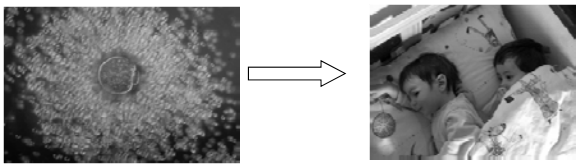
7th Workshop
Mammalian Folliculogenesis and Oogenesis

ESHRE Campus symposium
Stresa, Italy
19 - 21 April 2012

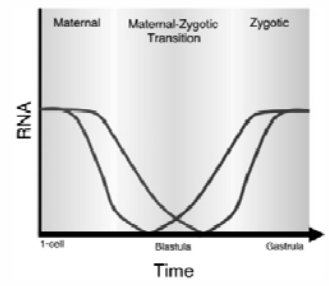
'Acquisition of the oocyte developmental competence'

Maurizio Zuccotti

Embryogenesis begins during oogenesis



mRNA degradation during the early stages of development



Embryonic genome activation

Mouse: 2-cell

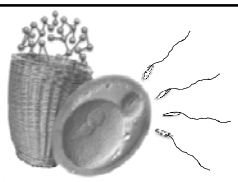
Human: 4-8 cell

Rabbit: 8-cell

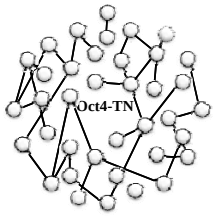
Sheep: 16-cell

What does make an egg good or bad?

Which is the transcriptional identity of the developmentally competent egg ?

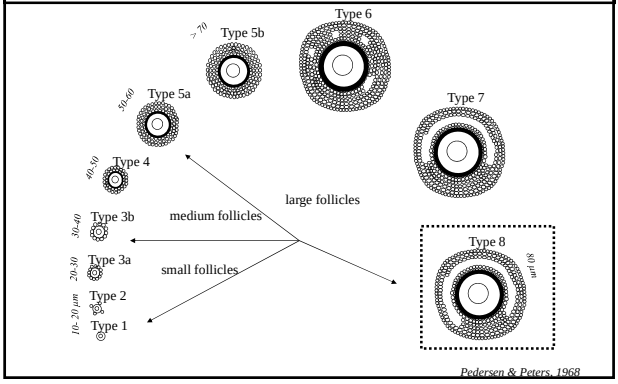


Identify the presence of an OCT4-transcriptional network (OCT4-TN) in oocytes

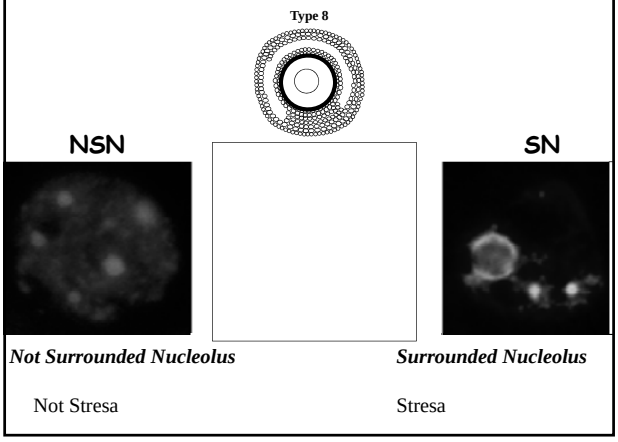


Use of a model study in which MII oocytes cease development at the 2-cell stage

Chromatin organisation of oocytes during folliculogenesis



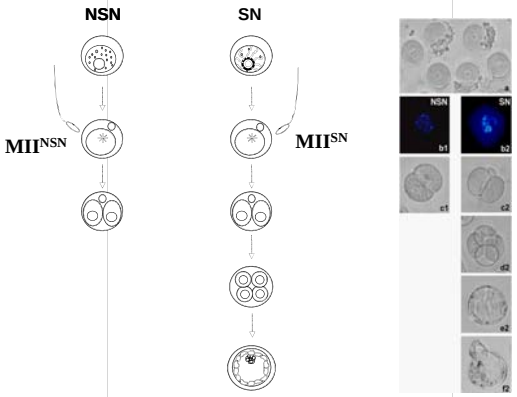
Pedersen & Peters, 1968



Not Surrounded Nucleolus ***Surrounded Nucleolus***

Not Stresa Stresa

DEVELOPMENTAL COMPETENCE



What does determine the 2-cell block in mouse MII^{NSN} oocytes?

