Ovine mastitis: Does early live nutrition influence immunity response in later life?

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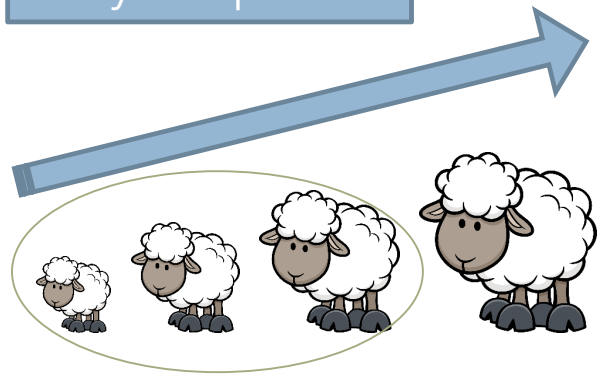
MEGA ule

EARLY LIFE CONDITIONS INFLUENCING IMMUNITY STATUS

Early live period



Sensitive life stage
Hardship conditions

Animal fitness
Immunity status

Food composition and availability

Immune system

(Fragkou et al., 2021)

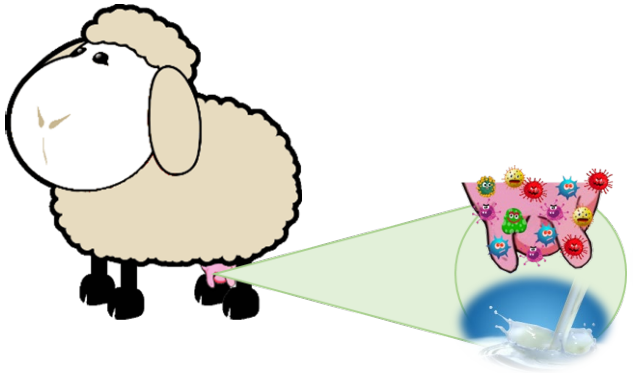
(Mugabo et al., 2010)

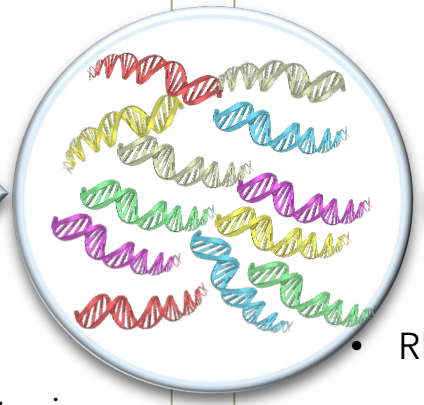
(Schmidt et al., 2015)

LACTATION, MASTITIS AND RNA-SEQ

Differences in immune response or immune system vulnerability can be more accused under stress situations, such as lactation in livestock animals

MASTITIS





- Inflammatory disease of the mammary gland
- Most commonly caused by environmental or contagious pathogenic microorganisms (*Escherichia coli*, *Streptococcus uberis* and *Staphylococcus aureus*)
- Economic losses

