whereas many animals are inbred
- Humans are subfertile whereas animals are fertile
- Species differences: data cannot always be extrapolated to the human

Animal models extrapolated to the human

- The human being is ‘unique’
  - → Ethical and legal issues

→ 

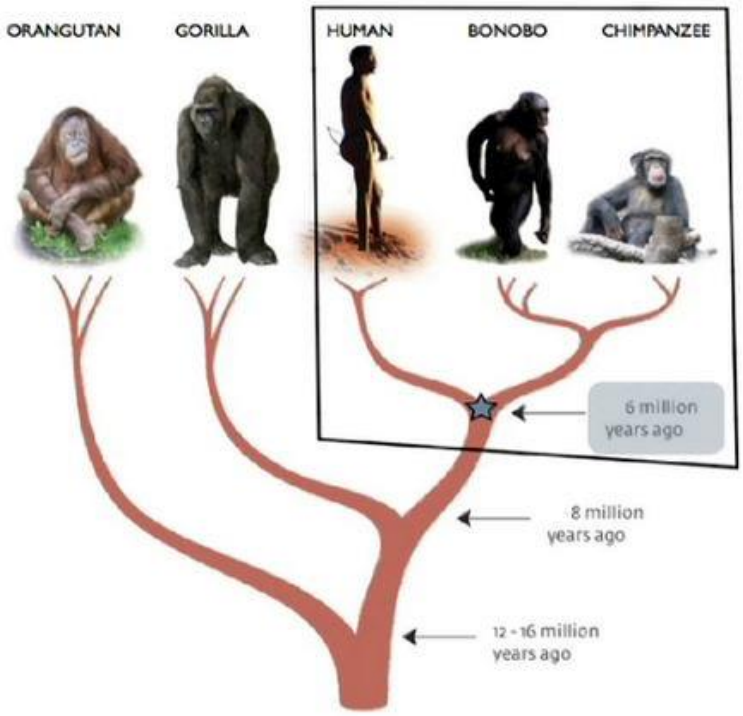
animalarium
stress
embryos after natural conception

→ 

cadavers
IVM oocytes

Animal models extrapolated to the human

- The human being is ‘unique’
  - → Higher primates
    - Similar ethical and legal issues
  - → Treat the human embryo with respect



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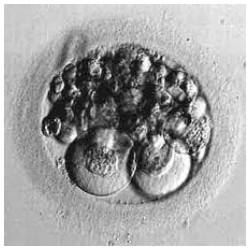
Research on human embryos

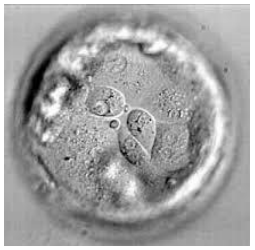
- Hypothesis
- Basic research in animal models
  - → Small animals (rodents)
  - → Large animals (cows and pigs)
- Basic research with human gametes and embryos donated to research
  - → Spare (supernumerary) embryos
  - → Embryos created for research

Research on spare human embryos

- Created for the couple undergoing IVF/ICSI treatment
- Supernumerary: not used for transfer in the fresh cycle
  - → Bad quality non-PGD/PGS and PGD/PGS
    - Not transferred
    - Not cryopreserved
  - → Good quality
    - Non-PGD/PGS
      - Cryopreserved (available after the legally determined period)



Day 3



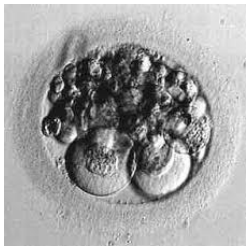
Day 6

Day 3

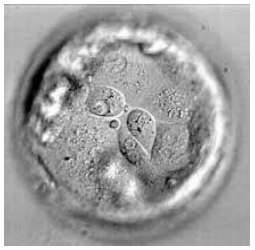
Day 6

Research on spare human embryos

- Created for the couple undergoing IVF/ICSI treatment
- Supernumerary: not used for transfer in the fresh cycle
  - → Bad quality non-PGD/PGS and PGD/PGS
    - Not transferred
    - Not cryopreserved
  - → Good quality
    - Non-PGD/PGS
      - Cryopreserved (available after the legally determined period)
    - PGD/PGS
      - Genetically abnormal
      - Fresh after Pb or blastomere biopspy
      - Cryopreserved after TE biopsy



