

Proteomic analyses reveal a role of cytoplasmic droplets as an energy source during sperm epididymal maturation

Shuiqiao Yuan^{a,b}, Huili Zheng^a, Zhihong Zheng^b, Wei Yan^{a,1}

^aDepartment of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, NV, 89557; and ^bDepartment of Laboratory Animal Medicine, China Medical University, Shenyang, 110001, China

¹ Corresponding author. Email: wyan@unr.edu

Supplemental Information contains one Figure (Figure S1), three Tables (Tables S1-S3) and two Videos (Videos S1 and S2) files.

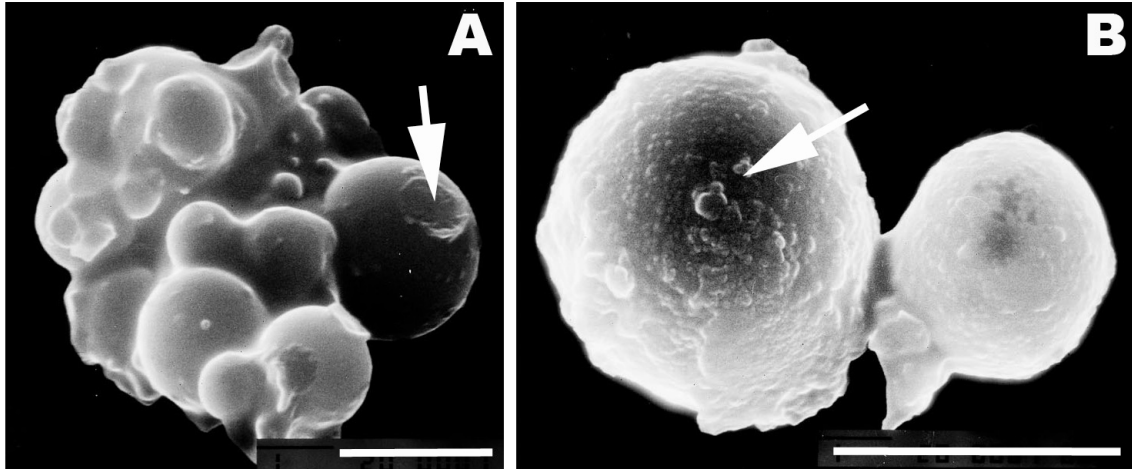


Figure S1. Scanning electron microscopic images of purified murine cytoplasmic droplets. Arrows point to indentations resembling the resealed defects at the detaching points when CDs come off the sperm flagella. Scale bar = 1 μ m

Table S1 Mass spectrometry-based identification of proteins highly enriched in murine cytoplasmic droplets.

