

# Blood serum extracellular vesicle coupled microRNAs expression analysis in metabolically divergent lactating cows

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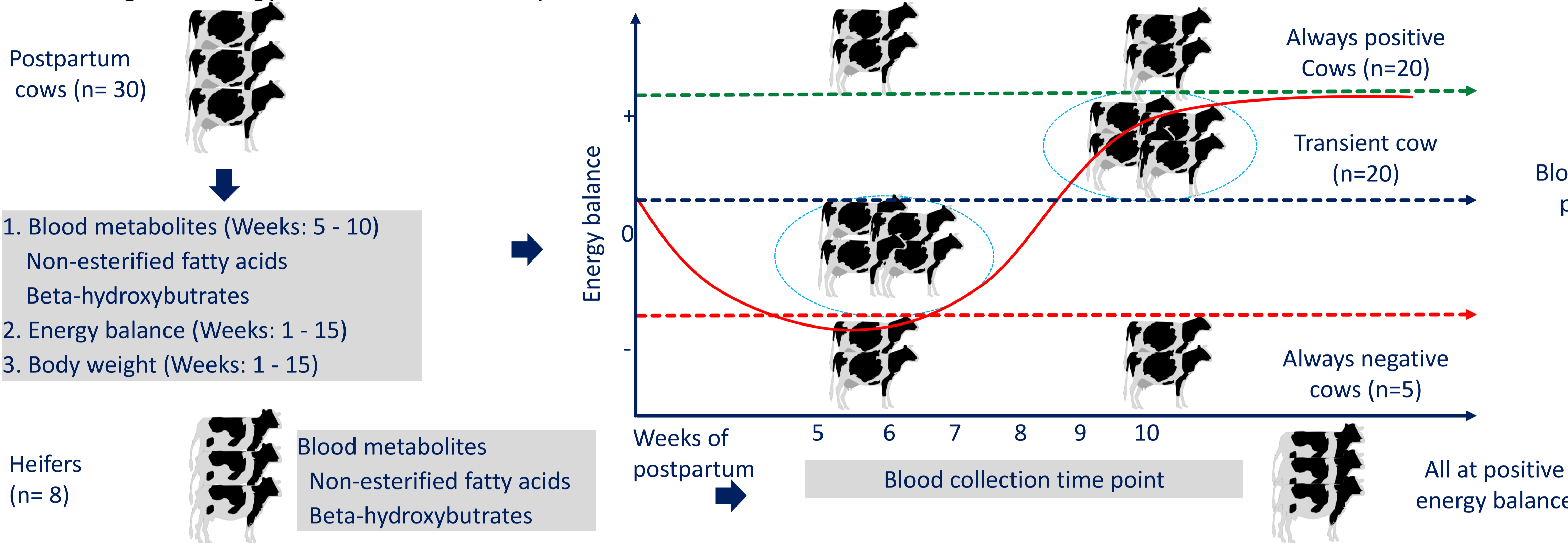


