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# Getting the measure of congenital, genetic and epigenetic risks for children born following ART: basic and clinical data

Special Interest Groups Safety and Quality in ART and Reproductive Genetics

9

1 July 2012
Istanbul, Turkey


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**Istanbul, Turkey**
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**Organised by**
**the Special Interest Groups Safety and Quality in ART & Reproductive Genetics**

# Contents

# Course coordinators

Petra de Sutter (Belgium) and Jan Kremer (The Netherlands)(SIG safety and quality in ART), Stéphane Viville (France) and Karen Sermon (Belgium) (SIG Reproductive Genetics)

# Course description

Safety is one of the major concerns in ART practice. The complexity of gametogenesis, fertilisation and early development renders risk assessment difficult, mainly because of the limits of our knowledge. The goal of this course is to update researchers and clinical practitioners on the latest developments in the field. Epigenetics and reproduction are more and more intertwined and recent insights have highlighted the importance of epigenetic phenomena even more. Imprinting and DNA methylation in relation to reproduction have been studied for some time now, so an update as to the importance for our patients is timely. MicroRNAs and non-coding RNAs are a major breakthrough in epigenetics of the last years, and have been found to contribute to almost all biological pathways, including gametogenesis and early development. Here too, an introduction of the recent findings will interest all participants. Another field that has transpired to be important in gametogenesis is the behaviour of retrotransposons, an overview of the major milestones in this research area will be given. Tackling the problem from the clinical side, an update will be brought on known risks in children born after ART, as obtained through epidemiological and clinical studies. Low birth weight, karyotype and congenital abnormalities and long term health implications will all be addressed. This is an advanced course for the interested professional: basic knowledge in genetics and embryology is necessary, but the talks will be mainly informative and educative rather than focusing on latest findings or finer points of basic research and will be of clinical relevance.

# Target audience

Reproductive physicians, embryologists and basic scientists in reproduction and development.

# Scientific programme

**Epigenetics: basic and clinical data**

Chair: Stephane Viville (France) and Karen Sermon (Belgium)

| | |
|---|---|
| 09.00 - 09.30 | Disturbed genetic imprinting and IVF: truth or myth? – ***Jorn Walter (Germany)*** |
| 09.30 - 09.45 | Discussion |
| 09.45 - 10.15 | Retrotransposons: a new player in gametogenesis – ***Deborah Bourc'his (France)*** |
| 10.15 - 10.30 | Discussion |
| 10.30 - 11.00 | Coffee break |
| 11.00 - 11.30 | miRNA: from junk DNA to major regulatory mechanism – ***Olivier Voinnet (Switzerland)*** |
| 11.30 - 11.45 | Discussion |
| 11.45 - 12.15 | Clinical aspects of epigenetic deregulation in IVF – ***Aafke van Montfoort (The Netherlands)*** |
| 12.15 - 12.30 | Discussion |
| 12.30 - 13.30 | Lunch |

**Health risks for children following ART**

Chair: Jan Kremer (The Netherlands) and Petra De Sutter (Belgium)

| | |
|---|---|
| 13.30 - 14.00 | Karyotype abnormalities in children born after ART – ***Maryse Bonduelle (Belgium)*** |
| 14.00 - 14.15 | Discussion |
| 14.15 - 14.45 | Congenital anomalies following ART – ***Karl Nygren (Sweden*****)** |
| 14.45 - 15.00 | Discussion |
| 15.00 - 15.30 | Coffee break |
| 15.30 - 16.00 | Low-birth weight after ART – ***Anja Pinborg (Denmark)*** |
| 16.00 - 16.15 | Discussion |
| 16.15 - 16.45 | Long term health implications of children after IVF and ICSI – ***Alastair Sutcliffe (United Kingdom)*** |
| 16.45 - 17.00 | Discussion |

# EPIGENETICS AND ITS ROLE IN EARLY DEVELOPMENT

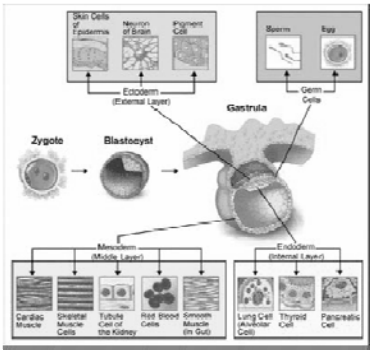
29/06/2012

PROF. DR. JÖRN WALTER
EPIGENETICS WORKGROUP
UNIVERSITÄT DES SAARLANDES

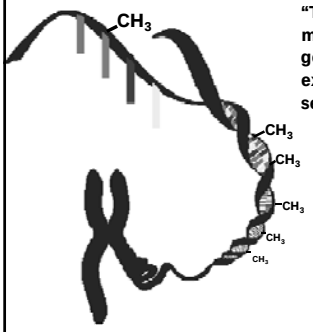
## WHAT IS EPIGENETICS?

CONRAD HAL WADDINGTON
(1905–1975)

## WHAT IS EPIGENETICS?

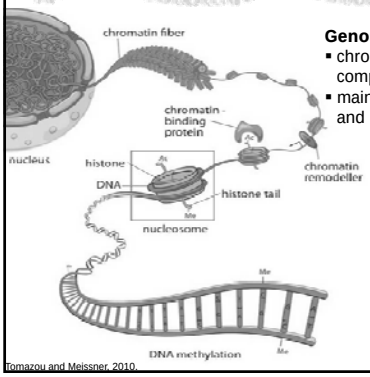


## WHAT IS EPIGENETICS?




**"The study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence"** (Riggs and Colleagues, 1996)

## EPIGENETIC CONTROL IS IMPORTANT FOR:

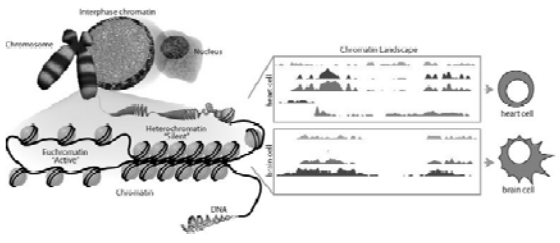



**Genome structure and function:**
- chromosome organization/ compaction
- maintenance of nuclear integrity and identity (mitosis/meiosis)

Tomazou and Meissner, 2010.

## EPIGENETIC CONTROL IS IMPORTANT FOR:

**Transcriptional memory and control:**
- Long term control of developmental processes, e.g. silencing of developmental regulators.




Sha and Boyer, StemBook, 2009.

## EPIGENETIC CONTROL IS IMPORTANT FOR:

**Genomic defence:**

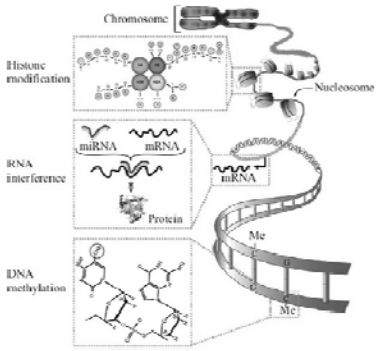
- Silencing of retroviral/transposable elements, to prevent reactivation of endoparasitic sequences that cause chromosomal instability, translocations and gene disruption.


Methylated repetitive sequence


Unmethylated repetitive sequence

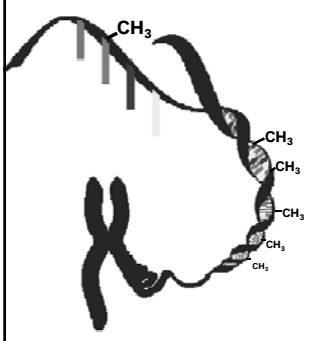
Portela and Esteller, *Nature Biotechnology*, 2010.

## EPIGENETIC MECHANISMS




Kim et al., *Pulmonary Circulation*, 2010.

## DNA METHYLATION

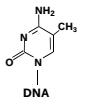



- **Refers to the enzymatic transfer of a methyl group to specific nucleotides within the DNA sequence**
- In mammals: mainly cytosine methylation in a CpG context

## *DNA METHYLATION*

**DNA-Methyltransferases**
DNMT1 (maintenance)
DNMT3A (*de novo*)
DNMT3B (*de novo*)

**Cytosine** → **5methylCytosine**




**SAM** **SAH**

Tahiliani et al. 2009
Kriaucionis and Heintz 2009

## *DNA METHYLATION AND TRANSCRIPTION*

CpG island methylation is generally associated with gene silencing!

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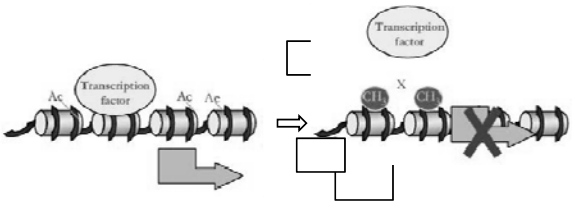
- Direct: Methylation of a CpG in the recognition site of a transcription factor inhibits binding resulting in gene silencing.

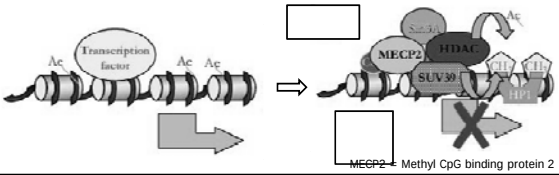


## *DNA METHYLATION AND TRANSCRIPTION*

CpG island methylation is generally associated with gene silencing!

Mechanisms:

- Direct: Methylation of a CpG in the recognition site of a transcription factor inhibits binding resulting in gene silencing.
- Indirect: Methylated DNA promotes recruitment of methyl-CpG-binding domain (MBD) proteins. Subsequently, MBD proteins recruit histone modifying and chromatin-remodeling complexes to the methylated sites.




MECP2 = Methyl CpG binding protein 2

## *DNA METHYLATION*

**DNA-Methyltransferases**
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DNMT3A (*de novo*)
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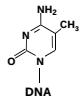
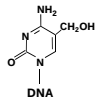
**Cytosine** → **5$^{methyl}$Cytosine** (SAM → SAH)




**Tet 1-3 oxygenases**

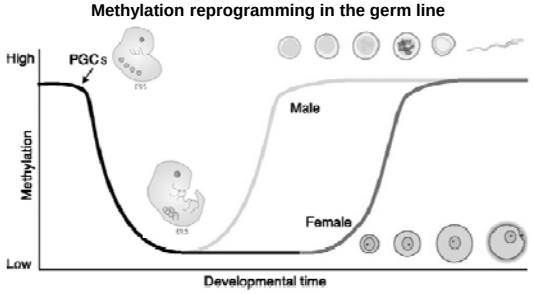
**(5$^{hydroxymethyl}$Cytosine)**




Tahiliani et al. 2009
Kriaucionis and Heintz 2009

## *DNA METHYLATION AND DEVELOPMENT*

**Methylation reprogramming in the germ line**




**PGCs = Primordial germ cells**

Reik *et al., Science*, 2001

## GENOMIC IMPRINTING

Genomic imprinting is an epigenetic phenomenon by which the expression of a gene is determined by its parental origin.

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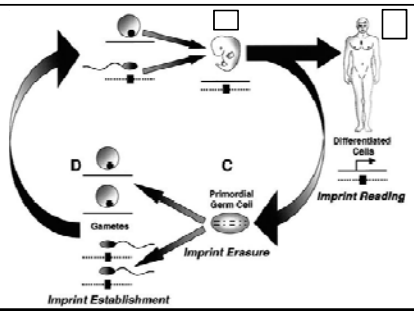
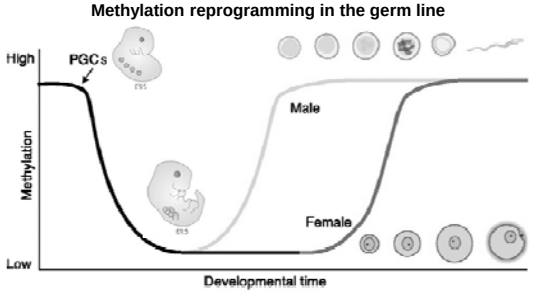



## DNA METHYLATION AND DEVELOPMENT

**Methylation reprogramming in the germ line**

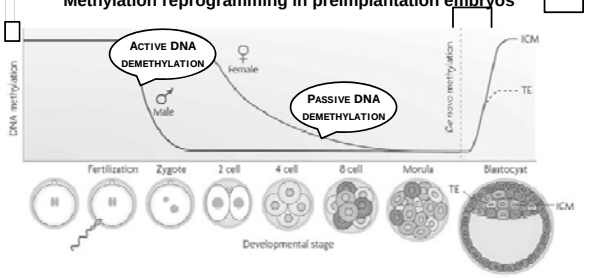



**PGCs = Primordial germ cells**

Reik *et al.*, *Science*, 2001

## *DNA METHYLATION AND DEVELOPMENT*

**Methylation reprogramming in preimplantation embryos**




Wu & Zhang, *Nature Reviews Molecular Cell Biology*, 2010

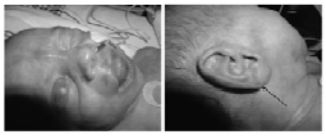
## *DNA METHYLATION AND DEVELOPMENTAL MISTAKES*

**DNA METHYLATION STUDIES ON IMPRINTED LOCI IN A MALE MONOZYGOTIC TWIN PAIR DISCORDANT FOR BECKWITH–WIEDEMANN SYNDROME**

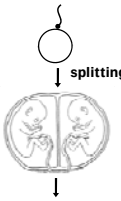
Tierling et al. *Clinical Genetics, 2011*

## *MZ MONOCHORIONIC MALE TWIN PAIR DISCORDANT FOR BWS*

- Incidence BWS: ~1 in 13,000 live births
- (mild) phenotype affected twin:
  - hypoglycaemia (at birth)
  - large protruding tongue
  - indented ears
  - mid-face hypoplasia
  - facial hemangioma



**MZ MC twins**

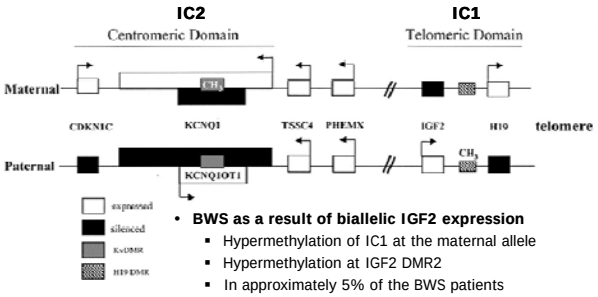


**splitting**

**Genetically identical**

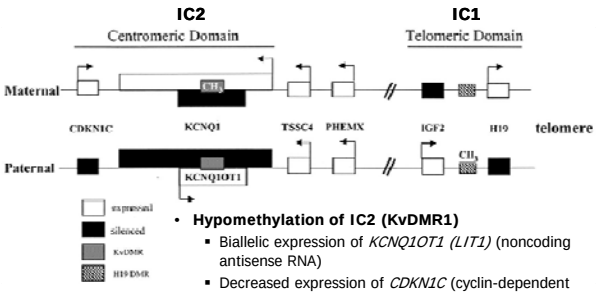
- 66% of all MZ twins
- 4-8 day post fertilisation
- 1 chorionic membrane
- 1 (shared) placenta
- share blood supply during prenatal development

## BECKWITH-WIEDEMANN SYNDROME 11P15



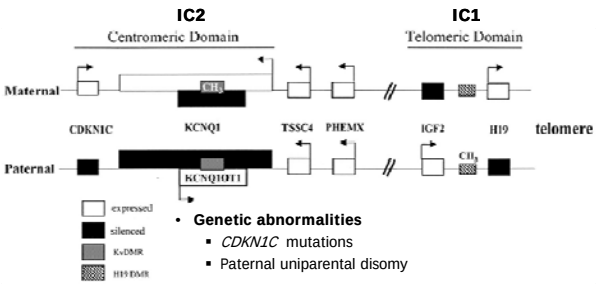

- **BWS as a result of biallelic IGF2 expression**
  - Hypermethylation of IC1 at the maternal allele
  - Hypermethylation at IGF2 DMR2
  - In approximately 5% of the BWS patients methylation defects at IC1 are observed

## BECKWITH-WIEDEMANN SYNDROME 11P15



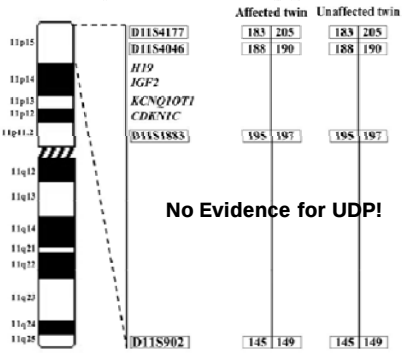

- **Hypomethylation of IC2 (KvDMR1)**
  - Biallelic expression of *KCNQ1OT1 (LIT1)* (noncoding antisense RNA)
  - Decreased expression of *CDKN1C* (cyclin-dependent kinase inhibitor 1C): cell cycle inhibitor
  - Most common aberration in BWS!

## BECKWITH-WIEDEMANN SYNDROME 11P15




- **Genetic abnormalities**
  - *CDKN1C* mutations
  - Paternal uniparental disomy
  - *KCNQ1OT1* microdeletions

## *UNIPARENTAL DISOMY (UPD) ANALYSIS FOR MICROSATELLITE MARKERS ON CHROMOSOME 11P15*




**No Evidence for UDP!**

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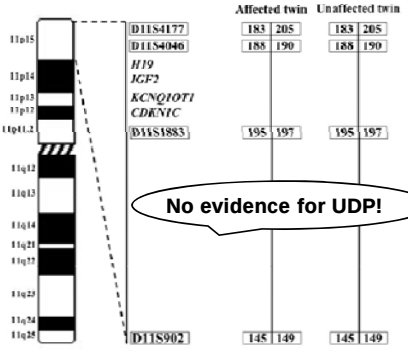


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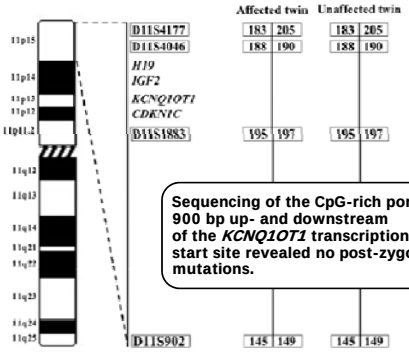
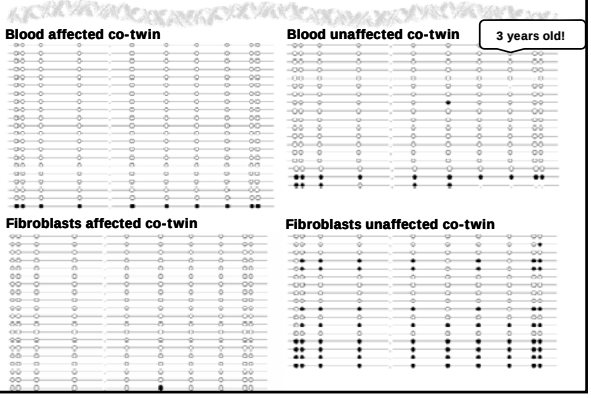



**Sequencing of the CpG-rich portion 900 bp up- and downstream of the *KCNQ1OT1* transcriptional start site revealed no post-zygotic mutations.**

## *BISULFITE SEQUENCING OF KVDMR1*

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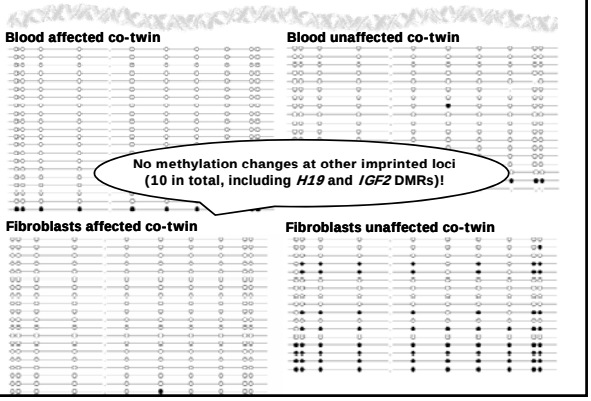



## *DNA METHYLATION AND DEVELOPMENTAL MISTAKES*

- Our results point to an exclusive role of KvDMR1 loss of methylation (LOM) in developing the BWS phenotype in the affected twin.
- **The discordant phenotype seems to be a result of a failure of the DNA methylation maintenance machinery during very early embryonic development!**
- Incidence of MZ twinning is dramatically increased in BWS and the majority displays KvDMR1 LOM!

