# Gamete Epigenetics and the Influence of Hormonal Stimulation

**Jacquetta Trasler**

Departments of Pediatrics, Human Genetics and
Pharmacology & Therapeutics, McGill University

Developmental Genetics Laboratory, McGill
University-Montreal Children's Hospital Research
Institute

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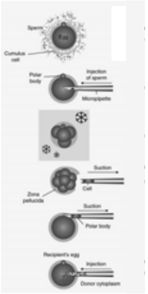
## Risks of Assisted Reproductive Technologies?



***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**

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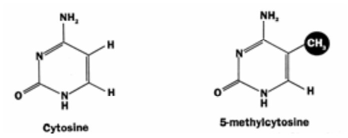
## OVERVIEW

- **DNA methylation pattern acquisition and maintenance- imprinted genes, DNMTs**
- **Effects of hormonal stimulation- animal models**

**If epigenetic programs are abnormal in oocytes, how does this affect the embryo and placenta?**

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

# DNA Methylation



- Methylation occurs at CpG dinucleotides
- 60-80% of all CpG dinucleotides are methylated
- ~20 million cytosines are methylated

(Bestor et al, 1984; Bird 1980)

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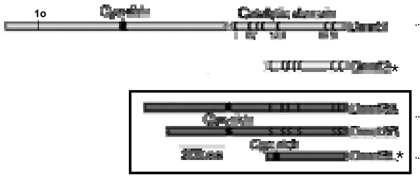
# The DNA Methyltransferases



**Maintenance methylation**

***De novo* methylation**

**No methylation activity**

*Adapted from Bourc'his et al. (2001) Science*

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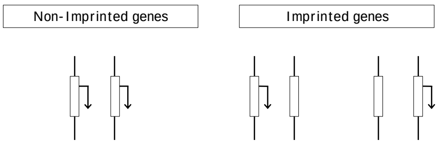
# Biological Roles of DNA Methylation

- Genomic Imprinting



Non-Imprinted genes

Imprinted genes

maternal

paternal

Biallelic expression

Monoallelic expression

- X-chromosome inactivation
- Repression of transposons

## Genomic Imprinting

Paternal Expression

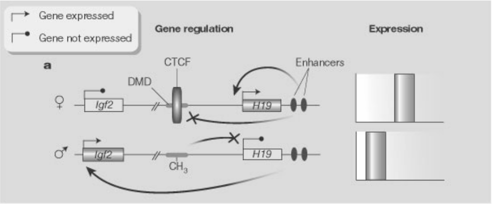
- ***Snrpn***
- *Peg3*
- *Peg1/Mest*
- *Igf2*
- ***Kcnq1ot1***
- *Nnat*
- *Ndn*
- *Ins2*
- *Dlk1*
- *Rasgrf1*

*Adapted from Falls et al. (1999) Am. J. Path.*

Maternal Expression

- ***H19***
- *Igf2r**
- *Ascl2*
- *Cdkn1c*
- *Ube3a*
- *Gtl2*
- *Kcnq1*
- *Slc22a3*
- *Tssc3*
- *Gnas*

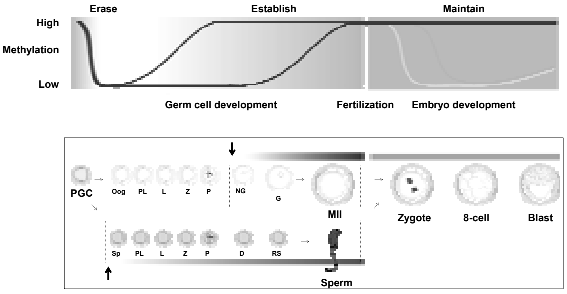
## *H19* imprinted region (distal chr. 7)



Loebel and Tam (2004) Nature **428:** 809-811

- *H19* is a non-coding RNA
- Main role is accessing enhancers to prevent *Igf2* expression

## DNA methylation in gametes and embryos



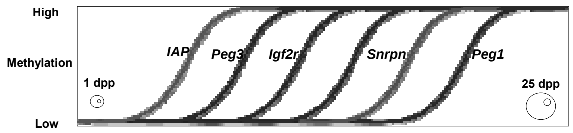
***Lucifero et al. (2004). Hum. Reprod. Update***

## Oogenesis - Mouse Studies

When are maternal methylation imprints established?

