

Supplementary Table S1. Sperm parameters, seminal plasma biochemical components and semen MC levels of the study participants ($n = 837$)

Outcomes	Percent detected (%)	Mean \pm SD	Median (25th, 75th)	Range (Min–Max)
Sperm motility parameters				
Total motility (%)	100	51.01 \pm 19.07	52.50 (38.70, 65.05)	1.3–96.2
Progressive motility (%)	100	39.94 \pm 17.50	40.30 (27.30, 52.85)	0–92.1
Sperm velocity parameters				
VCL ($\mu\text{m/s}$)	100	43.27 \pm 8.76	43.20 (38.50, 48.35)	0–65.9
VSL ($\mu\text{m/s}$)	100	19.50 \pm 4.64	19.60 (16.90, 22.20)	0–39.2
VAP ($\mu\text{m/s}$)	100	23.12 \pm 4.82	23.20 (20.60, 25.90)	0–41.1
ALH (μm)	100	2.30 \pm 0.56	2.30 (2.00, 2.60)	0–4.1
LIN (%)	100	44.74 \pm 8.55	44.50 (40.10, 50.00)	0–73.0
WOB (%)	100	53.15 \pm 8.09	53.00 (49.80, 57.80)	0–76.1
STR (%)	100	79.19 \pm 10.22	80.50 (76.60, 84.20)	0–95.5
Biochemical components				
Zinc (mmol/L)	100	2.65 \pm 1.17	2.53 (1.79, 3.41)	0.06–6.85
NAG (U/L)	100	22.27 \pm 12.35	19.67 (14.01, 27.42)	0.08–101.22
Citric acid (mmol/L)	100	22.68 \pm 14.26	20.10 (11.48, 30.77)	0.21–90.01
Fructose (mmol/L)	100	14.64 \pm 7.00	14.10 (9.68, 19.10)	0–40
Semen MCs ^a ($\mu\text{g/L}$)	88.6	0.16 \pm 0.07	0.16 (0.12, 0.20)	<LOD to 0.62

Note. VCL, curvilinear velocity; VSL, straight-line velocity; VAP, average path velocity; ALH, amplitude of lateral head displacement; LIN, linearity; WOB, wobble; STR, straightness; NAG, neutral α -glucosidase; SD, standard deviation; Min, minimum; Max, maximum; MCs, microcystins; LOD, limit of detection. ^aThe limit of detection (LOD) of microcystins is 0.1 $\mu\text{g/L}$. The concentrations of MCs below the LOD were imputed with a random forest model.

Supplementary Table S2. Spearman correlation coefficients between covariates and sperm motility, velocity parameters ($n = 837$)

Covariates	Total motility	Progressive motility	VCL	VSL	VAP	ALH	LIN	WOB	STR
Age	-0.025	-0.007	0.069*	0.071*	0.073*	0.040	0.044	0.036	0.027
BMI	-0.046	-0.052	-0.046	-0.041	-0.046	-0.043	-0.011	-0.008	-0.028
Race	-0.028	-0.033	0.002	0.024	0.019	-0.038	0.035	0.033	0.039
Abstinence time	0.014	-0.003	-0.049	-0.081*	-0.067	-0.046	-0.040	-0.025	-0.055
Educational level	0.039	0.053	0.080*	0.111**	0.110**	0.026	0.081*	0.083*	0.065
Income	0.005	0.006	0.019	0.019	0.016	-0.015	0.003	-0.003	0.006
Smoking status	-0.048	-0.046	-0.057	0.002	-0.019	-0.056	0.043	0.037	0.002
Drinking status	-0.016	-0.005	0.024	0.044	0.035	0.005	0.014	0.000	0.026
Tea/coffee consumption	-0.068*	-0.057	0.005	-0.044	-0.018	-0.011	-0.018	-0.037	0.001
Hypertension	-0.010	-0.026	-0.044	-0.018	-0.018	-0.022	0.033	0.044	0.002
Diabetes	0.006	0.001	-0.023	-0.022	-0.023	-0.008	0.023	0.022	0.024

Note. Estimates calculated using Spearman correlation. VCL, curvilinear velocity; VSL, straight-line velocity; VAP, average path velocity; ALH, amplitude of lateral head displacement; LIN, linearity; WOB, wobble; STR, straightness; BMI, body mass index. * $P < 0.05$, ** $P < 0.01$.

