Milking the dairy cattle epigenome: a co-methylation network to detect epigenetic biomarkers associated with metabolic stress in dairy calves

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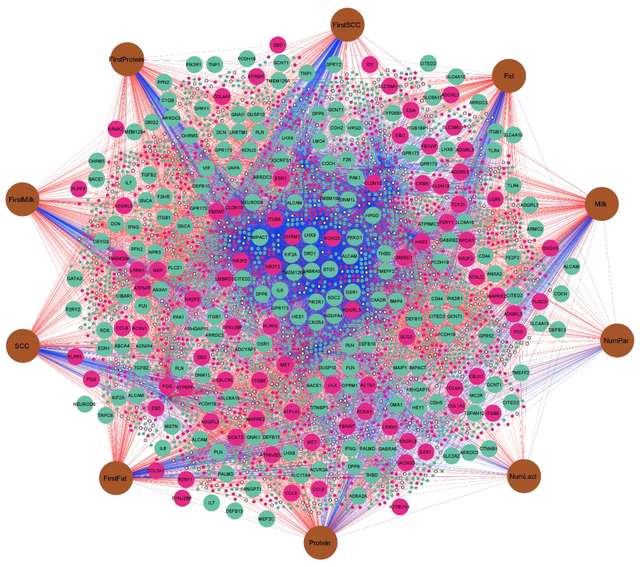
Introduction

- **Epigenetic** information can be used to explain part of the residual variance in **breeding programmes**
- Epigenetic marks can be **inherited** and **detected**
- In dairy cattle, **lactation** and **gestation** can **overlap**. The embryo competes with the mammary tissue for energy
- There can **epigenetic differences** between calves gestated by non-lactating **heifers (PP)** and by lactating **multiparous** cows **(MP)**



Objectives

1. **Find differentially methylated regions and cytosines in PP vs MP.**
2. **Determine the correlation between methylation frequencies in different genomic regions and the dam's phenotypes.**

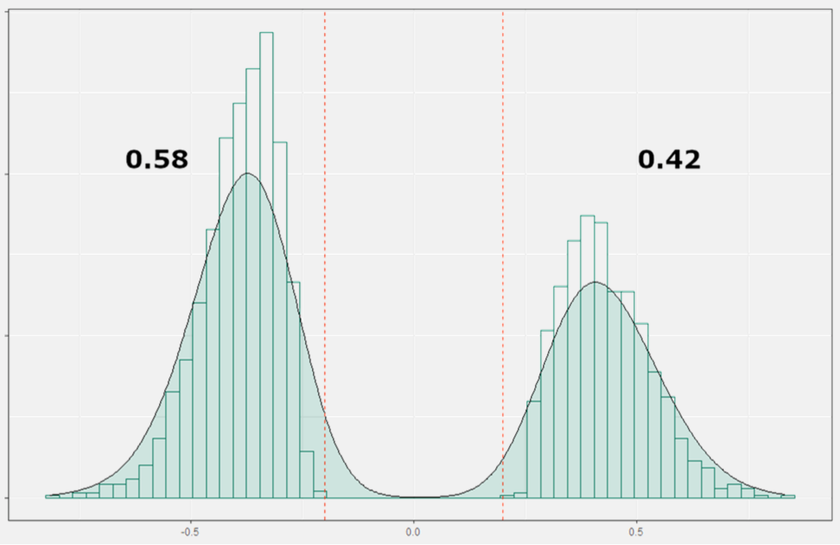
Material and Methods

6 blood samples from calves (3 PP & 3 MP) from a commercial farm:

- **DNA extraction**
  - **Monarch® HMW DNA Extraction Kit (T3050L) for Cells & Blood (New England Biolabs®)**
- **Sequencing**
  - **GridION sequencing. LSK-110 ligation kit. R9.4 flow cell**
- **Bioinformatics**
  - **Guppy v6.3.7. F5C v1.1**
  - **DSS (R package). Delta ≥ 0.2. FDR ≤ 0.05. Dist TTS ≤ 50,000 bp**
  - **PCIT algorithm + Cytoscape**

