Post-calving leukocyte immune-related genes are enhanced in Simmental compared with Holstein cows

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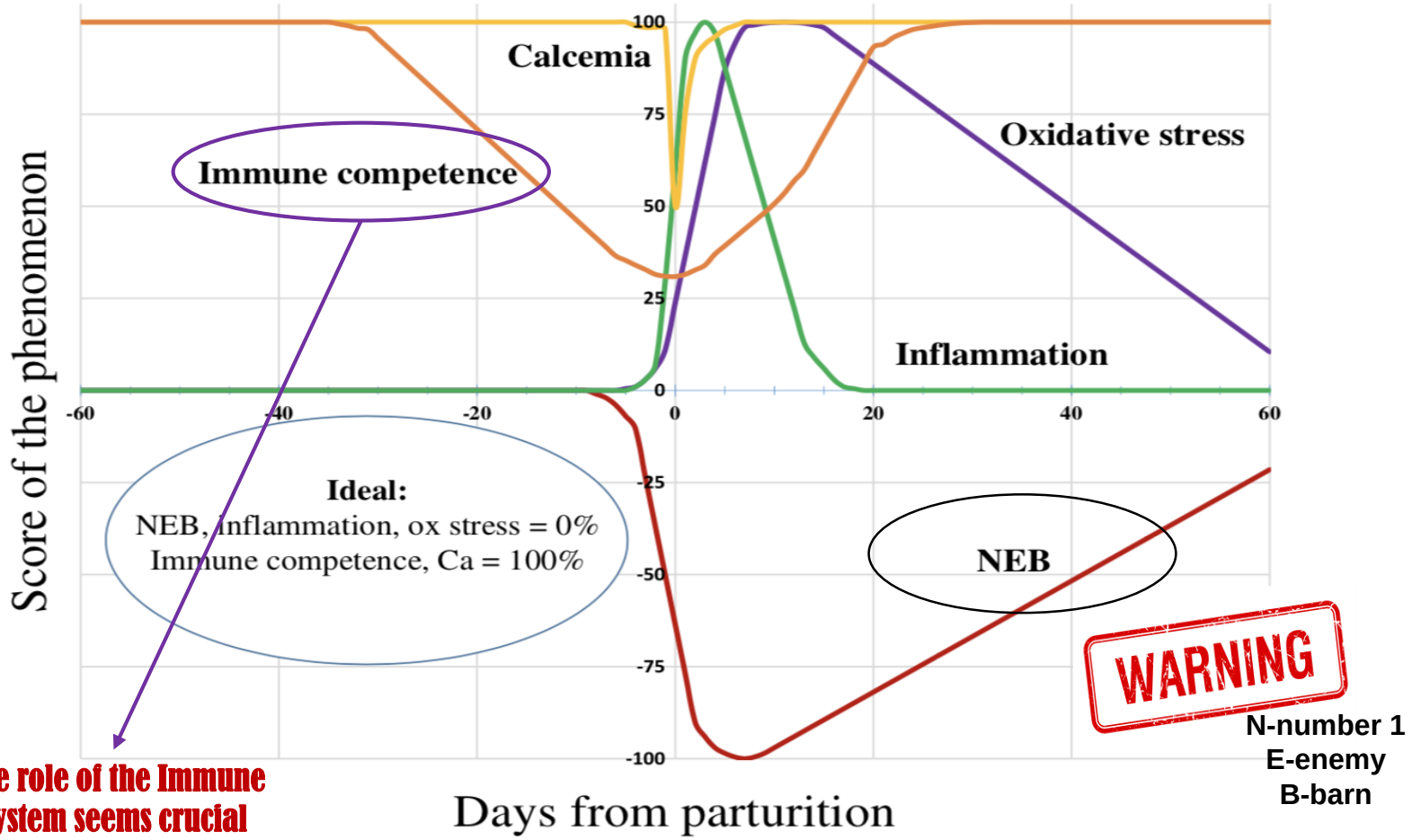
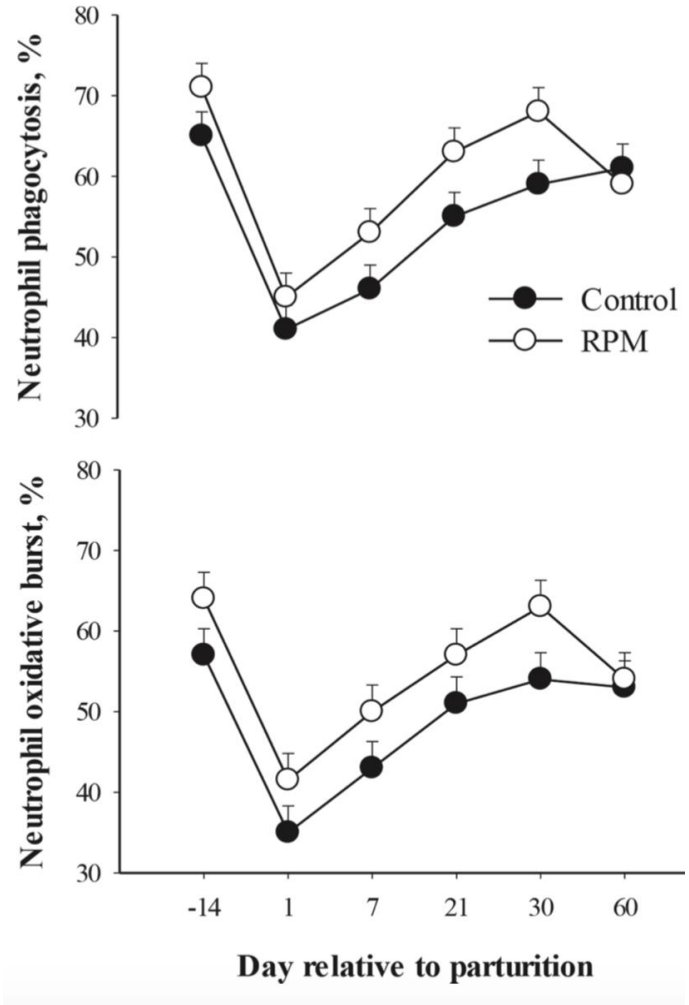
The Transition Period Scenario

