# Changes in Nuclear and Mitochondrial DNA Methylation in Cow Blood Associated with Age and Disease

**Lotfi Bouzeraa [1,2,*], Camila Bruna de Lima [1,2], Mohamed Oudihat [1,2], Helene Martin [1,2], Jessica C.S. Marques[3], Ronaldo Cerri[3], Marc-Andre Sirard [1,2,]**

*1 Centre for Research in Reproductive, Developmental and Intergenerational Health (CRDSI), Department of Animal Science, Faculty of Agricultural and Food Sciences, Laval University, Quebec, QC, Canada*

*2 Department of Molecular Medicine, Faculty of Medicine, Laval University, Quebec, QC, Canada*

*3 Faculty of Land and Food Systems, University of British Columbia, Vancouver, CB, Canada.*

** Presenting author*

# Concept of epigenetics

**Materials (genetic, Ex: genes)**


**Unlimited possibilities of what can be built.**

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**Architecture (epigenetics, Ex. methylation)**

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# Concept of epigenetics

Materials (genetic, Ex: genes)

Unlimited possibilities of what can be built.

Architecture (epigenetics, Ex. methylation)

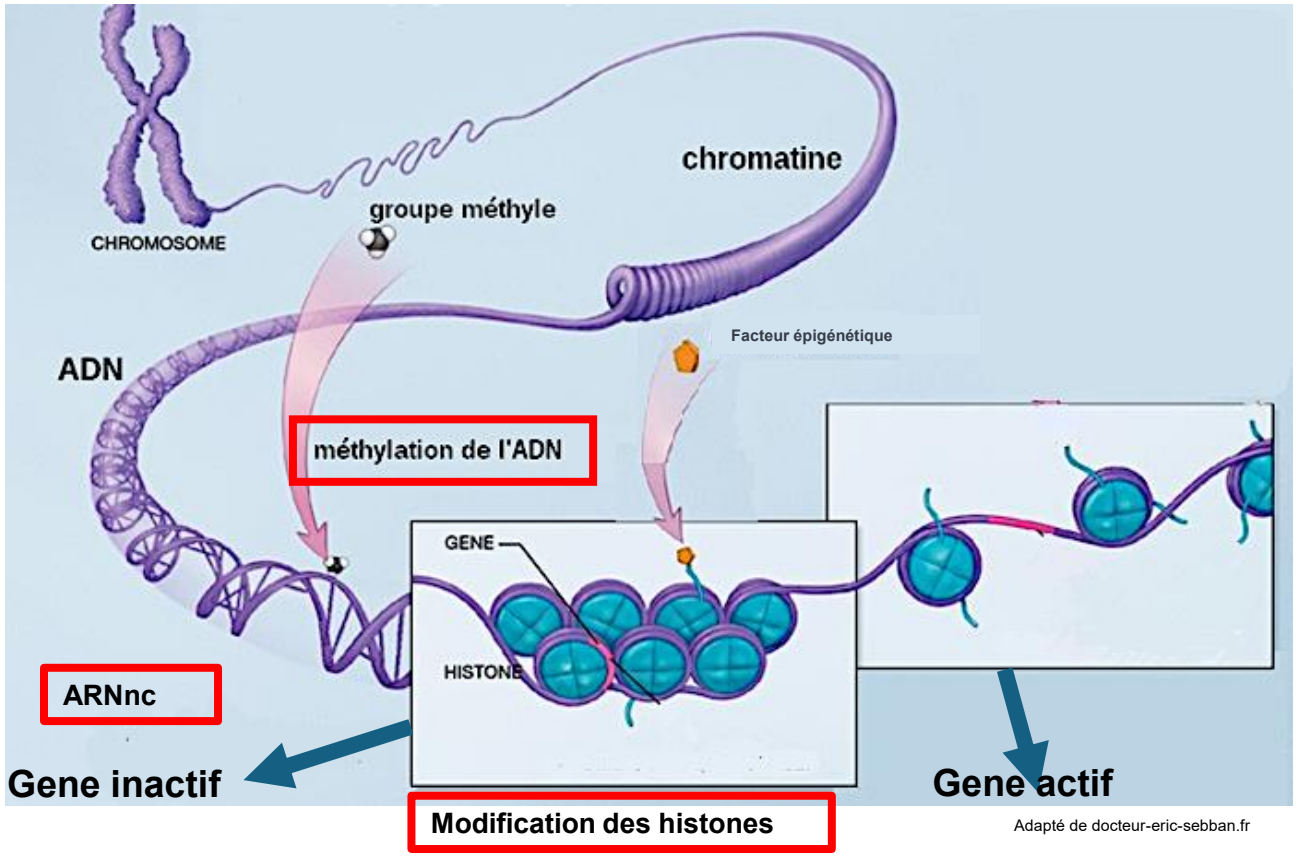
How building materials are used.

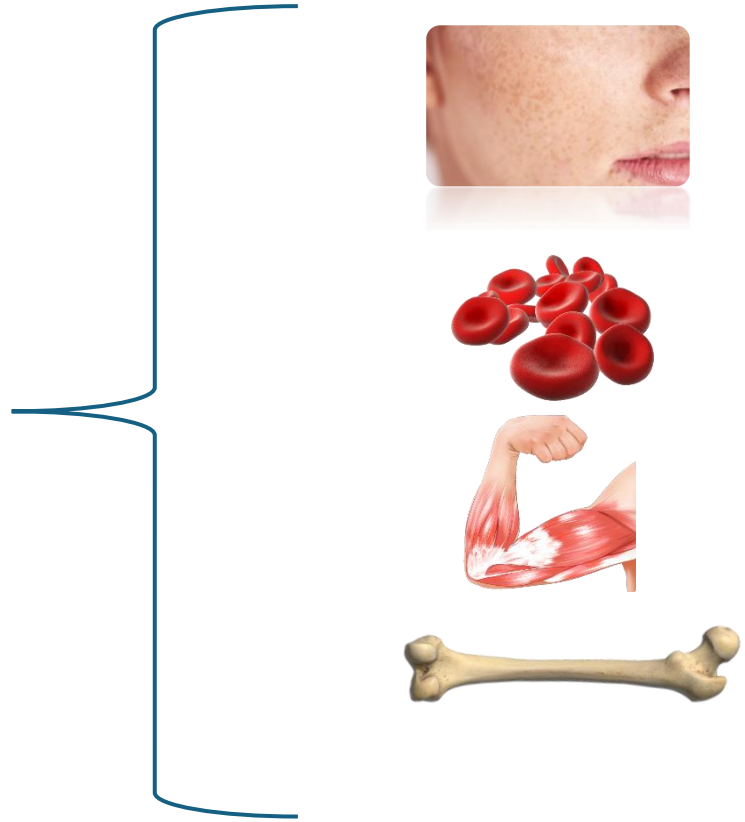
Home (phenotype, Ex. blood)

