

77° CONVEGNO SISVET**Stato: INVIATO - ID: 13119****The circulating microRNAs enter the reproductive landscape of a threatened sheep breed: a sequencing data analysis**I. Manenti¹, U. Ala¹, I. Viola¹, S. Miretti¹, E. Macchi¹, M. Baratta², E. Martignani¹¹Dept. of Veterinary Sciences, University of Turin, Turin – Italy²Dept. of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma – Italy

The preservation of locally endangered breeds during the current climate change is essential to the conservation of ecosystems, livestock biodiversity, and typical local products [1]. From this perspective, reproductive fitness becomes a crucial consideration. MicroRNAs (miRNAs) are small noncoding RNA molecules with a key role in the post-transcriptional regulation of a wide range of cellular processes, including development, immune responses and homeostasis [2]. When they are released into extracellular fluids from tissues, where they are synthesized, they are termed circulating miRNAs (c-miRNAs). C-miRNAs might be potential biomarkers, whose profile changes under conditions such as viral and bacterial infections, physiological states (eg. pregnancy) and environmental stressful stimuli [3].

There are several studies on tissue miRNAs' expression variations in ruminant oestrous cycles in the literature, but not many on c-miRNAs. The purpose of this work is to establish a connection between distinctive variations in c-miRNAs' expression and specific oestrous cycle moments in Frabosana-Roaschina sheep, an endangered Piedmontese sheep breed.

For this study, twenty Frabosana-Roaschina pluriparous ewes were synchronized with a standardised protocol, blood was sampled and ultrasound scans were performed during the induced oestrus cycle. Based on plasma hormonal trends (17 β -oestradiol and progesterone) and ultrasound images, two moments, the beginning of the follicular phase and the beginning of luteal phase, were identified for analyses. C-miRNAs of 6 selected animals were extracted from blood and sent to be sequenced (Illumina platform, single end 150-bp mode, smallRNA-seq analysis).

All sequenced samples shared seven miRNAs among the most expressed per sample: oar-miR-16b, -191, oar-let-7a, -7b, -7f, -7g and -7i; seven out of twelve samples shared also oar-miR-26b, -29a and -103 among the most expressed per sample. Further analyses on the sequencing data have highlighted twelve c-miRNAs differentially expressed: five miRNAs resulted upregulated, whereas seven miRNAs are downregulated. Some of these miRNAs are reported in literature to be expressed in ovarian cells and related to their endocrine regulation, development, growth and differentiation. Through TargetScan, a list of predicted target genes of these miRNAs was created. Since there isn't an archive for *Ovis aries*, orthologous miRNAs from *Bos taurus* and *Homo sapiens* have been used to perform an enrichment analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) databases. The objective of this analysis was to identify putative pathways that might be regulated by the identified c-miRNAs. Numerous different pathways were revealed by this investigation. Several of these can be linked to the regulation of the reproductive sphere: pathways of hormones' signalling (prolactin, oestrogen, oxytocin) from KEGG analysis, response pathways (response to hormone, response to endogenous stimulus) and behavioural pathways as biological processes from GO, pathways related to the circadian rhythm from both KEGG and GO databases. The investigation of these miRNAs' involvement in the regulation of the oestrous may prove to be useful for deepening the knowledge of physiology and reproductive efficiency in the Frabosana-Roaschina and other breeds.

[1] Leroy et al. Multifunctionality and provision of ecosystem services by livestock species and breeds at global level, *Animal*, 18:101048, 2024.

[2] O'brien et al. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation, *Front. Endocrinol.*, 9:402, 2018.

[3] Miretti et al. MicroRNAs as Biomarkers for Animal Health and Welfare in Livestock, *Front. Vet. Sci.*, 7: 578193, 2020.