

Supplementary Material: Tables and Table legends

Tables S1-3 – see supplementary Excel file.

Table S1: Fatty acids measured in this study.

Table S2: Lipid species measured in positive ionisation mode

Table S3: Lipid species measured in negative ionisation mode

Abbreviations used in tables S1-S3:

Ceramides (CERs), cholesterol (CHOL), cholesterol esters (CEs), diglycerides (DG), fatty acids (FAs), lysophosphatidylcholines (LPCs), lysophosphatidylethanolamines (LPEs), phosphatidylcholines (PCs), phosphatidylcholine-plasmalogens (PC-Os), phosphatidylethanolamines (PEs), phosphatidylethanolamine-plasmalogens (PE-Os), phosphatidylglycerides (PGs), phosphatidylinositols (PIs), phosphatidylserines (PSs), sphingomyelins (SMs), and triglycerides (TGs).

Supplementary Table S4. Characteristics of participants included in this study. Men were categorised into groups according to progressive motility (< or ≥ median; 50% progressive motility); sperm count (≤ or > median; 92 million/ ejaculate) and total semen volume (≤ or > median; 3.4 ml). Data are presented as median (range).

	All participants	Motility analysis		Sperm number analysis		Semen volume analysis	
		LOW MOTILITY	HIGH MOTILITY	LOW SPERM NUMBER	HIGH SPERM NUMBER	LOW SEMEN VOLUME	HIGH SEMEN VOLUME
	n=26	n=12	n=14	n=13	n=13	n=12	n=14
Age, years	35 (20 - 45)	36.5 (24 - 45)	32.5 (20 - 40)	32 (20 - 38)	37 (27 - 45)	35 (26 - 45)	34 (20 - 40)
BMI, kg/m ²	30.4 (24 - 36.5)	30.2 (24 - 36.5)	30.4 (26 - 33)	26 (24 - 36.5)	33 (30.2 - 34.4)	26 (26 - 33)	33 (24 - 36.5)
Abstinence, days	4 (2 - 9)	4 (3 - 8)	3 (2 - 9)	3 (2 - 9)	4 (3 - 8)	3 (2 - 8)	4 (3 - 9)
Semen volume, ml	3.4 (1.8 - 8.3)	4.35 (2.8 - 8.3)	3.2 (1.8 - 5.7)	3.1 (2.2 - 5.3)	4.7 (1.8 - 8.3)	2.8 (1.8 - 3.2)	4.7 (3.4 - 8.3)
Semen pH	8.5 (8 - 8.5)	8.5 (8 - 8.5)	8.5 (8 - 8.5)	8.5 (8 - 8.5)	8.5 (8 - 8.5)	8.25 (8 - 8.5)	8.5 (8 - 8.5)
Sperm concentration million/ml	26.75 (2.6 - 209)	21.2 (2.6 - 209)	27.25 (7.5 - 196)	13.3 (2.6 - 37.9)	38.4 (18.6 - 209)	28.95 (3.1 - 209)	26.25 (2.6 - 196)
Total motility %	56 (28 - 73)	45 (28 - 56)	62.5 (54 - 73)	61 (36 - 73)	56 (28 - 65)	58.5 (40 - 73)	53 (28 - 73)
Progressive motility %	50 (24 - 68)	40 (24 - 49)	58 (50 - 68)	50 (32 - 68)	50 (24 - 63)	53.5 (35 - 64)	49 (24 - 68)
Non-progressive motility %	5 (1 - 13)	6 (2 - 9)	5 (1 - 13)	6 (3 - 13)	4 (1 - 8)	5.5 (2 - 10)	5 (1 - 13)
Immotile %	44 (27 - 72)	55 (44 - 72)	38 (27 - 46)	39 (27 - 64)	44 (35 - 72)	41.5 (28 - 60)	47 (27 - 72)
Total sperm number millions/ejaculate	91.825 (8.68 - 921.2)	89.9 (8.68 - 606.1)	93.325 (24 - 921.2)	49.21 (8.68 - 90.65)	175.78 (93 - 921.2)	79.35 (8.68 - 606.1)	130.03 (13.78 - 921.2)
HbA1c mmol/mol	37 (28 - 49)	37.5 (28 - 49)	37 (32 - 42)	37 (28 - 42)	38 (32 - 49)	36 (33 - 49)	37.5 (28 - 41)
Random plasma glucose mmol/l	5.2 (4.4 - 8.2)	5.3 (4.4 - 8.2)	5.2 (4.7 - 6.5)	5.2 (4.4 - 6)	5.4 (4.8 - 8.2)	5.55 (4.7 - 8.2)	5.2 (4.4 - 5.6)