Observable Feature Set

# Epigenetics?

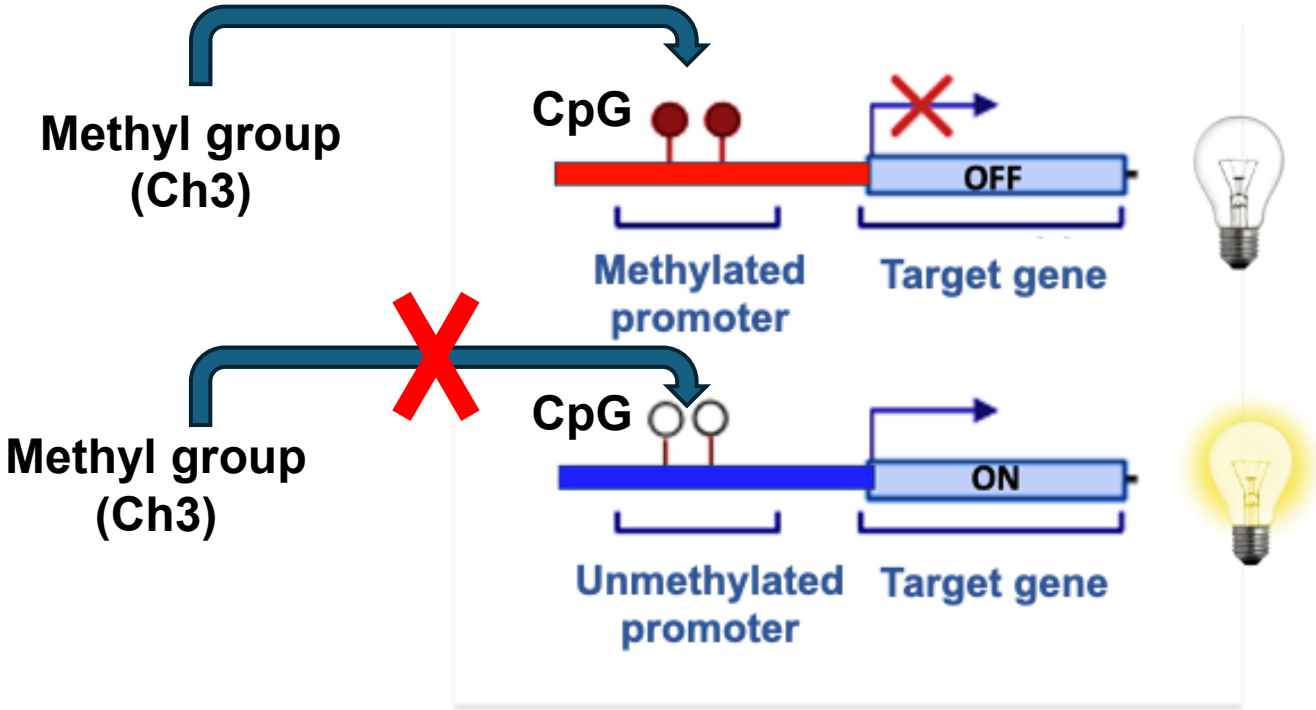
Set of marks that induce changes in gene expression without alteration of the DNA sequence.
(Berger et al., 2009)



