Electronic Supplementary Material (ESI) for Molecular BioSystems
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Supplementary Information on:

UPLC-MS Metabolic Profiling of Second Trimester Amniotic Fluid and Maternal Urine and Comparison with NMR Spectral Profiling for the Identification of Pregnancy Disorder Biomarkers

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Figure S1 – Base Peak Intensity (BPI) chromatograms obtained for the QC samples of 2nd trimester maternal urine using: a) HSS/ESI+, b) HSS/ESI-, c) HILIC/ESI+, d) HILIC/ESI-; and 2nd trimester amniotic fluid using: e) HSS/ESI+, f) HSS/ESI-, g) HILIC/ESI+, h) HILIC/ESI-.

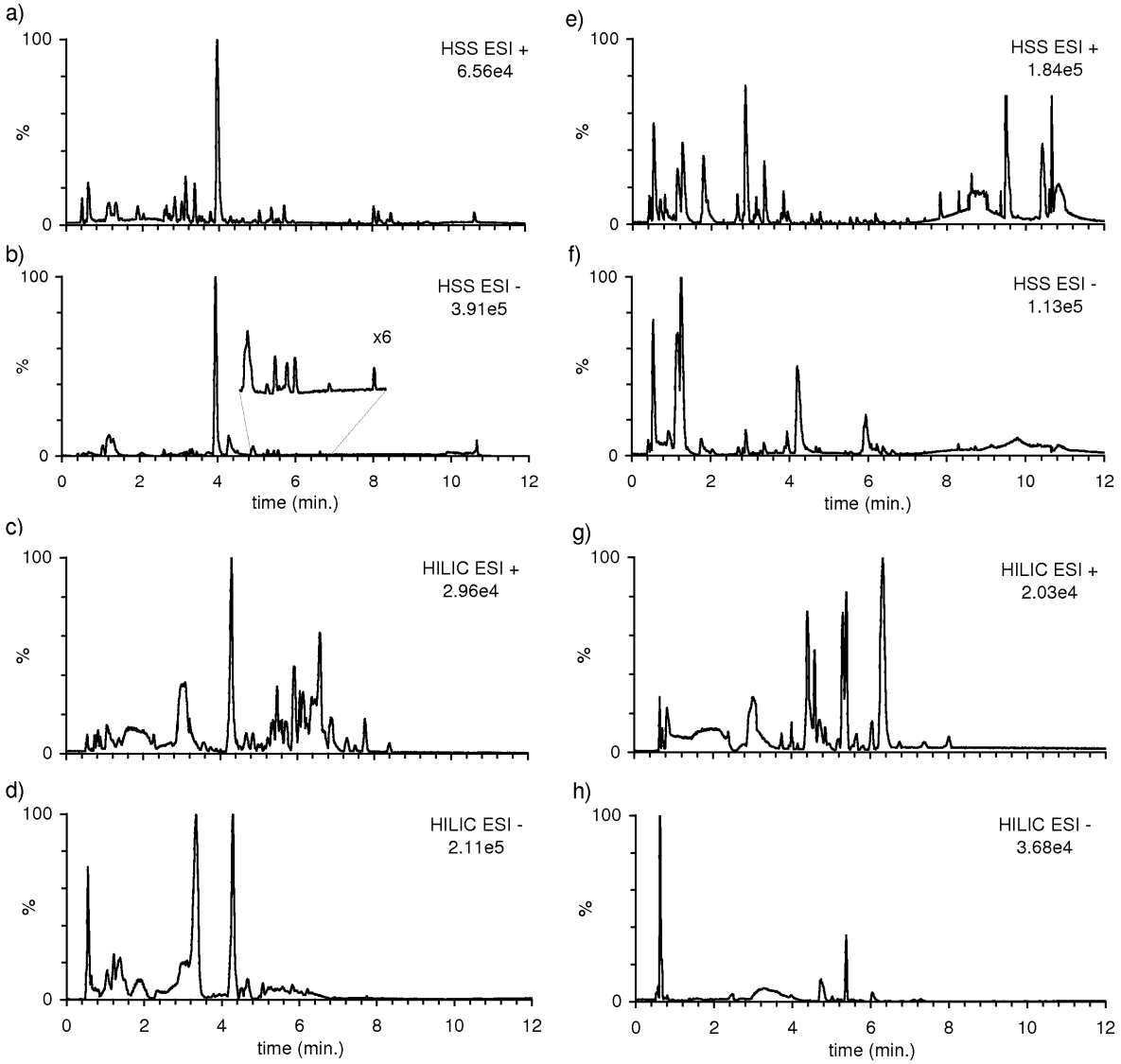

