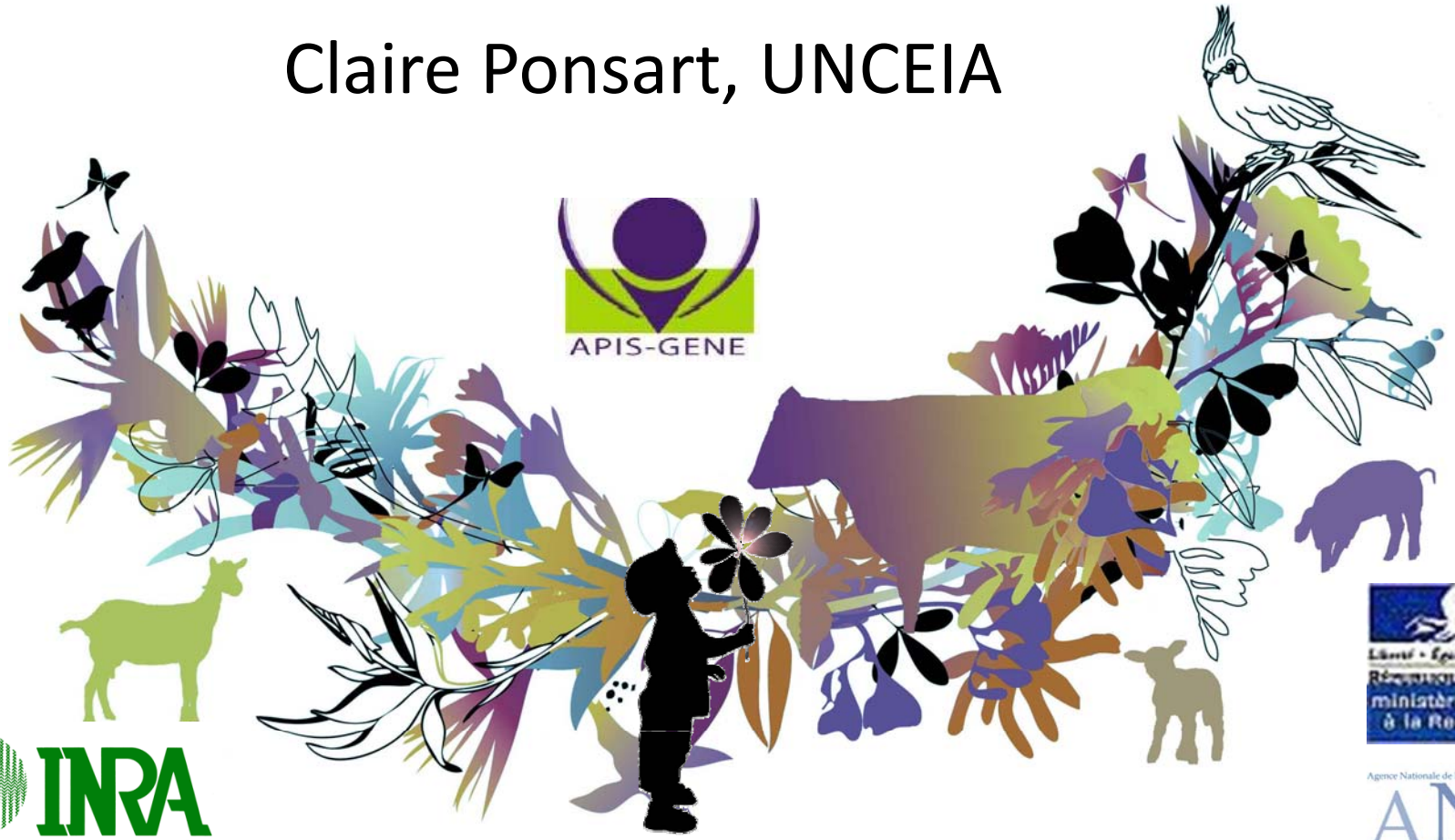
Phenotyping the reproduction function in cattle: inputs from functional genomics

Claire Ponsart, UNCEIA



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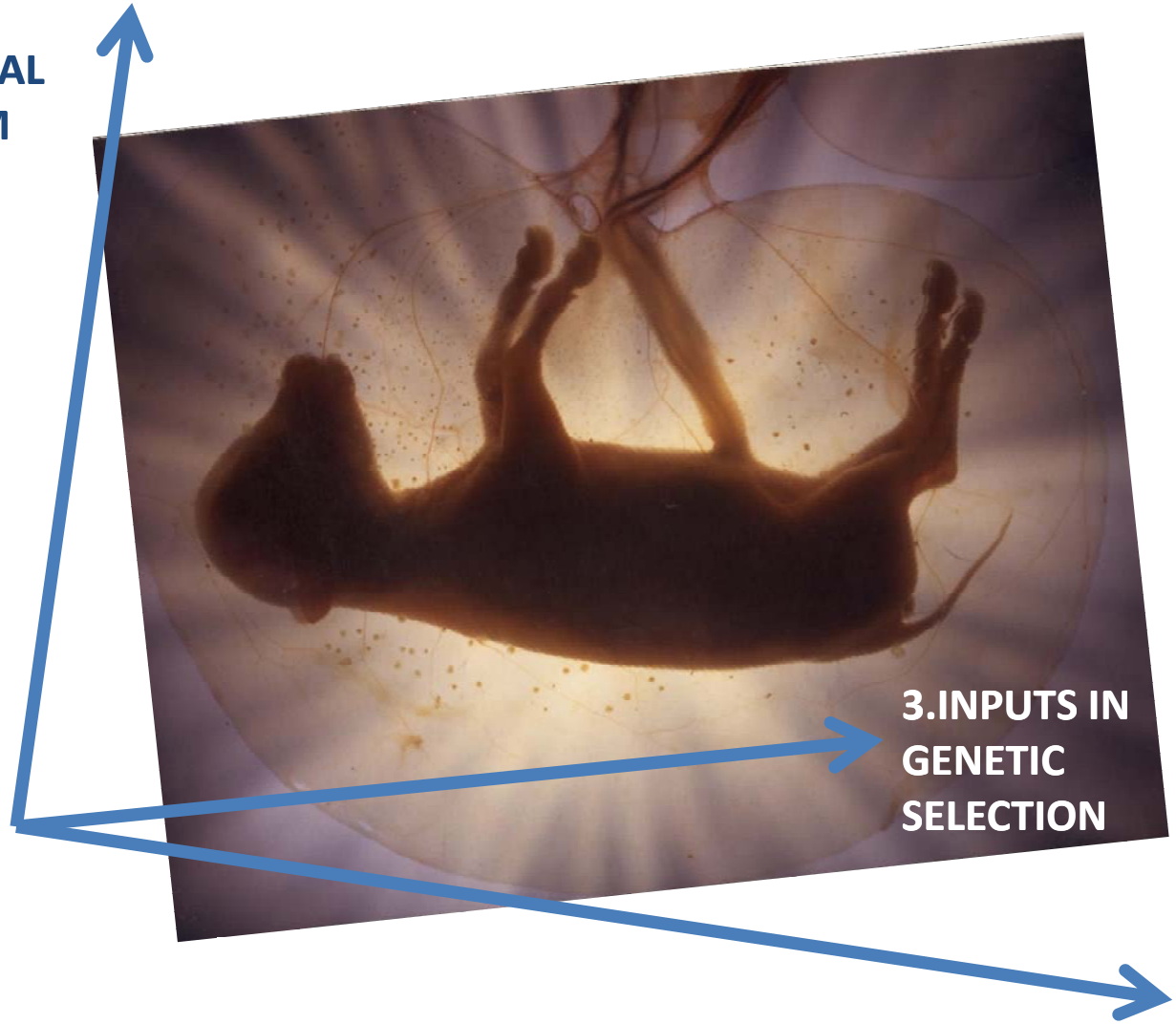
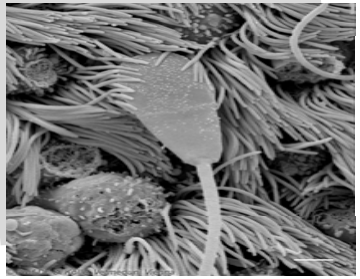
Agence Nationale de la Recherche
ANR