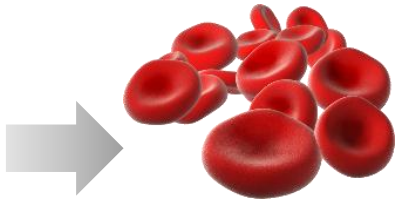



**Each cell type is characterized by a specific gene expression pattern supported by a specific epigenetic state.**

# Methylation controls gene expression




**CpG sites contribute to the differentiation of cell types**



**The promoter is essential for the regulation of gene expression**

# Epigenetic age vs chronological age

## Genetic modifications

- Ex: DNA sequence alterations

Hardly reversible

## Epigenetic modifications

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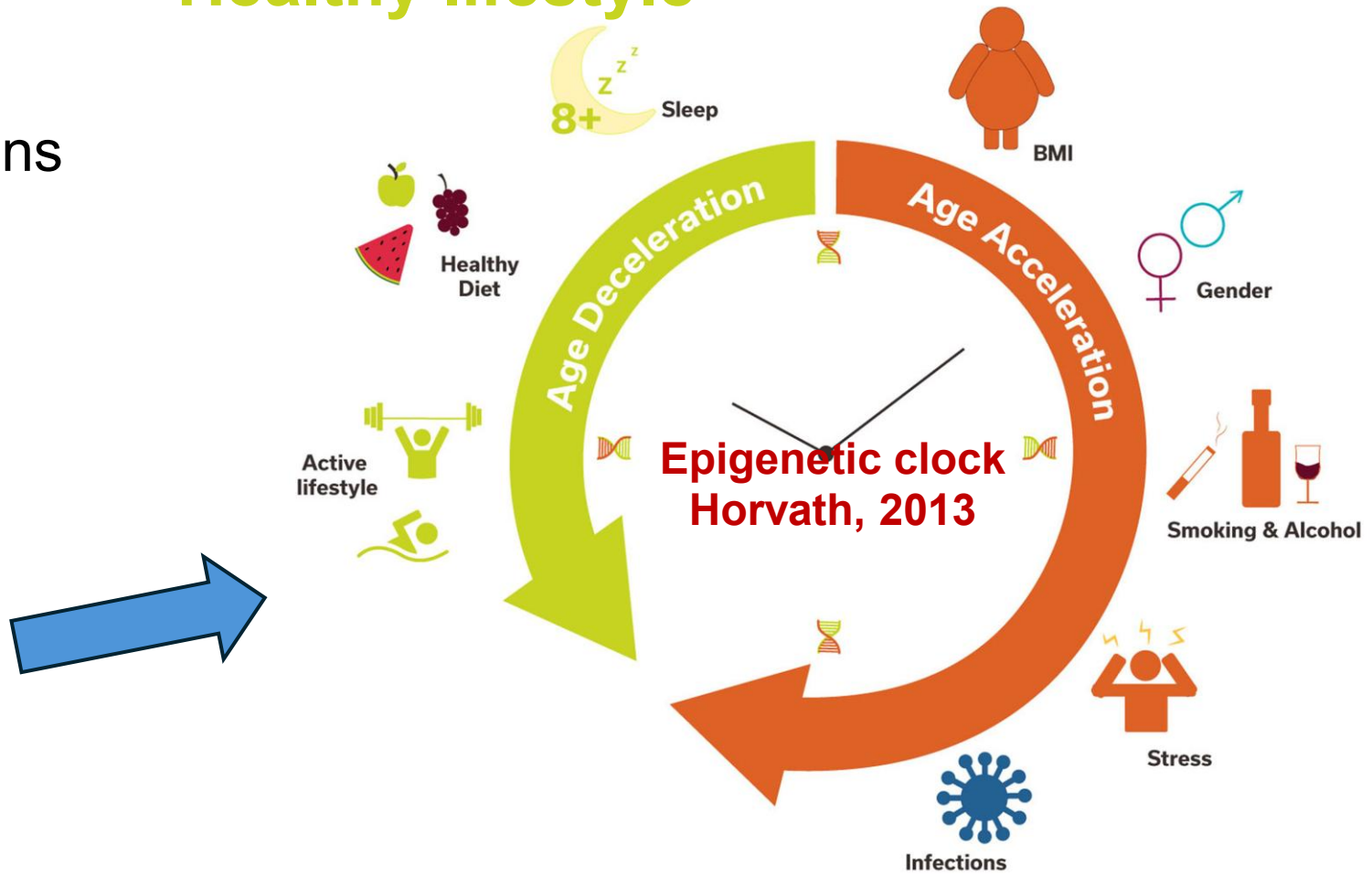





https://eremid.com/blog/the-epigenetic-clock-dna-methylation-and-aging/

# Objectives of the study

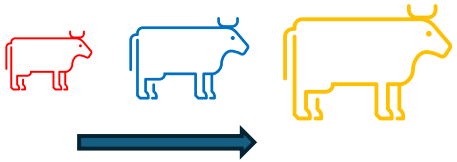
- **To identify changes in methylation associated with age and certain diseases in Holstein cows.**
- **Exploring the association of mitochondrial DNA methylation with age**
- **Explore potential epigenetic biomarkers.**

# Build a model

## Blood Sampling

Cross-sectional data

96 healthy cows



Age: 1.8 to 9.2 years

**~two years later**

48 diseased cows

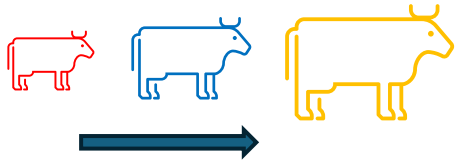
48 Control cows

# Build a model

## Blood Sampling

Cross-sectional data

96 healthy cows



Age: 1.8 to 9.2 years

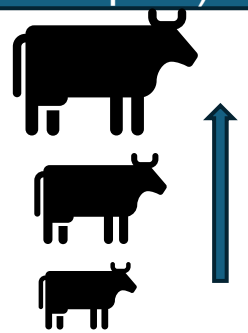
~two years later

48 diseased cows

48 Control cows

Longitudinal data

6 cows
(18 samples)



