Chapter IV

Result

1. **The effects of single amino acid (glycine, glutamine, and taurine), combination of either two amino acids (glycine plus glutamine, taurine plus glycine and taurine plus glutamine), and combination of all three amino acids (glycine plus glutamine plus taurine) on the development of eight-cell hamster embryos at 275 mOsmol.**

Groups of ten to twenty eight-cell hamster embryos were cultured in culture medium. Eight to ten replicate cultures were performed for each treatment. Results were summarized in table 4-5 and figure 5.

The eight-cell embryos cultured in HECM-10 containing a single amino acid (taurine, glutamine, glycine) and those in the control group developed to blastocyst stage after 24 hr. in culture (25.1%, 54.6%, 42.7%, and 36.2%, respectively) without any significant different in blastocyst formation between all treatments and control group. When culture period was extended to 48 hr., only embryos cultured in medium containing glutamine were significantly better developed to blastocyst (72.5%) compared to the control group (46.5%).

The percentage of blastocysts in taurine plus glutamine, taurine plus glycine, and glycine plus glutamine after 24 hr. in culture were 58.3%, 32.6%, and 55.3%. Blastocyst development in the medium containing taurine plus glutamine was significantly better than that of the control group (36.2%). By 48 hr. in culture, the blastocyst formations in medium containing taurine plus glutamine (80.4%) and glutamine plus glycine (84.0%) were significantly higher compared to the control (46.5%).

When eight-cell embryos were cultured in HECM-10 with taurine plus glutamine plus glycine for 24 hr., the percentage of blastocysts obtained in HECM-10 supplied with all three amino acid together (39.7%) was not significantly higher than

those of control group (36.2%). At 48 hr., the percentage of blastocyst obtained in HECM-10 supplemented with all three amino acids (54.8%) was not significantly higher than that of the control group (46.5%).

The percentages of blastocysts obtained in all treatments supplemented with amino acids (single amino acid, combination of either of two amino acids and combination of all three amino acid) at the end of 48 hr. in culture were compared. Blastocyst formations were significantly higher in groups supplemented with glutmine, glutamine plus taurine, and glycine plus glutamine than the other groups.

Table 4 **Effect of glutamine, glycine, and taurine on development of eight-cell hamster embryos in HECM-10 at 275 mOsmol after 24 hr in culture**

Culture medium	No. of eight-cell embryos at beginning of culture period	No. of morula (%mean+ SE)	No. of Blastocyst (%mean+SE)
HECM-10	107	55 (54.0±7.5)	40 (36.2±5.5)
HECM-10 + taurine	106	52 (49.6±7.5)	32 (25.1±7.6)
HECM-10 + glutamine	111	44 (39.9±7.5)	63 (54.6±3.9)
HECM-10 + glycine	112	58 (53.3±6.3)	50 (42.7±7.5)
HECM-10 + taurine + glutamine	117	41 (38.7±6.7)	71* (58.3±7.3)
HECM-10 + taurine + glycine	97	56 (59.1±6.7)	38 (32.6±8.4)
HECM-10 + glutamine + glycine	107	35 (34.6±6.4)	65 (55.3±7.4)
HECM-10 + taurine + glutamine + glycine	108	42 (36.±16.4)	43 (39.7±8.2)

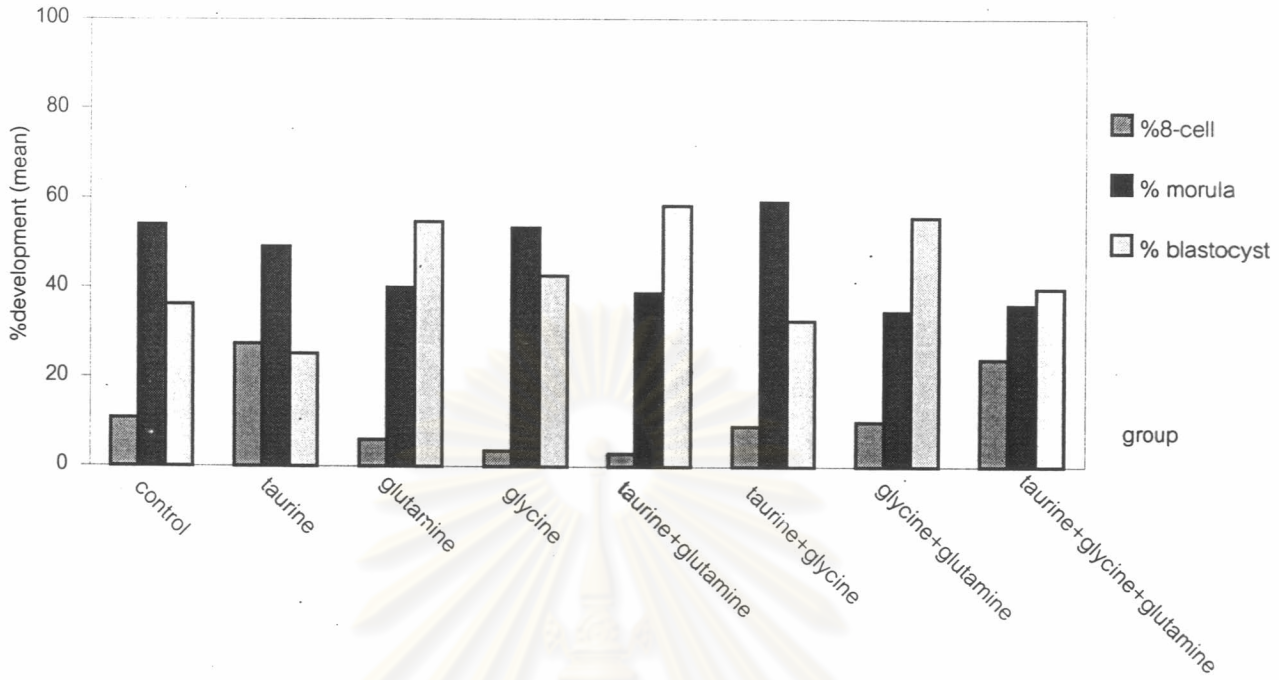
*significantly different compared to HECM-10 (control group)

