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## Effect of Magnesium Ion in the Culture Medium on the Development of Preimplantation Mouse Embryos *In Vitro*

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**Objective:** The present study was undertaken to examine the effects of magnesium ion in the culture medium on the development of mouse fertilized oocytes either before or after pronuclear formation, and to investigate whether the effect of magnesium ion is related with the redistributive change of mitochondria.

**Methods:** Fertilized oocytes obtained from the oviducts of mice at 15 hr after hCG injection before pronuclear formation (pre-PN) or 21 hr after hCG injection after pronuclear formation (post-PN) were used. The embryos were cultured for 3 days with basic T6 medium-magnesium free and various concentrations of magnesium ion, 0.0, 0.5, 1.0, 2.0, 4.0 or 8.0 mM, respectively. After culture, the developmental stages of embryos and the number of nuclei were evaluated. To observe the effects of magnesium ion on the mitochondrial distribution, fertilized oocytes were collected at 21 hr after hCG injection and cultured for 6 hr with various concentration of magnesium ion. As a control, fertilized oocytes with pronuclei at 27 hr after hCG injection were used.

**Results:** The concentration of magnesium ion to accelerate the *in vitro* development of mouse fertilized oocytes appeared to be at 2.0 mM for the pre-PN and the post-PN stage embryos. In the mitochondrial redistribution patterns, the embryos cultured in 2.0 mM concentration of magnesium ion showed the highest percentage (22.6%) of distinct perinuclear clustering pattern comparing to other experimental group.

**Conclusion:** The effect of magnesium ion may be related to the cytoplasmic redistribution of mitochondria. This relationship seems to connect the developmental competence of preimplantation mouse embryos *in vitro*. These results can suggest that higher concentration of magnesium ion (2.0 mM) than those of conventional culture medium (0.2~1.2 mM) is more suitable for *in vitro* culture of preimplantation mouse embryos.

**Key Words:** Magnesium ion, Culture medium, Preimplantation mouse embryos, Developmental competence, Mitochondrial distribution

junction communication,

가 가 1-10 (homeostasis) ,<sup>22</sup>

(*in vitro* culture) ,<sup>23</sup>

(cellular function) ,<sup>11,12</sup>

(calcium oscillation) , ATP가 가

<sup>13</sup> (abnormal control) , DNA ,<sup>24-27</sup>

communication 가 ,<sup>14,15</sup>

DNA uri- ,<sup>28</sup> Barnett Bavister <sup>29</sup>

dine 1- ,<sup>16,17</sup> Fisher <sup>18</sup>

(optimal stimulation)

