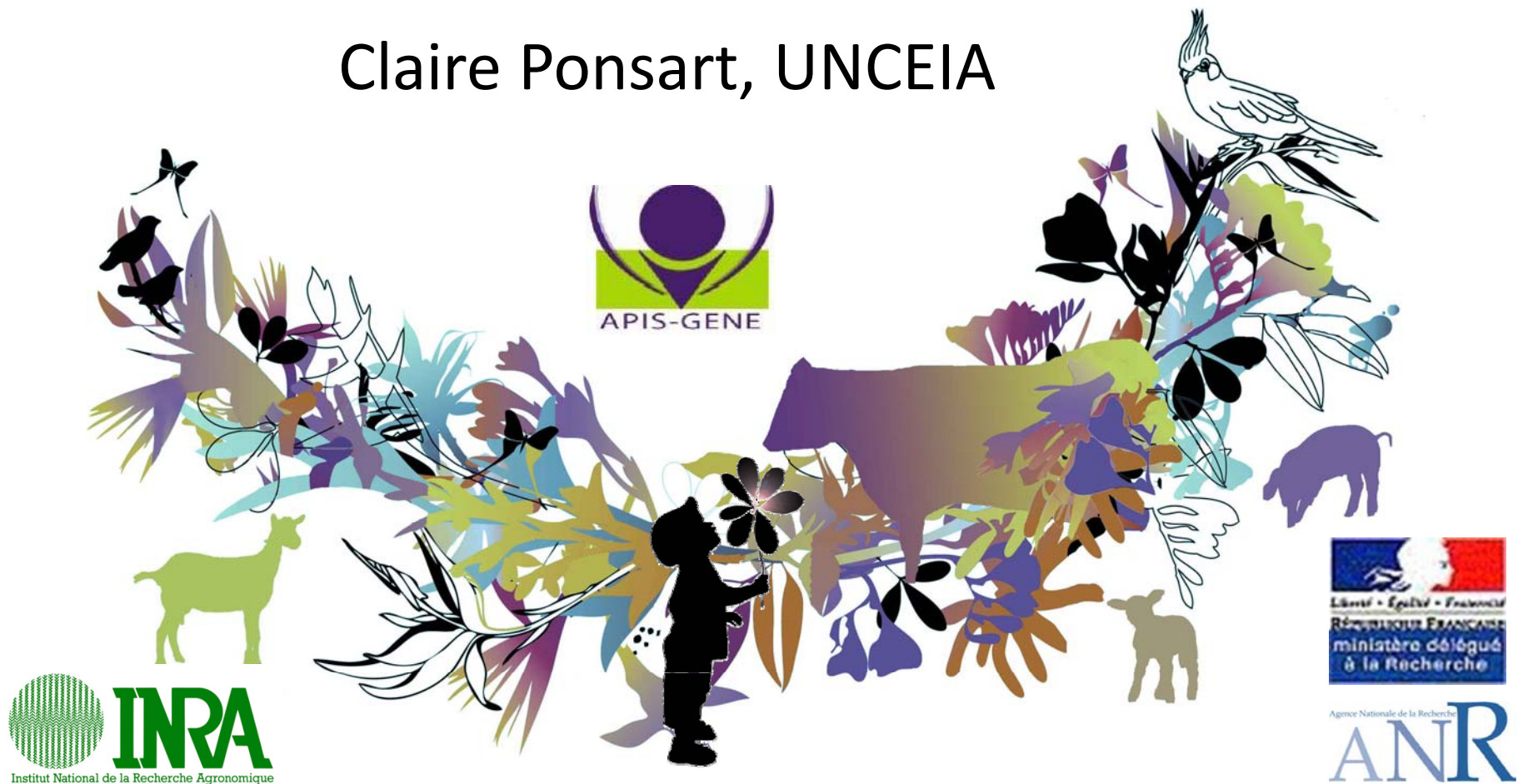


Phenotyping the reproduction function in cattle: inputs from functional genomics

Claire Ponsart, UNCEIA



Phenotyping the reproduction function : a multidimensional scale

**2.FUNCTIONAL
CONTINUUM**

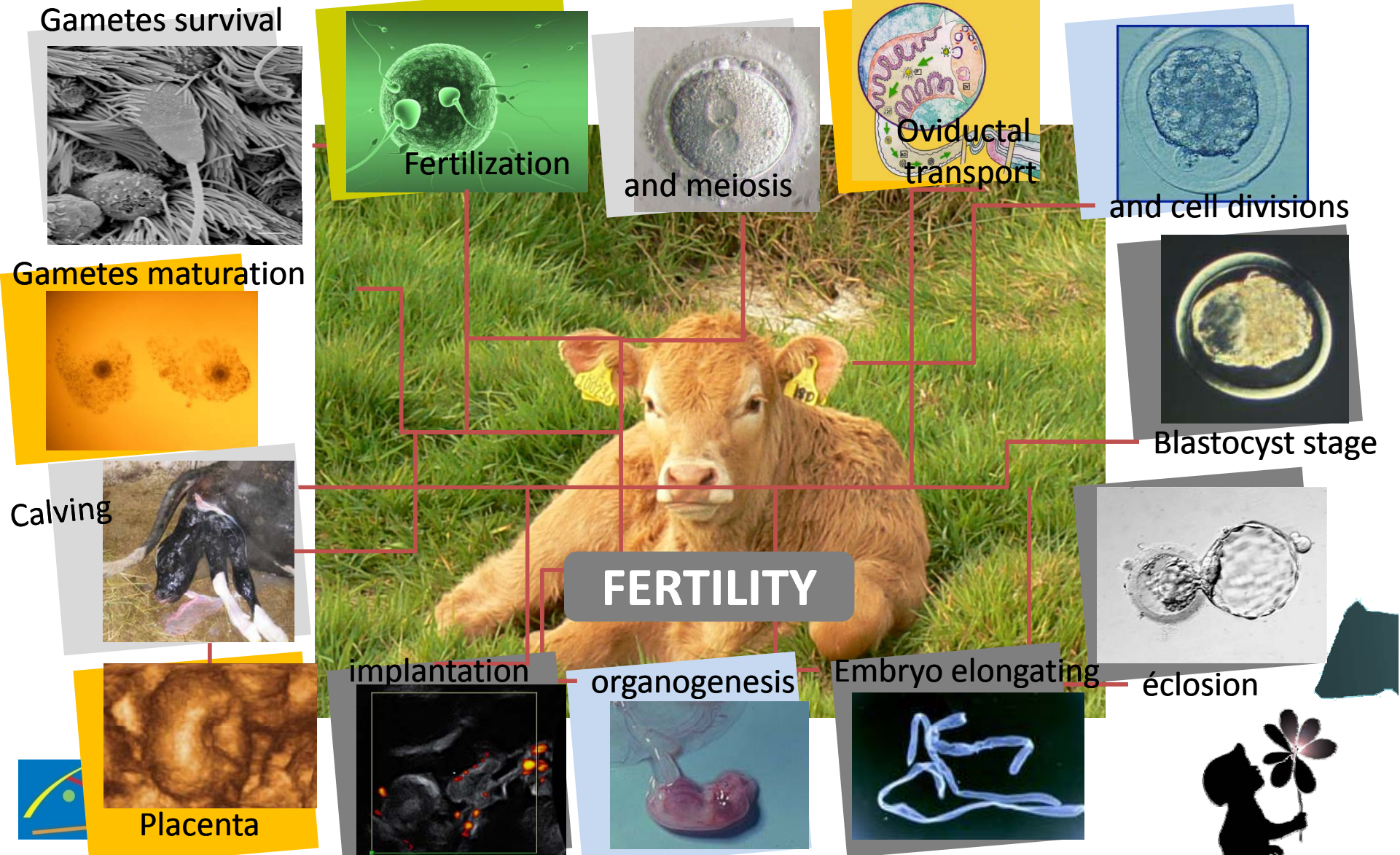


**3.INPUTS IN
GENETIC
SELECTION**

1.TIME-SPACE CONTINUUM



Reproduction function : a space-time continuum

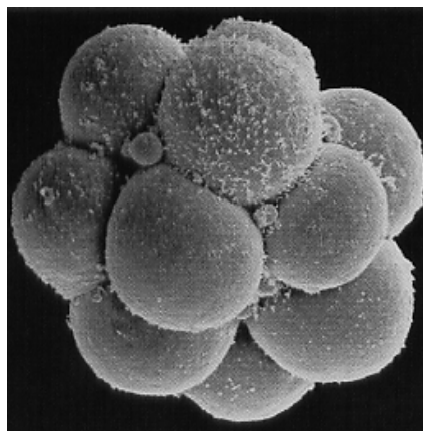
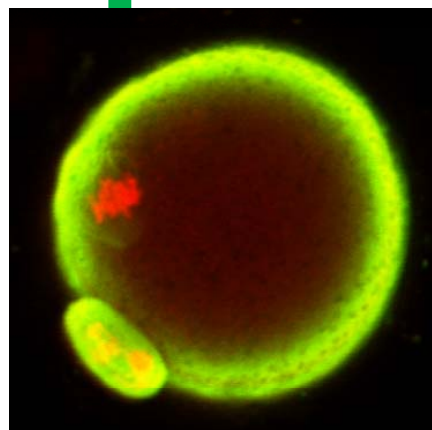
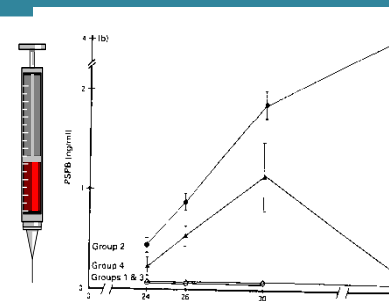


Phenotyping early embryo mortality : a black box remaining to be opened !

Breeding

Early : 75%
Milk Progesterone