## Introduction

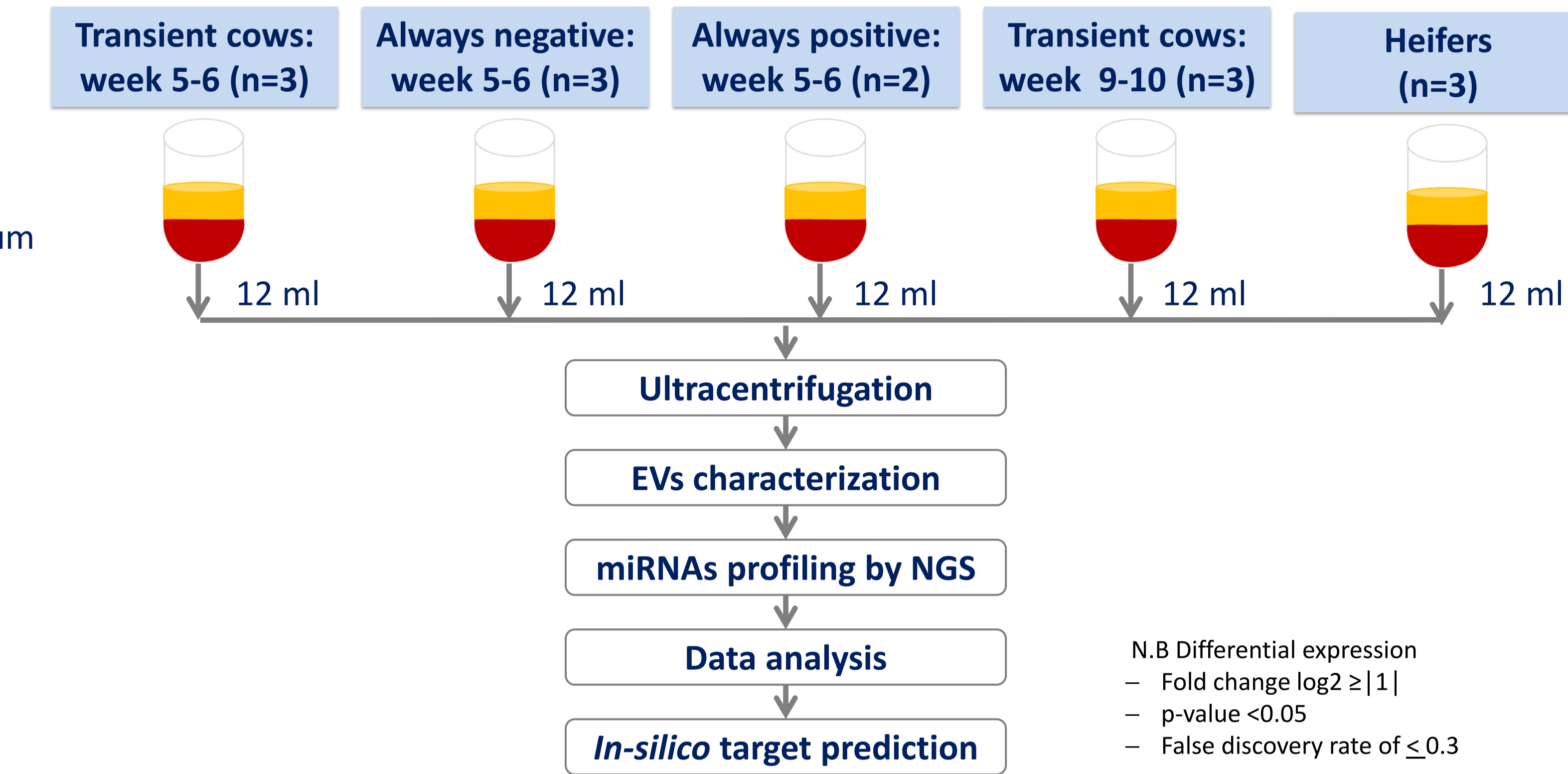
Negative energy balance during early lactation is the main cause of fertility reduction in dairy cows. Noninvasive marker development is vital for the future ARTs. Extracellular vesicles (EVs) are evolutionarily conserved communicasomes and transport various molecules including microRNAs. This study aimed to investigate the effect of postpartum metabolic stress on the dynamics of extracellular vesicle-coupled miRNAs content in blood serum as a potential noninvasive indicator of metabolic stress in Holstein-Friesian cows.

## Materials and Methods

Screening the energy balance status of experimental cows and heifers



Expression profiling of EV-coupled miRNAs in blood serum of postpartum cows



N.B Differential expression  
 - Fold change  $\log_2 \geq |1|$   
 - p-value  $< 0.05$   
 - False discovery rate of  $\leq 0.3$

## Results

Table 1: Blood serum metabolite analysis of a population of postpartum cows and heifers

Metabolic status	Week 5 (mmol/L)		Week 6 (mmol/L)		Week 7 (mmol/L)		Week 8 (mmol/L)		Week 9 (mmol/L)		Week 10 (mmol/L)	
	NEFA	BHO	NEFA	BHO	NEFA	BHO	NEFA	BHO	NEFA	BHO	NEFA	BHO
Transient	0.82±0.32	0.85±0.36	0.59±0.44	0.70±0.29	0.65±0.33	0.63±0.19	0.39±0.19	0.73±0.24	0.34±0.12	0.54±0.08	0.41±0.22	0.52±0.10
Always negative	0.69±0.08	0.78±0.17	0.76±0.28	0.90±0.28	0.87±0.13	0.71±0.28	0.59±0.24	0.88±0.54	0.96±0.15	0.61±0.06	1.05±0.06	0.98±0.02
Always positive	0.40±0.10	0.53±0.15	0.52±0.29	0.48±0.1	0.35±0.11	0.54±0.13	0.36±0.17	0.51±0.10	0.5±0.32	0.43±0.11	0.42±0.19	0.42±0.17
Heifers (5weeks)	0.19±0.13	0.38±0.14	0.16±0.08	0.42±0.05	0.28±0.13	0.39±0.05	0.07±0.03	0.5±0.06	0.07±0.03	0.46±0.05		

- A total of 179 known and 118 novel miRNAs were detected across all samples, of which 145 miRNAs were commonly detected. Several miRNAs were uniquely detected as the metabolic status is changed (figure 2).

