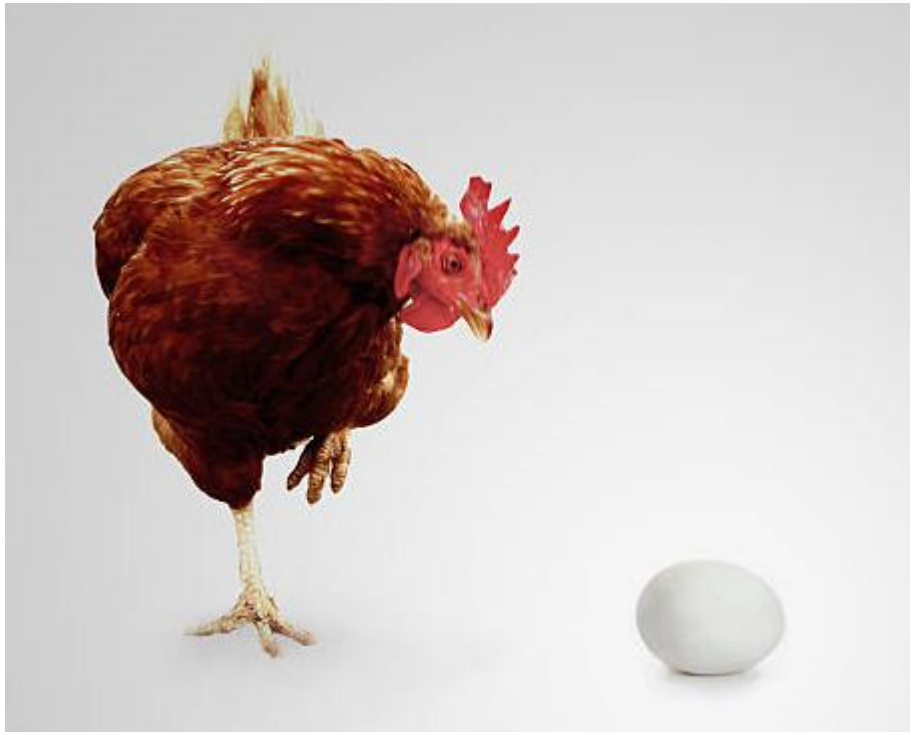
Problems with metabolism and immunity – the chicken or the egg?"



VEERkracht
Veterinaire Kenniscoöperatie voor de Melkveehouderij U.A.

Professor D. Claire Wathes

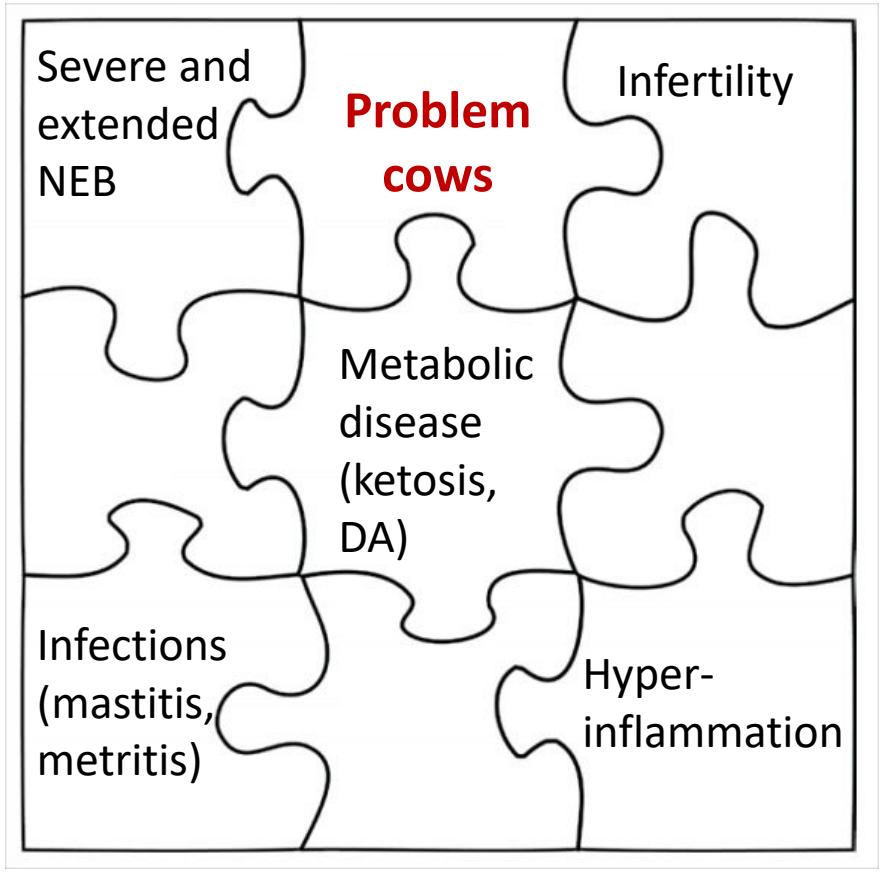
RVC Royal Veterinary College University of London

Adipose tissue mobilisation to support lactation is a normal physiological response

Inflammation is inevitable following calving

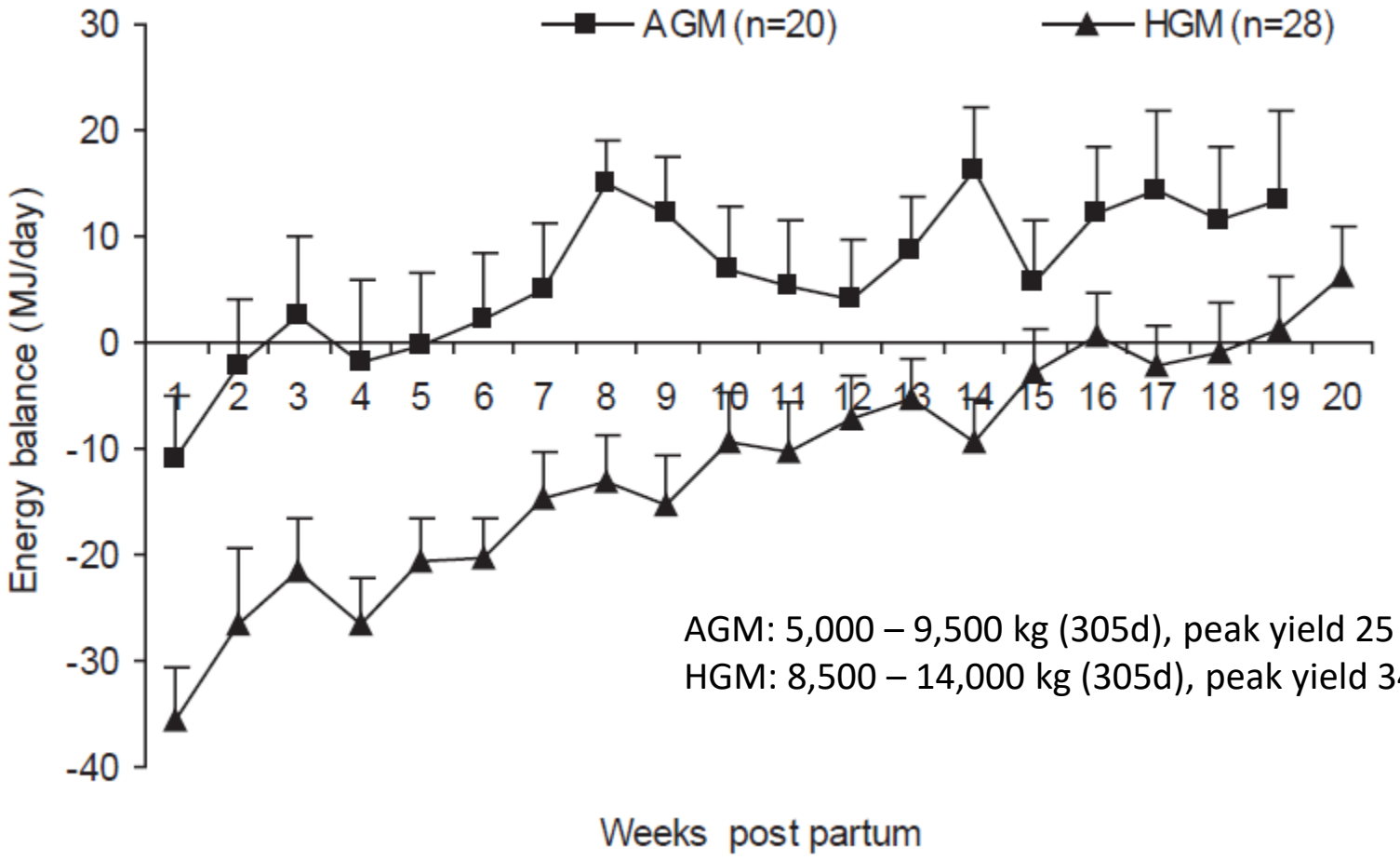
In a “normal” healthy cow these will both resolve within a few weeks in response to homeorhetic adaptations

Which cows?
What starts it?
Can it be prevented?



Calculated energy balances in average (AGM) and high (HGM) genetic merit multiparous Holstein cows

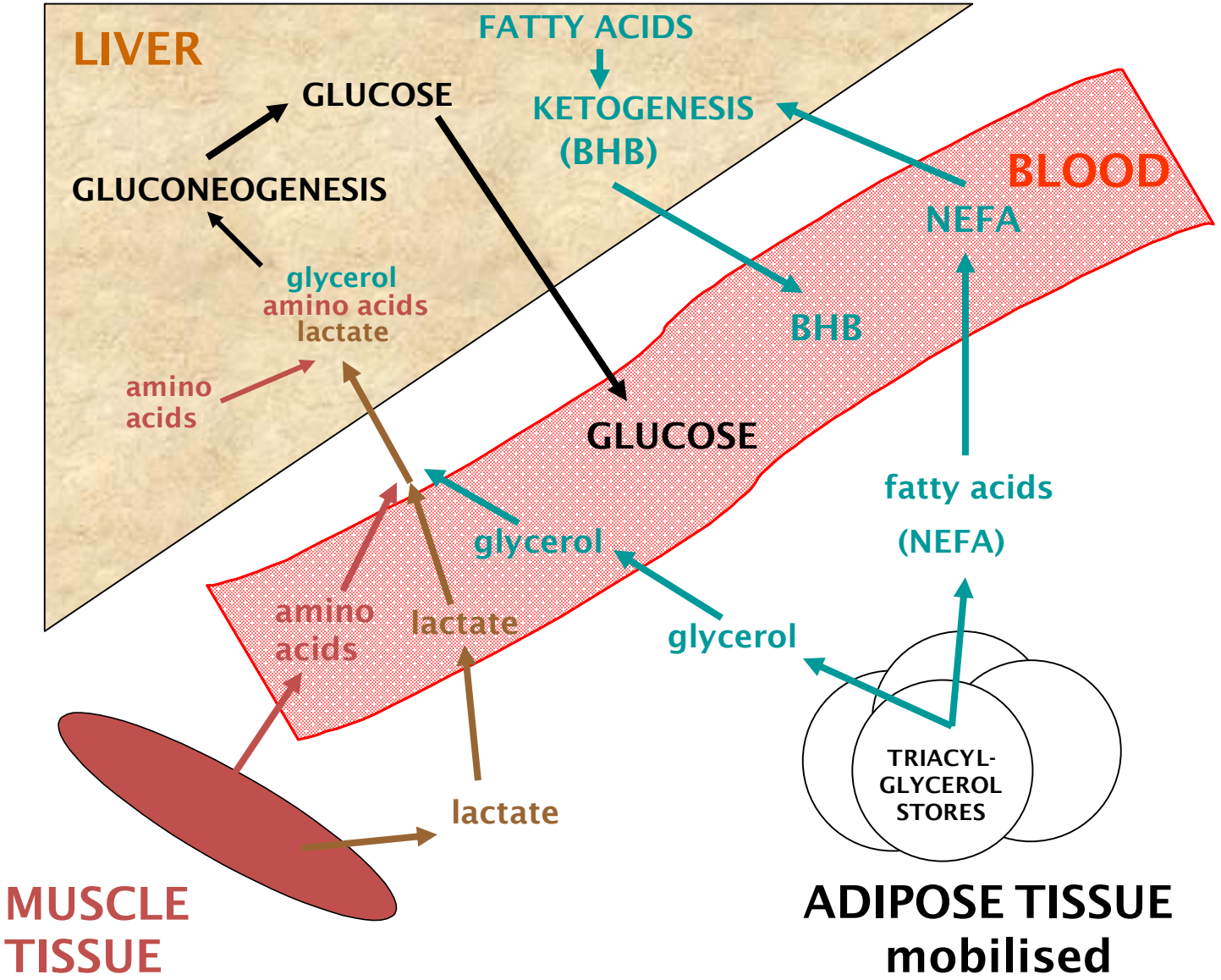
Figure 2. Energy balance (EB in MJ ME/day) for AGM (weeks 2 to 20) and HGM (weeks 1 to 20) cows (mean ± sem).



AGM: 5,000 – 9,500 kg (305d), peak yield 25 - 45 kg/day
HGM: 8,500 – 14,000 kg (305d), peak yield 34 - 65 kg/d

Taylor et al. (2003) In "Dairying, using science to meet consumer needs" pp 37-71. Eds E Kebreab, J Mills and D Beever. BSAS Occasional Publication No. ***29****. Nottingham University Press.*

Metabolic responses during negative energy balance (NEB)



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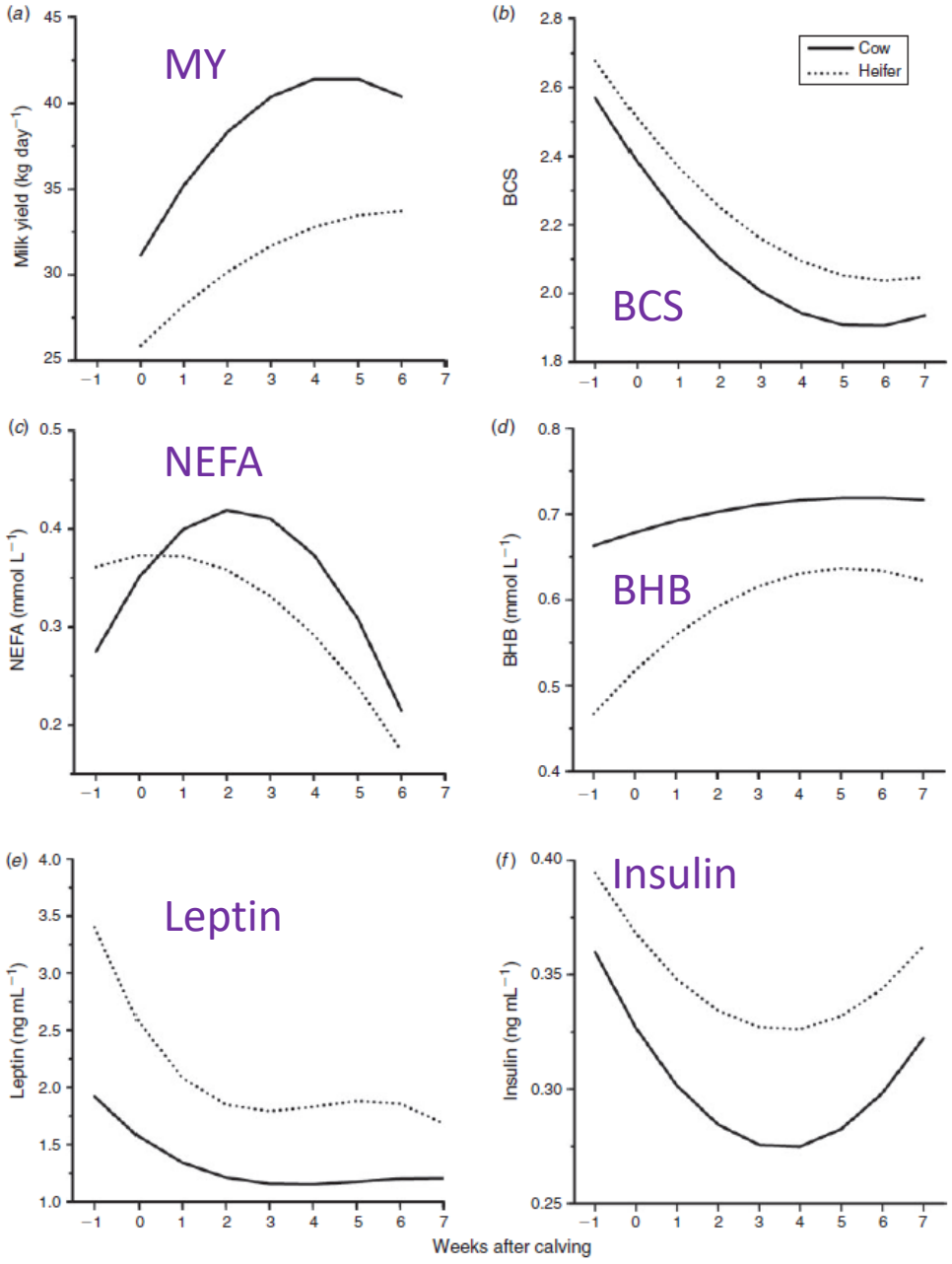
Metabolic changes around calving



Predicted LSM phenotypic values over time

Multiparous cows, n = 312

Primiparous cows, n = 188

Wathes et al. (2007) DAE 33:203

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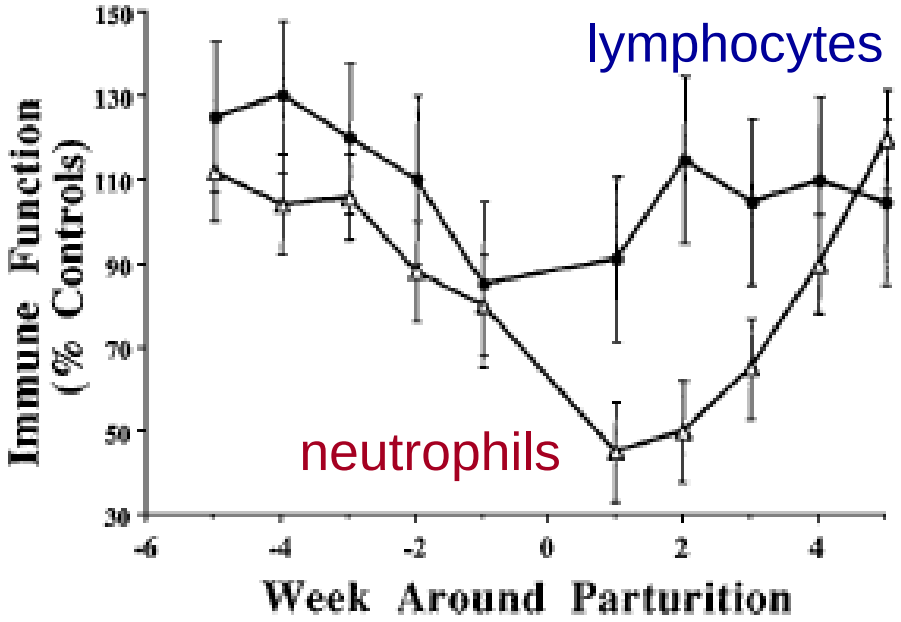
Immune system at calving

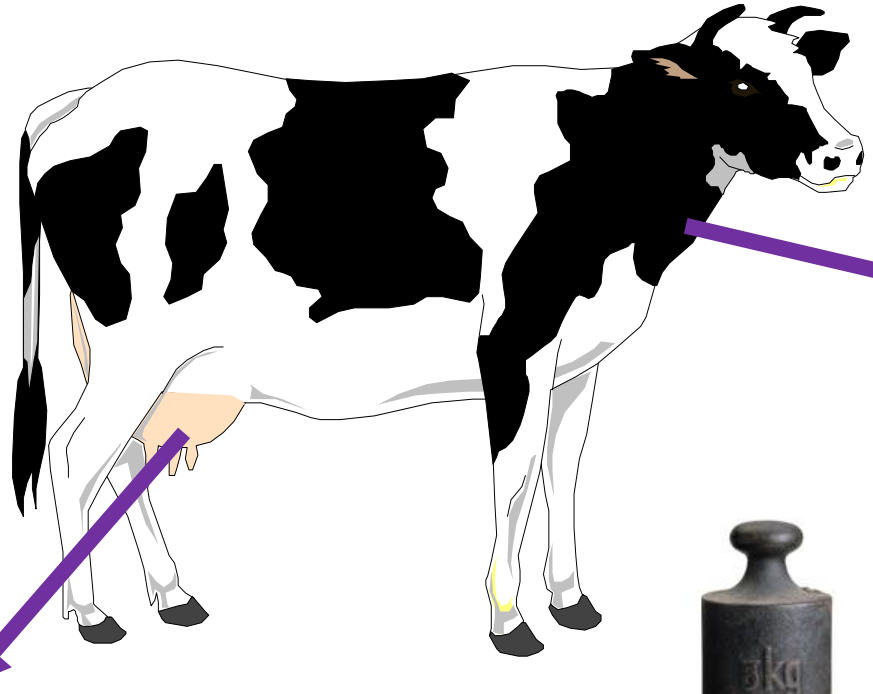
- Immune status is suppressed around calving.
- Blood neutrophil function declines.
- Circulating PMNs have reduced respiratory burst activity and cytotoxicity.