NB: WHO reference criteria for normal semen characteristics are:

Progressive motility 32-75%

Total sperm count 39-928 million / ejaculate

Semen volume 1.5-7.6 ml

Legends for Supplementary Figures

Figure S1: Distribution of lipid species across the three compartments. Lipid were included as follows: ceramides (CERs), cholesterol (CHOL), cholesterol esters (CEs), diglycerides (DG), fatty acids (FAs), lysophosphatidylcholines (LPCs), lysophosphatidylethanolamines (LPEs), phosphatidylcholines (PCs), phosphatidylcholine-plasmalogens (PC-Os), phosphatidylethanolamines (PEs), phosphatidylethanolamine-plasmalogens (PE-Os), phosphatidylglycerides (PGs), phosphatidylinositols (PIs), phosphatidylserines (PSs), polyunsaturated fatty acids (PUFAs), sphingomyelins (SMs), and triglycerides (TGs). Species were only included if $p \leq 0.001$.

HIGHER IN BLOOD, LOWER IN SEMINAL FLUID

LPC(15:0)	PC(33:0)	PC(37:2)	PC(41:6)	PC-O(40:4)	PI(35:0)	DG(34:3)	TG(52:05)
LPC(17:0)	PC(33:1)	PC(37:3)	PC(41:7)	PC-O(40:5)	PI(38:4)	DG(36:3)	TG(54:03)
LPC(18:1)	PC(33:2)	PC(37:4)	PC(42:2)	PC-O(40:6)	PI(39:0)	DG(36:4)	TG(54:04)
LPC(18:2)	PC(33:3)	PC(37:5)	PC(42:5)	PC-O(44:4)	PI-O(36:1)	TG(42:05)	TG(54:05)
LPE(20:0)	PC(34:1)	PC(37:6)	PC(43:4)	PE(36:2)	SM(34:02)	TG(46:01)	TG(54:10)
LPE(20:2)	PC(34:2)	PC(38:2)	PC(44:2)	PE(38:1)	SM(36:02)	TG(46:05)	TGox(45:5)
LPS(22:1)	PC(34:2)	PC(38:4)	PC(52:4)	PE(38:2)	SM(39:02)	TG(48:01)	TGox(45:6)
PA-O(42:2)	PC(35:1)	PC(38:5)	PC-O(34:01)	PE(40:3)	SM(40:02)	TG(48:02)	TGox(46:5)
PC(32:0)	PC(35:2)	PC(38:6)	PC-O(32:0)	PE(40:4)	SM(41:00)	TG(48:05)	TGox(46:6)
PC(34:1)	PC(35:3)	PC(39:5)	PC-O(35:4)	PE(40:39:2)	SM(41:01)	TG(50:01)	TGox(47:5)
PC(34:2)	PC(35:4)	PC(39:6)	PC-O(36:2)	PG(41:0)	SM(42:02)	TG(50:02)	TGox(47:6)
PC(34:3)	PC(35:5)	PC(40:2)	PC-O(36:4)	PG(42:2)	SM(42:03)	TG(50:03)	TGox(47:7)
PC(36:3)	PC(36:1)	PC(40:3)	PC-O(38:3)	PG(43:3)	SM(48:01)	TG(52:01)	TGox(48:6)
PC(36:4)	PC(36:2)	PC(40:4)	PC-O(38:4)	PG(44:4)	FA(16:2)	TG(52:02)	TGox(49:7)
PC(36:5)	PC(36:3)	PC(40:5)	PC-O(38:6)	PG-O(40:3)	FA(18:2)	TG(52:03)	TGox(50:8)
PC(38:5)	PC(36:4)	PC(41:2)	PC-O(38:7)	PI(34:2)	FA(20:4)	TG(52:04)	TGox(53:6)

