Human embryos made for research

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INSERM
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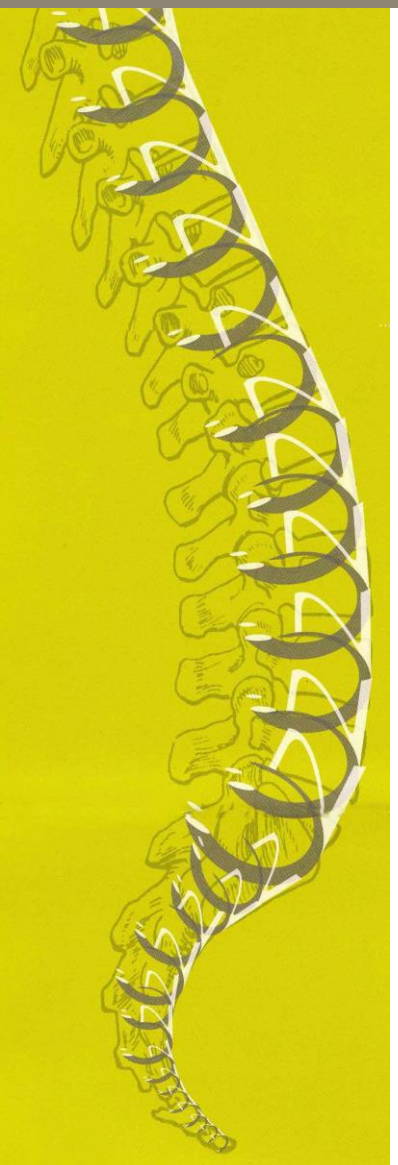
Conflict of interest

Nothing to declare

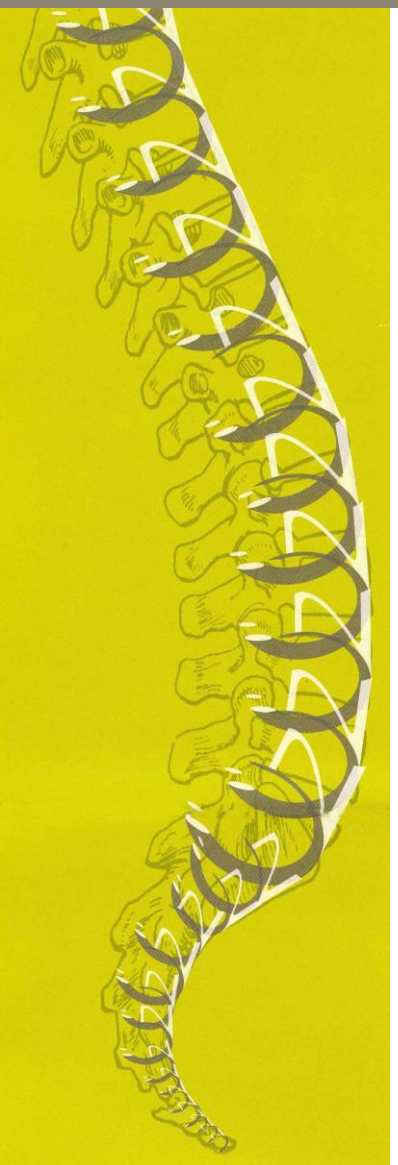
Outline



- A plea for research on human embryos
  - → ART procedures
  - → Animal models
  - → Belgium – UZ Brussel

- Examples
  - → Sperm cell factor
  - → Aneuploidy and mosaicism
  - → Totipotency and lineage segregation
  - → Epigenetic modifications
  - → Germ line therapy
  - → Gene editing

Outline



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Revolution in ART procedures

- 1978: 1st IVF baby born


Daily Mail

WORLD EXCLUSIVE

And here she is...

THE LOVELY LOUISE

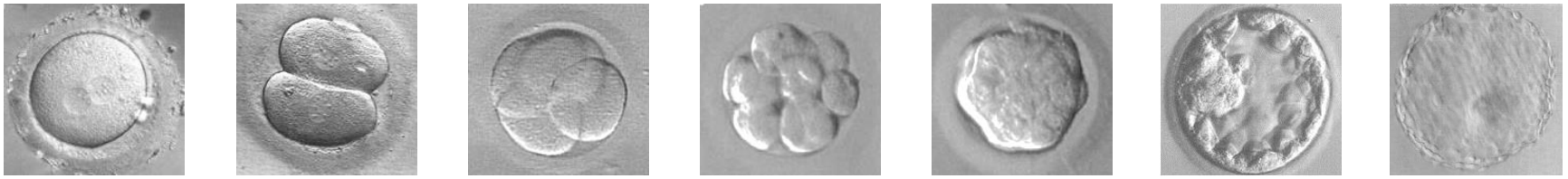
LOUISE BROWN, bright-eyed at 18 hours old: The test-tube baby in hospital yesterday

- 2015: >5 million ART babies born

- New (invasive) techniques
  - → ICSI
  - → Extended embryo culture
  - → Culture media supplemented with growth factors
  - → IVM
  - → Oocyte and embryo vitrification
  - → Genetic screening
  - → Mitochondrial transfer
  - → Gene editing

ART children

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



- New procedures are introduced without appropriate testing

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

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

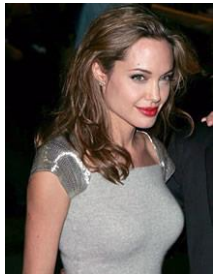
- Longterm children follow up

(Harper et al. 2012; Brison et al. 2013)

Preclinical research in animal models

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

Extrapolation from animal models to the human

			
Embryonic genome activation	4- to 8-cell stage	2-cell stage	8-cell stage
1st Lineage 2nd Lineage	? ?	Hippo signalling FGF4	? FGF4 and others
Implantation	Day 6 Invasion hCG Spontaneous decidualization	E4.5 Encapsulation LH	Day 15 Adhesion LH
Pregnancy	9 months	21 days	9 months

Preclinical research with human gametes and embryos donated to research

- Limited investigation on human gametes and embryos
  - → Scarcity of materials
  - → Legal aspects
  - → Ethical aspects
- Why?

Preclinical research with human gametes and embryos donated to research

- Limited investigation on human gametes and embryos
  - → Scarcity of materials
  - → Legal aspects
  - → Ethical aspects
- Why?
  - → Embryo > healthy child

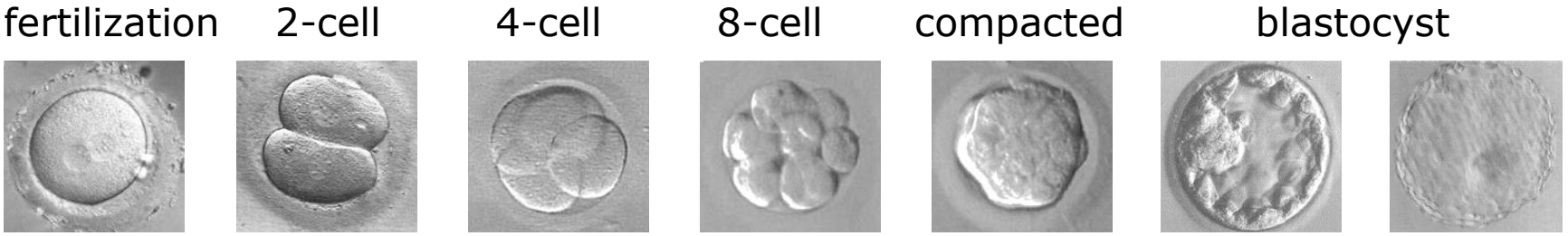
Preclinical research with human gametes and embryos donated to research

