

# Exosome-mediated microRNAs expression profile in follicular fluid of metabolically divergent postpartum cows

T. Hailay<sup>1</sup>, M. Hoelker<sup>1,2</sup>, S. Gebremedhn<sup>1</sup>, F. Rings<sup>1,2</sup>, M. M. Saeed-Zidane<sup>1</sup>, M. Poirier<sup>1</sup>, D. Salilew-Wondim<sup>1</sup>, C. Dauben<sup>1</sup>, E. Tholen<sup>1</sup>, C. Neuhoff<sup>1</sup>, K. Schellander<sup>1</sup>, D. Tesfaye<sup>1</sup>



<sup>1</sup> Institute of Animal Science, University of Bonn, Germany  
<sup>2</sup> Research Station Frankenforst, University of Bonn, Germany  
 Contact: dtes@itz.uni-bonn.de

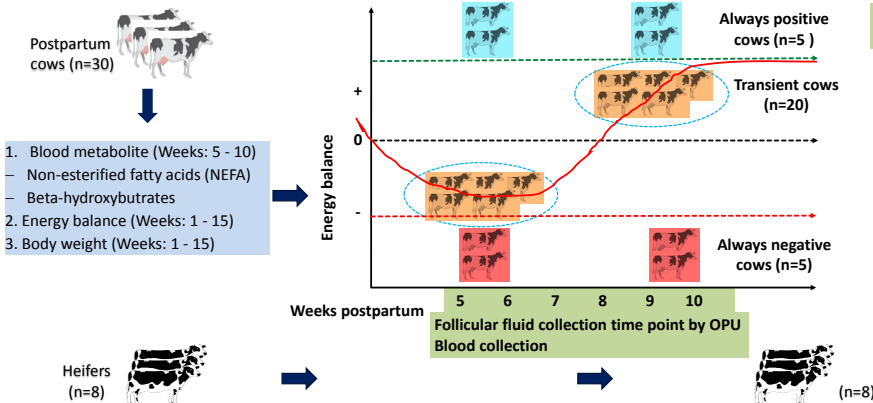


## Introduction

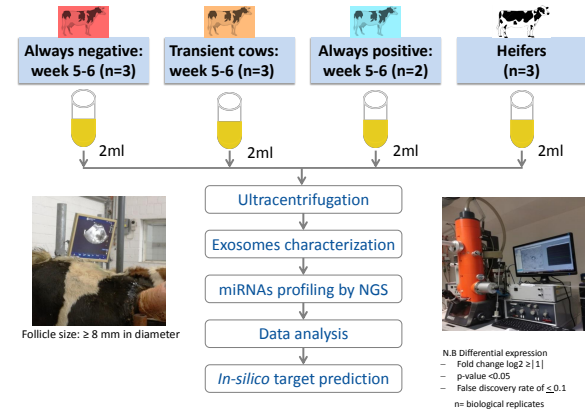
Most of the high-yielding dairy cows enter a state of negative energy balance during the early lactation period. This phenomenon disturbs the metabolic status of blood and the follicular fluid microenvironment resulting in disturbed fertility. Recent study showed that, calving rates in lactating cows declined to ~ 35 - 40% compared to heifers as high as ~ 55 - 60%. Exosomes (30-150 nm in diameter), evolutionarily conserved cargo molecules carrying RNA and proteins, are known to be involved in cell-to-cell communication. The aim of this study is to investigate the association between postpartum metabolic status and the expression of exosomal miRNAs in follicular fluid of Holstein-Friesian cows and heifers

## Materials and Methods

Experiment 1: Screening the energy balance status of experimental cows and heifers



Exp.2: Expression profiling of exosomal miRNAs in follicular fluid



## Results

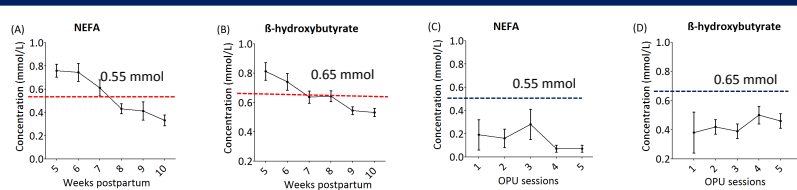


Figure 1: Blood serum metabolite analysis of a population of postpartum cows (A and B) and heifers (C and D)

A total of 356 known and 156 novel miRNAs were detected across all samples, of which 255 miRNAs were commonly detected. Several miRNAs were uniquely detected as the metabolic status is changed (figure 3).