Table 5 **Effect of glutamine, glycine, and taurine on development of eight-cell hamster embryos in HECM-10 at 275 mOsmol after 48 hr in culture**

Culture medium	No. of eight-cell embryos at beginning of culture period	No. of morula (%mean± SE)	No. of Blastocyst (%mean±SE)
HECM-10	107	26 (26.1±5.5)	51 (46.5±7.8)
HECM-10 + taurine	106	12 (14.5±5.5)	55 (48.8±7.8)
HECM-10 + glutamine	111	9 (10.1±5.5)	85*(72.5±7.8)
HECM-10 + glycine	112	29 (29.3±4.9)	67 (57.8±7.0)
HECM-10 + taurine + glutamine	117	7 (5.4±4.9)	96*(80.4±7.0)
HECM-10 + taurine + glycine	97	12 (13.1±4.9)	57 (57.7±7.0)
HECM-10 + glutamine + glycine	107	4 (3.6±4.7)	92*(84.0±6.7)
HECM-10 + taurine + glutamine + glycine	108	10 (6.2±4.7)	57 (54.8±6.7)

* significantly different compared to HECM-10 (control group)

a



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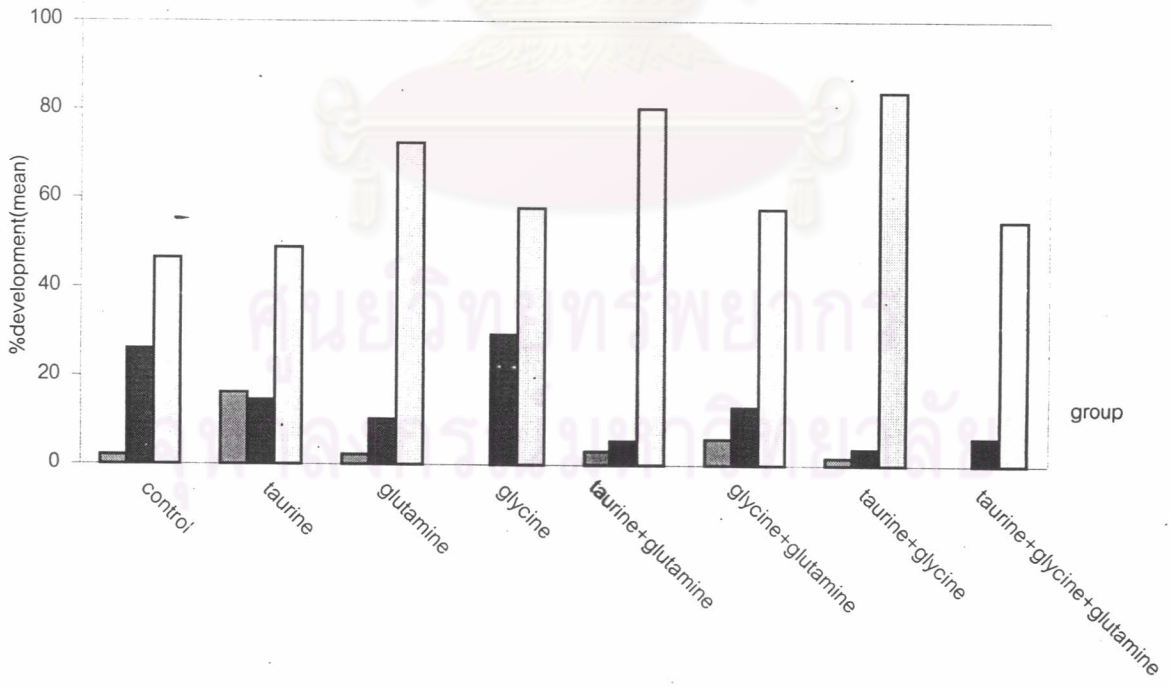

