

Supplementary Materials

Quantification of Analgesic and Anti-Inflammatory Lipid Mediators in Long-Term Cryopreserved and Freeze-Dried Preserved Human Amniotic Membrane

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Table S1. Concentrations of palmitoylethanolamide (PEA), oleoylethanolamide (OEA), and anandamide (AEA) (ng/g) in amniotic membranes in fresh, decontaminated (Deco), cryopreserved (Cryo), and freeze-dried (F-Dry) specimens. Statistical significance between decontaminated and other samples: fresh, cryopreserved, freeze-dried.

PEA							
Placenta	Fresh	Deco	Cryo 6 m	Cryo 1 y	Cryo 4 y	Cryo 10 y	F-Dry 1 y
P1	217.55	1487.36	1817.50	1362.33	991.33	267.00	2013.88
P2	239.34	1383.82	684.00	1273.33	569.83	351.00	906.99
P3	148.16	1218.37	895.67	497.07	243.12	667.74	603.46
P4	662.22	1369.13	627.75	247.97	261.30	433.21	1094.44
P5	395.33	1502.71	321.27	387.19	280.48	748.00	500.76
P6	171.08	1169.34	485.80	590.37	881.50	900.00	2195.56
P7	258.84	768.35	811.82	992.50	261.90	n/a	1347.16
P8	652.88	958.97	n/a	828.00	622.00	n/a	311.84
P9	169.26	1317.81	n/a	1137.00	363.00	n/a	n/a
AV	323.85	1241.76	806.26	812.86	497.16	561.16	1121.76
SD	202.68	245.07	485.69	403.27	285.76	248.25	692.50
Mann-Whitney U test							
Samples		P value	Significance				
Deco vs Fresh		<0.0001	****				
Deco vs Cryo 6 m		0.0311	*				
Deco vs Cryo 1 y		0.0180	*				
Deco vs Cryo 4 y		0.0003	***				
Deco vs Cryo 10 y		0.0008	***				
Deco vs F-Dry 1 y		0.5071	ns				
OEA							

Placenta	Fresh	Deco	Cryo 6 m	Cryo 1 y	Cryo 4 y	Cryo 10 y	F-Dry 1 y
P1	126.75	174.31	593.80	417.34	474.31	326.07	798.47
P2	115.26	324.58	211.28	462.89	266.73	236.90	239.01
P3	59.80	241.46	367.03	141.24	224.37	338.00	153.30
P4	150.75	294.67	489.60	134.50	223.04	454.44	349.36
P5	101.69	404.21	110.89	206.90	261.90	113.42	365.83
P6	65.57	591.05	186.17	210.90	187.25	n/a	n/a
P7	67.96	218.22	241.35	158.12	113.42	n/a	803.17
P8	223.06	353.50	n/a	352.09	171.88	n/a	372.64
P9	48.17	281.82	n/a	441.05	127.88	n/a	n/a
AV	106.56	320.42	314.30	280.56	227.87	293.77	440.25
SD	55.68	123.26	175.76	136.40	106.84	127.06	258.60
Mann-Whitney U test							
Samples		P value	Significance				
Deco vs Fresh		0.0002	***				
Deco vs Cryo 6 m		0.7577	ns				
Deco vs Cryo 1 y		0.4894	ns				
Deco vs Cryo 4 y		0.0625	ns				
Deco vs Cryo 10 y		>0.9999	ns				
Deco vs F-Dry 1 y		0.4079	ns				
AEA							
Placenta	Fresh	Deco	Cryo 6 m	Cryo 1 y	Cryo 4 y	Cryo 10 y	F-Dry 1 y
P1	11.67	20.43	109.17	58.46	33.14	30.28	317.23
P2	12.71	36.45	26.10	61.87	26.19	29.29	36.67
P3	4.98	11.74	59.98	24.84	13.94	39.19	24.23
P4	17.77	22.60	16.02	15.24	13.43	60.30	60.98
P5	9.28	37.47	19.04	27.21	35.08	n/a	223.28
P6 (P11)	17.92	87.07	29.61	31.03	7.52	n/a	n/a
P7	17.71	75.42	42.43	27.33	n/a	n/a	n/a
P8 (P10)	17.09	37.52	n/a	59.00	12.03	n/a	297.60
P9	24.69	63.51	n/a	88.35	29.12	n/a	n/a
AV	14.87	43.58	43.19	43.70	21.31	39.76	160.00
SD	5.82	26.02	32.75	24.09	10.74	14.39	134.99
Mann-Whitney U test							
Samples		P value	Significance				
Deco vs Fresh		0.0028	**				
Deco vs Cryo 6 m		0.8371	ns				
Deco vs Cryo 1 y		>0.9999	ns				
Deco vs Cryo 4 y		0.0464	ns				
Deco vs Cryo 10 y		>0.9999	ns				
Deco vs F-Dry 1 y		0.1447	ns				

Cryopreserved samples were stored for six months (m) and for one-, four-, and ten-years (y). Freeze-dried samples were stored for one year. Data are presented as an average value (AV). SD, standard deviation.

