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Epigenetic modifications induced by preclinical inflammation in the mouse mammary gland during successive lactations

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Introduction

Mastitis is among the main reasons women cease breastfeeding, which leads to them supplementing breast milk with artificial formula. In farm animals, mastitis results in significant economic losses and the premature culling of some animals. Nevertheless, knowledge related to inflammation's effect on the mammary gland is incomplete. One area that requires further study is the **epigenetic regulation of preclinical inflammation** in the **mammary gland** as it could potentially lead to early diagnosis and treatment of mastitis, preventing the onset of debilitating symptoms. The goal of this project is to study **epigenetic marks** involved in **mammary inflammation** and their persistence throughout **successive lactations**.

Methods

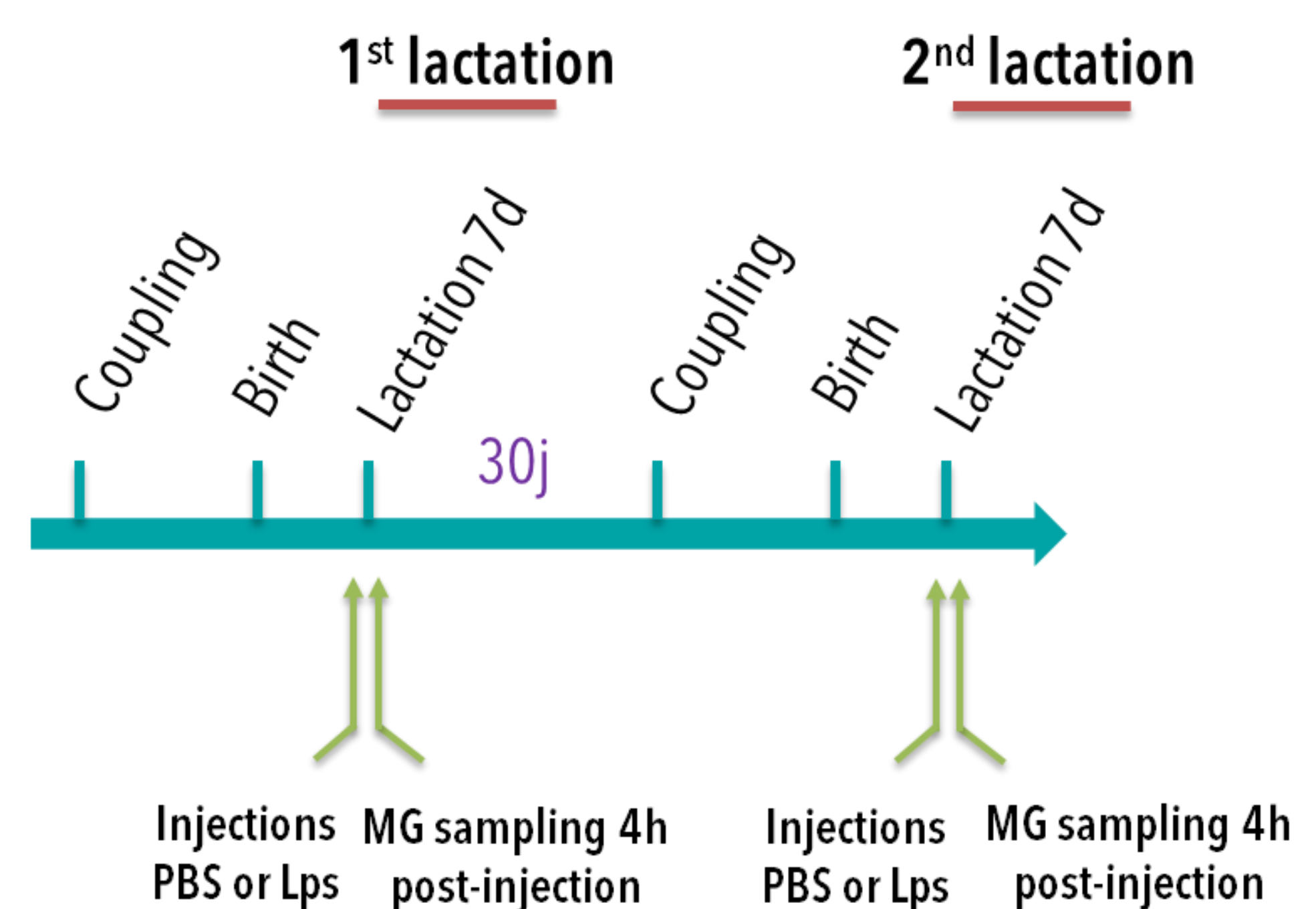
Proof of concept: **mouse** model of **early mammary inflammation** via intramammary LPS injections (tissue sampled **4 hours** post-injection).

