PRE-CONGRESS COURSE 11

Responsible innovation in medically assisted reproduction.

Special Interest Groups Ethics and Law
& Safety and Quality in ART
London - UK, 7 July 2013

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Responsible innovation in medically assisted reproduction

London, United Kingdom
7 July 2013

Organised by
The ESHRE Special Interest Groups Ethics and Law & Safety and Quality in ART

Contents

Course coordinators

Wybo Dondorp (The Netherlands), Guido De Wert (The Netherlands), Petra De Sutter (Belgium) and Willianne Nelen (The Netherlands)

Aim

To present an overview of issues, challenges and responsibilities relevant to the safety and quality of innovations in medically assisted reproduction

Background

Also in the light of reported subtle health effects in children born with the help of reproductive technologies, there is a growing awareness in the field that safety as a crucial aspect of innovation needs more systematic attention than it has sometimes received in the past. Relevant questions include (but are not limited to) the relation between practice adaption and research, the usefulness of research in animal models and embryos, the importance of registries and follow-up studies, the level of risk that should be regarded as acceptable and how the various stakeholders (including ESHRE) can contribute to making the field more accountable in this regard.

Target audience

Congress participants who as researchers, embryologists or clinicians have a professional interest in the safety and quality of new reproductive technologies, participants with a general interest in the ethics of assisted reproduction, policy makers, etc.

Scientific programme

Chairman: Willianne Nelen - The Netherlands

09:00 - 09:30	Possible subtle adverse health outcomes of medically assisted reproduction (MAR): a review *Arianna D'Angelo - United Kingdom*
09:30 - 09:45	Discussion
09:45 - 10:15	Innovation in MAR: lessons from the past (ICSI), challenges for the present (oocyte vitrification), perspectives on the future (artificial gametes) *Heidi Mertes - Belgium*
10:15 - 10:30	Discussion
10:30 - 11:00	Coffee break
11:00 - 11:30	What animal research may contribute to safety and quality in MAR? *Thomas D'Hooghe - Belgium*
11:30 - 11:45	Discussion
11:45 - 12:15	In vitro-maturation of oocytes: is there a case for preclinical embryo research? *Guido De Wert - The Netherlands*
12:15 - 12:30	Discussion
12:30 - 13:30	Lunch *Chairman: Veerle Provoost - Belgium*
13:30 - 14:00	When should practice adaptation be research? The case of culture media in IVF *John Dumoulin - The Netherlands*
14:00 - 14:15	Discussion
14:15 - 14:45	The health of the future child: what level of risk is acceptable in MAR? *Nils-Eric Sahlin - Sweden*
14:45 - 15:00	Discussion
15:00 - 15:30	Coffee break
15:30 - 16:00	Registries and follow-up studies: a joint responsibility of the field *Maryse Bonduelle - Belgium*
16:00 - 16:15	Discussion
16:15 - 16:45	Multidisciplinary forum debate aimed at formulating policy conclusions *Petra De Sutter - Belgium*
16:15 - 16:45	Multidisciplinary forum debate aimed at formulating policy conclusions *Wybo J. Dondorp - The Netherlands*
16:45 - 17:00	Discussion

eshre European Society of Human Reproduction and Embryology

Possible subtle adverse health outcomes of medically assisted reproduction (MAR): a review

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Conflict of interest

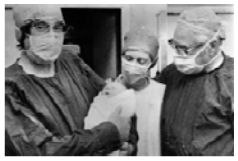
- None

Learning objectives

- Scale of the problem
- Health effects in women undergoing MAR
- Health effects in gametes/ embryos created through MAR
- Health effects in children born with the help of MAR
- Relation between practice adaption and research

Background

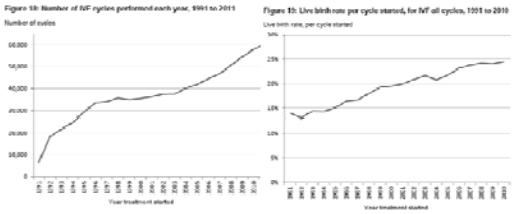
Louise Brown, the world's first "test tube baby", was born in July 1978, and the process has since helped many couples conceive. Robert Edwards, who is credited with developing IVF, won the Nobel Prize in Medicine for his work



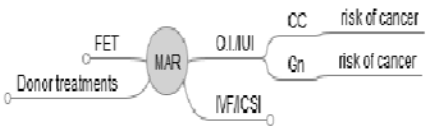
Background

- Since 1978, an estimated 5 million babies have been born worldwide after IVF
- In the UK 201,811 babies have been born between 1991 and 2010
- In the UK, nearly 60,000 cycles of fertility treatments are carried out each year
- The HFEA is the independent regulator of fertility treatment in the UK
- Between 2009 and 2010 the overall LBR/cycle has remained broadly steady, going from 24.1% to 24.5%.
- The overall MBR has declined
- Between 2008 and 2011 more embryos are being transferred at the blastocyst stage

Background



Key points: The number of IVF cycles performed each year has increased steadily since 1991. The age of women seeking fertility treatment has increased since 1991, reflecting the wider trend in society for couples to start their families later, but remained steady over the last 5 years. The live birth rate after IVF has increased from only 14% in 1991, to a quarter by 2010. In 2010, nearly 2% of all the babies born in the UK had been conceived through IVF treatment.

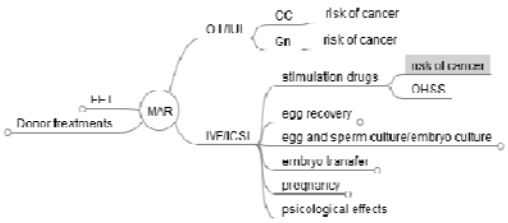


O.I/IUI

- CC and risk of cancer (Hughes E et al. Cochrane review 2010)
- A variety of publications have raised the question of increased ovarian cancer risks associated with clomiphene use (Rossing 1994; Whittemore 1992). The more rigorous of these studies (Rossing 1994) suggests that in women taking clomiphene for more than 12 cycles the incidence of invasive epithelial cancer increases approximately three-fold.

Gonadotropins

- Hilliard TS et al (2013) showed that pathways activated by FSH and LH in normal ovarian surface epithelium (OSE) grow in their microenvironment
- Gonadotropins increased proliferation in both three-dimensional (3D) ovarian organ culture and in a two-dimensional (2D) normal mouse cell line
- These data suggest that the Gn stimulate some of the same proliferative pathways in normal OSE that are activated in ovarian cancers



Risks on mums-to-be

Risk factors for ovarian cancer

- 3 epidemiological studies showed increased risks of ovarian cancer among women treated with fertility drugs, particularly nulliparous women and those who had used fertility drugs long term
- Two theories are usually used to explain how fertility drugs might affect the risk of ovarian cancer:
  - The incessant ovulation theory suggests that repeated, uninterrupted ovulation causes microtrauma to the ovarian epithelium, leading to malignant transformations.
  - The gonadotrophin theory suggests that exposure of the ovaries to endogenous or exogenous gonadotrophins is directly carcinogenic.

Whittemore et al. (1992); Rossing et al. (1992); Shushan et al. (1996)

Danish study (2009)

Jensen et al.

- Design: Population based cohort study
- Population: 54 362 women with infertility problems referred to all Danish fertility clinics during 1963-98.
- Main outcome measure: Effect of four groups of fertility drugs (Gn, CC, hCG and GnRH-a) on overall risk of ovarian cancer after adjustment for potential confounding factors.

Danish study (2009)

Jensen et al.

Results:

- No overall increased risk of ovarian cancer after any use of Gn (rate ratio 0.83, 95% confidence interval 0.50 to 1.37), CC (1.14, 0.79 to 1.64), hCG (0.89, 0.62 to 1.29), or GnRH-a (0.80, 0.42 to 1.51).
- No associations were found between all four fertility drugs and number of cycles of use, length of follow-up, or parity
- Analysis according to histological subtype showed 67% increased risk for serous ovarian cancer after use of CC primarily among women followed for 15 or more years

Danish study (2009)

Jensen et al.

- Limitation: Even though the follow-up period was long, the median age at the end of follow-up (47 years) was below the usual peak age (early 60s) for ovarian cancer.

Risk factors for breast cancer

- The aetiology of breast cancer is multifactorial where both endogenous and exogenous hormones have an important role.
- Nulliparity, late age at first birth, early menarche, and late menopause
- Only a limited number of epidemiologic studies have examined the possible association between use of fertility drugs and risk of breast cancer.
- Results, thus far, have been contradicting, as most studies found no association

Danish study (2007)

Jensen et al.

- Design: Population based cohort study
- Population: A cohort of 54,362 women with infertility problems referred to all Danish fertility clinics between 1963 and 1998
- Main outcome measured: effect of five groups of fertility drugs on breast cancer after adjustment for parity status.

Danish study (2007)

Jensen et al.

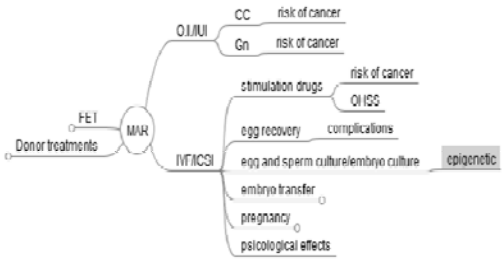
Results:

- 331 invasive breast cancers were identified in the cohort
- Analyses showed no overall increased breast cancer risk after use of Gn, CC, HCG, or GnRH-a
- Progesterone increased breast cancer risk (RR, 3.36; 95% confidence interval, 1.3-8.6).
- No relationships with number of cycles of use or years since first use of fertility drugs were found.
- Gn may have a stronger effect on breast cancer risk among nulliparous women (RR, 1.69; 95% confidence interval, 1.03-2.77).

Are infertility treatments a potential risk factor for cancer development? Perspective of 30 years of F/U

(Liat et al. 2012)

- Cancer development was assessed through linkage with the National Cancer Registry
- Population: 2431 women who were treated for infertility during the period 1964-1974
- Results: 18 ovarian cancer were observed as compared to 18.1 expected (SIR = 1.0; 95% CI = 0.59-1.57). For breast cancer, 153 cases were observed as compared to 131.9 expected (SIR = 1.16; 95% CI = 0.98-1.36), and for endometrial cancer, 30 cases were observed as compared to 17.8 expected cases (SIR = 1.69; 95% CI = 1.14-2.41)
- No excess risk associated with exposure to Gn was observed
- Infertility was found to be associated with significant increased risk for endometrial cancer and borderline increased risk for breast cancer



Epigenetic

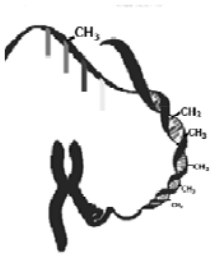
The science of studying changes in the pattern of gene activity, during embryo development and beyond, that do not involve alteration of the DNA sequence. These changes occur in response to conditions within the embryo or more generally

What is epigenetic control important for?

- Genome structure and function: chromosomal organization and maintenance of nuclear identity
- Transcriptional memory control: long term control of developmental processing
- Genomic defence: silencing retroviral elements

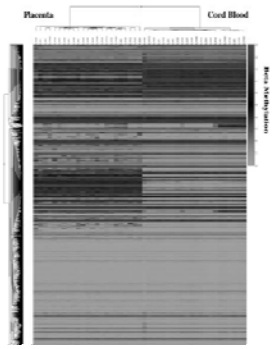
Epigenetic mechanism: DNA methylation

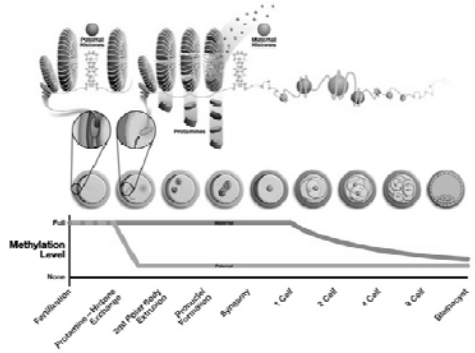
- Process by which genes not needed in certain tissues are 'switched off' by the attachment of molecules known as 'methyl groups' to the DNA.