## *REPROGRAMMING AND ART*

Incidence of imprinting disorders, especially BWS, is increased in children conceived by ART.

↓

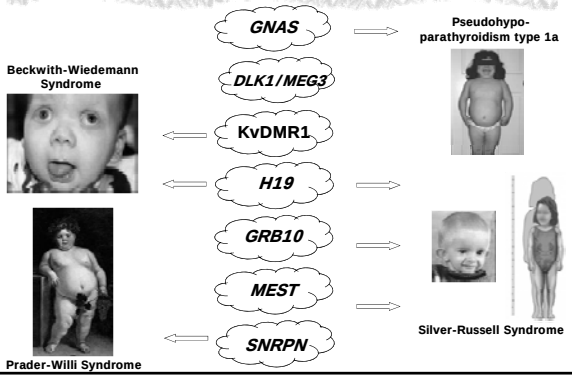
**DO ART PROCEDURES CAUSE AN INCREASED INSTABILITY OF GENOMIC IMPRINTS?**

Tierling et al. *Journal of Medical Genetics*, 2010

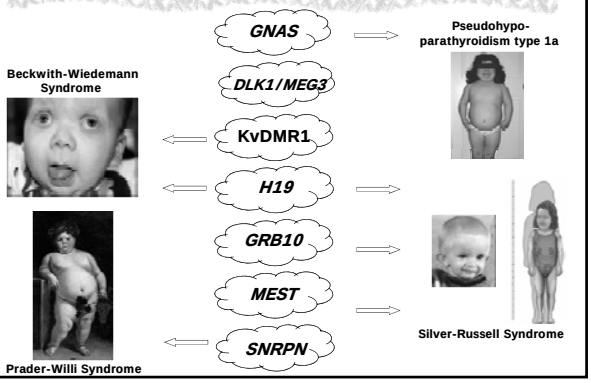
## *STUDY SAMPLE*

| Characteristic | Spontaneous | IVF | ICSI | p |
|---|---|---|---|---|
| Neonatal | | | | |
| N | 73 | 35 | 77 | |
| Gender: male (%) | 30 (41.1) | 20 (57.1) | 35 (45.5) | 0.29 |
| Twins (%) | 0 (0) | 20 (57.1)* | 14 (18.2)†,‡ | <0.0001 |
| Gestational age (weeks) | 39.5±1.5 | 38.2±2.0* | 38.8±2.1† | 0.004 |
| Birth weight (g) | 3399±504 | 2853±628* | 3142±590† | 0.008 |
| Birth length (cm) | 52.0±3.5 | 49.3±2.9* | 50.7±2.8† | 0.01 |
| Maternal | | | | |
| n | 73 | 25 | 70 | |
| Parity: primipara (%) | 39 (53.4) | 21 (84.0)* | 59 (84.3)† | <.0001 |
| Gravida: primigravida (%) | 35 (47.9) | 14 (56.0) | 43 (61.4) | 0.27 |
| Maternal age (years) | 31.7±5.7 | 34.8±4.0* | 35.3±4.3† | 0.0002 |
| Maternal body height (cm) | 167.8±7.0 | 167.3±6.7 | 168.8±5.9 | 0.51 |
| Maternal body mass (kg)§ | 63.3±12.8 | 64.0±12.2 | 66.2±10.5 | 0.11 |
| Maternal BMI (kg/m²)§ | 22.4±3.9 | 22.9±4.0 | 23.2±3.6 | 0.27 |

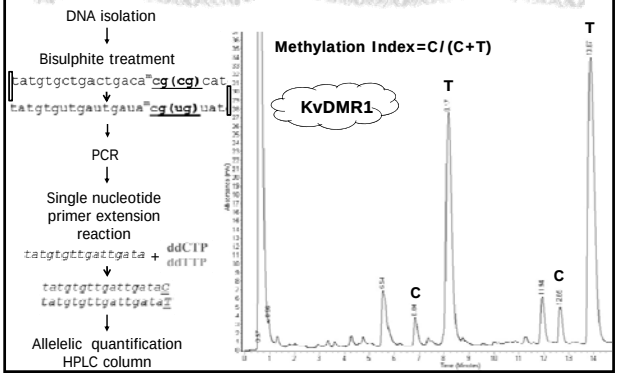
## *STUDIED LOCI*



## STUDIED LOCI




## METHOD




## RESULTS

| DMR | Maternal peripheral blood | | | | Umbilical cord blood | | | | Amnion/chorion tissue | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Spontaneous | IVF | HCSI | p | Spontaneous | IVF | HCSI | p | Spontaneous | IVF | HCSI | p |
| n° | 70 | 25 | 69 | | 71 | 25 | 72 | | 71 | 25 | 71 | |
| KvDMR1 | 0.50 ± 0.05 | 0.51 ± 0.03 | 0.52 ± 0.04 | 0.07 | 0.51 ± 0.05 | 0.52 ± 0.03 | 0.51 ± 0.04 | 0.72 | 0.51 ± 0.05 | 0.52 ± 0.04 | 0.52 ± 0.04 | 0.66 † |
| H19 | 0.41 ± 0.03 | 0.42 ± 0.03 | 0.42 ± 0.03 | 0.34 | 0.41 ± 0.03 | 0.41 ± 0.03 | 0.42 ± 0.03 | 0.44 | 0.44 ± 0.04 | 0.43 ± 0.05 | 0.43 ± 0.04 | 0.35 † |
| SNRPN | 0.42 ± 0.03 | 0.42 ± 0.03 | 0.43 ± 0.03 | 0.61 | 0.42 ± 0.02 | 0.42 ± 0.03 | 0.42 ± 0.03 | 0.25 | 0.43 ± 0.03 | 0.43 ± 0.04 | 0.43 ± 0.03 | 0.76 |
| MEST | 0.38 ± 0.04 | 0.40 ± 0.03 | 0.37 ± 0.04 | **0.003** | 0.38 ± 0.03 | 0.41 ± 0.03 | 0.38 ± 0.03 | **0.006** | 0.37 ± 0.04 | 0.39 ± 0.05 | 0.36 ± 0.05 | 0.05 |
| GRB10 | 0.49 ± 0.05 | 0.47 ± 0.03 | 0.49 ± 0.05 | 0.04 ‡ | 0.66 ± 0.06 | 0.65 ± 0.05 | 0.65 ± 0.05 | 0.86 | 0.61 ± 0.08 | 0.60 ± 0.08 | 0.62 ± 0.09 | 0.76 ± |
| DLK1/MEG3 IG-DMR | 0.51 ± 0.03 | 0.51 ± 0.03 | 0.52 ± 0.03 | 0.84 | 0.53 ± 0.02 | 0.52 ± 0.04 | 0.51 ± 0.02 | 0.02 | 0.48 ± 0.04 | 0.48 ± 0.05 | 0.48 ± 0.04 | 0.88 |
| GNAS NESP55 | 0.45 ± 0.06 | 0.44 ± 0.06 | 0.47 ± 0.06 | 0.07 | 0.45 ± 0.06 | 0.44 ± 0.06 | 0.47 ± 0.06 | 0.04 | 0.47 ± 0.07 | 0.46 ± 0.08 | 0.49 ± 0.06 | 0.61 |
| GNAS NESPlex | 0.45 ± 0.06 | 0.45 ± 0.06 | 0.45 ± 0.06 | 0.92 | 0.45 ± 0.07 | 0.45 ± 0.06 | 0.47 ± 0.06 | 0.02 | 0.43 ± 0.09 | 0.44 ± 0.10 | 0.41 ± 0.09 | 0.31 |
| GNAS XL alpha-s | 0.46 ± 0.03 | 0.46 ± 0.03 | 0.47 ± 0.03 | 0.21 | 0.44 ± 0.03 | 0.45 ± 0.03 | 0.46 ± 0.03 | 0.01 | 0.50 ± 0.06 | 0.51 ± 0.06 | 0.50 ± 0.06 | 0.82 † |
| GNAS Exon1A | 0.42 ± 0.04 | 0.43 ± 0.03 | 0.42 ± 0.03 | 0.50 | 0.42 ± 0.04 | 0.43 ± 0.03 | 0.41 ± 0.04 | 0.04 | 0.37 ± 0.07 | 0.40 ± 0.06 | 0.38 ± 0.06 | 0.21 |

## CONCLUSIONS

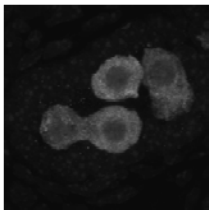
- Methylation at all ten DMRs (except MEST) are highly stable in umbilical cord blood and placenta of 185 children independent of conception type.
- Our data suggest that ART (standard conditions) do not cause an increased risk on imprint instability.

## DISCUSSION

- Study limitations
  - Technical: coverage and sensitivity
  - Sample size
- Other factors than ART procedures
  - Reprogramming in the germ line

## ACKNOWLEDGEMENTS

UNIVERSITÄT DES SAARLANDES

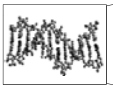


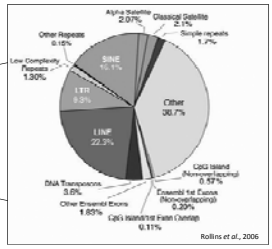
Retrotransposons: new players in gametogenesis

Déborah BOURC'HIS
Institut Curie, Paris
Dpt of Genetics and Developmental Biology

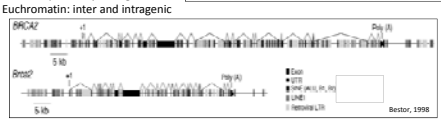
40% of the mammalian genome
made of transposable elements (at least…)

Genetic material





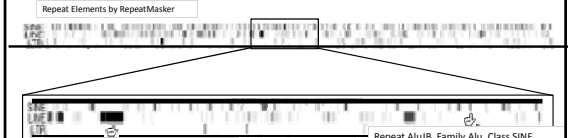

Close proximity with genes
Euchromatin: inter and intragenic




UCSC Genome Browser

Reference genes

Repeat Elements by RepeatMasker



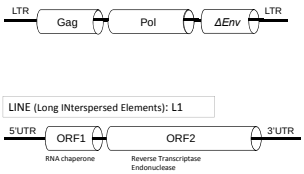
Repeat L1HS, Family L1, Class LINE
Divergence : 0.6%
Genomic size: 3371bp
View DNA for this feature
…

Repeat AluJB, Family Alu, Class SINE
Divergence : 18%
Genomic size: 312bp
View DNA for this feature
…

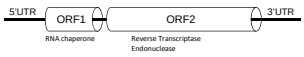
## Diverse families of mobile elements coexist within the same genome

DNA transposons: 3%, mostly fossils
Retrotransposons: at least 40%, LTR and non-LTR

| | Size | Copy number | Genome fraction |
|---|---|---|---|
| LTR-retrotransposons (classes I, II and III): IAP, ERV, Etn… | 5-7kb | >100 000 | 10% |
| LINE (Long INterspersed Elements): L1 | 6kb | >500 000 | 19% |
| SINE (Short INterspersed Elements): B1, B2 | 100bp | >900 000 | 2.6% |



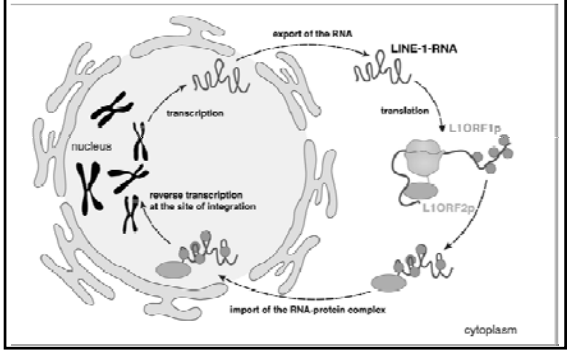

LINE (Long INterspersed Elements): L1

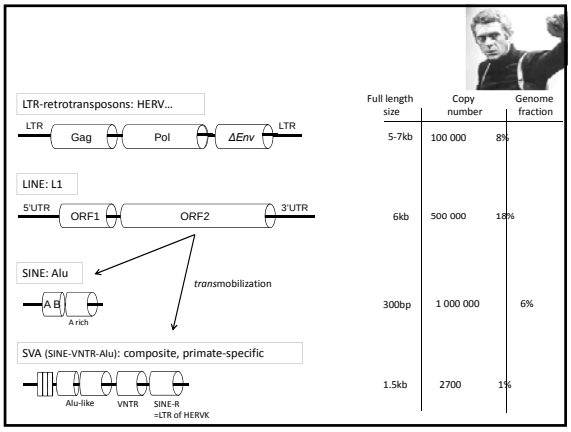



**Master transposons of mammalian genomes**
- Genome mass (20%)
- Ability to mobilize themselves (autonomous) and other transposons types (SINEs)

## Life cycle of a retrotransposon: LINE-1

TPRT: Target-site Primed Reverse Transcription






## Potential outcomes of transposon presence and activity in mammalian genomes

NEUTRAL → Most of the time

BENEFICIAL → Long term, upon purifying selection

DELETERIOUS → Short term

## Transposons as a source of genetic innovation and diversification

Transposon sequences as a source of genetic diversity

1-New coding sequences derived from transposon proteins
*Innate roles in nucleic acid biology (DNA binding, nicking, copying…) and protein processing and interactions*

« Molecular domestication »: Transposon functions captured by the cell
→RAG1: V(D)J joining, *DNA transposase*
→SYNCYTINS: fusion proteins for syncytiotrophoblast formation, *retroviral enveloppe*

2- New regulatory sequences provided by transposon sequences
*Widespread location and diversity*

→Binding sites for p53, Sox2 and CTCF embedded in distinct transposon types

## Transposons as a source of genetic innovation and diversification

Transposon activity as a source of genetic diversity

3- Duplication created by retrotransposons
*Cellular mRNAs highjacked by retrotransposon machinery and insertion into a new genomic location = intronless*

➔TRIM5/cyclophilin A fusion: resistance of the owl monkey to HIV infection
➔Number of imprinted genes generated this way

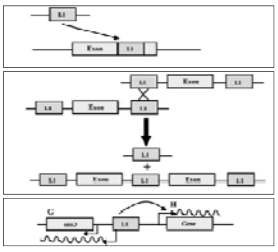
## Deleterious impact on genome architecture and function

Insertional mutagenesis
in a key gene

Unequal homologous recombination
= chromosome rearrangements

Position effects on genic expression
Abnormal expression pattern

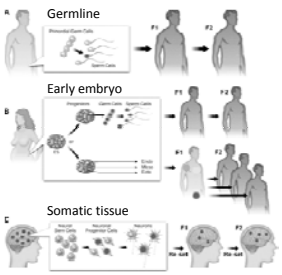
Etc….