Maternal-effect factors?

Down-regulation of maternal-effect gene expression blocks preimplantation development
(27 maternal-effect genes)

Stella (*Dppa3*)

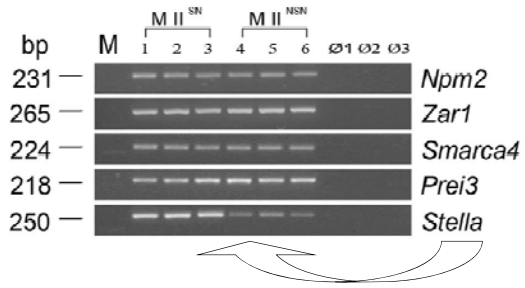
Zar1

Npm2

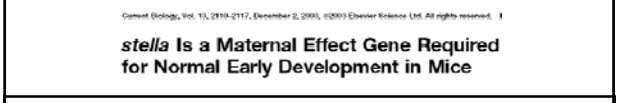
Smarca4 (*Brg1*)

Prei3

***Stella* gene expression is down-regulated in MII^{NSN} oocytes**



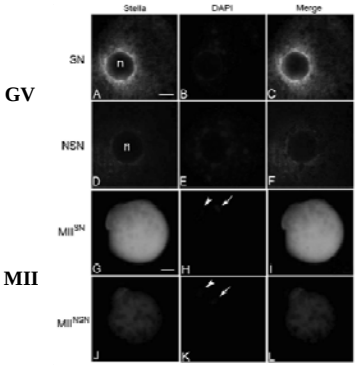
STELLA functions at the 1-cell stage to protect against demethylation of the maternal genome and some paternal imprinted genes



Lack of STELLA in MII oocytes blocks preimplantation development

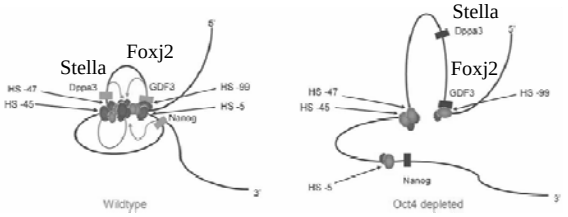
… and STELLA protein?

STELLA protein expression is down-regulated in GV-NSN and MII^{NSN} oocytes



In ES cells OCT4 regulates the expression of STELLA

Lack of OCT4 changes the chromatin organisation at the *Nanog* locus containing *Stella* and down-regulates its expression

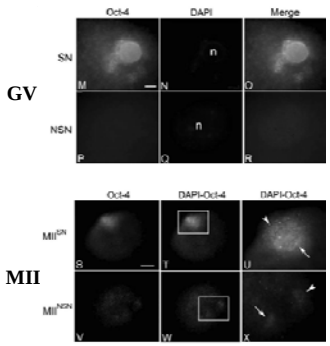


Levasseur et al., Genes & Dev. 22, 575, 2008

OCT4 is a marker of pluripotency

- maternal transcripts are necessary for preimplantation development
- Embryonic OCT4 transcription factor is first expressed at the 4- 8-cell stage, then is progressively confined to:
- the ICM in preimplantation blastocyst
- during gastrulation, to the PGCs
- in ES cells is down-regulated upon differentiation
-its function in the ovary is unknown

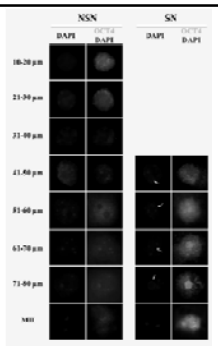
OCT4 protein expression is down-regulated in GV-NSN and MII^{NSN} oocytes

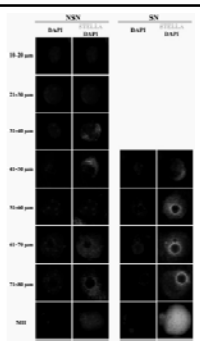


Profile of OCT4 and STELLA expression in NSN and SN oocytes during folliculogenesis

∅ µm	NSN %	SN %
10 - 20	100	-
20 - 30	100	-
30 - 40	100	-
40 - 50	95	5
50 - 60	85	15
60 - 70	65	35
70 - 80	50	50

