

Transcriptome comparison between the pubertal and adult testis in goat

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ABSTRACT: Reproduction is essential to goat and any other animal production. Understanding the molecular mechanism in male goat testis development and sperm formation will provide new insights in improving male reproduction. The objective of this study was to carry out a transcriptome comparison between two different development stages of testes, i.e. before and after sexual maturation, in goat using a RNA-seq approach. We generated approximately 50 million reads (75 bp/read) for each testis samples, and identified 5512 and 5216 genes/transcripts in the pubertal and adult testis, respectively. Our preliminary analysis identified a total of 796 differentially expressed genes between the two different development stages of testes, which were significantly enriched with GO terms related to testis development and male functions.

Key words: RNA-seq; VLDL; testis transcriptome, cashmere goat

Introduction

The male goats play an important role in the reproduction. The breeding and reproduction of the ram depended on the relative gene's mechanic on the testis-expressed genes. Testicular sperm undergo a series of major changes in sperm proliferation and differentiation from puberty to adult. Spermatogenesis is a complex cellular process in which male germ cells proliferate and differentiate into functionally specialized male gametes called spermatozoa. Spermatogenesis is divided into three phases: the mitotic phase (multiplication and differentiation of spermatogonia), the meiotic phase (meiotic cell division of spermatocytes) and the haploid phase (differentiation of spermatids into spermatozoa). In this process, testis-specific gene expression occurs, producing several enzymes and proteins that regulate spermatogenesis. When testis-specific gene abnormalities cause oligozoospermia, asthenozoospermia or sperm abnormalities occur.

But there were very limited researches in the testis-specific gene expression. And there is no relevant report about the different developmental stages of testicular gene expression in goat. The identification of expressed testis-specific genes is necessary to determine the molecular pathways and the signaling systems that control male gamete formation.

Materials and Methods

Testis tissues and RNA-seq. A 6-month-old and a 2-year-old goat testis were collected from Inner Mongolia AEBAS goat breeding farm (China). The animal care and sample collection protocols were approved by the Institutional Animal Care and Use Committee in the Inner Mongolia Agricultural University. The testis total RNAs were extracted and cDNAs were synthesized. Approximately 10 µg of sheared cDNA from each sample were prepared for Illumina sequencing according to the manufacturer's protocols. Libraries were prepared from a 150–200 bp size-selected fraction following adapter ligation and agarose gel separation. The libraries were sequenced in the Illumina Genome Analyzer with 1 x 75 bp reads. Data analysis and base calling were performed using the Illumina software. A Perl program was written to remove low sequences from the raw reads. After data mining, the high quality reads were de novo assembled with Velvet to construct unique consensus sequences.

Identification of differentially expressed genes.

To study the level of gene expression in different development stages of testes, we used the FPKM (reads per kilobase of transcript per million mapped reads) value, which was estimated from the clean reads without a reference genome[1,2].