Somatic cells: cancer
Germ line: genetic disorders

Ostertag and Kazazian, 2001

## Evolutionary consequences of retrotransposition in different cell types




New insertion passed to new generation

New insertion passed to new generation

Genetic mosaicism

Muotri *et al*.,2007

## Transposons and diseases

Genetic effects:

-L1, Alu and SVA are responsible for 0.27% of all human mutations to date (118/44,000) by insertion into key genes (Callinan and Batzer, 2006)
First case: 1991, Hemophilia A due to L1 insertion in factor VIII gene ()

-50 diseases created by ectopic recombination between repeats (Alu)

Epigenetic effects:

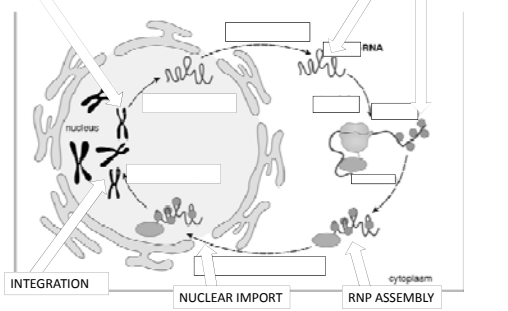
- Global loss of DNA methylation at transposons in cancer and aging cells (*erosion of repression*) (Iskow *et al.*, Cell 2010)
May participate to cancer onset and progression

-May also act as methylation variant, pure epigenetic effect
Childhood obesity resulting from repression of the POMC gene by methylation gain of an intragenic Alu. (Kuehnen *et al.*, Plos Genet 2012)

## Retrotransposon restriction

TRANSCRIPTION

POST-TRANSCRIPTION




INTEGRATION

NUCLEAR IMPORT

RNP ASSEMBLY

## Retrotransposon restriction

TRANSCRIPTION

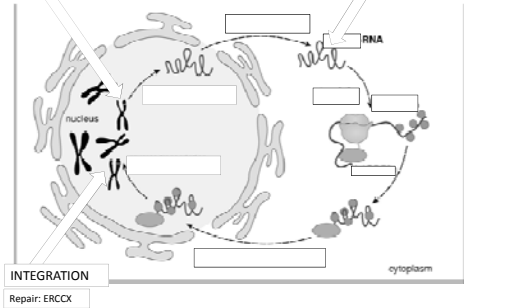
DNA methylation: DNMT3A, DNMT3L

H3K9 methylation: SetB1, KAP1

POST-TRANSCRIPTION

RNA editing: APOBECs

RNA interference: PIWI/piRNA




INTEGRATION

Repair: ERCCX

## The germline as an ecological niche for transposons

Transposons are non infectious: can not be passed horizontally, from cell to cell

Transposons strictly rely on a vertical mode of inheritance from generation to generation

↓

Their propagation requires some level of activity in the germline

↓

To maintain the fitness of a population, specific restricting strategies developed by the germline

## Germline-specific restriction factors

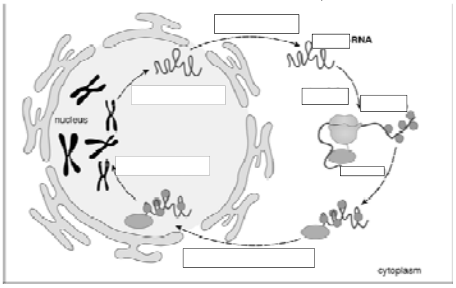
TRANSCRIPTION

DNA methylation: DNMT3L

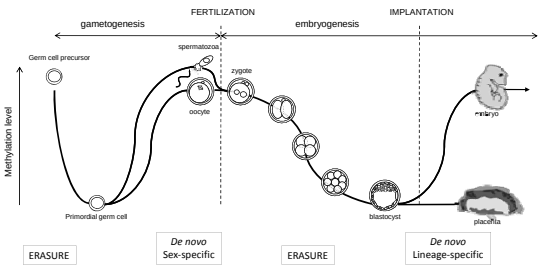
cooperation

POST-TRANSCRIPTION

RNA interference: PIWI/piRNA




## Dynamic changes of transposon methylation during development



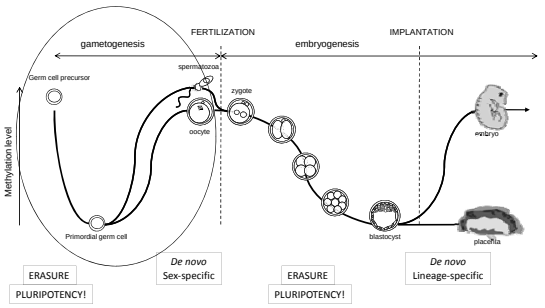

ERASURE

*De novo* Sex-specific

ERASURE

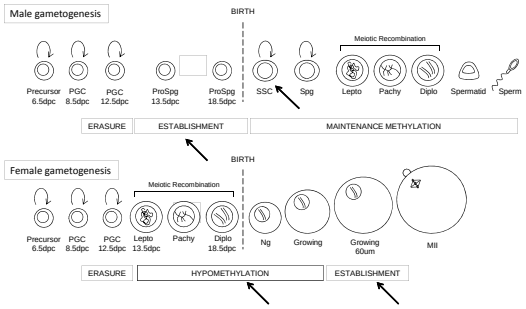
*De novo* Lineage-specific

## Dynamic changes of transposon methylation during development




Epigenetic relaxation coincident with pluripotency states

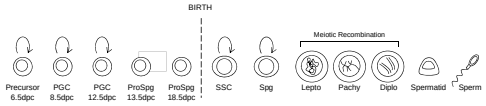
## Sexual dimorphism in gametogenesis kinetics and epigenetic setting




Late establishment of methylation in oocytes, transposons stay hypomethylated for most of the reproductive life

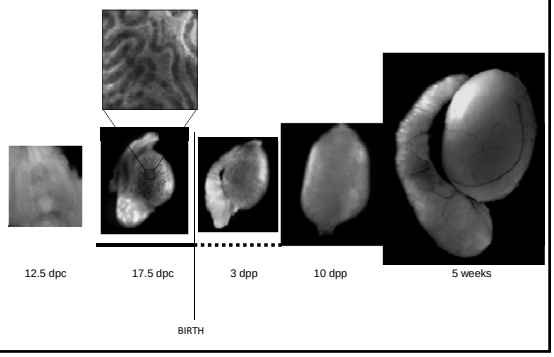
Existence of life-long proliferative stem cells in the male germline

## Male fetal germline
## cooperation between piRNA and DNA methylation machineries

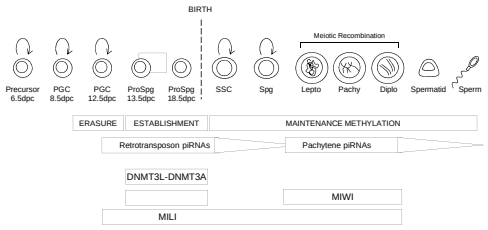



DNMT3L-DNMT3A

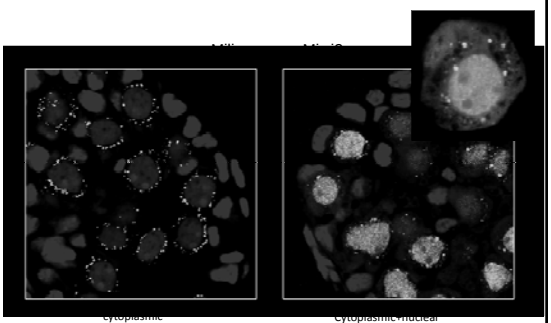
*Dnmt3L* specifically expressed in germ cells
*perinatal testis*




Male fetal germline
cooperation between piRNA and DNA methylation machineries

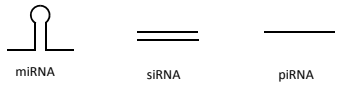



Mili-GFP and Miwi2-GFP expression in 16.5-17.5 dpc testes



A. Aravin

## 2006: The year of the piRNAs




Bigger: 26-30 nt (21-24 nt for miRNAs and siRNAs)

Single-stranded

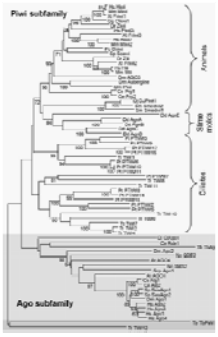
No secondary structure (distinct from miRNAs)

DICER independent (distinct from miRNAs and siRNAs)

Loaded by PIWI proteins (piRNA= PIWI-interacting RNAs)

Aravin *et al.*, 2006
Girard *et al.*, 2006
Grivna *et al.*, 2006
Laub *et al.*, 2006

## Piwi proteins: subfamily of Argonautes




-Argonaute family: RISC
(RNA induced silencing complex)
PAZ domain: ss nucleic acid binding
PIWI domain: RNase H fold, slicing activity

- present in a variety of species
Piwi, Miwi, Hiwi, Ziwi, Riwi, Chiwi, Friwi, Seawi….
except in plants and yeast
Mouse: 3 genes: Miwi, Mili, Miwi2
Human: 4 genes: PiwiL1 to L4

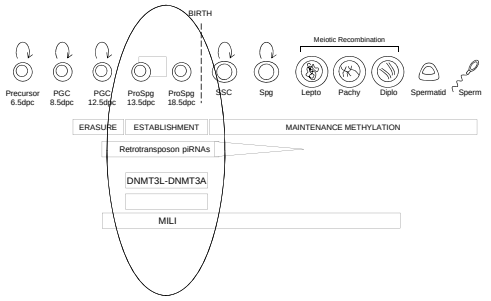
- germ line role: maintenance of germinal stem cells

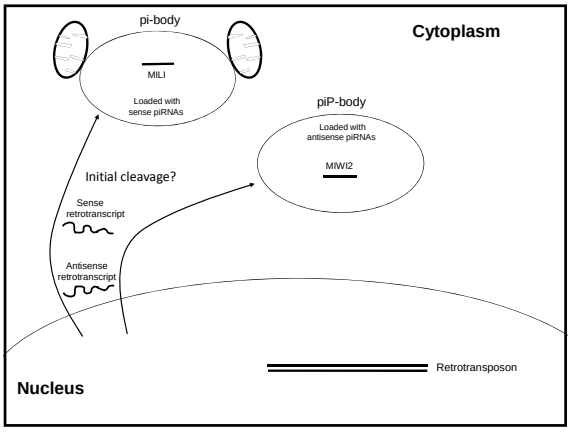
-associated with a specific class of small RNAs,
(Production and loading)
piRNAs (mammals)
rasiRNAs (flies)
scnRNAs (Tetrahymena)

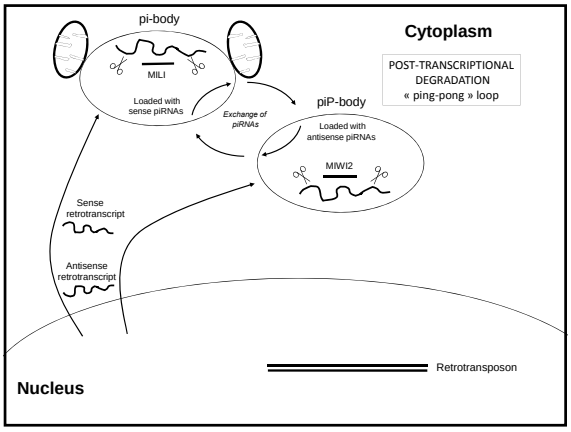
-Most often derive from retrotransposons
(not pachytene piRNAs)

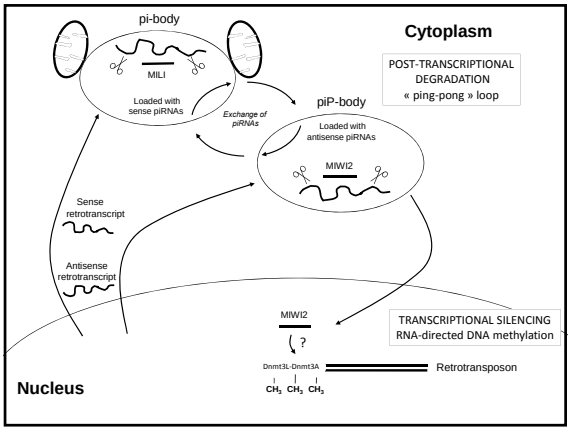
Seto *et al.*, 2007

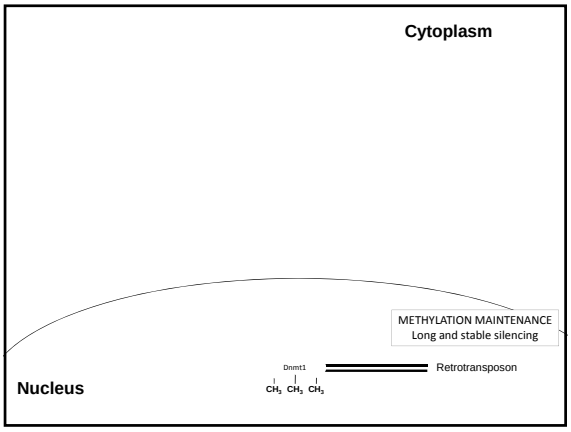
## Male fetal germline
## cooperation between piRNA and DNA methylation machineries

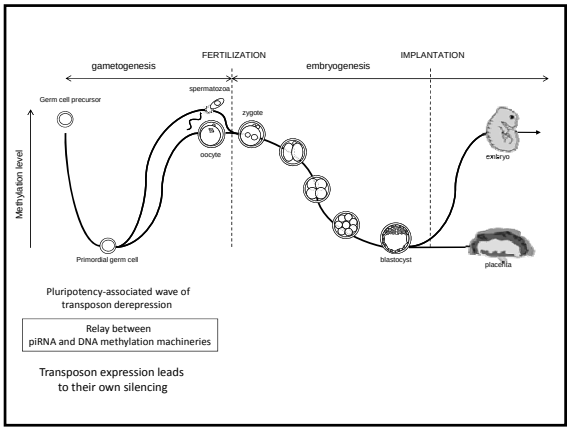


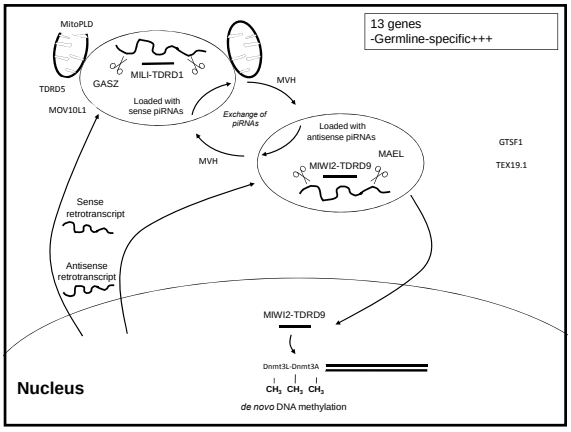

pi-body
Cytoplasm
MILI
Loaded with
sense piRNAs
piP-body
Loaded with
antisense piRNAs
MIWI2
Initial cleavage?
Sense
retrotranscript
Antisense
retrotranscript
Retrotransposon
Nucleus

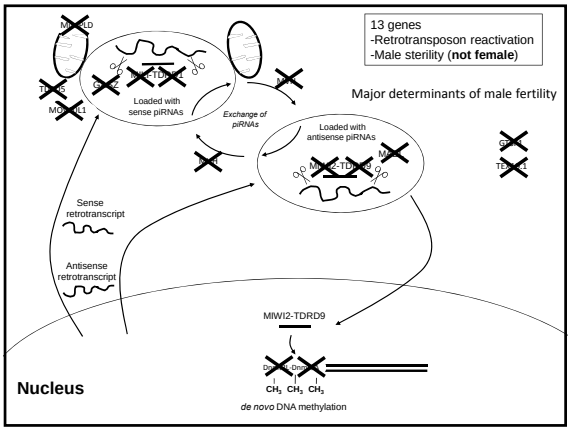

pi-body
Cytoplasm
POST-TRANSCRIPTIONAL
DEGRADATION
« ping-pong » loop
MILI
Loaded with
sense piRNAs
Exchange of
piRNAs
piP-body
Loaded with
antisense piRNAs
MIWI2
Sense
retrotranscript
Antisense
retrotranscript
Retrotransposon
Nucleus

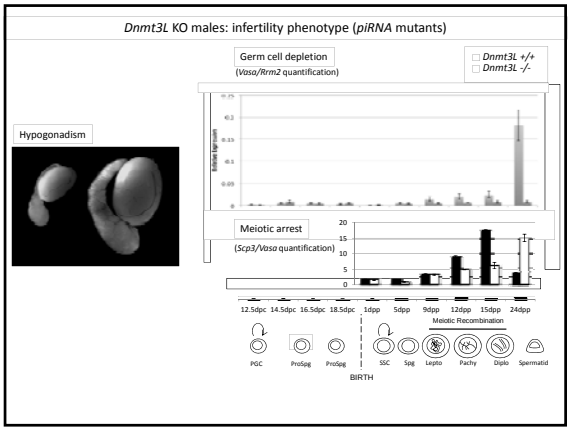

pi-body
Cytoplasm
POST-TRANSCRIPTIONAL
DEGRADATION
« ping-pong » loop
MILI
Loaded with
sense piRNAs
Exchange of
piRNAs
piP-body
Loaded with
antisense piRNAs
MIWI2
Sense
retrotranscript
Antisense
retrotranscript
MIWI2
?
TRANSCRIPTIONAL SILENCING
RNA-directed DNA methylation
Dnmt3L-Dnmt3A
CH3 CH3 CH3
Retrotransposon
Nucleus

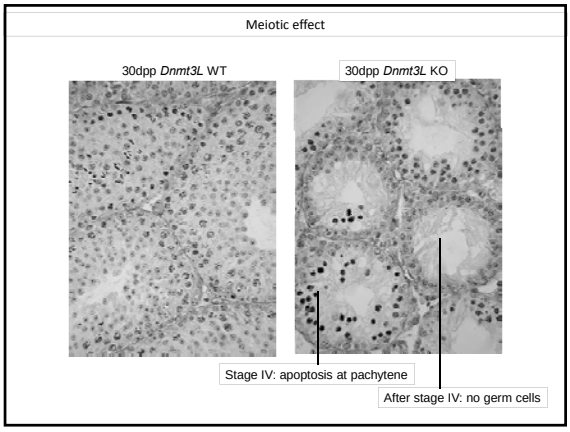

Cytoplasm
METHYLATION MAINTENANCE
Long and stable silencing
Dnmt1
Retrotransposon
Nucleus


FERTILIZATION
IMPLANTATION
gametogenesis
embryogenesis
spermatozoa
Germ cell precursor
zygote
oocyte
exsiryo
Methylation level
Primordial germ cell
blastocyst
placenta
Pluripotency-associated wave of transposon derepression
Relay between piRNA and DNA methylation machineries
Transposon expression leads to their own silencing


13 genes
-Germline-specific+++
MitoPLD
TDRD5
MOV10L1
GASZ
MILI-TDRD1
Loaded with sense piRNAs
MVH
Exchange of piRNAs
Loaded with antisense piRNAs
MAEL
MIWI2-TDRD9
GTSF1
TEX19.1
Sense retrotranscript
Antisense retrotranscript
MIWI2-TDRD9
Dnmt3L-Dnmt3A
de novo DNA methylation
Nucleus


13 genes
-Retrotransposon reactivation
-Male sterility (**not female**)
Major determinants of male fertility
Loaded with sense piRNAs
Exchange of piRNAs
Loaded with antisense piRNAs
Sense retrotranscript
Antisense retrotranscript
MIWI2-TDRD9
Nucleus
de novo DNA methylation

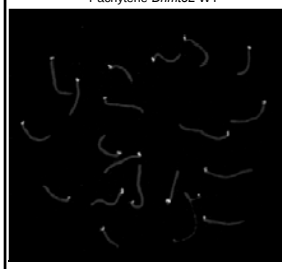

*Dnmt3L* KO males: infertility phenotype (*piRNA* mutants)
Germ cell depletion
(*Vasa/Rrm2* quantification)
*Dnmt3L +/+*
*Dnmt3L -/-*
Hypogonadism
Meiotic arrest
(*Scp3/Vasa* quantification)
12.5dpc 14.5dpc 16.5dpc 18.5dpc 1dpp 5dpp 9dpp 12dpp 15dpp 24dpp
Meiotic Recombination
PGC ProSpg ProSpg SSC Spg Lepto Pachy Diplo Spermatid
BIRTH


Meiotic effect
30dpp *Dnmt3L* WT
30dpp *Dnmt3L* KO
Stage IV: apoptosis at pachytene
After stage IV: no germ cells

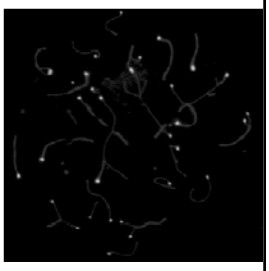
MEIOTIC EFFECT

Scp3: Synaptonemal complex
CREST: centromeres

Pachytene *Dnmt3L* WT

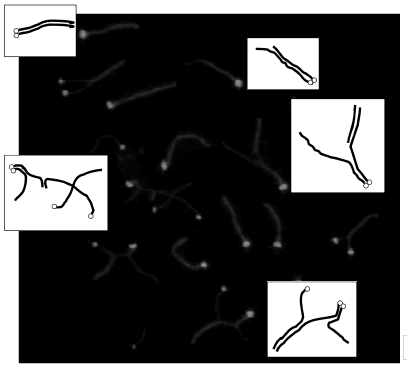


"Pachytene" *Dnmt3L* KO



19 pairs of autosomes
1 sex body (X and Y chromosomes)

