Beiträge der Funktionalen Genomanalyse zum Verständnis des komplexen Merkmals Fruchtbarkeit

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Fertility in livestock

Fruchtbarkeit der Nutztiere

- Trächtigkeit
- Befruchtung
- Erbgesundheit
- Hohe Ferkelzahl
- Empfänglichkeit
- Gesunder Nachwuchs
- Embryonalentwicklung
- Tiergesundheit

- Zwischenkalbezeit
- Anzahl Laktationen
- Sexualzyklus
- Trächtigkeitsrate
- IVF, Embryotransfer
- Steroidhormone

- Embryonaler Fruchttod
- Abort
- Nachgeburtsverhaltung
- Niedrige Ferkelzahl
- Mastitis
- Endometritis
- Uterine crowding
- Stille Brunst

Increase of early embryo death in Holstein

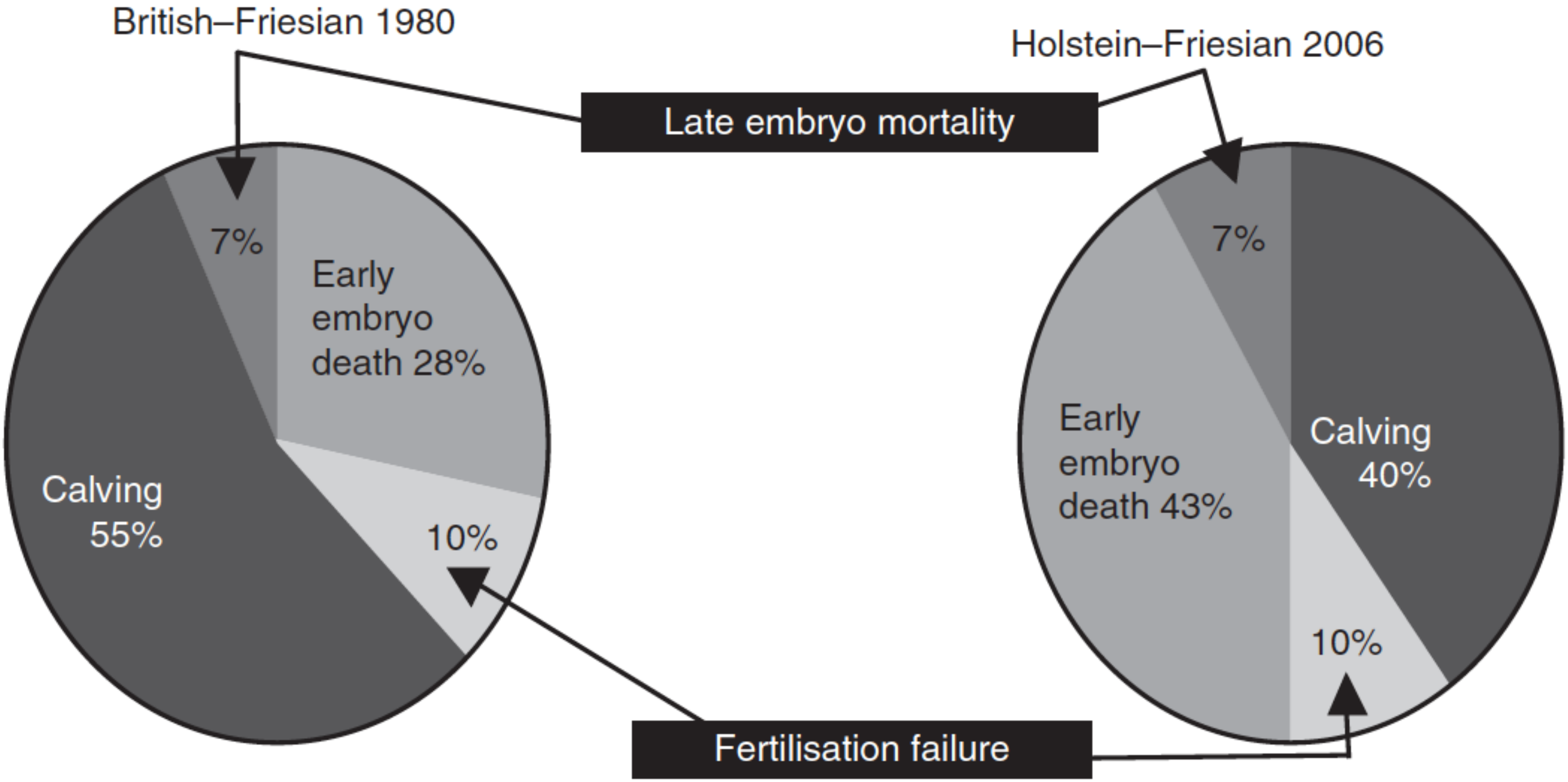
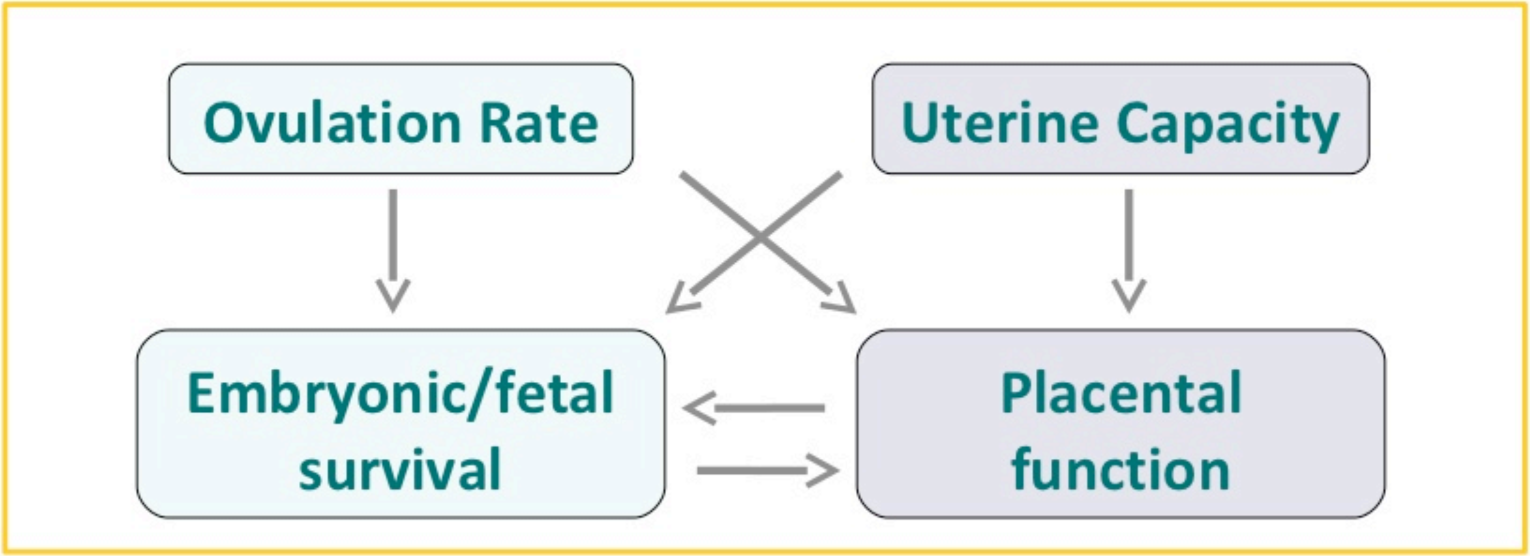


Fig. 1. Reproductive outcomes in British–Friesian *v*. Holstein–Friesian cows. (Source: Diskin *et al*. 2006). Late embryo death is defined as death of the embryo or fetus after Day 28 of gestation.

Low birth-weight phenotype

Genotype

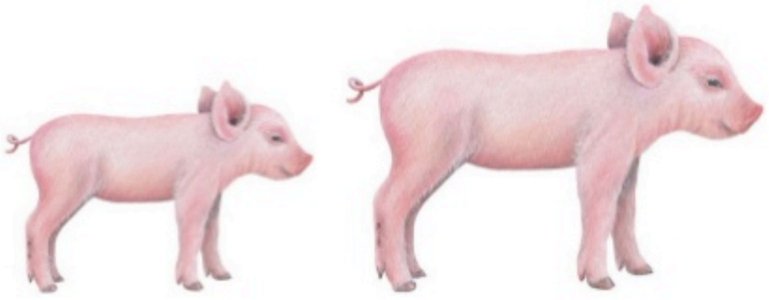


Ovulation Rate

Uterine Capacity

Embryonic/fetal survival

Placental function

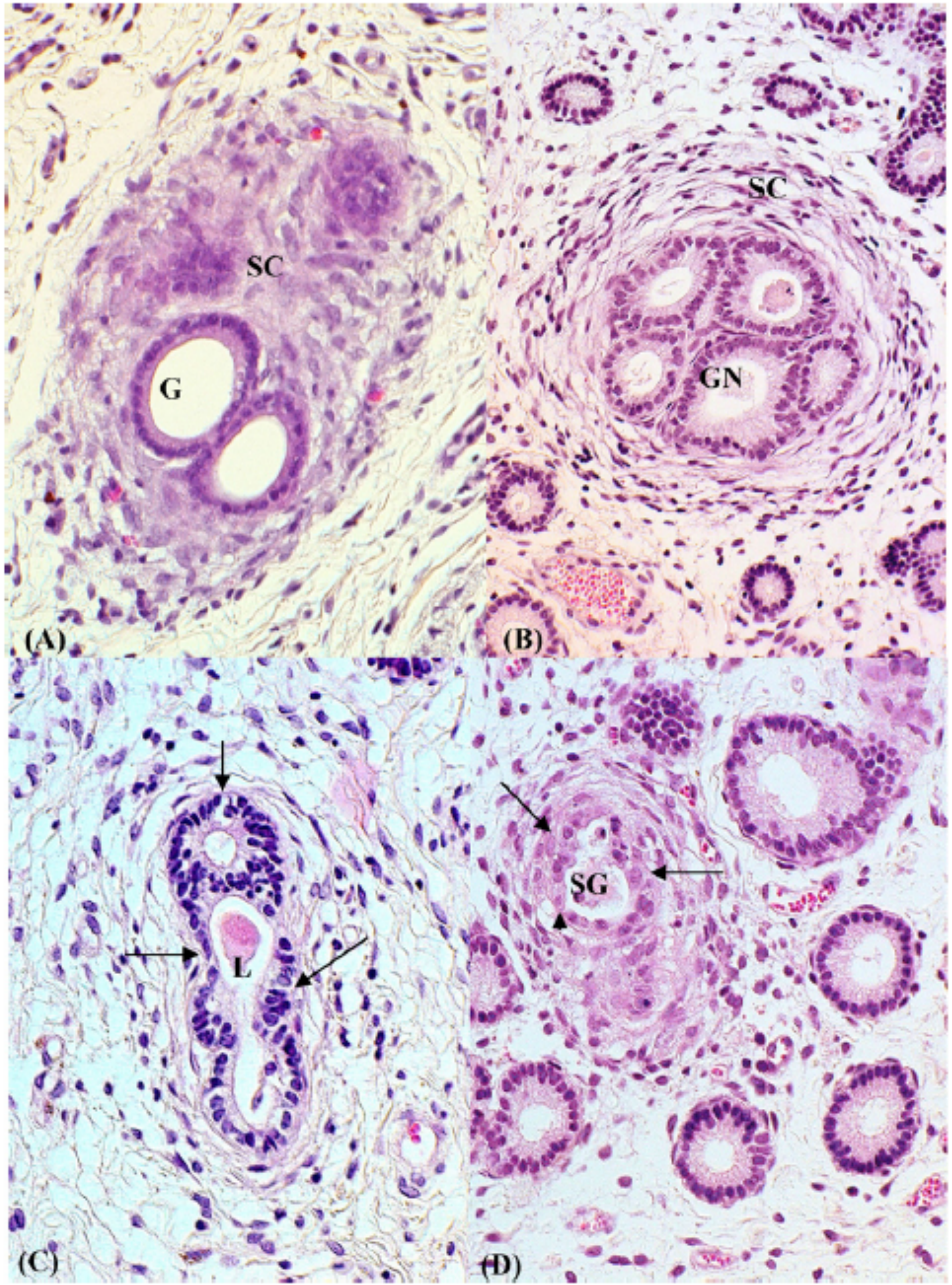


Phenotype

Repeatability of Litter Size in the Sow Population - Jenny Patterson, University of Alberta, Allen D. Leman Swine Conference, September 15-18 2012, St. Paul, Minnesota, USA.

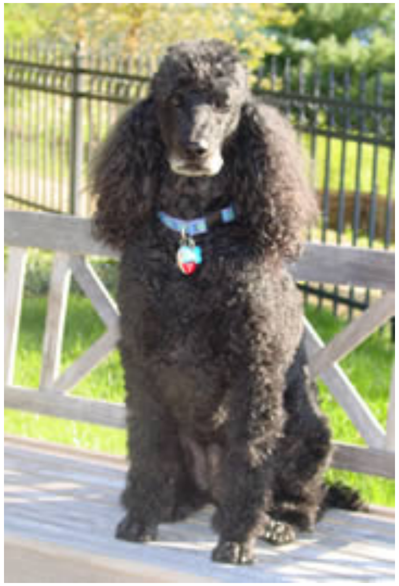
Stute:

Endometrose / Breeding-induced metritis


C. Hoffmann et al. / Animal Reproduction Science 111 (2009) 261–278

Impact of functional genomics tools

Genome research in livestock



Kirkness et al., Science (2003)

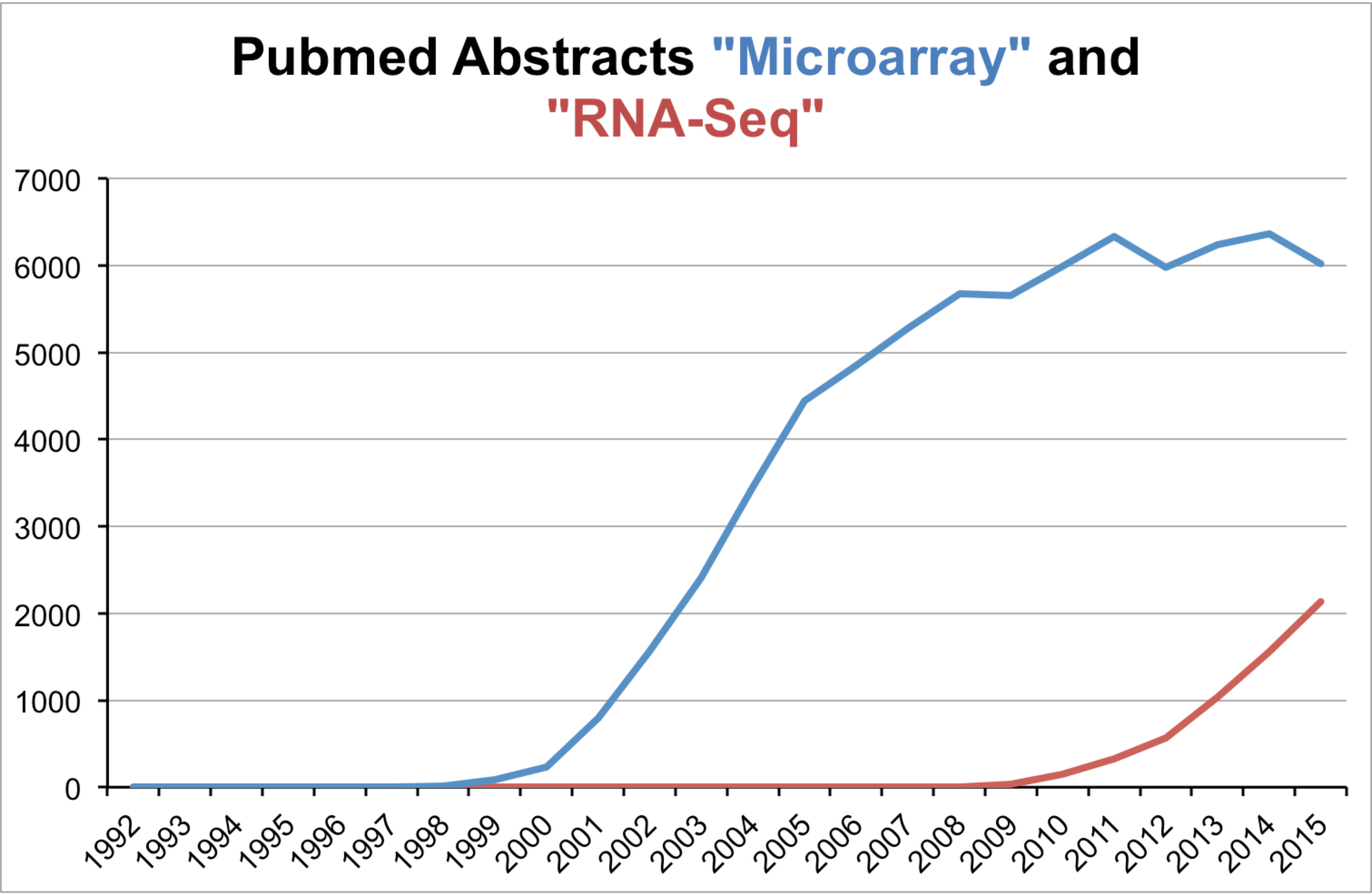
U.S. Livestock Genome Mapping Projects

Supported by

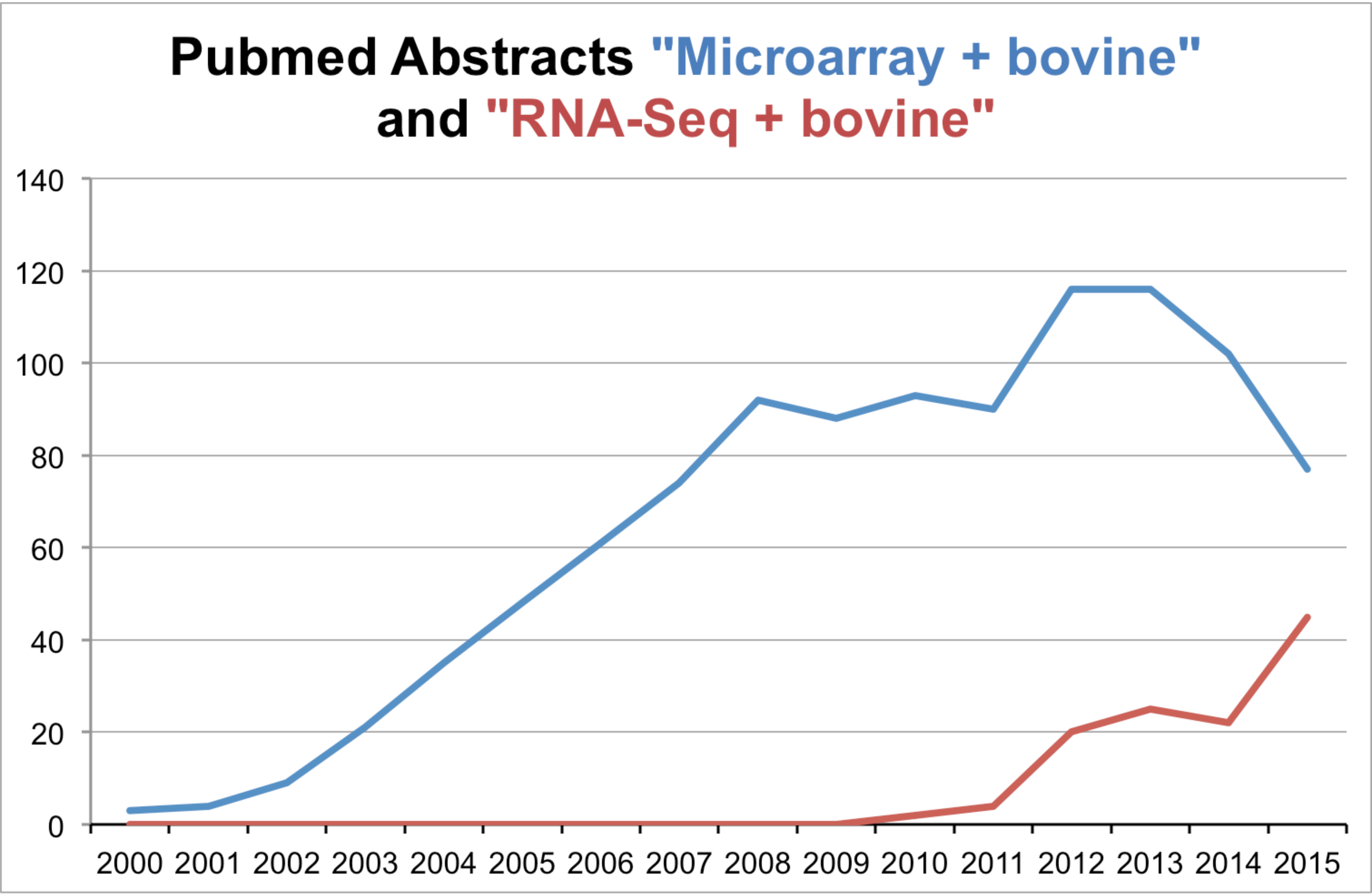
NATIONAL ANIMAL GENOME RESEARCH PROGRAM

© 1998-2004 National Animal Genome Research - Bioinformatics Coordination Program.

