













75<sup>th</sup> EAAP 2024 Annual meeting – Florence, Italy 03/09/2024

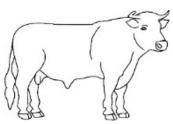
PhD student **Amrita Raja Ravi Shankar**amrita.raja-ravi-shankar@inrae.fr





### **Bovine selection**

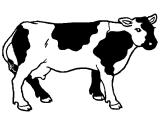
Father



Holstein bull selection

**Artificial Insemination** 

Daughter



Improve milk production, fertility, longevity...

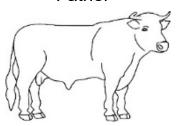
Boichard et al, 2012



### **Bovine selection**

Father

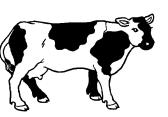
Context



Holstein bull selection

**Artificial Insemination** 

Daughter



Improve milk production, fertility, longevity...

Based on: Genetics → some of the phenotypic variability

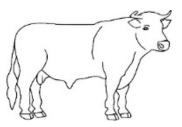
Boichard et al, 2012



### **Bovine selection**

Father

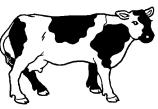
Context



Holstein bull selection

**Artificial Insemination** 





Improve milk production, fertility, longevity...

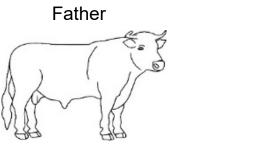
Based on:

Genetics → some of the phenotypic variability

**Sperm epigenetics** 

Boichard et al, 2012

# Impact of the father's environment on offspring's phenotype



Holstein bull selection

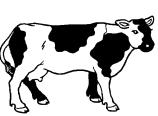


**Artificial Insemination** 

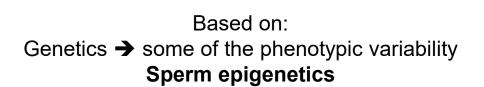


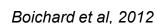
→ Ir

Daughter



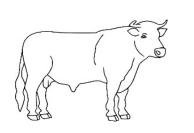
Improve milk production, fertility, longevity...







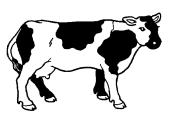
Environment



Father



DNA methylation modifications



Daughter's phenotype

Kiefer et al, 2021

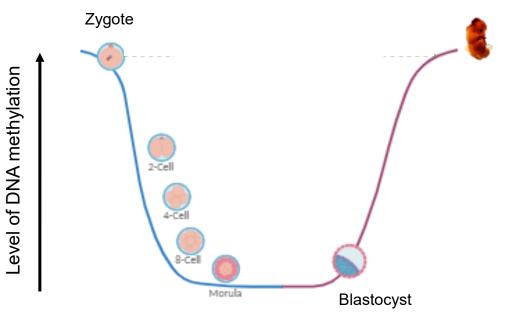
INRAe

Transmission of sperm DNA methylation patterns to the embryo in cattle: a genome-wide analysis 03/09/2024/Amrita Raja Ravi Shankar

Images from Biorender

## **Epigenetic reprogramming during embryogenesis**





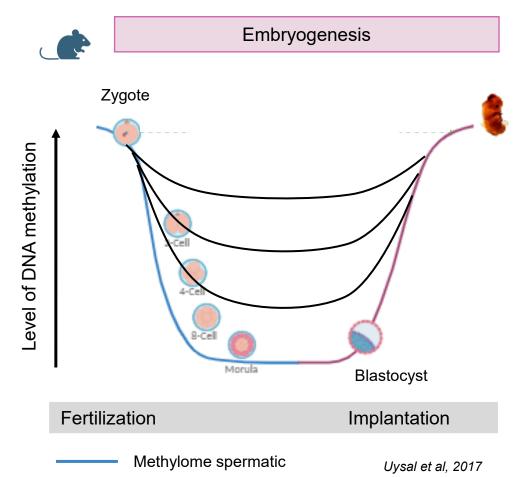
Fertilization	Implantation

Methylome spermatic Uysal et al, 2017



p. 6

## Some loci remain partially methylated during embryogenesis



Examples:

Transposons (Smith et al, 2012) [mouse]
Imprinted genes (Bartolomei, 2009)
Individual genes (Sambath Kumar et al, 2017) [mouse]



Intergenerational transmission (Takahashi et al, 2023) [mouse]

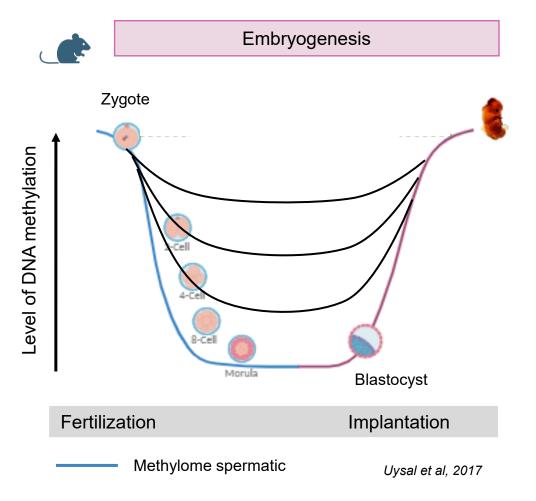


Offspring's phenotype (Takahashi et al, 2023) [mouse]



### INRAO

## Some loci remain partially methylated during embryogenesis



**Examples:** 

Transposons (Smith et al, 2012) [mouse] Imprinted genes (Bartolomei, 2009) Individual genes (Sambath Kumar et al, 2017) [mouse]

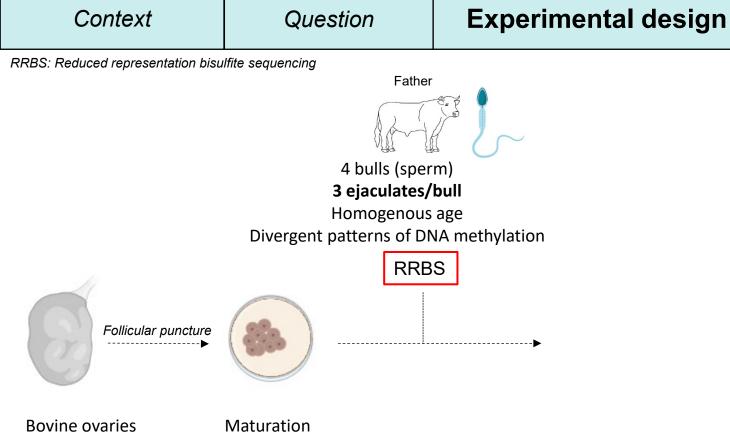


Intergenerational transmission (Takahashi et al, 2023) [mouse]



Offspring's phenotype (Takahashi et al, 2023) [mouse]

Which CpGs remain partially methylated in bovine embryos that could mediate paternal effects?





from

slaughterhouses

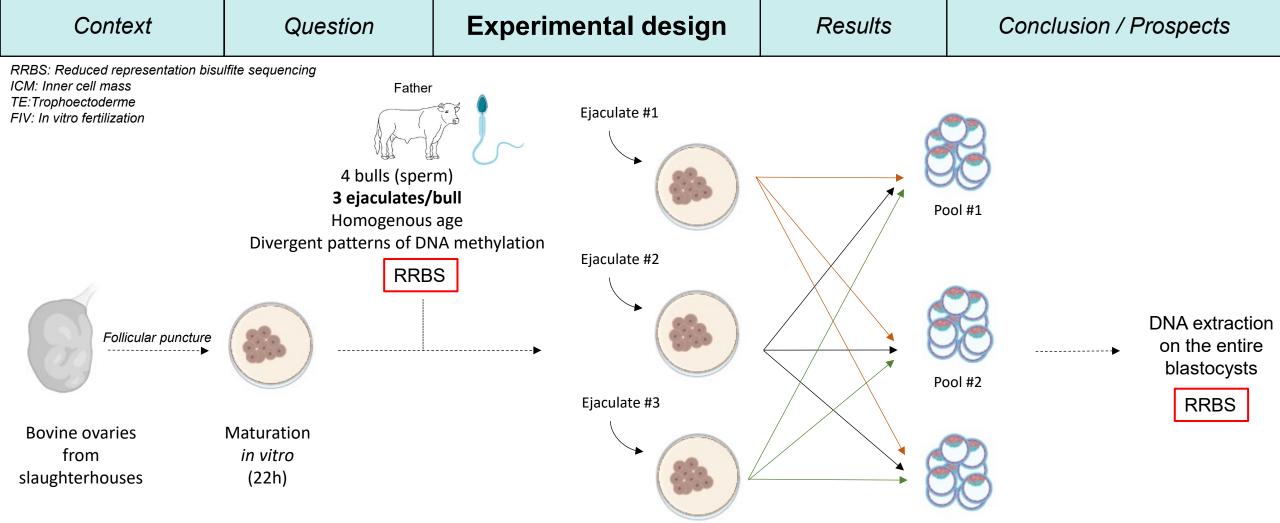
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in vitro