Figure 5 Effect of taurine, glutamine, and glycine on the development of eight-cell hamster embryos in HECM-10 at 275 mOsmol (a) after 24 hr. in culture (b) after 48 hr. in culture

a

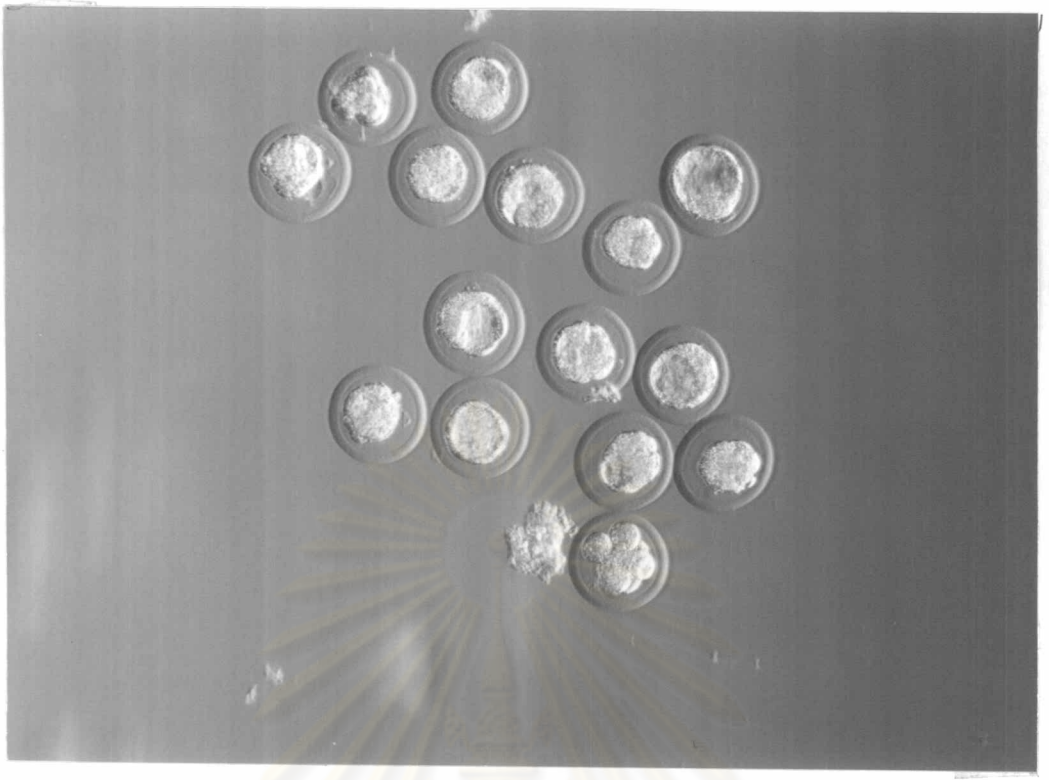

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Figure 6 The development of eight-cell hamster embryos in HECM –10 supplemented with glycine plus glutamine at 275 mOsmol (a) morula and blastocyst formation after 24 hr. in culture, X10 (b) blastocyst formation after 48 hr. in culture, X10

2. The effects of single amino acid (glycine, glutamine, and taurine), combination of either two amino acids (glycine plus glutamine, taurine plus glycine and taurine plus glutamine), and combination of all three amino acids (glycine plus glutamine plus taurine) on the development of eight-cell hamster embryos at 325 mOsmol.

Six to ten replicate cultures containing 15 to 20 eight-cell hamster embryos per culture medium were carried out to each treatment. Results were summarized in table 6-7 and figure 6

At 325 mOsmol, the percentages of blastocyst obtained in all seven treatments and control group scored after 24 hr. and 48 hr. in culture were decrease compared to 275 mOsmol.

When embryos were cultured in HECM-10 supplemented with taurine, glutamine or glycine alone, there was no significant in the percentage of blastocyst between these groups and the control group after 24 hr. and 48 hr. However, taurine and glutamine supplemented medium tended to yield higher percentages of blastocyst (44.1% and 36.8%) than that of control (30.3%) after 48 hr. culture.

The blastocyst developments of eight-cell embryo in taurine plus glutamine, taurine plus glycine, and glycine plus glutamine were not significantly different from the control at 24 hr., although the percentage of blastocyst in taurine plus glutamine supplemented medium (28.5%) was higher than that of the control (20.3%). Neither percentages of blastocyst formation in medium supplemented with any combination of two amino acids (29.3% to 34.0%) were higher than that of the control (30.3%) at 48 hr. in culture.

The percentage of blastocyst in medium containing all three amino acids was not significantly different from the control at 24 hr. (34.7%vs. 20.3%), and at 48 hr. (38.2% vs. 30.3%) after culture.

The percentages of blastocyst obtained in all treatments supplemented with amino acids (single amino acid, combination of either of two amino acids and combination of all three amino acid) at the end of 48 hr. in culture were compared. Although supplemented with taurine alone seems to result in high blastocyst formation, nonetheless, it was not significantly different from any other groups.

