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Supplementary Material

Preliminary comparative deep-metabolomic analysis of spermatozoa from zebu and crossbred cattle suggests associations between metabolites, sperm quality and fertility

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Table S1. Differentially expressed metabolites between dwarf zebu bull spermatozoa and crossbred bull spermatozoa

Bolded text in left-hand column indicates the selected '11 differentially expressed metabolites' (per Results section in the main article)

Compounds	**Fold Change**	**log2(FC)**
Hexachlorobenzene	637.03	9.32
Guanosine 2',3'-cyclic phosphate	216.38	7.76
4-2R,3S,4R,5R-5-6-amino-9H-purin-9-yl-4-hydroxy-3-phosphonooxyoxolan-2-ylmethoxyhydroxyphosphoryloxyhydroxyphosphoryloxy-2-hydroxy-N-2-2-2-4-,hydroxyphenylacetylsulfanylethyl-C-hydroxycarbonimidoylethyl-3,3-dimethylbutanimidic acid	154.38	7.27
p1,p6-Bis5'-adenosylhexaphosphate	141.60	7.15
Nitroprusside	117.12	6.87
2,4,6-Tribromophenol	82.21	6.36
beta-D-Glucosyl-N-docosanoylsphingosine	79.50	6.31
Guanosine 3'-diphosphate 5'-triphosphate	65.53	6.03
Cefalotin	64.30	6.01
17Z-N-4Z-1-2R,3R,4R,5R,6R-3,4-dihydroxy-6-hydroxymethyl-5-2R,3R,4S,5R,6R-3,4,5-trihydroxy-6-hydroxymethyloxan-2-yloxyoxan-2-yloxy-3-hydroxyoctadec-4-en-2-ylhexacos-17-enimidic acid	60.73	5.92
Sedoheptulose 1,7-bisphosphate	47.73	5.58
gamma-Hexachlorocyclohexane	31.74	4.99
5-Diphosphoinositol pentakisphosphate	10.20	3.35
Acrylyl-CoA	7.18	2.84
Methoxyflurane	6.30	2.65
dUDP	6.30	2.65
Tricosane	5.54	2.47
N-4Z-1-2R,3R,4R,5R,6R-3,4-dihydroxy-6-hydroxymethyl-5-2R,3R,4S,5R,6R-3,4,5-trihydroxy-6-hydroxymethyloxan-2-yloxyoxan-2-yloxy-3-hydroxyoctadec-4-en-2-yltetracosanimidic acid	3.50	1.81
Adrenorphin	3.48	1.80
2-5-hydroxy-6-hydroxymethyl-2-10,16,20-tetramethyl-22-azahexacyclo12.10.0.0,0,0,0,tetracos-4-en-7-yloxy-4-3,4,5-trihydroxy-6-hydroxymethyloxan-2-yloxyoxan-3-yloxy-6-methyloxane-3,4,5-triol	3.46	1.79
Glu-glu	3.07	1.62
Flecainide	3.07	1.62
Amantadine	2.89	1.53

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Halothane	2.63	1.39
3,5-Diiodo-4-hydroxyphenylpyruvic acid	2.55	1.35
1,1,1-Trichloroethane	2.52	1.34
Pentachlorophenol	2.52	1.34
Labetalol	2.34	1.22
PE200/2055Z,8Z,11Z,14Z,17Z	2.16	1.11
14-Dichlorobenzene	2.03	1.02
Cefotetan	0.49	-1.02
Purine	0.32	-1.63
Hydroxymethylbilane	0.26	-1.96
L-Cysteine	0.06	-4.04
1D-Myo-inositol 1,2-cyclic phosphate	0.06	-4.04
CerD181/260	0.05	-4.23
PC150/1619Z	0.05	-4.44
Levomethadyl acetate	0.04	-4.63
Acetyl-CoA	0.03	-5.00
Trichloroethene	0.03	-5.09
Phytosphingosine	0.02	-5.52
Thiabendazole	0.02	-5.63
Nadph	0.02	-5.64
2-3-docosanoyloxy-2-1Z,11Z-octadeca-1,11-dien-1-yloxypropyl phosphonatooxyethyltrimethylazanium	0.01	-6.44
2R-4-2-3-4-2R,3S,4R,5R-5-6-amino-9H-purin-9-yl-4-hydroxy-3-phosphonooxyoxolan-2-ylmethoxyhydroxyphosphoryloxyhydroxyphosphoryloxy-1,2-dihydroxy-3,3-dimethylbutylideneamino-1-hydroxypropylideneaminoethylsulfanyl-2-benzyl-4-oxobutanoic acid	0.01	-6.51
7,8-Dihydropteroic acid	0.01	-6.99
Meconic acid	0.01	-7.33
Trichloroacetic acid	0.01	-7.45
3-Nitropropanoic acid	0.00	-8.95

