74th EAAP ANNUAL MEETING, Lyon – France
Session 80 - "Animal behaviour"

MP-SANBA (SANté et Bien-être des Animaux en élevage)

Impact of environmental enrichment on the behavior and immune cell transcriptome of pregnant sows

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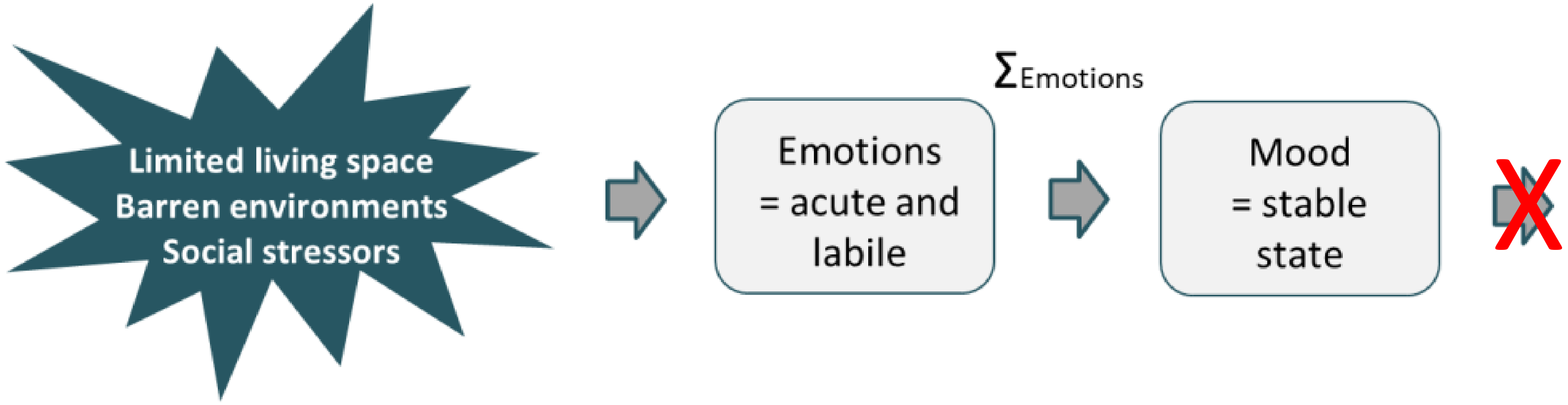
Introduction: welfare definition and challenges

Animal welfare definition

“The state resulting from the **positive mental** and physical state related to the satisfaction of its physiological and behavioral needs, as well as its expectations” *ANSES, 2018*

⚠ Need for valid, reliable and feasible animal-based welfare biomarkers



Limited living space
Barren environments
Social stressors

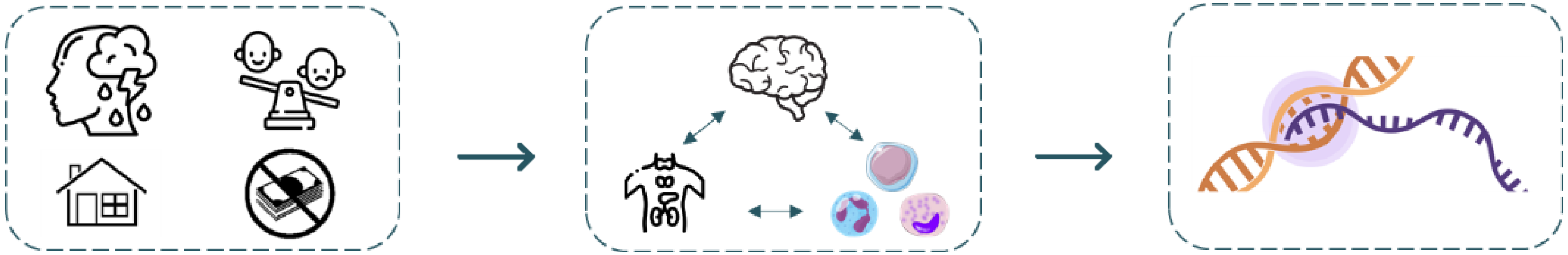
Emotions
= acute and labile

ΣEmotions

Mood
= stable state

Physiological tools to access mood

Introduction: gene expression x social-environment



Social-environment determinants → Brain x endocrine x immunity → Gene expression alterations

