

Standard substance

Folic acid, uridine, cytidine, deoxycytidine, N-phenylacetylaspartic acid, hexanoylcarnitine, carnitine 12:0, LysoPC (14:0), LysoPC (18:0), LysoPE (22:0)

Sample information

Two hundreds and three individuals collected from June 2015 to December 2015 were divided into 7 groups: one group containing 203 men who attended the Xiangya hospital because of conception failure for at least 12 months was defined as male infertility (MI). The MI patients included 45 erectile dysfunction patients, 56 asthenozoospermia patients and 36 oligozoospermia patients, 9 teratospermia patients, 4 necrospermia patients, 3 cryptozoospermia patients, 7 azoospermia patients. The other group containing 43 age-matched fertile men was defined as healthy controls. Referring to fifth edition World Health Organization (WHO) Laboratory Manual for the Examination and Processing of Human Semen, oligozoospermia was defined as total sperm number <40 million per ejaculate; asthenozoospermia was defined with the following ranges: rapid progressive motility (a)<25% or rapid progressive motility(a) + slow progressive motility (b)<50%. According to International Index of Erectile Function (IIEF), erectile dysfunction was defined as IIEF<21. Detailed sample information in Table S1. Hospital did not provide the relevant sample information about erectile function patients. Before participants were included in the analysis, it was confirmed that they did not have metabolic diseases.

Table S1. Clinical Data of Participant

	healthy control (43)	Asthenozoospermia (56)	Oligozoospermia (36)
Age	28.21±3.24	30.26±5.77	27.83±4.82
Sperm concentration(10 ⁶ per ml)	112.01±35.87	127.47±29.12	27.60±11.23
Total progressive motility (a + b, %)	73.15±17.43	36.54±8.54	47.15±16.83
Rapid progressive motility (a, %)	41.92±15.45	12.88±7.59	24.91±15.09
Slow progressive motility (b, %)	31.23±12.97	23.66±9.92	22.23±6.44
Deformity rate (%)	7.43±3.12	12.73±2.95	14.44±5.00

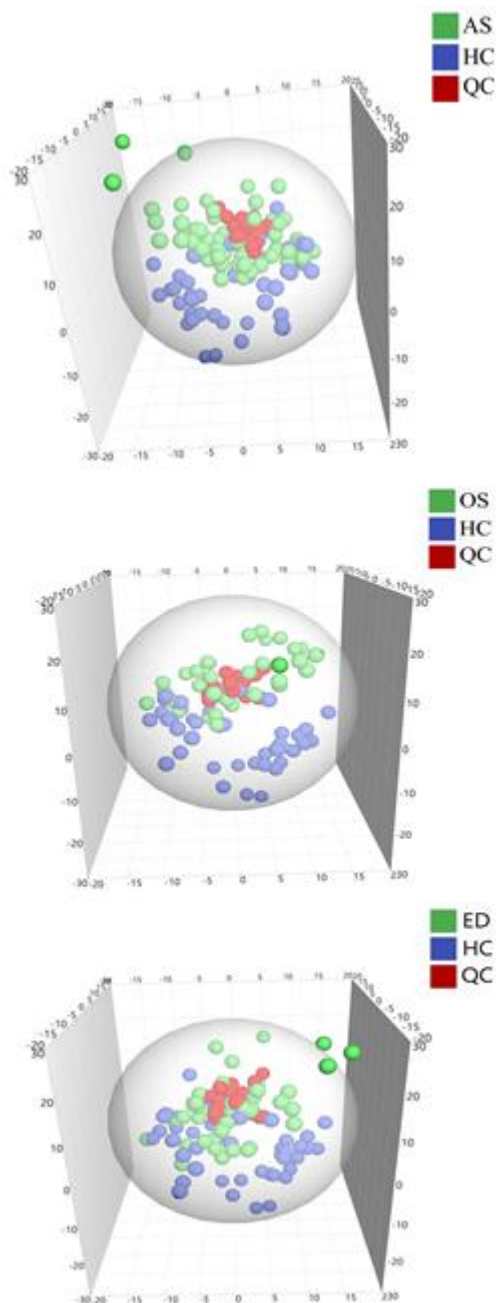


Fig.1S.The PCA score three-dimensional plots of (a) asthenospermia (AS) group, (b) oligozoospermia (OS) group and (c) erectile dysfunction (ED) group

Qualitative process

As for, m/z 524.3698

Possible formulas were generated using the molecular formula predict software of Shimadzu LCMSsolution. Element number restriction, lewis check, isotopic pattern and hydrogen/carbon element ratio check were used to reduce the number of candidate molecular formulas. Molecular formula calculation software to obtain $[M + H]$ the only possible formula is $C_{26}H_{54}NO_4P$. Further metabolites database search and found that the formula most likely corresponds to LysoPC (18:0).