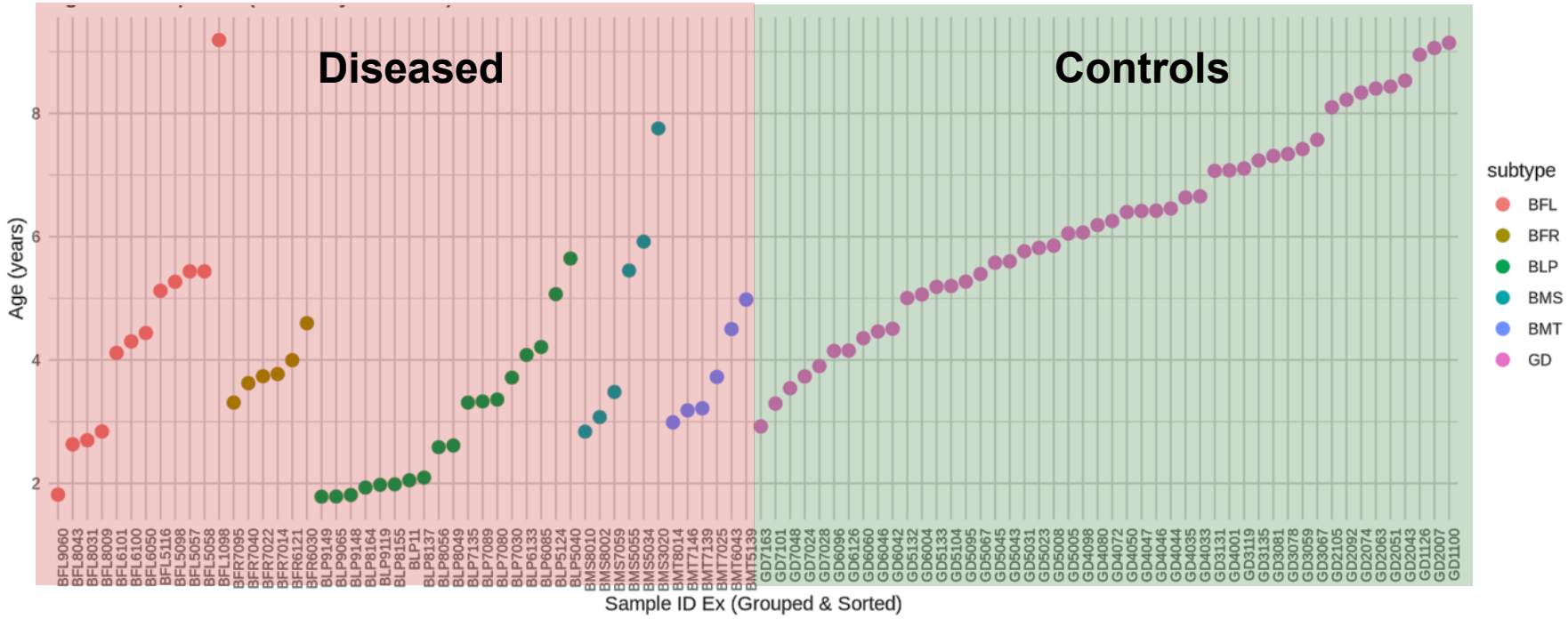
3 times

Age : -Newborn 4 days,
-Weaning 77 days
-Adult 3 years

## Sequencing

Methylome of blood markers
Enzymatic methyl-seq (EM-seq)
30X

# Cross-sectional data
# Age Distribution



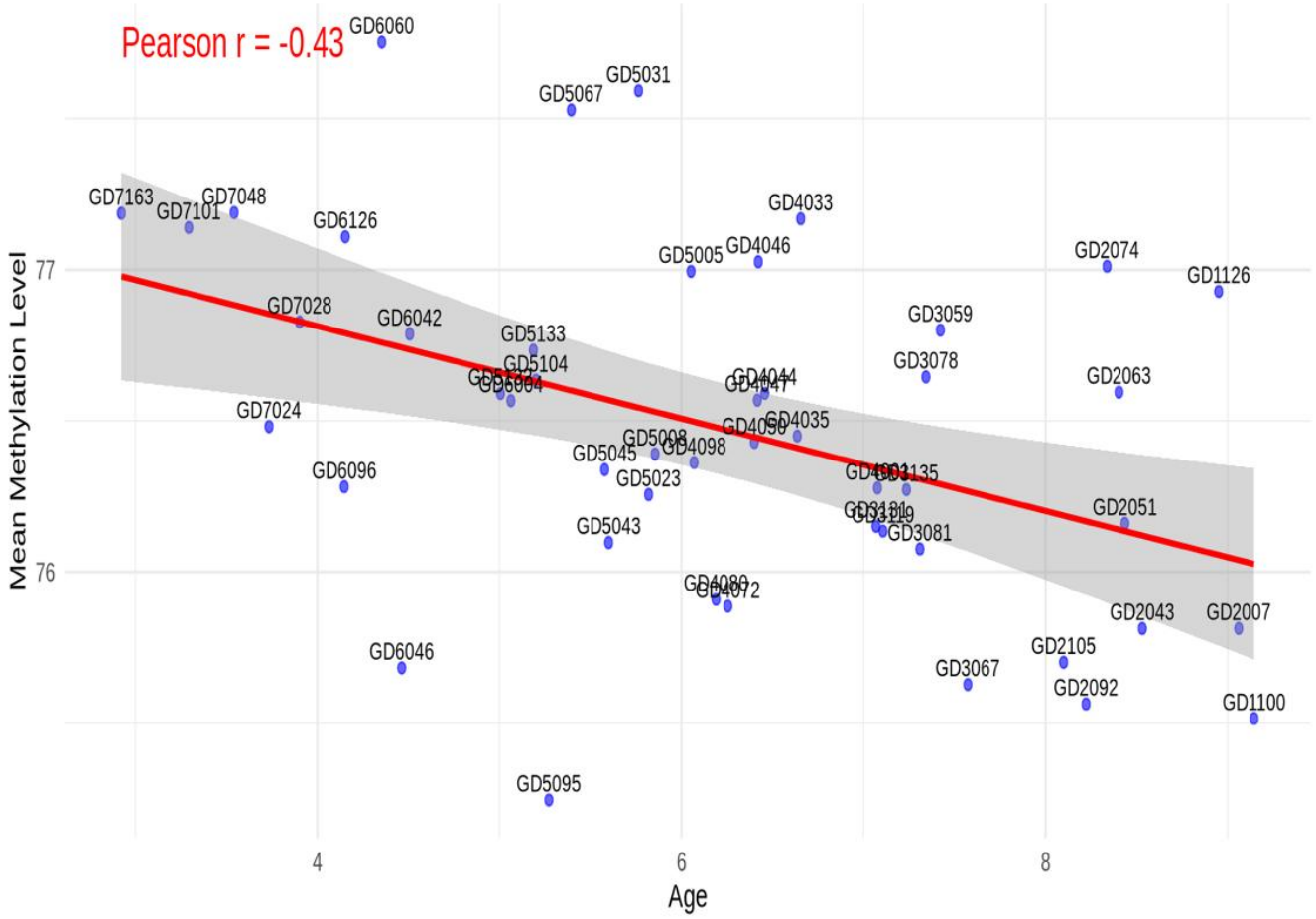

- Lameness 1.8 to 9.2 years,
- Fertility problems 3.3 to 4.6 years,
- Low production 1.8 to 5.6 years,
- Mastitis 2.8 to 7.8 years,
- Metabolic disorders 3.0 to 5.0 years.

- Control 2.9 to 9.1 years.

