Early Embryo Epigenetics and the Influence of Laboratory Culture

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OVERVIEW

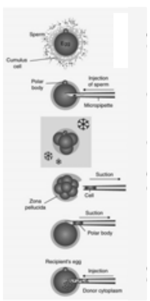
Epigenetic effects of embryo culture conditions

If epigenetic programs are abnormal in preimplantation embryos, how does this affect the postimplantation embryo and placenta or offspring postnatally?

How can we use this information to monitor/improve human ART?

Epigenetic basis for the developmental origins of adult disease?

Risks of Assisted Reproductive Technologies?

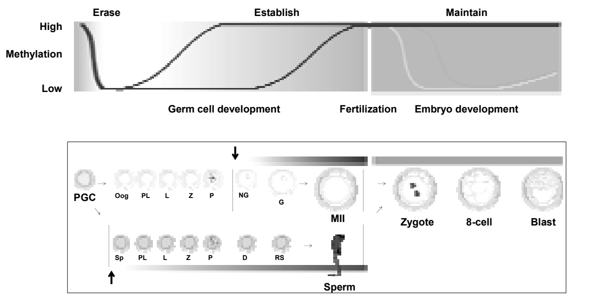


Adapted from Winston et al. (2002) Nat. Fert. Supplement

- Increased risk of birth defects and low birth weight in ART-conceived children
  - Hansen *et al.*, 2002; Schieve *et al.*, 2002
- Cases of IVF/ICSI-born children with the imprinting diseases Angelman syndrome and Beckwith-Wiedemann syndrome
  - Maher et al, 2003; Orstavik et al, 2003; Cox et al, 2002; DeBaun et al, 2003; Gicquel et al, 2003; Halliday et al, 2004

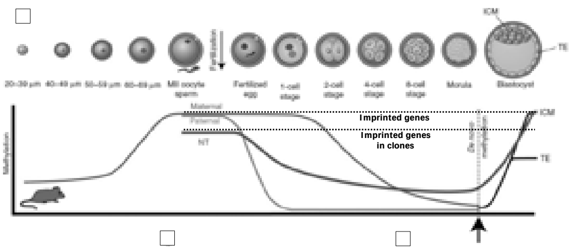
Mechanisms?
underlying infertility,
ovulation protocols,
gamete manipulation,
embryo culture

DNA methylation in gametes and embryos



Lucifero et al. (2004). Hum. Reprod. Update

DNA Methylation- normal and clones



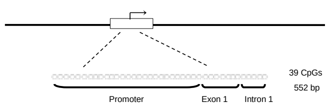
Yang et al (2007) Nat Genetics **39**: 295-302.

CLONING: Reprogramming Errors → Abnormal Placenta, Abnormal Organ Development → Birth of Clone (few survive) → Adult Phenotypes

Bovine *Snrpn* sequence and gene structure

```
TGTTACCCTG CAACAGGATA AGGTCACCAG TGGAAAGCCT GAGGAAACCT GAATAAGGTA CAGACTTTTG AATGTTAACA
AAAAAGAAAT ACCCAATTCC CACTCCACTG GGAGGTACCA CTCTGGGTTG AAGTACAACT GTTAACATTT GGTACAATTC
TAAGAGCCAT TAACTGCTGG CTCACGGTGT CCCTAGCGCT CCCCGACCCC AAAGGCCGTA GGACTTTGAN CACCGGGTG
GGGTAGACAC CATCCCCGGT TTCCCTGCCT GAGAGACGCG CGTCACCAGC AGNCGCAGCG CCTCCCACTG CGGCAAACAN
GANCTGCAGG CGCGGCAGAG TCTGGTCGCC AGGAGCGGNG CGNCTTGCCG CGCATGCTCA GGCGGAGTCT GCGGAGTGGT
GCCGTGGCGG TGGAGCANGC AGCGTGGTCT GCCAGCCGGA GATGCGTGAC GTTGTGTTCT GAGGAGCGGC CAGCGGCGCG
ATGGAGCGGG CCAGGTCAGC CGTGTGGTGG GCAACATTTT TTTTTTTTTT TAATCATCCT GAGCTTCCAG AACTAGAAGT
ATTTCGGTAT TTACTGGGGA TCTGGGCTCT CAAGTTCTTT GTTCGGTAGC CGGATGGGAC AACTTCTGGA GGCTTGGGGT
TGGACTGACT CTCCCGACCG TGTTTTGTCA GGTCTTGGGG GTATCTGCAC CGTCATGTTG CACCCCCTAA GATTCTCTGA
TTCTTTGTTC CGGTGGTTGA GGCCGCGACC GCGGCTGGCC ACGATGCAAC AGTCGGGGGA GGGCGCGGTT AGCGGCGTCC
ACAGCTGCCG
```