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Figure S2 – PCA of UPLC-MS datasets showing QC sample clustering for a) amniotic fluid, HSS/ESI+ log transformed, b) amniotic fluid, HILIC/ESI- log transformed, c) maternal urine, HSS/ESI+ UV scaled and d) maternal urine, HILIC/ESI- UV scaled. ■ QC samples, □ all samples.

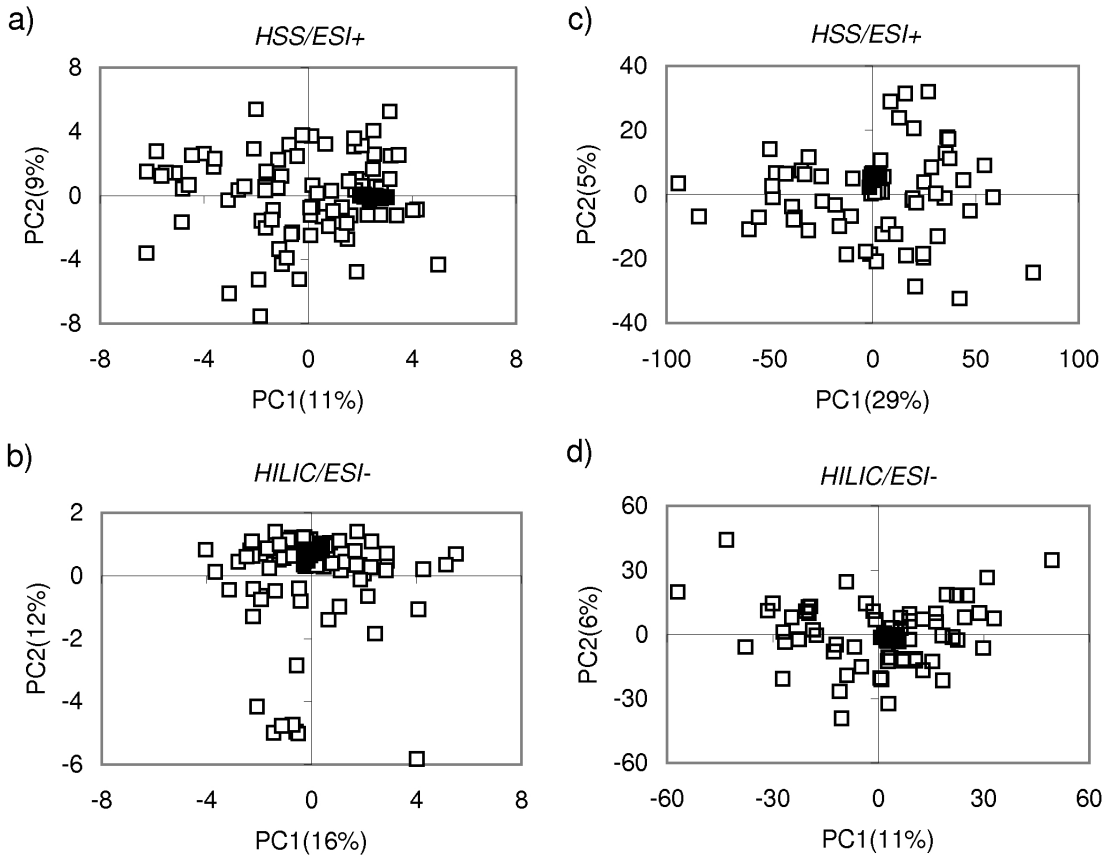

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Figure S3 – Distributions of Q^2 values of models from true and permuted classes obtained from Monte-Carlo cross-validation (MCCV): a) control *vs* pre-PTD HSS/ESI+ Pareto scaling for amniotic fluid; b) control *vs* FM HILIC/ESI- UV scaling for maternal urine.

