

The Functional Analysis of Developmentally Important Genes in Bovine Oocytes Using siRNA

Ernesto Orozco-Lucero

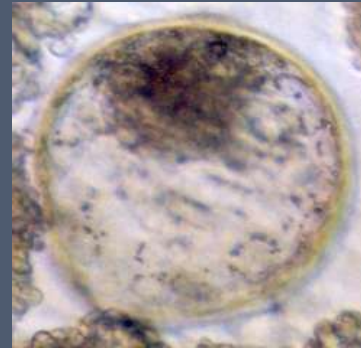
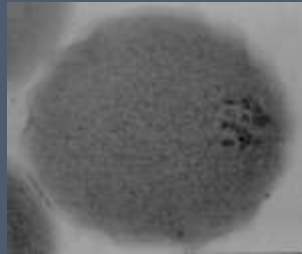
Director: Marc-André Sirard

Co-director: Claude Robert

EmbryoGENE 2011 Annual General Meeting

INTRODUCTION

Oocyte developmental competence



Transcripts



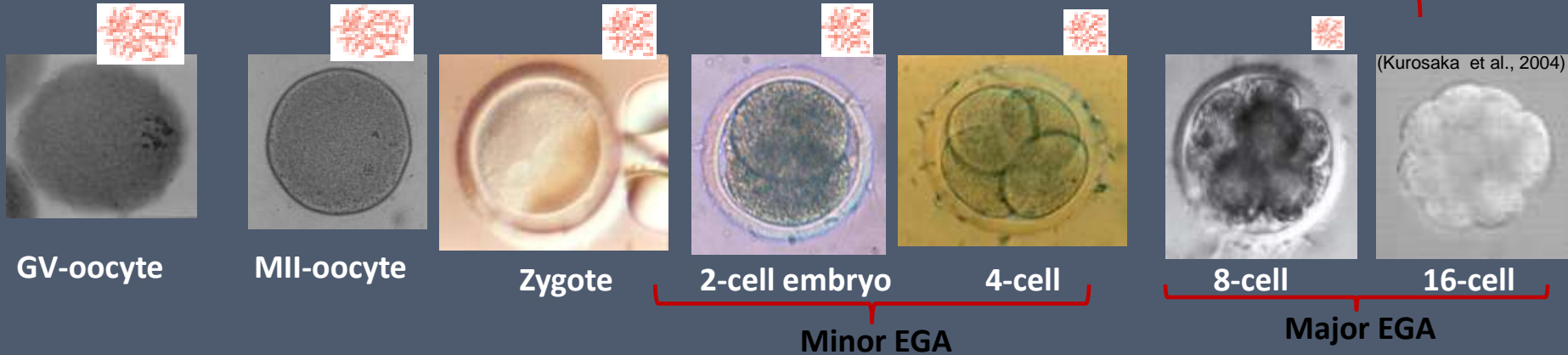
Proteins

Used during transcriptional silence period: GV-EGA

Gene products stored in oocyte cytoplasm

INTRODUCTION

Maternal mRNA use by the bovine oocyte/embryo



Transcriptional silence

Some stored gene products with high importance

Oocyte developmental competence markers

Could potentially be used to improve IVP programs in animals

Information still scarce

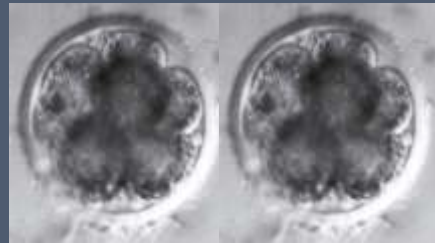
INTRODUCTION

Oocytes with differential developmental competence