Phenotyping the reproduction function : a multidimensional scale



Reproduction function : a space-time continuum

Gametes survival



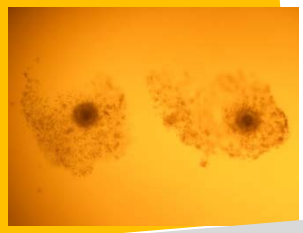
Fertilization

and meiosis

Oviductal transport

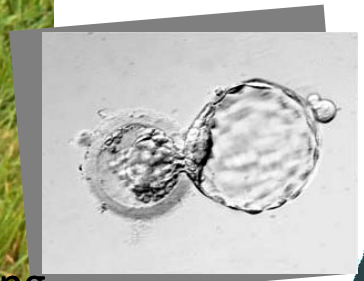
and cell divisions

Gametes maturation



Blastocyst stage

Calving

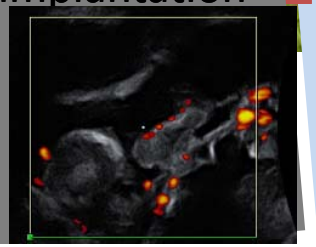


éclosion

FERTILITY

Placenta

implantation



organogenesis

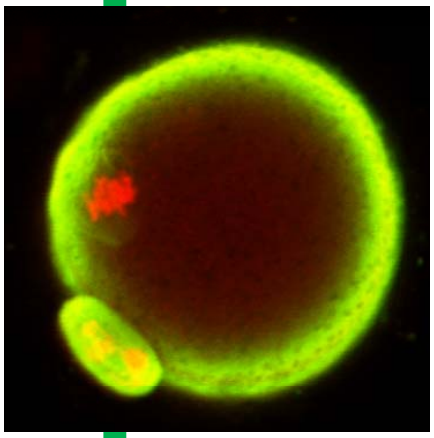
Embryo elongating

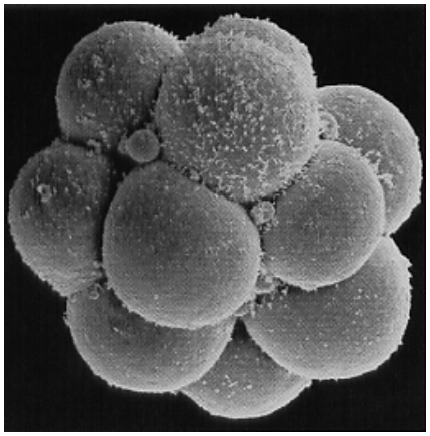
Phenotyping early embryo mortality : a black box remaining to be opened !

Breeding

Early : 75%

Milk Progesterone

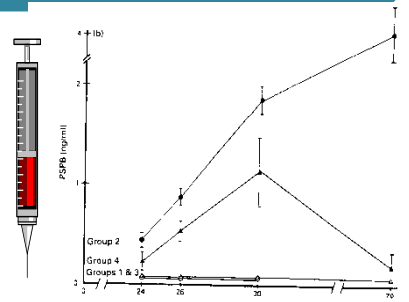




Gamete quality

Late: 25%

Proteins



Implantation

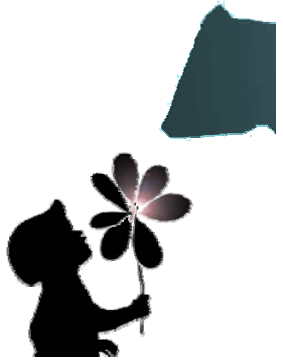
Time

Source HUMBLOT

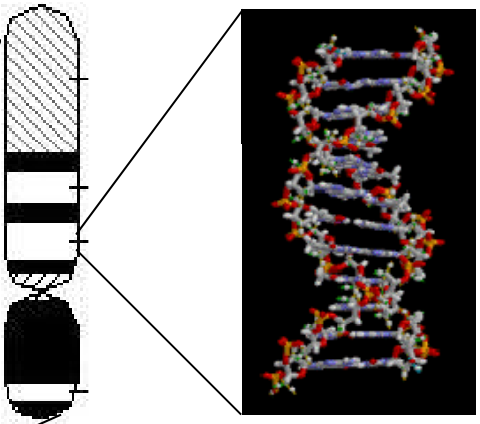
How may functional genomics help to characterise this time-space continuum ?

- New phenotyes refined
  - More precise : successive events can be identified / isolated
  - New phenotypes : « omics »

- Very powerful techniques : lots of information !

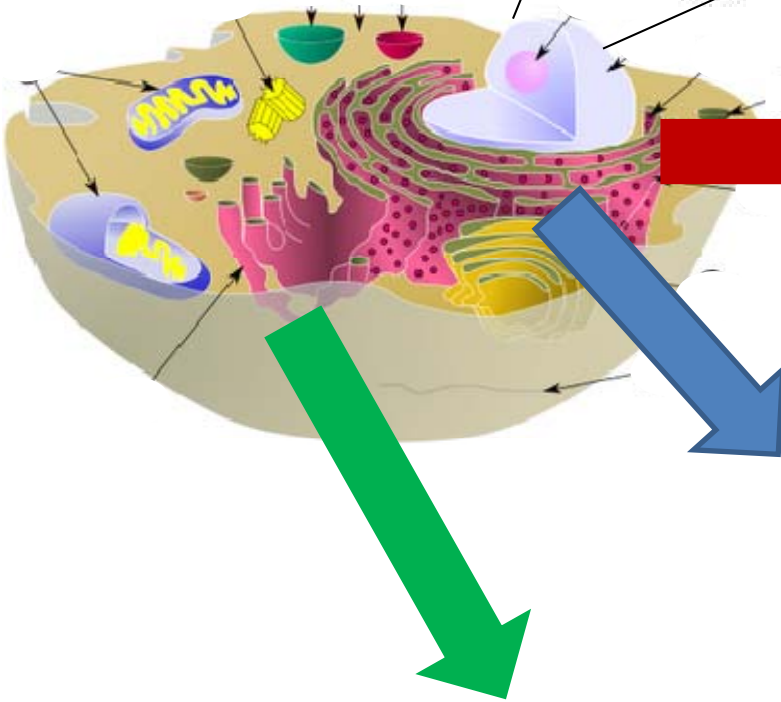


”OMICS”