Mammary gland samples from 1st or 2nd lactation, with or without inflammation (5 or 7 mice).

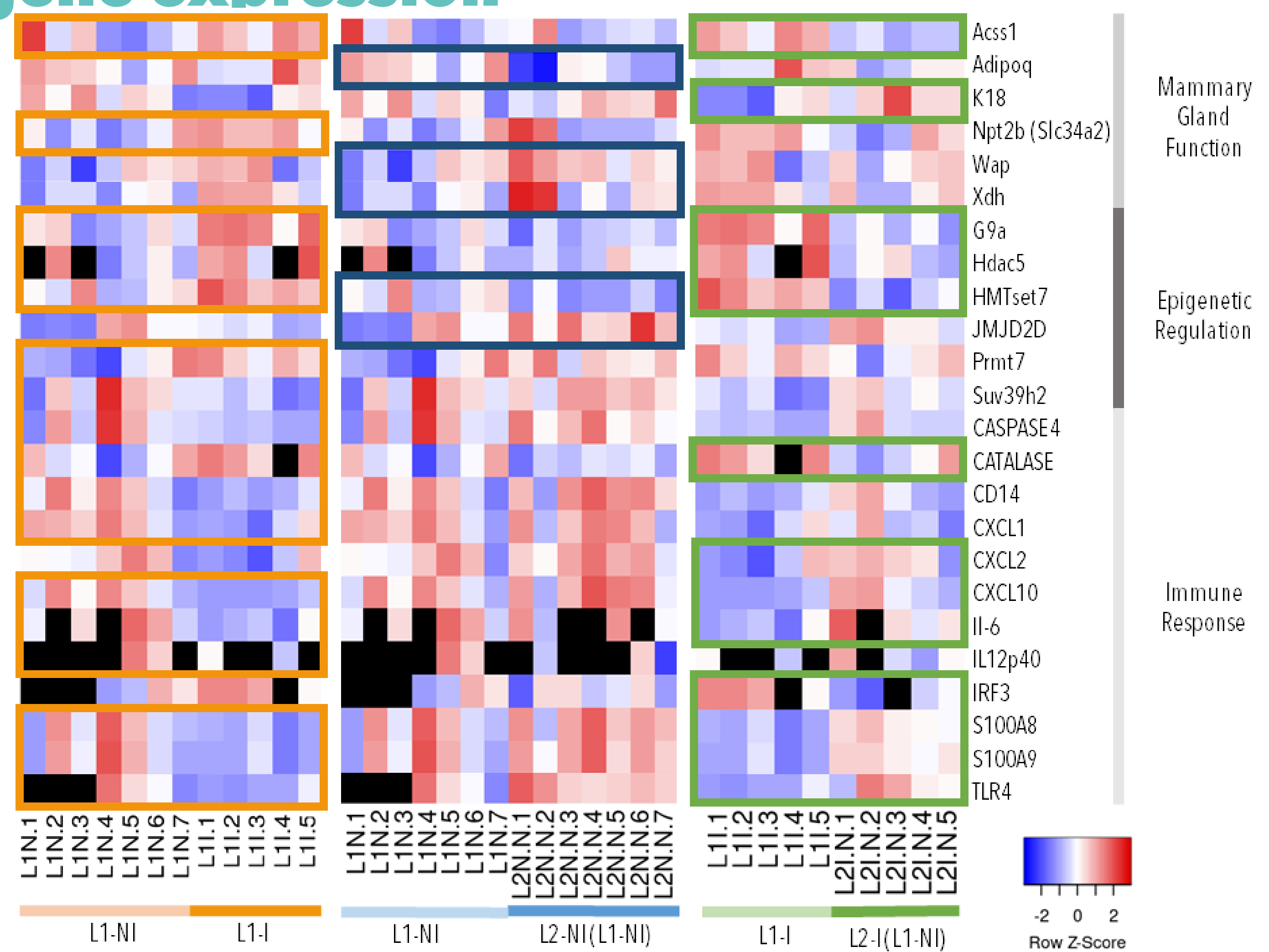
- **Gene expression** via RT-qPCR Fluidigm
- **DNA methylation** via RRBS