Katari et al. 2009

- Certain genes in babies conceived following IVF tended to have lower methylation levels in placental tissue and higher methylation levels among umbilical cord blood tissue, compared to babies conceived naturally.
- Epigenetic errors could have implications for susceptibility to cancer and other common diseases later in life

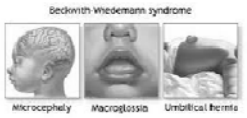




Timothy G. Jenkins, et al. Front Genet. 2012;3:143.

Beckwith-Wiedemann syndrome (BWS)

- Incidence: 1/15000
- Overgrowth Syndrome (>90th percentile)
- Enlarged tongue
- Abdominal wall defects
- Ear creases or ear pits
- Neonatal hypoglycemia
- Predisposition for embryonal tumours / Wilms' tumour



• Caused by genetic or epigenetic defects in an imprinted region on chromosome 11p15

Feinberg et al. 2003

- Reported the first evidence that ART is associated with BWS.
- Prospective study; the prevalence of ART was 4.6% vs the background rate of 0.8% in the United States.
- 7 children with BWS were born after ART
- 5 were conceived after ICSI.
- It is not clear whether these BWS cases were related to the infertility of their parents, or caused by something in the IVF process itself

Angelman Syndrome

- Incidence 1/10.000-30.000
- Primary developmental and neurologic disorder

- severe mental retardation
- ataxia
- "happy puppet syndrome"
- absence of speech

- Caused by genetic or epigenetic defects in an imprinted region on chr15q11-13

Imprinted genes

Functions imprinted genes

- Foetal growth
- Placental growth or function
- Intra-uterine growth defects

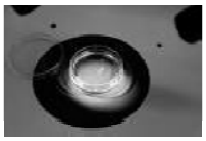
Functions Defects

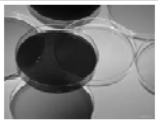
- Postnatal cognition and behaviour
- Brain development
- Abnormal maternal behaviour,
- Impaired memory
- Neurological disorders (autism, schizophrenia, epilepsy, Tourette syndrome)
- Cancers

Aafke van Montfoort PCC ESHRE 2012

Culture medium

- Certain qualities of media used in IVF procedures can affect the presence or absence of activity of certain groups of genes, and whether or not they become 'switched on' during development.





Effect of culture media on birth weight

Dumoulin JC et al. (2010)

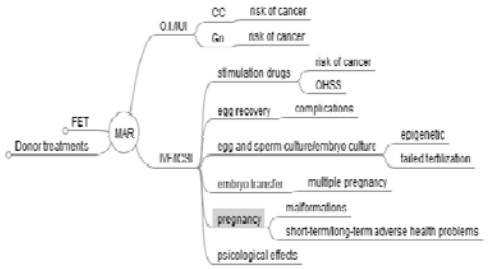
Vitrolife

- 110 live born from IVF treatment cycle
- 3453 + 53 g (*P* =0.003)

Cook

- 78 live born from IVF treatment cycles
- 3208 + 61 g (*P* =0.003)

Multiple linear regression showed that culture medium was significantly associated with birth weight (*P* =0.001)



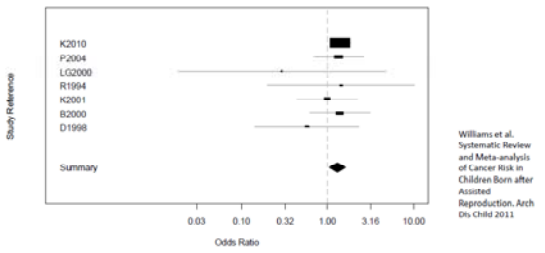
Risks on children

Risk of cancer in children born after ART

Williams et al. (2011)

- Searches of MEDLINE and EMBASE
- All relevant identified studies were reviewed independently by two authors.
- Studies were classified according to design and cohort study quality assessed by two reviewers prior to inclusion in the meta-analysis
- 11 cohort studies were included in the meta-analysis.

Risk of cancer in children born after ART



Possible small increase risk of cancer, further studies are needed.

Neurological and neurodevelopmental risk

- There is probably an increased risk of Cerebral Palsy in singletons conceived after IVF (OR from 1.3 to 1.85) (Lidegaard *et al.* 2005) (Strömberg *et al.* 2002)
- There is also a higher risk of epilepsy (OR=1.83) (Ericson *et al.* 2002)
- There may be an increased risk of Autistic spectrum disorders and ADHD (attention deficit hyperactivity disorder) (Källén *et al. 2011)*

Birth defects

J. Reefhuis et al. (2009)

- In 2009, an analysis of the data of the National Birth Defects Study in the US found that certain birth defects were significantly more common in babies conceived through IVF

- septal heart defects
- cleft lip with or without cleft palate
- esophageal atresia
- anorectal atresia

Table III Adjusted odds ratios for association between ART and birth defects stratified by plurality (National Birth Defects Prevention Study, 1997–2003)

	Singleton[a] AOR (95% CI)	Twins or higher[b] AOR (95% CI)
Anotia/microtia		4.0 (0.7–21.8)
Conotruncal heart defects	1.4 (0.6–3.2)	0.8 (0.3–2.6)
Tetralogy of Fallot	1.6 (0.6–4.3)	
Septal heart defects[c]	2.1 (1.1–4.0)	1.3 (0.6–2.8)
Perimembraneous VSD[c]	1.4 (0.6–3.3)	1.1 (0.4–2.8)
ASD secundum/NOS[c]	3.0 (1.5–6.1)	1.7 (0.7–3.9)
VSD and ASD[c]	2.8 (1.2–7.0)	1.3 (0.3–5.4)
Right outflow tract heart defects		1.0 (0.4–2.9)
Pulmonary valve stenosis		1.0 (0.3–3.1)
Left outflow tract heart defects		1.0 (0.4–2.7)
Coarctation of aorta		1.1 (0.4–3.6)
Cleft lip with or without palate	2.4 (1.2–5.1)	1.3 (0.5–3.4)
Cleft palate	2.2 (1.0–5.1)	1.4 (0.4–4.8)
Esophageal atresia	4.5 (1.9–10.5)	2.2 (0.7–7.3)
Anorectal atresia	3.7 (1.5–9.1)	1.5 (0.4–5.2)
Hypospadias, second or third degree	2.1 (0.9–5.2)	2.1 (0.7–6.4)
Craniosynostosis		2.3 (0.6–9.3)

J. Reefhuis et al. (2009)

Birth defects

Davies et al (2012)

- A new Australian study examined birth defects associated with different types of MAR.
- Gestational age at least 20/40 and birth weight at least 400g
- Comparison of risks of birth defects (before a child's 5th birthday) among pregnancies in women after MAR, spontaneous pregnancies, in women who had a previous birth with assisted conception, pregnancies in women with a record of infertility but no treatment and pregnancies in women with no record of infertility.

Table 3. Odds Ratio for Birth Defects According to Category of Defect and Multiplicity.*

Birth-Defect Category	Singleton Births			
	Assisted Conception (N=4333)	Spontaneous Conception (N=295,220)	Unadjusted Odds Ratio	Adjusted Odds Ratio†
	no. of births (%)			
Any defect	361 (8.3)	16,989 (5.8)	1.48 (1.32–1.65)	1.30 (1.16–1.45)
Multiple defects	95 (2.2)	4,690 (1.6)	1.38 (1.13–1.70)	1.24 (1.00–1.54)
Congenital abnormalities: ICD-9 codes 740–759	335 (7.7)	15,372 (5.2)	1.52 (1.35–1.70)	1.32 (1.17–1.48)
Cardiovascular abnormalities: BPA codes 74500–74799	78 (1.8)	3,472 (1.2)	1.54 (1.22–1.93)	1.36 (1.08–1.72)
Musculoskeletal abnormalities: BPA codes 75400–75699	130 (3.0)	4,776 (1.6)	1.87 (1.57–2.24)	1.50 (1.24–1.80)
Urogenital abnormalities: BPA codes 75200–75399	95 (2.2)	4,872 (1.7)	1.34 (1.09–1.65)	1.25 (1.01–1.55)
Gastrointestinal abnormalities: BPA codes 74900–75199	34 (0.8)	1,832 (0.6)	1.26 (0.89–1.78)	1.18 (0.83–1.68)
Central nervous system abnormalities: BPA codes 74000–74299	22 (0.5)	1,104 (0.4)	1.37 (0.89–2.09)	1.34 (0.86–2.07)
Respiratory abnormalities: BPA codes 74800–74899	3 (0.1)	455 (0.2)	0.41 (0.12–1.40)	0.36 (0.11–1.18)
Chromosomal abnormalities: BPA codes 75800–75899	23 (0.5)	1,088 (0.4)	1.43 (0.94–2.17)	0.87 (0.57–1.33)
Metabolic abnormalities: BPA codes 24390–27790	3 (0.1)	379 (0.1)	0.59 (0.19–1.79)	0.53 (0.16–1.74)
Hematologic abnormalities: BPA codes 28200–28699	5 (0.1)	225 (0.1)	1.38 (0.56–3.35)	1.61 (0.61–4.23)

Davies et al (2012)

Birth defects

Davies et al (2012)

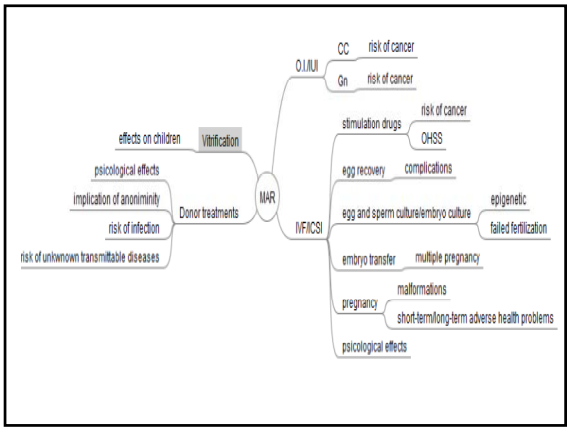
Conclusions:

- The increased risk of birth defects associated with IVF was no longer significant after adjustment for parental factors.
- The risk of birth defects associated with ICSI remained increased after multivariate adjustment, although the possibility of residual confounding cannot be excluded.

IMSI and gender association

A higher proportion of morphologically normal spermatozoa carries the X chromosome

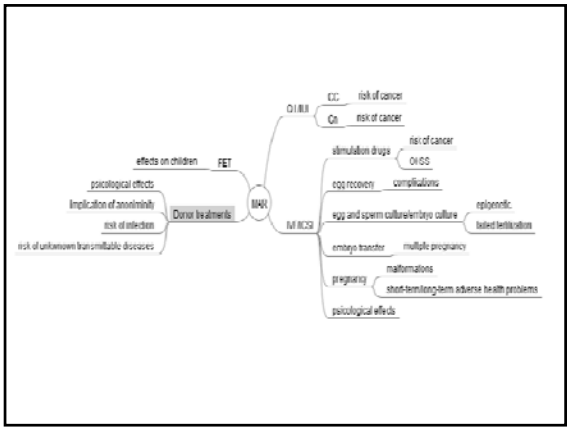
Setti et al. (2012)



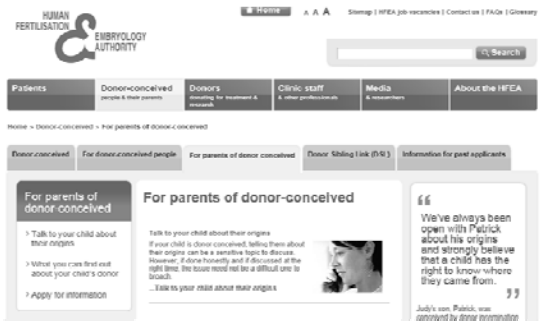
Perinatal and neonatal outcomes after Vitrification

Shi W. et al (2012)

Compared with fresh ETs, vitrified day 3 ET shows no significant differences in obstetrical and neonatal outcomes



Anonymity (HFEA)



What do the experts say…..