Adversity for extended periods leads to recurrent gene expression profile in immune cells
= **Conserved Transcriptional Response to Adversity (CTRA;** *Cole, 2019***)**

↑ Inflammation-related genes
(*ex: IL1B, IL6, IL8/CXCL8, COX2/PTGS2, and TNF*)

↓ Antiviral responses/antibody production genes
(*ex: IFI-, MX-, and OAS- family genes*)

Less research using transcriptomic tools in farm animals

- ☐ Weaning stress in cows (*O'Loughlin et al, 2012, Johnston et al, 2016*)
- ☐ Social stress and social hierarchy in pigs (*Oster et al, 2014 and 2015*)
- ☐ Environmental enrichment in horses (*Lansade et al, 2014*)

Is it also possible to observe blood transcriptomic differences when comparing animals with contrasted welfare states?

Objective

Evaluate the variations in immune cells transcriptome of farm animals as a welfare indicator

Use of pregnant sows housed in contrasted welfare conditions and of different parities

Why pregnant sows?

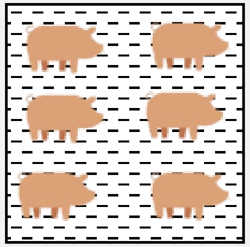
- ☐ Diverse housing systems → ≠ welfare levels
- ☐ Stable condition → moods?
- ☐ Group-housed → ≠ social statuses in a group
- ☐ Several gestations in the production system → ≠ parities

Experimental design

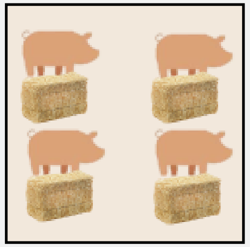
- ☐ Environmental enrichment with a known positive impact on the sows' welfare (*Merlot et al, 2022*)

↪ Use of **behavioral** tools and physiological variables (**cortisol**) for confirmation

During successive gestations:



Conventional (C)
- ☐ Concrete floor
- ☐ 2.4 m^2/sow



Enriched (E)
- ☐ Accumulated straw floor
- ☐ 3.5 m^2/sow

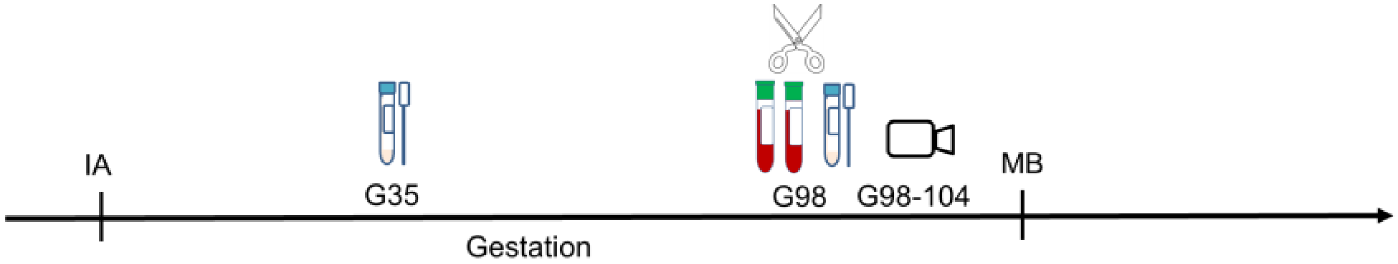
- ☐ 28 animals selected (2nd gestations or more)