#	MS/MS View/Identified Proteins (105)	Accession Number	Molecular Weight	Protein Grouping	Ambiguity	Dot_1	Dot_2	Dot_3	Dot_4	Dot_5	Dot_1_2	Dot_2_2	Dot_3_2	Dot_4_2	Dot_5_2
1	IP1P00467457.3 Tax_Id=10090 Gene_Symbol=Ldch L-lactate dehydrogenase C chain	IP00467457	36 kDa	TRUE		91%	100%	100%	100%	100%		100%	100%	100%	100%
2	IP1P00273320.2 Tax_Id=10090 Gene_Symbol=Putative uncharacterized protein	IP00047320	42 kDa	TRUE		75%	100%	100%	100%	100%	89%	100%	100%	100%	100%
3	IP1P00224181.7 Tax_Id=10090 Gene_Symbol=Akr1b7 Aldose reductase-related protein 1	IP00224181	36 kDa	TRUE					100%	100%				100%	100%
4	IP1P00228633.7 Tax_Id=10090 Gene_Symbol=Gp11 Glucose-6-phosphate isomerase	IP00228633	63 kDa				100%						100%	100%	100%
5	IP1P00134191.3 Tax_Id=10090 Gene_Symbol=Slc2a3 Solute carrier family 2, facilitated glucose transporter member 3	IP00134191	53 kDa			100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
6	IP1P00272690.2 Tax_Id=10090 Gene_Symbol=Ace Isoform Somatic of Angiotensin-converting enzyme	IP00272690	151 kDa			100%	100%	100%	89%	100%	100%	100%	100%	100%	89%
7	IP1P0029768.1 Tax_Id=10090 Gene_Symbol=Pgd Phosphoglycerate kinase 2	IP0029768	45 kDa			91%	100%	100%	100%	100%	100%	100%	100%	100%	100%
8	IP1P00320217.9 Tax_Id=10090 Gene_Symbol=Cct2 T-complex protein 1 subunit beta	IP00320217	57 kDa				100%	100%	89%				100%	100%	48%
9	IP1P00387281.1 Tax_Id=10090 Gene_Symbol=Oxct2a Succinyl-CoA:3-ketoadic-coenzyme A transferase 2A, mitochondrial	IP00387281	56 kDa	TRUE			100%	78%					100%		
10	IP1P00114593.1 Tax_Id=10090 Gene_Symbol=Actc1 Actin, alpha cardiac muscle 1	IP00114593 (+1)	42 kDa				100%	100%	89%	90%			100%	89%	92%
11	IP1P00138399.9 Tax_Id=10090 Gene_Symbol=Heatr7b2 hypothetical protein LOC223825	IP00138399	188 kDa			100%					100%				
12	IP1P00460693.2 Tax_Id=10090 Gene_Symbol=Pcyox1 Prenylcysteine oxidase	IP00460693	58 kDa				100%						100%		
13	IP1P00387289.3 Tax_Id=10090 Gene_Symbol=Cc3 Carbonyltransferase 3	IP00387289	62 kDa				100%						100%		
14	IP1P00331394.3 Tax_Id=10090 Gene_Symbol=Onpep aspartyl aminopeptidase isoform a	IP00331394	52 kDa			100%	100%						100%		
15	IP1P00125705.1 Tax_Id=10090 Gene_Symbol=Tex101 Testis-expressed protein 101	IP00125705	27 kDa						100%	100%				100%	100%
16	IP1P00319965.3 Tax_Id=10090 Gene_Symbol=Psmd6 26S proteasome non-ATPase regulatory subunit 6	IP00319965	46 kDa					100%					100%		
17	IP1P00122815.3 Tax_Id=10090 Gene_Symbol=P4hb Putative uncharacterized protein	IP00122815 (+1)	57 kDa			100%						100%	100%		
18	IP1P00125971.1 Tax_Id=10090 Gene_Symbol=Psmc2 26S proteasome regulatory subunit S10B	IP00125971	44 kDa										100%	100%	
19	IP1P002230108.6 Tax_Id=10090 Gene_Symbol=Pdl3 Protein disulfide-isomerase A3	IP002230108	57 kDa			100%							100%		
20	IP1P00554868.2 Tax_Id=10090 Gene_Symbol=Prps1 Ribose-phosphate pyrophosphokinase 1	IP00554868 (+4)	35 kDa	TRUE					100%					100%	
21	IP1P00228630.5 Tax_Id=10090 Gene_Symbol=Fbp1 Fructose-1,6-bisphosphatase 1	IP00228630	37 kDa						100%					100%	
22	IP1P00762897.2 Tax_Id=10090 Gene_Symbol=Uggt1 UDP-glucose glycoprotein glucosyltransferase 1	IP00762897	176 kDa			100%								100%	
23	IP1P00314430.3 Tax_Id=10090 Gene_Symbol=Psm14 26S proteasome non-ATPase regulatory subunit 3	IP00314430 (+1)	61 kDa					100%					100%		
24	IP1P00119495.3 Tax_Id=10090 Gene_Symbol=Prss21 Testin	IP00119495	36 kDa					100%	89%	100%				89%	92%
