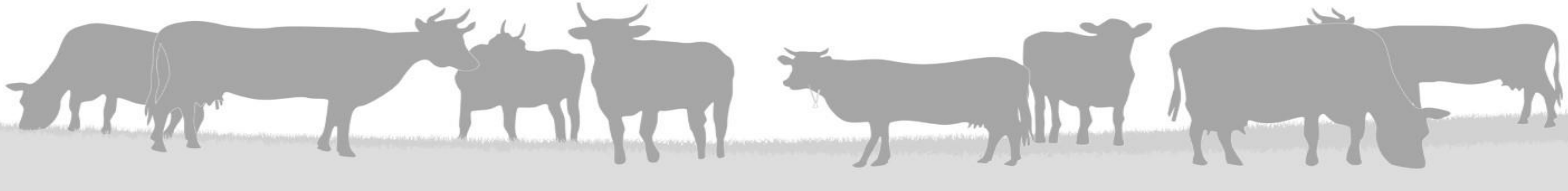
GplusE Genotype plus Environment
Integration for a more sustainable dairy production system

Royal Veterinary College
University of London

Associations between the blood transcriptomic profile in early lactation and subsequent fertility in Holstein dairy cows

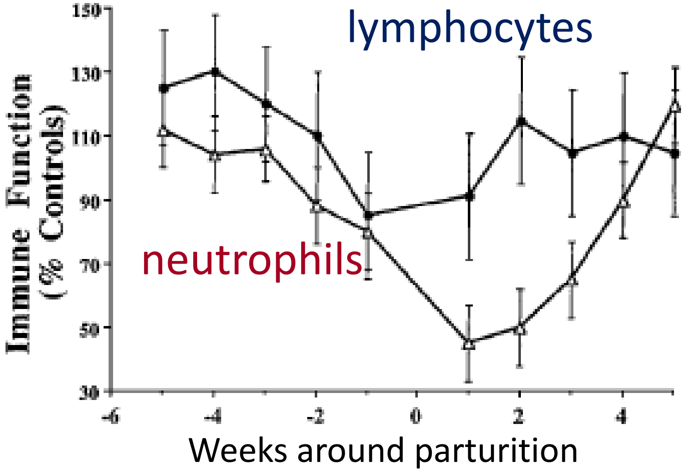
D C Wathes and GplusE consortium

Royal Veterinary College, London, UK

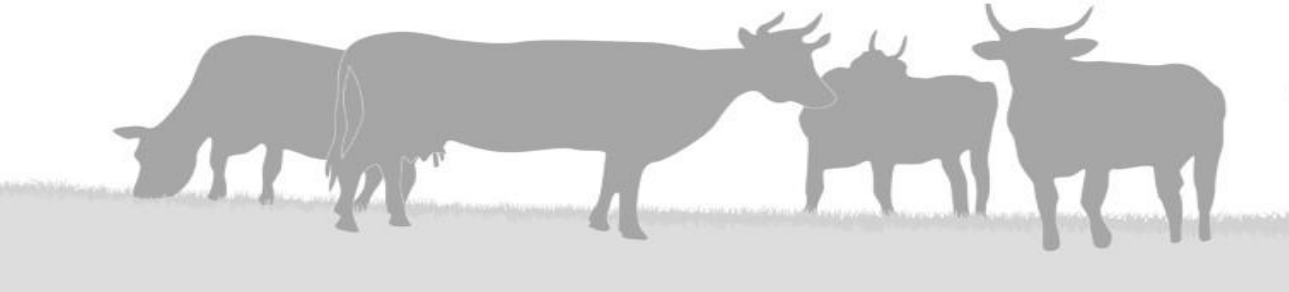


Introduction

In dairy cows immune status is suppressed around calving and circulating PMNs have reduced respiratory burst activity and cytotoxicity.

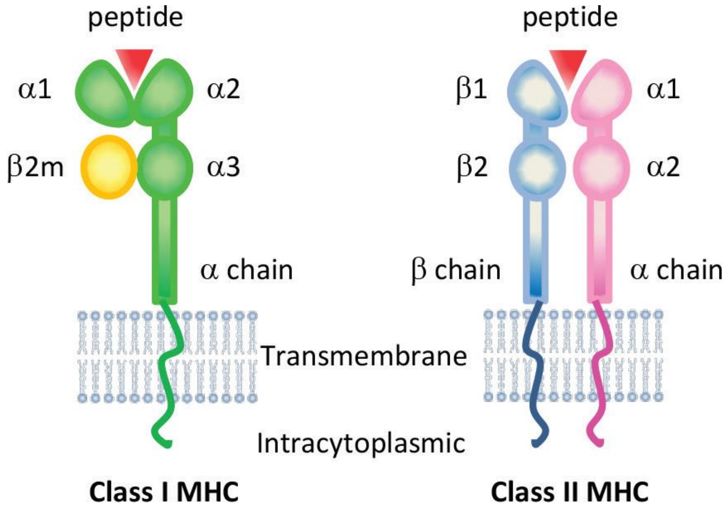


Goff & Horst (1997) JDS 80:1260



Bovine leukocyte antigen: BoLA

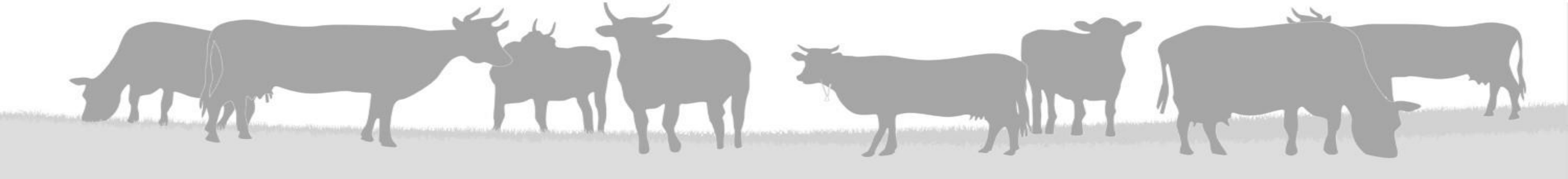
- The major histocompatibility complex (MHC) is a set of highly polymorphic genes that bind pathogen-derived peptides and present them for recognition by specialized T cells.
- Following pathogen recognition, T cells and B cells are activated, expanded and differentiated into effector cells.
- In cattle, this region is called the BoLA gene locus and contains over 150 predicted functional genes on BtA_23, subdivided into three gene classes:
  - BoLA class I and II both play a role in antigen presentation.
  - BoLA class III is associated with the complement system



