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Department of Environmental Systems Science

Investigating functional variation in testis tissue of pre- and postpubertal bulls

M. Osbahr*[1], X. M. Mapel[1], A. S. Leonard[1], H. Pausch[1]

[1]Animal Genomics ETH Zurich, * meret.osbahr@usys.ethz.ch

Introduction

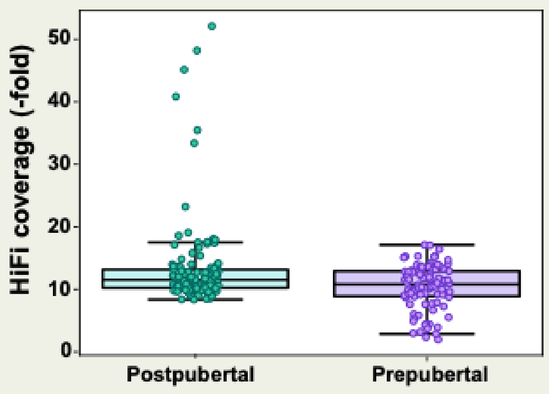
Gene expression is dynamically regulated by alternative splicing and DNA methylation. This process contributes to numerous molecular changes that occur during puberty and are essential for successful reproduction at a mature age.

Here, we examined the changes in gene expression, splicing, and methylation in a large cohort of pre- and postpubertal Braunvieh bulls.

Material and Methods

Total RNA (150bp paired-end) and HiFi-DNA (read length: 16.3 Kb) reads were collected from testis tissue of 213 bulls

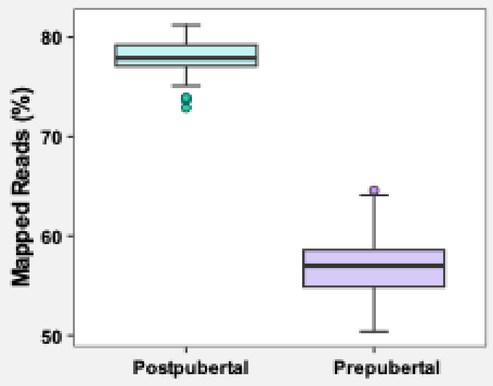
- prepubertal: 94
- postpubertal: 117



Analysis		Software used
Differential Expression	(DE)	DESeq2[1]
Differential Splicing	(DS)	LeafCutter[2], rMATS[3]
Differential Methylation	(DM)	pb-CpG-tools, MethBat

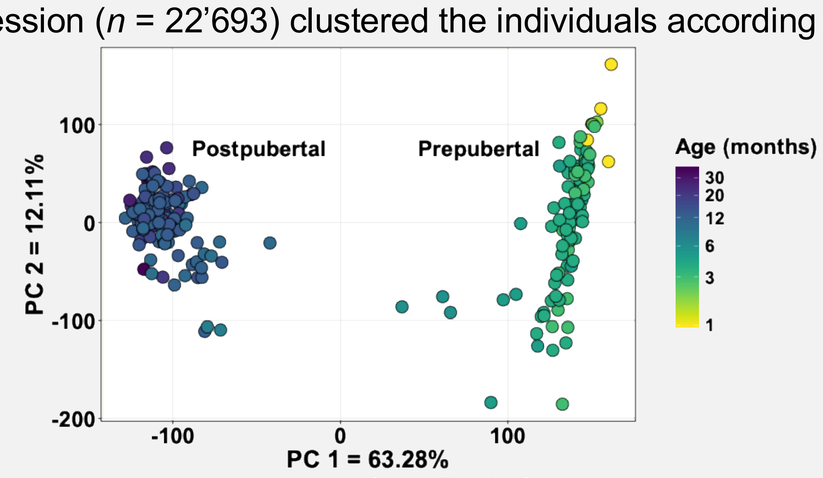
Alternative Splicing

- 17,527 genes DS
  - 86.1% mutually exclusive exons
  - 79.9% exon skipping
- Tendency to retain introns in prepubertal bulls (89.5%)
- Low mapping rate in prepubertal (57.2% vs. 78%) due to poor representation in transcript annotation