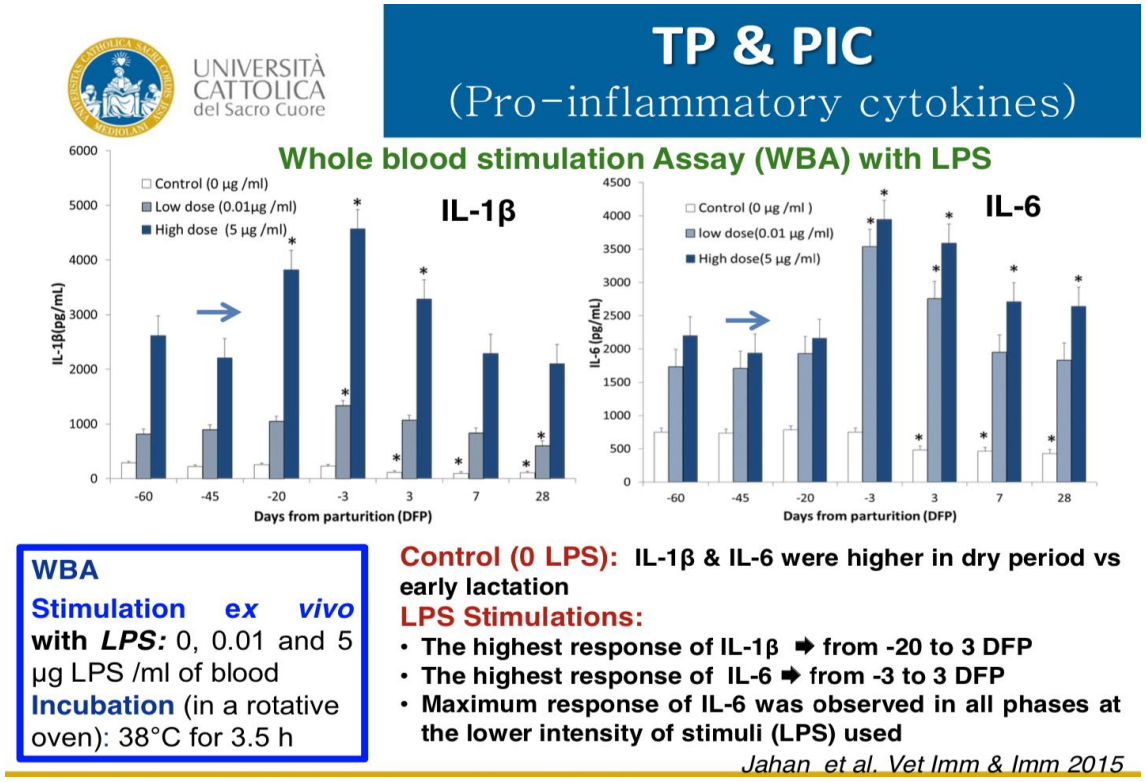


Theoretical pattern of changes in the main physiological aspects of healthy subjects during the transition period (Trevisi E. and Minuti A., Reseacrh in Veterinary Science 2018).

Immune-response after calving



(Batistel et al., 2018)

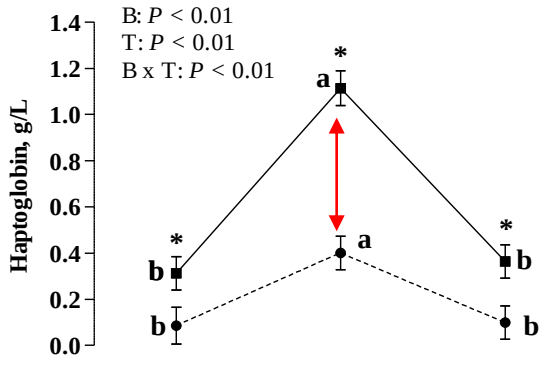


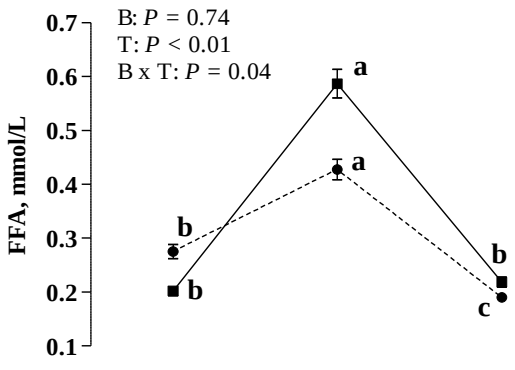
The entering into NEB status during the transition period represents an explanation for this reduced immune function also associated with increased concentrations of some blood metabolites as a result of tissue mobilization.

(Kehrli et al., 1989; Ingvartsen and Moyes, 2013; Mezzetti et al., 2019).

Simmental vs Holstein

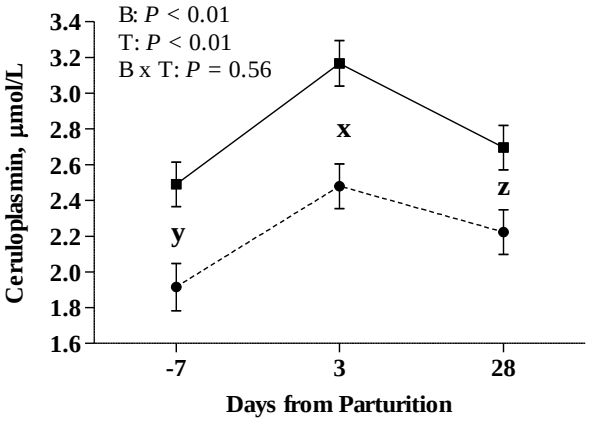
Different metabolic response during the Transition Period