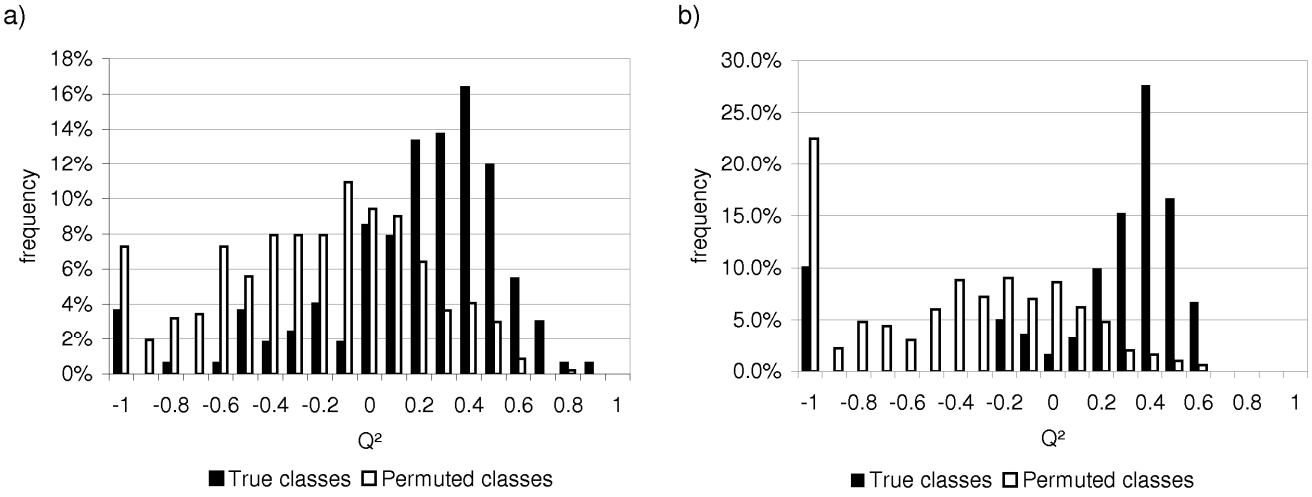

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Figure S4 – PLS-DA scores plots from controls *vs* pre-diagnostic-GDM models of 2nd trimester Maternal Urine: a) HSS/ESI- Pareto scaled model, b) HSS/ESI+ UV scaled model. ■ Controls (n=21), △ pre-diagnostic-GDM (n=20) samples.

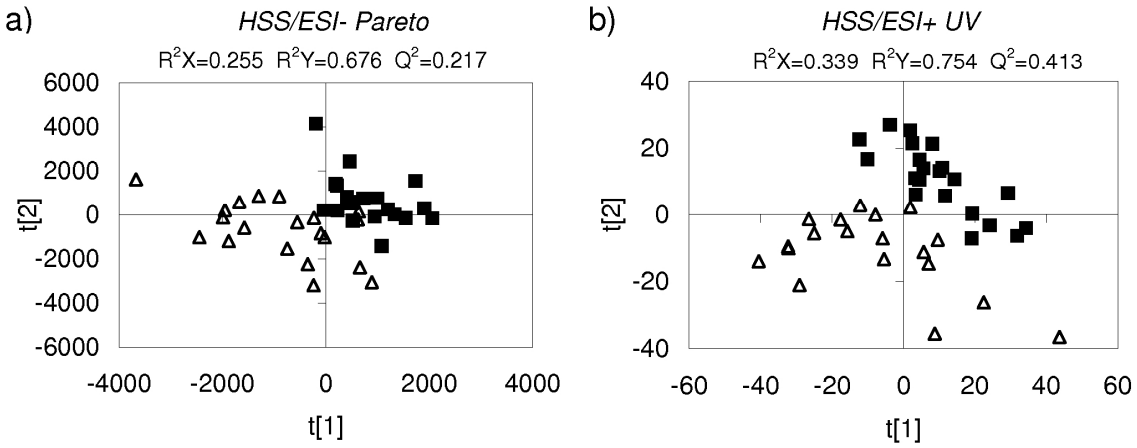

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Table S1 –Parameters describing the PLS-DA and OPLS-DA models obtained for 2nd trimester Amniotic Fluid and Urine UPLC-MS datasets. Values in bold and underlined correspond to the models with good predictive power; values only in bold correspond to models with lower predictive power that were considered for further interpretation.