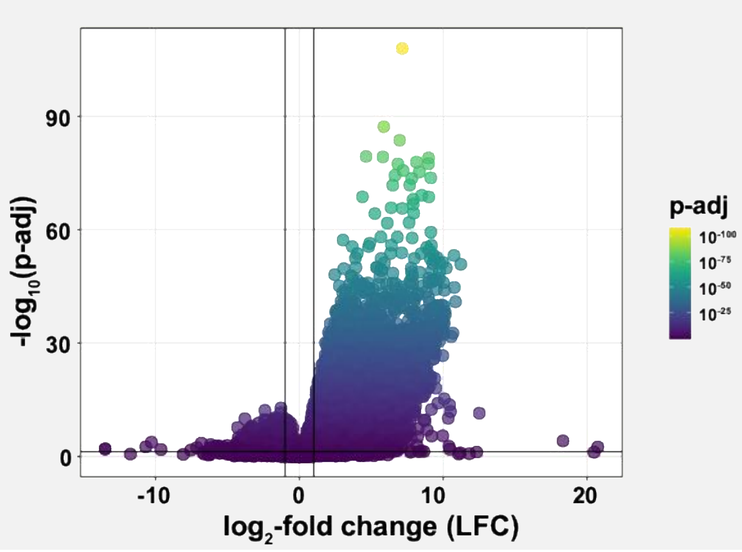


Gene Expression

Gene expression (*n* = 22’693) clustered the individuals according to age.

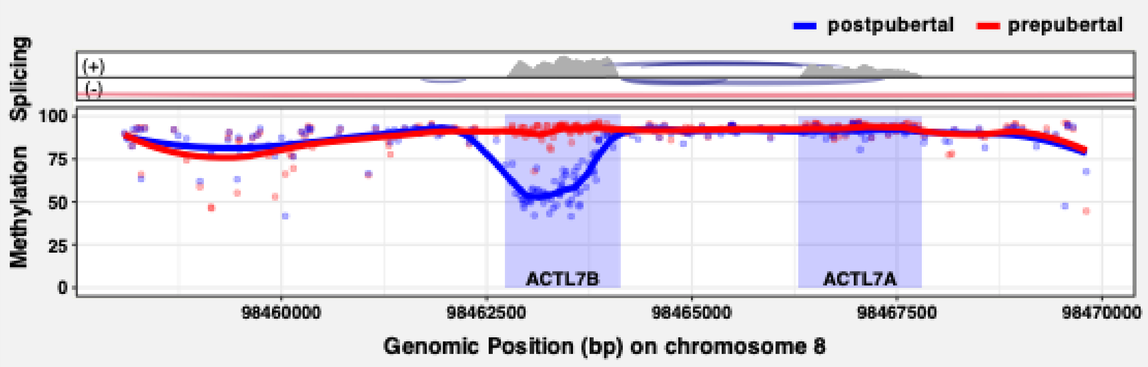


11,543 genes DE, with the majority (*n* = 7,262) being upregulated in postpubertal bulls. These included spermatogenesis-associated gene families (e.g., *PRM* and *SPATA*).



DNA Methylation

- 6,895 CpG islands DM
  - 88.6% hypermethylated in prepubertal bulls
  - 47.7% in exonic regions, including exons of DS spermatogenesis-associated genes, such as *ACTL7B*



Key Findings

- ~ 50% of genes are differentially expressed in testis before and after puberty. The majority of them are upregulated.
- The current transcript annotation does not capture prepubertal isoforms well, leading to low mapping rates.
- Differential methylation in selected genes was associated with DS and DE.

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References

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2. Li, Y., et al., 2018. Annotation-free quantification of RNA splicing using LeafCutter
3. Wang, Y., et al., 2024. rMATS-turbo: an efficient and flexible computational tool for alternative splicing analysis of large-scale RNA-seq data