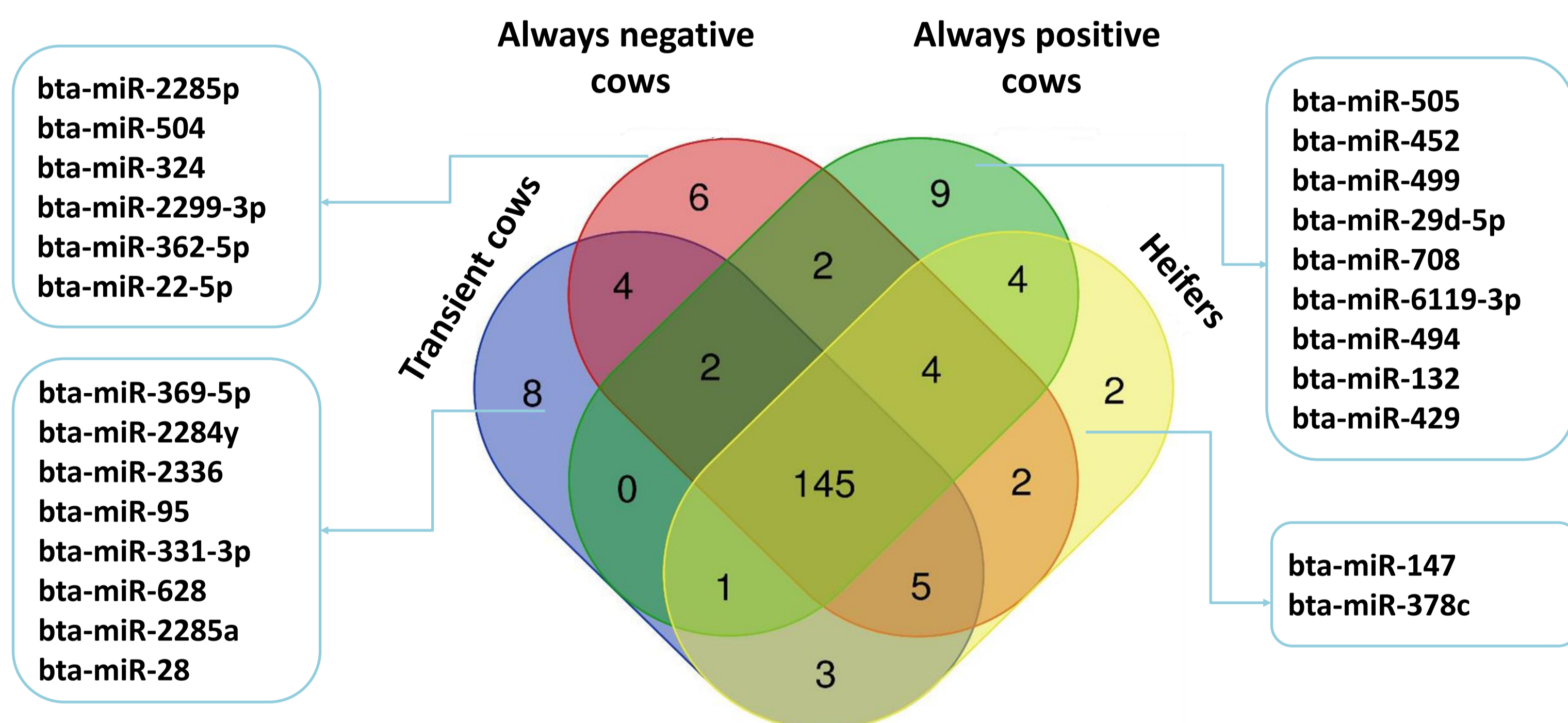


Figure 2: Detection of EVs coupled miRNAs in blood serum of different metabolic status cows

- Hierarchical clustering of EVs-coupled microRNAs' expression analysis showed that, downregulation of EVs coupled miRNAs in the blood serum of most biological replicates of always negative and transient cows (figure 3).

