DNA methylation dynamics during spermatogenesis in ruminants

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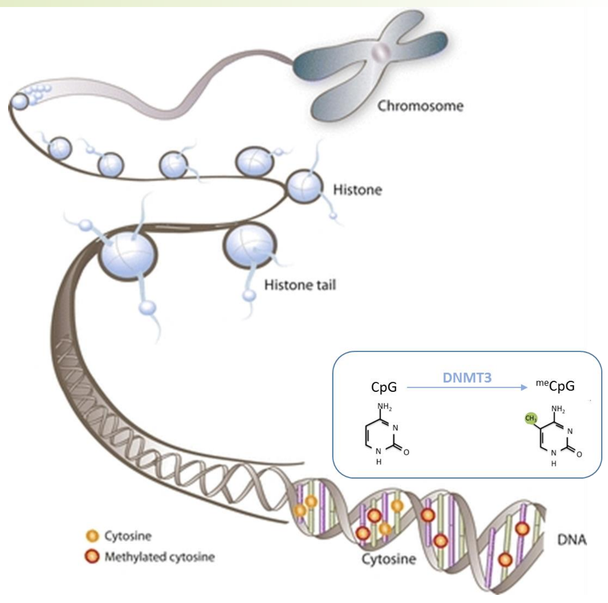
INTRODUCTION

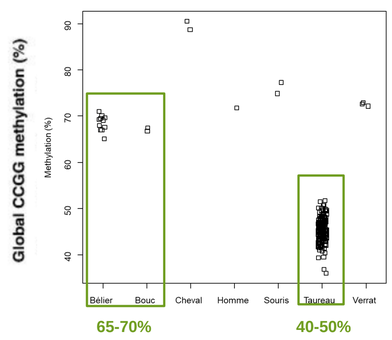
- DNA methylation of cytosines is a critical epigenetic modification in mammals that plays crucial roles in transcriptional regulation, chromatin remodelling and genomic imprinting. Dynamic erasure and reestablishment of DNA methylation marks are required for spermatogenesis and the normal function of mature sperm.

- DNA methylation is catalysed by DNA methyltransferase enzymes (DNMT) providing either maintenance (DNMT1) or *de novo* (DNMT3A/B/L) DNA methylation processes.



- DNA methylation dynamics during spermatogenesis has been previously described in mice and humans. Nothing is known in productive livestock.

- Recent study from our laboratory pointed DNA undermethylation of bull spermatozoa compared to other mammals such as humans, mice, sheep or goats [1]



[1] Perrier *et al.*, BMC Genomic. 2018

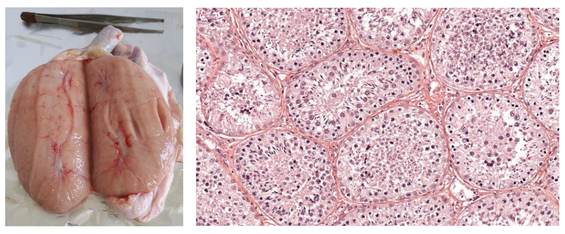
- DNA methylation description in ruminants would determine methylation state of male germ line during spermatogenesis and could be helpful to determine its impact on male fertily in productive livestock.

OBJECTIVES

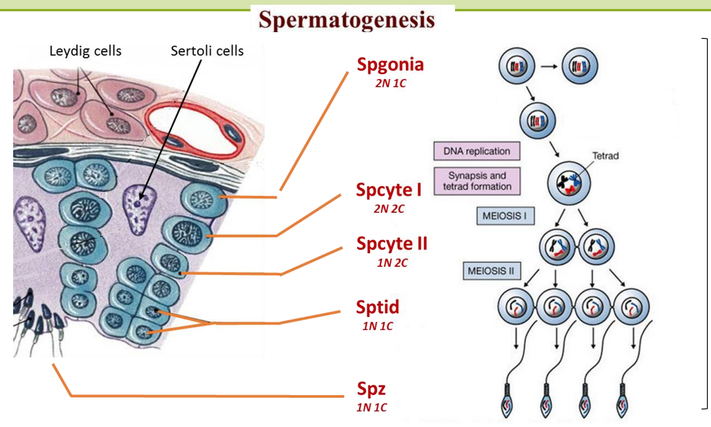
Determination and comparison of DNA methylation dynamics in bovine and caprine germ cells during spermatogenesis.