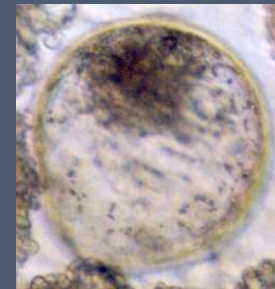


Late-cleaving oocytes

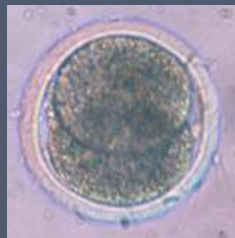
E.g. 27 hpi



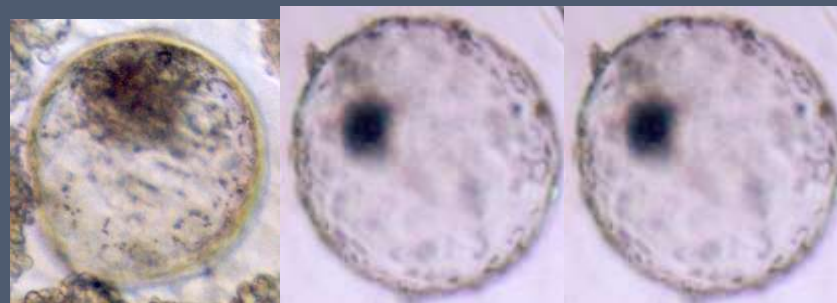
High number of 8-cell arrested embryos



Decreased blastocyst rate



Early cleaving oocytes



High blastocyst rate

Variation of stored transcripts in oocytes of distinct quality

(Mourot et al., 2006; Dode et al., 2006; Fair et al., 2004; Patel et al., 2007)

INTRODUCTION



Assessing the function of a specific stored transcript in the oocyte:

RNAi to study the function of a specific oocyte developmental competence marker



mRNA



Microarray analysis to evaluate gene expression differences (RNAi vs ctrl-)

(Tesfaye et al., 2010;)

HYPOTHESIS

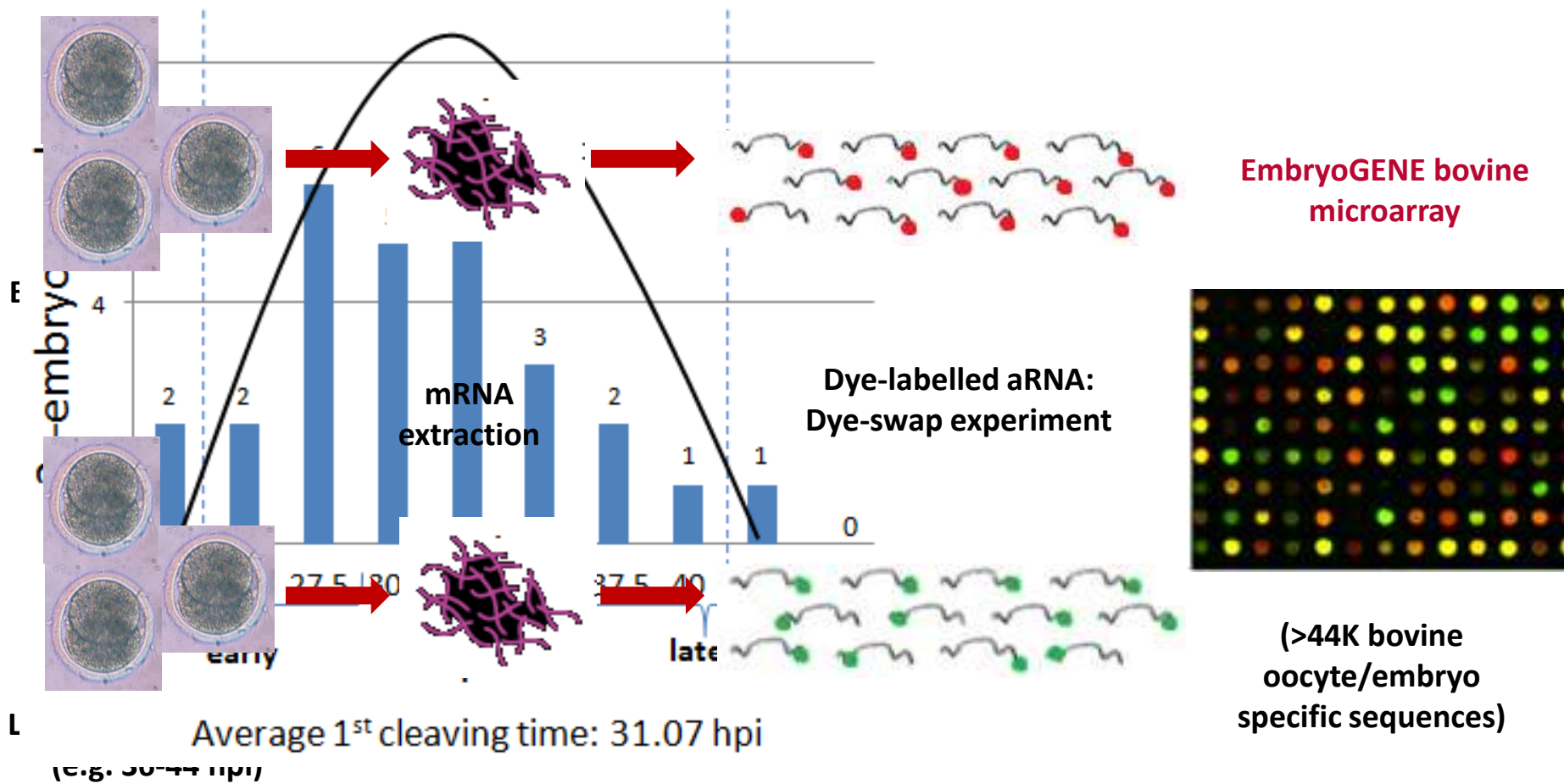
Oocytes of different developmental competence have varying levels in some stored transcripts whose knock down induces developmental arrest.