Late: 25%
Proteins



Gamete quality

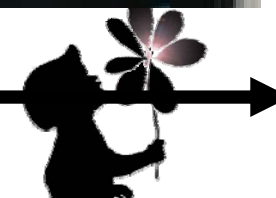


Implantation



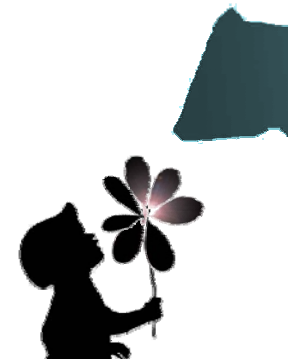
Time

Source HUMBLLOT



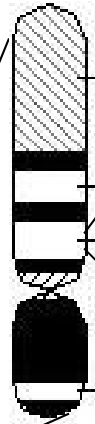
How may functional genomics help to characterise this time-space continuum ?

- New phenotypes refined
 - More precise : successive events can be identified / isolated
 - New phenotypes : « omics »
- Very powerful techniques : lots of information !





"OMICS"



ID /Position
GENOMICS

+

Gene Expression
TRANSCRIPTOMICS

+

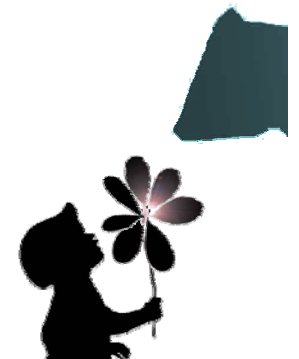
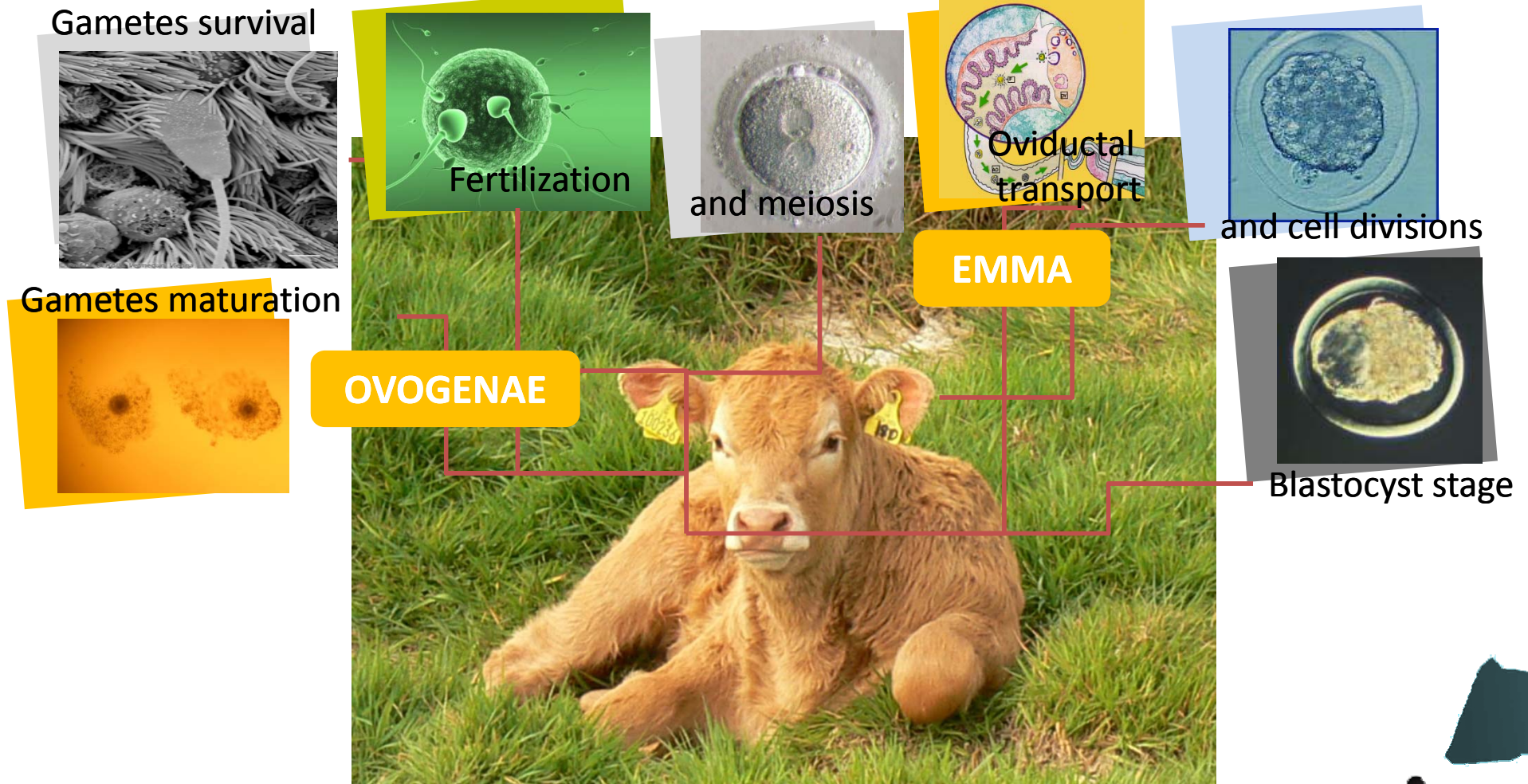
Proteins
PROTEOMICS

