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Supplemental Material

Profiles of Emerging and Legacy Per-/Polyfluoroalkyl Substances in Matched Serum and Semen Samples: New Implications for Human Semen Quality

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Table S8. Regression coefficients (95% confidence intervals) in semen quality parameters across serum-based PFAS quartiles (n = 664).

Figure S1. Regression coefficients and 95% confidence intervals for changes in semen parameters across PFAS quartiles (n = 664). First quartile (Q1) was used as a reference group. Models were adjusted for age, BMI, BMI^2, smoking, alcohol intake, and abstinence time. Semen volume was ln-transformed, sperm concentration and total sperm count were cubic-root transformed. Curvilinear velocity (VCL), straight-line velocity (VSL), and sperm morphology were included in the regressions untransformed. FDR-adjusted p-values were used for trends and are shown in blue (semen PFAS quartiles) and black (serum PFAS quartiles). See Table S7 and S8 for corresponding numerical data.

Competing financial interests: The authors declare no conflicts of interest.

Standards and reagents

The 16 target PFASs included perfluorobutanoate (PFBA), perfluoropentanoate (PFPeA), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDoDA), perfluorotridecanoate (PFTriDA), perfluorotetradecanoate (PFTeDA), perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), and chlorinated polyfluorinated ether sulfonates (6:2 and 8:2 Cl-PFESA). All native standards and their corresponding mass-labelled internal standards were purchased from Wellington Laboratories (Guelph, ON, Canada).

Tetra-*n*-butyl ammonium hydrogen sulfate (TBAS), ammonium acetate, sodium carbonate, and sodium bicarbonate were obtained from Sigma (St. Louis, MO, USA). LC-MS grade water and methanol and LC grade methyl *tert*-butyl ether (MTBE) were obtained from Fisher Scientific (Pittsburgh, PA, USA). Newborn bovine serum was purchased from Gibco Life Technologies (Paisley, UK). Standard reference material (SRM1957, organic contaminants in non-fortified human serum) was purchased from the National Institute of Standards and Technology (NIST, USA).

Sample extraction

Serum (200 μL) and semen (0.4–1.0 mL, depending on volume remaining after routine semen analyses) samples were spiked with 0.5 ng of mass-labelled extraction standard, 1 mL of tetra-*n*-butylammonium hydrogen sulfate solution (TBAS, 0.5 M), 2 mL of $NaHCO_3/Na_2CO_3$ buffer solution (pH = 10), and 4 mL of methyl *tert*-butyl ether (MTBE). After shaking and centrifugation, the supernatant was collected by a polypropylene Pasteur pipette, with the remaining residue extracted twice more with 4 mL of MTBE. All three extracts were combined and evaporated to dryness under nitrogen at 40 °C and reconstituted with 200 μL of methanol, with 0.5 ng of additional mass-labelled injection standard (containing $^{13}C_3$-PFBA, $^{13}C_2$-PFOA, $^{13}C_4$-PFOS, and $^{13}C_2$-PFDA, Table S1) then added before LC-MS/MS analysis.

Instrument analysis

An Acquity UPLC coupled to a Xevo TQ-S triple quadrupole mass spectrometer (Waters, Milford, MA, USA) was used to quantify target PFASs. Details of chromatographic column and instrument parameters are presented in Table S1.

Table S1. LC-MS/MS instrument parameters for the quantification of target analytes.

Instrument	Acquity I-Class UPLC coupled to a Xevo TQ-*S* triple quadrupole mass spectrometer (Waters, Milford, MA, USA)
Analytical column	Acquity BEH C18 column (100 mm × 2.1 mm, 1.7 µm, Waters, MA, USA)
Trap column	C18 column (50 mm × 2.1 mm, 3.0 µm, Waters, MA, USA)
Column temperature	40 °C
Injection volume	2 µL
Mobile phase	2 mM ammonium acetate in water (A) and methanol (B)

Gradient

	Time (min)	Flow rate (mL/min)	A (%)	B (%)	
	0.0	0.30	90	10	
	1.0	0.30	80	20	
	4.0	0.30	10	90	
	6.0	0.30	10	90	
	6.1	0.30	90	10	
	9.0	0.30	90	10	

Multiple reaction monitoring (MRM) transitions

Compound	Ion transitions	CV (V)	CE (V)	Internal standard
PFBA	213→169*	30	11	$^{13}C_4$-PFBA
PFPeA	263→219*	2	8	$^{13}C_5$-PFPeA
PFHxA	313→269*	14	10	$^{13}C_5$-PFHxA
	313→119	14	15	
PFHpA	363→319*	30	10	$^{13}C_4$-PFHpA
	363→169	30	19	
PFOA	413→369*	30	10	$^{13}C_8$-PFOA
	413→169	30	18	
PFNA	463→419*	28	1	$^{13}C_9$-PFNA
	463→169	32	20	
PFDA	513→469*	12	10	$^{13}C_6$-PFDA
	513→219	12	18	
PFUnDA	563→519*	30	10	$^{13}C_7$-PFUnDA
	563→319	30	17	
PFDoDA	613→569*	22	10	$^{13}C_2$-PFDoDA
	613→169	10	22	