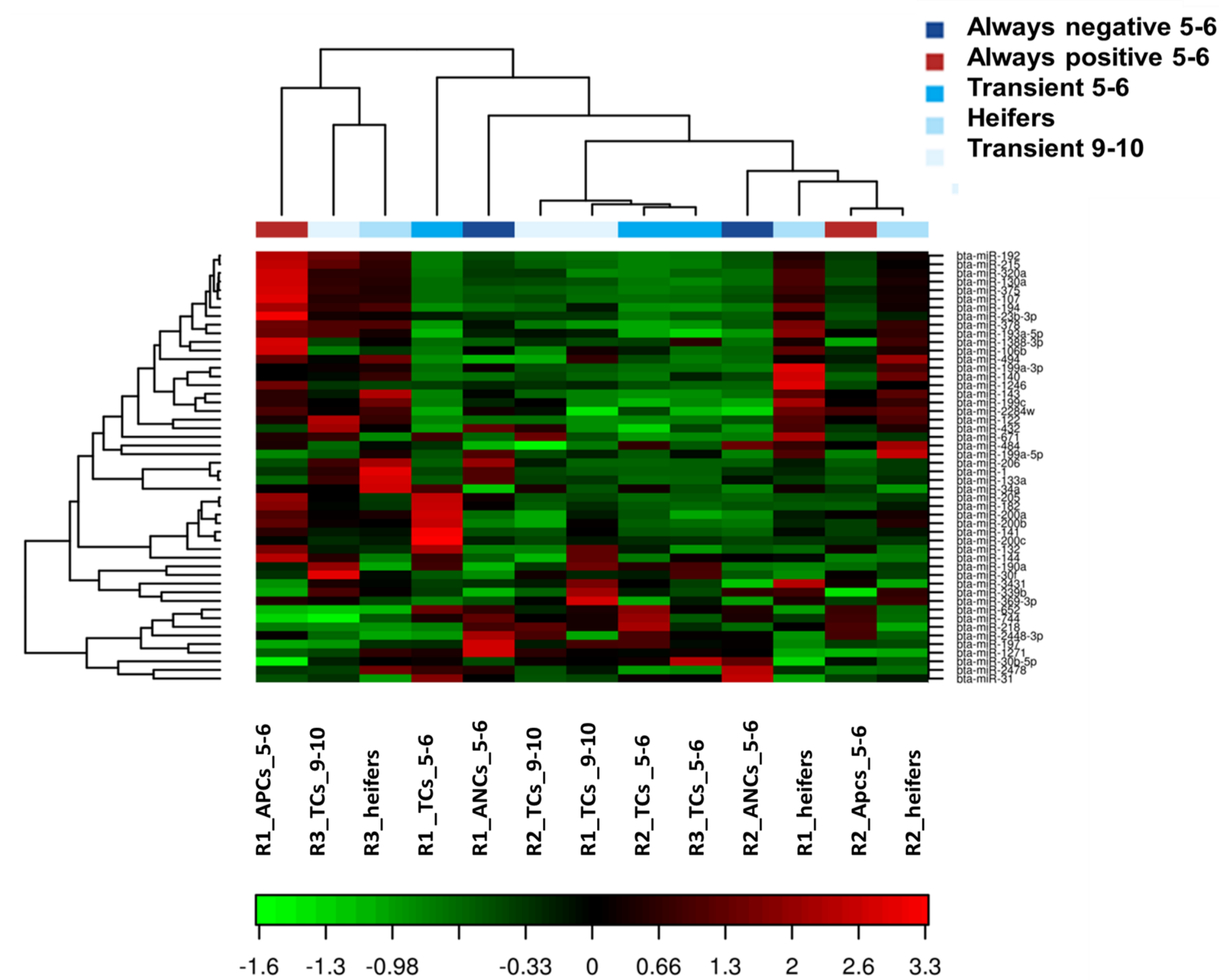


Figure 3: Hierarchical clustering of EVs-coupled microRNAs in the blood serum of different metabolic status cows and heifers