Supplementary Table S3. Spearman correlation coefficients between covariates and seminal plasma biochemical components ($n = 837$)

Covariates	Zinc	NAG	Citric acid	Fructose
Age	0.036	0.117**	0.063	0.010
BMI	0.069*	0.034	0.087*	-0.051
Race	0.033	0.019	0.030	0.049
Abstinence time	0.036	0.077*	0.043	-0.012
Educational level	0.044	0.059	0.066	-0.039
Income	-0.031	-0.031	-0.004	-0.005
Smoking status	-0.026	-0.012	-0.039	0.124**
Drinking status	0.016	0.014	-0.004	0.019
Tea/coffee consumption	-0.013	0.015	-0.015	-0.013
Hypertension	0.016	-0.007	0.016	0.005
Diabetes	-0.003	0.040	0.003	-0.005

Note. Estimates calculated using Spearman correlation. NAG, neutral α -glucosidase; BMI, body mass index. * $P < 0.05$, ** $P < 0.01$.

Supplementary Table S4. Regression coefficients [β (95% CI)] for changes in sperm motility and velocity parameters associated with seminal plasma biochemical components ($n = 837$)^a

Outcomes	Zinc		NAG		Citric acid		Fructose	
	Adjusted β (95% CI)	P -value ^b	Adjusted β (95% CI)	P -value	Adjusted β (95% CI)	P -value	Adjusted β (95% CI)	P -value
Total motility	1.478 (-1.966, 4.921)	0.400	2.936 (1.882, 3.991)	< 0.001	1.047 (0.185, 1.909)	0.017	-2.865 (-4.181, -1.549)	< 0.001
Progressive motility	1.810 (-1.351, 4.971)	0.261	2.423 (1.452, 3.395)	< 0.001	1.183 (0.393, 1.973)	0.003	-2.393 (-3.603, -1.182)	< 0.001
VCL	1.092 (-0.489, 2.673)	0.176	0.480 (-0.012, 0.972)	0.056	0.798 (0.405, 1.192)	< 0.001	-0.359 (-0.970, 0.251)	0.249
VSL	-0.692 (-1.528, 0.144)	0.105	-0.350 (-0.610, -0.090)	0.008	0.136 (-0.074, 0.346)	0.204	0.244 (-0.079, 0.567)	0.138
VAP	-0.296 (-1.167, 0.575)	0.505	-0.028 (-0.300, 0.243)	0.838	0.254 (0.036, 0.472)	0.022	0.023 (-0.313, 0.360)	0.893
ALH	0.129 (0.029, 0.230)	0.011	0.067 (0.036, 0.098)	< 0.001	0.050 (0.025, 0.075)	< 0.001	-0.042 (-0.081, -0.003)	0.034
LIN	-2.067 (-3.607, -0.526)	0.009	-0.908 (-1.386, -0.430)	< 0.001	-0.329 (-0.717, 0.059)	0.096	1.019 (0.425, 1.612)	0.001
WOB	-1.261 (-2.722, 0.201)	0.091	-0.271 (-0.727, 0.184)	0.243	-0.170 (-0.537, 0.197)	0.364	0.595 (0.031, 1.159)	0.039
STR	-0.484 (-2.331, 1.363)	0.607	-0.611 (-1.185, -0.037)	0.037	0.057 (-0.406, 0.521)	0.808	0.993 (0.283, 1.703)	0.006

Note. Estimates calculated using multiple linear regression models adjusted for age, BMI, abstinence time, smoking status and drinking status. CI , confidence interval; NAG, neutral α -glucosidase; VCL, curvilinear velocity; VSL, straight-line velocity; VAP, average path velocity; ALH, amplitude of lateral head displacement; LIN, linearity; WOB, wobble; STR, straightness. ^aZinc, NAG, citric acid and fructose were square root-transformed. Other parameters were not transformed. ^b $P < 0.05$ was considered as statistically significant.

