Circulating microRNAs and associated gene regulation in puerperal metritis in dairy cows
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Dairy cows that calved recently are prone to uterine and metabolic disorders due to suppressed immune function and negative energy balance. Metritis is one of the most common uterine diseases of dairy cows diagnosed during puerperal period (first 10 days in milk). It is defined as presence of fetid reddish-brown watery vaginal discharge, systemic signs of illness with fever (rectal temperature of 103°F or greater). Cows that suffer from metritis associated with poor reproductive performance, including irregular estrous cycles, lower conception rates and greater intervals from calving to pregnancy. Although uterine infection occurs most commonly after calving complicated by dystocia, retained fetal membranes, twins or stillbirth, cows with poor immune function are most likely to develop metritis. MicroRNAs (miRNAs) are small non-coding molecules that are partially complementary to one or more messenger RNA (mRNA). Their main function is to down-regulate gene expression in a variety of manners, including translational repression, mRNA cleavage and deadenylation. The objective was to compare circulating miRNAs and their integrated genes in cows suffering from metritis and normal cows. Dairy cows diagnosed with (n=4) or without (n=4) metritis from a single farm were included in this study. Blood samples were collected via coccygeal venipuncture at the time of diagnosis. In individual serum samples, we investigated 84 prioritized cow-specific miRNAs using RT-PCR method. Total RNA, including miRNAs, was isolated from frozen-thawed serum, complementary DNA was synthesized and mature miRNA expression profiling was performed using real time PCR. MiRNA-specific forward primer and universal reverse primer were used to amplify mature miRNAs. Caenorhabditis elegans miRNA, cel-miR-39-3p was used as endogenous control to normalize target miRNA expression. Data were analyzed using the $\Delta\Delta$CT method of relative quantification using the computational software at http://pcrdataanalysis.sabiosciences.com/mirna. Circulating miRNAs (n=34) were identified in differential abundance in cows with metritis compared to normal cows. Of those 34 miRNA, 18 were observed in abundance and 16 were scarce among cows with metritis compared to normal cows. Specifically several miRNA families were scarce, including bta-let-7f (-31.3), bta-miR-10a (-20.1), bta-miR-127 (-4.2) and bta-miR-148b-3p (-61.8); and several families were found to be in abundance, including bta-let-7a-5p (25.6), bta-miR-101 (88.2), bta-miR-142-3p (77.5), bta-miR-150 (16.4), bta-miR-16b (27.18), bta-miR-181a (4.2), bta-miR-191 (21.9), bta-miR-192 (8.2), bta-miR-21-5p (3.1), bta-miR-24-3p (2.8), bta-miR-25 (3.1), bta-miR-26b (169.3), bta-miR-30d (2.5) and bta-miR-30e-5p (4.0) in cows with metritis compared to normal cows (P<0.01). A considerable number of miRNAs were predicted to inhibit the expression of genes associated to proinflammatory and immune-related responses, angiogenesis, cell-cycle progression, and adhesion molecules. In most of the cases, the levels of these miRNAs were abundant in cows with metritis compared to those without metritis. In conclusion, the presence of distinct miRNA profiles between cows with metritis and normal cows indicates that miRNA may have a role in the pathophysiology of metritis. It is possible that these miRNA could be targeted for treatment using inhibitors and/or mimics.

Keywords: Dairy cows, postpartum, metritis; miRNA, genes