Gutierrez et al. (2017) http://www.intechopen.com/books/trends-and-advances-inveterinary-genetics

AIM

- Investigate the link between immune function in early lactation and subsequent fertility in Holstein dairy cows

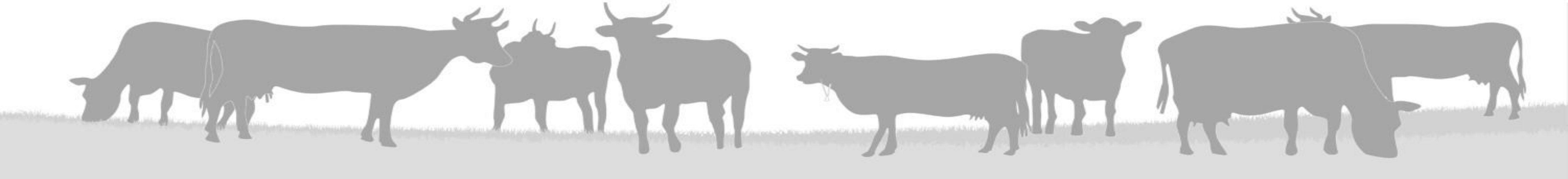


Materials and Methods 1: Cows and samples

Study 1. Single UK commercial dairy herd. All year round calving. About 400 cows in total of which 100 had samples taken for analysis. Blood samples taken into Tempus tubes at 8±6 days in milk (DIM, mean±SD).

Study 2. Total of 230 cows included, taken from 6 experimental herds in different EU countries (Belgium, Denmark, Germany, Ireland, Italy, UK). Blood samples taken into Tempus tubes at 14±3 DIM.

White blood cells (leucocytes) processed for total RNA-Seq using Illumina NextSeq 500 platform.



Materials and Methods: RNA-Seq

Total RNA from WBC extracted using Tempus Spin RNA isolation Kits and used for library preparation

Study 1

Samples analysed by Novogene (HK) Company Ltd.

250~300 bp insert strand specific library with rRNA removal using a Globin-zero Gold rRNA removal kit.

cDNA Libraries sequenced at 75nt to reach 30 million pair end reads/sample using Illumina PE150

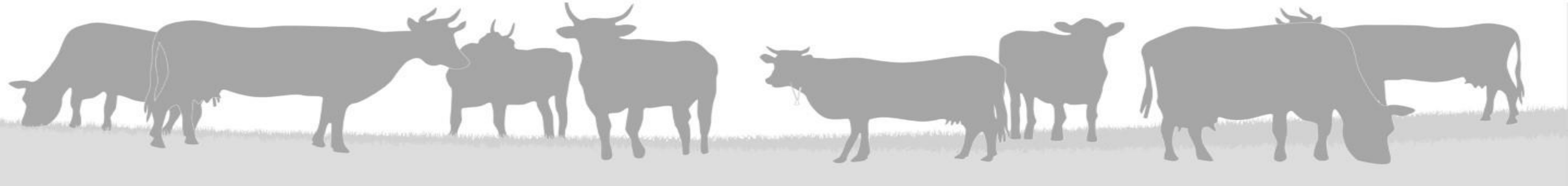
Study 2

Samples analysed by University of Liege (GIGA Research Facility).

Illumina TruSeq Stranded Total RNA Library Prep Ribo-Zero Gold kit

cDNA Libraries sequenced at 75nt to reach 30 million single end reads/sample using Illumina NextSeq 500 platform

- Fastq raw files were quality controlled and mapped onto *Bos taurus* ARS-UCD1.2
- Picard Tools used to remove PCR duplicates, sort by chromosome and create indexes.
- Reads per gene were counted with StringTie.
- Data normalized to RPKM (reads per kilobase of exon model per million reads mapped).