PFTriDA	663→619*	10	10	$^{13}C_2$- PFTeDA
	663→169	10	26	
PFTeDA	713→669*	8	15	$^{13}C_2$- PFTeDA
	713→169	10	22	
PFBS	299→80*	40	30	$^{13}C_3$-PFBS
	299→99	40	28	
PFHxS	399→99*	30	31	$^{13}C_3$-PFHxS
	399→80	45	33	
PFOS	499→99*	30	39	$^{13}C_8$-PFOS
	499→80	30	37	
6:2 Cl-PFESA	531→351*	12	24	$^{13}C_8$-PFOS
	531→83	12	22	
8:2 Cl-PFESA	631→451*	2	28	$^{13}C_8$-PFOS
	631→83	20	35	
Mass-labeled extraction standards				
$^{13}C_4$-PFBA	217→172	30	11	
$^{13}C_5$-PFPeA	268→223	2	8	
$^{13}C_5$-PFHxA	318→273	14	10	
$^{13}C_4$-PFHpA	367→322	30	10	
$^{13}C_8$-PFOA	421→376	30	10	
$^{13}C_9$-PFNA	472→427	30	10	
$^{13}C_6$-PFDA	519→474	12	10	
$^{13}C_7$-PFUnDA	570→525	30	10	
$^{13}C_2$-PFDoDA	615→570	22	10	
$^{13}C_2$-PFTeDA	715→670	10	14	
$^{13}C_3$-PFBS	302→99	10	14	
$^{13}C_3$-PFHxS	402→99	45	30	
$^{13}C_8$-PFOS	507→80	20	34	
Mass-labeled injection standards				
$^{13}C_3$-PFBA	216→172	30	11	
$^{13}C_2$-PFOA	415→370	30	10	
$^{13}C_2$-PFDA	515→470	12	10	
$^{13}C_4$-PFOS	503→80	20	34	

(*) represents quantitative ion transition of corresponding analyte

CV: cone voltage; CE: collision energy

Other mass	**<u>Xevo TQ-S, Waters</u>**

parameters	Capillary voltage, -0.5 kV Source temperature, 150 °C Desolvation temperature, 450 °C Desolvation gas flow, 850 L/h Cone gas flow, 150 L/h

Table S2. Limits of quantitation (LOQs), matrix spike recoveries, and matrix effects (means ± SD, %) of target PFASs (*n* = 5).

Analyte	Serum					Semen			
	LOQ (ng/mL)	Spike recovery (%)		Matrix effect (%)		LOQ (ng/mL)	Spike recovery (%)		Matrix effect (%)
		0.1 ppb	1 ppb				0.1 ppb	1 ppb	
PFBA	0.100	89.2 ± 6.2	99.0 ± 2.6	96.3 ± 2.1		0.030–0.075	86.3 ± 9.0	92.2 ± 1.8	91.9 ± 2.4
PFPeA	0.050	96.5 ± 1.9	105.1 ± 5.0	99.0 ± 2.4		0.020–0.050	93.1 ± 4.3	105.5 ± 2.0	100.1 ± 1.6
PFHxA	0.200	87.3 ± 4.0	107.5 ± 4.1	100.1 ± 1.8		0.040–0.100	84.2 ± 5.9	100.7 ± 2.1	95.2 ± 2.2
PFHpA	0.020	96.1 ± 2.6	109.2 ± 5.1	100.4 ± 3.2		0.004–0.010	101.6 ± 3.8	112.3 ± 2.5	104.3 ± 2.6
PFOA	0.020	90.8 ± 4.4	102.7 ± 5.6	98.3 ± 2.5		0.004–0.010	97.4 ± 9.6	106.1 ± 3.4	90.6 ± 6.1
PFNA	0.020	92.4 ± 4.1	105.2 ± 4.2	96.1 ± 2.1		0.004–0.010	105.5 ± 4.3	103.7 ± 3.4	92.2 ± 1.6
PFDA	0.020	94.6 ± 4.2	106.8 ± 5.4	97.7 ± 2.8		0.004–0.010	96.1 ± 8.2	108.9 ± 5.2	99.6 ± 2.4
PFUnDA	0.010	96.2 ± 1.7	106.2 ± 4.7	100.2 ± 1.9		0.002–0.005	110.0 ± 5.0	106.4 ± 2.3	100.5 ± 4.0
PFDoDA	0.010	92.8 ± 3.5	107.7 ± 7.0	101.0 ± 2.4		0.002–0.005	101.2 ± 6.1	112.2 ± 1.5	103.1 ± 1.8
PFTriDA	0.010	97.1 ± 2.1	102.2 ± 5.2	99.7 ± 2.2		0.002–0.005	104.3 ± 7.8	96.9 ± 3.2	97.6 ± 1.4
PFTeDA	0.010	101.0 ± 5.5	107.6 ± 5.8	100.9 ± 2.6		0.002–0.005	115.1 ± 2.5	106.1 ± 2.0	104.7 ± 3.5
PFBS	0.010	94.5 ± 3.2	106.1 ± 4.1	99.2 ± 1.7		0.004–0.010	106.7 ± 3.9	104.2 ± 3.1	97.2 ± 1.2
PFHxS	0.020	92.5 ± 3.0	104.5 ± 4.0	97.1 ± 2.2		0.008–0.020	98.3 ± 6.2	106.5 ± 2.4	99.8 ± 2.8
PFOS	0.020	93.2 ± 3.2	103.3 ± 6.3	99.5 ± 1.5		0.004–0.010	109.6 ± 5.0	105.2 ± 4.4	99.1 ± 5.3
6:2 Cl-PFESA	0.010	97.6 ± 4.0	110.8 ± 5.5	102.3 ± 3.4		0.002–0.005	83.9 ± 4.4	91.3 ± 4.8	79.0 ± 8.5
8:2 Cl-PFESA	0.010	92.2 ± 3.7	103.2 ± 6.4	98.3 ± 3.6		0.002–0.005	100.9 ± 5.1	104.5 ± 3.6	99.2 ± 3.4

Three criteria were used to evaluate LOQs: (1) Concentration resulting in a signal-to-noise ratio at or greater than 10 in matrices; (2) Lowest concentration of standard in the calibration curve with measured concentrations within ± 20% of its theoretical value; and, (3) Concentration factor. LOQs for semen were reported as a range because the concentration factor varied among semen samples (depending on remaining volume after routine semen analyses).