ID /Position
GENOMICS

+



Gene Expression
TRANSCRIPTOMICS

+

Proteins
PROTEOMICS

+

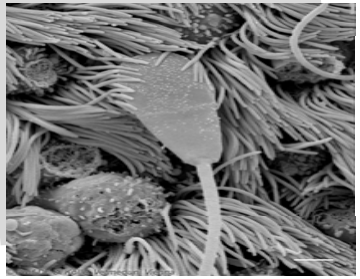
Small Molecules
METABOLOMICS

FUNCTION

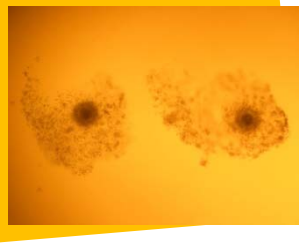
Source HUMBLOT

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

Gametes survival



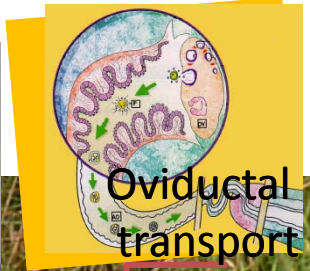
Gametes maturation



OVOGENAE

Fertilization

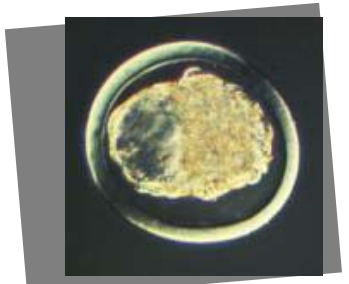
and meiosis



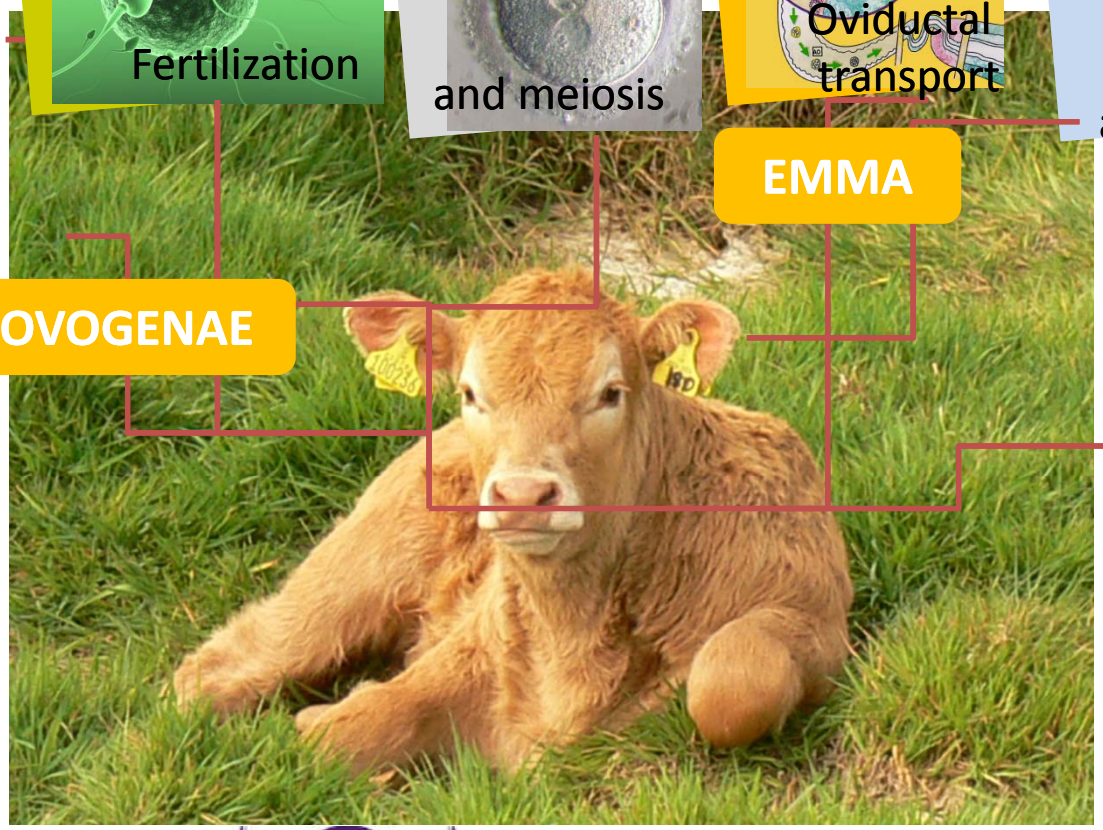
Oviductal transport

EMMA

and cell divisions



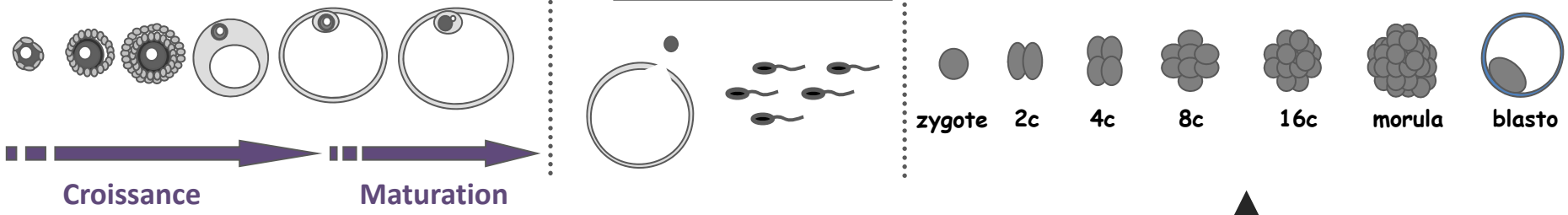
Blastocyst stage



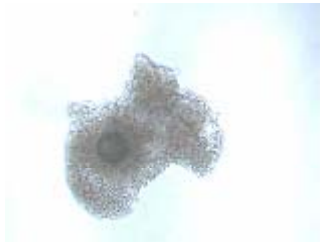
Oocyte quality impacts embryo development

Folliculogenesis | ***Ovulation*** | ***Embryo development***

Fertilization



Start of embryonic genome



MII

- **Nuclear maturation**
- Chromatine condensation
- Meiosis

- **Cytoplasmic maturation**

-Regulation of RNA and proteins synthesis

Source ANGULO Leslie

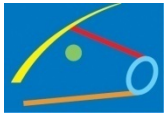
OVOGENAE : genes related to oocye quality

(Rozenn Trans-Dalbies, INRA)