RNA-Seq

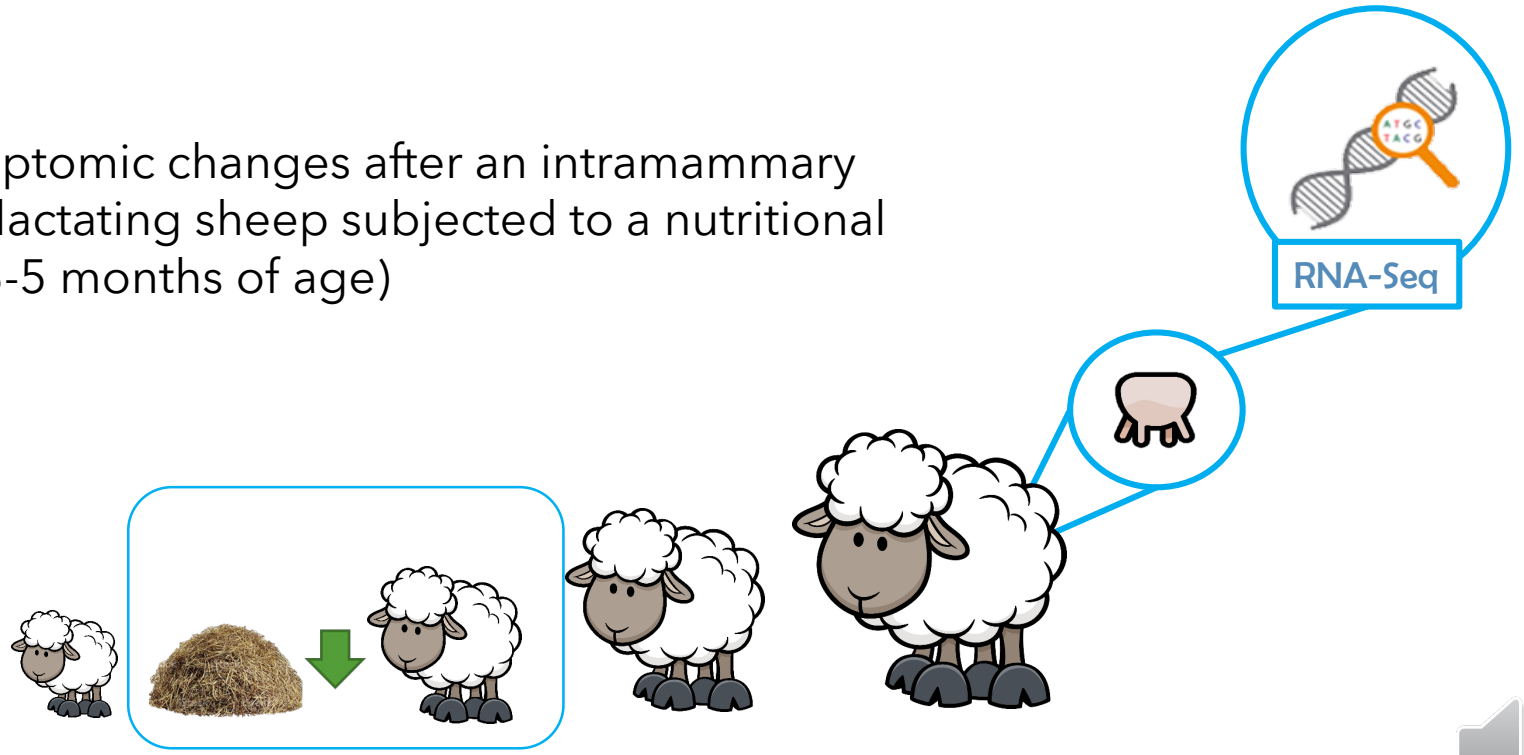
- Aims to characterize the transcriptome of a specific tissue at a specific time or condition

- RNA-Seq studies on Mastitis in dairy livestock:
  - More studied in dairy cows (Fang et al., 2017; Asselstine et al., 2019; Wang et al., 2020)
  - *Mycoplasma agalactiae* Infection in sheep (Chopra-Dewasthaly et al., 2017)

OBJECTIVE

The aim of this study was:

To evaluate mammary gland transcriptomic changes after an intramammary lipopolysaccharide (LPS) infusion in lactating sheep subjected to a nutritional challenge at the prepuberal stage (3-5 months of age)



EXPERIMENTAL DESIGN= NUTRITIONAL CHALLENGE

Nov-18	Dec-18	Jan-19	Feb-19	Mar-19	Apr-19	may-19	jun-19	Jul-19	Aug-19	Sep-19	Oct-19	Nov-19	Dec-19	Jan-20	Feb-20	Mar-20	Apr-20	May-20	Jun-20	Jul-20
M1	M2	M3	M4	M5	M6	M7	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18	M19	M20	M21	M22

Ewes´ birth

Allometric growth of the mammary gland

Artificial insemination

Lambing

Lactation

Inflammatory Challenge

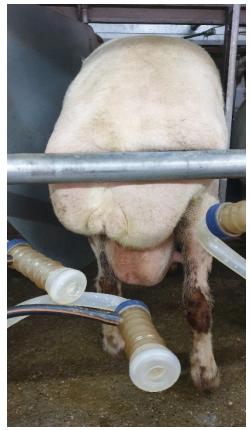
N=40

CSIC

Ad libitum barley straw + 1.3 kg of granulated feed/animal and day

Nutritional challenge= Differences in granulated feed

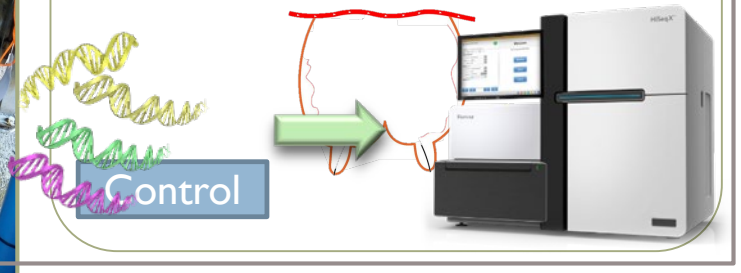
- Control (**n=20**)= 16% raw protein (soy cake)
- Challenge (**n=20**)= without soy cake, containing ~9% raw protein



15 Controls
15 NC

EXPERIMENTAL DESIGN= INFLAMMATORY CHALLENGE

Application of LPS
Healthier udder based on
SCC -96/72 h



Animals:

12 Nutritional Challenge (NC)

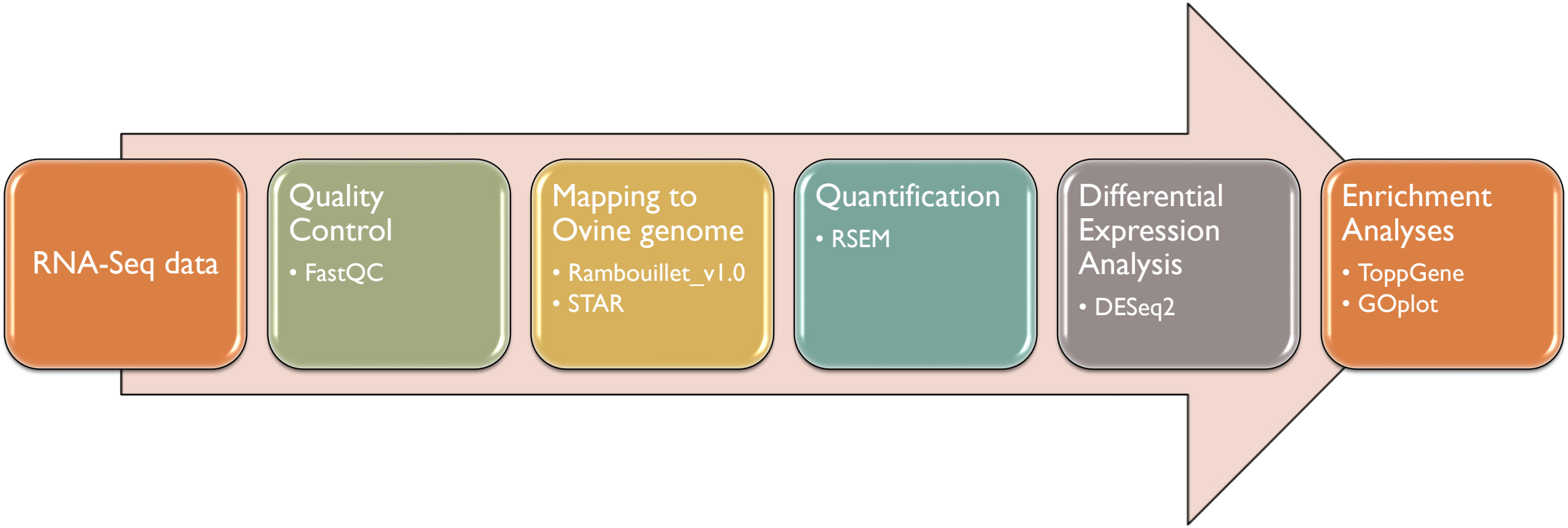
8 Control (C)

Time:

-96/72 h	-24 h	0 h	2 h	4 h	6 h	8 h	12 h	24 h	48 h	72 h	144 h
Milk	Milk Blood Serum Tª	Milk Serum Tª	Plasma Serum Tª	Plasma Serum Tª	Milk Blood Serum Tª	Plasma Serum Tª	Plasma Serum Tª	Milk Blood Serum Tª	Milk Serum Tª	Milk Serum Tª	Milk Serum Tª

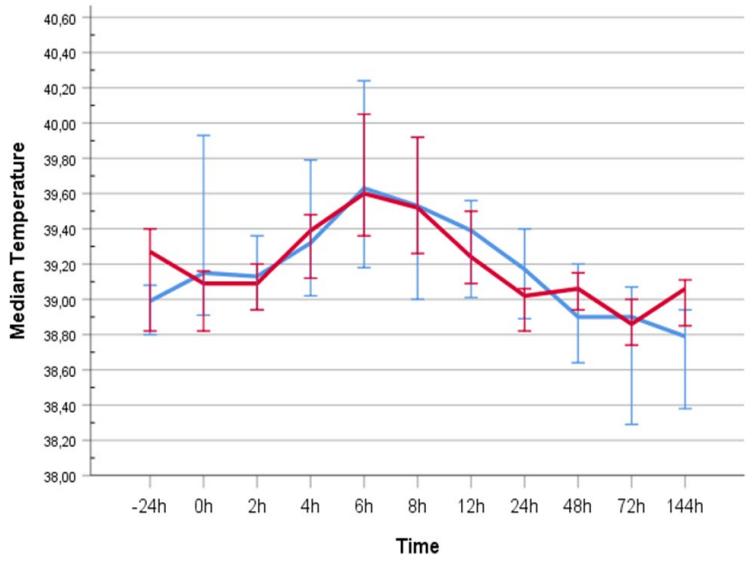
Samples:

RNA-SEQ BIOINFORMATIC PIPELINE



EFFECTS OF THE INFLAMMATORY CHALLENGE

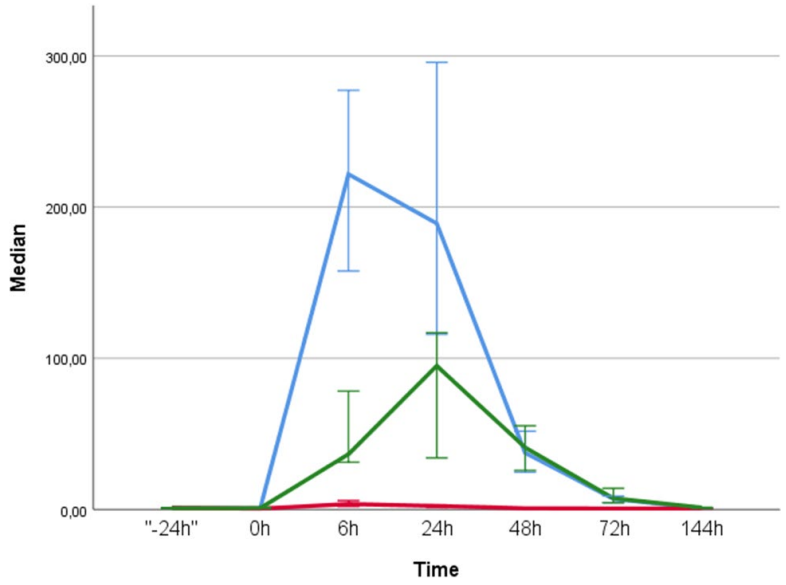
Temperature



Control

Nutritional Challenge

Somatic Cell Counts

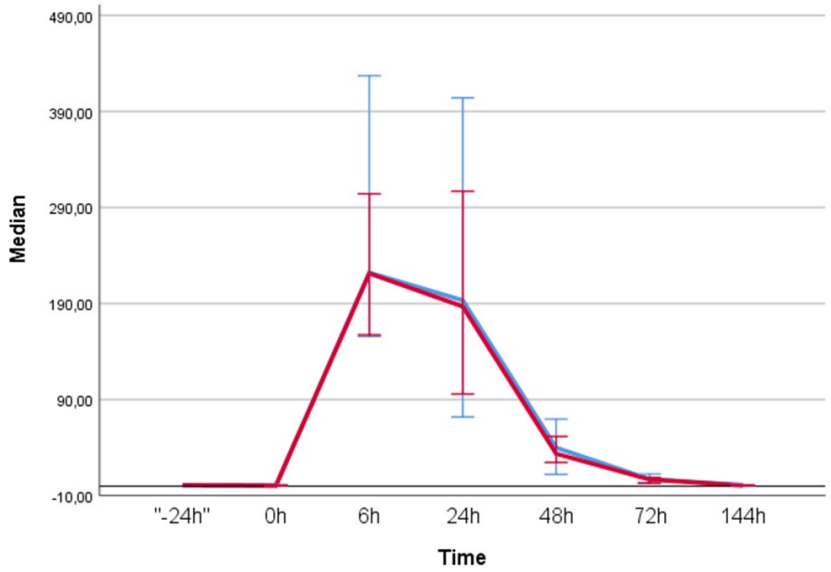


LPS innoculated udder

Control udder (not innoculated)

