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Title: Establishment of Repertoire of Placentome-Associated MicroRNAs and Their Appearance in

Blood Plasma Could Identify Early Establishment of Pregnancy in Buffalo (Bubalus bubalis)

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Abstract:

Precise early pregnancy diagnosis in dairy animals is of utmost importance for an efficient dairy production system. Not detecting a dairy animal pregnant sufficiently early after the breeding results to extending the unproductive time of their milk production cycle and causes substantial economic loss for a dairy producer. At present, the most conventional and authentic pregnancy confirmation practice in cows and buffaloes is rectal palpation of the reproductive organs at Days 35-40 after insemination, which sometime leads to considering an animal as false pregnant. Other alternative methods available for early pregnancy diagnosis lack either accuracy or reproducibility or require elaborate instrumentation and laboratory setup not feasible to practice at farmers' doorstep. The present study was aimed at establishment of the microRNA (miRNA) repertoire of the placentome in buffaloes, which could capture the event of the cross talk between a growing embryo and a dam, through fetal cotyledons and maternal caruncles, and thus could hint at the early pregnancy establishment event in ruminants. Total RNA was isolated from buffalo placentome tissues during early stages of pregnancy (at Day < 25 and Days 30-35), and global small RNA analysis was performed by using Illumina single-end read chemistry and Bubalus bubalis genome. A total of 2,199 miRNAs comprising 1,620 conserved and 579 non-conserved miRNAs were identified. Stringent functional miRNA selection criteria could predict 20 miRNAs worth evaluating for their abundance in the plasma of pregnant, non-pregnant, cyclic non-bred, and non-cyclic prepubertal animals. Eight of them (viz., miR-195-5p, miR-708-3p, miR-379-5p, miR-XX1, miR-XX2, miR-130a-3p, miR-200a-3p, and miR-27) displayed typical abundance patterns in the plasma samples of the animals on Day 19 as well as Day 25 post-insemination, thus making them ambiguous candidates for early pregnancy detection. Similarly, higher abundance of miR-200a-3p and miR130a-3p in non-pregnant animals was indicative of their utility for detecting the animals as not pregnant. Most interestingly, miR-XX1 and miR-XX2 were very characteristically abundant only in pregnant animals. In silico target prediction analysis confirmed that these two miRNAs are important regulators of cyclooxygenase-2 (COX-2) and cell adhesion molecule-2 (CADM-2), both of which play a significant role in the implantation process during fetomaternal cross talk. We interpret that circulatory miR-XX1 and miR-XX2 in blood plasma could be the potential biomarkers for early pregnancy detection in buffaloes.

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