# Cross-sectional data
# Correlation with age

53 million CpG sites

(r = –0.43)



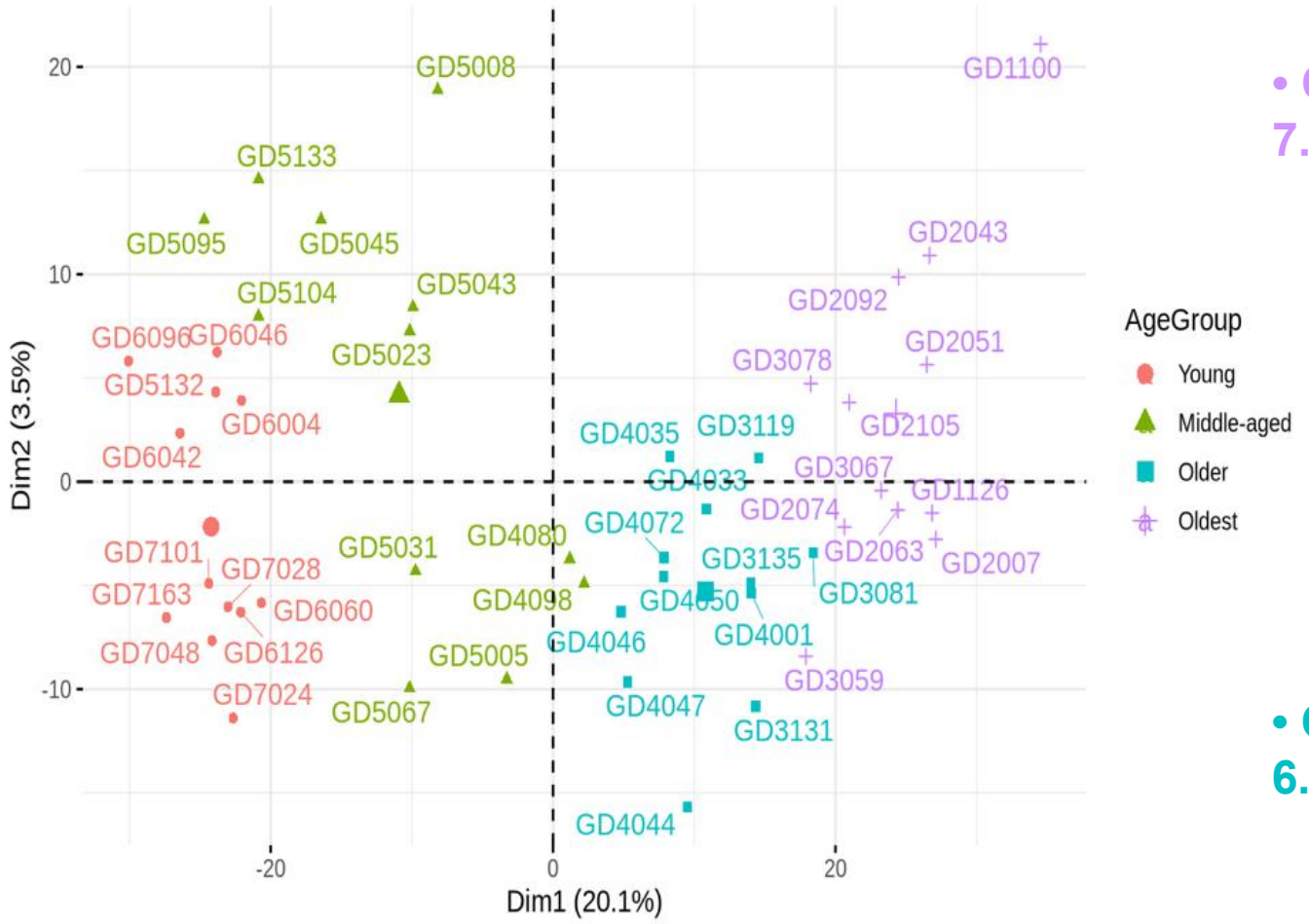

~ -1%

# Cross-sectional data
# ~2 000 age-correlated CpG sites

**Correlation coefficient> 0.5, qval < 0.01 and no missing data**

- **Middle-aged :**
  **5.2 à 6.2 years**

- **Young :**
  **2.9 à 5.0 years**




- **Oldest:**
  **7.3 à 9.2 years**

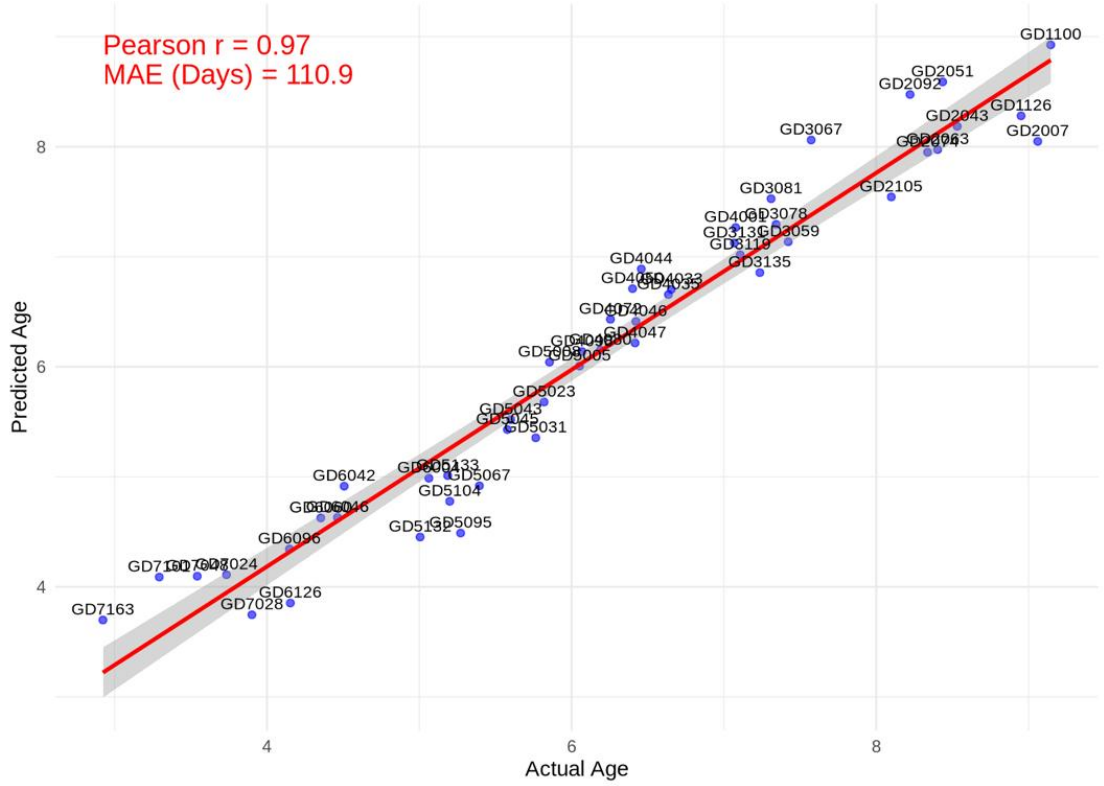
- **Old:**
  **6.3 à 7.3 years**

# Cross-sectional data

## Correlation between chronological age and predicted age

**Precision:**
**3 months and 21 days**

**~8 months for previous studies**



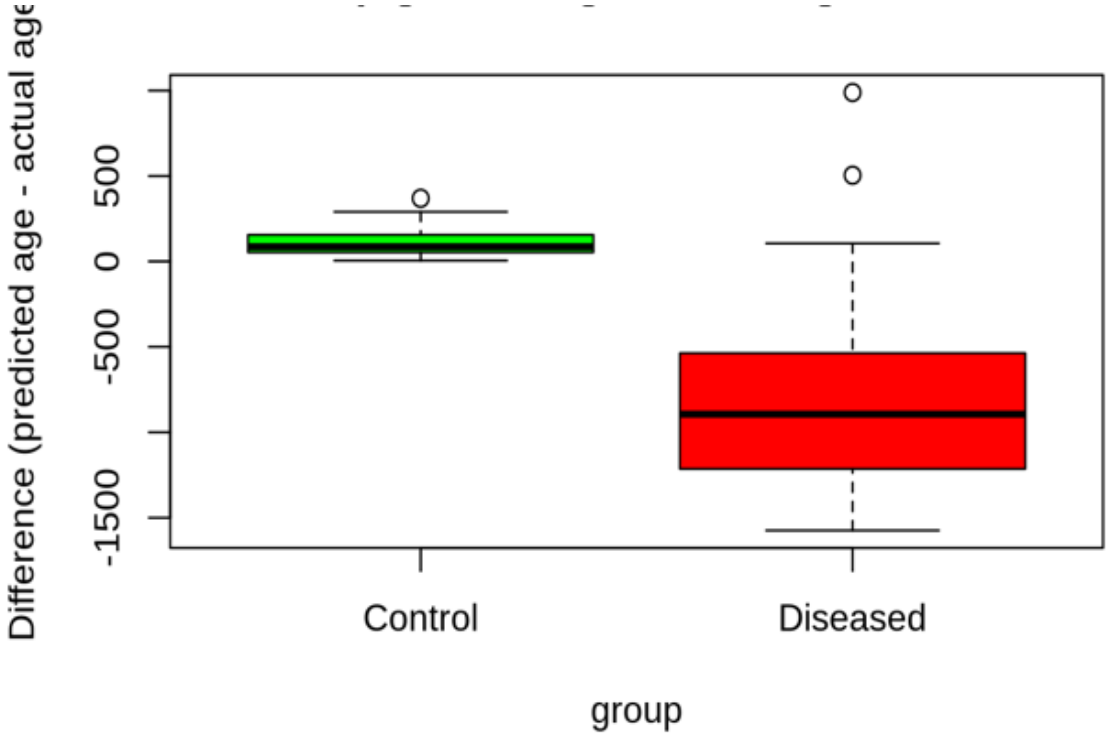