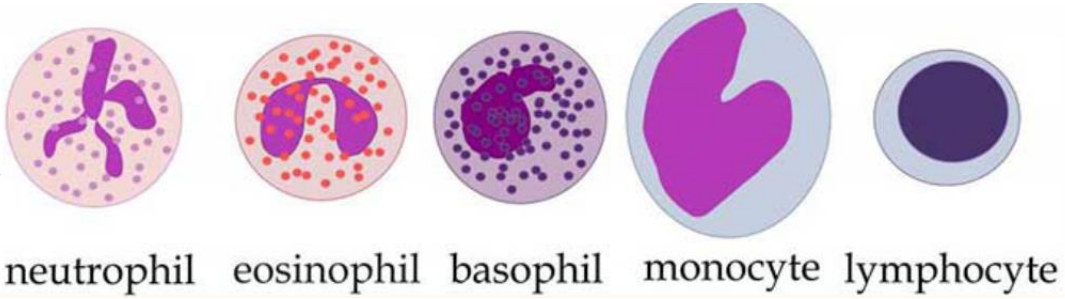
Goff & Horst (1997) JDS 80:1260



eg: Cai et al. 1994; Mallard et al. 1998; Hammon et al. 2006; Scalia et al. 2006

Competition for glucose





- Glucose is an essential component of milk lactose
- Mammary gland at peak lactation extracts **3-4 kg/d**

Non mammary tissue requires 200 g/d for maintenance
Hepatic gluconeogenesis generates about **3 kg/d**

- Immune cells require glucose, amino acids, fatty acids, cholesterol/oxysterols to proliferate and synthesise immune molecules.
- Neutrophils contain relatively few mitochondria, so rely on glucose to fuel glycolytic energy production.
- Macrophage glucose uptake increases during an inflammatory response.
- Fully activated immune system in dairy cows needs about **2.2-3.1 kg/d** glucose.

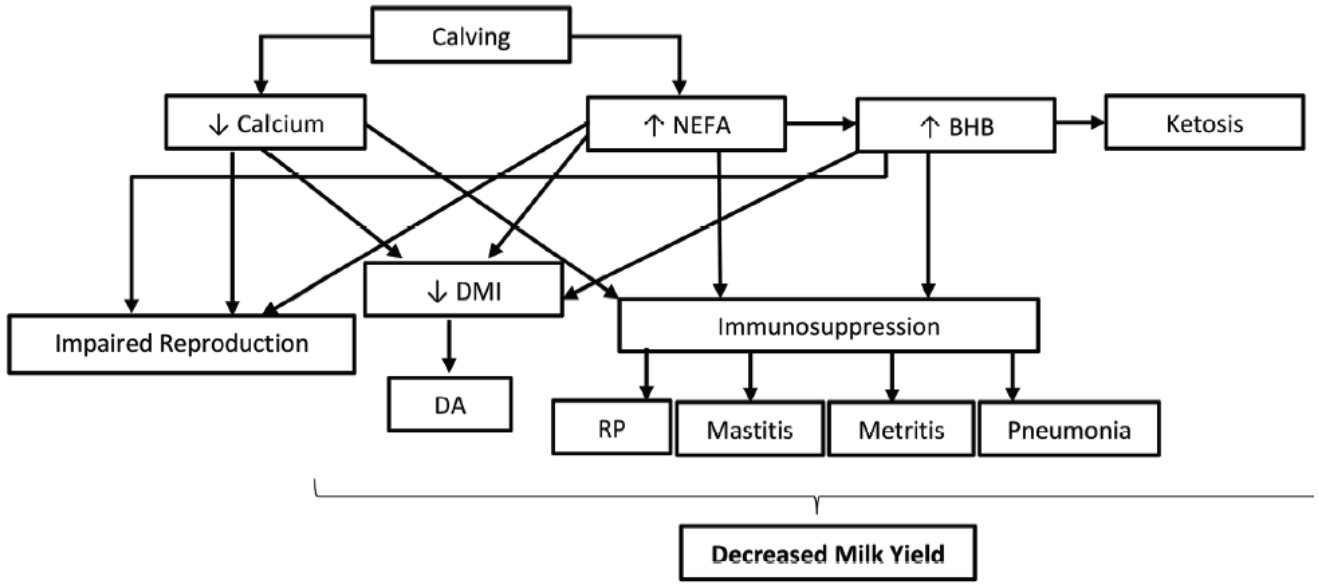
Some glucose obtained from post ruminal starch digestion

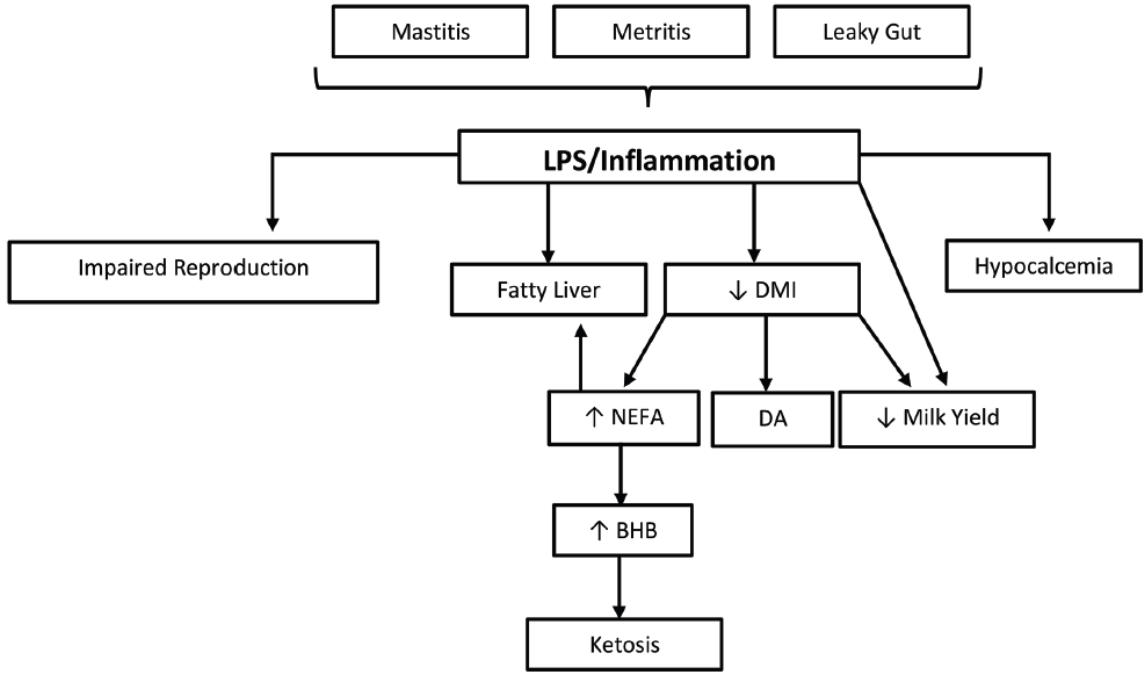
Reynolds, 2005; Aschenbach et al. 2010; Kvidera et al., 2017; Habel & Sundrum, 2020; Horst et al. 2021

J. Dairy Sci. 104:8380–8410
https://doi.org/10.3168/jds.2021-20330

Invited review: The influence of immune activation on transition cow health and performance—A critical evaluation of traditional dogmas

E. A. Horst, S. K. Kvidera, and L. H. Baumgard*
Department of Animal Science, Iowa State University, Ames 50011





Hypothesis 1. Hypocalcaemia and increased NEFA and BHB cause reduced DMI and immunosuppression

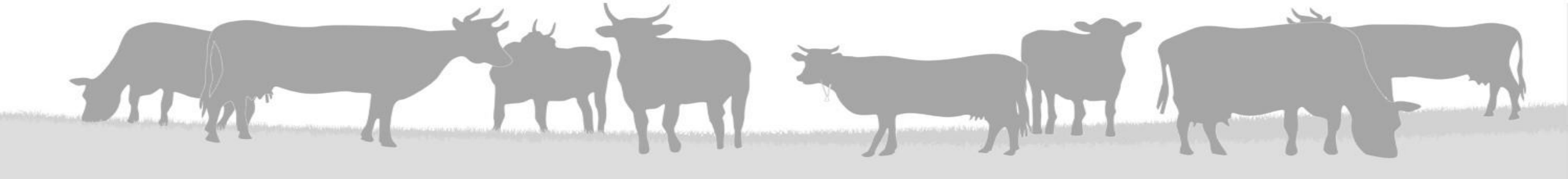
Hypothesis 2. Infection causes inflammation. Subsequent reduced DMI and fatty liver increase tissue mobilisation

GE PLUS

Genotype plus Environment

Integration for a more sustainable dairy production system

RNA-Seq analysis of blood and liver biopsy samples used to investigate gene expression in early lactation dairy cows



Materials and Methods: 1
Cows and phenotypes

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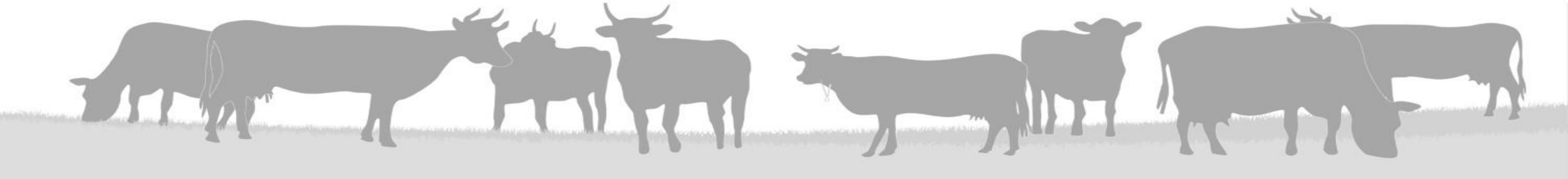
- Blood samples and liver biopsies collected at 14±3 days in milk (DIM) from 229 cows in 6 dairy herds.

- White blood cells (leucocytes), PBMC and hepatic biopsy samples processed for total RNA-Seq using Illumina NextSeq 500 platform.

- Phenotype data captured:
  - Body condition score (BCS) at calving, 14 and 35 DIM, change in BCS
  - Circulating metabolites and IGF1 at 14 and 35 DIM
  - Total milk yield and milk quality data 0-50 DIM
  - Fertility and health data recorded



Materials and Methods: 2. RNASeq

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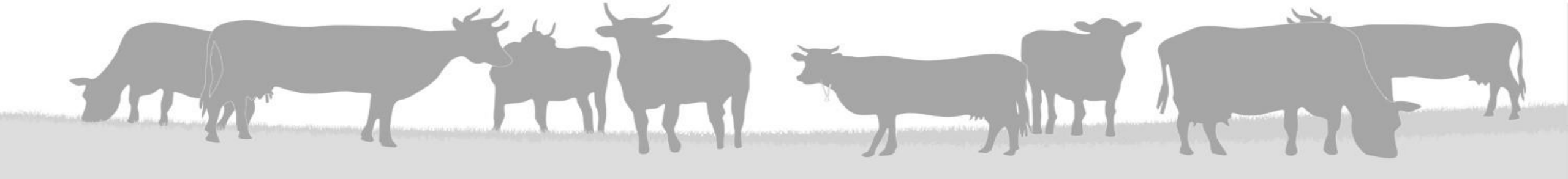
- Total RNA from leucocytes, PBMC and hepatic biopsies used for library preparation.
- Libraries sequenced at 75 nt length single end reads to reach average 30 million reads/sample using Illumina NextSeq 500 platform.
- Fastq raw files were quality controlled and mapped onto *Bos taurus* ARS-UCD1.2 (new annotation gene tracks). StringTie used to count isoforms based in the new genome annotation.
- DEG between groups identified using CLC Genomics Workbench V21 with a negative binomial generalized linear model in an ANOVA – like procedure. False discovery rates for multiple tests were adjusted with the Benjamini-Hochberg (**BH**) procedure.
- Gene ontology (**GO**) and Kyoto Encyclopedia of Genes and Genomes (**KEGG**) pathway analyses were carried out.



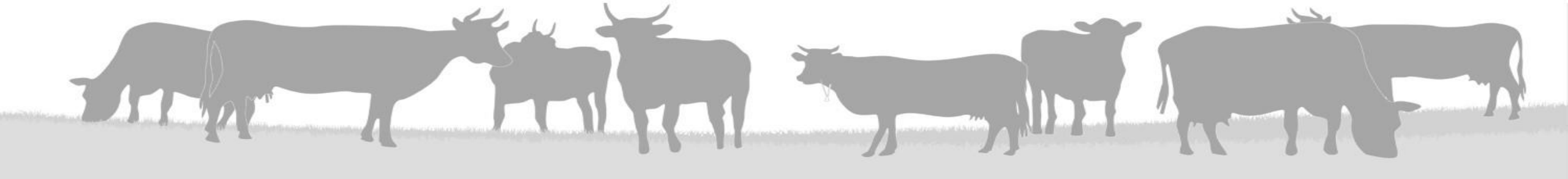
Experimental Analyses of Differentially Expressed Genes (DEG) in early lactation

1. Associations of DEGs in leucocytes from multiparous (MP) cows with circulating IGF1.

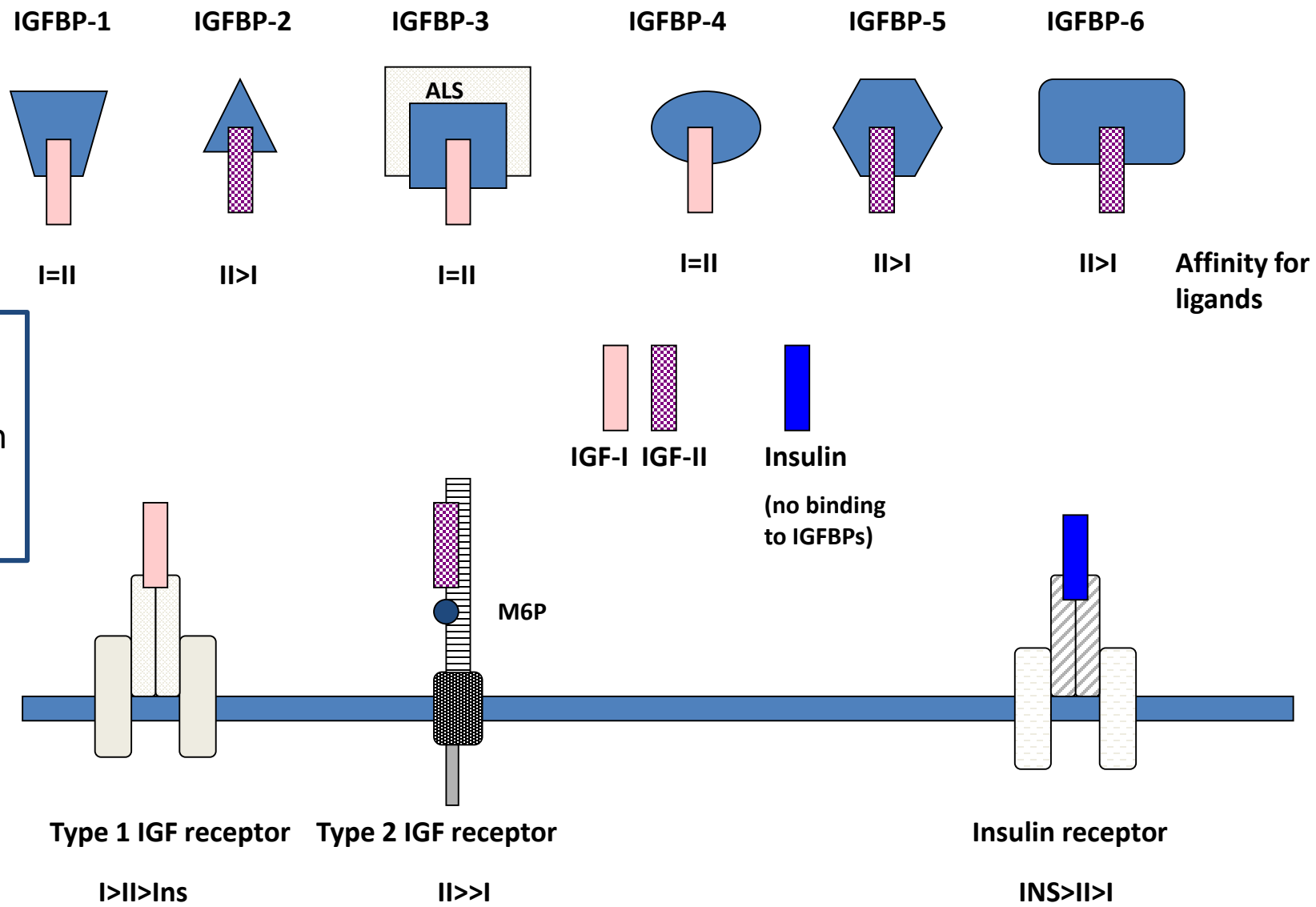
2. Associations of DEGs in peripheral blood mononuclear cells (PBMC) with circulating NEFA.

