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Effect of embryo culture on epigenetic adaptation

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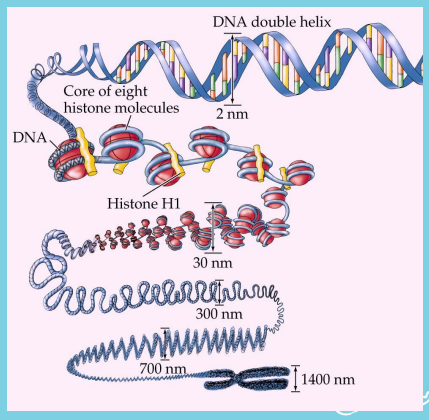
Epigenetics is the study of heritable changes in gene expression or cellular phenotype caused by mechanisms other than changes in the underlying DNA



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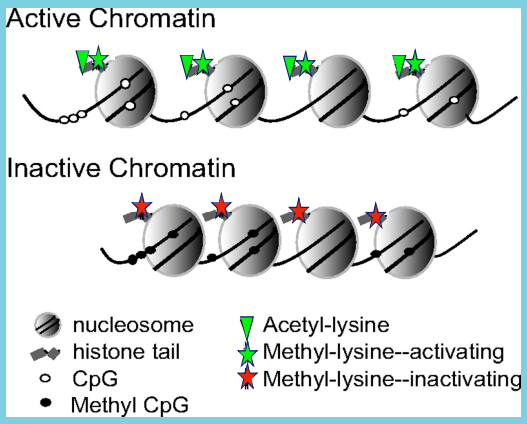
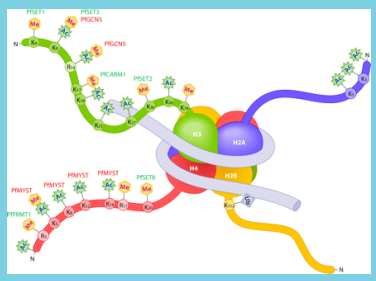
Epigenetics & chromatin





CpG methylation and histone modifications configure chromatin → affects structure and transcription factors access

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Can epigenetics explain ART related abnormalities?

ART ≠ spontaneously conceived children:

- Birth defects: Birth weight, preterm birth, perinatal mortality (Helmerhorst et al 04, McDonald et al 09)
- Congenital malformations : esophagal atresia, urogenital defects, neural tube defects etc (Kallen et al 05, 10)
- Physical development: changes in blood pressure, fasting glucose, fasting insulin, DHEAS, height (Ceelen et al 07, 08, 09)

(selected by van Montfoort et al, 2012)

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Function of Epigenetic adaptation

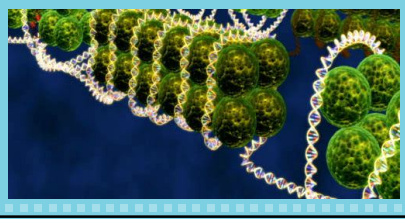
Optimal adaptation by embryo/placenta/young to environment.

But mismatch generates disease

→Barker hypothesis/ Thrifty phenotype (Barker, 04; Jirtle & Skinner, 07)

= Developmental origins of health and disease (DOHaD)

Example hunger winter pregnancy - heart disease (heijmans et al 08)



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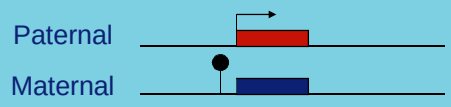
Special form of epigenetics: genomic imprinting

Epigenetic regulatory mechanisms:

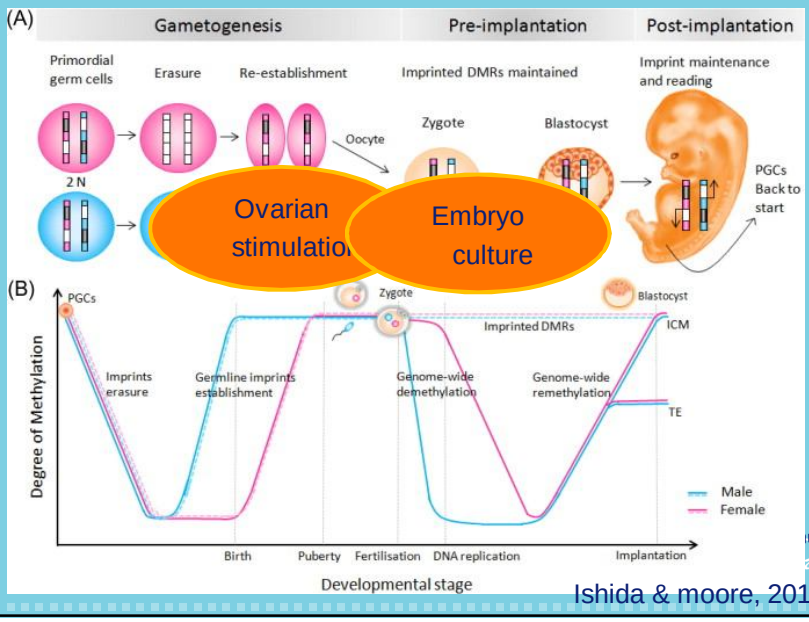
1. Individual gene regulation
2. X chromosome inactivation
3. Retroviral silencing
4. Genomic imprinting