Ratio infected udder/control udder

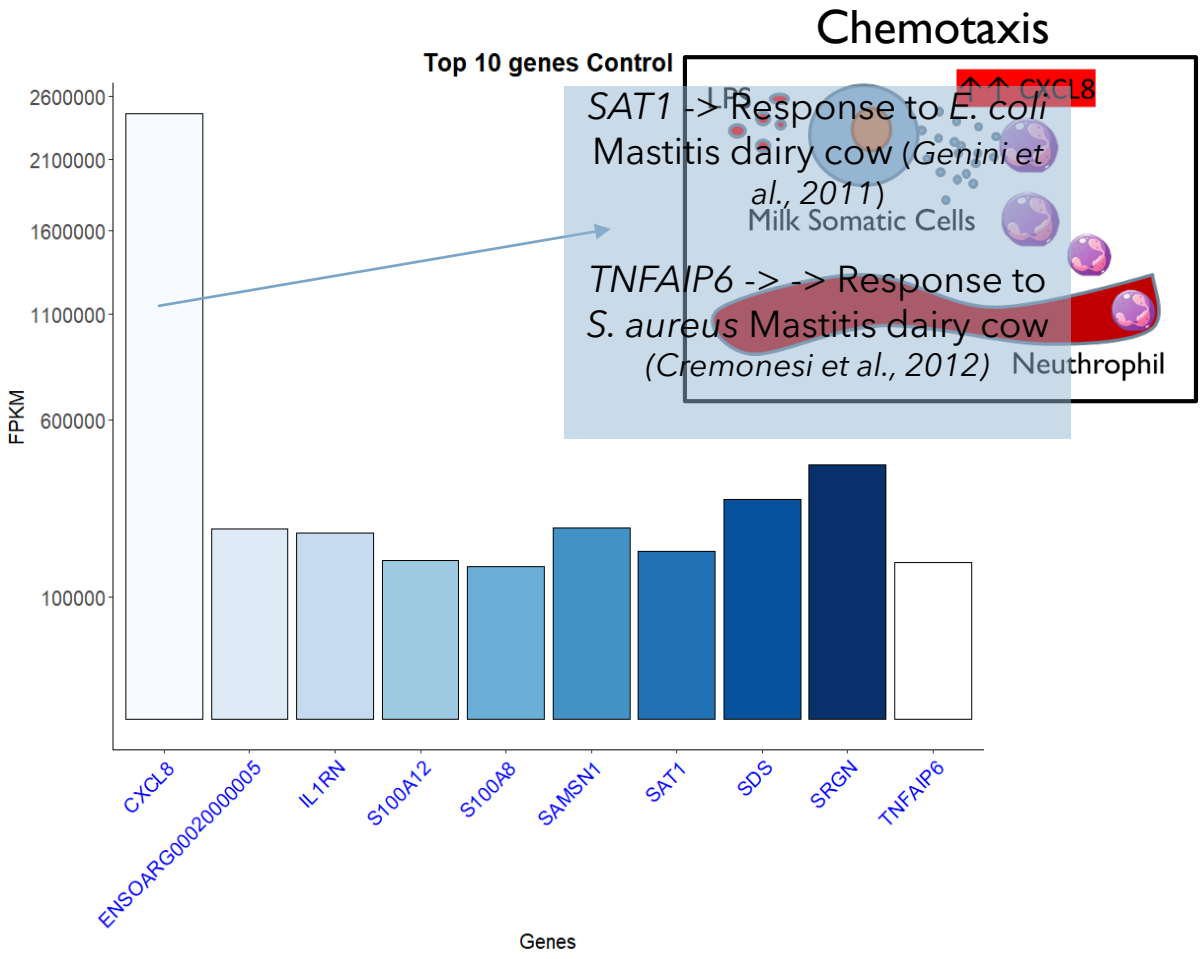
Somatic Cell Counts

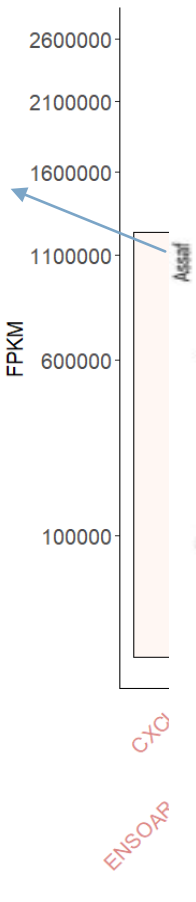


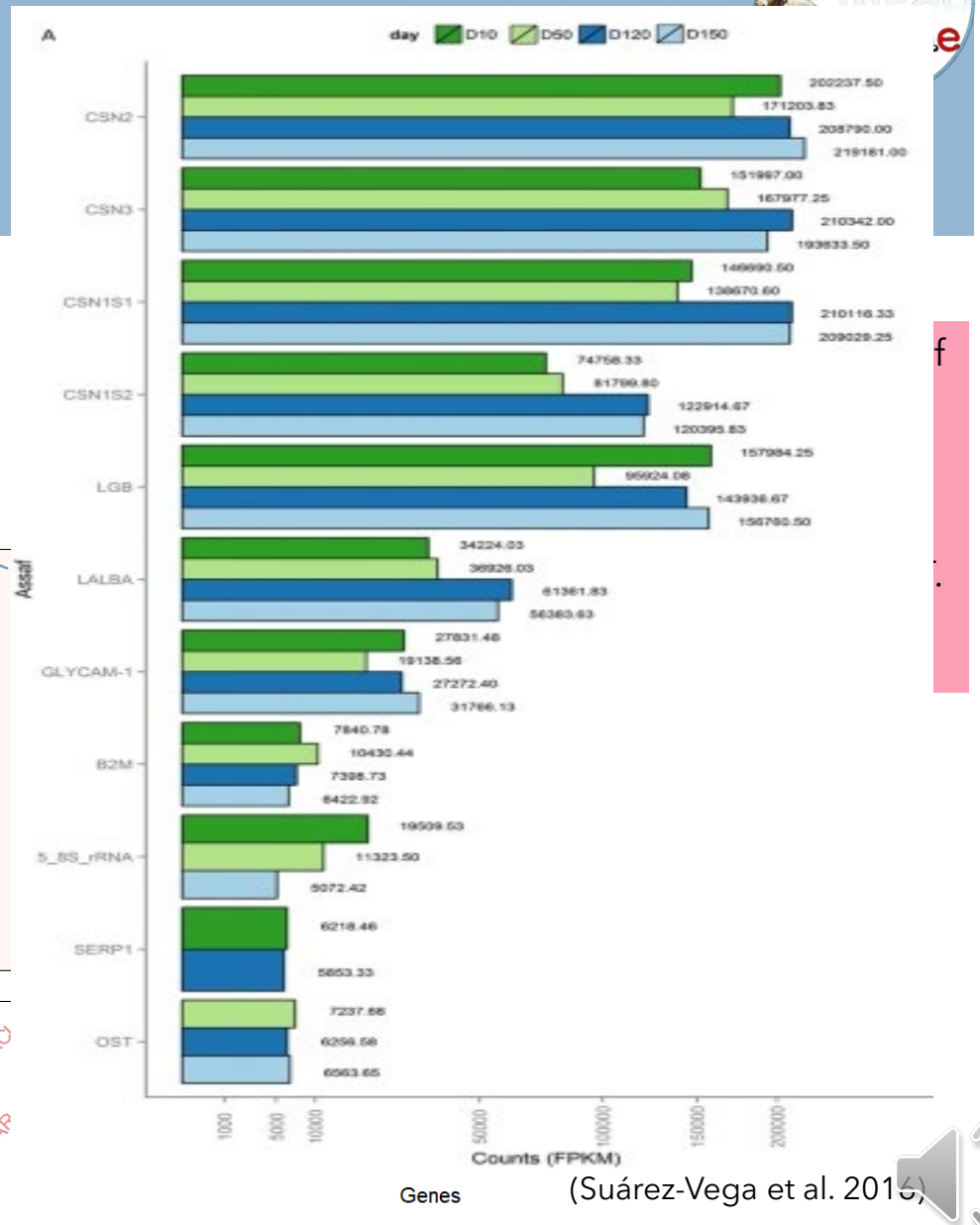
Control

