The epigenetic perspective of ART

Gabriele Gillessen-Kaesbach
Institut für Humangenetik Lübeck

1 % of all births
are associated to ART

Safety of the procedure?

Adverse health outcome after ART: The epigenetic perspective

- **Imprinting defects**

Angelman syndrome

Beckwith-Wiedemann syndrome

- **low birth weight**

- **malformations**

Oculo-Auriculo-Vertebral Spectrum

Three important questions:

Is ART associated with an increased risk of imprinting defects (AS, BWS)?

Do epigenetic effects contribute to low birth weight and malformations seen in ART children?

If the associations are genuine, what are the causes?

Adverse clinical outcome of ART

Malformations

Controverse studies

Risk due to the procedure itself ?

Risk factors in the parents ?

Adverse clinical outcome of ART

Malformations

Prospective study by Ludwig et al. (2002):

8.6% with major malformations

6,9% in a control cohort

Adverse clinical outcome of ART

Low birth weight

Increased frequency of
children who are born small for gestational age (SGA)

Methylation analysis in ICSI children who were born small for gestational age (SGA)

- Work in progress -

LIT1
IGF2
H19
PEG1
PEG3
GTL2
ZAC1

Adverse clinical outcome of ART

malformations

low birth weight

possibly: imprinting disorders

ART and imprinting defects

Angelman syndrome

Beckwith-Wiedemann syndrome

Angelman Syndrome (AS)

Microcephaly
Ataxia
Epilepsy
Abnormal EEG
Absence of speech
Severe mental retardation
Friendly behaviour

UBE3A mutation (mat)	10%
Deletion 15q11-q13 (mat)	70%
Uniparental Disomy 15 (pat)	1%
Imprinting defect (mat hypomethylation)	4%
Others	15%

ART and imprinting defects

Angelman syndrome

Cases reports/preliminary studies

Cox et al. *Am J Hum Genet* 71:162-164, 2002

Ørstavik et al. *Am J Hum Genet* 72:218-219, 2003

Sutcliffe et al. *Hum Reprod*, 21:1009-1011, 2006

To date 6 children with AS and an imprinting defect after ICSI

Beckwith-Wiedemann Syndrome (BWS)

Birth weight and length >90th percentile
Macroglossia
Hypoglycemia
Ear creases or pits
Exomphalos
Increased risk of Wilms tumour

CDKN1C mutation (mat)	4 %
Imprinting defect (mat IC2 hypomethylation)	60 %
Imprinting defect (pat IC1 hypermethylation)	15 %
Uniparental Disomy 11p15 (pat)	20 %
Duplication/Translocation 11p15 (pat)	1 %

ART and imprinting defects

Beckwith-Wiedemann syndrome

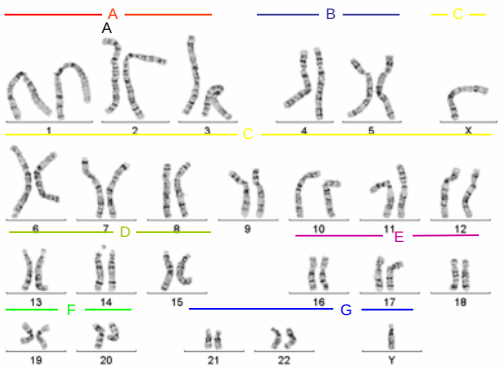
Three- to sixfold increased prevalence of IVF/ICSI in BWS

DeBaun et al. *Am J Hum Genet* 72:156-60, 2003
Maher et al. J *Med Gen* 39; 40:62-64, 2003
Gicquel et al. *Am J Hum Genet*, 72:1338-41, 2003
Sutcliffe et al. *Hum Reprod*, 21:1009-1011, 2006

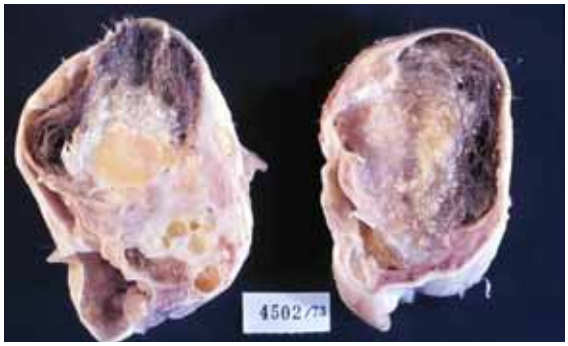
Ninefold increased risk for BWS after IVF/ICSI