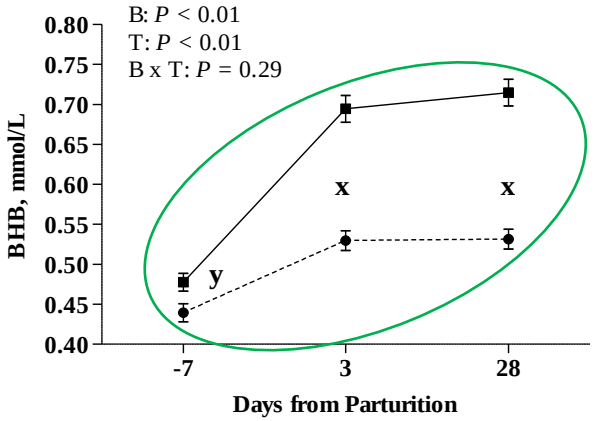


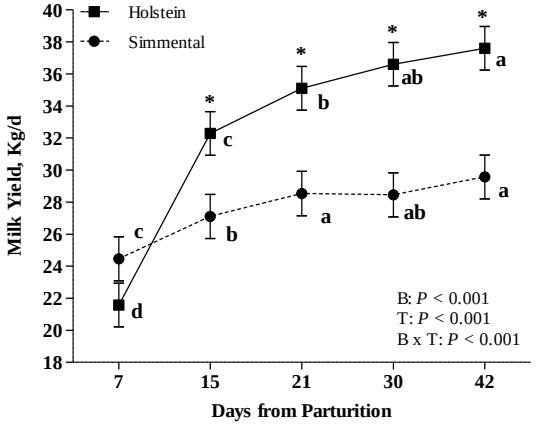


Simmental
Holstein

These findings clearly demonstrate a lower mobilization of body reserves and also a better oxidation of the fatty acids in the liver in cows belonging to breeds that did not undergo to selective pressure for increased milk production.







(Lopreiato et al., 2019 Journal of Dairy Science)

Our Mission

Where we started from:

During the periparturient period of dairy cows, functional studies at the cellular level reported neutrophil dysfunction.

What we aimed:

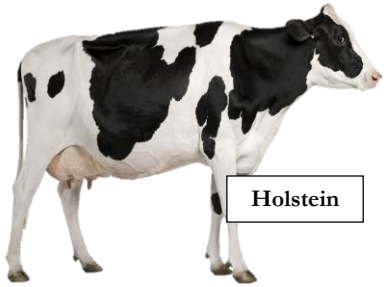
To widely explore several gene networks of whole blood leukocytes immediately after calving from Simmental, and Holstein.

Hypothesis:

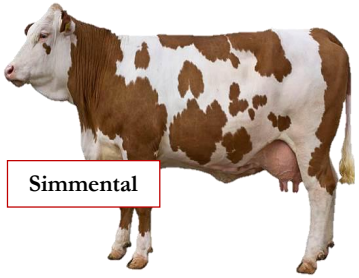
The specific genetic pattern and breeding history between Simmental, cows selected for meat and milk production, and Holstein, cows highly specialized for milk production cows led us to hypothesize a different innate and adaptive immune- response after calving when these breeds are managed under the intensive dairy system production.

Materials and Methods

Experimental Design



12 cows



13 cows

At 3 days after calving
Blood samples in PAXgene tubes for mRNA expression

- Quantitative PCR was performed using 4 µL of diluted cDNA combined with:
- In an ABI Prism 7900 HT SDS instrument (Applied Biosystems).
  - 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C (denaturation), and 1 min at 60°C (annealing + extension).

- The qPCR efficiency and quantification cycle values were obtained for each reaction using LinRegPCR, a program for the analysis of quantitative RT-PCR (qPCR) data resulting from monitoring the PCR reaction with SYBR green or similar fluorescent dyes.
- The expression of genes was normalized using the geometric mean of 3 internal control genes (*ACTB, SDHA, and YWHAZ*).