## DNA Methylation Establishment - Oocytes

Lucifero et al., Hum Mol Genet, 2004



⟵ *Dnmt3a* ⟶
*Dnmt3b*
*Dnmt3L*

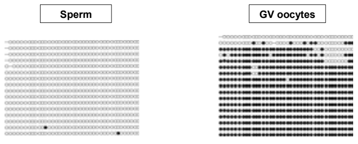
**85% DMR Methylation in Oocytes Sized:** **Hiura et al., Genes to Cells, 2006**

**55µm-** ***Igf2r*, *Zac1*, *Kcnq1ot1 (Lit1)***

**60µm-** ***Snrpn*, *Peg1*, *Impact*, *Meg1/Grb2***

## Bovine and Human *SNRPN* methylation

**BOVINE**



**Lucifero et al., Biol. Reprod. 2006**

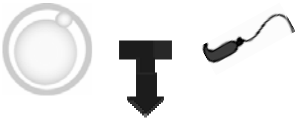
**HUMAN- Methylation imprints at *SNRPN* are present in germinal vesicle, metaphase I and metaphase II oocytes**

**Geuns et al., Hum. Mol. Genet. 2003**

**BECKWITH-WIEDEMANN SYNDROME: KvDMR1 (controls paternally expressed transcript KCNQ1OT1 or LIT1)- Methylation imprints present in GV, MI and MII oocytes**

**Geuns et al. J. Med. Genet. 2007**

## Epigenetic contributions of oocytes and sperm



**OOCYTE:** **Nucleus- female epigenetic 'marks'**

**: Cytoplasm- factors (DNMTs) that will maintain male and female 'marks' during preimplantation development**

**SPERM:** **Nucleus- male epigenetic 'marks'**

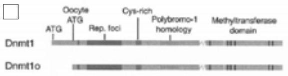
## Oogenesis-Mouse Studies

How are maternal and paternal methylation imprints maintained in the preimplantation embryo?

## *Dnmts* in Preimplantation Embryos

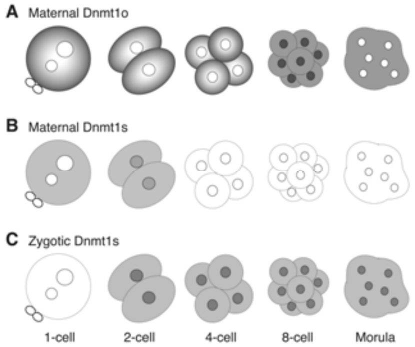
How are methylation imprints maintained during pre-implantation development?

☐



☆ ***Dnmt1o$^{-/-}$*** **Lack of maintenance of methylation imprints at the 8-cell stage; phenotypic variation** (Howell et al. Cell 2001)

## DNMTs in Preimplantation Embryos



Kurihara et al, Dev Biol 313:335, 2008

Cirio et al, BMC Dev Biol 8:9, 2008

## Upsetting Imprints

1. Adverse effects of hormonal stimulation
2. Evidence for epigenetic effects of hormonal stimulation
3. Effects of superovulation on the midgestation embryo and placenta: e.g. mouse

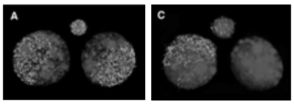
## Early studies

- Effect of superovulation on embryo growth and development:
  - delayed development
  - decreased implantation rate
  - increased postimplantation loss
  - smaller embryos (weight)

(Fossum *et al,* 1989; Ertzeid and Storeng, 1992, 2001; Van der Auwera and D'Hooghe, 2001)

# Superovulation and Epigenetics

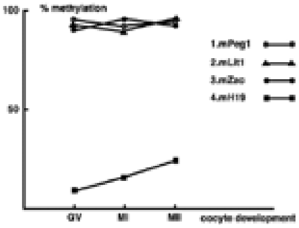
- Effect of superovulation on methylation or imprinting:
  - Abnormal 5-methylcytosine staining in 2-cell embryos (Shi and Haaf, 2002)



# Superovulation and Epigenetics

- Effect of superovulation on methylation or imprinting:

