Clinical epigenetics and its relation to ART

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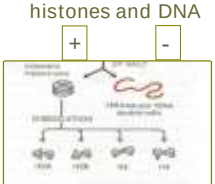
Epigenetics and ART

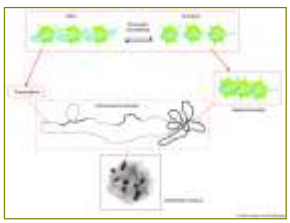
- Basics of epigenetics
- Genomic imprinting
- Human imprinting disorders
- Review data on imprinting disorders in ART children

What is epigenetics?

Chromatin structure: histones and DNA



What is epigenetics?

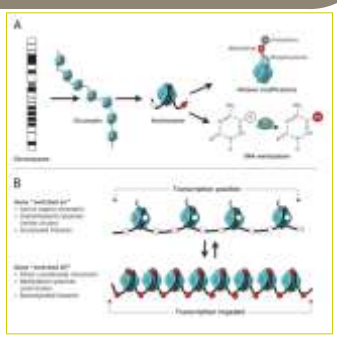


gene expression

gene silencing

Basic epigenetic mechanisms

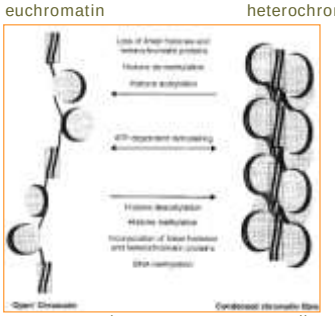
"epigenetics": refers to modifications on top of DNA and histones that will influence gene expression patterns without changes in the DNA code



Basic epigenetic mechanisms

euchromatin

heterochromatin



gene expression

gene silencing

What is epigenetics?

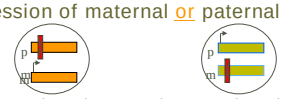
"epigenetics":
study of changes in gene expression that occur without changes in the DNA code

gene expression patterns
- inheritable through mitosis and meiosis
- changes during cell differentiation
- influenced by environmental factors

Genomic imprinting

most genes: biallelic expression or silencing
expression of maternal and paternal alleles

imprinted genes: monoallelic expression
expression of maternal or paternal alleles

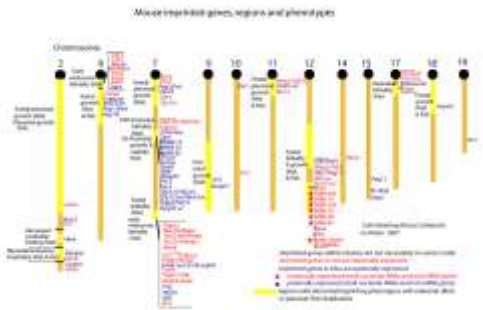


Maternal copy is expressed Paternal copy is expressed

Genomic imprinting

- > 80 imprinted genes (0.1-1% of all genes)
- Eutherian mammals, marsupials, higher plants
- Key role in embryonic growth and placental function, cognition and maternal behaviour
- Growth regulation: paternally expressed genes enhance growth, maternally expressed genes repress growth
- Defective imprinting involved in carcinogenesis and in human genetic diseases

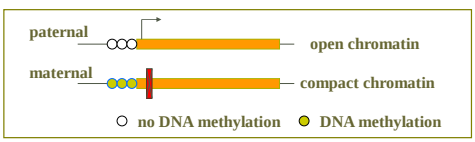
Genomic imprinting



http://www.mgu.har.mrc.ac.uk/research/imprinting/

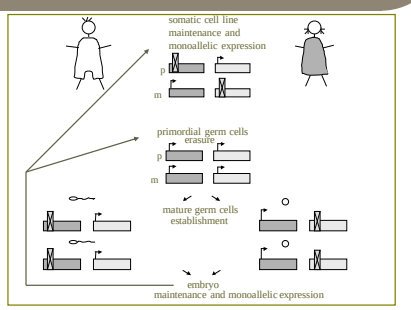
Genomic imprinting

- Imprint Control Regions (ICR) control imprinting of the clusters
- Maternal and paternal alleles carry different epigenetic modifications (“imprints”)



Genomic imprinting

gametogenesis
mprint resetting



early embryonic development
imprint maintenance

Epigenetics and ART

Does ART interfere with

- epigenetic reprogramming (imprint maintenance) in the early embryo?
- epigenetic reprogramming (imprint resetting) during gametogenesis?