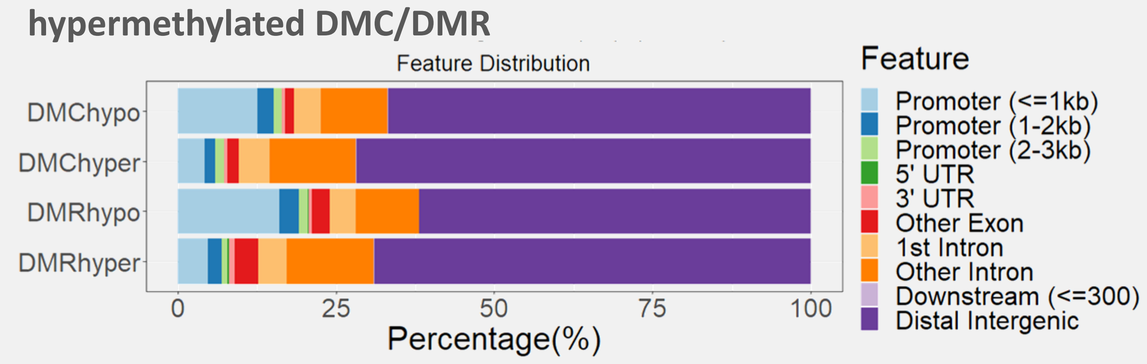
Results I

- **Of the 2,355 DMR detected, 58% were hypomethylated in PP**
- **We detected 53,499 DMC, 54% hypomethylated in PP**



Results II

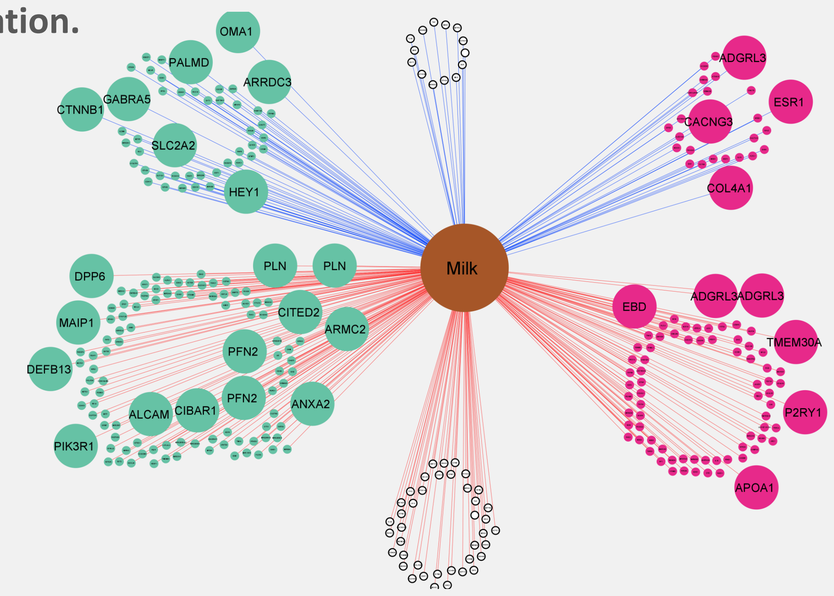
- **Using PP as the reference group. Hypomethylated DMC/DMR appeared associated to promoter regions in larger proportion than hypermethylated DMC/DMR**



- **An enrichment analysis of the regions in the networks showed genes involved in developmental processes.**

Gene set	Biological process	Adjusted p-value
GO:0032502	developmental process	4.39e-22
GO:0007275	multicellular organism development	8.5e-22
GO:0048856	anatomical structure development	1.14e-21
GO:0048519	negative regulation of biological process	2.01e-19
GO:0048518	positive regulation of biological process	4.12e-19
GO:0048523	negative regulation of cellular process	7.67e-19
GO:0048731	system development	8.44e-19
GO:0048522	positive regulation of cellular process	4.09e-17
GO:0009653	anatomical structure morphogenesis	4.17e-16
GO:0007399	nervous system development	5.73e-15

- **The dam's phenotypes (brown nodes) affect the methylation status of certain genomic regions of their calves. In light green and pink are represented hypo and hypermethylated regions, respectively. Blue lines represent a positive correlation and red lines a negative correlation.**



- **Genes like OMA1, ESR1 or PALMD, associated with milk yield, have been found in our networks. Their methylation status in calves could be partially explained by the dams' phenotypes.**
- **As well as CITED2, IL7 or SLC6A15, that are related to inflammatory and mastitis processes.**

Conclusions

- **Nanopore** sequencing enables the detection of **DMC/DMR** in **agrigenomics** and the **PCIT algorithm** eases the study of the effects of the dam's phenotypes over the calves' methylome**.**
- There is **a transgenerational epigenetic effect** that could explain part of the **differences in the performance** of individual born from heifers and multiparous cows.

Acknowledgments

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