  Superovulation → Normal methylation *Peg1*, *Kcnq1ot1*, *Zac* but ↑ *H19* methylation in oocytes



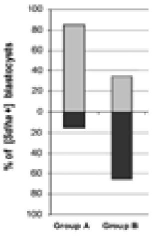
Sato *et al*, Hum. Reprod. 2007

# Superovulation and Epigenetics

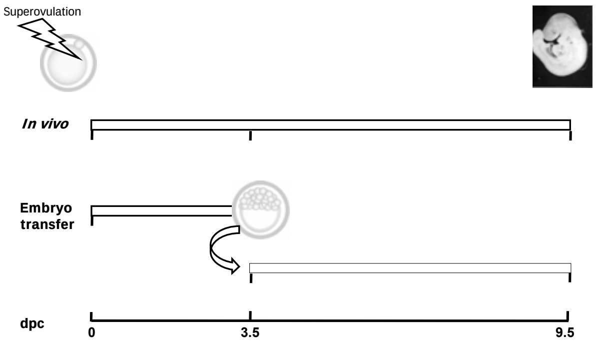
- Effect of superovulation on methylation or imprinting:

  Superovulation → ↓ number of blastocysts with *H19* expression, <u>no change</u> in methylation

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

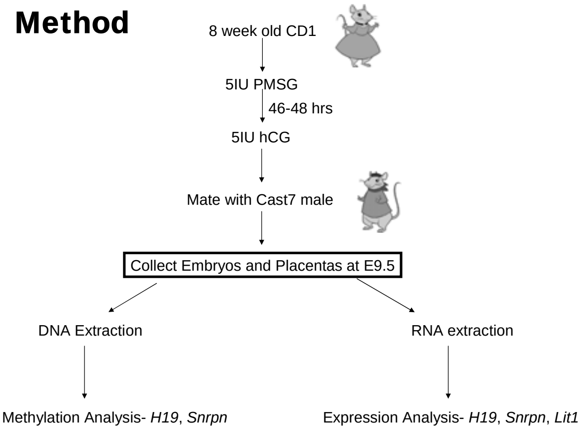


## Superovulation with two approaches: *in vivo* and embryo transfer methods



**Fortier et al. Hum. Mol. Genet. 2008**

## Method



8 week old CD1

5IU PMSG

46-48 hrs

5IU hCG

Mate with Cast7 male

Collect Embryos and Placentas at E9.5

DNA Extraction

Methylation Analysis- *H19*, *Snrpn*

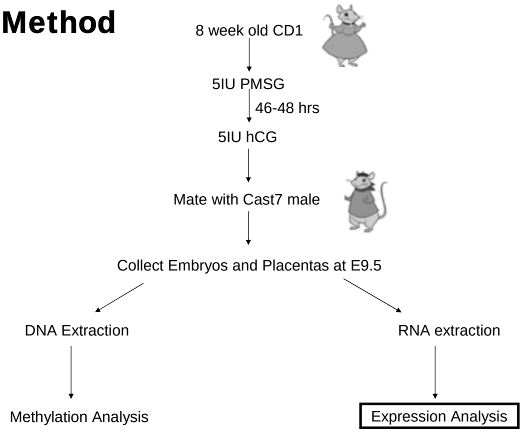
RNA extraction

Expression Analysis- *H19*, *Snrpn*, *Lit1*

## ***In vivo*** **development: superovulation increases resorption rates and delay**

- Increase in
  - resorption sites
  - delayed embryos
- No difference in
  - implantation rate
  - % viable embryos
  - gross abnormalities
  - embryo size (CR length)