Table S3. Pearson's correlations between ln-transformed PFAS concentrations in serum (n = 664).

	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTriDA	PFHxS	PFOS	6:2 Cl-PFESA	8:2 Cl-PFESA
PFOA	1									
PFNA	0.570	1								
PFDA	0.428	0.825	1							
PFUnDA	0.440	0.910	0.851	1						
PFDoDA	0.413	0.821	0.768	0.908	1					
PFTriDA	0.404	0.810	0.731	0.906	0.890	1				
PFHxS	0.340	0.361	0.327	0.341	0.282	0.348	1			
PFOS	0.429	0.740	0.688	0.760	0.675	0.686	0.548	1		
6:2 Cl-PFESA	0.593	0.752	0.702	0.762	0.700	0.652	0.317	0.730	1	
8:2 Cl-PFESA	0.445	0.665	0.659	0.741	0.704	0.649	0.279	0.674	0.844	1

Censored likelihood multiple imputation was used to impute PFAS concentrations below the LOQ.

$p \leq 0.001$ in all Pearson's correlations

Table S4. Pearson's correlations between ln-transformed PFAS concentrations in semen (*n* = 664).

	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTriDA	PFHxS	PFOS	6:2 Cl-PFESA	8:2 Cl-PFESA
PFOA	1									
PFNA	0.732	1								
PFDA	0.599	0.869	1							
PFUnDA	0.473	0.749	0.864	1						
PFDoDA	0.458	0.619	0.706	0.71	1					
PFTriDA	0.371	0.594	0.704	0.814	0.7	1				
PFHxS	0.542	0.536	0.493	0.425	0.406	0.371	1			
PFOS	0.588	0.757	0.798	0.755	0.609	0.631	0.573	1		
6:2 Cl-PFESA	0.696	0.761	0.795	0.699	0.614	0.575	0.506	0.785	1	
8:2 Cl-PFESA	0.549	0.605	0.693	0.642	0.672	0.567	0.454	0.653	0.780	1

Censored likelihood multiple imputation was used to impute PFAS concentrations below the LOQ.

$p \leq 0.001$ in all Pearson's correlations

Table S5. Estimated means (95% confidence intervals) for PFAS concentration ratios (semen versus serum) with and without imputation.

	With imputation [a]		Without imputation [b]	
	Mean	*n*	Mean	*n*
PFOA	3.07 (2.93, 3.20)	664	3.07 (2.93, 3.20)	664
PFNA	1.88 (1.77, 1.98)	664	2.07 (1.96, 2.18)	557
PFDA	2.01 (1.85, 2.17)	664	2.09 (1.95, 2.22)	554
PFUnDA	2.35 (2.25, 2.45)	664	2.57 (2.47, 2.68)	557
PFDoDA	5.07 (4.59, 5.54)	664	6.45 (5.48, 7.41)	192
PFTriDA	8.13 (7.48, 8.79)	664	8.39 (7.95, 8.82)	506
PFHxS	2.18 (1.99, 2.36)	664	4.03 (3.56, 4.51)	204
PFOS	1.33 (1.26, 1.40)	664	1.37 (1.30, 1.44)	638
6:2 Cl-PFESA	1.31 (1.22, 1.39)	664	1.31 (1.22, 1.39)	664
8:2 Cl-PFESA	3.90 (3.47, 4.33)	664	3.94 (3.23, 4.65)	201

[a] Censored likelihood multiple imputation was used to impute PFAS concentrations below the LOQ. All samples were included in analysis.

[b] Only paired serum-semen samples with PFAS levels both greater than the corresponding LOQs were included in analysis

Table S6. Estimated changes in semen quality parameters associated with both semen- and serum-based PFAS levels (*n* = 664).

