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Title

A medium-chain fatty acid as an alternative energy source in mouse preimplantation development

Author names

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Supplementary Table S1 Successful rates of blastocyst development in fatty-acid-containing KSOM, fatty-acid-deficient culture media, energy-depleted culture media or octanoate-supplemented culture media.

The experiment was repeated more than ten times.

Fatty-acid-containing KSOM

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	30	0	0	0	0	0
Two cell	0	30	1	0	0	0
Four cell	0	0	0	0	0	0
Eight cell	0	0	15	0	0	0
Morula	0	0	14	30	0	0
Blastocyst	0	0	0	0	26	10
Hatched blastocyst	0	0	0	0	3	19
Fragmentation	0	0	0	0	1	1

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	14	0	0	0	0	0
Two cell	0	14	0	0	0	0
Four cell	0	0	11	0	0	0
Eight cell	0	0	3	1	0	0
Morula	0	0	0	13	0	0
Blastocyst	0	0	0	0	8	4
Hatched blastocyst	0	0	0	0	5	9
Fragmentation	0	0	0	0	0	0

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	15	0	0	0	0	0
Two cell	0	15	1	0	0	0
Four cell	0	0	0	0	0	0
Eight cell	0	0	11	0	0	0
Morula	0	0	3	1	9	0
Blastocyst	0	0	0	13	5	4
Hatched blastocyst	0	0	0	0	0	10
Fragmentation	0	0	0	1	1	1

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	17	0	0	0	0	0
Two cell	0	17	0	0	0	0
Four cell	0	0	0	0	0	0
Eight cell	0	0	1	0	0	0
Morula	0	0	16	1	0	0
Blastocyst	0	0	0	16	14	4
Hatched blastocyst	0	0	0	0	3	13
Fragmentation	0	0	0	0	0	0

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	39	0	0	0	0	0
Two cell	0	39	0	0	0	0
Four cell	0	0	1	0	0	0
Eight cell	0	0	14	0	0	0
Morula	0	0	23	1	0	0
Blastocyst	0	0	0	37	34	10
Hatched blastocyst	0	0	0	0	4	28
Fragmentation	0	0	1	1	1	1

Fatty-acid-deficient culture media

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	26	4	4	2	1	0
Two cell	0	22	0	0	0	0
Four cell	0	0	6	0	0	0
Eight cell	0	0	12	0	0	0
Morula	0	0	4	22	3	0
Blastocyst	0	0	0	0	19	3
Hatched blastocyst	0	0	0	0	0	13
Fragmentation	0	0	0	2	3	10

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	40	8	7	6	1	0
Two cell	0	32	0	0	0	0
Four cell	0	0	19	0	0	0
Eight cell	0	0	14	0	0	0
Morula	0	0	0	30	7	2
Blastocyst	0	0	0	3	22	3
Hatched blastocyst	0	0	0	0	0	24
Fragmentation	0	0	0	1	10	11

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	20	0	0	0	0	0
Two cell	0	20	0	0	0	0
Four cell	0	0	14	0	0	0
Eight cell	0	0	6	2	2	0
Morula	0	0	0	17	3	0
Blastocyst	0	0	0	0	13	10
Hatched blastocyst	0	0	0	0	0	5
Fragmentation	0	0	0	0	2	5

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	30	3	3	0	0	0
Two cell	0	27	22	16	11	11
Four cell	0	0	5	4	6	3
Eight cell	0	0	0	6	0	0
Morula	0	0	0	3	5	0
Blastocyst	0	0	0	0	3	3
Hatched blastocyst	0	0	0	0	0	1
Fragmentation	0	0	0	1	5	12

50 μ M-octanoate -supplemented fatty acid deficient culture media

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	40	4	2	2	1	0
Two cell	0	36	2	0	0	1
Four cell	0	0	17	2	3	1
Eight cell	0	0	13	1	0	0
Morula	0	0	5	21	12	2
Blastocyst	0	0	0	13	22	4
Hatched blastocyst	0	0	0	0	0	24
Fragmentation	0	0	1	1	2	6

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	20	0	0	0	0	0
Two cell	0	20	5	0	0	0
Four cell	0	0	12	3	1	0
Eight cell	0	0	3	0	0	0
Morula	0	0	0	12	7	0
Blastocyst	0	0	0	3	12	12
Hatched blastocyst	0	0	0	0	0	4
Fragmentation	0	0	0	2	0	4