3. Age-related changes in leucocytes.

4. Diet-related changes in DEGs in liver of MP and primiparous (PP) cows.



Summary diagram of the IGF system



Binding proteins influence:

- Half life of IGF1 in the circulation
- Receptor binding

Hepatic mRNA expression of IGF system members at around 14 DIM is strongly influenced by negative energy balance

Gene	Mild NEB	Severe NEB	Difference (P)
IGF-I	36.8 ± 8.83	3.6 ± 0.47 ↓	<0.01
IGF-1R	0.8 ± 0.14	0.3 ± 0.03	<0.01
IGF-II	206.0 ± 49.51	100.3 ± 17.65	0.100
IGF-2R	2.3 ± 0.35	1.0 ± 0.12	<0.02
IGFBP-1	139.8 ± 71.81	85.6 ± 42.75	0.538
IGFBP-2	53.4 ± 7.71	109.5 ± 10.58 ↑	<0.01
IGFBP-3	20.2 ± 2.88	7.7 ± 1.04 ↓	<0.01
IGFBP-4	87.3 ± 23.69	33.9 ± 3.35	<0.05
IGFBP-5	0.6 ± 0.12	0.2 ± 0.02	<0.01
IGFBP-6	0.8 ± 0.06	0.4 ± 0.04	<0.01
IGFBP ALS	12.8 ± 3.63	0.9 ± 0.30 ↓	<0.02

fg mg reverse transcribed RNA^{-1}

- After calving there is a doubling in circulating IGFBP2 while IGFBP3 and ALS both decrease.

Fenwick et al. (2008) Dom Anim Endocrinol 34, 31–44

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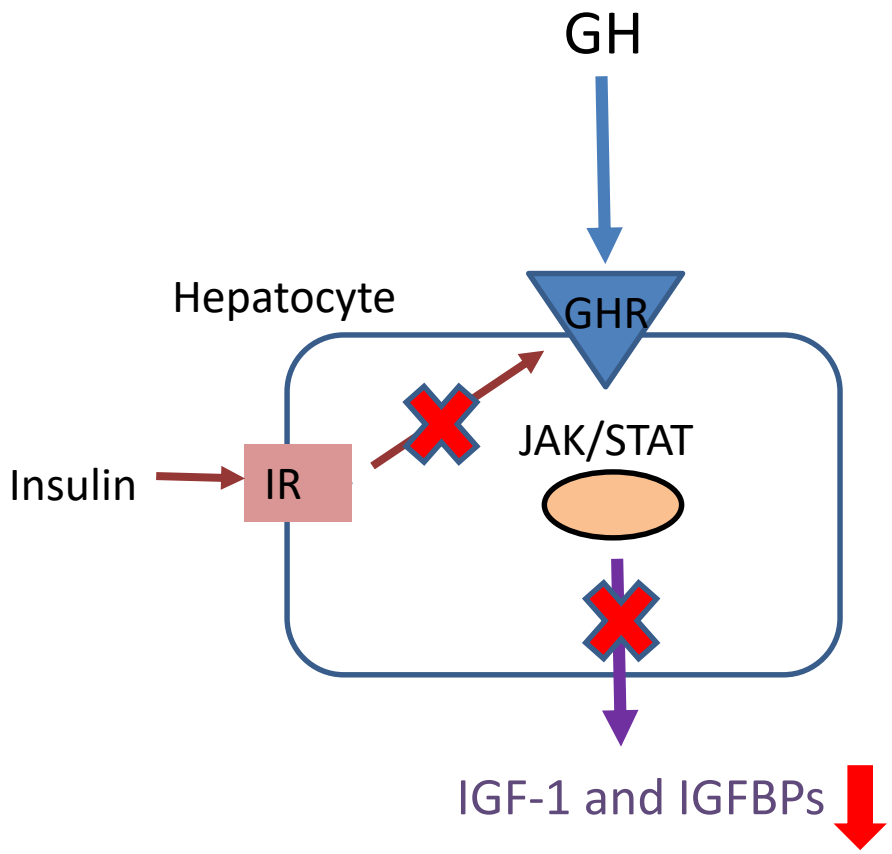
This is mainly achieved by down-regulation of hepatic GH receptor



	MNEB	SNEB	P
GHR total	62.6 ±12.2	18.8 ± 1.5	<0.01
GHR 1A (hepatic)	24.7 ± 4.8	0.9 ± 0.1	<0.01

fg mg reverse transcribed RNA^{-1}

Fenwick et al. (2008) Dom Anim Endocrinol 34, 31–44

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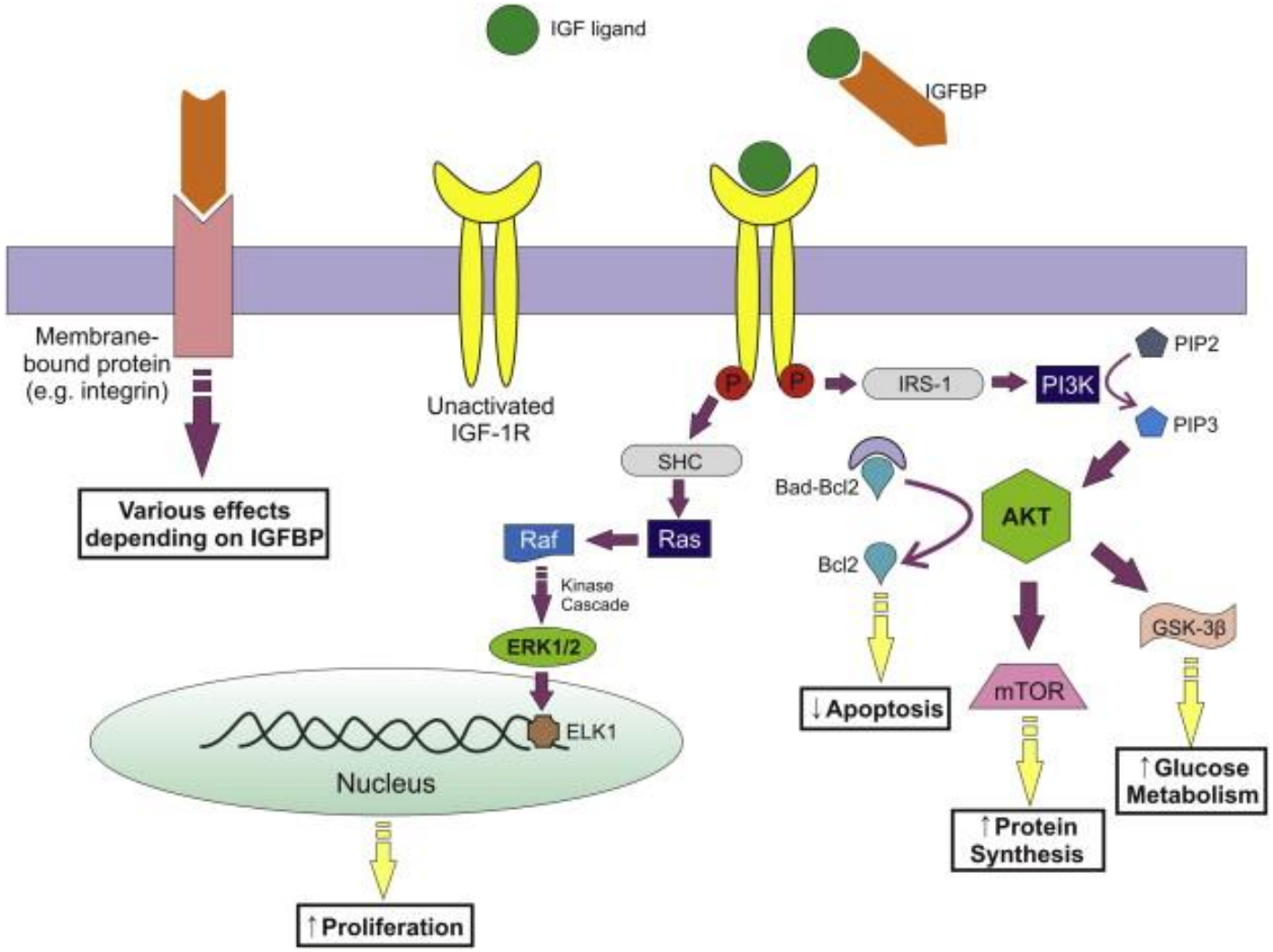
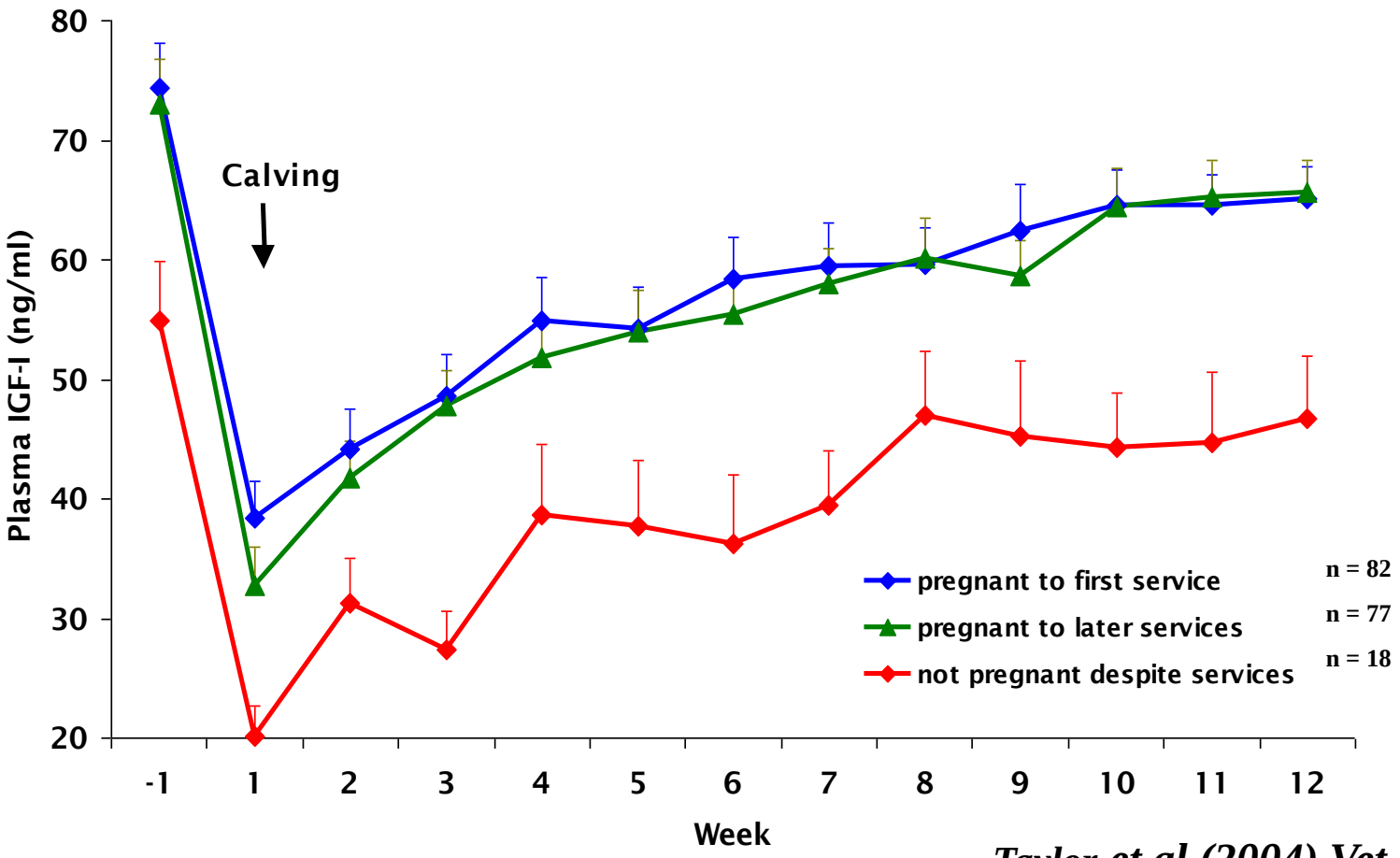
IGF signalling pathways



Galadari et al., Lipids Health Dis. 2013;12:98.

Relationship between IGF-I and conception in multiparous cows

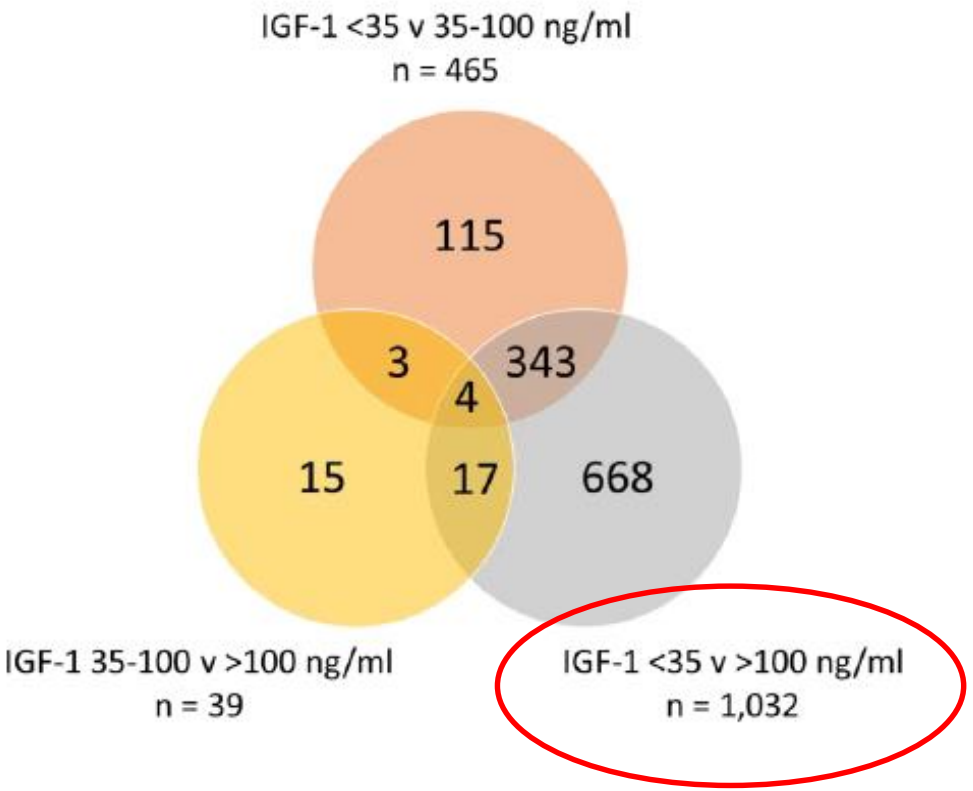


Taylor et al (2004) Vet Rec. 155, 583-588

Analysis based on circulating IGF1 in early lactation

Methodology

- Multiparous cows subdivided into three groups defined as low IGF-1 (<35 ng/ml, n=35), moderate IGF-1 (35-100 ng/ml, n=92) or high IGF-1 (>100 ng/ml, n=43) at 14 DIM.
- Leucocyte gene expression from RNASeq analysed using CLC Genomics Workbench V21
- Compared between groups using negative binomial generalized linear modelling. IGF-1 groups were set as the test variable and herd as the controlling variable.
- DEG selected using criteria P(BH)< 0.05 and FC>1.2



Wathes et al. (2021) *Ruminants* 1, 147–177.

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Lactation number, milk yield and milk quality data from second week of lactation according to the IGF-1 group at 14 days in milk.

	LOW <35 ng/ml	MOD 35-100 ng/ml	HIGH >100 ng/ml	P
Week 2				
n	32	82	31	
Lactation No.	3.9 ± 0.27[b]	3.2 ± 0.11[a]	2.6 ± 0.17[a]	<0.001
Milk yield (kg/d)	30.2±1.24[b]	33.3±0.79[ab]	36.6±1.13[b]	0.001
Fat (F%)	4.4±0.14	4.5±0.11	4.2±0.15	0.23
Protein (P%)	3.1±0.05[a]	3.2±0.03[a]	3.4±0.05[b]	<0.001

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Wathes et al. (2021) *Ruminants* 1, 147–177.

Blood metabolites, feed intakes and energy balance at 14 DIM according to the IGF-1 group at 14 days in milk.