Supplementary Table S5. Stratified analysis for the association between ln-transformed MC levels and sperm motility, velocity parameters and seminal plasma chemical components by stratification of age^a

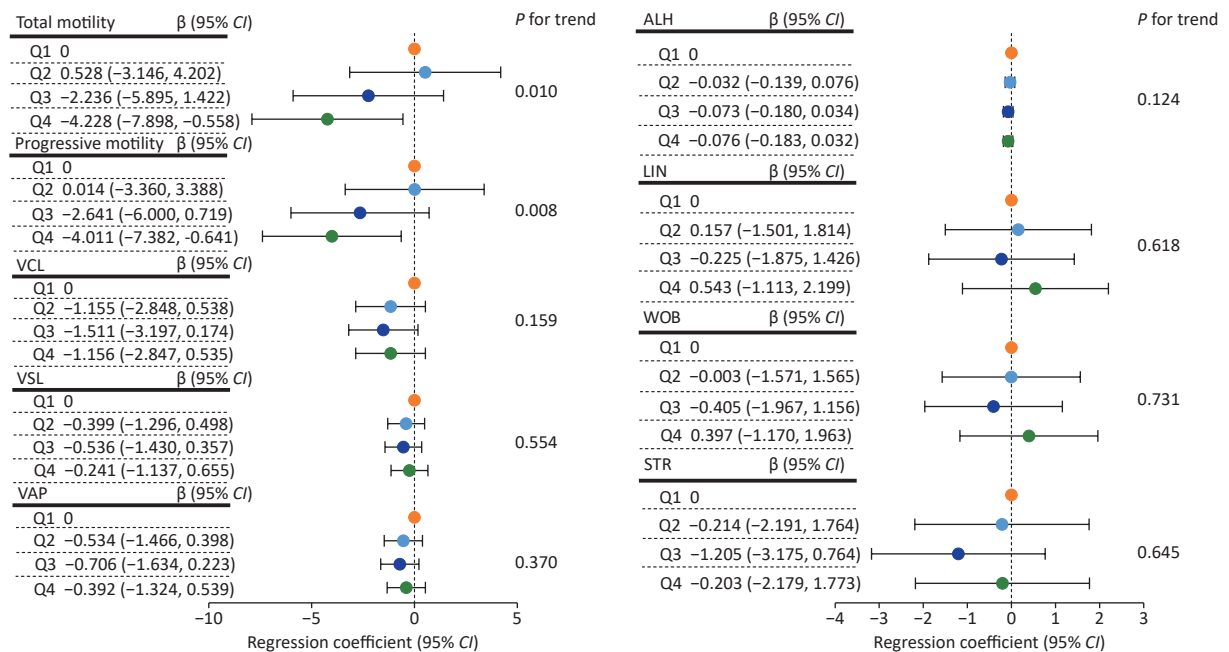
Outcomes	Semen MC levels			
	Age: 20–30 (n = 387)	P-value ^b	Age: 31–40 (n = 450)	P-value
	Adjusted β (95% CI)		Adjusted β (95% CI)	
Motility parameters				
Total motility	-6.720 (-11.129, -2.310)	0.003	-2.340 (-6.659, 1.978)	0.287
Progressive motility	-7.036 (-11.119, -2.953)	0.001	-2.296 (-6.220, 1.629)	0.251
Velocity parameters				
VCL	-3.282 (-5.458, -1.107)	0.003	-0.081 (-1.938, 1.777)	0.932
VSL	-1.415 (-2.511, -0.318)	0.012	0.116 (-0.922, 1.155)	0.826
VAP	-1.564 (-2.721, -0.407)	0.008	-0.010 (-1.073, 1.052)	0.985
ALH	-0.167 (-0.303, -0.032)	0.016	-0.024 (-0.145, 0.097)	0.695
LIN	-0.460 (-2.540, 1.621)	0.664	0.599 (-1.297, 2.496)	0.535
WOB	-0.232 (-2.232, 1.768)	0.820	0.423 (-1.343, 2.190)	0.638
STR	-1.562 (-4.134, 1.010)	0.233	0.402 (-1.786, 2.589)	0.718
Biochemical components				
Zinc	-0.093 (-0.179, -0.006)	0.036	-0.073 (-0.159, 0.013)	0.097
NAG	-0.480 (-0.766, -0.194)	0.001	-0.177 (-0.447, 0.093)	0.198
Citric acid	-0.501 (-0.838, -0.164)	0.004	-0.425 (-0.769, -0.081)	0.016
Fructose	0.055 (-0.175, 0.286)	0.637	0.094 (-0.126, 0.314)	0.401

