Milk fat globules as a source of mammary microRNA. D. Lago-Novais1,2, K. Pawlowski3, J. A. A. Pires4, L. Mobuchon1,3, S. Bes1, P. Martin1, and C. Leroux1, 1UMR1213 Herbivores, INRA, VetAgroSup, Saint-Genes-Champanelle, France, 2Universidade Federal da Bahia, CEP, Salvador-BA, Brazil, 3UMR1313 Gabi, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France.

Tissue for research on mammary gland (MG) gene expression is obtained via invasive and expensive methods (biopsy or post-mortem) that limit high throughput analyses. Milk fat globules (MFG) have been used to assess the mRNA content of the mammary epithelial cells in the bovine and goat (Brennau et al., 2012; Canovas et al., 2014) for gene expression studies. MFG is therefore a satisfactory alternative source of mammary mRNA. microRNAs (miRNA) are small stable noncoding RNAs involved in multiple aspects of mammary gland physiology. Whereas the use of MFG was reported in humans (Munch et al., 2013), until now MFG as the source of miRNA has not been studied in the bovine. The objective of this study was to assess MFG as a source of miRNA, and whether the latter are representative of MG miRNA expression, by comparing targeted miRNA in MFG and MG sampled from mid-lactation Holstein cows. Total RNAs were extracted from MFG (n = 6) and MG (n = 6) using TRIzol (ThermoFisher, Inc, USA). Nine miRNA (miR-29a, miR-125, miR-126, miR-141, miR-148a, miR-204, miR-223, miR-320, and miR-494) were studied by RT-qPCR. The results are expressed as fold change of MFG data relative to MG data using the 2−ΔΔCt method and U6 as internal reference. Statistical analyses were performed using a t test (DataAssist™ software) and P < 0.05 considered as significant. Among the nine miRNA chosen on the basis of the expression in MG, two were not detected in MFG whereas they were highly abundant in MG (miR-126 and miR-204), and three were significantly more abundant in MG than in MFG (miR-29a, miR-125b, and miR-148a, presenting a fold change value of 23.2, 13.9, and 8.7, respectively). Four miRNA were detected at the same level in both MFG and MG. Our results suggest that there are different mechanisms of miRNA transfer to milk. Nevertheless, it is possible that miRNA not present in MFG are not expressed in epithelial cells, but are present in other MG cell-types, and therefore not transferred to milk. In conclusion, MFG can be used as a non-invasive source of microRNA but do not reflect exactly the MG miRNAome. Further research is warranted on the composition of MFG miRNAome and modulation of their secretion in milk.

Key Words: bovine, microRNA, milk fat globule