1. DNA methylation marks observation → IF
2. Germ cells purification → flow cytometry
3. DNA methylation analyses → RRBS

Adult stage



1. DNA methylation mark detection in testis
⇨ *5-methylcytosine (5-mC) Immunofluorescence*



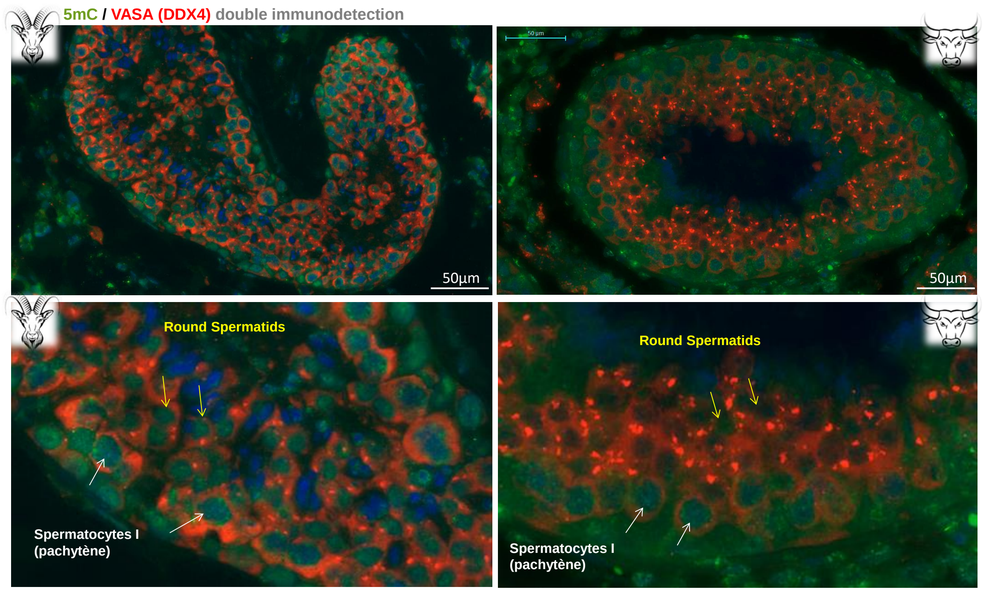
2. Spermatogenic cells purification from seminiferous tubules:
⇨ *flow cytometry-based method (Hoechst-FACS)*

3. DNA Methylation analysis on purified spermatogenic cells
⇨ *DNMT3s expression by real time PCR (RT-qPCR)*
⇨ *Reduced-Representation Bisulfite Sequencing (RRBS)*

RESULTS

1. DNA methylation in male germ line

❍ **5mC detection during spermatogenesis**

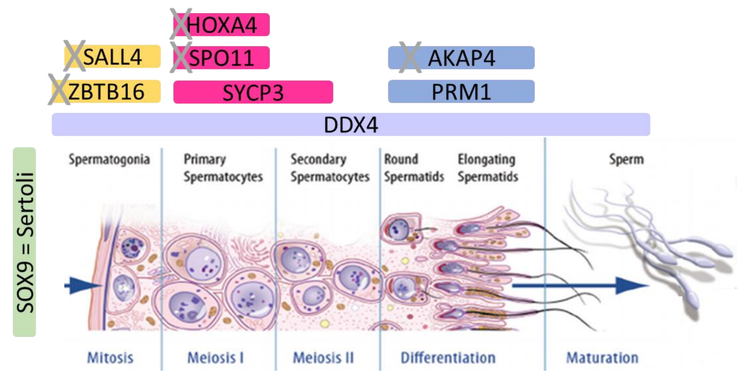


- In caprine testis , DNA methylation is highly detected in germ cells of all stages of spermatogenesis (VASA+).
- In bovine testis, DNA methylation is clearly detected in Spermatogonia and Spermatocyte I nuclei, while low to negative signal is observed at later stages.
- Differences in DNA methylation dynamics in spermatogenic cells between this two species of ruminants.