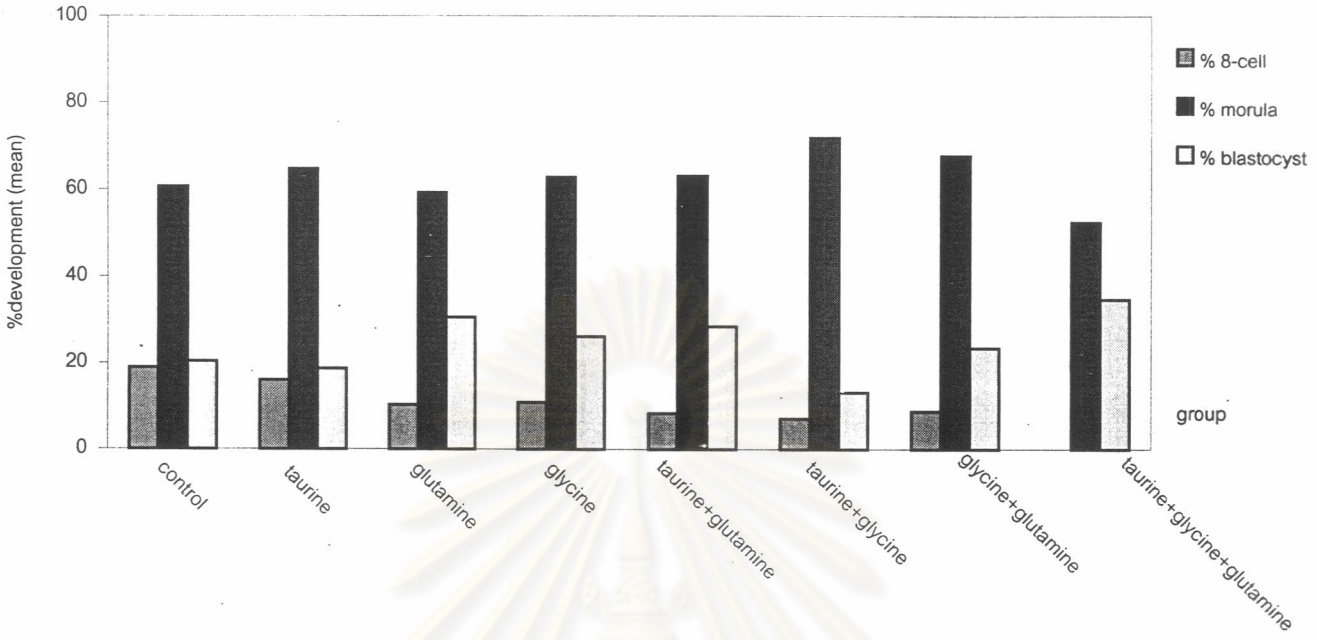
Table 6 **Effect of glutamine, glycine, and taurine on development of eight-cell hamster embryos in HECM-10 at 325 mOsmol after 24 hr. in culture**

Culture medium	No. of eight-cell embryos at beginning of culture period	No. of morula (%mean±SE)	No. of Blastocyst (% mean±SE)
HECM-10	111	69 (60.7 ±6.9)	19 (20.3 ±5.9)
HECM-10 + taurine	125	86 (64.7± 9.6)	21 (18.7 ±8.5)
HECM-10 + glutamine	116	70 (59.2± 6.8)	34 (30.2± 4.9)
HECM-10 + glycine	104	63 (62.8 ±8.9)	29 (26.1 ±7.1)
HECM-10 + taurine + glutamine	108	74 (63.1± 8.9)	27 (28.5± 10.6)
HECM-10 + taurine + glycine	103	73 (71.9± 4.9)	14 (13.2± 6.0)
HECM-10 + glutamine + glycine	93	62 (67.7 ±7.6)	25 (23.4 ±6.6)
HECM-10 + taurine + glutamine + glycine	88	47 (52.5 ±8.5)	18 (34.7 ±9.3)

Table 7 **Effect of glutamine, glycine, and taurine on development of eight-cell hamster embryos in HECM-10 at 325 mOsmol after 48 hr. in culture**

Culture medium	No. of eight-cell embryos at beginning of culture period	No. of morula (% mean±SE)	No. of Blastocyst (%mean±SE)
HECM-10	111	23 (21.3± 5.2)	34 (30.3 ±6.2)
HECM-10 + taurine	125	22 (19.9 ±5.9)	55 (44.1 ±12.1)
HECM-10 + glutamine	116	27 (21.6 ±4.9)	48 (36.8 ±11.7)
HECM-10 + glycine	104	22 (20.4 ±5.9)	35 (31.5± 6.3)
HECM-10 + taurine + glutamine	108	43 (38.4 ±7.2)	35 (29.3 ±8.9)
HECM-10 + taurine + glycine	103	28 (27.2 ±6.7)	30 (30.7± 8.6)
HECM-10 + glutamine + glycine	93	37 (46.8 ±8.2)	50 (34.0± 7.0)
HECM-10 + taurine + glutamine + glycine	88	10 (7.4± 4.9)	36 (38.2± 5.7)