↗ 14 Conventional (7 x 2 repetitions)
14 Enriched (7 x 2 repetitions)

→ 9 Dominant
11 Intermediary
8 Subordinate

↘ 14 Low Parity (LP; 2nd or 3rd gestation)
14 High Parity (HP; 4th or more gestations)

Methods: experimental timeline and measurements



Cortisol analysis

28 selected sows

Saliva: Acute stress (G35 and G98)

Hair: Chronic stress (G98)

Behavioral analysis

All sows, except those involved in their 1st gestation

Behavioral activity: Scan sampling with 10 min intervals (6h on G99, G101, G103)

Social interactions: Continuous sampling (3h on G99 to G103)

Dominance rank: ELO-rating method

RNA-seq analysis

28 selected sows

Blood sampling G98 (09:00 – 10:00) – Peripheral Blood Mononuclear Cells (PBMC)

Loss of 4 samples due contamination

Statistics

Fixed effects:

Housing x Parity

or

Housing x Dominance

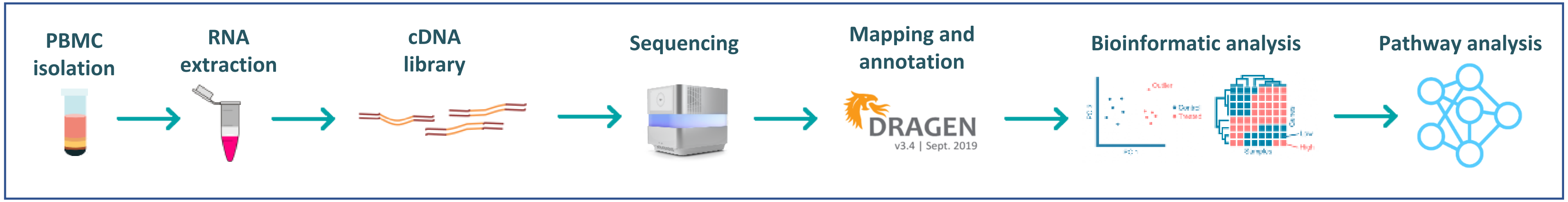
Random effect:

Repetition (R1 or R2)

	High Parity *(753 ± 80 days old)*	Low Parity *(456 ±71 days old)*
Dominant	6	0
Intermediary	4	6
Subordinate	1	7

Methods: RNA-seq overview

cDNA library preparation, sequencing and mapping performed by the PGTB platform (Bordeaux)



PBMC isolation → **RNA extraction** → **cDNA library** → **Sequencing** → **Mapping and annotation** → **Bioinformatic analysis** → **Pathway analysis**

- *Sus scrofa* reference genome GCF_000003025.6

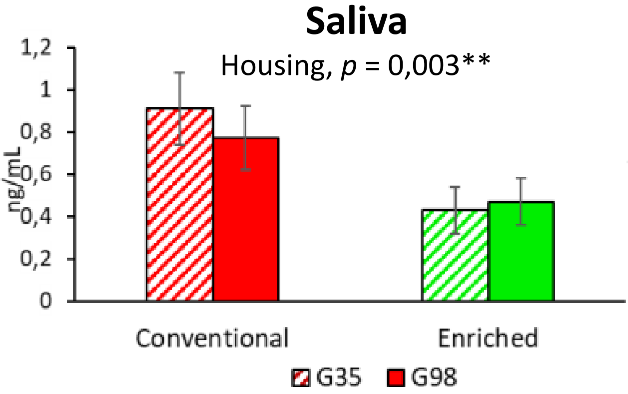
- Differential analysis in R package DESeq2
  - ~ Housing + Parity + Repetition
  - ~ Housing + Dominance + Repetition

- Comparisons extracted with contrast function DESeq2
  - Housing = “enriched” vs “conventional”
  - Parity = “high parity” vs “low parity”
  - Dominance = “dominant” vs “subordinate”

