Influence of maternal-embryonic transition on embryonic development

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The latest speakers..... Is not and easy job.....

Bad points:

Somebody more cleaver
have already presented of your talk

Delegates are enough satisfied about
The scientific information
(quality ad quantity)

Good points

The main part of the background
presentation has been well presented

There is the chance to make
connections with
the previously speakers

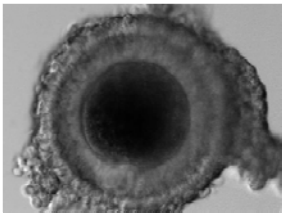
Oocyte competence and subsequent embryonic development in mammals are regulated by several orchestrated events that could be regrouped in two main activities

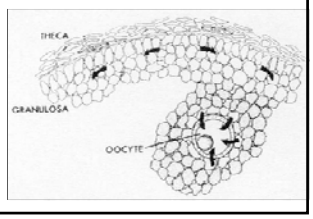
Inductive signals:

Reprogramming activity

Inductive signals

- FSH and LH: *LH delivery to the pre-ovulatory follicle in sheep influences the subsequent developmental competence*
- Steroid signals
- Growth factors
- Metalloproteinase(MMP) and tissue inhibitor of metalloproteinase(TIMP)
- Cyclic AMP in mammalian follicle cells and oocytes during maturation



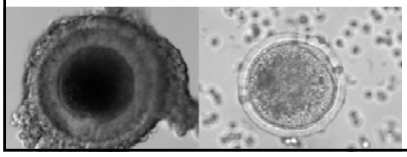


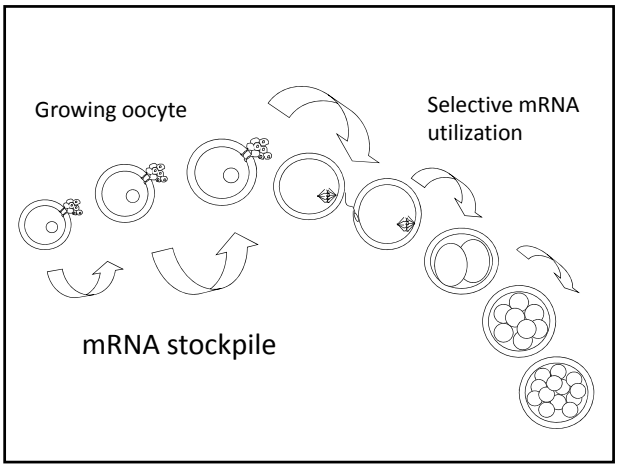
Some indications from these studies

- The developmental potential of the embryos can be compromised both by the administration of inappropriate form of gonadotropins or by non-physiological modes of LH administration.
- A functional link between steroid signals, oocyte reprogramming and subsequent development competence has been provided(Moor et al. 1998)

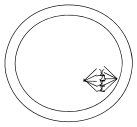
Reprogramming activity

- mRNA storage
- mRNA selective utilization
- Cromatine reorganization
- Protein synthesis
- Changes in protein phosphorylation
- Localization of protein





mRNA utilization or increase of degradation ?



A stockpile of maternal mRNA

mRNA probably is in a translationally inactive state (masked) in immature GV-stage

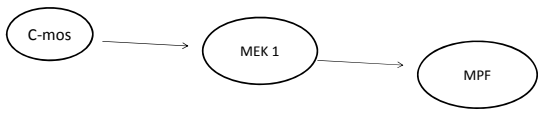
Cytoplasmic polyadenylation has been described as a way of initiating translation of stored maternal mRNA during oocyte maturation and embryo development

A simply example how is important the selective degradation of oocyte stored mRNA

The case of c-mos

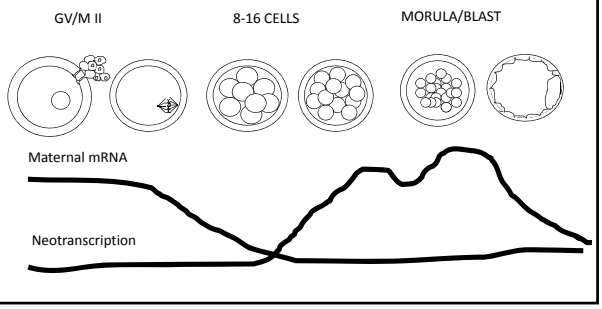
MOS/MEK1/MAPK/p90rsk signaling pathway regulates the cell cycle through a cascade of protein kinase phosphorylation