25	IP1P00273646.9 Tax_Id=10090 Gene_Symbol=Gm3272.L0C10042025.Gm12033.Gapdh.Gm10359.Gm2574.Gm5138 Glycerlaldehyde-3-phosphate dehydrogenase	IP00273646	36 kDa	TRUE			100%		100%				100%	100%	
26	IP1P00225945.1 Tax_Id=10090 Gene_Symbol=LdhlaB L-lactate dehydrogenase	IP00225945	42 kDa	TRUE			83%	100%	100%					90%	100%
27	IP1P00626662.3 Tax_Id=10090 Gene_Symbol=Aldh1a1 Retinal dehydrogenase 1	IP00626662	54 kDa				100%	100%	100%				100%	100%	87%
28	IP1P00124221.1 Tax_Id=10090 Gene_Symbol=Ct4 T-complex protein 1 subunit delta	IP00124221	32 kDa				100%	100%	100%	90%			100%	100%	100%
29	IP1P00115528.3 Tax_Id=10090 Gene_Symbol=Pfs1 Plastin-3	IP00115528 (+1)	71 kDa	TRUE			100%							100%	100%
30	IP1P00119113.3 Tax_Id=10090 Gene_Symbol=Atp6v1b2 V-type proton ATPase subunit B, brain isoform	IP00119113	57 kDa	TRUE			100%						100%		
31	IP1P00225961.5 Tax_Id=10090 Gene_Symbol=Pghd3 D-3-phosphoglycerate dehydrogenase	IP00225961	57 kDa				100%						100%		
32	IP1P00468124.1 Tax_Id=10090 Gene_Symbol=Ppp3cc Serine/threonine-protein phosphatase 2B catalytic subunit gamma isoform	IP00468124	59 kDa	TRUE			100%						100%		
33	IP1P00116277.3 Tax_Id=10090 Gene_Symbol=Ct4 T-complex protein 1 subunit delta	IP00116277	58 kDa				100%	100%			91%		100%	100%	
34	IP1P00228867.5 Tax_Id=10090 Gene_Symbol=G6pd2 Glucose-6-phosphate 1-dehydrogenase 2	IP00228867	59 kDa	TRUE									100%	100%	
35	IP1P00225668.1 Tax_Id=10090 Gene_Symbol=Fam78a Protein FAM78A	IP00225668	32 kDa						100%						
36	IP1P00114256.2 Tax_Id=10090 Gene_Symbol=Sypl Isoform 1 of Synaptophysin-like protein 1	IP00114256 (+1)	29 kDa			83%			100%				100%	100%	
37	IP1P00118037.1 Tax_Id=10090 Gene_Symbol=Dppe3 Dipeptidase 3	IP00118037	54 kDa			100%		100%	100%	100%			100%	100%	100%
38	IP1P00113262.1 Tax_Id=10090 Gene_Symbol=Psm14 26S proteasome non-ATPase regulatory subunit 14	IP00113262	35 kDa						100%					100%	
39	IP1P00221540.1 Tax_Id=10090 Gene_Symbol=Erln2 Erim-2	IP00221540	38 kDa					100%					100%		
40	IP1P00469307.2 Tax_Id=10090 Gene_Symbol=Lrap1 Alpha-2-macroglobulin receptor-associated protein	IP00469307	42 kDa					100%					100%		
41	IP1P00261627.1 Tax_Id=10090 Gene_Symbol=Sucla2 Succinyl-CoA ligase [ADP-forming] subunit beta, mitochondrial	IP00261627	50 kDa				100%		90%				100%		
42	IP1P00268308.5 Tax_Id=10090 Gene_Symbol=Prss46 1700112C13Rik protein	IP00268308	34 kDa				89%	100%	100%					100%	92%
43	IP1P00131204.1 Tax_Id=10090 Gene_Symbol=Ug2 Isoform 1 of UDP-glucose-1-phosphate uridylyltransferase	IP00131204 (+1)	57 kDa			100%							100%		
44	IP1P0030874850.1 Tax_Id=10090 Gene_Symbol=Car4 Carbonic anhydrase 4	IP0030874850	34 kDa						100%	100%	100%			100%	100%
45	IP1P00130280.1 Tax_Id=10090 Gene_Symbol=Atp5a1 ATP synthase subunit alpha, mitochondrial	IP00130280	60 kDa			100%							100%		
46	IP1P00379245.2 Tax_Id=10090 Gene_Symbol=Gnpd1 Glucosamine-6-phosphate isomerase 1	IP00379245 (+1)	33 kDa						100%	100%				100%	92%
47	IP1P00117705.1 Tax_Id=10090 Gene_Symbol=Odost Dolichyl-diphosphooligosaccharide-protein glycosyltransferase 48 kDa subunit	IP00117705 (+1)	49 kDa				100%						100%		
48	IP1P00112876.2 Tax_Id=10090 Gene_Symbol=Prss52 Testicular-specific serine protease 3	IP00112876 (+1)	36 kDa				100%	100%	90%				100%	100%	100%
49	IP1P00118930.1 Tax_Id=10090 Gene_Symbol=Napa Alpha-soluble NSF attachment protein	IP00118930	33 kDa				100%			100%				100%	100%
50	IP1P00330862.5 Tax_Id=10090 Gene_Symbol=LOC100041777.Er Ern	IP00330862	59 kDa										100%		
