

Submitted abstract:**Investigating functional variation in testis tissue of pre- and postpubertal bulls**

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Male puberty triggers a multitude of molecular changes, including altered gene expression, splicing, and DNA methylation, leading to distinct transcriptional profiles of reproductive tissues in immature and mature individuals. However, these transcriptional differences are not fully understood yet, as investigations of functional variation in reproductive tissue were often limited to a few candidate genes in selected species. To explore transcriptome-wide and epigenetic changes between pre- and postpubertal bulls, we sequenced total RNA with Illumina short reads and DNA with PacBio HiFi reads in 95 immature and 117 mature Braunvieh bulls. RNA pseudoalignment revealed substantially lower mapping rates for prepubertal individuals than for adults (57.2% vs. 78.0%), suggesting that prepubertal-specific transcripts are underrepresented in the current *Bos taurus* reference genome annotation. The first principal component of a gene expression matrix separated the individuals by age, with a distinct age-dependent structure within the prepubertal group. Differential expression analyses using DESeq2 identified 6828 differentially expressed genes (FDR < 0.05) between pre- and postpubertal animals. Most of these genes were highly expressed in adult individuals and associated with reproductive processes. We identified 17,527 differentially spliced genes, of which only 20% overlapped with differentially expressed genes. The most prevalent splicing events were mutually exclusive exons and exon skipping, affecting 86.1% and 79.7% of the differentially spliced genes, respectively. We assessed variation in 5mC methylation using MethBat and identified 6900 differentially methylated regions, with 91.2% hypermethylated in the prepubertal individuals. These regions included genes involved in reproductive processes that were upregulated in mature individuals, corroborating an inverse relationship between methylation and gene expression. This study provides a comprehensive overview of the transcriptional differences in testis tissue between immature and mature bulls and highlights the functional complexity of testicular development.

KEYWORDS

Functional Genomics

Transcriptome

Epigenomics

Cattle

Puberty