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—Review—

Time-Lapse Videomicrographic Analyses of Contractions in Mouse Blastocysts

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Abstract. Contraction has been observed in cultured blastocysts of many mammals, but little is known about the features of the contraction and its physiological role in blastocysts. The author analyzed contractions of a large number of cultured mouse blastocysts by time-lapse videomicrography. The results revealed that blastocysts repeated contractions of different degrees during the expanded stage from 10 h after blastocoel formation, and that the number of contractions was greater during the hatching period than in the periods pre- and post-hatching. The results also showed that the time needed for both contraction and re-expansion to the size before contraction tended to lengthen in blastocysts severely contracted. It was inferred that contractions of blastocysts occur physiologically in relation to myosin light chain kinase, but not due to an increase in permeability between trophectoderm cells in association with their division, or the influence of culture. Furthermore, it was inferred that re-expansion of contracted blastocysts occurs due to active transport and accumulation of Na^+ from the trophectoderm cells into blastocoelic fluid as a result of the action of Na^+/K^+-ATPase activated in the membrane of trophectoderm cells. Our results suggested that contractions are also present in blastocysts developed *in vivo*, and that weak contractions (less than 20% volume reduction) play an important role in hatching, whereas strong contractions (20% or more volume reduction) have the effect of inhibiting hatching. From our results on contractions of various blastocysts, it seems possible to evaluate the developmental ability of embryos, i.e. embryo quality, based on contractions of blastocysts.

Key words: Blastocyst, Contraction, Embryo quality, Hatching, Time-lapse videomicrography

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The presence of contractions in mammalian blastocysts was first reported by Lewis and Gregory in 1929 [1]. They observed cultured rabbit blastocysts for 8 days by time-lapse microcinematography, and they found that the blastocysts repeatedly contracted and re-expanded during the entire period, except for the stage of the early blastocyst. The contraction has since been observed in cultured blastocysts of cattle [2, 3], guinea pigs [4], rats [5], hamsters [6–8] and mice [9–15] by time-lapse microcinematography or time-lapse videomicrography, and also in cultured porcine blastocysts [16] by measuring time-sequence changes in diameter. In the mouse, it has been reported that early blastocysts contract 4 to 15 times during culture for 18 h, and a contraction so large that it may eliminate the blastocoel occurs after 3 or 4 small contractions [11, 12]. The time required by mouse blastocysts for contraction was 15 sec to 20 min [10–13, 15]. Cattle blastocysts required 13 to 17 min for contraction and 6 to 10 h for re-expansion, and the number of contractions in these blastocysts was 3 or fewer until completion of hatching [3]. It has also been reported that some blastocysts of mice and cattle can completely hatch with no contractions [2, 3, 14]. As described, the number of contractions and the time needed for

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contraction and re-expansion have been studied in blastocysts of mice and cattle, whereas only the presence of contractions has been observed in cultured blastocysts of the other mammals. Furthermore, since the largest number of blastocysts observed as to contractions was as small as 6 in the previous studies [1–15], detailed features of the contraction and its physiological role in blastocysts have not yet been clarified.

In this review article, the number and degree of contraction, the mechanisms of contraction and re-expansion, and physiological roles of contraction will be described, mainly based on the results of our observation of mouse blastocysts.

Detailed Features of Contraction in Cultured Blastocysts

Recently, the authors [17] have observed the contraction of a large number of mouse blastocysts by means of time-lapse videomicrography. The results revealed that blastocysts repeated contraction and re-expansion during the expanded stage from 10 h after blastocoel formation, and that the degree of contraction differed from contraction to contraction, even in the same blastocysts, varying from contractions with little expansion of the perivitelline space to those in which the blastocoel almost disappeared.

In order to analyze contractions, we classified the degree of contraction into the following two types according to the percentage of volume reduction at the time of contraction from the volume before contraction: weak when the volume reduction was less than 20% (Fig. 1a-e); strong when it was 20% or more (Fig. 1f-j). Light microscopically, enlargement of the perivitelline space and reduction of blastocoel were clear in blastocysts showing strong contraction (Fig. 1h), but not so clear in those showing weak contraction (Fig. 1c). The number of times of weak and strong contractions in 30 cultured mouse blastocysts completing hatching is shown in Table 1 [17]. In the pre-hatching period, 83.3% of blastocysts contracted 1.97 times on average, with a mean number of 0.09 times/h. During the periods of hatching and post-hatching until 10 h after hatching, all blastocysts contracted 8.80 and 3.47 times on average, respectively, with mean numbers of 0.41 and 0.35 times/h. Among

