



Gene expression profiling of blastocyst stage embryos from pLH treated gilts

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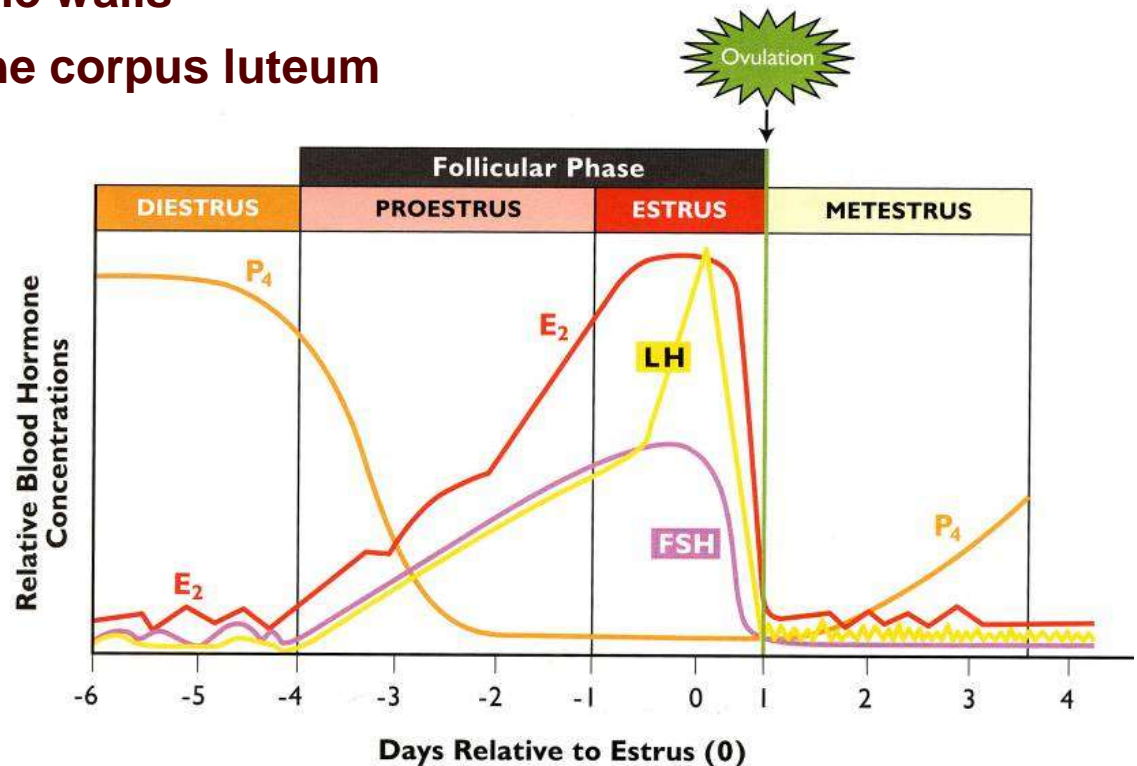


Introduction

Luteinizing hormone (LH)

Glycoprotein hormone secreted by the anterior lobe of the pituitary

- **Causes ovulation and subsequent development**
 - Increase follicular pressure
 - Weaken follicle walls
- **Maintenance of the corpus luteum**



Introduction

Exogenous porcine luteinizing hormone (pLH) treatment in pig:

Stimulate ovulation (synchronize ovulation)

- **Increase the efficiency of artificial insemination (AI)**
- **Reduce semen doses for AI**
- **Reduce labor requirement for AI**