Epigenetics and ART

Recent studies have presented (limited) evidence for a higher risk of imprinting disorders after ART

Imprinting disorders: altered expression of imprinted genes

- epigenetic mechanisms
- genetic mechanisms (large duplications or deletions, mutations in imprinted genes and ICRs, uniparental disomy)

Angelman syndrome

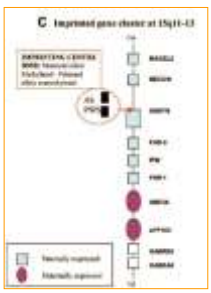
- OMIM 105830
- Incidence: 1/15.000
- Neurogenetic disorder
  - severe mental retardation
  - ataxia
  - “happy puppet syndrome”
  - absence of speech

Angelman syndrome

Angelman syndrome

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

=> loss of *UBE3A* expression



ART and AS

Syndrome	# cases	ART	country	reference
AS	2	ICSI	Germany	Cox et al., 2002
AS	1	ICSI	Norway	Orstavik et al., 2003

molecular analysis: an epigenetic defect (loss of maternal methylation at the IC of imprinted region on chr15q11-13) was found in all three cases
+ 1 case in Ludwig et al.,2005
+ 1 case in Sutcliffe et al.,2006

Beckwith-Wiedemann syndrome

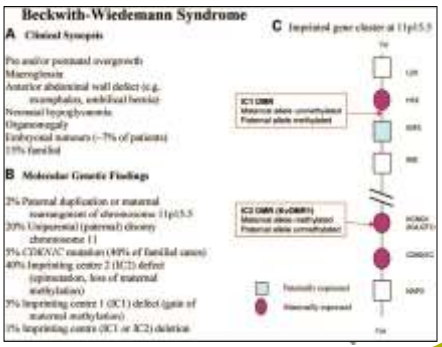
- Incidence: 1/13700

- Overgrowth syndrome with predisposition for embryonal tumours

- Caused by genetic or epigenetic defects in an imprinted region on chr11p15

- Inheritance: - 15% familial cases, autosomal dominant
  - 85% sporadic cases: - 20 % genetic (pUPD, mutations)
    - 60 % epigenetic (aberrant methylation)
    - 20 % unknown

Beckwith-Wiedemann syndrome



ART and BWS

ART/ cohort	study	#LOM/ #tested	#ICSI/ #IVF	RR	country	reference
4 3/65*	retro. prosp.	5/6	5/2	x 6	USA	DeBaun et al., 2003
6/149	retro.	2/2	3/3	x 4	UK	Maher et al., 2003
6/149	retro.	6/6	2/4	x 3	France	Gicquel et al., 2003
4/37	retro. case-contr.	3/3	1/3	x 9	Australia	Halliday et al., 2004
	retro.	24/25	13/12	-	UK	Lim et al., 2009
11/79	retro.	8/8	5/1/5HT		UK	Sutcliffe et al., 2006

RR = relative risk; HT = hormonal treatment
LOM = loss of maternal methylation * overlapping patients

ART and BWS

Association between
ART and BWS to be considered

→epidemiological data

→molecular analysis: an epigenetic defect (loss of maternal methylation at BWSIC2) in nearly all BWS patients after ART

→general population: epigenetic defect in 50-60% of cases

ART and imprinting disorders

- Insufficient evidence for association between ART and other imprinting disorders (SR, PWS, global hypomethylation syndrome, RB)
- limited evidence for association between ART and AS and BWS
- absolute risk is low

Cause of imprinting disorders after ART

* in-vitro culture systems

- Khosla et al. 2001: culture (serum) of preimplantation mouse embryos reduced fetal development (lower birthweight) and expression of imprinted genes
- Young et al. 1998: after exposure *in vitro* unusually large offspring syndrome (LOS) in relation with imprinted genes
- Young et al. 2000: epigenetic changes in *IGF2R* are associated with fetal overgrowth (LOS) after sheep embryo culture

The mammalian preimplantation embryo is very sensitive to culture conditions; epigenetic changes induced at the early stages may lead to altered phenotypes at later stages

