Gametes and embryo epigenetics and the influence of assisted reproductive technologies

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Epigenetics in ART, ESHRE Campus, LISBON 2008

ART Risks and children health

- Multiple pregnancies :
  Prematurity, low birth weight,
- Birth defects :
  Congenital malformations
  Chromosomal aberrations
- Childhood disabilities
- Adulthood disabilities
- Transgenerational inheritance

ART Risks

- Genitors (future parents):
  Infertility, age, pathologies….
- Gametes:
  Maturity
  Defective gametogenesis
  Treatment
    in-vivo: superovulation
    in-vitro: culture medium, PVP, freezing…
- Fertilization method
  IVF, ICSI…
- Processing of preimplantation embryo
  Transfer, culture, freezing, biopsy, hatching…
- Multiple pregnancy

TABLE 4. OBSERVED AND EXPECTED CASES OF LOW BIRTH WEIGHT AMONG TERM AND PRETERM SINGLETON INFANTS CONCEIVED WITH ASSISTED REPRODUCTIVE TECHNOLOGY IN 1996 AND 1997.*

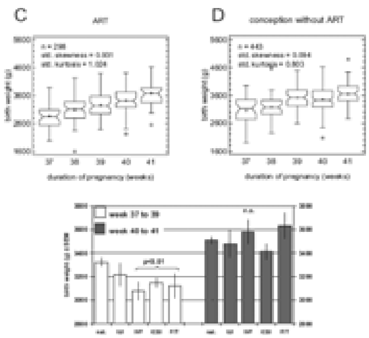
VARIABLE	TOTAL NO.	NO. OF CASES OBSERVED	NO. OF CASES EXPECTED†	STANDARDIZED RISK RATIO (95% CI)
Term low birth weight				
All infants	18,182	1180	455.2	2.6 (2.4–2.7)
Pregnancies with one fetal heart	16,530	1059	413.1	2.6 (2.4–2.8)
Use of donor oocytes, no diagnosis of male-factor infertility	1,390	80	42.4	1.9 (1.5–2.3)
Diagnosis of male-factor infertility	2,730	190	66.5	2.9 (2.5–3.3)
Use of gestational carrier	180	8	4.7	1.7 (0.5–2.9)
Preterm low birth weight				
All infants	18,182	1206	859.6	1.4 (1.3–1.5)
Pregnancies with one fetal heart	16,530	1011	780.3	1.3 (1.2–1.4)
Use of donor oocytes, no diagnosis of male-factor infertility	1,390	110	75.7	1.5 (1.2–1.7)
Diagnosis of male-factor infertility	2,730	131	126.1	1.0 (0.9–1.2)
Use of gestational carrier	180	8	8.5	0.9 (0.3–1.6)

*Term infants were defined as those born at or after 37 weeks of gestation, and preterm infants were defined as those born at less than 37 weeks of gestation. Ten infants with missing data on parity and 216 infants (1 percent) with missing data required to calculate gestational age were not included in these analyses; of the infants missing gestational-age data, 37 had low birth weight and 179 had normal birth weight. CI denotes confidence interval.

†The number of expected cases was calculated by applying the rates of low birth weight from the 1997 U.S. birth-certificate data to the population of infants conceived with assisted reproductive technology. Values were adjusted to account for differences in the distributions of age (in the following categories: 20 to 29 years, 30 to 34 years, 35 to 39 years, 40 to 44 years, and ≥45 years) and parity (0, 1, or ≥2) between the two populations.

Schieve et al, NEJM, 2002

Birthweight in singletons from after natural conception or ART in infertile women



Geyter et al, Hum Reprod, 2006

- In sheep and cattle epigenetic abnormalities have been shown to be involved in large offspring syndrome (LOS) (YOUNG et al, 1998)
  - Observed in case of preimplantation in vitro development of embryos
  - LOS related to the loss of imprinting of the IGF2 receptor gene (YOUNG et al, 2001)
- In mice, in vitro development of embryo may be responsible for overgrowth related to the abnormal expression of various imprited genes, particularly genes located at distal chromosome 7 (orthologous to the human 11p15 region) (HUMPHERYS et al, 2001)