100 μ M-octanoate -supplemented fatty acid deficient culture media

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	40	6	6	2	3	0
Two cell	0	34	1	0	0	0
Four cell	0	0	18	1	0	0
Eight cell	0	0	10	0	0	0
Morula	0	0	3	22	5	0
Blastocyst	0	0	0	11	24	3
Hatched blastocyst	0	0	0	4	0	24
Fragmentation	0	0	2	0	8	13

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	24	2	2	2	0	0
Two cell	0	17	2	0	0	0
Four cell	0	1	13	2	0	0
Eight cell	0	0	3	1	0	0
Morula	0	0	0	14	3	0
Blastocyst	0	0	0	1	15	8
Hatched blastocyst	0	0	0	0	0	9
Fragmentation	0	0	0	0	2	7

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	40	6	6	2	3	0
Two cell	0	34	1	0	0	0
Four cell	0	0	18	1	0	0
Eight cell	0	0	10	0	0	0
Morula	0	0	3	22	5	0
Blastocyst	0	0	0	15	24	3
Hatched blastocyst	0	0	0	0	0	24
Fragmentation	0	0	2	0	8	13

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	50	1	2	0	0	0
Two cell	0	49	0	0	0	0
Four cell	0	0	0	0	0	0
Eight cell	0	0	7	0	0	0
Morula	0	0	39	33	3	0
Blastocyst	0	0	0	13	36	20
Hatched blastocyst	0	0	0	0	3	22
Fragmentation	0	0	2	4	8	8

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	50	5	5	5	3	3
Two cell	0	45	0	0	0	0
Four cell	0	0	0	0	0	0
Eight cell	0	0	4	0	0	0
Morula	0	0	41	30	4	0
Blastocyst	0	0	0	15	38	28
Hatched blastocyst	0	0	0	0	3	13
Fragmentation	0	0	0	0	2	6

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	95	7	0	0	0	0
Two cell	0	60	0	0	0	0
Four cell	0	26	29	0	0	0
Eight cell	0	0	7	0	0	0
Morula	0	0	50	45	2	0
Blastocyst	0	0	0	33	76	16
Hatched blastocyst	0	0	0	0	0	58
Fragmentation	0	2	9	17	17	21

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	112	11	11	11	11	5
Two cell	0	71	0	0	0	0
Four cell	0	29	30	0	0	0
Eight cell	0	0	7	0	0	0
Morula	0	0	63	51	0	0
Blastocyst	0	0	0	40	89	8
Hatched blastocyst	0	0	0	0	0	80
Fragmentation	0	1	1	10	12	19

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	105	3	0	0	0	0
Two cell	0	97	0	0	0	0
Four cell	0	5	39	0	0	0
Eight cell	0	0	6	0	0	0
Morula	0	0	57	55	0	0
Blastocyst	0	0	0	40	89	10
Hatched blastocyst	0	0	0	0	0	79
Fragmentation	0	0	3	10	16	22

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	87	11	3	3	3	3
Two cell	0	54	0	0	0	0
Four cell	0	21	21	0	0	0
Eight cell	0	0	4	0	0	0
Morula	0	0	50	44	9	0
Blastocyst	0	0	0	30	63	6
Hatched blastocyst	0	0	0	0	0	57
Fragmentation	0	1	9	10	12	21

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	34	1	0	0	0	0
Two cell	0	25	0	0	0	0
Four cell	0	7	13	0	0	0
Eight cell	0	0	3	0	0	0
Morula	0	0	16	18	0	0
Blastocyst	0	0	0	12	29	6
Hatched blastocyst	0	0	0	0	0	20
Fragmentation	0	1	2	4	5	8

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	41	0	0	0	0	0
Two cell	0	14	0	0	0	0
Four cell	0	27	16	0	0	0
Eight cell	0	0	5	0	0	0
Morula	0	0	20	25	0	0
Blastocyst	0	0	0	12	37	10
Hatched blastocyst	0	0	0	0	0	27
Fragmentation	0	1	1	4	4	4

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	51	2	0	0	0	0
Two cell	0	23	0	0	0	0
Four cell	0	25	18	0	0	0
Eight cell	0	0	15	1	0	0
Morula	0	0	15	20	0	0
Blastocyst	0	0	0	24	44	5
Hatched blastocyst	0	0	0	0	0	38
Fragmentation	0	1	3	6	7	8

200 μ M-octanoate -supplemented fatty acid deficient culture media

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	36	1	1	1	0	0
Two cell	0	35	2	1	1	0
Four cell	0	0	21	0	1	0
Eight cell	0	0	10	1	0	1
Morula	0	0	2	31	14	0
Blastocyst	0	0	0	6	16	1
Hatched blastocyst	0	0	0	0	0	16
Fragmentation	0	0	0	0	8	18