Cause of imprinting disorders after ART

* use of hormonal ovarian stimulation

- mouse: comparison of methylation in 4 imprinted genes in normal vs superovulated GVs: no differences for maternally methyl. genes, but gain of methylation for H19 after superovulation
  Sato et al, 2007
- human: superovulated GV & MI oocytes show gain of H19 methylation (2/6) and loss of maternal methylation at PEG1 (6/16)
  Sato et al, 2007
- human: methylation analysis of KvDMR1 in GV, MI & MII superovulated oocytes shows loss of methylation in 1/16 oocytes
  Geuns et al., 2007

Cause of imprinting disorders after ART

* parental infertility

- Doornbos *et al.*, 2007: correlation between ART, fertility problems and epigenetic defects (cohort 220 AS, BWS, PWS patients)

	cohort	general population
Born after ART	6.4%	2.1%
Fertility problems (no ART)	6.8%	3.5%

Cause of imprinting disorders after ART

* parental infertility

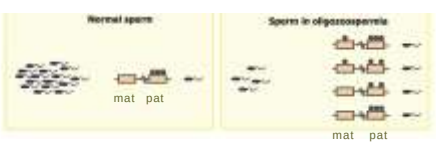
subfertile couples are predisposed to epigenetic defects
imprinting defects and subfertility have a common cause

- AS cohort study: subfertile couples have an increased risk of conceiving a child with an imprinting defect
TTP> 2 years, no therapy RR 6.25
TTP> 2 years, treatment RR 12.5 *Ludwig et al., 2005*

Cause of imprinting disorders after ART

* parental infertility

Link between imprinting errors and disruptive spermatogenesis



Filipponi et al., 2009

- Incomplete establishment of imprints (incomplete DNA methylation of *H19*) in sperm from men with fertility problems
Marques et al., 2004 & 2008 & 2009

Cause of imprinting disorders after ART

* parental infertility

Link between imprinting errors and disruptive spermatogenesis

- Aberrant DNA methylation at imprinted loci in men with fertility problems *Kobayashi et al., 2007*

- Aberrant DNA methylation at imprinted loci in 17/78 paired DNA samples (conceptus and father)
the DNA methylation errors were inherited from the father (7/17)
they were associated with DNA sequence variants in DNMT3L and more prevalent in oligozoospermic men *Kobayashi et al., 2009*

Epimutations associated with ART

- in-vitro culture systems
- hormonal stimulation
- parental infertility

imprinted genes: vulnerable

→ functionally haploid
→ differential epigenetic modifications
→ embryonic and placental development

Iceberg theory

epigenetic defects

- imprinting disorders (congenital syndromes) (tip of iceberg)
- neurodevelopmental delay
- cancer predisposition
- IUGR
- increased risk for cardiovascular diseases

Iceberg theory

- No higher risk of childhood cancer

  Doyle et al. 1998, (UK n= 2057), Bruinsma et al. 2000, (Australia, n = 5249), Klip et al. 2001, (the Netherlands, n = 9484), Lerner-Geva et al. 2000, (Israel n = 332)

- Lidegaard et al., 2005:
  - → systematic follow up of 6052 ART singletons (1995-2001) till 2002 vs a control group of 442.349 non-ART
  - → the incidence rate of childhood cancer, mental diseases, imprinting syndromes and developmental disturbances was equal in the two groups

Iceberg theory

- *Ceelen et al., 2008*: follow-up of IVF children vs controls born to subfertile couples
  - →IVF children show higher blood pressure levels
- ART is associated with an increased incidence of Low Birth weight

  maybe as a consequence of embryonic/fetal epigenetic programming in response to early adverse environmental factors and stress???

Iceberg theory

- *Katari et al., 2009:* changes in DNA methylation and gene expression patterns of imprinted and non-imprinted genes in cord blood and placenta of ART children (n=10) versus spontaneously conceived children (n = 13)

  -> phenotype?

  -> long term: increased risk for metabolic and cardiovascular diseases?

Conclusion and perspectives

- Epidemiological data: increased relative risk for AS and BWS after ART but absolute risk is low
- need for long-term follow up
- Prospective multi-centre studies and record data on infertility history, hormonal stimulation, *in vitro* culture conditions
- Need for basic epigenetic and imprinting research
  - → in animal models
  - → human gametes and embryos