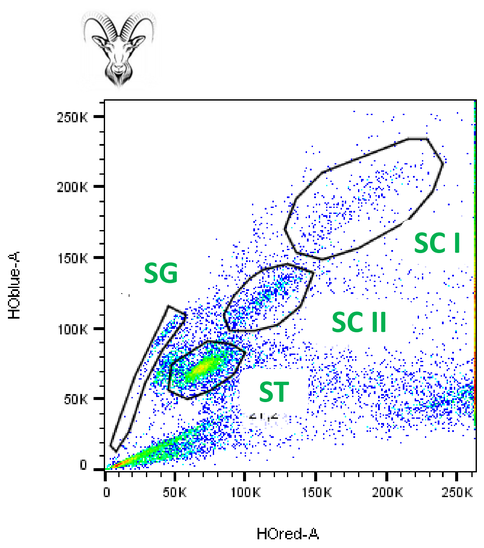
→ **What are the differentially methylated regions in testis?**

2. Male germ cells purification

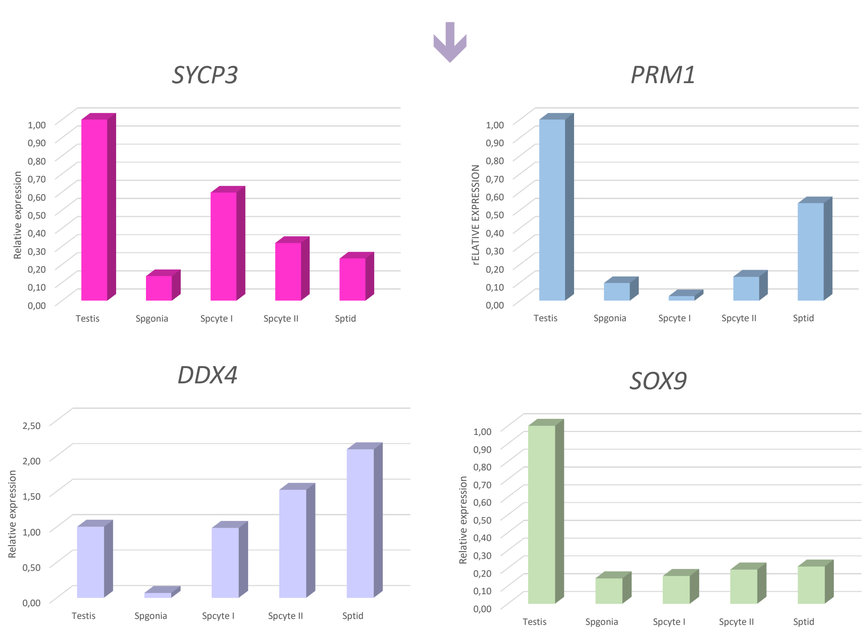
❍ **Flow cytometry isolation**
- Collection of fresh testicular tissue
- Cellular dissociation
- Cell staining (+Ho +PI)
- Fluorescence – Activated Cell Sorting