Note. Estimates calculated using multiple linear regression models adjusted for BMI, abstinence time, smoking status and drinking status. Stratified analysis divided age into two groups. MC, microcystin; CI, confidence interval; VCL, curvilinear velocity; VSL, straight-line velocity; VAP, average path velocity; ALH, amplitude of lateral head displacement; LIN, linearity; WOB, wobble; STR, straightness; NAG, neutral α -glucosidase. ^azinc, NAG, citric acid and fructose were square root-transformed. MCs were ln-transformed. Other parameters were not transformed. ^b $P < 0.05$ was considered as statistically significant.

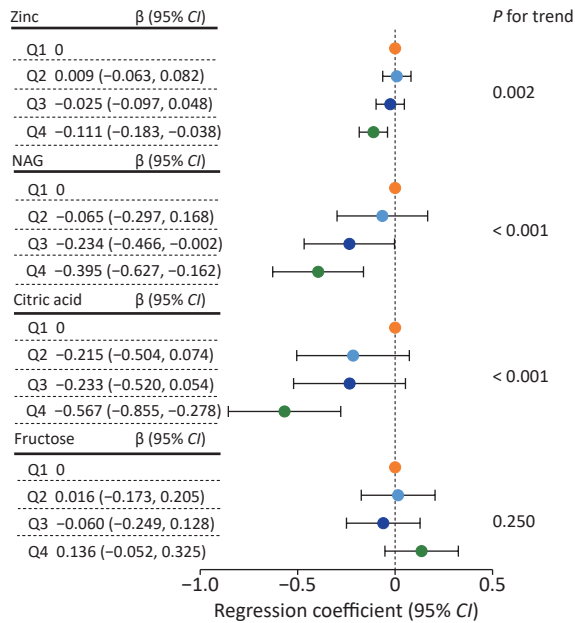
Supplementary Table S6. Stratified analysis for the association between seminal plasma biochemical components and sperm motility and velocity parameters by stratification of age^a