- Reproductive biology
  - → Basic knowledge
    - Preimplantation development
    - Implantation

- Reproductive medicine
  - → Infertility treatment
  - → Efficacy and safety

- Stem cell biology
  - → Model early embryogenesis
  - → Transplantation therapy
  - → Infertility treatment: germ cell differentiation
  - → Cancer

Human preimplantation embryo development

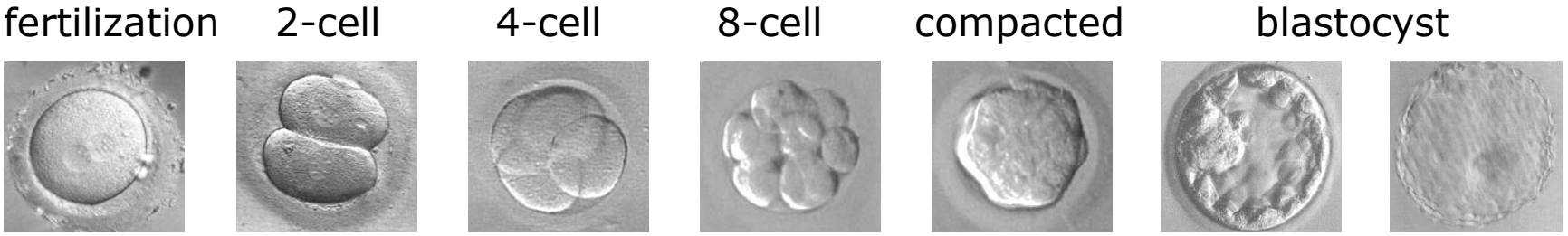


Epigenetic modifications

Embryonic genome activation

Differentiation
ICM vs TE
EPI vs PrE

Human preimplantation embryo development



Epigenetic modifications

Sperm cell factor
Mitochondria transfer
Gene editing

Embryonic genome activation

Aneuploidy
Mosaicism

Differentiation
ICM vs TE
EPI vs PrE

Signalling pathways
hESC derivation

Research on human embryos at the VUB

- Belgian law May 2003: research on human embryos *in vitro*
  - → Permission Local Ethical Committee
  - → Permission Federal Ethical Committee
- Brochure and informed consent

MEDICALLY ASSISTED PROCREATION

SCIENTIFIC RESEARCH

using human gametes and/or embryos

Centrum voor Reproductieve Geneeskunde

Advances in fertility medicine owe a great deal to the scientific research that is constantly taking place in this area. This would not be possible, however, without the help of patients who are willing to donate their tissues. This brochure explains more about the various research projects at UZ Brussel and your rights as a (participating) patient.

Universitair Ziekenhuis Brussel

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

Centrum voor Reproductieve Geneeskunde

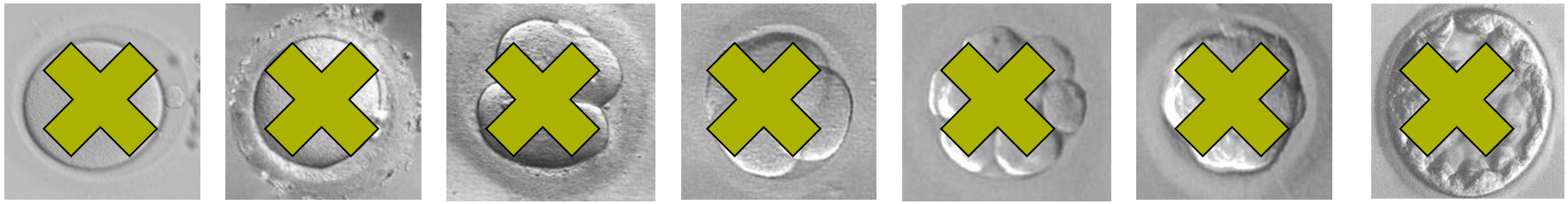
Research on human embryos at the VUB

- Brochure and informed consent
  - → Project and goal
  - → Benefit for science (reproduction and/or disease)
  - → No alternative research methodology
  - → No commercialization (no patents)
  - → No eugenetics
  - → No reproductive cloning
  - → Donor autonomy and privacy are respected
  - → Embryos are ultimately destroyed (not transferred)
    - *In vitro* development until day 14
  - → Particular permission: create embryos for research
    - Egg bank donors
    - One sperm donor

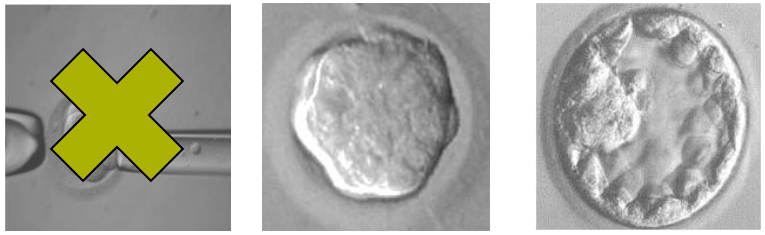
Research on human embryos at the VUB

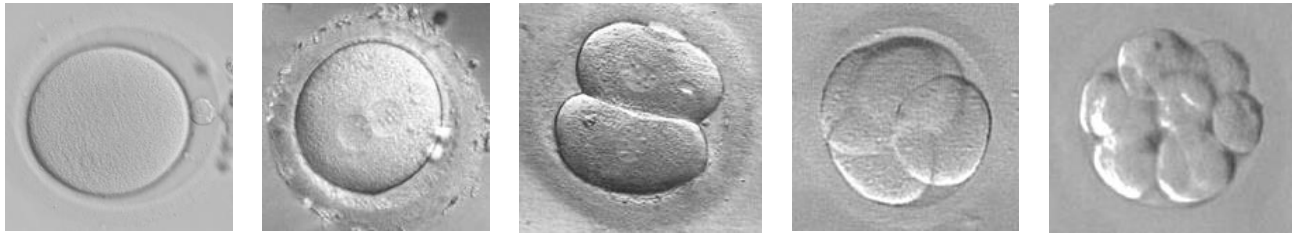
- Fresh embryos day 1 - day 7 (up to day 14)

Day 0 | Day 1 | Day 1,5 | Day 2 | Day 3 | Day 4 | Day 5



PGD: Genetically affected
(-1 or -2 cells, delayed)





Created for research
(egg bank/sperm bank)