(Chick embryo cell) 가 /

DNA , DNA 가 ,

(effector) 가 가

(modifier) <sup>19</sup> 가

<sup>20,21</sup>

Lane 14

<sup>22</sup> 1- 0.5 mM ~ 1. 5~6

4.0 mM 가 1) 가

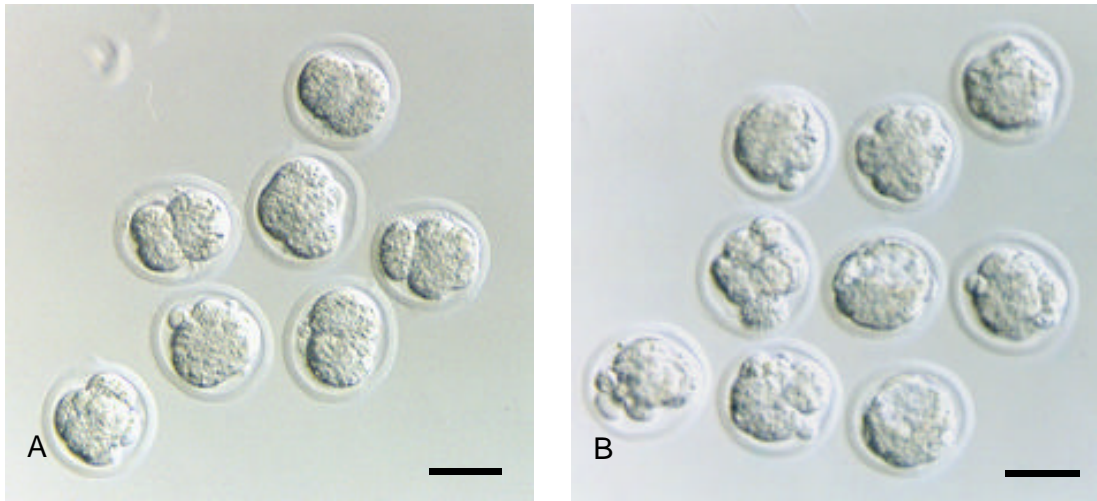
2.0 mM 가 , 10 12

가 가 2- (C57BL/6 × CBA F1)

가가 가 (homeo-

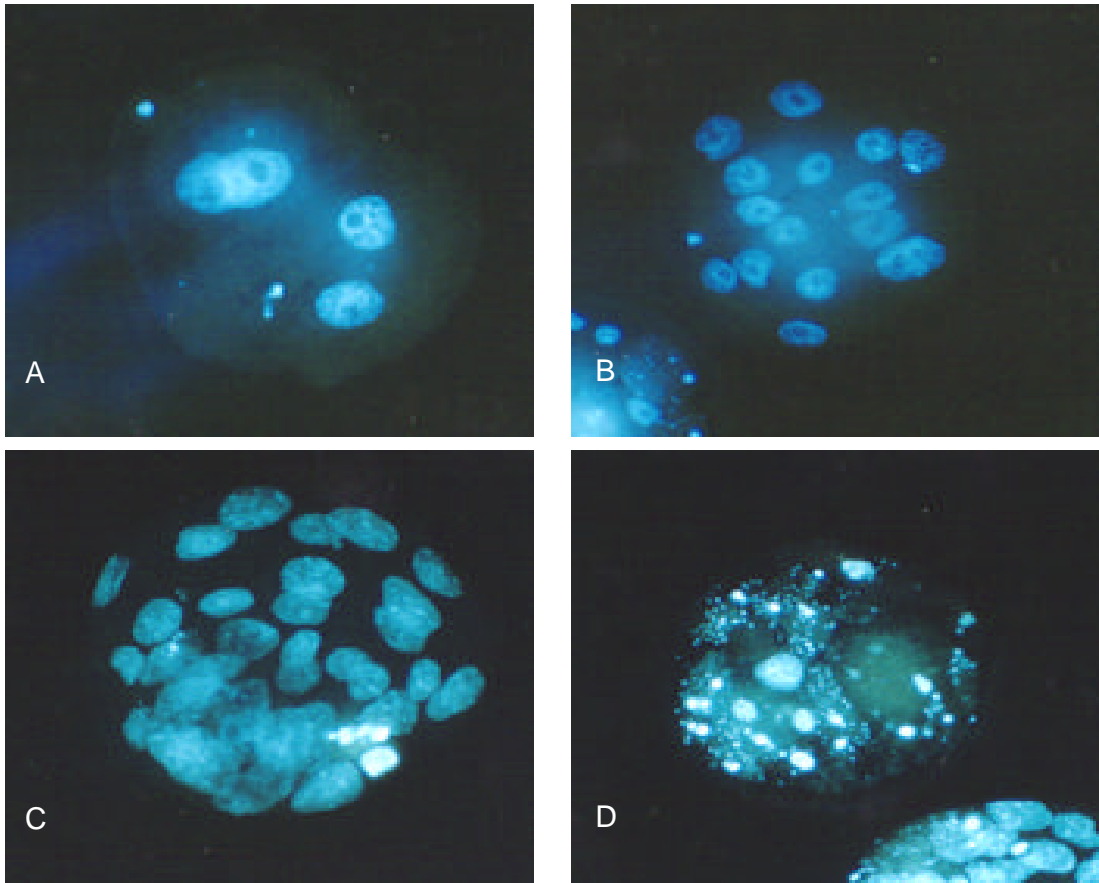
tasis) 5 IU (international unit) pe-

, gap pregnant mare's serum gonadotropin (PMSG, Sigma)