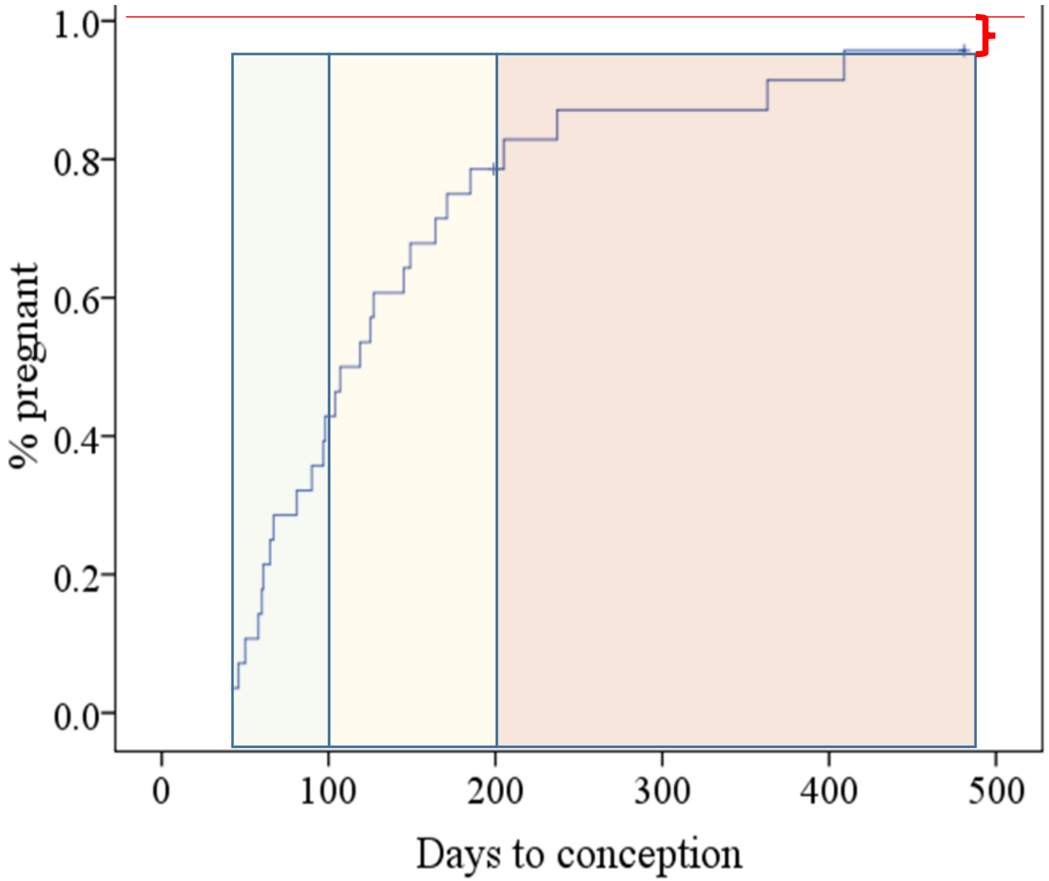


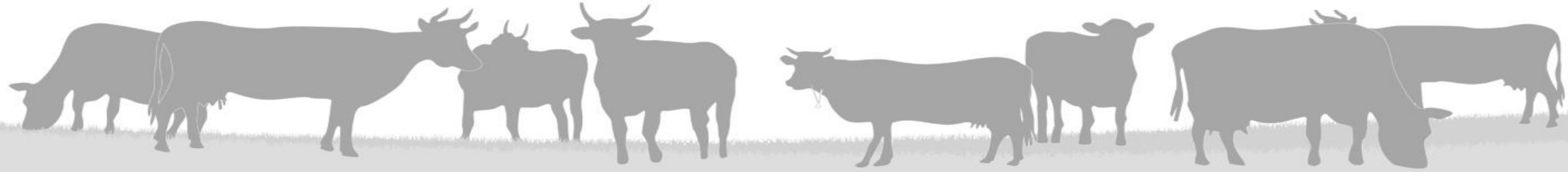
Materials and Methods: Fertility phenotypes

All service data were recorded. Monitoring continued until each cow was either in calf or classified as failed to conceive.

For subsequent analysis the cows were divided into four "in calf by" (ICB) categories:

1. Conceived at <100 DIM (Good fertility)
2. Conceived at 100-200 DIM (Medium fertility)
3. Conceived at >200 DIM (Poor fertility)
4. Failed to conceive despite being served at least once (Not fertile)





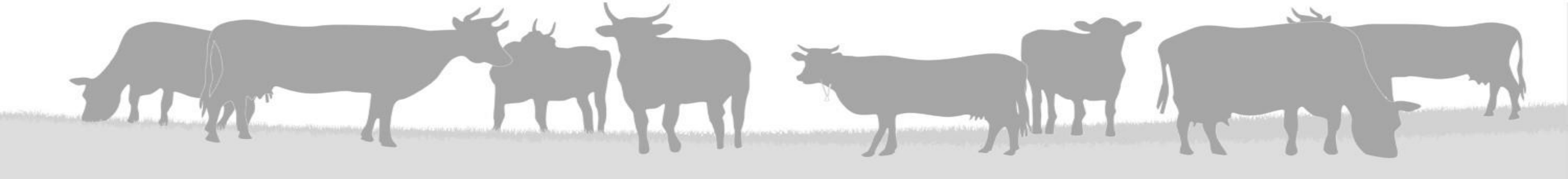
Materials and Methods: Data analysis

- In Study 2, the herd effect was removed using Partek Genomics Suite (PGS) Software V7.1.

- One way or mixed model ANOVA using log2 transformed data performed to compare ICB1 vs ICB2. In mixed model, age was included as a random effect.

- Differentially expressed genes (DEG) reaching a significance level of $P<0.05$ with a fold change >1.2 were selected.

- DEG list analysed using Panther and DAVID pathway analysis

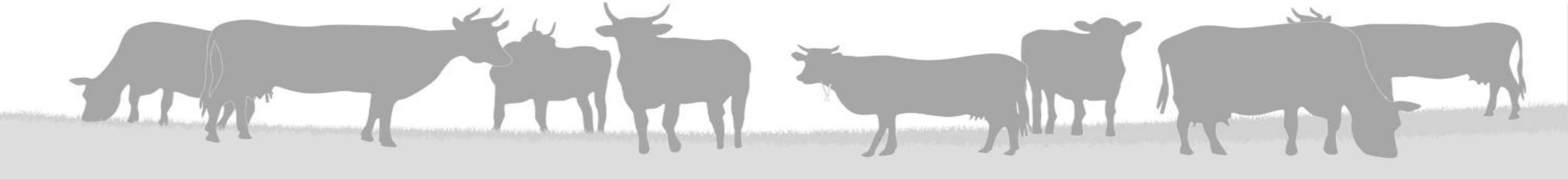


Study 1: One UK herd - Fertility data according to “in calf by” (ICB) category

	ICB1	ICB2	ICB3	ICB4	P
n	27 (31%)	34 (38%)	11 (12%)	20 (23%)	
Days open range	42 - 98	102 - 194	209 - 269	DNC	
Days open mean	72 ± 3.3^{a}	142 ± 5.1^{b}	243 ± 6.3^{c}	DNC	0.000†
S/C	1.4 ± 0.10^{a}	3.2 ± 0.23^{b}	6.0 ± 0.47^{c}	DNC	0.000
Lactation no.	1.8 ± 0.15^{a}	3.1 ± 0.28^{b}	2.1 ± 0.50^{a}	2.2 ± 0.28^{a}	0.002

Groups used in DEG comparison

a<b<c. † Determined using log values. Values are mean ± SEM.
DNC = did not conceive