Day 3



Day 6

Day 3

Day 6

Use of spare human embryos for research

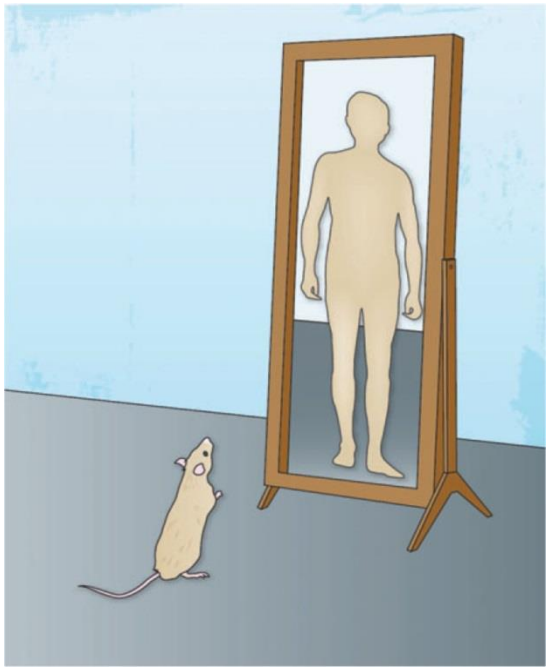
- A mouse is not a human being

  → First lineage differentiation

  → Second lineage differentiation

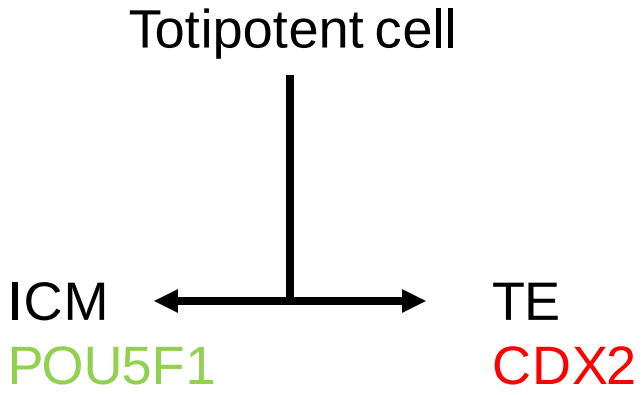
  → Implantation

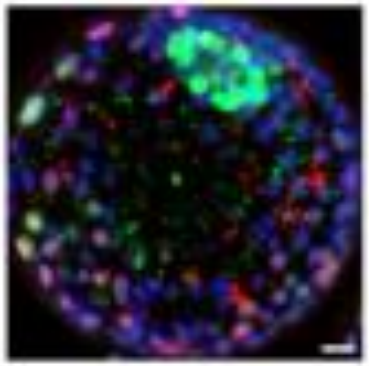
  → Embryonic stem cells (ESC)



First lineage differentiation

- Cell lineages are similar, timing and pathways are different
  - → Inner cell mass (ICM) → embryo proper
    - Extraembryonic endoderm, mesoderm, ectoderm
    - Embryonic endoderm, mesoderm, ectoderm
    - Germ cells
  - → Trophectoderm (TE) → trophoblast (TB)