Parameter	LOW <35 ng/ml	MOD 35-100 ng/ml	HIGH >100 ng/ml	P
n	34	88	41	
IGF-1 (ng/ml)	25±1.3[a]	63±1.9[b]	143±5.3[c]	<0.001
Glucose (mM)	3.2±0.07[a]	3.3±0.06[a]	3.5±0.07[b]	0.005
Fructosamine (µM)	246±3.0[a]	247±2.0[a]	258±2.7[b]	0.003
BHB (µM)	0.79±0.082	0.75±0.077	0.52±0.044	0.082
NEFA (µekv/L)	884±82[b]	740±47[ab]	633±57[a]	0.046
Urea (mM)	3.0±0.22[a]	2.9±0.12[a]	3.7±0.21[b]	0.004
n	18	61	38	
Weight (kg, 7 day average)	671 ±18.9	670 ± 9.5	657 ± 10.4	0.288
DMI (kg/d, 7 day average)	16.4 ± 0.60[a]	18.5 ± 0.38[b]	19.8 ± 0.54[b]	0.001
EB (MJoule/d 7d average)	-10.7±1.51[b]	-7.5±0.70[ab]	-5.0±1.09[a]	0.005

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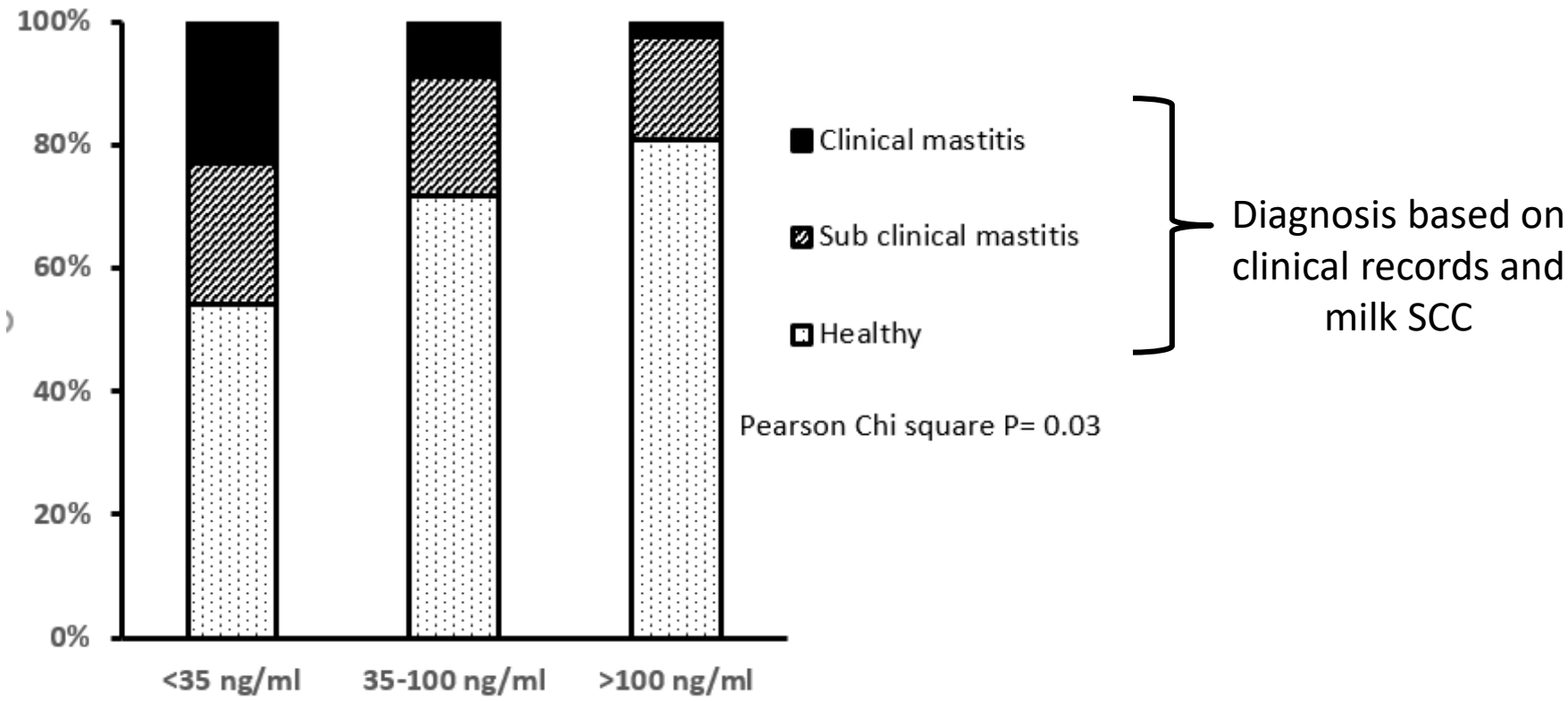
Wathes et al. (2021) *Ruminants* 1, 147–177.

Proportion of cows with mastitis in early lactation according to IGF-1 group at 14 DIM



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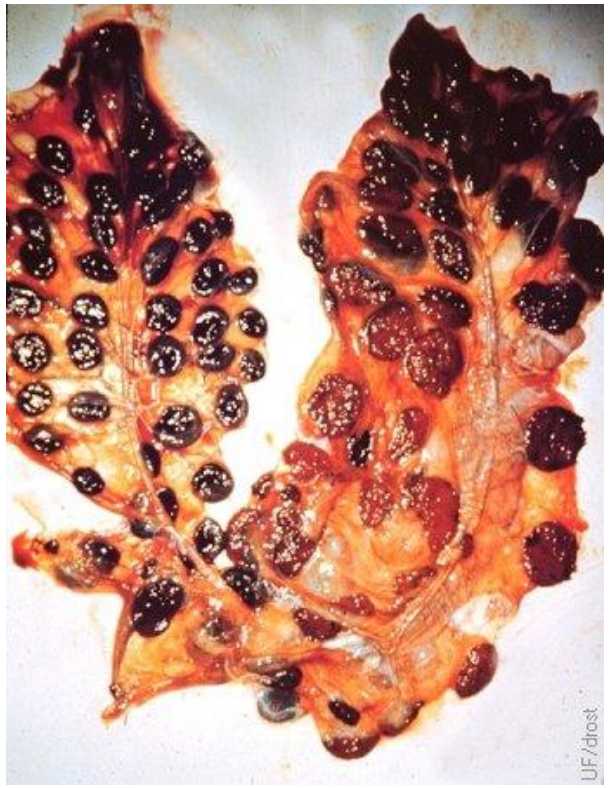
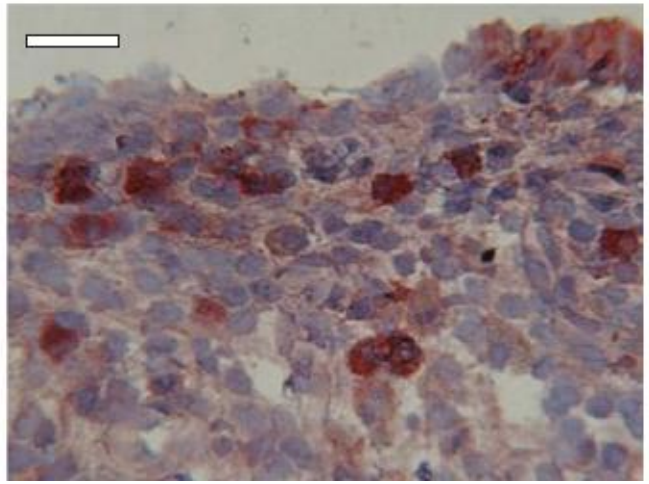
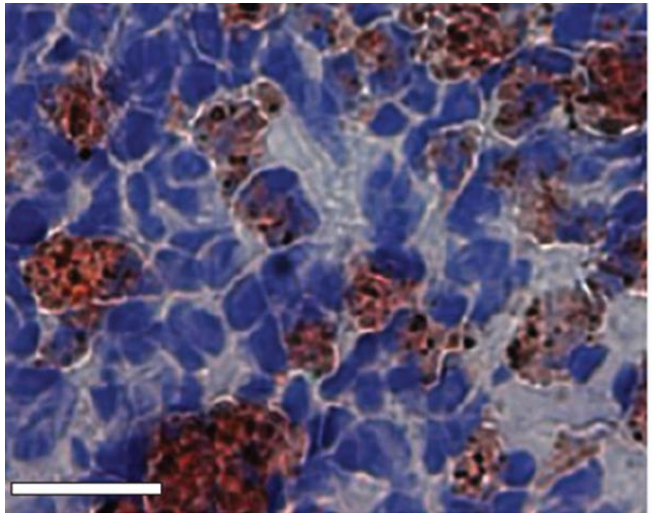
Wathes et al., (2021) Ruminants 1, 147–177.

Endometrium at 14 days postaprtum







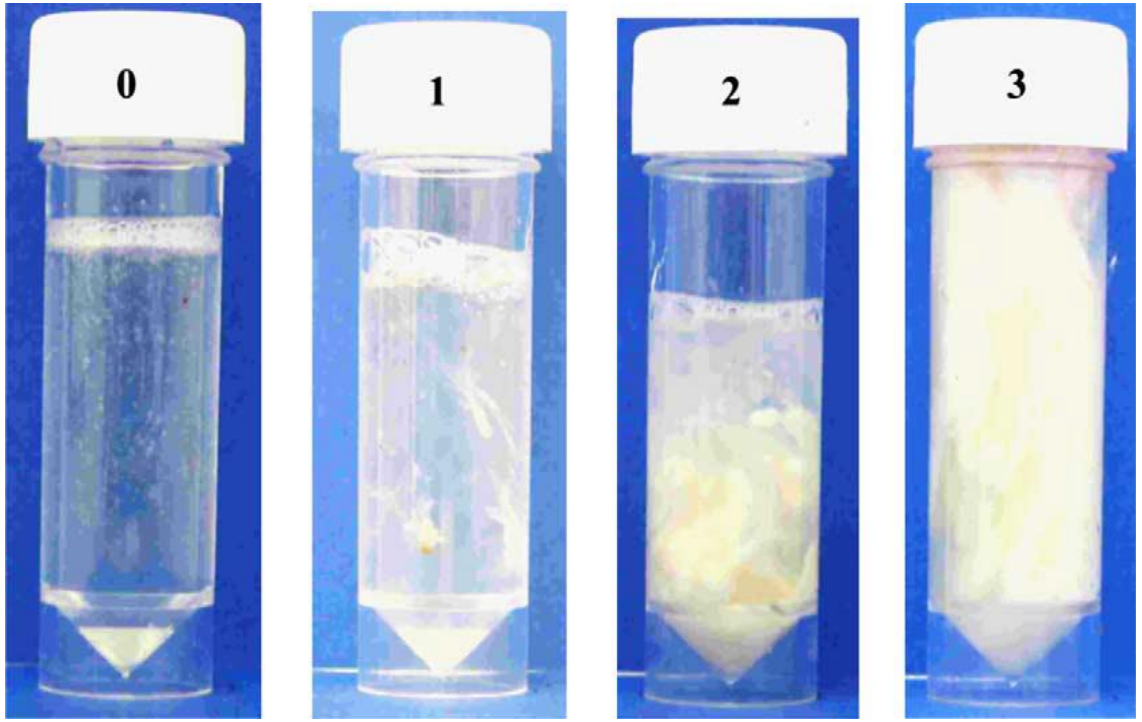
- Erosion of epithelium
- Influx of monocytes and granulocytes (CD172+)
- Macrophages take up lipid forming foam cells

- Metritis is present in 40% of cows in early postpartum period.
- 15% go on to develop persistent endometritis in the 3-6 weeks postpartum period

Sheldon et al. (2006) Theriogenology 65: 1516-1530.
Wathes et al. (2013) Reprod. Fert. Dev. 25, 1-14.

Scoring system for vaginal discharge

Williams, E.J. et al., Theriogenology **2005**, 63, 102–117.



Appearance

0: clear or translucent mucus;

1: mucus containing flecks of white or off-white pus;

2: <50 mL exudate containing 50% white or off-white mucopurulent material;

3: >50 mL exudate containing 50% purulent material, usually white or yellow, but occasionally sanguineous.

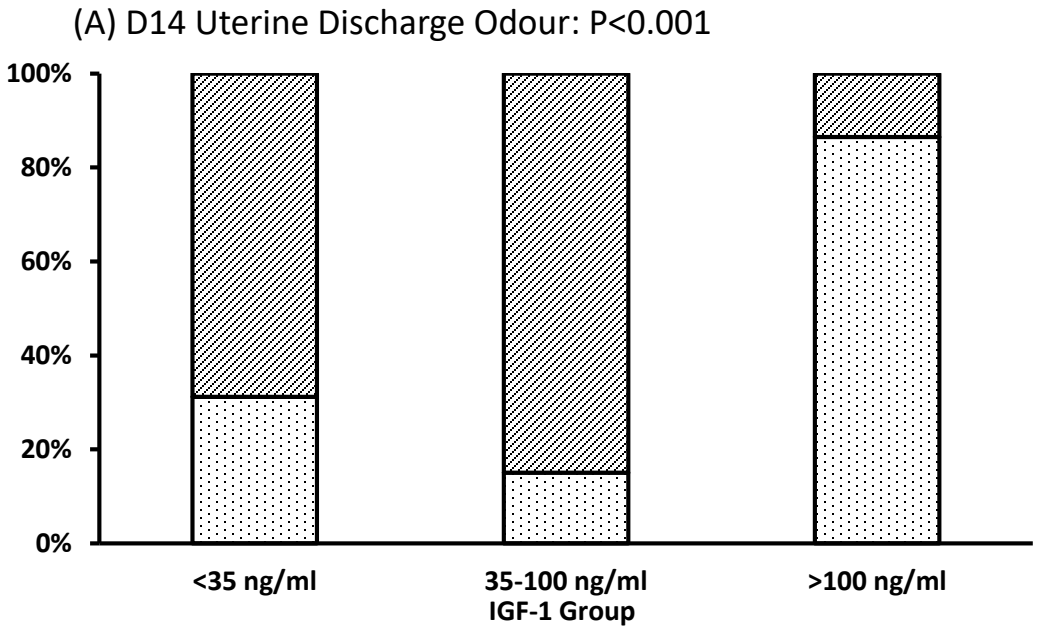
Samples taken at 14 & 35 DIM

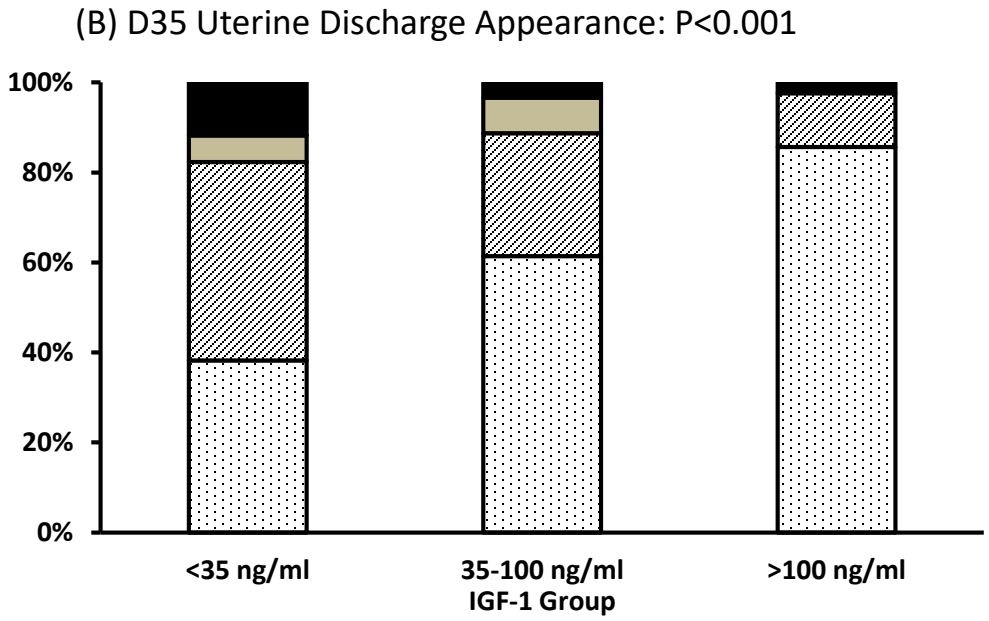
Odour

0: No smell

1: Fetid

Uterine odour and discharge scores according to the IGF-1 group





Odour

0: No smell 1: Fetid

Discharge appearance

0: 1: 2: 3:

Wathes et al., (2021) *Ruminants* 1, 147–177.

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KEGG pathway analysis based on all DEG in leukocytes collected from cows with LOW (<35 ng/ml) vs HIGH (>100 ng/ml) circulating IGF-1 concentrations, sampled at around 14 DIM

Term	Gene Count	Fold Enrich	P(BH)
bta04611:Platelet activation	24	3.61	3.23E-05
bta04640:Hematopoietic cell lineage	19	3.95	1.19E-04
bta04610:Complement and coagulation cascades	16	4.13	4.09E-04
bta04060:Cytokine-cytokine receptor interaction	26	2.28	0.010

Extensive evidence of more ongoing innate immunity and inflammatory responses in LOW IGF-1 cows

- Antimicrobial peptides (e.g. *PTX3*, *DMBT1,* S100A8, S100A9, S100A12).
- Iron homeostasis (e.g. *TF*, *HP, CYBRD1, STEAP1, STEAP2*, *HMOX1).*
- Vasodilation and platelet aggregation (e.g. VWF, *F5, FGA FGB*, *ITGA2, ITGB3, PLA2G4A*, *PTGIR, PTGS1, SELP, TBXAS1).*
- Complement cascade (e.g. *CFI*, *C1R, C2, C3AR1, C8G, CD55, CFB, CFI).*
- Cytokines and their receptors (e.g. *ACVR1, BMPR2, CSF1R, IL12B, IL1RAP, IL2RA, IL3RA, IL15RA, LTBR, TNFRSF1A).*
- Chemokines and their receptors (*CCL8, CXCL8, CCR1, CCR4*).

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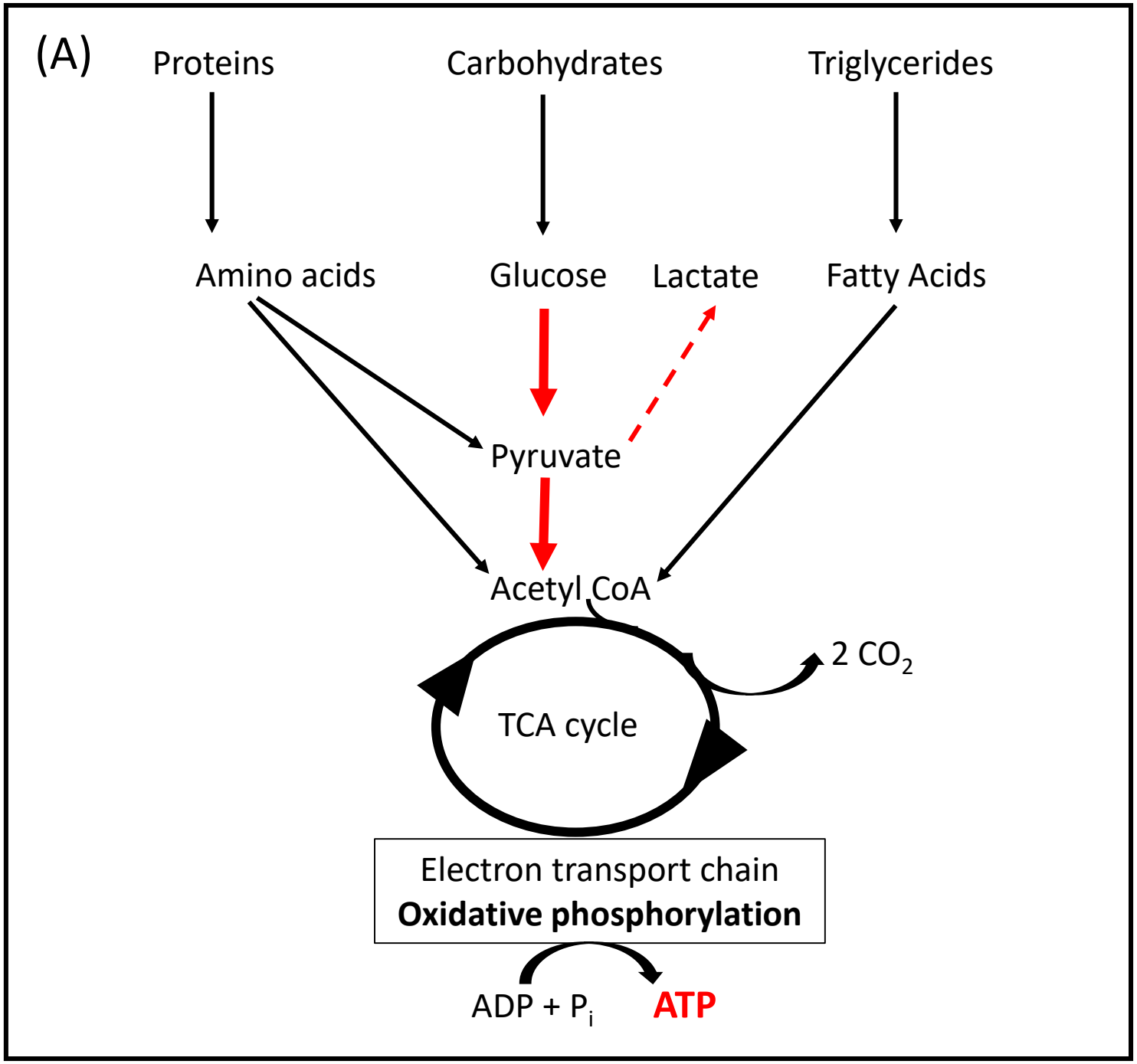
Wathes et al., (2021) *Ruminants* 1, 147–177.