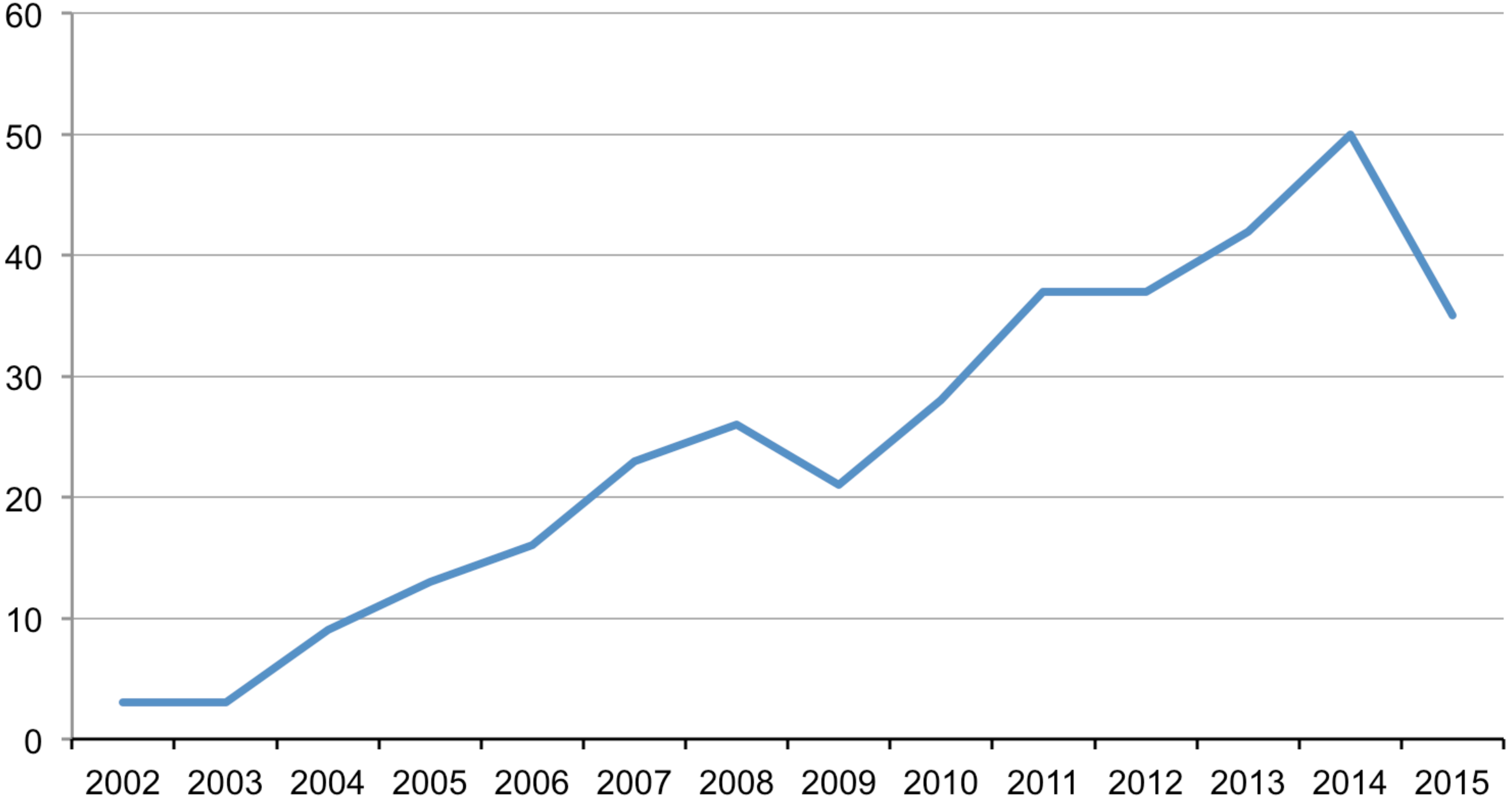
Omics-technologies – revolution of the exploration of biological systems



Application of Omics tools in cattle



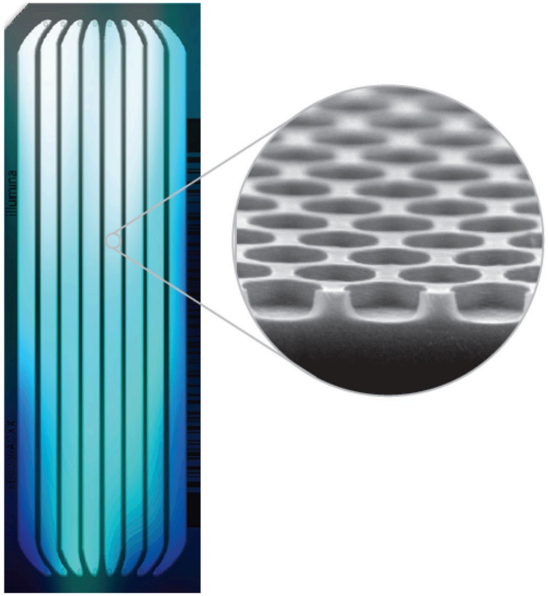
Pubmed Abstracts related to Reproduction for cattle, pig, and horse



Illumina HiSeq 3000/HiSeq 4000 instruments

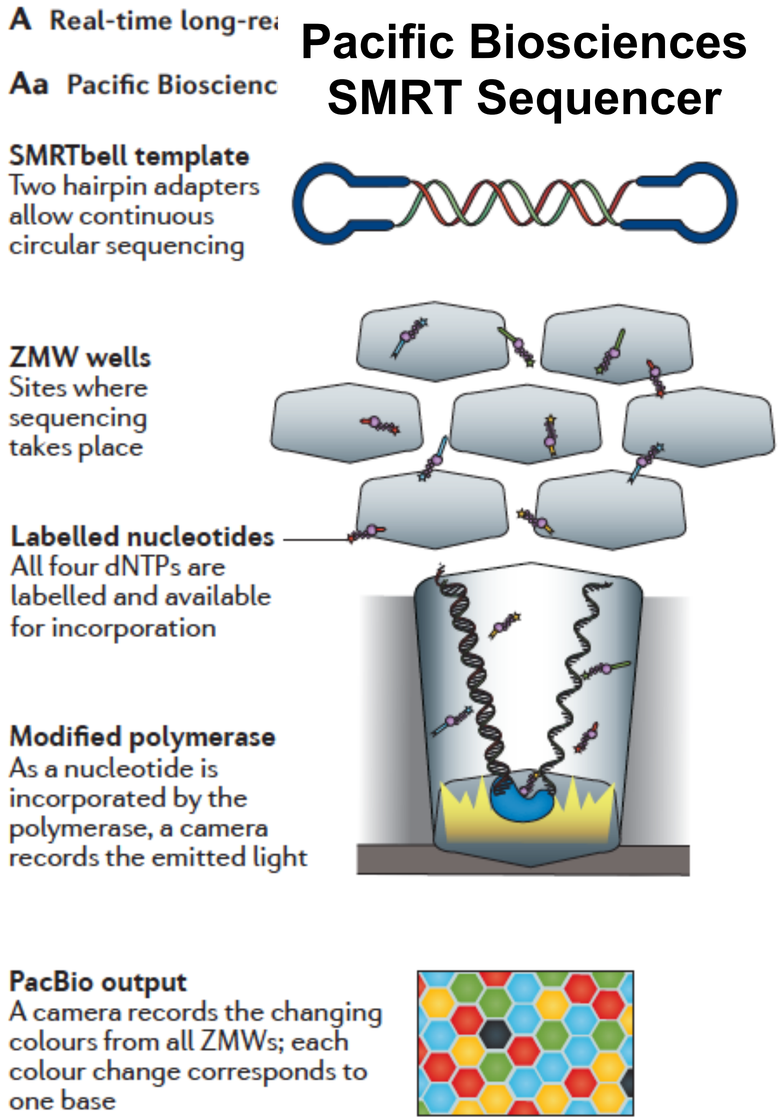
Per instrument run (3.5 days):

- Up to 1.5 Tera bases
- = 5 billion reads per run, 2x 150 bp
- = 12 genomes
- =100 transcriptomes
- =180 exomes



Third-generation sequencing – generation of ultralong reads

Pacific Biosciences SMRT Sequencer



SMRTbell template
Two hairpin adapters allow continuous circular sequencing

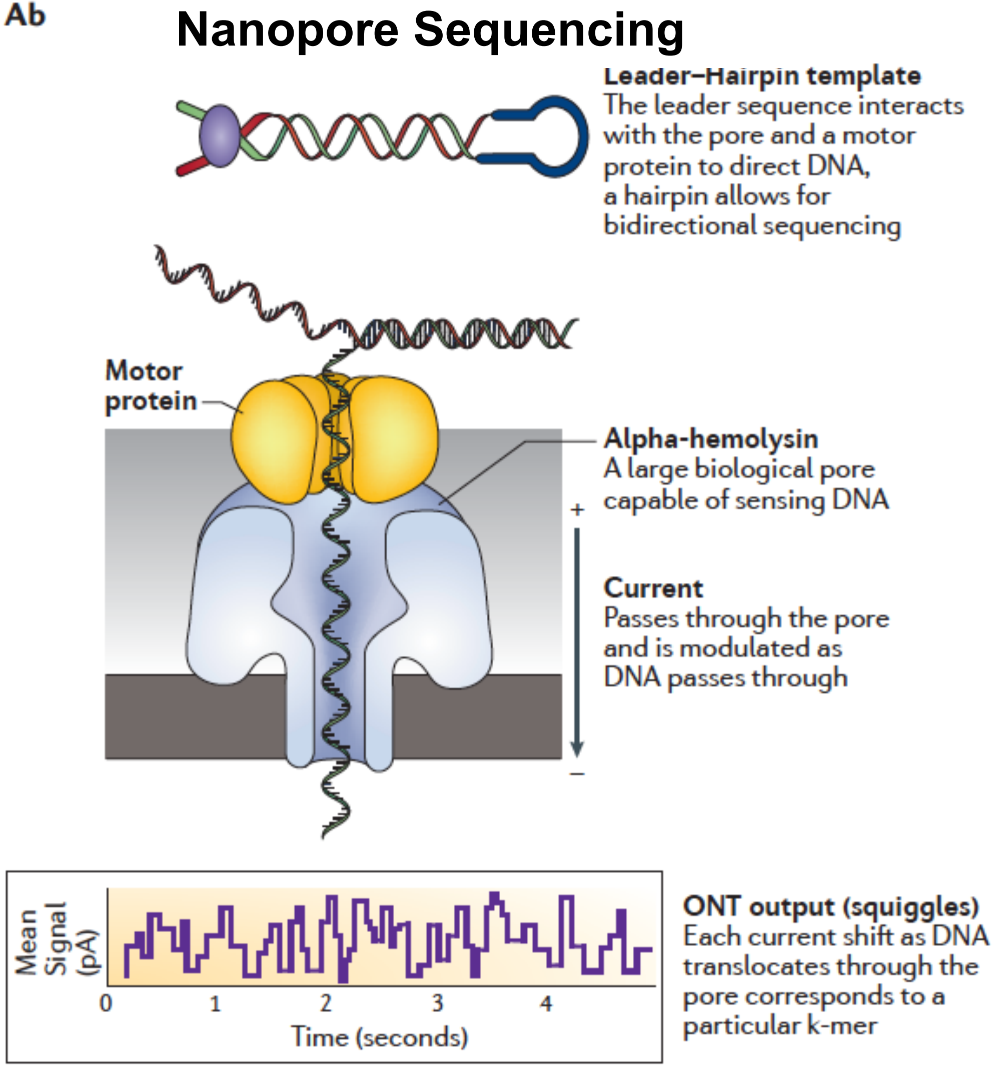
ZMW wells
Sites where sequencing takes place

Labelled nucleotides
All four dNTPs are labelled and available for incorporation

Modified polymerase
As a nucleotide is incorporated by the polymerase, a camera records the emitted light

PacBio output
A camera records the changing colours from all ZMWs; each colour change corresponds to one base

Oxford Nanopore Technologies Nanopore Sequencing



Leader–Hairpin template
The leader sequence interacts with the pore and a motor protein to direct DNA, a hairpin allows for bidirectional sequencing

Alpha-hemolysin
A large biological pore capable of sensing DNA

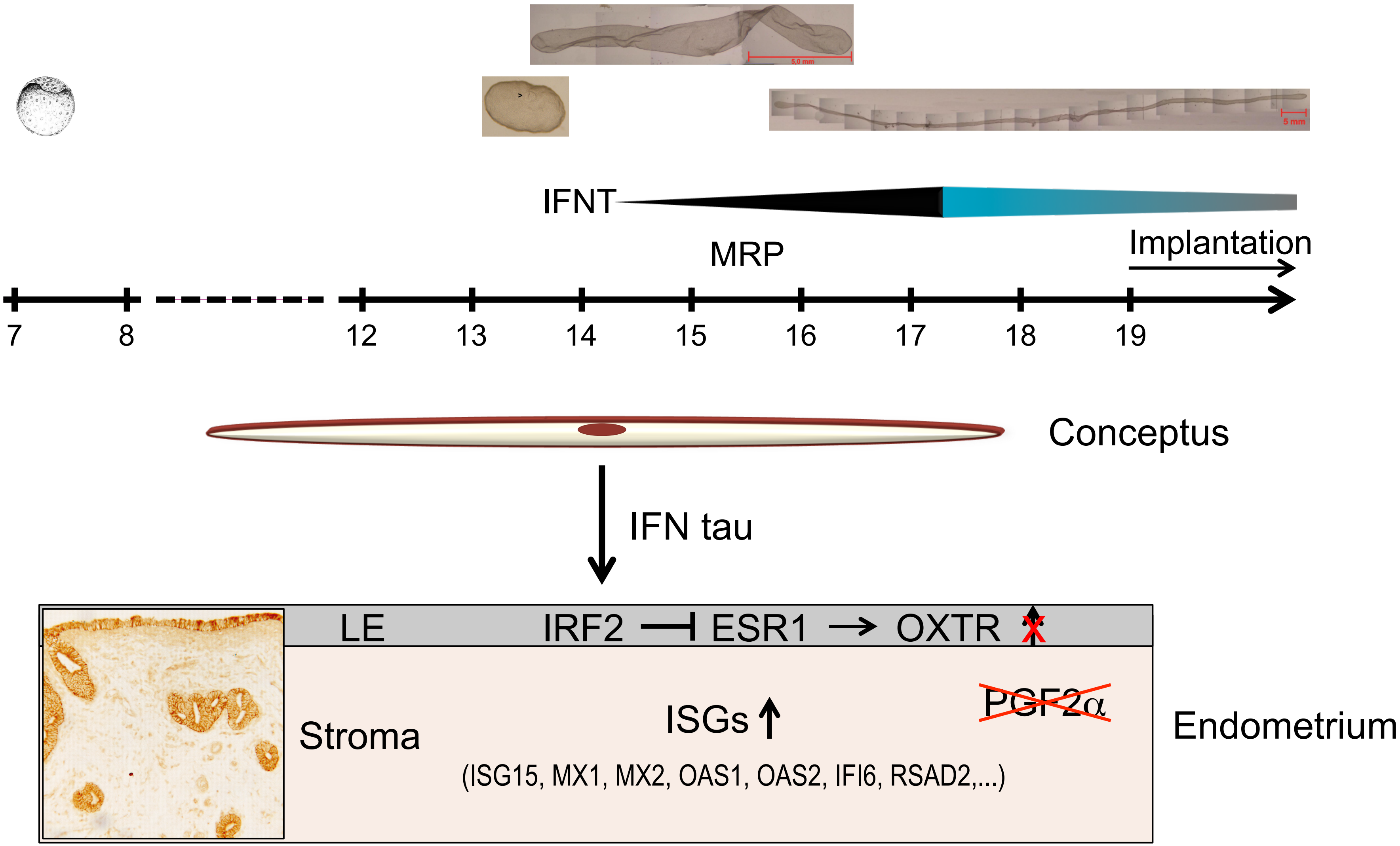
Current
Passes through the pore and is modulated as DNA passes through

ONT output (squiggles)
Each current shift as DNA translocates through the pore corresponds to a particular k-mer

Goodwin et al. Nat Rev Gen 2016

Approaches to explore differential gene expression related to the trait fertility

Embryo-maternal Cross-talk in the Bovine Uterus



IFNT

MRP

Implantation

7 8 12 13 14 15 16 17 18 19

Conceptus

IFN tau

LE IRF2 ⊣ ESR1 → OXTR ~~↑~~

Stroma

ISGs ↑

(ISG15, MX1, MX2, OAS1, OAS2, IFI6, RSAD2,...)