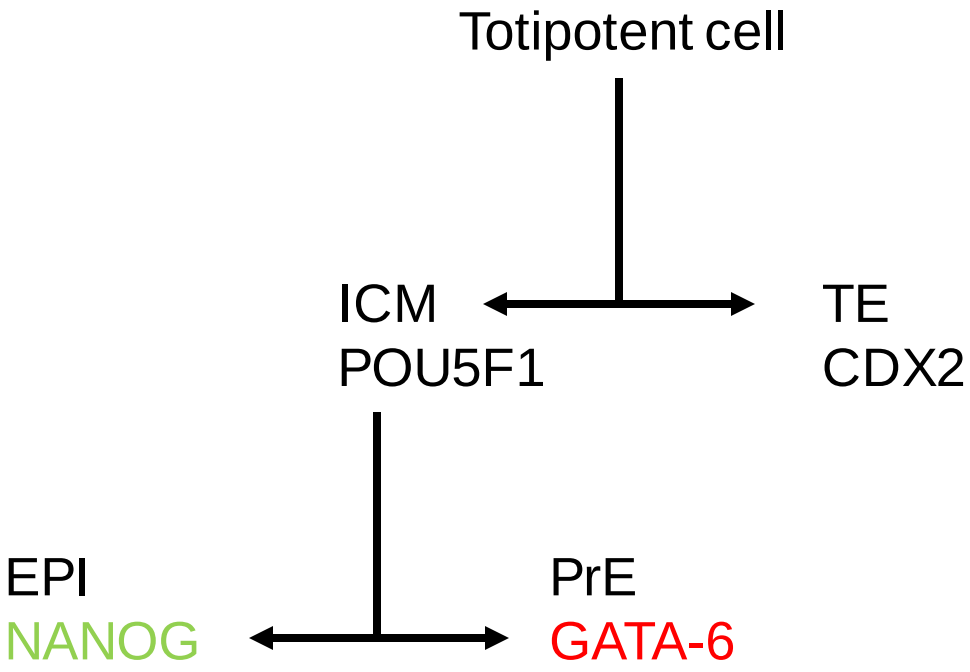
Niakan and Eggan, 2013

First lineage differentiation

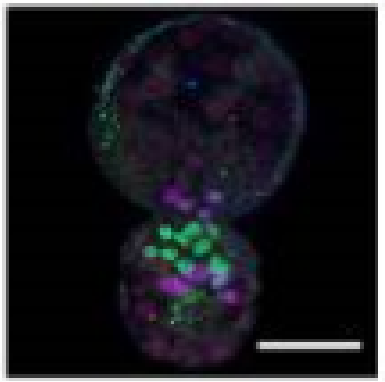
- Mouse
  - → KO mice, CRISPR/Cas9
  - → siRNA, morpholinos, small inhibitors
  - → TE: CDX2, GATA3, EOMES, ELF5, TCFAP2C
  - → Position, polarization, compaction (Hippo: TEAD4 and YAP)
- Human
  - → Descriptive studies
    - Immunocytochemistry (protein) (Cauffman et al. 2006 and 2009; Niakan and Eggan, 2013)
    - qPCR (mRNA) (Wong et al. 2010; Yan et al. 2013; Kleijkers et al. 2015; Blakely et al. 2015)
  - → Functional studies: proof of evidence is lacking
    - Small inhibitors (Krivega et al. 2015)
    - None with growth factors
    - None with genetic modifications

Second lineage differentiation

- Cell lineages are similar, timing and pathways are different
- Inner cell mass (ICM)
  - → Epiblast (EPI)
  - → Hypoblast or primitive endoderm (PrE)



Kuijk et al. 2012



Second lineage differentiation

- Mouse
  - → EPI: NANOG (FGF4)
  - → PrE: GATA6 (FGF2R)

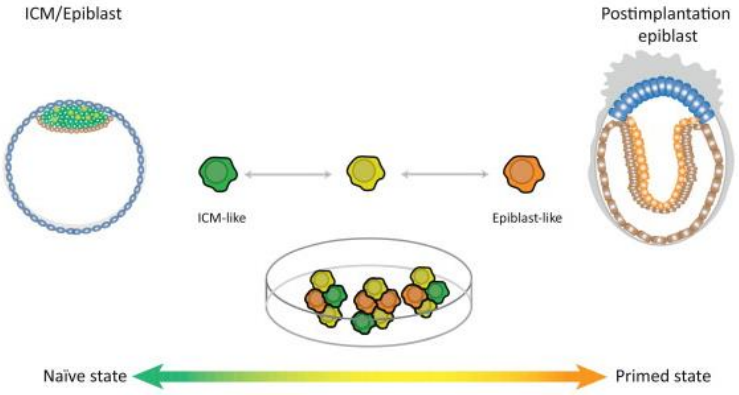
- Human
  - → Descriptive and functional studies (small inhibitors)
    - Not FGF4 (Kuijk et al. 2012; Roode et al. 2012)
    - TGFbeta (Van der Jeught et al. 2013)