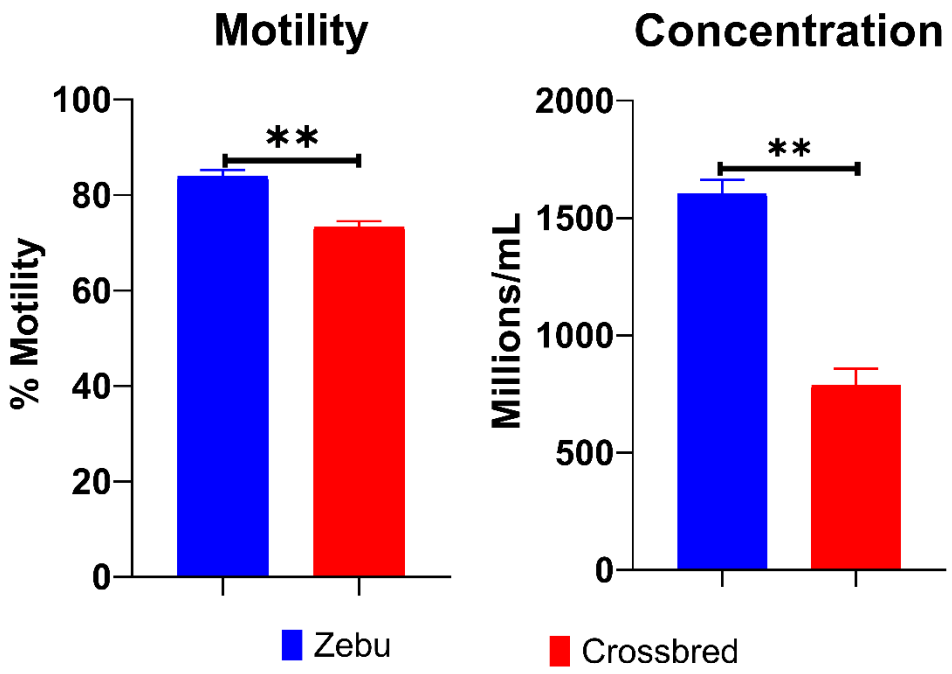

Fig. S1. Mean (±SEM) of sperm motility and concentration of zebu and crossbred bulls.

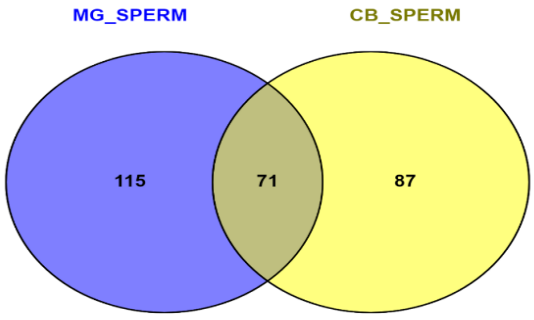

Fig. S2. The Venn diagram of total mapped metabolites in dwarf zebu bull spermatozoa and crossbred bull spermatozoa.