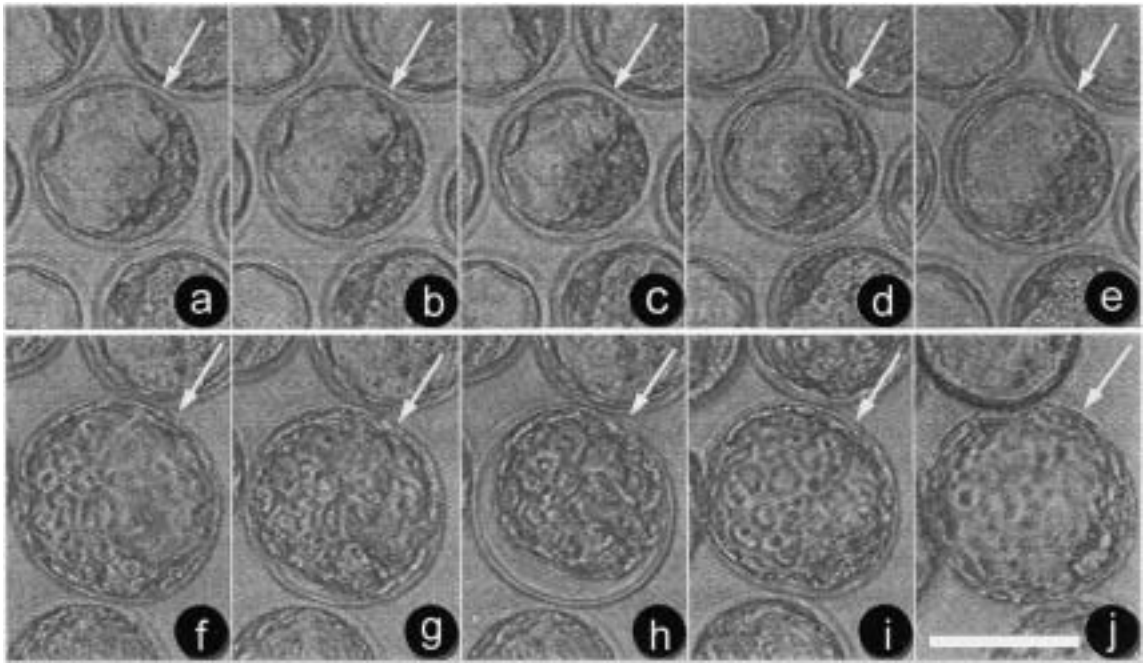

Fig. 1. Time-lapse videomicrographs of cultured mouse blastocysts (arrows) showing a weak contraction (a-e) and a strong contraction (f-j). The blastocysts showing the weak and strong contractions required 3 min (a to c) and 8 min (f to h) to contract maximally, and 78 min (c to e) and 396 min (h to j) to re-expand to the size before contraction, respectively. Scale indicates 100 μm. a, f: before contraction; b, g: during contraction; c, h: at the peak of contraction; d, i: during re-expansion; e, j: at the time of re-expansion.

Table 1. The number of contractions of hatched mouse blastocysts during the three periods

Blastocyst No.	Pre-hatching			Hatching			Post-hatching		
	Weak	Strong	Total	Weak	Strong	Total	Weak	Strong	Total
1	3	0	3	8	1	9	4	0	4
2	5	0	5	6	0	6	2	1	3
3	4	0	4	3	1	4	10	0	10
4	2	0	2	4	0	4	3	1	4
5	2	0	2	15	2	17	1	1	2
6	2	0	2	6	1	7	2	0	2
7	1	0	1	4	0	4	3	0	3
8	1	0	1	29	0	29	2	1	3
9	2	0	2	7	0	7	5	0	5
10	1	0	1	7	1	8	2	1	3
11	1	0	1	12	0	12	2	0	2
12	0	0	0	7	0	7	1	2	3
13	1	0	1	11	1	12	3	0	3
14	1	0	1	9	1	10	1	0	1
15	6	0	6	1	1	2	1	1	2
16	4	1	5	6	2	8	1	0	1
17	0	0	0	2	0	2	9	0	9
18	2	0	2	4	1	5	2	1	3
19	1	0	1	11	1	12	4	0	4
20	2	0	2	2	2	4	6	0	6
21	3	0	3	2	0	2	3	0	3
22	0	0	0	8	0	8	3	0	3
23	0	0	0	40	1	41	2	0	2
24	1	0	1	6	0	6	5	0	5
25	0	0	0	4	1	5	1	2	3
26	5	0	5	2	0	2	3	1	4
27	2	0	2	3	1	4	3	1	4
28	1	0	1	15	0	15	2	0	2
29	1	0	1	6	0	6	2	1	3
30	3	1	4	6	0	6	1	1	2
Mean No.	1.90	0.07	1.97	8.17	0.63	8.80	2.97	0.50	3.47

The degree of contraction was divided into two, based on the percentages of volume reduction of blastocysts; weak: less than 20%, strong: more than 20%.

these three periods, the total number of contractions and the number of contractions per hour were larger during the hatching period than in the pre- and post-hatching periods. In these 30 blastocysts, the lengths of time needed for weak and strong contractions were 30 sec to 6 min and 20 sec to 16 min, respectively. Those needed for re-expansion to the size before contraction were 75 to 440 min after weak contraction and 299 to 560 min after strong contraction, showing that the larger the contraction, the longer the duration of time required for re-expansion. Some examples of contraction in mouse blastocysts are shown in Fig. 2.