- Prof Nygren said,
  "…….if we ask is IVF safe, then the real answer must be 'safe enough from what we know'……
- "In some cases higher risks may be due to the IVF techniques themselves. But we suspect that in many cases the greater risks are due to the fact that people who come for IVF already have difficulties in reproducing, and so by definition, reproduction is more difficult for them. "

Take-home messages

- Counselling patients
- Continue research
- Linking databases
- Data collection and monitoring registries (EIM/HFEA/PGD consortium…)

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Innovation in MAR:
lessons from the past, challenges for the present, perspectives on the future

ICSI, oocyte vitrification & artificial gametes

Heidi Mertes, PhD
Bioethics Institute Ghent, Ghent University

29th annual meeting ESHRE
London, 7-10 July 2013

Bioethics Institute Ghent

Conflicts of interest

none

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Learning objectives

At the conclusion of this presentation, participants should be able to:

- Understand the ethical challenges of innovation in MAR
- Describe the issues involved in the introduction of IVF, ICSI, oocyte vitrification and artificial gametes into the clinic

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Evidence Based (reproductive) Medicine

- Current paradigm: Evidence Based Medicine (EBM):

  "Evidence based medicine is the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients. The practice of EBM means integrating individual clinical expertise with the best available external clinical evidence from systematic research." (Sackett et al, 1996)

- Gold standard to establish both efficiency and safety: prospective (double blind) RCTs + systematic reviews, meta-analyses,…

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Responsible innovation

Innovative procedures *per definition* lack a firm base of evidence.

How to deal with this?

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Responsible innovation

- Clinical research should be **preceded by pre-clinical research** in animals, cell cultures, embryos (Dondorp & de Wert, 2011)
- Innovative treatments should be **embedded in clinical research protocols** (with ethics committee oversight)
- **Safeguards** should be installed for early diagnosis of adverse outcomes (PGD, amniocentesis,…) and **follow up care** should be provided when needed

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Responsible innovation

- Even after all these steps, risks remain, but these are considered acceptable if outweighed by the expected **benefits** and if the patient gives **informed consent**.
- However in reproductive medicine, there are 2 important problems:
  - not only the patients are involved, but also future offspring; IC of resulting children is always absent
  - study of adverse effects on future generations requires a very long follow-up

=> Welfare of the child is always compromised

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Welfare of the future child

- Does this preclude innovation in MAR?
- General principles:
  - **proportionality**: in pathologies where life of the patient is already at risk, high risks are acceptable, for 'trivial' medical problems, low risks are acceptable
  - **welfare of vulnerable groups** should be protected: research on children is permitted when risks are minimal; if risks are greater than minimal, there must be a direct benefit to the child

=> Innovation is acceptable if risks are minimal…

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Welfare of the future child

… but what is the benchmark when non-existing (future) persons are involved?

Different positions are possible:

- **maximal threshold**: a child should be born in the best possible circumstances
- **minimal threshold**: quality of life cannot be worse than death
- **average welfare** (worldwide? nationwide? within social class?) (Green, 1997)
- **natural conception as benchmark** (why?)

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Welfare of the future child

- maximal threshold
- minimal threshold
- average welfare
- natural conception as benchmark
- **reasonable welfare standard:** "The provision of medical assistance in procreation is acceptable when the child born as a result of the treatment will have a reasonably happy life." (Pennings, 1999)

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In a nutshell

Responsible innovation in MAR is possible, BUT:

- level of acceptable risk is low as there is always an unconsenting future person involved
- pre-clinical research
- research protocols
- safeguards + follow-up care
- follow-up studies

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Lessons from the past: IVF & ICSI

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Lessons from the past
IVF

'60s: successful IVF in rabbit, hamster, mouse, guinea pig

1969: 7 human pronuclear-stage zygotes (Edwards & Steptoe)

1971: Edwards and Steptoe apply to the Medical Research Council for funding of IVF research. Application is denied:

- risks of laparoscopy for 'experimental research'
- need for infertility treatment? (overpopulation, 'not a serious health condition')
- primate studies needed

1978: birth of Louise Brown

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Lessons from the past
IVF

1978: MRC revises its position after merely 2 live births: IVF is now 'experimental treatment' instead of 'research' (Johnson et al, 2010)

2010: Edwards receives the Nobel Prize in Physiology or Medicine:

"IVF is an established therapy throughout the world. […] IVF is a safe and effective therapy. […] Approximately four million individuals have been born thanks to IVF. […] Today, Robert Edwards' vision is a reality and brings joy to infertile people all over the world."

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Lessons from the past
IVF

Is IVF safe?

- For the patient? -> OHSS, infections, risks associated with multiple pregnancy,…
- For the children? -> complications due to multiple pregnancies, but even in singleton pregnancies: adverse perinatal outcome, congenital malformations, rare epigenetic defects (Ceelen et al, Fertil Steril 2008); cardiovascular and metabolic risk factors (Hart & Norman, HR Update, 2013)

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Lessons from the past
ICSI

1992: First life birth ICSI


SHORT REPORT

Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte

2013: 2,5 million ICSI-children born

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Lessons from the past
ICSI

Is ICSI a safe medical intervention?

- Increased risk of congenital malformations (+30% compared to spontaneous conception)
- Little knowledge about long term risks
- Imprinting disorders?
- Transgenerational passage of male factor infertility?
- Y-chromosome deletion: why not only implant female embryos?

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Challenges for the present: oocyte vitrification

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Challenges for the present
oocyte vitrification

- 1999: first case report healthy live birth after oocyte vitrification (Kuleshova et al.)
- 2009: review Noyes et al: 301 live births after oocyte vitrification, no increase in congenital abnormalities
- 2010: Noyes et al plead to remove experimental label
- RCTs: Rienzi et al (2010, 2012), Cobo et al (2008, 2010),…
- Systematic reviews/meta-analyses of RCTs: AbdelHafez et al (2010), Cobo & Diaz (2011)
- **2012: ASRM removes experimental label**
- Ongoing discussion open versus closed systems
- NEXT: freeze-dried oocytes?

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Perspectives on the future: artificial gametes

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Perspectives on the future
Artificial gametes

Different approaches:

- **Haploidization** (Tesarik et al, 2002; Tateno et al, 2003; Heindryckx et al, 2004; Nagy, 2005)
- Gametes < **hESCs** (Hübner et al, 2003; Toyooka et al, 2004; Gheijsen et al, 2004; Nayernia et al, 2006; Pelosi et al, 2011)
- Gametes < **ASCs** (Drusenheimer et al, 2007)
- Gametes < **iPSCs** (Imamura et al, 2010; Eguizabal et al, 2011)
- Primordial germ cell like cells (**PGCLCs**) < iPSCs (Hayashi et al, 2012)

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Perspectives on the future
Artificial gametes from iPSCs

Manipulation is extensive, many questions linger regarding reprogramming, are these germ cells really 'rejuvenated'? What about epigenetic changes?

Probability of compromised health in resulting offspring appears higher than in other areas of reproductive medicine, not sure that one will be able to assess safety in animal models and embryo research, long term follow-up of offspring would be needed before large-scale implementation

One would expect a similar moratorium as for reproductive cloning or germ line gene therapy, as safety concerns are comparable.

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Perspectives on the future
Artificial gametes from iPSCs

- Most likely, gametes cultured in vitro will be less safe and more expensive than naturally produced gametes.
- The reproductive rights of people do not trump considerations for the welfare of future children
- Donor sperm and oocytes are an available and safe alternative

=> When do attempts to have genetically related children become unacceptable?

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Conclusion

Lessons from the past:
There are now an estimated 5 million children born thanks to IVF and ICSI. 30 (IVF)/20 (ICSI) years have passed since their introduction and adverse outcomes appear limited. However, this 20/20 hindsight should not prevent us from acknowledging that the outcome could have been far worse, given the limited pre-clinical research and the fast pace at which both were introduced into the clinic. Moreover, many uncertainties still linger. Thus, the fact that IVF & ICSI are 'established procedures' does not mean that efforts to improve safety (lowering OHSS rates, single embryo transfer,…) are unnecessary or that follow-up research is no longer needed.

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Conclusion

Challenges for the present:
Responsibly introducing new innovations into the clinic requires a lot of patience. Due to the fact that today's patient has easy access to information about new innovations in MAR, pressure on clinicians to provide 'state-of-the-art' treatments can be substantial. Nevertheless, the steps necessary to protect the welfare of the patient and future offspring should be respected: pre-clinical research (animals, embryos, cell cultures), small scale introduction within research protocols, RCTs, reviews and meta-analyses, long term follow-up research.

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Conclusion

Perspectives on the future:
As innovations in MAR require ever more manipulations and imply interventions of which the mechanisms and consequences are poorly understood, it is important not to be blinded by the noble objective of helping every possible infertile patient, but to stop and ask the most basic question, namely: **from which point on are interventions no longer justified?**

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Thank you.

Heidi.Mertes@UGent.be

What animal research may contribute to safety and quality in MAR?

Prof Dr TM D'Hooghe, MD, PhD

Leuven University (B) /Yale University (USA)

Institute of Primate Research (WHO CC),

Nairobi, Kenya

29th Annual Meeting – ESHRE 2013 – London,
United Kingdom, 7-10 July 2013
SIG Ethics and Law & SIG Safety and Quality in ART–
Pre-congress course 7 July 2013

Conflict of interest/potential bias

Full Professor Leuven University/Adjunct Prof Yale Univ.
Merck Serono Chair (2005-15) /Ferring Chair (2010-2016) Reproductive Medicine (Leuven University)

Research Associate and Chair International Advisory Board, Institute of Primate Research, Kenya

Fundamental Clinical Investigator for endometriosis, Belgian Research Foundation (1998-2009), Leuven University Hospital Clinical Research Fund (2010-2015)

Consultant/advisor: Bayer Pharma, Astellas, Novartis, Ferring, MSD, Roche, Proteomika, Pharmagen, Merck Serono, Gedeon Richter, …

Learning objectives

At the conclusion of this presentation, participants should be able to understand the:

1. Need for preclinical animal research to establish safety, quality and effectiveness of innovations in reprod. medicine
2. Preclinical relevance of reproductive biology of nonhuman primates when compared to rodents
3. Potential of the baboon model for research in ART and implantation, based on it's validation in endometriosis research
4. Opinion of patients and the role of scientists in the public debate on preclinical reproductive animal research.