GENERAL OBJECTIVE

To acquire information of the role and timing of genes related to bovine oocyte developmental capacity by knocking down developmentally important oocyte genes.

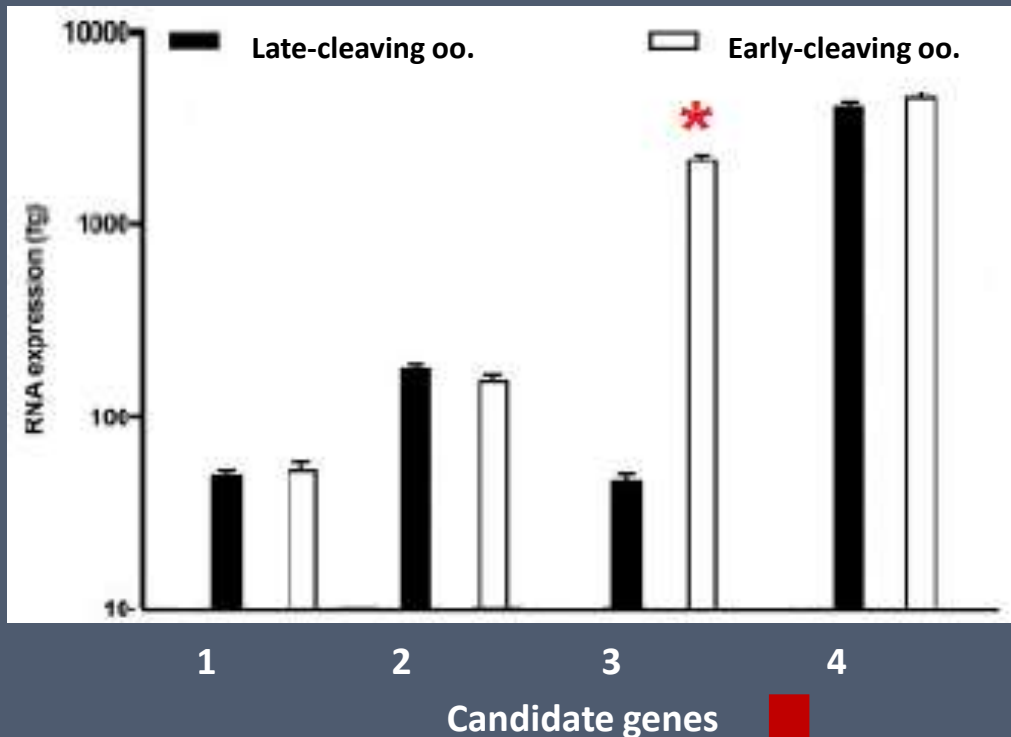
SPECIFIC OBJECTIVES

1. Transcriptomic analysis of bovine early embryos of different developmental abilities to identify differences in mRNA



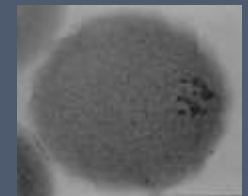
2. Selection of candidate genes and verification of the presence of their codified proteins in the immature oocyte

