

# MiRNA profile in milk extracellular vesicles is modulated by caprine $\alpha$ S1-casein genotype

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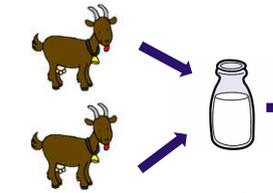
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**Introduction:** In addition to the major and well-known milk components, nutritional quality of milk is also influenced by recently detected components, such as miRNAs that regulate many biological processes. They were found in different fractions of milk including extracellular vesicles (EVs). Evidence of the presence of food-sourced miRNAs in human plasma suggests that they could influence human health [Zhou et al., 2012; Leroux et al., 2017; Kahn et al., 2018].

In goats, the gene encoding  $\alpha$ S1-casein (*CSN1S1*) exhibits a “naturally occurring KO” (*CSN1S1* O/O) with serious consequences to the mammary epithelial cell (MEC) secretory functions and therefore on milk composition [Grosclaude et al., 1994; Chanat et al., 1999]. We hypothesize that the absence of *CSN1S1* expression, which is known to disturb MEC functions, may influence milk vesicular cargos, including miRNA.

**Objectives:** 1) To characterize the miRNome from milk-derived EVs; 2) To compare the miRNomes of strong (AA) vs natural-KO (OO) *CSN1S1* genotypes and hypothesize on the putative effects on milk consumers.

Goats (n=5) homozygous A/A at the *CSN1S1* locus (strong allele)



Goats (n=5) homozygous O/O at the *CSN1S1* locus (null allele)

## Materials and Methods:

Milk EVs preparation then total RNAs extraction [Krupova et al., 2016]

RNA sequencing  
Illumina NextSeq500 (Exiqon)

Data processing

- Normalization
- Filtering
- Mapping (*Bos Taurus*, *Capra hircus*)
- Statistical analysis (DESeq2)

Bio-informatic analyses  
Using miRWalk & Metacore™ softwares

## Goat milk EV miRNomes:

- 247 miRNA identified from goat EVs.
- Comparison of the TOP 20 of the most abundant miRNAs with its human [Liao et al., 2017] counterparts revealed 10 miRNAs in common.

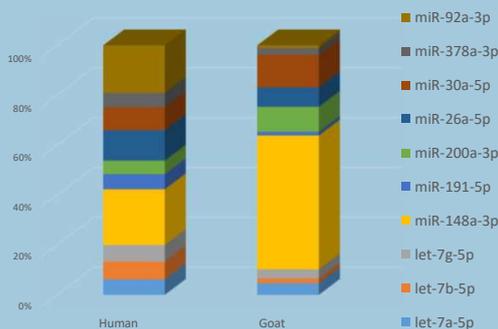
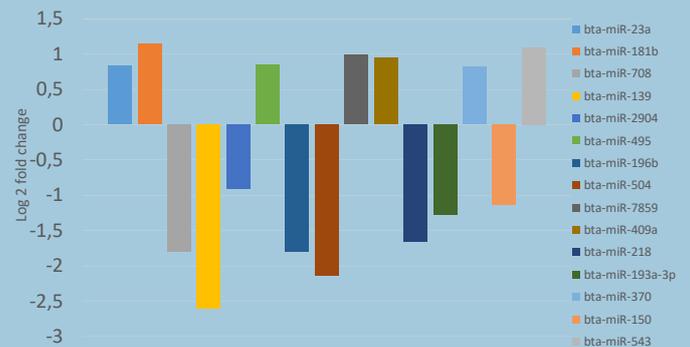


Figure 1 : Comparison between human [Liao et al., 2017] and goat most TOP 20 abundant miRNAs

## Results:

### Comparison between *CSN1S1* AA vs OO EV miRNomes

- revealed 15 differentially abundant miRNAs ( $P_{adj} \leq 0.05$ )



- Consideration of 5 of more abundant among the 15 miRNAs revealed their potential influence on process networks involved in development.

### Are differentially abundant miRNAs in milk EVs can be related to mRNA profile?

Comparison between potential mRNA targeted by the 15 DA miRNAs and mRNA differentially expressed ( $P_{adj} \leq 0.1$ ) identified from mammary epithelial cells isolated by laser microdissection from the same goats [Bevilacqua et al., in prep] identified:

- ✓ 1<sup>st</sup> pathway altered in common: **Cytoskeleton remodeling.**
- ✓ 14<sup>th</sup> rank: Apoptosis and **endoplasmic reticulum (ER) stress response (UPR)** pathway  
→ could be related to the ER dilatation observed in MEC of goats homozygous O/O at the *CSN1S1* locus.

**Conclusions :** Differentially abundant miRNAs were identified in goat milk EVs, according to the genotype at the *CSN1S1* locus. These differences were related to the UPR phenotype confirmed by transcriptome analyses. The potential effects of these differentially abundant miRNAs on offspring or infants consuming goat milk should be considered.