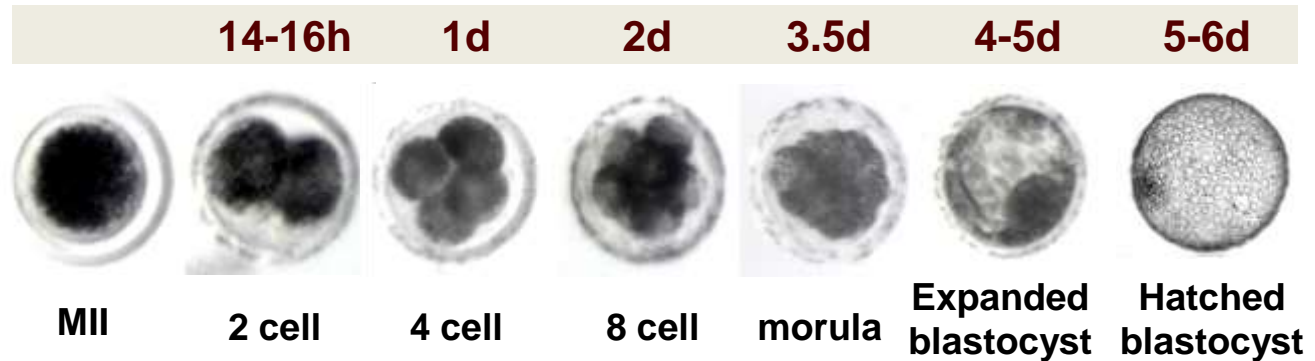
Affect the quality of embryos

- **Smaller follicle size**



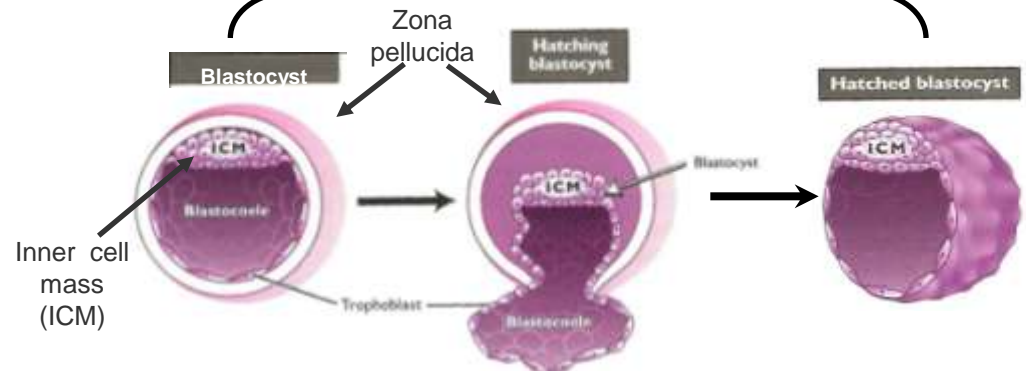
Introduction

Early porcine Embryo (in vivo)



Embryo genome activation

- Blastocyst**
- ◆ Critical embryonic stage
 - ◆ More materials to work with (compare to earlier stages)
 - ◆ Pre-implantation stage



Embryonic quality?

Embryos that are less viable or with molecular deviations appear the same as “normal” healthy embryos

Good ? Bad ?



Embryonic development and morphological criteria alone are not good enough to determine embryo quality

- Characterization of early porcine embryo transcriptome could give a hint to their embryonic quality

• Magnani, L., & Cabot, R. A. (2008). In vitro and in vivo derived porcine embryos possess similar, but not identical, patterns of Oct4, nanog, and Sox2 mRNA expression during cleavage development. *Molecular Reproduction and Development*, 75(12), 1726-1735.

• McHughes, C. E., Springer, G. K., Spate, L. D., Li, R., Woods, R., Green, M. P., et al. (2009). Identification and quantification of differentially represented transcripts in in vitro and in vivo derived preimplantation bovine embryos. *Molecular Reproduction and Development*, 76(1), 48-60.

Objective

Determine if induced ovulation with porcine Luteinizing hormone (pLH) treatment in gilts affect the embryonic quality of blastocysts?

- ◆ **Transcriptomic difference in blastocyst from pLH treated gilts ?**
- ◆ **How does the oocyte/embryo respond to the pLH treatment?**

Research Approach

Animal experiment

(A. Cameron, MSc project collaborate with BIONICHE)

- Cyclic gilts - synchronized estrous cycle with altrenogest
- Hormonal treatments
 - **CON**: Non-induced ovulation with single timed AI
 - **pLH**: pLH-induced ovulation with single timed AI
- Embryos collect at 5 days post-breeding

Bioniche - Fixed-time AI Protocols (pLH induced)

Bioniche Life Sciences has developed Fixed –time AI protocols in pigs using their products:

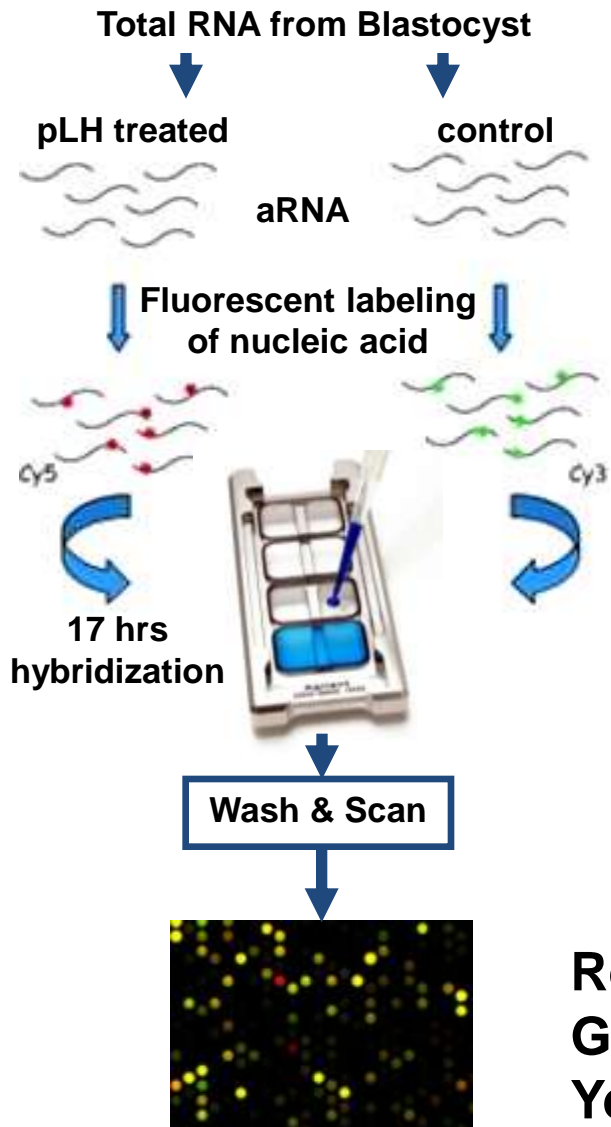
Lutropin-V:

porcine luteinizing hormone (pLH)



Research Approach

Two-color Microarray



EmbryoGene porcine microarray

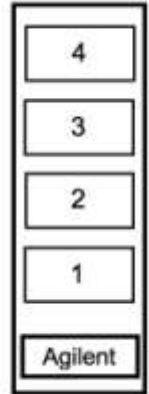
4x44k probes

Target: anti-sense RNA (aRNA)

Agilent Spike-IN: positive control

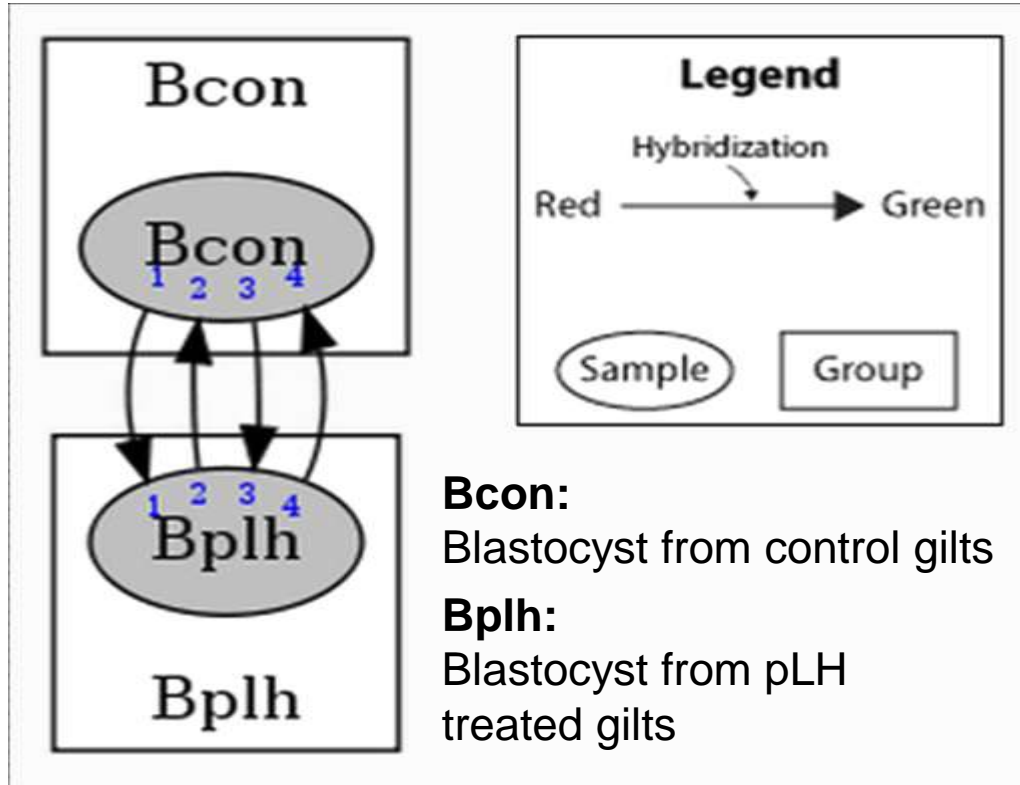
Gene expressions levels quantified by detection of fluorescent labeled **targets-probe hybridization**