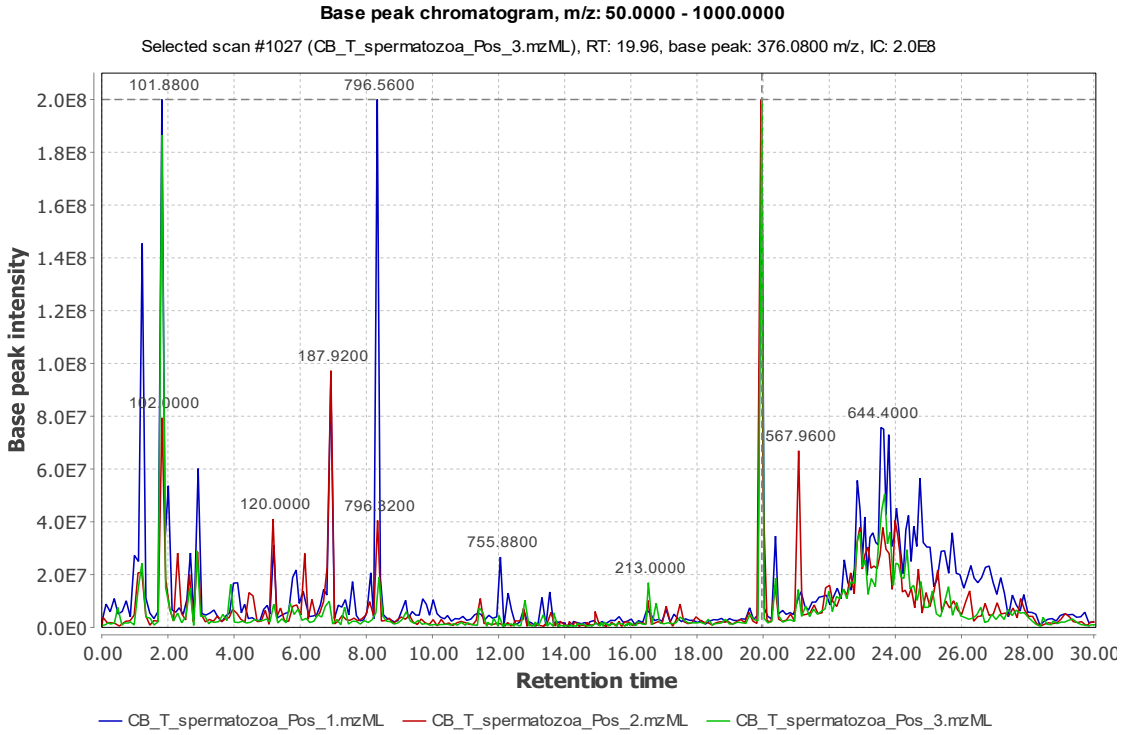

Fig. S3. Representative LC/MS MS chromatogram of select endogenous metabolites.

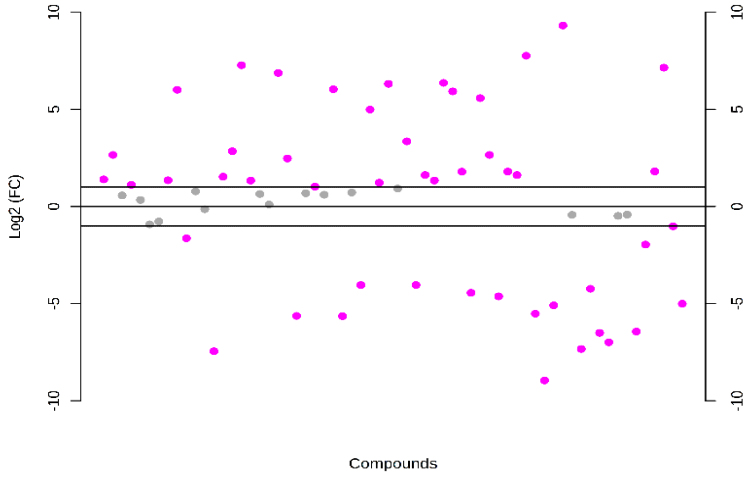

Fig. S4. Graphical representation of fold change with threshold 2 of differentially expressed features between dwarf zebu bull spermatozoa and crossbred bull spermatozoa. Fold change analysis is to compare the absolute value changes between two group means.