The *C-mos* mRNA is stored as maternal information in the growing oocytes and it is translated into protein, which initiates MAPK cascade phosphorylation during oocyte maturation

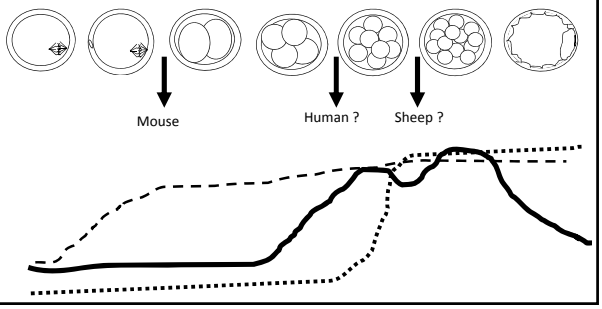


Translational control of c-mos mRNA by cytoplasmic polyadenylation is necessary for normal development.

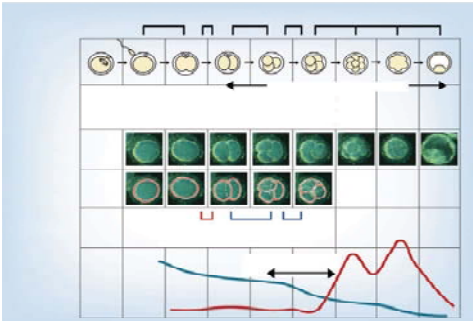
CRITICAL POINTS OF PREIMPLANTATION DEVELOPMENT in LARGE ANIMALS

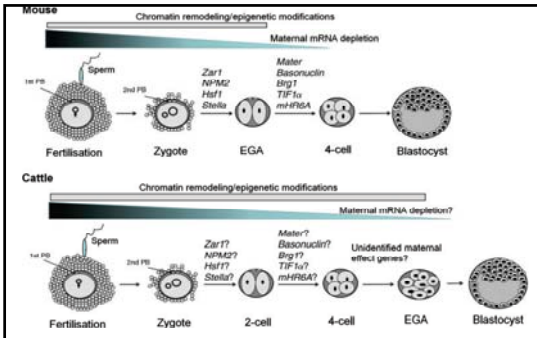


Embryonic Genomic activation(EGA)



Maternal embryonic transition in human embryos. From Wong et al. Nature Biotechnology on line published 3 October 2010 EGA is detected at 4-8 cell stage. Blastomeres showed a different temporal gene expression pattern.





So two points can be :considered:

EGA can occurs in a different time between species

Can occurs a different time in the blastomeres

Among the genes that are changing during the meiotic progression and preimplantation embryonic development great interest has been focused on:

Maternal Effect Genes(MEGs) or....
Ocoyte Specific Genes(OSGs)

How much they can influence the embryonic development ?

MEGs or OSGs gene expression and potential preimplantation development

- The correct embryonic development at the early stages(preimplantation embryos) largely depend to the mRNA resorvoir that is stored in the oocyte during the growing phase
- Amongst the mRNAs stored in the growing oocyte are the maternal effect genes (MEG), which are expressed predominantly in the oocyte and are involved in the early cleavage regulation.
- They express preferentially in the oocyte and usually are present in early embryos and then degraded at the time of MET, without compensation by embryonic transcription.

MEGs or OSGs gene expression and potential preimplantation development

- Studies in mouse have demonstrated that often the knock-out of MEG affects the oogenesis and leads to the incapacity of the embryo to develop beyond the first cleavages.
- These effects are more heavy or less dramatic according to the MEGs functions
- In large animals their specific functions in the oocyte and early embryo have not been yet elucidated even these information could be resemble more closely what can happen in human oocyte and embryos at early stages.

MEGs or OSGs gene expression and potential preimplantation development: open questions

Are they present in the oocyte and early embryonic stages in large animals ?

Are the expression patterns, during oocyte maturation and embryonic development, similar to other experimental animals ?

Are they related to different developmental competence ?

We have decide to you sheep oocyte as model of large animal: why ?

