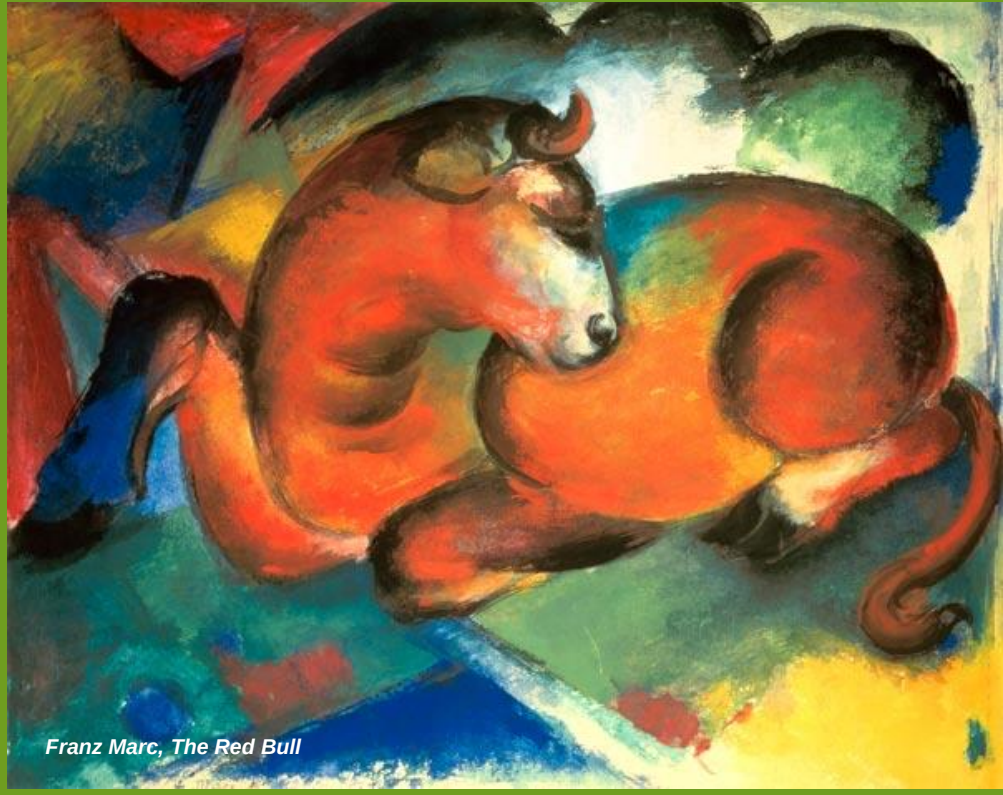


Long-term impact of early life nutrition on DNA methylation patterns in bull sperm

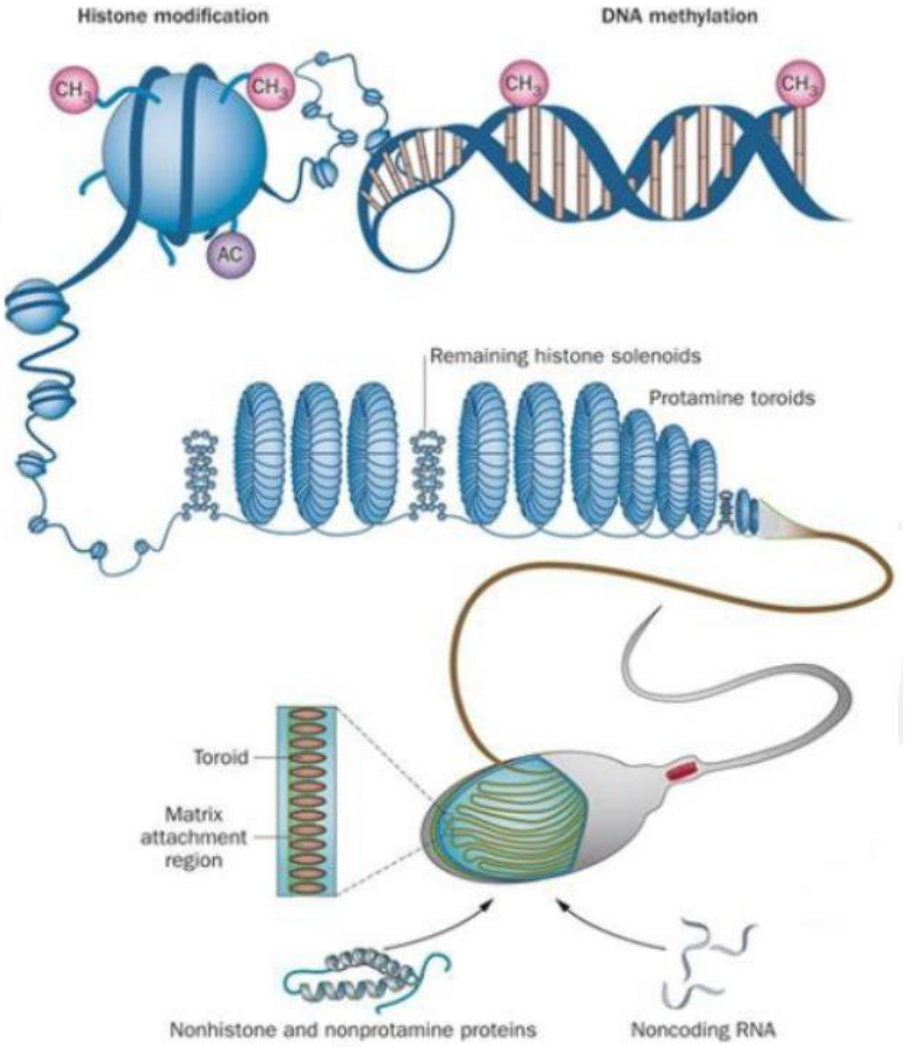
H. Kiefer, August 27, 2019

EAAP session 24, 'Epigenetics'