Age	Outcomes	Zinc		NAG		Citric acid		Fructose	
		Adjusted β (95% CI)	P-value ^b	Adjusted β (95% CI)	P-value	Adjusted β (95% CI)	P-value	Adjusted β (95% CI)	P-value
20–30	Total motility	3.935 (-1.237, 9.107)	0.135	3.635 (2.116, 5.154)	< 0.001	1.880 (0.567, 3.194)	0.005	-3.803 (-5.730, -1.877)	< 0.001
	Progressive motility	4.372 (-0.426, 9.170)	0.074	3.481 (2.071, 4.890)	< 0.001	1.979 (0.762, 3.197)	0.002	-3.295 (-5.090, -1.499)	< 0.001
	VCL	2.783 (0.240, 5.326)	0.032	1.398 (0.639, 2.157)	< 0.001	1.208 (0.565, 1.852)	< 0.001	-0.417 (-1.386, 0.552)	0.398
	VSL	0.335 (-0.950, 1.620)	0.608	-0.015 (-0.403, 0.372)	0.938	0.421 (0.094, 0.747)	0.012	0.288 (-0.198, 0.775)	0.245
	VAP	0.771 (-0.584, 2.126)	0.264	0.361 (-0.047, 0.768)	0.083	0.541 (0.198, 0.884)	0.002	0.069 (-0.446, 0.583)	0.793
	ALH	0.213 (0.055, 0.371)	0.008	0.120 (0.073, 0.166)	< 0.001	0.071 (0.031, 0.111)	0.001	-0.060 (-0.120, 0.000)	0.049
	LIN	-0.714 (-3.132, 1.705)	0.562	-0.878 (-1.602, -0.153)	0.018	0.058 (-0.562, 0.677)	0.855	1.371 (0.465, 2.278)	0.003
	WOB	0.055 (-2.270, 2.381)	0.963	-0.261 (-0.962, 0.440)	0.465	0.194 (-0.401, 0.790)	0.521	1.004 (0.128, 1.880)	0.025
	STR	1.518 (-1.474, 4.510)	0.319	-0.265 (-1.164, 0.634)	0.562	0.540 (-0.225, 1.305)	0.166	1.398 (0.271, 2.525)	0.015
	31–40	Total motility	-0.384 (-5.058, 4.290)	0.872	2.338 (0.856, 3.820)	0.002	0.456 (-0.712, 1.624)	0.443	-2.215 (-4.046, -0.384)
Progressive motility		-0.084 (-4.333, 4.165)	0.969	1.541 (0.187, 2.896)	0.026	0.630 (-0.431, 1.690)	0.244	-1.710 (-3.377, -0.043)	0.044
VCL		-0.212 (-2.228, 1.803)	0.836	-0.288 (-0.933, 0.358)	0.381	0.499 (-0.003, 1.000)	0.051	-0.207 (-1.001, 0.587)	0.609
VSL		-1.523 (-2.637, -0.410)	0.007	-0.615 (-0.970, -0.259)	0.001	-0.077 (-0.358, 0.203)	0.588	0.276 (-0.166, 0.718)	0.220
VAP		-1.171 (-2.314, -0.027)	0.045	-0.350 (-0.717, 0.016)	0.061	0.032 (-0.256, 0.319)	0.829	0.063 (-0.389, 0.516)	0.783
ALH		0.064 (-0.066, 0.194)	0.335	0.022 (-0.019, 0.064)	0.290	0.033 (0.001, 0.066)	0.044	-0.022 (-0.073, 0.029)	0.398
LIN		-3.174 (-5.203, -1.145)	0.002	-0.887 (-1.539, -0.235)	0.008	-0.614 (-1.123, -0.104)	0.018	0.790 (-0.015, 1.595)	0.054
WOB		-2.374 (-4.272, -0.477)	0.014	-0.255 (-0.867, 0.357)	0.412	-0.457 (-0.933, 0.019)	0.060	0.333 (-0.419, 1.086)	0.384
STR		-1.966 (-4.324, 0.392)	0.102	-0.840 (-1.594, -0.085)	0.029	-0.259 (-0.849, 0.332)	0.390	0.708 (-0.222, 1.638)	0.135

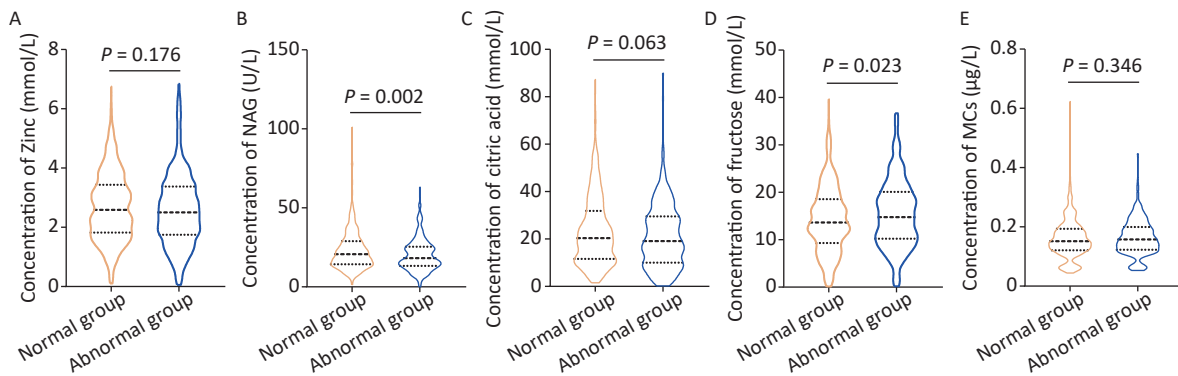
Note. Estimates calculated using multiple linear regression models adjusted for BMI, abstinence time, smoking status and drinking status. Stratified analysis divided age into two groups. *CI*, confidence interval; VCL, curvilinear velocity; VSL, straight-line velocity; VAP, average path velocity; ALH, amplitude of lateral head displacement; LIN, linearity; WOB, wobble; STR, straightness; NAG, neutral α -glucosidase. ^azinc, NAG, citric acid and fructose were square root-transformed. Other parameters were not transformed. ^b $P < 0.05$ was considered as statistically significant.