Research on human embryos at the VUB

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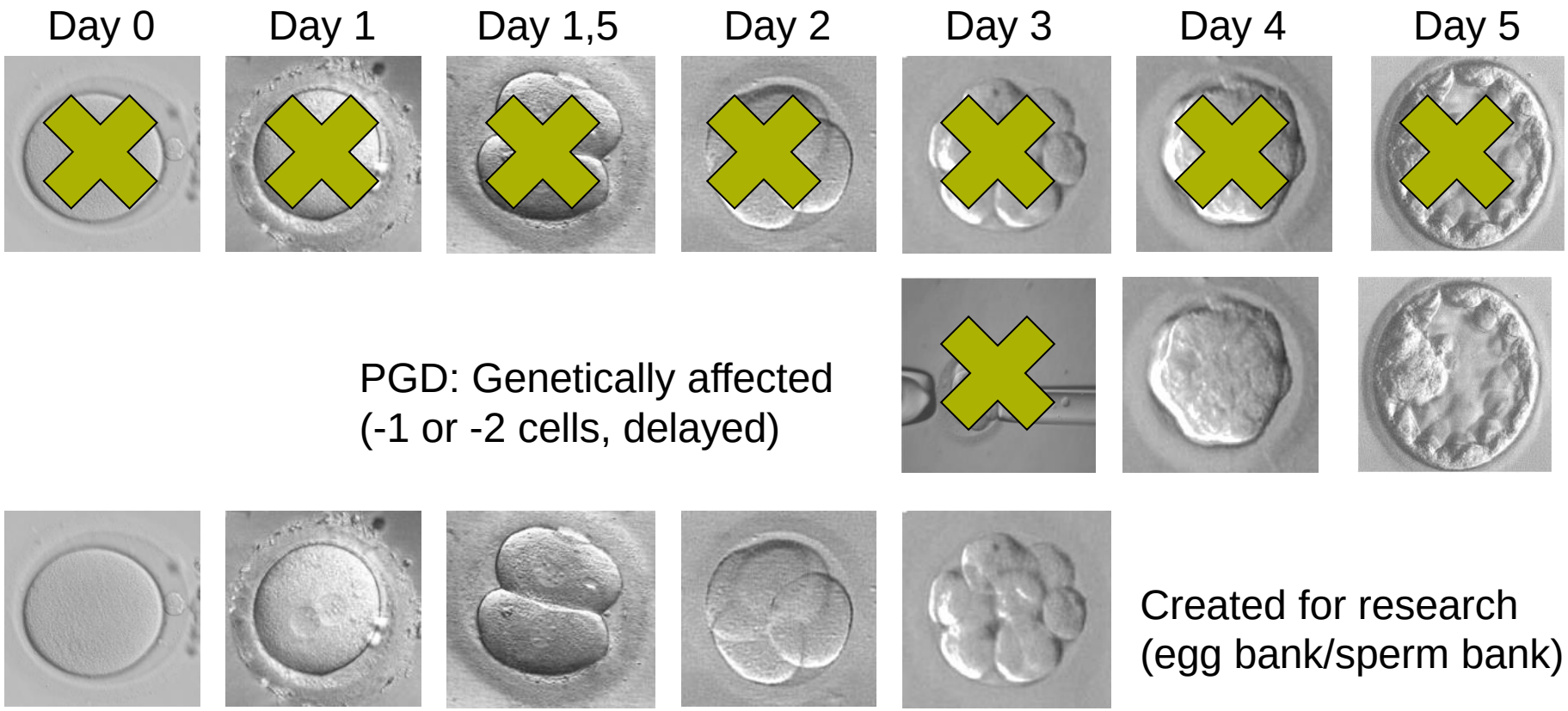
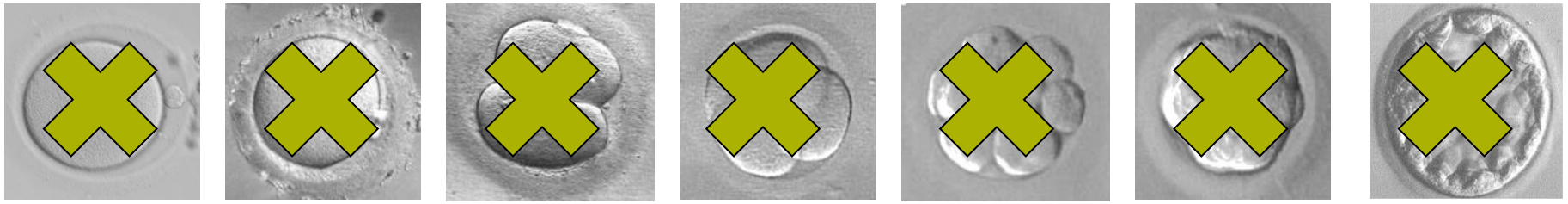


- Cryopreserved embryos (day 3 and day 5/6)

Research on human embryos at the VUB

- Fresh embryos day 1 - day 7 (up to day 14)

Day 0 | Day 1 | Day 1,5 | Day 2 | Day 3 | Day 4 | Day 5



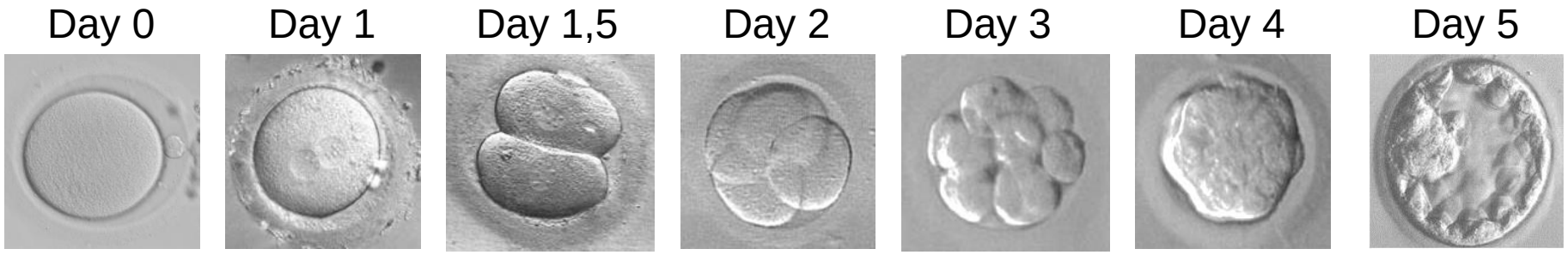
Created for research
(egg bank/sperm bank)

- Cryopreserved embryos (day 3 and day 5/6)

Research on human embryos at the VUB

- Embryos indended for reproduction (day 5/6)
  - → Poor quality fresh non-PGD (day 3 and day 6)
    - Setting up experiments
  - → Good quality fresh affected PGD (day 6)
    - Descriptive studies
    - hESC derivation
  - → Good quality cryopreserved non-PGD and PGD (day 3 and day 5/6)
    - Functional studies
    - hESC derivation
- Embryos intended for research (day 1- day 5)
  - → Good quality fresh
    - Manipulations
    - Descriptive studies
    - hESC derivation

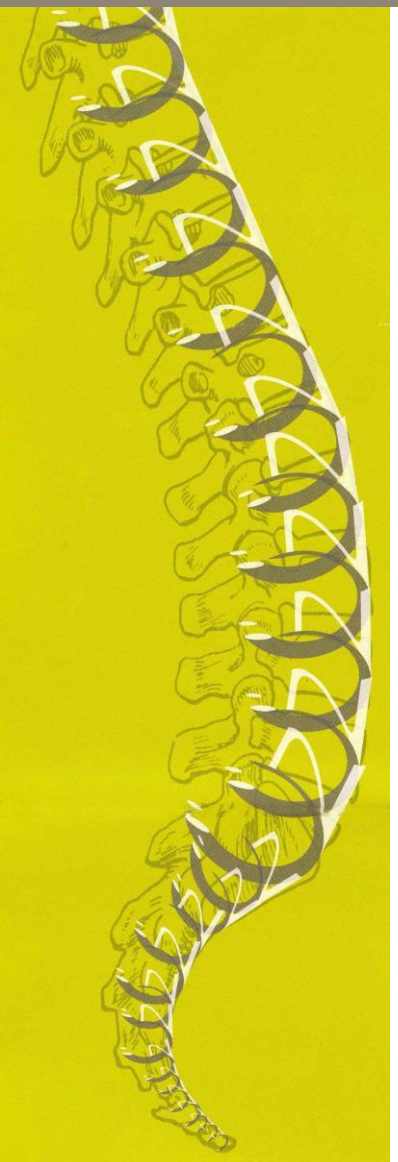
Research on human embryos at the VUB



Totipotency and differentiation (De Paepe and Krivega et al. 2014)