Presence of Contraction in Uterine Blastocysts

Checiu and Checiu [18] reported that 6 (1.5%) and 3 (0.7%) of 401 uterine mouse blastocysts contracted so strongly that their blastocoels disappeared or were diminished in size. Cole [12] also reported that uterine mouse blastocysts contracted to various degrees.

The authors [19] also collected mouse blastocysts from uteri and observed the presence or absence of contracting embryos. We confirmed that none of 513 blastocysts collected at 80, 85, 90 and 110 h after hCG injection contracted so severely that their blastocoels disappeared, but such marked contractions were observed in 1.0% (5/492) of blastocysts collected at 95 and 100 h after hCG injection. Of these 5 blastocysts, 4 and 1 were at the

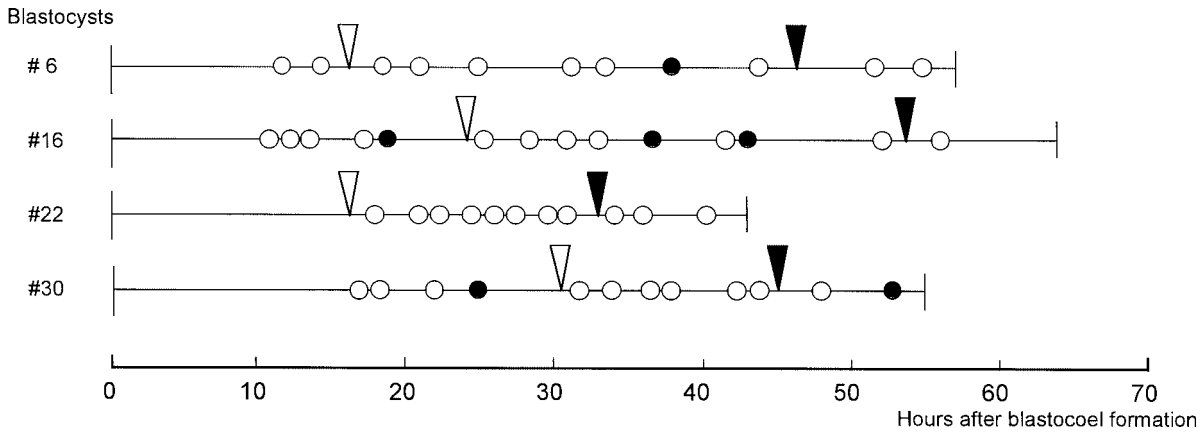

Fig. 2. A few examples of mouse blastocyst contraction during each hatching period. ○: Weak contraction, ●: strong contraction. ▽Onset of hatching, ▼Completion of hatching.

expanded stage before hatching and at the post-hatching stage, respectively. On the other hand, in 34 cultured mouse blastocysts developed from morulae, it was confirmed that 194 contractions were observed altogether until 32 h after blastocoel formation, and only 3 (1.5%) of these contractions were so severe that the blastocoel disappeared [20]. Therefore, contractions were thought to occur in blastocysts developed in uteri at the same frequency as that observed in cultured blastocysts.

Morphology of Contracted Blastocysts

Through observations with a scanning electron microscope, we [21] can confirm the formation of intercellular ridges on the surface of expanded blastocysts where the ends of two trophectoderm cells adhere (Fig. 3a). But in contracted blastocysts, there were depressions on the surface where two trophectoderm cells were touching each other, and it was confirmed that in such a case the intercellular ridges do not appear (Fig. 4a). Since these intercellular ridges are considered to be formed by strong adhesion between trophectoderm cells, the adhesion of trophectoderm cells is looser in contracted blastocysts than in expanded blastocysts. On the other hand, the perivitelline space was not apparent in expanded blastocysts, but was clearly identified in contracted blastocysts (Figs. 3b and 4b). In addition, the trophectoderm cells of expanded blastocysts were flattened and extensively stretched, whereas those of contracted blastocysts were compressed flat but not so stretched. Inner-cell-mass cells in expanded blastocysts appeared to be oval or cuboidal in shape, but those in contracted blastocysts were in most cases of indefinite shapes. Furthermore, intercellular spaces were larger in contracted blastocysts than in expanded blastocysts (Figs. 3b, 3c, 4b and 4c).