OCT4 and STELLA are expressed coincidentally at the beginning of the oocyte growth and are upregulated in SN oocytes



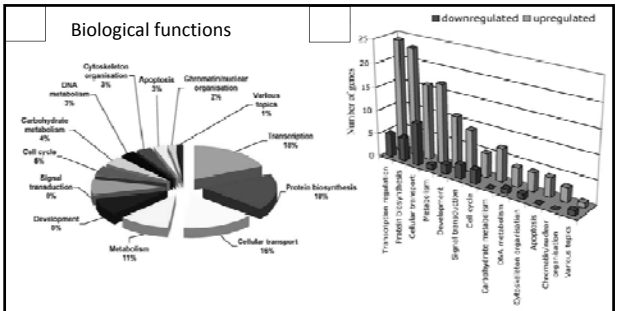


Does OCT4 down-regulation in MII^{NSN} oocytes modify the expression of other genes?

⇩

Microarray analysis of gene expression in MII^{NSN} and MII^{SN} oocytes

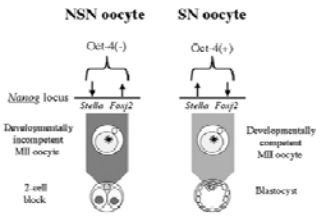
Biological functions



- Illumina microarrays: 39.450 genes analysed
- 380 regulated genes
  - 303 up-regulated in MII^{NSN} oocytes
  - 77 down-regulated in MII^{NSN} oocytes

Among the 380 genes, *Fox2* is located in the Nanog locus and is up-regulated

Foxj2 over-expression in zygotes, 2-cell block
(Martın-de-Lara et al., 2008)



25 of the 380 regulated genes are known for being OCT4-regulated and 18 are assigned to apoptosis or mitochondrial dysfunction

OCT4 is a component of a maternal regulatory transcriptional network (TN) that influences positively (when OCT4 is expressed) or negatively (when OCT4 is down-regulated) the oocyte quality.

Questions addressed next:

- **Which is the extension of this TN ?**

- **Is its presence circumscribed to the egg or**

- **is it maintained after fertilisation ?**

Microarrays comparison of the transcriptome of
MII^{NSN} vs. MII^{ctrl} oocytes
and 2-$cell^{NSN}$ vs. 2-$cell^{ctrl}$
brought up an expanded OCT4-transcriptional network present in both eggs and
2-cell embryos

MII^{NSN} vs. MII^{ctrl} 3102 regulated genes

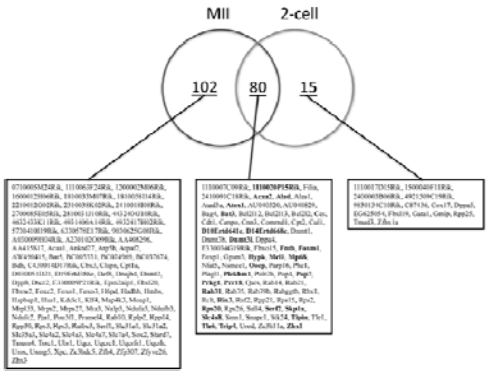
2-cell^{NSN} vs. 2-cell^{ctrl} 1887 regulated genes

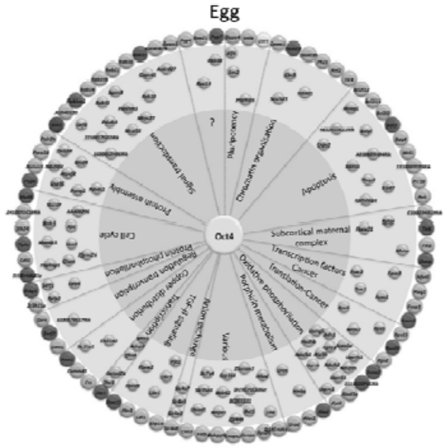
32 OCT4-regulated genes expressed in both MII and 2-cell