Genomic imprinting:

Mono-allelic parental specific expression



Link epigenetics, DOHAD and ART



Ishida & moore, 2012

Possible causes of ART effects on fysiology

- Subfertility, time to pregnancy
- Ovarian Hyperstimulation
- Embryo culture
- Hyperstimulated endrometrium
- Asynchronous transfer

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Animal manipulations of embryos affect outcome (diff species)

Influence:

- fetal growth and viability.
- reduced pregnancy rates,
- reduced viability and growth,
- increased developmental abnormalities,
- behavioral deviations,
- prone to metabolic and growth disorders (large offspring syndrome)
- display aberrant gene expression

Many of these correlated with epigenetic and genomic imprinting changes

Mann et al 2004, Amor Halliday 2008

Also see van Montfoort et al, 2012

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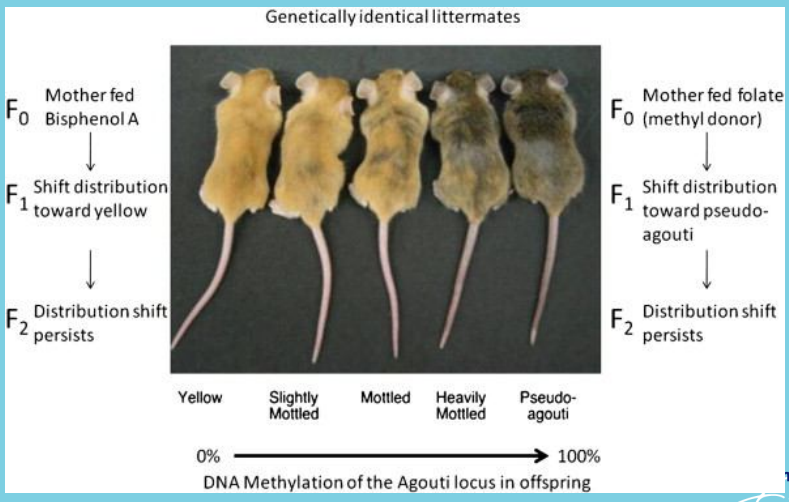
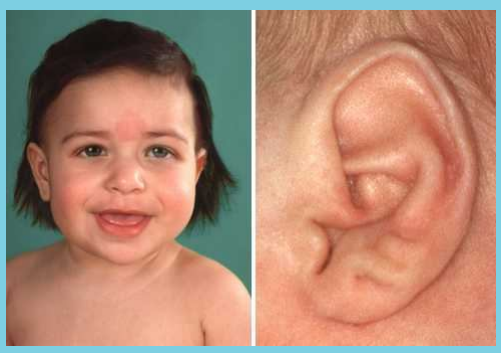
IVF defects due to adaptation or lack of methyl donors??



Wolstenholme et al, 2011

Human imprinting syndrome: Bechwith Wiedeman syndrome



- EMG triad (abdominal wall defect-macroglossia-pre- and postnatal growth abnormalities, earlobe pits or creases, facial nevus flammeus, hypoglycemia, renal abnormalities and hemihypertrophy (unilateral overgrowth)
- Phenotype normalizes during childhood