2nd trimester Amniotic Fluid											
				PLS-DA model parameters				**OPLS-DA model parameters**			
model	**UPLC column**	**detection mode**	**scaling or transformation**	**R^2X**	**R^2Y**	**Q^2**	**LV**	**R^2X**	**R^2Y**	**Q^2**	**LV**
	HSS	ESI+	Pareto	0.187	0.815	**<u>0.471</u>**	2	0.187	0.815	0.558	1+1
control	HSS	ESI+	Log	0.179	0.844	**<u>0.490</u>**	2	0.179	0.844	0.558	1+1
vs	HSS	ESI-	Log	0.201	0.859	**<u>0.589</u>**	2	0.201	0.859	0.591	1+1
FM	HILIC	ESI+	UV	0.243	0.819	**<u>0.623</u>**	2	0.243	0.819	0.535	1+1
	HILIC	ESI+	Log	0.271	0.770	**<u>0.538</u>**	2	0.271	0.770	0.571	1+1
	HILIC	ESI-	Log	0.286	0.690	**<u>0.438</u>**	2	0.286	0.690	0.470	1+1
	HSS	ESI+	Pareto	0.118	0.805	0.186	2	0.118	0.805	0.247	1+1
control	HSS	ESI+	Log	0.175	0.650	0.098	2	0.175	0.650	0.110	1+1
vs	HSS	ESI-	Log	0.110	0.844	0.096	2	0.110	0.844	0.040	1+1
pre-diagnostic	HILIC	ESI+	UV	0.223	0.741	0.248	2	0.223	0.741	-0.133	1+1
GDM	HILIC	ESI+	Log	0.230	0.647	-0.034	2	0.230	0.647	-0.199	1+1
	HILIC	ESI-	Log	0.217	0.650	-0.063	2	0.217	0.650	-0.250	1+1
	HSS	ESI+	Pareto	0.162	0.856	**0.384**	2	0.162	0.856	0.460	1+1
control	HSS	ESI+	Log	0.196	0.769	**0.202**	2	0.196	0.769	0.071	1+1
vs	HSS	ESI-	Log	0.122	0.929	**0.296**	2	0.122	0.929	0.282	1+1
pre-PTD	HILIC	ESI+	UV	0.292	0.953	**0.565**	3	0.249	0.825	0.121	1+1
	HILIC	ESI+	Log	0.351	0.899	**0.353**	3	0.275	0.795	0.031	1+1
	HILIC	ESI-	Log	0.153	0.870	0.108	2	0.153	0.870	0.125	1+1
2nd trimester Maternal Urine											
				PLS-DA model parameters				**OPLS-DA model parameters**			
model	**UPLC column**	**detection mode**	**scaling or transformation**	**R^2X**	**R^2Y**	**Q^2**	**LV**	**R^2X**	**R^2Y**	**Q^2**	**LV**
	HSS	ESI+	Pareto	0.772	0.242	0.242	2	0.256	0.772	0.182	1+1
	HSS	ESI+	UV	0.337	0.834	0.363	2	0.337	0.834	0.281	1+1
control	HSS	ESI-	Pareto	0.372	0.958	**<u>0.558</u>**	4	0.325	0.887	0.338	1+2
vs	HSS	ESI-	UV	0.340	0.996	**<u>0.736</u>**	4	0.194	0.906	0.427	1+1
FM	HILIC	ESI+	Pareto	0.248	0.795	0.331	2	0.248	0.795	0.116	1+1
	HILIC	ESI+	UV	0.251	0.822	0.313	2	0.251	0.822	0.061	1+1
	HILIC	ESI-	Pareto	0.294	0.980	**<u>0.552</u>**	4	0.175	0.850	0.256	1+1
	HILIC	ESI-	UV	0.248	0.998	**<u>0.673</u>**	4	0.130	0.952	0.494	1+1
	HSS	ESI+	Pareto	0.262	0.666	0.176	2	0.269	0.666	-0.034	1+1
control	HSS	ESI+	UV	0.339	0.754	0.413	2	0.339	0.754	-0.031	1+1
vs	HSS	ESI-	Pareto	0.255	0.676	0.217	2	0.255	0.676	0.141	1+1
pre-diagnostic	HSS	ESI-	UV	0.255	0.832	0.478	2	0.255	0.832	0.115	1+1
GDM	HILIC	ESI+	Pareto	0.262	0.670	0.164	2	0.262	0.670	0.044	1+1
	HILIC	ESI+	UV	0.239	0.776	0.389	2	0.239	0.776	-0.033	1+1
	HILIC	ESI-	Pareto	0.123	0.831	-0.038	2	0.123	0.831	0.062	1+1
	HILIC	ESI-	UV	0.150	0.883	0.322	2	0.15	0.883	0.112	1+1

