Correlation between microRNAs in amniotic fluid and mRNAs in maternal blood at 42 days of gestation in cattle

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Estimating normal pregnancy progression and foetal pathologies through maternal blood molecular profile in animals is scarcely explored. In a previous study (1), we employed an asynchronous transfer model in heifers to produce pregnancies of 10 male foetuses whose weight ranged from 1.25 to 1.69 gr at 42 days of gestation, and we used co-expression cluster analyses to identify a maternal blood expression signature that accurately predicted foetal weight (R^2=0.93). However, it is still unknown how the bovine foetus could influence the maternal blood transcriptome, given that the superficial placentation in ruminants limits the circulation of cell-free foetal RNA, as observed in humans. On the other hand, microRNAs (miRNAs), which are short, non-coding RNAs that regulate gene expression, may be involved in direct communication between foetus and maternal blood. Indeed, we have previously reported sexually dimorphic differences in the expression of miRNAs in amniotic fluid (AF) and maternal blood plasma in association with the process of sex determination and gonad differentiation in cattle (2). Therefore, we hypothesized that specific circulating miRNAs in the AF of foetuses at the end of organogenesis are correlated with mRNAs in maternal blood, which in turn, are influenced by the foetal weight. To test this hypothesis, AF samples were collected from the 10 foetuses obtained at slaughter on Day 42 of gestation (1). Samples were directly aspirated from the amnion and centrifuged. The supernatant was subjected to miRNA extraction, library preparation with the SMARTer Small RNA kit, and sequencing with a HiSeqXten platform, generating ~15 million raw reads per sample in FASQ format. Raw files were processed with miRDeep2, obtaining the reference sequences for the bovine from the miRBase database (https://www.mirbase.org/). The rest of the analyses were done with R packages, including in the analysis the transcriptomic information from the maternal blood, which was obtained as described previously (1). Correlations between the abundance of the 1030 mature bovine miRNAs in AF and the expression of 17501 mRNAs in maternal blood were evaluated using the non-parametric Kendall rank correlation coefficient. There were 130 miRNAs in AF whose abundance was correlated with the expression of 2768 mRNA in maternal blood ($p<0.01$, $R>0.75$), corresponding to 3554 miRNA-mRNA pairs. Next, we determined if the genes significantly correlated with AF miRNAs were influenced by foetal weight using the DESeq2 package. Of the 222 genes changing with foetal weight (FDR<0.1), 63 significantly correlated with AF miRNAs. Thus, there was a significant association between genes affected by foetal weight and those correlated with AF miRNAs, as determined by a chi-square test ($p<0.0001$). Functional analysis with the DAVID software (https://david.ncifcrf.gov/) of the 63 blood mRNAs revealed significant enrichment (FDR<0.1) of defence response to virus, given the presence of interferon-induced protein 44 and interferon regulatory factor 1, and antigen processing and presentation, because of MHC class II antigen (BLA-DQB) and proteasome activator subunit 1 and 2, among others. In conclusion, these results support the notion that miRNAs of foetal origin can influence the expression of certain genes in the maternal blood according to foetal phenotype characteristics, such as weight. Further studies will be aimed to determine if these AF miRNAs are also circulating in maternal blood.
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(1)Rabaglino et al., Biol Reprod. 109:749-758. 2023.
(2)Sánchez et al., Biol. Reprod. 105:345–358. 2021.