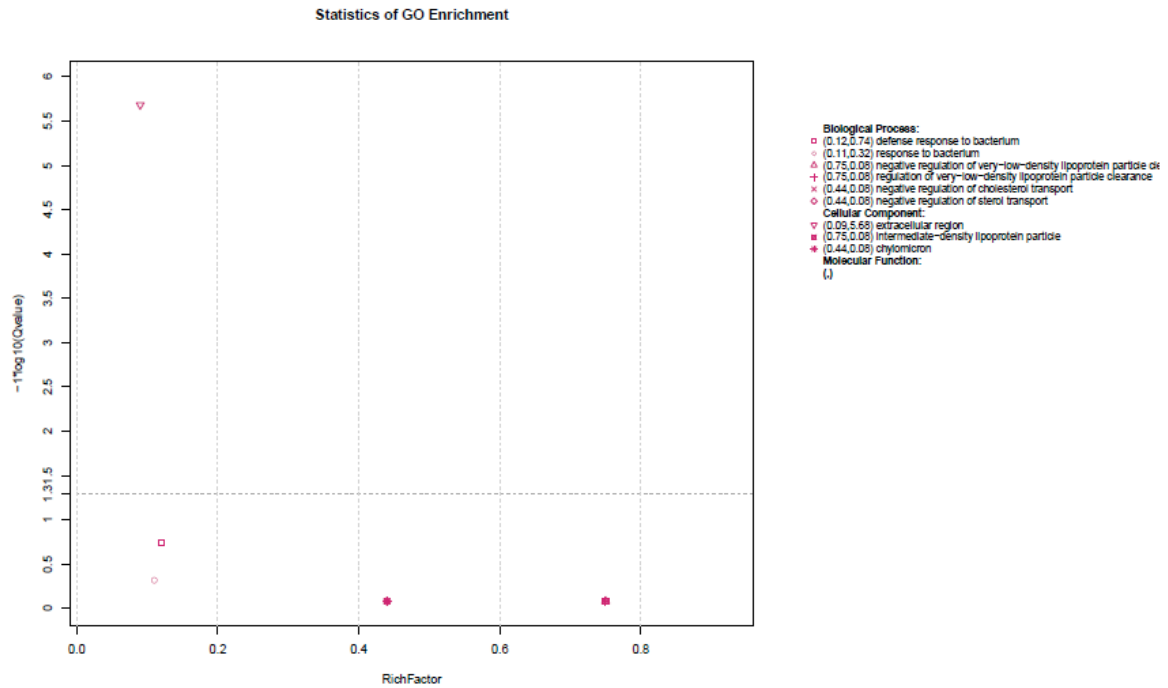


Figure 1. The volcano plot of the differentially expressed genes between the pubertal and adult testis in goat.

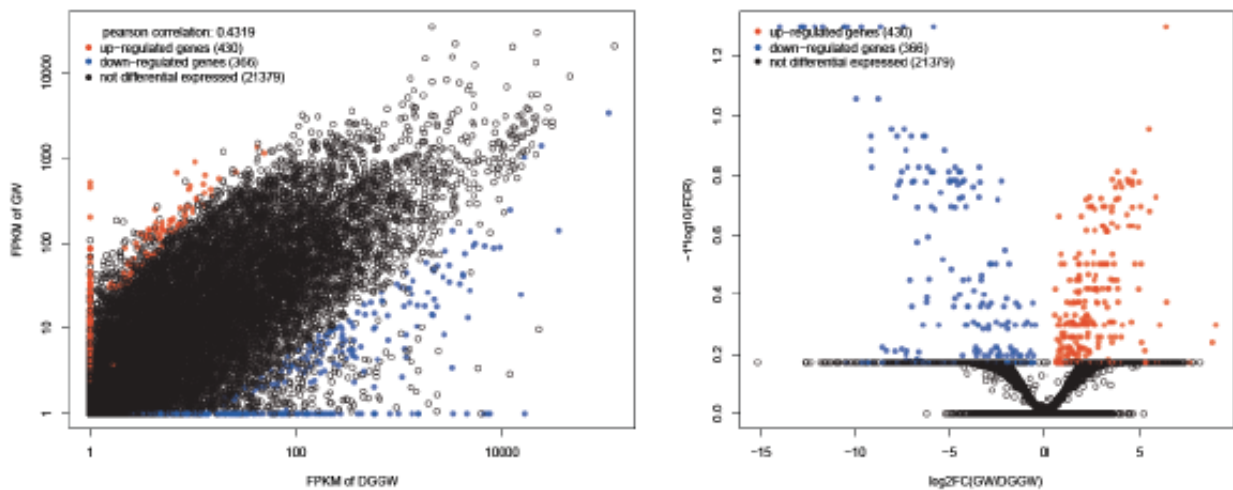


Figure 2 The GO term enrichment of the differentially expressed genes between the pubertal and adult testis in goat.

Gene ontology (GO) analysis. GO analysis was basically following the protocol described by Tang et al. [3]. The GO functional enrichment analysis was carried out using the Goatools (<https://github.com/tanghaibao/Goatools>) and Fisher's test method. In order to control and reduce the false positive rate, we used four kinds of multiple testing methods (Bonferroni, Holm, Sidak and false discovery rate) for P values correction [4,5,6,7]. It was considered as significant GO term enrichments when the corrected P value was less than 0.05

Results and Discussion

We obtained a total of 49,874,605 clean reads in pubertal goat testis and 51,307,979 in the adult goat testis. Our de novo assembly revealed that approximately 5212 and 5516 genes/transcripts were expressed in the pubertal and adult testis, respectively. Of which, 796 genes were differentially expressed genes (Figure 1). Further GO analyses of these differentially expressed genes, with the software Goatools revealed that the differentially expressed genes between the two different development

stages of testes, i.e. before and after sexual maturity, were functionally enriched in the GO terms. Although the GO results are still being analyzed, our preliminary data present here on the VLDL gene provided an example on the significance of this study. We found that the VLDL gene was significantly down-regulated in the mature testis. As shown in Figure 2, the GO were enriched with terms on negative regulation of very-low-density lipoprotein particle. The increased serum VLDL, total triglyceride, and testosterone values were significantly correlated with decreased sperm motility[8]. So, we speculated that intermediate density lipoproteins may be closely related to testicular function before and after the sexual maturity.

Conclusion

This is an initial transcriptome comparison between the two different development stages of testes before and after sexual maturation in goat. Our preliminary analysis identified a total of 796 differentially expressed genes, which were significantly enriched with GO terms related to testis development and male functions.

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