Energy consumption in macrophages

Resting Macrophages

- In resting macrophages, most pyruvate is directed to the TCA cycle via acetyl-CoA to generate ATP via oxidative phosphorylation, although some is converted to lactate.

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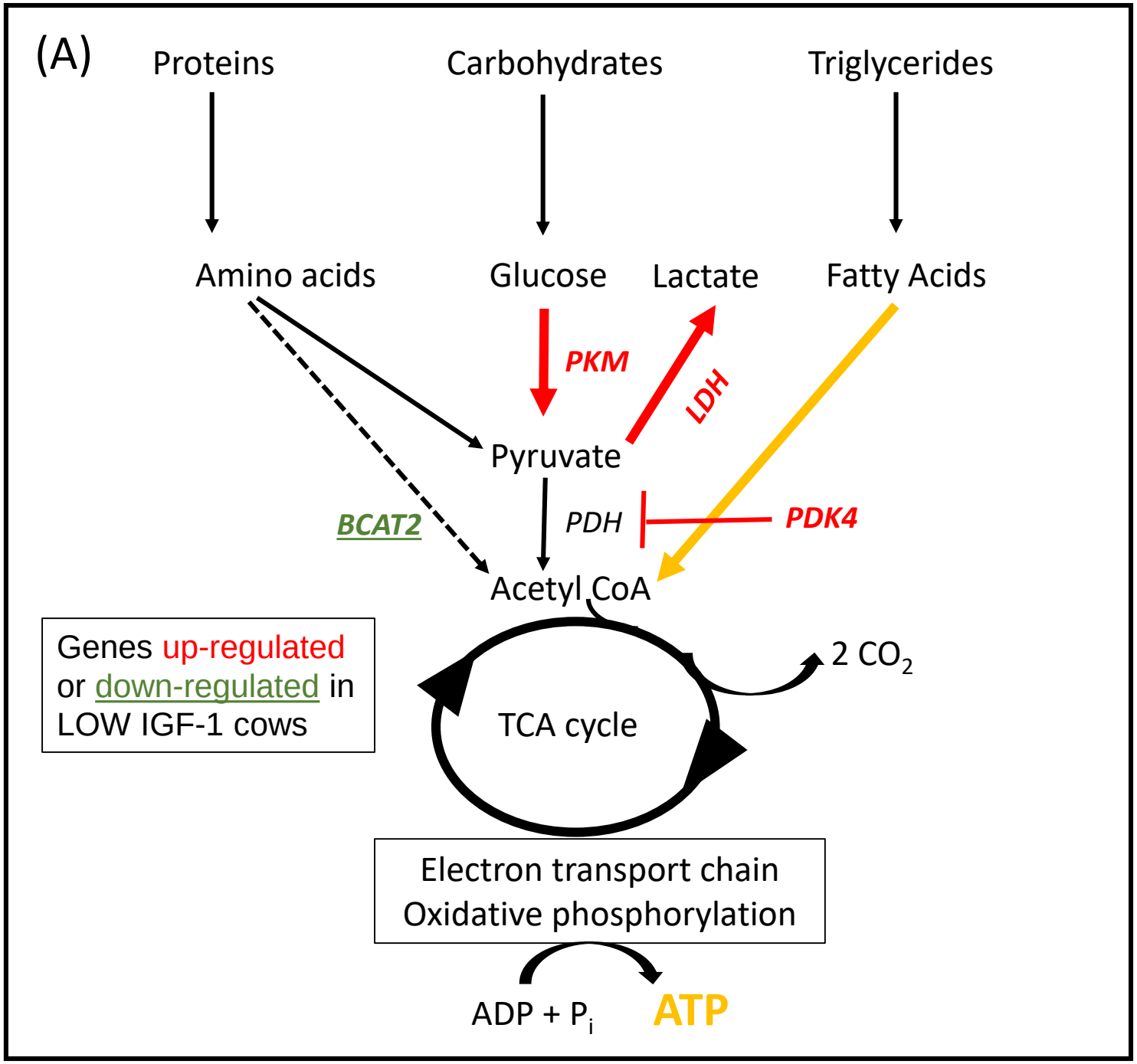

Activated immune cells in LOW IGF-1 cows

- Preferential production of lactate, even in the presence of oxygen (Warburg effect).
- Energy metabolism switches to aerobic glycolysis.
- PDK4 is up-regulated in response to increased lipid supply and inactivates the pyruvate dehydrogenase complex
- This conserves glucose by limiting conversion of pyruvate to acetyl Co-A
- In this situation fatty acids and glycolytic intermediates provide the source of ATP.

Wathes et al., (2021) Ruminants 1, 147–177.

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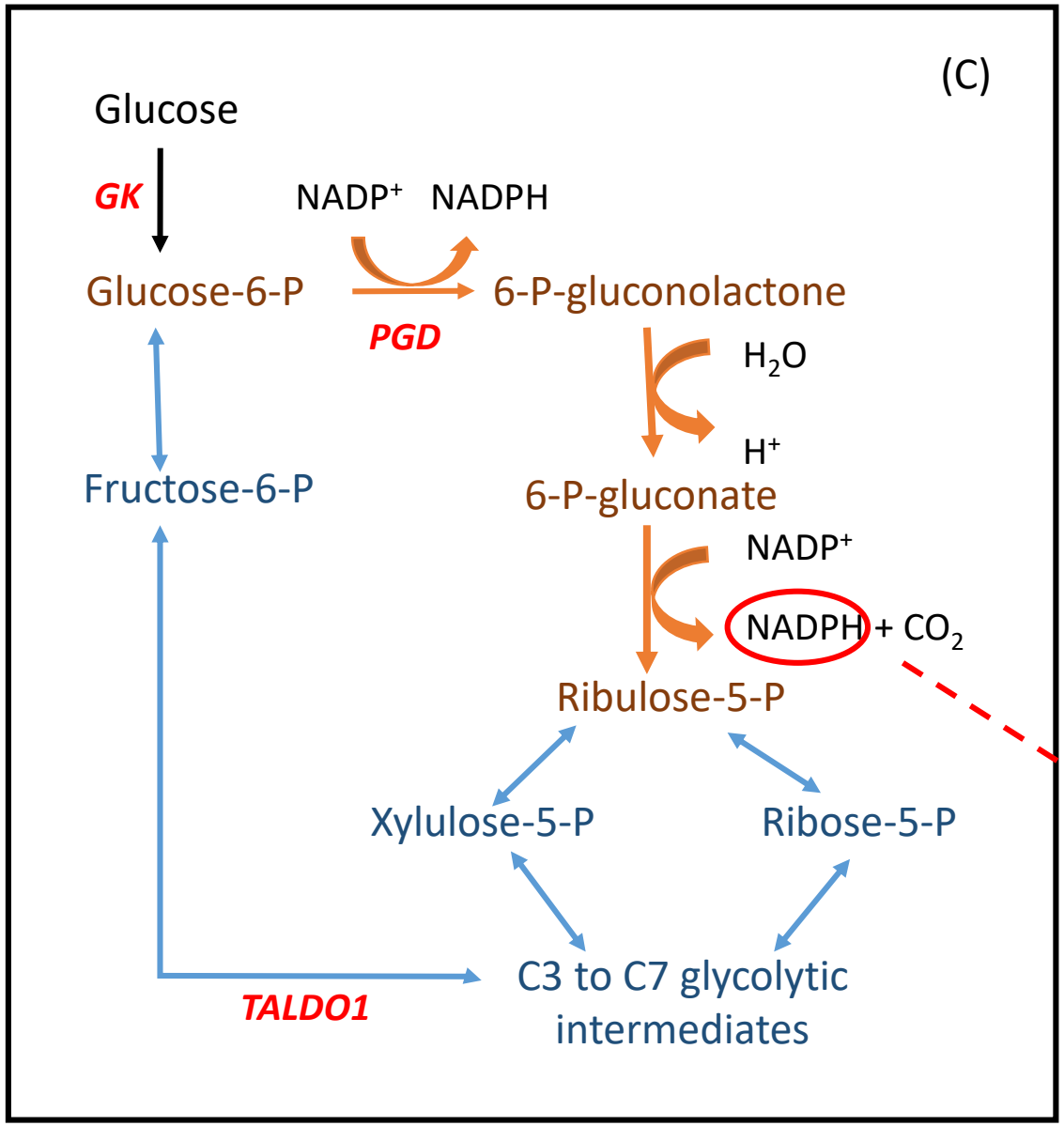

The pentose phosphate shunt in LOW IGF-1 Cows

Provides an alternative pathway for oxidation of glucose and has two phases:

- an oxidative phase, in which NADPH oxidase and ribulose-5-phosphate are generated.
- a non-oxidative phase, in which unused ribulose-5-phosphate is converted to other sugar intermediates and returned to the glycolytic pathway.

NADPH acts as a reducing agent in many biosynthetic pathways

- Important for ROS generation
- Provides anti-oxidant cellular defence in LPS-activated macrophages

Genes encoding enzymes which were up-regulated in LOW IGF-1 cows

Wathes et al., (2021) Ruminants 1, 147–177.

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Evidence for up-regulation of adaptive immunity in high IGF-1 cows

Key genes involved in T- and B-cell activation

- *BOLA-DQB,* antigen presentation to T-cells
- *CD52,* cell surface glycoprotein modulating T-cell activation.
- MS4A1 (CD20), development and differentiation of B-cells into plasma cells.
- *SPIB*, transcriptional activator regulating B-cell receptor signaling pathway
- *BANK1,* expressed in B-cells and involved in TLR signaling pathway and calcium mobilization while also preventing hyperactive B-cell responses

Wathes et al., (2021) Ruminants 1, 147–177.

Summary of the IGF-1 analysis

The metabolic status of individual cows is directly linked to the high incidence of health disorders during the critical peripartum period.

In this study 35/170 (20%) of all MP cows fell into the LOW IGF-1 category.

Most LOW IGF-1 cows are engaged in an energetically demanding battle against ongoing infection(s) involving the innate immune system.

- This reduces their DMI and milk yield.
- Previous evidence shows this will have an adverse effect on their subsequent fertility.

HIGH IGF-1 cows have higher nutrient availability and can use this to promote use of their adaptive immune system to better resist infection.

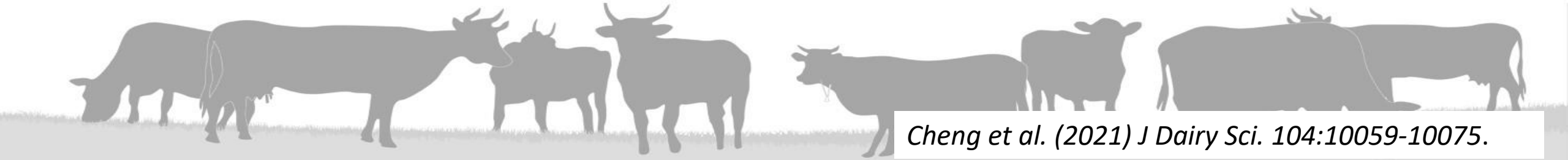
2. Associations with circulating NEFA in peripheral blood mononuclear cells (PBMC)

- PBMC isolated from blood of 24 multiparous Holstein Friesian cows at around 14 days in milk

- Processed for total RNA-Seq using Illumina NovaSeq 500 platform by Novogene Co. Ltd.

Data analysis:

- Cows subdivided by circulating NEFA concentration into low <500 µM, n =6; medium, 500-750 µM, n = 8; or high, >750 µM, n = 10.
- Main comparison was between Low NEFA or High NEFA



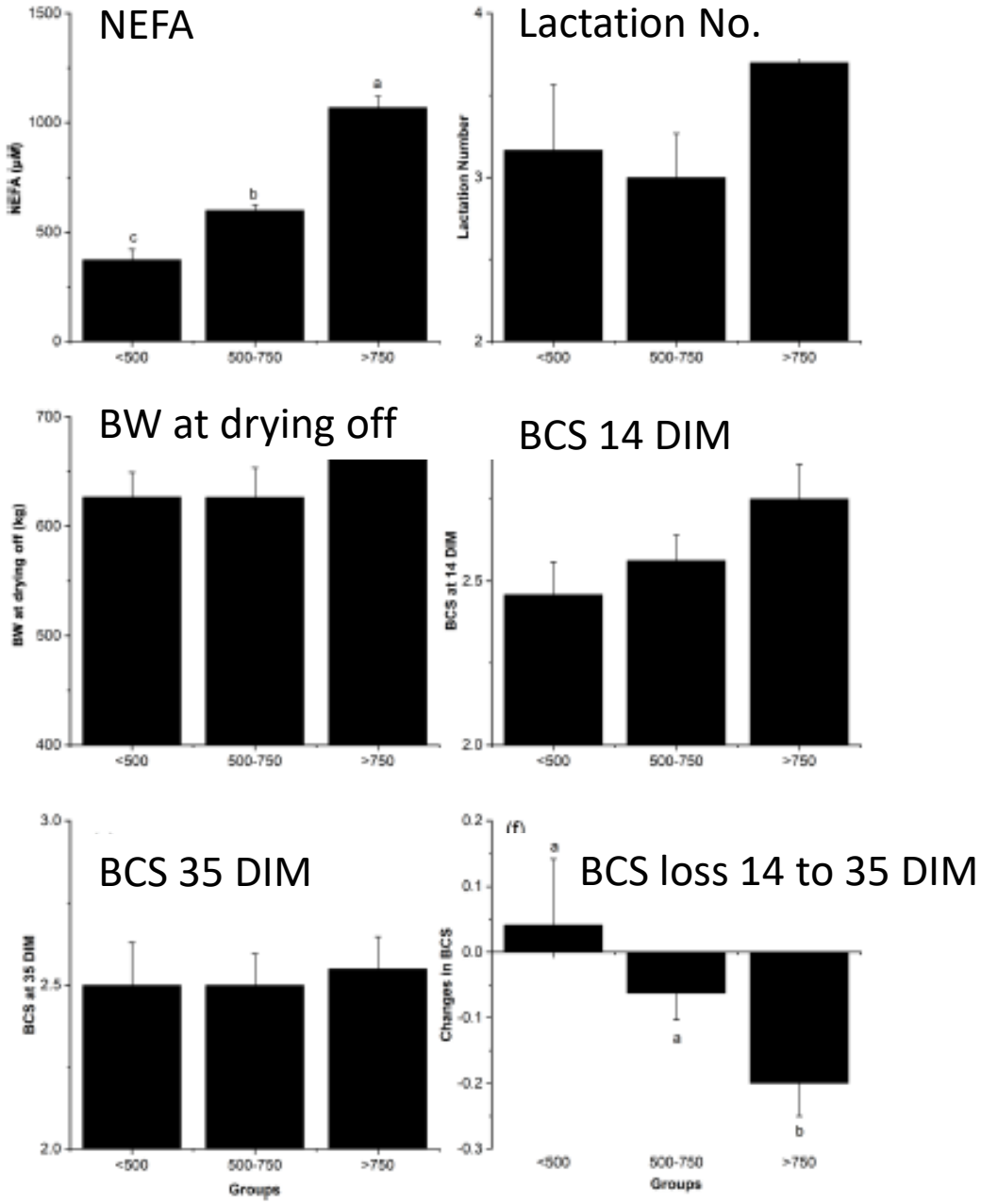
Cheng et al. (2021) J Dairy Sci. 104:10059-10075.