From our observations with the electron microscope, it was assumed that the contraction of blastocysts was caused by an efflux of blastocoelic fluid because of their loose cell bindings in trophectoderm. Nevertheless, with respect to the state of cell bindings of contracted blastocysts, it is as yet unknown and therefore to henceforth also ascertain the causes of contraction, this is assumed to be a dominant question that must be answered.

Mechanisms of Contraction in Blastocysts

It is generally thought that contraction of vascular endothelial cells is induced through activation of myosin light chain kinase (MLCK) by calcium-calmodulin and phosphorylation of MLC by the activated MLCK [22–24]. In order to determine whether MLCK is involved in contraction of blastocysts, we [25] have observed the contraction of mouse blastocysts treated with ML-9 [26], an inhibitor of MLCK. The results revealed that the total number of contractions was comparable in treated and non-treated blastocysts, whereas the number of weak and strong

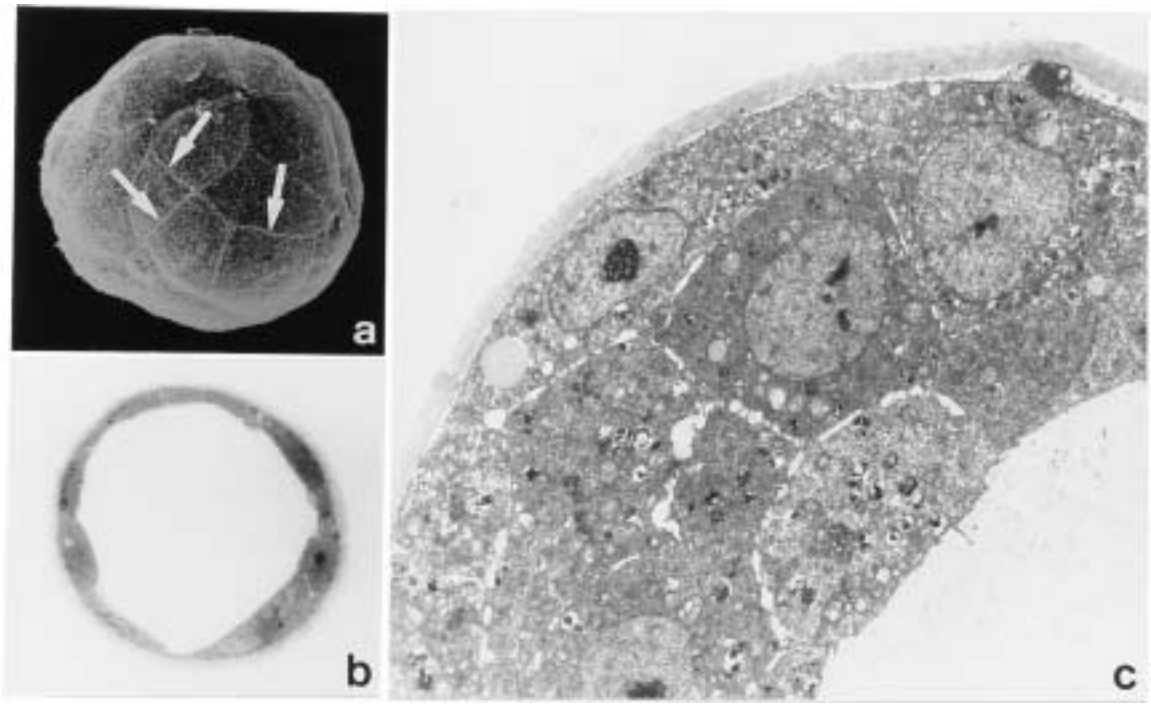

Fig. 3. Microphotographs of expanded mouse blastocysts. Fig.3a is a whole mount blastocyst seen under a scanning electron microscope, and Figs. 3b and 3c are sectioned blastocysts seen under a light microscope (3b) and a transmission electron microscope (3c). a. Intercellular ridges (arrows) are formed on the surface of a blastocyst where two trophectoderm cells adjoin. × 600. b. Perivitelline space and intercellular spaces are not apparent. × 500. c. Intercellular spaces between each pair of inner-cell-mass cells and of tophectoderm cells, and between a trophectoderm cell and an inner-cell-mass cell are narrow. × 2,200.

contractions in treated and non-treated blastocysts differed (Table 2). From the results, it is inferred that permeability between trophectoderm cells increases due to retraction of trophectoderm cells by the action of both actin and myosin having a light chain phosphorylated by MLCK, and this causes an efflux of blastocoelic fluid, leading to blastocyst contraction.