Nutritional Challenge

PROFILE OF THE MILK SOMATIC CELL TRANSCRIPTOME





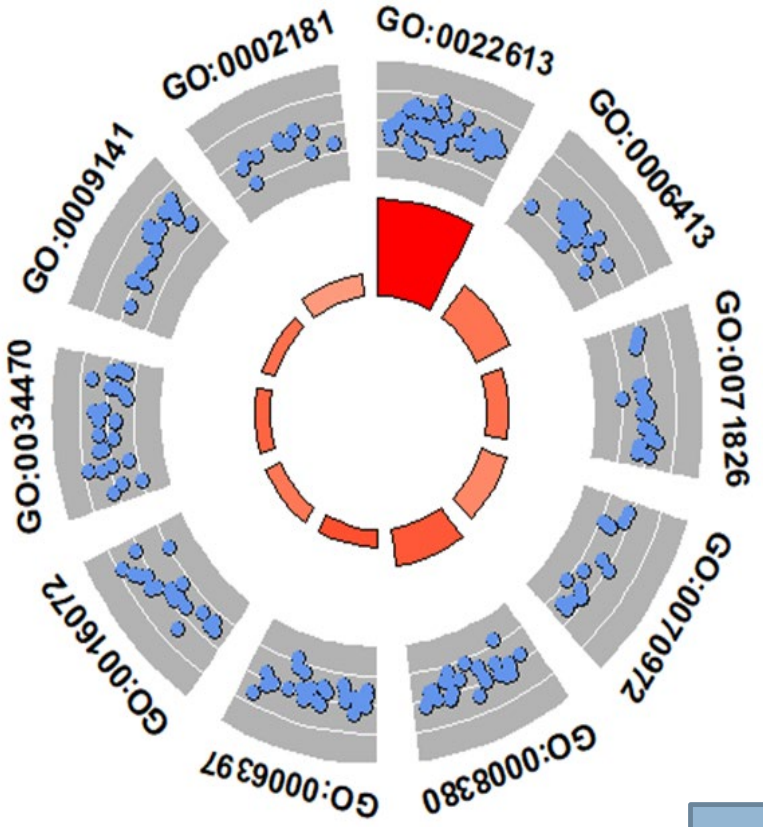


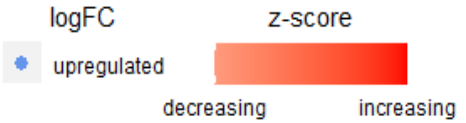
(Suárez-Vega et al. 2015)