51	IP1P00116331.1 Tax_Id=10090 Gene_Symbol=Sgt4 Isoform 1 of Small glutamine-rich tetrapeptide repeat-containing protein alpha	IP00116331 (+1)	34 kDa					100%						100%	
52	IP1P00307837.6 Tax_Id=10090 Gene_Symbol=Eef1a1 Elongation factor 1-alpha 1	IP00307837	50 kDa				100%	100%	90%				100%	100%	92%
53	IP1P00221402.7 Tax_Id=10090 Gene_Symbol=Aldoa Fructose-bisphosphate aldolase A	IP00221402 (+1)	39 kDa	TRUE				100%	90%				100%	100%	
54	IP1P00313475.1 Tax_Id=10090 Gene_Symbol=Atp5c1 ATP synthase subunit gamma, mitochondrial	IP00313475 (+2)	33 kDa									100%			100%
55	IP1P00118279.1 Tax_Id=10090 Gene_Symbol=Slc16a7 Monocarboxylate transporter 2	IP00118279	53 kDa			100%	99%	100%	100%	100%	91%	76%	90%	100%	100%
56	IP1P00355382.2 Tax_Id=10090 Gene_Symbol=Wars Isoform 2 of Tryptophanyl-tRNA synthetase, cytoplasmic	IP00355382	54 kDa				100%	89%	100%				100%	90%	100%
57	IP1P00116281.3 Tax_Id=10090 Gene_Symbol=Ct6a T-complex protein 1 subunit zeta	IP00116281	58 kDa						100%				100%	100%	
58	IP1P00122522.1 Tax_Id=10090 Gene_Symbol=Gt1 Gamma-glutamyltranspeptidase 1	IP00122522 (+1)	62 kDa				100%						100%		
59	IP1P00127841.3 Tax_Id=10090 Gene_Symbol=Slc25a5 ADP/ATP translocase 2	IP00127841	33 kDa	TRUE					89%	100%					100%
60	IP1P0012743.1 Tax_Id=10090 Gene_Symbol=Dars Aspartyl-tRNA synthetase, cytoplasmic	IP0012743 (+1)	57 kDa				100%						100%		
61	IP1P00118594.1 Tax_Id=10090 Gene_Symbol=Pfh2a2 Pyruvate dehydrogenase E1 component subunit alpha, testis-specific form, mitochondrial	IP00118594	43 kDa					100%					100%		
62	IP1P00329890.1 Tax_Id=10090 Gene_Symbol=BCD49730 cDNA sequence BCD49730	IP00329890	27 kDa						100%	100%				100%	100%
63	IP1P00116222.1 Tax_Id=10090 Gene_Symbol=Hibadh 3-hydroxyisobutyrate dehydrogenase, mitochondrial	IP00116222	35 kDa						100%	100%				100%	100%
64	IP1P00330480.1 Tax_Id=10090 Gene_Symbol= - 35 kDa protein	IP00330480	35 kDa	TRUE		100%	83%	99%	89%					89%	99%
65	IP1P00223757.4 Tax_Id=10090 Gene_Symbol=Akr1b3 Aldose reductase	IP00223757	36 kDa	TRUE										100%	
66	IP1P00127119.1 Tax_Id=10090 Gene_Symbol=Adad Delta-aminolevulinic acid dehydratase	IP00127119	36 kDa											100%	
67	IP1P00753038.1 Tax_Id=10090 Gene_Symbol=Sord Sorbitol dehydrogenase	IP00753038 (+1)	38 kDa				83%	100%	100%				100%	100%	
68	IP1P00114209.1 Tax_Id=10090 Gene_Symbol=Glul1 Glutamate dehydrogenase 1, mitochondrial	IP00114209	61 kDa				100%						100%	100%	
69	IP1P00132475.2 Tax_Id=10090 Gene_Symbol=Lman1 Putative uncharacterized protein	IP00132475	61 kDa				100%						100%		
70	IP1P00122344.1 Tax_Id=10090 Gene_Symbol=Cth Cystathionine gamma-lyase	IP00122344	44 kDa						100%				100%		
71	IP1P00469268.5 Tax_Id=10090 Gene_Symbol=Ct8 T-complex protein 1 subunit theta	IP00469268	60 kDa						100%				100%		
72	IP1P00114801.2 Tax_Id=10090 Gene_Symbol=Inpp1 Inositol polyphosphate 1-phosphatase	IP00114801	43 kDa					100%					100%		
73	IP1P00131887.3 Tax_Id=10090 Gene_Symbol=Ptgr1 Prostaglandin reductase 1	IP00131887	36 kDa						100%				100%	73%	
74	IP1P00114402.2 Tax_Id=10090 Gene_Symbol=Spam1 Hyaluronidase PH-20	IP00114402	58 kDa					100%	100%				100%	90%	100%
75	IP1P00134521.1 Tax_Id=10090 Gene_Symbol=Gapdh3 Glycerlaldehyde-3-phosphate dehydrogenase, testis-specific	IP00134521	48 kDa	TRUE			100%						100%		
76	IP1P00113669.1 Tax_Id=10090 Gene_Symbol=Big Isoform 2 of Basigin	IP00113669													