+

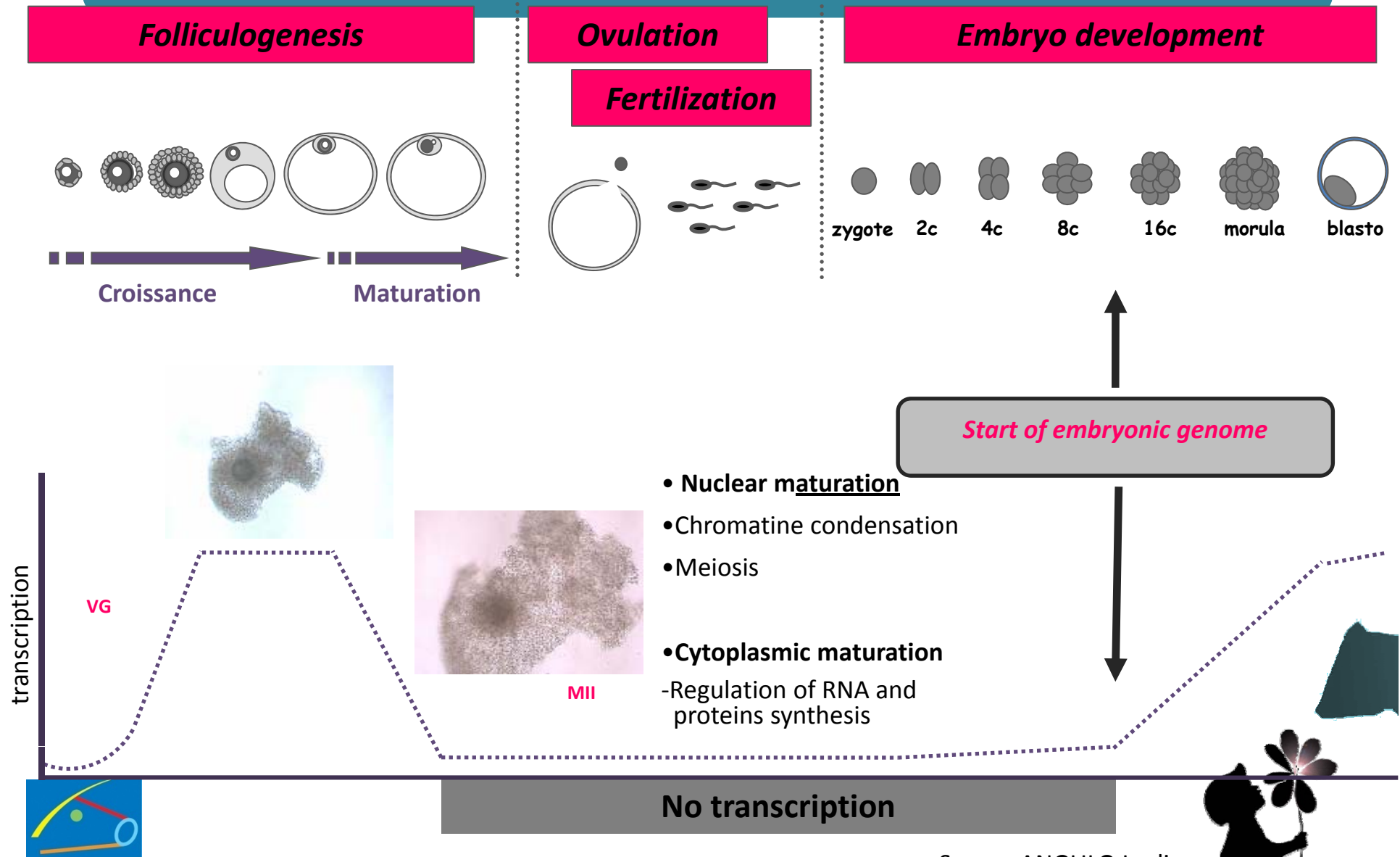
Small Molecules
METABOLOMICS

FUNCTION

Phenotyping early embryo mortality : focus on oocyte quality and early embryo development



Oocyte quality impacts embryo development



Source ANGULO Leslie

OVOGENAE : genes related to oocyte quality

(Rozenn Trans-Dalbies, INRA)

Gene expression profiles related to embryo development competence



Transcriptomics

Profiles compared in oocyte populations presenting contrasted development competence

Functional approach of candidate genes



Candidate genes

Functional approach of one gene specifically expressed in oocytes

Source ANGULO Leslie



Combining « omics » approaches and embryo related biotechnologies : a powerful model