- It has been used to standardised in vitro protocols as IVM, IVF and IVC that are now used largely in ART, including human (Edwards et al. (Nature, 1965) *Maturation in vitro of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes); Jagiello et al. (Biol. Reprod., 1974) Sheep oocytes matured in vitro.*
- Relative low cost and easy availability
- Large simlarities with human folliculargenesis
- Good endocrinological background *(Moor and Trounson (J. Reprod. Fert., 1977) Hormonal and follicular factors affecting maturation of sheep oocytes in vitro and their subsequent developmental capacity).*

Dynamic changes of ZAR 1, Mater, GDF 9 and BMP 15 in sheep oocyte and early cleavage embryos

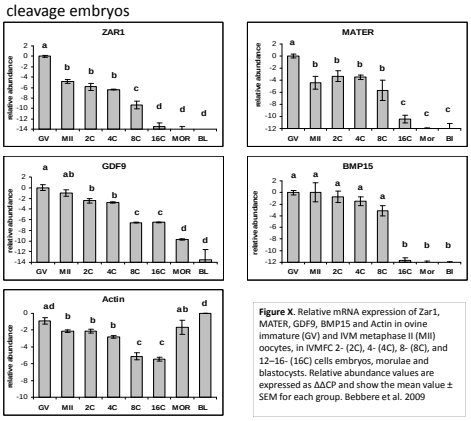
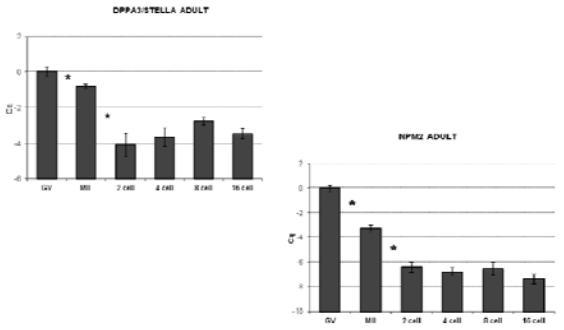
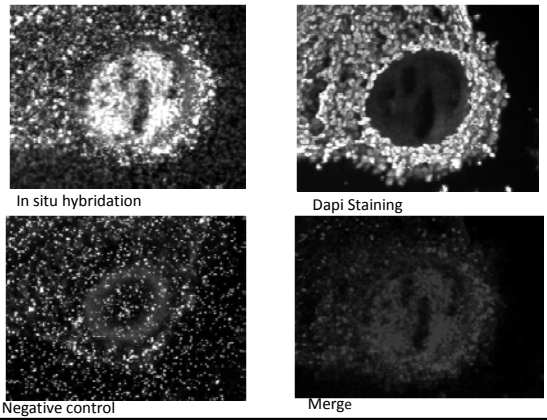


Figure X. Relative mRNA expression of Zar1, MATER, GDF9, BMP15 and Actin in ovine immature (GV) and IVM metaphase II (MII) oocytes, in IVMFC 2- (2C), 4- (4C), 8- (8C), and 12–16- (16C) cells embryos, morulae and blastocysts. Relative abundance values are expressed as ΔΔCP and show the mean value ± SEM for each group. Bebbere et al. 2009



Detection of ZAR 1 expression of cumulus complex oocyte by In situ Hubridation of sheeo ovary



In situ hybridation

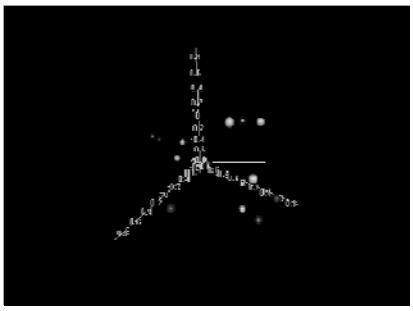
Dapi Staining

Negative control

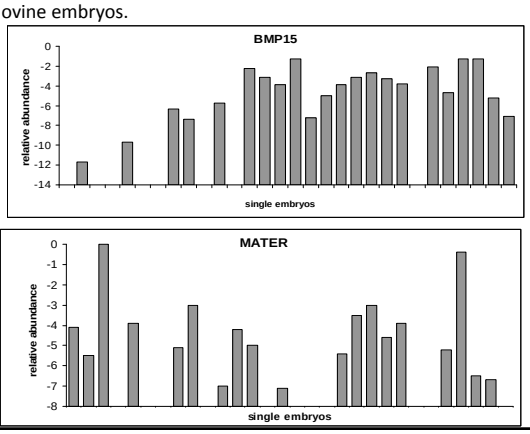
Merge

mRNA analysis of GV/M II oocyte from adult and prepubertal oocyte (Microarray analysis we sheep customised microchip(Illumina) show an important differences between samples due to hetereogenously molecular store

Number of genes that passed filtering criteria: 1536
Nominal significance level of each univariate test: 0.01
Number of genes significant at 0.01 level of the univariate test: 45

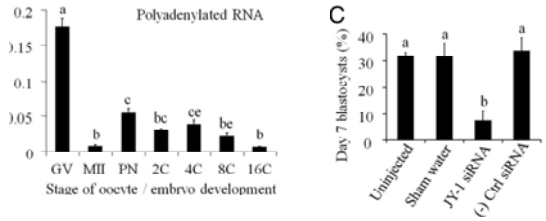


Relative expression of *BMP15* and *MATER* genes in single 8-16 cells IVF ovine embryos.