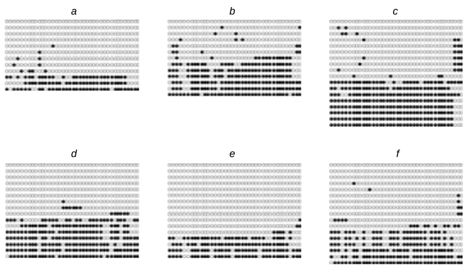
Blue: Primers;
Yellow: Exon 1



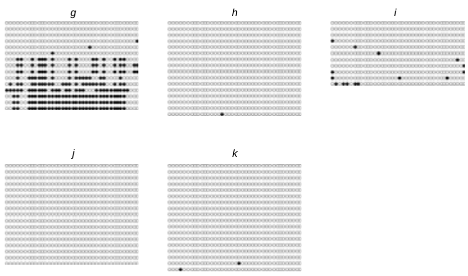
Lucifero et al. Biol. Reprod. 2006

In vivo Snrpn methylation

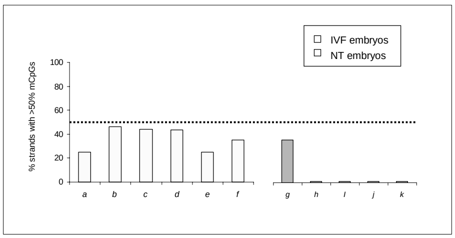
17 dpc IVP embryos *Snrpn* methylation



17 dpc NT embryos *Snrpn* methylation



17 dpc IVP and NT embryos methylation



Embryo Culture and Epigenetic Defects: Early Lessons from Animal Models

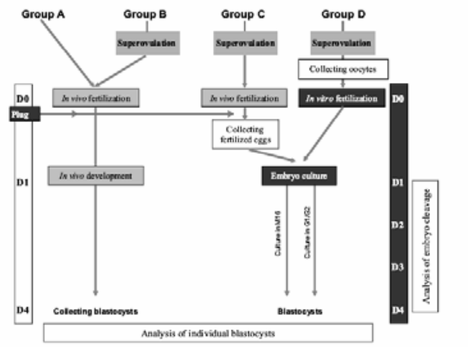
Young et al., 2001- Large offspring syndrome in sheep associated with in vitro culture (serum) and *Igf2r* hypomethylation (maternal).

Doherty et al. 2000- Mouse embryos cultured in Whitten's media (but not KSOM+AA) showed loss of imprinting of *H19* (hypomethylated)

Khosla et al., 2001- Addition of serum to mouse embryos cultured in M16 led to altered methylation of multiple imprinted genes and decreased fetal weight

Ecker et al. & Fernandez-Gonzalez et al., 2004- Behavioral defects following in offspring after culture of preimplantation mouse embryos

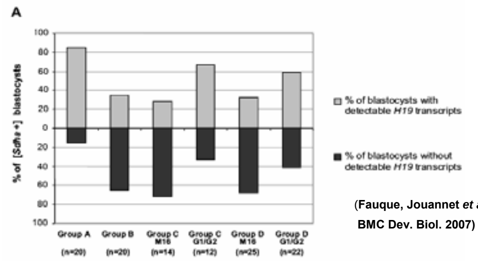
Culture and Epigenetics



(Fauque, Jouannet *et al*, BMC Dev. Biol. 2007)

Culture and Epigenetics

- Effect of superovulation, in vivo (C) or in vitro (D) fertilization, culture (M16, G1.2/G2.2) to blastocyst on *H19* methylation or imprinting:



(Fauque, Jouannet *et al*, BMC Dev. Biol. 2007)