**62 CpG markers**

To avoid overfitting and to assess the generalizability of the model, we applied Leave-one-Out (LOOCV) cross-validation. This approach involved iterative training of the model on all but one sample

# Cross-sectional data
# Age acceleration

the model showed a mean absolute error (MAE) of ~2.5 years and a correlation of 0.16 in diseased cows

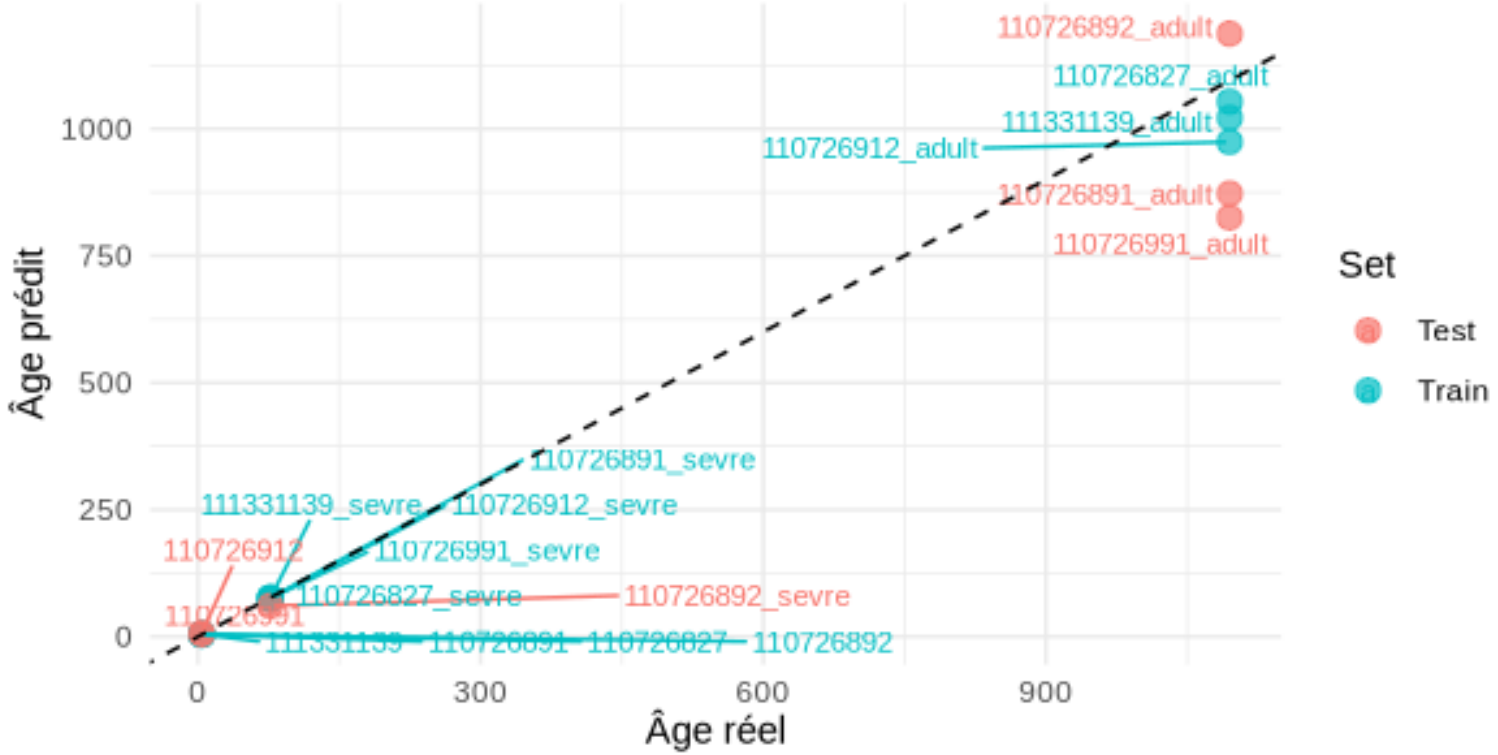
- Low production: ~3.1 years
- Metabolic disorders: ~2.4 years
- Fertility problems: ~2.0 years
- Lameness: ~1.5 years
- Mastitis: ~1.3 years