Halliday et al. *Am J Hum Genet* 75:526-528, 2004

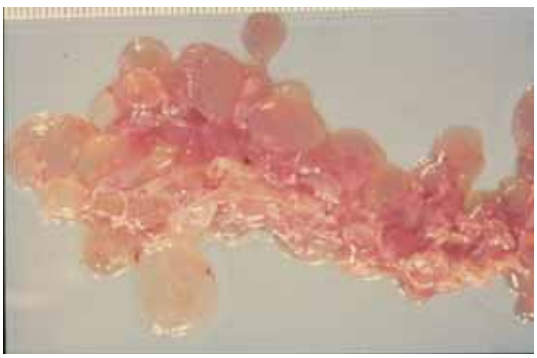
Metaphase-Chromosomes 46,XY



Teratoma: All chromosomes are maternally derived



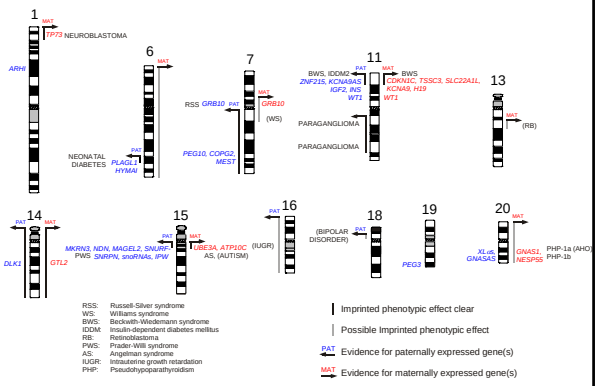
Mole: All chromosomes are paternally derived



Imprinting

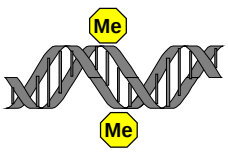
- **Imprinting** means an epigenetic process, in which specific parts of the chromosomes-while passing the male and female germline- get a specific mark (imprint). As a consequence, in somatic cells either the paternal or the maternal allele are active.
- Expression of some genes is dependent on whether they derive from father or mother .

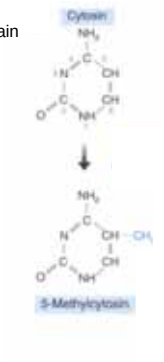
About 100 human genes are imprinted



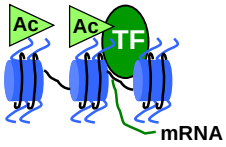
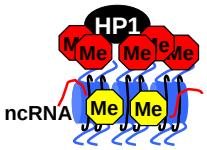
DNA-Methylation

Parent-specific methylation of certain CpG-dinucleotides and histone modification play a central role in the process of imprinting.

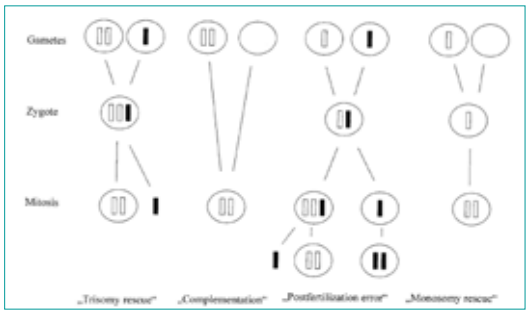




Imprinted genes have different, parent-of-origin specific epialleles

parental origin of allele	epigenetic state	functional state
paternal		gene on
maternal		gene off

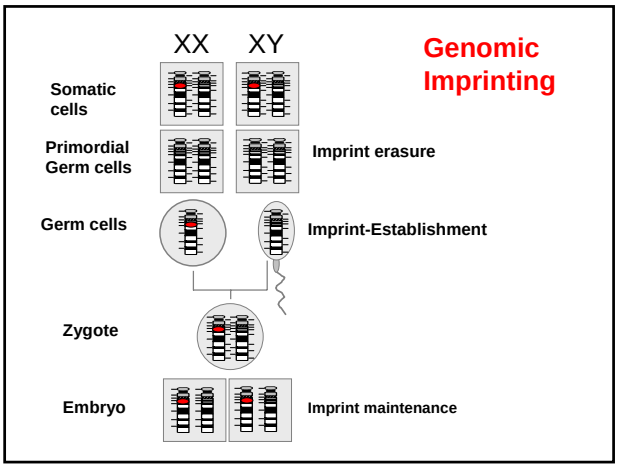
Mechanisms leading to Uniparental Disomy (UPD)