a



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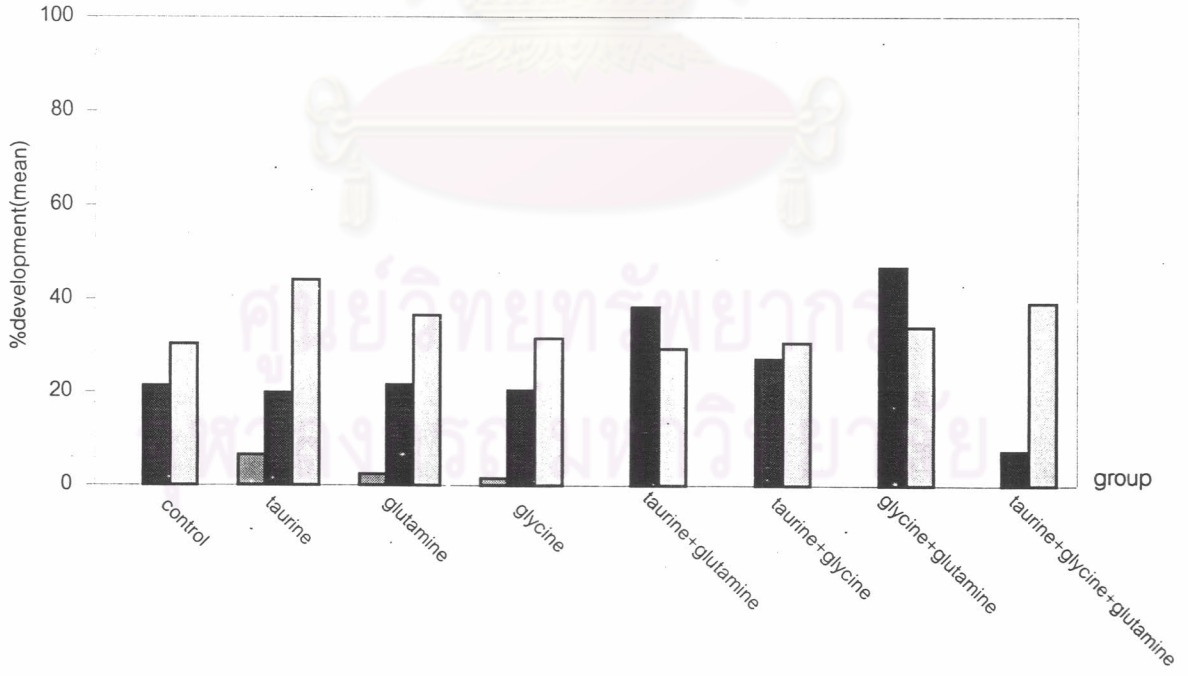
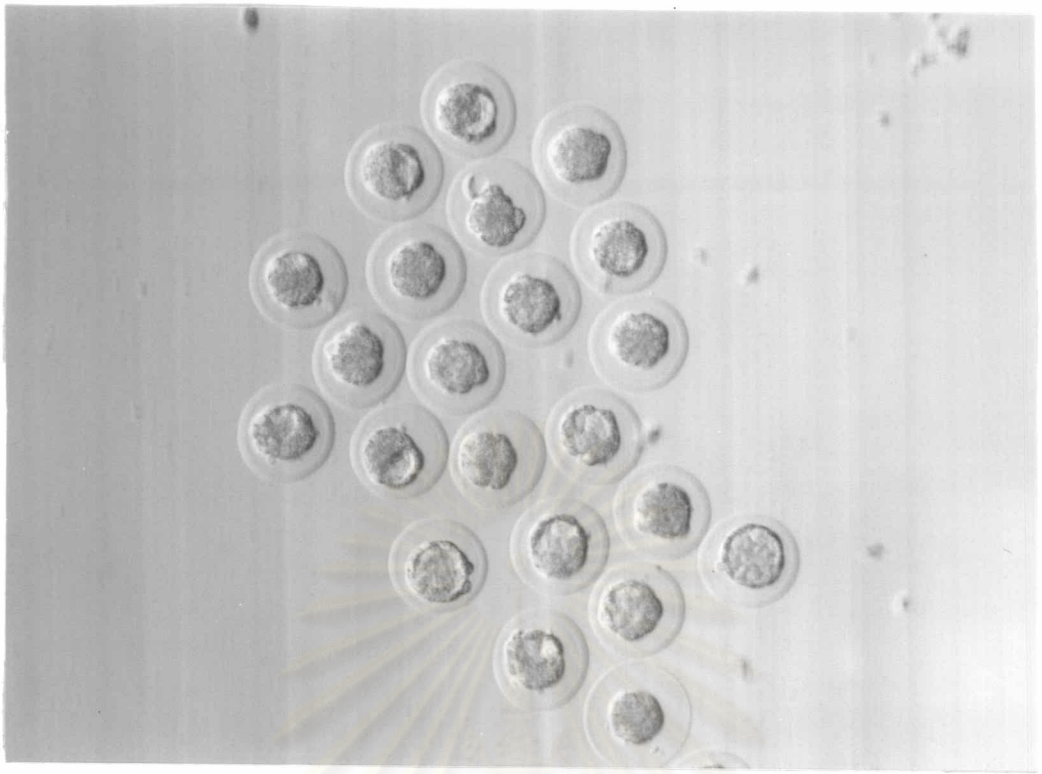


Figure 7 Effect of taurine, glutamine, and glycine on the development of eight-cell hamster embryos in HECM-10 at 325 mOsmol (a) at 24 hr. in culture (b)at 48 hr. in culture

a



b

Figure 8 The development of eight-cell hamster embryos in HECM –10 supplemented with taurine at 325 mOsmol (a) morula and blastocyst formation after 24 hr. in culture, X10 (b) blastocyst formation after 48 hr. in culture, X 20

3. The effects of single amino acid (glycine, glutamine, and taurine), combination of either two amino acids (glycine plus glutamine, taurine plus glycine and taurine plus glutamine), and combination of all three amino acids (glycine plus glutamine plus taurine) on the development of eight-cell hamster embryos at 375 mOsmol.

