10.1071/RD22246

Reproduction, Fertility and Development

Supplementary Material

Use of sensitivity-enhanced nuclear magnetic resonance spectroscopy equipped with a 1.7-mm cryogenically cooled micro-coil probe in identifying human sperm intracellular metabolites

Aswathi Cheredath^A, Shubhashree Uppangala^B, Ameya Jijo^A, R. Vani Lakshmi^C, G. A. Nagana Gowda^D, Guruprasad Kalthur^E, and Satish Kumar Adiga^{A,*}

^ACentre of Excellence in Clinical Embryology, Department of Reproductive Science, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal 576 104, India.

^BDivision of Reproductive Genetics, Department of Reproductive Science, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal576 104, India.

^cDepartment of Data Science, Prasanna School of Public Health, Manipal Academy of Higher Education, Manipal576 104, India.

^DNorthwest Metabolomics Research Centre, Anaesthesiology and Pain Medicine, University of Washington, Seattle, WA, USA.

^EDivision of Reproductive Biology, Department of Reproductive Science, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal576 104, India.

^{*}Correspondence to: Satish Kumar Adiga Centre of Excellence in Clinical Embryology, Department of Reproductive Science, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal 576 104, India Email: satish.adiga@manipal.edu Supplementary Table S1: Chemical shift values of the identified sperm intracellular metabolites

Metabolites	Chemical shift values	
Leucine	0.9657	
Isoleucine	1.0152	
Valine	1.0455	
Propylene glycol	1.1444	
Ethanol	1.1868	
Lactate	1.3304	
Alanine	1.4838	
Putrescine	3.0557	
Betaine	3.2708	
Methanol	3.3886	
Glycerol	3.5615/3.7809	
Tyrosine	6.9043	
Formate	8.458	

Supplementary Table S2. The different class of metabolites identified in the human sperm (Paiva *et al.* 2014; Engel *et al.* 2018; Zhao *et al.* 2018).

Aliphatic cyclic compounds	Carnitine and acetyl carnitine are
Carnitine	involved in the modulation of fatty
Methanol	acids and transfer acetyl groups into
O-Phosphocholine	mitochondria for beta-oxidation
Propylene glycol	
Putrescine	
Trimethylamine	
Creatinin	
Amino acids, peptides, and analogues	Can serve as chelating agents, in
2-Aminoadipate Aminoisobutyrate Aminobutyrate	particular for toxic metals, and/or as
Gamma-	oxidizable substrates for spermatozoa.
Alanine	Amino acids could also serve as
Arginine	endogenous substrates in human
Creatine	sperm cells
Creatine phosphate	
Glutamine	
Isoleucine	
Leucine	
Threonine	
Tyrosine	
Valine	

Tryphtophan	
Cysteine	
Proline s	
Norvaline	
Aminocaprylic acid	
5-Aminovaleric acid	
Asparagine	
Aspartate	
Citrulline	
Glutamate	
Glycine	
Histidine	
Lysine	
Methionine	
Ornithine	
Serine	
Valine	
Carbohydrates and carbohydrate conjugates	Indicate the active glycolysis in sperm
Glucose	cells
Glycerol	
Orotic acid	
Pipecolinic acid	
Lipids	Indicates the fatty acid oxidation.

