

SUPPLEMENTARY INFORMATION

**Distribution of an analgesic palmitoylethanolamide and other N-acylethanolamines in human placental membranes**

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**Supplementary Table S1:** Concentrations of PEA (ng/g; ng/ml for US) in various placental specimens - original data and statistics (AV, average; SD, standard deviation). The descriptive statistics for each data set was calculated using R package. The outliers were identified using the interquartile range criterion. Outlier values (strikethrough) were excluded from the Wilcoxon test. Only data with a p-value of  $\leq 0.05$  were considered statistically significant.

**Average PEA concentrations (ng/g; ng/ml for US) – original data**

	AM1	AM2	ACM	AM1-d	AM2-d	AC M-d	PL1	PL2	UC	VX	US
<i>PL 1</i>	140.53	198.00	238.39	1701.3 3	934.29	200. 10	<del>695.</del> <del>19</del>	<del>537.</del> <del>21</del>	82.6 2	52.3 7	1.8 6
<i>PL 2</i>	549.81	755.94	197.04	801.85	1116.0 9	124. 85	252. 24	113. 86	101. 33	44.3 3	2.3 0
<i>PL 3</i>	195.99	321.69	161.28	726.94	809.76	348. 18	102. 48	75.3 1	39.9 0	25.1 7	6.6 0
<i>PL 4</i>	142.57	199.59	136.75	1141.9 4	1196.7 5	289. 19	161. 90	129. 75	49.6 8	42.1 3	1.4 2
<i>PL 5</i>	<del>1613.5</del> 9	<del>1123.5</del> 1	352.15	<del>6064.9</del> 1	<del>2838.4</del> 3	655. 11	393. 55	287. 97	109. 84	20.7 7	2.0 3
<i>PL 6</i>	180.50	610.16	266.51	1591.1 9	1414.2 2	833. 44	152. 04	147. 13	60.9 8	55.8 0	1.3 5
<i>PL 7</i>	625.15	699.30	483.86	1497.0 2	1241.2 5	<del>1536</del> <del>63</del>	170. 39	165. 32	85.9 1	28.2 0	6.3 3
<i>PL 8</i>	109.04	176.34		896.86	1532.3 1	-	-	-	-	39.2 0	5.8 1
<i>PL 9</i>	-	-	-	-	-	-	-	-	-	20.3 0	7.5 8
<i>PL 10</i>	-	-	-	-	-	-	-	-	-	22.5 0	-
<b>AV</b>	<b>277.65</b>	<b>423.00</b>	<b>262.28</b>	<b>1193.8</b> 7	<b>1177.8</b> 1	<b>408.</b> 48	<b>205.</b> 43	<b>153.</b> 22	<b>75.7</b> 5	<b>35.0</b> 8	<b>3.9</b> 2
<b>SD</b>	<b>214.63</b>	<b>256.21</b>	<b>121.09</b>	<b>402.06</b>	<b>253.15</b>	<b>276.</b> 82	<b>104.</b> 07	<b>72.8</b> 2	<b>26.3</b> 0	<b>13.3</b> 7	<b>2.5</b> 8

**Statistics for PEA (calculated in R) - Wilcoxon test;  $P < 0.050^*$ ;  $P < 0.010^{**}$ ;  $P < 0.0010^{***}$**

	Wilcoxon		t-test	
<i>AM1 vs AM1-d</i>	0.0006	***	0.0005	***
<i>AM2 vs AM2-d</i>	0.0006	***	0.0001	***
<i>ACM vs ACM-d</i>	0.4452	ns	0.2716	ns
<i>AM1 vs AM2</i>	0.0973	ns	0.2730	ns
<i>PL1 vs PL2</i>	0.3095	ns	0.3405	ns

**Supplementary Table S2:** Concentrations of OEA (ng/g; ng/ml for US) in various placental specimens - original data and statistics (AV, average and SD, standard deviation). The descriptive statistics for each data set was calculated using R package. The outliers were identified using the interquartile range criterion. Outlier values (strikethrough) were excluded from the Wilcoxon test. Only data with a p-value of  $\leq 0.05$  were considered statistically significant.