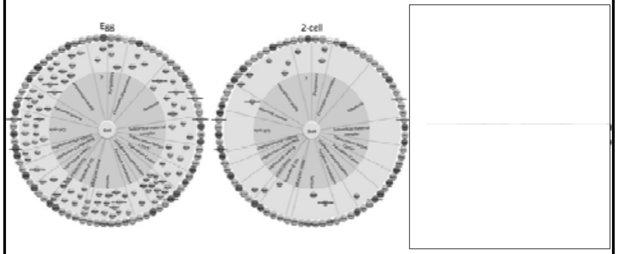
MII^{ctrl} down-regulated

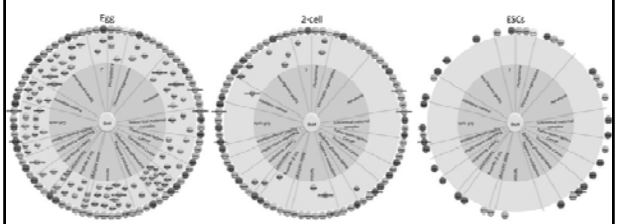
2-cell^{ctrl} up-regulated

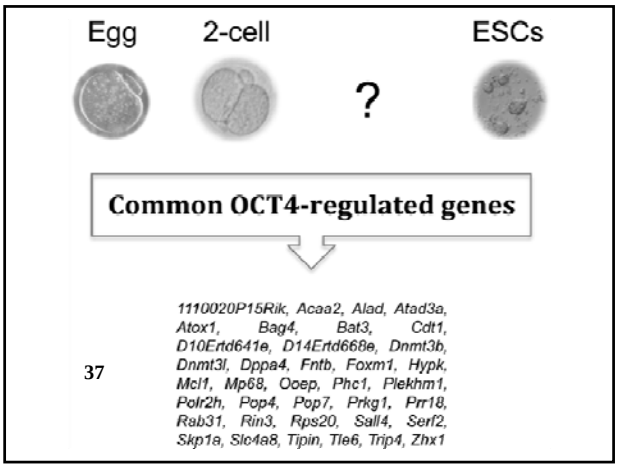
OCT4-TN: 197 genes




Egg
Oct4
Subcortical maternal complex
Transcription factors
Cancer
Apoptosis
Cell cycle
Signal transduction
Protein assembly


Egg
2-cell


Egg
2-cell
ESCs



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TAKE HOME MESSAGE

IN THIS MODEL STUDY:

- STELLA AND OCT4 (FOXJ2) ARE MARKERS OF THE OOCYTE DEVELOPMENTAL COMPETENCE;

- IDENTIFICATION IN OOCYTES OF AN OCT4-TN;

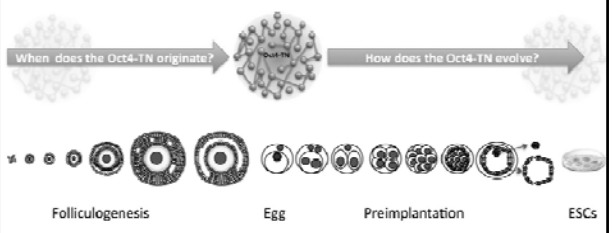
- OCT4-TN MAY CONTAIN TRANSCRIPTS/PROTEINS MARKERS OF THE OOCYTE QUALITY?

- OCT4-TN SURVIVES THE WIDE TRANSCRIPTS ERASURE THAT OCCURS AFTER FERTILISATION;

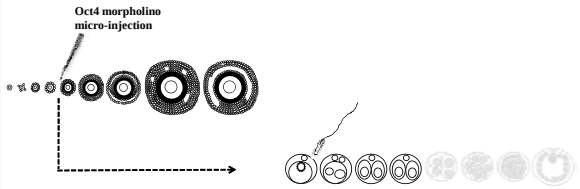
- IT IS PRESENT IN 2-CELL EMBRYOS AND IN ESCs.

- OCT4-TN MAY CONTRIBUTE TO THE ACQUISITION OF THE OOCYTE DEVELOPMENTAL COMPETENCE AND MAY REPRESENT THE EARLIEST MOLECULAR SIGNATURE OF THE ICM PLURIPOTENCY.

NEXT...



Does inactivation of *Oct4* expression influence the expression of genes of the OCT4-TN ?



Effects of *Oct4* knockdown on:
•oocyte maturation;
•Oct4-TN expression;
•oocyte-specific gene expression;
•developmental competence.

COLLABORATIONS !!!!!!

Publications

- BMC Developmental Biology, 8: 97, 2008.
- Human Reproduction 24: 2225, 2009.
- Reproductive BioMedicine Online, 19 Suppl. 3: 57-62, 2009.
- BMC Genomics 12: 345, 2011.
- Human Reproduction Update 17: 525, 2011.

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