# Method



8 week old CD1

5IU PMSG

46-48 hrs

5IU hCG

Mate with Cast7 male

Collect Embryos and Placentas at E9.5

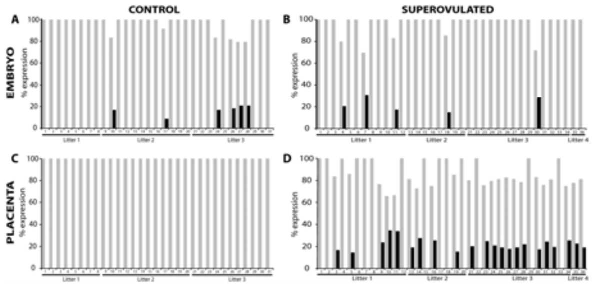
DNA Extraction

RNA extraction

Methylation Analysis

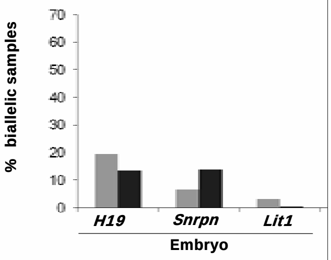
Expression Analysis

# *H19* expression



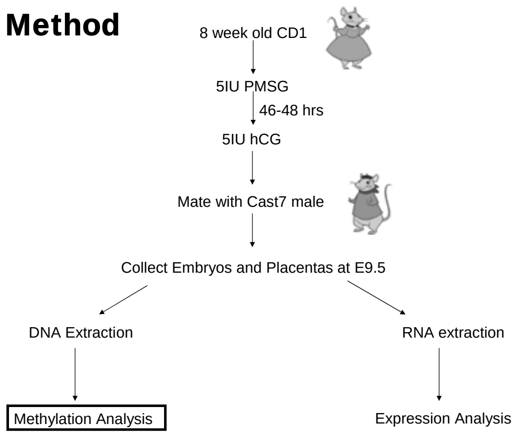
☐Paternal expression ☐Maternal expression

# Biallelic expression of *H19* and *Snrpn* in the placenta following superovulation



☐Control (n=31) ☐Superovulated (n=36)

## Method



8 week old CD1

5IU PMSG

46-48 hrs

5IU hCG

Mate with Cast7 male

Collect Embryos and Placentas at E9.5

DNA Extraction

RNA extraction

Methylation Analysis

Expression Analysis

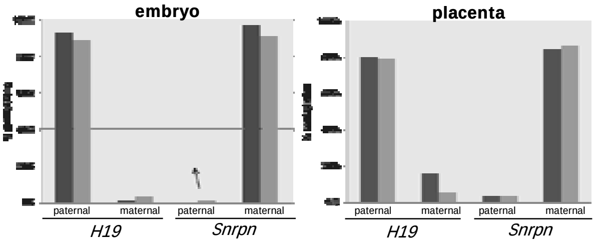
## *H19* and *Snrpn* regions examined

Maternally expressed
Methylation acquired in the male germline

Paternally Expressed
Methylation acquired in the female germline

- These regions have a well characterized role in **establishment** of imprints at these loci

## Superovulation does not affect the overall amounts of methylation at *H19* or *Snrpn* in the embryo or the placenta



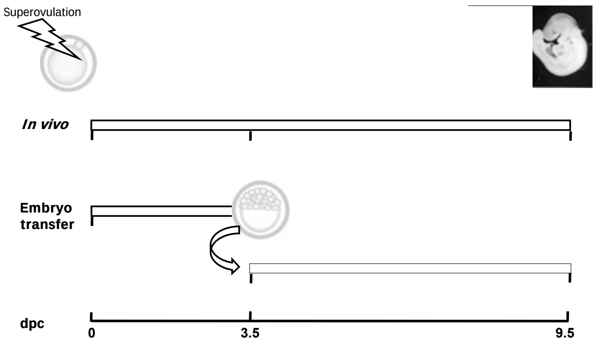
The region we examined may not be affected, but other regions may lose methylation (i.e. these regions may not be required for imprinting **maintenance**)

# Conclusions

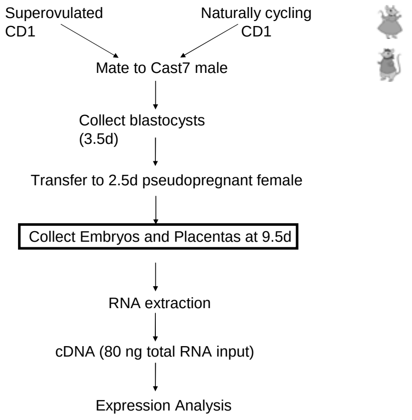
- Superovulation leads to biallelic expression of *Snrpn* and *H19* in the midgestation mouse placenta
- *Lit1 (Kcnq1ot1)* expression was not affected
- Overall levels of methylation at *Snrpn* and *H19* were not affected

