Identification of epigenetic markers predictive of late embryonic mortality in milk

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Introduction

Current industry standards of pregnancy diagnostics include transrectal palpation, ultrasound and measurement of pregnancy-associated glycoprotein (PAG) levels via ELISA; however, there is no available diagnostic that can predict embryonic mortality, which is a major contributor to reproductive failure (Inskeep and Dailey, 2005). Early embryonic mortality (EEM) occurs before day 30 of gestation and is generally associated with failures of implantation and maternal recognition of pregnancy (Pohler et al., 2016a; Pohler et al., 2016b; Thatcher et al., 2001). Late embryonic mortality (LEM) occurs between days 30 and 60 of pregnancy is generally attributed to placental insufficiency (Pohler et al., 2016b). LEM has an inordinate economic impact for the producer due to calving interval extension (Pohler et al., 2016; Silke et al., 2002).

Developmental processes enlist epigenetic factors that tightly control the timing and magnitude of gene expression within and between mother and fetus. Detection and quantification of soluble and circulating epigenetic factors such as microRNAs to determine embryogenic fidelity is at the forefront of modern molecular diagnostics as demonstrated in non-invasive prenatal testing (NIPT) within humans (Biró et al., 2018; Liu et al., 2017; Zhu et al., 2013). Circulating miRNAs in serum and milk have been shown to be reliable non-invasive biomarkers of acute animal physiology due to their stable, sensitive, and specific nature (Pohler et al, 2017; Etheridge et al., 2011; Gilad et al.,2008; Izumi et al., 2012). We hypothesized that LEM-specific microRNAs in milk are present, predictive and robust biomarkers.

Materials and Methods

Small non-coding RNA profiling is being accomplished via RNA-sequencing and RT-qPCR analysis. Milk samples are being taken from cows at 28-31 days post FTAI (P1) and 70-80 days post FTAI (P2). Cows who successfully maintained pregnancy and cows who lost their pregnancy by 80 days post FTAI; P1 milk samples are being sequenced to identify differentially expressed candidate miRNAs distinct for LEM. Quantitative PCR validation of candidate LEM miRNAs to demonstrate feasibility of a qPCR diagnostic platform. Calculation of relative expression between candidate miRNAs and endogenous controls provides a more robust qPCR readout.

MicroRNA extraction and RT-qPCR using stem-loop TaqMan miRNA assays (Thermo Fisher) are being optimized on DHI milk samples. Identification of endogenous controls and their robustness is being evaluated within DHI milk samples.

Predictability of candidate LEM miRNAs are being analyzed using TaqMan RT-qPCR on up to 2,000 paired P1 and P2 DHI milk samples that have been tested for pregnancy status (IDEXX PAG ELISA) and will be cross validated with the Mix-45 ELISA which has been shown to predict LEM in serum (Pohler Laboratory).

Results

MicroRNA-148a and miR-26a were successfully amplified and screened as potential endogenous controls due to their ubiquitous presence during the lactation cycle. U6 is long ncRNA and was screened as a potential endogenous control. MicroRNA-222 was amplified and screened as a potential immune response target. MicroRNA-25 was screened as a potential LEM candidate. All microRNAs were determined to be abundant and informative in milk.

Significance

This work will aid in the discovery of a milk-based diagnostic capable of predicting LEM; equipping producers and practitioners with a novel diagnostic that delivers enhanced knowledge about pregnancy status empowering them to make more profitable breeding decisions. Development of milk based assays that can reliably detect epigenetic biomarkers will create a platform for diagnostics that can deliver improved knowledge about cow physiology. Contributing to the development of a new paradigm in dairy diagnostics; reforming the value added to a milk sample for dairy producers and practitioners.