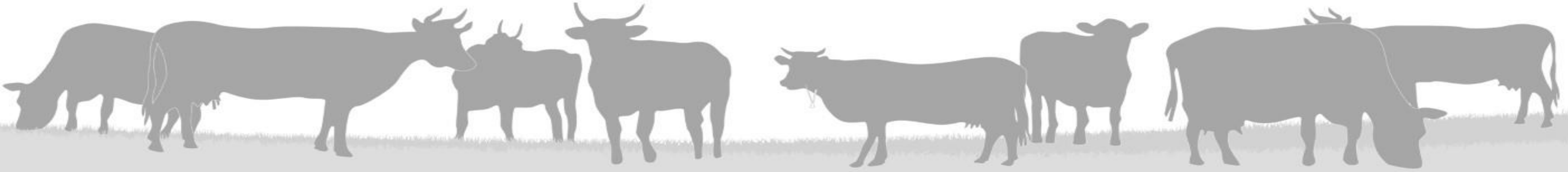


Study 2: Six EU herds - Fertility data according to “in calf by” (ICB) category

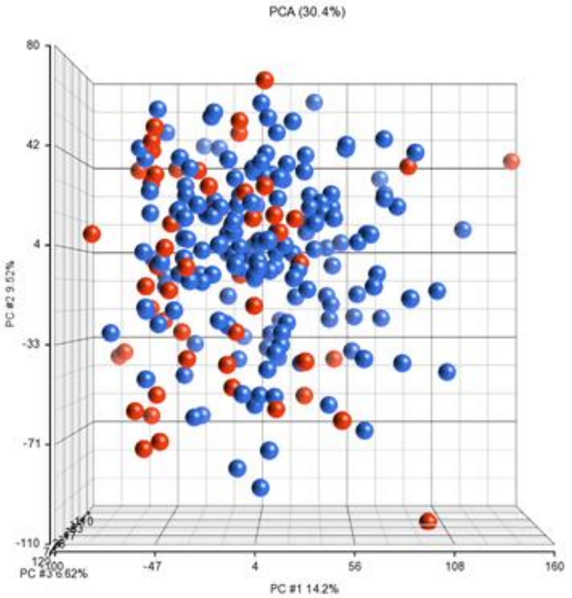
	ICB1	ICB2	ICB3	ICB4	P
n	82 (36%)	51 (23%)	26 (11%)	66 (29%)	
Days open range	42 - 99	100 - 198	201 - 528	DNC	
Days open	75 ± 1.5^a	142 ± 4.2^b	275 ± 13.4^c	DNC	0.000†
S/C	1.4 ± 0.07^a	2.4 ± 0.16^b	3.6 ± 0.40^c	DNC	0.000
Lactation no.	2.5 ±0.15^a	2.5 ± 0.18^a	2.5 ± 0.22^a	3.1 ± 0.20^b	0.033

Groups used in DEG comparison

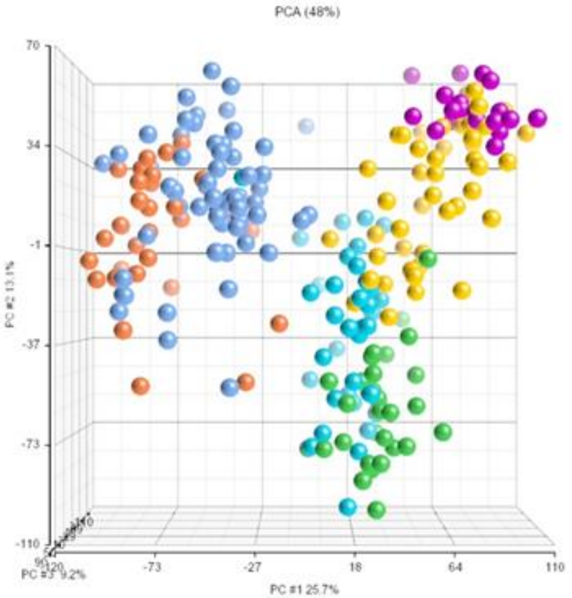
a<b<c. † Determined using log values. DNC = did not conceive