LOWER IN BLOOD, HIGHER IN SEMINAL FLUID

LPE(16:0)	PC(36:7)	PE-O(36:5)	PI(48:5)	FA(20:1)
LPE(18:0)	PC-O(33:1)	PE-O(38:4)	Cer(33:1)	FA(20:3)
LPE(18:1)	PC-O(35:0)	PE-O(38:6)	Cer(34:1)	FA(22:6)
LPE-O(18:1)	PC-O(35:1)	PE-P(38:6)	Cer(36:1)	DG(28:0)
LPE-P(16:0)	PC-O(37:1)	PE-P(40:6)	Cer(38:1)	DG(44:7)
LPG(35:0)	PC-O(29:1)	PG(34:0)	Cer(40:1)	TG(44:4)
PA(33:03)	PE(32:6)	PG(36:1)	Cer(42:1)	TG(46:4)
PA-O(36:0)	PE(40:7)	PG(45:7)	SM(33:1)	TG(48:4)
PA-O(42:0)	PE(34:1)	PG-O(35:0)	SM(34:1)	TGox(46:6)
PC(30:2)	PE(40:1)	PG-O(39:0)	SM(35:1)	TGox(46:3)
PC(32:2)	PE(42:1)	PG-O(41:0)	SM(37:1)	TGox(46:7)
PC(32:3)	PE-O(34:2)	PI(32:0)	SM(38:1)	TGox(46:8)
PC(34:3)	PE-O(34:3)	PI(34:0)	SM(39:1)	TGox(48:3)
PC(34:4)	PE-O(36:2)	PI(34:1)	SM(40:1)	TGox(48:9)
PC(36:0)	PE-O(36:3)	PI(36:1)	SM(41:0)	TGox(51:0)
PC(36:6)	PE-O(36:4)	PI(38:2)	SM(42:1)	