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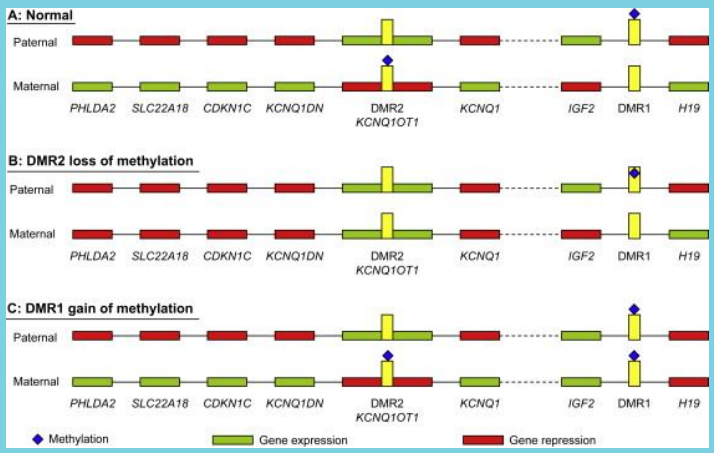
Imprinted locus 11p15 BWS



- Biallelic expression of *KCNQ1OT1* is seen in 50% of BWS patients = partial or complete loss of maternal allelic-specific methylation at KvDMR1, a CpG island upstream of *KCNQ1OT1*

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Experimental and clinical prove for hypothesis IVF-BWS

Table 1: Summary of studies of BWS after ART

Series	Gicquel et al 2003	Maher et al 2003	Debaun et al (2003) 2 registries		Halliday et al 2004
Nature of the study BWS definition	retrospective molecular	retrospective clinical	retrospective clinical	prospective	case-control clinical
N° in registry	149	149	-	65	37 BWS
N° of ART cases	6	6	4	3	4
Odds ratio (95% CI)	3.2 (1.7-7.3)	3.5 (1.5-8.8)	? ?	5.7 ?	17.8 (1.8-432.9)
Molecular analysis					
N° analyzed	6/6	2/6	6/7		3/4
Demethylation of KCNQ1OT	6/6	2/2	4/6		3/3
ART procedures					
Sperm	6/6 ejaculated	?	6 ejaculated/1testicular		?
ICSI	2	3	5		3
IVF	4	3	2		1
Frozen embryo	1/6	?	?		3/4
Transfer day 2/3/5	4/1/1	?	?		4/0/0

Imprinting disorders: 7/31850 (kallen et al, 2005, 2010)

0/6052 (lidegaard et al, 2005)

6 bws/15162 (viot et al, 2010)

Spontaneous 1/ 13700 (amor & halliday 2008)

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Imprint BWS region is late in mouse and human oocyte maturation

Germinal vesicle oocytes do not yet contain the proper imprint, most MII oocytes do (89-94%)

→ Thus, locus is possibly affected by hormonal stimulation, by timing and method final maturation treatment.

- Khoureiry et al, 2008
- Geuns et al, 2007

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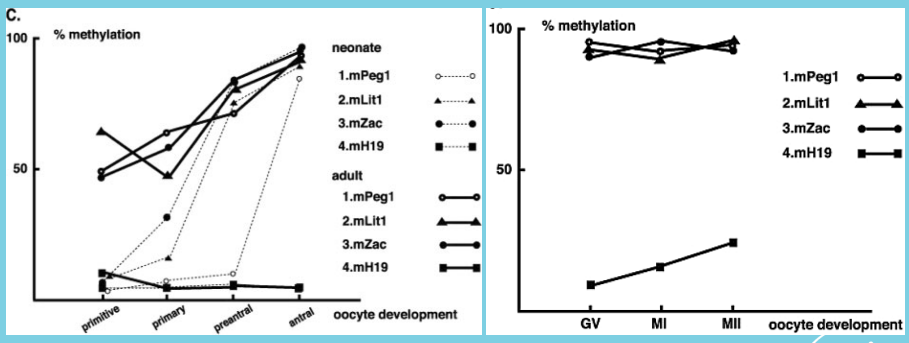
Effect ovarian hyperstimulation on imprinted loci

Human Reproduction Vol.22, No.1 pp. 26–35, 2007
Advance Access publication August 21, 2006.

Aberrant DNA methylation of imprinted loci in superovulated oocytes

A.Sato[1,*], E.Otsu[1,*], H.Negishi[1], T.Utsunomiya[1] and T.Arima[2,3]



Multilocus hypomethylation disorder

The epigenetic imprinting defect of patients with Beckwith—Wiedemann syndrome born after assisted reproductive technology is not restricted to the 11p15 region

S Rossignol, V Steunou, C Chalas, A Kerjean, M Rigolet, E Viegas-Pequignot, P Jouannet, Y Le Bouc, C Gicquel

J Med Genet 2006;43:902–907. doi: 10.1136/j

Both maternal and paternal alleles affected →

Maintenance of imprints affected

ol.24, No.3 pp. 741–747, 2009
on December 10, 2008 doi:10.1093/humrep/den406

ORIGINAL ARTICLE *Reproductive genetics*

Clinical and molecular genetic features of Beckwith–Wiedemann syndrome associated with assisted reproductive technologies

