

Supplementary material

Embryo structure reorganisation reduces the probability of apoptosis in preimplantation mouse embryos

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Group	blastomere volume (average) [um ³]	Embryo volume (average) [um ³]	N/C ratio
Embryos 1/2	57933.3 (96.2%)	57933.3 (48.1%)	0.0449
Embryos 2/1	94533.3 (157.1%)	94533.3 (78.5%)	0.0276*
Embryos 4n	102133.3 (169.8%)	102133.3 (84.8%)	0.0501*
Aggregates	60166.6 (100%)	240800 (200%)	0.0452
Control	60166.6 (100%)	120400 (100%)	0.0452

Supplementary Table S1: The average N/C ratio, blastomere and embryo volume at start of the experiment. Statistically significant results were marked compared to controls for a given stage. ($p < 0.05$; Kruskal-Wallis test and Dunn's post hoc test). Values in brackets are percentage comparison between volume in experimental and control blastomeres and embryos.*

Group	Morulae	Early Blastocysts	Late Blastocysts
Embryos 1/2	19.3 (69%)	24 (52%)*	53.5 (60%)*
Embryos 2/1	11.5 (41%)*	20.4 (44%)*	51.8 (58%)*
Embryos 4n	16.5 (59%)*	19.6 (42%)*	38.9 (44%)*
Aggregates	46.4 (166%)*	68.7(148%)	166.2(187%)*
Control	27.9 (100%)	46.2 (100%)	89.1 (100%)

Supplementary Table S2: The average number of cells in the embryos. Statistically significant results were marked compared to controls for a given stage. (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$; Kruskal-Wallis test and Dunn's post hoc test). Values in brackets are percentage comparison between cell number in experimental and control embryos.

Group	Early Blastocysts			Late Blastocysts		
	ICM	TE	ICM:TE	ICM	TE	ICM:TE
Embryos 1/2	7.7 (39.9%)**	14.3 (75.3%)	0.54	8.6 (31.6%***)	50.6 (69,2%)	0.17
Embryos 2/1	7.5 (38.9%)**	13 (68.4%)	0.58	10.6 (39%)**	40.3 (55,1%)	0.26
Embryos 4n	8 (41.5%)*	12.5 (65.8%)	0.64	15.6 (57.4%)	31.4 (43%)	0.5
Aggregates	33.8 (175.1%)	38.7 (203.7%)	0.88	28.7 (105.5%)	128.9 (176.3%)**	0.22
Control	19.3	19	1.01	27.2	73.1	0.37

Supplementary Table S3: Average number of cells in the ICM and TE in blastocysts (based on CDX2 staining). Statistically significant results were marked compared to controls for a given stage. (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$; Kruskal-Wallis test and Dunn's post hoc test). Values in brackets are percentage comparison between cell number in experimental and control embryos.

ICM	Group	% embryos containing at least 1 apoptotic cell	Chi square test p value comparing with control group	TE	Group	% embryos containing at least 1 apoptotic cell	Chi square test p value comparing with control group
Early blastocysts	Embryos 1/2	7.14	0.01	Early blastocysts	Embryos 1/2	0	0.065
	Embryos 2/1	17.02	0.074		Embryos 2/1	10.64	1.000
	Embryos 4n	0	0.01		Embryos 4n	0	0.072
	Aggregates	2.94	0.001		Aggregates	2.94	0.08
	Control	32.14	-		Control	14.28	-
Late blastocysts	Embryos 1/2	50	0.9	Late blastocysts	Embryos 1/2	6.66	0.0008
	Embryos 2/1	50	0.85		Embryos 2/1	25	0.3
	Embryos 4n	56.52	0.09		Embryos 4n	30.43	0.082
	Aggregates	65.22	1.000		Aggregates	34.78	0.6
	Control	63.63	-		Control	54.54	-

Supplementary Table S4: Apoptosis in blastocysts (based on nuclear staining and morphology). Percent of blastocysts in which at least one cell was apoptotic in a given embryonic cell lineage (Fisher's exact test).