Multiple Ovulation AI

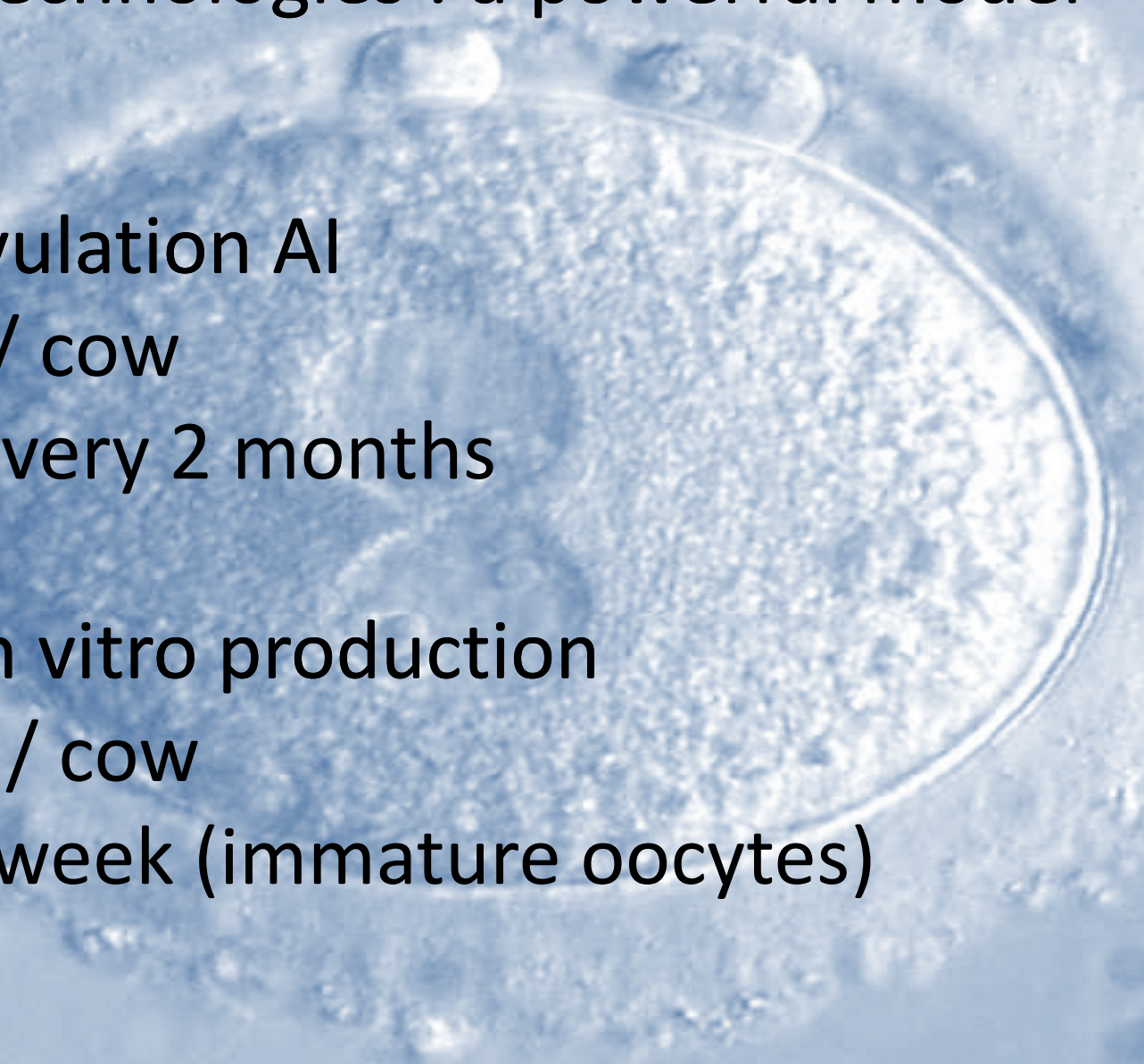
5 embryos / cow

1 flushing every 2 months

Repeated In vitro production

2 embryos / cow

1 session / week (immature oocytes)



Transcriptomic approach

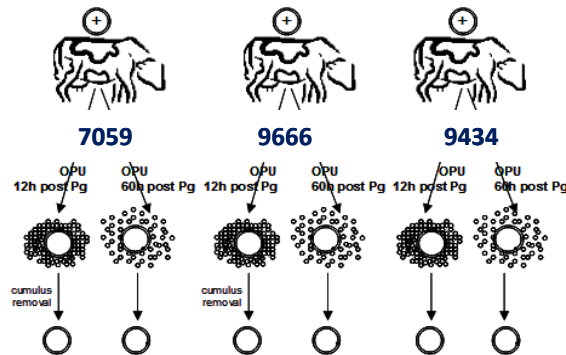
36% - 55%



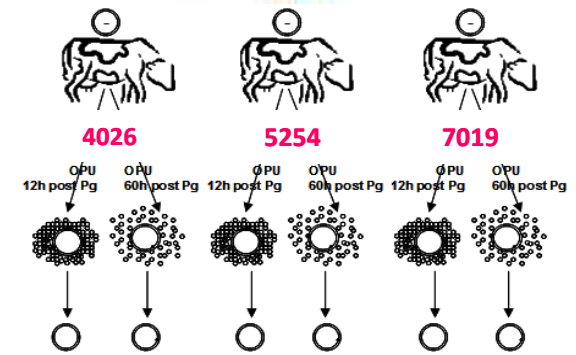
0% - 17%



Donor cows with contrasted embryo production (UNCEIA)



2 replicates ; 15 oocytes/stage/donor



RNA extraction

Amplification

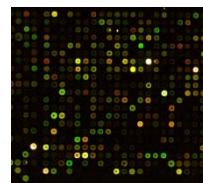
Cy 3
Cy 5

RT + P³³

ARNa ARNa

ADNc - P³³

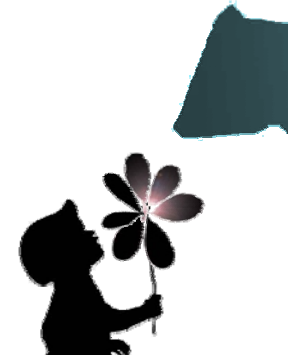
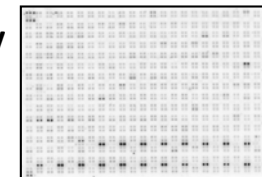
Source
ANGULO Leslie



Microarray 22k

+

Macroarray



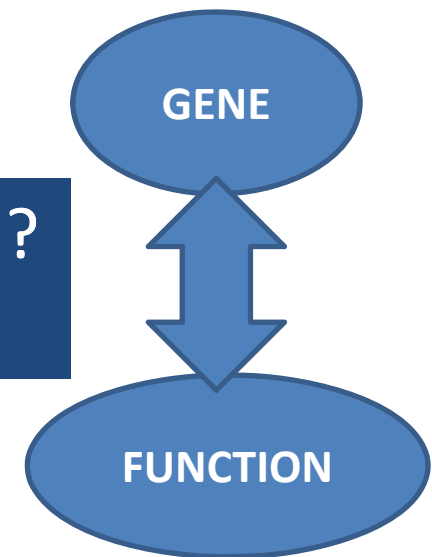
Transcriptomic approach

Mature oocytes

2212 spots / 2018 genes with different expression profiles

- 974 genes up-regulated in oocytes from donor cows with high development competence ($B < M$) :
- 1238 genes up-regulated in oocytes from donor cows with low development competence ($B > M$) :

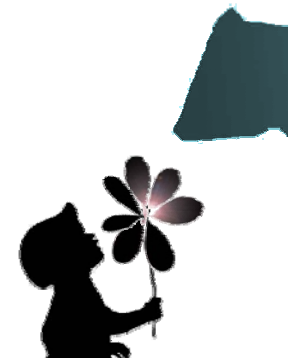
Implication in physiological pathways ?
Links with fertility QTLs ?



Oocyte stage	Genes in fertility QTLs	
	$B > M$	$B < M$
Immature	(108)	(123)
Mature	(180)	(149)

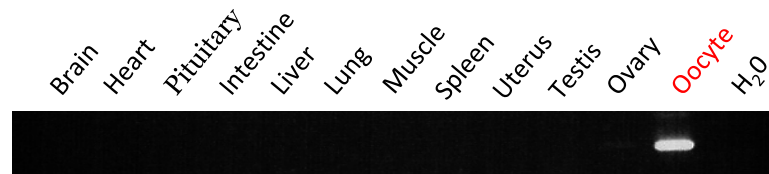


Source ANGULO Leslie

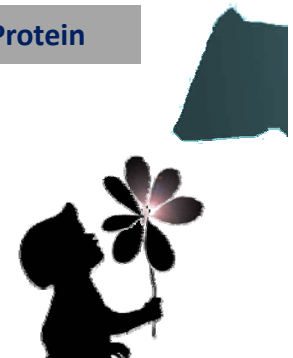
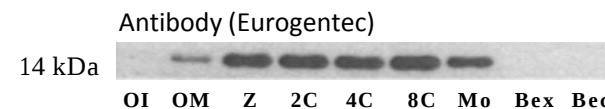
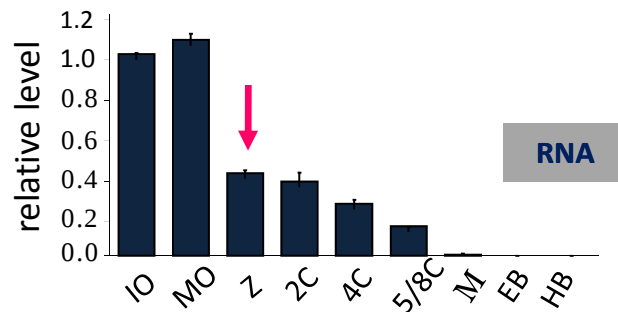