Outcome[a]	matrix	PFOA		PFNA		PFDA		PFUnDA		PFOS		6:2 Cl-PFESA	
		β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*
Semen volume (mL)[b]	semen	–0.028 (–0.070, 0.013)	0.4	–0.019 (–0.052, 0.015)	0.5	–0.010 (–0.046, 0.025)	0.8	–0.032 (–0.064, 0.000)	0.2	–0.006 (–0.038, 0.025)	0.9	–0.003 (–0.034, 0.029)	1.0
	serum	0.025 (–0.029, 0.079)	0.6	–0.013 (–0.058, 0.032)	0.8	–0.006 (–0.040, 0.028)	0.9	–0.010 (–0.047, 0.027)	0.8	–0.003 (–0.044, 0.037)	1.0	0.009 (–0.024, 0.041)	0.8
Sperm conc. (million/mL)[b]	semen	0.188 (0.068, 0.308)	0.04	0.096 (–0.004, 0.197)	0.2	0.049 (–0.057, 0.156)	0.6	0.093 (0.001, 0.186)	0.2	0.098 (0.007, 0.189)	0.2	0.072 (–0.019, 0.164)	0.3
	serum	0.037 (–0.118, 0.192)	0.9	–0.043 (–0.173, 0.087)	0.8	–0.015 (–0.114, 0.083)	0.9	–0.005 (–0.112, 0.102)	1.0	–0.003 (–0.120, 0.115)	1.0	–0.011 (–0.105, 0.082)	1.0
Total sperm count (million)[b]	semen	0.234 (0.046, 0.423)	0.08	0.115 (–0.042, 0.271)	0.4	0.056 (–0.109, 0.220)	0.8	0.087 (–0.059, 0.232)	0.5	0.135 (–0.008, 0.278)	0.2	0.105 (–0.039, 0.249)	0.4
	serum	0.097 (–0.147, 0.341)	0.7	–0.094 (–0.299, 0.110)	0.6	–0.037 (–0.192, 0.118)	0.9	–0.025 (–0.193, 0.143)	0.9	–0.014 (–0.199, 0.170)	1.0	–0.006 (–0.153, 0.141)	1.0
Progressive motile (%)	semen	–2.359 (–3.943, –0.775)	0.04	–1.979 (–3.274, –0.684)	0.04	–1.693 (–3.047, –0.339)	0.08	–1.857 (–3.091, –0.623)	0.04	–1.818 (–3.002, –0.633)	0.04	–1.870 (–3.080, –0.660)	0.04
	serum	–0.153 (–2.245, 1.938)	1.0	–0.273 (–2.353, 1.806)	1.0	1.028 (–0.293, 2.350)	0.3	0.858 (–0.574, 2.290)	0.5	0.918 (–0.654, 2.490)	0.5	0.993 (–0.259, 2.244)	0.3
VCL (μm/sec)	semen	–1.176 (–2.098, –0.254)	0.07	–0.798 (–1.554, –0.042)	0.2	–0.716 (–1.515, 0.083)	0.3	–0.606 (–1.319, 0.106)	0.2	–0.841 (–1.531, –0.151)	0.09	–0.727 (–1.433, –0.022)	0.2
	serum	0.179 (–1.038, 1.396)	0.9	0.083 (–1.129, 1.295)	1.0	1.015 (–0.007, 2.036)	0.2	0.616 (–0.219, 1.450)	0.4	0.570 (–0.345, 1.485)	0.5	0.532 (–0.198, 1.261)	0.4
VSL (μm/sec)	semen	–0.921 (–1.687, –0.115)	0.09	–0.682 (–1.316, –0.049)	0.2	–0.769 (–1.432, –0.106)	0.1	–0.782 (–1.376, –0.189)	0.07	–0.840 (–1.412, –0.267)	0.04	–0.779 (–1.364, –0.194)	0.07

	serum	0.004 (–1.008, 1.015)	1.0	–0.060 (–1.066, 0.946)	1.0	0.521 (–0.116, 1.159)	0.3	0.412 (–0.280, 1.104)	0.5	0.520 (–0.238, 1.279)	0.4	0.522 (–0.083, 1.126)	0.3
Morphologically normal (%)	semen	–0.064 (–0.306, 0.292)	1.0	–0.140 (–0.341, 0.062)	0.4	–0.155 (–0.368, 0.057)	0.4	–0.107 (–0.299, 0.084)	0.5	0.000 (–0.186, 0.185)	1.0	–0.020 (–0.209, 0.170)	1.0
	serum	–0.103 (–0.321, 0.289)	0.5	–0.045 (–0.318, 0.227)	0.9	–0.060 (–0.266, 0.146)	0.8	–0.093 (–0.316, 0.131)	0.7	–0.035 (–0.281, 0.211)	0.9	–0.015 (–0.211, 0.181)	1.0
DFI (%)	semen	0.130 (0.057, 0.204)	0.03	0.112 (0.051, 0.172)	0.03	0.089 (0.025, 0.152)	0.05	0.052 (–0.006, 0.110)	0.3	0.090 (0.035, 0.145)	0.04	0.085 (0.029, 0.141)	0.04
	serum	0.047 (0.050, 0.144)	0.6	–0.040 (–0.121, 0.041)	0.6	–0.028 (–0.089, 0.034)	0.6	–0.020 (–0.087, 0.047)	0.8	–0.023 (–0.096, 0.051)	0.8	–0.019 (–0.077, 0.039)	0.8
HDS (%)	semen	0.006 (–0.062, 0.074)	1.0	–0.018 (–0.074, 0.038)	0.8	0.045 (–0.013, 0.104)	0.3	0.003 (–0.057, 0.063)	1.0	0.010 (–0.049, 0.069)	0.9	–0.012 (–0.076, 0.051)	0.9
	serum	0.056 (–0.036, 0.147)	0.5	0.128 (0.049, 0.207)	0.04	0.037 (–0.023, 0.096)	0.5	0.098 (0.026, 0.170)	0.06	0.047 (–0.036, 0.131)	0.5	0.086 (0.019, 0.154)	0.07

For each outcome, the estimates for semen PFAS levels are shown on the first row and those for serum PFASs levels are shown on the second row. Other covariates included in the regression models were age, BMI, BMI^2, smoking, alcohol intake, and abstinence time. Censored likelihood multiple imputation was used when seminal PFAS levels were below LOQ. *P*-values were false discovery rate (FDR)-adjusted to reduce overall testing error rate. VCL, curvilinear velocity; VSL, straight-line velocity; DFI, DNA fragmentation index; HDS, high DNA stainability.

[a] Semen volume, DFI, and HDS were ln transformed, sperm concentration and total sperm count were cubic-root transformed, other parameters were not transformed. [b] Subjects reporting spillage during semen collection (*n* = 21) were excluded from these analyses.

Table S7. Regression coefficients (95% confidence intervals) in semen quality parameters across semen-based PFAS quartiles (n = 664).