(22h)

Conclusion / Prospects

Results

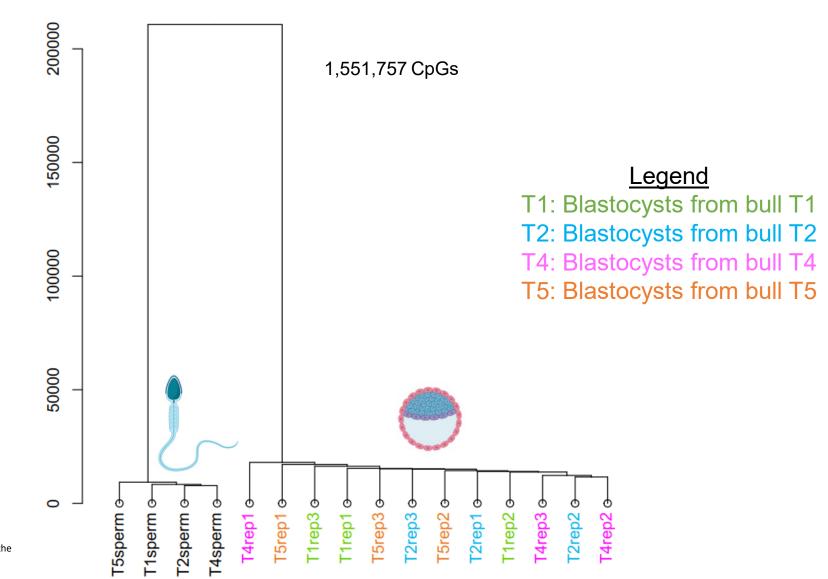


3 IVFs/bull (22h) Pool of expanded blastocysts (D6-7)
Homogenous stage
[14-16 blastocysts/pool]
(100 cells/blastocyst)

Pool #3

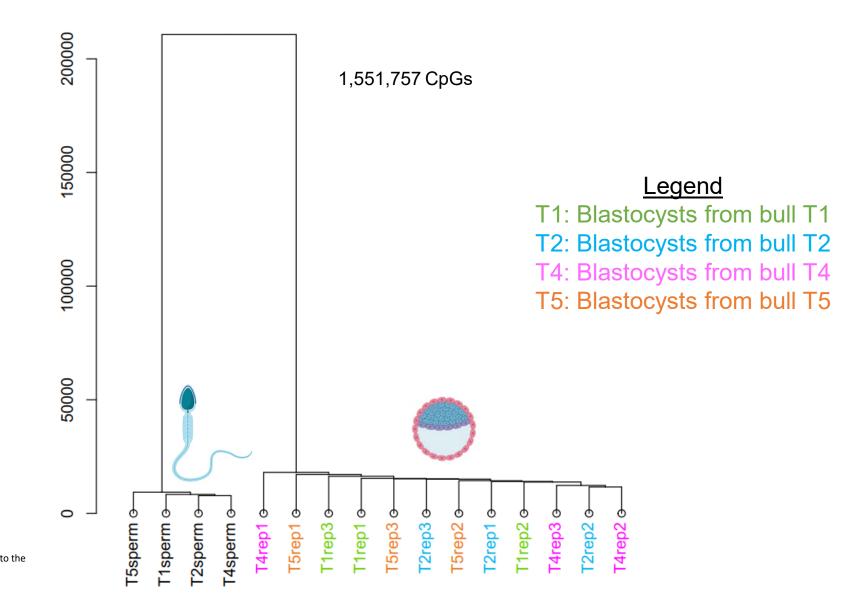