**Low-production cows showed the greatest acceleration in age**

# Longitudinal data
## Correlation between chronological age and predicted age

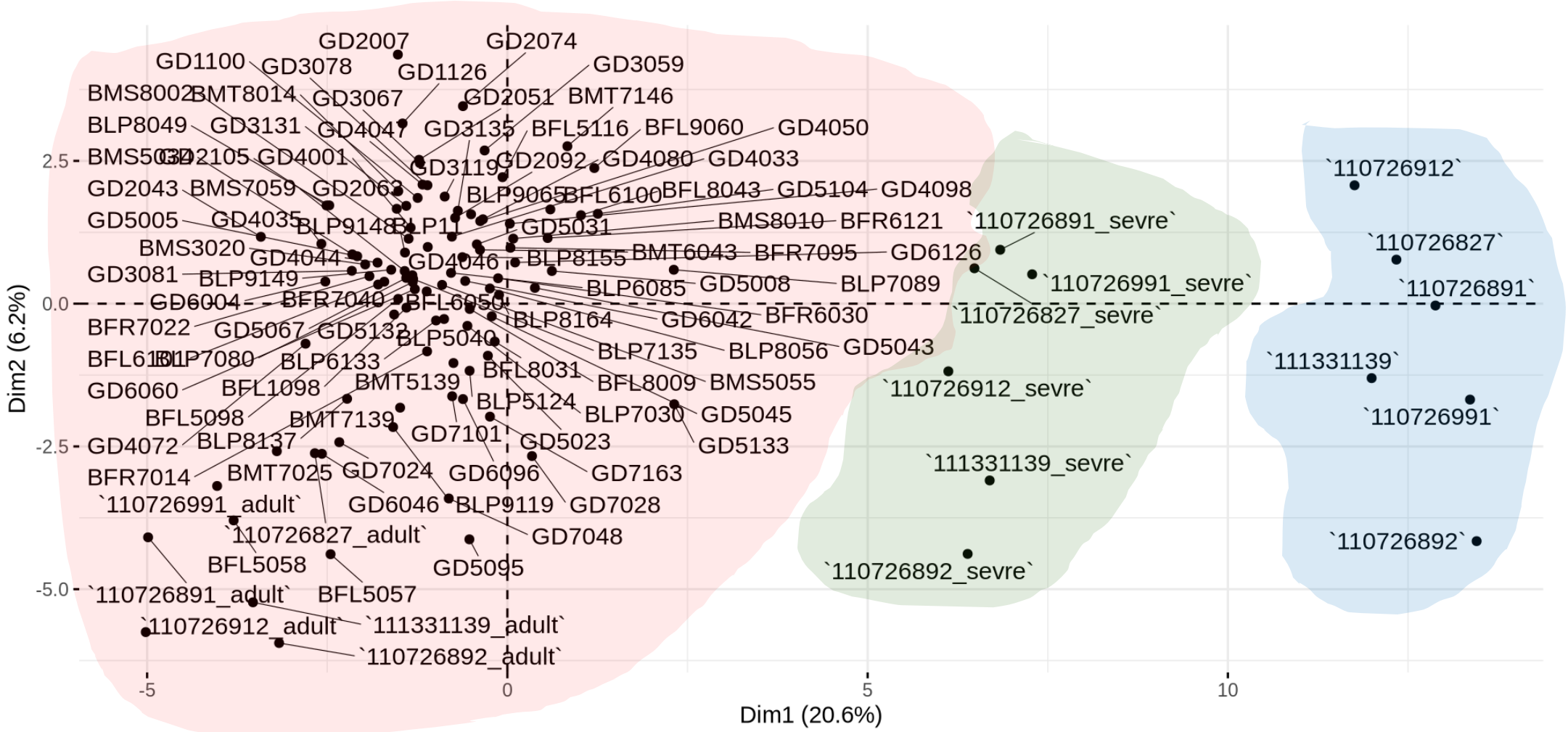
**Precision: 100 days**




**35 CpG markers**

**The high variability of ages was reduced through a logarithmic transformation.**

# Longitudinal and Cross-Sectional DNA Methylation Markers




**Adult**
> 2 years

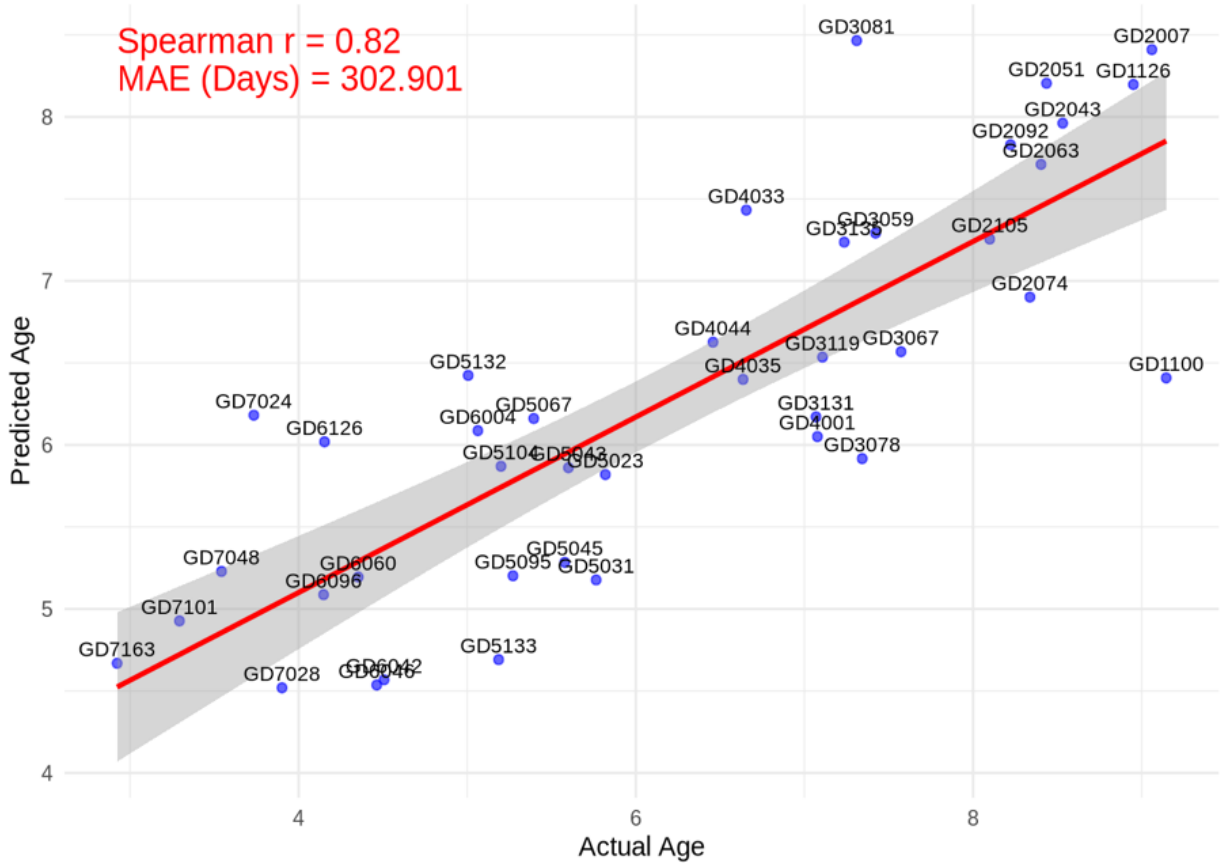
**Weaning**
~ 3 months

Newborn
4 days

# Age and mitochondrial DNA methylation

Among 6k mitochondrial sites, 61 sites with a negative correlation rho = -0,77 )

**Precision:**
**10 months**




**10 CpG markers**

# Differentially methylated regions (DMRs)

## 197 DMRs were identified

Methylation difference > 10% and qvalue < 0.05

| SYMBOL | width | num.cpgs | diff | FDR | distanceToTSS | GENENAME |
|---|---|---|---|---|---|---|
| MAB21L1 | 348 | 63 | -14.55 | 1.48E-02 | -2158 | mab-21 like 1 |
| MAB21L1 | 260 | 54 | -14.68 | 6.64E-04 | -1887 | mab-21 like 1 |
| NSD1 | 135 | 41 | -14.67 | 2.11E-02 | -2664 | nuclear receptor binding SET domain protein 1 |
| INKA2 | 155 | 32 | -14.67 | 4.94E-03 | -1517 | inka box actin regulator 2 |
| NUDT10 | 91 | 21 | 11.48 | 1.33E-02 | -108 | nudix (nucleoside diphosphate linked moiety X)-type motif 10 |
| GABRQ | 70 | 20 | -10.21 | 1.92E-02 | -330 | gamma-aminobutyric acid type A receptor subunit theta |
| HOXB9 | 189 | 20 | -12.32 | 3.31E-02 | -1392 | homeobox B9 |
| RBM33 | 488 | 20 | 16.84 | 8.36E-04 | -2042 | RNA binding motif protein 33 |
| INSIG1 | 29 | 15 | 12.05 | 2.99E-02 | -986 | insulin induced gene 1 |