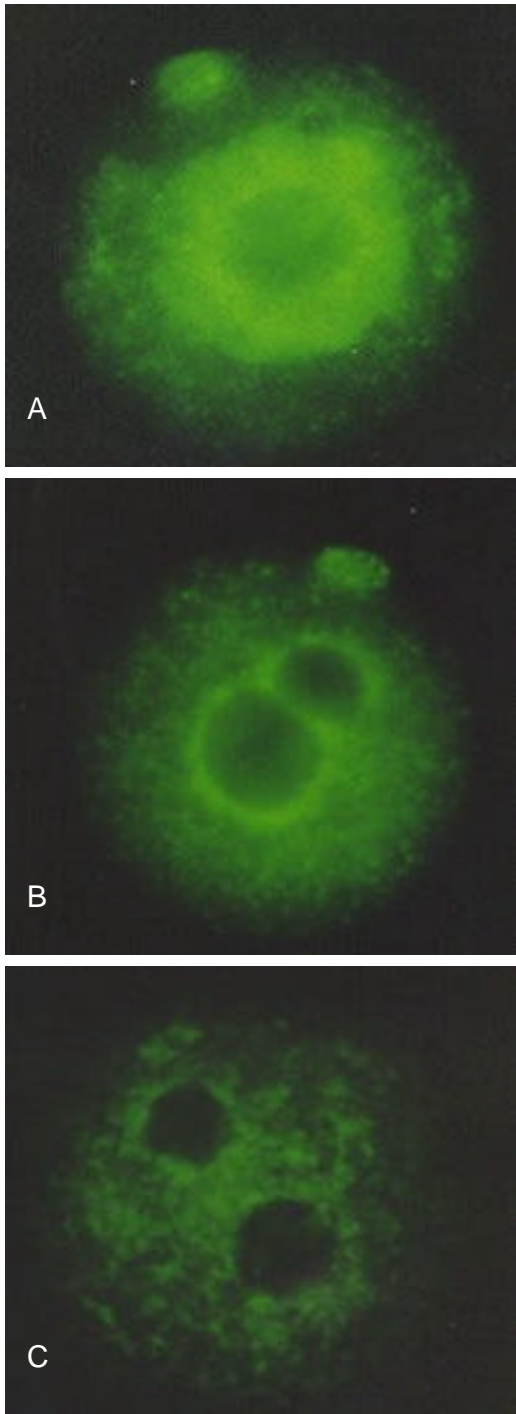
**Figure 1.** Microphotographs of mouse embryos collected from oviduct 21 hr after hCG injection followed by culture for 75 hr. Magnification,  $\times 200$ . Bar indicates 50  $\mu$ m. For details, see Table 2. **A**, 0.0 mM  $Mg^{2+}$  concentration, 1.78 mM  $Ca^{2+}$  concentration; **B**, 2.0 mM  $Mg^{2+}$  concentration, 1.78 mM  $Ca^{2+}$  concentration

48 human chorionic gonadotropin (hCG, (Falcon 3002) 0.0, Sigma) 0.5, 1.0, 2.0, 4.0, 8.0 mM 가  
(vaginal plug) 30,31 20  $\mu$ l  
2) (pre - PN) mineral oil (Sigma)  
hCG 15 3  
0.4% bovine serum albumin (BSA, 37 5%  $CO_2$   
Gibco) 가 modified  
Tyrode's solution-Magnesium free (T6-Mg free ) hCG 96  
(blastocyst) (degenerated embryo)  
0.1% hyaluronidase (Sigma) (Figure 1).  
mucin (cumu- 3.  
lus cell) (pronucleus) hCG 96  
3) (post - PN) 1% glutaraldehyde , 10  
hCG 21  $\mu$ g/ml Hoechst 33342 (bisbenzimidazole solution, Sigma)  
phosphate buffered saline (PBS)  
0.4% polyvinyl-pyrrolidone (PVP, 32  
Sigma)-PBS mounting  
(Nikon, Japan) (400 $\times$ )  
2. (Figure 2).  
microdroplet