# Segregation between sperm and blastocysts by unsupervised hierarchical clustering





Context

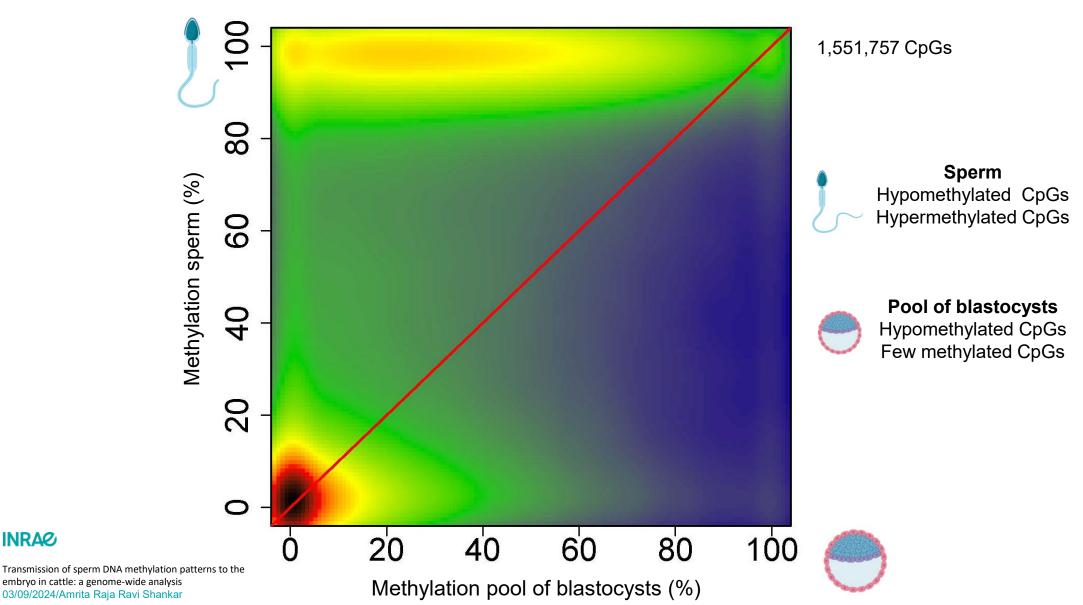


Context

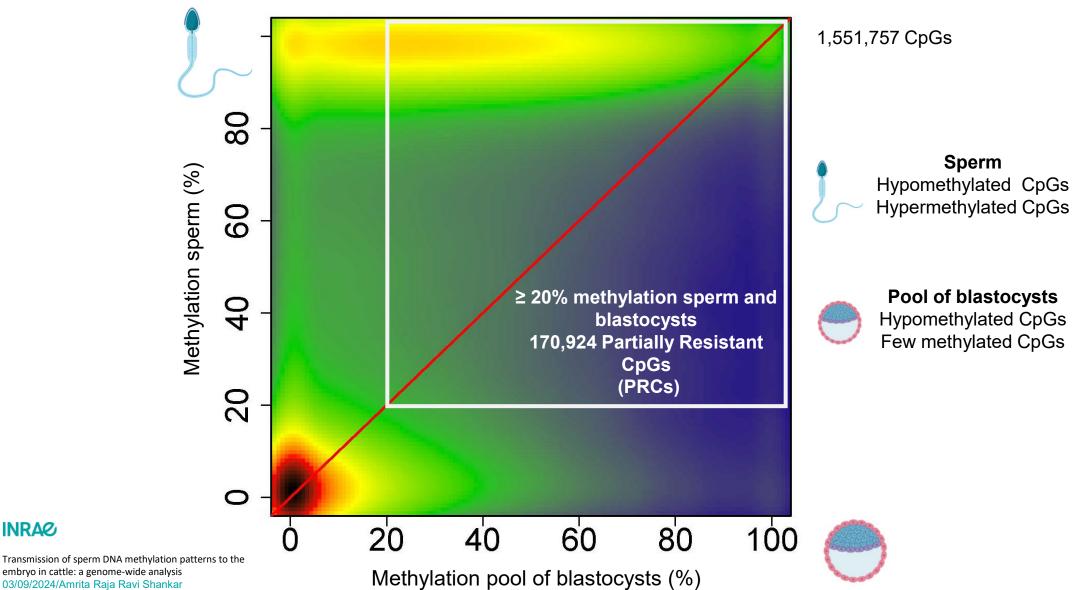
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# **Methylation status of CpGs in sperm and blastocysts**