- Are they related to different developmental competence ?

Experiments in Bovine using JY-1 siRNA showed the role of MEGs/OSGs in the preimplatation development(Bettegowda et al. PNAS 2007)



Large animal model : sheep

Low competence oocyte versus high competence oocyte…..
: sheep oocyte from prepubertal (low) VS adult(high)

Our previously data showed :

IVM – no significant different
IVF - no significant different
IVC - early stages : slow early cleavage(12 h later 2 – 4 cell stage and lower and delayed blastocyst formation
Later development : reduce viability and high rate of abortion

Timers of meiotic progression and embryonic division: MPF and MAP kinases

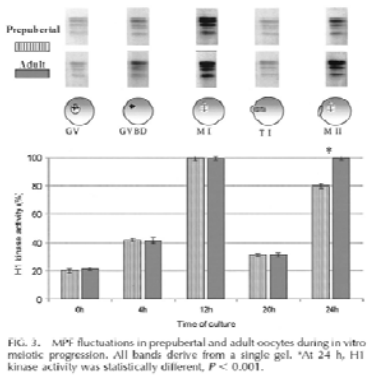
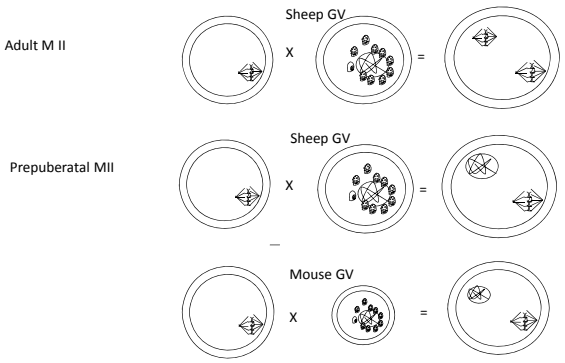


FIG. 3. MPF fluctuations in prepubertal and adult oocytes during in vitro meiotic progression. All bands derive from a single gel. *At 24 h, H1 kinase activity was statistically different, $P < 0.001$.

Comparation of MPF activity between adult and prepubertal oocyte

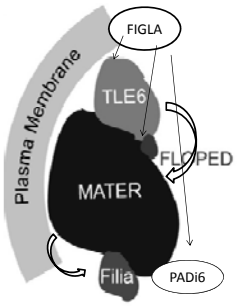


A Subcortical Maternal Complex Essential for Pre-implantation Mouse Embryogenesis

Lei Li1,2, Boris Baibakov1, and Jurrien Dean1

1*Laboratory of Cellular and Developmental Biology, NIDDK, National Institutes of Health, Bethesda, MD 20892-8028.* *Dev Cell. 2008 September ; 15(3): 416–425.*

Subcortical Maternal Complex(SCMC)



SCMC is located in the subcortex of eggs but is excluded from region of cell contact during early cleavage

Transcription enconding these protein are degradated during meiosis and ovulation but protein persist in the early embryos stages

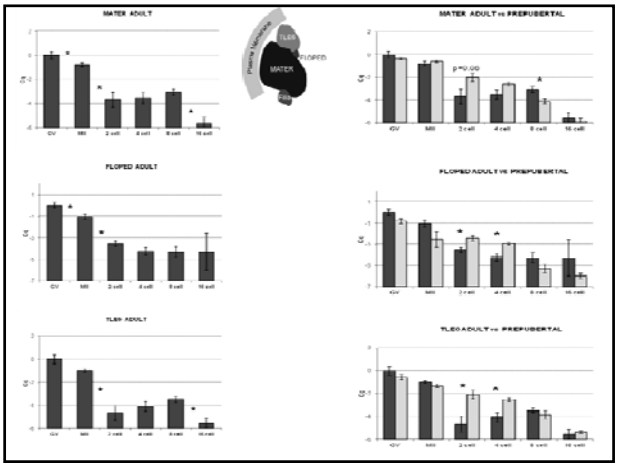
The expression of the genes that encode at least four of these proteins *Floped, Mater, Tle6, Padi6) is regulated by FIGLA* ***(Factor in the germline, alpha), a bHLH transcription factor***