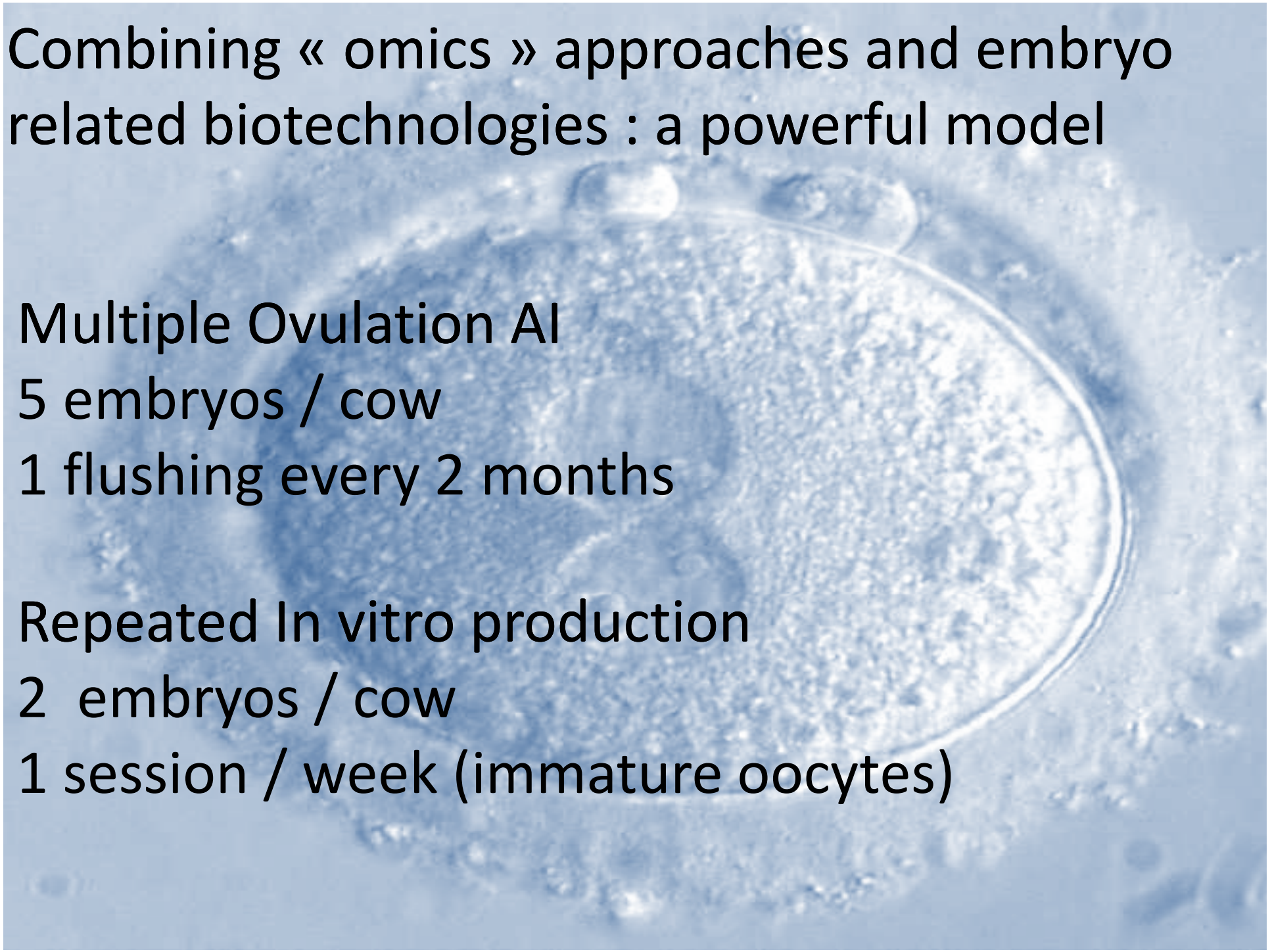
Gene expression profiles related to embryo development competence → **Transcriptomics**: Profiles compared in oocyte populations presenting contrasted development competence

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



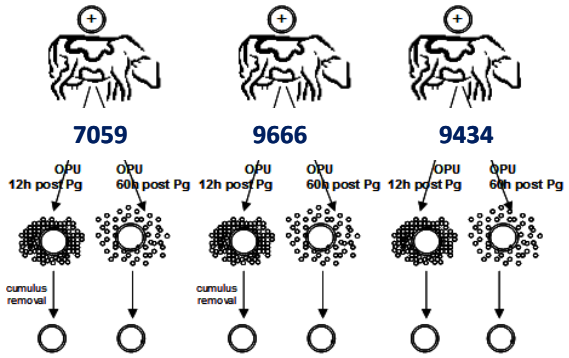
Multiple Ovulation AI
5 embryos / cow
1 flushing every 2 months

Repeated In vitro production
2 embryos / cow
1 session / week (immature oocytes)

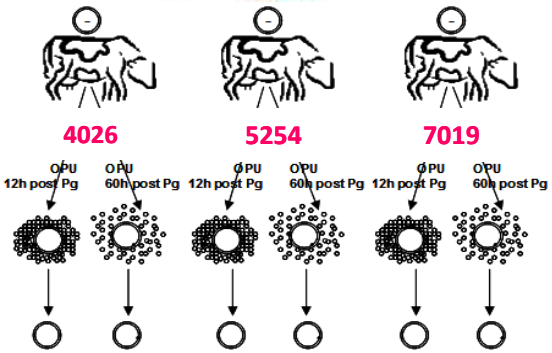
Transcriptomic approach

36% - 55%

0 % - 17 %



Donor cows with contrasted embryo production (UNCEIA)



2 replicates ; 15 oocytes/stage/donor

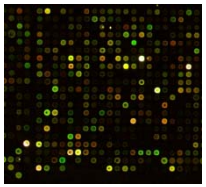
RNA extraction

Amplification

Cy 3
Cy 5

RT + P^{33}

ARNa **ARNa**

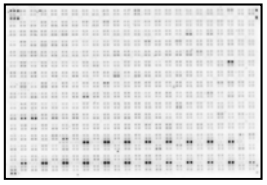


Microarray 22k

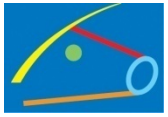
+

Macroarray

ADNc - P^{33}



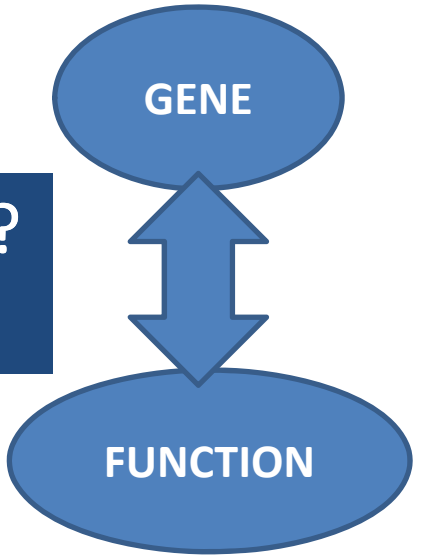
Source
ANGULO Leslie



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) :**



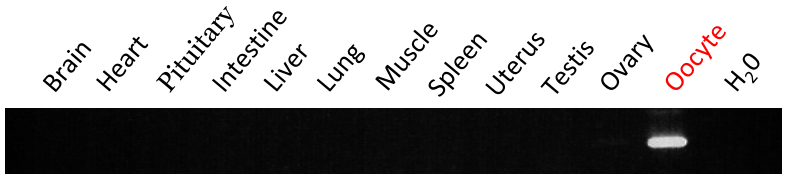
Implication in physiological pathways ?
Links with fertility QTLs ?