Circulating metabolite concentrations in MP cows at around 14 DIM according to their circulating NEFA

Group	n	NEFA (µM)	BHB (mmol/L)	Glucose (mmol/L)	Urea (mmol/L)
<500 µM	6	373 ± 49	0.65 ± 0.10	3.57 ± 0.11	2.93 ± 0.32
500-750 µM	8	600 ± 27	0.47 ± 0.05	3.46 ± 0.10	3.36 ± 0.35
>750 µM	10	1070 ± 53	0.62 ± 0.07	3.41 ± 0.08	3.32 ± 0.25
P		P<0.001	NS	NS	NS

Values are mean ± SEM

Cheng et al. (2021) J Dairy Sci. 104:10059-10075



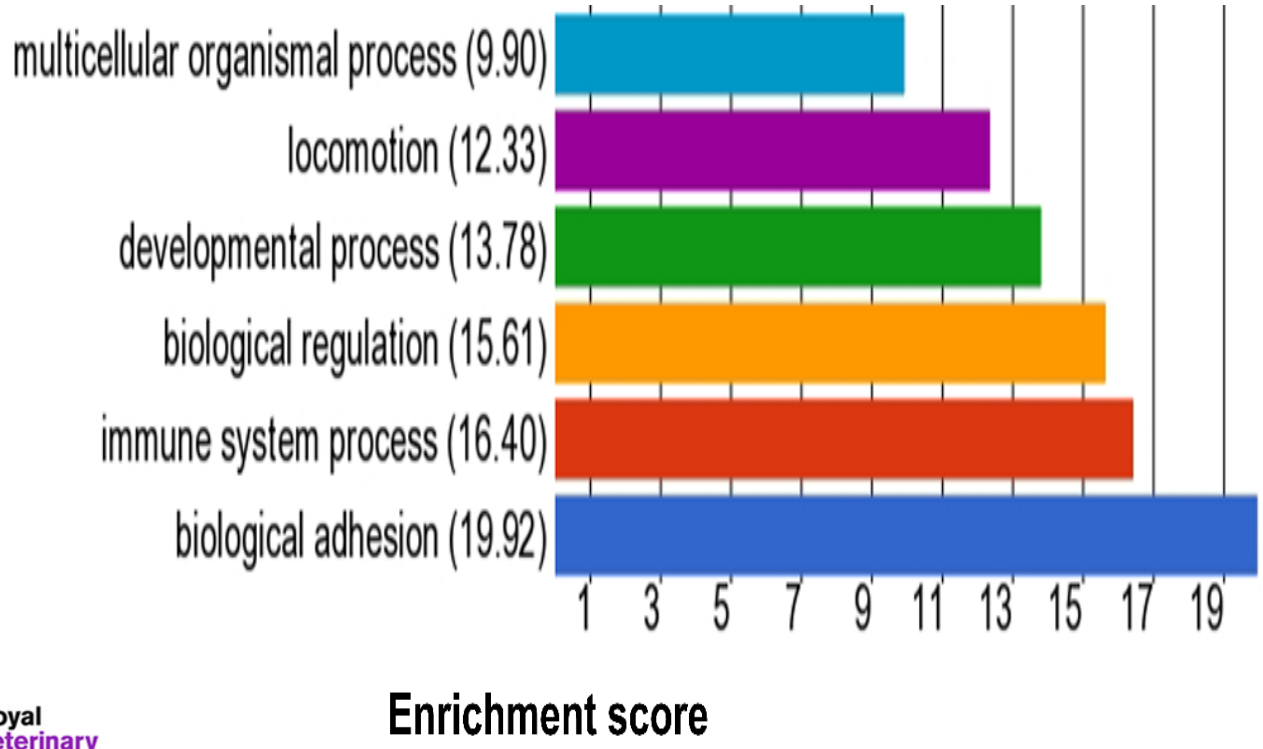
High NEFA cows tended to be older, heavier at drying off and they lost more BCS after calving

Cheng et al. (2021) J Dairy Sci. 104:10059-10075

Gene Ontology enrichment analysis between the cows with High (>750 µM, n = 10) and Low (<500 µM, n = 6) circulating NEFA concentrations in circulating PBMC

The PBMC population includes lymphocytes (T-cells, B-cells, NK cells), monocytes and dendritic cells.

DEG: 118 upregulated and 186 downregulated.



High NEFA concentrations were associated with inhibited cellular adhesion by down-regulating 20/26 genes (by up to 17 fold) related to this process

ADAMTS9, ANGPT1, CLDN4, COL12A1, CYP1B1, DDR1, DSG2, EPHB4, FERMT2, ICAM1, ICAM5, IL1B, ITGAD, KIRREL1, JAM2, PDGFRA, PLEK, SSPO, TNC, TNF,
CD4, CD36, EPDR1, GAS6, ITGA4, PCDHGA5

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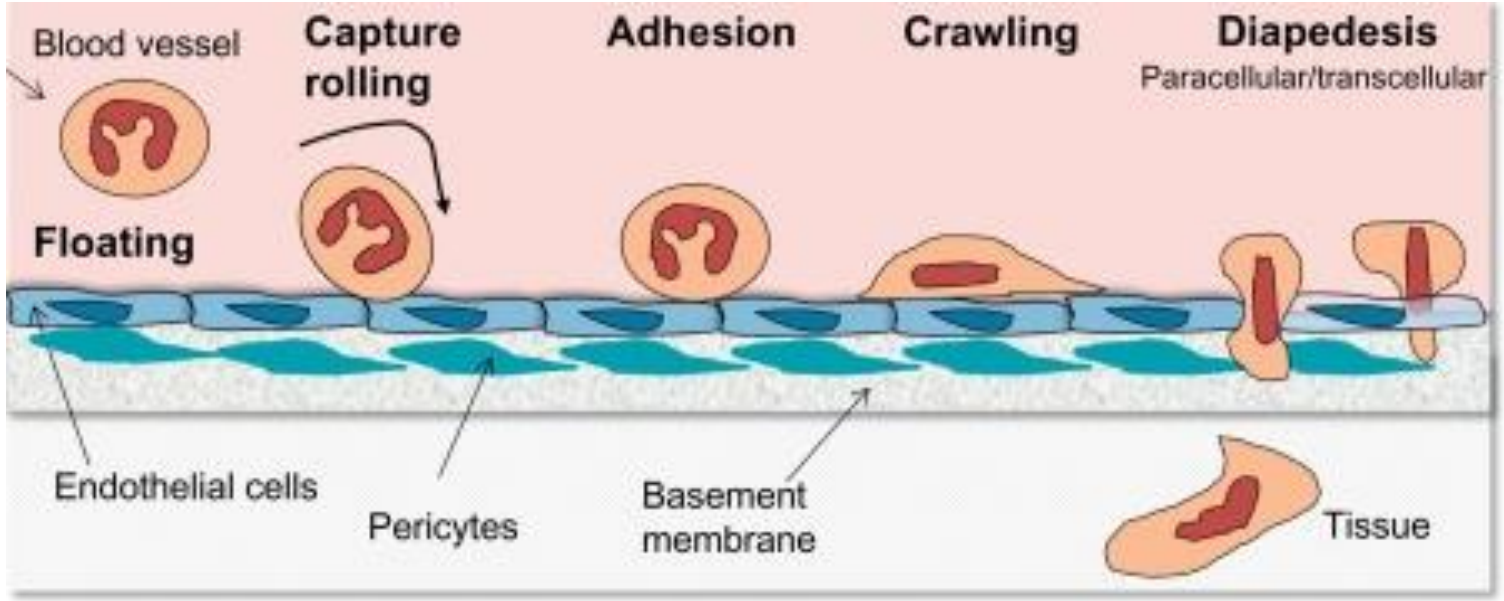
CONCLUSIONS



Fillippi (2016) Adv. Immunol. 129 25-53

- Leukocyte adhesion to the vascular endothelium and diapedesis contributed to the top signaling pathway of candidate genes associated with responses to mastitis (Chen et al., 2015).
- Many other pro-immune mediators in the PBMC were significantly downregulated in the cows with high circulating NEFA.
- Inhibition of key immune functions in the PBMC associated with high circulating NEFA concentrations during early lactation may therefore reduce resilience to infectious diseases.

Age-related changes and survival

•Cattle can potentially live for over 20 years.

•Average UK lifespan in dairy cows is currently around 6 years.

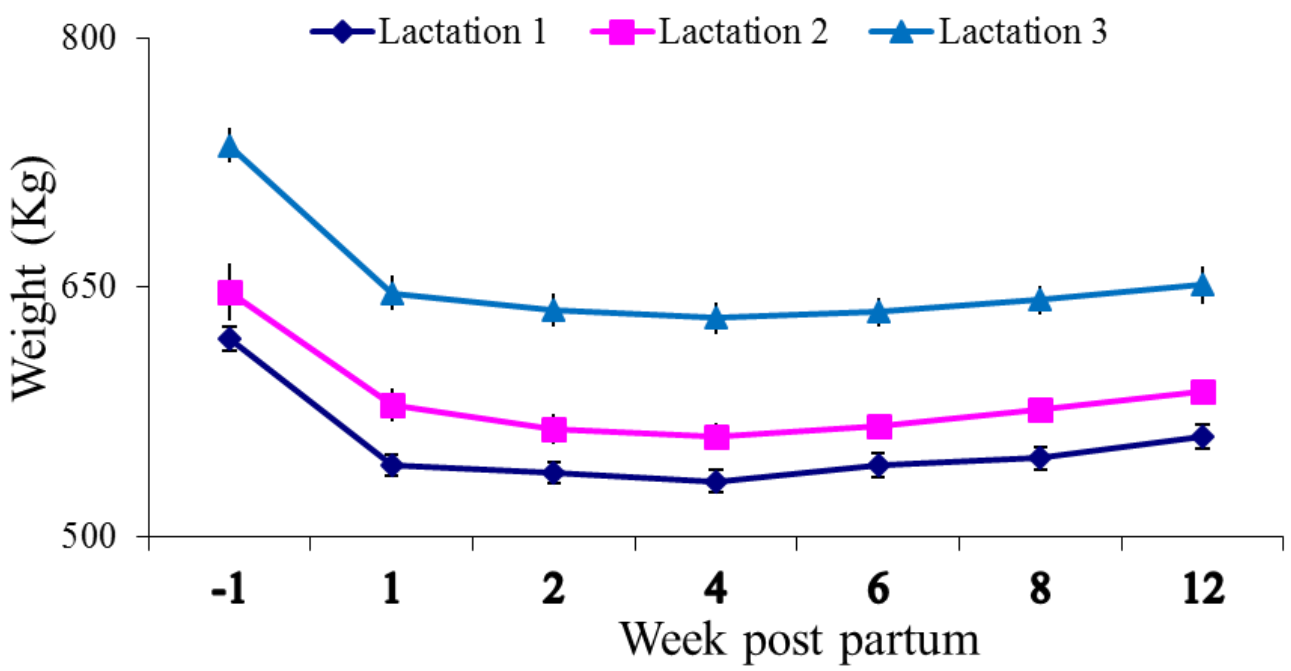
•Cull rate is about 25% per lactation

Average lifespan is around 3 lactations per cow

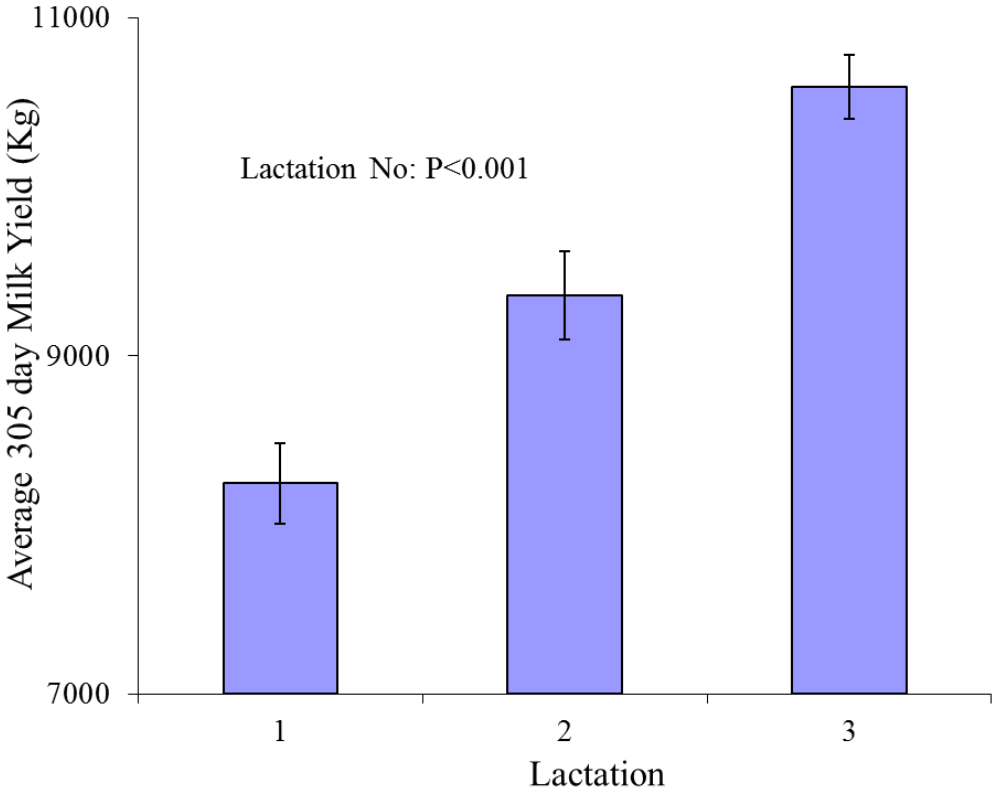
- Growth
- Pregnancy
- Lactation
- Exposure to pathogens

Age related changes

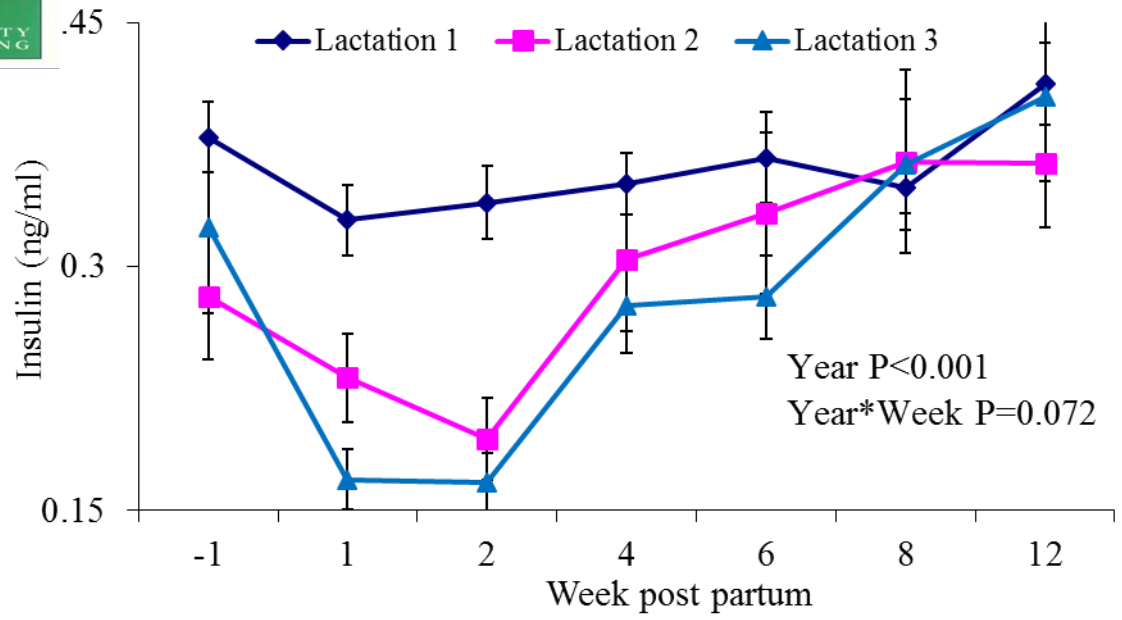
Body Weight

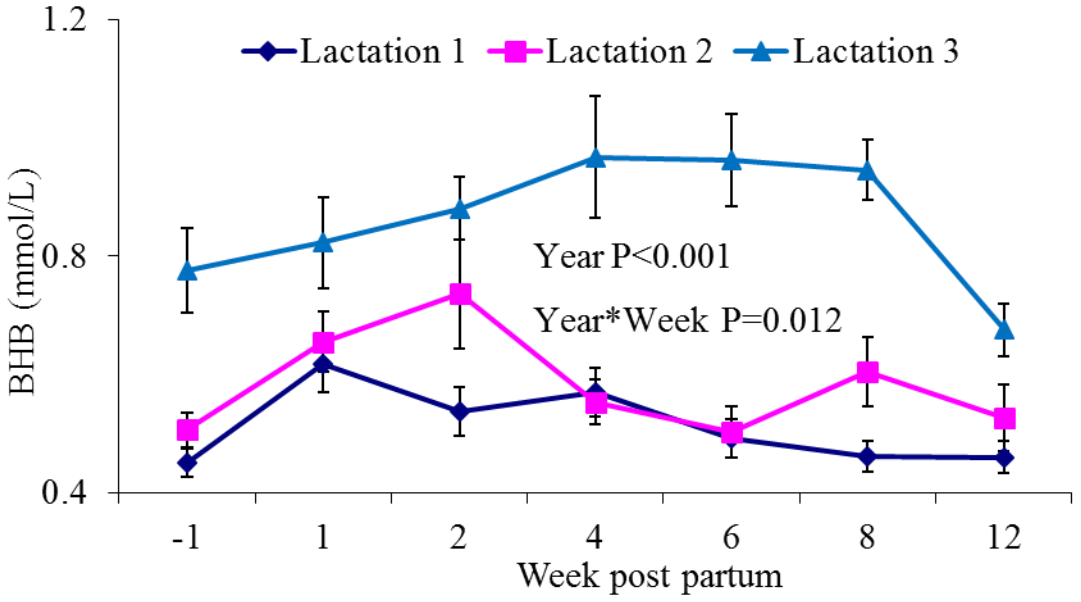


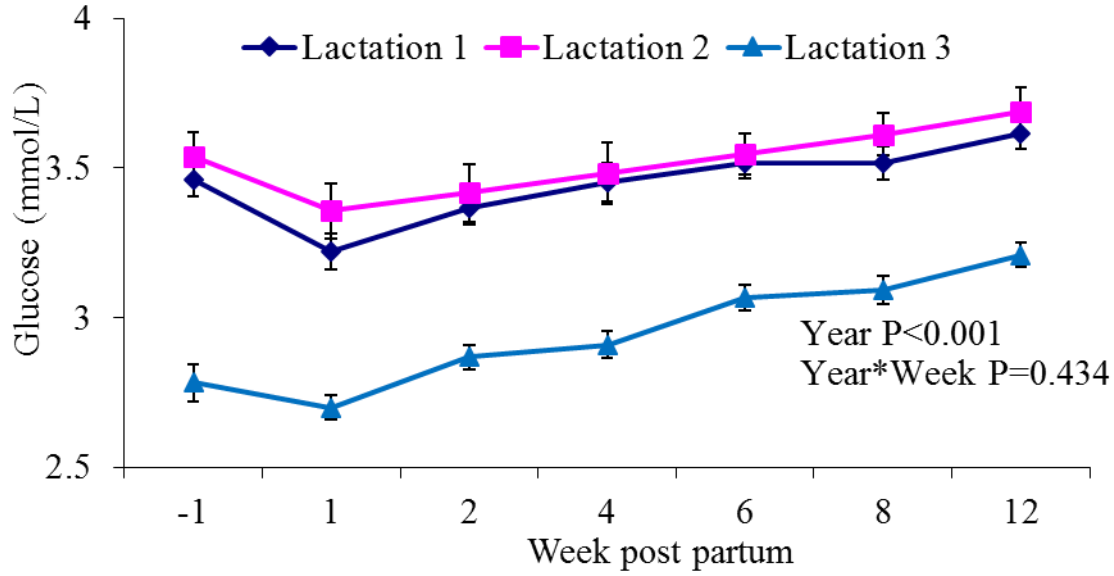
Milk yield

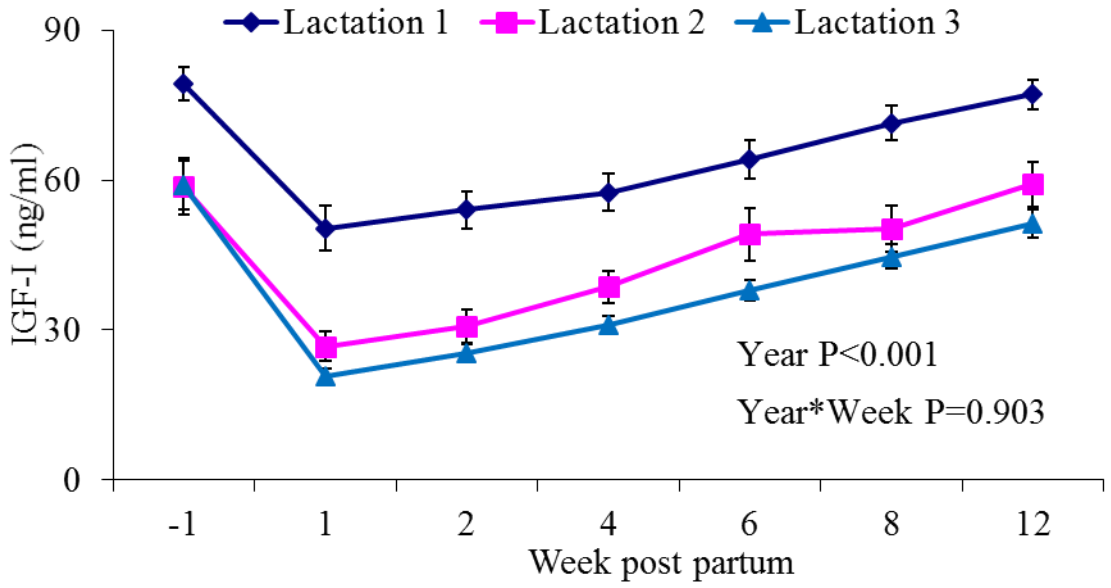


Data are from 47 cows monitored over their three first three lactations


Lactation 1
Lactation 2
Lactation 3
Insulin (ng/ml)
.45
0.3
0.15
Year P<0.001
Year*Week P=0.072
-1
1
2
4
6
8
12
Week post partum