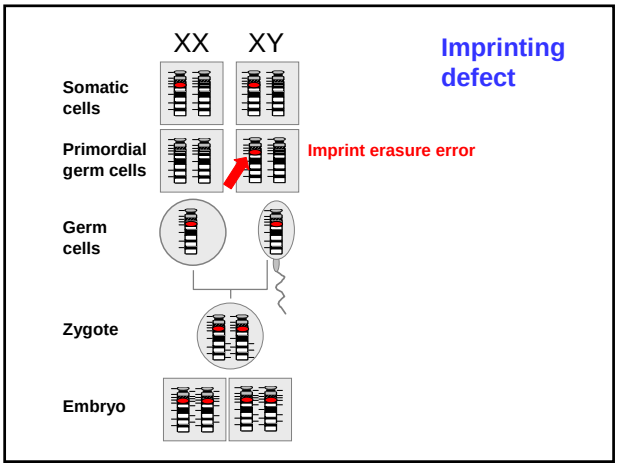


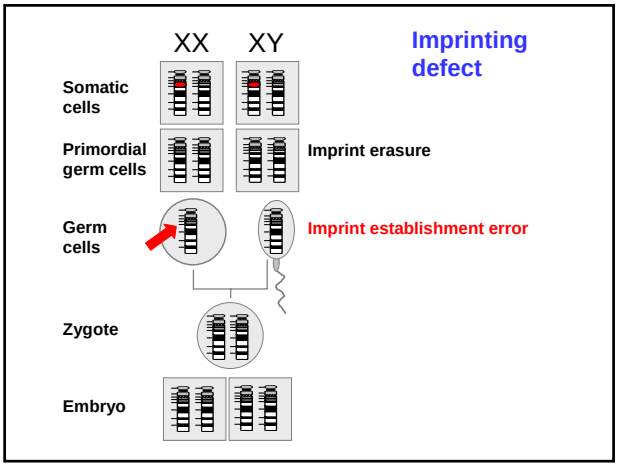
Kotzot et al. 2005

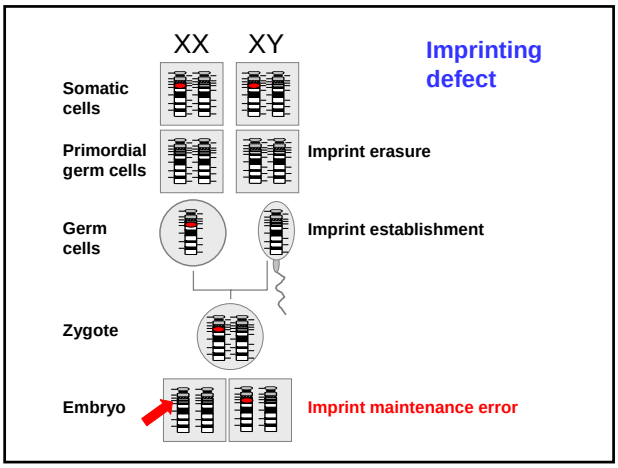
Conditions due to UPD and Imprinting

- **pUPD 6** Transient neonatal diabetes
- **mUPD 7** Russell-Silver syndrome
- **pUPD 11p** Beckwith-Wiedemann syndrome
- **mUPD 14** IUGR, precocious puberty, short stature
- **pUPD 14** narow thorax, „coat hanger" ribs
- **mUPD 15** Prader-Willi syndrome (PWS)
- **pUPD 15** Angelman syndrome
- **mUPD 16** IUGR, malformations, MR