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

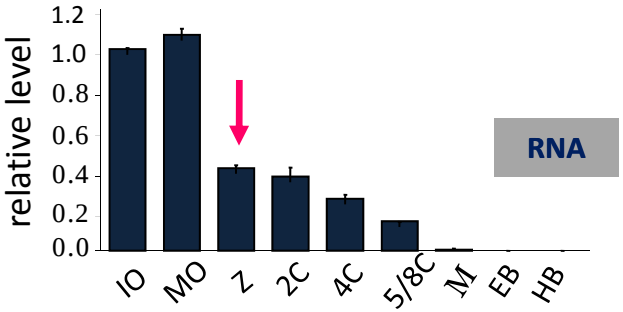
Source ANGULO Leslie

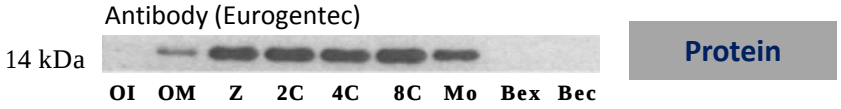
Functional approach : one gene specifically expressed in oocytes

- Gene specifically expressed in oocytes



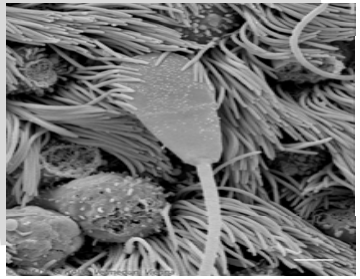
- Transcripts and proteins abundance investigated among early steps of development



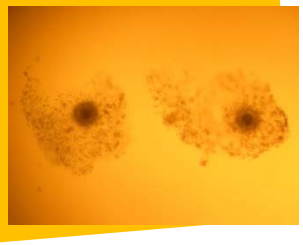


Phenotyping early embryo mortality : focus on oocyte quality and early embryo development

Gametes survival



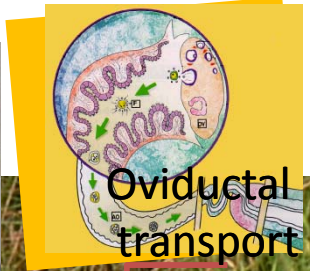
Gametes maturation



OVOGENAE

Fertilization

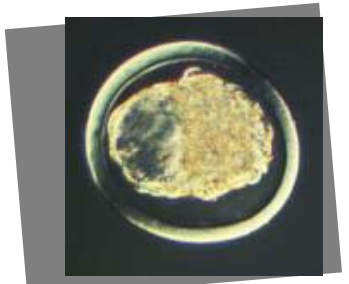
and meiosis



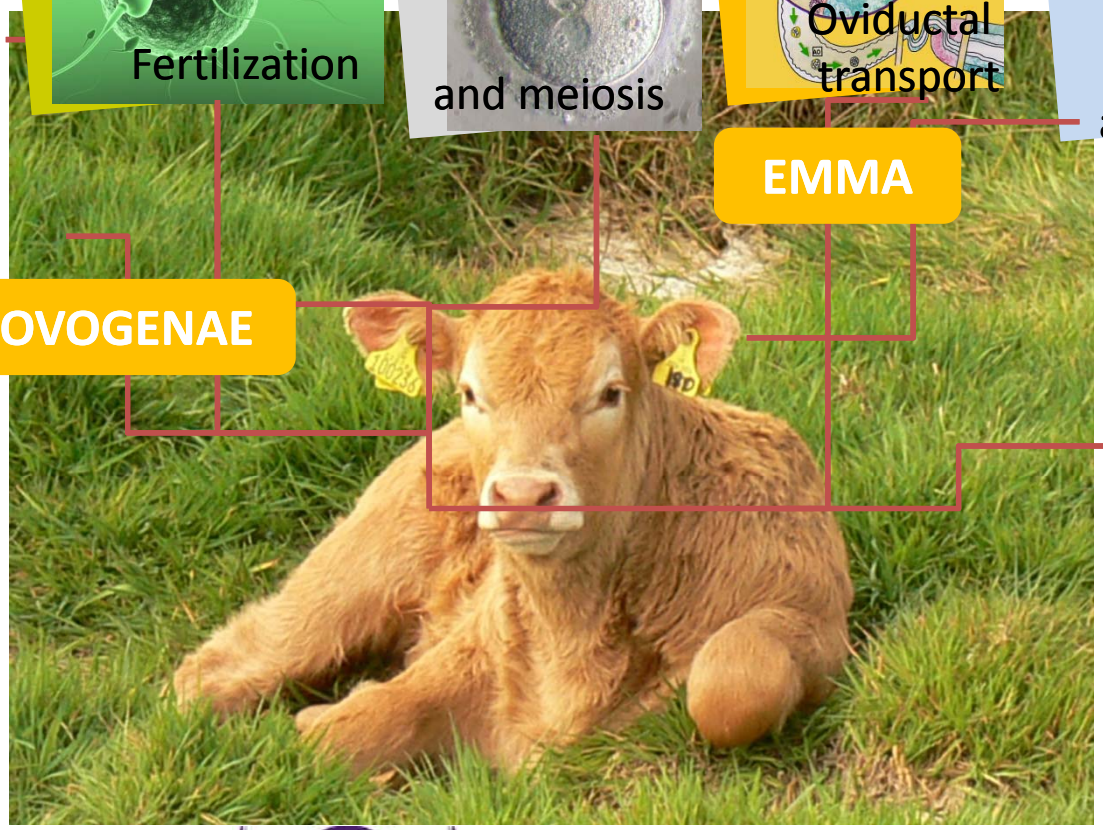
Oviductal transport

EMMA

and cell divisions



Blastocyst stage

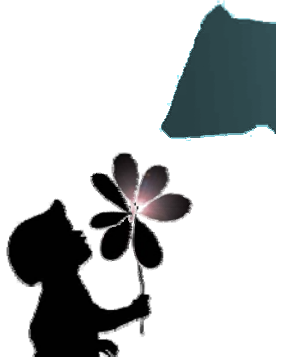


Transport in oviduct : genes related to embryo-maternal communication (P Mermillod, INRA PRC)