**Average OEA concentrations (ng/g; ng/ml for US) – original data**

	AM1	AM2	ACM	AM1-d	AM2-d	ACM-d	PL1	PL2	UC	VX	US
<i>PL 1</i>	35.03	61.30	100.3	507.67	274.23	86.80	<del>1383.7</del>	<del>1156.8</del>	50.3	5.93	24.7
			3				3	0	0		3
<i>PL 2</i>	198.1	248.1	57.83	289.41	417.59	60.30	273.71	195.62	40.9	7.88	28.7
	2	5							7		7
<i>PL 3</i>	36.04	99.89	70.69	218.58	217.86	132.0	93.48	45.14	14.7	9.47	11.9
						5			3		0
<i>PL 4</i>	53.78	77.35	57.54	676.88	505.22	159.6	209.26	133.15	21.6	1.60	17.3
						3			9		3
<i>PL 5</i>	<del>372.4</del>	<del>276.7</del>	148.5	<del>1538.5</del>	<del>1058.8</del>	334.8	291.98	303.30	40.1	2.63	8.33
	2	4	5	6	5	7			4		
<i>PL 6</i>	67.39	136.0	99.44	446.60	361.82	404.0	285.75	250.15	24.1	1.99	39.9
		0				8			7		7
<i>PL 7</i>	146.2	155.2	149.6	295.97	293.37	568.0	292.30	260.47	21.5	6.14	14.0
	5	4	7			7			4		7
<i>PL 8</i>	44.43	72.56	-	243.06	374.01	-	-	-	-	9.01	15.1
											3
<i>PL 9</i>	-	-	-	-	-	-	-	-	-	15.2	8.00
										9	
<i>PL 10</i>	-	-	-	-	-	-	-	-	-	-	13.5
											0
<b>AV</b>	<b>83.01</b>	<b>121.4</b>	<b>97.72</b>	<b>382.60</b>	<b>349.16</b>	<b>249.4</b>	<b>241.08</b>	<b>197.97</b>	<b>30.5</b>	<b>18.1</b>	<b>6.66</b>
		8				0			1	7	
<b>SD</b>	<b>63.70</b>	<b>65.56</b>	<b>39.22</b>	<b>167.78</b>	<b>96.42</b>	<b>190.0</b>	<b>78.83</b>	<b>95.17</b>	<b>13.1</b>	<b>9.58</b>	<b>4.39</b>
						9			7		

**Statistics for OEA (calculated in R) - Wilcoxon test;  $P < 0.050^*$ ;  $P < 0.010^{**}$ ;  $P < 0.0010^{***}$**

	Wilcoxon		t-test	
<i>AM1 vs AM1-d</i>	0.0006	***	0.0025	**
<i>AM2 vs AM2-d</i>	0.0012	**	0.0004	***
<i>ACM vs ACM-d</i>	0.0973	ns	0.0805	ns
<i>AM1 vs AM2</i>	0.1282	ns	0.2873	ns
<i>PL1 vs PL2</i>	0.3939	ns	0.4135	ns

**Supplementary Table S3:** Concentrations of AEA (ng/g; ng/ml for US) in various placental specimens - original data and statistics (AV, average, and SD, standard deviation). The descriptive statistics for each data set was calculated using R package. The outliers were identified using the interquartile range criterion. Outlier values (strikethrough) were excluded from the Wilcoxon test. Only data with a p-value of  $\leq 0.05$  were considered statistically significant. AEA was under detection limit (UDL) in VX.