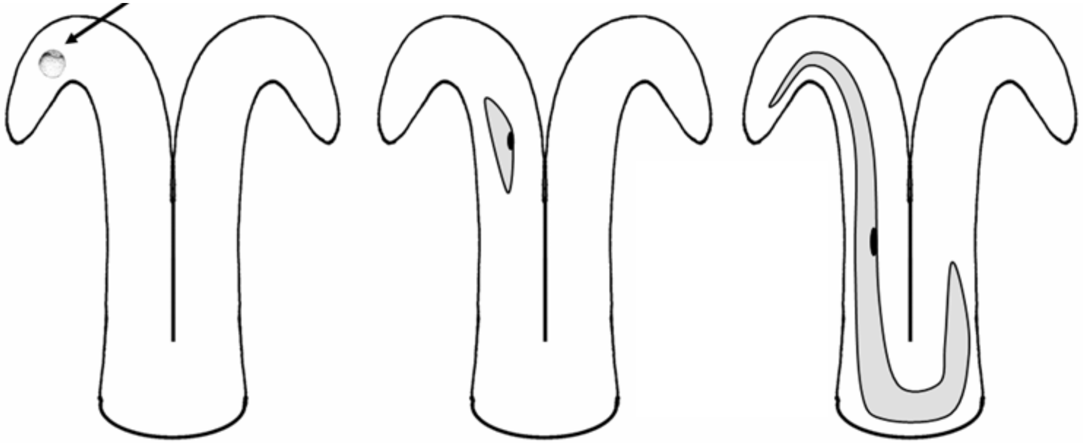
~~PGF2α~~

Endometrium

Spencer et al., *Endocrinology* 1995; Klein et al., *Biol Reprod* 2006

Study design – analysis of early pregnancy

- Artificial insemination (AI) after cycle synchronization or ET
- Slaughter and sample collection on Days 12 to D18 of pregnancy
- Controls: inseminated with supernatant of centrifuged sperm or sham ET
- Analysis with microarrays or RNA-Seq



Blastocyst
∅ 1 mm

Conceptus
length 3-5 cm

Conceptus
length >15 cm

Day 12

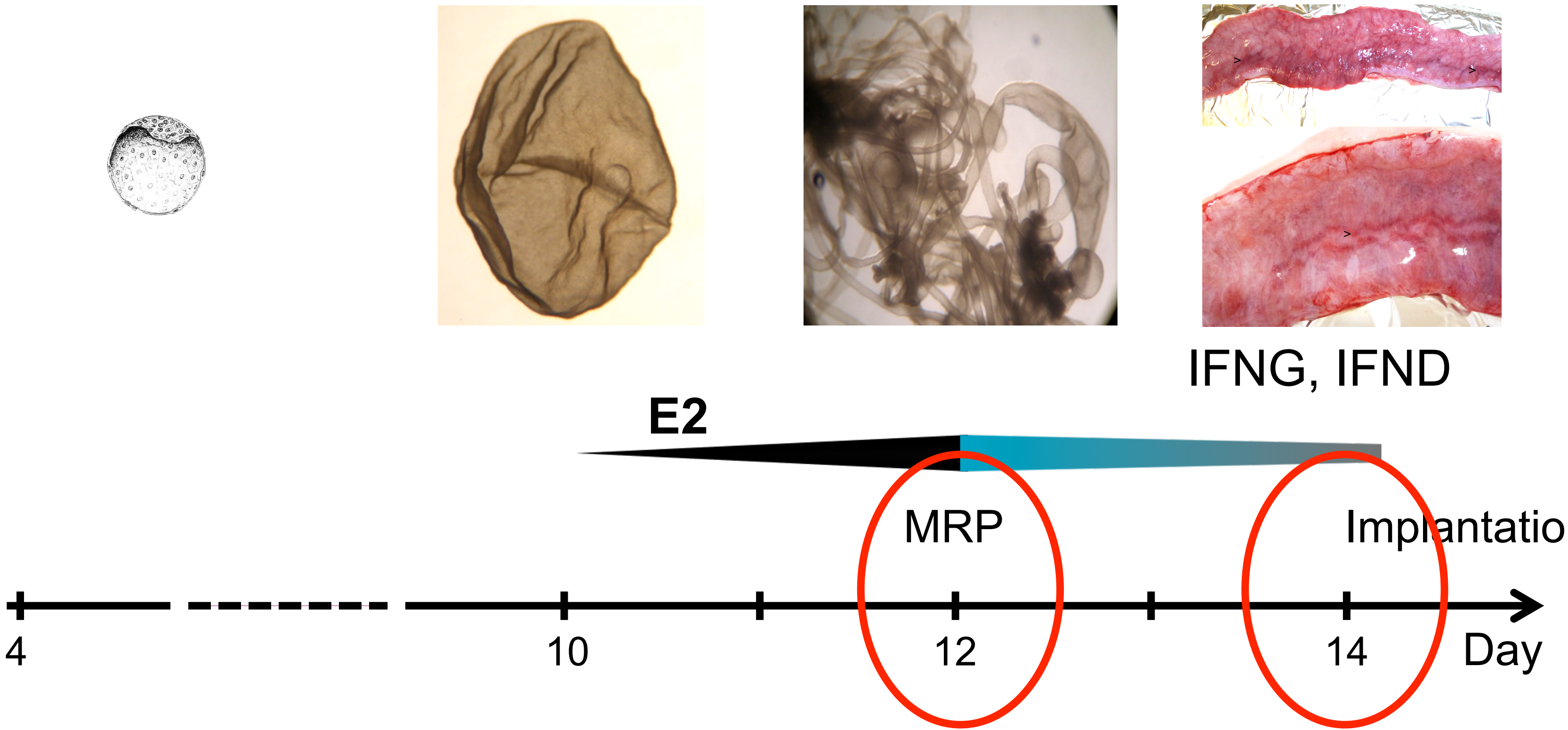
Day 15

Day 18

Day	Expression	Genes	Platform	Study
13	downregulated	7	Illumina RNA-seq	Forde et al. 2012
15	downregulated	59	Affymetrix	Bauersachs et al. 2012
15	downregulated	72	Illumina RNA-seq	Bauersachs et al. 2012
16	downregulated	161	Affymetrix	Forde et al. 2011
16	downregulated	159	Illumina RNA-seq	Forde et al. 2012
17	downregulated	460	Agilent	Walker et al. 2010
18	downregulated	68	SSH+cDNA array	Bauersachs et al. 2006
18	downregulated	328	Affymetrix	Bauersachs et al. 2012
18	downregulated	915	Illumina RNA-seq	Bauersachs et al. 2012
13	upregulated	9	Illumina RNA-seq	Forde et al. 2012
15	upregulated	247	Affymetrix	Bauersachs et al. 2012
15	upregulated	286	Illumina RNA-seq	Bauersachs et al. 2012
16	upregulated	340	Affymetrix	Forde et al. 2011
16	upregulated	229	Illumina RNA-seq	Forde et al. 2012
17	upregulated	540	Agilent	Walker et al. 2010
18	upregulated	80	SSH+cDNA array	Klein et al. 2006
18	upregulated	99	SSH+cDNA array	Bauersachs et al. 2006
18	upregulated	585	Affymetrix	Bauersachs et al. 2012
18	upregulated	863	Illumina RNA-seq	Bauersachs et al. 2012
hIFNA	upregulated	169	Affymetrix	Bauersachs et al. 2012

Pre-implantation phase in pigs

Day 14 endometrium, hyperemic zones

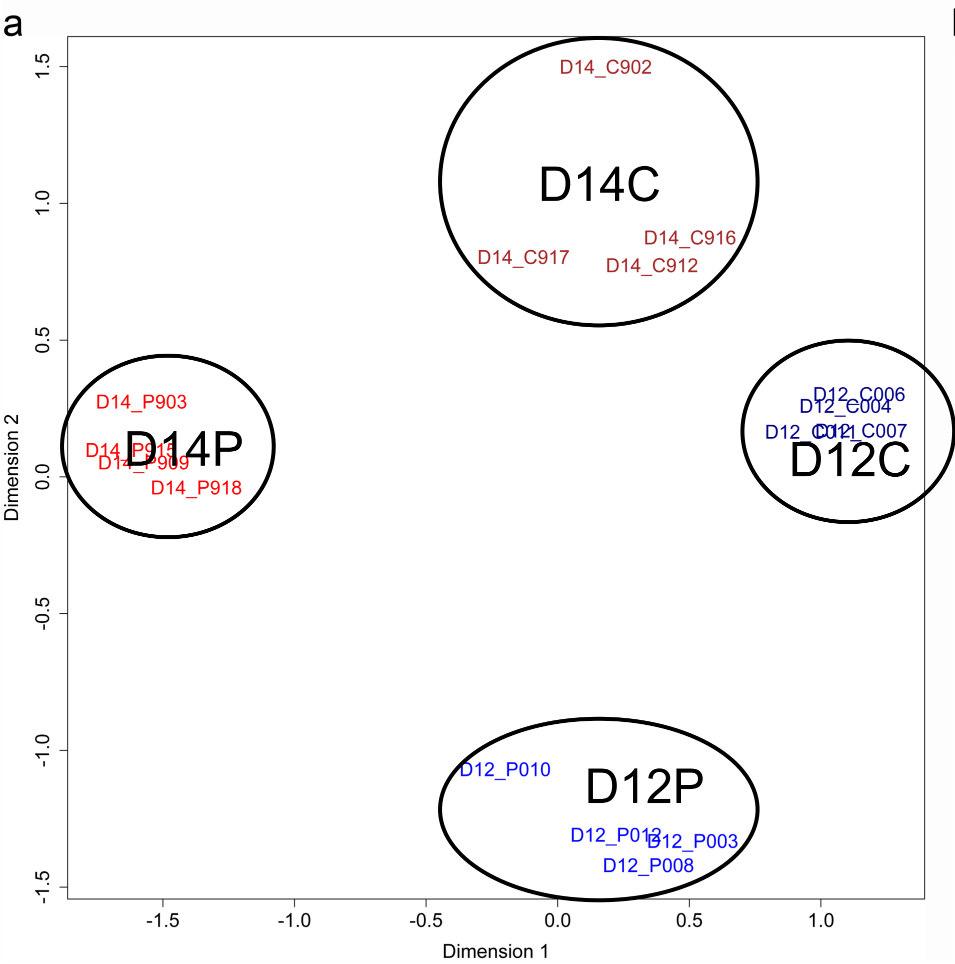


- Day 10-12: transition from spherical to filamentous blastocysts
- Increase of conceptus estrogen synthesis with conceptus elongation
- Day 14: Conceptus attachment, increased vascularization at implantation zones, initiation of epitheliochorial placentation

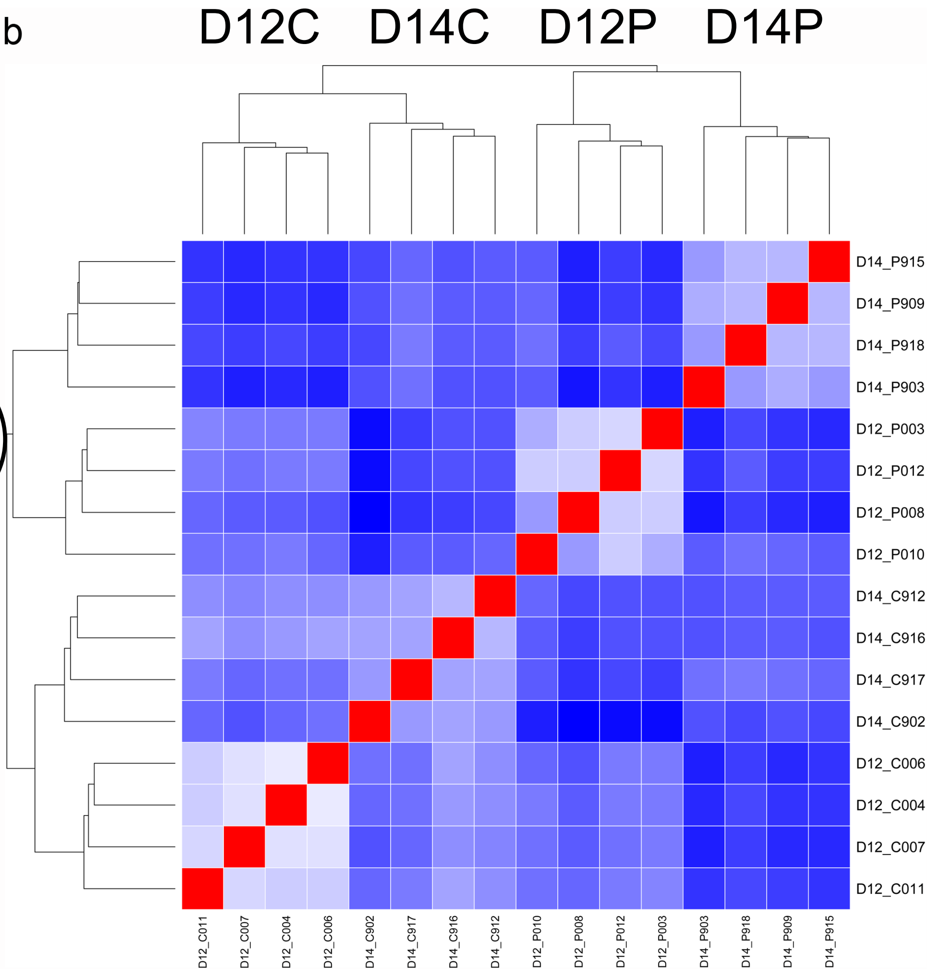
ETH zürich

Combined analysis of Days 12 and 14

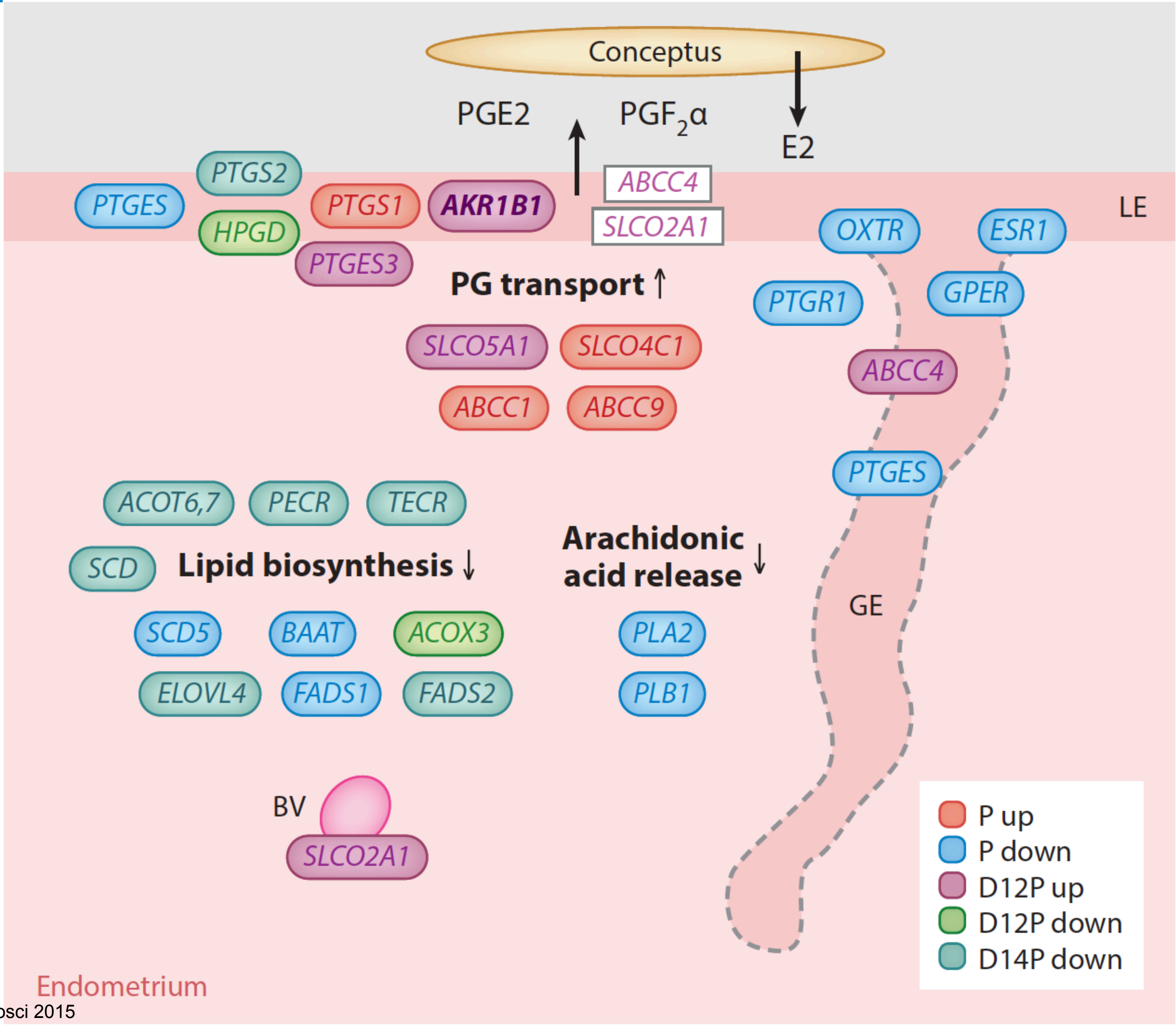
PCA



Distance heatmap



Genes related to PG metabolism



Bauersachs & Wolf Annu Rev Anim Biosci 2015

Comparison of combined bovine data sets to porcine endometrium from Day14 of pregnancy

- Bovine data sets (3300 genes): 505 out of 1512 DEGs in pig endometrium
- More than 80% of overlapping genes showed similar regulation in both species

Genes upregulated in both species:

- Mainly functional terms related to immune response
- Genes involved in regulation of apoptosis