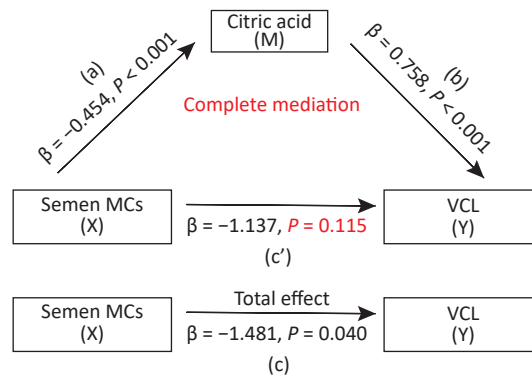
Supplementary Figure S1. Regression coefficients [β (95% CI)] for changes in sperm motility and velocity parameters across semen MC quartiles ($n = 837$). First quartile (Q1) was used as a reference group. Multivariable linear regression models were adjusted for age, BMI, abstinence time, smoking and drinking status. Semen MC levels were ln-transformed. Other parameters were not transformed. Test for trend was performed by entering the median value of each quartile of semen MCs as a continuous variable in the models. CI, confidence interval; BMI, body mass index; MCs, microcystins; VCL, curvilinear velocity; VSL, straight-line velocity; VAP, average path velocity; ALH, amplitude of lateral head displacement; LIN, linearity; WOB, wobble; STR, straightness; Q, quartile.