Derek Lim[1,2], Sarah C. Bowdin[1,2], Louise Tee[1], Gail A. Kirby[1], Edward Blair[3], Alan Fryer[4], Wayne Lam[5], Christine Oley[1,2], Trevor Cole[1,2], Louise A. Brueton[1,2], Wolf Reik[6], Fiona Macdonald[2], and Eamonn R. Maher[1,2,7]

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Many parameters in IVF culture

- Embryo culture: media, CO2, temp, VOC, oil…
- Media: composition: salts, nutrients, osmolarity, pH, macromolecules, chelators
- Single step, sequential composition, waste,
- Manipulation/handling
- Light, stability of pH, temp (in & out incubator)
- Timing retrieval~hCG, denudation, fertilization, follicle size retrieval, timing ET, rinsing hyase, mops, hepes?

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Quiet embryo hypothesis

- Henry Leese, 2008: optimal embryo environment results in quiet top embryo's with lower oxygen and nutrient consumption compared to less quality embryos
- Adaptation to environment: initial responses to metabolic environment, confirmed by epigenetic markers
- Metabolic state early embryo ~ physiology adult (DOHAD)
- ART might interfere with genomic imprinting, by altering the erasure, the acquisition or the maintenance of imprints

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Literature study van Montfoort et al, 2012: effect ART on expression + imprinting

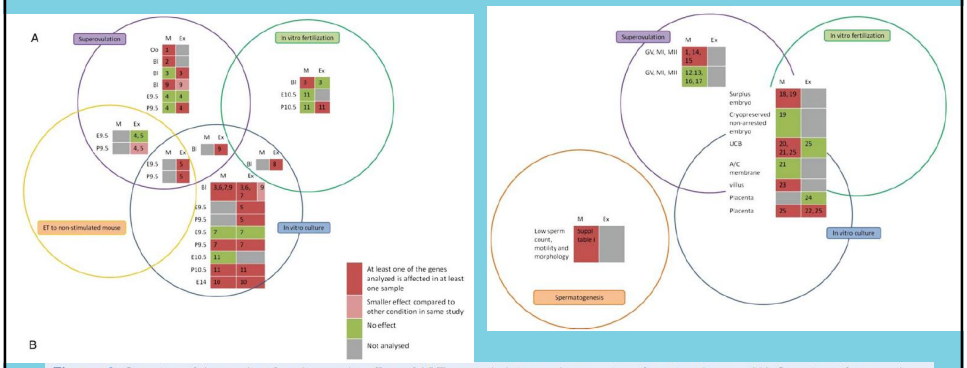
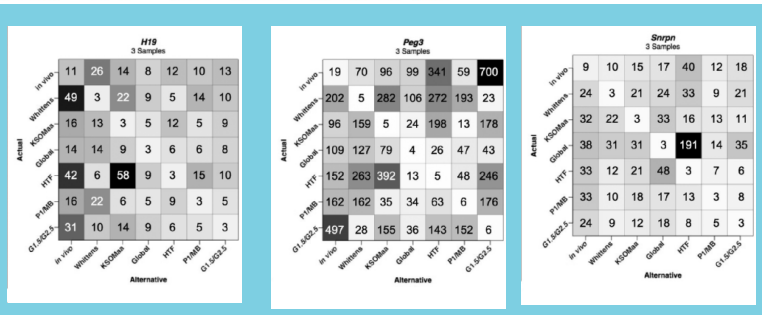


Figure 6 Overview of the results of studies on the effect of ART on methylation and expression of imprinted genes. (**A**) Overview of mouse data. (**B**) Overview of human data. A/C = Amnion/Chorion, Bl = Blastocyst, E = embryo with the age, Ex = Expression which indicates either the level of expression or the allelic expression, ET = embryo transfer, GV = germinal vesicle oocyte, M = methylation, MI = oocyte in meiosis I, MII = oocyte in meiosis II, P = placenta with the age, UCB = umbilical cord blood. The numbers refer to the studies: 1. Sato *et al.* (2007), 2. Market-Velker *et al.* (2010a), 3. Fauque *et al.* (2007), 4. Fortier *et al.* (2008), 5. Rivera *et al.* (2008), 6. Doherty *et al.* (2000), 7. Mann *et al.* (2004), 8. Li *et al.* (2005), 9. Market-Velker *et al.* (2010b), 10. Khosla *et al.* (2001), 11. Fauque *et al.* (2010a), 12. Geuns *et al.* (2003), 13. Geuns *et al.* (2007b), 14. Khoueiry *et al.* (2008), 15. El-Maarri *et al.* (2001), 16. Borghol *et al.* (2006), 17. Geuns *et al.* (2007a) 18. Chen *et al.* (2010), 19. Ibala-Romdhane *et al.* (2011), 20. Gomes *et al.* (2009), 21. Tierling *et al.* (2010), 22. Turan *et al.* (2010), 23. Zechner *et al.* (2010), 24. Zhang *et al.* (2010), 25. Katari *et al.* (2009).