**Figure 2.** Fluorescence micrographs of mouse embryo stained with Hoechst 33342 (bisbenzimidazole solution). Magnification,  $\times 400$ . For details, see Table 1 & 2. **A**, 4-cell embryo; **B**, Morula; **C**, Early Blastocyst; **D**, Degenerated embryo

4.		Rh123 stock	(1 $\mu$ g/ml; final concentration 10 $\mu$ g/ml)	
			(culture condition)	15
	transmembrane electrical potential	rhodamine 123 (Rh123, Molecular probes R-302, Eugene, USA)	slide	B-2A filter (200 $\times$ )
		Rh123 stock solution	cover slip (Nikon, Japan)	
		methanol 10 mg/ml		
		-20		
			10,29,33	
			(distinct perinuclear clustering),	
			(perinuclear clustering),	
			(dispersed throughout the cytoplasm)	
			(Figure 3).	
			5.	
				Student's t-test



**Figure 3.** Fluorescence micrographs of the mitochondria in the one-cell embryo stained with Rhodamine 123. Magnification,  $\times 400$ . For details, see Table 3. **A**, distinct perinuclear clustering; **B**, perinuclear clustering; **C**, dispersed throughout the cytoplasm

1.

1 -  
 0.0 mM  
 25.7% , 2.0, 4.0, 8.0 mM  
 62.0%, 55.6%, 47.1%  
 (p<0.01). 2.0 mM  
 가

Hoechst 33342

2.0, 4.0, 8.0 mM 25.2, 25.2, 22.5  
 0.0 mM (17.4)  
 (p<0.01), 2.0 4.0 mM 가  
 가 (Table 1).

1 -  
 가 0.0 mM (25.1%)  
 2.0 4.0 mM 71.3%, 64.4%  
 , 2.0 mM 가  
 (p<0.01).

1.0, 2.0, 4.0 mM 20.2, 21.0,  
 18.8 0.0 mM (15.5)  
 (p<0.05), 2.0 mM 가  
 가 (Table 2, p<0.01).

2.

hCG  
 27 64.4% 가  
 ,  
 6  
 (p<0.01).  
 2.0 mM  
 22.6% 0.0, 8.0 mM  
 4.3%, 6.0% (Table  
 3, p<0.05).

**Table 1.** Effect of various magnesium concentrations in the culture medium on the *in vitro* development of pre-PN stage embryos

Magnesium concentration (mM)	Total no. of embryos	Developmental stage reached after 3 days of culture (%)			Cell number of Mo. and Bl.
		Morula (Mo)	Blastocyst (Bl)	Mo. + Bl.	
0.0	66	25.7 ± 3.9	0.0 ± 0.0	25.7 ± 3.9	17.4 ± 1.2
0.5	62	33.6 ± 3.7	8.1 ± 6.5	41.7 ± 6.2	19.8 ± 1.5
1.0	64	34.8 ± 3.2	5.2 ± 3.4	39.9 ± 3.3*	18.9 ± 1.0
2.0	71	48.6 ± 6.5	13.4 ± 3.1	62.0 ± 5.5**	25.2 ± 1.1**
4.0	67	41.7 ± 6.8	8.7 ± 3.4	55.6 ± 5.2**	25.2 ± 0.9**
8.0	68	47.1 ± 4.8	0.0 ± 0.0	47.1 ± 4.8**	22.5 ± 1.0**

The concentration of calcium ion was adjusted to 1.78 mM. The results were obtained by pooling of seven replicates (Student's t-test). P values significantly differ from the 0.0 mM concentration group (\*\*p<0.01, \*p<0.05). Embryos were flushed at 15 hr after hCG injection and those of PN formed embryos at 26~28 hr after hCG were further culture in the present study. Data are mean ± SEM.