The authors [27] have also examined the relationship between the number of contractions and the frequency of divisions of trophectoderm cells in cultured mouse blastocysts during each period of hatching, to determine whether the increase in permeability between trophectoderm cells due to their division causes blastocyst contraction. The results revealed that contractions occurred 0.09, 0.21 and 0.20 times/h during the periods of pre-hatching, hatching and post-hatching, respectively, showing a smaller number in the pre-hatching period. Nevertheless, the frequencies of cell division of trophectoderm in the three periods were 4.19, 2.87 and 2.65%/h, respectively, showing a higher incidence in the pre-hatching period. Therefore, it is suggested that contractions of blastocysts are not caused by division of trophectoderm cells.

On the other hand, Checiu and Checiu [18] have reported that almost all blastocysts cultured in chambers for time-lapse microcinematography contracted, but only 8.9% of such blastocysts could complete hatching. From the results, they have thought that contractions of blastocysts are not physiological and are induced due to the influence of culture. The authors [28] compared the number of contraction times in cultured mouse blastocysts developed from 1-cell embryos and morulae by using culture chambers for time-lapse videomicrography, to determine whether contractions of blastocysts occurred due to the influence of culture. The hatching rates and the number of both weak and strong contractions did not differ in these blastocysts (Table 3). As mentioned previously, because Checiu and Checiu [18] reported a low frequency of 1.5% among uterine mouse blastocysts contracting so severely that their blastocoels disappeared, frequent

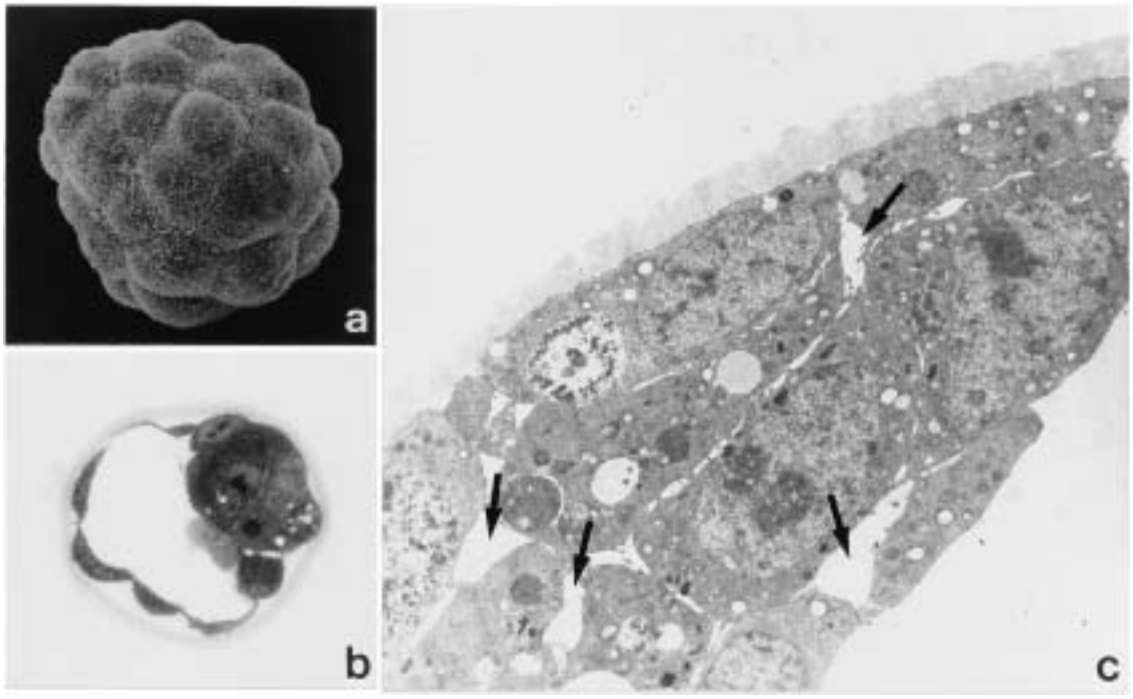

Fig. 4. Microphotographs of contracted mouse blastocysts. Fig.4a is a whole mount blastocyst seen under a scanning electron microscope, and Figs. 4b and 4c are sectioned blastocysts seen under a light microscope (4b) and a transmission electron microscope (4c). a. Intercellular ridges are not present on the surface of a blastocyst where two trophectoderm cells adjoin. × 600. b. Perivitelline space and intercellular spaces are clearly seen. × 500. c. Wide spaces (arrows) are seen between each pair of inner-cell-mass cells and of tophectoderm cells, and between a trophectoderm cell and an inner-cell-mass cell. × 2,900.