DIFFERENTIAL EXPRESSION ANALYSES: GENES UPREGULATED IN CONTROL

585 Differentially expressed genes (padj≤0.05)

495 upregulated in Control animals

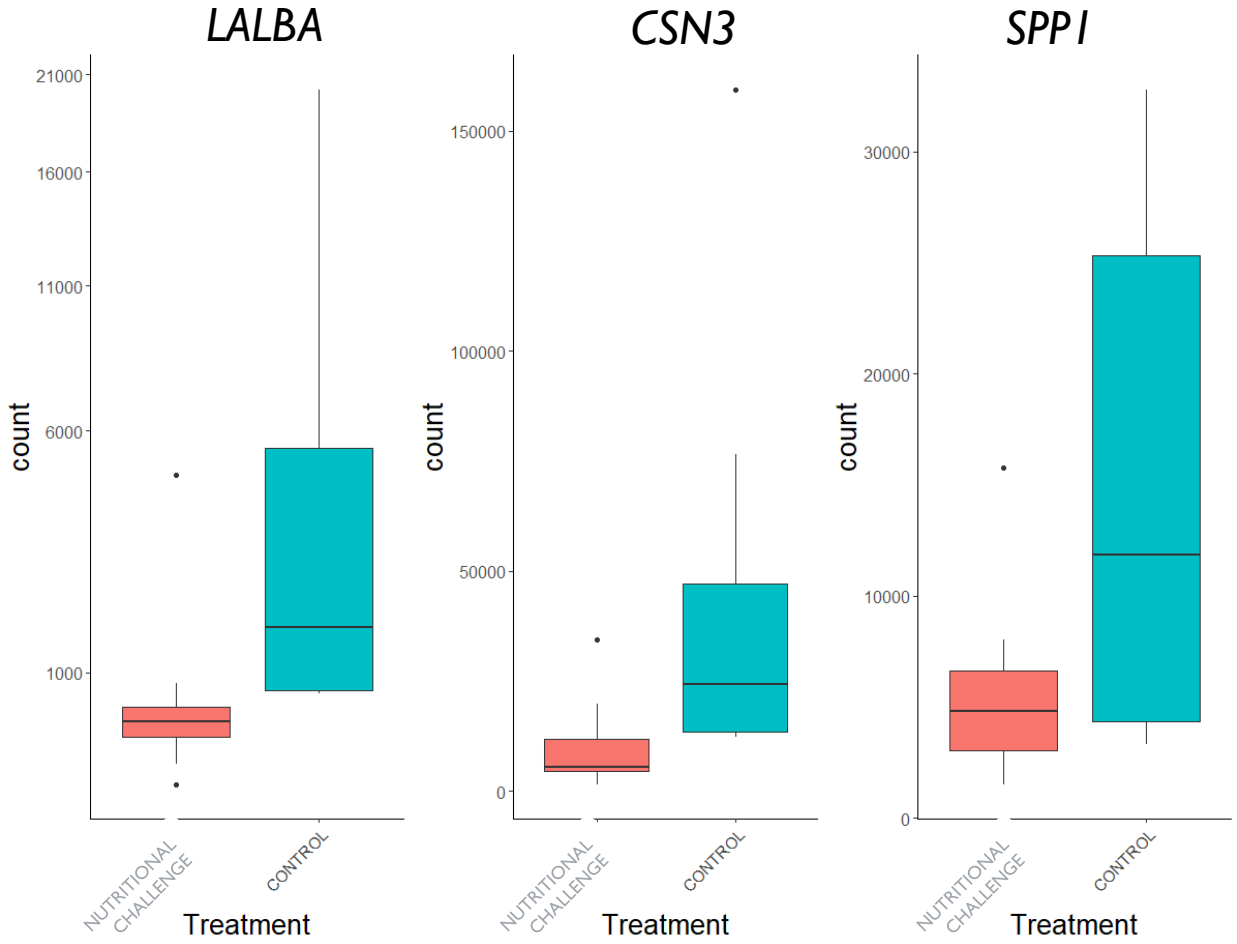




ID	Description
GO:0022613	ribonucleoprotein complex biogenesis
GO:0006413	translational initiation
GO:0071826	ribonucleoprotein complex subunit organization
GO:0070972	protein localization to endoplasmic reticulum
GO:0008380	RNA splicing
GO:0006397	mRNA processing
GO:0016072	rRNA metabolic process
GO:0034470	ncRNA processing
GO:0009141	nucleoside triphosphate metabolic process
GO:0002181	cytoplasmic translation

Transcriptional and translational regulation, protein synthesis

DIFFERENTIAL EXPRESSION ANALYSES: GENES UPREGULATED IN CONTROL



Transcriptional and translational regulation, protein synthesis

Synthesis of lactation proteins

Higher number of Milk Epithelial Cells in Controls??