Outcome[a]		PFOA	PFNA	PFDA	PFUnDA	PFOS	6:2 Cl-PFESA
Semen volume (mL)[b]	Q1	Ref	Ref	Ref	Ref	Ref	Ref
	Q2	–0.04 (–0.11, 0.04)	–0.07 (–0.14, 0.01)	0.03 (–0.05, 0.11)	0.00 (–0.08, 0.07)	0.03 (–0.05, 0.11)	0.01 (–0.06, 0.09)
	Q3	–0.10 (–0.17, –0.02)	–0.08 (–0.16, 0.00)	–0.08 (–0.16, 0.00)	–0.07 (–0.15, 0.01)	–0.04 (–0.12, 0.04)	–0.06 (–0.14, 0.02)
	Q4	–0.06 (–0.14, 0.02)	–0.06 (–0.14, 0.03)	–0.01 (–0.09, 0.07)	–0.07 (–0.16, 0.01)	–0.02 (–0.10, 0.07)	0.01 (–0.07, 0.09)
	p-Trend	0.4	0.5	0.6	0.1	0.6	0.9
Sperm conc. (million/mL)[b]	Q1	Ref	Ref	Ref	Ref	Ref	Ref
	Q2	0.30 (0.08, 0.52)	–0.02 (–0.24, 0.20)	0.05 (–0.18, 0.27)	–0.05 (–0.27, 0.18)	0.03 (–0.20, 0.25)	–0.01 (–0.24, 0.21)
	Q3	0.06 (–0.16, 0.28)	0.05 (–0.18, 0.28)	0.20 (–0.03, 0.43)	0.11 (–0.12, 0.33)	0.19 (–0.05, 0.42)	0.06 (–0.18, 0.29)
	Q4	0.36 (0.13, 0.59)	0.16 (–0.07, 0.40)	0.04 (–0.20, 0.28)	0.17 (–0.07, 0.40)	0.17 (–0.07, 0.41)	0.14 (–0.10, 0.37)
	p-Trend	0.1	0.3	0.9	0.2	0.4	0.4
Total sperm count (million)[b]	Q1	Ref	Ref	Ref	Ref	Ref	Ref
	Q2	0.37 (0.02, 0.71)	–0.15 (–0.50, 0.20)	0.13 (–0.23, 0.49)	–0.06 (–0.41, 0.29)	0.10 (–0.25, 0.46)	0.00 (–0.35, 0.36)
	Q3	–0.08 (–0.43, 0.27)	–0.07 (–0.43, 0.29)	0.18 (–0.18, 0.54)	0.05 (–0.31, 0.40)	0.20 (–0.16, 0.57)	–0.02 (–0.39, 0.34)
	Q4	0.42 (0.06, 0.78)	0.15 (–0.22, 0.52)	0.05 (–0.33, 0.42)	0.13 (–0.24, 0.51)	0.23 (–0.15, 0.61)	0.21 (–0.16, 0.58)
	p-Trend	0.2	0.4	1.0	0.5	0.5	0.4
Progressive motile (%)	Q1	Ref	Ref	Ref	Ref	Ref	Ref
	Q2	0.31 (–2.65, 3.27)	0.00 (–2.98, 2.99)	–0.29 (–3.34, 2.76)	–1.60 (–4.61, 1.40)	–2.30 (–5.27, 0.68)	–1.37 (–4.39, 1.64)
	Q3	–1.49 (–4.48, 1.50)	–2.02 (–5.08, 1.03)	–1.71 (–4.79, 1.36)	–2.78 (–5.78, 0.22)	–1.53 (–4.61, 1.56)	–3.06 (–6.17, 0.04)
	Q4	–4.26 (–7.30, –1.22)	–4.13 (–7.30, –0.97)	–2.87 (–6.03, 0.29)	–4.80 (–7.96, –1.64)	–5.54 (–8.72, –2.36)	–3.94 (–7.04, –0.83)
	p-Trend	0.02	0.03	0.2	0.03	0.01	0.1
VCL (μm/sec)	Q1	Ref	Ref	Ref	Ref	Ref	Ref
	Q2	–1.65 (–3.38, 0.07)	0.87 (–0.86, 2.61)	–1.22 (–2.99, 0.55)	0.25 (–4.61, 1.40)	–1.60 (–1.50, 2.01)	0.41 (–1.34, 2.16)
	Q3	–1.61 (–3.35, 0.12)	–0.52 (–2.30, 1.25)	–1.23 (–3.02, 0.55)	–0.65 (–5.78, 0.22)	–2.78 (–2.40, 1.10)	–1.86 (–3.66, –0.06)
	Q4	–2.64 (–4.41, –0.87)	–1.48 (–3.33, 0.36)	–1.01 (–2.84, 0.83)	–1.12 (–7.96, –1.64)	–4.80 (–2.97, –0.72)	–1.03 (–2.84, 0.77)