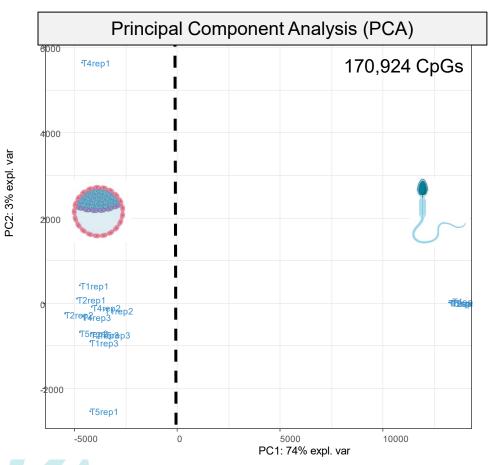
### 170,924 CpGs remain partially methylated in blastocysts



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## PRCs' methylation status allows segregation between sperm and blastocysts

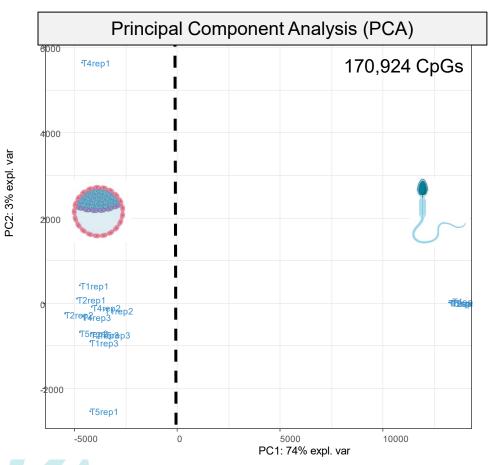
### Unsupervised method



Context

# PRCs' methylation status doesn't allow segregation between blastocysts according to the bull

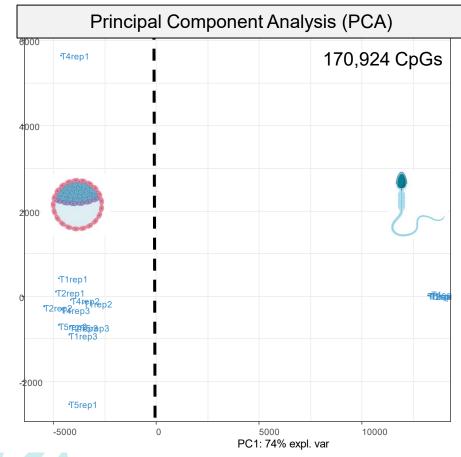
### Unsupervised method



# Switch to a supervised method: Partial Least Square –Discriminant Analysis on sperm and blastocysts according to the bull

Unsupervised method

Supervised method



PC2: 3% expl. var

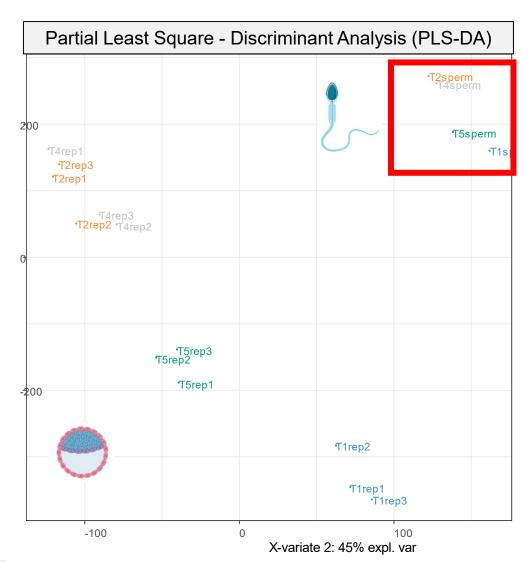
Partial Least Square - Discriminant Analysis (PLS-DA)