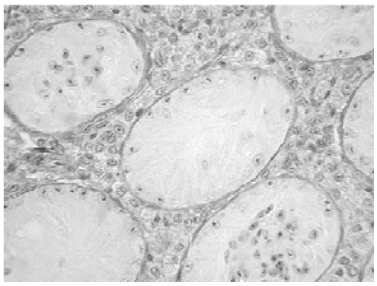
Non-homologous associations



*Dnmt3L-/-*

Progressive loss of germ cells
Spermatogonial stem cells (SSC): self-renewal

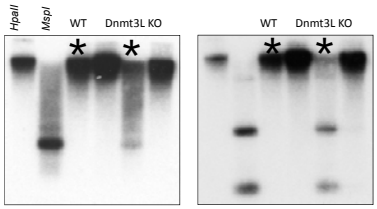
5 months *Dnmt3L* KO testis



No germ cells in seminiferous tubules
"Sertoli-only" phenotype

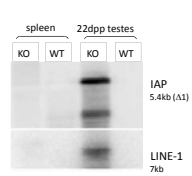
## Methylation defect at retrotransposons in *Dnmt3L* KO

-Flow-sorted germ cells (GCNA antibody)
-Methyl-sensitive Southern blot



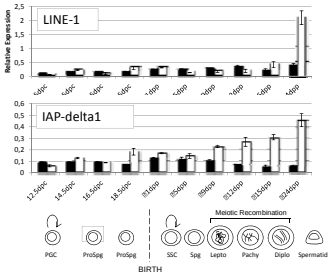

IAP 5'LTR LINE-1 5'UTR

## Reactivation of hypomethylated transposons



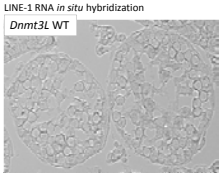


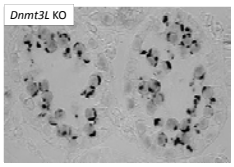
Electron microscopy
Perinuclear accumulation of dense material

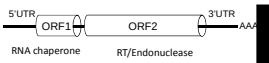



## Perinuclear accumulation of LINE-1 transcripts and proteins

LINE-1 RNA *in situ* hybridization

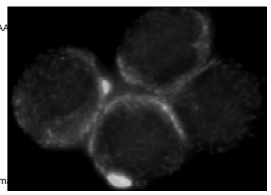


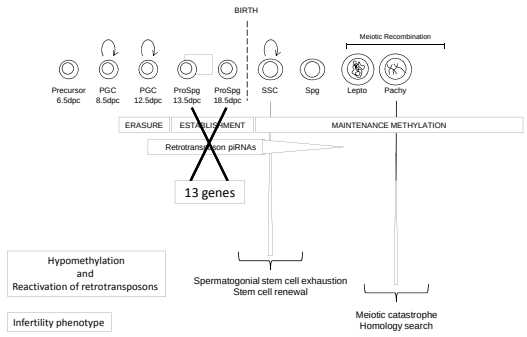

RNA chaperone RT/Endonuclease

Ability to retrotranspose?
Genetic vs epigenetic effect?



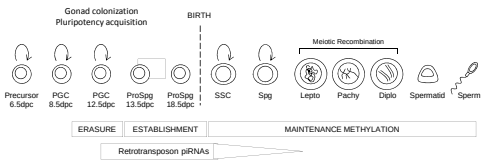
LINE ORF1
Scp3 (sperm

## DNA methylation/piRNA pathway: male sterility phenotype




## In human?

## 




Fetal period of male germ cell development crucial for the epigenetic setting of male gametes

>> piRNA/DNA methylation pathway

>> integrity of the genetic material through transposon control

>> safeguards of male fertility, female gametogenesis not sensitive

>> more subtle effects: risk of hereditary insertional mutations

>> Major cause of infertility and reproductive failure? (environment and genetic origins)

## miRNA: from junk DNA to major regulatory mechanism – Olivier Voinnet

Contribution not submitted by speaker

# Clinical aspects of epigenetic deregulation in IVF

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Center for Reproductive Medicine
Maastricht UMC+



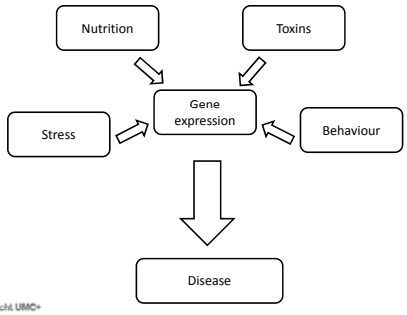
Conflict of interest: none



## Outline / learning objectives

- Indications for an IVF effect on epigenetic regulation
- Clinical effects in human and animal
  - Genomic imprinting disorders
  - Birth weight
  - Postnatal effects
- Cause
  - Subfertile population
  - IVF technique

## Gene – environment interaction

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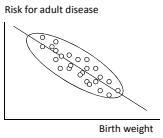
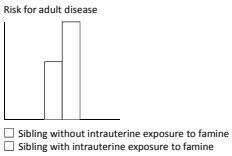
## Fetal environment and disease

Barker-studies




Dutch famine - WWII

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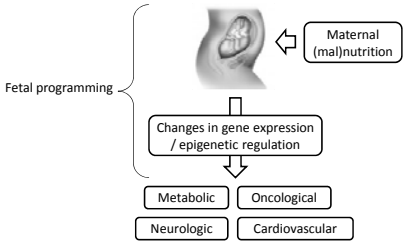
## Effect environment on fetal gene expression

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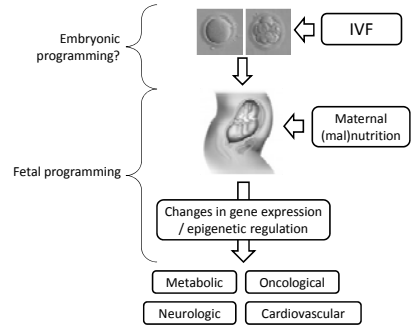
## Effect pre-implantation environment?

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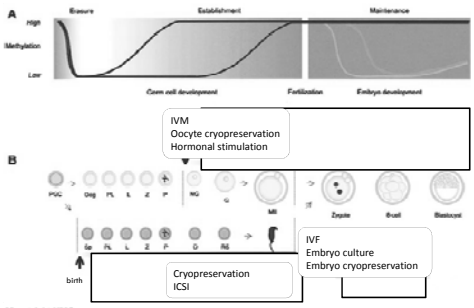
## Epigenetic reprogramming at the time of IVF

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Adjusted from Lucifero *et al.* 2004

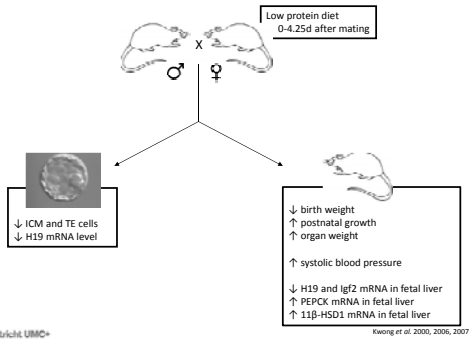
## Effect Dutch famine in early pregnancy

| Early | Mid | Late |
|---|---|---|
| Glucose metabolism | Glucose metabolism | Glucose metabolism |
| Obesity | Obstructive airways disease | |
| Lipid profile | Microalbuminurea | |
| Coagulation | Reproductive succes | |
| Coronary heart disease | | |
| Breast cancer | | |
| Stress responsiveness | | |
| Food preference | | |
| Depression | | |
| Cognition | | |
| Schizophrenia | | |
| Reproductive success | | |

TJ Roseboom

**IGF2 methylation at age 60+** *(Heijmans et al. 2008)*

## Preimplantation embryo is sensitive to environment




Kwong *et al.* 2000, 2006, 2007

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## Epigenetic deregulation in IVF?

Genomic Imprinting Disorders | Birth | Postnatal

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## Angelman Syndrome (AS)

- Incidence: 1/10000 -1/30000
- Primary developmental & 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, → loss of *UBE3A expression*

Kindly provided by Dr. S. Frints

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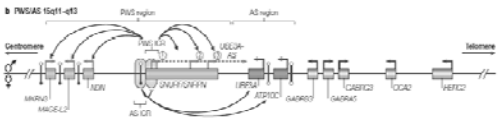
## Angelman Syndrome



Inheritance: most cases are sporadic
85-90 % genetic defects
< 5 % epigenetic defects

Robertson 2005

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## IVF and Angelman syndrome

| Literature | Type of study | N° AS | %ART in AS | % ART in ref pop | Type of ART | Molecular defect |
|---|---|---|---|---|---|---|
| Cox *et al.* 2002 | Case series | 2 | - | - | ICSI | 2/2 LOM SNRPN |
| Orstavik *et al.* 2003 | Case report | 1 | - | - | ICSI | 1/1 LOM SNRPN |
| Ludwig *et al.* 2005 | Survey | 79 | 3.8% | - | ICSI | 1/3 LOM SNRPN<br>2/3 mat deletion 15q11 |
| Sutcliffe *et al.* 2006 | Survey | 384 | 0 | 0.8 | - | |
| Doornbos *et al.* 2007 | Survey | 63 | 0 | 0.92 | - | |

LOM=loss of methylation

→ No evidence for a higher risk

→ In 4/6 cases an epigenetic defect was found

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## Silver Russell syndrome (SRS)

- Prevalence: 1: 100000 (?)
- Genetically heterogeneous
- Main defects:
  - 44% H19 DMR hypomethylation
  - 5-10% uniparental disomy chromosome 7

Binder *et al.*2011

| Literature | Type of study | N° SRS | Type of ART | Molecular defect |
|---|---|---|---|---|
| Svensson *et al.* 2005 | Case series | 2 | ICSI | ? |
| Kagami *et al.* 2007 | Case report | 1 | IVF | Hypermeth MEST |
| Galli-Tsinopoulou *et al.* 2008 | Case report | 1 | IVF | ? |

→ No case control studies, no evidence for increased risk

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## Prader-Willi Syndrome and Retinoblastoma

| Literature | Type of study | N | %ART in PWS | % ART in ref pop | Estimated risk | Type of ART | Defect |
|---|---|---|---|---|---|---|---|
| **Prader-Willi Syndrome** | | | | | | | |
| Sutcliffe et al. 2006 | Survey | 522 | 0.4-1.2% | 0.8% | - | ICSI | 2/2 paternal deletion 15q11.2 |
| Doornbos et al. 2007 | Survey | 86 | 2.3% | 0.92% | 2.5 | IVF and ICSI | 1/2 deletion<br>1/2 unknown |
| | | | | | | | |
| **Retinoblastoma** | | | | | | | |
| Moll et al. 2003* | Case series | 5 | - | - | 4.9-7.2 | IVF and ICSI | 2/5 de novo RB1 mutation<br>3/5 unknown |
| Bradbury et al. 2004 | Survey | 24 | 0% | 0.007% | - | - | - |
| Marees et al. 2009* | Survey | 162 | 4.3% | - | 2.5 | IVF and ICSI | 3/7 de novo RB1 mutation<br>5/7 unknown |

*Overlapping data

→ No epigenetic defect found

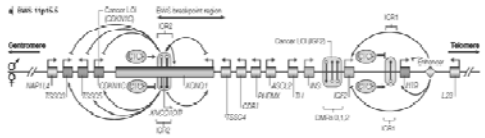


## Beckwith-Wiedemann Syndrome (BWS)

- 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
- Hard to identify BWS
- At adult stage: normal size, no symptoms
- Caused by genetic or epigenetic defects in an imprinted region on chromosome 11p15



## Beckwith-Wiedemann Syndrome




Inheritance
- 15% familial cases, autosomal dominant
- 85% sporadic cases:
  - -20% genetic (UPD, ICR epimutation)
  - -10% *CDKN1C* gene defect
  - -50% epigenetic (aberrant methylation)
  - -10% unknown

Paternal expression | Unmethylated | Promoter (not imprinted)
Maternal expression | Methylated | ICR or DMR



Robertson 2005

## IVF and Beckwith-Wiedemann syndrome

| Literature | Type of study | Nº BWS | %ART in BWS | % ART in ref | Estimated risk | Type of ART |
|---|---|---|---|---|---|---|
| De Baun *et al.* 2003 | Case series | 65 | 4.6 | 0.8 | 6.1 | IVF/ICSI |
| Maher *et al.* 2003 | Case series | 149 | 4 | 1.0 | 4.0* | IVF/ICSI |
| Gicquel *et al.* 2003 | Case series | 149 | 4 | 1.3 | 3.1* | IVF/ICSI |
| Halliday *et al.* 2004 | Case control | 37 | 10.8 | 0.67 | 16.1* | IVF/ICSI |
| Chang *et al.* 2005 | Case series | 341 | 5.6 | NA | - | IVF/ICSI |
| Sutcliffe *et al.* 2006 | Survey | 209 | 2.9 – 7.6 | 0.8 | 3.6 - 9.5* | IVF/ICSI |
| Doornbos *et al.* 2007 | Survey | 71 | 5.6 | 0.92 | 6.1* | IVF/ICSI |

* $P < 0.05$

→Statistical evidence for increased risk

→Molecular defect: In 25/26 cases loss of methylation KCNQ1OT1 (ICR2 region)

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## Conclusion I

→ limited evidence for an increased risk of imprinting disorders after ART, mainly BWS

→ Absolute risk still low

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## "Minor" epigenetic defects after IVF

| Ref | ART | N (ART) | sample | CpGs analysed | result |
|---|---|---|---|---|---|
| Kanber *et al.* 2009 | ICSI + SGA | 19 | Buccal smear | Imprinted | 1/19 hypermeth KCNQ1OT1 and MEST |
| Gomes *et al.* 2009 | IVF + ICSI | 18 | Peripheral blood, UCB or placenta | Imprinted | 3/18 hypometh KCNQ1OT1 |
| Katari *et al.* 2009 | IVF | 10 | UCB, placenta | Imprinted + non-imprinted | CpG sites differed in methylation, impr genes not extra vulnerable compared to non-impr |
| Tierling *et al.* 2010 | IVF + ICSI | 112 | UCB amnion membrane | Imprinted | Slight hypermeth of MEST in IVF |
| Zechner *et al.* 2010 | IVF + ICSI | 42 | Chorion villi | Imprinted | Hypometh KCNQ1OT1 in IVF |
| Turan *et al.* 2010 | IVF (ICSI?) | 45-98 | UCB, cord, placenta | Imprinted | Higher variation in methylation |
| Van Montfoort *et al.* 2011 | IVF + ICSI | 35 | placenta | Imprinted | Hypomethylation at H19 and MEST |
| Feng *et al.* 2011 | IVF + ICSI | 60 | UCB | Imprinted | Expression of PHLDA2 down and of PEG10 and L3MBTL up |

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Derived from van Montfoort et al. 2012

## Functions imprinted genes

| Functions | Defects |
|---|---|
| • Foetal growth<br>– foetal growth itself *(IGF2, H19, MEST)*<br>– placental growth or function *(IGF2, PHLDA2, MEST)* | • Intra-uterine growth defects |
| • Postnatal cognition and behaviour *(MEST, PEG3, UBE3A)* | • Abnormal maternal behaviour, impaired memory |
| • Brain development *(UBE3A, NDN)* | • Neurological disorders (autism, schizophrenia, epilepsy, Tourette syndrome) |
| • Tumour suppressor gene *(DIRAS3, MEG3)* | • Cancers |

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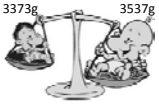
## Intra-uterine growth defects after IVF

Risk for IVF (ICSI) babies:

- VLBW: RR 2.7-3.8
- LBW: RR 1.4-1.8
- SGA: RR 1.4-1.6

(derived from meta-analyses of Helmerhorst *et al.* 2004, Jackson *et al* 2004 and McDonald *et al.* 2009)

- Lower birth weight after fresh ET (not after frozen ET) (Pinborg *et al.* 2009) but still within normal ranges

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## Growth related imprinted genes and IVF

| Ref | N (ART) | Tissue | M/E | Epigenetic change IVF? | Difference in BW? |
|---|---|---|---|---|---|
| Tierling *et al.* 2010 | 112 | UCB amnion membrane | M | Yes | Yes* |
| Wong *et al.* 2011 | 77 | placenta | M | No | Yes |
| Turan *et al.* 2010 | 45-98 | UCB Cord placenta | M+E | Yes | Yes |
| Van Montfoort *et al.* 2010, 2011 | 35-74 | placenta | M+E | Yes | No |
| Katagiri *et al.* 2010 | 48 | placenta | E | No | Yes |
| Feng *et al.* 2011 | 60 | UCB | E | Yes | No |

*Twins included
M/E=Methylation/Expression

→Growth related genes not always affected in IVF

→ If affected, not always effect on fetal growth

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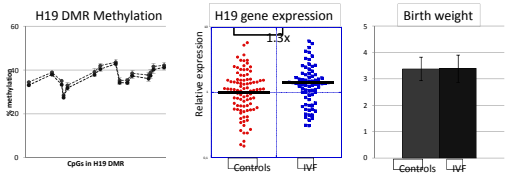
## No relation between deregulation of imprinted genes and birth weight




Van Montfoort *et al* 2010, 2011
Nelissen *et al*, submitted

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## IUGR and imprinted gene expression

IUGR=birth weight < 10th percentile for gestational age

| Gene | Fold change |
|---|---|
| PHLDA2 | 1.27 |
| MEST | 0.72 |
| MEG3 | 0.52 |
| GATM | 0.57 |
| PLAGL | 0.67 |

McMinn *et al.* 2006

| Gene | Fold change |
|---|---|
| PHLDA2 | 2.8 |
| ILK2 | 2.3 |
| NNAT | 2.3 |
| CCDC86 | 2.5 |
| PEG10 | 2.6 |
| PLAGL | 0.23 |
| DHCR24 | 0.35 |
| ZNF331 | 0.31 |
| CDKAL1 | 0.52 |

Diplas *et al.* 2009

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## Or non-imprinted genes?

→ Analysis on IVF and control placentas

**Gene expression**
*No imprinted genes*
Immune response
Transmembrane transport
Metabolism
Oxidative stress
Cell differentiation

**Proteomics**
*No imprinted genes*
Nucleic acid processing
Transmembrane transport
Metabolism
Stress response
Cytoskeleton

**Ultrastructure**
Thicker placental barrier
Less syncytiotrophoblast apical microvilli
More vacuoles in syncytiotrophoblast

Zhang *et al.* 2008, 2010, 2011

Effect IVF on maternal-fetal exchange in placenta via non-imprinted genes??