ANGELMAN SYNDROME

Severe mental retardation, motor defects, lack of speech

COX et al, 2002 : 2 cases with demethylation of SNRPN

ORSTAVIK et al, 2003 : 1 case with demethylation of SNRPN

BECKWITH – WIEDEMANN SYNDROME AND ART

Organ overgrowth, abdominal wall defects and increased riskof embryonal tumor associated with abnormalities of imprinted genes on chromosome 11p15

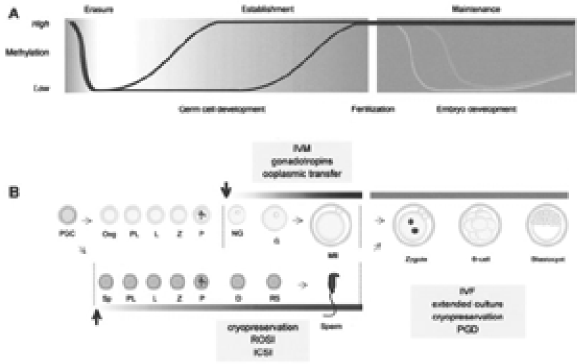
AUTHORS	Number of cases	Born after ART	Born after ART in general population
DE BAUN et al, 2003	65	3 (4.6 %)	0.76 %
MAHER et el al, 2003	149	6 (4.0 %)	1.0 %
GICQUEL et al, 2003	149	6 (4.0 %)	1.3 %

DE BAUN et al : Isolated demethylation of KvDMR1 in 4 / 6 patients
Hypermethylation of H19 in 1 / 6 patient

MAHER et al : Isolated demethylation of KvDMR1 in 2 / 6 patients

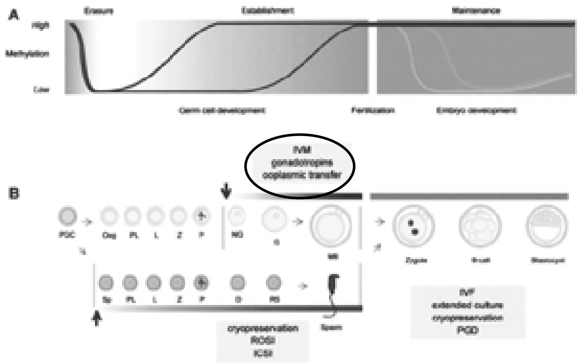
GICQUEL et al : Isolated demethylation of KvDMR1 in 6 patients

Methylation dynamics of imprinted genes and ART



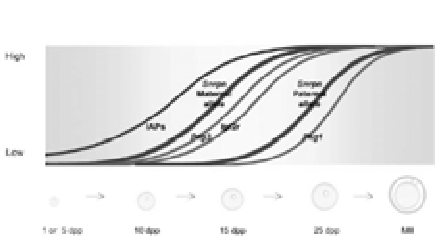
Lucifero et al, Hum Reprod update, 2004

Methylation dynamics of imprinted genes and ART



Lucifero et al, Hum Reprod update, 2004

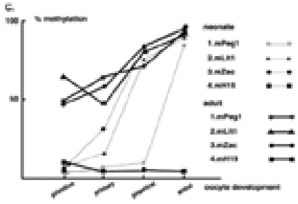
Methylation acquisition during postnatal oogenesis



Lucifero et al, Hum Mon genet, 2004

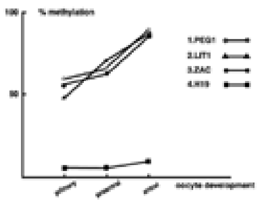
Methylation in imprinted loci in mouse and human oocytes