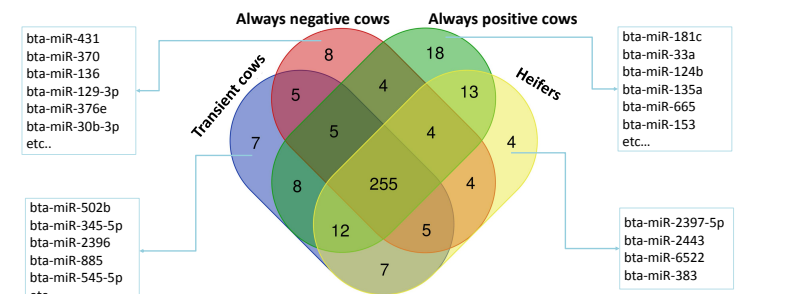


Figure 3: Detection of exosomal miRNAs in follicular fluid of cows and heifers of different metabolic status

Downregulation of 14 and upregulation of 3 miRNAs was observed in always negative cows compared to heifers and are predicted to target different metabolism related pathways (figure-5).

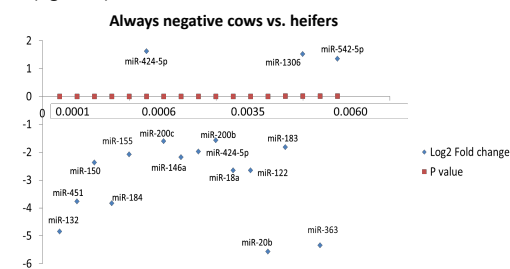


Figure 5: Differentially expressed miRNAs in always negative cows compared to heifers

- Targeted pathways**
- Insulin signaling pathway
  - Estrogen signaling pathway
  - MAPK signaling pathway
  - Vitamin B6 metabolism
  - Fatty acid biosynthesis
  - Fatty acid metabolism
  - Fatty acid elongation
  - Progesterone-mediated oocyte maturation

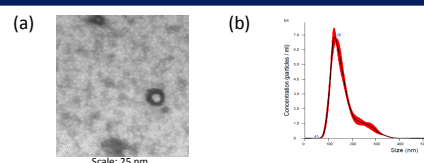


Figure 2: Electron microscopy (a) and nanoparticle tracking of exosomes (b)

Analysis of miRNAs expression showed that, massive downregulations in always negative cows compared to heifers and the opposite was observed in always positive cows (figure 4).

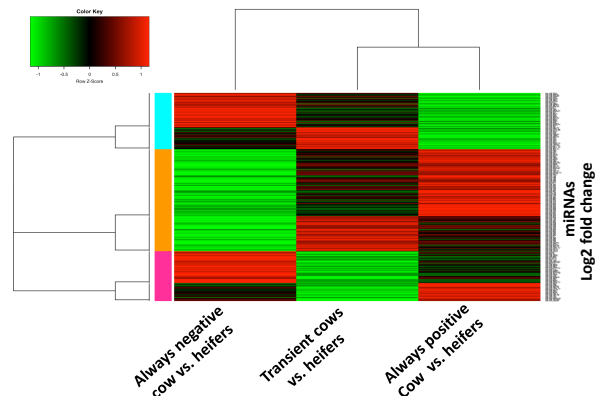


Figure 4: Heat map of differentially expressed exosome-mediated miRNAs in follicular fluid of cows of different metabolic status

Table 1: Always negative vs. Always positive cows

MicroRNAs	Log2 Fold change	P value
bta-miR-2285	-6.08	0.0011
bta-miR-451	-4.89	0.0001
bta-miR-132	-4.87	0.0005
bta-miR-34c	-3.17	0.0065
bta-miR-486	-3.02	0.0046
bta-miR-874	-2.37	0.0033

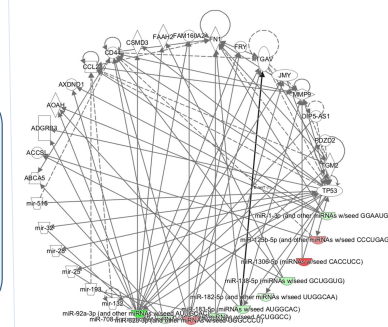


Figure 6: miRNA-gene network of differentially expressed miRNAs in always negative cows vs heifers

- Targeted pathways**
- Fatty acid biosynthesis
  - Hippo signaling pathway
  - TGF-beta signaling pathway
  - Cell cycle
  - Apoptosis

## Conclusion

Negative energy balance in postpartum dairy cows was associated with suppressed exosomal miRNAs expression in follicular fluid. This divergence of exosome-mediated miRNA expression in the follicular fluid of metabolically stressed cows could be associated with the reduced fertility of high-yielding dairy cows.