Learning objectives

At the conclusion of this presentation, participants should be able to understand the:

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5/19/2013

Need for NH Primate Models for the Study of Embryo Implantation

- **"TO UNDERSTAND THE ABNORMAL THERE IS A NEED TO STUDY THE NORMAL" (Hertig and Rock, Ward Burdick Award Address, 1967)**

- **ETHICAL LIMITS IN HUMANS:**

IN VIVO EMBRYO IMPLANTATION =

THE BLACK BOX OF HUM REPROD

Background IVF

- Over 1Million IVF/ART babies born
- First IVF baby born July 25; 1978 (30yrs old)
- IVF outcomes/efficiency (30yrs): Overall low (Blake et al., 2007;Syst. Rev.)
  - Pregnancy rates (PR): low <30%
  - Implantation rates (IR): low<20%
  - Success rates/birth rate: low <30% (high with multiple ET)
- Combination of factors lead to inefficiency of IVF technique
  - Embryo viability (cessation of dev't, fragmentation, perturbed gene expression, metabolic anomalies, aneuploidy)
  - Embryo selection-Subjective; depends on embryologist
  - Uterine receptivity

Improvements of IVF

- =>Improve quality of in vitro produced (IVP) embryo
  - -No information on normal human embryo? Mosaicism, aneuploidy
  - -Embryo quality is a combination of:
    - (egg quality, sperm quality, culture media, resulting embryos)
- =>Improve selection criteria for in vitro produced (IVP) embryos for ET
  - -metabolism? gene activation? compare to normal?
- =>Address safety of the new innovative techniques such as OT, GVT,PGD,NT,ICSI-abnormal/immature sperm? cryopreservation-toxicity? (Schatten et al., 2002)
- =>Evaluate uterine receptivity-invasive & non-invasive techniques: effect of OS regimens on uterine? (Nyachieo et al., 2007)

Why animal models

- Ethical, moral and religious concerns for use of human embryos:
  - -Embryo flushing to study in vivo embryos??
  - -Deliberate use of 'good quality' embryos for research?
  - -Terminate pregnancy for biopsies for biomarker study??
- Birth defect reports for IVF children? (anorectal atresia, septal heart defects, oesophangeal atresia, preterm births, low birth wts etc)
- Future uncertainties: quality and safety of IVF techniques (OT,ICSI,etc)
- Historical reasons
  - -Human IVF based on previous studies in animal models
    - -Sperm capacitation (Yanagimachi and Cheng, 1953)
    - -IVF (Yanagimachi and Cheng, 1963, 1964)
    - -Role of pH in IVF (Bavister, 1969)

Need for animal models for ART

Needed Studies	Status in human
Test new embryo culture media for safety/efficiency	Improve embryo quality and IR/PR
Preclinical evaluations of new ART techniques (e.g OT, NT, etc)	Safety concerns
Evaluate new markers for embryo selection	Selection based on morphology
New methods with non-toxic/low toxicity cryoprotectant formulations for vitrification	Vitrification (embryos and gametes): toxicity concerns
Derivation and study of ESC	Ethical concerns; ESC quality?
Immunological compatibility studies during ESC transplantation	Ethically impossible in humans

(Pool et al., 2005; Blake et al (2005, 2007); Oktay et al., 2008)

Learning objectives

At the conclusion of this presentation, participants should be able to understand the:

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	Rodents	NHPs	Humans
Genetically close to humans	-	+	+
Repro anatomy close to humans	-	+	+
Estrus behavior	+	-	-
Repro cycle	5 days	28-33 days	28-30 days
Embryonic aneuploidy	-	?	+
Optional diapause	+	-	-
Multiple implantations	+	-	-
Embryonic control of endometrium	+	-	-
Invasive implantation	-	+	+
Menstruation	-	+	+
Spont Endo	-	+	+
Spt+Ind Endo similar to humans	-	+	+
Spont PE	-	+	+

Rodent models

- **Advantages: low cost**

- **Disadvantages:**
- **Wide phylogenetic gap,**
- **Different reproductive endocrinology**
- **Different reproductive anatomy**
- **No menstrual cycle**
- **Different implantation process**

Selection of animal model for ART

- Rodent model
  -Easy to handle, cost, gene knock-out etc
- Bovine model
  -Easy to obtain oocytes from slaughter houses for IVM, paracrine and autocrine studies etc
- Limitations
  - Phylogenetically different from human
  - EGA occurs in 2 cell (mouse); bovine (8 cell); human (4 cell);
  - Different metabolic patterns
  - Differences in frequencies of aneuploidies (2%: mouse; 36%: bovine, 83% human)

(Menezo and Herubel, 2002; Vanneste et al., 2009)

NEED FOR NHP MODELS FOR THE STUDY OF REPRODUCTIVE BIOLOGY AND ART

- Advantages when compared to humans:

1. Very narrow phylogenetic gap (baboons: 20 autosomes + X,Y)
2. Human-based monoclonal Abs, RT PCR, microarrays usable
3. Comparable reproductive endocrinology and reproductive anatomy,
4. Menstruation
   (baboon, rhesus, not all other NHPs)

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Selection of animal model for ART

- Non-human primate models
  - Chimpanzees and gorilla are endangered species
  - Baboons and rhesus monkeys can be used for IVF/ART research
  - Currently only the rhesus monkey is characterized model for IVF/ART
    - Rhesus monkeys also used for research in HIV/AIDs and toxicology
  - Baboons need to be characterized as supplementary animal model

(Nyachieo et al., 2009; Wolf, 2008; Zelinski-Wooten 2004; Bavister 2004)

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BABOON BEST NHP MODEL REPRO RESEARCH

1. Noninvasive monitoring of menstrual cycle:

- Perineal inflation= Foll. Phase
- Perineal deflation=Luteal phase
- Ovulation = perineal deflation minus 2 days

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Baboon Reproductive Endocrinology

- **Menarche: 4.5 yrs (wild) or 3-3.5yrs (food abundance or captive)**
- **Lifespan: 27 yrs (wild) or 33 yrs (capt)**
- **Cycle: 32-34 (SD 2-4) days**
  - **proliferative phase: 16.4 +/- 2.4 days (18)**
  - **luteal phase: 16.4 +/- 3.7 days (15)**
  - **menstruation: 3.7 (2-7) days**

Baboon Reproductive Endocrinology

- **E2 peak level : 350 pg/ml, during FF same as in women (no second peak luteal phase in baboons, in contrast with women)**
- **P secretion increases after the onset of LH surge and prior to LH peak**
- **P max level luteal phase: 6-7 ng/mL**
- **LH peak: 25 ng/mL, sharp**

Baboon Reproductive Endocrinology

- **Interval between E2 peak and ovulation: 41 +/- 2 hours (34 +/- 2 in rhesus and 34+/- 3 in humans)**
- **Interval between E2 peak and LH peak: 23 +/- 2 hours (12 +/- 2 hours Rhesus and 24 +/3 hours humans)**
- **Interval between LH peak and ovulation: 18 +/-4 hours (rhesus: 22 +/- 2 hours; Human 9 +/- 2 hours)**

BABOON BEST NHP MODEL REPRO RESEARCH

2. Continuous breeding in captivity
(>< rhesus)

3. Size (12-15kg) and Strength (>rhesus>cynomolgus)
-repetitive blood sampling
(hourly during 24 hr in chair; daily)
-repetitive surgery
(every 2-3 days; D'Hooghe et al, 1996)

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BABOON BEST NHP MODEL REPRO RESEARCH

4. Spontaneous peritoneal fluid (PF)

about 2 mL after ovulation (>< rhesus)
(D'Hooghe et al, 1991)

5. Vaginal transcervical uterine access.

-endometrial biopsy **(D'Hooghe et al, 1991)**

-embryo transfer

-preimplantation embryo flushing

-hysteroscopy

(D'Hooghe et al, 1991; 1995; 1996; 2004; Nyachieo et al. 2007, Chai et al., 2007).

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Baboon model

- **6. Human assays can be used for endocrine and immunological markers (ELISA, IH, real-time RT-PCR, …) (D"Hooghe et al, 1996-2004; Fazleabas et al, 2002-2005)**

Baboon embryology and implantation

- **Embryo development and placentation well known (Enders, 1997; Enders, 2000)**
- **Uterine receptivity: 3 distinct phases (Fazleabas et al, 2004)**

Phase I: D8-10 PO: columnar epith + microvilli + stromal cell proliferation + pinopods + increased Muc-1 expression + reduced SMM II expression

Phase II: induced by blastocyst signalling

Phase III: following attachment+implantation: increased permeability subepith capillaries + glandular hypertrophy + decidualization

Baboon = model for invasive research in implantation

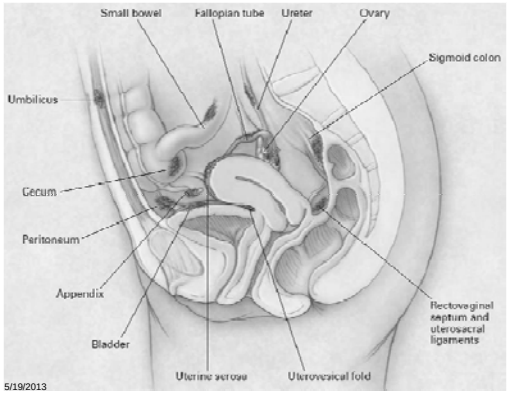
In vivo model for study of implantation sites by visual inspection and biopsy during laparotomy and hysterotomy during early pregnancy (Fazleabas et al, 1993-2005)

- Days 15-22 of pregnancy
- Region 1 = under implantation site
- Region 2 = immediately adjacent to implantation site
- Region 3 = opposite implantation site (non-implantation site, control)
- Hysterectomy to study uterine tissue
- Disadvantage: invasive, hysterectomy, no repetitive assessment

Baboon = model for noninvasive research in implantation

- **IN VIVO TRANSCERVICAL UTERINE STUDIES: Accessibility of the uterine cavity via the cervix**
- **EM biopsy (D'Hooghe et al, 1991)**
- **Embryo flushing (Bambra et al, 1990)**
- **Embryo transfer (D'Hooghe et al, 2004)**
- **Hysteroscopy (D'Hooghe et al, 2005)**

LESS INVASIVE, FOLLOW-UP POSSIBLE



Endometriosis

- EM (glands/stroma) outside uterus
- Prevalence
  - 7-15% of reproductive age women
  - up to 50% patients with pelvic pain/infertility
- Endo cost higher than cost of Crohn or migraine in USA (2002: 22 billion USD, Simoens et al., 2007)
- Diagnosis: laparoscopy (+ histology) (Kennedy et al, 2005; ESHRE guidelines) → diagnostic delay
- Estrogen dependent
  - rare before menarche or after menopause
- Progressive
  - >50% women/baboons after 1-2 years
- Retrograde menstruation/Sampson Hypothesis -1927

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NEED FOR ANIMAL MODELS IN ENDOMETRIOSIS RESEARCH

1.Unknown duration of endo at diagnosis

2.Inadequate study design: nl controls needed

- **pelvic condition (endo, nl pelvis, other)**
- **symptoms (none, infertility, pain, other)**

3.Impossible to address cause-effect relationships

4. Preclinical testing of new medical treatment (safety and efficiency)

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Baboon model endo

Institute of Primate Research **(Nairobi, Kenya)**
National Museums Kenya

WHO Collaborating Center

Research areas:
Reproduction
Infectious Diseases
Ecology and Conservation

5/19/2013

NEED FOR NHP MODELS FOR THE STUDY OF ENDOMETRIOSIS

NHPs:

- **Very close to humans (>< rodent models)**

1. **Very narrow phylogenetic gap (baboons: 20 autosomes + X,Y)**
2. **Human-based monoclonal Abs, RT PCR, microarrays usable**
3. **Comparable reproductive endocrinology/anatomy,**
4. **Menstruation (baboon, rhesus, not all other NHPs)**
5. **Spontaneous endometriosis 5-10% (! Bowel obstruction)**
6. **Induced endometriosis by intrapelvic injection of eutopic EM in pelvis (baboons, rhesus, cynomolgus)**
7. **Both spontaneous and induced endometriosis: similar phenotype, histology and localization as human peritoneal endometriosis (adapted ASRM classification)**
8. **Endometriosis-associated subfertility. Pain?**