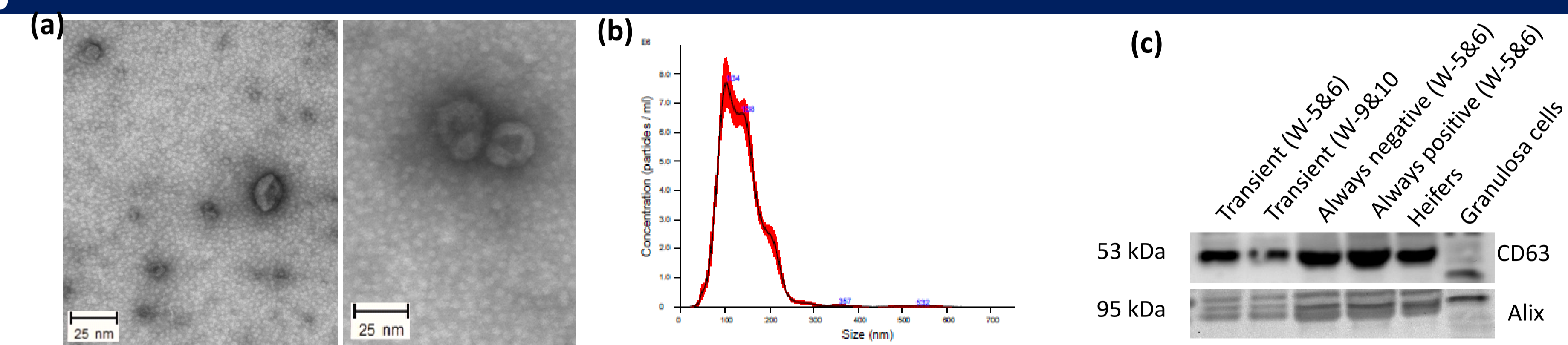


Figure 1: Morphological and molecular characterization of EVs recovered from blood serum different metabolic status cows and heifers; Electron microscopy (a), nanoparticle tracking (b) and protein marker detection (c)

- Downregulation of 17 miRNAs and upregulation of 3 miRNA differentially expressed was observed in always negative cows (ANCs) compared to heifers and Transient cows (TCs) compared to Heifers (figure 4). Their target prediction showed to involve in different pathways (figure 5).