99	IPI:IP00338561.4	Tax_id=10090	Gene_Symbol=Sil1	Nucleotide exchange factor Sil1	IP00338561	52 kDa	100%		100%	
100	IPI:IP00110224.1	Tax_id=10090	Gene_Symbol=Hsd17b11	Isoform 1 of Estradiol 17-beta-dehydrogenase 11	IP00110224 (+1)	33 kDa		100%		100%
101	IPI:IP00230139.5	Tax_id=10090	Gene_Symbol=FKBP4	FK506-binding protein 4	IP00230139	52 kDa	100%		100%	
102	IPI:IP00411056.1	Tax_id=10090	Gene_Symbol=Atg8b3	SAPLT	IP00411056 (+1)	152 kDa	100%		91%	
103	IPI:IP00283264.2	Tax_id=10090	Gene_Symbol=Arad5	Arrestin domain-containing protein 5	IP00283264	37 kDa		89%		100%
104	IPI:IP00129319.3	Tax_id=10090	Gene_Symbol=Ppp1r7	Protein phosphatase 1 regulatory subunit 7	IP00129319	41 kDa		100%		100%
105	IPI:IP00475154.1	Tax_id=10090	Gene_Symbol=Rpn2	Dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit 2	IP00475154 (+2)	69 kDa	100%		76%	