ALIMENTATION
AGRICULTURE
ENVIRONNEMENT

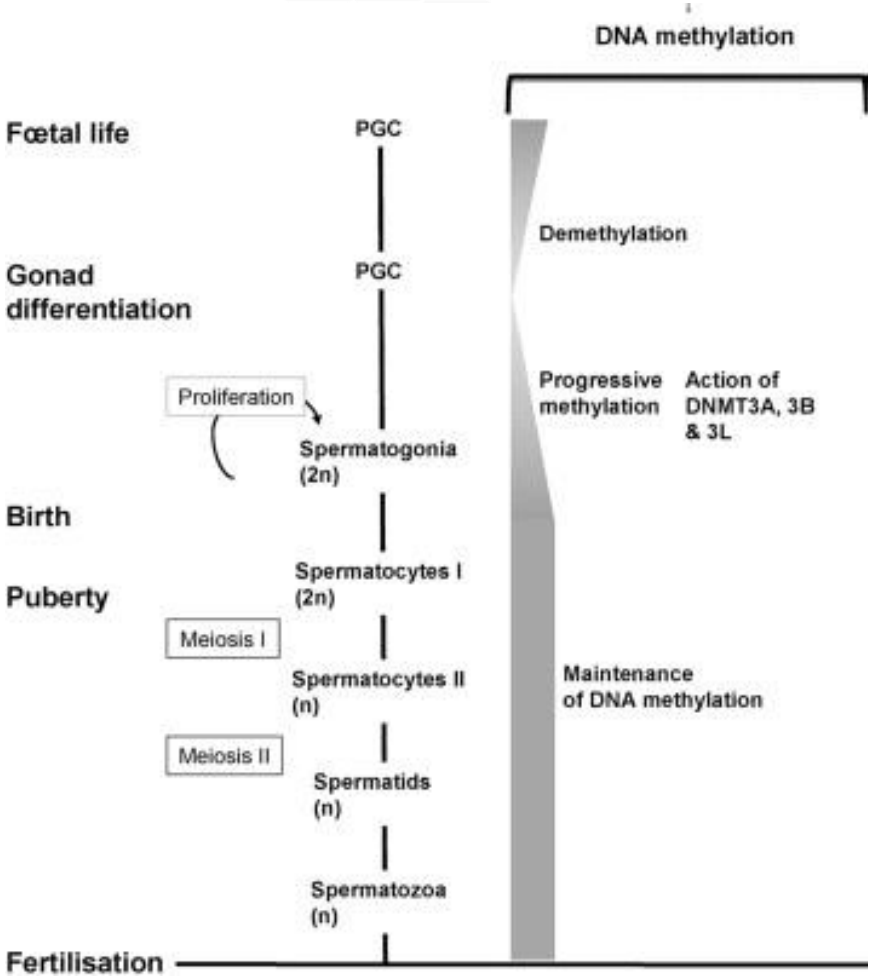
The differentiation of sperm cells requires an unique epigenetic reprogramming

- Compaction of paternal genome
- Morphological features
- Protection against oxydation (epididymis, female genital tract)
- Carry an epigenetic information important for embryo development and offspring phenotype



Schagdarsurengin *et al.*, 2012
Carrell, 2012
Champroux *et al.*, 2018

Dynamics of epigenetic reprogramming in male germ cells and influence of environmental factors



In utero & post-natal environment:

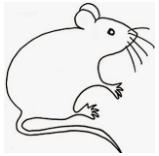
- DNA methylation erasure
- *De novo* DNA methylation

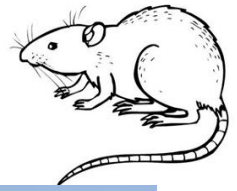
Environment during spermatogenesis:

- Maintenance of DNA methylation across mitosis/meiosis
- Crosstalk with other epigenetic marks