	p-Trend	0.08	0.1	0.6	0.3	0.1	0.4
VSL (μm/sec)	Q1	Ref	Ref	Ref	Ref	Ref	Ref
	Q2	–1.68 (–3.11, –0.24)	0.60 (–0.84, 2.04)	–1.52 (–2.99, –0.05)	–0.04 (–1.50, 1.41)	–1.00 (–2.44, 0.45)	0.15 (–1.30, 1.60)
	Q3	–0.87 (–2.32, 0.57)	–0.34 (–1.82, 1.13)	–1.72 (–3.20, –0.25)	–1.21 (–2.66, 0.24)	–1.40 (–2.89, 0.09)	–1.49 (–2.99, 0.00)
	Q4	–2.13 (–3.60, –0.66)	–1.37 (–2.89, 0.16)	–1.46 (–2.98, 0.07)	–1.60 (–3.13, –0.07)	–2.06 (–3.60, –0.52)	–1.27 (–2.77, 0.22)
	p-Trend	0.1	0.1	0.4	0.1	0.1	0.2
Morphologically normal (%)	Q1	Ref	Ref	Ref	Ref	Ref	Ref
	Q2	0.32 (–0.14, 0.78)	0.23 (–0.23, 0.69)	0.16 (–0.32, 0.63)	–0.02 (–0.49, 0.45)	0.31 (–0.15, 0.78)	0.07 (–0.40, 0.54)
	Q3	0.06 (–0.40, 0.53)	–0.02 (–0.50, 0.45)	0.03 (–0.44, 0.51)	0.08 (–0.39, 0.55)	0.13 (–0.35, 0.62)	–0.36 (–0.84, 0.13)
	Q4	–0.21 (–0.68, 0.27)	–0.36 (–0.86, 0.13)	–0.31 (–0.80, 0.18)	–0.24 (–0.73, 0.25)	0.03 (–0.46, 0.53)	–0.15 (–0.63, 0.33)
	p-Trend	0.3	0.1	0.2	0.5	0.8	0.6
DFI (%)	Q1	Ref	Ref	Ref	Ref	Ref	Ref
	Q2	0.05 (–0.09, 0.19)	–0.08 (–0.22, 0.06)	0.07 (–0.07, 0.22)	0.06 (–0.08, 0.20)	0.03 (–0.11, 0.17)	0.07 (–0.07, 0.21)
	Q3	0.14 (0.00, 0.28)	0.06 (–0.08, 0.21)	–0.02 (–0.16, 0.12)	0.04 (–0.10, 0.18)	0.08 (–0.07, 0.22)	0.14 (0.00, 0.29)
	Q4	0.21 (0.07, 0.35)	0.19 (0.05, 0.34)	0.20 (0.06, 0.35)	0.16 (0.01, 0.30)	0.25 (0.10, 0.40)	0.17 (0.02, 0.31)
	p-Trend	0.03	0.01	0.05	0.1	0.01	0.1
HDS (%)	Q1	Ref	Ref	Ref	Ref	Ref	Ref
	Q2	0.02 (–0.08, 0.11)	–0.02 (–0.12, 0.07)	0.04 (–0.06, 0.13)	0.06 (–0.04, 0.15)	0.00 (–0.09, 0.10)	0.10 (0.00, 0.19)
	Q3	0.06 (–0.04, 0.15)	0.01 (–0.09, 0.10)	0.02 (–0.07, 0.12)	0.09 (0.00, 0.19)	0.04 (–0.06, 0.14)	0.14 (0.04, 0.23)
	Q4	0.04 (–0.06, 0.14)	0.10 (0.00, 0.20)	0.14 (0.04, 0.24)	0.17 (0.07, 0.27)	0.08 (–0.02, 0.18)	0.14 (0.04, 0.23)
	p-Trend	0.6	0.1	0.04	0.01	0.2	0.1

Estimates calculated using linear regression models adjusted for age, BMI, BMI^2, smoking, alcohol intake, and abstinence time. *P*-Trend was the false discovery rate (FDR)-adjusted *p*-value for linear trend across quartiles. See Figure 3 and Figure S1 for corresponding graphical forms. VCL, curvilinear velocity; VSL, straight-line velocity; DFI, DNA fragmentation index; HDS, high DNA stainability.

[a] Semen volume, DFI, and HDS were ln transformed, sperm concentration and total sperm count were cubic-root transformed, other parameters were not transformed.

[b] Subjects reporting spillage during semen collection (n = 21) were excluded from these analyses.

Table S8. Regression coefficients (95% confidence intervals) in semen quality parameters across serum-based PFAS quartiles (n = 664).

Outcome[a]		PFOA	PFNA	PFDA	PFUnDA	PFOS	6:2 Cl-PFESA
Semen volume (mL)[b]	Q1	Ref	Ref	Ref	Ref	Ref	Ref
	Q2	0.05 (–0.03, 0.12)	0.04 (–0.03, 0.12)	0.04 (–0.04, 0.12)	0.03 (–0.04, 0.11)	0.02 (–0.06, 0.09)	0.04 (–0.04, 0.12)
	Q3	0.01 (–0.07, 0.08)	–0.03 (–0.11, 0.05)	0.02 (–0.05, 0.10)	0.05 (–0.03, 0.12)	0.06 (–0.02, 0.14)	0.02 (–0.06, 0.10)
	Q4	0.06 (–0.02, 0.14)	0.05 (–0.03, 0.13)	0.09 (0.01, 0.17)	0.04 (–0.04, 0.12)	0.05 (–0.03, 0.13)	0.10 (0.02, 0.18)
	p-Trend	0.4	0.5	0.1	0.6	0.4	0.1
Sperm conc. (million/mL)[b]	Q1	Ref	Ref	Ref	Ref	Ref	Ref
	Q2	-0.01 (-0.23, 0.21)	–0.08 (–0.30, 0.14)	-0.18 (–0.40, 0.05)	–0.10 (–0.33, 0.12)	0.22 (–0.01, 0.44)	–0.21 (–0.43, 0.02)
	Q3	-0.09 (–0.31, 0.13)	-0.06 (–0.29, 0.17)	0.06 (–0.17, 0.28)	0.12 (–0.11, 0.34)	-0.01 (–0.24, 0.22)	0.02 (–0.21, 0.25)
	Q4	0.11 (-0.11, 0.34)	-0.12 (–0.35, 0.11)	-0.28 (–0.51, -0.05)	-0.11 (–0.34, 0.13)	0.01 (–0.22, 0.25)	-0.25 (–0.48, -0.02)
	p-Trend	0.4	0.5	0.1	0.7	0.6	0.2
Total sperm count (million)[b]	Q1	Ref	Ref	Ref	Ref	Ref	Ref
	Q2	0.05 (-0.30, 0.40)	–0.06(–0.41, 0.29)	-0.22(–0.57, 0.13)	–0.11 (–0.46, 0.25)	0.37 (0.01, 0.72)	-0.26 (–0.61, 0.10)
	Q3	–0.14 (–0.49, 0.21)	–0.14 (–0.50, 0.22)	0.14 (–0.22, 0.49)	0.26 (–0.09, 0.62)	0.09 (–0.27, 0.46)	0.07 (–0.29, 0.43)
	Q4	0.27 (-0.08, 0.62)	-0.10 (–0.47, 0.26)	-0.28 (–0.65, 0.08)	-0.09 (–0.46, 0.28)	0.10 (–0.27, 0.47)	-0.21 (–0.57, 0.15)
	p-Trend	0.3	0.7	0.4	0.9	0.9	0.6
Progressive motile (%)	Q1	Ref	Ref	Ref	Ref	Ref	Ref
	Q2	0.62 (–2.34, 3.58)	1.49 (–1.49, 4.47)	0.86 (–2.15, 3.87)	0.23 (–2.80, 3.26)	1.50 (–1.54, 4.54)	-0.26 (–0.61, 0.10)
	Q3	0.61 (–2.36, 3.58)	–1.25 (–4.29, 1.79)	1.41 (–1.63, 4.44)	0.47 (–2.57, 3.51)	0.63 (–2.50, 3.76)	0.07 (–0.29, 0.43)
	Q4	–2.16 (–5.14, 0.82)	1.06 (–2.03, 4.15)	1.16 (–1.94, 4.26)	-0.13 (–3.27, 3.02)	0.14 (–3.01, 3.29)	-0.21 (–0.57, –0.15)
	p-Trend	0.2	0.8	0.7	0.9	0.9	0.7
VCL (μm/sec)	Q1	Ref	Ref	Ref	Ref	Ref	Ref
	Q2	0.37 (–1.35, 2.10)	1.02 (–0.71, 2.75)	0.63 (–1.11, 2.38)	0.94 (–0.82, 2.70)	0.62 (–1.13, 2.38)	1.47 (–0.28, 3.22)
	Q3	0.54 (–1.19, 2.27)	0.55 (–1.22, 2.32)	1.40 (–0.36, 3.16)	0.89 (–0.87, 2.65)	2.49 (0.69, 4.30)	0.57(–1.19, 2.34)
	Q4	–0.08 (–1.82, 1.66)	0.59 (–1.21, 2.39)	1.95 (0.16, 3.75)	1.12 (–0.70, 2.94)	0.78 (–1.04, 2.61)	1.50 (–0.28, 3.28)