- Pathway analysis in DAVID software (v2022q2) using Homo sapiens database as background (minimum 3 genes/term and FDR < 0,05)

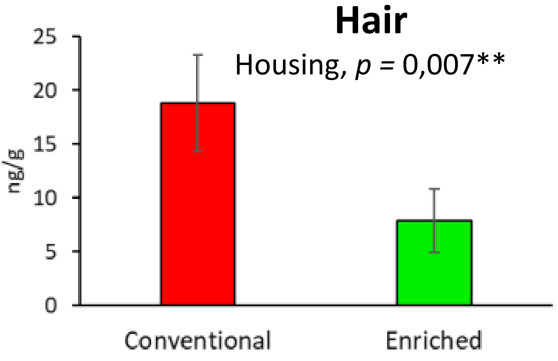
Results: cortisol and behavioral analyses

Cortisol



Saliva

Housing, p = 0,003**

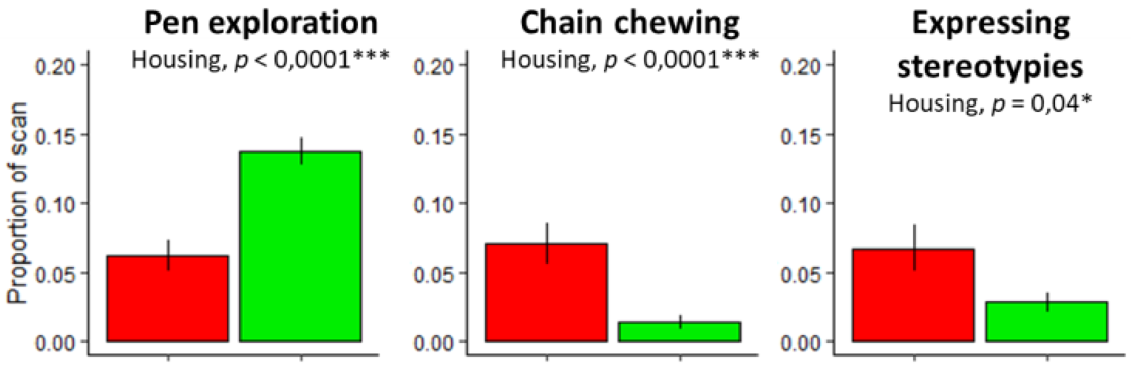


Hair

Housing, p = 0,007**

☐ ↓ cortisol concentration in E sows → in accordance with our previous studies *(Merlot et al, 2019 and 2022)*

Behavioral activity



Pen exploration

Housing, $p < 0{,}0001$***

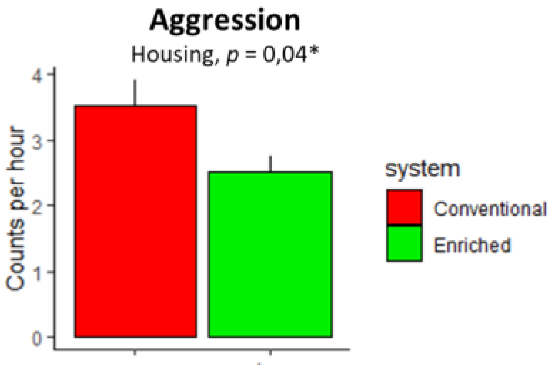
Chain chewing

Housing, $p < 0{,}0001$***

Expressing stereotypies

Housing, p = 0,04*

Social interactions



Aggression

Housing, p = 0,04*

☐ ↑ space + straw in E sows:

- ➢ ↑ exploratory behavior
- ➢ ↓ reduce frustration
- ➢ ↓ aggression

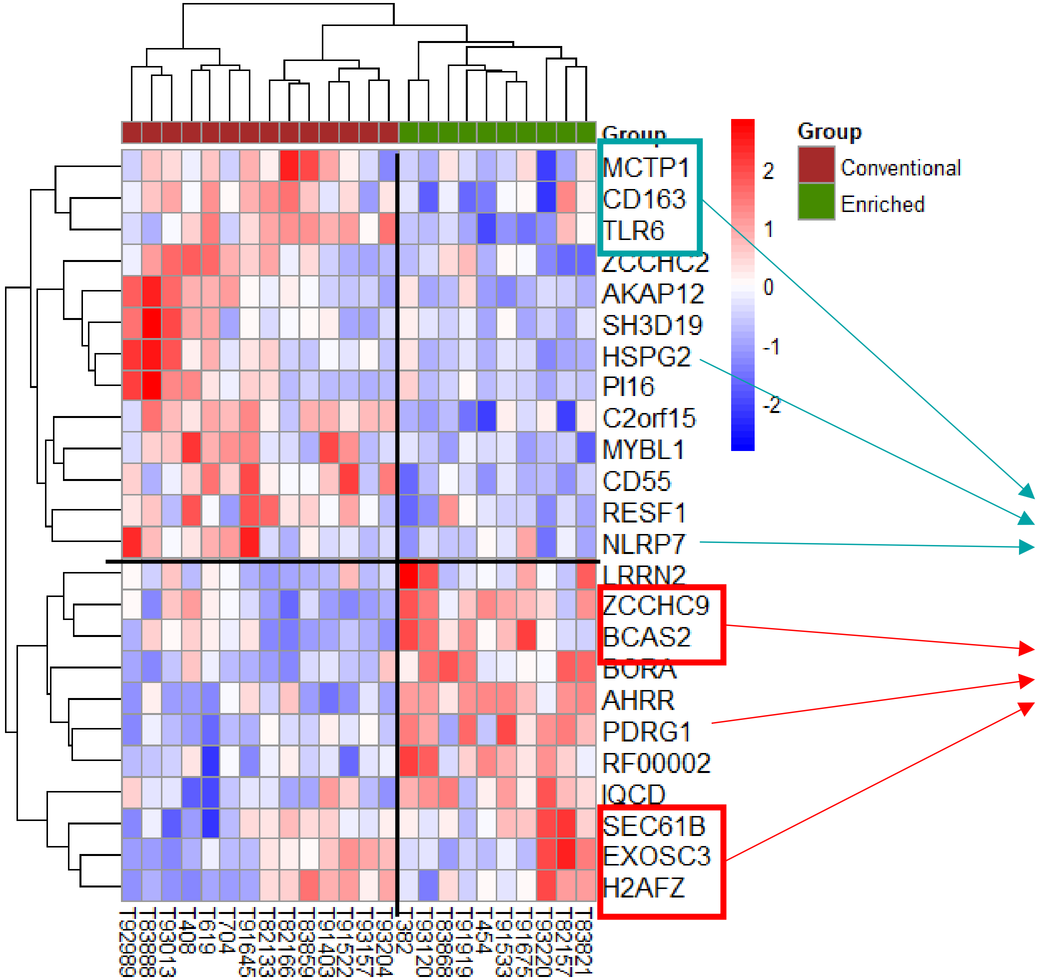
Confirmation of the positive influence of the enriched system on sows' welfare

Results: housing influence on PBMC transcriptome

Differential analysis

Enriched (E, n=10)

vs **Conventional (C, n=14)**



- ☐ **13** down-regulated and **11** up-regulated genes in E (p-adj < 0.1 and FC ratio > 1.2 or <0.83)
- ☐ ↓ Genes related to monocytes and plasmacytoid DC;
- ☐ ↑ Genes related to RNA and protein expression/processing.