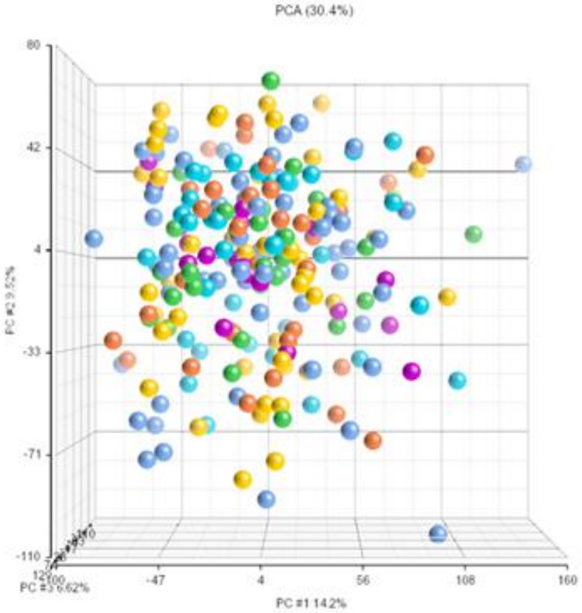
Results – Study 2, PCA analysis



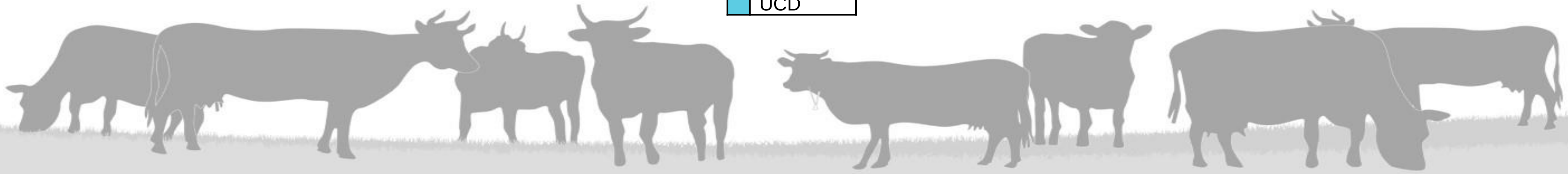
	Parity
🟦	MP
🟥	PP



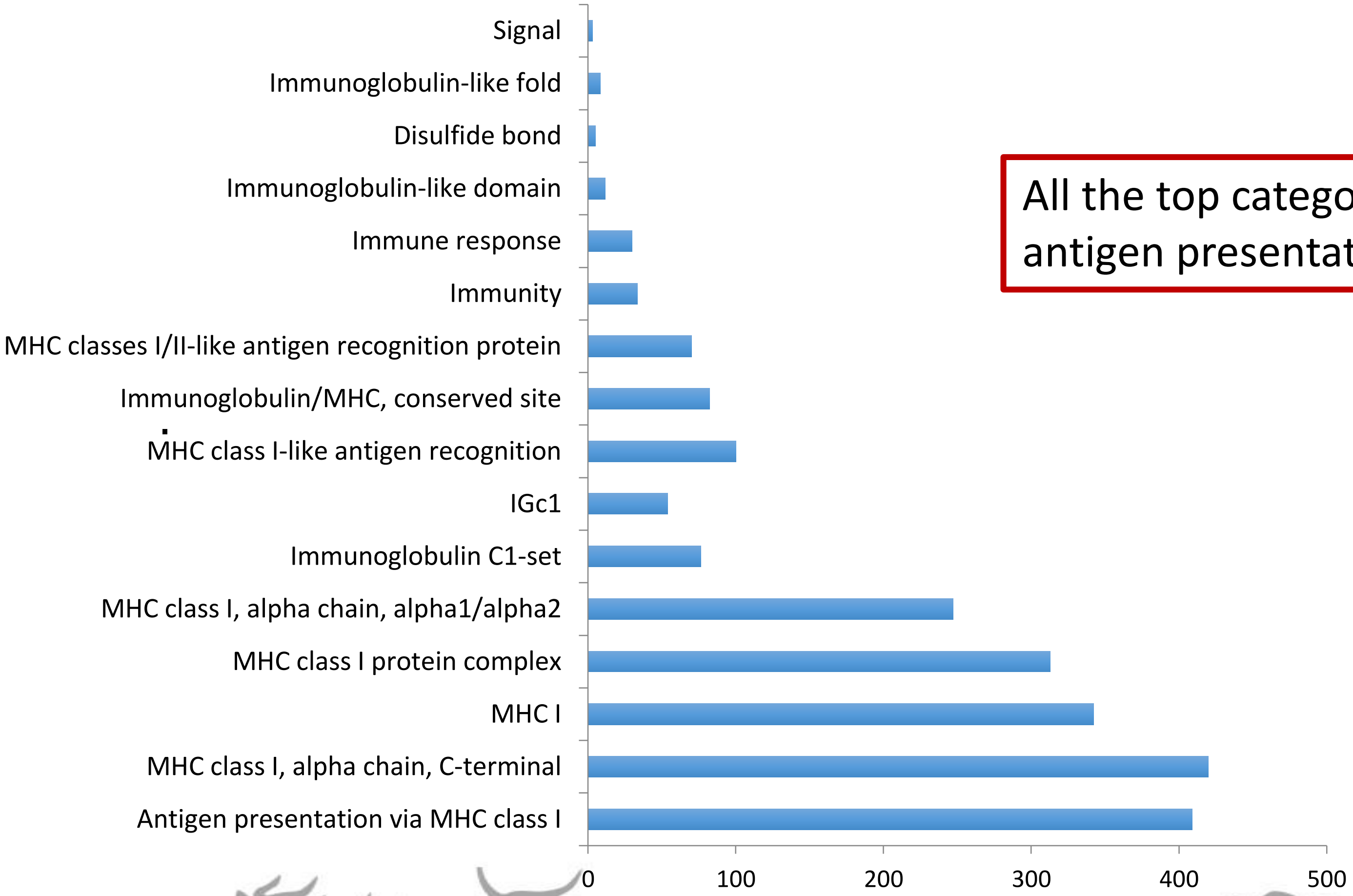
	Herd
🟦	AFBI
🟧	AU
🟨	CRA Italy
🟩	CRA-W
🟪	FBN
🟦	UCD



Herd effect removed using
Partek Genomics Suite
(PGS) Software V7.1

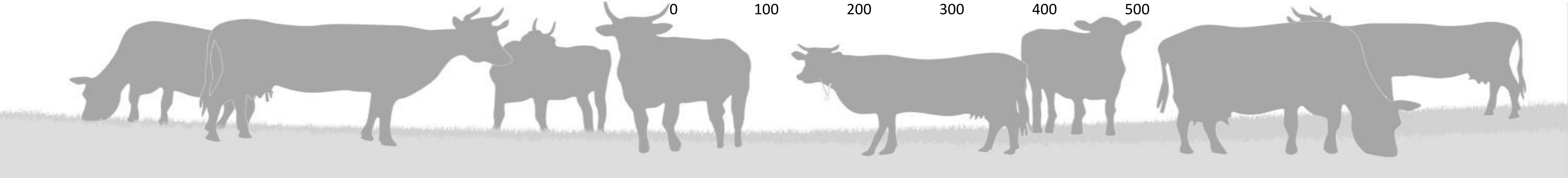


Study 1: Fold Enrichment of DEGs based on Annotation Clustering using DAVID analysis