Table S2. Proteins with known or potential enzymatic activities enriched in murine cytoplasmic droplets

Gene symbol	Enzymatic activity	Function in sperm and in male reproduction	Known inhibitors
Acat1	metal ion binding	NA	None
Ace	regulation of blood pressure;dipeptidyl-carboxypeptidase activity	Male fertilization; zona pellucida binding	Ramipril;Captopril; lisinopril;Fosinopril; Enalapril; Quinapril; Cilazapril
Akr1b3	alditol:NADP+ 1-oxidoreductase activity; glyceraldehyde oxidoreductase activity	NA	GP-1447 (3-[(4,5,7-trifluorobenzothiazol-2-yl)methyl]-5-methylphenyl acetic acid); WJ-38; β -glucogallin
Akr1b7	detoxifying processes; aldehyde reductase activity	No phenotype for male	None
Alad	porphobilinogen synthase activity; identical protein binding	biochemical parameters of semen quality	None
Aldh1a1	retinal dehydrogenase activity; 3-chloroallyl aldehyde dehydrogenase activity	NA	None
Aldh1a2	3-chloroallyl aldehyde dehydrogenase activity	a promising target for the development of a reversible, nonhormonal male contraceptive; testicular retinoic acid biosynthesis	bisdichloroacetyldiamine WIN 18,446
Aldoa	glycolysis for fructose-bisphosphate aldolase activity	support sperm motility	None
Amdhd2	N-acetylglucosamine-6-phosphate deacetylase activity;hydrolase activity	NA	None
Atp1b3	sodium:potassium-exchanging ATPase activity	NA	None
Atp5a1	ATP binding	NA	None
Atp5c1	hydrogen ion transporting ATPase activity, rotational mechanism	NA	None
Atp6v1b1	hydrogen ion transmembrane transporter activity	major contributor to luminal acidification	Bafilomycin A1
Atp6v1b2	hydrogen ion transporting ATPase activity, rotational mechanism	establish an acidic luminal pH in epididymis and vas deferens	None
Atp8b3	ATP binding	Acrosome development and/or in sperm function during fertilization	None
Car4	carbonate dehydratase activity; lyase activity; zinc ion binding	acidification of the epididymal fluid that prevents premature sperm activation and regulation of sperm maturation and motility	acetazolamide (AZ); 2-(4-sulfamoylphenyl-amino)-4,6-dichloro-1,3,5-triazine (TR1); 4-[3-(N,N-dimethylaminopropyl)thioereidophenylsulfonylaminoethyl]benzenesulfonamide (GA15); Sulthiame
Ces3	carboxylesterase activity, fatty-acyl-ethyl-ester synthase activity	NA	None
Cpne3	protein serine/threonine kinase activity	NA	None
Cth	pyridoxal phosphate binding;calmodulin binding	NA	DL-propargyl glycine;DL-propargylglycine
Dars	aspartate-tRNA ligase activity	NA	chloramphenicol analogs
Ddost	dolichyl-diphosphooligosaccharide-protein glycotransferase activity	NA	None
Dnpep	aminopeptidase activity; zinc ion binding; proteolysis	NA	None
Dpep3	metal ion binding;proteolysis	interactions with the Ts4 antigens, including TEX101, in testis	None
Eef1a1	GTP binding	Localization in the midpiece of the sperm tail, involve in PASKIN-dependent phosphorylation	Gamendazole
Fbp1	metal ion binding; AMP binding; catalytic activity	Key substrate cycle enzyme and control of glycolysis in spermatid	adenosine monophosphate (AMP); MB07803
Fkbp4	peptidyl-prolyl cis-trans isomerase activity; copper-dependent protein binding; GTP binding	fertilizing capacity and sperm morphology;	None
G6pd2	glucose-6-phosphate dehydrogenase activity; NADP binding	probably related to X chromosome inactivation during spermatogenesis;	beta-naphthoquinone-4-sulfonic acid; Cu ²⁺ ;Dehydroepiandrosterone (DHEA)
G6pdx	protein homodimerization activity;carbohydrate binding	NA	None
Gapdhs	glyceraldehyde-3-phosphate dehydrogenase (NAD ⁺) (phosphorylating) activity, NAD binding	sperm motility and male fertility	Koningic acid (heptelidic acid); alpha-Chlorohydrin;ornidazole
Ggt1	gamma-glutamyltransferase activity;	sperm GGT may be a limiting factor for male pronuclear formation in polyspermic oocytes	serine/borate; OU749; acivicin
Glud1	glutamate dehydrogenase [NAD(P) ⁺] activity; positive regulation of insulin secretion; ADP binding	NA	glutamate dehydrogenases
Glul	ATP binding	maintaining an optimal microenvironment for sperm maturation	methionine sulfoximine
Gm3272;LOC100042025; Gm12033;Gapdh;Gm10293;Gm10359;Gm2574;Gm5138	glyceraldehyde-3-phosphate dehydrogenase (phosphorylating) activity	sperm motility	ornidazole; alpha-chlorohydrin; 1-chloro-3-hydroxypropanone.
Gnpda1	glucosamine-6-phosphate deaminase activity	NA	None
Gpi1	glucose-6-phosphate isomerase activity	involved in sperm agglutination	None
Hibadh	NAD binding; pentose-phosphate shunt	NA	None
Hsd17b11	steroid biosynthetic process; estradiol 17-beta-dehydrogenase activity; nucleotide binding	NA	None
Inpp1	metal ion binding; inositol-1,4-bisphosphate 1-phosphatase activity	NA	None

