Urinary ^{1}H NMR-based Metabolomics can Distinguish Sub-fertility Buffalo Bulls

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Introduction: NMR-based metabolomics is a non-invasive technique to study metabolic profiles in bio-fluids and metabolites associate with physiologic and pathologic states can be determined. Sub-fertility in bulls is a major problem and results into huge economic loss. Identification of potential metabolic biomarkers to determine the fertility at earlier developmental stage accurately is important. In present study we used ^{1}H NMR technique to investigate the metabolic profile differences in urine samples to differentiate between buffalo bulls of good fertility and poor fertility. To our knowledge this is the first ^{1}H NMR metabolomics study of urine in buffalo bulls to investigate metabolic difference in relation to fertility.

Method: Urinary metabolic profiles were acquired with the use of ^{1}H NMR spectroscopy. Urine samples were collected from 7 good fertility and 17 poor fertility buffalo bull and stored at- 80° C until NMR experiments were performed. For NMR experiments 400µl urine, 30µl TSP (0.5mM) and 170µl phosphate buffer were added in D_2O, containing 1mM sodium azide, making total volume to 600µl for NMR experiments on a 700 MHz (Agilent) spectrometer. The chemical shifts of resonances referenced to TSP. 1D spectrum with water suppression was acquired using a single 90° pulse with following parameters: spectral width, 10504.2 Hz; scans, 64; relaxation delay, 14sec. The data is processed using the agilent software, Vnmrj 2.3A. PLS-DA multivariate analysis was carried out using MetaboAnalyst, a web-based metabolomics data processing tool.

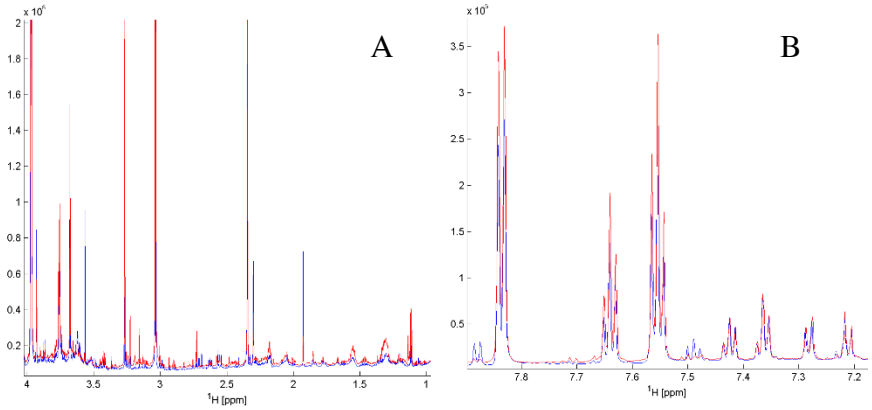

Figure 1 Representative ^{1}H NMR spectra of urine sample obtained from bull of good fertility (red) and poor fertility (blue). (A) and (B) expanded regions of NMR spectra from 1ppm to 4 ppm and 7.2ppm to 7.9ppm, respectively.

Results: Figure 1 shows representative ^{1}H NMR spectra obtained from urine samples of bull having good fertility (red) and poor fettility (blue) on same scale. Fig. 1A and 1B are expanded regions of the spectra, from 1ppm to 4ppm and 7.2ppm to 7.9 ppm, respectively. The differences in the metabolic profiles as evident from the resonance peaks between the two categories is clearly seen. Multivariate partial least square discriminate analysis (PLS-DA) analysis showed as score plot in Figure 2 depicts clear separation between two categories of samples (red-good fertility and green-poor fertility).

Discussion: Bulls with good and poor fertility show altered metabolism evident in urine that can be detected by NMR spectroscopy. The results of the PLS-DA revealed that sub-fertile bulls be differentiated from fertile bulls based on altered urinary metabolic profiles. Further analysis will be carried out to identify potential metabolic biomarkers which could be used for fertility assessment. It should be noted that urine included the effects of metabolism contributions from different organs and metabolic processes of body. Nevertheless, the present study was able to show the potential of NMR based metabolomics in bull fertility. It is inferred that the expression of certain metabolites in urine varied with bull fertility.

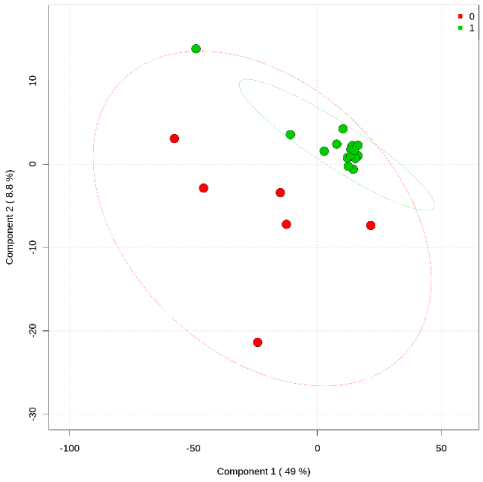

Figure 2 PLS-DA scores plot of ^{1}H NMR spectra of Bull urine obtained from two categories, good fertility (red) and poor fertility (green).