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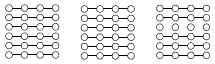
## Conclusion II

→ Relation between methylation/expression defects of imprinted genes and the reduced birth weight in IVF not clear yet



50% 38% 38%

- Minor methylation defects no effect?
- BW effect is dependent on multiple genes?
- Other regulatory/compensatory mechanisms?
- BW IVF are within normal range

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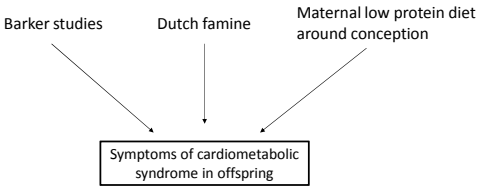
## Postnatal outcome of fetal/embryonic programming

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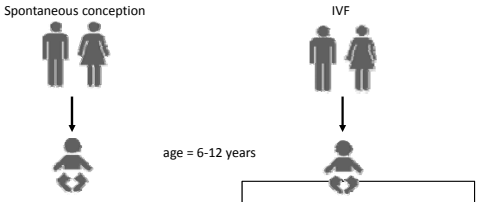
## Postnatal effects




Ceelen *et al.* 2007, 2008, 2009; Sakka *et al.* 2010; Miles *et al.*2007

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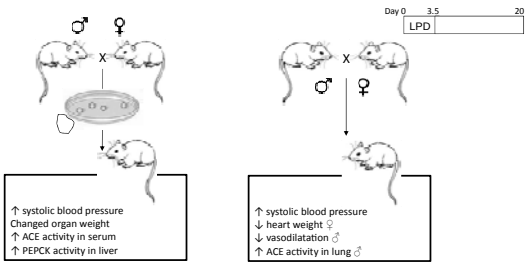
## Long-term effects of embryo environment

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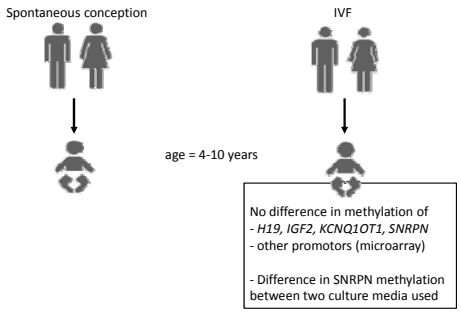
Watkins *et al. 2004*, 2008, 2010

## Epigenetic changes in IVF children?




Miles *et al.*2007, Oliver *et al.* 2012

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## Conclusion III

→ After human IVF signs of symptoms of metabolic syndrome are seen

→ Differences are small. Unknown how this will develop to adult stage

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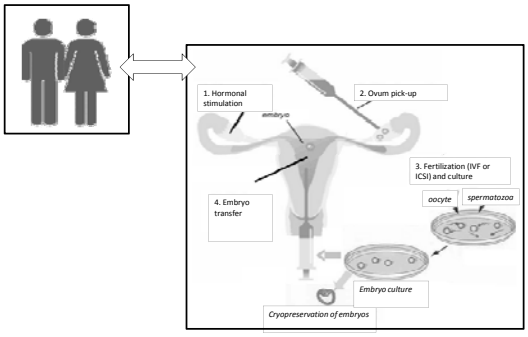
## What might be the cause?

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## BWS - Not related to a specific procedure

- Irrespective of cause of subfertility
- IVF/ICSI
- Fresh / frozen embryo transfer
- Day of transfer
- Different levels of hormonal stimulation

- Hormone stimulation +/- IUI

In common:
-Ovarian stimulation
-Embryo culture

-Subfertile patients

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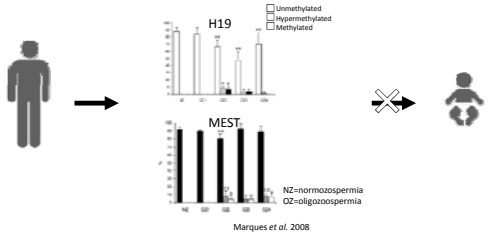
## Methylation defects in spermatozoa




Marques *et al.* 2008

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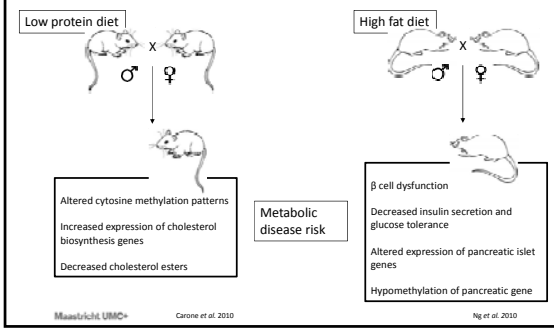
## Paternal epigenetic transmission effect

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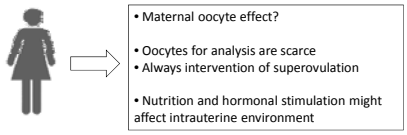
Carone *et al.* 2010

Ng *et al.* 2010

## Maternal transmission or superovulation?



- Maternal oocyte effect?
- Oocytes for analysis are scarce
- Always intervention of superovulation
- Nutrition and hormonal stimulation might affect intrauterine environment

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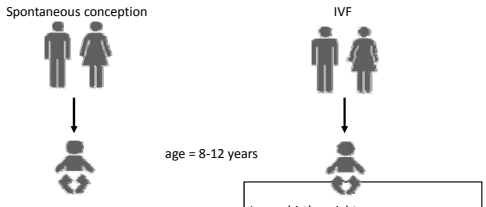
## Physiological effects – subfertile population




Ceelen *et al.* 2007, 2008, 2009

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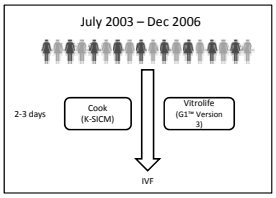
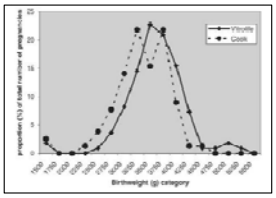
## Culture medium and birth weight




Dumoulin *et al.* 2010

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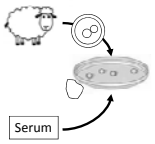
## Large offspring syndrome




Reduced methylation
and expression *Igf2R*

high birth weight
breathing difficulties
organ overgrowth,
skeletal anomalies
sudden neonatal death

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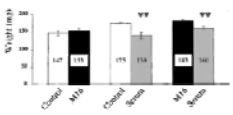
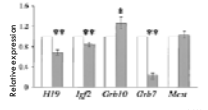
## Epigenetic effects

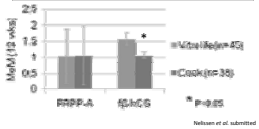
**Mice - Serum**




Khosla *et al.* 2001

**Human ?**




Nelissen *et al.* submitted

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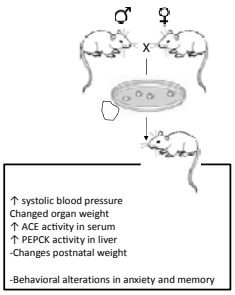
## Other culture effects in mice studies



↑ systolic blood pressure
Changed organ weight
↑ ACE activity in serum
↑ PEPCK activity in liver
-Changes postnatal weight

-Behavioral alterations in anxiety and memory

Watkins *et al.* 2007; Fernandez-Gonzalez *et al.* 2004

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## Conclusion IV

→ Indications from human and mice studies that IVF can affect physiologic outcome in offspring

→ Culture (medium) at risk

→ Some indications for an effect of superovulation and subfertile patient population

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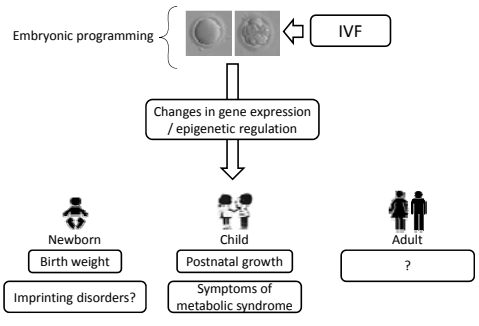
## Effect pre-implantation environment

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# Karyotype anomalies in children born after ART

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25-!

## Conflict of interest

- Prof M Bonduelle's institution (UZBrussel)

  has received educational grants from, IBSA, Ferring, Organon, Merck, Merck Belgium, Shering-Plough…



## Learning objectives

- Infertile couple: Risk for karyotype anomalies in men and women ?
- Risk for karyotype anomalies in children after ICSI
- Guidelines for prenatal diagnosis
- Guidelines for karyotyping in patients undergoing ART

## Introduction of IVF and ICSI

- 1978 Louise Brown
  - little concern about chromosomal anomalies, data collection on children through registers
- 1991 introduction of ICSI at the UZBrussel
  - concerns about health of the children
- concerns related to
  - type of sperm used
  - bypassing of natural selection
  - invasiveness of the procedure
- 1995 first child born after TESE



## ICSI / ART increased genetic risk?

- Risk due to the type of gametes used
  - Male gametes carrying
    - DNA anomalies : breaks, Y-deletions or structural changes
    - Chromosomal anomalies : de novo sex, aneuploidy, structural anomalies in peripheral blood
    - Chromosomal anomalies in sperm in severe infertility
  - Female gametes
    - Chromosomal anomalies in infertile women?
    - Suboptimal female gametes due to hormonal stimulation?
    - More chromosomal anomalies in stimulated oocytes?



## ICSI / ART increased genetic risk?

- Bypassing of natural selection ?
  - Little evidence of natural selection against chromosomally abnormal sperm
- Risk due to the invasiveness of the procedure ?
  - damage to the ooplasma or meiotic spindle and its DNA repair status -> sperm DNA lesions transmitted

## Outline lecture

- **Causes of Male infertility**
  - Chromosomal anomalies in blood/in sperm
- Causes of Female infertility
  - Chromosomal anomalies in blood
- Risk for the children if karyotype anomalies
- Prenatal testing in ICSI results
- Indications for prenatal testing



## Causes of male infertility

- In severe male infertility
  - **Genetic origin** of infertility in **10-15%** of cases
    - –Chromosomal
    - –Y deletions
    - –Single gene disorders
      - – CBAVD and CF mutations, …



## Causes of male infertility

Genetic screening in 750 oligozoospermic men before ICSI (Foresta et al, 2005)

| | Patients | | Sperm count (million/ml) | Controls | |
|---|---|---|---|---|---|
| Chromosomal aberrations | 42/750 | 5.6%* | 1.9 ± 1.4 | 1/295 | 0.3% |
| Y Chromosome microdeletions | 45/750 | 6.0%* | 1.6 ± 1.3 | 0/210 | |
| CFTR gene mutations | 9/750 | 1.2% | 2.0 ± 1.1 | 3/303 | 1.0% |
| AR gene mutations | 8/750 | 1.1% | 1.7 ± 0.8 | 0/188 | |

*Statistical significant $p < 0.001$ versus controls

## Chromosomes in male infertility

- Severe male factor infertility
  - Chromosomal anomaly in +/- **5%**
  - Inversely related to sperm count: **2-10%**
  - In azospermic men: **up to 15%**
    - Klinefelter syndrome (majority): 5-10%
    - Sex chromosomal anomalies: 0.1-0.2%
    - Structural chromosomal anomalies: 0.5-1%
    - ring chromosome, translocations, inversions



## Chromosomes in male infertility

Fenotypes associated with male infertility (from Ferlin et al. 2004)

| | **Phenotype** | **Prevalence %** |
|---|---|---|
| Chomosomal aberration | Azo- to normospermia | 2-10% |
| Klinefelter syndrome | Azo- to severe oligosp | 5-10% azospermia<br>2-5% severe oligo |
| Other sex chrom aberration | Azo- to normospermia | 0.1-0.2% |
| Robertsonian translocations | Azo- to severe oligosp. | 0.5-1% |
| Reciprocal translocations | Azo- to severe oligosp. | 0.5-1% |



## Chromosomal anomalies in sperm in severe oligo- and azospermia

- Presence of more chromosomal anomalies in sperm from oligozoospermic men reported
  - 5-38%, in relation to severity
  - a threefold increase reported by most studies
    - Martin et al. 2000, Bernardini et al. 2000, Vegetti et al. 2000, Levron et al. 2001, Calogero et al. 2001, Burello et al. 2002, Palermo et al, 2002
  - Compared to 3-5%in fertile men
- Presence of more (sex) chromosome anomalies in OAT and obstructive azospermia
  - Pfeffer et al.1999, Levron et al. 2001, Sbracia et al. 2002

## Chromosomal anomalies in sperm with abnormal morphology

- Sperm aneuploidy associated with ICSI failure
- Screening for sperm aneuploidy not routinely performed, however some case reports indicate we need to be able to investigate the risk
- In practice, sperm aneuploidy rarely explored



## Outline lecture

- Causes of Male infertility
  - Chromosomal anomalies in blood / in sperm
- **Causes of Female infertility**
  - Chromosomal anomalies in blood / in oocytes
- Risk for the children if karyotype anomalies
- Prenatal testing in ICSI results
- Indications for prenatal testing



## Chromosomes in female infertility

- Recurrent miscarriage
  - Higher risk of structural chromosomal anomaly
- Premature menopauze
  - Higher risk of Turner mozaiscism
  - Risk of Fragile X syndrome
- Contradictory data on karyotype anomalies in female partners of infertile couple
  - Papanikolaou et al. 2005 No increase in normovulatory women seeking infertility treatment?.
  - Schreurs et al. Increased frequency of chromosomal abnormalities in female partners of couples undergoing in vitro fertilisation or intracytoplasmic sperm injection

## Chromosomes in female infertility

- Contradictory data on karyotype anomalies in female partners of infertile couple
  - Papanikolaou et al. 2005
    - No increase in normovulatory women seeking infertility treatment.
    - Higher frequency of karyotype anomalies in women with secondary infertility
  - Schreurs et al. 2000
    - Increased frequency of chromosomal abnormalities in female partners of couples undergoing in vitro fertilisation or intracytoplasmic sperm injection



## Chromosomal anomalies in oocytes

- Increased risk for aneuploidy with maternal age
- 10-15% of all pregnancies end in spontaneous abortion and 60% of these have chromosomal anomalies



## Risk for the offspring related to chromosomal anomalies

- **Klinefelter syndrome**
- Other sex chromosomal anomalies
- Structural chromosomal anomalies
  - Robertsonian translocations
  - Reciprocal translocations
  - Other structural anomalies
- Mozaiscism

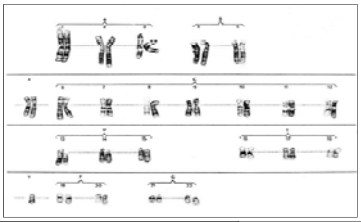
## Outline lecture

- Causes of Male infertility
  - Chromosomal anomalies in blood / in sperm
- Causes of Female infertility
  - Chromosomal anomalies in blood / in oocytes
- **Risk for the children if karyotype anomalies**
- Prenatal testing in ICSI : results
- Indications for prenatal testing



## Klinefelter syndrome: 47,XXY

- Most common chromosome abnormality
- 1 in 1000 (1/500 of live-born males)



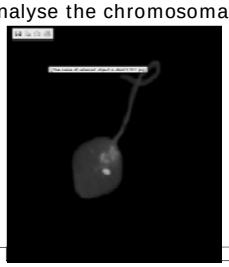


## Klinefelter syndrome

- Non-mosaic 47,XXY
  - Very few cases of naturally conceived offspring of proven paternity reported
    (Laron et al., 1982, Terzoli et al., 1992)
  - The majority of patients: infertile
    - severe oligozoospermia
    - azoospermia
    - focal spermatogenesis and testicular sperm may be recovered and used for ICSI
      (Tournaye et al., 1996; 1997)

## Klinefelter syndrome

- Genetic risk
- cytogenetic techniques to analyse the chromosomal content of
  - spermatozoa
  - embryos
- multicolor FISH



Disomic XY sperm
(X:green, Y:red, chrom 1 blue)



## Klinefelter syndrome
## FISH on Spermatozoa

- Aneuploidy of the gonosomes increased:
  24,XX and 24,XY
  - Non-mosaic 47,XXY : 2% → 45%
    (Rives et al. 2000, Estop et al. 1998)
  - Mosaic 47,XXY : 1.5% → 7%
    (Lim et al., 1999, Kruse et al. 1998)
- Disomic autosomes increased
  (Hennebicq et al., 2001; Morel et al., 2003)

⇨ Increased aneuploidy rate



## Klinefelter syndrome:
## FISH on PGD embryo's

| | Klinefelter Age <38y | Control Age <38y |
|---|---|---|
| Total number of embryo's analysed | 113 | 578 |
| Total number of normal embryo's | 61 | 446 |
| % normal embryo's | 54%* | 77.2%* |

*significant

Staessen et al., Hum Reprod Update 2003; Vol.9, No4.;319-330

| | Klinefelter <= 38 year | Controls <= 38 year |
|---|---|---|
| I. Sex chromosomal abnormalities No. of sex chromosome abnormalities / no. of embryos analysed (%) | 15/113 (13.2)* | 18/578 (3.1)* |
| II. Autosomal abnormalities No. of autosomal abnormalities / no. of embryos analysed (%) | 17/109 (15.6)* | 30/578 (5.2)* |
| III Ploidy status abnormalities No. of ploidy status abnormalities / no. of embryos analysed (%) | 12/113 (10.6)* | 25/578 (4.3)* |
| IV Combined abnormalities No. of embryos with comb. abnormalities / total no. of embryos analysed (%) | 10/113 (8.8)* | 59/578 (10.2)* |

* significant



## Klinefelter syndrome: Risk for the offspring

- ICSI : valid option
- The genetic risk in the offspring of 47,XXY is presumably low, but this risk concerns sex chromosomal as well as autosomal aneuploidy
- A cautious approach is warranted in advising couples with non-mosaic Klinefelter's syndrome.
- The use of ICSI with PGD or prenatal diagnosis should be carefully considered



## Risk for the offspring related to chromosomal anomalies

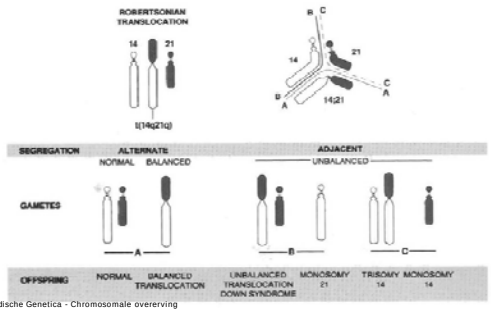
- Klinefelter syndrome
- Other sex chromosomal anomalies
- **Structural chromosomal anomalies**
  - Robertsonian translocations
  - Reciprocal translocations
  - Other structural anomalies
- Mozaiscism