Materials and Methods

Gene Networks Investigated

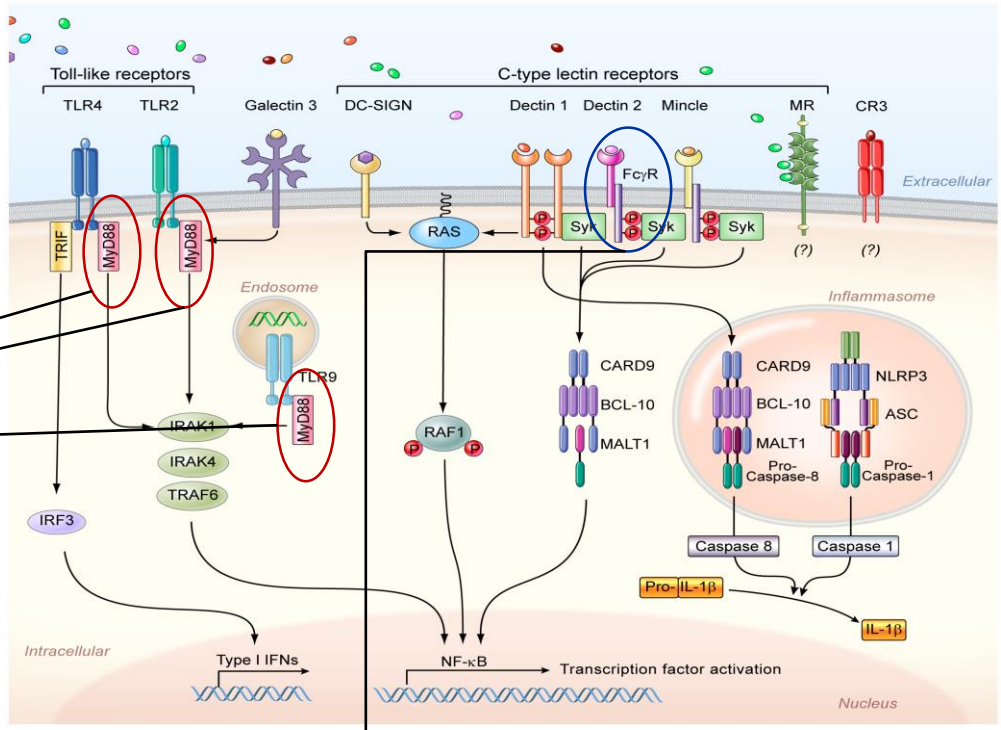
- Recognition and immune modulation: ***CD14***, ***CD16***, ***MYD88***, ***TLR2***, and ***TLR4***
- Migration and cell adhesion: ***CCR2***, ***CX3CR1***, ***ITGB2***, ***ITGAL***, ***TLN1***, ***TLN2***, ***SELL***, ***SELPLG***, ***CD44***, and ***LGALS8***
- Antimicrobial strategies: ***MMP9***, ***LTF***, ***LYZ***, ***MPO***, ***LCN2***, and ***IDO1***
- Inflammatory cascade: ***CASP1***, ***TNFRSF1A***, ***IL1B***, ***IL1R***, ***IL8***, ***IL18***, ***IRAK1***, ***TNFA***, ***NLRP3***, ***S100A8***, and ***RPL13A***
- Oxidative pattern: ***SOD1*** and ***SOD2***
- Leukotrienes pathway: ***ALOX5*** and ***ALOX15***
- Glucose-metabolism: ***GAPDH***

Results

Pattern Recognition Receptors

Table 1. Effect of breed on mRNA gene expression of leukocytes from Simmental and Holstein cows for genes related to recognition and immune mediation functions.

Target	Simmental (vs Holstein)
CD16 Fc Fragment of IgG Receptor IIIa	up
TLR2 Toll Like Receptor 2	down
MYD88 Myeloid Differentiation Primary Response 88	up



Greater expression of *MYD88*, a key adapter in the downstream signalling, which lead to the translocation of NF-κB to the nucleus initiating the transcription of proinflammatory cytokines (Akira and Hoshino, 2003).