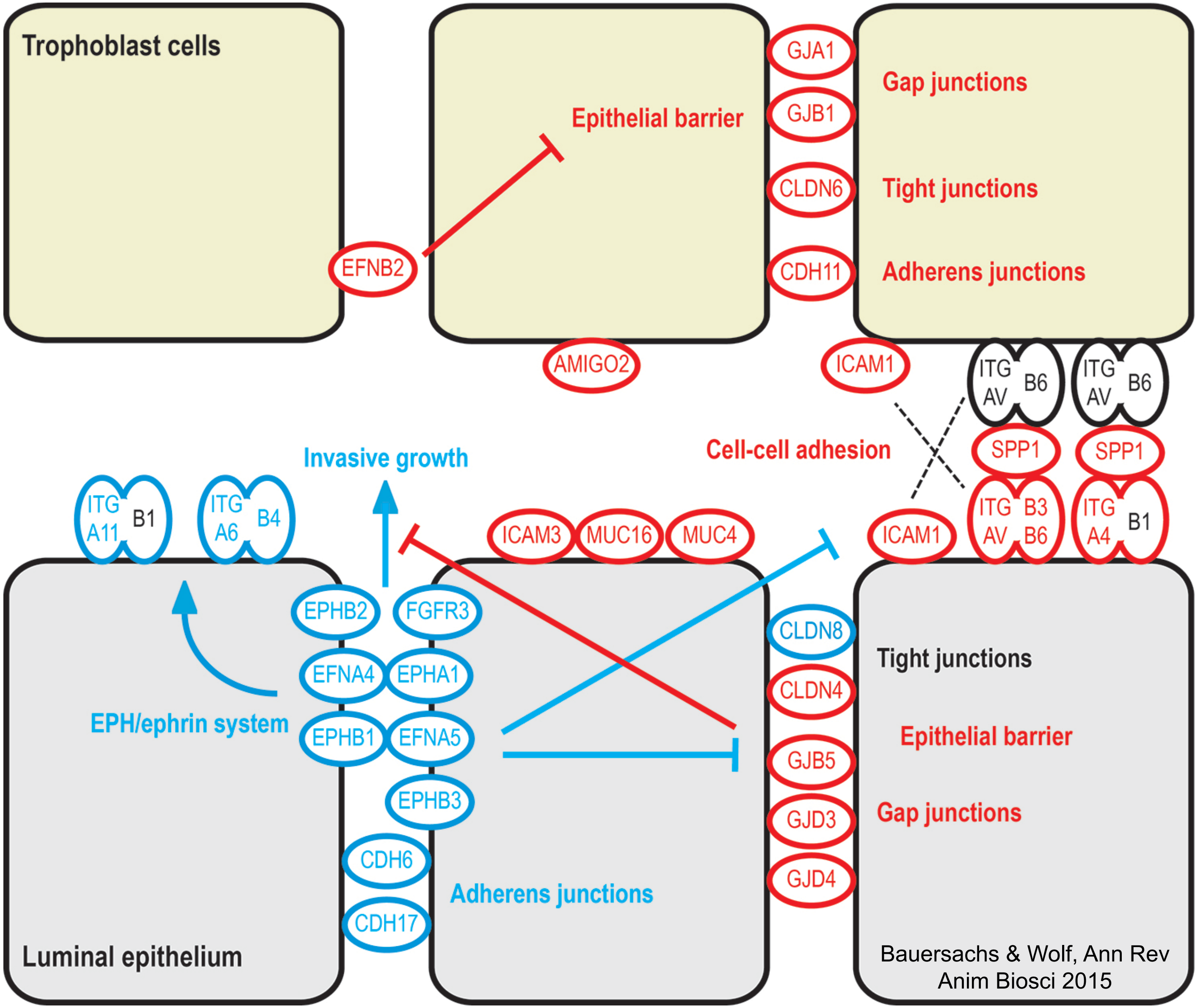
Genes downregulated in both species:

- Extracellular matrix
- Calcium ion binding
- Transcription factor activity
- Members of the HOXB family: *HOXB2*, *HOXB3*, *HOXB4*, *HOXB5*, *HOXB6*, *HOXB8*

Genes with opposite regulation:

- Extracellular region, cell adhesion, cytoplasmic membrane-bounded vesicle, extracellular matrix, and cell motion

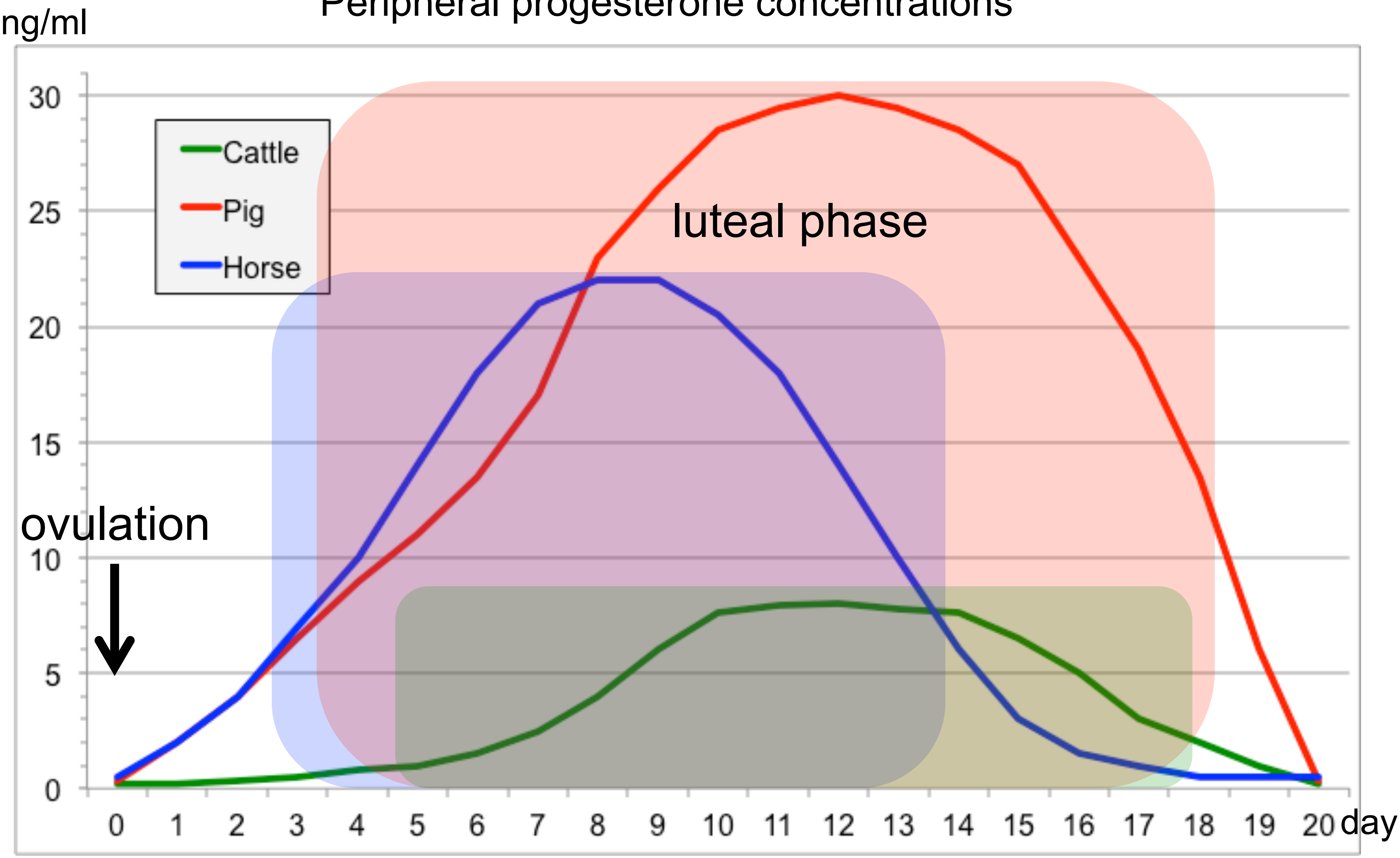
Regulation of trophoblast attachment



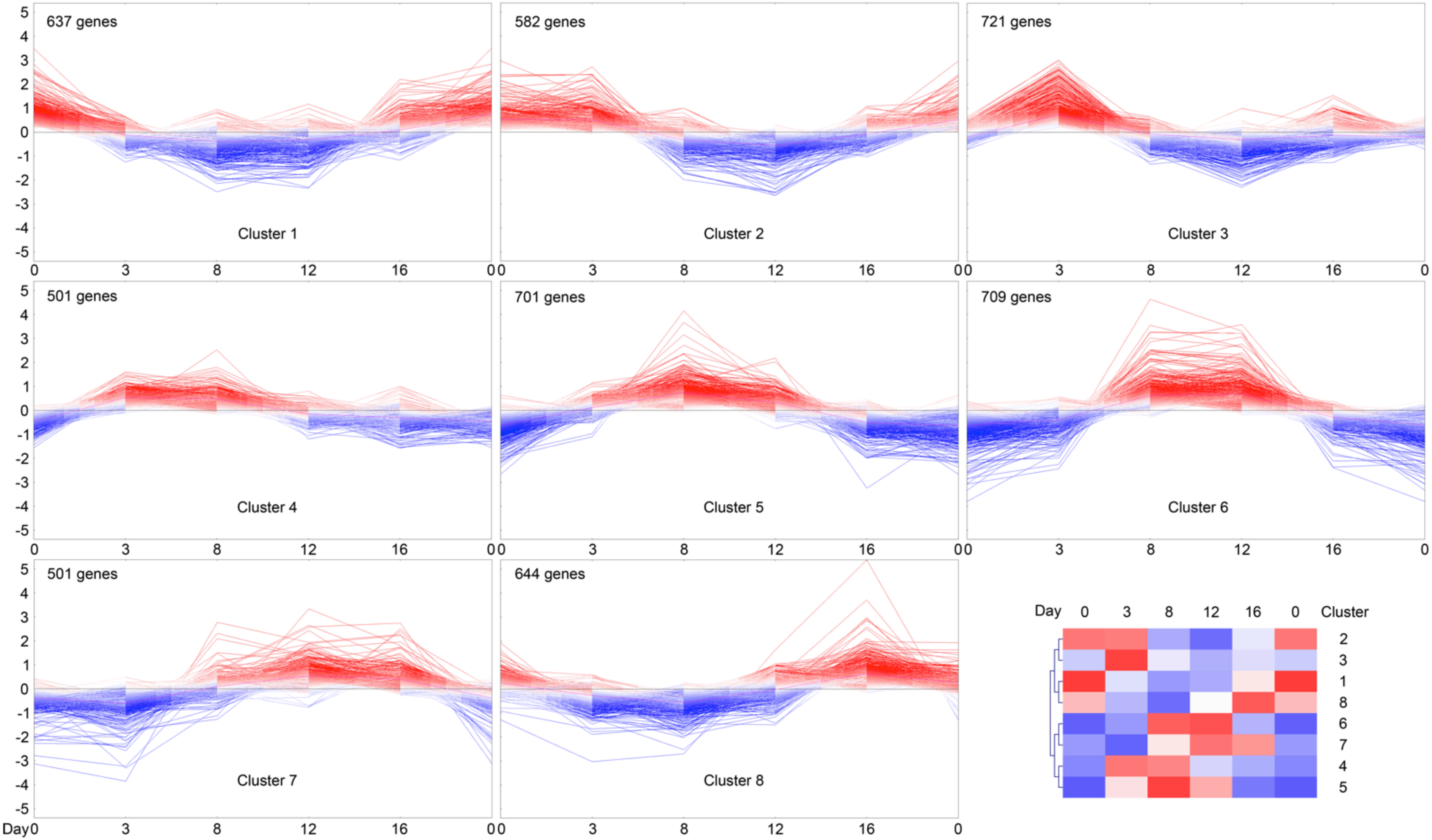
Bauersachs & Wolf, Ann Rev Anim Biosci 2015

Reproductive (estrous) cycle

Peripheral progesterone concentrations

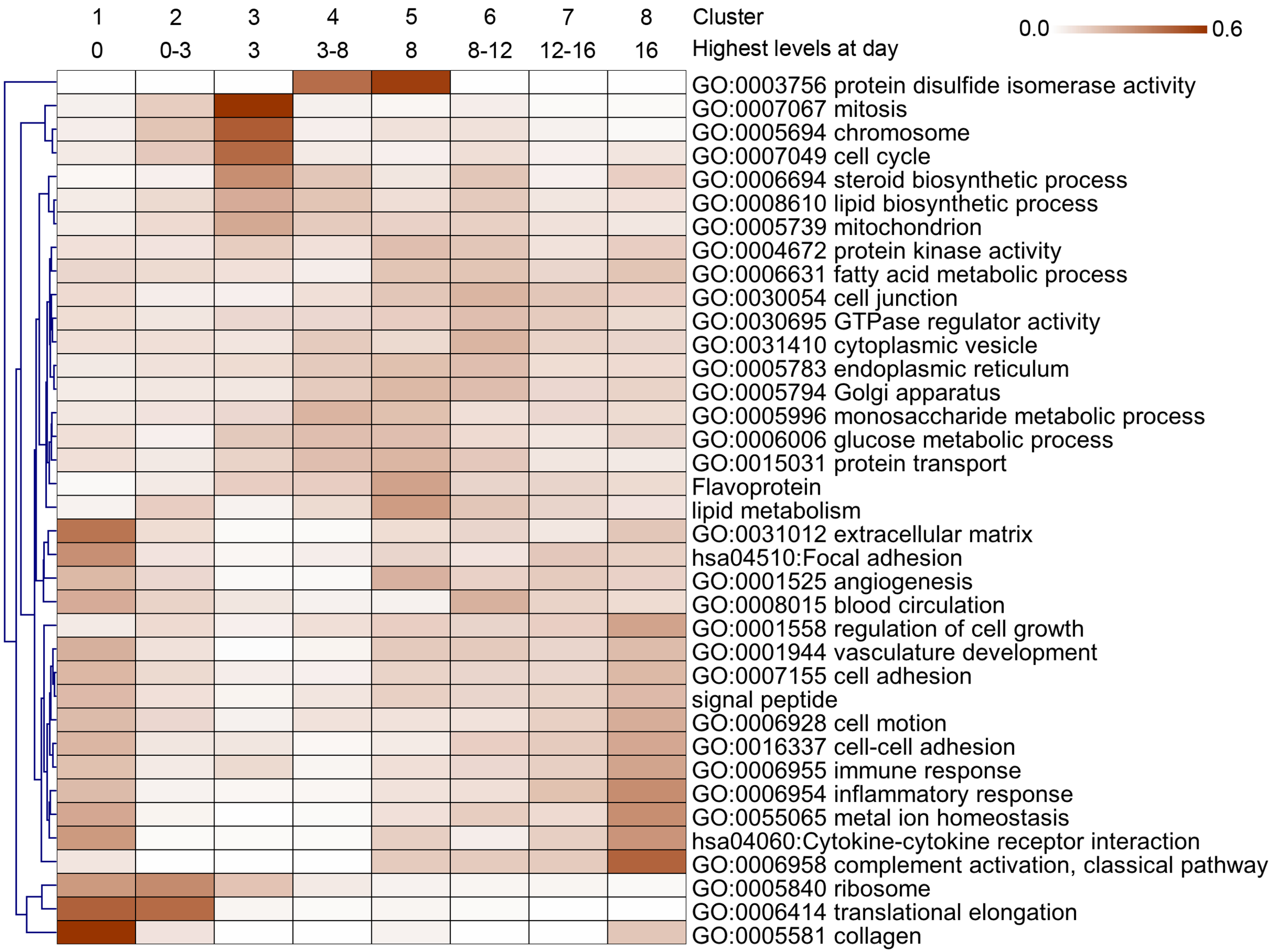


Expression profiles during the estrous cycle



Gebhardt et al. Biol Reprod 2012

Selected functional categories

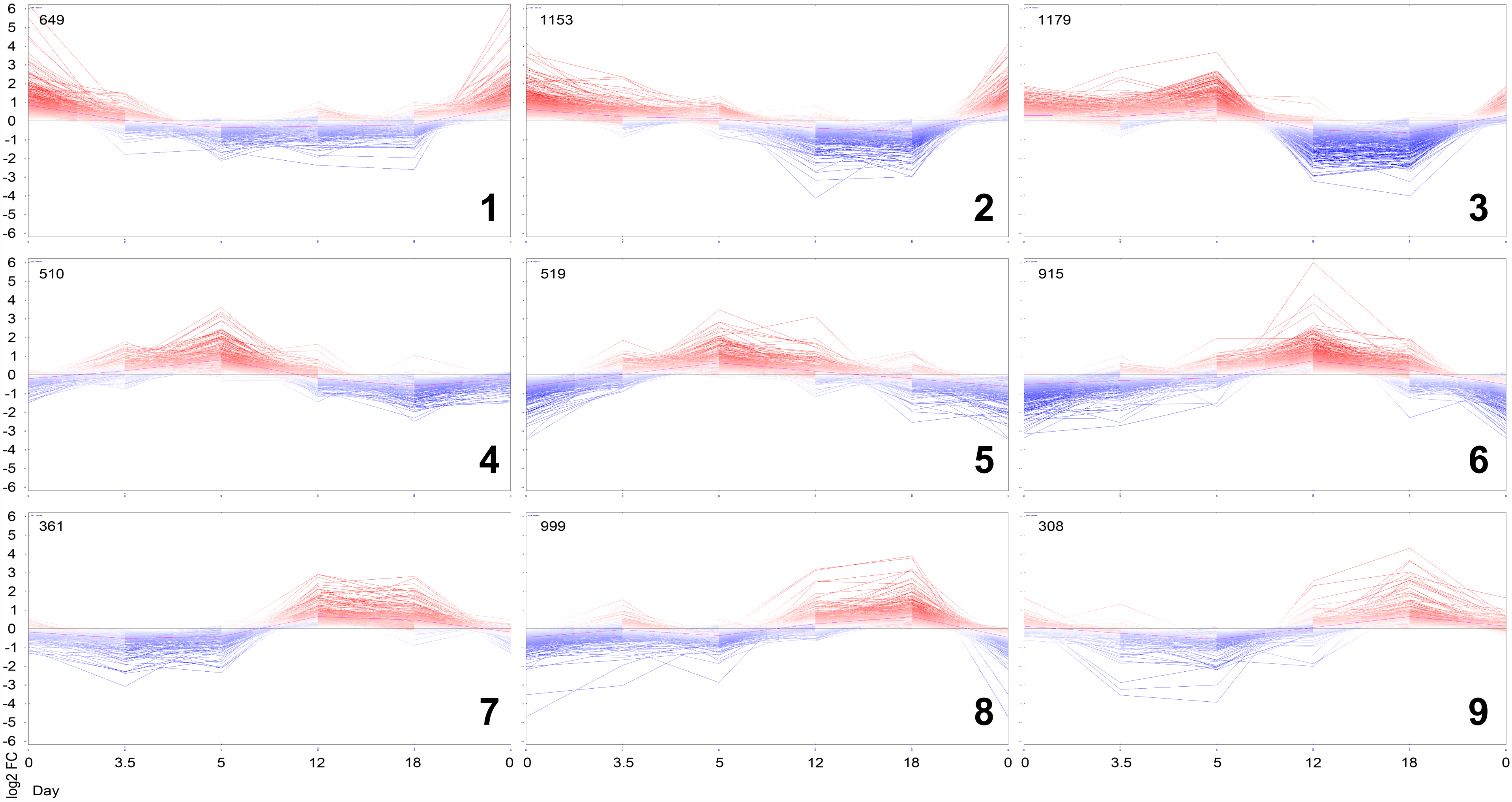


Gebhardt et al. Biol Reprod 2012

Results of dynamic gene expression analysis

- 5000-7000 differentially expressed genes (DEGs)
- Overlap: 1,900-2,350 DEGs (pairwise overlaps)
- >1,070 genes differential in all three species