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Table S2 – Unassigned significant features obtained from models of both AF and Maternal Urine datasets.

Amniotic Fluid - Fetal Malformations (FM)								
UPLC column	**detection mode**	**m/z**	**RT(min.)**	**feature intensity**	**model scaling/ transformation**	**direction of variation**	**magnitude of variation**	***p*-value**
HSS	ESI+	125.97	0.4	low	Pareto	↑	2.8	1.01×10^{-05}
HSS	ESI+	143.99	0.4	low	Pareto	↑	2.7	1.61×10^{-05}
HSS	ESI+	185.02	0.4	low	Pareto	↑	2.3	5.61×10^{-07}
HSS	ESI+	167.01	0.4	high	Pareto	↑	2.4	4.85×10^{-07}
HSS	ESI+	122.53	0.4	low	Log	↑	2.7	5.61×10^{-07}
HSS	ESI+	185.02	0.4	low	Log	↑	2.3	2.02×10^{-05}
HSS	ESI+	239.96	0.4	low	Log	↑	2.7	2.52×10^{-05}
HSS	ESI+	242.00	0.4	low	Pareto	↑	1.5	5.36×10^{-05}
HSS	ESI+	507.23	0.5	low	Log	↓	3.7	8.96×10^{-05}
HSS	ESI+	288.92	0.5	low	Log	↑	2.1	3.10×10^{-04}
HSS	ESI+	248.08	0.8	very low	Log	↑	1.8	4.85×10^{-04}
HSS	ESI+	209.05	1.0	low	Pareto	↑	2.0	2.25×10^{-05}
HSS	ESI+	245.07	1.0	low	Pareto	↑	1.8	1.80×10^{-05}
HSS	ESI+	267.06	1.0	low	Pareto	↑	1.7	5.49×10^{-06}
HSS	ESI+	281.14	1.2	low	Log	↓	1.9	2.02×10^{-05}
HSS	ESI+	137.03	1.4	low	Pareto	↑	2.4	4.84×10^{-06}
HSS	ESI+	170.07	1.7	low	Log	↓	1.7	3.10×10^{-04}
HSS	ESI+	153.03	1.7	low	Pareto	↑	1.7	3.40×10^{-04}
HSS	ESI+	749.29	2.6	low	Log	↑	2.8	4.44×10^{-04}
HSS	ESI+	387.17	2.6	low	Log	↑	2.1	8.93×10^{-06}
HSS	ESI+	328.14	2.9	low	Log	↓	1.5	2.83×10^{-03}
HSS	ESI+	310.13	2.9	low	Log	↓	1.6	1.14×10^{-06}
HSS	ESI+	410.18	3.4	low	Log	↓	2.3	2.59×10^{-04}
HSS	ESI+	819.35	3.4	low	Log	↓	3.5	2.91×10^{-06}
HSS	ESI+	261.14	3.5	high	Log	↓	2.4	2.67×10^{-07}
HSS	ESI+	363.22	5.7	low	Log	↑	1.7	7.43×10^{-04}
HSS	ESI+	541.27	6.7	very low	Log	↑	2.0	1.43×10^{-05}
HSS	ESI+	285.26	6.7	low	Pareto	↑	2.3	4.34×10^{-05}
HSS	ESI-	402.93	0.4	low	Log	↑	2.2	3.90×10^{-05}
HSS	ESI-	516.90	0.4	low	Log	↑	2.2	7.31×10^{-05}
HSS	ESI-	548.85	0.4	low	Log	↑	2.6	3.50×10^{-05}
HSS	ESI-	494.86	0.5	low	Log	↑	2.4	1.67×10^{-07}
HSS	ESI-	597.30	1.9	low	Log	↓	2.1	1.96×10^{-06}
HSS	ESI-	626.25	1.9	low	Log	↓	1.9	4.84×10^{-06}
HSS	ESI-	624.26	2.7	low	Log	↑	2.7	9.90×10^{-05}
HSS	ESI-	817.31	3.4	low	Log	↓	3.8	4.84×10^{-06}
HSS	ESI-	407.22	5.6	low	Log	↑	2.5	4.79×10^{-09}
HSS	ESI-	564.29	6.7	low	Log	↑	2.6	3.14×10^{-05}
HILIC	ESI+	752.56	0.8	very low	UV	↑	1.6	2.55×10^{-04}
HILIC	ESI+	123.06	0.9	very low	UV	↓	1.3	9.02×10^{-04}
HILIC	ESI+	718.58	4.0	low	UV	↑	2.3	1.80×10^{-06}
HILIC	ESI+	701.56	4.2	low	UV	↑	2.5	3.49×10^{-03}
HILIC	ESI+	260.19	4.9	low	UV	↓	2.0	4.01×10^{-05}
HILIC	ESI+	177.07	4.9	low	UV	↓	1.8	8.85×10^{-07}
HILIC	ESI+	397.13	4.9	low	Log	↓	2.7	3.07×10^{-07}
HILIC	ESI+	367.09	5.2	low	UV	↓	1.4	1.16×10^{-07}
HILIC	ESI+	299.10	5.2	very low	UV	↓	1.6	4.06×10^{-08}