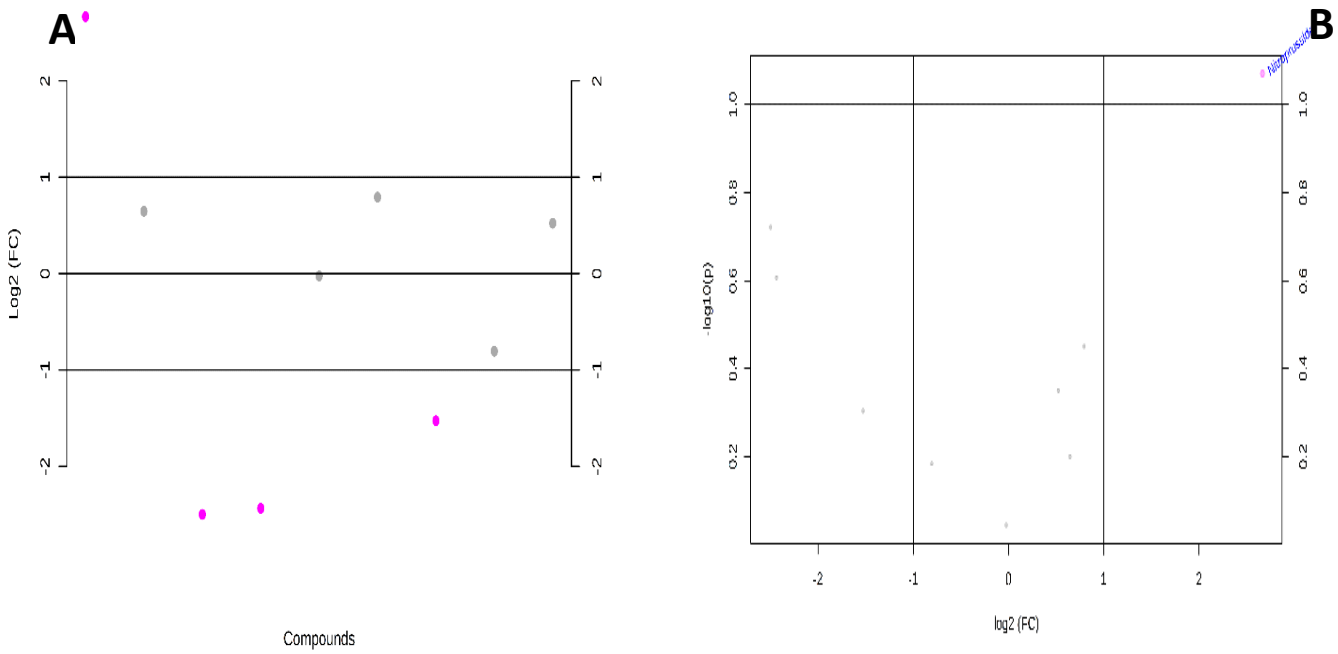

Fig. S5. A) Important features identified by fold change analysis on log2 scale) between dwarf zebu bull spermatozoa and crossbred bull spermatozoa. B) Nitroprusside identified by volcano plot analysis (combination of t-test and fold change analysis of means).

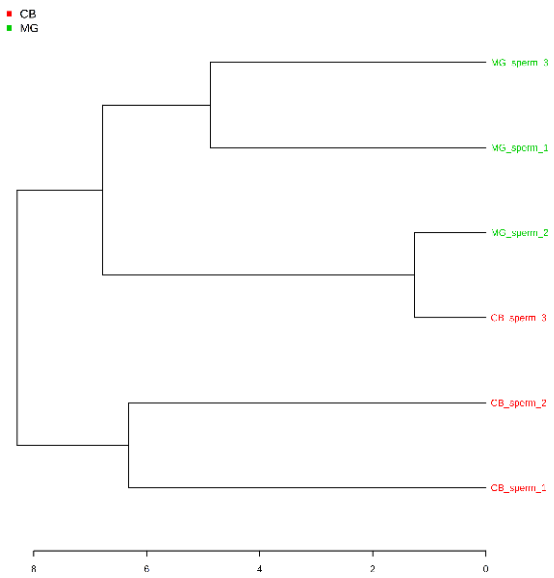

Fig S6. Hierarchical dendrogram clustering between dwarf zebu bull spermatozoa and crossbred bull spermatozoa.