BIOLOGY OF REPRODUCTION **83,** 938–950 (2010)
Published online before print 11 August 2010.
DOI 10.1095/biolreprod.110.085480

Side-by-Side Comparison of Five Commercial Media Systems in a Mouse Model: Suboptimal In Vitro Culture Interferes with Imprint Maintenance[1]

B.A. Market-Velker,[3,4,5] A.D. Fernandes,[6,7] and M.R.W. Mann[2,3,4,5]



KSOMaa ~Global

HTF no aa~Whitten,

Rest has aa + P1 taurine, P1/MB citrate, G1/2 vitamins

(G1 also taurine, HPLC, unpubl. HvD)

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Effect medium culture on epigenetics

- Gene expression and imprinting affected by culture in mouse media and commercial IVF media
- Need to know composition of commercial media (**Niemitz & Feinberg '04....)**
- Need to know composition of oviductal fluid post ovulation (but also of microenvironment, not easy to study or to mimic)
- Also improvements pH regulation, osmolar optimization and temperature needed to reduce stress and possibly metabolic rates

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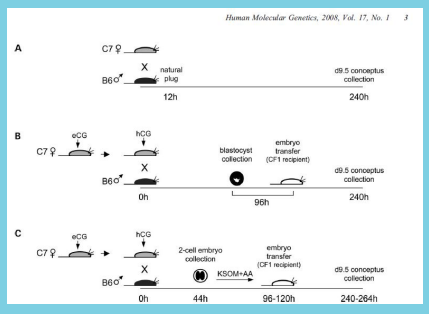
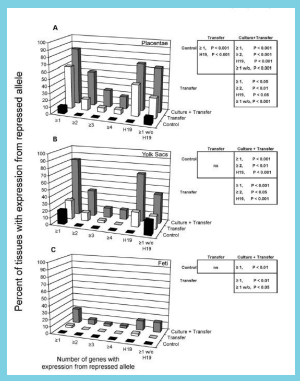
Effect embryo transfer on imprinted loci?





Rivera et al, 2008

Embryo pipeting activates the stress kinases MAPK8/9 and SAPK/JNK (46,47).

JNK kinases down-regulate Polycomb Group proteins (PcGs), which confer repressive histone modifications at imprinted loci. Xie et al 2006, 2007

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Questions??

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ZFP57 gene is involved in establishment and mainly maintenance of most maternal and paternal imprints.

Homozygous and compound heterozygous *ZFP57* mutations have been identified in approximately 50% of TNDM1 with MHD, mostly in consanguineous families (6).

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Grandma's curse

- Some see this as contrary to Darwinism, since it would permit characteristics acquired during an organism's lifetime to be passed on to its offspring, as suggested by a rival theory of evolution put forward by Jean-Baptiste Lamarck

Perinatal nicotine exposure induces asthma in second generation offspr

an, **Jie Liu**, **Erum Naeem**, **Jia Tian**, **Reiko Sakurai**, **Kenny Kwong**, **Omid Akba**

BMC Medicine 2012, **10**:129

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Doherty, bartolom 2000,

Fernandez-gonzalez gutierrez-adan, 2004 pnas

Alles waar mouse strain specificity voor te vindne is, zoals bv osmolarity sensitivity 2 cell block, blastocysts outgrowth, kan ook patient sensitivity geven

Rossignol

Table 1. Epigenetics and embryonic metabolism
Evidence for epigenetic regulation of preimplantation embryo metabolism

Epigenetic marks link genome with environment via regulation of gene expression

In vitro environment alter embryonic metabolism
In vitro environment alters embryonic DNA methylation patterns

Culture substrates alter metabolic pathways
Individual culture media differentially alter Dmnt expression

DNA damage induces increased metabolic activity
ROS associated with delayed embryonic development
ROS increase histone acetylation