Boissonnas *et al.*, 2013
Ly *et al.*, 2015
Donkin & Barrès, 2018

Nutritional programming and DNA methylation in sperm





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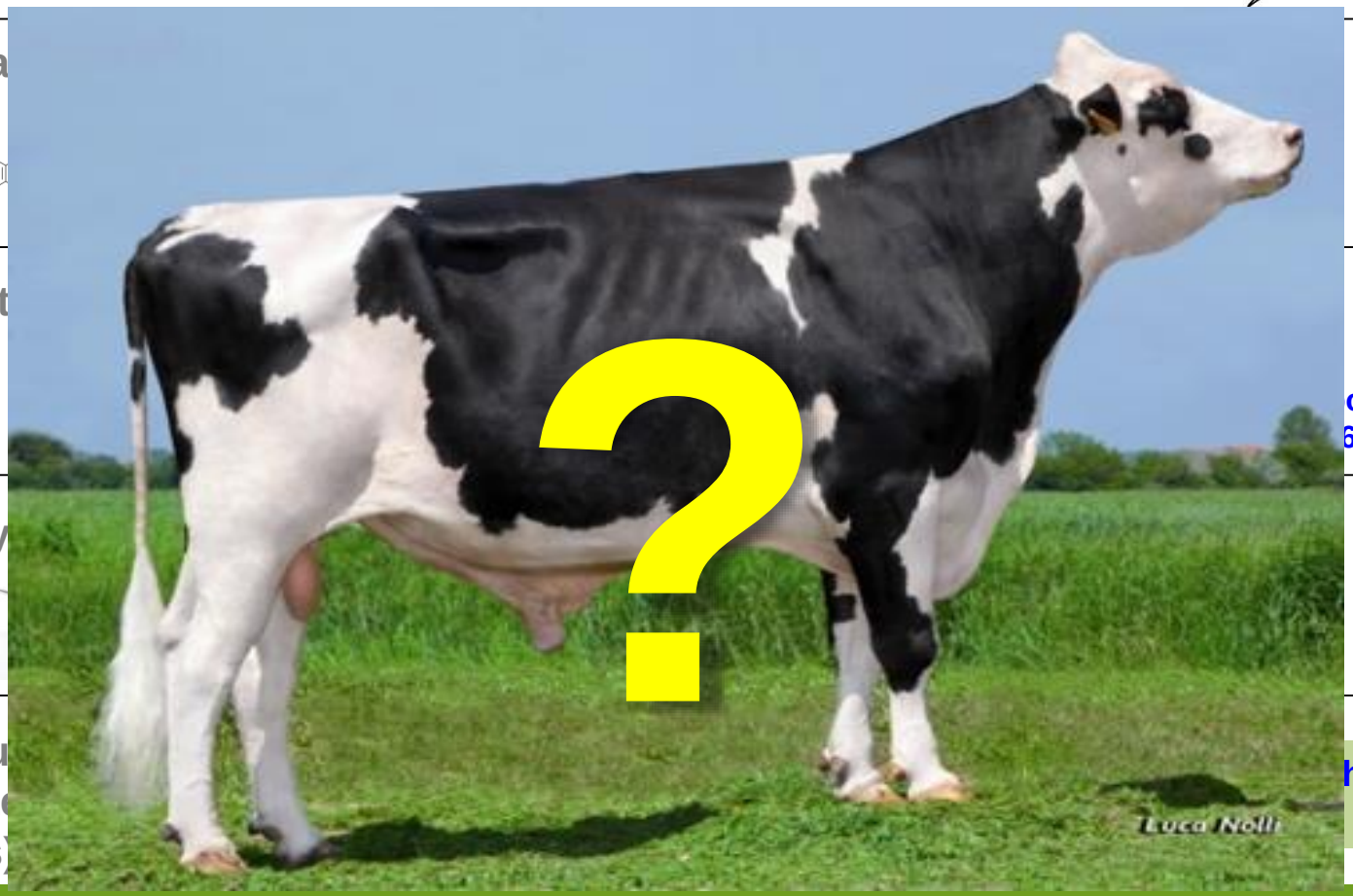


Bull semen is an important product for breeders and artificial insemination (AI) industry…

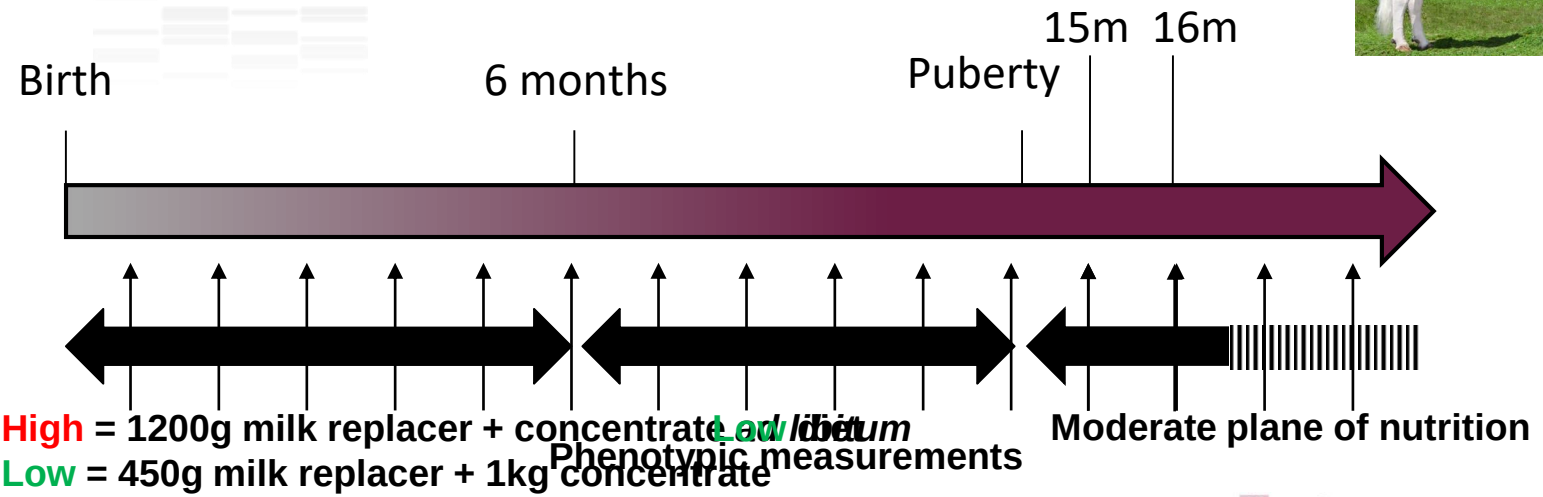
- 7 millions AI performed in France in 2016 (ruminants), ~100 million worldwide
- Success of AI = important issue for breeders
- AI allows the diffusion of valuable genotypes

…Genomic selection has considerably modified breeding practices within AI sector

- The breeding value of bulls is determined during early life
- Increased demand for semen from sires at a younger age
- Enhanced nutrition during early life hastens age at puberty **(Dance *et al.*, 2015; Byrne *et al.*, 2018a; 2018b)**

Impact of enhanced nutrition and hastened puberty on sperm DNA methylome?