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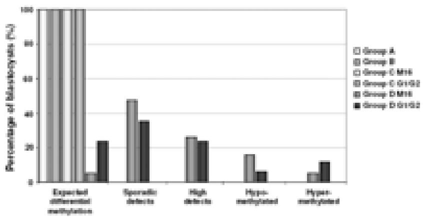


(Fauque, Jouannet *et al*, BMC Dev. Biol. 2007)

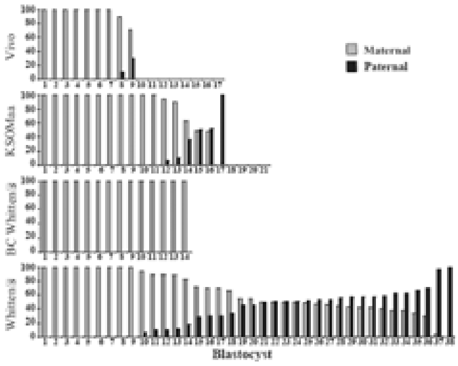
Culture and Epigenetics

Do alterations in imprinting detected in blastocysts persist into the postimplantation period?

If so, are the embryo and placenta affected in a similarly?

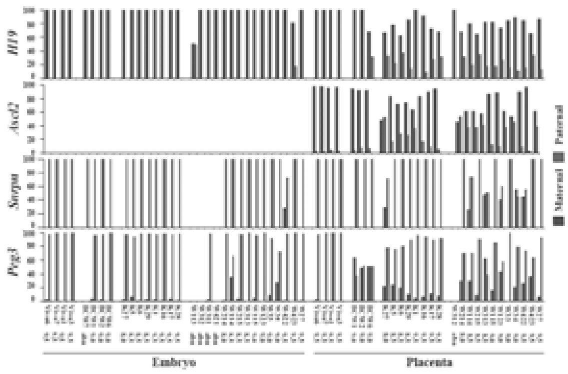
Culture and Epigenetics

H19



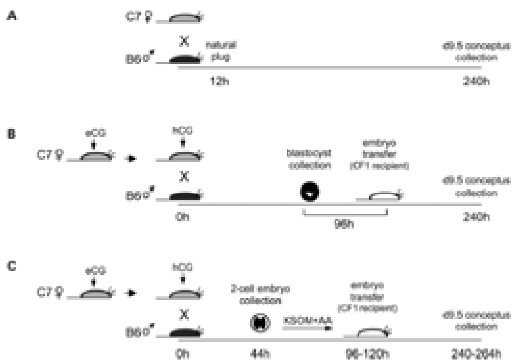
(Mann *et al*, Development 2004)

Culture and Epigenetics



(Mann *et al*, Development 2004)

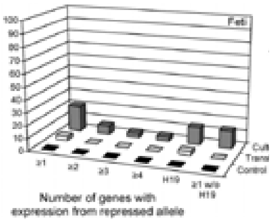
Culture and Embryo Transfer

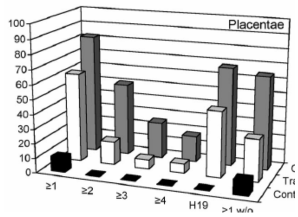


Rivera et al. Hum. Mol. Genet., 2008

Culture and Embryo Transfer

- Control, Superovulation/embryo transfer, Superovulation/in vitro culture/embryo transfer

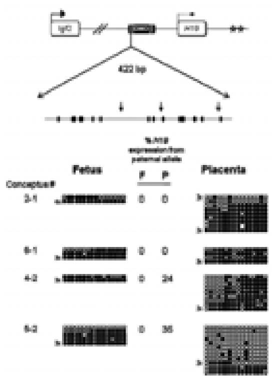




Culture: KSOM+AA, 10 Imprinted genes

Rivera et al. Hum. Mol. Genet., 2008

Culture and Embryo Transfer



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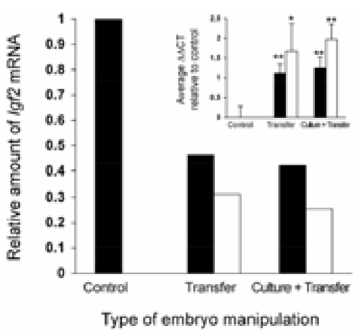
Culture and Embryo Transfer



↑ ***Ascl2*** **– maternal expression/ role in spongiotrophoblast development**

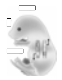
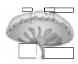
Rivera et al. Hum. Mol. Genet., 2008

Epigenetic Defects - Susceptibility of Placenta

- *H19* particularly affected
- Outer position of trophectoderm in blastocyst (?YS)
- Cells of placenta (and yolk sac) epigenetically distinct
- Stress response of embryo

What is seen at later stages of development? Do the abnormalities in the placenta compromise fetal growth and development?

