Association of uterine fluid metabolites with the occurrence of metritis in dairy cows: A case-control study

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Introduction

Metritis is highly prevalent (10-30%) in dairy cows leading to decreased reproductive performance and economic losses. Recent studies demonstrated that aerobic and anaerobic bacteria are present in the uterine lumen of all cows immediately after parturition. Although the etiology of uterine diseases is mainly attributed to bacterial infection, bacterial contamination alone is not sufficient for the development of metritis. Reduced immune function and impaired regulation of inflammation are other components in the pathogenesis of metritis. Therefore, the objective of this study is to investigate the association of the uterine fluid biomarkers at the day of calving and metritis incidence within the first 2 weeks postpartum. We hypothesize that a disturbed uterine fluid metabolic landscape at the day of calving contributes to the development of metritis in dairy cows.

Materials and Methods

A case-control study was conducted on a commercial farm. All cows (n = 68) calving during the experiment period were eligible to be enrolled. Matched pairs were selected after uterine samples were collected and metritis cases diagnosed. Cows that developed metritis (n = 11) were matched to cows that did not develop metritis (n = 22) based on parity, date of calving, and calving difficulty. Within 24 h of calving, uterine samples were collected using double-guarded culture swabs (Jorgensen Labs, Loveland, CO) after the vulva was cleaned with 70% alcohol. Samples were immediately placed on a 50% aqueous acetonitrile extraction solution and stored at -112°F (-80°C) until further processing. All cows were visually observed daily until 14 days-in-milk (DIM) for the diagnosis of metritis. Metritis was identified by observation of at least 2 of the following symptoms: watery red-brownish fetid vaginal discharge, fever, and systemic illness (i.e., dullness, inappetence, decreased milk production). For metabolomic analysis, the extracts were precipitated to remove proteins and injected into an ultra-performance liquid chromatography and quadrupole-time-of-flight mass spectrometry system (SYNAPT-G2-Si, Waters) using the methods that cover both hydrophilic and hydrophobic metabolites. The concentrations of amino acids and short-chain fatty acids (SCFAs) in uterine samples were quanitified by targeted analysis. For untargeted analysis, a data matrix was constructed based on mass-charge ratio (m/z), retention time (RT), and signal intensity collected from analyzing uterine samples. The metabolites associated with metritis were identified by multivariate data analysis and chemical identities were determined by accurate mass measurement, elemental composition analysis, MS/MS fragmentation, authentic standards, and database search.

Results

Both metritic cows and their paired healthy controls had time-dependent changes in their uterine fluid metabolomes during the first 10 d of lactation. Between metritic cows and the controls, dramatic differences were observed from the day of calving to 3 DIM due to the presence of a higher abundance of SCFAs (acetic acid, propionic acid, and butyric acid), C20 fatty acids (C20:1, C20:2, and C20:3), lysophosphatidylcholines, ammonia, glycine, and bacterial metabolites in metritic cows.

Significance

The results of this pilot study showed that uterine fluid contains a unique metabolome that is different from other biofluids in dairy cows. The metabolomic analysis was capable of distinguishing the uterine fluid metabolome of metritic cows from that of healthy controls, especially from cows that develop metritis in the early days of lactation (before 5 DIM). The identification of SCFAs, C20 fatty acids, lysophosphatidylcholines, ammonia, glycine, and bacterial metabolites as the metritis-associated metabolites revealed the potential metabolic association of bacterial infection and inflammatory responses, as well as disrupted intermediary metabolites in metritis. All these observations warrant further mechanistic investigations.