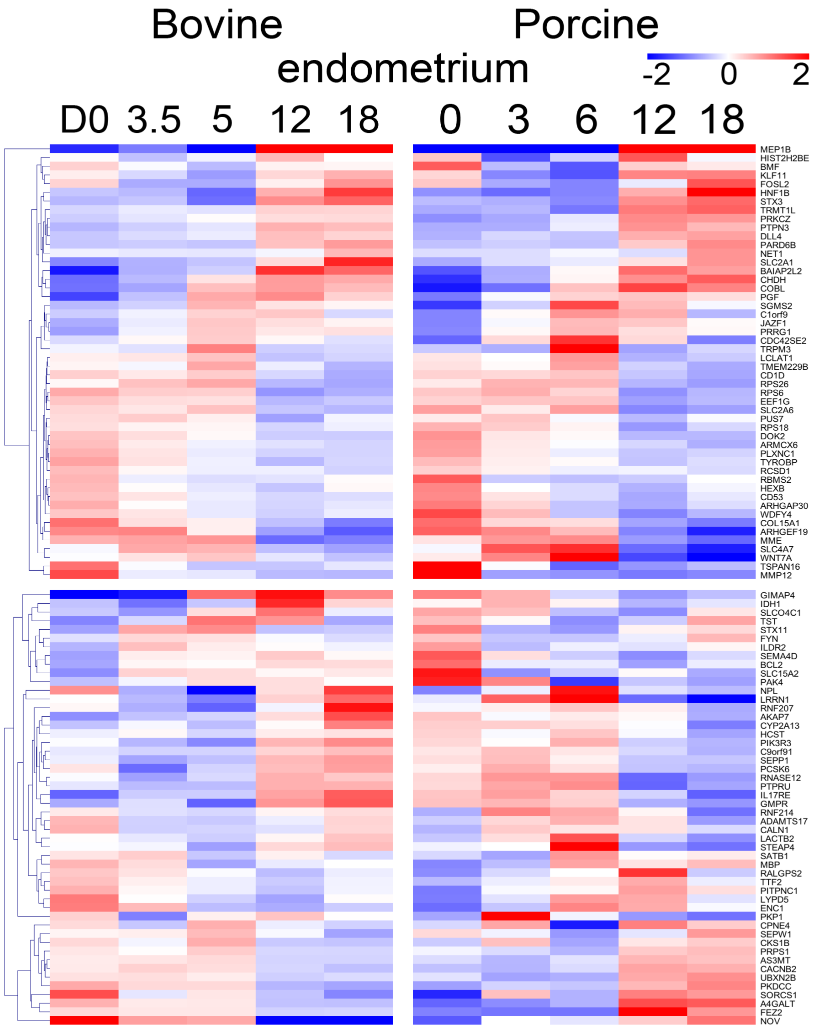
Clusters of genes with similar expression profiles



Correlation of cyclic expression changes

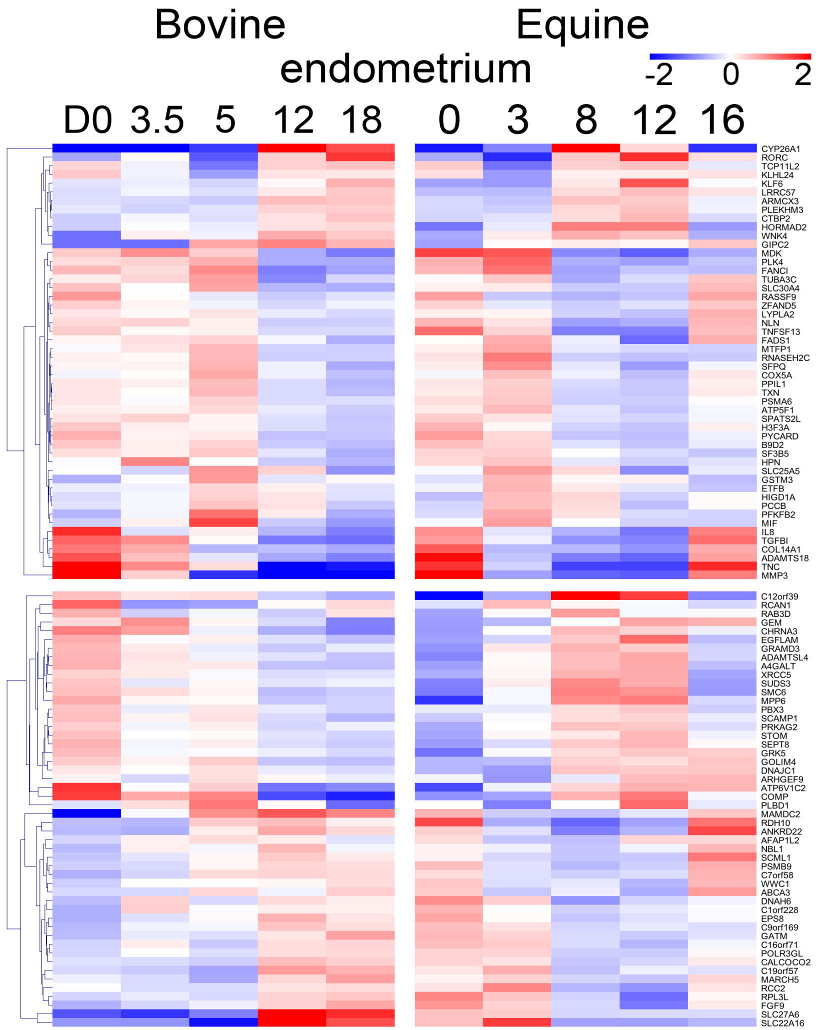
Comparison to porcine endometrium

>2350 overlapping DEGs



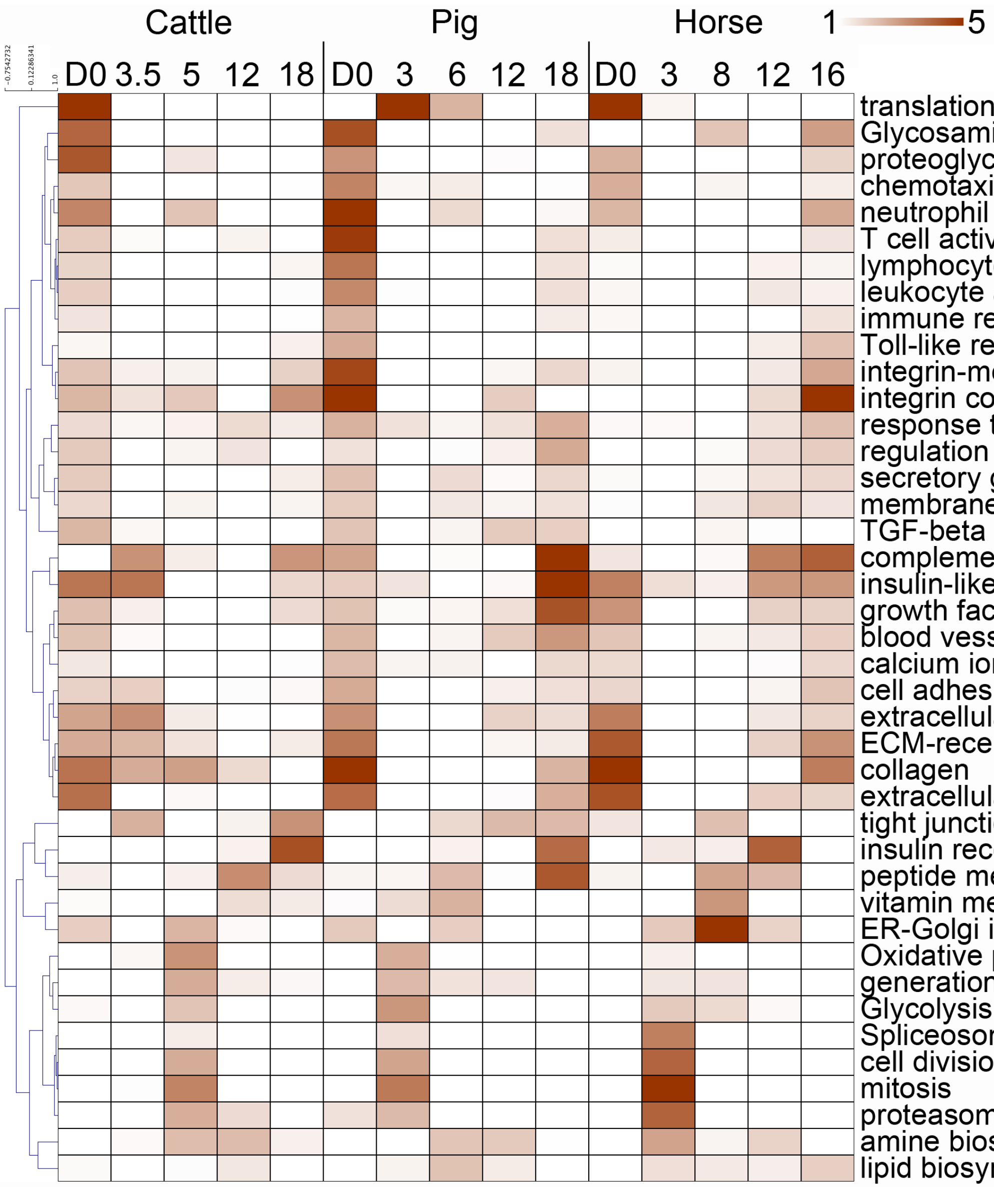
Comparison to equine endometrium

>2340 overlapping DEGs



Data from Gebhardt et al. 2012 and Bauersachs et al. (unpublished)

Correlation of overrepresentation of selected functional categories

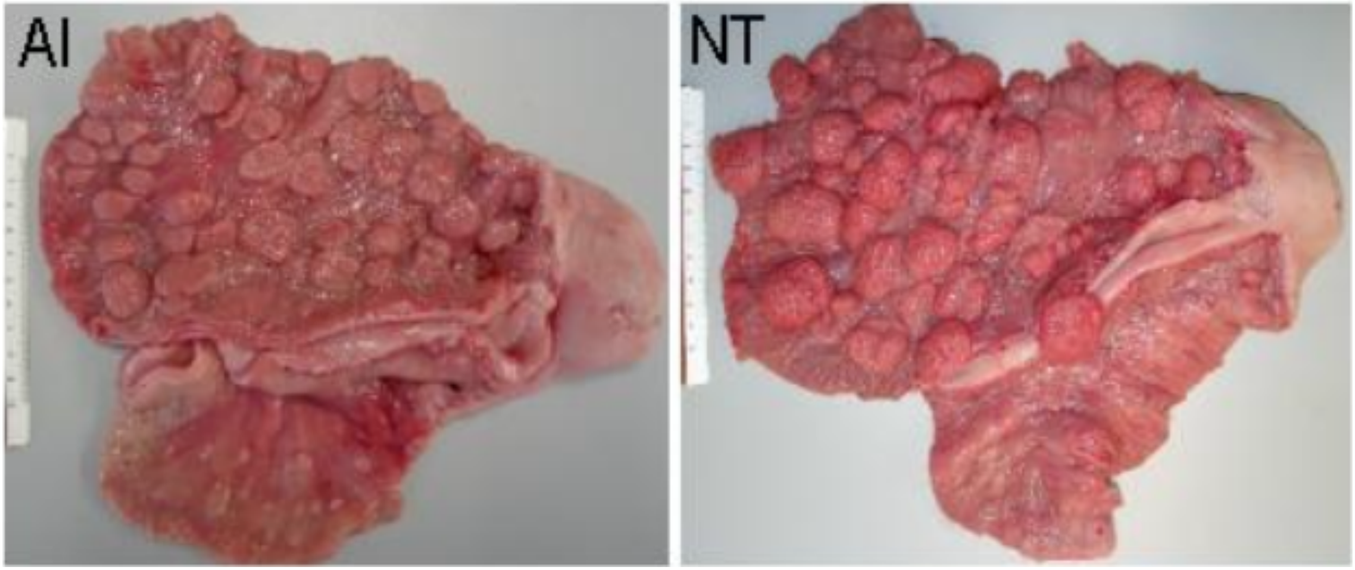


Functional term	Correlation		
	Bta-Ssc	Bta-Eca	Ssc-Eca
translational elongation	-0.347	0.989	-0.192
Glycosaminoglycan degradation	0.910	-0.412	-0.642
proteoglycan biosynthetic process	0.936	0.818	0.850
chemotaxis	0.878	0.699	0.639
neutrophil chemotaxis	0.892	0.816	0.765
T cell activation	0.890	0.861	0.806
lymphocyte activation	0.874	-0.026	0.361
leukocyte activation	0.856	0.384	0.575
immune response	0.834	0.819	0.765
Toll-like receptor signaling pathway	0.760	0.292	-0.106
integrin-mediated signaling pathway	0.771	0.847	0.410
integrin complex	0.451	0.451	-0.662
response to steroid hormone stimulus	0.056	-0.188	0.043
regulation of cell growth	0.190	-0.952	-0.167
secretory granule	0.814	-0.831	-0.894
membrane-bounded vesicle	0.839	-0.973	-0.942
TGF-beta signaling pathway	0.889	-0.413	-0.417
complement activation, classical pathway	0.760	0.316	0.827
insulin-like growth factor binding	0.247	0.911	0.273
growth factor binding	0.484	0.990	0.503
blood vessel development	0.712	0.661	0.675
calcium ion homeostasis	0.576	0.866	0.670
cell adhesion	0.877	0.805	0.863
extracellular matrix	0.855	0.790	0.978
ECM-receptor interaction	0.799	0.816	0.978
collagen	0.631	0.722	0.968
extracellular matrix organization	0.819	0.867	0.982
tight junction	0.607	-0.642	-0.279
insulin receptor signaling pathway	0.965	0.733	0.536
peptide metabolic process	-0.250	0.813	-0.264
vitamin metabolic process	-0.745	0.456	-0.511
ER-Golgi intermediate compartment	0.900	-0.249	-0.629
Oxidative phosphorylation	-0.070	0.993	0.966
generation of precursor metabolites and energy	-0.185	0.767	0.780
Glycolysis / Gluconeogenesis	-0.468	0.585	0.778
Spliceosome	-0.295	0.979	0.984
cell division	-0.322	0.997	0.983
mitosis	-0.227	0.992	0.997
proteasomal protein catabolic process	-0.696	0.981	0.842
amine biosynthetic process	0.892	0.939	0.522
lipid biosynthetic process	-0.027	0.305	0.826

Investigation of diseased states

Placental abnormalities in SCNT pregnancies

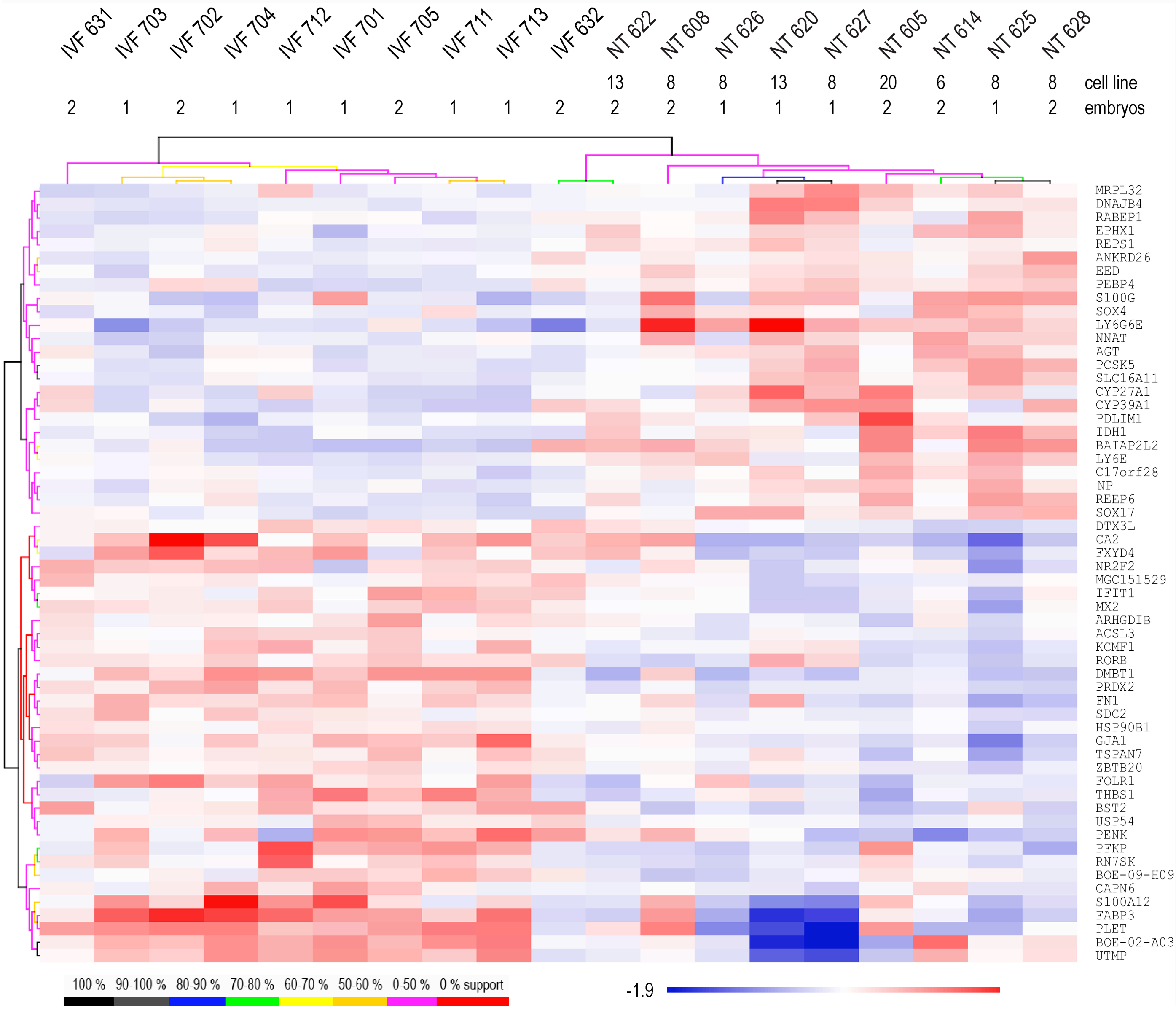
- High rate of pregnancy failure in recipients of cloned embryos has been linked to structural and functional abnormalities of the placenta [1].
- Fewer but larger placentomes in somatic cell nuclear transfer (SCNT) recipient cows at day 60 of gestation, suggesting abnormal placental/fetal growth in these animals [2]
- Placental abnormalities are thought to be the major cause of mortality in first-trimester SCNT cloned bovine fetuses