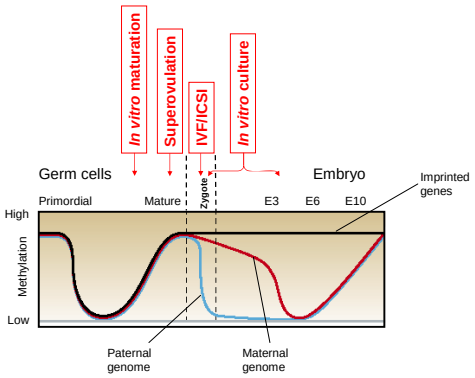

XX
XY
Genomic Imprinting
Somatic cells
Primordial Germ cells
Imprint erasure
Germ cells
Imprint-Establishment
Zygote
Embryo
Imprint maintenance


XX
XY
Imprinting defect
Somatic cells
Primordial germ cells
Imprint erasure error
Germ cells
Zygote
Embryo


XX
XY
Imprinting defect
Somatic cells
Primordial germ cells
Imprint erasure
Germ cells
Imprint establishment error
Zygote
Embryo



Epigenetic reprogramming and assisted reproductive technology (ART)



ART-A big problem ?

Incidence of AS: 1/15.000
Imprinting defects: 4%
-> Incidence of AS-ID: 1/375.000

assuming a 3-fold increased risk, the incidence of AS-ID is 1/125.000 in ART conceived children

Incidence of BWS: 1/12.000
Imprinting defects: 60%
-> Incidence of BWS-ID: 1/20.000

assuming a 3-fold increased risik, the incidence of BWS-ID is 1/7.000 in ART conceived children

Follow-up studies addressing the risk of imprinting defects after ART

Manning M, Lissens W, Bonduelle M, Camus M, De Rijcke M, Liebaers I, Van Steirteghem A (2000)
Study of DNA-methylation patterns at chromosome 15q11-q13
in children born after ICSI reveals no imprinting defects.
Mol Hum Reprod 6:1049-53

"In all 92 children …, a regular DNA-methylation pattern was found in the PWS/AS region."

Follow-up studies addressing the risk of imprinting defects after ART

Lidegaard O, Pinborg A, Andersen AN (2005)
Imprinting diseases and IVF: Danish National IVF cohort study.
Hum Reprod 20:950-954

"During the 7-year study period, 442 349 singleton non-IVF and 6052 IVF children were born. … We found no indication of an increased risk of imprinting diseases after IVF."

Follow-up studies addressing the risk of imprinting defects after ART

Chang AS, Moley KH, Wangler M, Feinberg AP, DeBaun MR
Association between Beckwith-Wiedemann syndrome and
assisted reproductive technology: a case series of 19 patients
in children born after ICSI reveals no imprinting defects.
Fertility and Sterility 83:349-354,2005

"The only consistent finding was that all 12 women received some type of ovarian medication."

So far no evidence for increased risk for

Transient neonatal diabetes m. (mat hypomethylation)*
(however: only very few cases studied)

Prader-Willi syndrome (pat hypermethylation)*

Silver-Russell syndrome (pat IC1 hypomethylation)
(two reported cases of unknown molecular defect)

*Sutcliffe *et al. Hum Reprod*, 21:1009-1011, 2006

Is the adverse outcome of ART mainly due to an oocyte problem?

ART and AS

Ludwig M et al, *J Med Genet* 42:289-91, 2005

Increased prevalence of imprinting defects in patients with Angelman syndrome born to subfertile couples.

Group	N	ID	RR*	[95% CI]
Time to pregnancy >2 yrs OR treatment	16	4	6.25	[1.7;16.0]
Time to pregnancy >2 yrs, no treatment	8	2	6.25	[0.7;22.6]
Treatment	8	2	6.25	[0.7;22.6]
Hormone treatment only	5	1	5.00	[0.1;27.8]
ICSI	3	1	8.33	[0.1;46.4]
Time to pregnancy >2 yrs AND treatment	4	2	12.50	[1.4;45.1]

*assuming that 4% of all AS patients have an imprinting defect

"Subfertile couples have an increased risk to conceive a child with an imprinting defect. Superovulation appears to increase the risk further."

Possible causes of ART-associated imprinting defects

Infertility-linked risk

Technique-related risk
- **Hormone stimulation***
- ***In vitro* culture of gametes and preembryo***
- **ICSI**

*Evidence from animal studies

A causal relationship cannot be excluded

Six children with AS and an imprinting defect after ICSI, although imprinting defects are rare in AS

The prevalence of IVF/ICSI in children with BWS is increased by a factor of 3-6.