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UNICITY OF BABOON MODEL

9. In vivo culture model for study of early endometrial-peritoneal interaction (after induction)

10. Preclinical model for study of cause-effect relationships in endometriosis (after induction)

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IDEAL ANTI-ENDOMETRIOSIS DRUG

1. Prevent the development of endometriosis
2. Cures existing endometriosis, also after cessation of treatment
3. No interference with menstrual cycle
4. No side effects
5. Safe for women who wish to become pregnant

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UNICITY OF BABOON MODEL

11. Evaluate new drugs for prevention of endometriosis (TNF-alpha inhibitors, D'Hooghe et al, 2006; TZDs Lebovic et al, 2009)

12. Evaluate new drugs for treatment of endometriosis (TNF-alpha inhibitors, Falconer et al, 2006; ROSI, Lebovic et al, 2007)

13. Well defined endometriosis outcome variables in prevention or treatment trials: lesion N, size, phenotype, localization, adhesions, classification (D'Hooghe et al, 2006; Falconer et al, 2006; Lebovic et al, 2007)

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14. General and reproductive safety in prevention or treatment trials (D'Hooghe et al, 2006; Falconer et al, 2006; Lebovic et al, 2007)

15. Model Endometriosis-associated infertility (D'Hooghe et al, 1994 and 1996)

16. Treatment of endometriosis-associated subfertility standardized for stage of endo, ovulation, mating, male factor (Falconer et al, 2007)

17. Endometriosis-associated pain (+ Oregon Primate Center)

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VALIDATION OF BABOON ENDOMETRIOSIS MODEL

- **Pub Med** (updated 11th May 2013):
- **Baboon AND Endometriosis N=85**
  - **about 40% Leuven-IPR Nairobi group (T. D'Hooghe)**
  - **about 40% Chicago group (A. Fazleabas)**
  - **about 10% San Antonio Group and others**

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5 observations
BABOON ENDOMETRIOSIS MODEL

- Uninterrupted retrograde menstruation causes endometriosis
- Endometriosis causes pelvic inflammation + systemic immunomodulation
- Endometriosis causes secondary endometrial changes
- General immunosuppression does not cause or cure endometriosis
- Specific immunomodulation may prevent and/or cure endometriosis

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Specific immunomodulation may prevent and/or cure endometriosis

- PPAR-gamma activators reduce and prevent induced endometriosis (Lebovic et al, 2007; 2009)
- J-kinase inhibitors reduce induced endometriosis (Hussein et al, 2009)
- TNF alpha antagonists prevent and reduce spontaneous or induced endometriosis, mainly via an effect on active red peritoneal lesions (3 independent studies Barrier et al, 2004; D'Hooghe et al, 2006; Falconer et al, 2006)

MAJOR CONCERN: GENERAL AND REPRODUCTIVE SAFETY

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Overall conclusions

- NHPs = most relevant preclinical models for endo research
- Among NHPs, baboons represent
  - the most relevant and
  - the best validated model for endo research

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Overall conclusions

Most important areas of endometriosis research in baboons:

1. Early pathogenesis
2. Cause-effect relationship studies
   may lead to discovery of new biomarkers
   and therapeutic targets
3. Test new drugs in prevention or treatment of endometriosis and endometriosis-associated subfertility
4. Test new endometriosis drugs with respect to general and reproductive safety
5. Validation baboon model for pelvic pain

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Ethics of endometriosis research in baboons at IPR

1. Baboons are not an endangered species
   but represent a threat to agriculture in Africa
2. Baboons live in their natural habitat at IPR (WHO CC)
3. Lack of other clinically relevant
   preclinical animal models to study cause-effect relationships:
   Only NHPs do have spontaneous/induced endo similar to peritoneal endo in women
4. Ethical need to show safety + efficiency of new drugs before application in women

Ethics of endometriosis research in baboons at IPR

5. Double approval for most projects by ethical committees from both IPR and from Leuven University
6. Supported by majority of Leuven patients (Reproductive Sciences, in press)
7. Professional Contract-based collaboration

Ethics of endometriosis research in baboons at IPR

8. **Research potentially beneficial for clinical care of baboons and other NHPs with spontaneous endometriosis in zoos, baboon colonies, etc…**
9. **Global level:**
   Capacity building of Primate Research Center important aspect of North-South collaboration (emerging markets…)
10. **Preparation for International accreditation IPR in line with USA (later EU) legislation (F1 bred, cages)**

IPR International Advisory Board

- **Established 2007**
- **Initiative by NMK/IPR +**
  supported by WHO (P. Van Look)
- **Aim:**

- **advise Kenyan leaders about**
  long term development of IPR
  into African Center of Excellence

- increase international research collaboration

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IPR International Advisory Board

- 12 experts in areas of
  reproduction, infectious diseases
  ecology and conservation
- Chair T. D'Hooghe
- Annual meetings,
  (August + December 07, February 09,
  July 10)

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Slide 45

x1 x292720; 9/11/2007

EU Directive October 2010 (1/1/2013)

- NHPs in scientific procedures still necessary in biomedical research
- Only allowed in essential biomedical areas: basic research, preservation of NHP species, avoidance, prevention, diagnosis or treatment of -potentially life-threatening conditions,

  -debilitating conditions (substantial impact on day to day ability to function normally physically/psychologically):

  Including endometriosis and infertility

BABOON ART (PhD Nyachieo, 10 papers)

- Long protocol and Depot protocol may be useful for baboon OS
- Baboon cycle synchronization is possible using an oral contraceptive for easier OS planning
- ICSI better than IVF (fert. rates 23-71%)
- Baboon serum (BS), dcAMP, caffeine improve baboon sperm motility and binding ability, whereas Khat reduces sperm motility, chromatin integrity and serulm testosterone levels
- Embryo culture system successful up to day 3 (80-100% survival; 50-60% (4cell-8cell embryos)
- Non-surgical transcervical ET is possible in baboons
- Baboon is useful for ovarian tissue cryopreservation studies by vitrification
- Baboon can be useful for IVF/ICSI studies

Assisted Reproductive Technologies (ART) With Baboons Generate Live Offspring: A Nonhuman Primate Model for ART and Reproductive Sciences

Calvin R. Simerly, PhD[1], Carlos A. Castro, MD[1], Ethan Jacoby, BS[2], Kevin Grund, BS[1], Janet Turpin, BS[1], Dave McFarland, BS[3], Jamie Champagne, BS[3], Joe B. Jimenez Jr, BS[4], Pat Frost, DVM[4], Cassondra Bauer, DVM[4], Laura Hewitson, PhD[1], and Gerald Schatten, PhD[1]

Reproductive Sciences
17(10) 917–930

5/19/2013

Simerly et al, 2010

- ICSI and preimplantation development to blastocyst stage
- Motility during fertilization assessed by Time Lapse Video Monitoring
- Delivery of male non-identical twins
- Impact for ART and embryonic stem cell research

5/19/2013

Learning objectives

At the conclusion of this presentation, participants should be able to understand the:

1. Need for preclinical animal research to establish safety, quality and effectiveness of innovations in reprod. medicine
2. Preclinical relevance of reproductive biology of nonhuman primates when compared to rodents
3. Potential of the baboon model for research in ART and implantation, based on it's validation in endometriosis research
4. Opinion of patients and the role of scientists in the public debate on preclinical reproductive animal research.

5/19/2013

Acceptability of Preclinical Research on Nonhuman Primates in Reproductive Medicine: The Patient Perspective

Eline A.F. Dancet, MSc[1], Carl Spiessens, PhD[1], Rebecca Vangenechten, MSc[2], Jaak Billiet, PhD[3], Johan De Tavernier, PhD[4], Myriam Welkenhuysen, MSc[1], and Thomas M. D'Hooghe, PhD[1,5]

Reproductive Sciences 2011 18: 70 originally published online 27 September 2010
DOI: 10.1177/1933719110380277

5/19/2013

First study Pt Perspective NHP repro research (Dancet et al, 2010)

- N=299, RR 80%
- 71% accept preclinical reproductive research in NHPs if no alternative and if optimal care
- Positively correlated with trust in researchers and previous pregnancy
- Negatively correlated with having a pet and being member of nature organization
- Take home lesson for researchers: gain trust!

BIOLOGY OF REPRODUCTION (2010) 83(2):181–183
DOI 10.1095/biolreprod.110.085928

Editorial

The Role of Scientists and Clinicians in Raising Public Support for Animal Research in Reproductive Biology and Medicine[1]

Dancet EAF, Brannstrom M, Brasky K, Chai D, Chan AWS, Conn PM, Else J, Falconer H, Fazleabas AT, Farah IO, Goddeeris BM, Golos TG, Hau J, Hearn JP, Kariuki TM, Kyama CM, Lebovic DI, Mwenda JM, Ndung'u J, Nyachieo A, Parker J, Slayden OvD, Stouffer RL, Strauss JF, Taylor HS, Vanderpoel S, Westergaard JG, Zelinski M, **D'Hooghe TM**.

Dancet et al, 2012

- Advise for animal researchers worldwide to comply with USA or EU legislation and to obtain AAALAC accreditation
- Communication to public:

  - as a group

  -seek partners: patients, celebrities, politicians, international educational/professional organizations, academic authorities

  -justify need for animal model, type of disease, benefits, species and numbers, care, pain relief, …

Dancet et al, 2012

- Inform public how animal research has advanced understanding, diagnosis and treatment of human disease
- Inform public about highest quality of care provided according to international criteria, after peer review of research protocols, and regular external auditing
- Use lay language, compassionately, including emotion and personal experience (>< scientific language)

Dancet et al, 2012

- Tailored approach for specific target groups (patients, general public, animal rights organizations, …)
- Create information opportunities:
  -tours in research institutions
  -present research findings in schools and colleges
  -websites, newsletters, brochures, social media
  - involve patients in ethical debates and legislation

Conclusions

1. Preclinical animal research is needed to establish safety, quality and effectiveness of innovations in reprod. medicine
2. The preclinical relevance of reproductive biology research in nonhuman primates (NHPs)may be higher than that of rodents in view of their closeness to humans
3. NHPs including baboons are important models for research in ART and embryo implantation, and have been used successfully in other areas of reproductive medicine research like endometriosis
4. The opinion of patients and the role of scientists in the public debate on ethics and legislation of preclinical reproductive animal research is important

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Baboon IVF published articles (PhD A. Nyachieo)

Published papers

- 1.Nyachieo et al., 2009 Fertil Steril 91(2):602-10 (Ovarian stimulation regimens and IVF)
- 2. Nyachieo et al., 2009 J. Med primatology 38(2):145-50 (Baboon sperm capacitation)
- 3. Nyachieo et al., 2009 Animal Reprod. Science 110(3-4):187-206 (Ovarian stimulation regimens)
- 4. D'Hooghe et al., 2008 Human Reprod. Monograp 2008:102-107; doi:10.1093/humarep/den164
- 5.Nyachieo et al., 2007 Gynecol. Obstet. Invest. 64(3):149-55 (Baboon embryo-uterine receptivity)
- 6.Chai et al., 2007 J. Medical primatology 36(6):365-9 (Baboon uterine access techniques)
- 7. D'Hooghe et al., 2004 Gynecol. Obstet. Invest. 57(1):23-26 (pilot baboon IVF)
- 8. Nyachieo et al, 2010 Separate and combined effects of caffeine and dbcAMP on olive baboon (Papio anubis) sperm. J Med Primatol 2010;39(3):137-42
- 9. Nyachieo et al, 2011. Randomized comparison of different ovarian stimulation regimens for assisted reproductive technology in baboons (*Papio anubis).* Fertil Steril 2011;95(4):1354-9
- 10. Nyachieo et al, 2012 Effect of Khat (Catha edulis) on baboon sperm (Gynecol Obstet Invest, 2012)
- 11. Nyachieo et al, 2013. Baboon ovarian cryopreservation by vitrification (Gynecol Obstet Invest 2013, in press).
- 12. Nyachieo et al, 2012. Baboon spermatology: basic assessment and reproducibility in olive baboons (*Papio anubis*). J Med Primatol 2012;41(5):297-303.