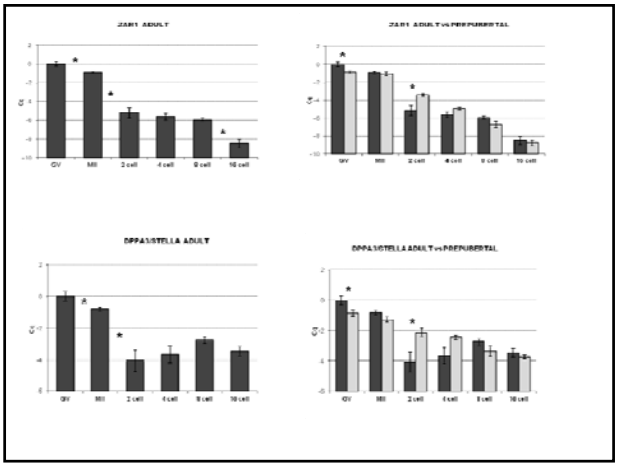
Floped knockout mouse showed normal ovulation and fertilization but 1 –to 2 cell division was delayed 6 -8 h hours

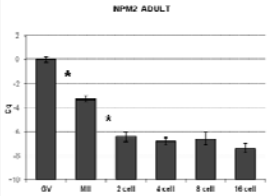
Low competence oocyte versus high competence oocyte: SCMC

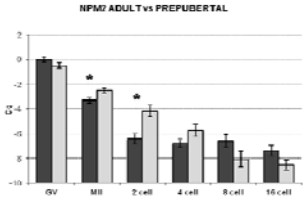
The presence and dynamic changes of Mater, Floped and TLE6, components of SCMC have been determined in sheep oocyte and early cleavage embryos up to 16 cell stage(time of EGA)

What we have observed ?









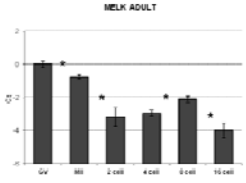
Low competence oocyte versus high competence oocyte: Stella, ZAR 1, MELK and NPM2

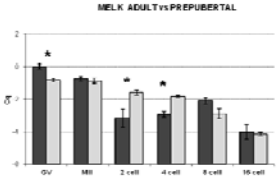
Expression pattern of DPP£/Stella, ZAR 1, Melk and NPM2 followed what described for the other MEGs and reported in mouse. They progressively decrease from GV stage to 16 cell stage embryos

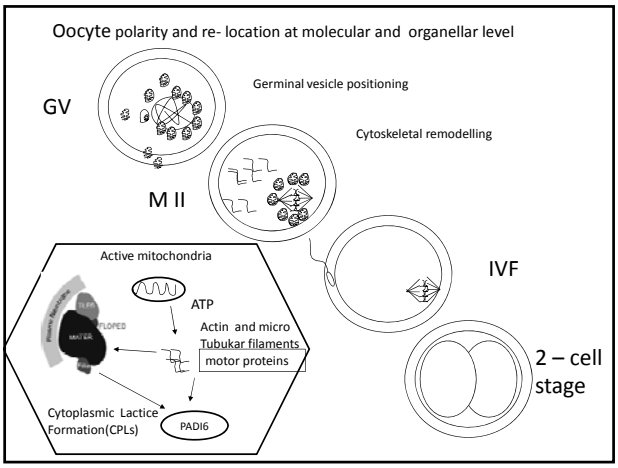
Low competence GV oocyte showed a lower mRNA level of DPP3/Stella, ZAR 1 and Melk

Analysis of mRNa quantity of all the four genes were significantly different in lower competence oocyte compared to adult sheep oocyte.

Maternal Embryonic Leucine Zipper Kinase
Member of Snf1/AMPK serine/threonine Kinase Family
Provide protection to cell aganist enviromental stress and may control biosyntetic pathways of carbon metabolism







Conclusion

- Understand the molecular mechanisms of the embryo quality and a chance to optimize in vitro embryo production systems in human embryology area.
- In fact. limitation in human experiments are due to the scarcity of material as well the heterogeneity of sources (young and aged patients, different style of life, ecc.), that could overcame performing large and controlled studies.
- In our animal model we observed that the expression patterns of the analysed MEGs or OSGs seem partially followed what has been already reported n mouse supporting the hypothesis of an involvement of these transcripts in the transition from oocyte to embryonic genome activation in large animals too.
- We found differences of MEGs or OSGs amount during specific stage of the cleavage, in the same model of different developmental competence represented by prepubertal (low competence) and adult(high competence).
- Even the experiments are under close control we should consider that there is a significant variation

Thank you for your attention