1. Hill et al., Biol Reprod 63, 1787-1794 (2000)
2. Hashizume et al., Cloning Stem Cells 4, 197-209 (2002)

Cluster analysis of differently abundant transcripts

- No correlation of expression patterns and clone origin
- Higher variance of expression values in the SCNT group
- Only few genes differentially regulated in endometrium of all SCNT pregnancies

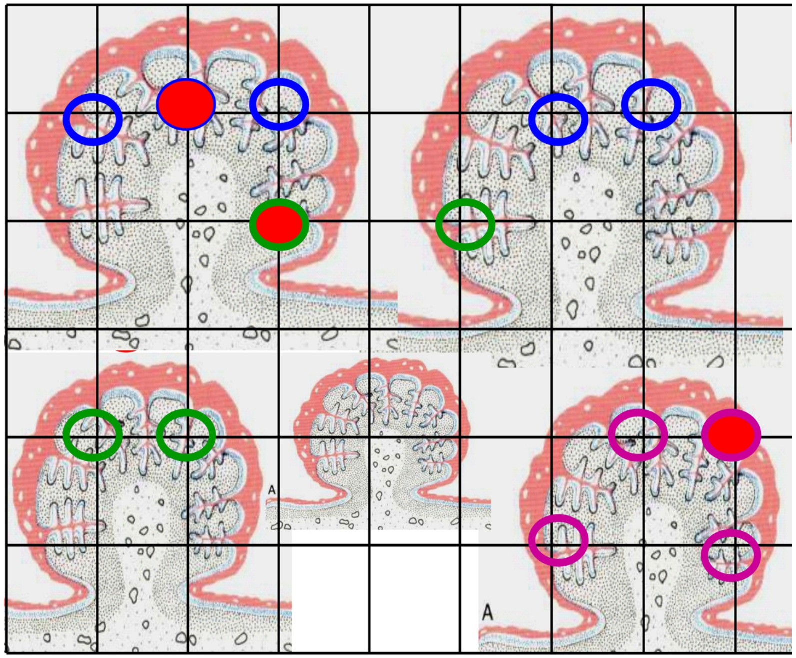


Support tree, MEV 4.1

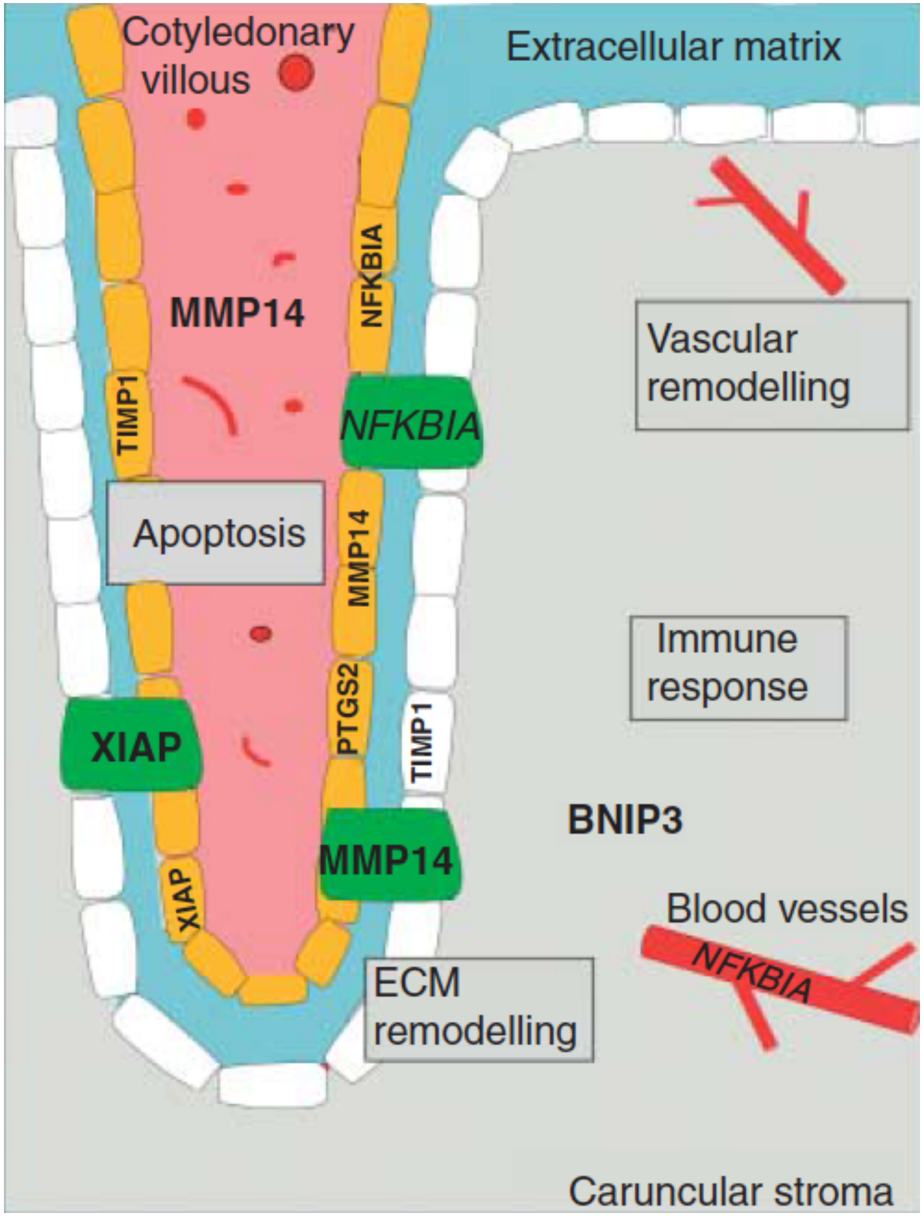
Bauersachs et al. PNAS 14: 5681-5686 (2009)

Release of fetal membranes

- Analysis of contact zones fetal and maternal tissue
- 7-10 d ante partum vs. 15 min after calving
- >1.200 differentially expessed genes



Streyl *et al.*, *Reproduction* (2011)



Mastitis

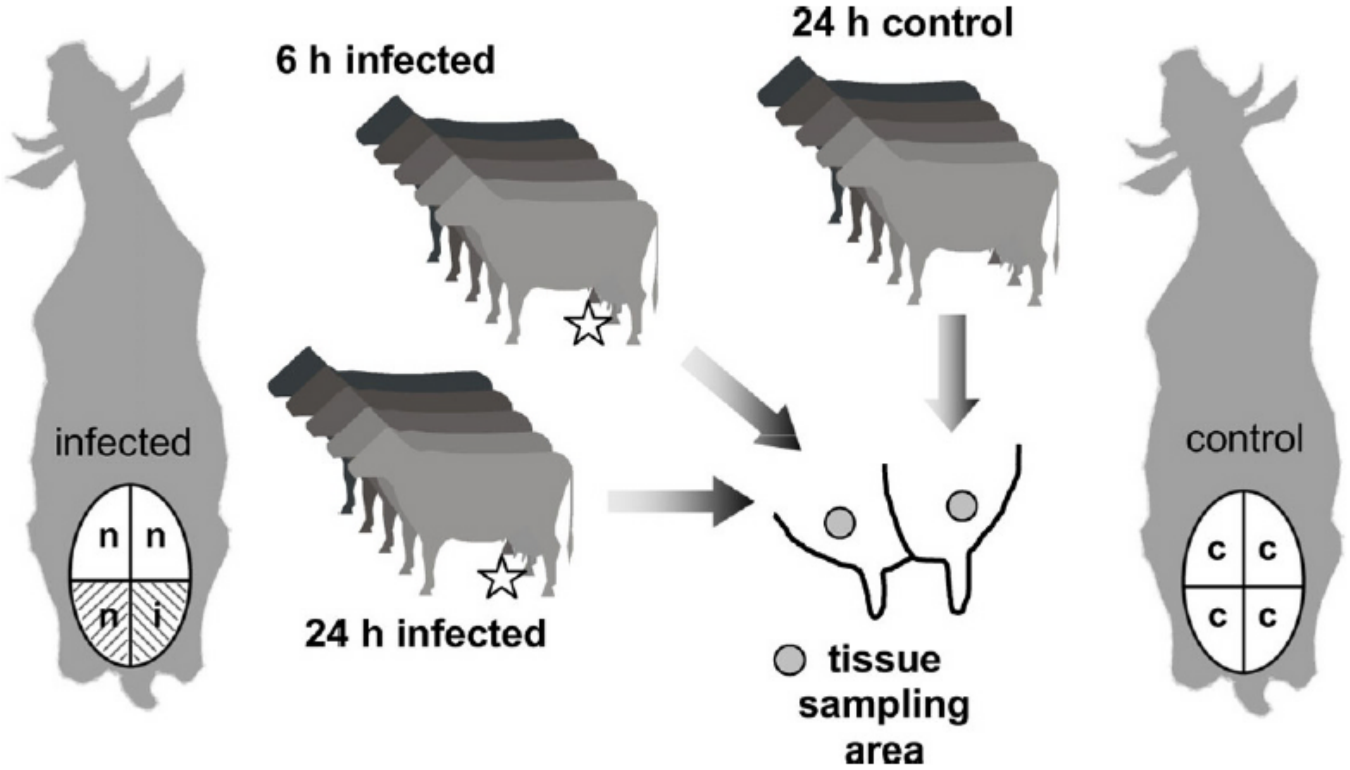


Figure 1 Experimental setup of infection with *E. coli* 1303. During estrus, 10 synchronized dairy cows were infected on one quarter with *E. coli* 1303 (star, i) in saline solution (shaded). One of the neighboring quarters (n) was treated with sterile saline solution (shaded). Five synchronized healthy animals were slaughtered 24 h after onset of estrus and served as external control (c). Tissue samples were collected from marked areas of the bovine udder.

Mitterhuemer et al. BMC Genomics 2010

Regulation of immune response genes

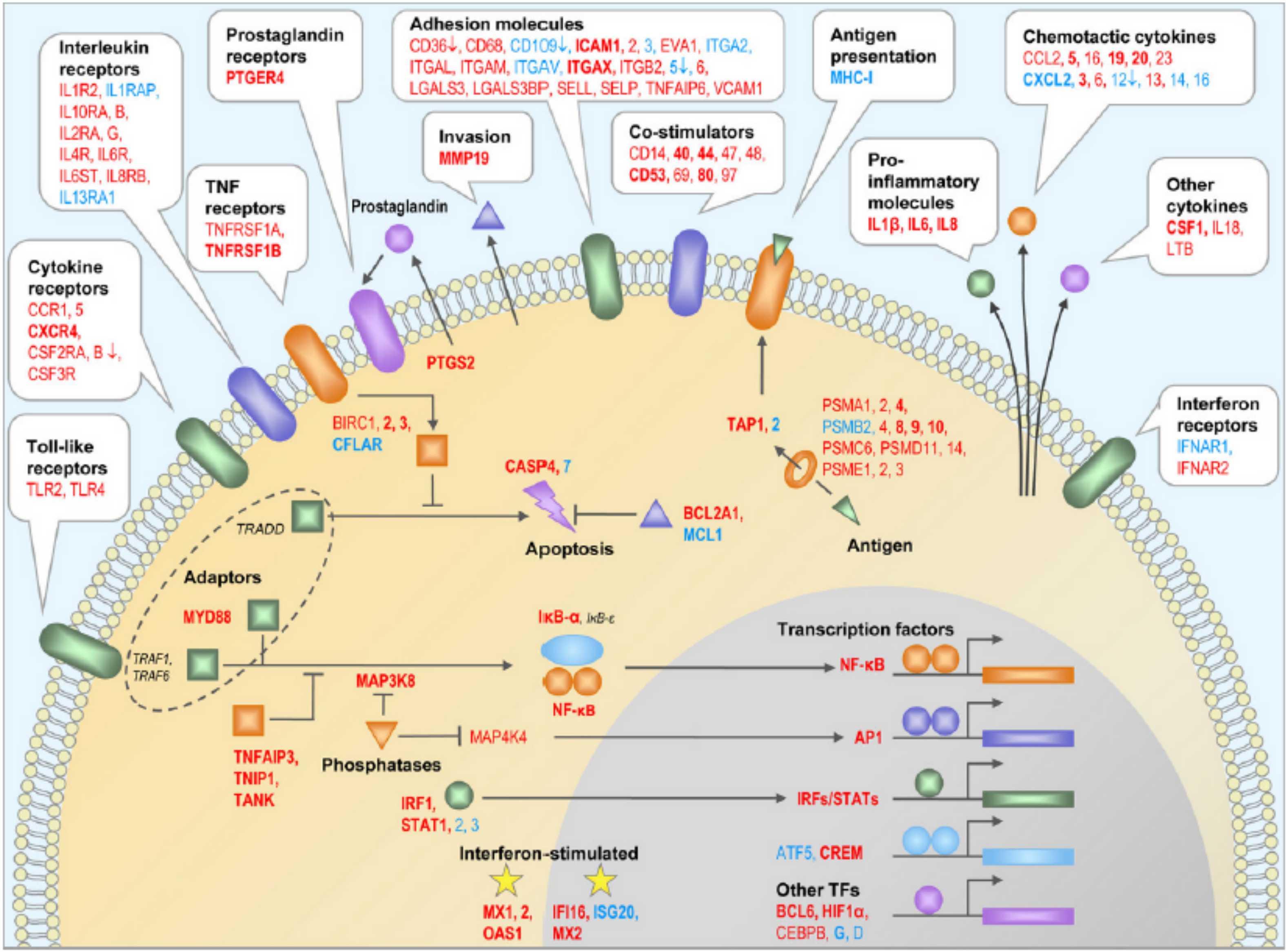
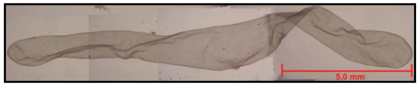


Figure 6 Local and systemic immune response of the bovine udder 24 h after infection. A graphical representation of a robust response

Mitterhuemer et al. BMC Genomics 2010

New modes of embryo-maternal interaction

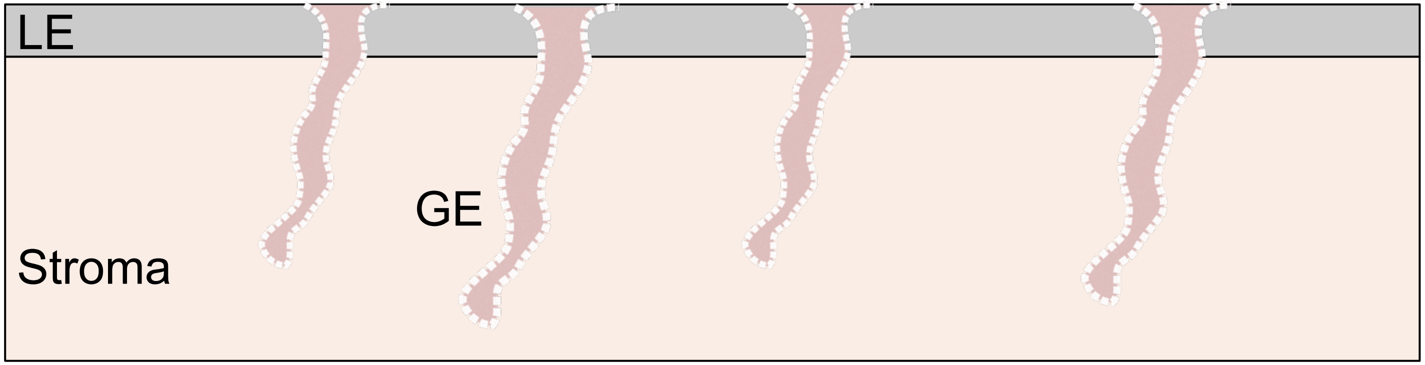
Classical model for recognition and establishment of pregnancy