Red: Gene up-regulated in pLH treat group
Green: Gene down-regulated in pLH treat group
Yellow: Equal expression in both group



Research Approach

Microarray data analysis:



Balanced block design

5 embryos per pool

4 biological replicates from each group
(control and pLH treated)

Dye swap between biological replicates

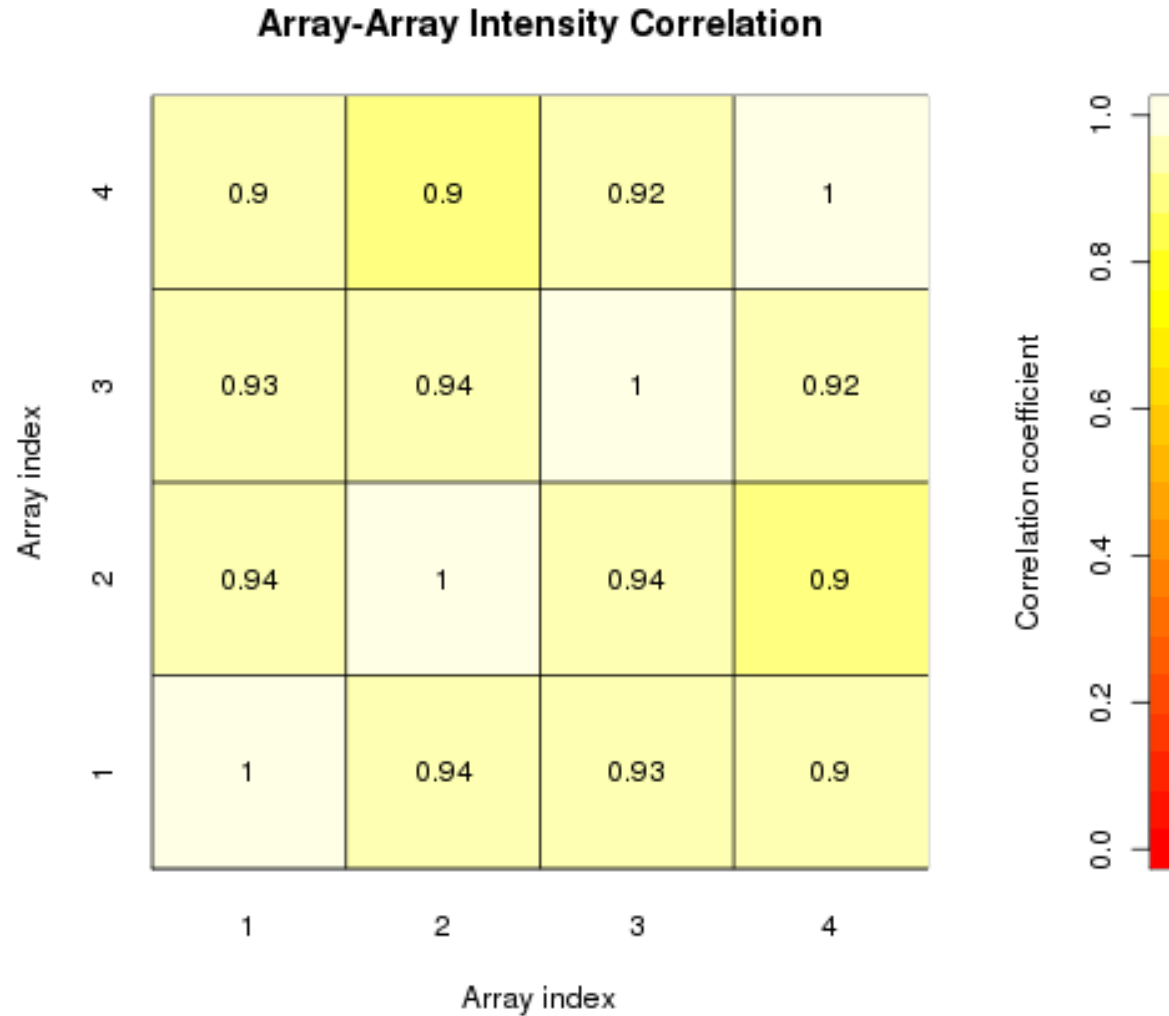
Gyde QC module (<https://www.gyde.com/embryogene/qc/accounts/profile/>)