Fifteen to twenty eight-cell hamster embryos were cultivated in culture medium. Each treatment was repeated five to nine times. Results were summarized in table 8-9 figure 7.

At 375 mOsmol, the percentages of blastocyst either of control group or HECM-10 supplemented with amino acids at 24 hr. and 48 hr. in culture were decreased compare to 275, and 325mOsmol. Most eight-cell in all treatments failed to develop beyond the morula stage after 24 hr. culture. Moreover, a few blastocysts which developed in the first 24 hr. culture degenerated when the culture was extended to 48 hr.

Not a single eight-cell embryos developed to blastocyst in medium supplemented with taurine alone, while only two of 96 and four of 106 embryos in medium supplemented with glutamine and glycine alone respectively, developed to blastocyst stage. However, the percentage of morulae in medium supplemented with glutamine alone (57.0%) was significantly higher than that of the control (20.5%).

When eight-cell embryos were cultured in medium supplemented with two amino acids, very few embryos developed to blastocyst stage, i.e. one of 116, one of 93 and eleven of 118 eight-cell embryos in medium with taurine plus glutamine, taurine plus glycine and glycine plus glutamine respectively, while none of embryos in the control group could develop to the blastocyst stage. However, development of eight-cell embryos to morula stage in medium supplemented with taurine plus glutamine (44.2%), and glutamine plus glycine (48.9%) were significantly higher than those in the control group (20.5%).

Two of 107 eight-cell embryos cultured in HECM-10 supplemented with all three amino acids developed to blastocyst stage whereas none in the control group could. However, development of these embryos to morula stage in medium supplemented with all three amino acids was significantly higher than that in the control group (20.5%).

Most eight-cell embryos failed to develop to blastocyst and all of them degenerated at 48 hr. in culture. Thus the percentages of morula obtained in all treatments supplemented with amino acids (single amino acid, combination of any of two amino acids, and combination of all three amino acids) at the end of 24 hr. in culture were compared. Medium supplemented with glutamine, taurine plus glutamine, glycine plus glutamine, glycine plus glutamine plus taurine promoted the development of eight-cell embryos to morula stage significantly higher than the other groups. However, There was no significant difference in morula formations among all promoted groups.

Table 8 **Effect of glutamine, glycine, and taurine on development of eight-cell hamster embryos in HECM-10 at 375 mOsmol after 24 hr in culture**

Culture medium	No. of eight-cell embryos at beginning of culture period	No. of morula (%mean ± SE)	No. of Blastocyst (%mean ± SE)
HECM-10	103	22 (20.5± 5.3)	0 (0)
HECM-10 + taurine	94	17 (19.4 ± 3.1)	0 (0)
HECM-10 + glutamine	96	58*(57.0 ± 6.9)	2 (2.3 ± 1.6)
HECM-10 + glycine	106	29 (23.8 ± 5.4)	4 (2.8 ± 1.5)
HECM-10 + taurine + glutamine	116	49*(44.2± 8.8)	1 (1.7 ± 1.1)
HECM-10 + taurine + glycine	93	23 (26.9± 4.1)	1 (1.8± 1.7)
HECM-10 + glutamine + glycine	118	63*(48.9± 5.4)	6 (10.0 ± 3.4)
HECM-10 + taurine + glutamine + glycine	107	62* (57.5 ± 9.6)	2 (2.5 ± 1.6)

* significantly different compared to HECM-10 (control group)

Table 9 **Effect of glutamine, glycine, and taurine on development of eight-cell hamster embryos in HECM-10 at 375 mOsmol after 48 hr in culture**

Culture medium	No. of eight-cell embryos at beginning of culture period	No. of morula (%mean ± SE)	No. of Blastocyst (% mean± SE)
HECM-10	103	0	0
HECM-10 + taurine	94	0	0
HECM-10 + glutamine	96	0	0
HECM-10 + glycine	106	0	0
HECM-10 + taurine + glutamine	116	0	0
HECM-10 + taurine + glycine	93	0	0
HECM-10 + glutamine + glycine	118	0	0
HECM-10 + taurine + glutamine + glycine	107	0	0