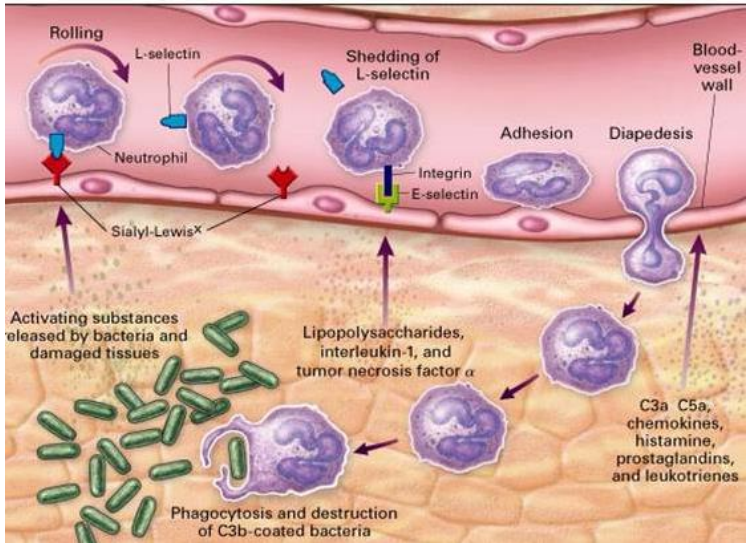
Greater abundance of *CD16*, representing a receptor of IgG antibody-coated cells and upon recognition it delivers a potent signal to natural killers cells leading in turn to eliminate targets through direct killing and cytokine production
(Romee et al., 2013).

Results

Migration and Adhesion of Leukocytes

Table 2. Effect of breed on mRNA gene expression of leukocytes from Simmental and Holstein cows for genes related to migration and cell adhesion functions mediation functions.

Target	Simmental (vs Holstein)
CX3CR1 C-X3-C Motif Chemokine Receptor 1	up
ITGB2 Integrin Subunit Beta 2	up
SELPLG Selectin P Ligand	up
CD44 Hematopoietic Cell E- And L-Selectin Ligand	up
LGALS8 Lectin, Galactoside-Binding, Soluble 8	up



- ✓ The adhesion of leukocytes is guaranteed by the expression of selectins, which bind to adhesion molecules located on the surface of endothelial cells near the inflamed tissues (Kansas, 2004).
- ✓ After selectins allow for binding between leukocytes and endothelial cells, β2-integrins interact with intercellular adhesion molecules, thereby allowing attaching more firmly with endothelial cells (Engelhardt and Wolburg, 2004).

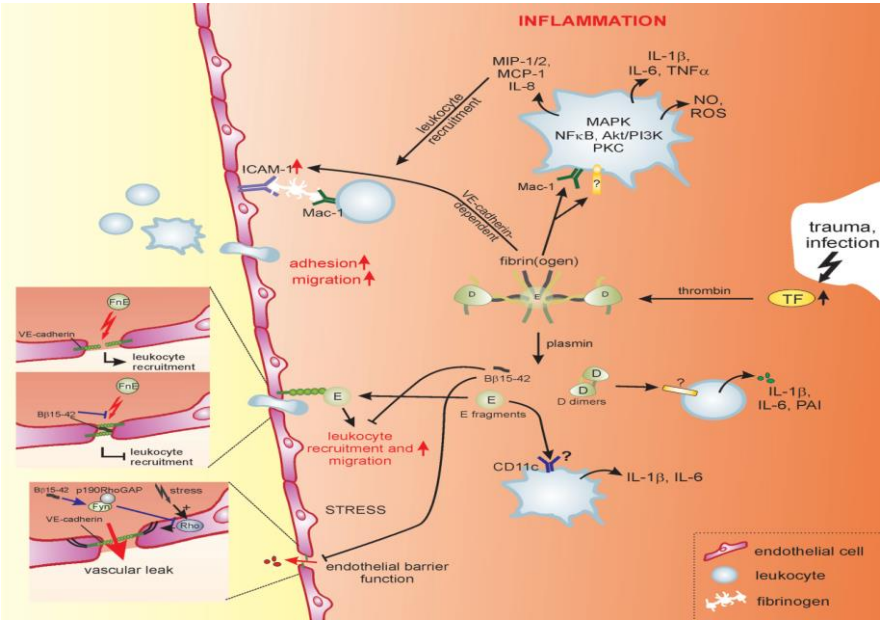
In our opinion

Together, our results suggest that Simmental cows (upregulation of *ITGB2*, *CD44*, *CXC3CR1*, and *LGALS8*) after calving are characterized by leukocytes with a greater degree of functional leukocytes.