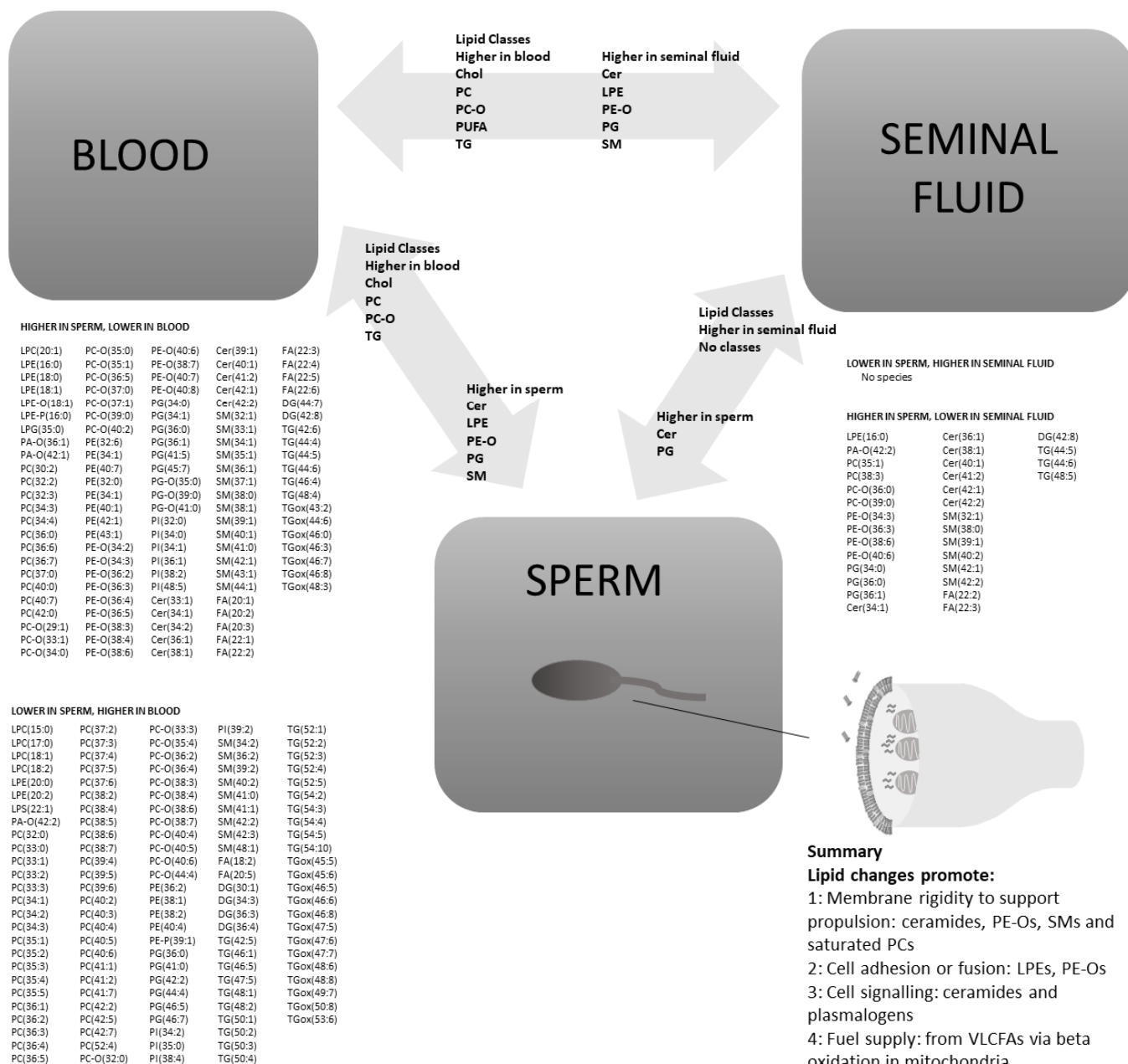
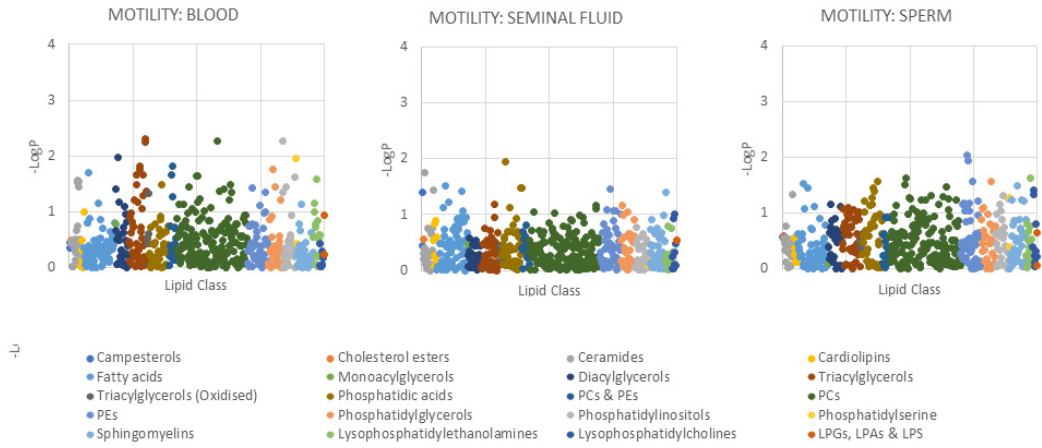


Figure S2: Distribution of lipids across three compartments shown in manhattan plots and heatmaps of relative abundance (a-c) and significance (d-f). Manhattan plots: lipid metabolites were tested for significance ($-\log P$) and fold change between low motility and high motility groups within each compartment using unpaired t tests. Heatmap 1: Lipid metabolites were compared for relative abundance raw counts (a-b) and normalised to blood (c-d). Heatmap 2: Lipid metabolites were tested for significance ($-\log P$) between low motility and high motility groups within each compartment using paired t tests. Lipid were identified as follows: ceramides (CERs), cholesterol (CHOL), cholesterol esters (CEs), diglycerides (DG), fatty acids (FAs), lysophosphatidylcholines (LPCs), lysophosphatidylethanolamines (LPEs), phosphatidylcholines (PCs), phosphatidylcholine-plasmalogens (PC-Os), phosphatidylethanolamines (PEs), phosphatidylethanolamine-plasmalogens (PE-Os), phosphatidylglycerides (PGs), phosphatidylinositols (PIs), phosphatidylserines (PSs), polyunsaturated FAs (PUFAs), sphingomyelins (SMs), triglycerides (TGs).