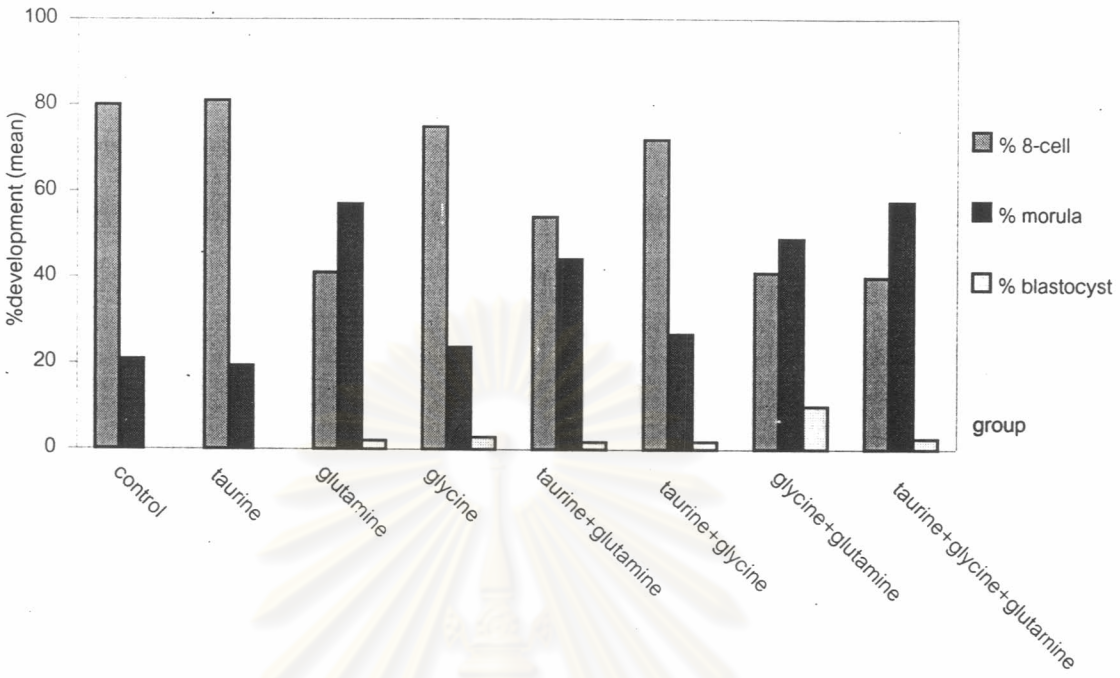


Figure 9 Effect of taurine, glutamine, and glycine on the development of eight-cell hamster embryos in HECM-10 at 375 mOsmol (a) after 24 hr. in culture

a

b

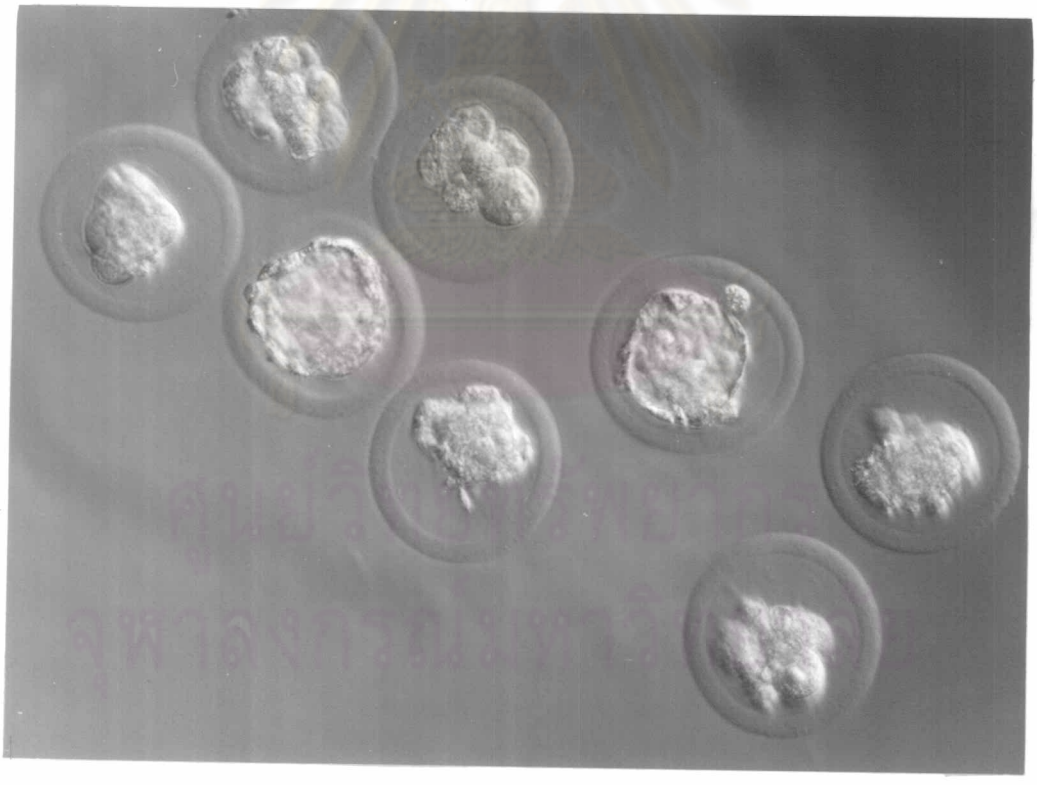


Figure 10 The development of eight-cell hamster embryos in HECM –10 supplemented with glycine plus glutamine plus taurine at 375 mOsmol (a) morula formation after 24 hr. in culture, X20 (b) blastocyst formation after 24 hr. in culture, X20

4. **The effect of single amino acid (glycine, glutamine, and taurine), combination of either two amino acids (glycine plus glutamine, taurine plus glycine and taurine plus glutamine) , and combination of all three amino acids (glycine plus glutamine plus taurine) on the development of two-cell hamster embryos at 275 mOsmol**

Groups of ten to fifteen two-cell embryos were cultivated in medium without amino acid (control) and with amino acids supplemented (treatments). Six to ten replicates were performed for each treatment. Results were summarized in table 10 and figure 8.