Table S2. Concentrations of palmitoylethanolamide (PEA), oleoylethanolamide (OEA), and anandamide (AEA) (ng/g) in amniotic membranes in fresh, decontaminated (Deco), cryopreserved (Cryo), and freeze-dried (F-Dry) specimens. Statistical significance between fresh and other samples (cryopreserved, freeze-dried).

PEA							
Placenta	Fresh	Deco	Cryo 6 m	Cryo 1 y	Cryo 4 y	Cryo 10 y	F-Dry 1 y
P1	217.55	1487.36	1817.50	1362.33	991.33	267.00	2013.88
P2	239.34	1383.82	684.00	1273.33	569.83	351.00	906.99
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P4	662.22	1369.13	627.75	247.97	261.30	433.21	1094.44
P5	395.33	1502.71	321.27	387.19	280.48	748.00	500.76
P6	171.08	1169.34	485.80	590.37	881.50	900.00	2195.56
P7	258.84	768.35	811.82	992.50	261.90	n/a	1347.16
P8	652.88	958.97	n/a	828.00	622.00	n/a	311.84
P9	169.26	1317.81	n/a	1137.00	363.00	n/a	n/a
AV	323.85	1241.76	806.26	812.86	497.16	561.16	1121.76
SD	202.68	245.07	485.69	403.27	285.76	248.25	692.50
Mann-Whitney U test							
Samples		P value	Significance				
Deco vs Fresh		<0.0001	****				
Fresh vs Cryo 6 m		0.0079	**				
Fresh vs Cryo 1 y		0.0078	**				
Fresh vs Cryo 4 y		0.0770	ns				
Freshvs Cryo 10 y		0.0256	*				
Fresh vs F-Dry 1 y		0.0042	**				
OEA							
Placenta	Fresh	Deco	Cryo 6 m	Cryo 1 y	Cryo 4 y	Cryo 10 y	F-Dry 1 y
P1	126.75	174.31	593.80	417.34	474.31	326.07	798.47
P2	115.26	324.58	211.28	462.89	266.73	236.90	239.01
P3	59.80	241.46	367.03	141.24	224.37	338.00	153.30
P4	150.75	294.67	489.60	134.50	223.04	454.44	349.36
P5	101.69	404.21	110.89	206.90	261.90	113.42	365.83
P6	65.57	591.05	186.17	210.90	187.25	n/a	n/a
P7	67.96	218.22	241.35	158.12	113.42	n/a	803.17
P8	223.06	353.50	n/a	352.09	171.88	n/a	372.64
P9	48.17	281.82	n/a	441.05	127.88	n/a	n/a
AV	106.56	320.42	314.30	280.56	227.87	293.77	440.25
SD	55.68	123.26	175.76	136.40	106.84	127.06	258.60
Mann-Whitney U test							
Samples		P value	Significance				
Deco vs Fresh		0.0002	***				
Fresh vs Cryo 6 m		0.0052	**				
Fresh vs Cryo 1 y		0.0019	**				
Fresh vs Cryo 4 y		0.0040	**				
Fresh vs Cryo 10 y		0.0120	*				
Fresh vs F-Dry 1 y		0.0003	***				
AEA							
Placenta	Fresh	Deco	Cryo 6 m	Cryo 1 y	Cryo 4 y	Cryo 10 y	F-Dry 1 y
P1	11.67	20.43	109.17	58.46	33.14	30.28	317.23
P2	12.71	36.45	26.10	61.87	26.19	29.29	36.67

P3	4.98	11.74	59.98	24.84	13.94	39.19	24.23
P4	17.77	22.60	16.02	15.24	13.43	60.30	60.98
P5	9.28	37.47	19.04	27.21	35.08	n/a	223.28
P6 (P11)	17.92	87.07	29.61	31.03	7.52	n/a	n/a
P7	17.71	75.42	42.43	27.33	n/a	n/a	n/a
P8 (P10)	17.09	37.52	n/a	59.00	12.03	n/a	297.60
P9	24.69	63.51	n/a	88.35	29.12	n/a	n/a
AV	14.87	43.58	43.19	43.70	21.31	39.76	160.00
SD	5.82	26.02	32.75	24.09	10.74	14.39	134.99

**Mann-Whitney U
test**

Samples	P value	Signifi- cance
Deco vs Fresh	0.0028	**
Fresh vs Cryo 6 m	0.0052	**
Fresh vs Cryo 1 y	0.0008	***
Fresh vs Cryo 4 y	0.2766	ns
Fresh vs Cryo 10 y	0.0028	**
Fresh vs F-Dry 1 y	0.0008	***

Footer: Cryopreserved samples were stored for six months (m) and for one-, four-, and ten-years (y). Freeze-dried samples were stored for one year. Data are presented as an average value (AV). SD, standard deviation.