Experimental design



Bulls on the High diet were 1 month younger at puberty

Bulls on the High diet had an increased:

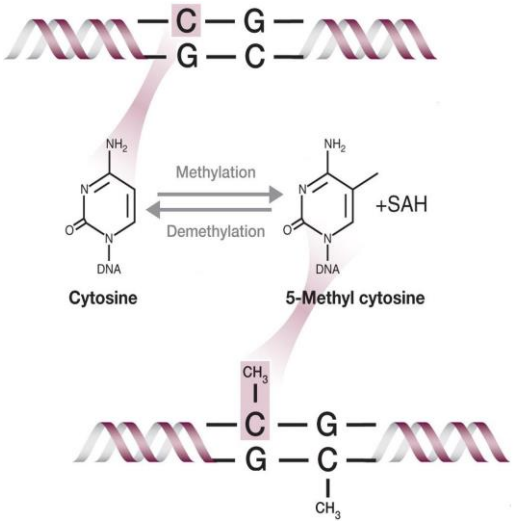
- Average daily gain
- Weight
- Scrotal skin thickness and temperature
- Testosterone level

But no effects on semen quantity or quality

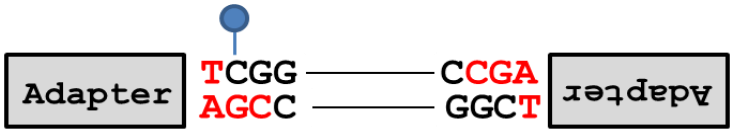
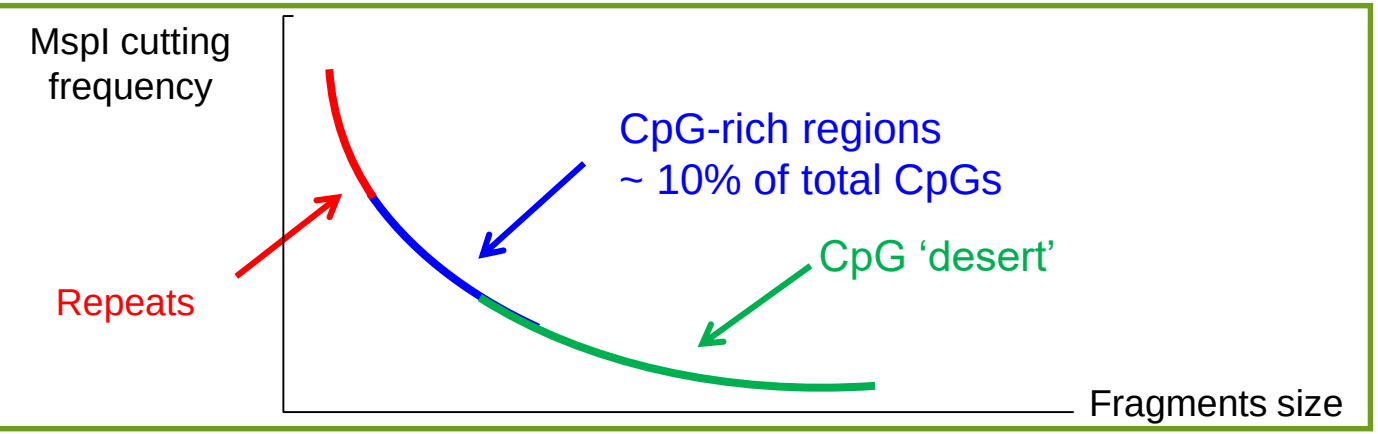
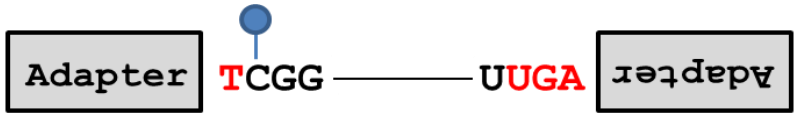
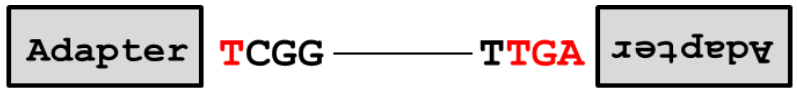
- Same physiological age

DNA methylation analyses on sperm:

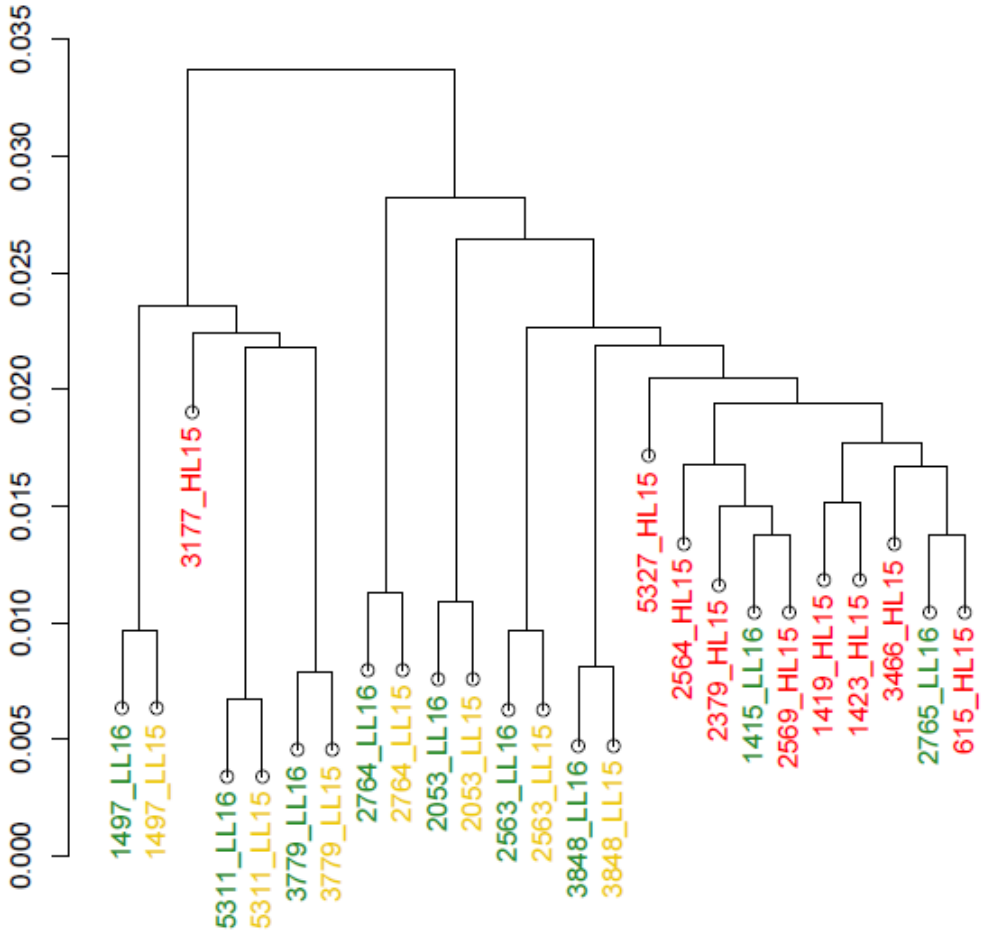
- **High diet at 15m (HL15, n=9)**
- **Low diet at 15m (LL15, n=7)**
- **Low diet at 16m (LL16, n=9)**



RRBS method

Enzymatic digestion	
End-repair / A tailing / Adapters ligation	
Size selection	
Bisulfite conversion	
Amplification	

Samples collected on the same bull cluster together independently of age



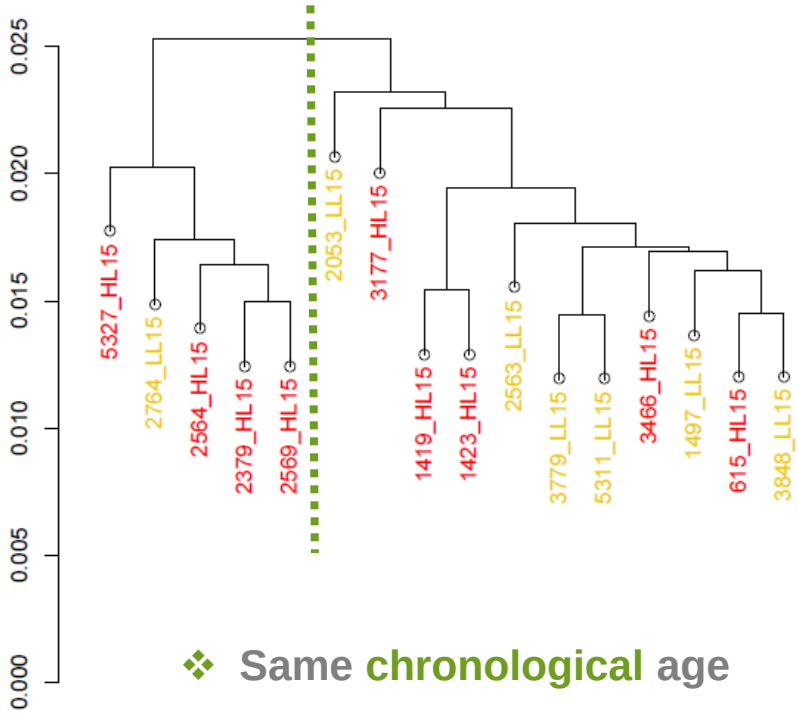
Correlation clustering on 1,328,693 CpGs

- Interpretation is complicated by the presence of paired samples in the **LL** group

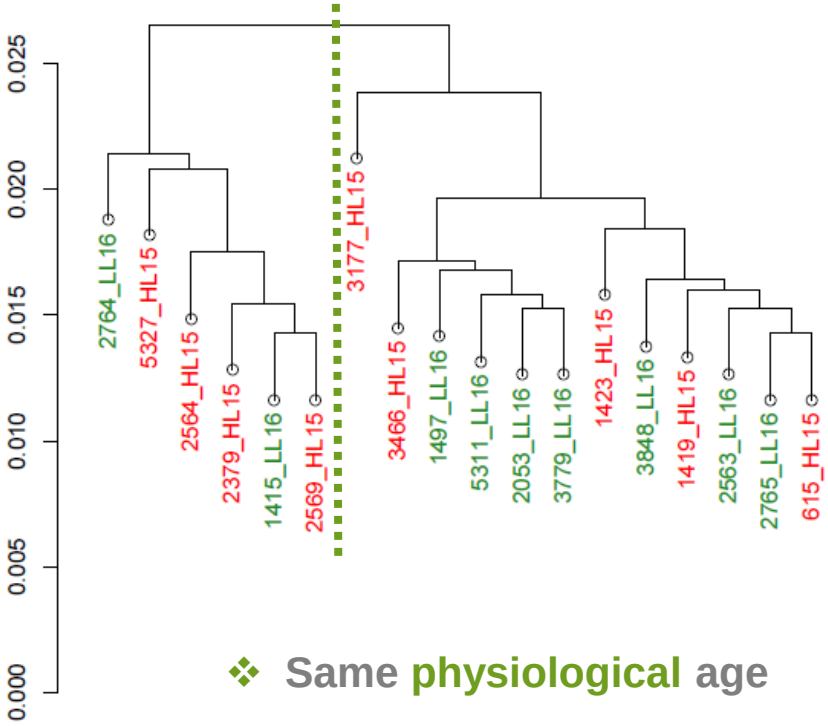
High diet at 15m (HL15, n=9)
Low diet at 15m (LL15, n=7)
Low diet at 16m (LL16, n=9)