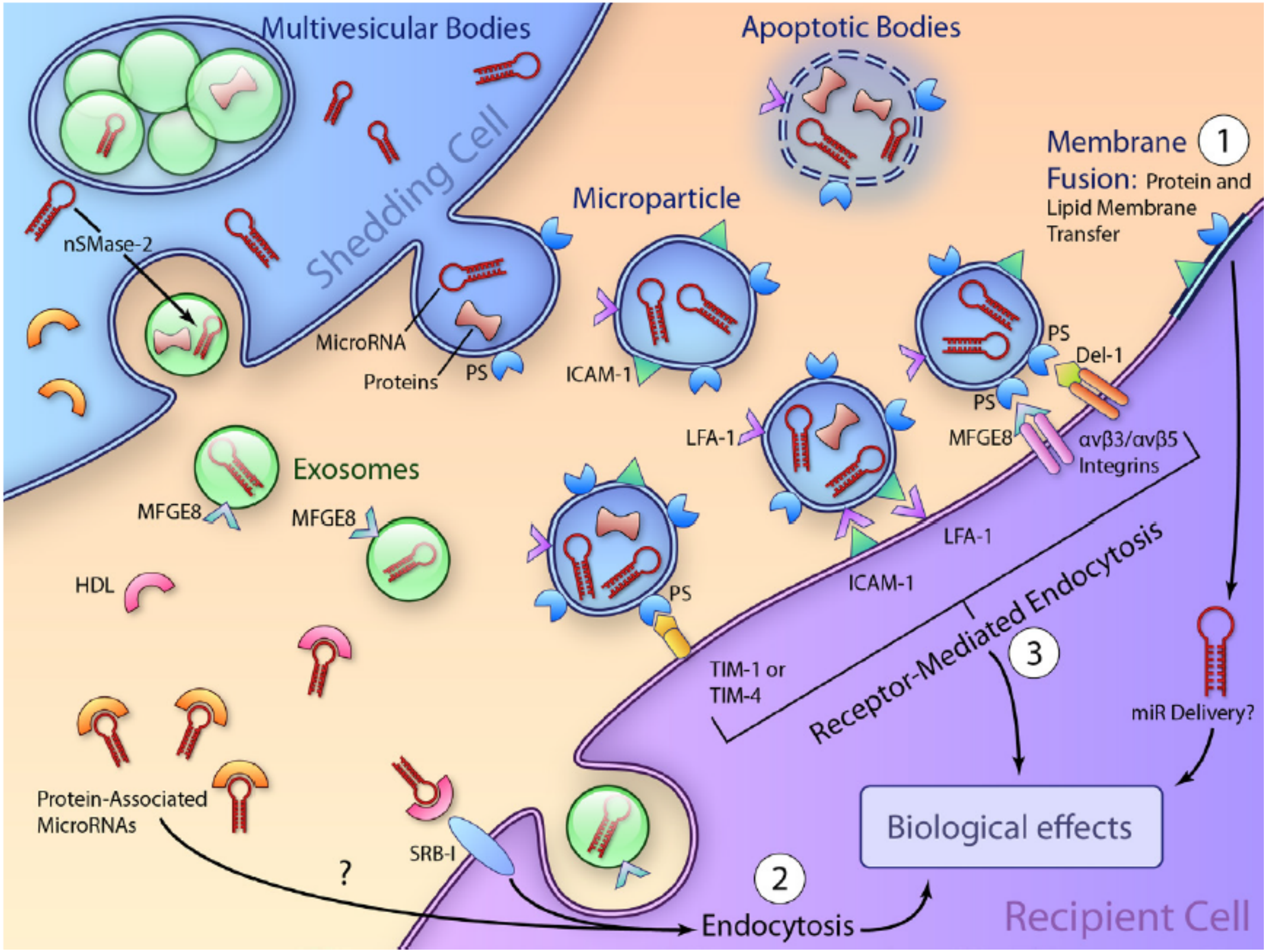
↓ signaling molecule(s) for MRP and preparation for implantation

secreted to the uterine lumen

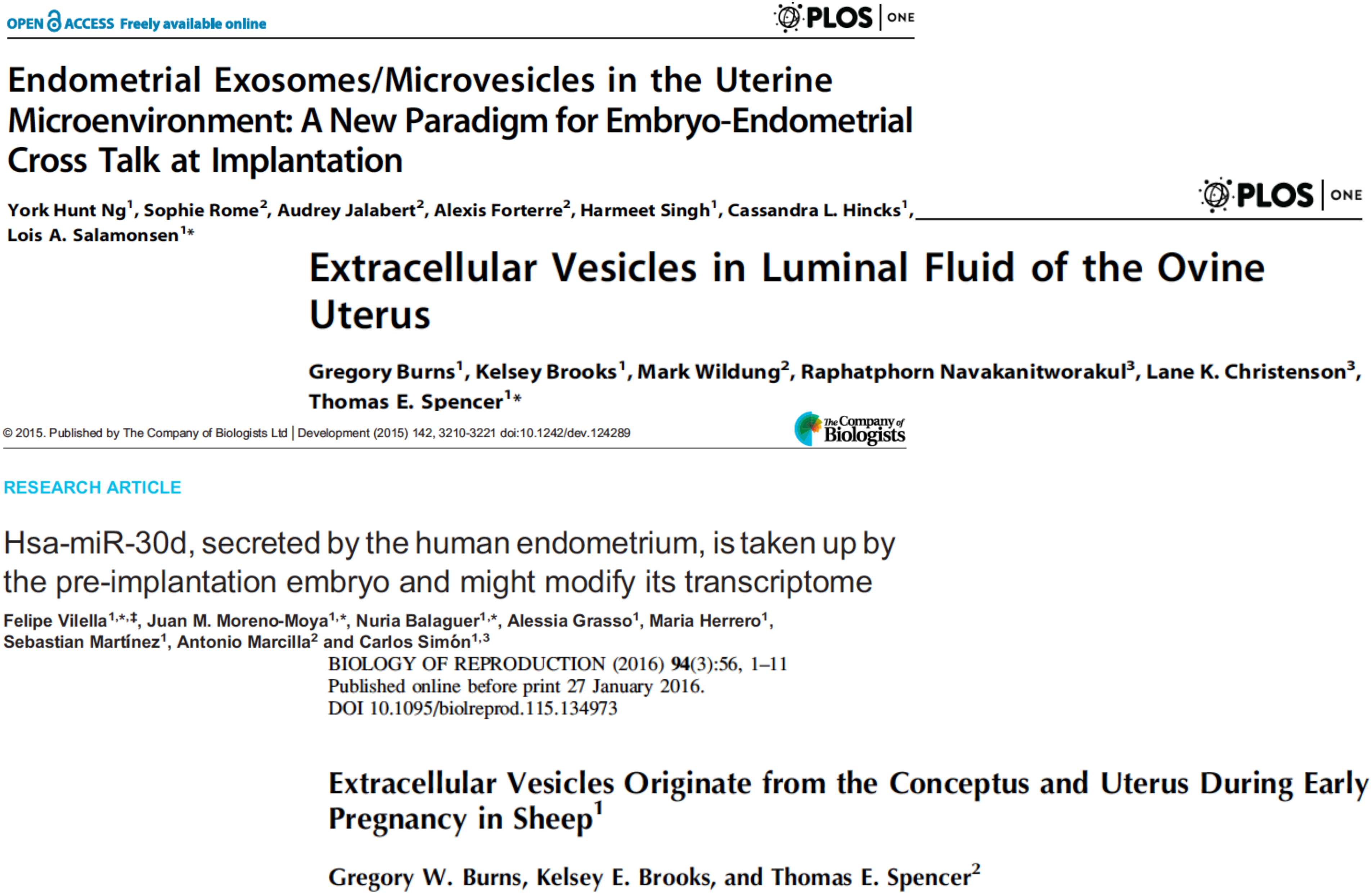
↑ growth factors and nutrients



Exosomes in embryo-maternal interaction



Exosome research in the reproductive tract



OPEN ACCESS Freely available online

PLOS | ONE

Endometrial Exosomes/Microvesicles in the Uterine Microenvironment: A New Paradigm for Embryo-Endometrial Cross Talk at Implantation

York Hunt Ng[1], Sophie Rome[2], Audrey Jalabert[2], Alexis Forterre[2], Harmeet Singh[1], Cassandra L. Hincks[1], Lois A. Salamonsen[1]*

PLOS | ONE

Extracellular Vesicles in Luminal Fluid of the Ovine Uterus

Gregory Burns[1], Kelsey Brooks[1], Mark Wildung[2], Raphatphorn Navakanitworakul[3], Lane K. Christenson[3], Thomas E. Spencer[1]*

© 2015. Published by The Company of Biologists Ltd | Development (2015) 142, 3210-3221 doi:10.1242/dev.124289

The Company of Biologists

RESEARCH ARTICLE

Hsa-miR-30d, secreted by the human endometrium, is taken up by the pre-implantation embryo and might modify its transcriptome

Felipe Vilella[1,*,‡], Juan M. Moreno-Moya[1,*], Nuria Balaguer[1,*], Alessia Grasso[1], Maria Herrero[1], Sebastian Martínez[1], Antonio Marcilla[2] and Carlos Simón[1,3]

BIOLOGY OF REPRODUCTION (2016) **94**(3):56, 1–11
Published online before print 27 January 2016.
DOI 10.1095/biolreprod.115.134973

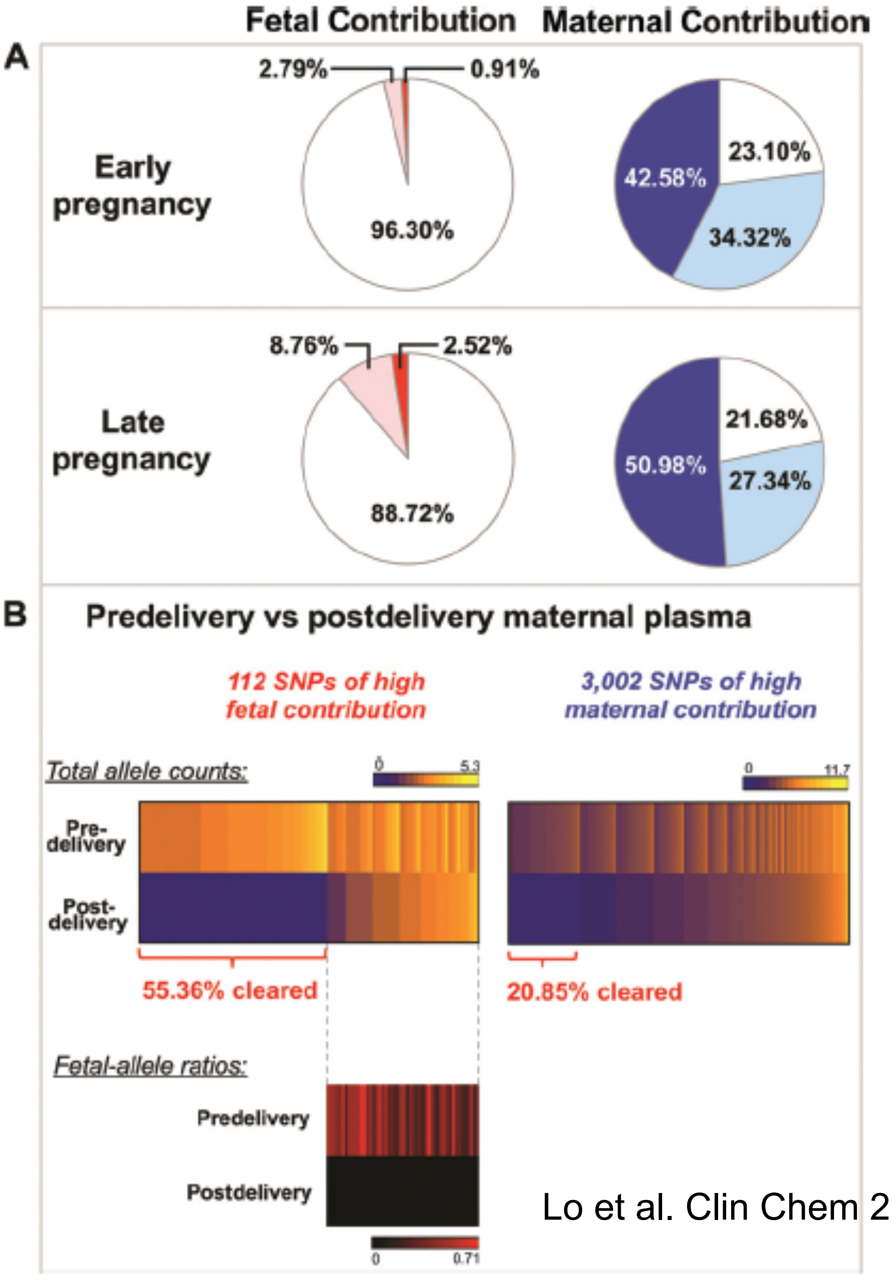
Extracellular Vesicles Originate from the Conceptus and Uterus During Early Pregnancy in Sheep[1]

Gregory W. Burns, Kelsey E. Brooks, and Thomas E. Spencer[2]

Maternal plasma RNA sequencing for genome-wide transcriptomic profiling and identification of pregnancy-associated transcripts

- Plasma transcriptome profiling in first-, second- and third-trimester pregnant women, and after delivery
- Genotyping of amniotic fluid, placental tissues, and maternal blood cells using exome-enriched sequencing
- Relative proportions of fetal and maternal contributions to plasma RNAs by examination of polymorphic differences
- Identification of pregnancy-associated genes by direct examination of the maternal plasma transcriptomic profiles before and after delivery

Lo et al. Clin Chem 2014



Lo et al. Clin Chem 2014

Fig. 1. Fetal and maternal contributions in maternal plasma transcriptome.

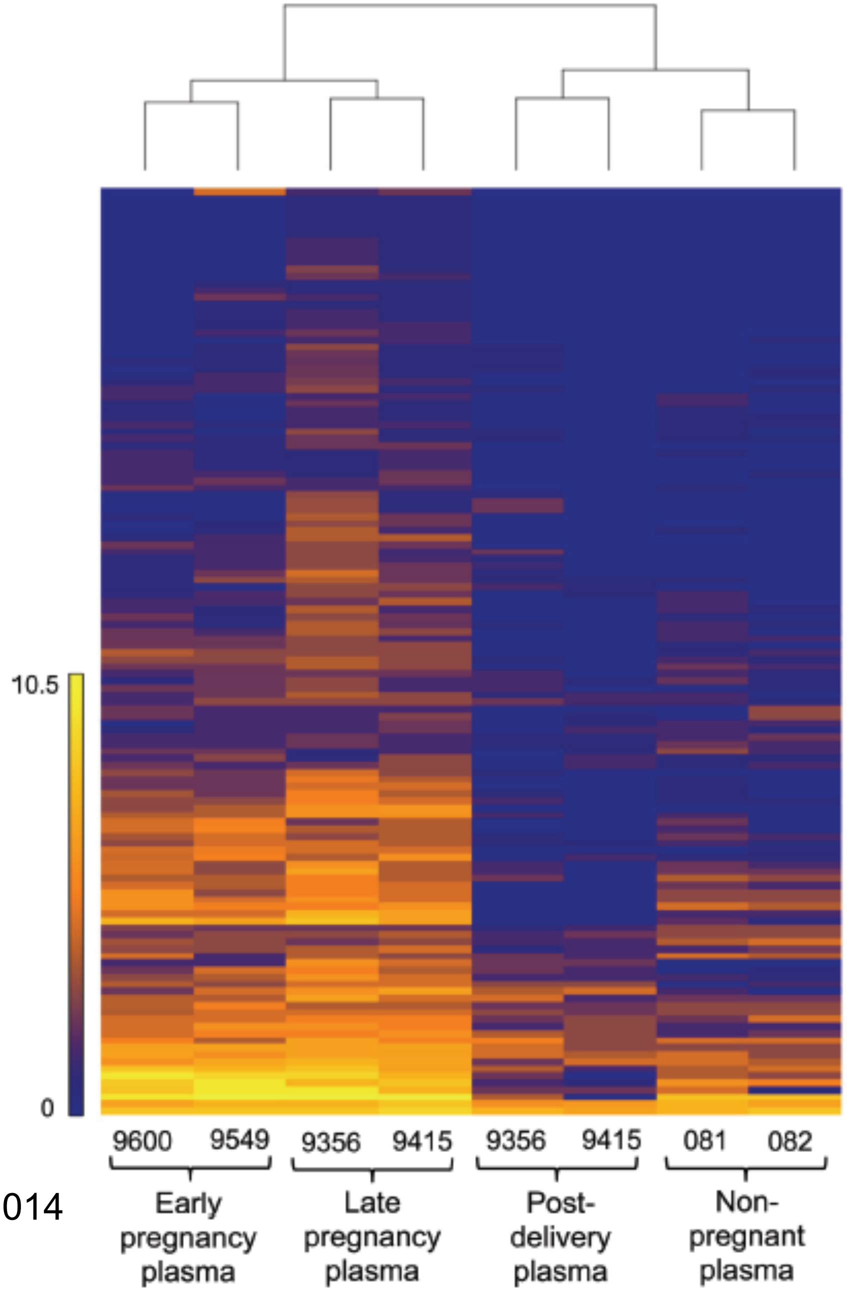


Fig. 2. Hierarchical clustering of plasma samples using the 131 pregnancy-associated genes.

Maternal plasma RNA sequencing for genome-wide transcriptomic profiling and identification of pregnancy-associated transcripts

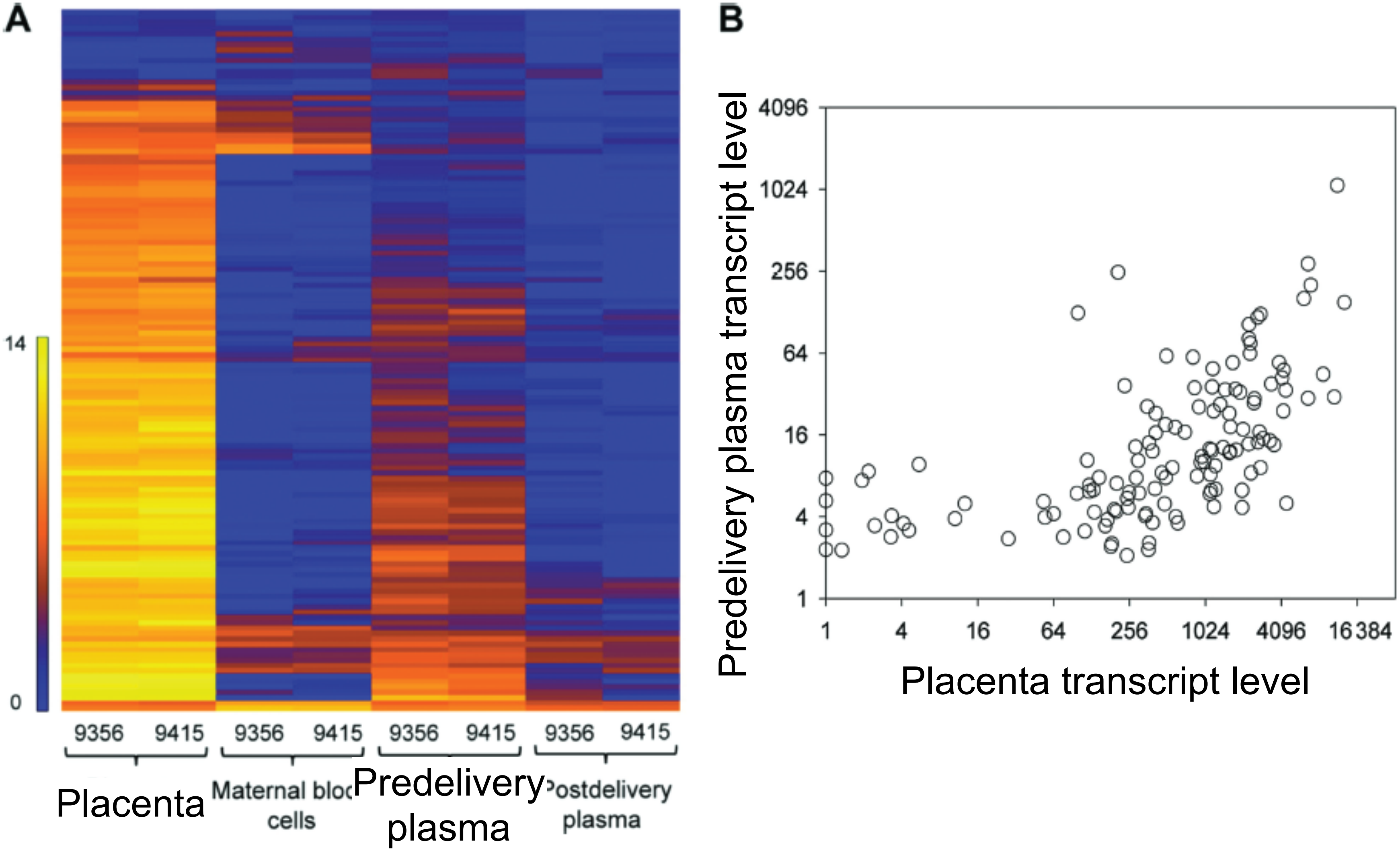


Fig. 3. Expression levels of 131 pregnancy-associated genes in the placenta, maternal blood cells, and maternal plasma.