Low impact of the housing on the transcriptome of immune cells

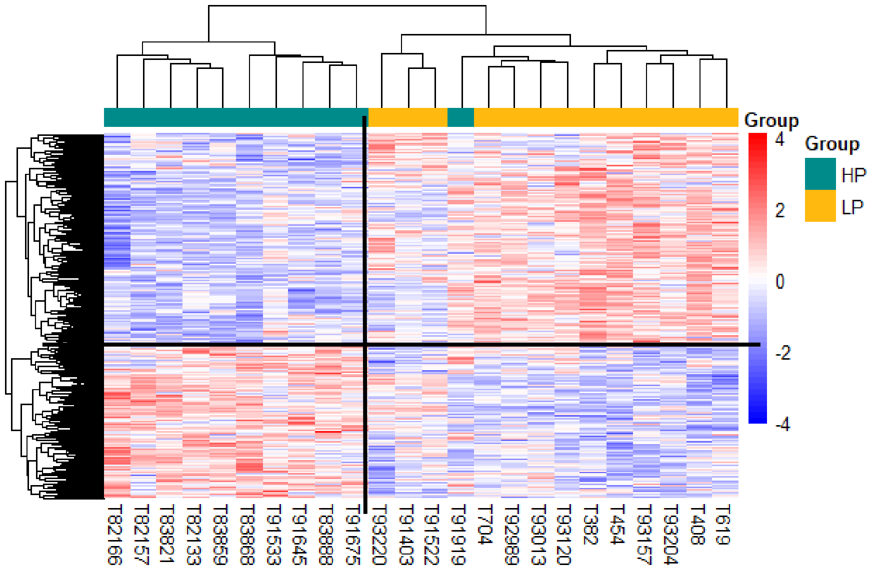
Results: parity influence on PBMC transcriptome

High parity (HP, n=11)

vs **Low parity (LP, n=13)**

Differential analysis

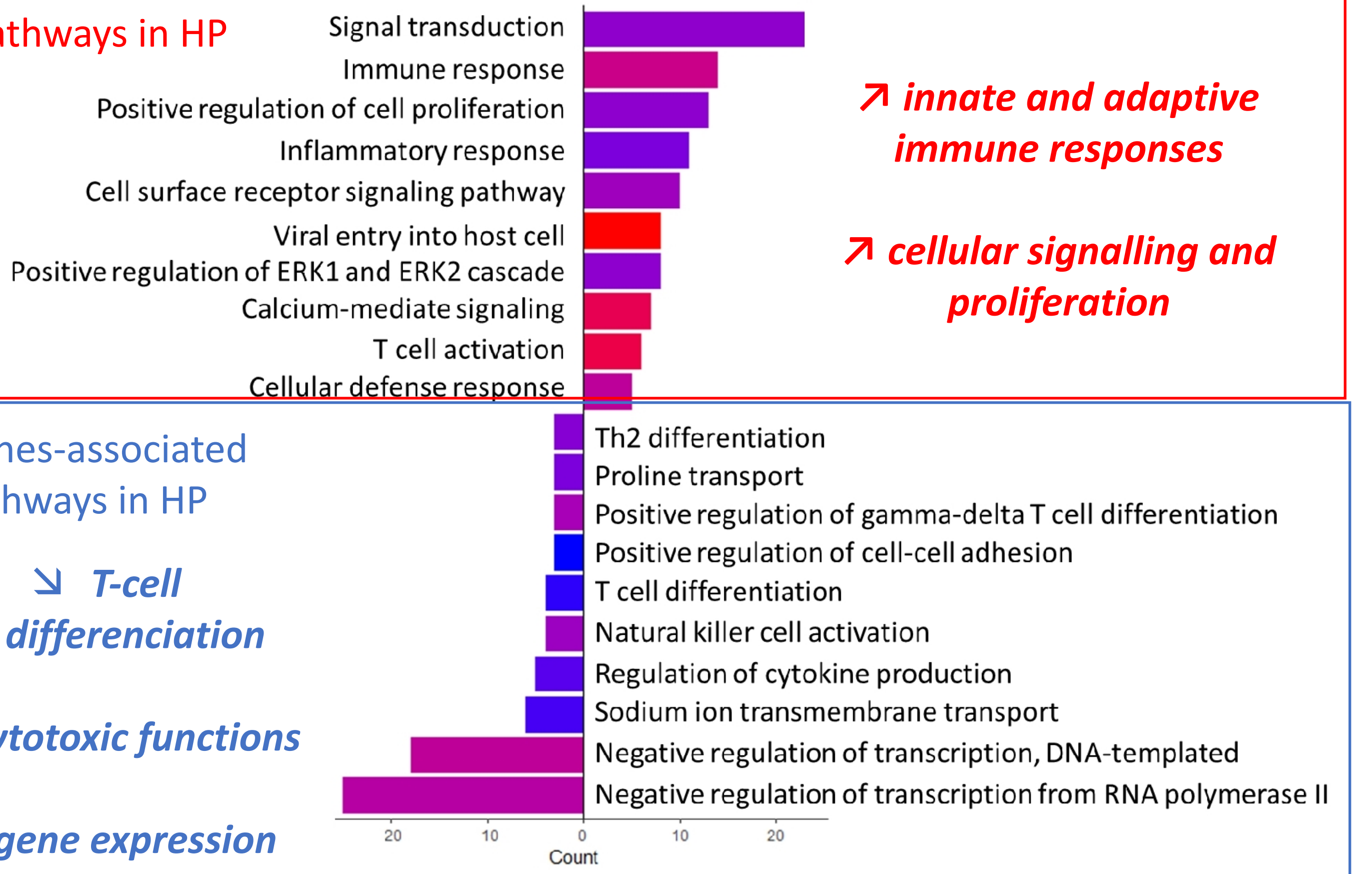
312 down-regulated and
225 up-regulated genes in HP
(p-adj < 0.1 and FC ratio > 1.2 or <0.83)