A qRT-PCR validation of the microarray results



Selection of "gene 3" (e.g) as potential oocyte developmental capacity marker

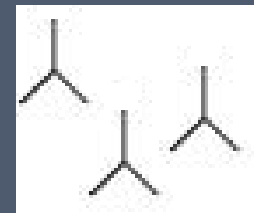
B Protein analysis



GV-oocytes

Protein extraction

MW GV oocyte Ctrl -



"Gene 3"-codified protein specific antibody



Western blot

3. Candidate genes siRNAs synthesis and GV oocytes injection

(bio.davidson.edu/Courses/)



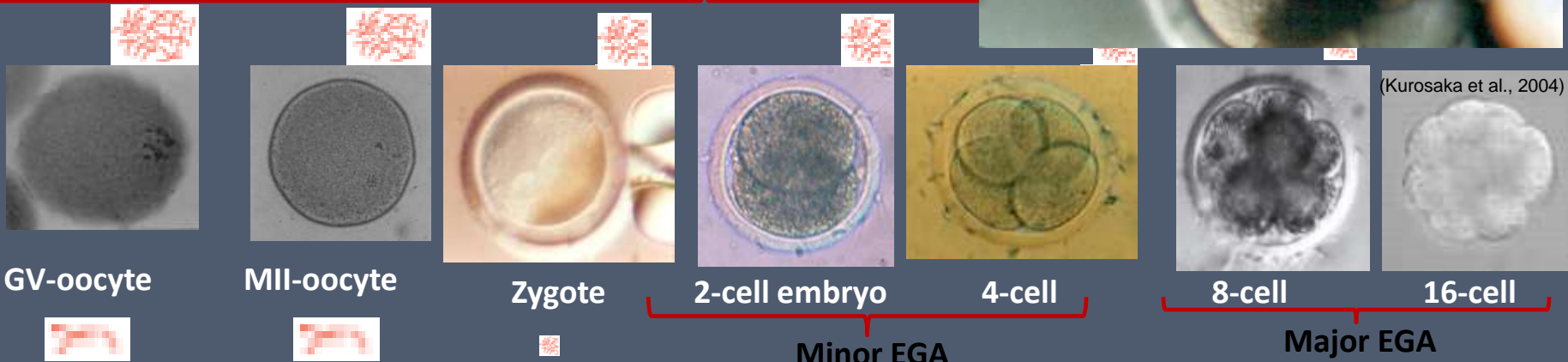
siRNA synthesis



Microinjection



Maternal mRNA use by the oocyte/embryo



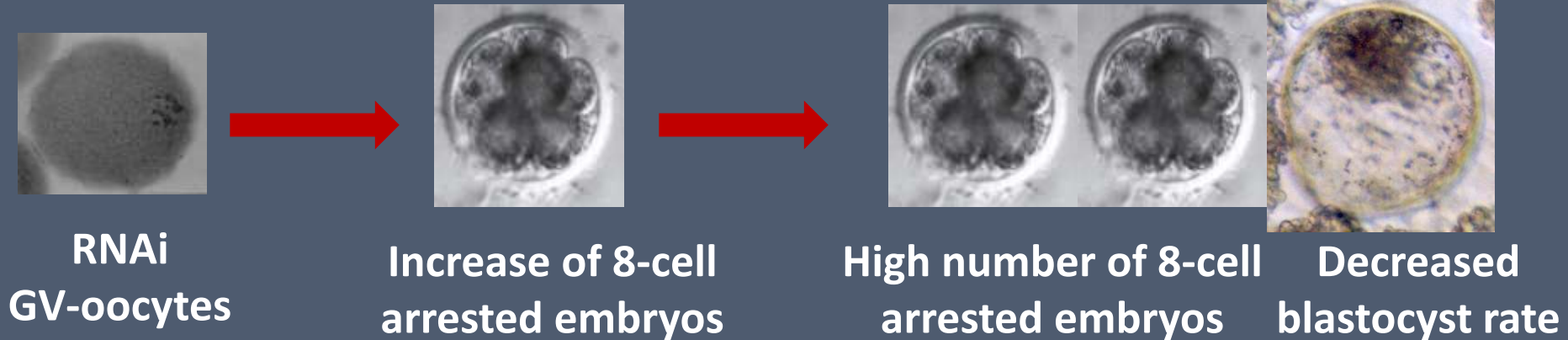
(Kurosaka et al., 2004)