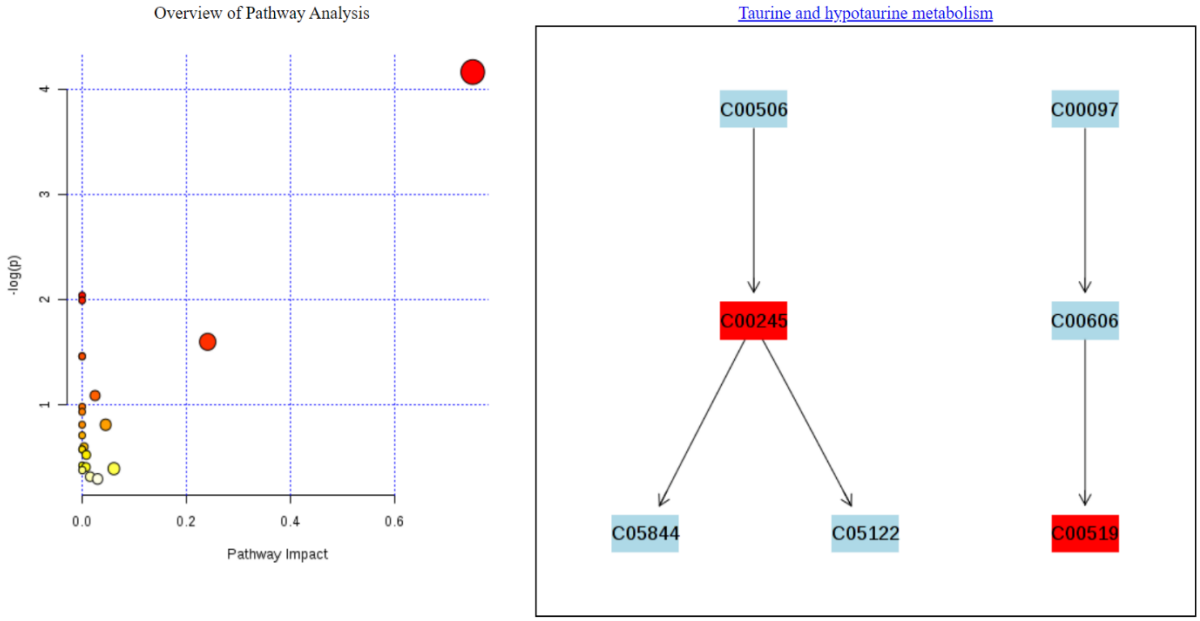

Fig S7. The metabolome view of the graphical output of significant pathways of dwarf zebu bull spermatozoa.