→ Impose a point of view to cluster blastocysts according to the bull

Numeric data: 170,924 CpGs Categorical dataset: bull Context

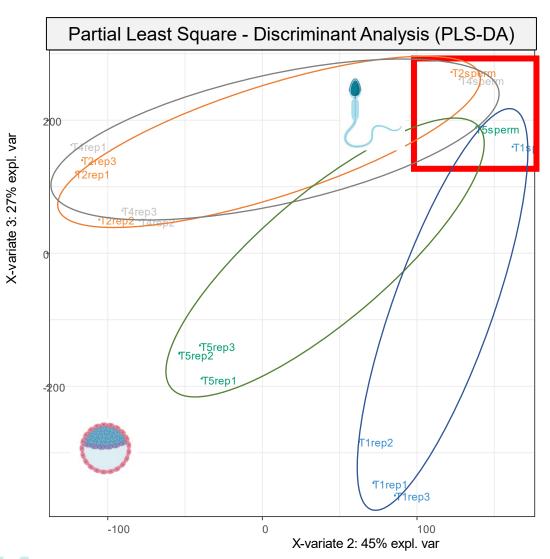
X-variate 3: 27% expl. var

# PRCs' methylation status allows segregation between sperm and blastocysts



170,924 CpGs

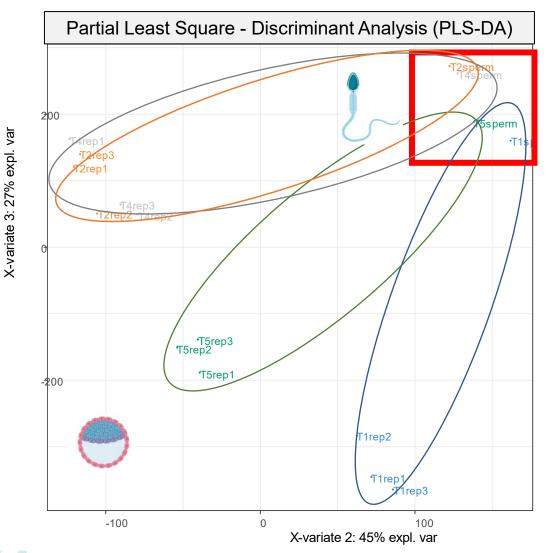
## PRCs' methylation is divergent in blastocysts from bulls T5, T1