Selective degradation of the maternal "gene 3" transcripts

Developmental window targeted

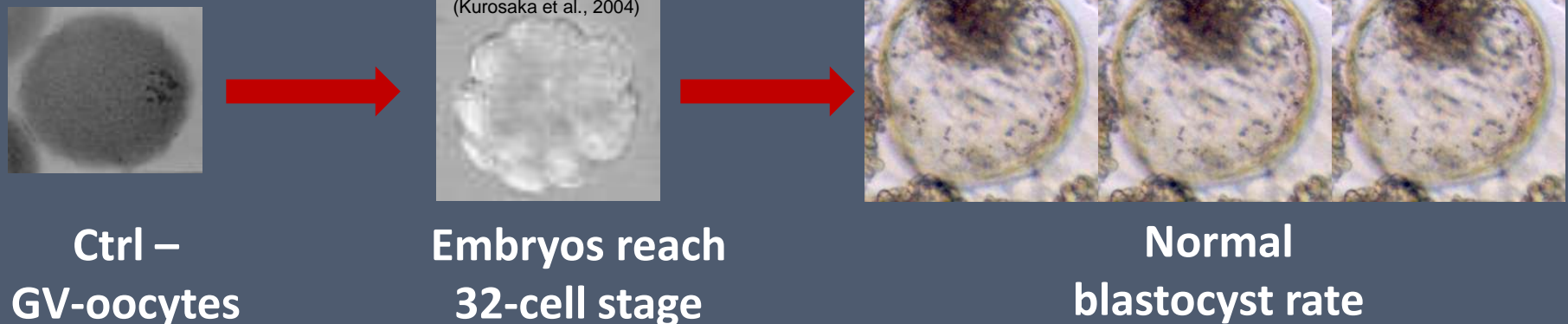
4. Phenotypic evaluation of RNAi embryos; qRT-PCR analysis of the target genes