MANHATTAN PLOTS



HEATMAP 1 – RELATIVE ABUNDANCE

a	HIGH MOTILITY			b	LOW MOTILITY			c	HIGH MOTILITY			d	LOW MOTILITY			Key
	BLOOD	SF	SPERM		BLOOD	SF	SPERM		BLOOD	SF	SPERM		BLOOD	SF	SPERM	
CER	2.21	34.30	51.67	CER	5.04	42.23	53.72	CER	1.00	15.48	23.33	CER	1.00	8.38	10.66	Low Abundance Medium Abundance High Abundance
CER +	0.34	57.46	75.29	CER +	0.53	60.08	77.88	CER +	1.00	169.40	221.98	CER +	1.00	114.31	148.18	
CFA	21.18	27.39	33.21	CFA	24.00	25.46	39.60	CFA	1.00	1.29	1.57	CFA	1.00	1.06	1.65	
CHOL +	126.90	23.07	5.35	CHOL +	123.21	15.04	27.23	CHOL +	1.00	0.18	0.04	CHOL +	1.00	0.12	0.22	
EFA	4.12	5.88	7.41	EFA	4.26	5.17	8.46	EFA	1.00	1.43	1.80	EFA	1.00	1.22	1.99	
FA-OH	11.89	13.06	10.94	FA-OH	14.60	9.82	11.71	FA-OH	1.00	1.10	0.92	FA-OH	1.00	0.67	0.80	
LPC	81.41	39.89	34.78	LPC	87.20	35.14	29.78	LPC	1.00	0.49	0.43	LPC	1.00	0.40	0.34	
LPC +	175.34	177.60	188.01	LPC +	161.97	155.44	140.70	LPC +	1.00	1.01	1.07	LPC +	1.00	0.96	0.87	
LPE	157.24	375.24	360.36	LPE	162.49	362.55	320.31	LPE	1.00	2.39	2.29	LPE	1.00	2.23	1.97	
LPE +	0.38	44.83	58.90	LPE +	0.11	34.98	35.85	LPE +	1.00	118.40	155.59	LPE +	1.00	307.26	314.84	
MFA	98.99	111.29	128.80	MFA	113.35	117.21	162.51	MFA	1.00	1.12	1.30	MFA	1.00	1.03	1.43	
PC	323.56	73.87	79.60	PC	316.99	67.02	74.23	PC	1.00	0.23	0.25	PC	1.00	0.21	0.23	
PC +	393.27	74.74	80.62	PC +	343.95	65.01	63.88	PC +	1.00	0.19	0.20	PC +	1.00	0.19	0.19	
PC-O	177.19	38.59	36.75	PC-O	177.35	33.29	35.09	PC-O	1.00	0.22	0.21	PC-O	1.00	0.19	0.20	
PE	106.29	99.59	90.51	PE	102.95	107.27	95.17	PE	1.00	0.94	0.85	PE	1.00	1.04	0.92	
PE +	4.08	58.20	44.07	PE +	3.97	80.00	59.82	PE +	1.00	14.26	10.79	PE +	1.00	20.16	15.07	
PE-O	31.82	130.57	137.38	PE-O	27.95	125.18	148.77	PE-O	1.00	4.10	4.32	PE-O	1.00	4.48	5.32	
PG	5.71	12.57	17.09	PG	5.47	13.62	21.44	PG	1.00	2.20	2.99	PG	1.00	2.49	3.92	
PG +	4.25	3.44	3.34	PG +	3.38	10.04	2.71	PG +	1.00	0.81	0.79	PG +	1.00	2.97	0.80	
PI	36.76	35.50	37.33	PI	31.91	38.67	37.40	PI	1.00	0.97	1.02	PI	1.00	1.21	1.17	
PS +	5.05	13.98	10.30	PS +	6.32	9.34	13.40	PS +	1.00	2.77	2.04	PS +	1.00	1.48	2.12	
PUFA	69.71	44.06	49.71	PUFA	86.02	44.57	60.44	PUFA	1.00	0.63	0.71	PUFA	1.00	0.52	0.70	
SFA	818.32	830.71	809.49	SFA	784.76	827.53	764.28	SFA	1.00	1.02	0.99	SFA	1.00	1.05	0.97	
SM	74.65	154.61	149.02	SM	78.96	168.46	177.77	SM	1.00	2.07	2.00	SM	1.00	2.13	2.25	
SM +	94.18	385.48	405.30	SM +	90.02	419.38	434.03	SM +	1.00	4.09	4.30	SM +	1.00	4.66	4.82	
TG +	195.72	160.14	128.58	TG +	266.11	150.52	144.02	TG +	1.00	0.82	0.66	TG +	1.00	0.57	0.54	

