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## Details of the Collaborative Activity

2020-21

**Name of the Collaborating Institute:** ICAR-National Dairy Research Institute (NDRI), South Regional Station, Bengaluru.

**Name of Collaborating Department:** Yenepoya Research Centre

### Activities:

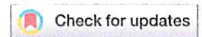
Collaborative research work in the area of genomics and proteomics at YRC.

### Joint Publications:

- Ashokan M, Ramesha KP, Swetha H, Gayathree K, Rana E, Azaruddin N, Raj R, Jeyakumr S, Kumaresan A, ....., TS Keshavaprasad. Differences in milk metabolites in Manad gidda (*Bos indicus*) cows reared under pasture-based feeding system. *Scientific Reports*. 2021.
- Ramesha KP, Praseeda M, Kannegundla U, Thota LN, Gopalakrishnan L, Rana E, Azharuddin N, Mangalaparathi KK, Kumar M, Dey G, Patil A, Saravanan K, Behera SK, Jeyakumar S, Kumaresan A, Katakaltware MA, Prasad TSK. Deep Proteome Profiling of Semen of Indian Indigenous Malnad Gidda (*Bos indicus*) Cattle. *Journal of Proteome Research*. 2020
- Saraf KK, Kumaresan A, Dasgupta M, Karthikkeyan G, Prasad TSK, Modi PK, Ramesha K, Jeyakumar S, Manimaran A. Metabolomic fingerprinting of bull spermatozoa for identification of fertility signature metabolites. *Molecular Reproduction and Development* 2020; 1-12

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OPEN

# Differences in milk metabolites in Malnad Gidda (*Bos indicus*) cows reared under pasture-based feeding system

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The milk and milk products from cows reared under grazing system are believed to be healthier and hence have high demand compared to milk from cows reared in the non-grazing system. However, the effect of grazing on milk metabolites, specifically lipids has not been fully understood. In this study, we used acetonitrile precipitation and methanol:chloroform methods for extracting the milk metabolites followed by liquid chromatography–tandem mass spectrometry (LC–MS/MS) run to identify the different metabolites between the milk of grazing and non-grazing early lactating Malnad Gidda cows. Various carbohydrates, amino acids, nucleosides and vitamin derivatives were found to be differentially abundant in grazing cows. A total of 35 metabolites were differentially regulated (fold change above 1.5) between the two groups. Tyrosyl-threonine, histidinyl-cysteine, 1-methyladenine, L-cysteine and selenocysteine showed fold change above 3 in grazing cows. The lipid profile of milk showed a lesser difference between grazing and non-grazing cows as compared to polar metabolites. To the best of our knowledge, this is the largest inventory of milk metabolomics data of an Indian cattle (*Bos indicus*) breed. We believe that our study would help to emerge a field of Nutri-metabolomics and veterinary omics research.

India is blessed with a large number of cattle (193.46 million) population<sup>1</sup> and ranks first in the world in total milk production (187.7 MT)<sup>2</sup>. Along with quantity, the quality of bovine milk is equally important because it is an important entity in human nutrition as well<sup>3</sup>. The understanding of the molecular composition of bovine milk in different stages and conditions would help in integrative molecular biology and nutrition-based studies<sup>4–7</sup>. Bovine milk composition and quality are mainly influenced by various factors including genetics, diet (feeding system), stage of lactation, season and somatic cell count<sup>4,8</sup>. The difference in the feeding systems significantly influences the milk composition in cows<sup>9–11</sup>. It has been reported that forages like alfalfa hay, corn stover<sup>12</sup>, corn silage, fresh forage and hay<sup>13</sup> significantly alter the metabolomics profile of the cow milk. Pasture-based (grazing) feeding system is traditionally the most adopted management practice by small-holder dairy farmers (about 86% of all farmers) in India, where the indigenous cattle graze on a natural pasture of native flora. Recently, intensification in the dairy industry resulted in the inclination of the feeding practices towards non-grazing (zero-grazing/stall-fed) system where cows are housed indoor and mainly fed with paddy straw and cultivated green fodder along with concentrates which enables the farmers to have better control over nutrition, health and management of lactating animals<sup>6</sup>.

It has been reported that the nutritional value of paddy straw is lower than alfalfa hay and is a highly lignified material<sup>14</sup>. There is a perception that milk and milk products derived from cows reared in the grazing system are natural and healthier, and hence have high demand in the dairy market<sup>15</sup>. Milk derived from the pasture system has been found to have higher unsaturated fatty acids and more levels of  $\alpha$ -tocopherol and  $\beta$ -carotene compared to Total Mixed Ration (TMR) diets<sup>16</sup>. O'Callaghan et al.<sup>6</sup> have also observed the beneficial influence of the grazing system on the nutritional composition of milk and milk products (butter and cheese) both at macronutrient and fatty acid levels (conjugated linoleic acid and omega 3 fatty acid). Therefore, it could be interesting to observe

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## Deep proteome profiling of semen of an Indian indigenous Malnad Gidda (*Bos indicus*) cattle

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*J. Proteome Res.*, **Just Accepted Manuscript** • DOI: 10.1021/acs.jproteome.0c00237 • Publication Date (Web): 06 Jun 2020

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## RESEARCH ARTICLE

# Metabolomic fingerprinting of bull spermatozoa for identification of fertility signature metabolites

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**Abstract**

The objective of the study was to identify the fertility-associated metabolites in bovine spermatozoa using liquid chromatography-mass spectrometry (LC-MS). Six Holstein Friesian crossbred bulls (three high-fertile and three low-fertile bulls) were the experimental animals. Sperm proteins were isolated and protein-normalized samples were processed for metabolite extraction and subjected to LC-MS/MS analysis. Mass spectrometry data were processed using iMETQ software and metabolites were identified using Human Metabolome DataBase while, Metaboanalyst 4.0 tool was used for statistical and pathway analysis. A total of 3,704 metabolites belonging to various chemical classes were identified in bull spermatozoa. After sorting out exogenous metabolites, 56 metabolites were observed common to both the groups while 44 and 35 metabolites were found unique to high- and low-fertile spermatozoa, respectively. Among the common metabolites, concentrations of 19 metabolites were higher in high-fertile compared to low-fertile spermatozoa (fold change > 1.00). Spermatozoa metabolites with variable importance in projections score of more than 1.5 included hypotaurine, D-cysteine, selenocystine. In addition, metabolites such as spermine and L-cysteine were identified exclusively in high-fertile spermatozoa. Collectively, the present study established the metabolic profile of bovine spermatozoa and identified the metabolomic differences between spermatozoa from high- and low-fertile bulls. Among the sperm metabolites, hypotaurine, selenocystine, L-malic acid, D-cysteine, and chondroitin 4-sulfate hold the potential to be recognized as fertility-associated metabolites.

**KEYWORDS**

fertility, metabolomic profile, spermatozoa

## 1 | INTRODUCTION

Male fertility is characterized by the ability to produce spermatozoa competent enough to fertilize the oocyte and ultimately produce viable offspring (Wood, Foulkes, Shaw, & Melrose, 1986). In dairy industry, females are inseminated artificially using semen

collected from breeding bulls to explore full potential of the bull genetics to get maximum production. Researchers around the globe are striving hard to develop quick, cost effective, and noninvasive tests/methods for prediction of bull fertility. Sperm kinematic and sperm functional attributes like membrane integrity (Petrunkina & Harrison, 2011; Singh et al., 2016), phospholipid scrambling