A) Phenotype analysis

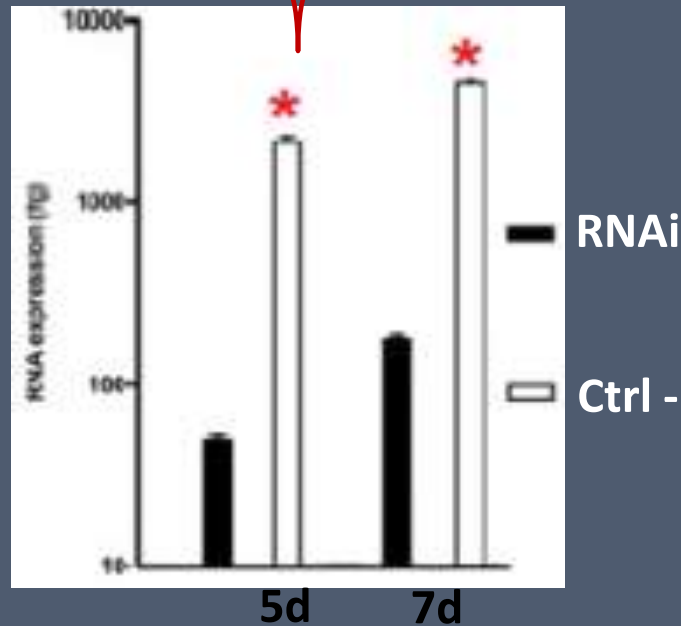
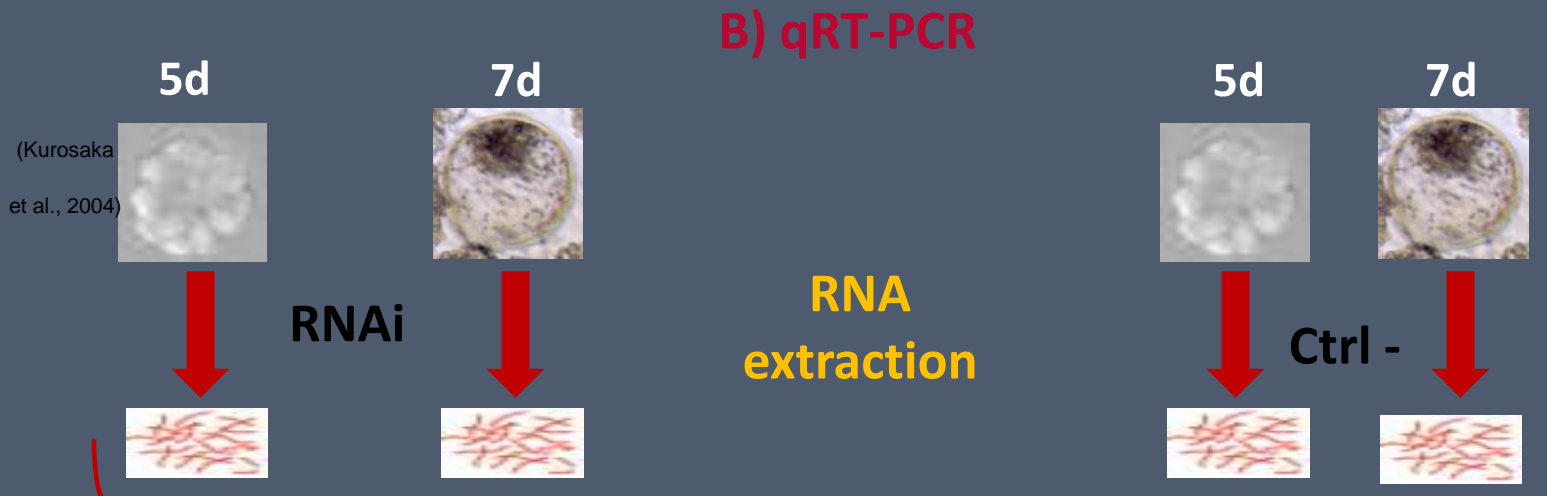


5d of culture

7d of culture



4. Phenotypic evaluation of RNAi embryos; qRT-PCR analysis of the target genes



5. Protein decrease validation

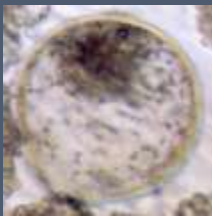
(Kurosaka et al., 2004)

5d



RNAi

7d



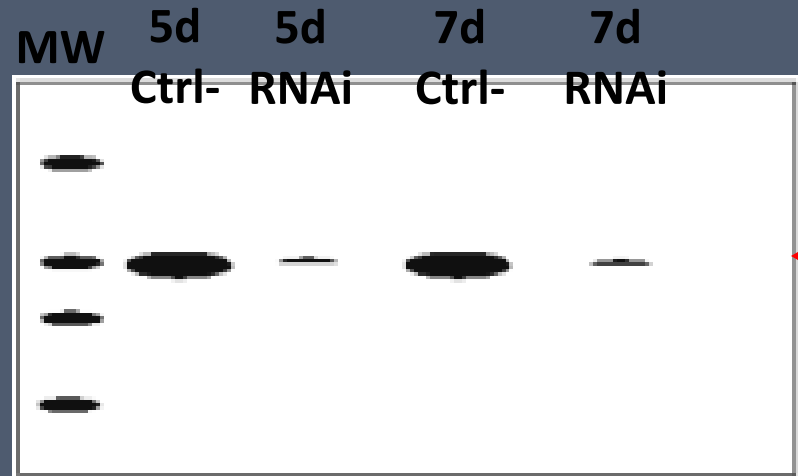
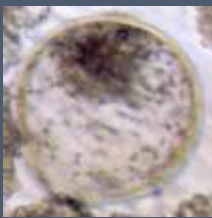
Protein extraction