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	20	1	0	0	0	0
Two cell	0	19	3	0	0	0
Four cell	0	0	17	4 (3C-2)	0	0
Eight cell	0	0	0	1	0	0
Morula	0	0	0	15	8	0
Blastocyst	0	0	0	0	11	8
Hatched blastocyst	0	0	0	0	0	8
Fragmentation	0	0	0	0	1	4

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	20	2	1	1	0	0
Two cell	0	17	0	0	0	0
Four cell	0	1	3	1	0	0
Eight cell	0	0	3	0	0	0
Morula	0	0	12	13	1	0
Blastocyst	0	0	0	4	1	2
Hatched blastocyst	0	0	0	0	13	13
Fragmentation	0	0	1	1	5	5

400 μ M-octanoate -supplemented fatty acid deficient culture media

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	40	2	1	0	1	0
Two cell	0	38	2	0	0	0
Four cell	0	0	28	6	7	0
Eight cell	0	0	5	2	0	0
Morula	0	0	4	27	14	0
Blastocyst	0	0	0	3	15	0
Hatched blastocyst	0	0	0	0	0	20
Fragmentation	0	0	0	2	3	20

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	29	0	2	1	1	0
Two cell	0	29	16	1	1	0
Four cell	0	0	11	8 (3C-2)	0	0
Eight cell	0	0	0	6	0	0
Morula	0	0	0	6	24	0
Blastocyst	0	0	0	14	4	7
Hatched blastocyst	0	0	0	0	0	2
Fragmentation	0	0	0	1	1	20

800 μ M-octanoate -supplemented fatty acid deficient culture media

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	40	4	4	4	0	0
Two cell	0	36	9	3	5	0
Four cell	0	0	26	13	6	0
Eight cell	0	0	0	3	0	0
Morula	0	0	0	16	9	0
Blastocyst	0	0	0	0	4	1
Hatched blastocyst	0	0	0	0	0	4
Fragmentation	0	0	1	1	16	35

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	20	0	0	0	0	0
Two cell	0	20	0	0	0	0
Four cell	0	0	3	0	0	0
Eight cell	0	0	9	0	0	0
Morula	0	0	8	18	2	0
Blastocyst	0	0	0	2	15	4
Hatched blastocyst	0	0	0	0	0	12
Fragmentation	0	0	0	0	3	4

1000 μ M-octanoate -supplemented fatty acid deficient culture media

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	30	4	4	3	5	0
Two cell	0	24	22	8	4	0
Four cell	0	0	2	13 (3C-8)	10	0
Eight cell	0	0	0	5	0	0
Morula	0	0	0	1	4	0
Blastocyst	0	0	0	4	4	4
Hatched blastocyst	0	0	0	0	0	0
Fragmentation	0	2	2	1	3	26

1700 μ M-octanoate -supplemented fatty acid deficient culture media

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	21	1	1	0	0	0
Two cell	0	20	2	1	2	2
Four cell	0	0	7	2	2	0
Eight cell	0	0	9	7	0	0
Morula	0	0	1	9	5	0
Blastocyst	0	0	0	0	7	1
Hatched blastocyst	0	0	0	0	1	8
Fragmentation	0	0	1	2	4	10

3400 μ M-octanoate -supplemented fatty acid deficient culture media

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	20	2	0	0	0	0
Two cell	0	18	18	18	18	18
Four cell	0	0	0	0	0	0
Eight cell	0	0	0	0	0	0
Morula	0	0	0	0	0	0
Blastocyst	0	0	0	0	0	0
Hatched blastocyst	0	0	0	0	0	0
Fragmentation	0	0	2	2	2	2

5000 μ M-octanoate -supplemented fatty acid deficient culture media

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	33	33	3	3	3	3
Two cell	0	0	0	0	0	0
Four cell	0	0	0	0	0	0
Eight cell	0	0	0	0	0	0
Morula	0	0	0	0	0	0
Blastocyst	0	0	0	0	0	0
Hatched blastocyst	0	0	0	0	0	0
Fragmentation	0	0	30	30	30	30

Media lacking glucose, pyruvate and fatty acids (energy-depleted media)

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	30	4	5	4	4	3
Two cell	0	26	20	2	5	4
Four cell	0	0	5	18	5	1
Eight cell	0	0	0	1	6	0
Morula	0	0	0	1	8	1
Blastocyst	0	0	0	0	1	3
Hatched blastocyst	0	0	0	0	0	0
Fragmentation	0	0	0	1	1	17