Lap3	metal ion binding;aminopeptidase activity; proteolysis	NA	Bestatin
Ldhal6b	L-lactate dehydrogenase activity, oxidoreductase activity	NA	None
Ldhc	L-lactate dehydrogenase activity; oxidation reduction	required for male fertility and sperm function	sodium oxamate
Oxct2a	3-oxoacid CoA-transferase activity; ketone body catabolic process	testicular haploid germ cell-specific gene	None
P4hb	electron carrier activity, isomerase activity	esponsible for spermatogenesis	testosterone undecanoate; levonorgestrel
Pcyox1	prenylcysteine catabolic process; oxidoreductase activity,	NA	None
Pdha2	pyruvate dehydrogenase (acetyl-transferring) activity; oxidoreductase activity	tyrosine phosphorylated in capacitated spermatozoa and required for sperm hyperactivation and acrosome reaction	bromopyruvate (BP)
Pdia3	protein disulfide isomerase activity; electron carrier activity	play a critical role in gamete fusion;	None
Pgk2	ATP binding; kinase activity	associated with male fertility and sperm function	2-oxo-1,5-bisphosphonopentane
Phgdh	NAD binding	NA	Koningic acid; l-Serine
Ppp1cc	protein serine/threonine phosphatase activity; protein amino acid dephosphorylation; metal ion binding	required for spermiogenesis	Phosphatidic acid (PA)
Ppp3cc	phosphoprotein phosphatase activity; calmodulin binding	regulation of flagellar motility	None
Prps1	protein homodimerization activity; ribose phosphate diphosphokinase activity; ADP binding	NA	aminopyrimidopyrimidine nucleotide 4-amino-8-(beta-D-ribofuranosylamino)pyrimido[5,4-d]pyrimidine[-5' -monophosphate (APP-MP)
Prps11l	kinase activity; metal ion binding	NA	None
Prps2	protein homodimerization activity;ribose phosphate diphosphokinase activity; ADP binding	NA	aminopyrimidopyrimidine nucleotide 4-amino-8-(beta-D-ribofuranosylamino)pyrimido[5,4-d]pyrimidine[-5' -monophosphate (APP-MP)
Prss21	serine-type endopeptidase activity; catalytic activity	regulation of epididymal sperm cell maturation and sperm-fertilizing ability	None
Prss46	molecular function unknown	NA	None
Prss52	serine-type endopeptidase activity; proteolysis	NA	None
Psmc6	ATP binding; nucleoside-triphosphatase activity; protein catabolic process	have a role in the remodeling of the outer dense fibers and other tail components during epididymal transit; degrade an ubiquitinated ZP (glyco)protein during fertilization	MG132
Psmd14	metal ion binding; ubiquitin thiolesterase activity	NA	None
Psmd3	regulation of protein catabolic process;enzyme regulator activity	NA	None
Psmd6	unknown	NA	None
Ptgr1	2-alkenal reductase activity;13-prostaglandin reductase activity; 15-oxoprostaglandin 13-oxidase activity	NA	None
Rpn2	dolichyl-diphosphooligosaccharide-protein glycotransferase activity; ribosome binding	NA	None
Sgta	protein heterodimerization activity; protein homodimerization activity; binding	NA	None
Slc25a5	transporter activity; binding	NA	None
Sord	L-iditol 2-dehydrogenase activity; identical protein binding	sperm motility and protein tyrosine phosphorylation	CP-642,931; SDI-711
Spam1	hyaluronoglucosaminidase activity; catalytic activity	function in sperm entry into the cumulus and sperm penetration through the cumulus matrix during fertilization	polyphenols (tannic acid, apigenin and quercetin) ; sodium cromoglycate and sodium auro-thiomalate
Sucla2	ATP binding; succinate-CoA ligase (ADP-forming) activity;	NA	None
Ugg1	UDP-glucose:glycoprotein glucosyltransferase activity; protein amino acid glycosylation	NA	None
Ugp2	metal ion binding; UTP:glucose-1-phosphate uridylyltransferase activity	NA	None
Vdac3	porin activity; nucleotide binding	Function in sperm motility and epididymal axonemes structure	None
Wars	ATP binding; tryptophan-tRNA ligase activity	NA	None