Lactation 1
Lactation 2
Lactation 3
BHB (mmol/L)
1.2
0.8
0.4
Year P<0.001
Year*Week P=0.012
-1
1
2
4
6
8
12
Week post partum


Lactation 1
Lactation 2
Lactation 3
Glucose (mmol/L)
4
3.5
3
2.5
Year P<0.001
Year*Week P=0.434
-1
1
2
4
6
8
12
Week post partum


Lactation 1
Lactation 2
Lactation 3
IGF-I (ng/ml)
90
60
30
0
Year P<0.001
Year*Week P=0.903
-1
1
2
4
6
8
12
Week post partum

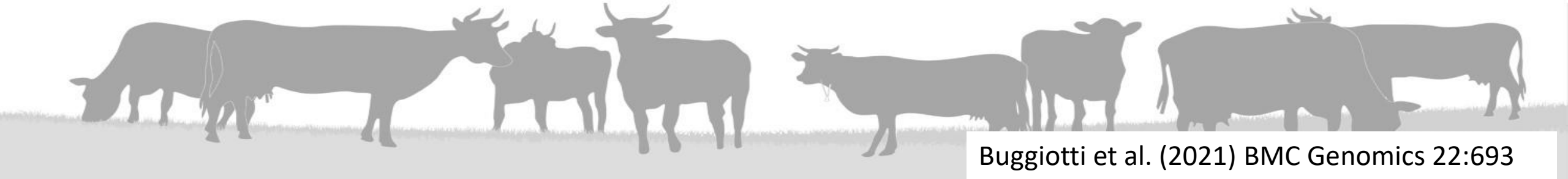
3. Age-related changes in leucocytes

Blood samples (229 cows, 6 herds) taken into Tempus tubes at 14±3 days in milk (DIM).

Leucocytes processed for total RNA-Seq using Illumina NextSeq 500 platform.

Data analysis:

- Cows subdivided by lactation no: 1 (PP), n =53; 2-3 (MP), n = 121; 4-7 (MP>3), n = 55.
- Analytical procedure using DESeq2 followed by weighted gene co-expression network analysis (WGCNA)
- Main comparison between young (primiparous) and old (multiparous >3)



Buggiotti et al. (2021) BMC Genomics 22:693

Hallmarks of cellular ageing

Previous studies in humans and model organisms (*C. elegans, Drosophila*, mice) have identified candidate genes whose expression is consistently associated with cellular ageing. These have been classified into six categories:

- downregulation of genes encoding mitochondrial proteins,
- downregulation of the protein synthesis machinery,
- dysregulation of immune system genes,
- reduction in growth factor signalling,
- constitutive responses to stress and DNA damage,
- dysregulation of gene expression and mRNA processing.

- 38/170 (22.3%) of these candidate genes were present in our list of DEGs from ageing cows

Frenk S, Houseley J. Gene expression hallmarks of cellular ageing. Biogerontology. 2018;19:547–66
Peters MJ, et al. The transcriptional landscape of age in human peripheral blood. Nat. Commun. 2015;6:1–14.

DAVID functional annotation cluster analysis of the leukocyte DEG in the modules related to lactation number

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Cluster	Higher expression in PP cows	Higher expression in MP>3 cows
1	ES 16.2, n=56 Krueppel-associated box Zinc finger C2H2-type/integrase DNA-binding domain	ES 29.7, n=471 Membrane component
2	ES 7.0, n=142 Zinc-finger Metal ion binding	ES 19.9, n=435 Signal peptide Disulfide bond Glycoprotein
3	ES 6.8, n=18 Transcription regulator SCAN	ES 7.3, n=254 Transmembrane region
4	ES 5.0, n=61 Nucleotide binding	ES 3.9, n=27 Glycolysis / Gluconeogenesis
5	ES 2.0, n=31 BTB/POZ fold	ES 3.7, n=25 C-type lectin fold
6	ES 1.9, n=50 Immunoglobulin-like domain	ES 3.3, n=45 Innate immunity

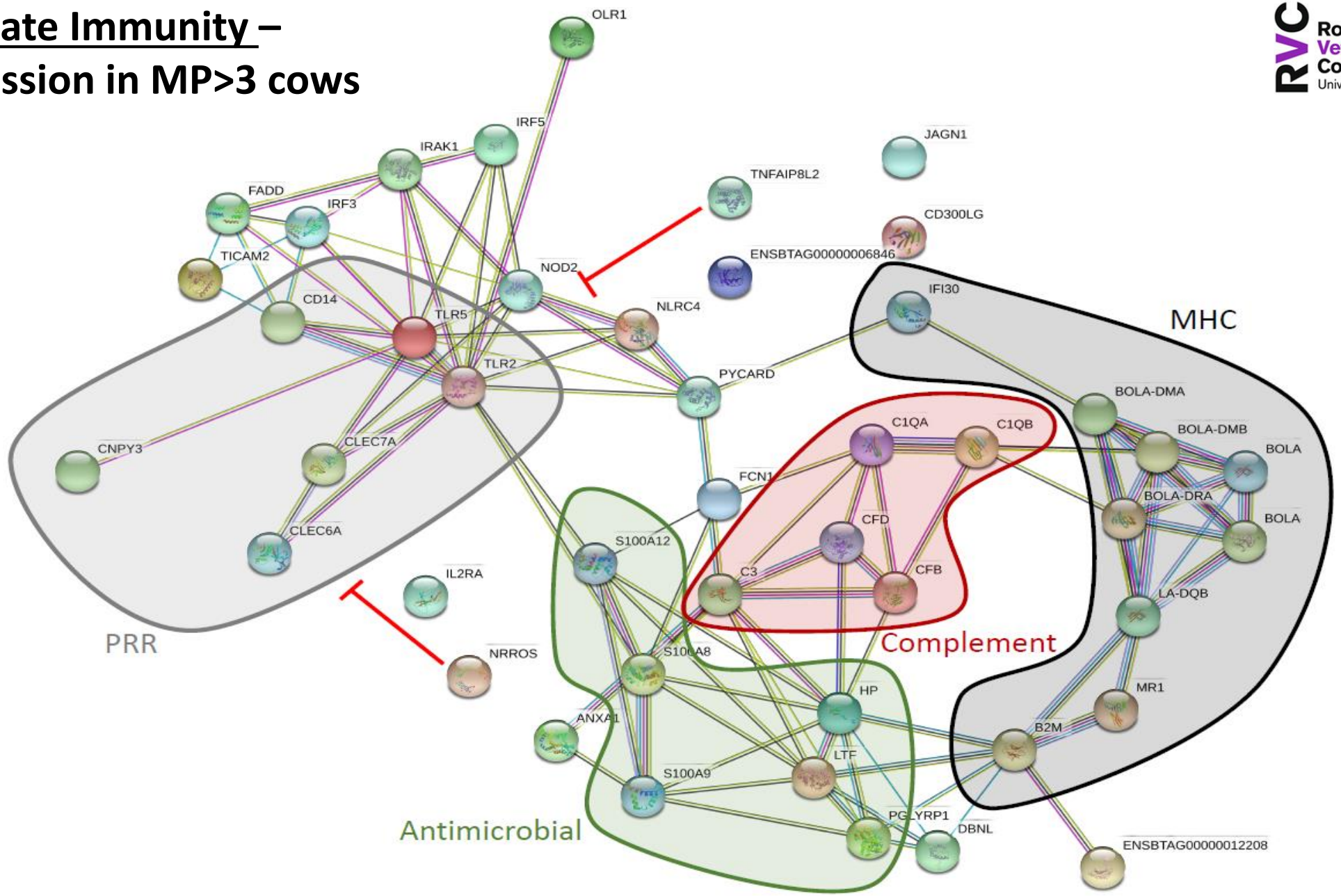
Regulation of gene transcription

Membrane

ES: enrichment score, n =No. DEG in cluster

Buggiotti et al. (2021) BMC Genomics 22:693

Cluster 6 Innate Immunity – higher expression in MP>3 cows



Buggiotti et al. (2021) BMC Genomics 22:693

Genes involved in adaptive immunity were up-regulated in the PP cows

- The GO term *immune system process* was enriched
- Up-regulation of genes associated with T-cell development and function (*CCR7, CD27, IL7R, CAMK4, CD28, ITK, LCK*)
- Down-regulation of genes involved in the *regulation of cytokine secretion* (*IL10, CD14, FGR, IL17RC*).

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Summary of key biological pathways in leukocytes which alter as cows age

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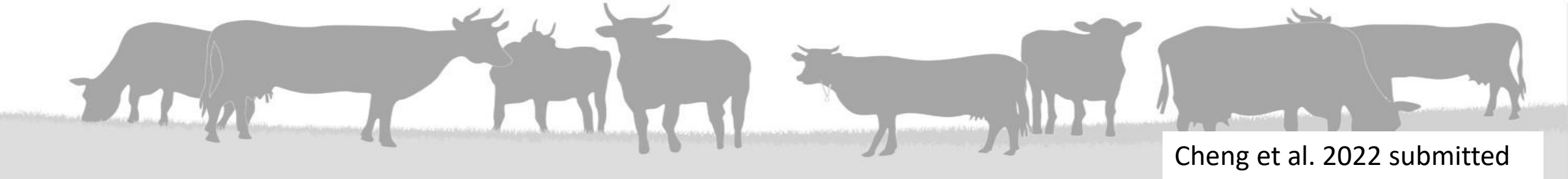
- Ribosome biogenesis, transcriptional regulation and DNA replication, elongation and repair: all higher in PP cows.
- Differential expression of immune pathways: adaptive immunity higher in PP cows, innate immunity higher in MP>3 cows.
- Changes in pathways supplying leukocytes with energy: increased expression of genes encoding enzymes involved in beta-oxidation of fatty acids in PP cows whereas genes involved in glycolysis up-regulated in MP>3 cows.
- Changes in mitochondrial function: differential expression of genes encoding mitochondrial ribosomal proteins between age groups.

- Buggiotti et al. (2021) BMC Genomics 22:693

4. Diet-related changes in liver

- Hepatic biopsy samples taken from Holstein-Friesian cows at around 14 days in milk.
- Processed for total RNA-Seq using Illumina NextSeq 500 platform at GIGA Institute, University of Liège
- Cows offered isonitrogenous grass silage-based diets from calving with different proportions of concentrates: 1) low concentrate (LC, 30% concentrate + 70% grass silage); 2) medium concentrate (MC, 50% concentrate + 50% grass silage), or 3) high concentrate (HC, 70% concentrate + 30% grass silage).
- Within each dietary group cows were subdivided by lactation number into primiparous (n=18) and multiparous (n=40) groups.
- Main comparison between HC and LC diets for primiparous and multiparous cows, analysed separately.



Cheng et al. 2022 submitted

Chemical composition of the grass silage and concentrates offered (Mean ± SD)

		Treatment Concentrate			
	Grass silage	LC	MC	HC	Concentrate in parlour
DM (g/kg)	206 ± 30.8	891 ± 5.5	895 ± 8.0	894 ± 8.7	898 ± 11.3
CP (g/kg DM)	137 ± 9.6	182 ± 5.6	168 ± 6.6	162 ± 2.7	166 ± 5.1
ME (MJ/kg DM)	11.4 ± 0.32	13.1	13.4	13.4	13.4

	Ration		
Ratio concentate:silage	LC 30:70	MC 50:50	HC 70:30
CP (g/kg DM)	152	152	154
ME (MJ/kg DM)	12.0	12.4	12.8
ERDP (g/cow/d, offered)	1556	1997	2420
ERDP (g/cow/d, required)	1235	1700	2175
DUP (g/cow/d, offered)	559	733	888
DUP (g/cow/d, required)	790	788	857
MP (g/cow/d, offered)	1346	1817	2275
MP (g/cow/d, required	1577	1872	2262

- Offered as partial mixed ration.
- Fed using Calan Broadbent feeding system.
- Offered at 107% previous day's intake.
- Formulated to achieve a common total diet CP.
- Only the HC diet was calculated to fully meet the DUP and MP requirements of the cows.

afbi Agri Food and Biosciences Institute

Little et al. (2019) Animal 13:799-809.

Effect of diet on production parameters in multiparous cows at 14 DIM

Diet	LC	MC	HC
n	13	12	15
Total DMI (kg/d)	16.3 ± 0.8[c]	20.4 ± 0.9[b]	23.6 ± 0.8[a]
BW (kg)	656 ± 21	643 ± 14	669 ± 12
BCS	2.6 ± 0.12	2.4 ± 0.10	2.5 ± 0.07
MY (kg/d)	33.4 ± 1.4[b]	35.9 ± 1.5[ab]	37.9 ± 1.1[a]
ECM (kg/d)[2]	34.8 ± 1.6[b]	36.4 ± 1.5[ab]	40.4 ± 1.4[a]
EBAL (MJ/d)[2]	-11.0 ± 1.5[b]	-4.6 ± 1.4[a]	-1.3 ± 1.3[a]

At the time of hepatic biopsy, the MP cows on HC diet vs a LC diet had:

- greater DMI,
- higher milk yield,
- less negative energy balance

Effect of diet on production parameters in primiparous cows at 14 DIM

Diet	LC	MC	HC
n	6	6	6
Total DMI (kg/d)	12.9 ± 0.6[b]	15.8 ± 0.5[a]	17.1 ± 0.6[a]
BW (kg)	534 ± 16	536 ± 13	525 ± 17
BCS	2.9 ± 0.11	2.9 ± 0.09	3.0 ± 0.15
MY (kg/d)	20.7 ± 1.1	20.5 ± 2.0	23.3 ± 1.1
ECM (kg/d)[2]	21.0 ± 1.2	22.0 ± 2.1	24.2 ± 1.2
EBAL (MJ/d)[2]	-4.5 ± 1.7[b]	0.1 ± 1.4[ab]	1.6 ± 1.6[a]

At the time of hepatic biopsy, the PP cows on HC diet vs a LC diet had:

- greater DMI
- positive energy balance
- milk yield was numerically greater but NS

Blood metabolites and IGF-1 measurements in multiparous and primiparous cows at around 14 days after calving

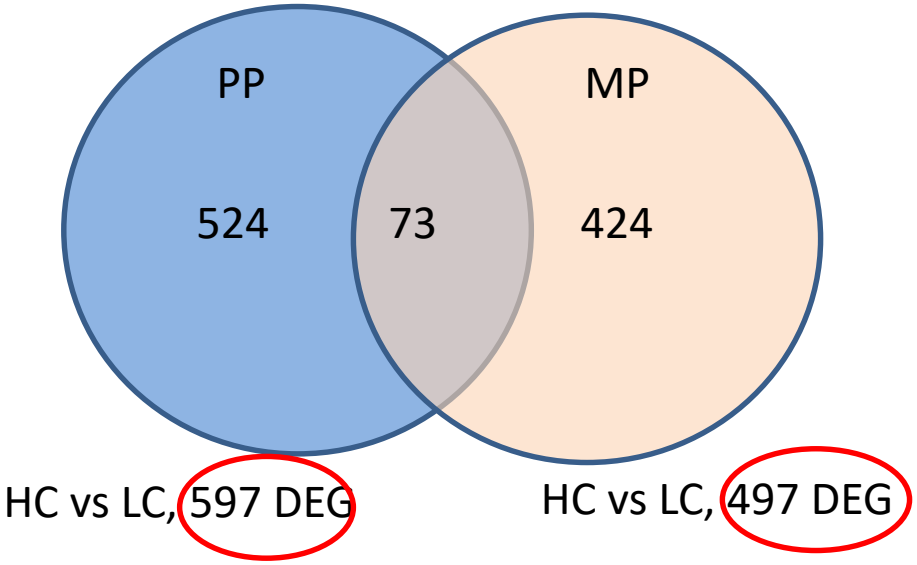
	MP			PP		
Diet	LC	MC	HC	LC	MC	HC
n	13	12	15	6	6	6
Glucose (mmol/l)	3.3 ± 0.08[b]	3.4 ± 0.09[b]	3.6 ± 0.06[a]	3.8 ± 0.14	3.9 ± 0.10	4.0 ± 0.06
Urea (mmol/l)	3.5 ± 0.17[a]	2.9 ± 0.28[b]	2.5 ± 0.11[b]	4.4 ± 0.26[a]	2.7 ± 0.15[b]	2.2 ± 0.15[b]
BHB (mmol/l)	0.63 ± 0.09	0.58 ± 0.05	0.42 ± 0.05	0.44 ± 0.03	0.40 ± 0.04	0.33 ± 0.04
NEFA (mmol/l)	775 ± 110	692 ± 108	608 ± 81	501 ± 149	350 ± 99	462 ± 119
Cholesterol (mmol/l)	2.9 ± 0.19	3.1 ± 0.14	3.1 ± 0.14	3.0 ± 0.12	2.5 ± 0.22	2.6 ± 0.21
IGF-1 (ng/ml)	56 ± 7.3[b]	55 ± 7.1[b]	103 ± 10.1[a]	110 ± 21.7	160 ± 20.5	167 ± 29.2

Values are mean ± SEM.

a > b within rows for means of multiparous (MP) or primiparous (PP) cows, $P < 0.05$.