In vitro-maturation of oocytes: is there a case for preclinical embryo research? - **Guido De Wert (The Netherlands)**

Contribution not submitted by the speaker

When should practice adaptation be research? The case of culture media in IVF

John CM Dumoulin, PhD
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DISCLOSURE

JCM Dumoulin does not have any commercial and/or financial relationship with manufacturers of culture media.

Presentation outline and learning objectives:

- **Introductions of innovations in ART**
- **Effectiveness of culture media**
- **Safety of culture media: partly responsible for adverse perinatal outcome in IVF children?**
  - **Evidence in followup studies**
  - **Evidence for epigenetic disturbance**
- **Evaluation of effectiveness and safety of culture media:**
  - **Role of ART professionals**
  - **Role of ESHRE**
  - **Role of manufacturers**

Progress in medical science is characterized by a continuous cycle of innovation and research

Any new technology should be evaluated for:
(i) effectiveness,
(ii) safety and
(iii) costeffectiveness

Primary outcome in ART:
healthy live birth with lifelong health

(Harper *et al*., 2012)

Ideal introduction of a new technique

1) Hypothesis developed and tested in animal models, including small rodent (mouse) and large animal (bovine and pig)
2) Tested in human embryos donated to research
3) Tested in small scale single site clinical IVF-study
4) Tested in larger multi-site clinical study
5) Assess clinical and cost effectiveness

(Harper *et al*., 2012)

Introduction of major innovations in ART

- IVF
- Cryopreservation of embryos
- ICSI
- PGD
- PGS
- In Vitro Maturation
- Cryopreservation of oocytes

→ Introduced in clinical practice without much preclinical research
→ Often, no suitable animal models available
→ RCTs often not appropriate: non-treatment control not available
→ Little/late evaluation of safety issues in children

(Dondorp *et al*., 2011; Harper *et al*., 2012)

Introduction of other innovations in ART:

small adaptation of everyday practice or major innovation?

- Blastocyst vs cleavage stage transfer
- Vitrification vs slowfreezing
- Laser vs Acid Tyrodes biopsy
- Different types of culture media
- 5% vs 20% oxygen
- Small changes in pH or osmolality
- IMSI vs ICSI
- Different types of Embryo Transfer catheters
- Different types of culture dishes
- Different types of ICSI needles

→ Introduced in clinical practice without much preclinical research
→ Expected differences small → large sample sizes needed
→ Proper introduction often practically impossible

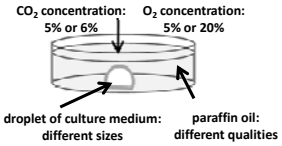
Culture media - culture systems

Composition

- **water**
- **ions**
- **bufferingsystem**
- **energy source**
- **protein (albumin)**
- **antibiotics**
- **amino acids**
- **vitamins**
- **growth factors**

- **pH**
- **temperature**
- **osmolality**

- **flushing media**
- **sperm washing media**
- **cumulus removal media**
- **ICSI (PVP) media**
- **culture for 2-6 days:**



- **sequential or single step media**
- **embryo transfer media**

Innovations in embryo culture media

- 1950-1970s: mouse embryo culture: Whitten, Biggers, Whittingham, Brinster
- 1970-1980s: somatic cell media also used (Ham's F10)
- 1985: Quinn: HTF based on oviduct fluid
- 1989: CZB (Chatot, Ziomek, Bavister): 2-cell block overcome
- 1991: Lawitts & Biggers: KSOM simplex optimized media
- 1990s: inhibitory effect glucose on early development
- 1990s: amino acids improve embryo development
- Glutamine → glycyl-glutamine to avoid ammonium
- 1990s: switch from serum to albumin preparations
- 1990s: co-culture with somatic cells
- 1990-2000s: sequential and single-step media
- 1990-2000s: commercial media: precise composition unknown
- pH: 7.4 → 7.2 for early cleavage
- Osmolality: 285 → 260 → 280
- Growth factors

Question:

should the introduction of a new, or modified, culture medium be considered a small adaptation of everyday practice or a major innovation?

- **Effectiveness?**
- **Safety?**
- **Primary outcome in ART: healthy live birth with lifelong health**

Effectiveness of embryo culture media

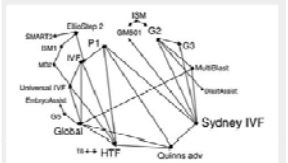


Figure 2 Schematic representation of the comparisons made in the included studies. The font size represents the number of studies on each medium.

22 RCTs included that evaluated 31 different comparisons.

"Conventional meta-analysis was not possible as nearly all trials compared different culture media. It is yet unknown what culture medium leads to the best success rates in IVF/ICSI."

Mantikou *et al*., 2013

Effectiveness of embryo culture media

Optimization of culture media for human embryo development practically almost impossible:

- Large numbers of embryos are needed (2200 mouse zygotes, Lawitts and Biggers, 1991)
- 3000 patients needed to detect 5% difference in life birth rate between 2 media

Safety:

Is there reason for concern for ART children?

- **At present: 5 million ART babies born!**

Differences in outcome of pregnancies between spontaneous and IVF conceptions:

Higher risk of adverse perinatal outcome in IVF singletons when compared with matched controls

- Preterm birth (<37 weeks): RR = **1.5** - **2.0**
- Perinatal mortality: RR = **1.7** - **2.2**
- Low birthweight (<2500 g): RR = **1.6** - **1.8**
- Small for gestational age: RR = **1.4** - **1.6**
- Congenital abnormalities: RR = **1.7**

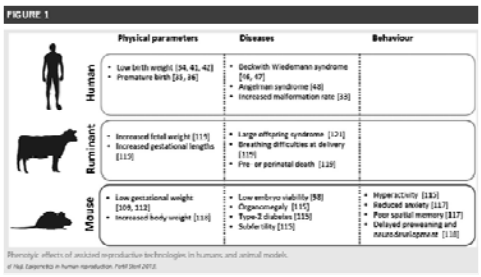
Helmerhorst, 2004 ; Jackson, 2004 ; McDonald, 2009 ; Pandey, 2012

Differences in outcome of pregnancies between spontaneous and IVF conceptions:

Changes in phenotype of IVF children compared to controls

- different blood lipid profiles (Miles, 2007; Sakka, 2010)
- higher fasting glucose levels (Ceelen, 2008)
- increases in blood pressure (Belva, 2007; Ceelen, 2008; Sakka, 2010)
- different weight and height (Koivurova, 2003; Green, 2013).
- generalized vascular dysfunction (Scherrer, 2012)

Differences in outcome of pregnancies between spontaneous and IVF conceptions:



el Hajj, 2013

Differences in outcome of pregnancies between spontaneous and IVF conceptions:

No changes in phenotype of IVF children compared to controls

- no increases in blood pressure (Belva, 2012)
- no increases in glucose levels (Miles, 2007; Sakka, 2010)
- no differences in weight and height (Bonduelle, 2005; Basatemur, 2010).
- comparable cognitive and motor development (Leunens, 2008)

Differences in outcome of pregnancies between spontaneous and IVF conceptions:

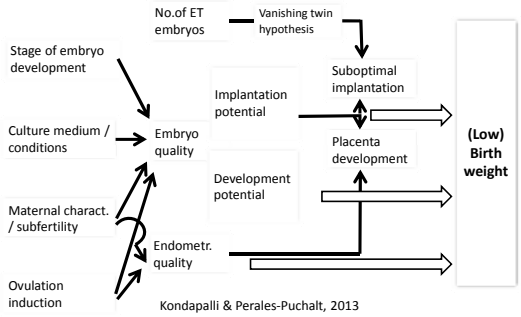
What could be the underlying cause?

- Patient related factors such as subfertility?
- IVF technique related factors: ovarian stimulation?
- IVF technique related factors: in vitro culture?

Outcome parameters?

- Birth weight, Low Birth Weight, Large for Gestational Age?
- Congenital abnormalities?
- Placental abnormalities?
- Long term followup of children: blood pressure, etc.?
- Parameters of epigenetic disturbance in oocytes and embryos?

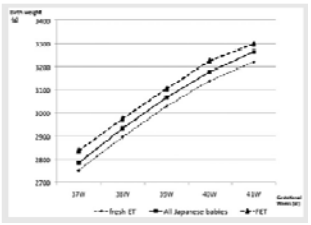
Many factors influence birthweight



Kondapalli & Perales-Puchalt, 2013

IVF technique partly responsible for differences
effect of hormonal stimulation or cryopreservation?

FIGURE 1



25,777 singletons after IVF,

11,374 after fresh ET
14,403 after cryo ET

Nakashima et al., 2013

IVF technique partly responsible for differences
effect of embryo culture?

Table II Adjusted risks for adverse outcomes in singleton births from IVF/ICSI in Canada, 2001–2009, after transfer on Day 3 (reference) or Day 5/6.

Outcome	OR (95% CI) Day 5/6 versus Day 3[a]
All preterm births (<37 weeks)	**1.32 (1.17–1.49)**
Early preterm births (<32 weeks)	1.09 (0.84–1.42)
Low birthweight (<2500 g)	0.99 (0.85–1.15)
Very low birthweight (<1500 g)	0.93 (0.66–1.32)
Congenital anomalies	1.13 (0.85–1.50)

[a]Adjusted for the year of treatment, maternal age, parity, infertility diagnosis category, number of oocytes retrieved, insemination method, number of embryos transferred and the presence of a vanishing twin.
Bold indicates the main findings.

12,712 singletons,

9506 Day 3 ET
3206 Day 5/6 ET

Dar et al., 2013

IVF technique partly responsible for differences
effect of in vitro culture?

Maastricht UMC - Vitrolife vs Cook study (2003 – 2006)

- Lab: randomisation of all consecutive cycles by alternating between the two media
- Clinic: planned OPUs, unaware of randomisation
- Vitrolife G1.3 culture medium: 414 patients, 715 cycles
- Cook culture medium: 412 patients, 717 cycles
- Culture: 5% O_2, 6% CO_2, 89% N_2
- Except from medium: identical IVF procedures
- Embryo transfer on day 2 or 3: eSET based on female age (<38 yrs) and embryo quality

Dumoulin *et al.*, 2010; Nelissen *et al.*, 2012

IVF technique partly responsible for differences
effect of in vitro culture?

Maastricht UMC - Vitrolife vs Cook study (2003 – 2006)

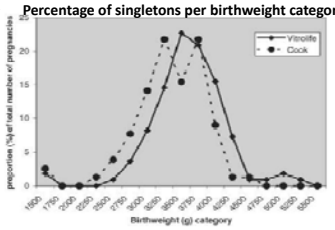
	Vitrolife (n=168)	Cook (n= 126)	*P*-value
Gestational age (GA) at birth	39.6 ± 0.1	39.4 ± 0.2	NS
Preterm birth (<37wks)	6 (3.6)	8 (6.4)	NS
Birthweight (g)	3436 ± 44	3253 ± 50	0.006
Z-score	0.05 ± 0.08	-0.27 ± 0.08	0.007
Low birthweight (<2500g)	4 (2.4)	12 (9.5)	0.006
Low birthweight with GA ≥ 37 wks	2 (1.2)	8 (6.4)	0.015
High birthweight (>4500g)	5 (3.0)	0	NS

Nelissen *et al*., 2012

IVF technique partly responsible for differences
effect of in vitro culture?