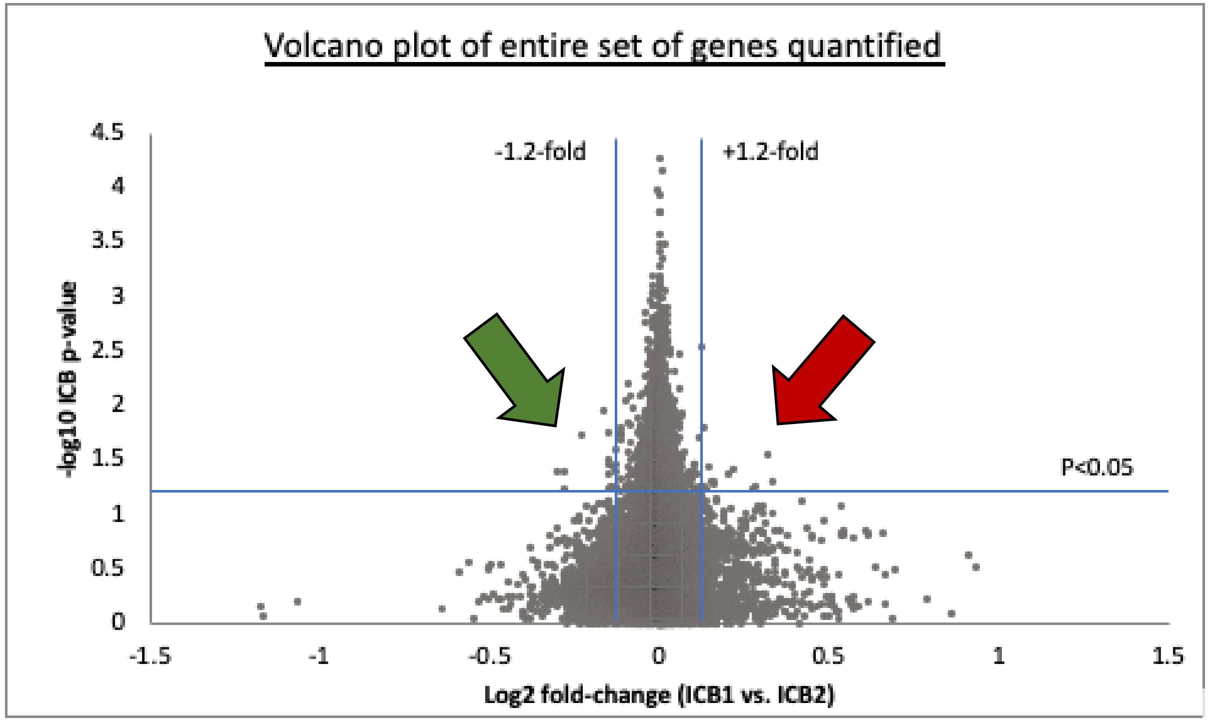


All the top categories identified relate to antigen presentation and MHC

Benjamini $P<0.05$



Study 1: Results



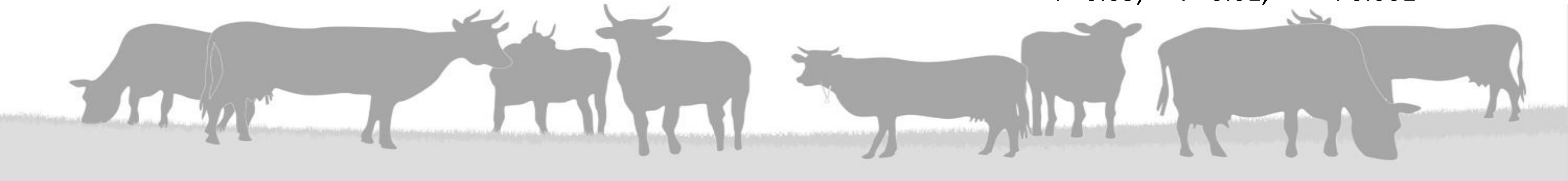
Red denotes up-regulation in more fertile cows

Green denotes down-regulation in more fertile cows

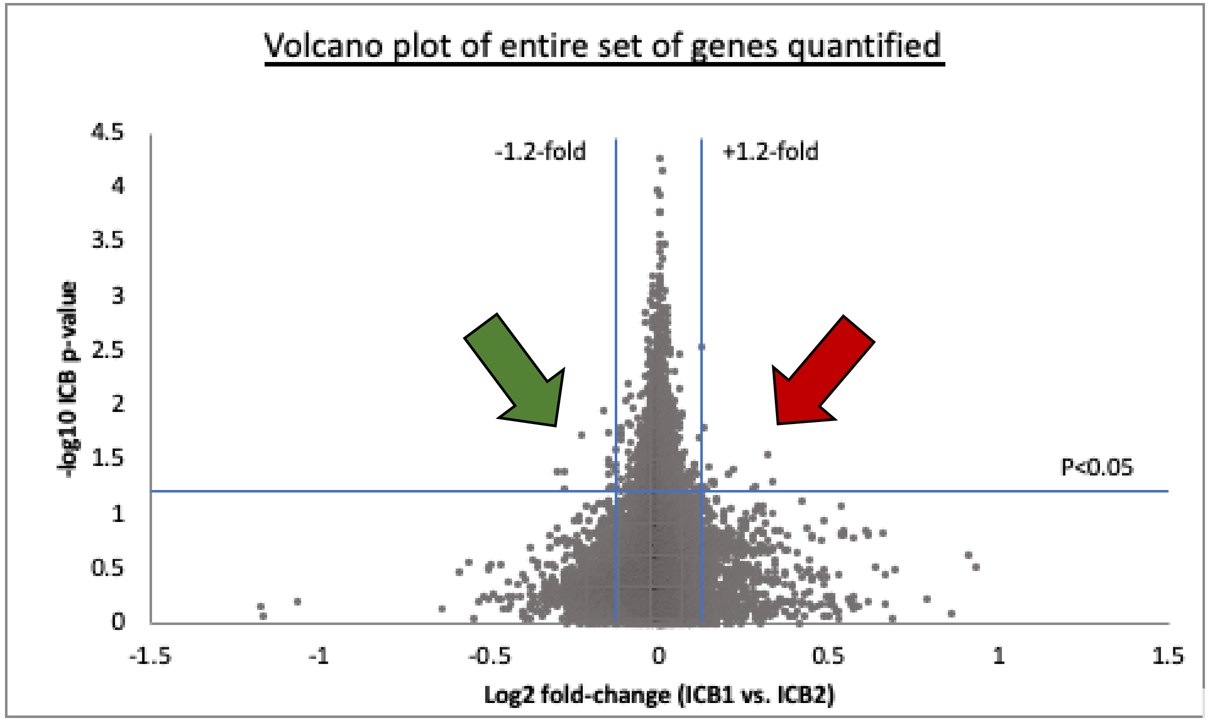
DEG between ICB1 (n=27) v ICB2 (n=34)

Gene symbol	Fold-change	P-value
		ICB1 vs. ICB2
LOC505306	1.42	*
IGF2BP3	1.39	***
LOC520016	1.33	*
PF4	1.33	*
LOC100335205	1.32	**
PLCL1	1.29	**
SLC40A1	1.27	*
LOC786297	1.26	*
LGALSL	1.25	*
EMID1	1.23	*
LAMA4	1.23	*
NREP	1.20	*
ADRB3	-1.24	**
KLRG1	-1.24	**
LOC521214	-1.28	*
GBP5	-1.30	*
LOC618633	-1.30	*
MX1	-1.31	*
NELL2	-1.31	*
BOLA-DRB2	-1.43	*
BOLA	-2.24	*