Differential hepatic gene expression between MP and PP cows on different concentrate diets

	MP cows		PP cows	
Diets	Upregulated	Downregulated	Upregulated	Downregulated
HC vs LC	200	297	406	191



- Most differences found in the HC v LC diet comparison
- More significant DEG in the PP than the MP cows
- Little overlap in the responses to diet between the MP and PP cows

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Summary of GO enrichment analysis for DEG in hepatic biopsy samples, HC vs LC diet comparison

Multiparous cows on HC diet:

Up-regulated: Metabolic processes for lipids, cholesterol, alcohol

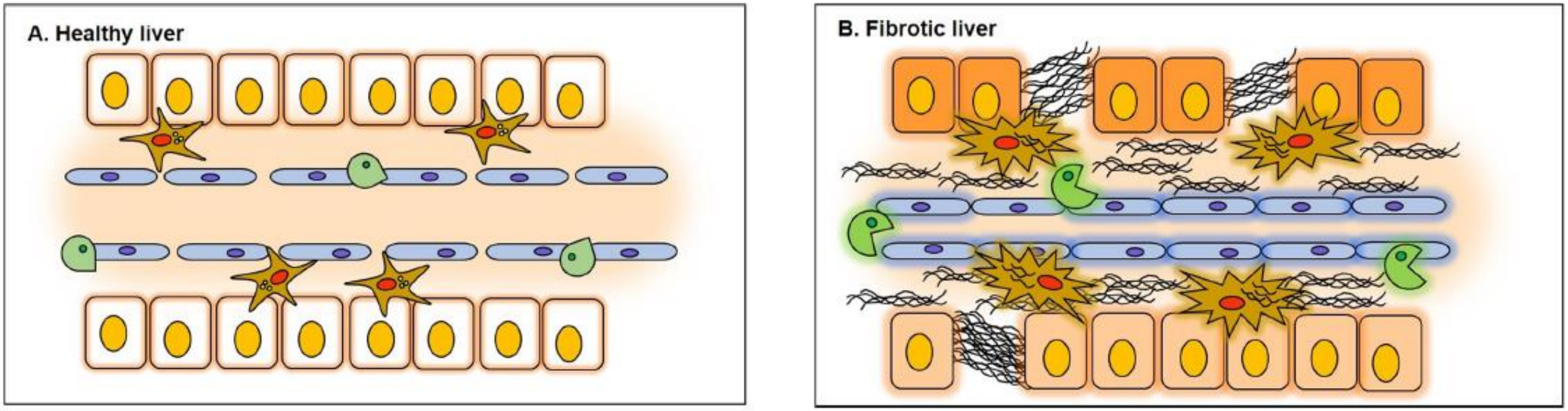
Down-regulated: acute phase response, amino acid metabolism, acute inflammatory response, response to stress

Primiparous cows on HC diet:

Up-regulated: Extra-cellular matrix structure an organisation, collagen, cell adhesion

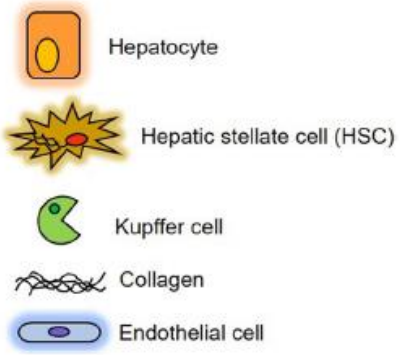
Down-regulated: a variety of amino acid metabolic processes

Development of hepatic fibrosis in humans



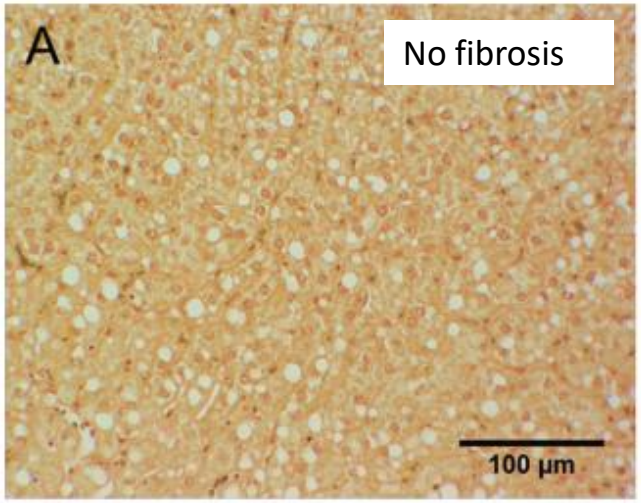
Inflammation

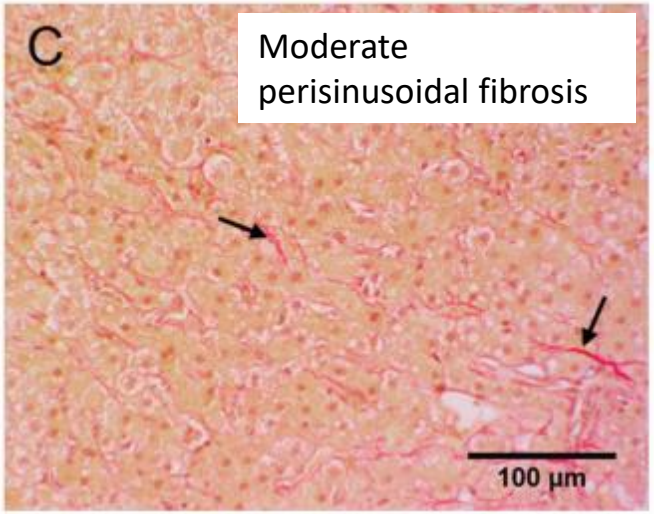
Rise in hepatic blood pressure



Ortiz et al. Extracellular matrix remodelling in chronic liver disease (2021) Curr. Tissue Microenviron. Rep. 2:41–52

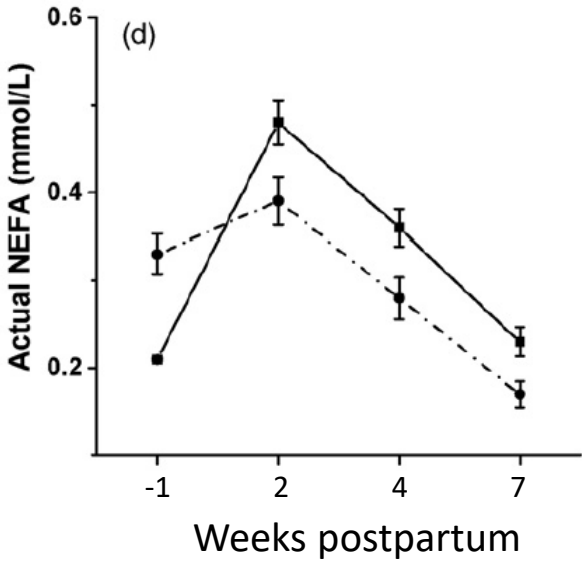
Pietsch et al. (2021) Aspects of transition cow metabolomics-Part II: Histomorphologic changes in the liver parenchyma throughout the transition period…. J. Dairy Sci. 104:9227





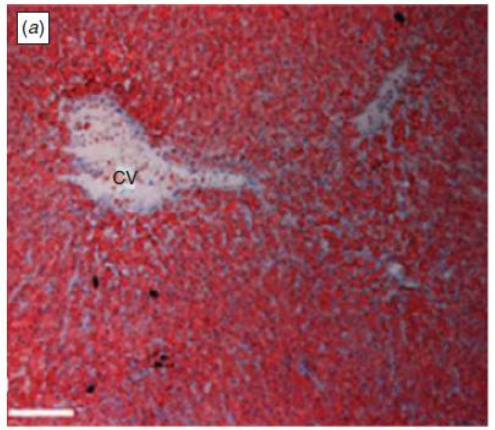
Picrosirius red stain for collagen

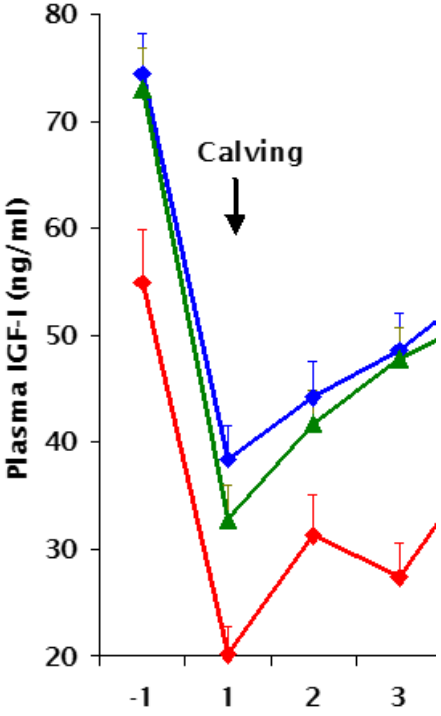
- No primiparous cows included in this study. 60% of MP cows had evidence of fibrosis.
- Perisinusoidal fibrosis more common in cows with lactation numbers ≥5 and in animals classified as Metabiotype B (more severe NEB, livers with higher fat deposition, glycogen depletion, and increased degenerative, inflammatory, fibrotic, and proliferative changes).
- Associated with <milk protein%, >milk fat%, >BCS loss, >disease incidence.
- Raised circulating concentrations of bilirubin, fatty acids, triglycerides and gamma-glutamyltransferase (GTT).
- Raised GTT suggests damage to bile ducts.
- The authors speculated that perisinusoidal fibrosis was caused by the increased blood pressure due to higher postpartum blood flow which compressed the liver sinusoids, a situation which would be exacerbated in cows with fatty livers.



Lipids from NEFA can (directly or indirectly):

- Promote fatty liver disease
- Cause vascular dysfunction
- Impair immune cell function
- Cause systemic insulin resistance
- Activate TLR4 signalling directly, causing inflammation
- Dysregulate the somatotrophic axis





Infectious disease can exacerbate this by:

- Activating TLR signalling pathways
- Inhibiting DMI

As leukocytes age they:

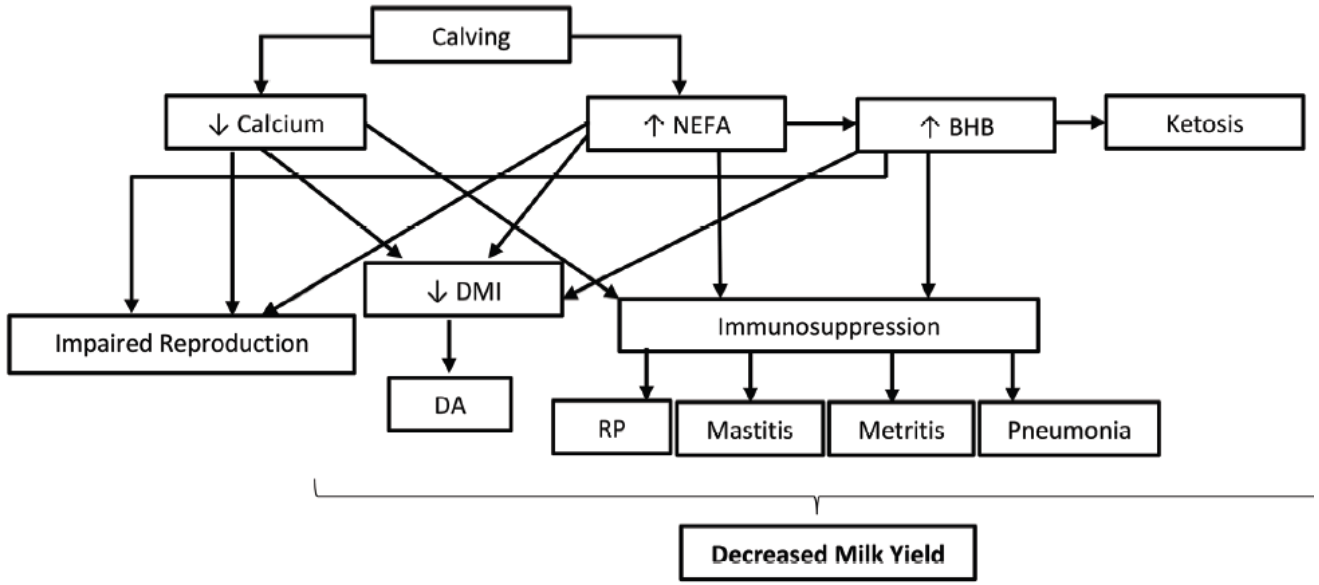
- Experience more damage to mitochondria and DNA
- Downregulate their capacity for mRNA processing and protein synthesis
- Become less capable of mounting an adaptive immune response

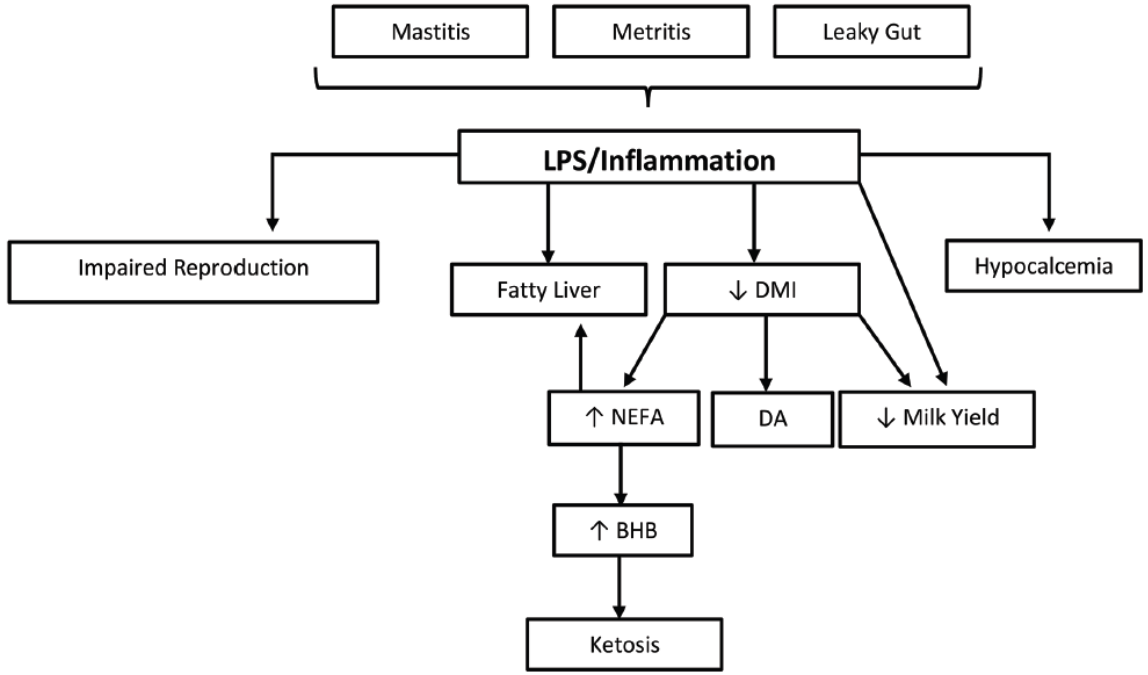
J. Dairy Sci. 104:8380–8410
https://doi.org/10.3168/jds.2021-20330

Invited review: The influence of immune activation on transition cow health and performance—A critical evaluation of traditional dogmas

E. A. Horst, S. K. Kvidera, and L. H. Baumgard*
Department of Animal Science, Iowa State University, Ames 50011

- Problems start before calving
- Can have inflammation and insulin resistance without an infection

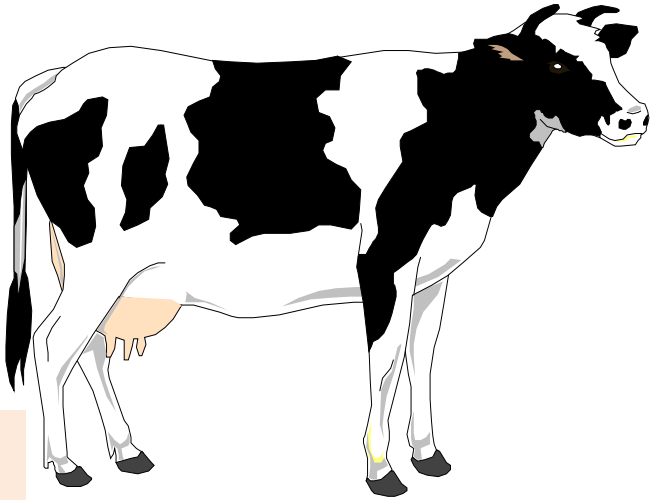




Hypothesis 1. Hypocalcaemia and increased NEFA and BHB cause reduced DMI and immunosuppression

Hypothesis 2. Infection causes inflammation. Subsequent reduced DMI and fatty liver increase tissue mobilisation

Which cows?
What starts it?
Can it be prevented?



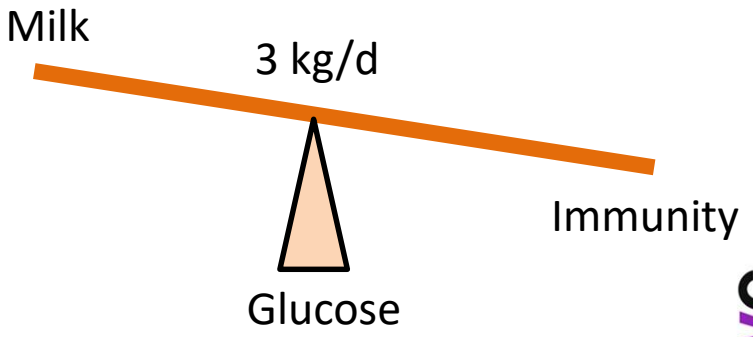
Which cows?

- Excessive genetic selection to promote tissue mobilisation
- Multiparous - Poor dry cow management
- Primiparous – Poor rearing management
- Pre-existing health issues e.g. mastitis, BRD.
- Risks become higher in older cows

- Pre-existing inflammation
- Lipid mobilisation
- Insulin resistance
- Down-regulated somatotropic axis
- Reduced immunity

Increased likelihood of calving problems

- Metabolic imbalance
- Increased likelihood of clinical disease
- Reduced likelihood of conception



Prevention

- Appropriate dry cow therapy
- Correct BCS pre-calving
- Appropriate pre-calving and fresh cow nutrition
- Stress free and clean calving facilities
- Housing design supports high DMI

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