Maastricht UMC - Vitrolife vs Cook study (2003 – 2006)

Percentage of singletons per birthweight category



Dumoulin *et al*., 2010

IVF technique partly responsible for differences
effect of in vitro culture?

Maastricht UMC - Vitrolife vs Cook study (2003 – 2006)

Many factors affect birthweight of singletons

- Gestational age at birth ***P<0.001***
- Fetal gender ***P<0.001***
- Parity ***P<0.001***
- Maternal and paternal height and weight ***P=0.008***
- Age of the mother
- Pregnancy related factors: gestational diabetes and hypertension
- Lifestyle factors such as smoking
- History of subfertility (duration of subfertility)
- Number of transferred embryos (vanishing twins)
- Type of culture medium ***P=0.031***

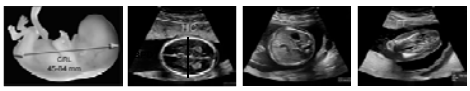
Lineair Regression analysis

Dumoulin *et al*., 2010; Nelissen *et al*., 2012

IVF technique partly responsible for differences
effect of in vitro culture?

Maastricht UMC - Vitrolife vs Cook study (2003 – 2006)
Fetal development of 294 singletons after fresh ET

- Ultrasound examination at 8, 12 and 20 weeks' gestation
- First-trimester serum markers (fβ-hCG, PAPP-A)



CRL: crown-rump length | BPD: biparietal diameter ("from ear to ear") HC: head circumference | AC: abdominal circumference | FL: femur length

Nelissen *et al.*, in press

IVF technique partly responsible for differences
effect of in vitro culture?

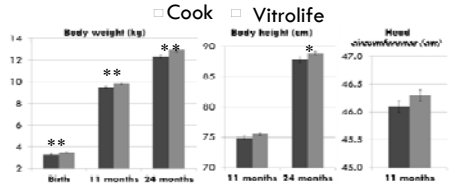
Maastricht UMC - Vitrolife vs Cook study (2003 – 2006)
Fetal parameters at 20 weeks of pregnancy

Sonographic markers	**Vitrolife group (n=115)**	**Cook group (n=91)**	**Adjusted mean difference**	***P*-value**
BPD (biparietal diameter)	50.2	49.8	0.5	0.07
HC (head circumference)	177.3	175.9	1.8	0.03
AC (abdominal circumference)	152.1	151.2	0.8	0.43
FL (femur length)	32.7	32.8	-0.1	0.81

Nelissen *et al.*, in press

IVF technique partly responsible for differences
effect of in vitro culture?

Maastricht UMC - Vitrolife vs Cook study (2003 – 2006)
Postnatal development up to 2 years



*** P < 0.05 raw data** *** P < 0.05 data adjusted for possible confounders**

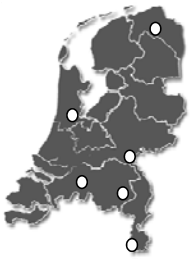
Kleijkers *et al.*, 2012

IVF technique partly responsible for differences
effect of in vitro culture? At present: too early to tell

Publication	Study design	No. of singletons after fresh ET	Significant difference found?
Dumoulin, 2010; Nelissen, 2012	Quasi-randomized, 2 media (Cook and Vitrolife G1.3)	168 and 126	Yes
Eaton, 2012	Not randomized, 3 media: G1.3 (2003-2005), Global (2005-2007), G1.5 (2007-2008)	102, 53 and 43	No
Vergouw, 2012	Not randomized, 2 media: HTF (2008), Sage (2009-2010)	99 and 259	No

500-700 babies in each arm of a RCT needed to detect:
- a doubling of the Low Birth Weight (LBW) rate
- a difference of 100 gram in mean birthweight
- a doubling of the Large for Gestational Age (LGA) rate

IVF technique partly responsible for differences
effect of in vitro culture?
Dutch multi-center culture medium trial (2011 – 2014)



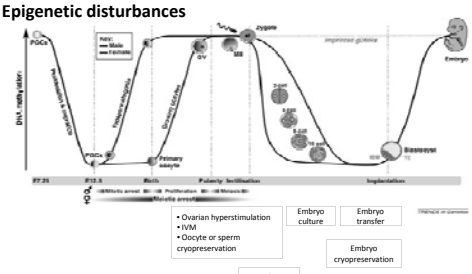
- 800 patients randomised over 2 culture media: Vitrolife version 5 versus HTF (Lonza)
- Inclusion completed in May 2012

Outcome parameters:
- IVF results after 1 year of treatment
- Foetal growth
- Perinatal outcome (birthweight)
- Postnatal outcome and growth

- Epigenetic effects

IVF technique partly responsible for differences
effect of in vitro culture?

Epigenetic disturbances



Adjusted from Smallwood *et al.* (2012)

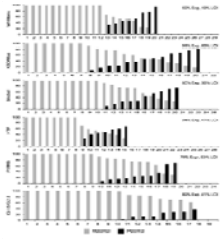
IVF technique partly responsible for differences

Epigenetic disturbances after in vitro culture of mouse embryos in different media

Methylation levels

	H19	Snrpn	Peg3
in vivo:	82%	92%	100%
Whitten:	61%	58%	54%
KSOMaa:	75%	73%	93%
HTF:	52%	54%	85%
Global:	65%	72%	77%
P1/MB:	65%	65%	75%
G1/G2:	55%	64%	50%

Allelic expression levels

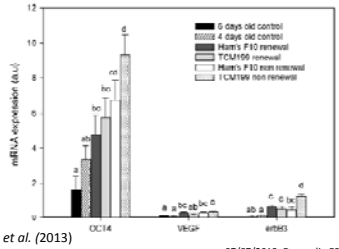


Market-Velker *et al. (*2010)

IVF technique partly responsible for differences

Epigenetic disturbances after in vitro culture?

Gene expression in rabbit embryos cultured in different media compared to in vivo controls

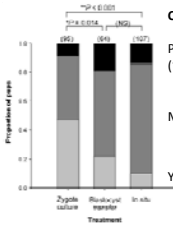


Saenz-de-Juan *et al. (*2013)

IVF technique partly responsible for differences

Epigenetic disturbances after in vitro culture?

Preimplantation embryo's growth environment can affect postnatal expression of a defined epigenetically sensitive allele

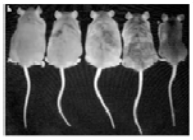


Coat color:

Pseudoagouti ("wild-type")

Mottled

Yellow



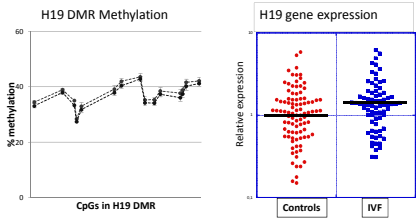
Zygote culture: 48 h in Sydney IVF Cleavage Media (Cook) then 48 h in Sydney IVF Blastocyst Media

Morgan *et al. (*2008)

IVF technique partly responsible for differences

Epigenetic disturbances after in vitro culture in human?

Aberrant DNA methylation and gene expression in IVF/ICSI conceived versus naturally conceived placentas



Nelissen *et al.* (2013)

Introduction new culture medium: small adaptation of everyday practice or major innovation?

- **Possibility that media and other culture conditions are partly responsible for adverse perinatal outcome in IVF children should not be ignored**
- **Ideal introduction will be difficult**
- **More relevant preclinical testing is needed**
- **Followup of pregnancies and children is needed**

Working Group on Culture media (ESHRE)

- companies should disclose the composition and preferably the formulation of each medium,
- new formulations should have a scientific foundation,
- a standard minimum QC certificate should be shared by all companies that should use the same SOPs,
- a more relevant test than the mouse embryo assay should be designed

Role of manufacturer in evaluation of effectiveness and safety of culture media.

European Union - Medical Device Directive 93/42/EEC (1993):
" 'medical device' means any …. , material or other article, …. , intended by the manufacturer to be used for human beings for the purpose of:
— …….
— control of conception,
and which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means."

Manual on borderline and classification in the community regulatory framework for medical devices Version 1.13 (10-2012):
"Although case by case analysis should always be performed, media intended for use in the IVF process to support the growth / storage of the embryo may generally be considered to be Class III medical devices."

Role of manufacturer in evaluation of effectiveness and safety of culture media.

Medical Devices Directive 93/42 and directive 2007/47/EC :

CLINICAL EVALUATION

- *Clinical investigations shall be performed for Class III devices unless it is duly justified to rely on existing clinical data,*
- *Evaluation of this data is referred to as 'clinical evaluation',*
- *Clinical evaluation should be part of the technical documentation.*
- *Notified Bodies are obliged to assess the clinical evaluation as part of their conformity assessments.*
- *Emphasis on risk analysis and gathering sufficient clinical data to ensure compliance with essential requirements.*

Role of manufacturer in evaluation of effectiveness and safety of culture media.

EU Guidance document to MDD 93/42 and 2007/47 (2012): Guidelines for conformity assessment of IVF and ART products.

IV. Hazards/Risks

- *each risk related to each component should be considered and should constitute an acceptable risk when weighed against the benefit to **the gamete, to the embryo or to the mother-to-be.***
- *In order to evaluate the overall risk due to the IVF/ART products, pre-clinical testing is necessary. If appropriate, the manufacturer should include the following biocompatibility testing:*
  - *Tests for genotoxicity, carcinogenicity and reproductive toxicity*
  - *Tests for in vitro cytotoxicity*
  - *Toxicokinetic study design for degradation products*

Role of manufacturer in evaluation of effectiveness and safety of culture media.

EU Guidance document (2012) to MDD 93/42 and 2007/47: Guidelines for conformity assessment of IVF and ART products.

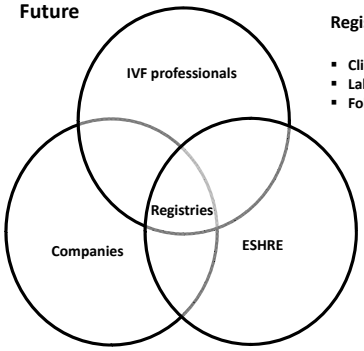
VIII. Preclinical data and clinical data supporting CE marking including Post- Market Clinical Follow-up (PMCF)

- *A PMCF programme must be planned and can take the form of clinical investigations and/or registries. Postmarket surveillance should be carried out in "all comers" registries to better provide the clinical safety and performance data on the IVF/ART solutions' use in the clinical practice.*

IX. IVF/ART solutions' modification

When the manufacturer does some subsequent modifications to an already marketed IVF/ART products, it is essential to evaluate, based on a risk analysis, preclinical data and clinical data, the impact of the modified characteristics. The results of this evaluation will determine the need for any new or additional testing.

Future



Registries including:

- **Clinical M&M**
- **Laboratory M&M**
- **Followup outcome**

Thank you for your attention

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The health of the future child: what level of risk is acceptable in medically assisted reproduction (MAR)?

Nils-Eric Sahlin - Sweden

(I have no conflicting interests to declare)

What is risk?

What is the risk to get a child with Down syndrome? At maternal age 20, the probability is low, very low. At age 45 the probability is 0,05. But probabilities are not the same as risks. A probability becomes a risk if we put a value on the outcome, if the outcome is negative, is below a given level of aspiration. Thus, an answer to the question can be: There is no risk. Risks are, to put it in a different way, value laden probabilities.

Epistemic risk

Examining some contemporary risk debates we find that the evaluation of epistemic uncertainty seems as problematic as the assessment of the disutility of potential negative outcomes. The epistemic uncertainty causes what might be called epistemic risks, making it hard to identify, assess, and manage the outcome risks, i.e., the different negative things that might or might not happen as the result of our decisions.