Specific markers of spermatogenic cells analyzed in RT-qPCR



SP = Spgonia; SC I= Spcyte I
SC II= Spcyte II; ST=Sptid

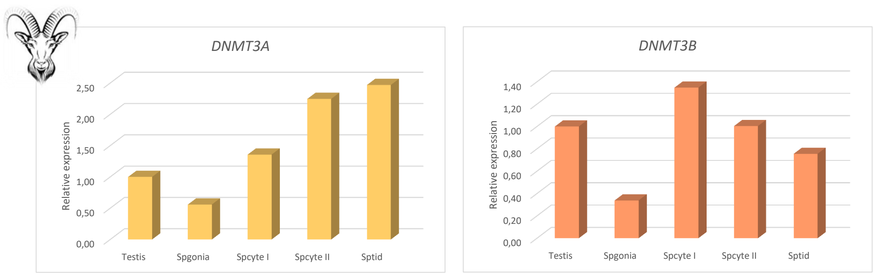


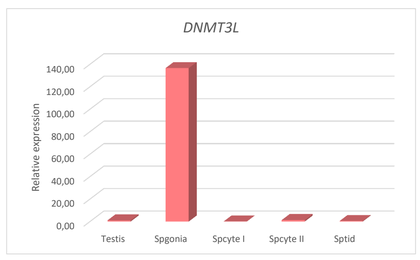
❖ *SYCP3* and *PRM1* expression seems to be higher in Spermatocyte I and Spermatid cells respectively indicating a specific enrichment of these spermatogenic cells in each fraction.

❖ *DDX4* is expressed at all stages of spermatogenesis and the quantification of *SOX9* expression shows that purified cells are mostly germ cells.

3. DNA methylation analysis in spermatogenic cells

❍ ***DNMT3s* expression quantification – *De novo* methylation**





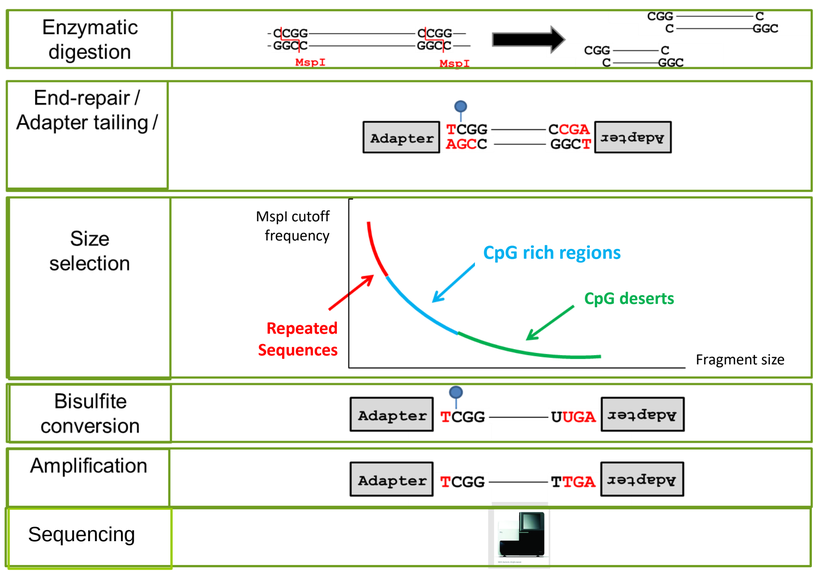
❖ *DNMT3A/B* = *de novo* DNA methyltransferase

❖ *DNMT3L* = catalytically inactive regulatory factor of *DNMT3A* and *DNMT3B.* Specifically expressed in the germline of both sexes.

❖ Expression level of *DNMT3s* fluctuates within stages of spermatogenic cells indicating methylation variations during spermatogenesis progression.

Quantification of DNMT3s by RT-qPCR on FACS- purified spermatogenic cells.

❍ **RRBS analysis – Protocol (Gu et *al.*, 2011)**



CONCLUSION

☑ Caprine testis
IF = high 5mC staining at all stages of spermatogenesis
→ Stability in DNA methylation ?
RT-qPCR = DNMT3s expression variation
→ New events of DNA methylation
→ Specific regions ?

☑ Bovine testis
IF = 5mC staining only in Spgonia and Spcyte I
→ Strong variation in DNA methylation ?
→ Studies on purified germ cell fractions are highly required,

PERSPECTIVES

❑ Spermatogenic cells purification
→ Optimisation of the flow cytometry-based method (Hoechst-FACS)
→ Caprine and bovine testis (X4)
→ Enrichment analysis

❑ DNA methylation analyses of purified spermatogenic cells
→ RRBS analysis
→ RNA sequencing

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