# MicroRNA and circular RNA profiling in the deposited fat tissue of sunite sheep

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**Objectives:** The fat tail is the most typical deposited fat and helps Sunite sheep (SS) adapt to harsh conditions such as cold, drought, and food shortages, making them more adaptable than other breeds. As a by-product of mutton, tail fat is widely used as a raw ma- terial for the production of various daily necessities. Also, it can be an important source of dietary fat, providing the human body with the energy it needs. However, the regulatory mechanisms of microRNA (miRNA) and circular RNA (circRNA) in tail fat de- velopment remain unclear.

**Materials and Methods:** In this study, nine castrated sunite rams tail fat at 6 (6M, n=3), 18 (18M, n=3), and 30 months of age (30M, n=3) were slected to total RNA extraction, and miRNA and circRNA expression profiles were characterized by using Illumina HiSeq 2500 and Illumina HiSeq 4000. The differential expression of miRNAs were determined by $P < 0.05$, and differentially ex- pressed (DE) circRNAs were selected based on $|\log2 \text{ (fold change)}| > 1$ and $P < 0.05$. The ceRNA relationship network with oar- miR- 27a_R-1 and oar-miR-29a as the core was validated by using dual-luciferase gene reporter analysis.

**Results and Discussion:** We compared miRNAs and circRNAs in the tail fat of SS at three different stages (30M vs 6M, 30M vs 18M, and 18M vs 6M). The largest number of DE miRNAs were obtained for the 30M vs 6M comparison, with a total of 110 DE miRNAs, including four novel DE miRNAs. The 30M vs 18M comparison yielded 88 DE miRNAs, including 3 novel DE miR- NAs. For 18M vs 6M, 95 DE miRNAs were obtained, including seven novel DE miRNAs. Furthermore, 10 overlapping DE miRNAs were identified between the three comparison groups. These were highly expressed in 6M SS, and their expression decreased with age. A total of 93, 89, and 66 DE circRNAs were obtained for 30M vs 6M, 30M vs 18M, and 18M vs 6M, respectively. None of these were found to overlap among the three comparison groups. KEGG pathway analysis of the miRNA target genes was per- formed, and the top 15 KEGG pathways are shown in a scatter plot. A total of 19 KEGG pathways were significantly enriched. Among these, we noticed that the Rap1 signaling pathway, adherens junction, tight junction, cell adhesion molecules, and regula- tion of actin cytoskeleton were enriched in the different growth stages. The significant enrichment of these pathways suggests that DE miRNAs may play prominent roles in sheep tail adipogenesis through cell-to-cell interactions. In addition, no studies have suggested a role of the Rap1 signaling pathway in adipose tissue. The present study may provide a theoretical basis for future research in this direction. Furthermore, butanoate metabolism was enriched in both the 30M vs 6M and 30M vs 18M comparisons. This in- dicates that the miRNA-mediated regulation of butyric acid may be a potential regulator of SS tail adipose tissue growth. The KEGG pathway analysis of the circRNA host genes was performed. There were 22 significantly enriched KEGG pathways, among which several fat-related pathways were identified. These processes included propanoate metabolism, fatty acid metabolism, fatty acid biosynthesis, unsaturated fatty acid biosynthesis, and fatty acid elongation. Fatty acid metabolism was the only pathway enriched in all three groups. Host genes ACACA (circRNA382, circRNA392, and circRNA394) and HADHA (circRNA10888) were enriched in these pathways, indicative of the involvement of these circRNAs in fatty acid metabolism within tail fat. We constructed co-expression networks based on the mRNAs, miRNAs, and circRNAs identified in sheep fat tails. A total of 101 (down-up- down) and 46 (up-down-up) mRNA-miRNA-circRNA co-expression patterns were obtained. This indicates that the down-up-down co-expression predominated the network, suggesting that upregulated miRNAs in the ceRNA network play a central regulatory role in tail fat. Acording to the coexpression networks, we sought to validate the ceRNA regulatory relationship between oar-miR-27a_ R-1 and oar-miR-29a as the core, that is, between ACSL-oar-miR-27a_R-1-circRNA1985 and GPAM-oar-miR-29acircRNA3539. Dual-luciferase gene reporte results suggest that oar-miR-27a_R-1 can decrease ACSL4 expression by targeting the ACSL4-3'- UTR, and circRNA1985 can competitively bind with oar-miR-27a_R-1 and thus regulate the expression of ACSL4. The same con- clusion was drawn for GPAM-oar-miR-29acircRNA3539. Overall, we verified two ceRNA expression networks and suggested multiple functions related to fat metabolism in sheep tail fat development. However, the involvement of these ceRNAs in sheep tail-fat metabolism requires further investigation. The current results provide a theoretical basis for the identification of molecular markers related to sheep tail fat metabolism and growth. Our findings highlight potential ceRNAs involved in sheep tail fat devel- opment and provide a theoretical basis for byproduct utilization.

**Key words:** Sunite sheep, Tail fat, Micro RNA, Circular RNA, Competing endogenous RNA