Table S3. Species with epididymal sperm bearing cytoplasmic droplets.

Species	Literatures
Human	(1-4)
Monkey	(5, 6)
Marmoset	(7)
Stallion	(8)
Donkey	(9)
Boar	(10-14)
Bovine	(15, 16)
Goat	(17)
Ram	(18, 19)
Llamas	(20)
Cheetah	(21)
Tiger	(21)
Leopard	(21)
Puma	(21)
Cat	(22)
Wolf	(23)
Dog	(24-28)
African elephant	(29, 30)
Rabbit	(19, 31, 32)
Guinea pig	(33-35)
Hamster	(19, 36, 37)
Rat	(19, 38-40)
Mouse	(18)
Bat	(41)
Macropus	(42)
Trichosurus vulpecula	(43, 44)
Bandicoot	(45)
Echidna	(46)
Platypus	(47)
Ostrich	(48)
Hagfish	(49)

References cited in Table S3:

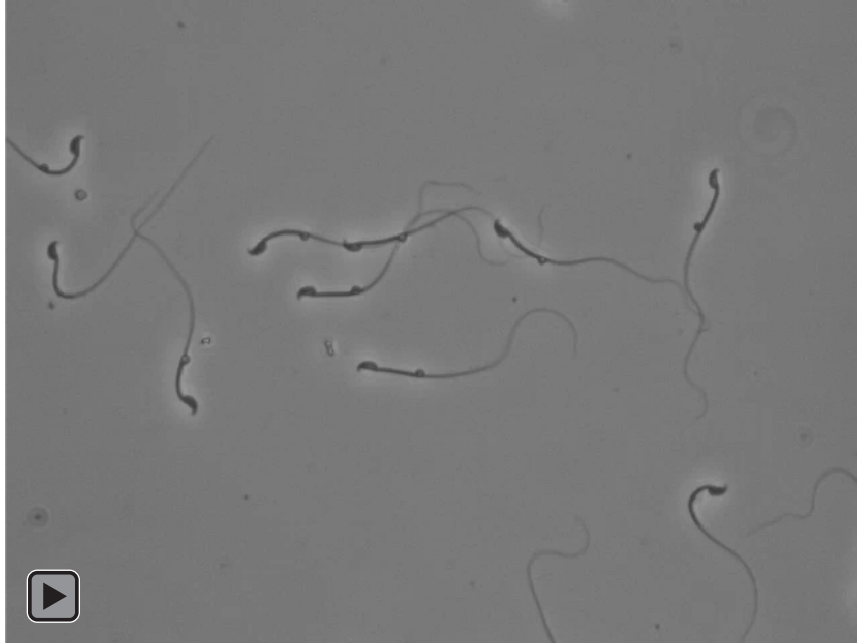
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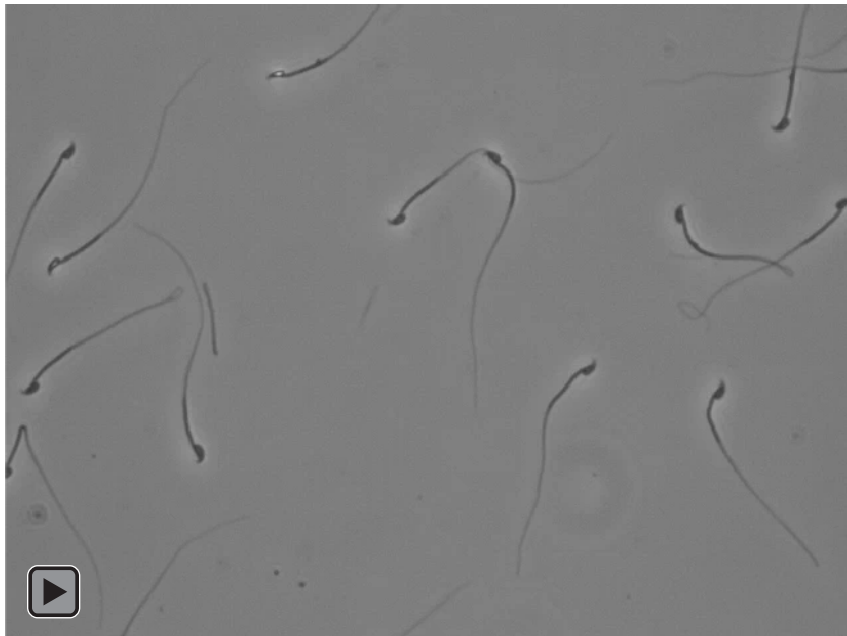
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Supporting Videos:



Supporting Video S1. CD-bearing murine epididymal spermatozoa displayed vigorous motility after activation in HTF-HEPES medium at 37°C for 30 minutes.



Supporting Video S2. “CD-less” murine epididymal spermatozoa developed no motility after activation in HTF-HEPES medium at 37°C for 30 minutes.