Genetic Imprinting in Oocyte and Ovarian Stimulation

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The role of assisted reproduction technologies in the increased frequency of imprinting disorders has not been clarified. To understand this association, it is important to perform studies about the imprinting mechanism and its critical moments. We propose to study deeply the genetic imprinting in the oocyte by the methylation study of three imprinted genes (*Impact, Zrsr1* and *Mest*) in oocytes obtained after ovarian stimulation with different gonadotropins dose in order to establish a suitable hormone dose.

Background

Genetic imprinting is an epigenetic mechanism that involves, for determinate loci, a sex-specific differential allele DNA methylation pattern. In addition, genetic imprinting is associated with histone modifications and non-coding RNA[1,2].

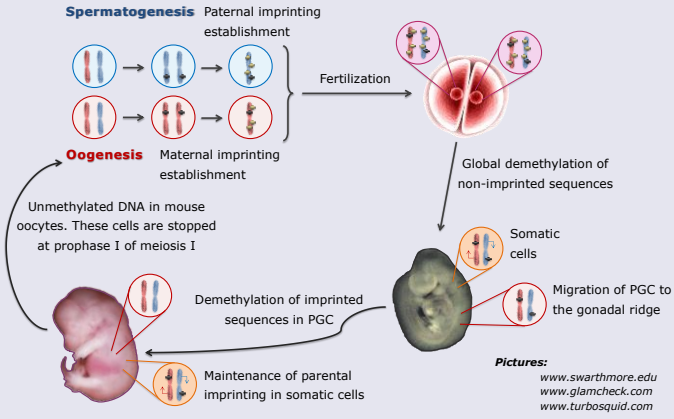
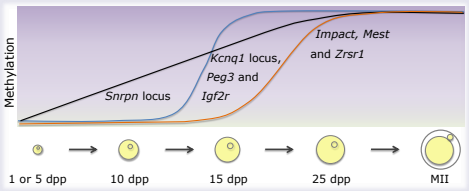


Figure 1. The genomic imprinting mechanism (PGC = Primordial germ cell). During the oogenesis and spermatogenesis the parental epigenetic pattern is erased and new sex-specific epigenetic marks are acquired[3].

Genetic imprinting in the oocyte is established during its growth phase but not at the same time for all the imprinted gene[4]:

Figure 2. Establishment of methylation pattern in different maternal imprinted loci (dpp = days pospartum).



Adapted from Lucifeo, D. *et al*. *Hum Mol Genet* 2004;**13**:839-49
with the results from Obata, Y. *J Reprod Dev* 2011;**57**:1-8

By **Induced ovulation** less mature oocytes are obtained. The fertilization of these oocytes with less stable imprints may contribute to increase the frequency of imprint disorders in humans. But also infertility itself may be associated with problems in the correct imprints establishment. Furthermore, combination of infertility and assisted reproductive technologies (ARTs) may impose greater risk for inducing epigenetic errors[1].

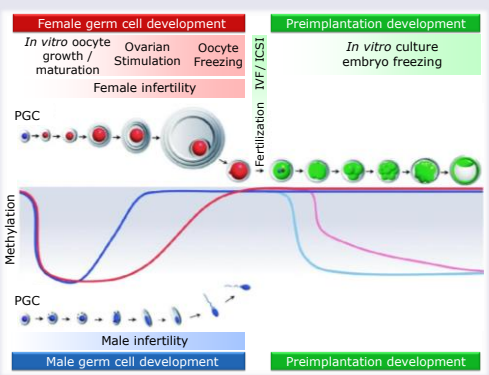


Figure 3. Methylation level during gametogenesis and preimplantation development. Imprinting is capable to be altered in various of these stages where ARTs can be performed.

Reproduced from Denomme, M.M and Mann M.R. *Reproduction* 2012;**144**:393-409

Materials and Methods

1. **Animal model.** *Mus musculus* – C57BL/6J. The procedures to be performed with mouse must be approved by the appropriate Ethics Committee, according to current legislation.
2. **Ovarian stimulation.** Study groups (weight range: 10.5g – 14g) and gonadotropins dose:
   a. Group 1.– Untreated females
   b. Group 2.– 2.5 IU dose females
   c. Group 3.– 3.5 IU dose females
   d. Group 4.– 5 IU dose females[5]

   – **Treatment**: Single PMSG (pregnant mare serum gonadotropin) injection followed 46-49h later by injection of hCG (human chorionic gonadotropin) with the same dosage.
3. **Oocyte collection.** Oocyte retrieval by ovarian puncture – 22h after hCG administration.
4. **Imprinted genes methylation study.** CpG methylation analysis by Pyrosequencing with an initial bisulfite conversion.

   – **Primer design**: PCR and sequencing primers will be designed using the Pyrosequencing Assay Design Software (Biotage) with gene sequences (Table 1).

Table 1. Information about the study genes and sequences code access to database.

	Impact	*Zrsr1*	*Mest*
Name	imprinted and ancient	zinc finger (CCCH type), RNA binding motif and serine/arginine rich 1	mesoderm specific transcript
Mouse Locus	Chr. 18: 12,972,252-12,992,948	Chr. 11: 22,972,005-22,976,496	Chr. 6: 30,723,547-30,748,455
Human Locus	18q11.2-12.1	5q22.2	7q32
NCBI_GenBank	NC_000084.6	NC_000077.6	NC_000072.6
ENSEMBL	ENSMUSG00000024423	ENSMUSG00000044068	ENSMUSG00000051855
UCSC	uc008eda.1	uc007iem.2	uc009bfs.2

Expected results

It aims to improve ovarian stimulation technique. We hope to induce more than 10 mature oocytes with a low hormone dose and without oocyte epigenome changes.

If a loss of imprinting (LOI) is observed in high dose treatment oocyte, but not in the low dose treatment oocyte, a clinical trial will be proposed.

References

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5. Luo, C. *et al*. Superovulation strategies for 6 commonly used mouse strains. *J Am Assoc Lab Anim Sci* **50**, 471-8 (2011).