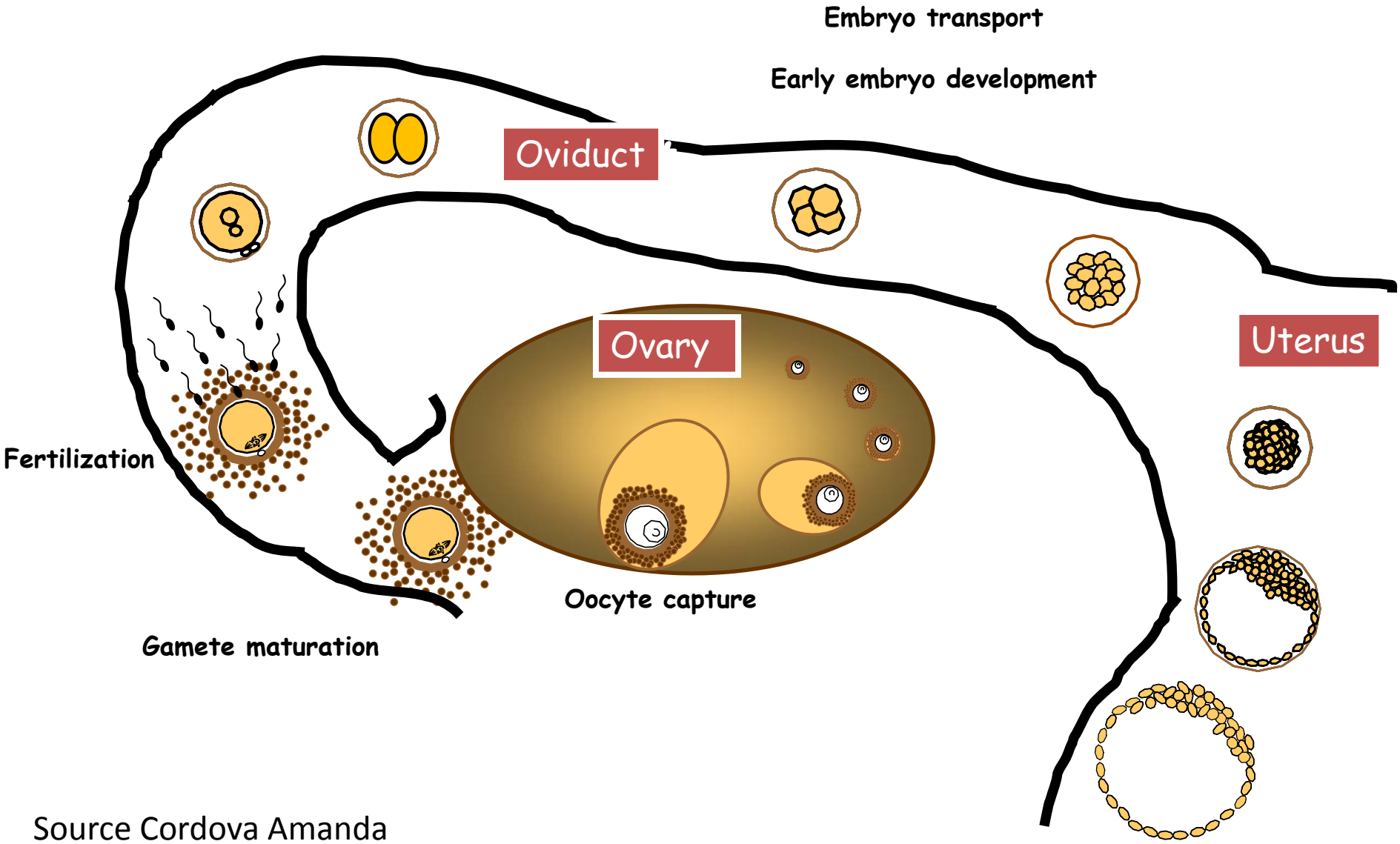
- Functional approach
  - Specific functions of the oviduct
  - Signals related to embryo-maternal communication
- Transcriptomics
  - Oviduct + embryo viability
- Proteomics
  - Investigation of proteins related to embryo-maternal communication

Institut National de la Recherche Agronomique

APIS-GENE



Oviduct : different successive events / functions in different oviduct parts

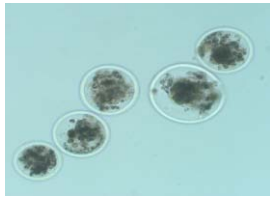


Source Cordova Amanda

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

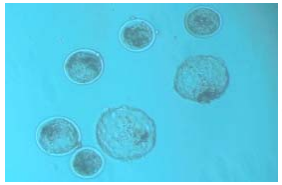
- Use of an original in vitro embryo culture system

- with BOEC (bovine oviduct epithelial cells)
- compared to classical culture system (SOF)

