MicroRNAs (miRNAs) are small, endogenous non-coding RNAs, approximately 22 nucleotides in length responsible for regulating gene expression at the transcriptional and post-transcriptional level. MiRNAs are highly conserved, with close to 90% sequence similarity between humans and animals and play a critical role in many cellular processes, such as spermatogenesis and sperm physiology, including fertilization, oocyte activation and embryo development. Several studies suggest miRNA repression occurs during spermatogenesis; therefore, there is a proposed association between aberrant miRNA levels and male infertility. Stable seminal plasma-specific miRNAs present may serve as a diagnostic biomarker and potential predictor for male infertility in both humans and animals. A comprehensive spectrum of seminal plasma miRNAs and their regulation on spermatogenesis-associated genes have not been well elucidated. The objective of this study was to profile and differentiate seminal plasma miRNAs in high and low fertile Holstein bulls. It was hypothesized that bull seminal plasma miRNA levels vary between high and low fertility groups. Sire conception rate (SCR; fertility index) was used for Holstein bull selection, where SCR estimates were based on at least 500 services. To accomplish the described objectives, semen samples from low (SCR -4; n=3) and high (SCR +7; n=3) fertile Holstein bulls were collected using an artificial vagina. Seminal plasma was separated using centrifugation steps, aliquoted, and stored at -80°C until miRNA profiling. Total RNA, including miRNA, was isolated from frozen-thawed seminal plasma, complementary DNA was synthesized, and mature miRNA expression profiling was performed using real time PCR for each individual sample. MiRNA-specific forward primers and universal reverse primers were used to amplify mature miRNAs. Data were analyzed using the ΔΔCT method of relative quantification using the computational software at http://pcrdataanalysis.sabiosciences.com/mirna. The software calculated the standard deviation for CT and delta CT values when more than one animal per treatment group was used. Caenorhabditis elegans miRNA (cel-miR-39-3p) was used as an endogenous control to normalize target miRNAs' expression. Interestingly, it was found that eighty four prioritized bovine-specific miRNAs were present in seminal plasma. Thirty two miRNAs were differentially seen at a 5-fold level in the seminal plasma of low and high fertile bulls. Twenty miRNAs, including bta-miR-214 (23.68 fold), bta-miR-199a-5p (20.11 fold), bta-miR20b (17.78 fold) and bta-miR-21-3p (13.48 fold) were highly abundant at a significant level (n = 3, p < 0.05) in the seminal plasma of high fertile bulls. Twelve miRNAs, including bta-miR-16 (-32.60 times), bta-miR-29c (-14.58 times), bta-miR-200a (-11.03 times) and bta-miR-101 (-10.99 times) were significantly lower in abundance (n = 3, p< 0.05) in seminal plasma of high fertile bulls compared to the levels in low fertile seminal plasma. The coefficient of variation values between bull samples were found to be similar. The amplified measurements of miRNAs in seminal plasma provide a novel, non-invasive approach to categorizing bulls in terms of fertility and can be used for diagnosing male infertility and reproductive pathology in both animals and humans.

Keywords: MicroRNA, seminal plasma, bull fertility, biomarkers