- Cell cycle features (Krivega et al. 2015)
- Molecular determinants (Cauffman et al. 2006, 2009; Verloes et al. 2011; Krivega et al. 2013)
- Embryonic genome activation (Cauffman et al. 2005)
- Signalling pathways (Krivega et al. 2014)
- Epigenetic modifications (Petrussa et al. 2014; De Munck and Petrussa et al. 2015)
- Implantation (Aberkane; Essahib)

Outline



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Sperm cell factor

- Vitality – motility
  - → Spermobile (Ebner et al. 2013)

- Fertilization failure
  - → PLCzeta (Swann and Lai, 1997)
  - → Activation of the oocyte
    - Ionophore (Ebner et al. 2015; Kim et al. 2015)
  - → Safety?
    - Miscarriage rate
    - Minor and major congenital malformations
    - Epigenetic modifications
  - → A plea for caution and more research in the ‘experimental’ use (van Blerkom et al. 2015)

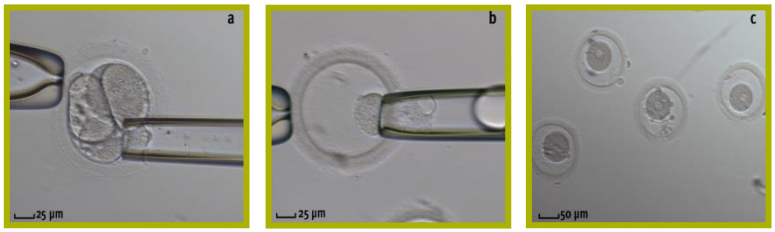
Sperm cell factor

- Embryo development
  - → Maternal factors
    - mRNA and proteins
    - Embryonic genome activation (EGA)
      - Major wave 4- to 8-cell stage (Braude et al. 1998; Dobson et al. 2004; Cauffman et al. 2005; Wong et al. 2010)
      - Minor waves (Zhang et al. 2009; Vassena et al. 2010)
  - → Paternal factors
    - DNA and protamines
    - Histones (Hammoud et al. 2009)
    - mRNA (Miller et al. 2011; Hamatani et al. 2012; Neff et al. 2014)
    - Proteins (Amaral et al. 2014; Azpiazu et al. 2014)
    - MicroRNA (Abu-Halima et al. 2014 ; Pantano et al. 2015; Yao et al. 2015)

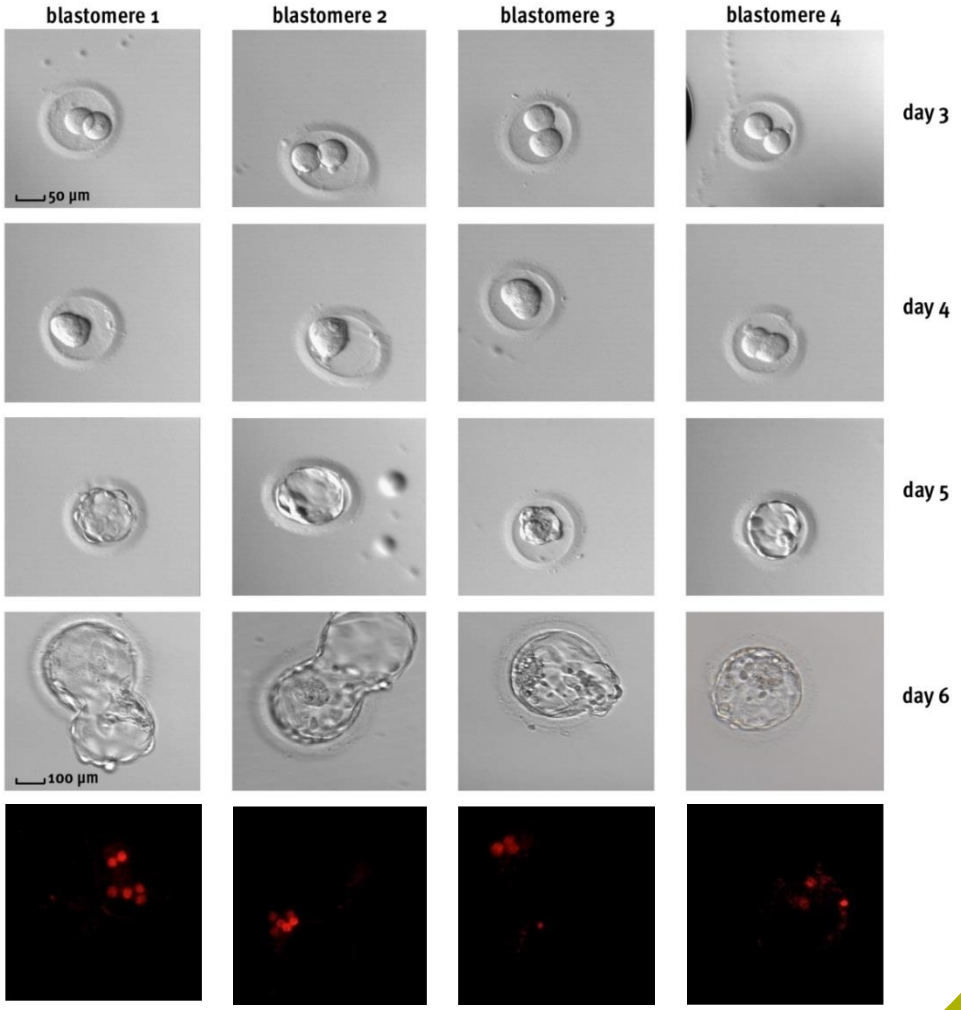
Sperm cell factor

- Poor embryo development
  - → < day 3
    - Oocyte problem → donor oocytes
    - And/or sperm problem
  - → > day 3
    - Sperm cell factor → donor sperm
    - And/or oocyte problem
  - → Couple prefers own genetic offspring
    - Personalized medicine
    - Fundamental research is required

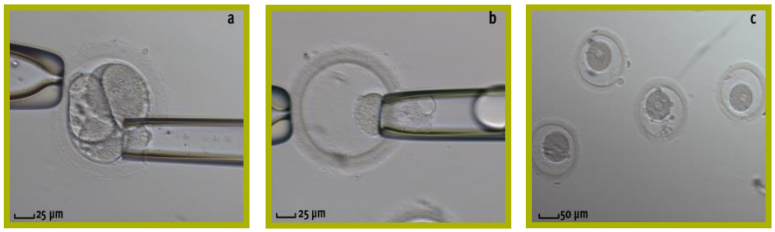
Totipotency and lineage segregation



4-cell stage blastomeres are plenipotent
(Van de Velde et al. 2008)