## Structural karyotype anomalies in parents

- Risk of chromosomal inbalanced offspring
  - most will end in miscarriages
  - mental retardation
  - congenital anomalies
- Risk of infertility
  - if balanced translocation
  - mostly male infertilty



## Robertsonian translocations segregation patterns

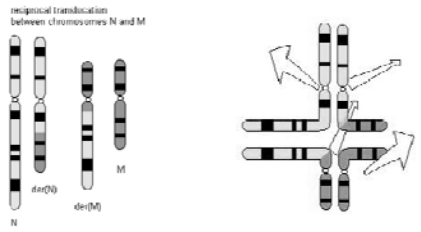





## Robertsonian translocations risk for offspring

| Translocation | Carrier | Chrom. anomaly in offspring |
|---|---|---|
| t(14,21) | mat | 10-15% |
| t(14,21) | pat | 2.5% |
| t(21,22) | mat | 10-15% |
| t(21,22) | pat | <1% |
| t(21,21) | mat | 100% |
| t(21,21) | pat | 100% |

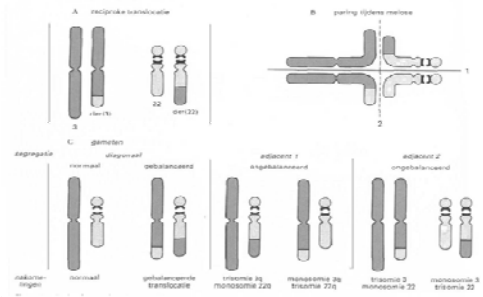
## Reciprocal trnslocations








## Reciprocal trnslocations



## Reciprocal translocations risk for offspring

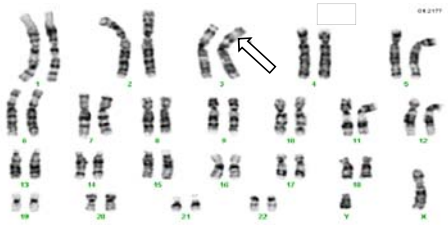
- Risk of abnormal gametes will depend on the segregation pattern and is diffenrent for each translocation
- General risk for imbalanced gametes:
  30-80%
- Miscarriage rate
  - Higher if translocated segment is large
- Lifeborn rate of children with MR/MCA
  - Higher if translocated segment is small

## Robertsonian and reciprocal translocations

- Risk for the offspring examined through PGD embryo's
  - 54-70% of embryo's from Robertsonian (Kuliev et al. 2010, Munné 2005)
  - 75-82% of embryo's from Reciprocal translocations were unbalanced (Kuliev et al. 2010, Munné 2005)



## Structural anomalies Paracentric inversion



Karyotype : 46,XY,inv(3) met G-Bandering



## Inversions risk for the offspring

- Paracentric inversions
  - General risk for imbalanced gametes: +/-3%
- Pericentric inversions
  - Problems of pairing at meiosis
  - Chromosomes are forced to form "inversion loop sduring meiosis
  - Risk comparable with translocations

⇨ Amniocentesis advisable

## Interchromosomal effect (ICE) risk for the offspring

- ICE refers to the abnormal behaviour of one or more chromosomes , not involved in the rearrangement
  - Robertsonian translocations 58% ICE
  - Reciprocal translocations 64% ICE
    (Martin RH, H Reprod Update 2008, 14, 379-90)



## Risk for the offspring related to chromosomal anomalies

- Klinefelter syndrome
- Other sex chromosomal anomalies
- Structural chromosomal anomalies
  - Robertsonian translocations
  - Reciprocal translocations
  - Other structural anomalies
- **Mozaiscism**



## Mozaicism Risk for the offspring

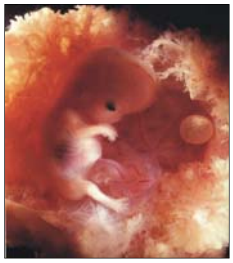
- Compatable with normal phenotype
  - Klinefelter mozaicism
    - indication for prenatal testing, low risk
  - Turner Mozaicism
    - Indication for prenatal testing, ICE
    - Follow-up of the mother for cardio vascular and metabolic risk
  - Mozaics of autosomes
    - Indication for prenatal testing
    - Often clinically abnormal

## Outline lecture

- Causes of Male infertility
  - Chromosomal anomalies in blood / in sperm
- Causes of Female infertility
  - Chromosomal anomalies in blood / in oocytes
- Risk for the children if karyotype anomalies
- **Prenatal testing in ICSI results**
- Indications for prenatal testing



## Prenatal diagnostic testing of ART children





## Follow-up studies at the UZ Brussel

- **AIM** Evaluate the risk of ICSI /TESE to the offspring
  - Overall risk
    - **genetic** constitution of the fetuses [1]
    - **perinatal** problems
    - **development** of the children
  - Procedure-related risk
    - comparison ICSI / IVF
  - Sperm-related risk
    - sperm quality / sperm origin

[1] Bonduelle et al, 2002

## Prenatal diagnosis in 1586 ICSI foetuses

| Abnormal results | n | % | Confidence Interval | % General population[1, 2, 3] |
|---|---|---|---|---|
| ■ *De novo* | 25 | **1.6%*** | 1.02 - 2.32 % | 0.45 - 0.87% |
| Sex chrom | 10 | **0.6%*** | 0.30 - 1.16 % | 0.19 - 0.27% |
| Autosomal | 15 | 0.9% | 0.53 - 1.56 % | 0.26 - 0.60% |
| Numerical | 8 | 0.5% | 0.22 - 0.99 % | 0.14 - 0.33% |
| Structural | 7 | 0.4% | 0.18 - 0.91 % | 0.11 - 0.22% |
| ■ Inherited | 22 | **1.4%*** | 0.87 - 2.09 % | 0.47 - 0.37% |
| Total | 47 | 3.0% | 2.19 - 3.92 % | 0.92% |

[1] Jacobs, 1992 on 34 910 newborns [2] Ferguson-Smith, 1984 on 52 965 prenatal samples
[3] Hook, 1981, 1984, 1987 on prenatal samples * significant



## Prenatal diagnosis in 1586 ICSI foetuses[1]

Non-inherited *de novo* anomalies **1.6%**

- Significantly higher than general population (with same age) but absolute risk low
- Related to sperm characteristics
- Severity is variable (termination not always chosen)
- Sex chromosomal anomalies 0.6%

Infertility in future generation (47,XXY and 45,X)

[1]Bonduelle et al. 2002



## Prenatal diagnosis in 1586 ICSI foetuses[1]

Inherited abnormalities **1.4%**

- Known risk related to the chromosomal anomalies in the parents (6.3%)
- 17/22 cases paternally inherited
- Preimplantation > prenatal diagnosis

Transmission of infertility to next generation

[1]Bonduelle et al. 2002

## Prenatal diagnosis in 1586 ICSI foetuses[1] *de novo* anomalies, sperm parameters

- Sperm count (72%)
  < $20.10^6$ / ml ⇒ **2.1 %** chromosomal abnormalities
  Fisher Exact 2 tailed test p < 0.05
- Sperm motility (83%)
  < 50 % N motility ⇒ **1.9%** chromosomal abnormalities
  Fisher Exact 2 tailed test p < 0.05
- Sperm morphology ⇒ **no influence**
  abn < 14 % N or abn ≥ 14 % N morphology

[1]Bonduelle et al. 2002



## Karyotypes in ICSI fetuses / anomalies in relation to sperm origin[1]

| | *de novo* | inherited |
|---|---|---|
| • Ejaculated sperm[1] | 1.7%* | 1.4% |
| • n =1469 | (25) | (20) |
| • Epididymal sperm[2] | 0%* | 0.0% |
| • n = 74 | (0) | (0) |
| • Testicular sperm[2] | 1.5%* | 0.5% |
| • n = 195 | (3) | (1) |

[1] Belva et al., 2011 * no significant differences



## Karyotypes in TESE foetusses[1]

| | TESE | Non-Ejaculated | Ejaculated | General pop |
|---|---|---|---|---|
| | 195 | 269 | 1721 | |
| Inherited anomaly | 0.5% | 0.4% | 1.3%[a] | 0.47%[b] |
| *De novo* | **1.5%** | 1.1% | **1.7%**[c] | **0.45%**[d] |

| | |
|---|---|
| a non-ejaculated vs ejaculated inherited | OR 0.3; 95%CI 0.03-2.0 |
| b non-ejaculated vs general population | OR 0.8; 95%CI 0.1-5.7 |
| c non-ejaculated vs ejaculated de novo | OR 6.3; 95%CI 0.2-2.1 |
| d non-ejaculated vs general population | OR 2.5; 95%CI 0.8-7.8 |

[1]F Belva et al. H Reprod 2011

## Karyotypes in ICSI literature

| | Bonduelle et al (2002) | Jozwiak et al (2004) |
|---|---|---|
| Number of foetusses | 1586 | 1136 |
| Karyotype anomalies | 47 | 17 |
| *De novo*: autosomes + sex | **1.6 %** 15 +10 | **1.2 %** 7 +7 |
| Inherited | **1.4 %** 22 | **0.3 %** 3 |
| TOTAL (95%CI) | 2.96% (2.19-3.92) | 1.50% (0.87-2.39) |

Karyotype anomalies in general population 0.45 - 0.87%



## Outline lecture

- Causes of Male infertility
  - Chromosomal anomalies in blood / in sperm
- Causes of Female infertility
  - Chromosomal anomalies in blood / in oocytes
- Risk for the children if karyotype anomalies
- Prenatal testing in ICSI results
- **Indications for prenatal testing**



## Indications for prenatal testing

- In all ICSI pregnancies?
  - A slight increase *de novo* anomalies in all ICSI pregnancies ⇒ 1.6%)
  - < $20.10^6$ / ml ⇒ **2.1 %**
- In all TESE and NOA pregnancies
- In ART pregnancies when karyotype anomaly detected in one of the future parents
- In ART if maternal age indication or US anomaly

## Conclusions

- Higher risk for chromosomal anomalies in male factor infertility
  - All men with motile sperm count < 1 million sperm /ml
  - All men with non-obstructive azospermia
- Screening before treatment
  - All men before ICSI treatment
  - Y chrom. deletion screening < 5-10 million/ml
  - Screening for sperm aneuploidy not routinely performed, however indication need to be investigated



## Conclusions

- Higher risk for female chromosomal anomalies in infertile couples
  - Karyotype of women entering IVF and ICSI?
  - Karyotype of women if reproductive failure (failed IVF, miscarriages)
- If chromosomal structural or numerical anomaly detected in man or woman before IVF /ICSI
  - Higher risk for the offspring

⇨ Prenatal diagnosis and/or PGD should be discussed and offered



## Conclusions

- Chromosomal anomalies in ICSI offspring
  - A slight increase (1.6%) in *de novo* anomalies
  - More *de novo* chromosomal anomalies were found in TESE compared to the general newborn population.
  - No significant differences were found in OA versus NOA subgroups in TESE children
- Karytotype in IVF offspring ??
  - No data available

## Conclusions

- Indications for prenatal diagnosis
  - In ICSI, TESE and NOA
    - if concentration < $20.10^6$ / ml or abnormal motility
  - In IVF: no actual data on prenatal diagnosis
  - In ART: if karyotype anomaly in the parent



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S. De Smyttere

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Centrum voor Medische Genetica

Centrum voor Reproductieve Geneeskunde

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# Congenital anomalies following ART

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## Learning objectives:

- To appreciate that access to data on birth defects after IVF, with appropriate popuation based controls, is limited to a small fraction of the close to 5 million children born after IVF, so far.
- That generalizations to other settings are uncertain due to differences over time in patient mix, clinical practice and technologies.
- Reasons behind the risk increases of around 25% reported are multi-factorial, non-iatrogenic but possibly also iatrogenic.

## Disclosures:

- I have no financial interests towards any stakeholder in IVF.
- I am not an embryologist, geneticist, statistcian or epidemiologist - I am a clinician with some experience in IVF outcome research

## Birth defects has been perceived differently over time.

- First, the fear of ”un-natural” children with malformations was the main concern.
- Later, medical risks due to multiple pregnancy came into focus. Birth defects lost in relative importance.
- The forthcoming SET era will again reverse the focus back to birth defects!
- And now epigenetics!

## Background and IVF additive risks

1. Basic risks for birth defects in the population
   no zero-level
   differs with time and place
2. Additional risks from sub-fertility status, non-iatrogenic
   parental, maternal or paternal
3. Additional iatrogenic IVF risks, on top:
   the method per se, clinic or lab, e.g. epigenetic risk
   clinical policy, e.g. patient selection / ET

Country specific risk profile.

## So, characteristics of data on IVF birth defects are complex:

-**Inequity of access to national data**
- **Sensitive to time and place** due to changeing patient mix, methodology and country-specific factors

- **Additive risks** to background risks
- Crusial for all **stakeholders**
- One of several indicators of ”**treatment benefits**” (efficacy, safety, quality, time, cost)

”**Established**” vs ”**experimental**” procedures

## Data is lacking in most settings

- A very small proportion (2% ?) of the estimated 5 million IVF-children born so far have been systematically followed-up and reported for their medical safety, including birth defects.
- Only few countries have high quality registration (population based) of birth defects.
- Data are ”time-sensitive”, new factors are continously added, so monitoring needs to be continuous.

## Data collection difficulties:

- Definitions, minor vs major, ”weeded” etc
- Coverage
- Control groups
- Validation
- Lost-to-follow-up
- Time lag

## Early reports on birth defects following IVF

1987: 9 years after Louise Brown:

- *Birth defects after IVF*, Paul Lancaster, The Lancet.

1999: Only 13 years ago:

- *Deliveries and children born after IVF*, Bergh, Ericsson, Hillensjö, Nygren and Wennerholm, The Lancet.

## The Swedish example

- Each person has a personal identification number, a PIN code.
- Several population-based health registers since 30 years back:

  Medical Birth register, Hospital Diagnosis register, Cancer register, Malformation register, Causes of Death register, Drug register
- IVF register, for cross linkage.

## Swedish data

- Largest national data-set.
- Two consequtive time periods 1982-2001 and 2001-2006
- 16,000 and 16,000 = 32,000 IVF children, all.
- Individual PIN-codes and crosslink to register.
- 2,4 million controls from the same register.

## References.se

Congenital Malformations in Infants Born after IVF in Sweden.
Källén et al, Birth Defects Research (Part A) 88: 137-143 (2010).

Blastocyst versus cleavage stage transfer in IVF: differences in neonatal outcome?
Källén et al, Fertil.Steril. 2010, Oct 94 (5): 1680-3

## Birth defects / Sweden

- Swedish children: 16,280 + 15,570 = 31,850
- Simliar OR over time, 25% risk increase, but sub-group change over time
- Parental characteristics is the main reason
- Independent of multiplicity and IVF/ICSI
- Increased risk for monocygoty diappeared !
- Epi-genetics, imprinting
- ”Blasto-genetic” birth defects and mono-cygots (Halliday et.al. HR 2010 )
- Similar levels e.g. in US, Europe, Australia ( Reefhuis, Bounduelle, Sutcliff, Hansen )

## IVF vs Pop total

- Pop vs IVF, all malformations 4.4% vs 5.3%
- ”relatively severe” 3.0% vs 3.7%

  OR crude 1.27

  OR adjusted 1.25 (1.17-1.39)

## Five groups of increased risk of birth defects

- CNS
- Cardio-vascular
- Kidney agenesis
- Limb reduction
- Syndromes

## Sub-group change of birth defects, first 16.000 vs last 16.000 children

- Same risk level: (cardiovascular, limb reductions
- Decreasing: NTD (spina bifida), atresia of the oesophagus
- Disappeared risk increase: hypospadias, bowel atresias, monocygots

## Blastocyst transfer

- 1300 babies vs, 12000, blasto- vs cleavage-
  OR 1.3 for prematurity
  OR 1.3 for birth defects

No increase in monocygotic twninning
No change in sex ratio
Small numbers, further studies needed

## Syndromes

- OR 2 , IVF vs pop
  ( 14 cases from 31,850 children)

7 of these: assoc with imprinting problems:
- Prader-Willy 4 - 1,
- Russel - Silver 2 - 0
- Beckwith-Wiedeman 1- 1
- Zellweger 1 – 1

Total 7- 3, actual imprinting errors not known

## Why 25 % more birth defects?

When adjustments were made for year of birth, maternal age, parity, smoking and year of childlessness the increase disappeared.

- ”The increase, similar for IVF and ICSI, fresh and frozen embryos, were mainly a consequence of parental characteristics”
- Maybe this is not entirely the case ?

## Changes in patient mix and methods over time

Shorter period of infertility
ICSI women: less infertile.
Increasing BMI
Subfertile men for treatment
Smoke less

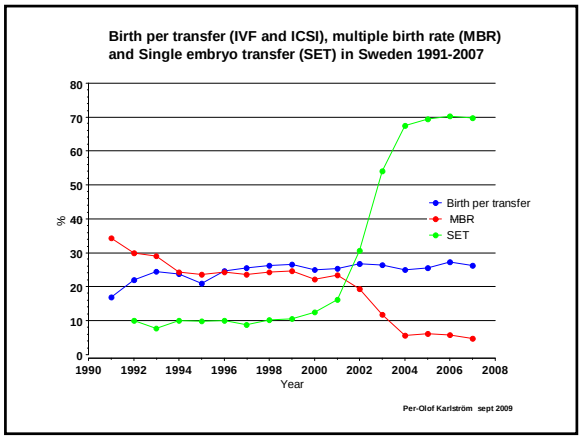
Drugs developments
Stimulation policies
Lab procedures
SET

## Maternal characteristics: IVF vs pop

Marked deviations from other parae:

Older, more first parity, less smokers, more low BMI, more high BMI, less work outside home, more previous abortions, *different drug use*.

These differences were less pronouced for ICSI women




## Three factors, possibly country specific for Sweden.

- 1/ Markedly different drug use during pregnancy for IVF mothers vs controls.
- 2/ Reluctance to abort a twin pregnany with one healthy and one damaged fetus.
- 3/ Birth defects discovered on ultrasound may be aborted to an unidentified proportion.

## IVF birth defects, summary.se

- OR 1.25 from 3 % to 3.7 %
- Variation over time.
- Parental characteristics and possibly iatrogenic
- ”Blasto-genetic” defects (?) by epigenetic mechanism and imprinting disturbances.
- IVF and ICSI similar risk
  but blastocyst higher (?), freezing lower (?)