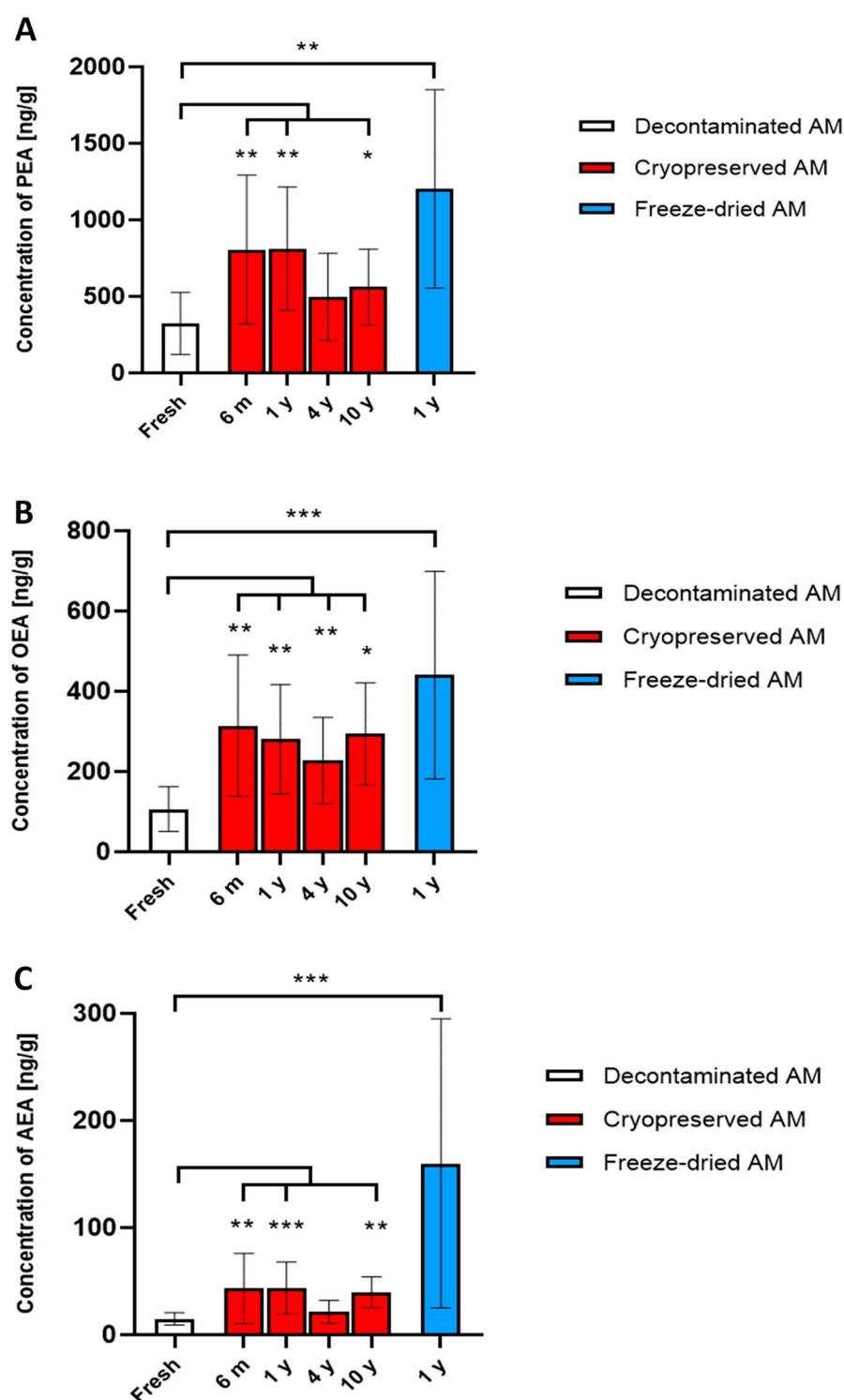


Figure S1. The levels of N-acylethanolamines in fresh, cryopreserved and freeze-dried amniotic membranes. The average concentrations of palmitoylethanolamide (PEA) (A), oleoylethanolamide (OEA) (B), and anandamide (AEA) (C) are expressed in ng/g. P-value: $P < 0.05^*$; $P < 0.01^{**}$; $P < 0.001^{***}$; $P < 0.0001^{****}$, m = months, y = years. Statistical significance between fresh and other samples (cryopreserved, freeze-dried). Cryopreserved samples were stored for six months (m) and for one-, four-, and ten-years (y). Freeze-dried samples were stored for one year. Data are presented as an average value (AV). SD, standard deviation.