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Table S2 (cont.).

Amniotic Fluid - Fetal Malformations (FM)								
UPLC column	**detection mode**	**m/z**	**RT(min.)**	**feature intensity**	**model scaling/ transformation**	**direction of variation**	**magnitude of variation**	***p*-value**
HILIC	ESI+	438.16	5.2	low	UV	↓	2.0	2.07×10^{-06}
HILIC	ESI+	543.15	5.4	low	Log	↓	1.5	2.79×10^{-02}
HILIC	ESI+	521.17	5.4	low	Log	↓	1.5	5.87×10^{-02}
HILIC	ESI+	277.06	5.4	low	Log	↓	1.8	4.66×10^{-03}
HILIC	ESI+	500.19	5.4	low	Log	↓	2.2	8.12×10^{-03}
HILIC	ESI+	176.04	5.6	low	UV	↓	1.4	3.72×10^{-04}
HILIC	ESI+	154.06	5.7	low	UV	↓	1.3	1.73×10^{-04}
HILIC	ESI+	307.11	5.7	very low	UV	↓	1.5	1.57×10^{-04}
HILIC	ESI-	465.31	0.6	high	Log	↑	2.3	1.96×10^{-06}
HILIC	ESI-	413.20	0.7	low	Log	↑	2.1	1.67×10^{-07}
HILIC	ESI-	385.17	0.7	low	Log	↑	2.0	7.02×10^{-06}
HILIC	ESI-	401.16	0.7	low	Log	↑	2.7	8.10×10^{-05}
HILIC	ESI-	198.07	5.0	low	Log	↓	1.9	2.29×10^{-07}
HILIC	ESI-	497.17	5.4	low	Log	↓	1.7	1.89×10^{-02}
HILIC	ESI-	237.09	5.4	high	Log	↓	1.6	2.66×10^{-02}
Amniotic Fluid - pre-Premature Delivery (PTD)								
UPLC column	**detection mode**	**m/z**	**RT(min.)**	**feature intensity**	**model scaling/ transformation**	**direction of variation**	**magnitude of variation**	***p*-value**
HSS	ESI+	331.12	0.6	low	Log	↑	1.8	6.10×10^{-03}
HSS	ESI+	353.10	0.6	very low	Log	↑	1.9	1.58×10^{-02}
HSS	ESI+	309.16	0.6	low	Log	↑	1.7	1.93×10^{-02}
HSS	ESI+	226.12	0.7	low	Log	↑	1.7	4.69×10^{-02}
HSS	ESI-	597.09	0.7	low	Log	↑	1.8	3.39×10^{-03}
HSS	ESI+	192.03	0.8	low	Pareto	↓	1.4	1.04×10^{-03}
HSS	ESI+	222.04	1.0	low	Log	↓	2.0	6.83×10^{-03}
HSS	ESI+	390.98	1.3	low	Pareto	↑	1.3	5.45×10^{-03}
HSS	ESI+	247.14	1.4	high	Log	↓	2.2	1.37×10^{-03}
HSS	ESI+	202.18	2.0	high	Log	↑	1.6	3.64×10^{-04}
HSS	ESI+	749.29	2.6	very low	Log	↑	1.9	2.33×10^{-02}
HSS	ESI+	247.16	2.9	low	Pareto	↑	1.3	6.10×10^{-03}
HSS	ESI-	371.06	3.1	low	Log	↓	1.9	2.63×10^{-04}
HSS	ESI-	248.06	3.4	low	Log	↓	2.9	6.72×10^{-04}
HSS	ESI+	316.21	3.9	low	Log	↑	1.2	3.06×10^{-02}
HSS	ESI-	920.87	4.2	high	Log	↑	2.7	1.05×10^{-02}
HSS	ESI+	361.20	5.7	very low	Log	↑	1.5	1.59×10^{-04}
HILIC	ESI+	397.20	0.7	low	UV	↓	1.2	4.92×10^{-02}
HILIC	ESI+	136.08	0.7	low	UV	↓	1.2	1.18×10^{-02}
HILIC	ESI+	147.07	0.7	low	UV	↓	1.2	1.18×10^{-02}
HILIC	ESI+	756.55	4.0	very low	Log	↑	1.4	4.74×10^{-03}
HILIC	ESI+	812.68	4.3	very low	UV	↑	1.7	9.49×10^{-03}
HILIC	ESI+	785.66	4.4	very low	Log	↑	1.8	6.01×10^{-03}
HILIC	ESI+	787.67	4.4	very low	Log	↑	1.4	1.46×10^{-02}
HILIC	ESI+	260.19	4.9	low	UV	↓	1.8	4.19×10^{-03}
HILIC	ESI+	169.99	4.9	low	UV	↓	1.4	4.19×10^{-03}
HILIC	ESI+	246.17	5.3	low	UV	↓	1.6	1.27×10^{-03}
HILIC	ESI+	527.25	7.2	low	UV	↓	1.7	6.01×10^{-03}

