Thèse LeukoBEA
Projet BEPPI (Bien-Etre des Porcs et Programmation Immunitaire)

The influence of environmental enrichment on the DNA methylation profile of sows' blood immune cells depends on their parity rank

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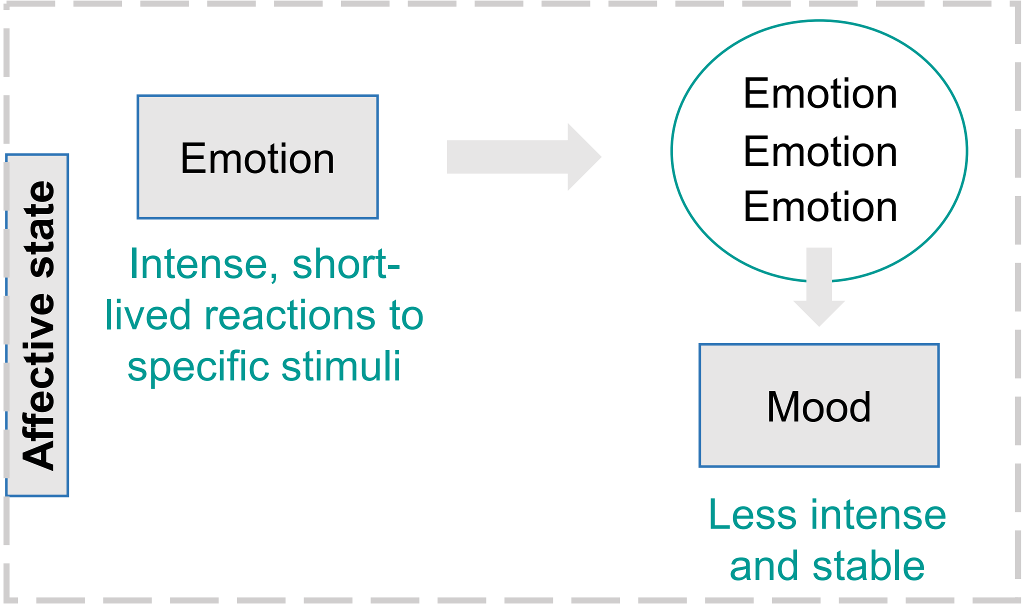
What is “affective state” and how can we assess it?

- An important component of **animal welfare**
- We can assess it by evaluating some components:
  - Behavior
  - Cognition
  - Neurophysiology
- However, the available tests are time-consuming and difficult to implement at the farm level



Affective state

Emotion

Intense, short-lived reactions to specific stimuli

Emotion
Emotion
Emotion

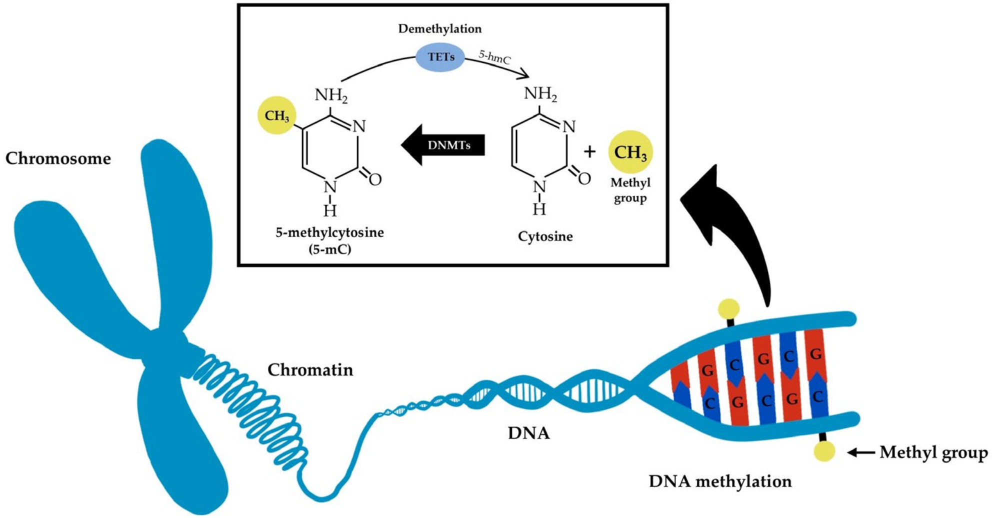
Mood

Less intense and stable

The development of reliable, feasible, and practical physiological markers that reflect the affective states of farm animals would be valuable for animal welfare science

Human social genomics research has demonstrated that the gene expression and **DNA methylation** profiles in immune cells can reflect affective states

