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Multi-omics analysis unravels candidate biomarkers associated with pregnancy outcomes in beef heifers

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Reproductive efficiency is a key factor in the success and sustainability of beef production systems. Although reproductively mature and healthy, between 5 to 15% of heifers fail to conceive within a designated breeding season. The underlying mechanisms preventing these heifers from achieving pregnancy remain elusive. In this study, we investigated the differential expression of genes (DEGs) and abundance of proteins (DAPs) associated with pregnancy outcomes in beef heifers. Angus-Simmental crossbred heifers (n = 106) were subjected to an estrus synchronization and fixed-time artificial insemination (AI) protocol (7-day CO-Synch + CIDR), followed by a 60-day natural bull breeding. Blood samples were collected at the time of AI to isolate Peripheral White Blood Cells (PWBCs). Heifers were classified as pregnant (P) and non-pregnant (NP) following the pregnancy check. Subsequently, six animals per group were randomly selected for total RNA and protein isolation from the PWBC samples. Total RNA was extracted, and genome-wide expression was assessed through RNA sequencing. After read quality control and mapping, DEGs were identified using DESeq2. Proteins were digested using LysC/trypsin, and peptide samples were analyzed using nano-flow LC-MS/MS system. MS data was searched against the Bovine Uniprot database with Proteome Discoverer 2.5 software utilizing Sequest analysis. Differential protein abundance was determined using a moderated *t*-test on Protti R-package. Functional over-representation analysis was performed using WebGestalt. Our approach revealed 230 DEGs, comprising 102 upregulated and 128 downregulated genes in the NP group (p-value ≤ 0.05 and $|\log_2FC| > 0.5$). Noteworthy DEGs, such as *BOLA-DQB*, *PRKAR1B*, *CALML4*, and *JUP*, were involved with key pathways reported downregulated during inflammatory response times. Proteomics analysis unveiled 70 DAPs, with 27 and 43 being upregulated and downregulated in the NP group (p-value ≤ 0.05). Over-represented KEGG pathways among DAPs included progesterone-mediated oocyte maturation and insulin signaling. Biological processes encompassed the regulation of the JAK-STAT cascade and response to estradiol. These findings suggest that there are transcriptomic and proteomic signatures underlying pregnancy outcomes in beef heifers. Further integrative analyses and mechanistic investigations are warranted to validate these targets and assess their potential as biomarkers.

Keywords: beef heifer, fertility, immune response, proteomics, transcriptomics

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