↑ influence immune genes/pathways than housing

Pathway analysis: top 10 enriched biological terms

↑ genes-associated pathways in HP



*↗ **innate and adaptive immune responses***

*↗ **cellular signalling and proliferation***

↓ genes-associated pathways in HP

*↘ **T-cell differenciation***

*↘ **cytotoxic functions***

*↘ **gene expression regulation***

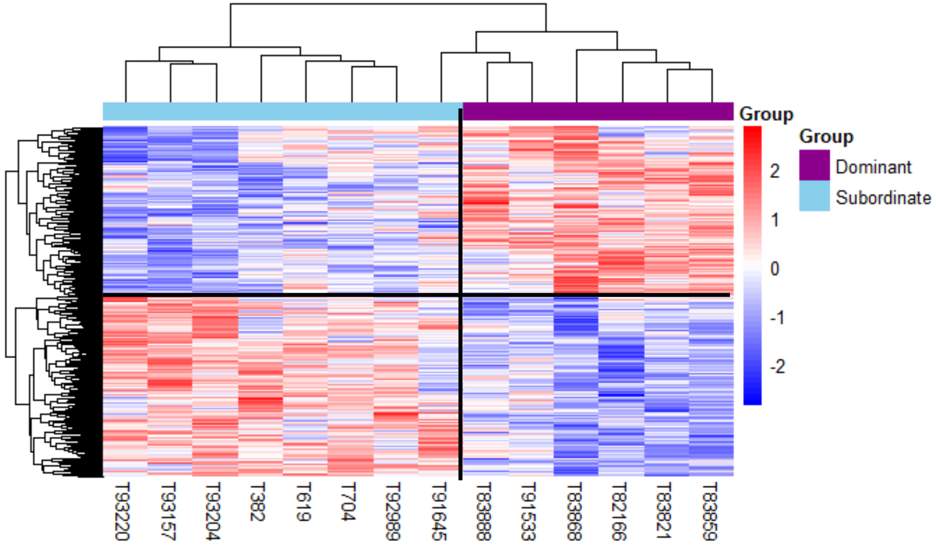
Results: social status influence on PBMC transcriptome