ELMA (EmbryoGENE LIMS and Microarray Analysis <http://elma.embryogene.ca>)

FlexArray (<http://www.gqinnovationcenter.com/services/bioinformatics/flexarray/index.aspx?l=e>)

DAVID (Database for Annotation, Visualization and Integrated Discovery functional classification tools)

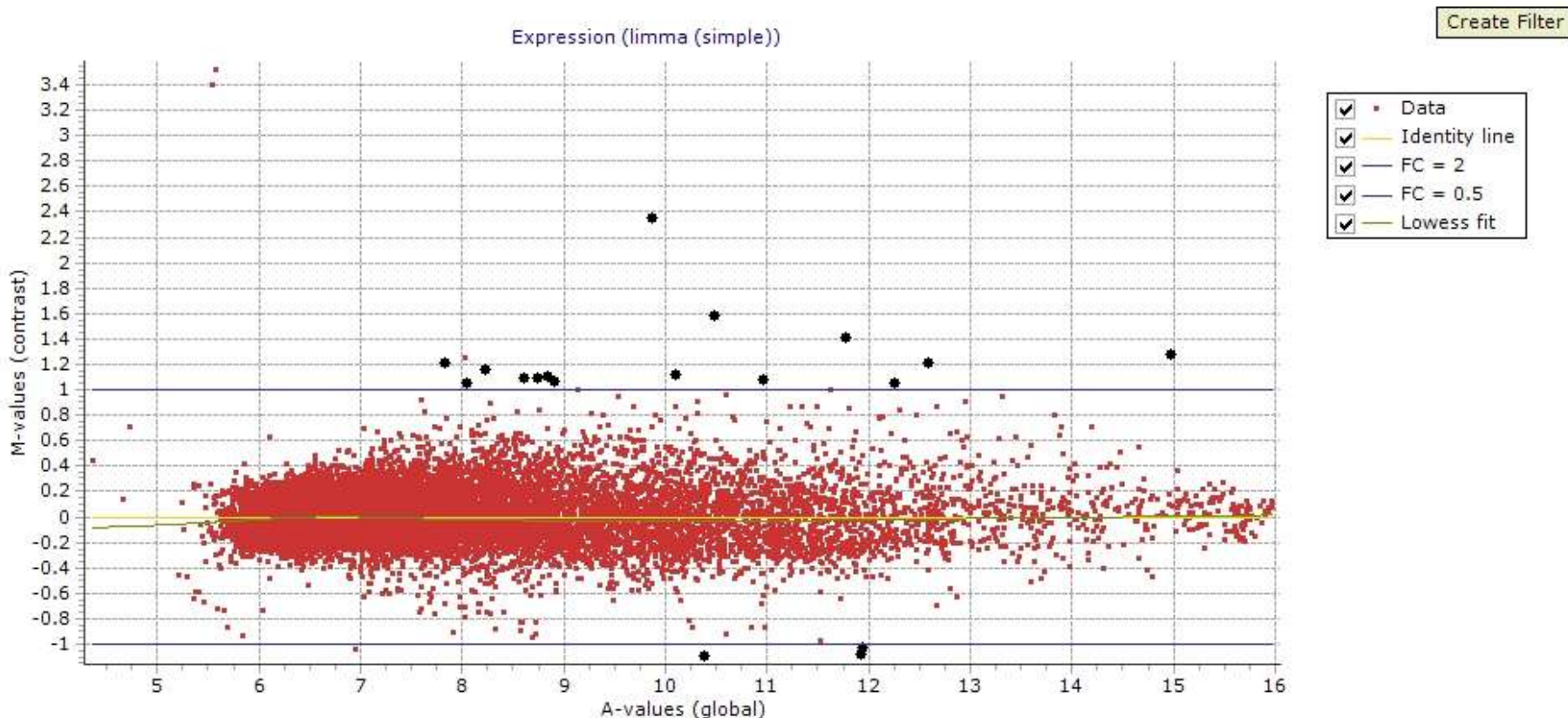
Result and Discussion



<https://www.gydle.com/embryogene/qc/accounts/profile/>

Result and Discussion

Contrast: pLH treated vs. control

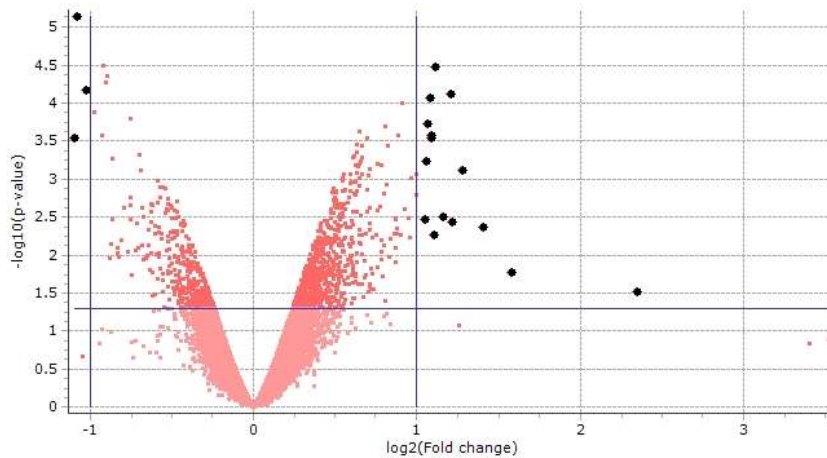


