**Could NMR-based metabolomic approach be a precise tool to unveil potential biomarkers for pregnancy diagnosis in buffaloes!**

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Ensuring the precise and timely delivery of nutrients through maternal metabolic regulation is crucial for proper fetal development. This study was targeted on metabolomic approach to examine the dynamic changes in urinary metabolites during early pregnancy and in non-pregnant animals in healthy Murrah buffaloes (*Bubalus bubalis*), thus to determine potential biomarker as a potential tool in early pregnancy diagnosis. Additionally, the diversity of metabolites between pregnant and non-pregnant animals could be harnessed to identify metabolic signatures of early pregnancy in Murrah buffaloes. We conducted analysis of urinary metabolites nuclear magnetic resonance (NMR) analysis taking pregnant (n=6) and non-pregnant (n=6) buffalo heifers using and further validation by gas chromatography-mass spectrometry (GC-MS). Urine samples were collected on days 0 (estrus), 10, 18, 35, and 42 of pregnancy (referred to as days post-insemination) from pregnant animals and on days 0, 10, and 18 from non-pregnant animals. Multivariate analysis of the metabolite data revealed changes in the concentration of twenty-three metabolites in pregnant animals during early pregnancy. Notably, several metabolites associated with tryptophan metabolism, such as 3-Hydroxykynurenine, Anthranilate, and 5-Hydroxytryptophan, exhibited a significant and consistent increasing trend from day 0 to day 42 of pregnancy. Furthermore, metabolic profiling showed significant variations in the levels of 3-Hydroxykynurenine, Tyramine, and 5-Hydroxytryptophan between pregnant and non-pregnant animals, suggesting their diagnostic potential as biomarkers for early pregnancy in buffaloes. Current metabolomics analyses aided in elucidation of signature metabolites associated with progression in on-site pregnancy diagnosis in buffaloes.

**Key words**: Buffaloes, urine, pregnancy, metabolites, NMR

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