**Could superovulation affect the uterine milieu? Does crowding cause problems?**

# Two approaches: *in vivo* and embryo transfer methods



# Method



Superovulated CD1

Naturally cycling CD1

Mate to Cast7 male

Collect blastocysts (3.5d)

Transfer to 2.5d pseudopregnant female

Collect Embryos and Placentas at 9.5d

RNA extraction

cDNA (80 ng total RNA input)

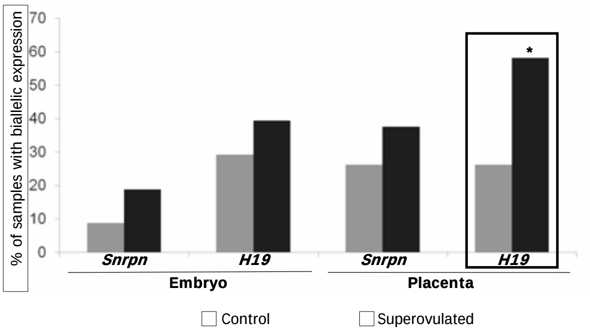
Expression Analysis

## Superovulation affects implantation rate and embryo development

- Decrease in
  - implantation rate
- Increase in
  - number of delayed embryos

- No difference in
  - % viable embryos
  - resorption sites
  - gross abnormalities
  - embryo size (CR length)

## Biallelic expression of *H19* in the placenta following superovulation and embryo transfer



* *P* < 0.03

## Conclusions

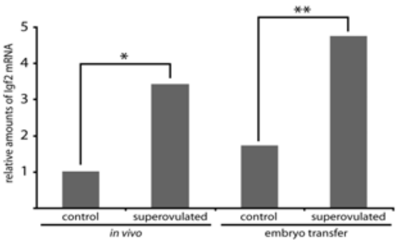
- Superovulation followed by embryo transfer resulted in decreased implantation rates and increase in the proportion of delayed embryos

- Superovulation followed by embryo transfer caused biallelic expression of *H19*

***?Igf2***

## Effects on *Igf2*

- Examined *Igf2* expression by qRT-PCR in the placenta



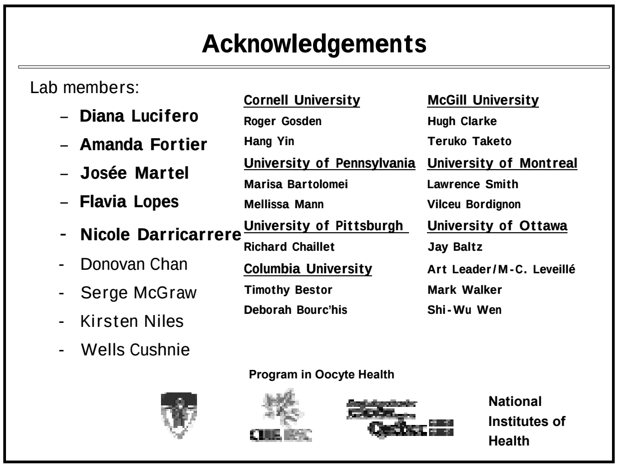
- The placenta does not play a critical role in supporting the embryo prior to 9.5dpc (Guillemot *et al,* 1995; Anson-Cartwright *et al,* 2000; Voss *et al*, 2000; Ono *et al,* 2006)

## Consequence of increased expression of *Igf2*

- Overexpression of *Igf2* in mice leads to overgrowth of the placenta (Leighton *et al*, 1995; Eggenschwiler *et al*, 1997; Sun *et al*; 1997)
- Increased levels of *Igf2* expression have been linked to fetal growth restriction in humans and sheep (Street et al, 2006; De Vrijer et al, 2006)
- Also linked to early embryonic lethality in bovine clones (Oishi et al, 2006)

## Conclusions

- Superovulation results in abnormal, biallelic expression of 2 imprinted genes (*H19* and *Snrpn)* in the placenta and increased levels of *Igf2* expression in the placenta
- Maintenance of imprinted expression may be affected by the exogenous hormones
- Placenta- a sensitive indicator of perturbations?



## 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

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