*Average AEA concentrations (ng/g; ng/ml for US) – original data*

	AM1	AM2	ACM	AM1-d	AM2-d	AC M-d	PL1	PL2	UC	VX	US
<i>PL 1</i>	13.66	21.76	25.26	102.92	47.91	24.2	<del>175.</del> 9	<del>146.</del> 27	11.4	UDL	0.9 6
<i>PL 2</i>	26.45	37.95	14.06	43.85	83.16	12.7	33.6 1	34.6 8	6.82	UDL	2.6 0
<i>PL3</i>	11.25	22.94	18.70	35.33	39.72	20.5	26.5 8	18.8 4	6.05	UDL	0.4 7
<i>PL 4</i>	16.91	18.92	10.73	101.03	73.11	27.2	40.1 2	28.3 8	5.84	UDL	0.5 3
<i>PL 5</i>	<del>48.77</del>	<del>32.72</del>	22.28	156.88	<del>152.84</del>	48.9	40.3 9	44.3 4	6.08	UDL	0.6 9
<i>PL 6</i>	5.21	13.34	14.61	43.29	31.65	62.2	27.0 2	24.4 4	5.86	UDL	1.5 1
<i>PL7</i>	15.32	20.23	28.87	23.18	22.02	66.9	21.2 1	21.2 6	3.93	UDL	2.1 0
<i>PL 8</i>	3.58	6.31	-	9.50	21.64	-	-	-	-	UDL	1.7 6
<b><i>AV</i></b>	<b>13.20</b>	<b>20.20</b>	<b>19.22</b>	<b>64.50</b>	<b>45.60</b>	<b>37.5</b>	<b>31.4</b>	<b>28.6</b>	<b>6.58</b>	-	<b>1.3</b> <b>3</b>
<b><i>SD</i></b>	<b>7.68</b>	<b>9.72</b>	<b>6.57</b>	<b>50.43</b>	<b>24.26</b>	<b>21.5</b>	<b>7.84</b>	<b>9.51</b>	<b>2.33</b>	-	<b>0.7</b> <b>9</b>

*Statistics for AEA (calculated in R) - Wilcoxon test; P < 0.050\*; P < 0.010\*\*; P < 0.0010\*\*\**

	Wilcoxon		t-test	
<i>AM1 vs AM1-d</i>	0.017	*	0.0331	*
<i>AM2 vs AM2-d</i>	0.017	*	0.0335	*
<i>ACM vs ACM-d</i>	0.128	ns	0.0677	ns
<i>AM1 vs AM2</i>	0.165	ns	0.1616	ns
<i>PL1 vs PL2</i>	0.589	ns	0.5833	ns

**Supplementary Table S4:** Reference concentrations (Ref. conc.) of PEA, OEA and AEA (ng/g) in amnion (AM) and placental disc (PL). The values from the central (1) and peripheral part (2) of AM and PL were averaged (AV), because the differences in NAEs concentrations between AM1 vs AM2 and PL1 vs PL2, respectively, were not statistically significant. SD, standard deviation.

<i>Ref. conc.</i> (ng/g)	<i>PEA</i>		<i>OEA</i>		<i>AEA</i>	
	AM (1+2)	PL (1+2)	AM (1+2)	PL (1+2)	AM (1+2)	PL (1+2)
	140.53	252.24	35.03	273.71	13.66	33.60
	549.81	102.48	198.12	93.48	26.45	26.54
	195.99	161.90	36.04	209.26	11.25	40.18
	142.57	393.55	53.78	291.98	16.91	40.34
	180.50	152.04	67.39	285.75	5.21	27.04
	625.15	170.39	146.25	292.30	15.32	21.26
	109.04	113.86	44.43	195.62	3.58	34.68
	198.00	75.31	61.30	45.14	21.76	18.86
	755.94	129.75	248.00	133.15	37.95	28.32
	321.69	287.97	99.89	303.30	22.94	44.35
	199.59	147.13	77.35	250.15	18.92	24.41
	610.16	165.32	136.00	260.47	13.34	21.22
	699.30	-	155.24	-	20.23	-
	176.34	-	72.56	-	6.31	-
<i>AV</i>	<b>350.3</b>	<b>179.3</b>	<b>102.2</b>	<b>219.5</b>	<b>16.7</b>	<b>30.1</b>
<i>SD</i>	<b>239.3</b>	<b>89.9</b>	<b>65.2</b>	<b>86.3</b>	<b>9.2</b>	<b>8.4</b>

**Supplementary Table S5:** NAEs concentrations in AM samples stored in two additional solutions: standard cell culture medium (DMEM) or physiological saline (PS), at three different temperatures: 4 °C, room temperature (about 25 °C), 37 °C. AV, average; SD, standard deviation; PL, placenta.