5d

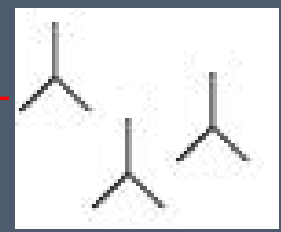


Ctrl-

7d



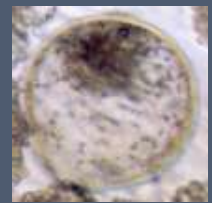
Western blot



"Gene 3"-
codified protein
specific
antibody

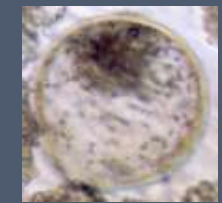
5. Protein decrease validation

7d



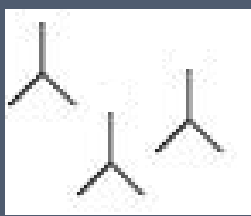
RNAi

7d



Ctrl-

Blastocyst
tissue sections



Gene 3-codified
protein specific
antibody



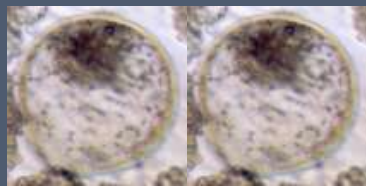
(Kliem et al., 1998)



Immunohistochemistry

6. Microarray analysis of the RNAi embryos to evaluate the effect of knock down, if surviving phenotype

(2nd year and beyond)