Dominant (Dom, n=6)

vs **Subordinate (n=8)**

Differential analysis

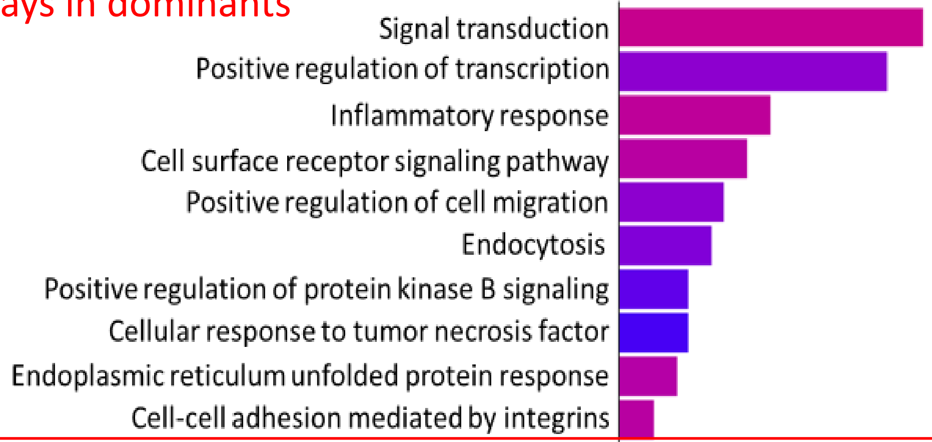
233 down-regulated and
216 up-regulated genes in HP
(p-adj < 0.1 and FC ratio > 1.2 or <0.83)



↑ influence of social status;
≠ effect from parity
Parity x social status: 179 common genes

Pathway analysis: top 10 enriched biological terms

↑ genes-associated pathways in dominants

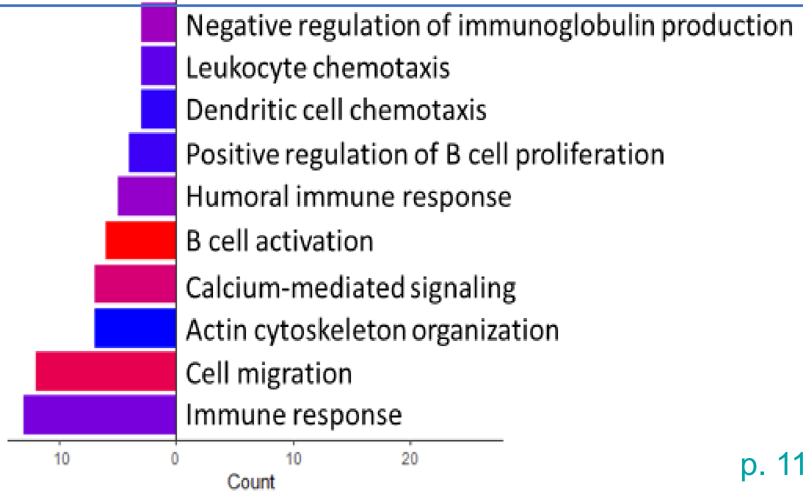


↗ Signaling (receptors, and protein kinase B (Akt))

↗ Inflammation

↗ Immune cell adhesion/migration

↓ genes-associated pathways in dominants



↘ ***Adaptive immunity (B cell and humoral immunity)***

↘ ***Signaling (calcium signaling)***

↘ **Immune cell migration**

Conclusions and perspectives

- Long-term environmental enrichment with straw and bigger space has a positive influence on sows' well-being;
- The blood transcriptome is a poor indicator of the effects of housing enrichment;
- Ageing or reproductive cycles have a strong impact on blood transcriptome;
- Social status in the group also strongly influences immune cell functioning

Next steps

- Detailed genes/pathways information for better understanding
- Methylome data (presented at session 10): data integration to elucidate the observed effects and determine the best welfare biomarkers!

Thank you!

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