In both conditions, the maternal chromosome is hypomethylated.

A causal relationship cannot be excluded

ART procedures are being done when major epigenetic events take place during germ cell and preembryo development, possibly interfering with the proper establishment (in gamete culture) and maintenance (in preembryo culture) of genomic imprints.

In vitro cultivation of animal preembryos leads to hypomethylation and increases the risk of overgrowth and malformations

large offspring syndrome.

Conclusions and recommendations

The absolute risk for Beckwith-Wiedemann and Angelman syndrome after ART

is very low (<1/1,000)

Genetic counseling before ART is recommended

no need for prenatal testing for these diseases

Conclusions and recommendations

Until reliable scientific data are available, **ART with IVM oocytes** should be performed in prospective studies only

Subfertility seems to a risk factor to conceive a child with an imprinting defect

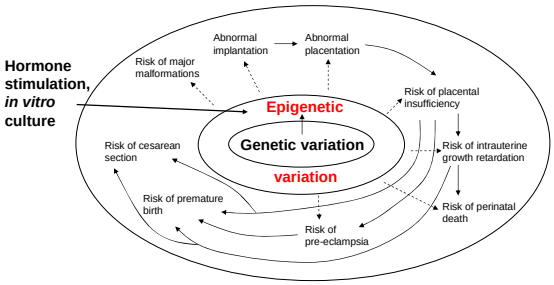
Superovulation may increase the risk further.

Are we seeing the tip of an iceberg ?

Acknowledgement

Bernhard Horsthemke
Institut für Humangenetik Essen

The role of epigenetic variation in the adverse clinical outcome of ART



Animal studies

Ertzeid and Storeng (2001) The impact of ovarian stimulation on implantation and fetal development in mice. *Hum Reprod* 16:221-225

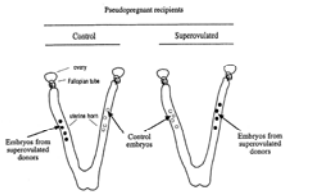


Table I. Stage of embryo development of normal embryos transferred on day 4 after in-vitro culture from day 2

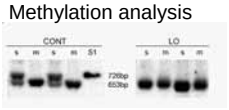
Embryo donor mice	Compacted morulae *n* (%)	Blastocysts *n* (%)
Control	97/250 (39)	153/250 (61)[a]
Superovulated	336/570 (59)	234/570 (41)[a]

[a]$P < 0.001$.

Young et al. (2001) Epigenetic change in *IGF2R* is associated with fetal overgrowth after sheep embryo culture. *Nat Genet* 27:153-154

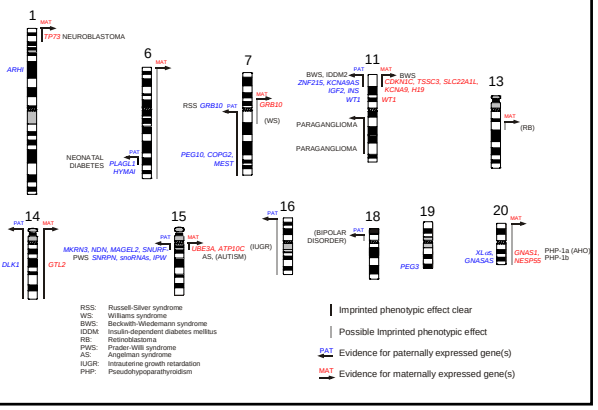
RT-PCR

Methylation analysis



Human imprinting map

http://greallyoffice.aecom.yu.edu/





Three important questions:

- **Is ART associated with an increased risk of imprinting defects (AS, BWS)?**
- **Do epigenetic effects contribute to low birth weight and malformations seen in ART children?**
- **If the associations are genuine, what are the causes?**

Adverse clinical outcome in ART

- **malformations ("risk not increased")***
- **low birth weight ("risk increased")***
- **imprinting defects ("risk possibly increased")***

***** ART Children's Health Panel, Baltimore 2004

Possible causes of ART-associated epigenetic defects

- **Infertility-linked risk**
- **Technique-related risk**
  - **Hormone stimulation***
  - ***In vitro* culture of gametes and preembryo***
  - **ICSI**

*Tentative evidence from animal studies

The End