Totipotency and lineage segregation



4-cell stage blastomeres are plenipotent
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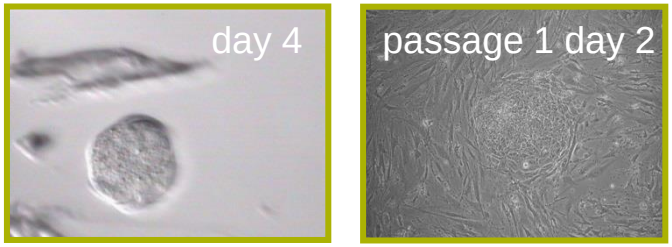
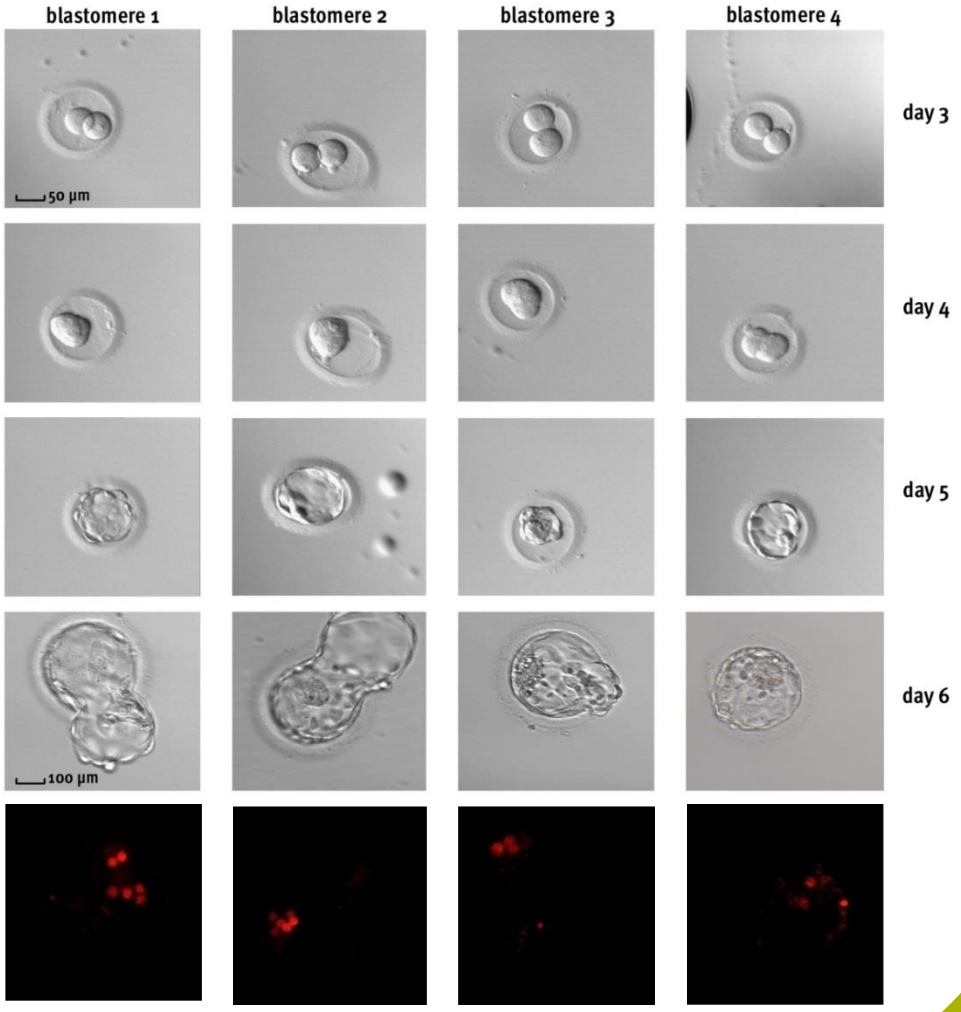


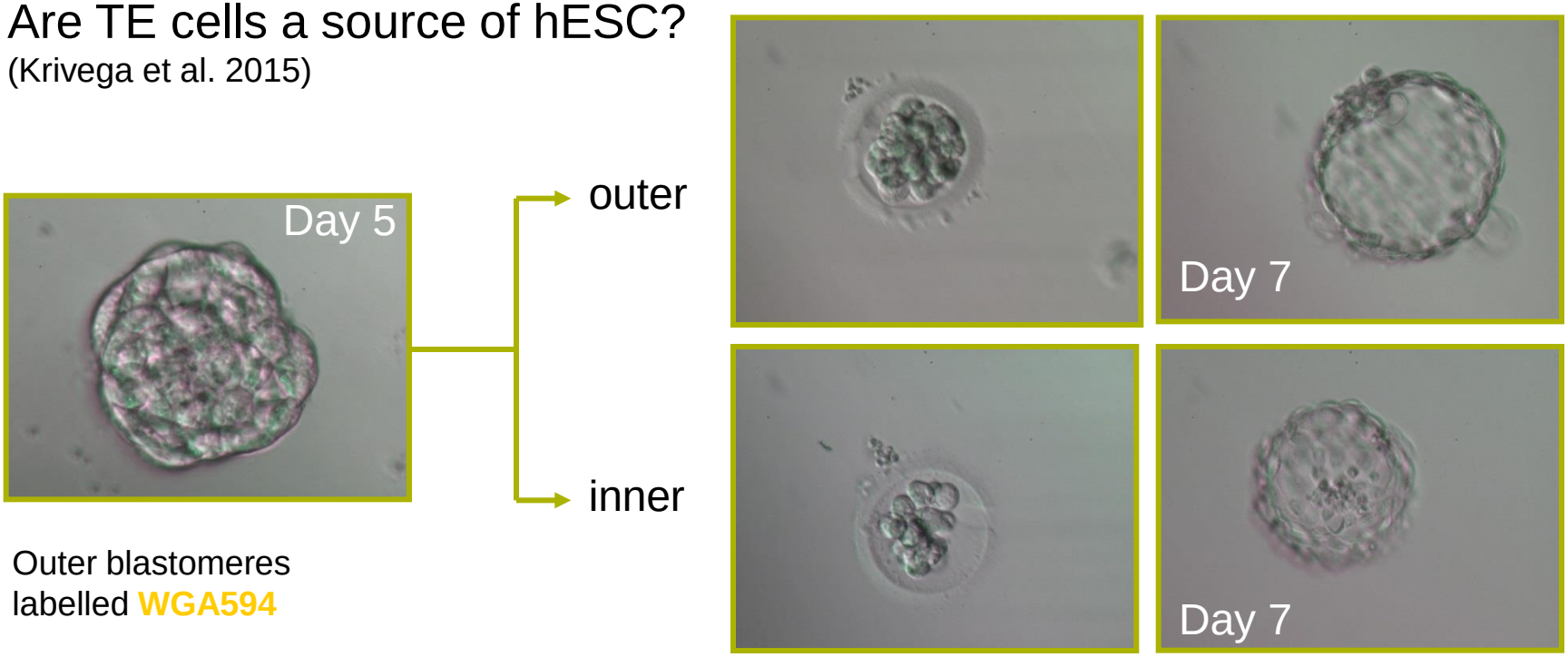
hESC derivation without embryo destruction (Geens et al. 2009)