	Lactation 1 (L1)	Lactation 2 (L2)
Lot 1	NI	NI
Lot 2	I	NI
Lot 3	I	I
Lot 4	NI	I

NI: non-inflamed
I: inflamed



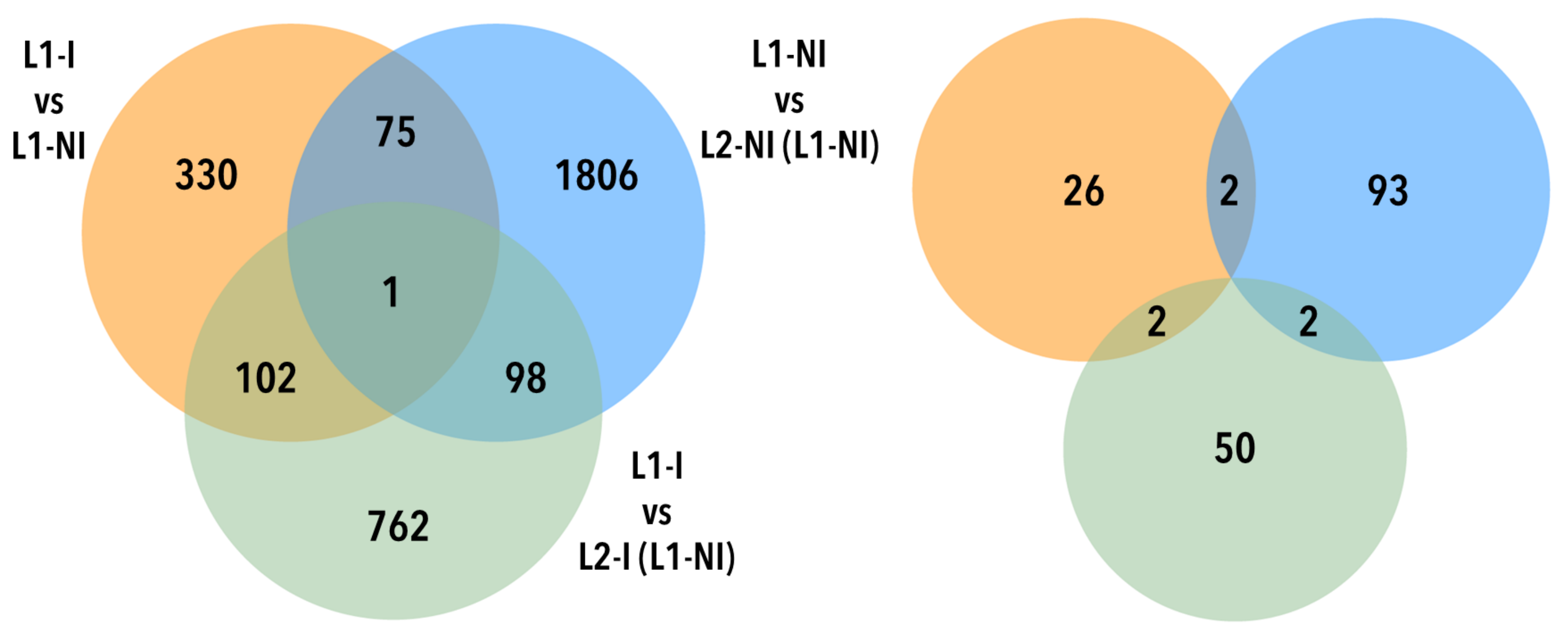
Lactation and early inflammation affect gene expression