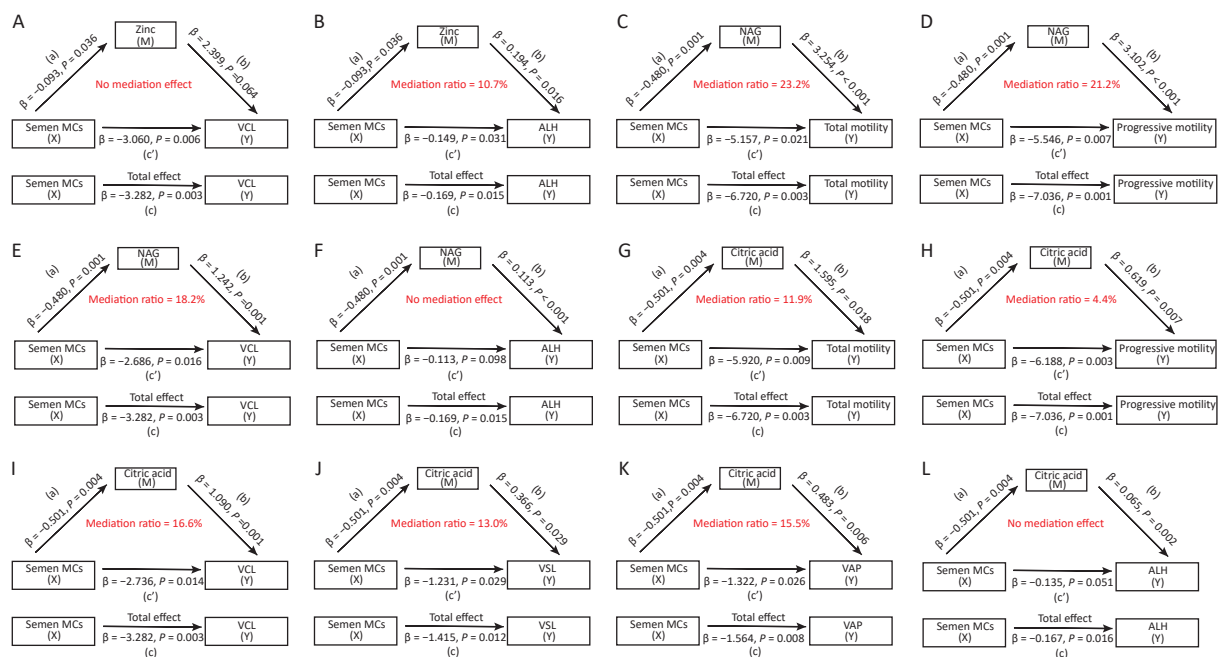
Supplementary Figure S2. Regression Coefficients [β (95% CI)] for changes in seminal plasma biochemical components across MC quartiles ($n = 837$). First quartile (Q1) was used as a reference group. Multivariable linear regression models were adjusted for age, BMI, abstinence time, smoking and drinking status. Zinc, NAG, citric acid and fructose were square root-transformed. Semen MC levels were ln-transformed. Test for trend was performed by entering the median value of each quartile of semen MCs as a continuous variable in the models. CI, confidence interval; MCs, microcystins; BMI, body mass index; NAG, neutral α -glucosidase.



Supplementary Figure S3. The concentrations of seminal plasma zinc (A), NAG (B), citric acid (C), fructose (D), and MCs (E) in normal group ($n = 552$) and abnormal group ($n = 285$). The abnormal semen quality parameters referred to the sperm concentration, count, total motility, and progressive motility lower than $15 \times 10^6/\text{mL}$, $39 \times 10^6/\text{ejaculate}$, 40%, and 32%, respectively. When all the four parameters were higher than the WHO standards, the participant was assigned to the normal group or the participant was assigned to the abnormal group. Mann-Whitney U test was used to analyze the difference between the two groups. P-value < 0.05 was considered statistical significance. NAG, neutral α -glucosidase; MCs, microcystins.



Supplementary Figure S4. Mediation analysis model exploring the mediating role of citric acid in the association between semen MC exposure and VCL ($n = 837$). Mediation analysis models were adjusted for age, BMI, abstinence time, smoking and drinking status. Semen MC levels were ln-transformed. Citric acid was square root-transformed. VCL was not transformed. a) the effect of semen MCs on citric acid; b) the effect of citric acid on VCL; c') the direct effect of semen MCs on VCL; c) the total effect of semen MCs on VCL ($c = ab + c'$). The mediation ratio was calculated as $(a \times b)/c$. MCs, microcystins; BMI, body mass index; VCL, curvilinear velocity.



Supplementary Figure S5. Mediation analysis model exploring the mediation effects of seminal plasma biochemical components in the effects of semen MCs on sperm motility and velocity parameters in the population aged from 20 to 30 years. Mediation analysis models were adjusted for BMI, abstinence time, smoking and drinking status. Semen MC levels were ln-transformed. Zinc, NAG and citric acid were square root-transformed. Other parameters were not transformed. a) the effect of semen MCs on seminal plasma biochemical components; b) the effect of seminal plasma components on sperm motility or velocity parameters; c') the direct effect of semen MCs on sperm motility or velocity parameters; c) the total effect of semen MCs on sperm motility or velocity parameters ($c = a \times b + c'$). The mediation ratio was calculated as $(a \times b)/c$. MCs, microcystins; BMI, body mass index; NAG, neutral α -glucosidase; VCL, curvilinear velocity; ALH, amplitude of lateral head displacement; VSL, straight-line velocity; VAP, average path velocity.