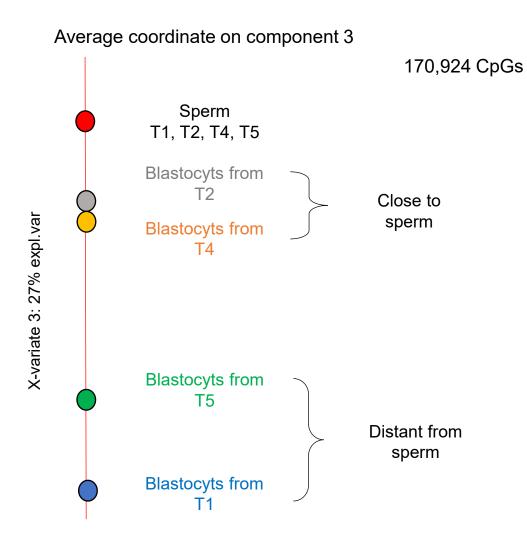


170,924 CpGs

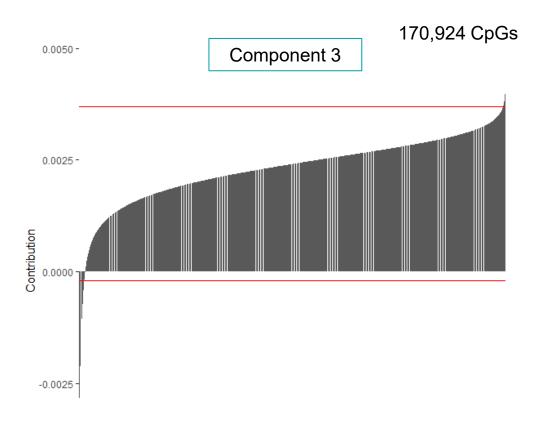
Context

## PRCs differentially reprogrammed in blastocysts according to the bull





# PRCs' contribution to segregate blastocysts according to the bull

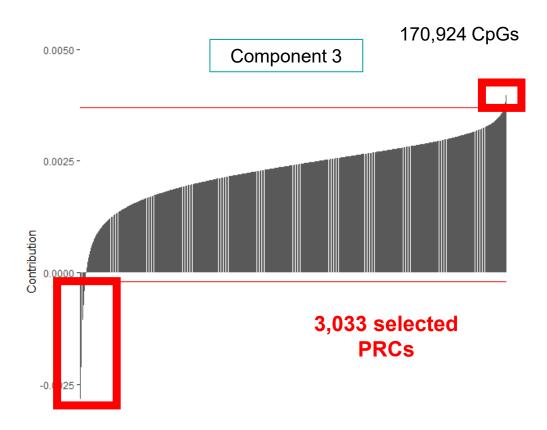


reorder(Variable, Contribution)



Context

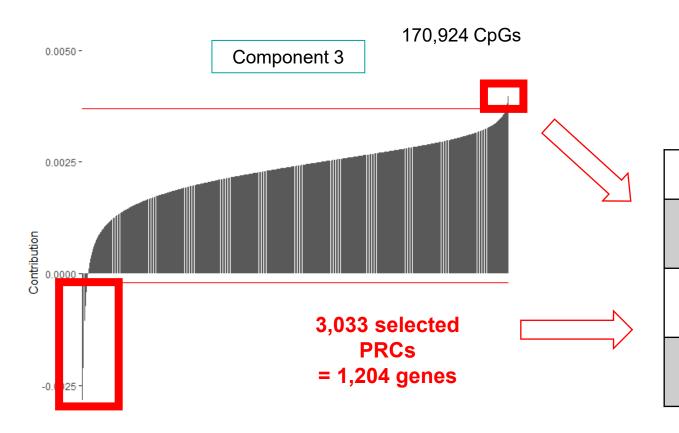
# Selection of 3,033 PRCs contributing to segregate blastocysts according to the bull



reorder(Variable, Contribution)



### The 3,033 selected PRCs contribute to several molecular functions



Database for Annotation, Visualization and Integrated Discovery (DAVID)

#### **GO Molecular functions**

**Protein Binding** (avp=3.6\*10<sup>-4</sup>) [191 genes]

ATP binding  $(avp=2.7*10^{-2})[122 genes]$ 

Guanyl-nucleotide exchange factor activity (avp=4.2\*10<sup>-2</sup>) [29 genes]

avp: adjusted p-value (Benjamini correction)

reorder(Variable, Contribution)



### Conclusion

Context

- Identification of 170,924 Partially Resistant CpGs (PRCs)
- → may escape epigenetic reprogramming
- Blastocysts from bull T5, T1 have divergent methylation patterns at 3,033 PRCs compared to T2 and T4
- → Interindividual variability between bulls in the ability to be reprogrammed after fertilization
- 3,033 PRCs are enriched in molecular functions

### **Prospects**

- As genetic contribute to interindividual variation What are the molecular mechanisms underlying interindividual variability between bulls? (genetics?)
- Do PRCs have an impact on the offspring's methylation status and phenotype?

These 3,033 PRCs are present in the RUMIGEN Epichip array



### Context

### Acknowledgment

### **Team members**

Hélène Kiefer
Aurélie Chaulot-Talmon
Olivier Dubois
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Sohary Jean-René
Ludivine Laffont
Catherine Archilla
Valentin Costes ELIANCE
Corentin Fouéré
Chrystelle Le Danvic ELIANCE
Hélène Jammes
Véronique Duranthon





















# Thank you for your attention

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