Reproduction - Implantation

- Mouse
  - → Polyestrous cycle (4-5 days)
  - → Short day breeder, “in heat”
  - → No menstruation (the endometrium is reabsorbed)
  - → Decidualization after implantation (in presence of an embryo)
  - → Embryo encapsulation
  - → LH
- Human
  - → Menstrual cycle (28 days)
  - → Continuous breeder, hidden ovulation
  - → Menstruation (endometrium is shed)
  - → Spontaneous decidualization (in absence of an embryo)
  - → Embryo invasion
  - → hCG

Embryonic stem cells

- Naive ESC
  - → Originate from preimpantation ICM/EPI
  - → Ground state in mice (permissive strains)
  - → Flat colonies
  - → BMP4 and LIF
  - → Sperm cells (Zhou et al. 2016)
  - → Oocytes (Hikabe et al. 2016)
- Primed ESC: EpiSC
  - → Originate from postimplantation EPI
  - → Ground state in human (outbred)
  - → Pilled up colonies
  - → FGF2 and Activin A
  - → Review (Hendriks et al. 2015)



Research on human embryos in Belgium

- Belgian law May 2003: research on human embryos *in vitro*
  - → Permission Local Ethical Committee (LEC)
  - → Permission Federal Committee Embryo (FCE)
- Do's
  - → Project and goal
  - → Benefit for science (reproduction and/or disease)
  - → No alternative research methodology
- Don'ts
  - → Commercialization (patents)
  - → Eugenetics
  - → Reproductive cloning
- Donor autonomy and privacy are respected
- Embryos are ultimately destroyed (not transferred)
  - → *In vitro* development until day 14

Research on human embryos at the VUB

- Brochure and informed consents


Universitair Ziekenhuis Brussel

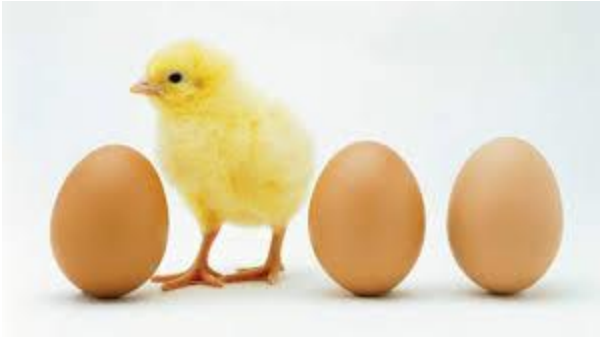
CONSENT FOR SCIENTIFIC RESEARCH ON FRESH GAMETES THAT CANNOT BE USED IN YOUR TREATMENT

Information on scientific research*

Centrum voor Reproductieve Geneeskunde

Research on human embryos at the VUB

- Brochure and informed consents
  - → Particular permission LEC and FCE
    - Create embryos if there is no alternative way to answer the research question
  - → Specific informed consent
    - Sperm
      - One consenting sperm donor
    - Eggs
      - No sperm found in TESE
        No oocyte vitrification
      - Egg bank donors



Need to create human embryos for research

- Cryopreserved embryos
  - → Good quality
  - → Available after the legally determined period of cryopreservation
    - 5 years in Belgium
  - → Slow freezing protocols ☹
  - → Vitrification ☺
- But ...