HEATMAP 2 - SIGNIFICANCE

a	HIGH MOTILITY GROUP			b	LOW MOTILITY GROUP			Key
	-LogP	SF	Blood		-LogP	SF	Blood	
CE+	2.35	SF	0.45	Sperm	2.86	Blood	1.38	0.00 2.00 4.00 6.00 8.00 10.00 12.00
CER+	4.11	SF	2.28	Sperm	8.20	Blood	4.52	
CHOL+	3.57	SF	0.64	Sperm	5.63	Blood	3.03	
EFA	1.15	SF	2.85	Sperm	2.77	Blood	1.66	
FA-OH	0.23	SF	4.62	Sperm	4.95	Blood	0.71	
LCFA	1.21	SF	3.63	Sperm	6.27	Blood	1.46	
LPC	4.36	SF	7.82	Sperm	8.42	Blood	5.35	
LPC+	0.18	SF	0.43	Sperm	0.87	Blood	0.10	
LPE	7.77	SF	6.31	Sperm	0.06	Blood	6.78	
LPE+	2.49	SF	1.10	Sperm	4.01	Blood	3.19	
LPI+	0.80	SF	0.72	Sperm	0.82	Blood	0.92	
MFA	0.57	SF	4.48	Sperm	4.78	Blood	0.88	
PC	5.41	SF	3.31	Sperm	8.66	Blood	4.72	
PC+	5.17	SF	0.59	Sperm	6.33	Blood	5.93	
PC-O	11.60	SF	5.77	Sperm	9.89	Blood	9.44	
PE	2.96	SF	2.03	Sperm	2.01	Blood	4.18	
PE+	3.45	SF	0.80	Sperm	3.69	Blood	4.01	
PE-O	1.01	SF	6.65	Sperm	8.28	Blood	0.79	
PG	1.58	SF	7.11	Sperm	0.39	Blood	2.19	
PG+	0.19	SF	0.04	Sperm	0.34	Blood	1.48	
PI	7.51	SF	8.95	Sperm	3.31	Blood	6.40	
PI-O	8.63	SF	8.97	Sperm	7.49	Blood	8.18	
PS+	1.34	SF	1.74	Sperm	0.83	Blood	0.41	
PUFA	0.87	SF	0.92	Sperm	2.84	Blood	1.10	
SFA	0.09	SF	8.88	Sperm	11.47	Blood	0.20	
SM	9.57	SF	0.00	Sperm	0.00	Blood	8.42	
SM+	6.05	SF	1.54	Sperm	8.73	Blood	6.62	
TG+	0.90	SF	1.56	Sperm	1.39	Blood	3.31	

Figure S3: Men with lower motility often have other conditions affecting fertility, including low spermatozoa count and low semen volume (categorised below median for each).