## Can data be generalized to other settings?

Not suprisingly, there is a variation of estimates of the proportion of IVF birth defects in different settings (Hansen *et.al.* 2005), possibly *due to country specific factors*:

- Genetic, nutritional, environmental etc differences.
- Differences in data collection
- Differences in ART technology and policies.
- Differences in pregnancy and obst care.

## National data are needed…

- An overall, international, estimation of the ”true” risk increase may not be that clinically meaningfull.
- Recorded, national differences may carry a message!
- National data more meaningfull

## So, is IVF safe?

- No, not totally safe
- But currently safe enough to use, provided efforts are made to further reduce additive iatrogenic risks and distinguish experimental methodology.
- Safety needs to be protected.
- Stakeholders need information.

## Protection of safety

- SET as the norm
- Milder ovarian stimulation
- Monitor safety (birth defects incl) continously
- Distinguish experimental technology
- Regulate lab methodology and equipments
- Intensify research on epigenetics

## Suggested information to patients:

…..current (limited) data indicates that :
”after IVF there is a moderate but significant risk increase of a birth defect, similar after IVF and ICSI, to a large extent due to parental characteristics, still corresponding to a low individual risk.”

## General Conclusions

- There is an association between IVF and birth defects.
- This is thought to be due to parental characteristics rather than the technique, but *this currently challanged* !
- The incidence will vary with time and place.
- Continous monitoring to *”keep a finger on the pulse”* is necessary to maintain confidence.
- Time lag in reporting is crusial.
- Correct information to patients is crusial!

Thank you !

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# Low birth weight after ART

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## Learning objectives

- Twinning
- Low birth weight in ART singletons
- The influence of:
  - Subfertility
  - "Vanishing twins"
  - Frozen embryo transfer (FET)
  - Sibling studies
  - Blastocyst transfer and culture media



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## ART children

- 2008: IVF children 4.8% and IUI 3.2% = Total ART 8%
- Multiples in DK: ART =16% ; IUI = 8%
- 150.00 ART children in the Nordic countries
- > 3 million ART children worldwide

## Twin birth rates

- The far most important health risk for IVF children
- Overall twin rates increased two-fold over the past decades
- ART and increasing maternal age
- ART increases MZ twinning 2-fold
- Multiples after IUI is still a challenge





## IVF twins vs. singletons

**Higher risk of**

- Preterm birth
- Low birth weight
- Mean BW 1000 gram lower
- SGA
- Perinatal mortality
- NICU

**(Pinborg, Hum Reprod Update 2005)**





**A comparison of the "optimal end-point"**
**The cumulative delivery rates per initiated cycle after fresh and FER**

| | Initiated cycles IVF and ICSI | Deliveries, "fresh" cycles IVF and ICSI | Multiple Deliveries fresh | FER cycles (thawings) | Deliveries FER | Multiple deliveries FER | Deliveries fresh | Cumulative Deliveries, Fresh and FER | All multiples |
|---|---|---|---|---|---|---|---|---|---|
| **Finland** | 4776 | 989 | 123 | 3561 | 541 | 50 | **20.7%** | **32.0%** | **11.3%** |
| **Sweden** | 10088 | 2341 | 141 | 4659 | 856 | 50 | **23.2%** | **31.7%** | **5.9%** |
| **UK** | 33818 | 8276 | 1970 | 7943 | 1388 | 261 | **24.5%** | **28.6%** | **23.1%** |
| US | 99199 | 28404 | 8720 | 22023 | 5797 | 1402 | **28.6%** | **34.5%** | **29.6%** |
| **Canada** | 8972 | 2584 | 781 | 3224 | 576 | 139 | **28.8%** | **35.2%** | **29.1%** |

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## ART vs. non-ART singletons

| AOR (95%CI) | Helmerhorst Br Med J, 2004 | Jackson Am J Obs Gyn, 2004 |
|---|---|---|
| N | 5.361 | 12.283 |
| <2500 gram | 1.7 (1.5-1.9) | 1.8 (1.4-2.2) |
| <1500 gram | 3.0 (2.1-4.4) | 2.7 (2.3-3.1) |
| SGA | 1.4 (1.2-1.7) | 1.6 (1.3-2.0) |
| <37 uger | 2.0 (1.8-2.3) | 2.0 (1.7-2.2) |
| <32 uger | 3.3 (2.0-5.3) | - |
| Mortalitet | 1.7 (1.1-2.6) | 2.2 (1.6-3.0) |

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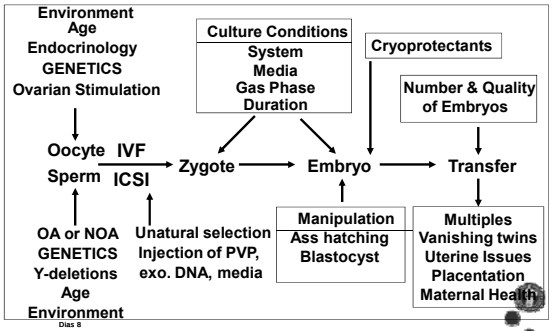
## Possible Causes of adverse Outcomes




Dias 8

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## ART versus non-ART mothers

- Mean maternal age is higher
- More nulliparous
- Fewer smokers
- Higher socio-economic status ?
- BMI ?
- *Less reproductive healthy*

Dias 9

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## Subfertility

**"Time-to-pregnancy" >1 year**

| | AOR (95%CI) |
|---|---|
| Low birth weight | 1.8 (1.2-2.7) |
| SGA | 1.2 (1.1-1.4) |
| Preterm delivery | 1.5 (1.2-1.8) |
| Malformation | 1.2 (1.1-1.4) |
| Neonatal mortality | 3.3 (1.5-7.5) |

(Basso, Hum Reprod 2003; BMJ 2005; Zhu, BMJ 2006; Obstet Gynecol 2007; Henriksen, Obstet Gynecol 1997; Draper, Lancet 1999; Pandian, Hum Reprod 2001)

Dias 10

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## Conclusions

**Subfertile couples conceiving spontaneously have a higher risk of low birth weight and small-for-gestational age babies than couples with normal fertility**

Dias 11

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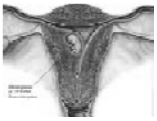
## Controlled Ovarian Stimulation

Endometrial receptivity
Implantation
Early placental development



PAPP-A is used in prenatal screening for trisomy 21
PAPP-A is associated with preterm delivery, preeclampsia, IUGR, stillbirth (Kirkegaard, 2010)
PAPP-A is lower after fresh IVF/ICSI (Amor 2009; Gjerris 2009)
PAPP-A similar in Cryo and non-ART (Gjerris 2009)

Dias 12

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## Vanishing twins

(AOR (95%CI) for maternal age, parity and treatment method)

| | | |
|---|---|---|
| Birth weight | <2500g | 1.7 (1.2-2.2) |
| Birth weight | <1500g | 2.1 (1.3-3.6) |
| Gestational age | <37 wks | 1.3 (1.0-1.7) |
| Gestational age | <32 wks | 2.3 (1.4-4.0) |
| SGA | | 1.5 (1.0-2.3) |
| Perinatal mortality | | 3.6 (1.7-7.6) |

(Pinborg et al., Hum Reprod 2005, 2007)

Dias 13

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## Vanishing twins
### Summary

- 10% IVF singletons is survivor of a "vanishing twin"
- SGA ↑ prematurity ↑ LBW ↑
- The higher gestational age at foetal demise the higher the risk for the survivor
- "Vanishing twins" cause poorer outcome in IVF singletons

Dias 14

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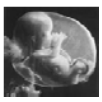
## Frozen embryo transfer



**Risks**

- Cryoprotectants
- Freezing/thawing procedure

**Advantages**

- Only the best embryos survive ~ embryo filter
- Positive patient selection
- Without ovarian stimulation
  - Only one corpus luteum
  - "Natural" hormone profile

Dias 15

ESHRE Istanbul July 2012

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## Obstetric outcome in FET vs. Fresh

| AOR (95%CI)<br>Study period<br>Singletons, N | Sweden<br>1982-1995<br>1055 | Denmark<br>1995-2006<br>1271 | Finland<br>1995-2006<br>2293 |
|---|---|---|---|
| PTB <37 weeks | 0.69 (0.50-0.95) | 0.70 (0.53-0.92) | 0.83 (0.71-0.97) |
| BW <2500 gram | 0.49 (0.02-0.75) | 0.63 (0.45-0.87) | 0.74 (0.62-0.88) |
| Malformations | 0.94 (0.74-1.21) | 0.92 (0.71-1.19) | - |

(Källen et al., Fertil Steril 2005)
(Pinborg et al., Fertil Steril 2010)
(Pelkonen et al., HR 2010)

Dias 16 16

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## Large offspring syndrome (LOF)

- Freezing/thawing procedures ?
- Epigenetic changes
  - Extended culture time
  - Asynchrony
  - Culture media
  - In-vitro culture prone to larger weight but fresh cycles prevent this

Dias 17 17

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## Large-for-gestational age

**Pelkonen HR 2010 (N=1803)**

**Large-for-gestational age in FET vs. fresh AOR 1.70 (1.21-2.40)**

Dias 18 18

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| | Singletons, N | LGA | *P* |
|---|---|---|---|
| Frozen embryo transfer | 910 | 16.9% | |
| Fresh embryo transfer | 9603 | 10.3% | <0.001 |
| Naturally conceived | 4565 | 11.4% | <0.001 |

FET vs. Fresh IVF/ICSI AOR 1.6 [1.3-1.9]*
FET vs. Naturally conceived AOR 1.5 [1.2-1.9]*

*Adjusted for maternal age, parity, child gender and year of birth

Dias 19 19

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## Birth weight > 4500 gram

| | Singletons, N | BW >4500 g | *P* |
|---|---|---|---|
| Frozen embryo transfer | 910 | 5.6% | |
| Fresh embryo transfer | 9603 | 2.8% | <0.001 |
| Naturally conceived | 4565 | 3.4% | <0.001 |

Dias 20 20

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| | Singletons, N | SGA | *P* |
|---|---|---|---|
| Frozen embryo transfer | 910 | 9.2% | |
| Fresh embryo transfer | 9603 | 14.8% | <0.001 |
| Naturally conceived | 4565 | 11.3% | 0.07 |

FET vs. Fresh IVF/ICSI AOR 0.6 [0.5-0.8]*

Adjusted for maternal age, parity, child gender and year of birth

Dias 21 21

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**Outcome in Singletons born after FET versus fresh**
(2348 FET and 8944 fresh singletons born 2002-2006)

| OR (95% CI) | Crude | Adjusted |
|---|---|---|
| 1500 g | 1.00 (0.66–1.51) | 1.07 (0.70–1.63) |
| 2500 g | 0.71 (0.57–0.89) | 0.76 (0.60–0.95) |
| 4500 g | 1.48 (1.18–1.87) | 1.46 (1.15–1.85) |
| 5500 g | 1.27 (0.13–12.2) | 0.84 (0.08–8.62) |
| SGA | 0.72 (0.54–0.96) | 0.78 (0.58–1.04) |
| LGA (>+2 SD) | 1.74 (1.39–2.16) | 1.59 (1.26–1.99) |
| LGA (>+3 SD) | 1.41 (0.85–2.33) | 1.24 (0.74–2.07) |

(Sazonova HR 2012)

Dias 22

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# I. Key message

**Children born after frozen embryo transfer have mean birth weight similar to naturally conceived children**

Dias 23 23

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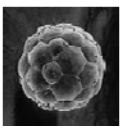
# II. Key message



**However, singletons born after FET have an increased risk of being large-for-gestational age (LGA) and BW >4500 gram**

Dias 24 24

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# Advantages with FET

- Mild and patient friendly – mimics the natural cycle
- Less multiple pregnancies
- No risk of OHSS
- Overall 19.1% delivery rate per transfer in Europe 2006
- But why LGA?



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## Sibling studies

**Differentiating between parental factors and the IVF techniques per se**

- Romundstad, Lancet 2008
- Henningsen, Fertil Steril 2010



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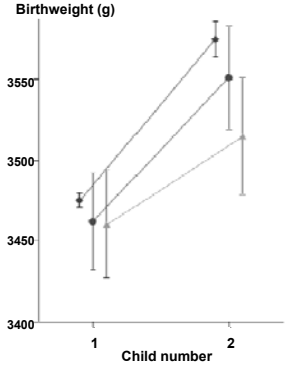


**Mean birthweight in two consecutive singleton siblings**

◇ Spo-Spo
○ ART-Spo
△ Spo-ART

Romundstad et al., Lancet 2008

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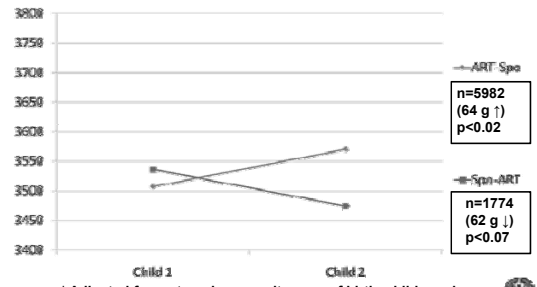
**Birthweight (g), adjusted***




* Adjusted for maternal age, parity, year of birth, child gender



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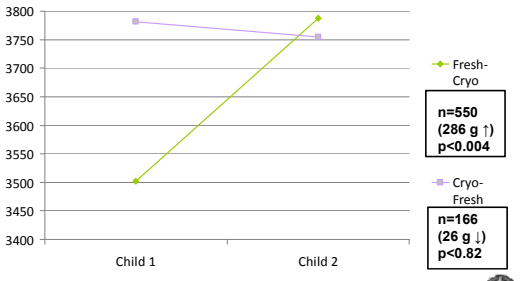
**FET: Birthweight (g), adjusted***




*Adjusted for maternal age, parity, year of birth, sex



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## Summary of sibling studies

The controlled ovarian stimulation (COS) and/or the in-vitro technique *per se* did influence the birth weight of ART singletons in the Danish study

- Norwegian study showed similar outcome in ART and non-ART sibling singletons
- Cryo singletons have a higher birth weight than children born after fresh embryo transfer, thus COS may have negative influence on the outcome

**(Romundstad 2008; Henningsen 2010)**

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## Blastocyst versus cleavage stage

Swedish Birth Register 2002-2007
1311 blastocyst and 12,562 cleavage stage transfer
Adj: Year of birth, maternal age, parity, smoking, BMI

| | |
|---|---|
| GA <37 weeks | AOR 2.3 (1.4-3.7) |
| Low birth weight | AOR 2.4 (1.3-4.2) |
| Low APGAR | AOR 3.0 (1.4-6.1) |
| Congenital malformations | AOR 1.5 (1.2-1.9) |

(Källén B, Fertil Steril 2010)



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## Blastocyst versus cleavage stage

Australia 2004-2009
Days 5-6 transfers (n=2486) vs. Days 2-4 transfers (n=1716)

| OR (95%CI) | Crude | Adjusted |
|---|---|---|
| VLBW | 0.95 (0.60–1.51) | 0.73 (0.35–1.52) |
| LBW | 1.02 (0.80–1.29) | 0.91 (0.62–1.33) |
| SGA | 0.95 (0.76–1.19) | 1.05 (0.73–1.52) |
| LGA | 0.99 (0.81–1.21) | 1.17 (0.83–1.64) |

Fernando, Fertil Steril 2012



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## Culture media and birth weight

826 IVF first treatment cycles
Live-born singletons
110 Vitrolife vs. 78 Cook
VL 3453 ± 53 g vs. Cook 3208 ± 61 g ($P$ =0.003)
After correction for gender and GA by z-score ($P$ =0.001)
Multiple linear regression showed that culture medium was significantly associated with birth weight ($P$ =0.001)

**(Dumoulin JC, HR 2010)**

## Take home messages

- Twins still remains the far most important health risk for IVF children
- Causes for the lower birth weight after ART
  - Subfertility
  - Vanishing twins
  - Controlled ovarian stimulation (COS) ?
  - Length of culture durations and culture media ?
- What are the mechanisms behind being LGA in Cryo singletons ? - and can it be prevented?





## Thank you for your attention

# Long term health implications of children after IVF and ICSI

Dr Alastair Sutcliffe
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Institute of Child Health
University College London

ESHRE 2012

What is known about the 400+ children born after oocyte cryopreservation?

**A. Very Little!!!**

What is known about the children born after PGD

**A. A little bit more**

What is known about the children born after Embryo Cryopreservation?

**A. Quite a bit more**

What is known about the children born after ICSI and 'standard' IVF?