Mouse



Unstimulated neonatal and adult ovaries

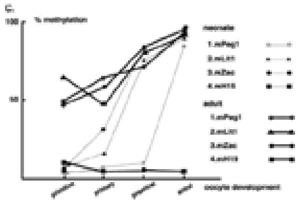
Human



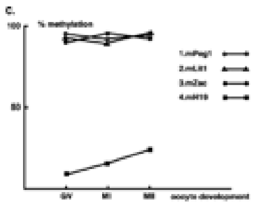
Unstimulated adult ovaries

Sato et al, Hum Reprod, 2007

Methylation in imprinted loci in mouse oocytes



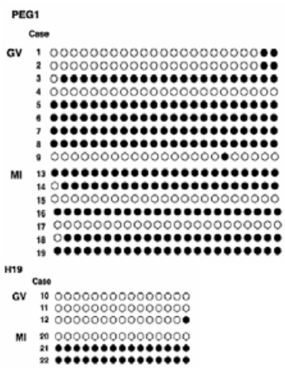
Unstimulated neonatal and adult ovaries



Superovulated oocytes

Sato et al, Hum Reprod, 2007

Methylation errors in GV an M I superovulated human oocytes



Sato et al, Hum Reprod, 2007

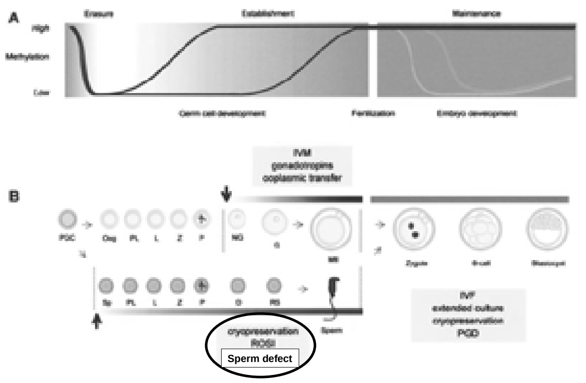
Methylation analysis of KvDMR1 in human oocytes

Bisulphite sequencing technique optimised for analysis of single cells

	Number	Clones (n)	1	2	3	4	5	6	7	8	9	10	11	12	Percentage
GV	1	11													100.00
	2	15													91.67
		1													100.00
	3	16													91.67
	4	Direct													91.67
	5	Direct													100.00
	6	Direct													100.00
MI	7	12													100.00
	8	8													100.00
	9	2													91.67
		1													91.67
	10	Direct													100.00
	11	Direct													0
	12	Direct													83.33
MII	13	5													100.00
	14+PB	4													75.00
		11													66.00
	15	8													100.00
	16+PB	Direct													100.00

Geuns et al, J Med Genet, 2007

Methylation dynamics of imprinted genes and ART

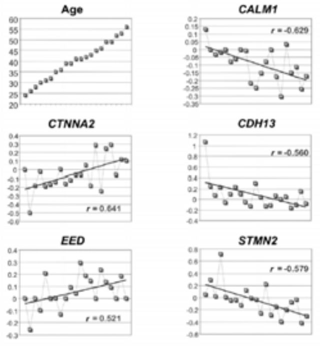


Lucifero et al, Hum Reprod update, 2004

Alterations of methylation state in human sperm

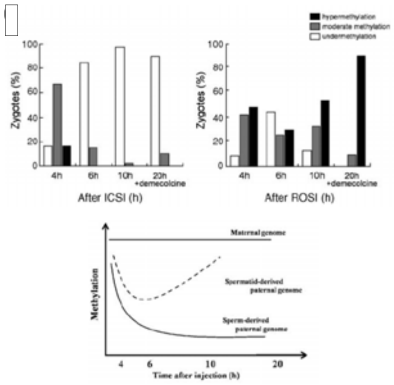
	Global/Gene	Sperm	Methylation imprinting Maternal (MI) - Paternal (PI)
Manning et *al.*, 2001	*SNRPN*	mixed	57-63% abnormal MI
Benchaib et *al.*, 2003	Global	mixed	in teratozoospermia
Marques et *al.*, 2004	*MEST* *H19*	normal normal oligo	Unmethylated, normal MI Methylated, normal PI 17-30% incomplete PI
Houshdaran et *al.*, 2007	*NTF3, MT1A* *PAX8, PLAGL1*	mixed	Hypermethylation
Kobayashi et *al.*, 2007	*H19, GTL2* *PEG1, LIT1, ZAC* *PEG3, SNRPN*	oligo	14 % abnormal PI 21 % abnormal MI
Marques et *al.*, *2008*	*MEST* *H19* *H19*DMR6thCTCF	oligo oligo oligo	14 % abnormal MI 47% abnormal PI
Chalas unpublished	IGF2/H19	terato oligo	56 % sporadic loss 59 % severe loss