Need to create human embryos for research

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  - → Vitrification ☺
- But
  - → Exposed to cryoprotectants and stored in liquid N2
    - Bias in the study
    - Overnight culture before use
  - → Day 5/6 > day 3 >> day 2 >>> day 1 (zygotes)
    - Stock of cryopreserved zygotes will be exhausted

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- Need to create fresh zygotes/early embryos

Need to create human embryos for research at the VUB

- Van de Velde H, Cauffman G, Tournaye H, Devroey P and Liebaers I. The four blastomeres of a 4-cell stage human embryo are able to develop into blastocysts with inner cell mass and trophectoderm. Hum. Reprod. 23: 1742-1747, 2008
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- Petrussa L, Van de Velde H, De Rycke M. 1. Dynamic regulation of DNA methyltransferases in human oocytes and preimplantation embryos after assisted reproductive technlogies. Mol. Hum. Reprod. 2014.20: 861-874, 2014
- Krivega M, Geens M, Van de Velde H. Differential CAR expression in human embryos and embryonic stem cells illustrates its role in pluripotency and tight junction formation. Reproduction.148: 531-544, 2014
- De Munck N, Petrussa L, Verheyen G, Staessen C, Vandeskelde Y, Sterckx J, Bocken G, Jacobs K, Stoop D, De Rycke M, Van de Velde H. Chromosomal meiotic segregation, embryonic developmental kinetics and DNA (hydroxyl)methylation analysis consolidate the safety of human oocyte vitrification. Mol. Hum. Reprod. 21: 535-44, 2015
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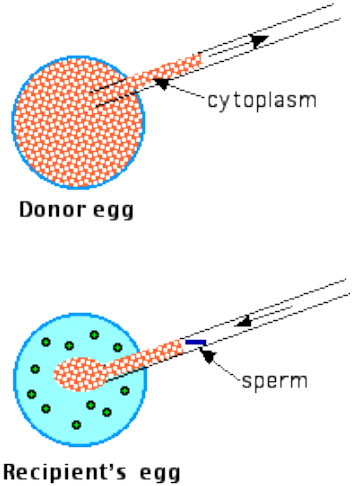
- Petrussa L, Van de Velde H and De Rycke M. DNA methylation and DNA hydroxymethylation follow similar kinetics during human preimplantation development in vitro. Mol. Dev. Rep. 83: 594-605, 2016

Need to create human embryos for research

- Germ line modification
  - → Mitochondria replacement therapy
  - → Genome editing
- Embryonic genome activation
- Mosaicism

Mitochondrial replacement therapy

- Cytoplasmic transfer to iuvenate oocytes
  - → Mitochondria
  - → IVF failure
  - → Allogeneic cytoplasm (Cohen et al. 1997)
    - 1996-2001
    - 17 babies born
    - 2 implantations with XO
    - Follow up
    - Ethical concerns (3 parent babies)

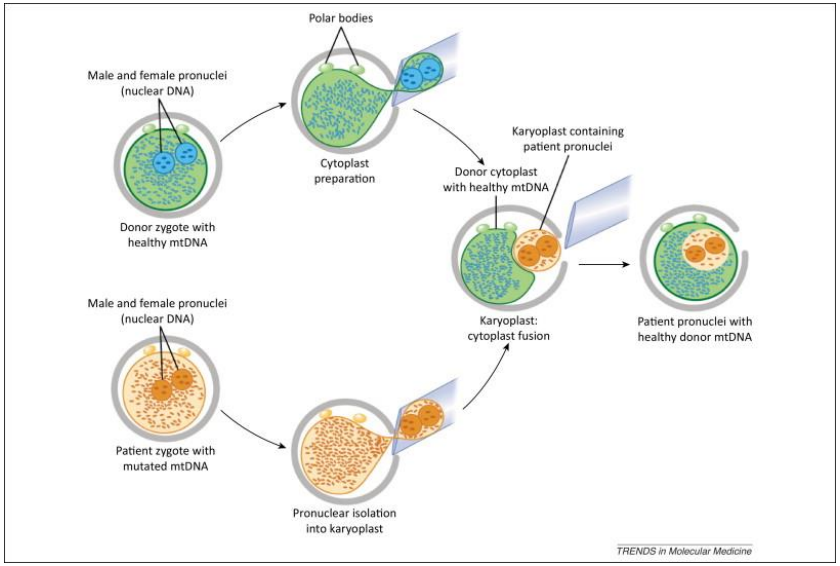


  - → Autologous mitochondria from ovaria (Fakih et al. 2015)
    - Stem cells?
    - Efficacy and safety concerns