**A. Quite a lot**

## Neurodevelopmental Outcome

**Case-control studies with cohort size > 100**

| Author | Cohort Size | Age | Study Findings |
|---|---|---|---|
| Sutcliffe *et al*. 2001 | 208 ICSI singleton<br>221 SC singleton | 1 to 2 years | No difference in Griffiths Mental Development Score |
| Koivurova *et al*. 2003 | 299 IVF<br>558 SC | Various points up to 3 years | No difference in Bayley developmental scores, in singleton and twin group analysis |
| Ponjaert-Kristoffersen *et al*. 2005 | 511 ICSI singleton<br>424 IVF singleton<br>488 SC singleton | 5 years | No difference in Wechsler scales of intelligence and McCarthy scales of motor abilities between the three groups |
| Ludwig *et al*. 2006 | 276 ICSI singleton<br>273 SC singleton | 5.5 years | No difference in motor assessment using Zimmer / Volkamer test, or intelligence assessed using Kaufman-Assessment Battery |
| Leunens *et al*. 2006 & 2008 | 151 ICSI singleton<br>153 SC singleton | 8 years, followed up at 10 years | No difference in Wechsler scales of intelligence, or motor assessment using Movement Assessment Battery for children (ABC) |

## Neurodevelopmental Outcome (2)

**Other recent studies**

| Author | Study design | Study Findings |
|---|---|---|
| Hvidtjørn et al. 2011 | Population study of 588 967 children born in Denmark from January 1995 to December 2003 | No risk of Autistic Spectrum Disorder in children born after assisted conception. |
| Källén *et al.* 2011 | Case control study of 28 158 children born after IVF compared with 2 417 886 controls | Weak but statistically significant association with ADHD found (OR = 1.18, 95% CI 1.03–1.36). Not significant if adjusted for length of childlessness |

## Neurodevelopmental Outcome (3)

**Studies reporting concerns**

| Author | Cohort Size | Age | Study Findings | Comments |
|---|---|---|---|---|
| Bowen *et al*. 1998 | 84 IVF<br>89 ICSI<br>80 SC | 1 year | Significantly lower mean Bayley mental development index (MDI) scores in ICSI children compared to IVF & SC children | • Size<br>• Follow up study of same cohort at age 5 found no difference in development between the groups (Leslie *et al.* 2003) |
| Knoester *et al.* 2008 | 86 ICSI<br>83 IVF<br>85 SC (singletons) | 5 to 8 years | • Lower mean IQ in ICSI children than SC controls (statistically significant).<br>• Lower mean IQ in ICSI than IVF children (not statistically significant). | • Size<br>• Multiple examiners |
| Zachor et al. 2011 | 507 patients with ASD | 9m-18y | 54 (10.7%) were conceived by ART, significantly higher than the number of ART pregnancies in Israeli newborn cohort of 3.06% | |

## Neurological Outcome – (1)

| Author | Age (y) | Study Findings |
|---|---|---|
| Strömberg *et al.* 2002 | 1 to 14 | • Increased risk of Cerebral Palsy (CP) in IVF singletons vs. SC controls (OR: 2.8, 95% CI 1.3-5.8)<br>• No difference in CP risk in IVF and SC twins (OR: 0.9, 95% CI 0.4-1.8)<br>• Increased CP risk in IVF singletons largely accounted for by low birthweight & premature birth |
| Ericson *et al.* 2002 | 1 to 11 | • Increased risk of hospitalisation with CP (OR: 1.69, 95% CI 1.06-2.68) and epilepsy (OR: 1.5, 95% CI 1.10-2.15) in IVF children compared to SC controls |
| Pinborg *et al.* 2004 | 2 to 7 | • No difference in risk of CP (OR:0.8, 95% CI 0.4-1.6) or neurological sequelae overall (OR: 0.9, 95% CI 0.6-1.4) between IVF twins & SC twins |
| Lidegaard *et al.* 2005 | 4 | • Increased incidence of cerebral palsy (CP) in IVF children compared to SC children (RR = 1.8 (95% CI 1.2 - 2.8) |

## Neurological Outcome – (2)

| Author | Age (y) | Study Findings |
|---|---|---|
| Källén *et al.* 2005 | Up to 6 | • Increased risk of hospitalisation with CP (OR: 1.89, 95% CI 1.37-2.60) and epilepsy (OR: 1.52, 95% CI 1.30-1.92) in IVF children compared to SC controls<br>• Statistical significance lost when only term children included, or after adjustment for various maternal factors (years of unwanted childlessness, parity, age, smoking) |
| Hvidjørn *et al.* 2006 | 1 to 7 | • Increased risk of CP in IVF children vs. SC controls (RR: 1.61, 95% CI 1.13-2.3), after adjustment for gender, maternal age, education level & parity<br>• Independent effect of IVF treatment on CP risk not significant after additional adjustment for multiplicity or gestational age |
| Sun *et al.* 2007 | Up to 6 | • Increased risk of presentation to hospital with epilepsy in SC singletons born to subfertile couples (time to conception > 12 months) compared to fertile couples (TTC < 6 months) (OR: 1.38, 95% CI 1.00-1.89)<br>• Increased risk of presentation to hospital with epilepsy in IVF/ICSI singletons born to subfertile couples compared to fertile couples (OR: 1.83, 95% CI 1.09-3.06).<br>• Above lost significance when preterm deliveries excluded. |

## Neurological Outcome – (3)

| Author | Age (y) | Study Findings |
|---|---|---|
| Reid et al. 2009 | 3-16 | Case control study with 1241 children with CP and 2482 without.<br>No significant increase in the odds of children with CP being conceived using ART (adjusted odds ratio 1.19, 95% confidence interval (CI) 0.63, 2.24) |
| Källén *et al.* 2010 | Up to 28 | • Increased risk of CP after IVF looking at cohort from 1982–2007 (OR 1.81 95% CI 1.52–2.13) but looking at 2004-2007 when twinning rate fell to <10%, odds ratio fell to 0.97 (95% CI 0.57–1.66).<br>• Data obtained from inpatient discharges and outpatient coding from 2,623,517 total infants, 31,587 born after IVF. |
| Zhu et al. 2010 | 4-13 | • Children born after IVF/ICSI had an increased risk of CP, even after adjustment for preterm birth and multiplicity (hazard ratio 2.30, 95% confidence interval 1.12–4.73)<br>• Unlike some other studies, no increased risk of CP with increasing time of childlessness however single subgroup of all childlessness >12 months |
| Hvidjørn *et al.* 2011 | Up to 15 | • Children born after ART had an increased risk of a CP, crude hazard rate ratio 1.90 (95% CI: 1.57-2.31)<br>• When adjusted for multiplicity and gestational age in multivariate models, the risk of CP in assisted conception disappeared |

## Growth & Physical Health – (1)

| Author | Cohort Size | Age | Study Findings |
|---|---|---|---|
| Wennerholm *et al.* 1998 | 255 IVF<br>252 SC | Up to 18 months | • No difference in weight / height / head circumference (HC)<br>• No difference in prevalence of common & chronic illnesses |
| Brandes *et al.* 1992 | 116 IVF<br>116 SC | 1 to 3 years | • No difference in weight / height / HC |
| Koivurova *et al.* 2003 | 299 IVF<br>558 SC | Up to 3 years | • Significantly lower weight in IVF singletons compared to SC controls<br>• IVF children more likely to have experienced a significant illness, in particular regarding respiratory illnesses & diarrhoea. |
| Banerjee *et al.* 2008 | 49 PGD<br>66 SC | 3 months - 4 years | • No difference in weight / height / HC |

## Growth & Physical Health – (2)

| Author | Cohort Size | Age | Study Findings |
|---|---|---|---|
| Bonduelle *et al.* 2005 | 540 ICSI 437 IVF 538 SC (singletons only) | 5 years | • No difference in weight / height / HC<br>• No difference in physical examination<br>• ICSI & IVF children more likely to have experienced a significant illness or had surgical intervention (in particular genito-urinary) |
| Ludwig *et al.* 2008 | 276 ICSI<br>273 SC (singletons) | 5.5 years | • No difference in weight / height / HC<br>• No difference in physical examination<br>• No difference in incidence of common & chronic illnesses<br>• Increased incidence of urogenital surgery in ICSI group |
| Knoester *et al.* 2008 | 81 ICSI<br>81 IVF<br>85 SC (singletons) | 5 to 8 years | • No difference in weight / height / HC<br>• No difference in incidence of common & chronic illnesses |

## Growth & Physical Health – (3)

| Author | Cohort Size | Age | Study Findings |
|---|---|---|---|
| Belva *et al.* 2007 | 150 ICSI<br>147 SC (singletons) | 8 years | • No difference in weight / height / HC<br>• No difference in physical examination<br>• No difference in incidence of common & chronic illnesses, or incidence of surgery |
| Miles *et al.* 2007 | 69 IVF<br>71 SC (singletons) | 4 to 10 years | • IVF children significantly taller than SC controls<br>• Significantly higher levels of IGF-II in IVF children |
| Makhoul et al. 2009 | 334 VLBW children (83 IVF, 45 ovulating agents and 203 SC) | 6-10 years | Childhood height standard deviation scores were greatest in IVF (−0.12 (SD 1.25); p<0.022) and insignificantly greater in OA (−0.37 (SD 1.02)) compared to SC (−0.58 (SD 1.36)) |
| Basatemur et al. 2010 | 143 IVF, 166 ICSI, 173 SC | Birth-12 years (measured at birth, 5 years, 7–9 years and 10–12 years | No significant differences were observed regarding head circumference, height and weight between the three groups at any of the time points |

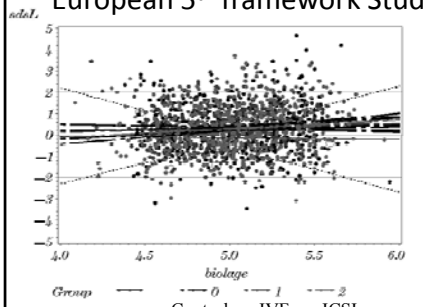
**Growth & Physical Health – (5)**

## European 5th framework Study

- Comprehensive assessment of 1550 children app. equal between ICSI/IVF and NC

**Growth & Physical Health – (6)**

## European 5th framework Study

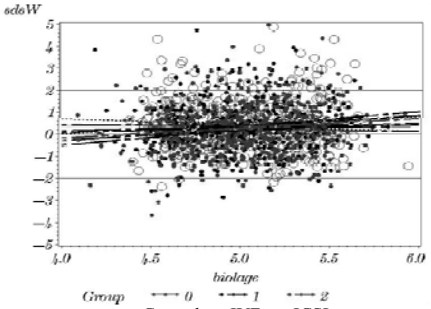



Controls IVF ICSI

No difference seen in length

**Growth & Physical Health – (5)**

## European 5th framework Study




Controls IVF ICSI

No difference seen in weight

# Cardiovascular risks

- Implications of Barker hypothesis on ART children as low birthweight more common

| Author | Cohort Size | Study Findings |
|---|---|---|
| Belva et al. 2012 | 217 ICSI, 223 SC | Pubertal females have significantly increased central, peripheral and total adiposity compared to SC<br>Males in later pubertal stages have increased peripheral adiposity |
| Ceelen et al. 2008 | 225 IVF, 225 SC | Systolic and diastolic blood pressure levels higher in IVF children (109 ± 11 *vs.* 105 ± 10 mm Hg, $P < 0.001$; and 61 ± 7 *vs.* 59 ± 7 mm Hg, $P < 0.001$)<br>Higher fasting glucose levels were observed in pubertal IVF children (5.0 ± 0.4 *vs.* 4.8 ± 0.4 mmol/liter in controls; $P = 0.005$). |
| Sakka et al. 2010 | 106 IVF and 68 SC, aged 4–14 years | Significantly higher systolic and diastolic blood pressures and triglycerides than controls<br>No significant differences in biochemical indices of insulin resistance, circulating adipokines, and inflammatory markers |

## Healthcare Utilisation – (1)

| Author | Cohort Size | Age | Study Findings |
|---|---|---|---|
| Leslie *et al.* 1998 | 95 IVF<br>79 SC | 4 to 12 months | • No difference in number of visits to healthcare providers (e.g. GP, outpatients dept, A&E) |
| Ericson *et al.* 2002 | 9,056 IVF<br>1,417,166 SC | 1 to 11 years | • IVF singletons more likely to have been admitted to hospital than SC controls (OR: 1.40, 95% CI 1.32-1.48 |
| Bonduelle *et al.* 2004 | 300 ICSI<br>266 SC (singletons) | 5 years | • ICSI children more likely to have received therapy (physiotherapy, speech & language, orthoptic, dietary, psychological therapy) |
| Bonduelle *et al.* 2005 | 540 ICSI<br>437 IVF<br>538 SC (singletons) | 5 years | • ICSI & IVF children more likely to have been admitted to hospital<br>• ICSI & IVF children more likely to have received therapy (e.g. physiotherapy, speech & language therapy) |

## Healthcare Utilisation – (2)

| Author | Cohort Size | Age | Study Findings |
|---|---|---|---|
| Källén *et al.* 2005 | 11,283 IVF<br>4,949 SC | Mean 5.5 years | • IVF children more likely to have been admitted to hospital than general population (OR: 2.09, 95% CI 2.02-2.16) up to age of 6 years<br>• No difference in hospital admission between IVF & ICSI children |
| Koivurova *et al.* 2007 | 303 IVF<br>567 SC | Up to 7 years | • IVF children more likely to have been admitted to hospital<br>• Post-neonatal health care costs 2.6 times greater in IVF children |
| Belva *et al.* 2007 | 150 ICSI<br>147 SC (singletons) | 8 years | • No difference in hospital admission.<br>• No difference in use of therapy (physiotherapy, speech, psychological therapy) |
| Ludwig *et al.* 2008 | 276 ICSI<br>273 SC | Mean 5.5 years | • ICSI children more likely to have been admitted to hospital |
| Knoester *et al.* 2008 | 81 ICSI<br>81 IVF<br>85 SC | 5 to 8 years | • No difference in hospital admission, number of GP visits, or treatment by specialists |

## Fertility

- Concerns over potential for inherited infertility
- ICSI raises particular concerns over Y chromosome microdeletions

| Author | Cohort Size | Study Findings |
|---|---|---|
| Mau Kai et al. 2007 | 264 IVF/ICSI fathers and sons, and in 168 fertile men | AZFc deletions/polymorphisms significantly more frequent in ART father s than controls. All deletions were transmitted to the sons. AZTc associated with infertility. |
| Belva et al. 2011 | 58 ICSI, 62 SC aged 14 | Salivary testosterone levels the same between groups including those whose fathers had severe oligozoospermia |

## Psychological and emotional wellbeing

| Author | Cohort Size | Study Findings |
|---|---|---|
| Van balen. 1996 | 45 IVF, 35 formally infertile, 35 fertile | No negative differences found in parent-child relationships |
| Golonbuk et al. 2001 and 2009 | 111 donor insemination, 116 IVF, 120 SC, 115 adopted aged 4-8 followed to 12yrs and then to 18years | More positive relationship with their children at 12 years.<br><br>Increased warmth between mothers and 18 yr olds in IVF and DI families than adopted. No difference in warmth was found between IVF and NC. |
| Barnes et al. 2004 | 439 IVF, 540 ICSI, 542 SC | No negative impact on parent-child relationships or family |
| Wagenaar et al. 2011 | 86 IVF, 97 controls | No differences in behaviour or socioemotional functioning self-reported by young people |

## Psychological and emotional wellbeing (2)

| Author | Cohort Size | Study Findings |
|---|---|---|
| Beydoun et al. 2010 | 173 IVF aged 18-26 | Young adults conceived by IVF were found be similar to the U.S. general population on most risk factors for chronic disease development but excess psychological problems.<br>Depression: 15.9% v 12.7% expected<br>ADHD: 27.1% v 3%–5% expected |
| Zhu et al 2011 | 25059 SC, 2765 subfertility, 2361 infertility treatment, 5766 unplanned pregnancies | Teachers: higher total difficulties score for children born after infertility treatment (but no significant differences seen on any subscales). Mothers: no differences on total score (some higher on peer problems subscale). Self-reported: no difference. NB – no consideration of gestational age although twins/triplets excluded. |

## Long Term Outcomes after In vitro Maturation

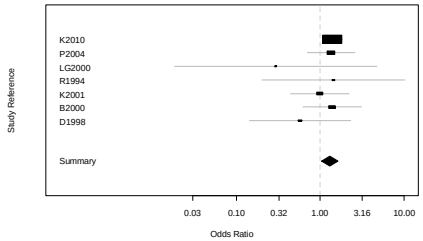
- Emerging field so limited data thus far
- Further research on possible epigenetic changes needed

| Author | Subjects | Study Findings |
|---|---|---|
| Söderström-Anttila 2006 | 46 IVM<br>No controls | Growth compared to national means:<br>- Mean height age 2 years: girls=+0.1 SD, boys=+0.2 SD<br>- Mean height-related weight age 2 years: girls=+1.1 percentile, boys=−1.3 percentiles<br>Bayley Scale at age 2: Normal in 34/35 children, Mild developmental delay in 1/35. |
| Shu-Chi 2006 | 21 IVM<br>21 SC | Bayley Scale at 6–24 months:<br>- Mean Mental Development Index scores not significantly different between IVM (92.7) or SC (97.2) groups (p=0.07).<br>- Mean Psychomotor Development Index scores not significantly different between IVM (96.7) or SC (96.2) groups (p=0.82). |
| Buckett 2007 | 55 IVM<br>338 SC | Birth weight: IVM=3.48kg, SC=3.26kg |

## Long Term Outcomes after Preimplantation Genetic Diagnosis

| Author | Cohort Size | Age | Study Findings |
|---|---|---|---|
| Banerjee *et al.* 2008 | 49 PGD<br>66 SC | 3 months to 4 years | No difference in Griffiths Mental Development Scores between the two groups. |
| Nekkebroeck *et al.* 2008 | 70 PGD singleton<br>70 ICSI singleton<br>70 SC singleton | 2 years | No difference in Bayley Scales of Infant Development scores between the three groups |

## Cancer risk in children born after ART




Williams et al. Systematic Review and Meta-analysis of Cancer Risk in Children Born after Assisted Reproduction. Arch Dis Child 2011

- Possible small increased risk of childhood cancer after ART
- Further, larger studies warranted

## Childhood Cancer

## Childhood Cancer

## Summary of where knowledge is today

## Neurological/neurodevelopmental Outcomes

- There is probably an increased risk of Cerebral Palsy (OR from 1.3 to 1.85)
- There is also a higher risk of epilepsy (OR of 1.83)
- There may be an increased risk of Autistic spectrum disorders and ADHD

## Growth and physical health

- There is a higher risk of hospital admission and accessing health care, therapists etc. ( OR 2.09)
- Growth is probably not affected

## Psychological

- No concerns exist about family relationships and psychosocial issues after ART conception.
- There may be an increased incidence of psychiatric diagnoses in adolescents

## Cardiovascular

- There may be an increase in adverse cardiovascular risk factors
- This may be associated with low birth weight

## Overall

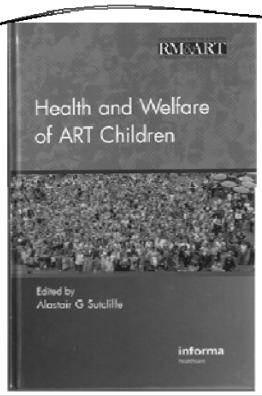
- Mature term babies born after ART progress healthily in relation to naturally conceived children.
- Little evidence exists about other health problems in children born after ART.
- However this is an emerging field of evidence with long term threats unanswered.

## Higher Order Births

- Remains the main threat of ART at present!
- BUT this is a changing field.

## Further reading

**Review Article**
**Sutcliffe** AG , **Ludwig** M
Outcome of assisted reproduction.
Lancet. 2007;370:351-9.




## Final Message
## (for fertility specialists!)

- 'Please think of the children'
- 'Prima di tutto pensa ai bambini'
- 'Denk aan de Kinderen alstublieft'
- 'Lutfen cocuklari dikkat!
- Thankyou
  a.sutcliffe@ucl.ac.uk

**Mark your calendar for the upcoming ESHRE Campus events**

- Basic Semen Analysis Course in Greek Language
  4-7 September 2012 - Athens, Greece
- Basic Genetics for ART practitioners
  7 September 2012 - Rome, Italy
- Regulation of quality and safety in ART – the EU Tissues and Cells Directive perspective
  14-15 September 2012 - Dublin, Ireland
- Basic Semen Analysis Course in Spanish language
  18-21 September 2012 - Galdakano,Vizcaya
- GnRH-antagonists in ovarian stimulation
  28 September 2012 - Hamburg, Germany
- The best sperm for the best oocyte
  6-7 October 2012 - Athens, Greece
- Basic Semen Analysis Course in Italian language
  8-11 October 2012 - Rome, Italy
- Accreditation of a preimplantation genetic diagnosis laboratory
  11-12 October 2012 - Istanbul, Turkey
- Endoscopy in reproductive medicine
  21-23 November 2012 - Leuven, Belgium
- Evidence based early pregnancy care
  29-30 November 2012 - Amsterdam, The Netherlands

www.eshre.eu
(see "Calendar")

Contact us at info@eshre.eu

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