| SULT1C4 | 42 | 12 | -11.54 | 5.74E-03 | -248 | sulfotransferase family, cytosolic, 1C, member 4 |
| SYTL2 | 78 | 12 | -13.81 | 5.74E-03 | -943 | synaptotagmin like 2 |
| TBCE | 62 | 12 | -13.64 | 1.70E-02 | -1360 | tubulin folding cofactor E |
| IPO7 | 90 | 10 | -11.22 | 4.65E-02 | -1968 | importin 7 |
| TMSB4 | 104 | 10 | -25.42 | 4.65E-02 | -966 | thymosin beta 4, X-linked |

**MAB21L1 gene contained two DMRs associated with the promoter:**

**First DMR: 260 bp region with 54 CpG, showing a 14.68% increase in methylation in older cows**

**Second DMR: 348 bp region with 63 CpG, showing a 14.55% increase in methylation in older cows**

# Conclusion

- This study highlights the value of blood methylation profiles in investigating age and disease in cattle, paving the way for applications in genomic selection and precision breeding.

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- This study highlights the value of blood methylation profiles in investigating age and disease in cattle, paving the way for applications in genomic selection and precision breeding.

- We propose a novel integrative framework called **ChronoMeth**, which combines nuclear and mitochondrial cytosine methylation profiles to explore molecular signatures of aging and disease susceptibility.

- This two-level approach enables refined age prediction and offers new insights into the interplay between epigenetic regulation, aging processes, and vulnerability to disease.

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**Collaborators:**

- **Camila Bruna De Lima**
- **Mohamed Oudihat**
- ***Helene Martin***
- **Jessica C.S. Marques**
- ***Ronaldo Cerri***

# Thank you