***PEA (ng/g) in AM***

<i>Cultivation condition</i>	4 °C		25 °C		37 °C	
	PS	DMEM	PS	DMEM	PS	DMEM
<i>PL1</i>	1486.3	903.4	2037.8	4657.8	2683.2	1855.0
<i>PL2</i>	1937.8	643.8	2722.9	3101.5	2516.2	1931.3
<i>PL3</i>	2725.0	868.9	3489.9	5484.5	2106.0	1821.4
<i>PL4</i>	993.6	2048.4	1150.0	3280.0	1267.4	682.5
<i>PL5</i>	1080.8	3537.5	1119.0	3557.1	4555.6	192.9
<i>PL6</i>	1402.5	2264.4	1992.7	5545.5	1686.5	600.0
<b><i>AV</i></b>	<b>1604.3</b>	<b>1711.1</b>	<b>2085.4</b>	<b>4271.1</b>	<b>2469.1</b>	<b>1180.5</b>
<b><i>SD</i></b>	<b>643.3</b>	<b>1118.6</b>	<b>916.4</b>	<b>1105.1</b>	<b>1148.0</b>	<b>773.3</b>

***AEA (ng/g) in AM***

<i>Cultivation condition</i>	4 °C		25 °C		37 °C	
	PS	DMEM	PS	DMEM	PS	DMEM
<i>PL1</i>	41.7	31.5	63.5	126.9	86.1	36.6
<i>PL2</i>	47.6	20.9	74.5	92.8	96.0	49.1
<i>PL3</i>	65.1	30.8	99.6	129.6	76.9	45.1
<i>PL4</i>	30.9	79.0	78.0	82.8	53.3	39.5
<i>PL5</i>	24.7	135.0	63.8	125.0	251.1	16.4
<i>PL6</i>	45.1	84.8	85.1	194.5	128.6	31.3
<b><i>AV</i></b>	<b>42.5</b>	<b>63.7</b>	<b>77.4</b>	<b>125.3</b>	<b>115.3</b>	<b>36.3</b>
<b><i>SD</i></b>	<b>14.1</b>	<b>44.1</b>	<b>13.7</b>	<b>39.2</b>	<b>71.0</b>	<b>11.6</b>

***OEA (ng/g) in AM***

<i>Cultivation condition</i>	4 °C		25 °C		37 °C	
	PS	DMEM	PS	DMEM	PS	DMEM
<i>PL1</i>	434.0	324.3	484.8	951.7	559.5	403.4
<i>PL2</i>	483.6	200.7	649.6	566.3	663.8	427.3
<i>PL3</i>	732.7	231.1	723.0	1081.5	503.2	452.9
<i>PL4</i>	325.3	1165.2	356.0	882.7	252.2	211.8
<i>PL5</i>	373.6	2436.7	356.2	778.6	1588.9	75.7
<i>PL6</i>	445.7	958.5	590.2	1136.4	683.8	188.7
<b><i>AV</i></b>	<b>465.8</b>	<b>886.1</b>	<b>526.6</b>	<b>899.5</b>	<b>708.6</b>	<b>293.3</b>
<b><i>SD</i></b>	<b>142.3</b>	<b>860.4</b>	<b>153.4</b>	<b>208.7</b>	<b>458.3</b>	<b>155.2</b>

**Supplementary Table S6:** Parameters for the mass spectrometry detection.

<b>Analyte</b>	<b>MRM (m/z)</b>	<b>Dwell time (ms)</b>	<b>CE (V)</b>	<b>CXP (V)</b>	<b>Quantification/Confirmation transition</b>
PEA	300.17 / 62.10	10	17	8	Quantification
	300.17 / 57.20	5	51	26	Confirmation
OEA	326.30 / 62.10	10	35	28	Quantification
	326.30 / 55.10	5	67	30	Confirmation
AEA	348.30 / 62.10	10	40	28	Quantification
	348.30 / 91.10	5	57	10	Confirmation
PEA-d4	304.17 / 61.20	10	17	8	Quantification