*P<0.05, **P<0.01, ***<P0.001



Study 1: Results



Red denotes up-regulation
Green denotes down-regulation

DEG between ICB1 v ICB2

	Gene symbol	**Fold-change**	**P-value**
			ICB1 vs. ICB2
T cell receptor	*LOC505306*	1.42	*
	IGF2BP3	1.39	***
	LOC520016	1.33	*
	PF4	1.33	*
T cell receptor	*LOC100335205*	1.32	**
	PLCL1	1.29	**
	SLC40A1	1.27	*
T cell receptor	*LOC786297*	1.26	*
T cell regulator	*LGALSL*	1.25	*
	EMID1	1.23	*
	LAMA4	1.23	*
	NREP	1.20	*
	ADRB3	-1.24	**
T cell regulator	*KLRG1*	-1.24	**
	LOC521214	-1.28	*
Immune function	*GBP5*	-1.30	*
Immune function	*LOC618633*	-1.30	*
Immune function	*MX1*	-1.31	*
	NELL2	-1.31	*
	BOLA-DRB2	-1.43	*
	BOLA	-2.24	*

*P<0.05, **P<0.01, ***<P0.001

Study 2: Results

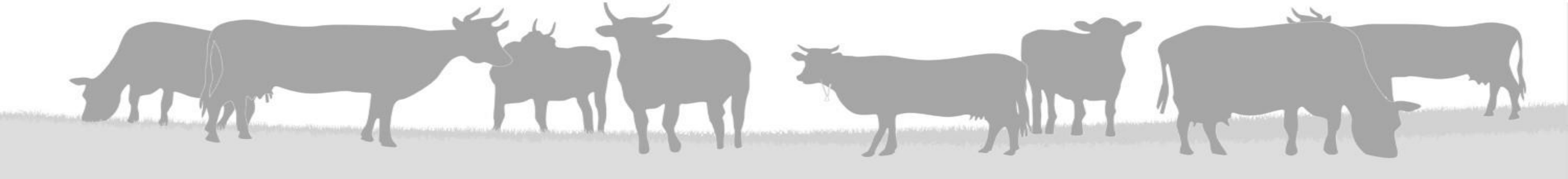
DEG between ICB1 (n=82) v ICB2 (n=51)

	Gene symbol*	Fold change	P value ICB1 vICB2
	OVCA2	1.44	**
	LOC104969934	1.41	*
	LOC785168	1.31	**
	STEAP4	1.26	*
	LOC616782	1.23	*
	DPH1	-1.21	**
	ALPL1	-1.24	*
Interacts with MHC ⇨	*CLEC1B*	-1.29	**
	DMBT1	-1.39	**
Antimicrobial peptide ⇨	*LOC104968634*	-1.45	*
	TUBA1D	-1.67	**
⇨	*BOLA-DQB*	-1.70	*

*P<0.05, **P<0.01,

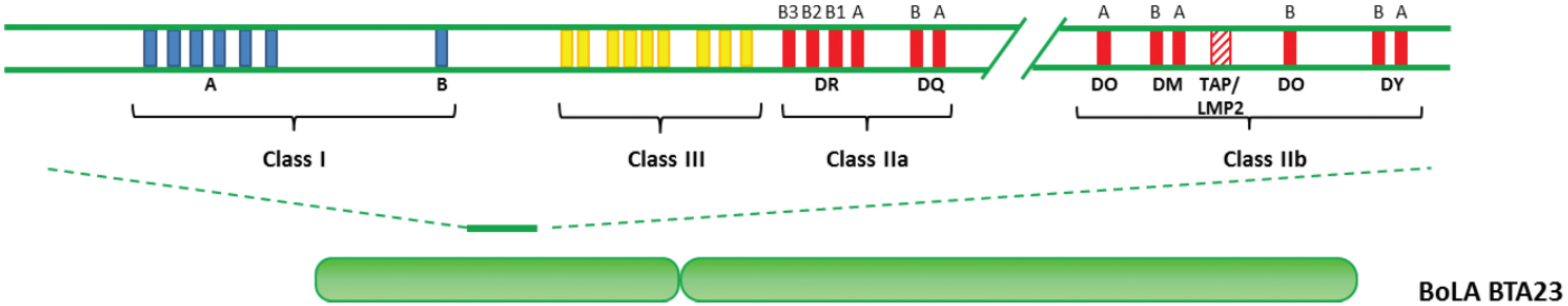
Up-regulated in more fertile cows

Down-regulated in more fertile cows

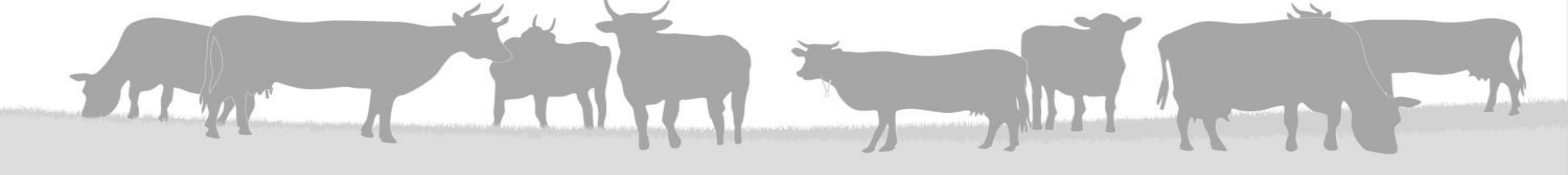


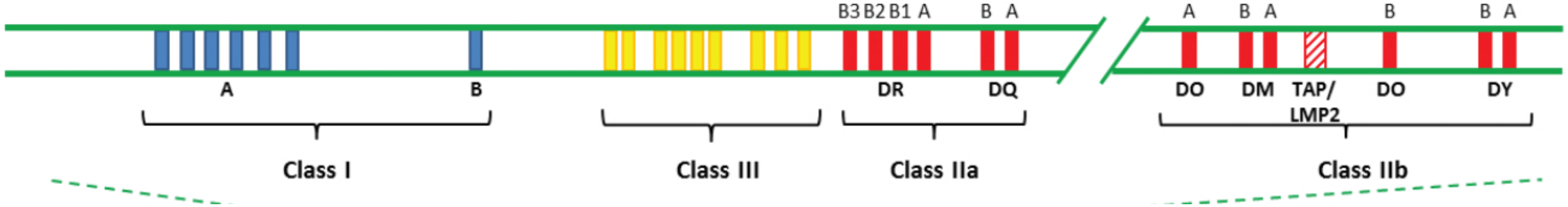
Summary of BoLA genes with potential associations with traits for bovine fertility