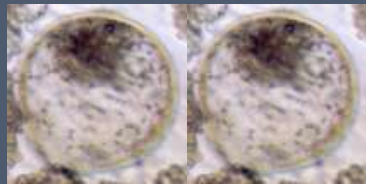


7d RNAi embryos



mRNA extraction

Dye-labelled aRNA:
Dye-swap experiment



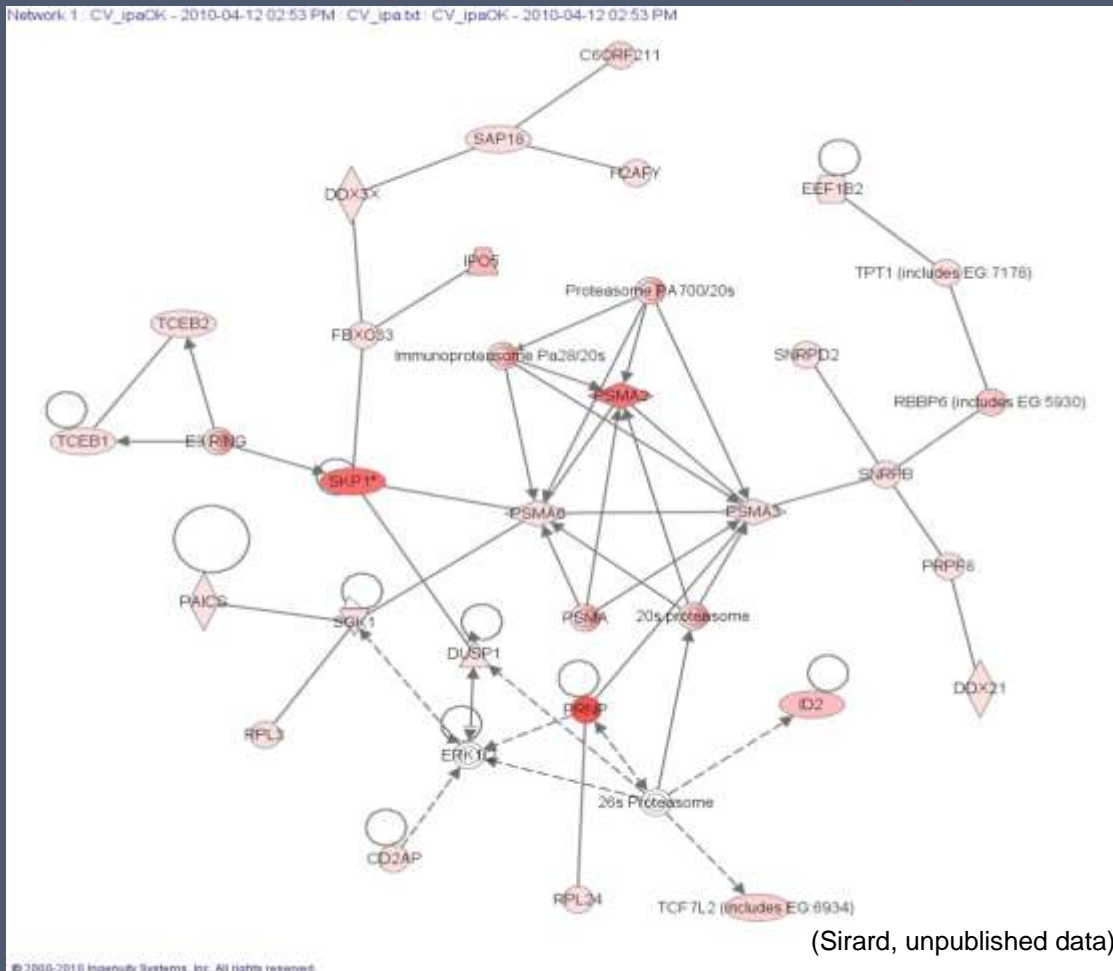
7d Ctrl - embryos



EmbryoGENE
bovine
microarray

6. Microarray analysis of the RNAi embryos to evaluate the effect of knock down, if surviving phenotype

Interpretation of microarray data through GO term studies



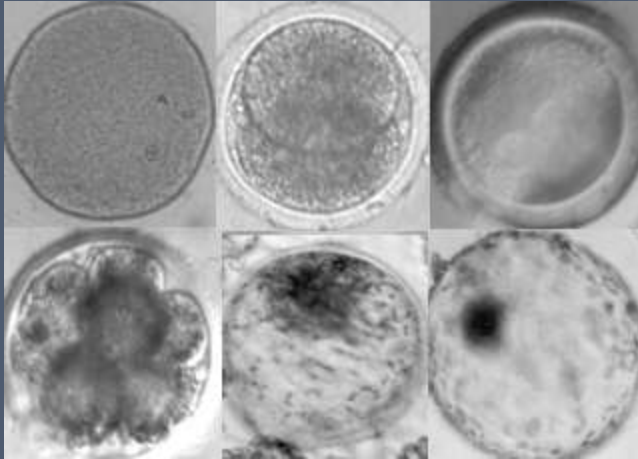
Example of an IPA-generated gene network analysis specific to the bovine maternal–embryonic gene control transition

OUTCOME

Comprehension of bovine early embryogenesis at the molecular level



Better understanding of the mammalian 1st week of life



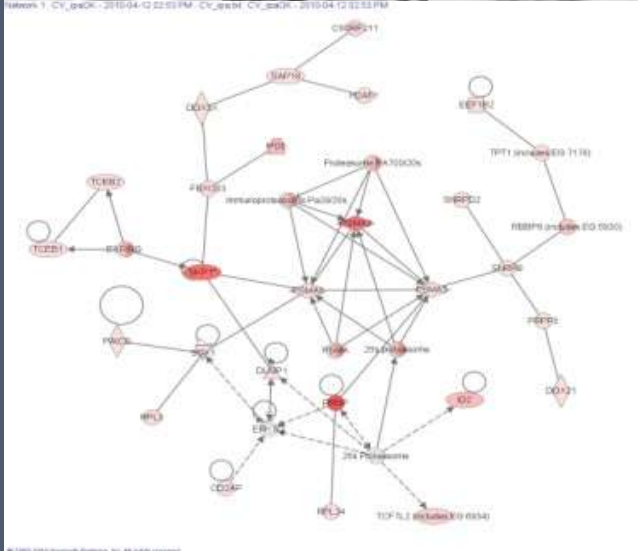
Explanations for low developmental competence of oocytes from slaughterhouse ovaries



Possible treatment



Oocytes obtained from valuable animals through OPU



THANKS

got embryo?



!!