A value: \log of the average intensities ($A = 1/2 * (\log_2 (\text{expanded intensity}) + \log_2 (\text{hatched intensity}))$) ;
M value: \log_2 intensities ratios of the pLH treated over control blastocyst
($M = \log_2(\text{pLH treated intensity} / \text{control intensity})$).
FC: fold change

Result and Discussion

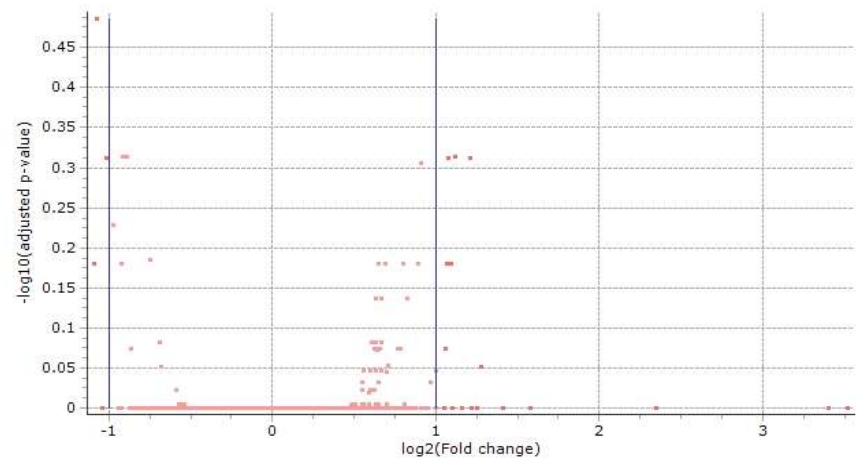
Volcano plot of p-value of pLH group vs. control group

Before p-value adjust
FDR (Benjamini Hochberg)



Up regulate: 15 probes
Down regulate: 3 probes

After p-value adjust
FDR (Benjamini Hochberg)