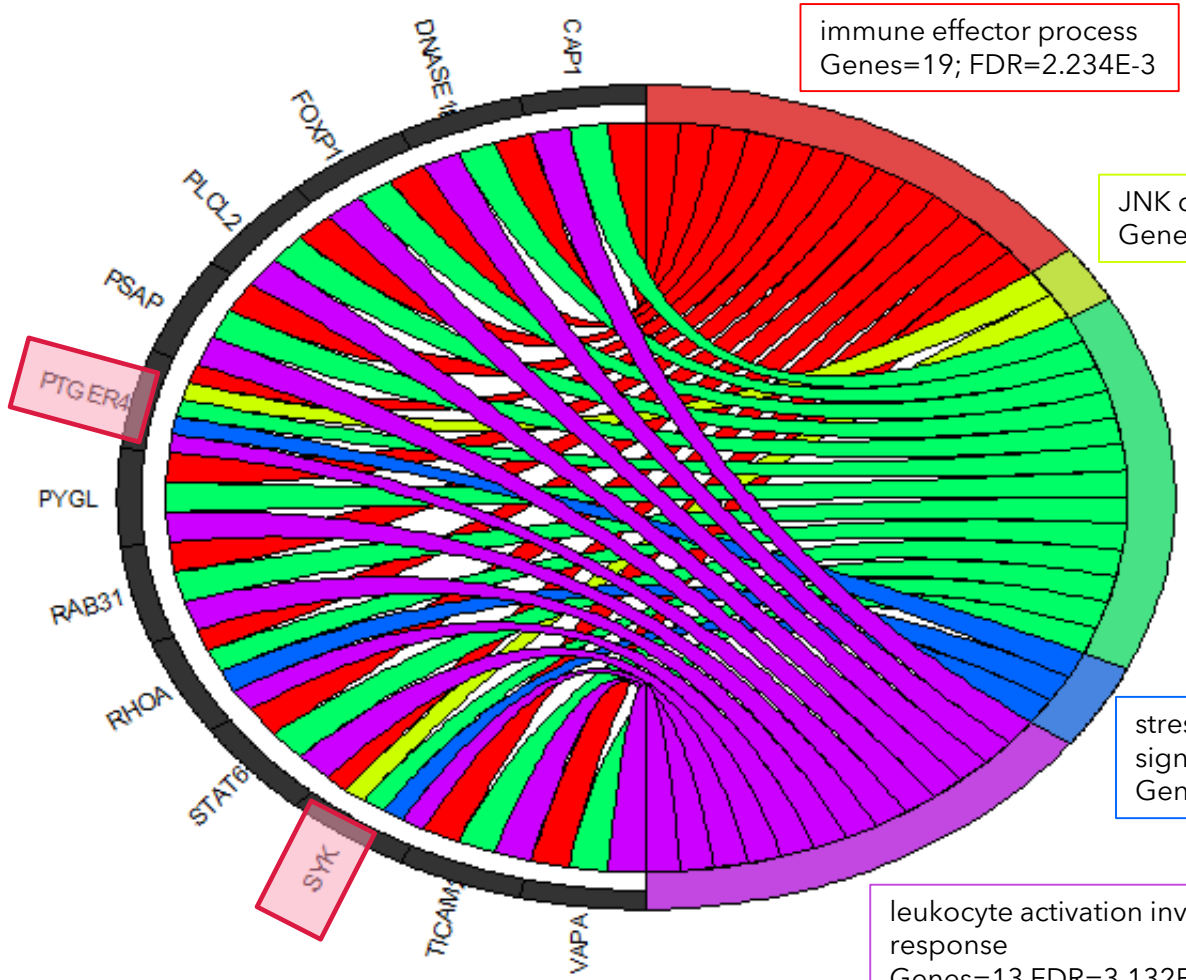
DIFFERENTIAL EXPRESSION ANALYSES: GENES UPREGULATED IN NUTRITIONAL CHALLENGE

585 Differentially expressed genes (padj≤0.05)

90 upregulated in Nutritional Challenge animals

(Mugabo et al., 2010)

Susceptibility/tolerance to mastitis in dairy cows and sheep (Banos et al., 2017; Oget et al., 2019; Yang et al., 2019)



TOP 5 GO BP

immune effector process
Genes=19; FDR=2.234E-3

JNK cascade
Genes=8; FDR=2.234E-3

cell activation
Genes=20; FDR=2.234E-3

stress-activated protein kinase signaling cascade
Genes=9; FDR=2.633E-3

leukocyte activation involved in immune response
Genes=13 FDR=3.132E-3

TAKE HOME MESSAGE

- The LPS inflammatory challenge has induced a change in the transcriptome profile of the milk somatic cells
  - Top 10 genes are related to immune response and not to milk protein synthesis
  - The majority of genes differentially expressed were upregulated in the control group
  - Genes overexpressed in controls were implicated in transcriptional and translational processes, and main lactation proteins were upregulated. Consequently, we hypothesize controls had a higher proportion of mammary epithelial cells that nutritional challenge sheep at 6 hours post LPS inoculation
  - In the nutritional challenge animals, the differential expressed genes were mainly related to immunity processes suggesting that protein restriction during the growth period could lead to a higher inflammatory immune response

Thank you for your attention

SMARTER PARTNERS

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