Age-related DNA methylation changes in the sperm



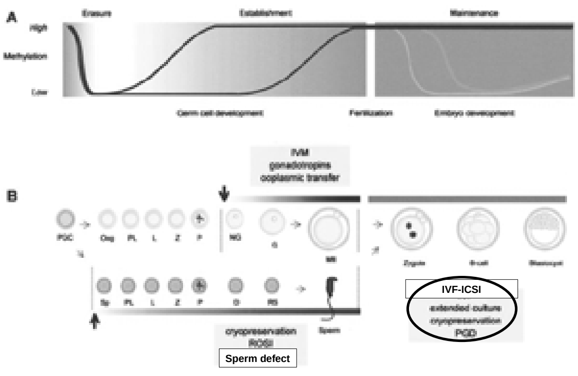
Flanagan et al, Am J Hum Genet, 2006

Aberrant DNA methylation in spermatic-derived zygotic paternal genome



Kishigami et al, Develop Biol, 2006

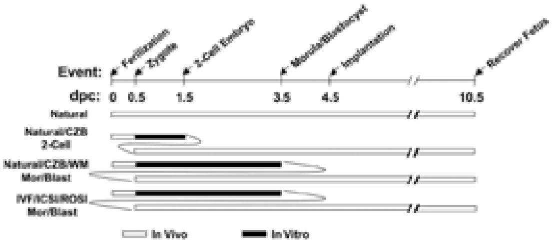
Methylation dynamics of imprinted genes and ART



Lucifero et al, Hum Reprod update, 2004

ART do not alter mutation frequency or spectrum

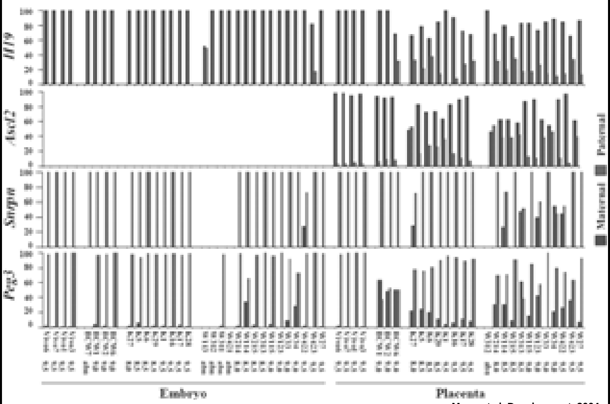
Big Blue transgenic mouse model and mutagenesis assay used to analyse the frequency and spectrum of rare point mutation



Frequency of de novo mutation 1 x 10^{-5}
No increase in the occurrence of de novo point mutations

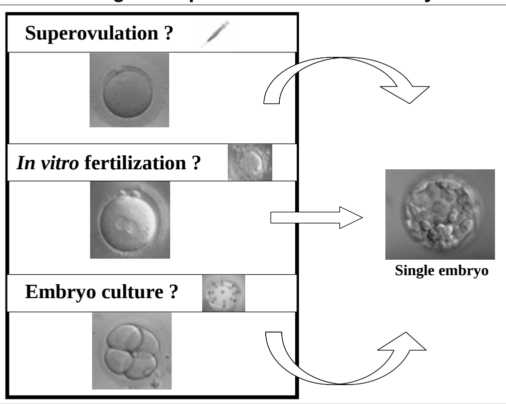
Caperton et al, PNAS, 2007

Selective loss of imprinting in the placenta following preimplantation development in culture