Functional approach : one gene specifically expressed in oocytes

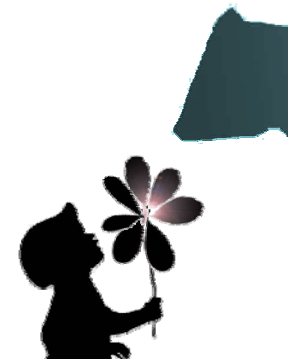
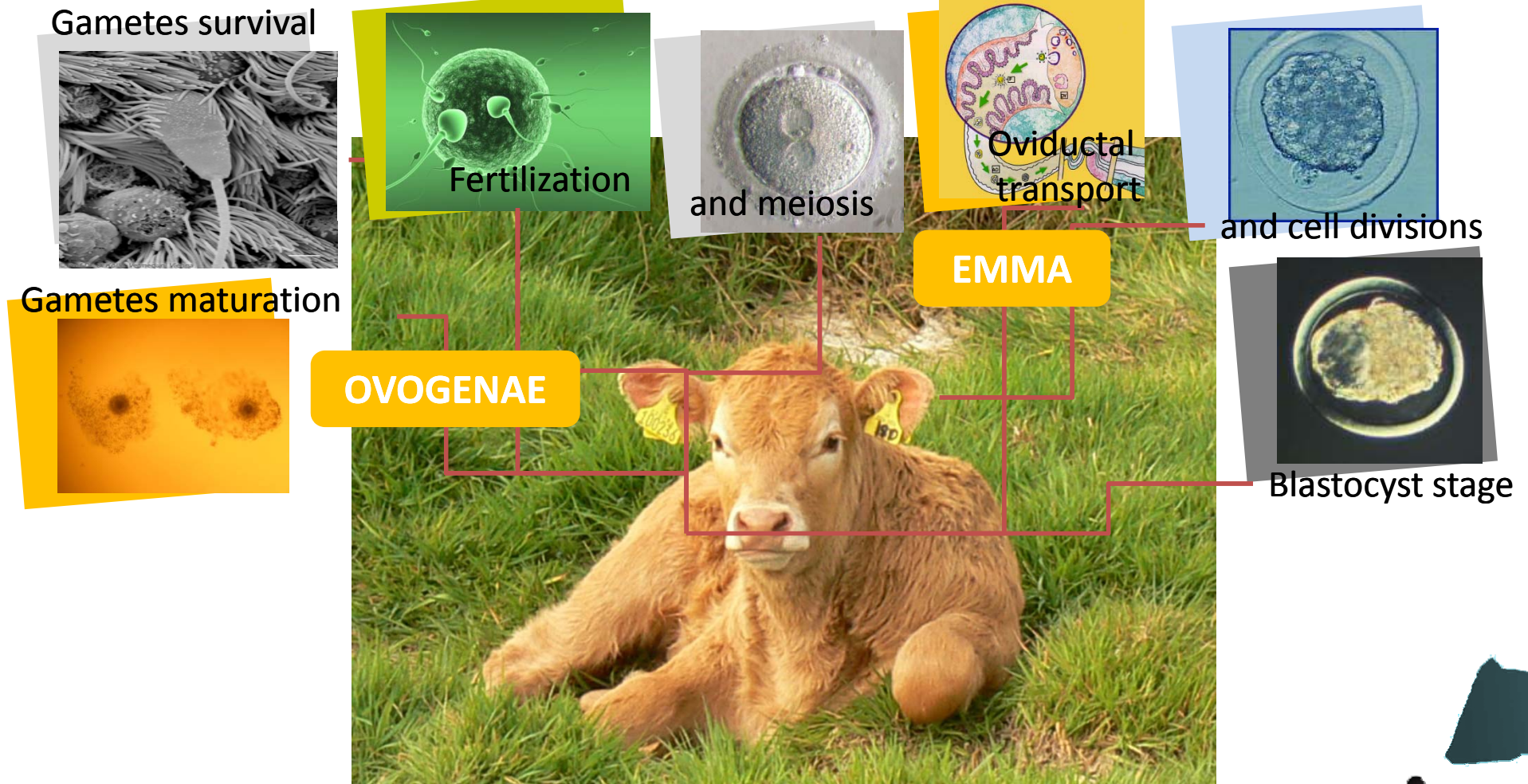
- Gene specifically expressed in oocytes



- Transcripts and proteins abundance investigated among early steps of development



Phenotyping early embryo mortality : focus on oocyte quality and early embryo development