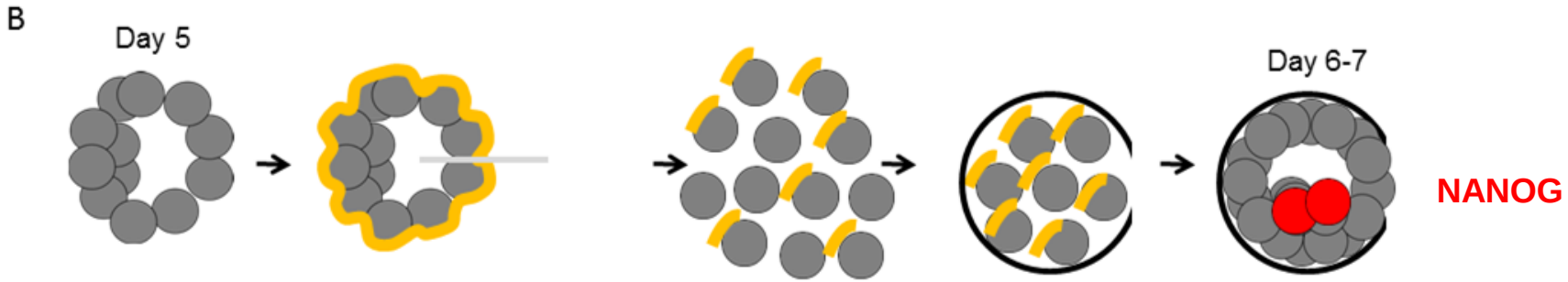


Totipotency and lineage segregation

- Full blastocyst TE cells are not committed (De Paepe et al. 2013)

Are TE cells a source of hESC?
(Krivega et al. 2015)





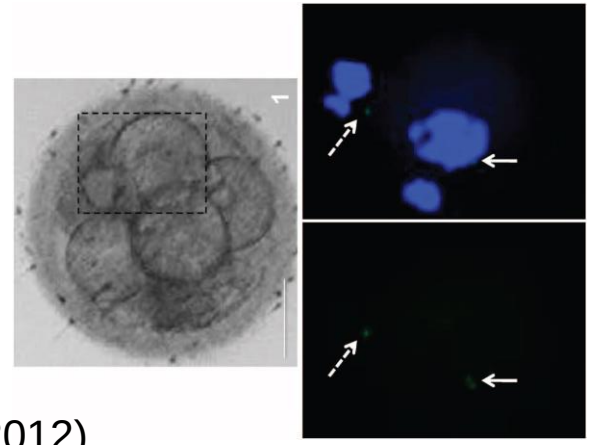
Aneuploidy

- 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

  - → Origin?

Aneuploidy

- Aneuploidy and mosaicism
  - → Less at blastocyst stage
  - → Self-correction?
    - Preferential growth euploid cells?
    - Preferential allocation euploid cells to ICM?
    - Elimination of aneuploid cells during compaction?
- Solving the problem without knowing the cause
  - →PGS and CCH
  - →RCTs (Scott et al. 2013)
    - Multiple pregnancy rate ↓
    - Time to pregnancy ↓
- Cause?
  - →Fragments with micronuclei (Chavez et al. 2012)



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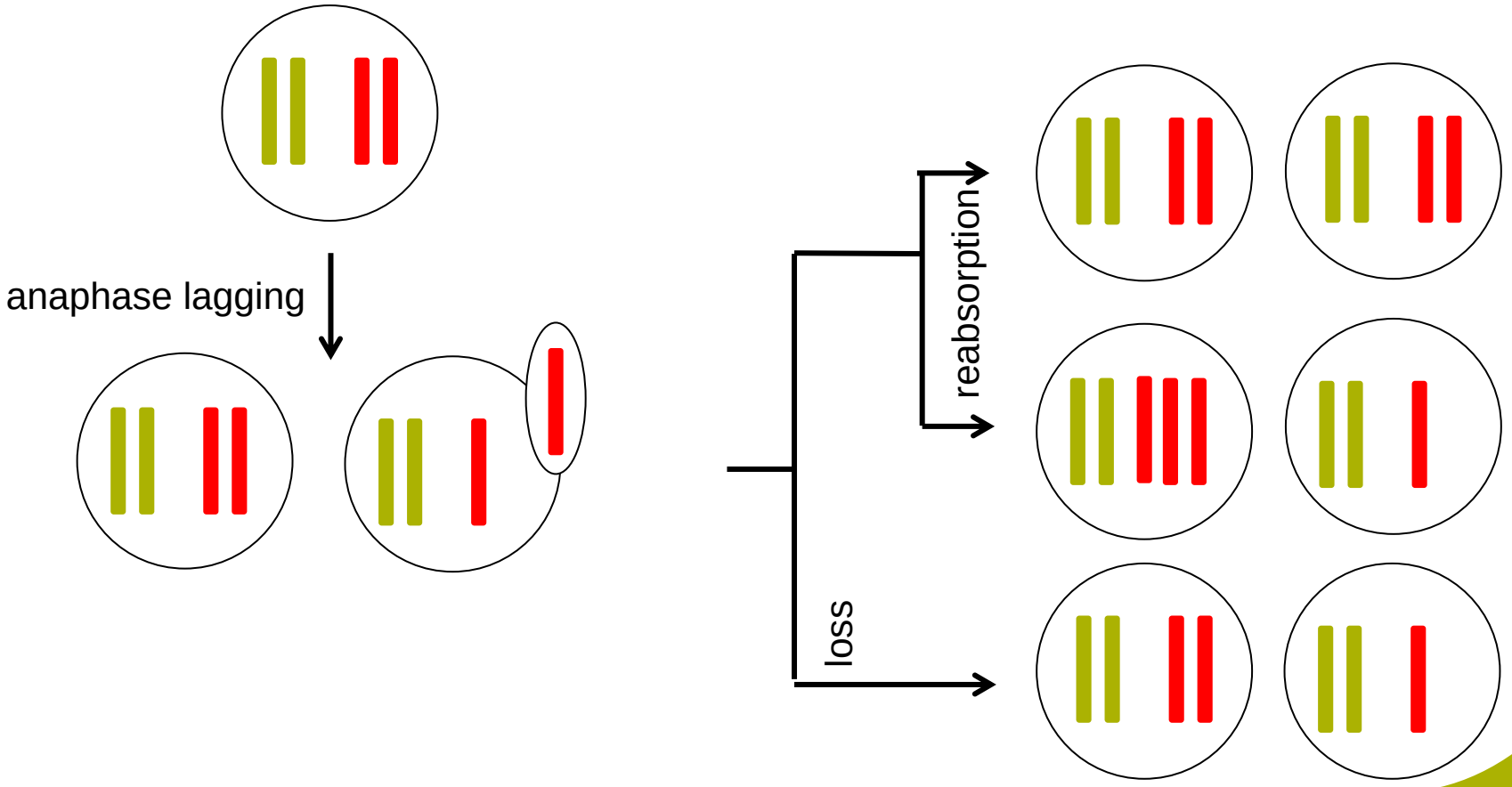