Gene expression ($\Delta\Delta C_t$) in 3 comparisons: **inflammation during 1st lactation**, **physiological 1st vs 2nd lactation**, and **inflammation in 1st vs 2nd lactation**. The genes listed are differentially expressed ($p < 0,05$) in at least one comparison (color-coded circles). Each column represents one individual. Lowest gene expression is represented in blue, highest - in red.

Early inflammation provokes **important gene expression changes** independent of lactation rank, with **similar** changes observed when comparing two **1st lactations** or **1st vs 2nd lactation**.

DNA methylation changes caused by mammary inflammation and lactation



The total number of **differentially methylated cytosines (DMCs)** in all 3 comparisons. The total number of **differentially methylated regions (DMRs)** (DMRs → minimum 3 DMCs, <100 bp from each other) in all 3 comparisons.

Lactation rank provokes the **most DNA methylation changes**, followed by inflammation in 1st vs 2nd lactation and inflammation in one lactation. **Inflammation** provokes **different marks** depending on **lactation rank**.

	L1-NI vs. L1-I	L1-NI vs. L2-NI (L1-NI)	L1-I vs. L2-I (L1-NI)
GO: MF	adjusted p value	adjusted p value	adjusted p value
protein binding	1.9 x 10 ⁻⁶	2.8 x 10 ⁻²⁷	5.3 x 10 ⁻¹⁰
DNA-binding transcription activator activity	1.0	1.3 x 10 ⁻¹	1.0
DNA-binding transcription repressor activity	1.0	2.9 x 10 ⁻²	1.0
GO: BP			
anatomical structure morphogenesis	2.6 x 10 ⁻⁴	2.7 x 10 ⁻²²	2.3 x 10 ⁻⁵
cell differentiation	2.1 x 10 ⁻⁴	3.6 x 10 ⁻²¹	9.4 x 10 ⁻⁷
transcription, DNA-templated	1.0	8.3 x 10 ⁻⁷	1.0
KEGG			
Wnt signaling pathway	1.0	4.1 x 10 ⁻²	9.4 x 10 ⁻¹
cAMP signaling pathway	1.0	7.8 x 10 ⁻²	1.0
Wiki Pathways			
adipogenesis genes	1.0	1.2 x 10 ⁻³	1.0

Enrichment analysis (gProfiler) using DMCs identified in all 3 comparisons. MF: molecular function BP: biological process

Lactation rank is **more important** than **inflammation** for epigenetic changes related to **transcription, Wnt signaling, and adipogenesis**.

Our results show that **differentially expressed genes** were **not differentially methylated**, suggesting other pathways for transcription regulation are involved, possibly **acetylation** as **HDAC** is differentially expressed when **inflammation** is present.

Conclusion

In this study, we found that **lactation rank** provokes more **important DNA methylation changes** compared to inflammation. The changes observed due to **lactation rank** were involved in **more biological processes** and **signaling pathways** compared to those caused by **inflammation**. Moreover, we found that inflammation in **1st lactation** is **not comparable** to one in **2nd lactation** in terms of **cytosine methylation**; however, it is **similar** when it comes to **gene expression changes**.



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