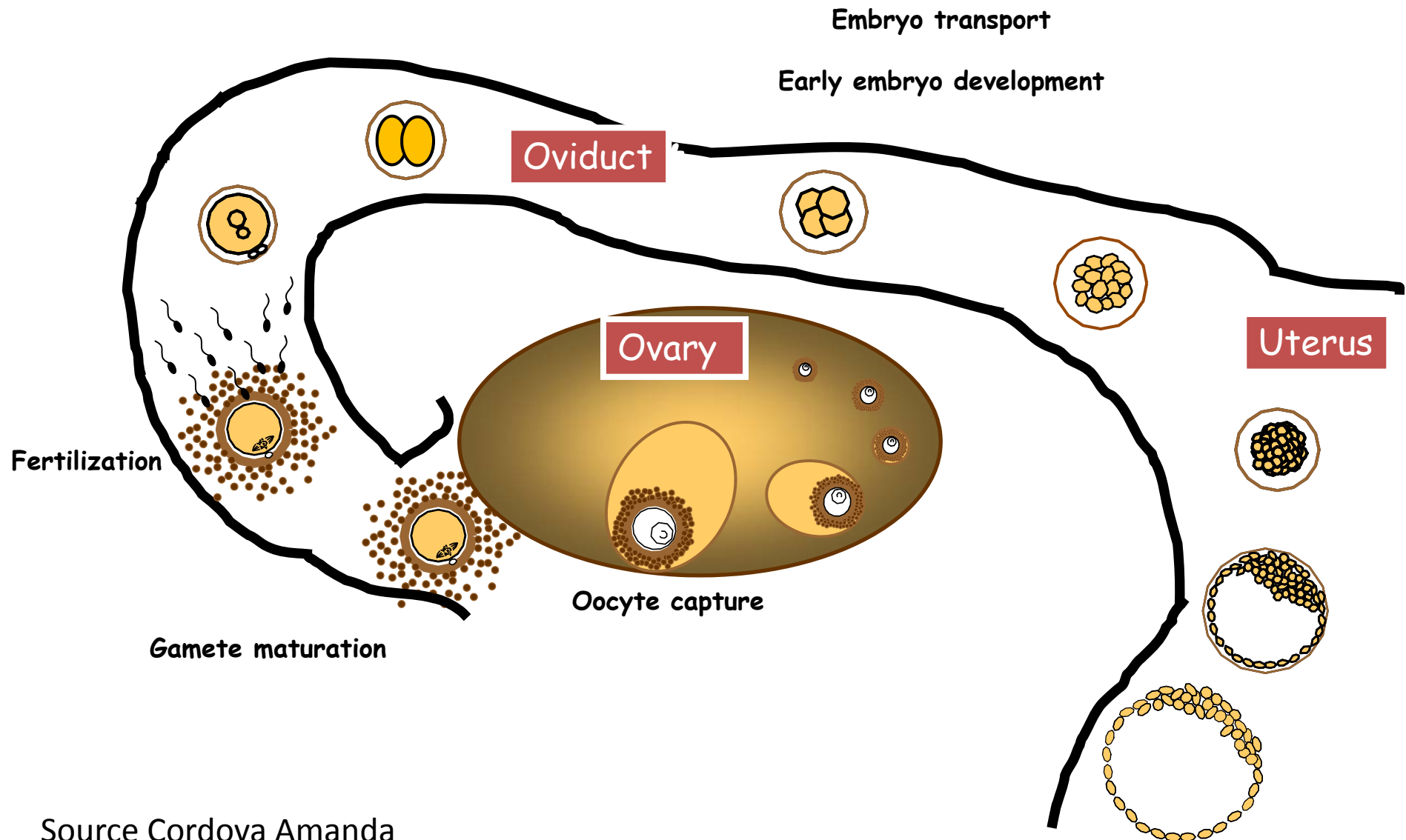


Transport in oviduct : genes related to embryo-maternal communication (P Mermillod, INRA PRC)

- Functional approach
 - Specific functions of the oviduct
 - Signals related to embryo-maternal communication
- Transcriptomics
 - Oviduct + embryo viability
- Proteomics
 - Investigation of proteins related to embryo-maternal communication



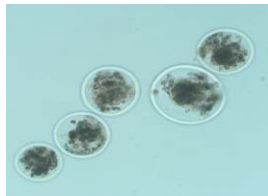
Oviduct : different successive events / functions in different oviduct parts



Source Cordova Amanda

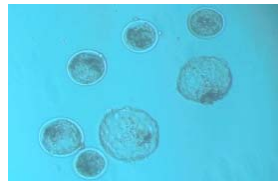
Transport in oviduct : genes related to embryo-maternal communication (P Mermillod, INRA PRC)

- Use of an original in vitro embryo culture system
- with BOEC (bovine oviduct epithelial cells)
- compared to classical culture system (SOF)