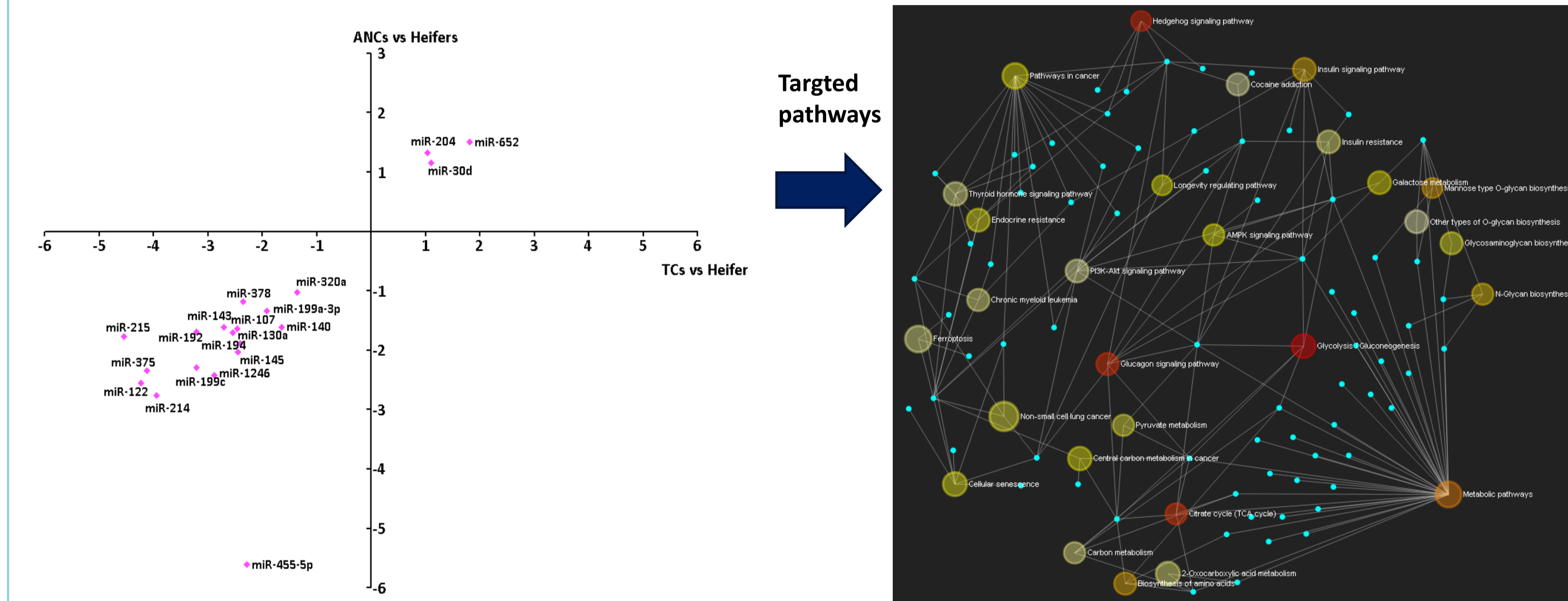


Figure 4: Differentially expressed miRNAs common to ANCs vs heifers and TCs vs heifers

Figure 5: Target prediction of commonly differentially expressed miRNAs in ANCs vs heifers and TCs vs heifers

- Analysis of EV-coupled miRNAs expression as an indicator of recovery by comparing the ANCs vs TCs showed that, all 9 differentially expressed miRNAs were upregulated in cows which remained at negative energy balance for longer time compared to the one which recover early, the TCs. This was supported by comparing the TCs at week 5-6 vs week 9-10 (figure 6).

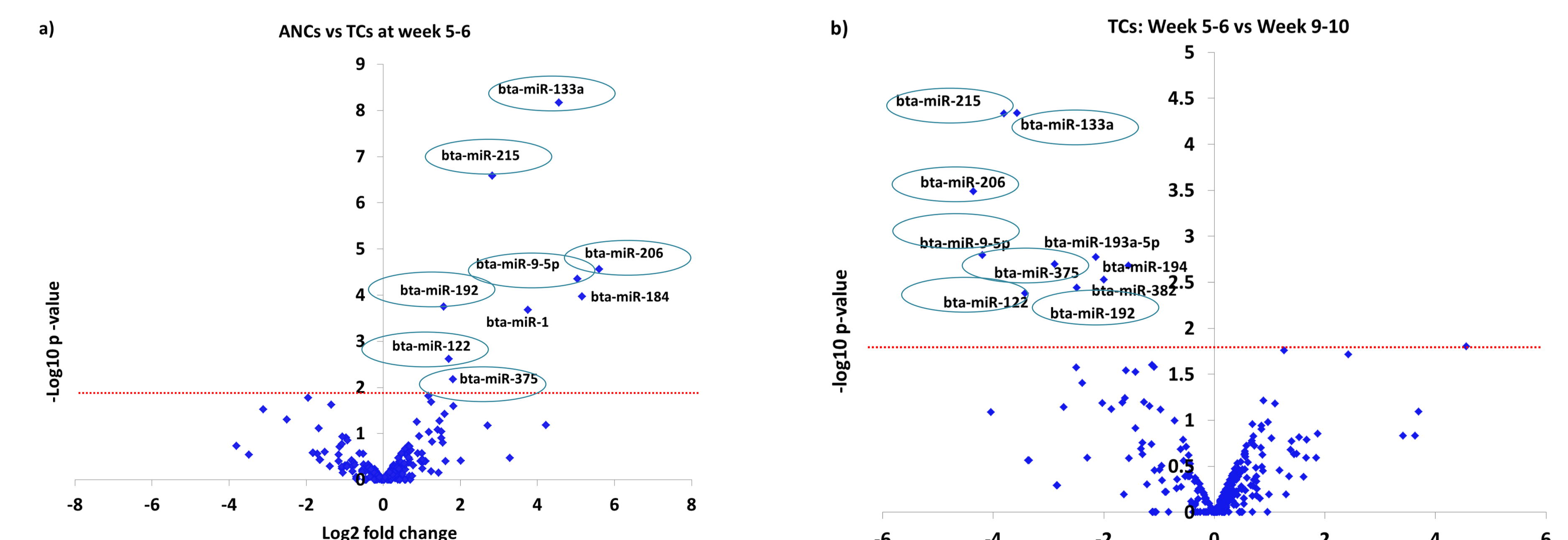


Figure 6: Differentially expressed miRNAs analysis of ANCs vs TCs (a) and TCs at week 5-6 vs week 9-10 (b)

## Conclusion

EVs-coupled miRNA hierarchical and differential expression is most likely to be associated with suppression of miRNA in the blood serum of metabolically stressed cows.