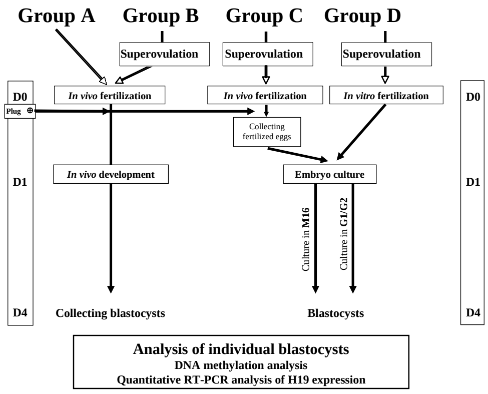


Mann et al, Development, 2004

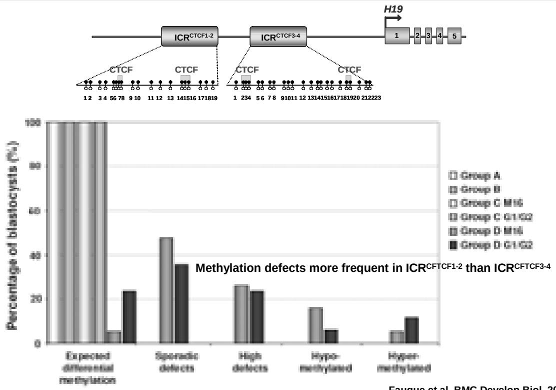
Influence of ART manipulations on H19 ICR methylation and H19 gene expression in mouse embryo



Study design



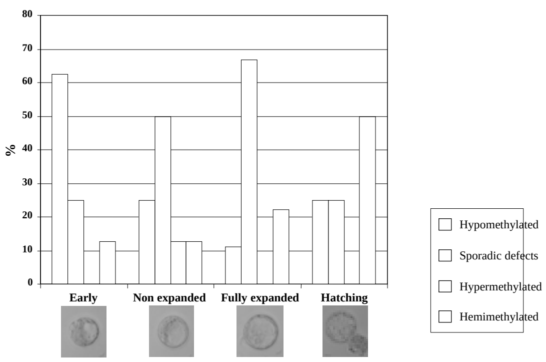
Methylation status of H19 ICR for individual blastocysts



Fauque et al, BMC Develop Biol, 2007

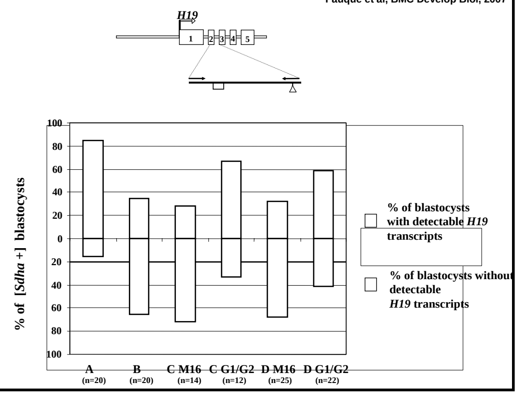
Methylation status of *H19* ICR according to the blastocyste maturity

Fauque et al, BMC Develop Biol, 2007



Expression level of *H19* RNA

Fauque et al, BMC Develop Biol, 2007



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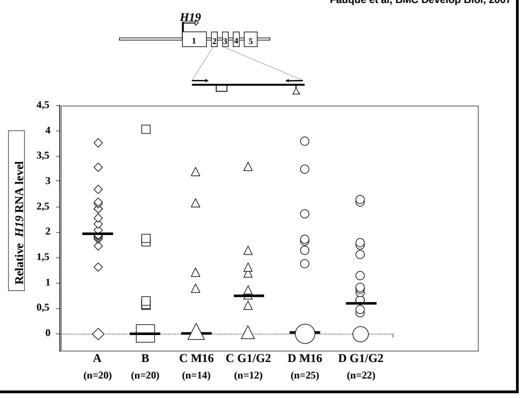

Conclusion

- **Important interblastocyst variability**
- **Each step of ART acts in a distinct manner**
  - **Superovulation induces a disruption of *H19* gene expression**
    - defects of oocyte maturation ?
  - ***In Vitro* Fertilization affects the methylation of *H19* ICR**
    - fertilization step could be a key period for epigenetic changes
  - **Role of culture media**

***H19* could be used as a sensor to investigate more deeply each of the parameters in the mouse system in order to improve human experimental procedures**

ART Epigenetic Risks

- Genitors (future parents):
  Infertility, age, pathologies….

- Gametes:
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  Defective gametogenesis
  Treatment
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- Fertilization method
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- Multiple pregnancy