…a small reminder of DNA methylation



- Establish and preserve cell identity;
- Occurs mostly at Cytosine-phosphate-Guanine (CpG) sites;
- Impact gene expression;
  - Can ↙ gene expression when located at regulatory regions
  - Can ↗ gene expression when located at the gene body
- Several factors impact DNA methylation in immune cells (physiological state, age, stress,…)

1. **What are the DNA methylation variations on immune cells of pregnant sows with contrasted affective states?**
2. **Does the sows' parity also impact DNA methylation as observed in our transcriptomic results?**

The housing environment impact the affective states of pigs

- Research has demonstrated the positive effects of environmental enrichment on the different components of the affective state (Beattie et al., 1995; Brajon et al., 2017; Douglas et al., 2012; Rault et al., 2018)

CRECOM farm of the Chambre Regional d'Agriculture de Bretagne (CRAB):

- Pregnant sows remain during the gestation and their entire productive lives in either a conventional or enriched environment:

Conventional
- Concrete floor
- Minimum space/sow

Enriched
- Accumulated straw
- More space/sow

- Decreased stereotypies, blood neutrophils, salivary cortisol, plasma hydroperoxide concentration, and increased behavioral investigation in enriched sows (Merlot et al., 2019, 2022) = **contrasted affective states**

General procedure

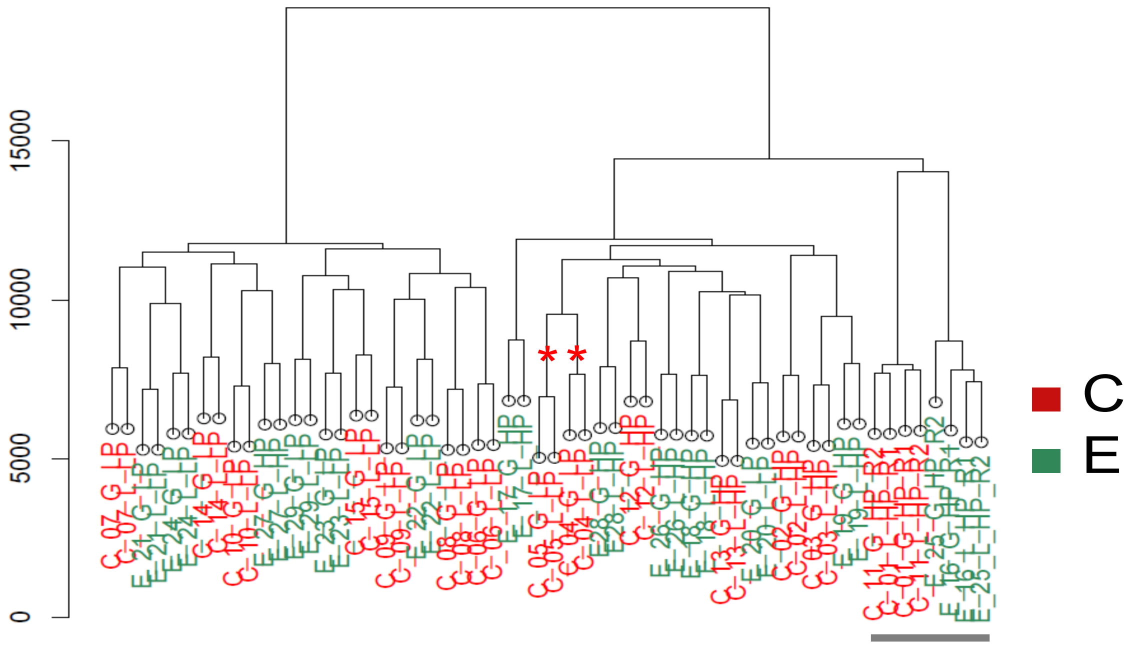
- Experiment at CRECOM farm (two replicates):
  - Two groups of sows housed in contrasted housing environments: conventional (C, n = 15) and enriched (E, n = 14)
  - Blood sampling at the end of gestation (**G98**) and three weeks later (beginning of lactation; **L12**)
- Isolation of immune cell fraction (PBMC), genomic DNA extraction, and reduced bisulfite sequencing (RRBS)
- Data processing:

Initial (raw) background 3,418,048 CpGs → Selection of common CpGs to all libraries 1,473,450 CpGs → Filtration of genetic effects (SNPs) 1,433,115 CpGs Final dataset