Table 2. The number of contractions of cultured mouse blastocysts

Kinds of media	No. of blastocysts observed	Hatching rates (%)	Degrees of contraction		
			Weak	Strong	Total
M16 medium	30	43.3[a]	5.63[a]	0.63[b]	6.26[a]
M16 medium containing 10^{-6}M ML-9	30	18.7[b]	3.33[b]	2.20[a]	5.53[a]

The number of contractions was counted over a span of 32 h after blastocoel formation. The degree of contraction was divided into two, based on the percentages of volume reduction of blastocysts; weak: less than 20%, strong: more than 20%. Values with different superscripts in the same column are significantly different ($P<0.05$).

contractions that they observed in cultured blastocysts are thought to result from their unsuitable culture condition, and we believe that contractions are not induced due to the influence of the culture if culture is carried out in a proper condition.

Mechanisms of Re-expansion in Contracted Blastocysts

The authors [29] have investigated the mechanisms of re-expansion in contracted blastocysts by using various inhibitors of Na^+ transporters in epithelial cells. Namely, we have observed contractions of mouse blastocysts treated with ouabain [30], benzamil [31] and 5-(*N*-ethyl-*N*-isopropyl)-amiloride (EIPA) [32], an inhibitor of

Table 3. The number of contractions of cultured mouse blastocysts

Developmental stages of culture initiation	No. of blastocysts observed	Hatching rates (%)	Degrees of contraction		
			Weak	Strong	Total
1-cell	30	43.3[a]	4.00 ± 0.40*[a]	1.40 ± 0.16[a]	5.40 ± 0.43[a]
Morula	34	52.9[a]	4.38 ± 0.28[a]	1.32 ± 0.15[a]	5.71 ± 0.29[a]

* Values represent mean ± SE. The number of contractions was counted over a span of 32 h after blastocoel formation. The degree of contraction was divided into two, based on the percentages of volume reduction of blastocysts; weak: less than 20%, strong: more than 20%. Values with different superscripts in the same column are significantly different ($P<0.05$).

Table 4. The number of contractions and the time needed for re-expansion of cultured mouse blastocysts

Kinds of media	No. of blastocysts observed	Hatching rates (%)	No. of times of contraction	Time required for re-expansion (min)
M16 medium	30	53.3[a]	6.73 ± 2.10*[a]	293 ± 103.2[b] (86-460)
M16 medium containing 100 μM ouabain	30	16.2[b]	5.68 ± 2.08[b]	369 ± 135.0[a] (144-639)
M16 medium	30	60.0[a]	6.87 ± 2.19[a]	283 ± 94.8[b] (110-503)
M16 medium containing 20 μM benzamil	30	30.0[b]	5.53 ± 1.59[b]	339 ± 90.0[a] (122-499)
M16 medium	30	53.3[a]	6.93 ± 2.63[a]	281 ± 106.2[b] (96-458)
M16 medium containing 10 μM EIPA	30	23.3[b]	5.43 ± 1.69[b]	346 ± 119.4[a] (104-559)

* Values represent mean ± SD. The number of contractions was counted over a span of 32 h after blastocoel formation. The degree of contraction was divided into two, based on the percentages of volume reduction of blastocysts; weak: less than 20%, strong: more than 20%. Values with different superscripts in the same column in each experimental lot are significantly different ($P<0.05$).

Na^+/K^+-ATPase, Na^+ channel or Na^+/H^+ exchanger, respectively. The results showed that the lengths of time needed to re-expansion to the size before contraction are always prolonged in blastocysts treated with either one of the inhibitors, compared with non-treated blastocysts (Table 4). The activity of Na^+/K^+-ATPase has also been observed histochemically to be higher in contracted blastocysts than in expanded blastocysts. From these results, it is inferred that re-expansion of contracted blastocysts occurs by active transport and accumulation of Na^+ from the trophectoderm cells into blastocoelic fluid as a result of the action of Na^+/K^+-ATPase activated in the membrane of trophectoderm cells. In addition, Na^+ necessary to re-expand of blastocysts was thought to be transported into trophectoderm cells through Na^+ channel and Na^+/H^+ exchanger from the outside of blastocysts.