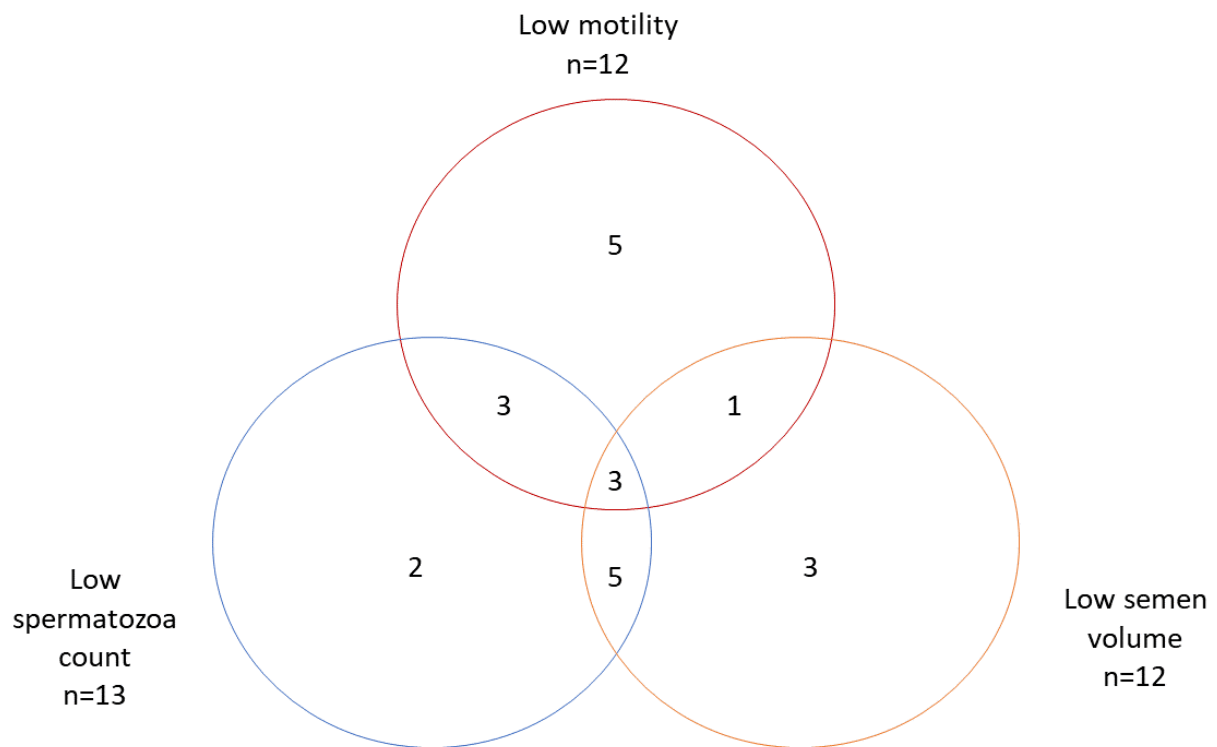


Figure S4: Significant differences in individual lipid species in association with sperm number between compartments. Lipid were included as follows: ceramides (CERs), cholesterol (CHOL), cholesterol esters (CEs), diglycerides (DG), fatty acids (FAs), lysophosphatidylcholines (LPCs), lysophosphatidylethanolamines (LPEs), phosphatidylcholines (PCs), phosphatidylcholine-plasmalogens (PC-Os), phosphatidylethanolamines (PEs), phosphatidylethanolamine-plasmalogens (PE-Os), phosphatidylglycerides (PGs), phosphatidylinositols (PIs), phosphatidylserines (PSs), polyunsaturated fatty acids (PUFAs), sphingomyelins (SMs), and triglycerides (TGs). Species were only included if $p \leq 0.001$.