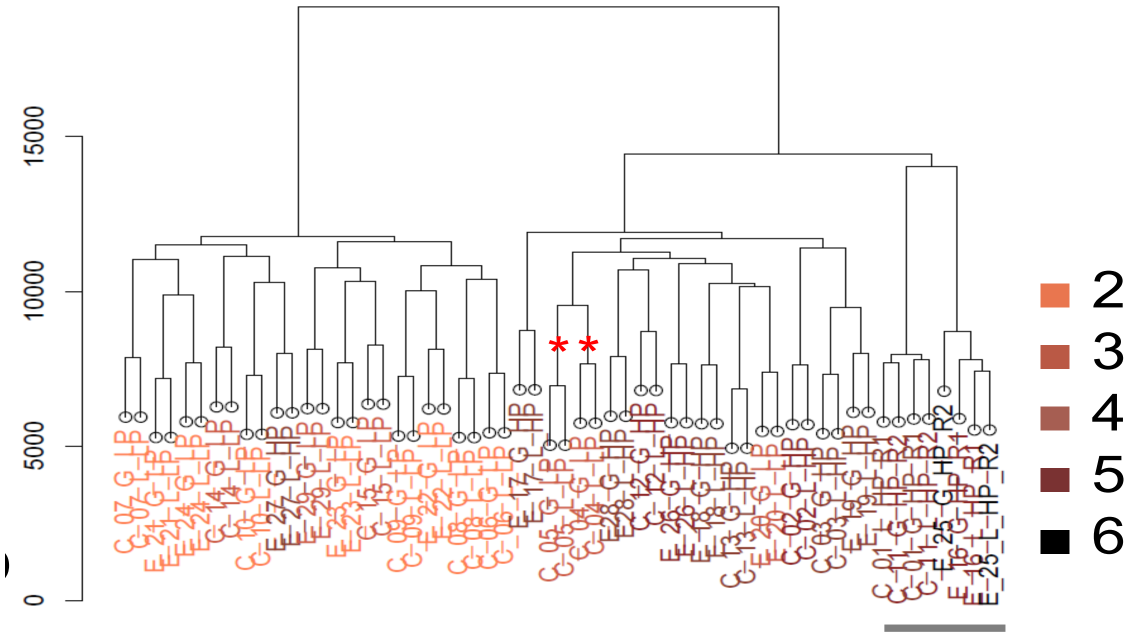
- Bioinformatic analysis:
  - Descriptive analysis: dendrograms
  - Differential analysis: differentially methylated cytosine (DMC; p-adj < 1% and Δ15%); differentially methylated region (DMR; > 3 DMCs with less than 100 bps of distance on the same gene)
  - Pathway analysis of the genes overlapping with a DMC using DAVID software

The impact of different factors on DNA methylation profiles

- Euclidian Clustering of all samples (G98 and L12) based on methylation level of the final dataset



Housing environment



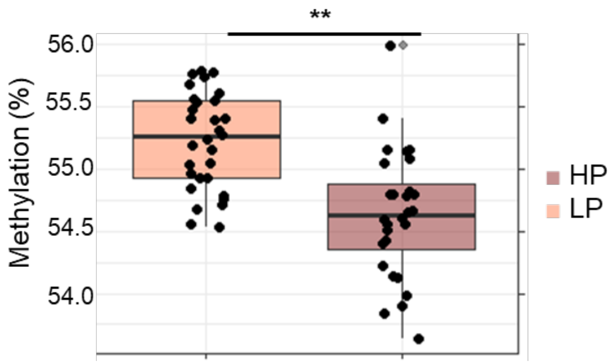
Parity

- ➢ Sample clustering does not discriminate according to the housing environment;
- ➢ Parity better explains the clustering profiles => high impact on DNA methylation profile

The influence of parity on DNA methylation profiles

- Based on clustering patterns, sows were categorized into two groups:
  - Low-parity (**LP**, n = 15): 2nd and 3rd gestations
  - High-parity (**HP**, n = 14): 4th and more gestations

- We calculated the methylation mean (%) for each sample:



*mod = lmer(global methylation mean (%) ~ housing * parity * state + (1|Sow) + (1|Replicate), data = final_background)*

> ➢ Significant **hypomethylation** in **HP** sows, which is commonly observed in the aging process, even though there were no major differences in the age of the animals (547 ± 73 days old in **LP** and 860 ± 163 days old in **HP**)

- Differential analysis: **HP** vs. **LP**

DMCs:

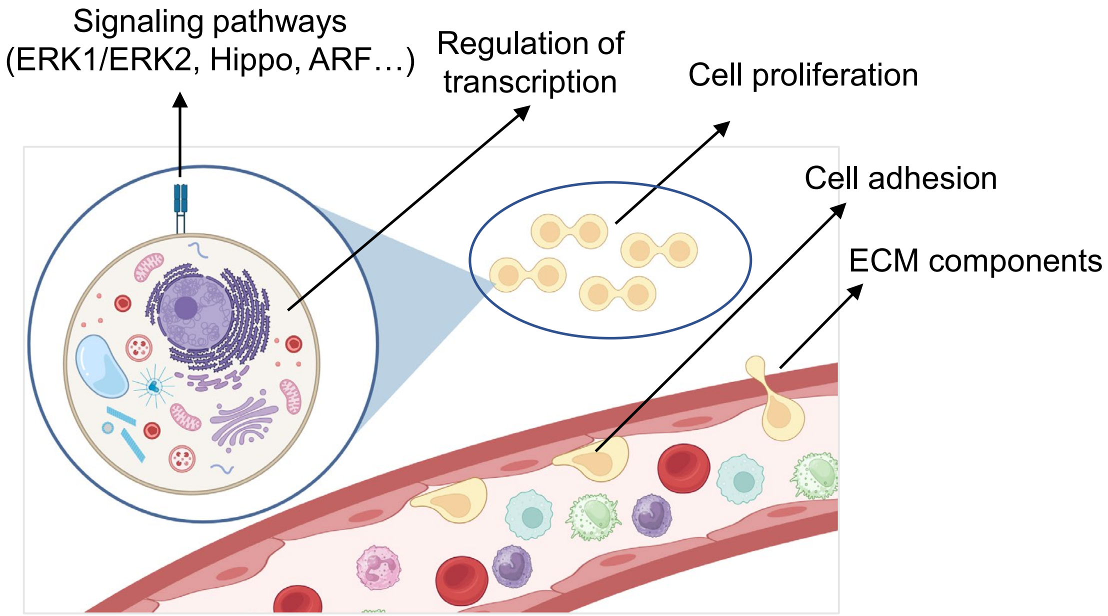
	Hypo in HP	**Hyper in HP**	**Total**
G98	1,011	347	1,358
L12	552	128	680

DMRs:

60 DMRs at G98
24 DMRs at L12

Parity impacted important immune-related functions and genes

- Biological processes enriched with genes overlapping with a DMC:
- Important genes overlapping with a DMR:
  - Epigenetic processes: *DNMT3A, KDM8*, and *HDAC4*
  - Immunity: ↗ *CD2, CAMK4, CMIP, TNFRSF1B*, and ↙ *SEC14L1, SGK1, SKI, PACS1* in HP sows
- A total of 28 genes were affected by parity at both **transcriptomic** and **methylome** levels



- ➢ Parity is indeed an important factor regulating immune function even among multiparous sows and should be considered in future studies;
- ➢ We hypothesized that the accumulation of subsequent gestations can cause similar effects to those of aging;
- ➢ Another hypothesis is based on a possible “imprinting” of gestation on immune cells

The impact of environmental enrichment (EE) in the different parity groups

- Due to the significant differences between LP and HP sows, we decided to investigate the effects of EE separately for both groups:
  - LP: **E**, n = 6 vs. **C**, n = 9
  - HP: **E**, n = 8 vs. **C**, n = 6

- Differential analysis: E vs. C

DMCs:

	LP			**HP**		
	Hypo	**Hyper**	**Total**	**Hypo**	**Hyper**	**Total**
G98	25	35	60	23	19	42
L12	18	17	35	46	35	80

DMRs: 0 for both groups and time-points

- No common DMCs between the contrasts

- ➢ EE did not have a major influence on DNA methylation in immune cells, despite its positive impact on the components of affective states (cortisol and behaviors - results showed last seminar);
- ➢ The effects of EE seems to differ between parity groups

Nevertheless, we were able to identify some genes that were already associated with affective states in humans and can be potential targets for future studies…

LP sows

SHANK2

Deficiency of this gene has been linked to mood disorders in a mice model (Pappas et al., 2017)

SNPs in this gene were related to depressive disorders in a GWAS study (Ciuculete et al., 2020)

MAD1L1

It has been linked to the reward system functioning, anxiety regulation, and psychiatric diseases (Snijders et al., 2020; Sokolov et al., 2023; Trost et al., 2016; Zhao et al., 2018; Starnawska et al., 2019)

HP sows

LINGO3

Found hypermethylated in postmortem brain samples of humans with depression (Lutz et al., 2017)

SORCS2

Receptor of BDNF (Glerup et al., 2016)

Implicated in brain plasticity and mood disorders (Jin et al., 2019)

Take home message

1. Parity is an important modulator of the DNA methylation in immune cells of multiparous pregnant sows, similar to our transcriptomic results;
2. Environmental enrichment exerts a modest effect on DNA methylation profiles in immune cells;
3. Some genes can be associated with positive affective states in pigs and should be further investigated

Thank you for your attention!

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