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	17	3	2	1	1	0
Two cell	0	12	10	5	5	0
Four cell	0	0	2	2	0	0
Eight cell	0	0	2	0	0	0
Morula	0	0	0	4	2	0
Blastocyst	0	0	0	0	3	5
Hatched blastocyst	0	0	0	0	0	0
Fragmentation	0	2	1	5	6	12

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	16	2	3	3	0	0
Two cell	0	14	12	12	0	0
Four cell	0	0	1	1	0	0
Eight cell	0	0	0	0	0	0
Morula	0	0	0	0	1	0
Blastocyst	0	0	0	0	0	1
Hatched blastocyst	0	0	0	0	0	0
Fragmentation	0	0	0	0	15	15

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	20	0	0	1	2	0
Two cell	0	20	17	6	7	0
Four cell	0	0	3	9 (3C-4)	8	0
Eight cell	0	0	0	1	1	0
Morula	0	0	0	2	0	0
Blastocyst	0	0	0	0	0	0
Hatched blastocyst	0	0	0	0	0	0
Fragmentation	0	0	0	1	3	20

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	18	0	1	3	0	0
Two cell	0	18	12	9	2	0
Four cell	0	0	5	3C-1	3	0
Eight cell	0	0	0	5	0	0
Morula	0	0	0	0	0	0
Blastocyst	0	0	0	0	0	3
Hatched blastocyst	0	0	0	0	0	0
Fragmentation	0	0	0	0	13	15

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	20	12	11	11	11	0
Two cell	0	8	8	5	5	0
Four cell	0	0	0	3 (3C-1)	3	0
Eight cell	0	0	0	0	0	0
Morula	0	0	0	0	0	0
Blastocyst	0	0	0	0	0	0
Hatched blastocyst	0	0	0	0	0	0
Fragmentation	0	0	1	1	1	20

50 μ M-octanoate supplemented energy-depleted media

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	15	5	1	1	0	0
Two cell	0	10	0	0	0	0
Four cell	0	0	2	0	0	0
Eight cell	0	0	1	0	0	0
Morula	0	0	11	10	0	0
Blastocyst	0	0	0	1	5	3
Hatched blastocyst	0	0	0	0	1	3
Fragmentation	0	0	0	3	9	9

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	15	1	1	0	0	0
Two cell	0	13	1	0	0	0
Four cell	0	0	5	1	0	0
Eight cell	0	0	2	0	0	0
Morula	0	0	5	11	0	0
Blastocyst	0	0	0	1	11	1
Hatched blastocyst	0	0	0	0	1	11
Fragmentation	0	1	1	2	3	3

100 μ M-octanoate supplemented energy-depleted media

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	15	2	1	0	0	0
Two cell	0	13	0	1	0	0
Four cell	0	0	4	1	0	0
Eight cell	0	0	5	0	0	0
Morula	0	0	5	8	1	0
Blastocyst	0	0	0	4	8	1
Hatched blastocyst	0	0	0	0	4	12
Fragmentation	0	0	0	1	2	2

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	15	5	1	0	0	0
Two cell	0	3	0	0	0	0
Four cell	0	6	0	0	0	0
Eight cell	0	0	0	0	0	0
Morula	0	0	10	1	0	0
Blastocyst	0	0	0	8	4	1
Hatched blastocyst	0	0	0	0	5	8
Fragmentation	0	0	5	6	6	6