Role of Contractions in Blastocyst Hatching

When the number of contractions was compared in mouse blastocysts having completed hatching and those failing to complete hatching, it was confirmed that the number of weak contractions until 32 h after blastocoel formation was similar, whereas strong contractions occurred more in blastocysts which failed to complete hatching (Table 5) [17]. It was also determined that the number of weak contractions until 32 h after blastocoel formation was similar in zona-intact and zona-free mouse blastocysts, whereas strong contractions were observed more in zona-free blastocysts (Table 5) [33]. Mouse blastocysts treated with indomethacin (IM), which is an inhibitor of the biosynthesis of prostaglandins (PGs) related to blastocyst hatching [34, 35], had the same number of weak contractions as that observed

Table 5. The number of contractions of cultured mouse blastocysts

Blastocysts	No. of blastocysts observed	Degrees of contraction		
		Weak	Strong	Total
Hatched	30	6.30[a]	0.23[b]	6.53[a]
Unhatched	30	5.57[a]	1.10[a]	6.67[a]
Zona-intact	30	5.63[a]	0.63[b]	6.26[a]
Zona-free	30	4.53[a]	1.33[a]	5.86[a]

The number of contractions was counted over a span of 32 h after blastocoel formation. The degree of contraction was divided into two, based on the percentages of volume reduction of blastocysts; weak: less than 20%, strong: more than 20%. Values with different superscripts in the same column in each experimental lot are significantly different ($P<0.05$).

Table 6. The number of contractions of mouse blastocysts cultured in media of different kinds

Kinds of media	No. of blastocysts observed	Hatching rates (%)	Degrees of contraction	
			Weak	Strong
M16 medium	28	46.4[a]	4.71 ± 2.62*[ab]	0.86 ± 0.97[bc]
M16 medium containing 10^{-4}M IM	28	17.9[b]	3.64 ± 2.68[b]	2.00 ± 1.09[a]
M16 medium containing 10^{-4}M IM and 10^{-6}M $PGF_{2\alpha}$	30	43.3[a]	6.03 ± 3.34[a]	1.10 ± 1.18[b]
M16 medium containing 10^{-4}M IM and 10^{-8}M PGE_2	30	46.7[a]	5.37 ± 2.88[a]	0.43 ± 0.73[c]
M16 medium	31	64.5[a]	3.81 ± 0.30[a]	1.13 ± 0.14[b]
M16 medium containing 0.4 μg/ml CB	30	6.7[b]	4.47 ± 0.39[a]	3.43 ± 0.23[a]
M16 medium	30	63.3[a]	4.50 ± 0.26[a]	1.30 ± 0.17[a]
M16 medium containing 1.0 mg/ml STI	32	15.6[b]	2.94 ± 0.28[b]	1.28 ± 0.22[a]

* Values represent mean ± SE. The number of contractions was counted over a span of 32 h after blastocoel formation. The degree of contraction was divided into two, based on the percentages of volume reduction of blastocysts; weak: less than 20%, strong: more than 20%. Values with different superscripts in the same column in each experimental lot are significantly different ($P<0.05$).

in non-treated blastocysts, but the number of strong contractions was larger and the hatching rate was lower in the IM-treated blastocysts [36]. When blastocysts were treated with $PGF_{2\alpha}$ or PGE_2, in combination with IM, the hatching rate and the number of weak and strong contractions were comparable to those observed in non-treated blastocysts (Table 6) [36]. We also examined the number and degree of contractions in mouse blastocysts whose hatching ability had been suppressed by cytochalasin B (CB) or soybean trypsin inhibitor (STI), in order to determine the role of contraction in blastocyst hatching. The results showed that the hatching rates were lower in both treated blastocysts, and that more strong contractions and fewer weak contractions were observed in CB-treated and STI-treated blastocysts, respectively (Table 6).

From these results, it is inferred that weak contractions play some important roles in hatching, whereas strong contractions have the effect of inhibiting hatching.

Evaluation of Embryo Quality According to Contraction

We have evaluated the quality of mouse blastocysts derived from *in vitro* fertilization as well as parthenogenetic mouse blastocysts, based on contractions observed until 32 h after blastocoel formation. It has been confirmed that the number of contractions or the hatching rate is similar in blastocysts derived from both *in vitro* and *in vivo*

Table 7. The number of contractions of cultured mouse blastocysts

Blastocysts	No. of blastocysts observed	Hatching rates (%)	Degrees of contraction		
			Weak	Strong	Total
In vivo-fertilized	30	43.3[a]	4.00 ± 0.40*[a]	1.40 ± 0.16[a]	5.40 ± 0.43[a]
In vitro-fertilized	32	34.4[a]	3.97 ± 0.42[a]	1.84 ± 0.19[a]	5.81 ± 0.38[a]
Fertilized	30	50.0[a]	4.0 ± 0.01[a]	1.4 ± 0.01[b]	5.4 ± 0.01[a]
Parthenogenone	30	23.3[b]	3.0 ± 0.01[a]	2.4 ± 0.01[a]	5.4 ± 0.01[a]

*Values represent mean ± SE. The number of contractions was counted over a span of 32 h after blastocoel formation. The degree of contraction was divided into two, based on the percentages of volume reduction of blastocysts; weak: less than 20%, strong: more than 20%. Values with different superscripts in the same column in each experimental lot are significantly different ($P<0.05$).