Differences in DNA Methylation between Embryo and Placenta

		EMBRYO	PLACENTA
	Overall levels of methylation	Higher	Lower
Imprinted Genes	Promoter methylation	Important	Less Important
	Chromatin modifications	Important	More Important
	Parental expression	Maternal OR Paternal	Maternal (~12)

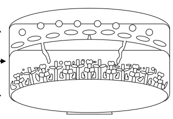
References: Gama-Sosa *et al*, 1983; Chapman *et al*, 1984; Rossant *et al*., 1986; Wagschal and Feil, 2006; Lewis *et al*, 2006; Umlauf *et al*, 2006

The Placenta – More susceptible or more tolerant of epigenetic disruption?

Embryo Culture

Superovulation

Cloning



Placental Defects

Tolerant?

Susceptible?

SUMMARY & CONCLUSIONS

If epigenetic patterns are abnormal in preimplantation embryos, how does this affect the embryo and placenta? → abnormalities in embryo and placenta

How can we use this information to monitor/improve human ART?

- identify all imprinted genes → screen for abnormalities (implications: imprinted gene defects can result in growth and neurobehavioral abnormalities and cancer)
- genome-wide epigenetic analysis → other sequences that will affect the placenta or postnatal development (?tissue/cell)
- **placenta→ gene expression and DNA methylation arrays**

Acknowledgements

Lab members:

- **Diana Lucifero**
- **Amanda Fortier**
- **Josée Martel**
- **Flavia Lopes**
- **Nicole Darricarrere**
- Donovan Chan
- Serge McGraw
- Kirsten Niles
- Wells Cushnie

Cornell University
Roger Gosden
Hang Yin

University of Pennsylvania
Marisa Bartolomei
Mellissa Mann

University of Pittsburgh
Richard Chaillet

Columbia University
Timothy Bestor
Deborah Bourc'his

McGill University
Hugh Clarke
Teruko Taketo

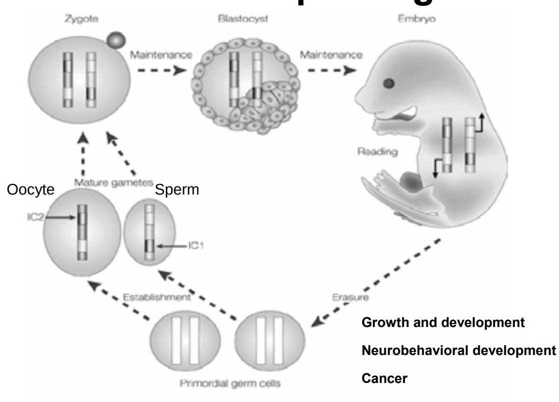
University of Montreal
Lawrence Smith
Vilceu Bordignon

University of Ottawa
Jay Baltz
Art Leader/M-C. Leveillé
Mark Walker
Shi-Wu Wen

Program in Oocyte Health

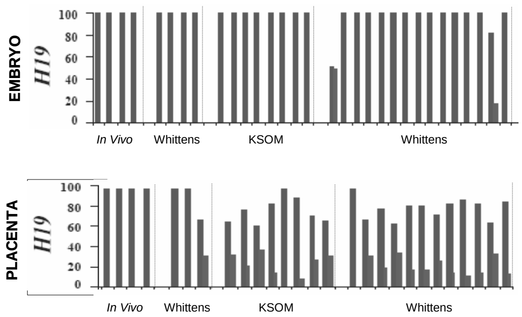
National Institutes of Health

Genomic Imprinting



Reik et al. (2001) Nat. Rev. Genet.

Loss of Imprinting in the Placenta



Mann *et al.* Development 131: 3727, 2004