Inter-individual variability unrelated to diet dominates DNA methylation profiles

Correlation clustering on 1,359,905 CpGs



Correlation clustering on 1,401,664 CpGs



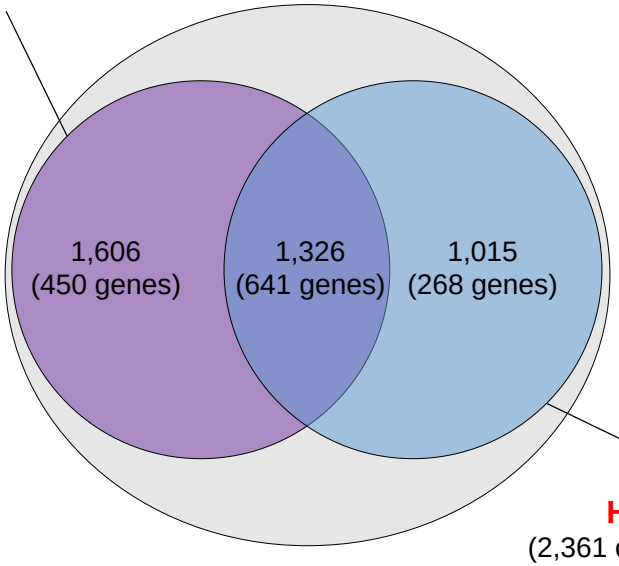
- Same **chronological** age
- No massive effect of nutritional programming on sperm methylome
- Same **physiological** age

High diet at 15m (**HL15**, n=9)
Low diet at 15m (**LL15**, n=7)
Low diet at 16m (**LL16**, n=9)

Identification of diet- and age-related Differentially Methylated CpGs (DMCs)

Diet-related DMCs

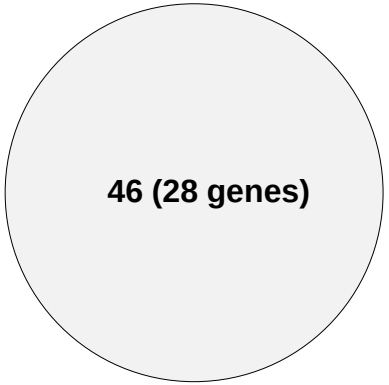
HL15 *vs* LL15
(2,932 over 1,669,560 CpGs)



HL15 *vs* LL16
(2,361 over 1,772,219 CpGs)

Total = 3,947 (1,359 genes)

LL15 *vs* LL16
Paired samples



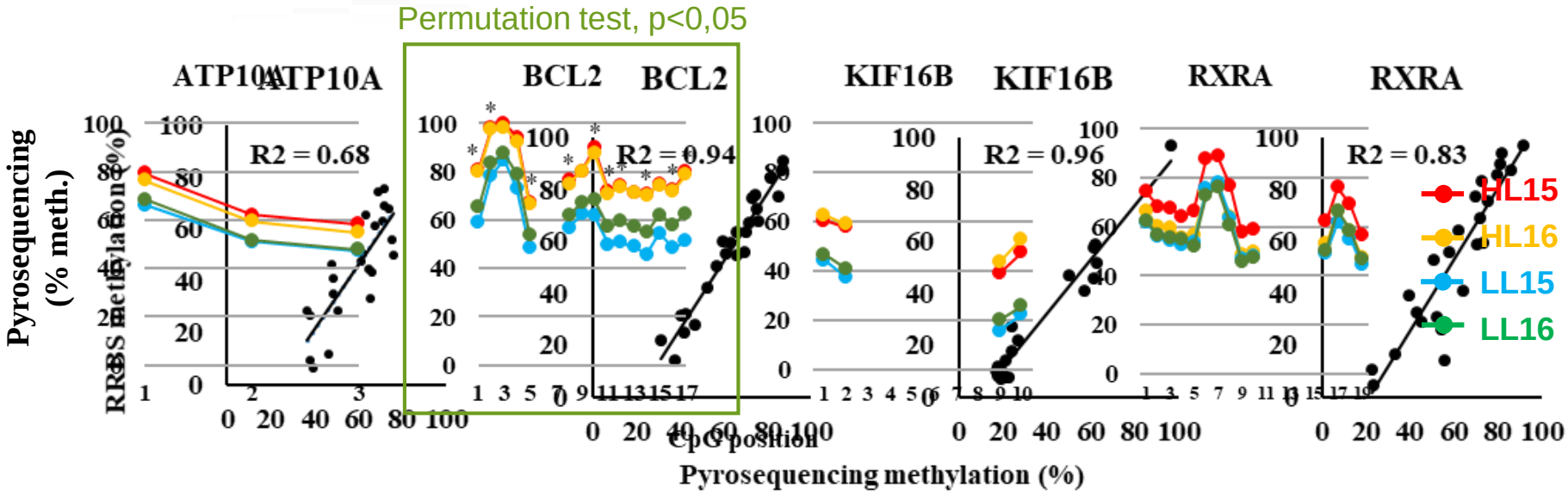
- **GO enrichment analysis:**
  ATP binding process, Focal adhesion

- **Consistent with earlier study on bull sperm reporting no methylation change between 12m and 16m (Lambert *et al.*, 2018)**

Differential analysis on CpGs covered by ≥10 reads in at least 4 samples per group
methylKit software, qvalue<0.01, methylation difference between groups ≥25%