A good example is evidence base decisions and rare diseases. Two more examples are different type of medically assisted reproduction, and stem cell research. MAR (medically assisted reproduction) promises come with a risk. It is impossible to say what the risk of MAR is. There is no such (single, overall) risk. What can be assessed is the risk of individual innovations, inventions, tools and methods.

There are four paradigmatic types of decision. The complete picture is, of course, greatly more complicated than a simple four-by-four matrix.

In **Type 1** situations the decision maker has extensive knowledge and information, expressed in terms of precise probability estimates. He or she has also clear and distinct preferences and values. An example of a Type 1 situation: In *The Foundations of Statistics* Savage asks us to consider a situation in which your wife or husband has just broken five good eggs into a bowl. A sixth egg lies beside the bowl and you have to decide what to do with it. You can break it into the bowl, break it onto a plate for inspection, or throw it away without inspection. If you know your eggs you know the probability that the sixth egg is also good. There is no epistemic uncertainty. And clearly a six-egg omelet is better than a five-egg omelet and the chore of washing up a plate, which in turn is better than five destroyed eggs and no omelet at all.

Many every day medical decisions are of this kind.

In **Type 2** situations the quality and quantity of information is poor, and it is difficult to represent the underlying uncertainty in terms of probability. On the other hand, the decision problem is one in

connection with which the decision maker still has clear and distinct preferences and values: he knows what he wants and desires.

An example of a Type 2 situation: Many of the most well-known, contemporary risk issues fall within this category: stem cell research and therapies, synthetic biology, uterus transplantations and different type of IVF. We all want the good things that the new technologies promise. But we do not want the risks, and given our present state of knowledge it is also hard to say with precision what, and how great, the probabilities (risks) are. The possible risks of uterus transplantations and egg donation are but two example. But what are the risks? How great are they? What does our present epistemic state look like?

In **Type 3** situations the quality and quantity of information is good – good enough to assess precise probabilities. However, the decision maker lacks harmonious, clear and distinct preferences and values. Perhaps his appetites are out of keeping with his valuations.

An example of a Type 3 situation: ICDs (Internal Cardiac Defibrillators) are implants monitoring the patient's heart rhythm. If life threatening arrhythmias are detected by the ICD device it gives the patient a stinker of an electric shock. Before the device is implanted the patient is informed about the risks and benefits. A problem is that the device can be triggered when the patient has no arrhythmia. The probabilities relevant to a decision are known with reasonable precision, but they are known at type, not token, level. This is a decision-situation where our preferences and utilities are blurred or all but indistinct. We lack experience. An experience we hope we never will have. A special problem is that we have to choose between a quick and painless death, on the one hand, and a long drawn-out and maybe not especially painless ending, on the other.

A very different example is the risk of follicle-stimulating hormones (FSH). The probabilities of different type of adverse effects are fairly well-known. But the values, the preferences of the donor may be anything but distinct. There are of course many more examples, "egg-sharing" is one of them. In this case the relevant probabilities are well known, but values and preferences may be conflicting.

In **Type 4** situations both information and preferences are unfixed or unreliable.

An example of a Type 4 situation: Imagine a 3-year-old child with high-risk neuroblastoma. She has been treated according to the standard protocols used by pediatricians: she has been given hematopoietic stem cell transplantation, for example – a treatment not without complications and considerable suffering. But not all children respond to stem cell treatment, and let us assume this is a case of recurring neuroblastoma. What are the odds that a hematopoietic stem cell transplantation that failed to succeed the first time will work at the third or fourth attempt? With the number of refractory instances, the relevant probabilities become harder and harder, if not impossible, to estimate; and as our epistemic state deteriorates, our desires and values become more and more unstable.

One might argue that nuclear transfer to avoid the transmission of mitochondrial disease fall within this category. There are plenty of unknowns and all but clear values and preferences. In a recent report, *Novel techniques for the prevention of mitochondrial DNA disorders: an ethical review*, Nuffiel Council on Bioethics emphasize that the risks of transmitting mitochondrial DNA disorders are complex and difficult to predict. A problem is that the relevant probabilities will differ

”depending on the proportion of mutated mitochondria carried by the affected woman’’s egg. In general, women with homoplasmic mutations pass on a homoplasmic mutant load to their children, whereas the load passed on by women with heteroplasmic mutations is variable and unpredictable. How severely a child may be affected, if at all, may be very hard to determine before birth, as this will also depend on the particular mutation, and in what way the mutation affects the functioning of the body and at what age these symptoms manifest”.

		Epistemic uncertainty: Low	Epistemic uncertainty: High
Value uncertainty	Low	Type 1	Type 2
	High	Type 3	Type 4

A Socratic approach

Type 1 situations are readily detected in the paradigmatic cases with which classical theories of rational choice and decision-making deal. But the traditional theories are ill equipped to handle the three other types of situation. In Types 2, 3 and 4 the traditional theories of risk analysis are no guides to action. Here we need theories that help us to represent unreliable or indeterminate beliefs and imprecise values. We must introduce more complex, but also more complete, decision procedures. ‘‘Maximize expected utility,’’ the mantra of the classical theories, is, for simple mathematical reasons, no longer an available option.

Examples involving nuclear power plants, BSE, GMOs, electro-magnetic fields, nano-technology, and MAR have taught us that various kinds of factor create epistemic uncertainty. Good risk analysis requires careful inspection of our present epistemic state. It is simply not enough to identify and evaluate outcome risks, i.e., the negative consequences of our actions. An estimation and evaluation of current levels of ignorance is crucial. We must know what type of knowledge we have and the value situation we are in (Type 1, 2, 3 or 4).

If we cannot use the traditional methods for rational decision-making and risk analysis, what shall we do? What we should do is promote a Socratic approach to risk analysis. What does this mean?

In Plato's Apology Socrates declares: ''Probably neither of us knows anything really worth knowing: but whereas this man imagines he knows, without really knowing, I, knowing nothing, do not even suppose I know. On this one point, at any rate, I appear to be a little wiser than he, because I do not even think I know things about which I know nothing''.

Socrates tells us that it is important that we monitor the epistemic uncertainties we have. This means that we should not let statistics and traditional methods of decision analysis force us to pretend that we are in Type 1 situations when in fact we are glued in the opposite corner dealing with Type 4 decisions. Instead we shall give an as-frank-as-possible picture of the epistemic situation – trying our best to say what is known, describing the stability of our knowledge, and honestly conceding what we do not know.

This is the only way to avoid the prospect of the new technologies becoming stigmatized, and loss of public trust. The bottom line is that we have a moral obligation to give an honest, complete description of our epistemic state.

But how is this goal to be achieved? We must identify factors that produce epistemic uncertainty. Here are some important and well-known factors producing epistemic uncertainty and value uncertainty.

The unreliable research process. Research is a mechanism which, off-and-on, gives us incorrect or indeterminate results. Sometimes the machinery works flawlessly, sometimes chance has an unfavorable effect on the results, and sometimes the investigation does not work at all. To accept the results without asking whether or no they are the result of a working mechanism is to wink at all forms of epistemic uncertainty. A Socratic approach to risk analysis demands of us that we carefully scrutinize the strength of the different pieces of evidence we have, that we assess their evidentiary value and find out if they corroborate, or conflict with, each other.

The fact of irrationality. Contemporary psychologists have taught us a great deal about the way we perceive risks, and about the way affects and emotions influence our behavior. Their research has shown that, as decision makers, as risk-assessors, and as risk-controllers, we are shortsighted, one-eyed, and prone to serious errors of refraction. We generate too few, and too narrow, hypotheses. We gather information, or evidence, in favor of our guesses that is too narrow, readily available, and skewed in favor of preferred beliefs. Once we have a pet hypothesis, we look for confirmatory material, neglecting countervailing evidence. We are simply not rational – not in the way our theories of rationality (logic, probability and decision- making) assume, at any rate. This is an alarming fact, considering the serious risk-assessment and risk-management tasks that lie ahead of us. The fact of irrationality then pushes us in the direction of epistemic uncertainty. It sends us into Type 2 and Type 4 situations. This must be avoided, and it can only be avoided if we take a Socratic approach to what we are doing.

The choice and lack of theories. Traditionally, toxic risks are understood in terms of dose-response ratios. However, nanoparticles do not behave like the chemicals with which we are more familiar. For them, dose, understood in terms of concentration and mass, is no longer an applicable model. Factors like specific surface, dimension, number of particles, and surface properties are often far more important, and it must not be forgotten that the properties of the nanoparticles can change or become totally different when the particle size decreases or increases, or when particles lump or

interact. For similar reasons it seems that in the case of nanoparticles the step from in vitro to in vivo might be bigger than usual. We need new models. And, adding to the epistemic uncertainty, we do not know much about species differences. Today's deficiency in models, theories and data simply yields a considerable amount of epistemic uncertainty – an uncertainty that we should take into account when making decisions, but one that should also arouse our curiosity and inspire new research.

Unrealizable research. We might get caught in situations where, for moral or practical reasons, it is difficult to carry out controlled experimental studies. As a result we might have to rely on indirect evidence rather than solid, direct empirical evidence.

The best way to discover the effects of toxic substances on humans is to conduct experiments on humans, not animals. But that is often impossible, because the tests would be unethical, so we end up relying on animal experimentation. Our moral commitments, then, create uncertainty. They produce epistemic risk. But that is a fact that we have to accept.

Time, a problem. Time causes a particular problem when it comes to risk assessment and risk management. How do new treatments affect human beings over the longer term? It is difficult, sometimes impossible, to say with any precision what will happen in the long run. It is also, for as obvious reasons, difficult to study this type of problem or phenomenon.

Uncertainty and morality

Why, from a moral perspective, is it significant to know what we do not know – especially when it comes to risk assessment and risk management? Why is this the best way to do risk analysis?

First, **neglecting epistemic robustness and value uncertainties can lead to a loss of trust**. The patient may lose his or her trust in the doctor, the public lose their trust in health care, and the citizen lose trust in the society at large.

Psychologists have shown that trust is an important commodity— particularly when issues requiring effective risk communication and risk management are at stake. Among other things, it has been found that it takes time to win someone's trust, and even then it is very easily eradicated by one, or just a few, foolhardy acts.

There is also evidence that events eroding trust tend to be more "explicit" than the factors that create and maintain it, and this is quite simply due to our all-too-human readiness to spot another's mistakes and frequent tardiness in appreciating others' achievements. We can carry out a hundred good deeds without anyone noticing them; a single mistake is always eagerly noted. The argument concludes that one trust-breaking occurrence carries more weight than the trust-creating process itself. Given that bad news is considered more reliable than good news, the bad news carries enormous weight when it comes to the breakdown of trust. If you have been untrustworthy on one occasion, then, fairly or unfairly, you will be marked with the same untrustworthiness on another. It is difficult to gain and maintain trust when our knowledge is robust and our preferences clear. It is far more difficult when we have epistemic uncertainty. What we can do is to be honest. If we do not know. We should say it.

Second, **epistemic uncertainties can create unfairness**. If epistemic uncertainty and value uncertainty means that we can not use the traditional tools for risk analysis and rational decision making, for example well-known methods of cost-effectiveness analysis, then we should not pretend we have better knowledge and more precise values than we do have just to be able to use these methods. Doing so may, of course, produce unfairness.

Third, **epistemic uncertainty can do harm**. Not taking what we do not know into account can lead to the introduction of new methods that in fact harm patients.

Fourth, **we may do things that have no effect**.

Fifth, **epistemic uncertainty can lead to new methods are stigmatised**. This is a well-known effect. The classical example is GMO. The question is if this might happen in the general area of medically assisted reproduction (MAR).

Suggestion for further reading

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Registries and follow-up studies: a joint responsibility of the field - **Maryse Bonduelle (Belgium)**

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