Epigenetic modifications

- Culture medium
  - → Effect on the birth weight of ART singletons
    - Yes (Dumoulin et al. 2010; Nelissen et al. 2012, 2013; Eskild et al. 2013; Hassani et al. 2013; Kleijkers et al. 2014)
    - No (Carrasco et al. 2013; Lin et al. 2013; De Vos et al. 2014)
  - → Confounding factors? BMI mother

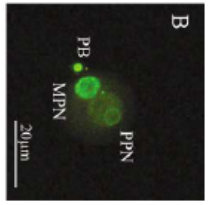
- Embryo cryopreservation (Pinborg et al. 2014)
  - → Singletons are large for gestational age
  - → Adjusted for BMI mother

- Research on embryos created for research
  - → Oocyte vitrification (De Munck et al. 2013; De Munck et al. 2015)
  - → IVM (Spitz et al. 2015)

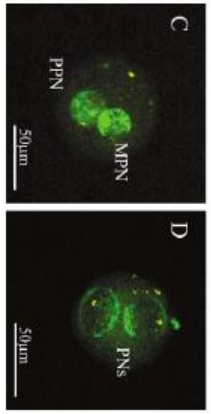
Epigenetic modifications

- DNA methylation after fertilization
  - → Mouse (Dean *et al.,* 2001; Santos *et al.,* 2002; Seisenberger *et al.,* 2013)
    - Global DNA demethylation after fertilization
    - Global DNA remethylation peri-implantation
    - Asymmetry in zygotes ♀ > ♂
    - Difference ICM > TE
  - → Human ≠ mouse (Fulka et al. 2004; Petrussa unpublished data)
    - No asymmetry in zygotes
    - DNA methylation/hydroxymethylation day1-day7
      - No global DNA remethylation at day 7
      - No difference ICM vs TE
      - Pattern disturbed in poor quality embryos

mouse

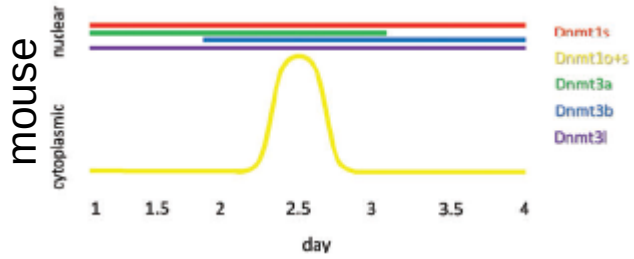


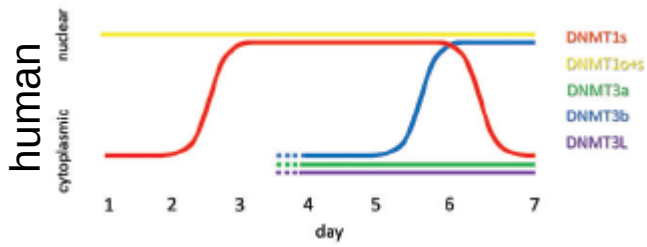
human



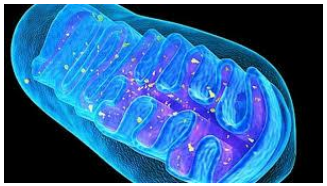
Epigenetic modifications

- Expression of DNA methyltransferases (DNMTs) during human preimplantation development (Huntriss et al. 2004; Petrussa et al. 2014)
  - → Human ≠ mouse
  - → Pattern disturbed in cryopreserved human embryos





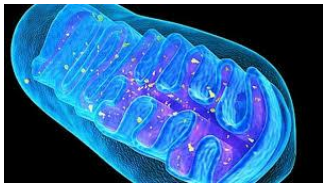
Germ line therapy



- Iuvenate oocytes/embryos by transfering mitochondria
- Animal models (Li et al. 2005; Hua et al. 2007; Cheng et al. 2009)
- Poor prognosis patients
  - → Cytoplasmic transfer from young donor eggs (Cohen et al. 1998)
    - 50 babies
    - Third parent (Levy et al. 2004; St John et al. 2010)
      - Ethical concerns
  - → Autologous mitochondria transfer
    - Granulosa cells (Tzeng et al. 2001)
    - Oogonial stem cells in ovarian cortex

      (Johnson et al. 2004; Kerr et al; 2009; Zou et al. 2009; White et al. 2012)
      - Source of young mitochondria
      - Safety (AUGMENT)? (Amato et al. 2014; Fakih et al. 2015)

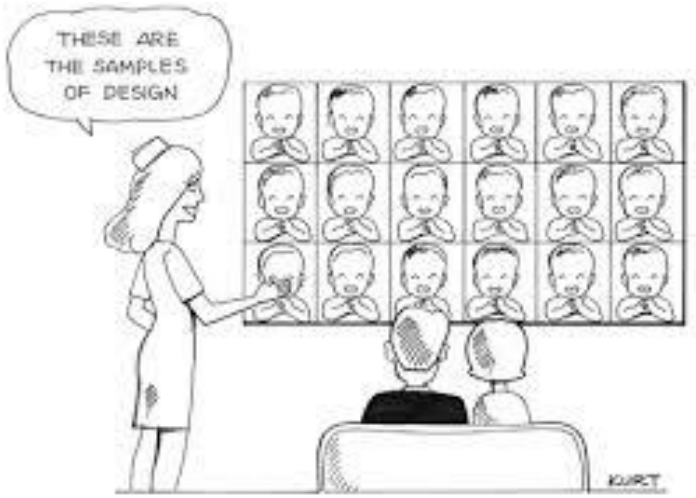
Germ line therapy



- Avoid mitochondrial diseases
  - → 1/5000
  - → Heart stroke, dementia, blindness, deafness
  - → Conventional PGD (Steffann et al. 2006)
    - Heteroplasmy: difficult to predict mutation load
    - Future generation still at risk → transfer boys
  - → Animal models
    - Pronuclear transfer (Newcastle): PNT (Craven et al. 2010)
    - Maternal spindle transfer (Oregon): MST (Tachibana et al. 2009)
  - → UK