Lo et al. Clin Chem 2014

Maternal plasma transcriptome analysis as a new tool for monitoring pregnancies?

- Analysis of circulating nucleic acids as a novel noninvasive tool for prenatal diagnosis of genetic diseases
- Monitoring of pregnancy-associated disorders based on the expression of placenta-derived transcripts present in plasma
- In addition, analysis of complete fetal genome in maternal plasma possible
- In combination with new sequencing technologies on-site genome analysis possible

Bauersachs Clin Chem 2014

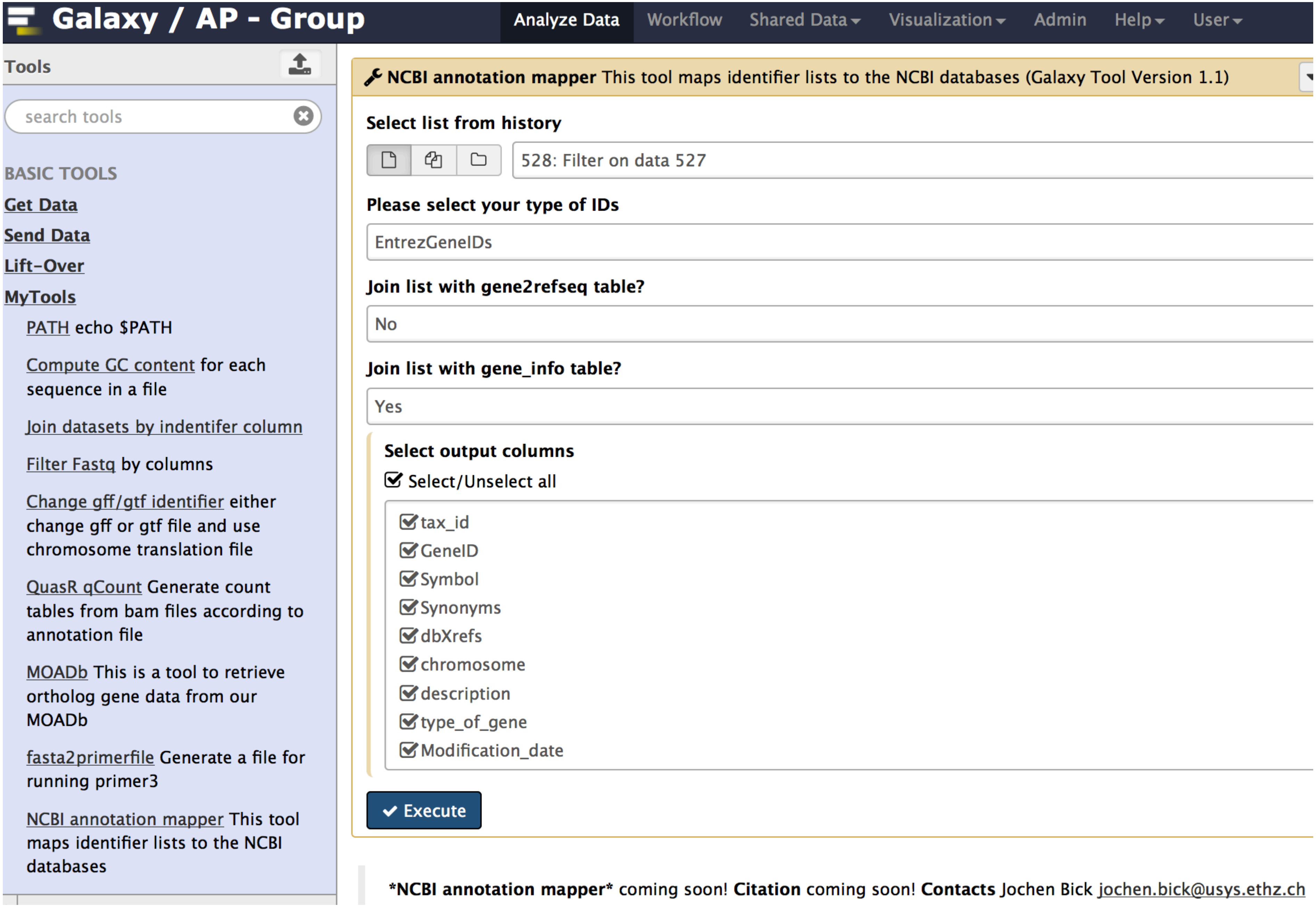
Analysis of exosomes derived from body fluids as biomarkers for physiological processes and for diseases

Analysis of protein or RNA markers of exosomes isolated from:

- Blood plasma
- Urine
- Milk

Ongoing research and future perspectives

“Deep annotation”



BASIC TOOLS

Get Data

Send Data

Lift-Over

MyTools

- PATH echo $PATH
- Compute GC content for each sequence in a file
- Join datasets by indentifer column
- Filter Fastq by columns
- Change gff/gtf identifier either change gff or gtf file and use chromosome translation file
- QuasR qCount Generate count tables from bam files according to annotation file
- MOADb This is a tool to retrieve ortholog gene data from our MOADb
- fasta2primerfile Generate a file for running primer3
- NCBI annotation mapper This tool maps identifier lists to the NCBI databases

NCBI annotation mapper This tool maps identifier lists to the NCBI databases (Galaxy Tool Version 1.1)

Select list from history

528: Filter on data 527

Please select your type of IDs

EntrezGeneIDs

Join list with gene2refseq table?

No

Join list with gene_info table?

Yes

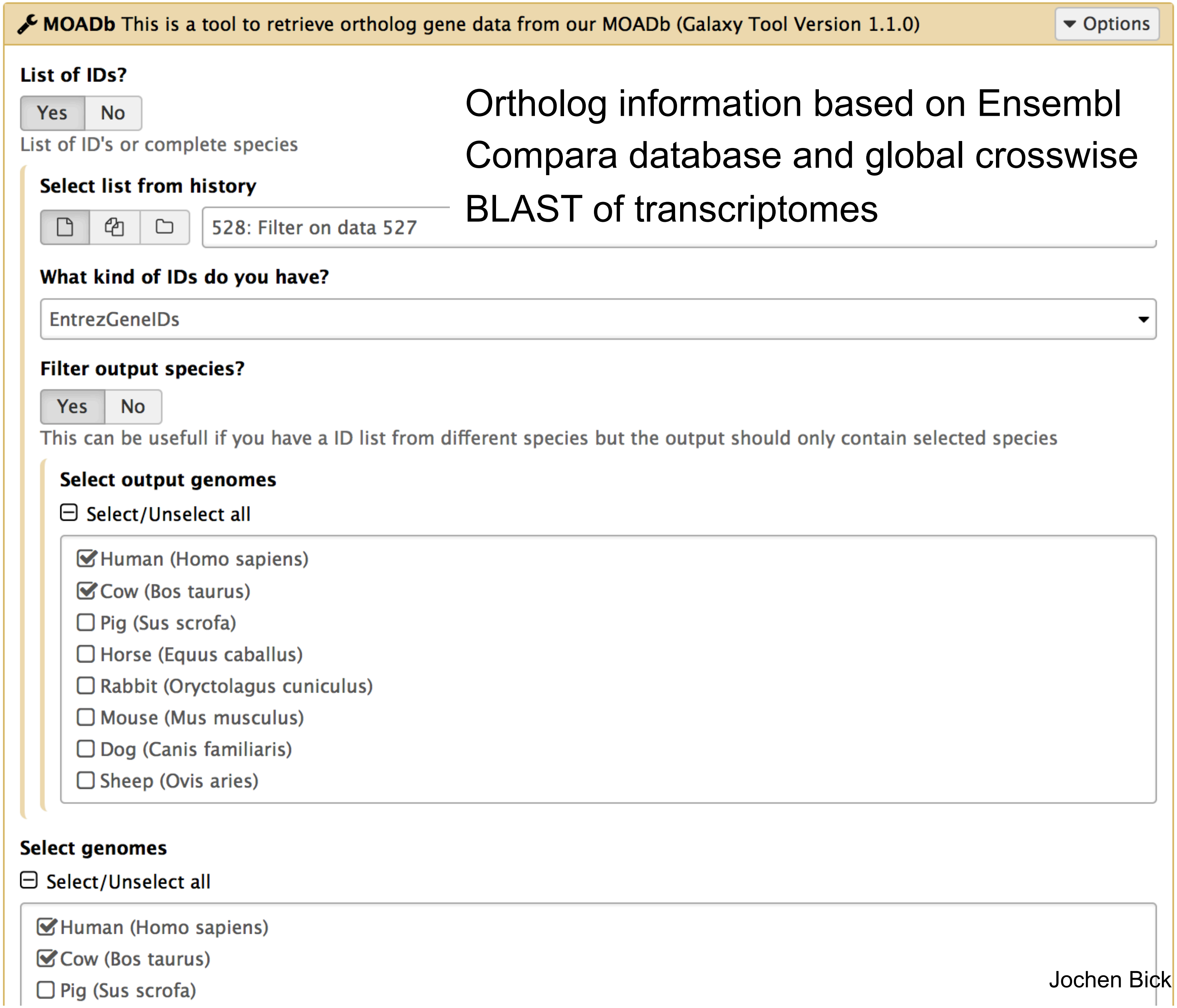
Select output columns

☑ Select/Unselect all

- ☑ tax_id
- ☑ GeneID
- ☑ Symbol
- ☑ Synonyms
- ☑ dbXrefs
- ☑ chromosome
- ☑ description
- ☑ type_of_gene
- ☑ Modification_date

NCBI annotation mapper coming soon! **Citation** coming soon! **Contacts** Jochen Bick jochen.bick@usys.ethz.ch

Mammalian ortholog and annotation database



MOADb This is a tool to retrieve ortholog gene data from our MOADb (Galaxy Tool Version 1.1.0)

List of IDs?

[✓ Yes] No

List of ID's or complete species

Select list from history

528: Filter on data 527

What kind of IDs do you have?

EntrezGeneIDs

Filter output species?

[✓ Yes] No

This can be usefull if you have a ID list from different species but the output should only contain selected species

Select output genomes

Select/Unselect all

- ☑ Human (Homo sapiens)
- ☑ Cow (Bos taurus)
- ☐ Pig (Sus scrofa)
- ☐ Horse (Equus caballus)
- ☐ Rabbit (Oryctolagus cuniculus)
- ☐ Mouse (Mus musculus)
- ☐ Dog (Canis familiaris)
- ☐ Sheep (Ovis aries)

Select genomes

Select/Unselect all

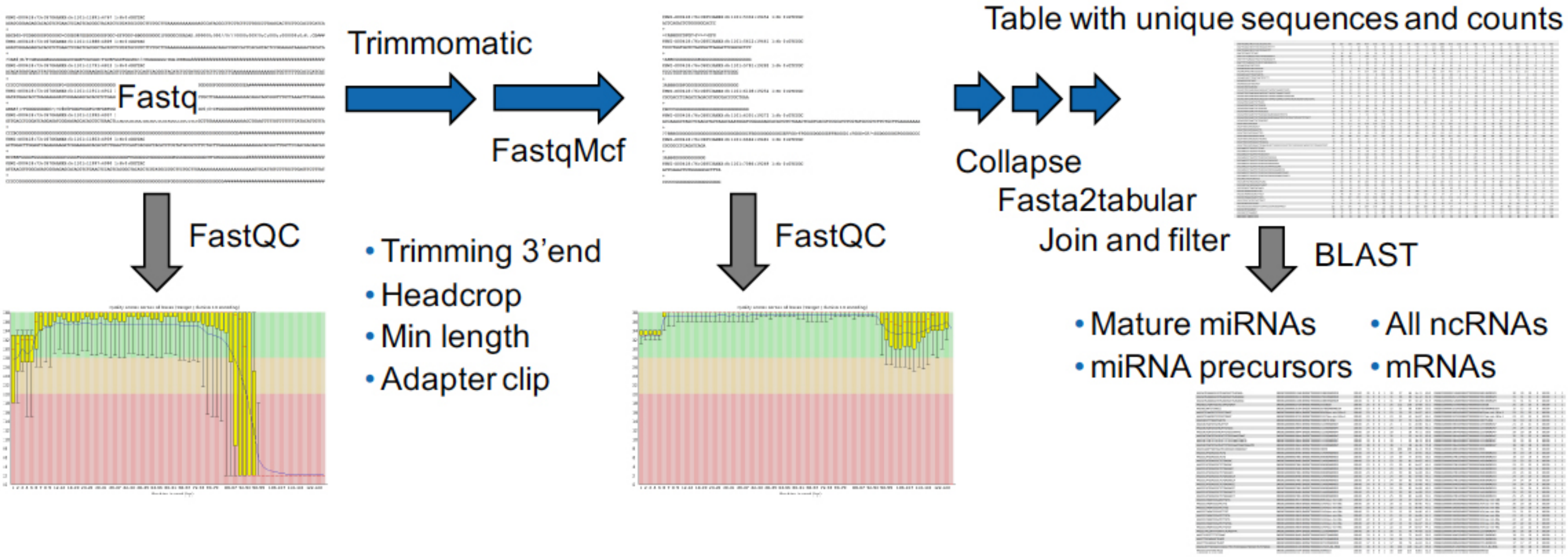
- ☑ Human (Homo sapiens)
- ☑ Cow (Bos taurus)
- ☐ Pig (Sus scrofa)

Ortholog information based on Ensembl Compara database and global crosswise BLAST of transcriptomes

Jochen Bick unpublished

Tailored data analysis pipelines

Analysis of smallRNA-Seq data from cattle and pig



Fastq

Trimmomatic

FastqMcf

FastQC

- Trimming 3’end
- Headcrop
- Min length
- Adapter clip

FastQC

Collapse

Fasta2tabular

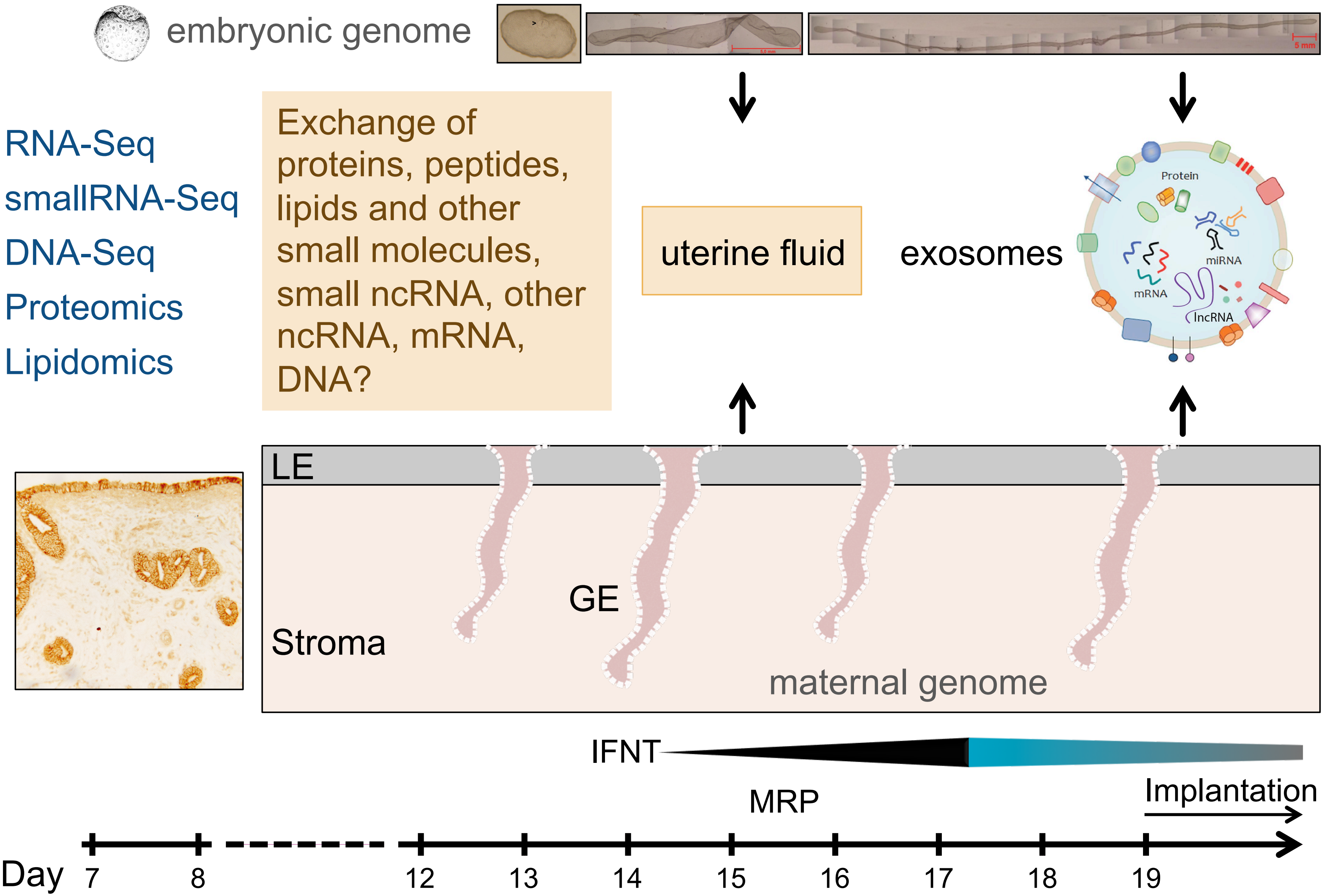
Join and filter

Table with unique sequences and counts

BLAST

- Mature miRNAs
- miRNA precursors
- All ncRNAs
- mRNAs

Systems biology approach of embryo-maternal interaction



embryonic genome

- RNA-Seq
- smallRNA-Seq
- DNA-Seq
- Proteomics
- Lipidomics

Exchange of proteins, peptides, lipids and other small molecules, small ncRNA, other ncRNA, mRNA, DNA?

uterine fluid

exosomes