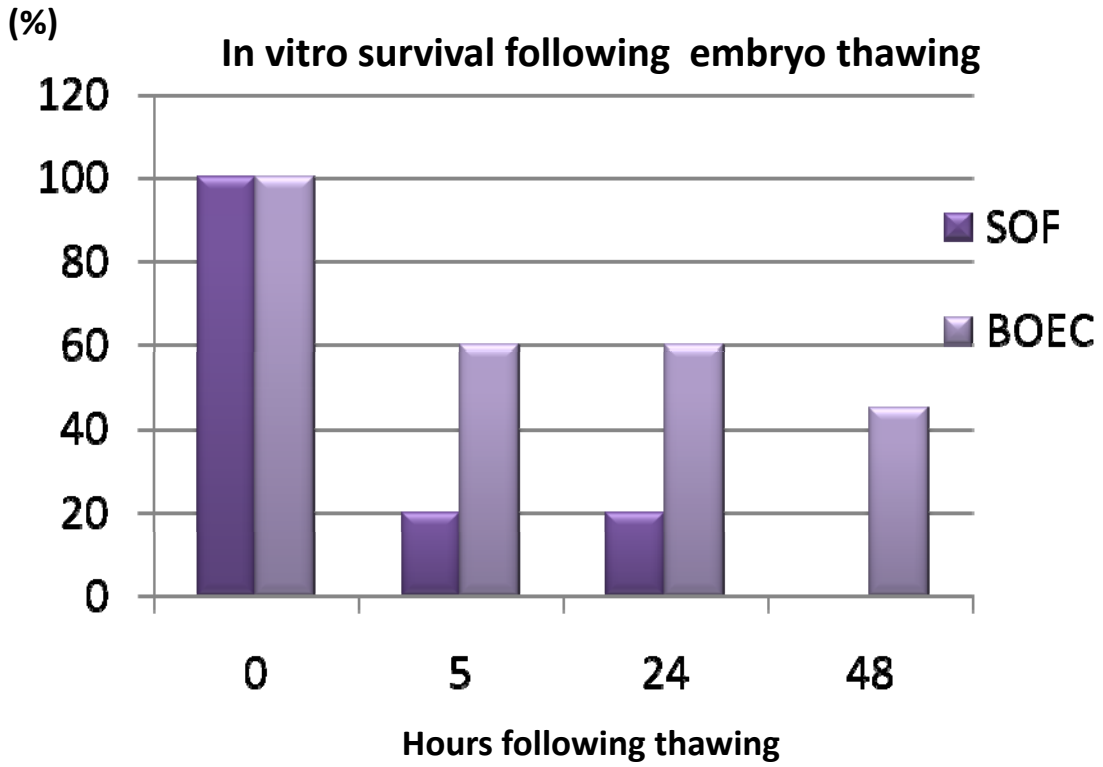


SOF

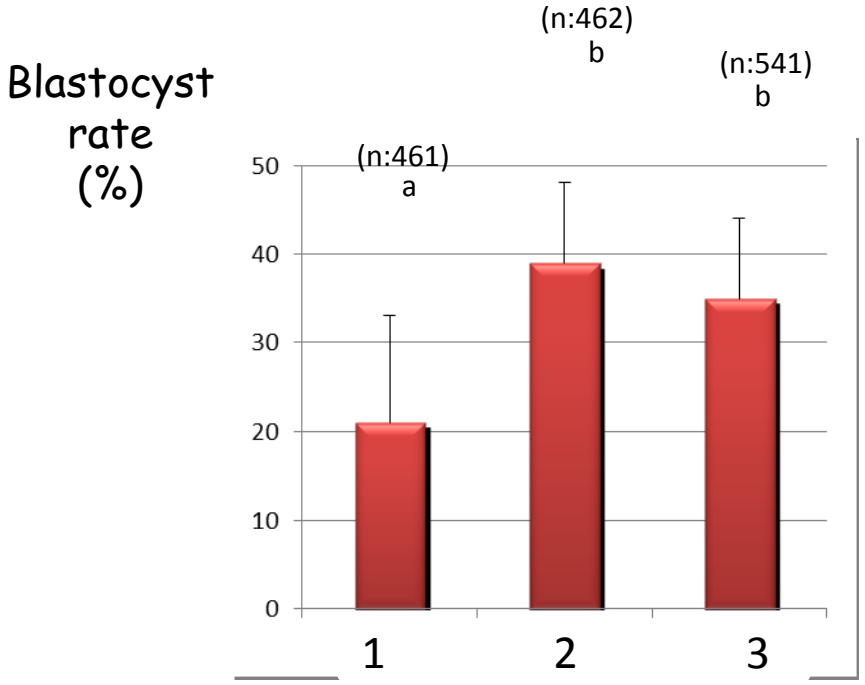
5 h following thawing



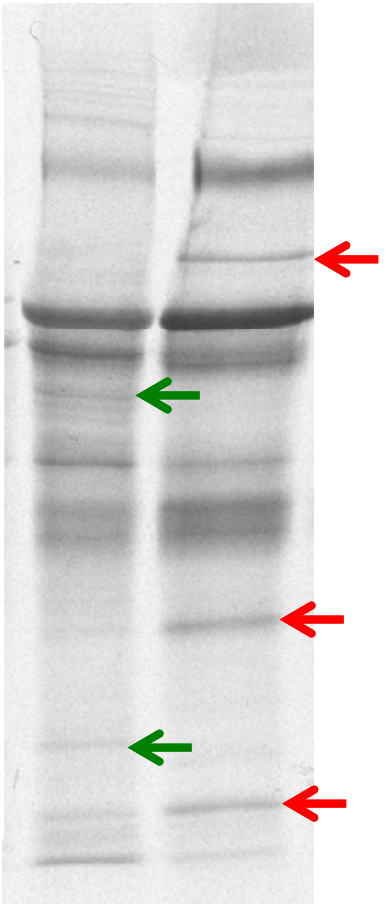
SOF + Boec



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

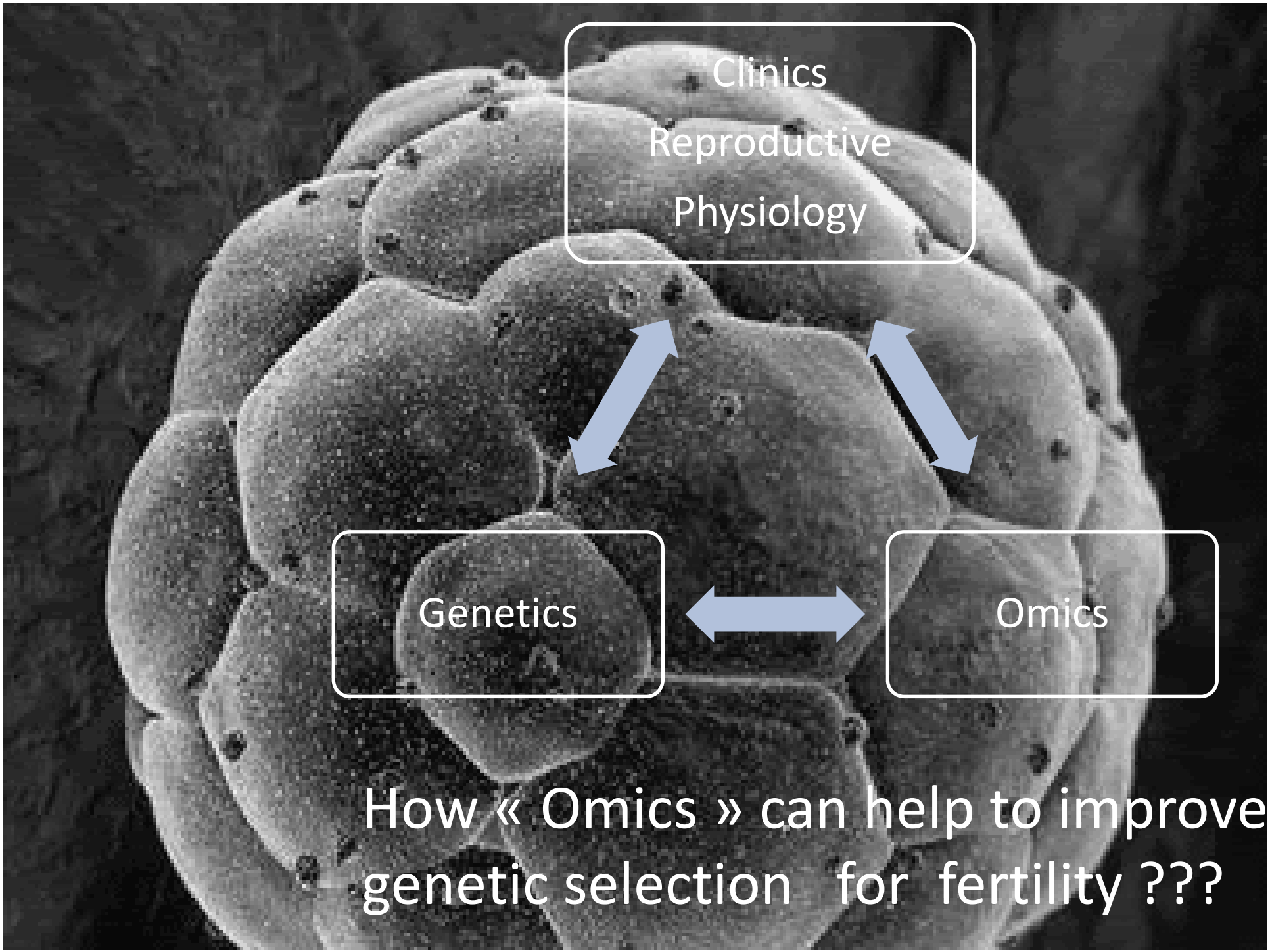


Cells issued from different oviduct parts



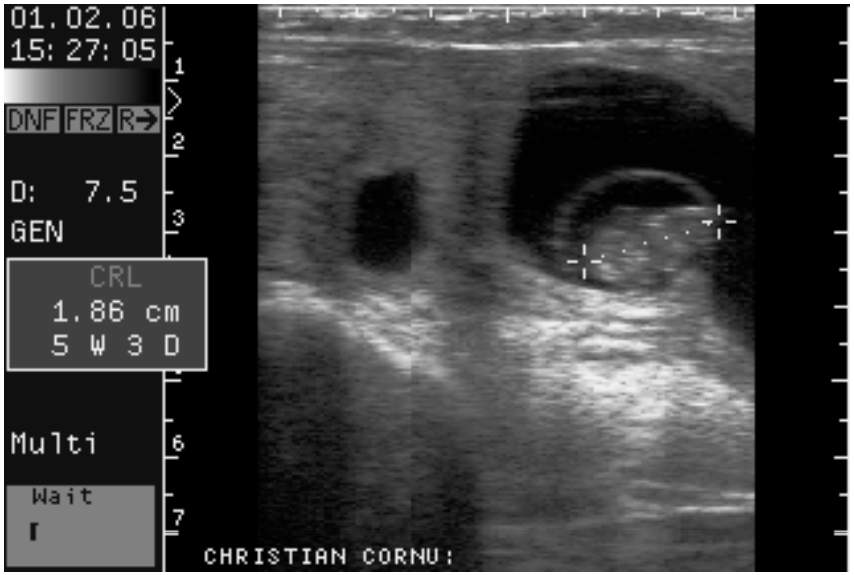
Proteins synthetised using an *in vitro* BOEC culture system with cells issued from different oviduct parts

Source Cordova Amanda


Clinics
Reproductive
Physiology
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