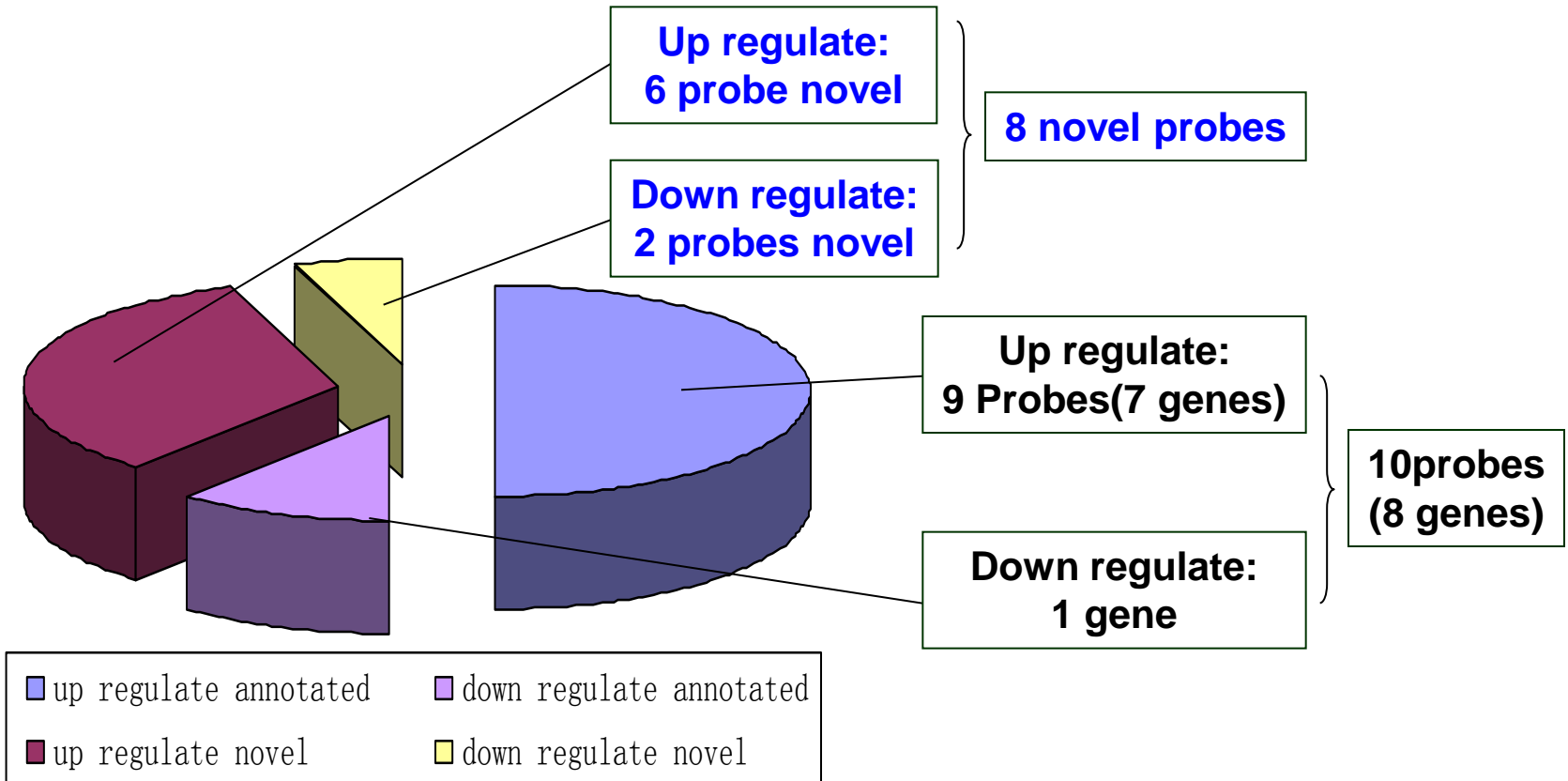


No significant differential
expressed genes

M value: \log_2 intensities ratios of the pLH treated over control blastocyst
($M = \log_2(\text{pLH treated intensity} / \text{control intensity})$).

Result and Discussion

18 probes have differential expression between pLH treated group and control group:



No enriched pathway observed

Result and Discussion

Differential expressed gene list

Probe ID	Description	Gene Symbol	Gene Function	
EMPV1_17330	Asparagine synthetase (glutamine-hydrolyzing)	ASNS	Asparagine synthase (glutamine-hydrolyzing) activity, ligase activity	+
EMPV1_24958	Dipeptidyl-peptidase 4	DPP4	Proteolysis, peptidase activity	+
EMPV1_22948	Glucosamine-6-phosphate isomerase 1-like	LOC100512094 (GNPDA1-like)	Catalytic activity, trigger calcium oscillations that trigger egg activation and early embryo development in mammalian	+
EMPV1_38049	Macrophage-expressed gene 1 protein-like	LOC100518469 (MPEG1-like)	Signal peptide	+
EMPV1_13639	Regucalcin-like	LOC100523295		+
EMPV1_17154	Regucalcin-like	LOC100523295	Calcium ion binding, ion binding,	+
EMPV1_08543	Regucalcin (senescence marker protein-30)	RGN	cation binding, metal ion binding,	+
EMPV1_16864	Regucalcin (senescence marker protein-30)	RGN		+
EMPV1_36757	18S ribosomal RNA	Rn18s	Ribosomal RNA	+
EMPV1_17384	Stratifin (SFN)	SFN	Adapter protein, signal transduction	-

Note: “ + ”: Up-regulate gene; “ - ”: Down-regulate gene

10 differential expressed probes (8 genes)

Conclusion

◆ **18 probes have differential expression between pLH treated group and control group:**

■ **Up regulate: 15 probes**

Annotated: 9 probes (7 genes, 2 splice forms)

Novel: 6 probes

■ **Down regulate: 3 probes**

Annotated: 1 gene

Novel: 2 probes

◆ **No enriched pathway observed**

QPCR verification of gene expression will be performed

pLH treatment in gilts exhibit limited effect on the transcriptome of blastocyst stage embryos