**Table 2.** Effect of various magnesium concentrations in the culture medium on the *in vitro* development of post-PN stage embryos

Magnesium concentration (mM)	Total no. of embryos	Developmental stage reached after 3 days of culture (%)			Cell number of Mo. and Bl.
		Morula (Mo)	Blastocyst (Bl)	Mo. + Bl.	
0.0	77	23.7 ± 5.1	1.4 ± 1.3	25.1 ± 5.9	15.5 ± 1.0
0.5	74	42.1 ± 9.3	0.0 ± 0.0	42.1 ± 9.3	15.3 ± 0.6
1.0	79	39.6 ± 6.2	7.7 ± 6.9	47.3 ± 10.3	20.2 ± 1.4*
2.0	74	60.7 ± 7.7	10.5 ± 3.2	71.3 ± 10.2**	21.0 ± 1.2**
4.0	76	59.0 ± 3.5	5.5 ± 2.6	64.4 ± 5.9**	18.8 ± 0.9*
8.0	77	32.1 ± 8.8	0.0 ± 0.0	32.1 ± 8.8	16.3 ± 0.9

The concentration of calcium ion was adjusted to 1.78 mM. The results were obtained by pooling of five replicates (Student's t-test). P values significantly differ from the 0.0 mM concentration group (\*\*p<0.01, \*p<0.05). Embryos were flushed at 21 hr after hCG injection and those of PN formed embryos were further culture in the present study. Data are mean ± SEM.

**Table 3.** Effect of various magnesium concentrations on mitochondrial distribution of one-cell embryos *in vitro* culture

Magnesium concentration	Total No. of embryos	Pattern of mitochondrial distribution (%)		
		Distinct Perinuclear clustering	Perinuclear clustering	Dispersed throughout the cytoplasm
Control	67	64.4 ± 4.4 <sup>a</sup>	26.5 ± 3.0	9.2 ± 4.1
0.0	74	4.3 ± 1.9 <sup>b</sup>	28.6 ± 4.3	67.0 ± 3.1
2.0	79	22.6 ± 4.7 <sup>c</sup>	35.6 ± 4.2	41.7 ± 3.0
8.0	77	6.0 ± 2.7 <sup>d</sup>	39.9 ± 5.2	54.1 ± 4.9

The concentration of calcium ion was adjusted to 1.78 mM. The results were obtained by pooling of seven replicates (student's t-test). P values significantly differ from the control group (ab, ac, ad p<0.01); P values significantly differ from the 2.0 mM concentration group (bc, p<0.01, cd p<0.05). Control, all embryos were flushed at 27 hr after hCG injection. The other group, all embryos were flushed at 21 hr after hCG injection and 6 hr further culture were done *in vitro* system. Data are mean ± SEM.

가<sup>34,36</sup>  
가  
buffer 가  
1- 2.0,  
4.0, 8.0 mM 가 0.0 mM Myocardial cell  
(p<0.01), 2.0 mM 가  
가  
2.0, 4.0, 8.0 Myocardial cell  
mM 가 가 2.0 mM  
(Table 1). 1- buffering  
가 2.0 4.0 mM  
가 (8.0 mM) (cytoplasm)  
가  
1.0, 2.0, 4.0 mM kom Runner<sup>24</sup>  
2.0 mM 21.0 가 가 spindle  
(Table 2).  
2- Muggleton-Harris  
Mckiernan<sup>4</sup> Bavister Brown<sup>26</sup> '2-cell block'  
Golden<sup>34</sup>  
McKiernan 0.5 mM ~0.125  
mM, Bavister 0.1 mM ~0.8 mM  
Lane<sup>22</sup> 1- 2.0 mM 가 가 2.0 mM 가 22.6%  
0.5 mM ~4.0 mM 가  
Bavister가<sup>35</sup> Barnett 가  
3 (Table 3). spindle  
9 16% 64%  
가 , 8.0 mM (in vivo) 가 가  
가 hCG 27 가  
(6 )  
1- (dispersed throughout the cytoplasm)  
가

- (perinuclear clustering)  
(distinct perinuclear clustering)
- 2.0 mM      가      가
- 가      ,
- 가      .
- 0.2 mM ~ 1.2 mM      가
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