	p-Trend	0.8	0.8	0.1	0.5	0.6	0.4
VSL (µm/sec)	Q1	Ref	Ref	Ref	Ref	Ref	Ref
	Q2	0.71 (–0.72, –2.14)	0.34 (–1.10, 1.78)	0.28 (–1.17, 1.73)	0.58 (–0.88, 2.04)	0.13(–1.34,1.60)	1.17 (–0.29, 2.62)
	Q3	0.77 (–0.67, 2.20)	0.31 (–1.16, 1.78)	0.48 (–0.98, 1.94)	–0.35 (–1.82, 1.11)	1.01 (–0.49, 2.52)	0.18 (–1.29, 1.64)
	Q4	-0.39 (–1.83, 1.05)	-0.03 (–1.52, 1.47)	1.02 (–0.48, 2.51)	0.30 (–1.21, 1.81)	0.33 (–1.19, 1.85)	0.84 (–0.64, 2.32
	p-Trend	0.5	0.9	0.4	1.0	0.8	0.7
Morphologically normal (%)	Q1	Ref	Ref	Ref	Ref	Ref	Ref
	Q2	0.03 (–0.43, 0.50)	0.14 (–0.32, 0.60)	0.08 (–0.39, 0.55)	–0.09 (–0.56, 0.38)	0.12 (–0.36, 0.59)	0.13 (–0.33, 0.60)
	Q3	0.00 (–0.46, 0.46)	–0.18 (–0.66, 0.29)	0.26 (–0.21, 0.73)	0.08 (–0.40, 0.55)	–0.07 (–0.56, 0.41)	–0.02 (–0.49, 0.46)
	Q4	–0.25 (–0.71, 0.21)	–0.38 (–086, 0.10)	–0.20 (–0.68, 0.28)	–0.25 (–0.74, 0.24)	–0.12 (–0.61, 0.37)	–0.23 (–0.70, 0.25)
	p-Trend	0.4	0.2	0.5	0.5	0.6	0.4
DFI (%)	Q1	Ref	Ref	Ref	Ref	Ref	Ref
	Q2	0.06 (–0.08, 0.20)	–0.04 (–0.18, 0.10)	0.00 (–0.14, 0.14)	–0.03 (–0.17, 0.12)	–0.03 (–0.17, 0.11)	–0.02 (–0.16, 0.12)
	Q3	–0.02 (–0.16, 0.11)	–0.06 (–0.20, 0.09)	–0.11 (–0.25, 0.03)	–0.15 (–0.29, –0.01)	0.05 (–0.09, 0.20)	0.04 (–0.11, 0.18)
	Q4	0.07 (–0.07, 0.21)	–0.03 (–0.17, 0.11)	–0.04 (–0.19, 0.10)	–0.02 (–0.16, 0.13)	0.07 (–0.08, 0.22)	0.01 (–0.13, 0.16)
	p-Trend	0.6	0.9	0.6	0.9	0.4	0.8
HDS (%)	Q1	Ref	Ref	Ref	Ref	Ref	Ref
	Q2	0.06 (–0.03, 0.16)	0.03 (–0.06, –0.12)	0.07 (–0.02, 0.16)	0.08 (–0.01, 0.18)	0.07 (–0.02, 0.17)	0.11 (0.01, 0.20)
	Q3	0.08 (–0.01, 0.18)	0.08 (–0.01, 0.18)	0.04 (–0.05, 0.14)	0.07 (-0.02, 0.17)	0.08 (–0.02, 0.18)	0.12 (0.03, 0.22)
	Q4	0.08 (–0.01, 0.18)	0.17 (0.07, 0.27)	0.17 (0.07, 0.27)	0.18 (0.08, 0.28)	0.13 (0.03, 0.22)	0.18 (0.09, 0.28)
	p-Trend	0.3	0.01	0.01	0.01	0.1	0.01