Mitochondrial replacement therapy

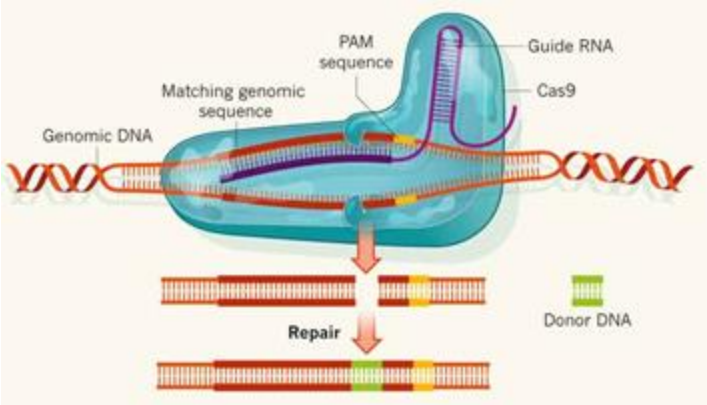
- Nuclear (GV, spindle, PN, Pb) transfer
  - → To avoid mitochondrial diseases: baby born (Zhang unpublished)
  - → To treat infertility: block at 2-cell stage (Zhang et al. 2016)
  - → Efficacy and safety concerns

- Basic research in models
  - → Animals
    - Mice and monkeys
  - → hESC (Tachibana et al. 2013)



Genome editing

- Proof of principle CRISPR/Cas9 (Liang et al. 2015)
  - → Easy and cheap
  - → 3PN embryos
  - → Beta-thalassemia (beta-globin)
  - → Safety concerns
    - Inefficient
    - Mosaic
    - Off-target mutations



- Ethical concerns
  - → Moratorium for human reproduction
    - > HIV receptor (CCR5Δ32) (Kang et al. 2016)
  - → Replace PGD only in very rare cases
- KO human embryos for basic research
  - → Study key mediators early embryogenesis

Embryonic genome activation

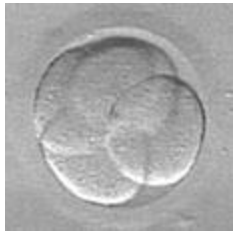
- Mouse
  - → 1- to 2-cell stage (Hamatani et al. 2004; Wang et al. 2004)
- Human
  - → Minor wave 2- to 4-cell stage (Dobson et al. 2004; Vassena et al. 2011)
  - → Major wave 4- to 8- cell stage (Braude et al. 1988; Vassena et al, 2011)

Maternal RNA
Maternal proteins
degradation

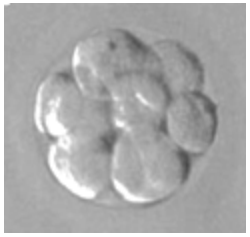
Embryonic genome
activation

Embryonic genome activation

- Human
  - → Poor embryo development < day 3
    - Oocyte problem
    - Donor oocytes
  - → Poor embryo development > day 3
    - Sperm problem
    - Donor sperm



EGA →



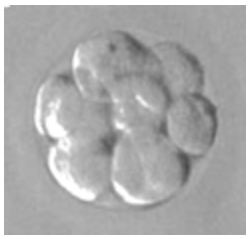
Maternal RNA
Maternal proteins
degradation

Embryonic genome
activation

Embryonic genome activation

- Human
  - → Poor embryo development < day 3
    - Oo[illegible]blem
    - Do[illegible]tes
  - → Poor e[illegible]evelopment > day 3
    - Sperm problem
    - Donor sperm