Implication

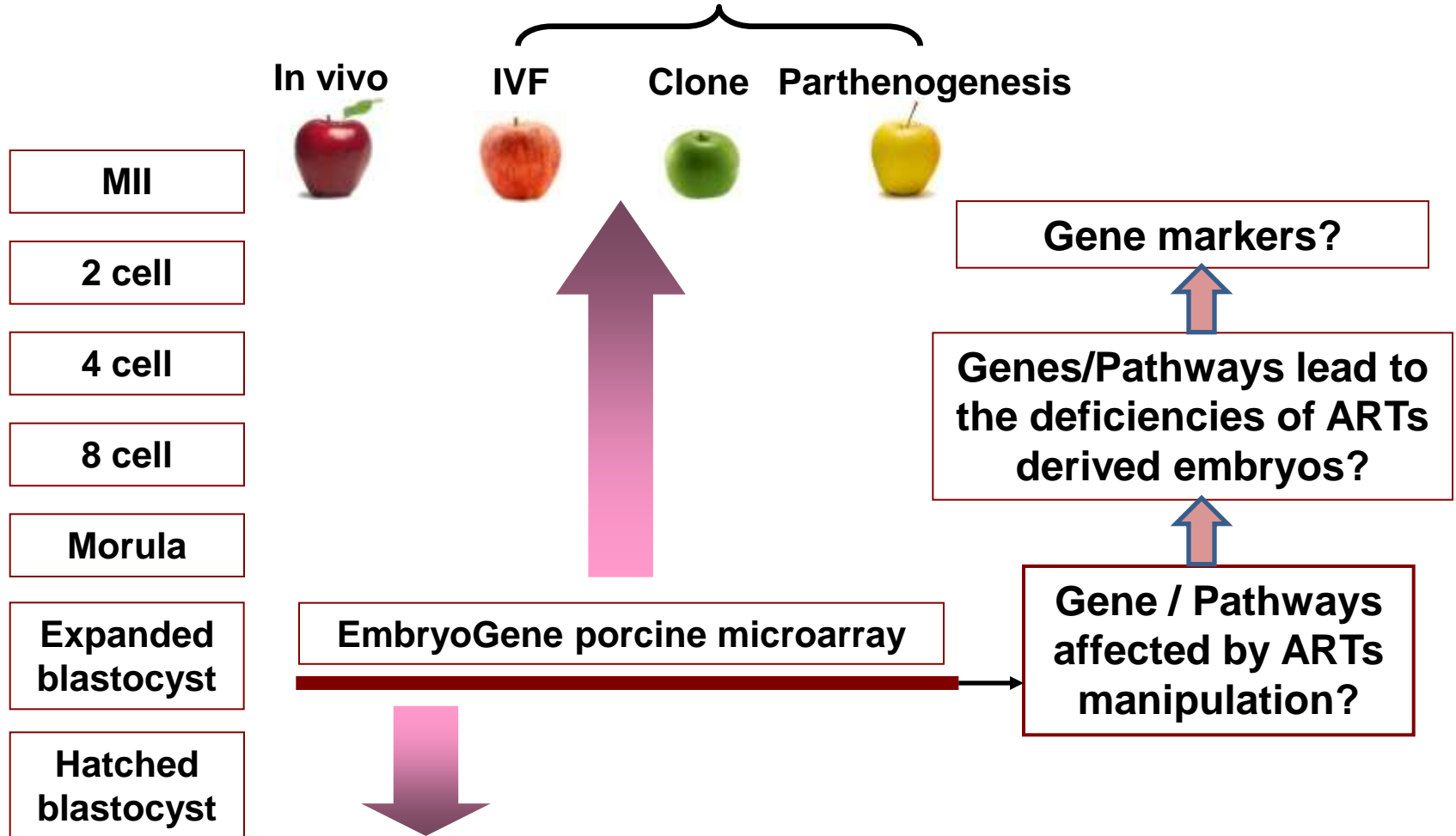
This study identified few important genes that differential expressed in blastocyst from pLH treated gilts

- Increase our understanding of how exogenous hormone treatment affect the gene expression in blastocyst stage embryos.
- Identify genes that potentially respond to exogenous hormone treatment in porcine embryo
- Provide useful information for the application of utilizing pLH in the ovulation synchronization in gilt as part of artificial insemination protocol



Next

Embryos manipulated with lab based ARTs



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Thank You ~ ~

Questions?