	Early Blastocysts		Late Blastocysts	
	TE	ICM	TE	ICM
Embryos 1/2	0/431 (0%)	4/308 (1.2%)*	2/1287 (0.1%)**	27/313 (8.6%)\$\$\$
Embryos 2/1	5/653 (0.8%)	7/303 (2.3%)*	6/819 (0.7%)	12/216 (5.5%)\$\$
Embryos 4n	0/173 (0%)	0/102 (0%)*	9/617 (1.5%)	22/275 (8%)\$\$\$
Aggregates	0/1438(0%)**	4/898 (0.4%***)	14/3164(0.4%)	50/669 (7.5%)\$\$\$
Control	8/846 (0.8%)	24/448 (5.3%)\$\$	14/1352 (1%)	31/520 (6%)\$\$

Supplementary Table S5: Apoptosis in blastocyst cells (based on nuclear staining and morphology). Percentage of all cells undergoing apoptosis in a given cell lineage relative to all cells of a given lineage in a given experimental group. ($p < 0.05$. ** $p < 0.01$. *** $p < 0.0001$. Fisher's exact test. control groups compared to experimental groups; \$ $p < 0.05$. \$\$ $p < 0.01$. \$\$\$ $p < 0.0001$ indicates significant difference between percentage of apoptotic cells comparing TE and ICM within the same experimental group).*

Embryo cell structure alteration*	Overall			Within TE across stages			Within ICM across stages			At early blastocyst across cell lines			At late blastocyst across cell lines			
	LSM	se	Related frequency#	LSM	se	Related frequency	LSM	se	Related frequency	LSM	se	Related frequency	LSM	se	Related frequency	
1	-5.37	0.56	0.0046	-7.55	1.05	0.0005	-3.19	0.28	0.0412	-5.96	0.74	0.0026	-4.78	0.52	0.0084	
2	-4.01	0.21	0.0182	-4.67	0.32	0.0093	-3.34	0.28	0.0355	-4.10	0.30	0.0167	-3.92	0.30	0.0199	
3	-15.12	0.34	2.7E-07	-15.97	0.50	1.2E-07	-14.26	0.35	6.4E-07	-27.01	0.56	1.9E-12	-3.22	0.23	0.0399	
4	-5.06	0.28	0.0064	-6.46	0.41	0.0016	-3.65	0.24	0.0260	-6.47	0.51	0.0015	-3.64	0.16	0.0263	
5	-3.68	0.18	0.0253	-4.89	0.32	0.0075	-2.46	0.15	0.0851	-3.86	0.25	0.0212	-3.50	0.22	0.0302	
Significance† of differences between the alteration group means																
Alteration	2	3	4	5	2	3	4	5	2	3	4	5	2	3	4	5
1	*	***	ns	**	***	***	ns	*	ns	***	ns	*	ns	**	*	*
2	***	***	**	***	***	***	ns	**	***	***	***	***	ns	ns	ns	ns
3	***	***	***	***	***	***	***	***	***	***	***	***	***	***	ns	ns
4	***	***	***	**	***	***	***	***	***	***	***	***	***	***	ns	ns

Supplementary Table S6: Least-squares means (LSM) and their standard errors (se) of apoptosis log frequencies for overall, across cell lines†, and embryo development stages for apoptosis occurrences, as affected by the embryo cell structure alteration (based on nuclear staining and morphology).

‡/ - TE - trophoblast; ICM - inner cell mass;

* / - 1 - removal of blastomere from a 2-cell embryo; 2 - enucleation of one blastomere of a 2-cell embryo, followed by fusion of both blastomeres; 3 - fusion of both blastomeres of the 2-cell embryo, resulting in a single-cell tetraploid embryo; 4 - aggregation of two 2-cell embryos, resulting in chimeric 4-cell embryos; 5 - no alteration

/ - Related frequency = e^{LSM}.

† / - ns - not significant; significant: * - at p<0.05; ** - at p<0.01; *** - at p<0.001.

Development stage	Overall development stage effect			Development stage within the TE			Development stage within the ICM		
	LSM	se	Related frequency [#]	LSM	se	Related frequency	LSM	se	Related frequency
Early blastocyst	-9.48	0.29	7.6E-05	-10.79	0.48	2.1E-05	-8.17	0.23	0.0003
Late blastocyst	-3.81	0.14	0.0221	-5.04	0.25	0.0065	-2.59	0.12	0.0750

Supplementary Table S7: Least-squares means (LSM) and their standard errors (se) of apoptosis log frequencies for overall development stage effect and development stage effect across cell lines[‡] (based on nuclear staining and morphology).

[‡]/ – TE – trophoblast; ICM – inner cell mass;

[#]/ – Related frequency = e^{LSM} .

Cell line	LSM	se	Related frequency [#]
TE	-7.91	0.30	0.0004
ICM	-5.38	0.13	0.0221

Supplementary Table S8: Least-squares means (LSM) and their standard errors (se) of apoptosis log frequencies for overall cell line[‡] effect. (based on nuclear staining and morphology).

[‡]/ – TE – trophoblast; ICM – inner cell mass;

[#]/ – Related frequency = e^{LSM} .