Estimates calculated using linear regression models adjusted for age, BMI, BMI^2, smoking, alcohol intake, and abstinence time. *P*-Trend was the false discovery rate (FDR)-adjusted *p*-value for linear trend across quartiles. See Figure 3 and Figure S1 for corresponding graphical forms. VCL, curvilinear velocity; VSL, straight-line velocity; DFI, DNA fragmentation index; HDS, high DNA stainability.

[a] Semen volume, DFI, and HDS were ln transformed, sperm concentration and total sperm count were cubic-root transformed, other parameters were not transformed.

[b] Subjects reporting spillage during semen collection (n = 21) were excluded from these analyses.

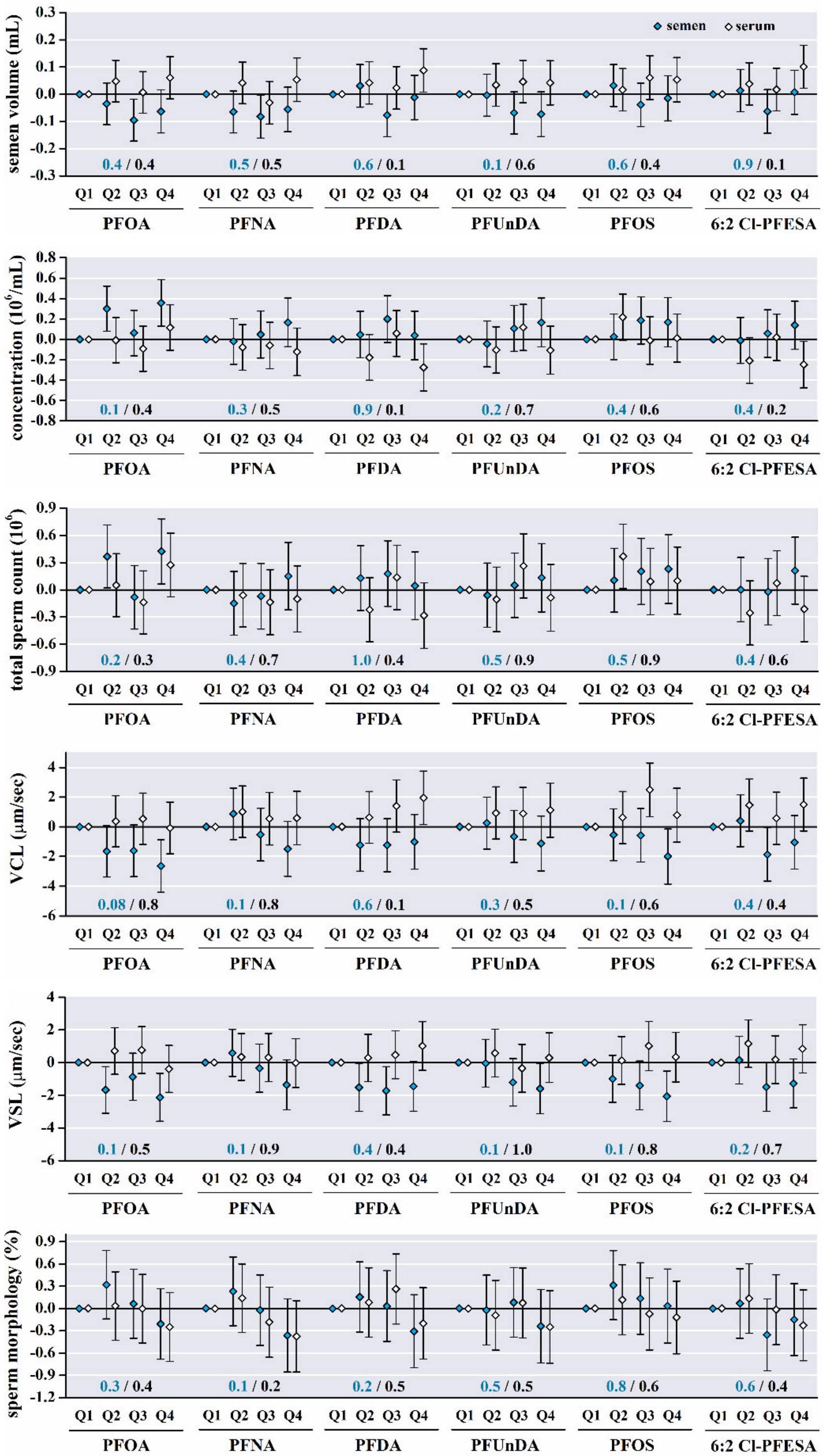

Figure S1 Regression coefficients and 95% confidence intervals for changes in semen parameters across PFAS quartiles (n = 664). First quartile (Q1) was used as a reference group. Models were adjusted for age, BMI, BMI^2, smoking, alcohol intake, and abstinence time. Semen volume was ln-transformed, sperm concentration and total sperm count were cubic-root transformed. Curvilinear velocity (VCL), straight-line velocity (VSL), and sperm morphology were included in the regressions untransformed. FDR-adjusted *p*-values were used for trends and are shown in blue (semen PFAS quartiles) and black (serum PFAS quartiles). See Table S7 and S8 for corresponding numerical data.