From the MS/MS spectra, the metabolites have obvious characteristic phosphatidylcholine compounds m/z 184.0735, 208.5433, 448.2923, 465.2359 and 507.3857. Finally, the MS/MS fragment of the LysoPC

(18:0) chemical standard is consistent with the metabolite, including to 184.0744, 208.5434, 448.2877, 465.2234 and 507.3608. The compound was further confirmed to be LysoPC (18:0). According to the above ideas, 23 potential biomarkers were finally identified, of which 10 metabolite identified and validated by the authorized chemical standard. Others are putatively identification by detailed comparisons with metabolite databases.

Table S2. Identification and fragmentation ions by liquid chromatography coupled with electrospray Ionization mass spectrometry

	Exact mass	Formula	Metabolite	MS/MS fragments	Matched MS/MS fragments
1	467.3117	C ₂₂ H ₄₆ NO ₇ P	LysoPC(14:0)	184.0732, 391.2249 450.2928	184.0738, 391.2257 450.2979
2	523.3724	C ₂₆ H ₅₄ NO ₇ P	LysoPC(18:0)	184.0735, 208.5433, 447.2923, 465.2359 506.3857	184.0744, 208.5434, 447.2877, 465.2234 5076.3608
3	529.2065	C ₂₇ H ₄₈ NO ₇ P	LysoPE(22:4)	287.2730, 315.2680, 389.3050, 469.2710	no
4	605.8496	C ₃₂ H ₆₄ NO ₇ P	LysoPC(24:1)	184.0735, 307.3360, 349.3460, 407.3887, 423.3830, 588.4390	no
5	537.7196	C ₂₇ H ₅₆ NO ₇ P	LysoPE(22:0)	203.1807, 333.2806 399.3256, 475.4163 519.4070	203.1829, 333.2851 399.3259, 475.4172 519.4092
6	569.3612	C ₃₀ H ₅₂ NO ₇ P	LysoPC(22:5)	184.0737, 321.2087 457.2089, 501.2421	no
7	251.2387	C ₁₂ H ₁₃ NO ₅	N-phenylacetylaspartic acid	192.0660, 206.0810 234.0760, 252.0870	192.0671, 206.0817 234.0767, 252.0884
8	425.1131	C ₁₃ H ₂₂ N ₄ O ₈ S ₂	S-Glutathionyl-L-cysteine	219.9889, 364.1604 350.0641	no
9	481.6480	C ₂₅ H ₃₉ NO ₆ S	N-Acetyl-LTE4	186.0161, 341.2067	no
10	441.0869	C ₁₉ H ₁₉ N ₇ O ₆	Folic acid	176.0540, 120.0457 295.0947	176.0556, 120.0462 295.0949
11	260.1854	C ₁₃ H ₂₅ NO ₄	Hexanoylcarnitine	99.0813, 146.1191 162.1134, 214.1834 242.1762	99.0867, 146.1189 162.1127, 214.1854 242.1771
12	344.2805	C ₁₉ H ₃₈ NO ₄	Carnitine 12:0	115.0563, 184.0732 211.0633, 265.2718 303.2189	115.0574, 184.0767 211.0646, 265.2746 303.2194
13	481.3149	C ₂₃ H ₄₈ NO ₇ P	LysoPC(15:0)	184.0725, 339.2893 405.2541, 457.73444	no
14	503.3391	C ₂₅ H ₄₆ NO ₇ P	LysoPE(20:3)	163.0155, 265.2724 367.1881, 445.2690 461.2639	no
15	505.3572	C ₂₅ H ₄₈ NO ₇ P	LysoPE(20:2)	163.0174, 311.2935 365.2712, 447.2899	no

16	368.4874	C ₁₉ H ₂₈ O ₅ S	Testosterone sulfate	171.9059, 242.9423 271.2060	no
17	370.5071	C ₁₉ H ₃₀ O ₅ S	5 α -Dihydrotestosterone sulfate	232.9095, 242.9423 268.0499, 273.0923	no
18	227.0788	C ₉ H ₁₃ N ₃ O ₄	Deoxycytidine	112.0507, 186.9537	112.0534, 186.9521
19	243.2190	C ₉ H ₁₃ N ₃ O ₅	Cytidine	112.0482, 244.0934	112.0476, 244.0937
20	251.0210	C ₁₀ H ₁₂ N ₄ O ₄	Deoxyinosine	137.0456, 253.0916	no
21	244.2338	C ₉ H ₁₂ N ₂ O ₆	Uridine	96.0123, 113.0367	96.0141, 113.0354
22	483.3078	C ₂₆ H ₄₅ NO ₅ S	Lithocholytaurine	331.3015, 353.6796 359.2940, 405.2383 464.3150	no
23	488.1828	C ₁₄ H ₂₆ N ₄ O ₁₁ P ₂	Citicoline	134.3948, 175.5719 407.1243, 425.1273	no