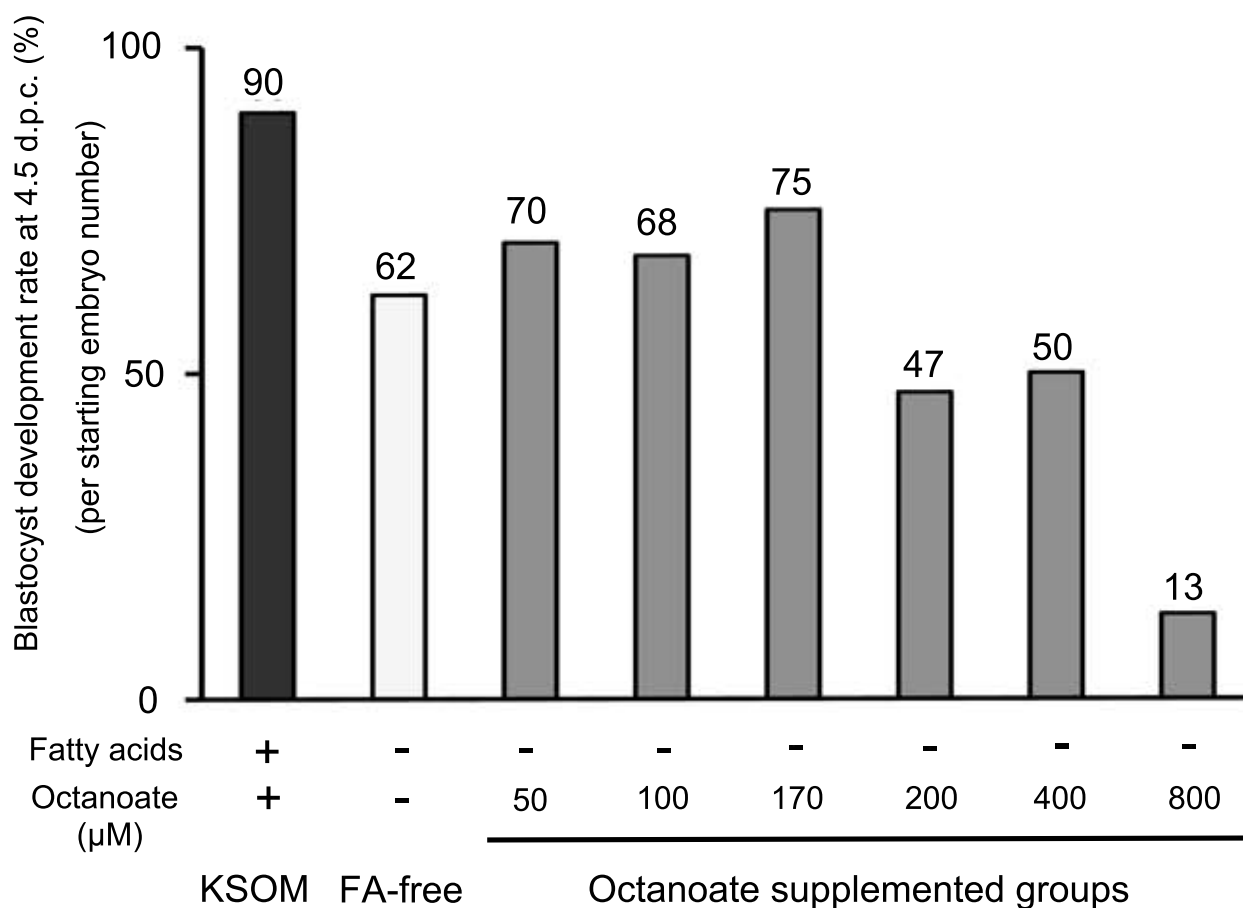
	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	15	0	1	0	0	0
Two cell	0	13	1	2	0	0
Four cell	0	0	5	0	0	0
Eight cell	0	0	6	1	0	0
Morula	0	0	0	7	1	0
Blastocyst	0	0	0	1	7	4
Hatched blastocyst	0	0	0	0	1	5
Fragmentation	0	2	2	4	6	6

200 μ M-octanoate supplemented energy-depleted media

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	15	0	1	0	0	0
Two cell	0	13	1	2	0	0
Four cell	0	0	5	0	0	0
Eight cell	0	0	6	1	0	0
Morula	0	0	0	7	1	0
Blastocyst	0	0	0	1	7	4
Hatched blastocyst	0	0	0	0	1	5
Fragmentation	0	2	2	4	6	6

	0.5dpc	1.5dpc	2.5dpc	3.5dpc	4.5dpc	5.5dpc
One cell	15	1	1	0	0	0
Two cell	0	14	6	0	0	0
Four cell	0	0	3	1	0	0
Eight cell	0	0	4	0	0	0
Morula	0	0	0	11	1	0
Blastocyst	0	0	0	1	11	4
Hatched blastocyst	0	0	0	0	1	2
Fragmentation	0	0	1	2	2	9

Supplementary Table S2 Successful rates of blastocyst development in media with fatty acid-free BSA (FA-free) or in the FA-free media supplemented with various concentrations of octanoate. When excessive octanoate was added to the FA-free culture media, only 13% of embryos reached the blastocyst stage. Interestingly, a series of studies performed by Nonogaki et al. showed that the addition of high levels of fatty acids to culture media resulted in toxicity with inhibition of the normal development of pronuclear fertilized eggs to blastocysts⁴⁴. Their finding of the detrimental effect of high concentrations of palmitic and stearic acids in culture media on preimplantation embryos echoes our experimental result.



Supplementary Table S2