2-Hydroxy-3-1H Butyrate1H Caprate Capric acid1H Caprylate Caprylic acid1H O-Acetylcarnitine L-sn-Glycero-3-phosphocholineMethyl heptadecanoateZymosterol (Zhao et al., 2018)cis-Gondoic acidPhytosphingosineNucleosides, nucleotides, and analoguesADPAdenosine monophosphate Inosinic acidCytidineGuanosineOrganic acids and derivatives3-Hydroxyisobutyrate2-Oxoglutarate	2-Methylglutarate	
1H Caprate Capric acid1H Caprylate Caprylic acid1H O-Acetylcarnitine L-sn-Glycero-3-phosphocholineMethyl heptadecanoateZymosterol (Zhao et al., 2018)cis-Gondoic acidPhytosphingosineNucleosides, nucleotides, and analoguesADPAdenosine monophosphate Inosinic acidCytidineGuanosineOrganic acids and derivatives3-Hydroxyisobutyrate	2-Hydroxy-3-	
1H Caprylate Caprylic acid1H Caprylate Caprylic acid1H O-Acetylcarnitine L-sn-Glycero-3-phosphocholineMethyl heptadecanoateZymosterol (Zhao et al., 2018)cis-Gondoic acidPhytosphingosineNucleosides, nucleotides, and analoguesADPAdenosine monophosphate Inosinic acidCytidineGuanosineOrganic acids and derivatives3-Hydroxyisobutyrate	1H Butyrate	
1H O-Acetylcarnitine L-sn-Glycero-3-phosphocholineMethyl heptadecanoateZymosterol (Zhao et al., 2018)cis-Gondoic acidPhytosphingosineNucleosides, nucleotides, and analoguesADPAdenosine monophosphate Inosinic acidCytidineGuanosineOrganic acids and derivatives3-Hydroxyisobutyrate	1H Caprate Capric acid	
sn-Glycero-3-phosphocholine Methyl heptadecanoate Zymosterol (Zhao et al., 2018) cis-Gondoic acid Phytosphingosine Nucleosides, nucleotides, and analogues ADP Adenosine monophosphate Inosinic acid Cytidine Guanosine Organic acids and derivatives 3-Hydroxyisobutyrate	1H Caprylate Caprylic acid	
Methyl heptadecanoateZymosterol (Zhao et al., 2018)cis-Gondoic acidPhytosphingosineNucleosides, nucleotides, and analoguesADPAdenosine monophosphate Inosinic acidCytidineGuanosineOrganic acids and derivatives3-Hydroxyisobutyrate	1H O-Acetylcarnitine L-	
Zymosterol (Zhao et al., 2018)cis-Gondoic acidPhytosphingosineNucleosides, nucleotides, and analoguesADPAdenosine monophosphate Inosinic acidCytidineGuanosineOrganic acids and derivatives3-Hydroxyisobutyrate	sn-Glycero-3-phosphocholine	
cis-Gondoic acid Phytosphingosine Nucleosides, nucleotides, and analogues ADP Adenosine monophosphate Inosinic acid Cytidine Guanosine Organic acids and derivatives 3-Hydroxyisobutyrate	Methyl heptadecanoate	
PhytosphingosineNucleosides, nucleotides, and analoguesADPAdenosine monophosphate Inosinic acidCytidineGuanosineOrganic acids and derivatives3-Hydroxyisobutyrate	Zymosterol (Zhao et al., 2018)	
Nucleosides, nucleotides, and analoguesADPAdenosine monophosphate Inosinic acidCytidineGuanosineOrganic acids and derivatives3-Hydroxyisobutyrate	cis-Gondoic acid	
ADPAdenosine monophosphate Inosinic acidCytidineGuanosineOrganic acids and derivatives3-Hydroxyisobutyrate	Phytosphingosine	
Adenosine monophosphate Inosinic acidCytidineGuanosineOrganic acids and derivatives3-Hydroxyisobutyrate	Nucleosides, nucleotides, and analogues	
Cytidine Image: Cytidine Guanosine Image: Cytidine Organic acids and derivatives Image: Cytidine 3-Hydroxyisobutyrate Image: Cytidine	ADP	
Guanosine Organic acids and derivatives 3-Hydroxyisobutyrate	Adenosine monophosphate Inosinic acid	
Organic acids and derivatives 3-Hydroxyisobutyrate	Cytidine	
3-Hydroxyisobutyrate	Guanosine	
	Organic acids and derivatives	
2-Oxoglutarate	3-Hydroxyisobutyrate	
	2-Oxoglutarate	
3-Hydroxybutyrate	3-Hydroxybutyrate	
Acetate	Acetate	
Azelate		
Formate	Azelate	

Glycolate	
Isobutyrate	
Lactate	
Ethanolamine	
Picolinic acid	
Benzoic acid	
Guanidinosuccinic acid	
pnylethylamine	
Glutamic acid	
Dithioerythritol	
Others	
2-Aminoethanethiol	
Monoolein	
DL-dihydrosphingosine	
N-(3-aminopropyl)-morpholine	
alpha-Tocopherol	
Deoxyerythritol	
Methylmercaptopurine	
Amino-1-	
5-Aminovaleric acid	
Phosphoglycerate	