High diet at 15m (HL15, n=9)
Low diet at 15m (LL15, n=7)
Low diet at 16m (LL16, n=9)

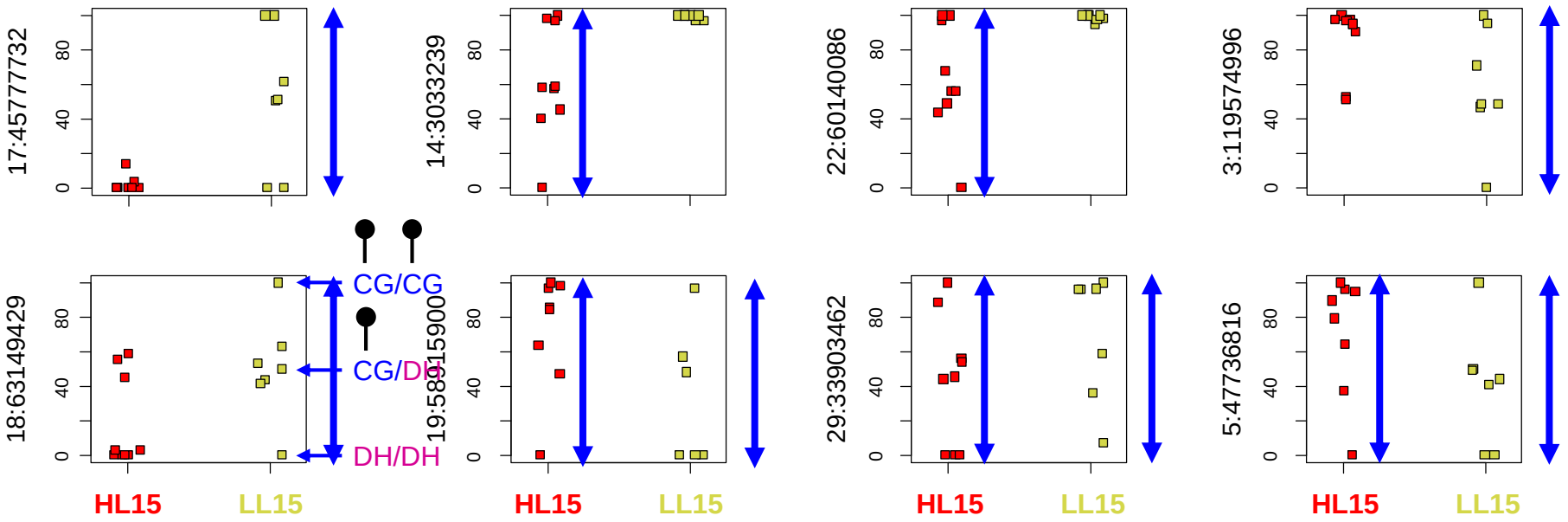
Validation of RRBS results by bisulfite-pyrosequencing



- Selection of 4 DMRs (including ≥ 3 DMCs)
- The methylation data produced by RRBS and bisulfite-pyrosequencing are in good agreement → **technical validation OK**
- But only 1 region shows significant methylation differences between groups → **statistical method to detect DMCs is not validated**

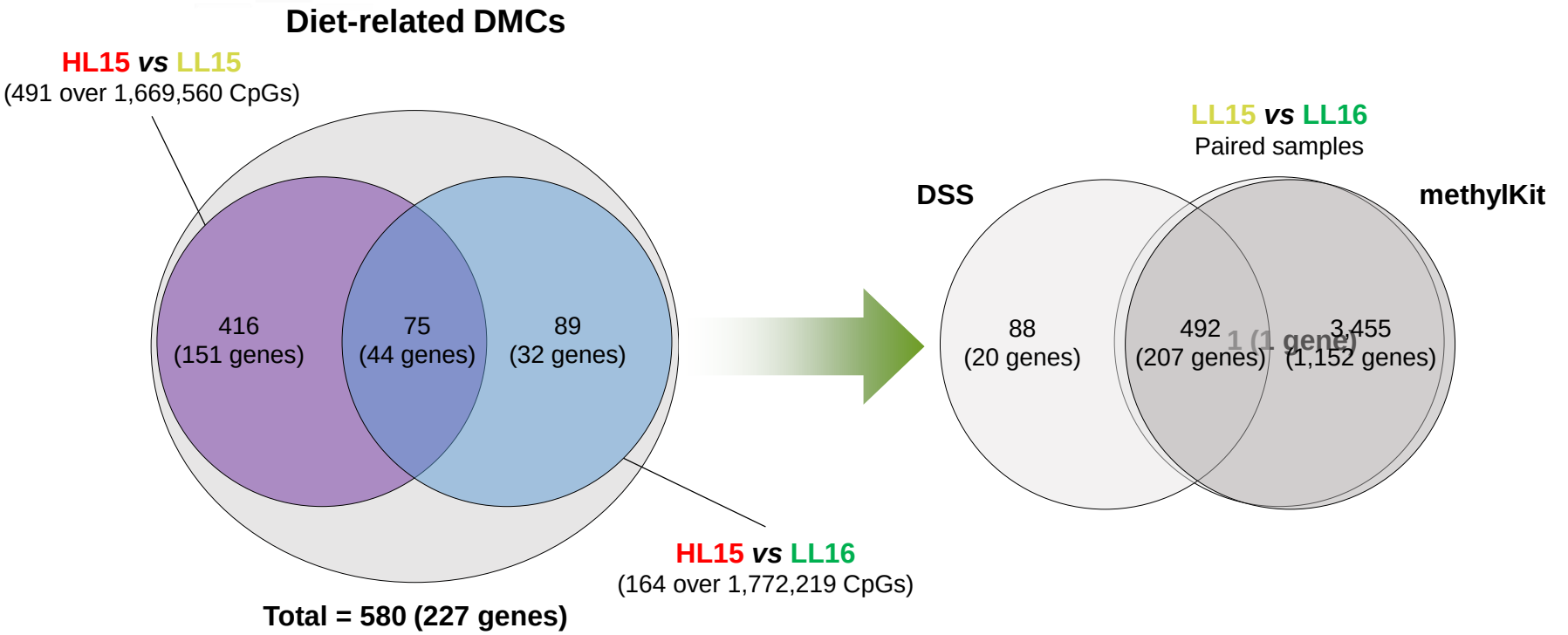
The statistical approach (methylKit) does not take account of inter-individual variability