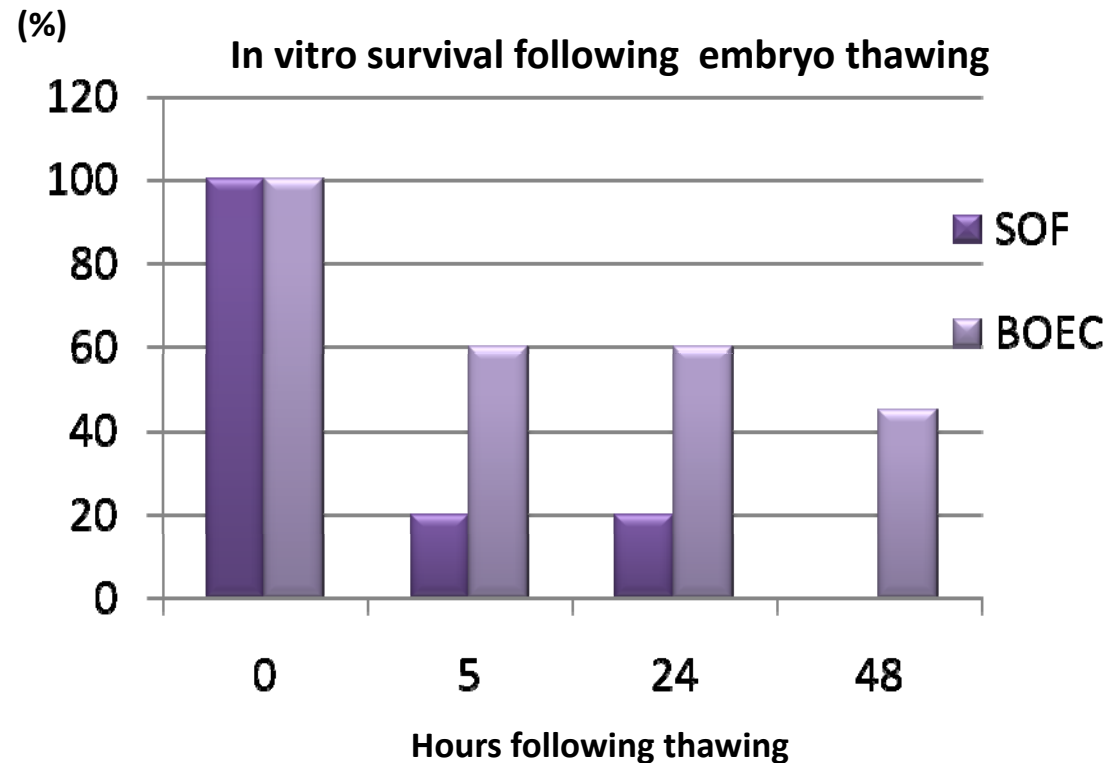


SOF

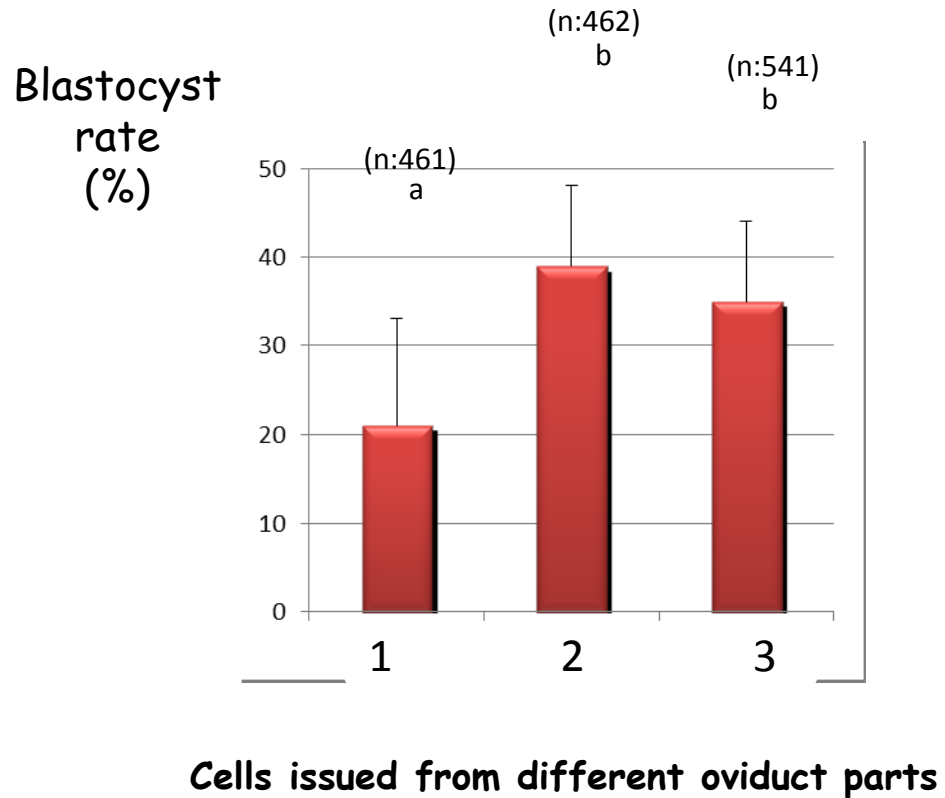
5 h following thawing



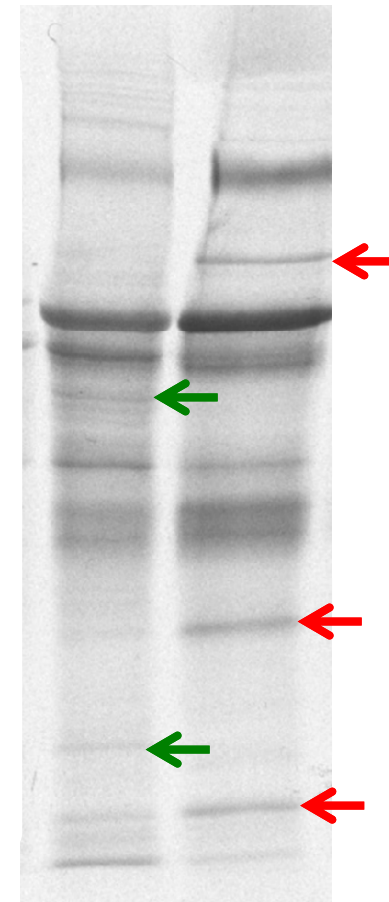
SOF + Boec



PROTEOMICS : different proteins are synthesised in specific parts of the oviduct



Source Cordova Amanda



Proteins synthesised using an *in vitro* BOEC culture system with cells issued from different oviduct parts

Clinics
Reproductive
Physiology

The diagram features a grayscale background image of a cluster of cells, likely an embryo. Overlaid on this is a conceptual diagram. At the top is a rounded rectangle containing the text 'Clinics', 'Reproductive', and 'Physiology'. Below this are two more rounded rectangles, one on the left labeled 'Genetics' and one on the right labeled 'Omics'. A double-headed blue arrow connects the 'Genetics' box to the 'Omics' box. Two additional double-headed blue arrows originate from the 'Omics' box, one pointing towards the 'Clinics' box and another pointing towards the 'Genetics' box, forming a triangular relationship.

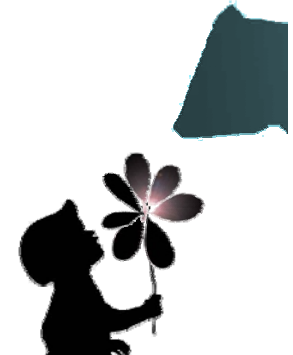
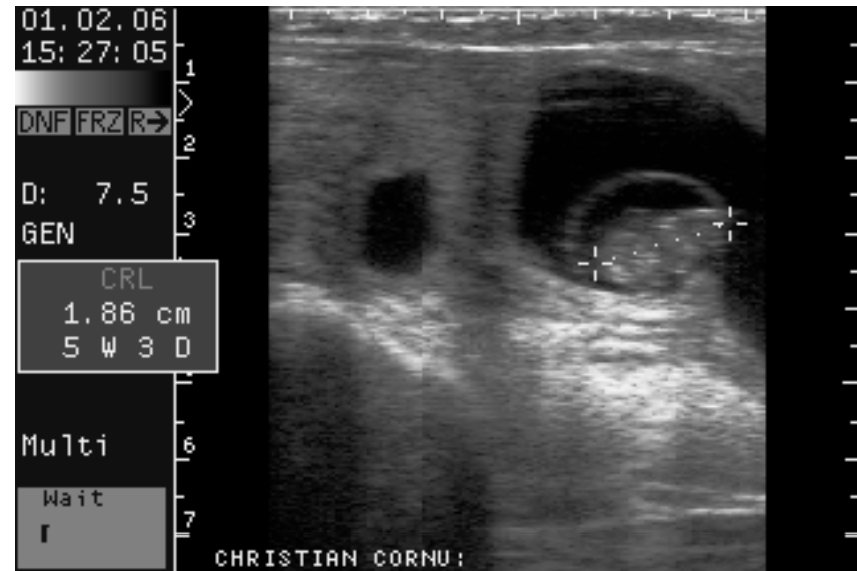
Genetics

Omics

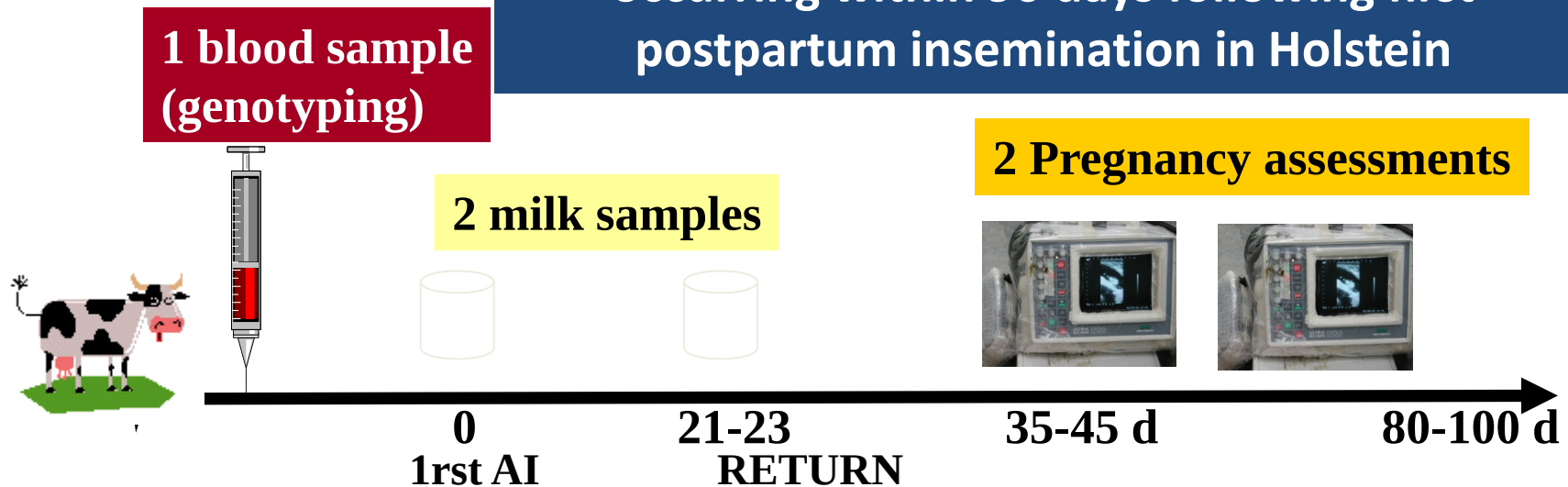
How « Omics » can help to improve
genetic selection for fertility ???