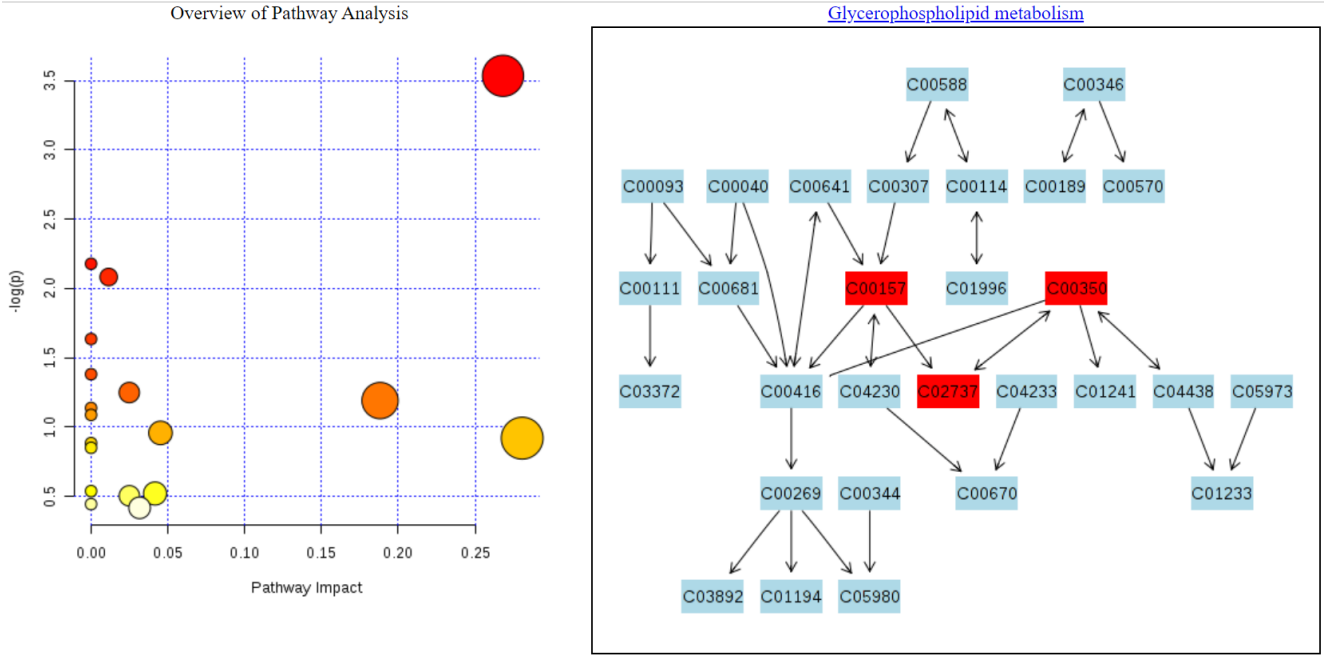

Fig. S8. The metabolome view of the graphical output of significant pathways of crossbred sperm.

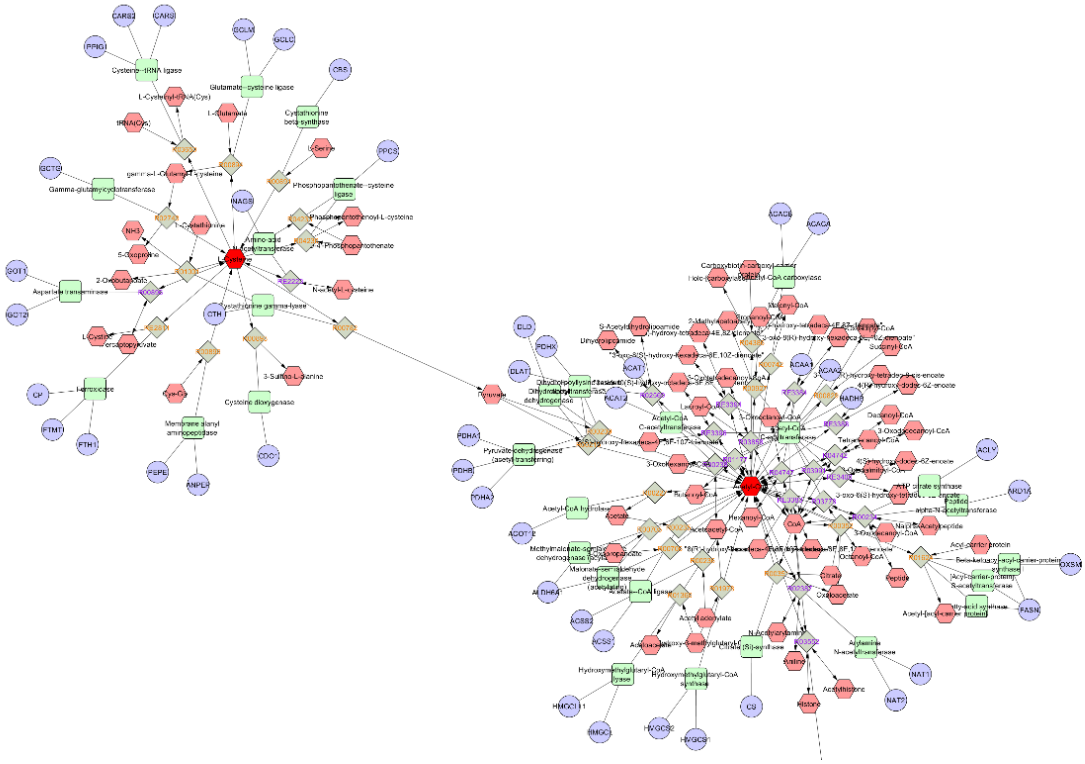

Fig. S9. Metabolic network based on compound -reaction (Met scape).