Methylation % at sampled CpGs identified as DMCs using methylKit:



- Part of this inter-individual variability may rely on genetic polymorphism

Identification of diet- and age-related Differentially Methylated CpGs (DMCs)



- **85%** of the DMCs identified using DSS were also found by methylKit
- **ATP binding** GO term is enriched in both lists
- Most CpGs found by methylKit do not reflect the effect of the treatment

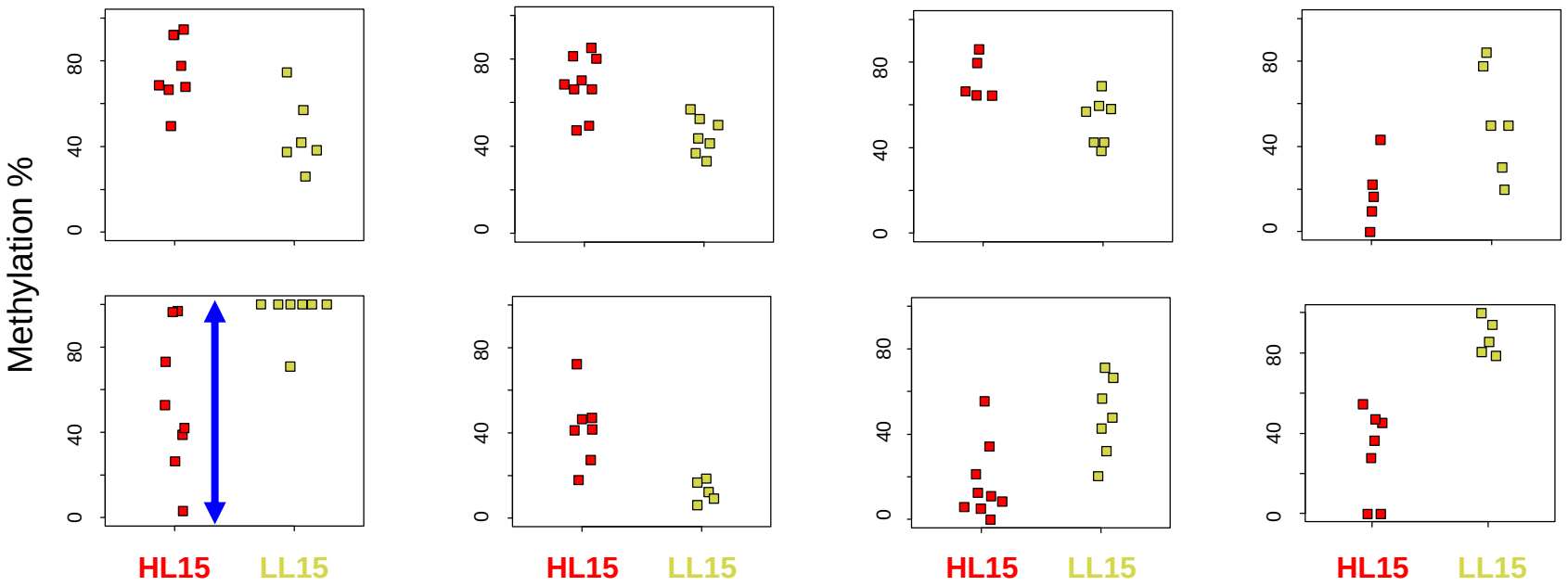
Differential analysis on CpGs covered by ≥10 reads in at least 4 samples per group
DSS software, FHW adj. pvalue<0.1, methylation difference between groups≥10%

High diet at 15m (HL15, n=9)
Low diet at 15m (LL15, n=7)
Low diet at 16m (LL16, n=9)

DSS: Feng *et al.*, 2014
methylKit: Akalin *et al.*, 2012

The distribution of methylation at DMCs found by DSS is more consistent with the effect of a diet challenge

Sampled CpGs identified as DMCs using DSS:



- Inter-individual variability within each group is lower than with methylKit

Conclusion

- Using two statistical approaches, we obtained two lists of **candidate CpGs potentially responsive to a nutritional challenge**. The list obtained using DSS is more restricted, but seems to better reflect the effect of diet

- In publications about DNA methylation, it is now usual to skip the validation step. A **careful examination of candidate CpGs responsive to environmental factors is required**, to make sure that differential methylation is not due to confounding factors (i.e, genetic polymorphism)

- Although with different magnitude, both statistical methods lead to the same conclusion: **a memory of the diet challenge that took place during early life is recorded in the sperm methylome**

- The validated DMR in ***BCL2*** is of particular interest, since spermatogenesis requires a dynamic balance of germ cells regeneration and elimination through **apoptosis** **(Sofikitis *et al.*, 2008)**

- Aurélie Chaulot-Talmon
- Luc Jouneau
- Anne Aubert
- Hélène Jammes

- Eli Sellem
- Laurent Schibler

- Jean-Philippe Perrier
- David Kenny
- Colin Byrne
- Patrick Lonergan
- Sean Fair