Genifer : Phenotyping pregnancy failure occurring within 90 days following first postpartum insemination in Holstein

- **3508 Holstein** (62% primiparous), 984 herds, 17 insemination centers
- 12 sires pooled in three **FI** groups, **low**: [-0.7; -0.5]; **average**: [-0.1;+0.3]; **high**: [0.5;+1.0]
- **Phenotypes of pregnancy failure between first AI (D0) and D90 (failure):**
- Environment and milk production data recorded

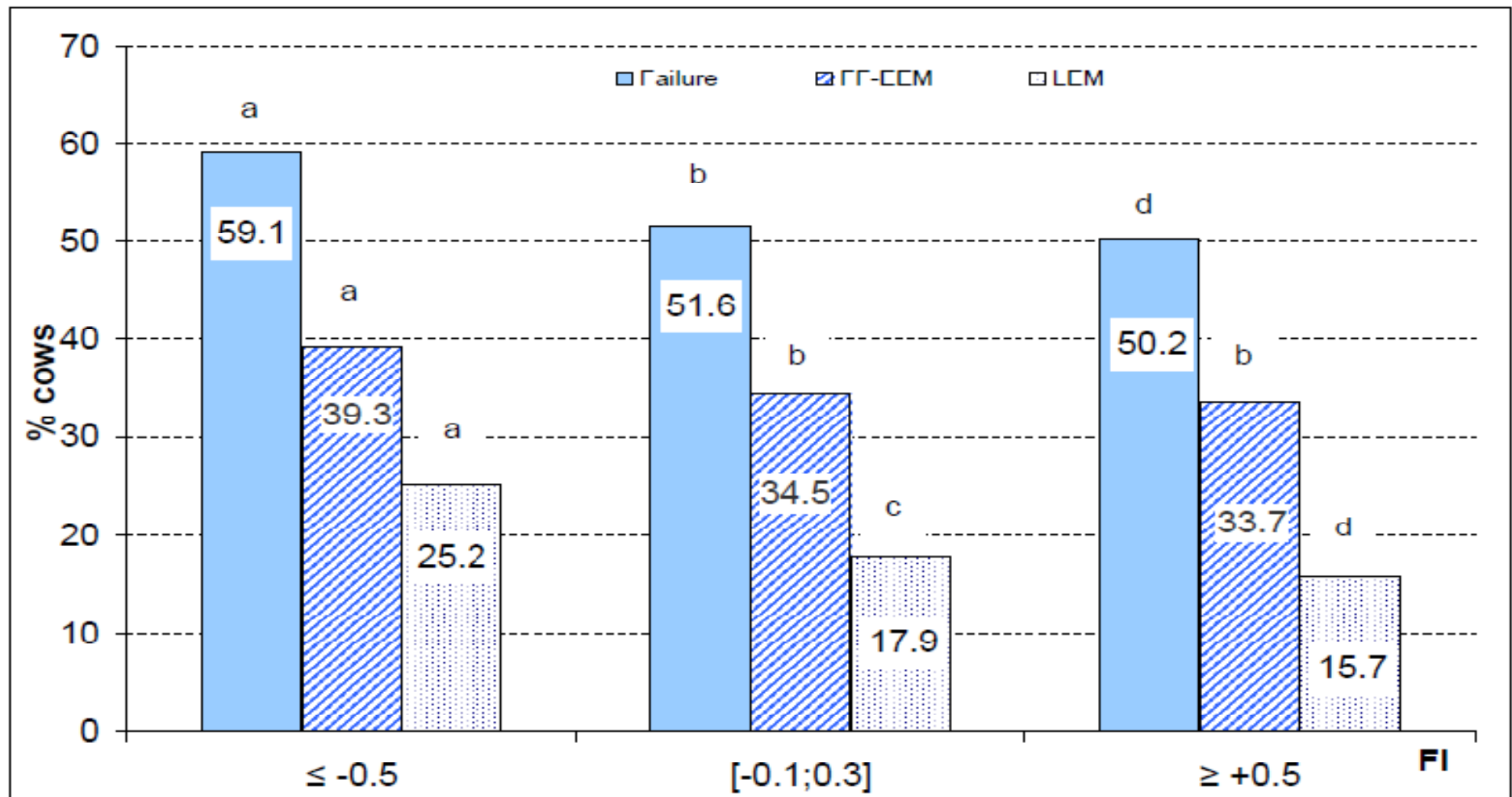


Genifer : Phenotyping pregnancy failure occurring within 90 days following first postpartum insemination in Holstein



AI during luteal phase	6,3 %	-	-	-	-
Early emb. death	35,4 %	-	-	Regular	-
Late emb. death	16,7 %	-	+	delayed	Not pregnant
Foetal death	3,6 %	-	+	delayed	Pregnant
Pregnancy	37 %	-	+	No return	Pregnant

Estimated incidence of pregnancy failure between D0 and D90, a vs b: $p \leq 0.05$; a vs c: $p \leq 0.01$; a vs d: $p \leq 0.001$



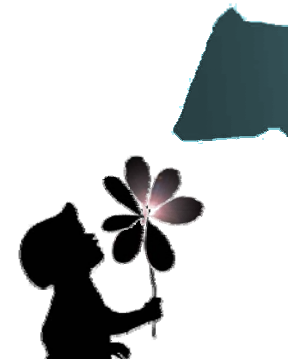
Source Ledoux 2011

Impacts on genomic selection

- 7 traits (type of pregnancy failure) analysed in 2669 females with 306 SNPs (from 13 chromosomes).
- Fertility QTL on chromosome 3 more precisely located
- Effects of QTLs confirmed / isolated
 - 6 on calving rate
 - 3 on EEM, 3 on LEM, 8 on global EM,
 - 2 on FM
 - 3 on abortions

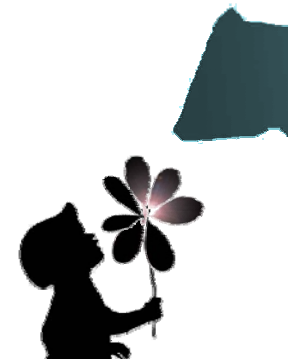


Source : Lefevre, 3R 2011



Conclusion

- Use of « omics » : lots of positive interactions with genomic selection
 - Refining new phenotypes : blood markers, genes, proteins...
 - Better evaluation of performances → reliability, genetic improvement
- More direct applications : prevention, treatment (for fertility ?)



Thanks !

- INRA PRC, Nouzilly
 - Rozenn Dalbiès-Tran, Xavier Druart, Joelle Dupont, Pascal Mermillod
- INRA BDR, Jouy
 - ,Véronique Duranthon, Isabelle Hue, Hélène Jammes, Svetlana Uzbekova, Fabienne Nuttinck, Gilles Charpigny, Olivier Sandra
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- INRA GABI, Jouy
 - Didier Boichard, Mekki Boussaha, Rachel Lefevre
- ENVA
 - Bénédicte Grimard, Dorothee Ledoux
- UNCEIA
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 - Catherine Joly, Brigitte Leguienne, Pascal Salvetti
 - Patrice Humblot (SLU)