EGA



Maternal RNA
Maternal proteins
degradation

Maternal genome
Paternal genome
activation

Embryonic genome activation

- Human
  - → Paternal factors
    - DNA and protamines
    - Centriole
    - Histones (Hammoud et al. 2009)
    - mRNA (Miller et al. 2011; Hamatani et al. 2012; Neff et al. 2014)
    - miRNA (Abu-Halima et al. 2014 ; Pantano et al. 2015; Yao et al. 2015)
    - Proteins (Amaral et al. 2014; Azpiazu et al. 2014)
  - → Somatic cell nuclear transfer
    - Therapeutic cloning
    - Often arrest at 4- to 8-cell stage (Noggle et al. 2011; Egli et al. 2011; Tachibana et al. 2013)

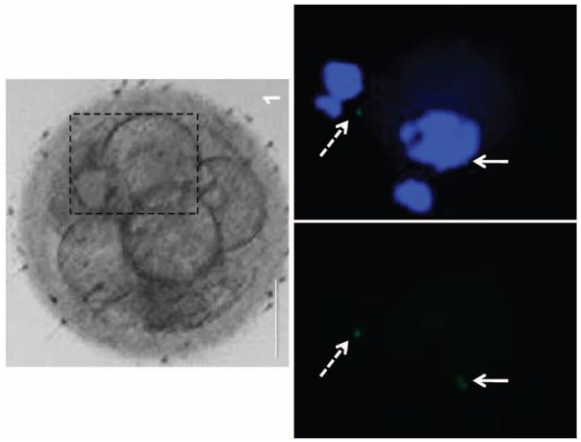
Aneuploidy

- Aneuploidy and mosaicism (Vanneste et al. 2009; Chavez et al. 2012; Mertzanidou et al. 2012 and 2013)
  - → Mainly mitotic errors
  - → 50-80% at cleavage stages and compaction
  - → No cell cycle check points proteins before EGA (Kiessling et al. 2010)
  - → Anaphase lagging and non-disjunction during mitosis in the early cleavage stages
  - → Origin?

Aneuploidy

- Aneuploidy and mosaicism
  - → Less at blastocyst stage
  - → Self-correction?
- Solving the problem without knowing the cause
  - → TE biospy + PGS/CCH (Scott et al. 2013)
    - Multiple pregnancy rate ↓
    - Time to pregnancy ↓
    - Healthy babies after transfer of mosaic embryos (Greco et al. 2015)
    - RCTs?
- Origin?
  - → Fragments with micronuclei

    (Chavez et al. 2012)



Aneuploidy

- Aneuploidy and mosaicism
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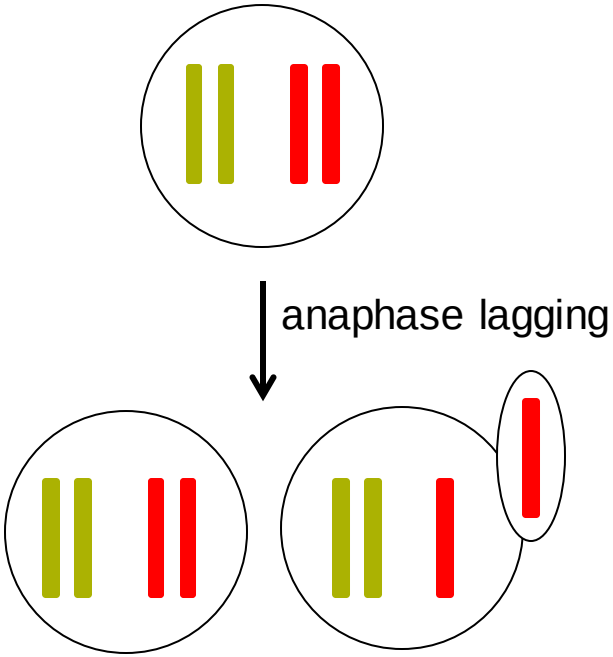