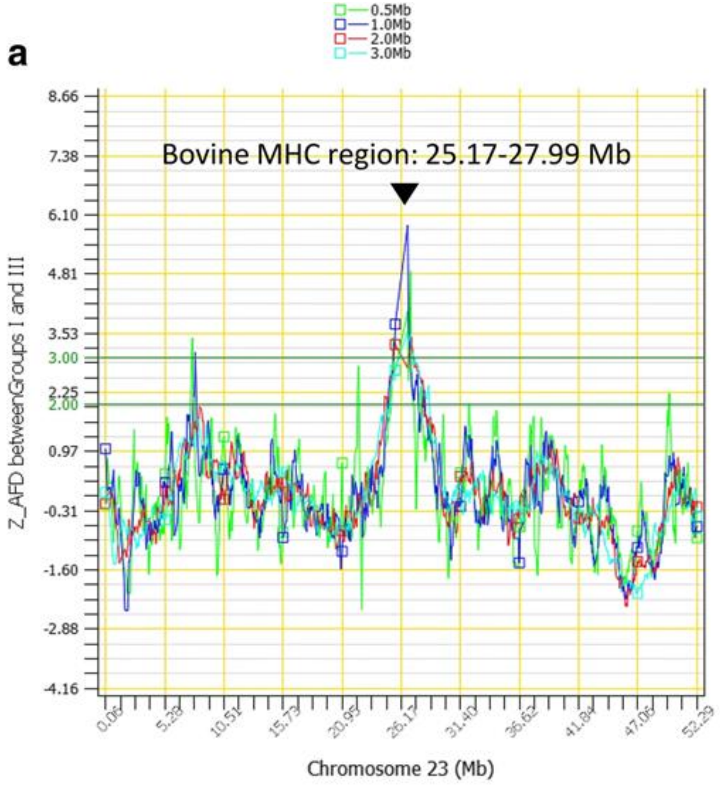
Gene	Full name	Reference(s)
BOLA	Bovine leucocyte antigen-I (Class I)	This study
BOLA-DRB2	Bovine leucocyte antigen-I (Class II)	*Ma et al. (2019) BMC Genomics, 20, 128*; this study
BOLA-DQB	Bovine leucocyte antigen-DQB (Class II)	*Dickinson et al. (2018) BMC Genomics, 19, 129*; this study



Gutierrez et al. (2017) http://www.intechopen.com/books/trends-and-advances-inveterinary-genetics

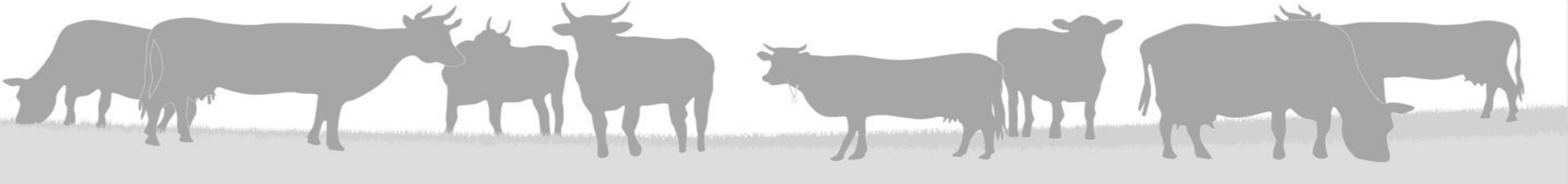






Ma *et al.* (2019) Genome changes due to artificial selection in U.S. Holstein cattle *BMC Genomics, 20(1), 128*;

- Compared whole genomes of Holsteins unselected since 1964 with modern animals
- Looking for reasons behind negative correlations between milk yield increase and fertility decline
- Bovine MHC region identified as showing a significant decrease in heterozygosity over this period.

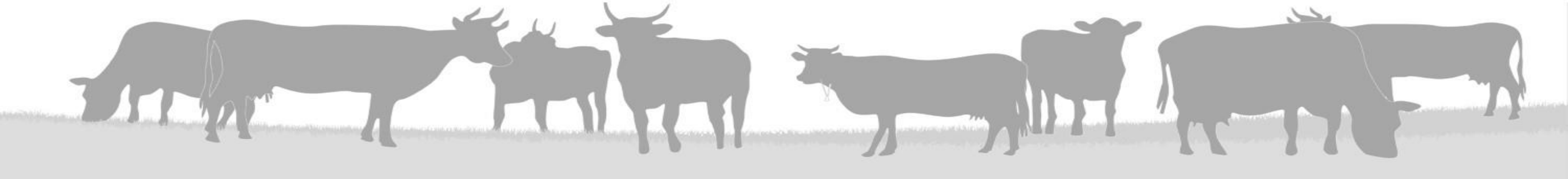


Conclusions

- Our data support other evidence that differential expression of MHC genes in leukocytes in early lactation is associated with subsequent fertility in dairy cattle.

- Genetic selection may have decreased heterozygosity in BoLA genes, making cows exposed to disease in the peripartum period less able to mount a robust immune response.

- Differential expression of BoLA genes could also indicate differences in exposure to inflammatory mediators between more and less fertile cows



Acknowledgments and Disclaimer:

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FBN

AARHUS UNIVERSITY

ICBF.com

crea

afbi Agri Food and Biosciences Institute

cra-w

Université de Liège ULg

UNIVERSITEIT GENT

DDW Dairy Data Warehouse

LANDBRUG FØDEVARER VIDENCENTRET FOR LANDBRUG

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