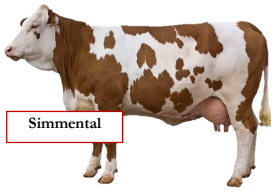
Inflammatory Cascade

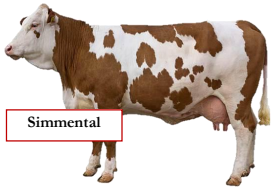
Table 4. Effect of breed on mRNA gene expression of leukocytes from Simmental and Holstein cows for genes related to inflammatory cascade.

Target	Simmental (vs Holstein)
CASP1 Caspase 1	
IL1B Interleukin 1 Beta	up
TNFRSF1A TNF Receptor Superfamily Member 1A	up
TNF Tumor Necrosis Factor Alpha	up
IL1R Interleukin 1 Receptor Type 1	up
S100A8 S100 Calcium Binding Protein A8	down
RPL13A Ribosomal Protein L13a	up



Greater expression of cytokines- and cytokine receptor-related genes functioning as **checkpoint regulators** of leukocytes **recruitment, trafficking, and also maturation** during the inflammatory response (Schmidt et al., 2013; Heiser et al., 2018).

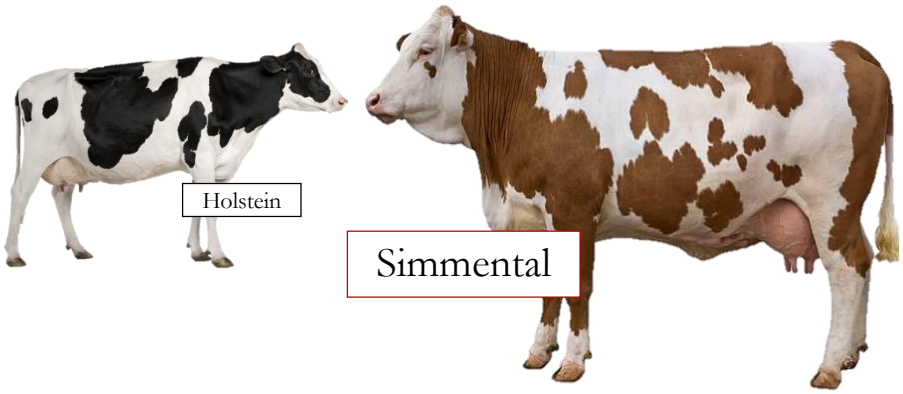




A more activated state of leukocytes (with cytokines that help to mediate and enhance the immune function) in response to parturition and consequently an increased exposure of damaged internal epithelial surfaces to potentially pathogenic microorganisms.

Take Home Messages

Milestone in discovering the mechanisms of the immune system in different breeds.



- Evidence of different expression marks between immune cells of Simmental cows (dual-purpose breed for milk and meat production) and Holstein cows (milk production) immediately after parturition.

- Suggesting an enhanced capacity of transepithelial migration of leukocytes and adhesion to microvascular endothelial cells in Simmental cows.

- Furthermore, compared with Holstein, the higher expression of inflammatory mediators in Simmental cows is supportive of a greater capacity from leukocytes to recruit and activate mainly neutrophils and monocytes.

- Taken together, the data support the hypothesis that Simmental cows are prone for mounting a better immune response to the homeorhetic adaptation of the new lactation.

- Accounting for important biological insights and functional information into the immune-function differentiation among cattle breeds.

Which Questions arose?

What could be the role of the energy status in these differences?

NEB could represent a key to understand differences in leukocytes function between the two breeds investigated herein, since Simmental cows were also characterized by a lower milk production compared with Holstein (Lopreiato et al., 2019).

However, further efforts should be addressed in order to ascertain that, even when different breeds are compared for immunological studies during the transition period, the interaction between metabolic condition and immune function still exists or maybe other mechanisms breed-specifics are involved.

Differences also during the entire peripartum?

Further investigation should be focused on temporal changes in order to provide additional evidence of variation in whole blood leukocytes function between Simmental and Holstein cows in response to the transition period and the homeorhetic adaptation to the onset of a new lactation, and whether differences pointed out herein are still observed before parturition and over the first or second week after parturition.

Thanks for your attention!

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