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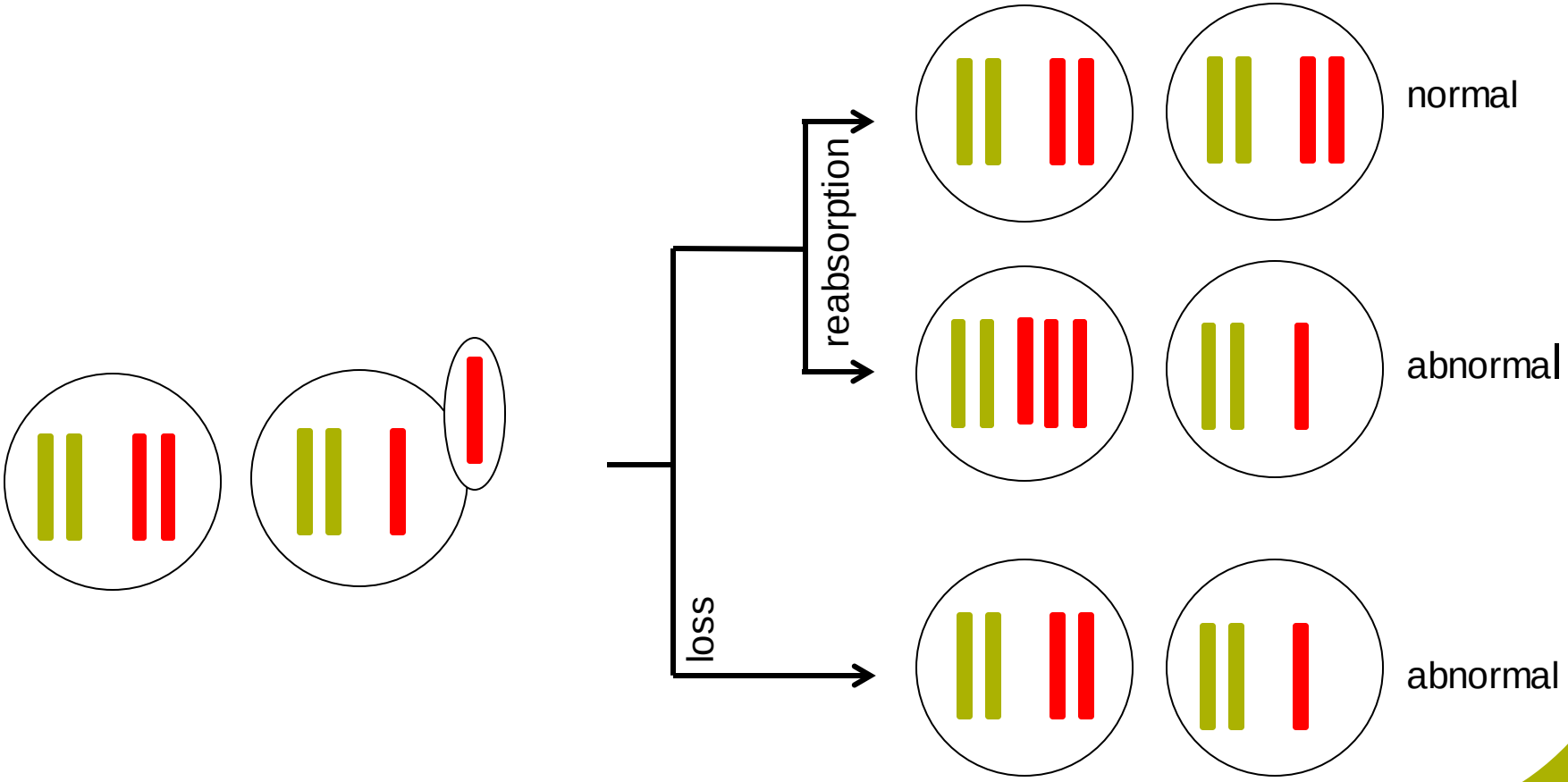

Conclusion

- Research on human embryos is needed because humans are “unique”
- It is necessary to create fresh human zygotes/early embryos for research because those stages are not available