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Table S2 (cont.).

Maternal Urine - Fetal Malformations (FM)								
UPLC column	**detection mode**	**m/z**	**RT(min.)**	**feature intensity**	**model scaling**	**direction of variation**	**magnitude of variation**	***p*-value**
HSS	ESI-	203.07	0.63	very low	UV	↑	1.5	7.39×10^{-03}
HSS	ESI-	895.34	3.25	very low	UV	↑	1.5	3.11×10^{-03}
HSS	ESI-	239.07	3.3	very low	UV	↑	1.4	2.73×10^{-03}
HSS	ESI-	507.07	3.37	very low	UV	↑	2.7	1.37×10^{-03}
HILIC	ESI-	109.04	0.55	low	UV	↓	2.8	2.02×10^{-04}
HILIC	ESI-	879.36	0.69	very low	UV	↑	3.7	1.69×10^{-04}
HILIC	ESI-	769.35	0.70	very low	UV	↑	3.2	4.00×10^{-04}
HILIC	ESI-	799.39	0.70	very low	UV	↑	4.9	5.69×10^{-06}
HILIC	ESI-	813.38	0.71	low	UV	↑	4.0	1.09×10^{-06}
HILIC	ESI-	443.19	0.71	low	UV	↑	1.8	2.39×10^{-03}
HILIC	ESI-	829.38	0.71	low	UV	↑	3.7	1.96×10^{-06}
HILIC	ESI-	771.35	0.72	very low	UV	↑	2.6	6.47×10^{-04}
HILIC	ESI-	385.17	0.72	high	UV	↑	1.9	1.16×10^{-02}
HILIC	ESI-	857.42	0.72	very low	UV	↑	4.3	7.30×10^{-06}
HILIC	ESI-	859.42	0.74	very low	UV	↑	3.4	6.60×10^{-05}
HILIC	ESI-	281.08	1.35	very high	Pareto	↑	1.6	1.60×10^{-02}
HILIC	ESI-	617.21	5.16	low	UV	↑	1.8	4.02×10^{-03}