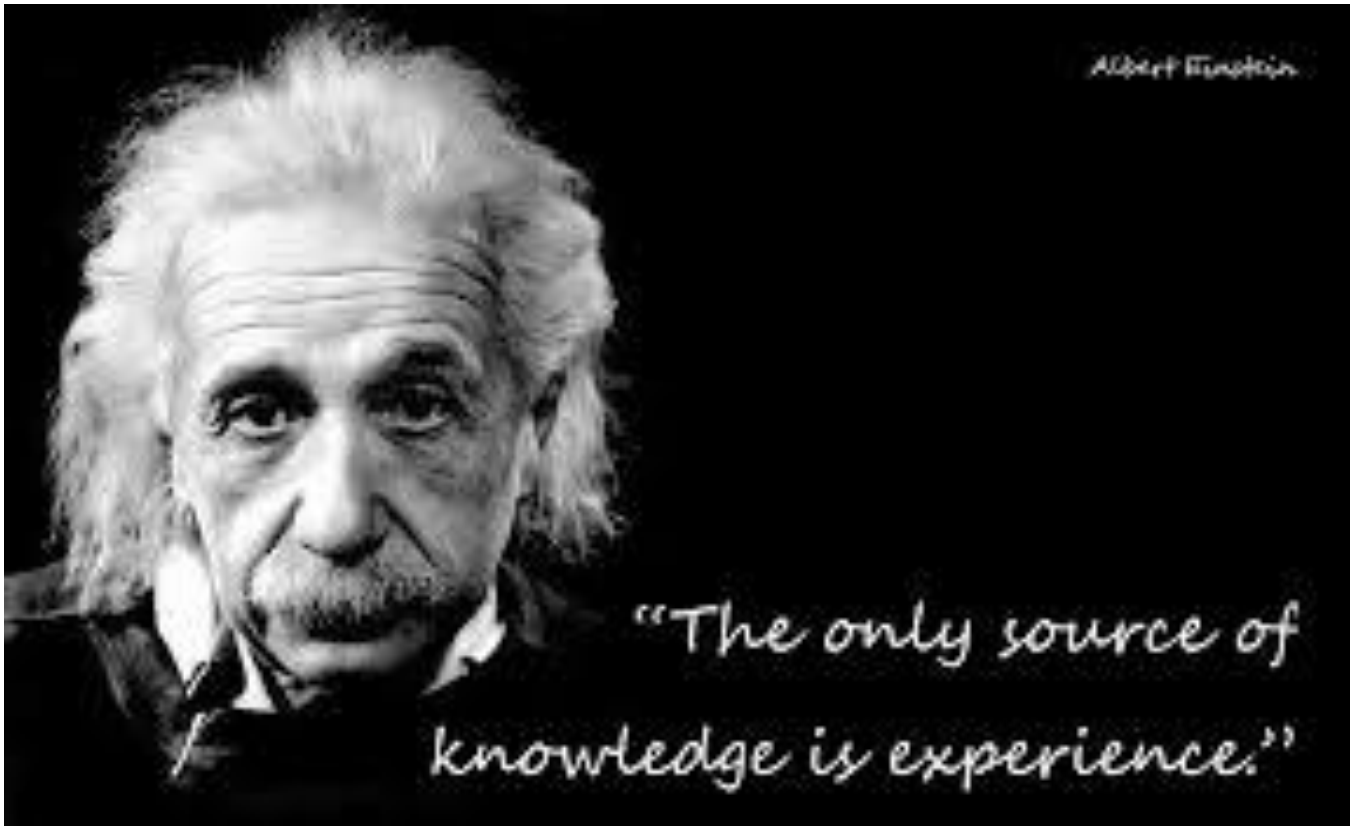
Gene editing

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



- Replace PGD only in very rare cases
- KO human embryos for basic research
  - → Study key mediators early embryogenesis

Conclusion


Albert Einstein
"The only source of knowledge is experience."

Thanks


Caroline De Paepe


Maria krivega


Greet Cauffman


Johan Sterckx


An Verloes


Wafaa Essahib


Asma Aberkane


Shari Mackens


Neelke De Munck


Laetitia Petrussa


Martine De Rycke


Mieke Geens


Ileana Mateizel


Karen Sermon


Inge liebaers

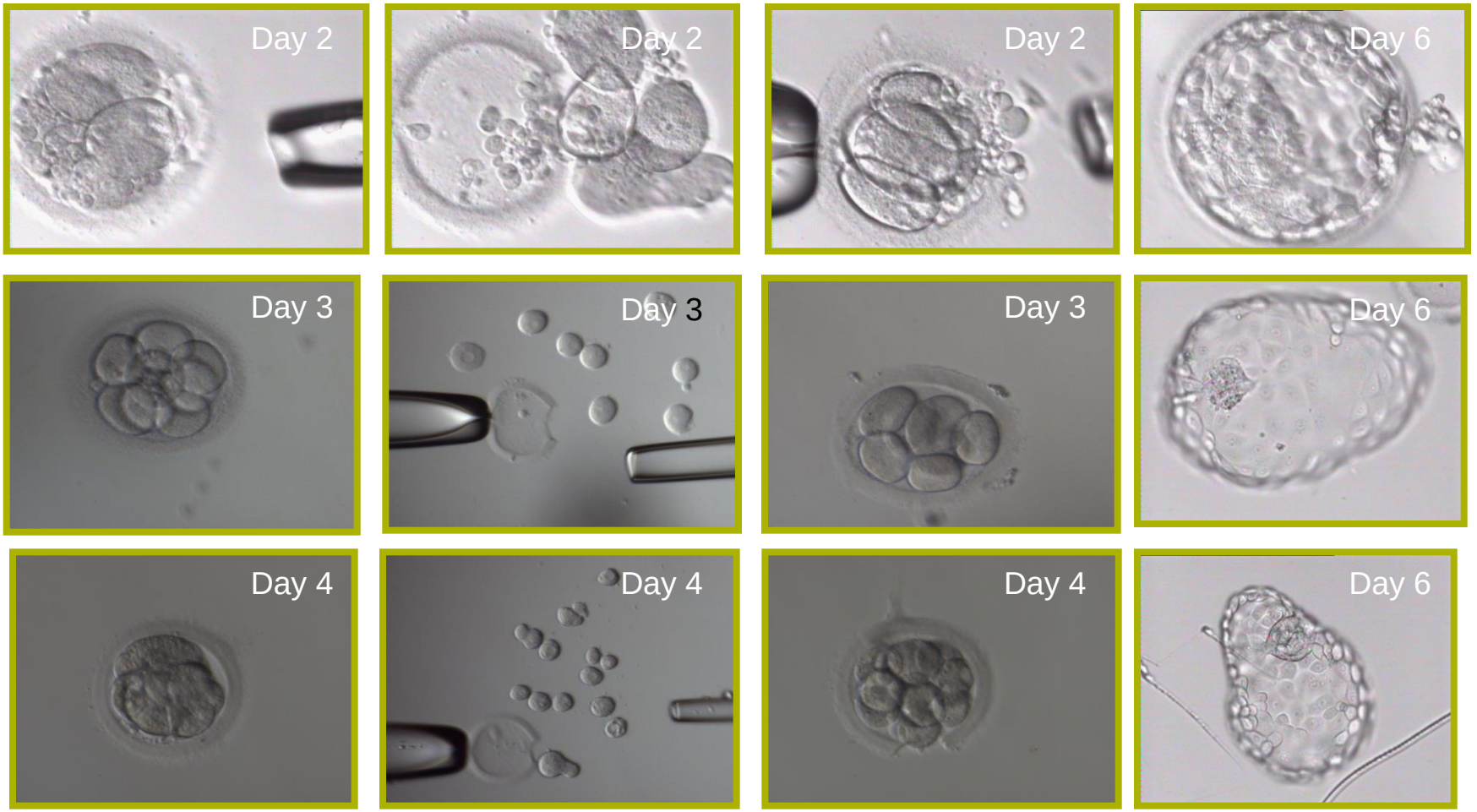
Research on human embryos worldwide

- Ethical and legal restrictions
- Scarcity of materials
- Investigated embryos cannot be transferred

- *In vitro* development into blastocysts
  - → Descriptive
    - Immunocytochemistry (protein)
    - qPCR (mRNA) (Wong et al. 2010; Kleijkers et al. 2015; Blakely et al. 2015)
  - → Functional proof of evidence is lacking
    - One with genetic modifications (Liang et al. 2015)
    - Few with small molecules inhibiting signalling pathways (Kuijk et al. 2012; Roode et al. 2012; Krivega et al. 2015)
    - None with growth factors

Totipotency and lineage segregation

- Change position blastomeres (micromanipulation)



Regulative development

- Change position blastomeres (micromanipulation) (8/8)