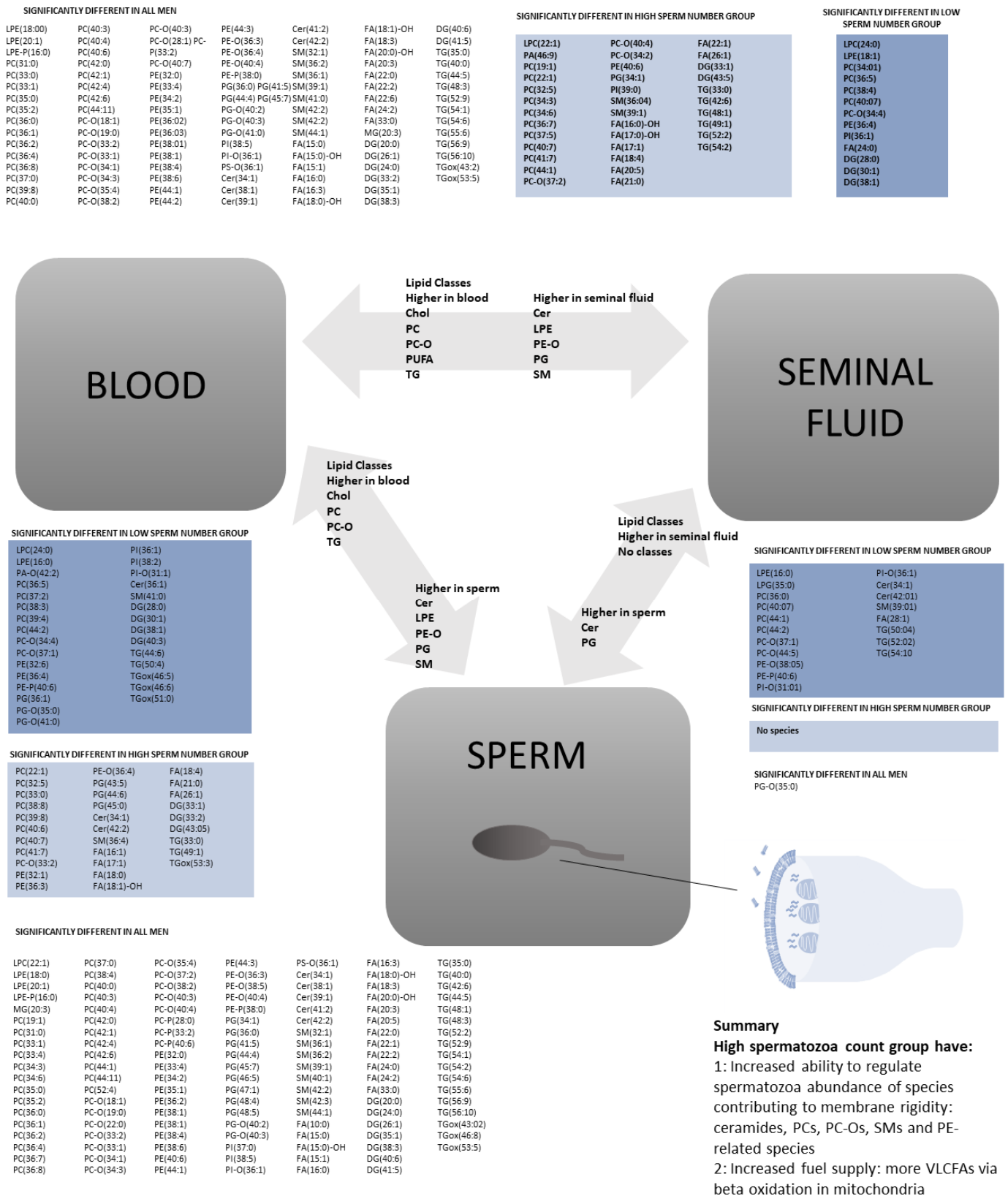


Figure S5: Significant differences in individual lipid species in association with semen volume between compartments. Lipid were included as follows: ceramides (CERs), cholesterol (CHOL), cholesterol esters (CEs), diglycerides (DG), fatty acids (FAs), lysophosphatidylcholines (LPCs), lysophosphatidylethanolamines (LPEs), phosphatidylcholines (PCs), phosphatidylcholine-plasmalogens (PC-Os), phosphatidylethanolamines (PEs), phosphatidylethanolamine-plasmalogens (PE-Os), phosphatidylglycerides (PGs), phosphatidylinositols (PIs), phosphatidylserines (PSs), polyunsaturated fatty acids (PUFAs), sphingomyelins (SMs), and triglycerides (TGs). Species were only included if $p \leq 0.001$.