Table 8. The length of time needed for cultured mouse blastocysts to contract and re-expand

Blastocysts	Weak contraction			Strong contraction		
	No. of contractions examined	Time required for contraction (min)	Time required for re-expansion (min)	No. of contractions examined	Time required for contraction (min)	Time required for re-expansion (min)
Fertilized	30	5.0 ± 0.02*[a]	107.7 ± 0.36[b]	30	19.4 ± 0.11[a]	242.6 ± 0.82[b]
Parthenogenone	30	3.9 ± 0.06[a]	147.7 ± 0.40[a]	30	24.0 ± 0.24[a]	345.0 ± 0.80[a]

* Values are mean ± SE. Weak and strong contractions examined were randomly selected in 30 blastocysts until 32 h after blastocoel formation. The degree of contraction was divided into two, based on the percentages of volume reduction of blastocysts; weak: less than 20%, strong: more than 20%. Values with different superscripts in the same column are significantly different ($P<0.05$).

fertilization (Table 7), suggesting no difference between these blastocysts in quality [28]. It has been determined that the hatching rate is lower in parthenogenetic blastocysts than in fertilized blastocysts, and that the number of weak contractions is similar in parthenogenetic and fertilized blastocysts, whereas strong contractions more often occur in parthenogenetic blastocysts (Table 7) [37]. It has also been clarified that the lengths of time needed for re-expansion after both strong and weak contractions are longer in parthenogenetic blastocysts than in fertilized blastocysts (Table 8). From these results, the lower hatching ability in parthenogenetic blastocysts is thought to be due to frequent strong contractions that require a much longer time for re-expansion [37].

The authors [38] have also investigated changes in embryo quality with age in dams from the standpoint of the relationship between developmental ability and contractions. Although the rate of development from 2-cell embryos to blastocysts did not differ with the dams' ages, the hatching rate was lower in the blastocysts from young and aged mice than in those from 60 to 90-day-old mice. The results also revealed that the number of weak contractions is fewer in blastocysts from mice aged 360 days, and the number of strong contractions is larger in blastocysts from 30-day-old mice (Table 9). From these results, it was confirmed that embryos collected from young and aged mice are able to develop to blastocysts at a high rate, but their ability to hatch is poor. A low hatching ability is thought to be due to frequent strong contractions observed in blastocysts from young mice, and to the small number of weak contractions seen in blastocysts from aged mice [38].

Concluding Remarks

As mentioned, contractions of blastocysts occur in relation to MLCK, but not due to an increase in permeability between trophectoderm cells in association with their division, or by the influence of culture. Nevertheless, the possibility that contractions are due to the efflux of blastocoelic fluid caused by degeneration or death of trophectoderm cells cannot be excluded [1, 2, 10, 12]. The state of cell binding of trophectoderms in

Table 9. The number of contractions in cultured blastocysts collected from mice of different ages

Age of mice (days)	Developmental rates of 2-cell embryos to blastocysts (%)	No. of blastocysts observed	Hatching rates (%)	Degrees of contraction		
				Weak	Strong	Total
30	87.5[a]	30	31.3[b]	5.53 ± 0.49*[a]	2.03 ± 0.23[a]	7.57 ± 0.49[a]
60–90	87.2[a]	30	53.8[a]	5.50 ± 0.42[a]	1.37 ± 0.18[b]	6.87 ± 0.41[a]
360	83.3[a]	30	25.0[b]	4.23 ± 0.85[b]	1.50 ± 0.17[ab]	5.73 ± 0.28[b]

* Values represent mean ± SE. The development of 2-cell embryos to blastocysts was observed in 48 h of culture, and the completion of hatching was observed until 140 h after blastocyst formation. The number of contractions was counted over a span of 32 h after blastocoel formation. The degree of contraction was divided into two, based on the percentages of volume reduction of blastocysts; weak: less than 20%, strong: more than 20%. Values with different superscripts in the same column are significantly different ($P<0.05$).

contracted blastocysts has also remained obscure [39], so that these issues should be further investigated. On the other hand, since contractions were also observed in blastocysts developed *in vivo*, such movements are thought to be physiological. Contractions of various cultured blastocysts particularly suggest that weak contractions play some positive roles in hatching, whereas strong contractions inhibit hatching. It seems to be possible to evaluate the developmental ability of embryos, i.e. quality of embryos, based on contractions of blastocysts. If embryo quality can be evaluated within a shorter period through analysis of contractions of blastocysts, this method will serve as a useful tool to select embryos for transfer after the frozen-thawed procedure or nuclear manipulation.

Since contractions are also observed in hatched blastocysts, it is thought that contractions may serve as a physical stimulus to evoke maternal recognition of the presence of blastocysts, but since no results of experiments which support this hypothesis have been obtained, further studies are required.

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