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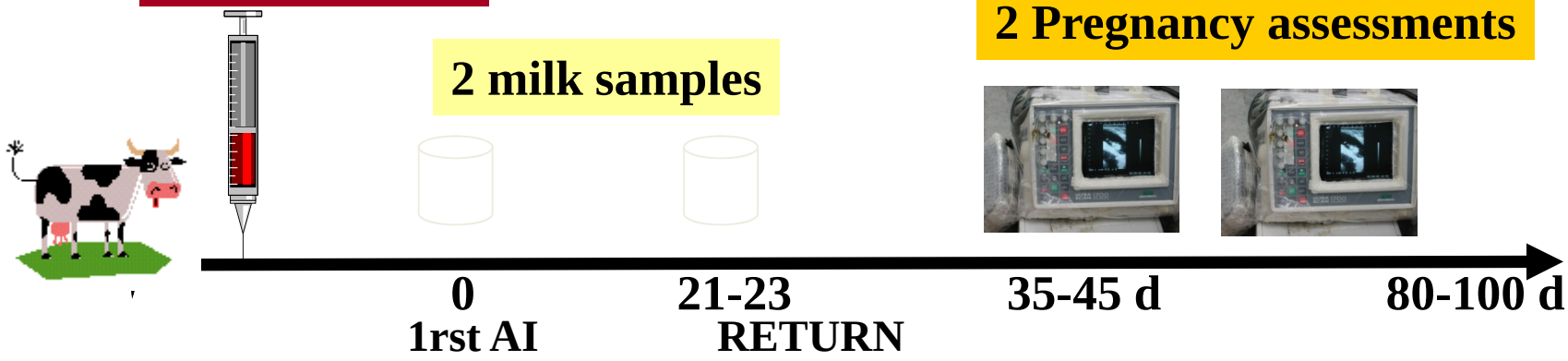
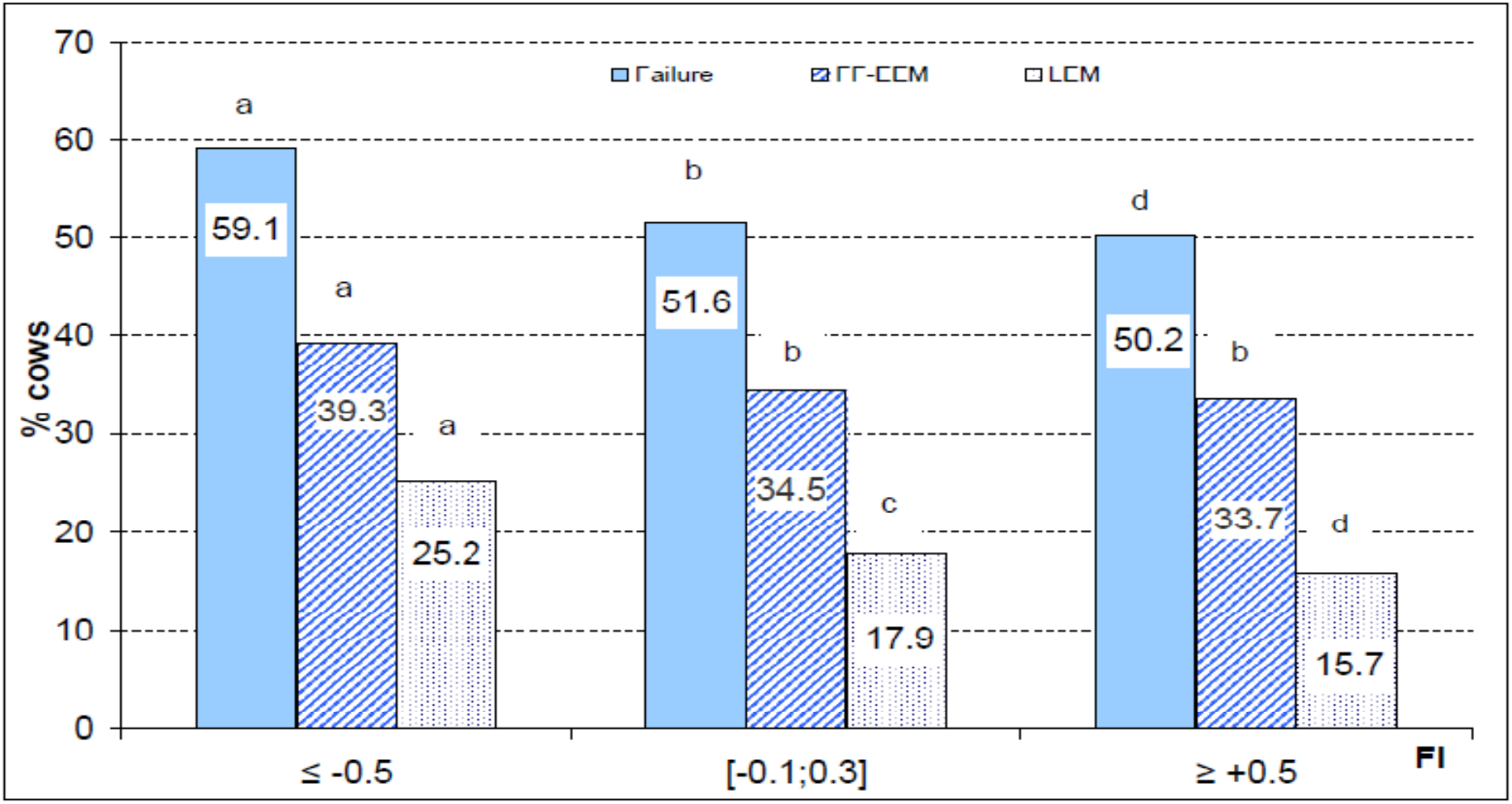


AI during luteal phase (6,3 %)	+				
Early emb. death (35,4 %)	-	-	Regular		
Late emb. death (16,7 %)	-	+	delayed	Not pregnant	
Foetal death (3,6 %)	-	+	delayed	Pregnant	Not pregnant
Pregnancy (37 %)	-	+	No return	Pregnant	Pregnant

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

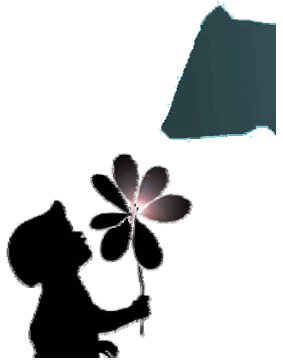


Source Ledoux 2011

Impacts on genomic selection

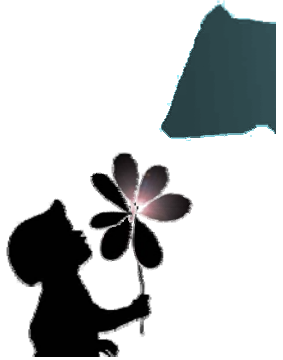
- 7 traits (type of pregnany failure) analysed in 2669 females with 306 SNPs (from 13 chromosomes).
- Fertility QTL on chromosome 3 more precisely located
- Effects of QTLs confirmed / isolated
  - 6 on calving rate
  - 3 on EEM, 3 on LEM, 8 on global EM,
  - 2 on FM
  - 3 on aborts

Source : Lefevre, 3R 2011



Conclusion

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



Thanks !

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  - 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
  -
- INRA GABI, Jouy
  - Didier Boichard, Mekki Boussaha, Rachel Lefevre
- ENVA
  - Bénédicte Grimard, Dorothée Ledoux
- UNCEIA
  - Sébastien Fritz, Aurélien Capitan,
  - Catherine Joly, Brigitte Leguienne, Pascal Salvetti
  - Patrice Humblot (SLU)