By 24 hr. of culture, two-cell embryos cultured in control group, experimental groups supplemented with taurine, glutamine and glycine developed no further than the eight-cell stage. Control group produced 48.9% four-cell embryos. Taurine supplemented group produced 69.9% four-cell embryos and 6.6% eight-cell embryos. Glutamine supplemented group produced 74.4% four-cell embryos and 6.0% eight-cell embryos. Glycine supplemented group produced 92.1% four-cell embryos. The percentage of four-cell embryos obtained in medium supplemented with glycine was significantly higher compared with control group. No further development of these embryos was observed at the end of 48 hr. culture. Some of them started to degenerate.

After 24 hr. in culture, high proportion (68.4%, 67.9%, and 85.6%) of two-cell embryos developed to four-cell stage in medium supplemented with taurine plus glutamine, taurine plus glycine, and glycine plus glutamine, respectively. Low proportions (9.7%, 2.0% and 1.7%) of eight-cell embryos were also obtained. At the end of 48 hr., none of these embryos further developed to morulae and blastocysts, and both four-cell embryos and eight-cell embryos stopped their development. Number of four-cell embryos developed in medium containing combination of glycine and glutamine (85.8%) was significantly higher than that of the control group (48.9%).

Five of 116 two-cell embryos were able to develop to eight-cell stage in medium supplemented with taurine plus glutamine plus glycine when culture was extended to 48 hr. The combination of all three amino acids did not significantly stimulated the development of two-cell embryos to four-cell stage when compare to the control group (71.4% vs. 48.9%) at 24 hr.

The percentages of four-cell embryos obtained in all treatments supplemented with amino acids (single amino acid, combination of any of two amino acids, and combination of all three amino acids) at the end of 24 hr. culture were compared. No significant difference was observed among them. However, culture medium supplemented with amino acids promoted the development of two-cell embryos to four-cell stage greater than the control group. Since two-cell embryos failed to develop past the four-cell stage at 275 mOsmol which is the normal osmolarity. Thus the studies of the effects of glutamine, taurine, and glycine on the development of two-cell embryos to blastocyst stage at 325 and 375 mOsmol were not performed.

Table 10 **Effect of glutamine, glycine, and taurine on development of two-cell hamster embryos in HECM-10 at 275 mOsmol after 24 hr in culture**

Culture medium	No.of two-cell embryos at beginning of culture period	No.of four-cell (%mean±SE)	No.of eight-cell (%mean±SE)
HECM-10	82	40 (48.9 ±6.0)	2 (3.06±3.0)
HECM-10 + taurine	98	66 (69.9 ±5.8)	7 (6.6 ±6.6)
HECM-10 + glutamine	137	101 (74.4 ±10.2)	10 (6.0± 3.9)
HECM-10 + glycine	96	87*(92.1 ±5.5)	0 (0)
HECM-10 + taurine + glutamine	102	68 (68.4± 6.9)	15 (9.7 ±6.6)
HECM-10 + taurine + glycine	92	58 (67.9 ±10.2)	2 (2.0 ±2.0)
HECM-10 + glutamine + glycine	95	81*(85.8± 7.4)	2 (1.7± 1.7)
HECM-10 + taurine + glutamine + glycine	116	79 (71.4 ±7.5)	0 (0)

* significantly different compared to HECM-10 (control group)

Table 11 **Effect of glutamine, glycine, and taurine on development of two-cell hamster embryos in HECM-10 at 275 mOsmol after 48 hr in culture**

Culture medium	No.of two-cell embryos at beginning of culture period	No.of four-cell (%mean±SE)	No.of eight-cell (%mean±SE)
HECM-10	82	40 (48.9 ±6.0)	2 (3.06±3.0)
HECM-10 + taurine	98	66 (69.9 ±5.8)	7 (6.6 ±6.6)
HECM-10 + glutamine	137	76 (56.9±9.2)	14 (8.7± 4.7)
HECM-10 + glycine	96	75*(81.8 ±8.5)	10 (14.7± .3)
HECM-10 + taurine + glutamine	102	66 (66.7± 5.8)	17 (11.4 ±6.4)
HECM-10 + taurine + glycine	92	58 (67.9 ±10.2)	2 (2.0 ±2.0)
HECM-10 + glutamine + glycine	95	81*(85.8± 7.4)	2 (1.7± 1.7)
HECM-10 + taurine + glutamine + glycine	116	80 (72.7±7.5)	5 (5.62±3.4)

* significantly different compared to HECM-10 (control group)

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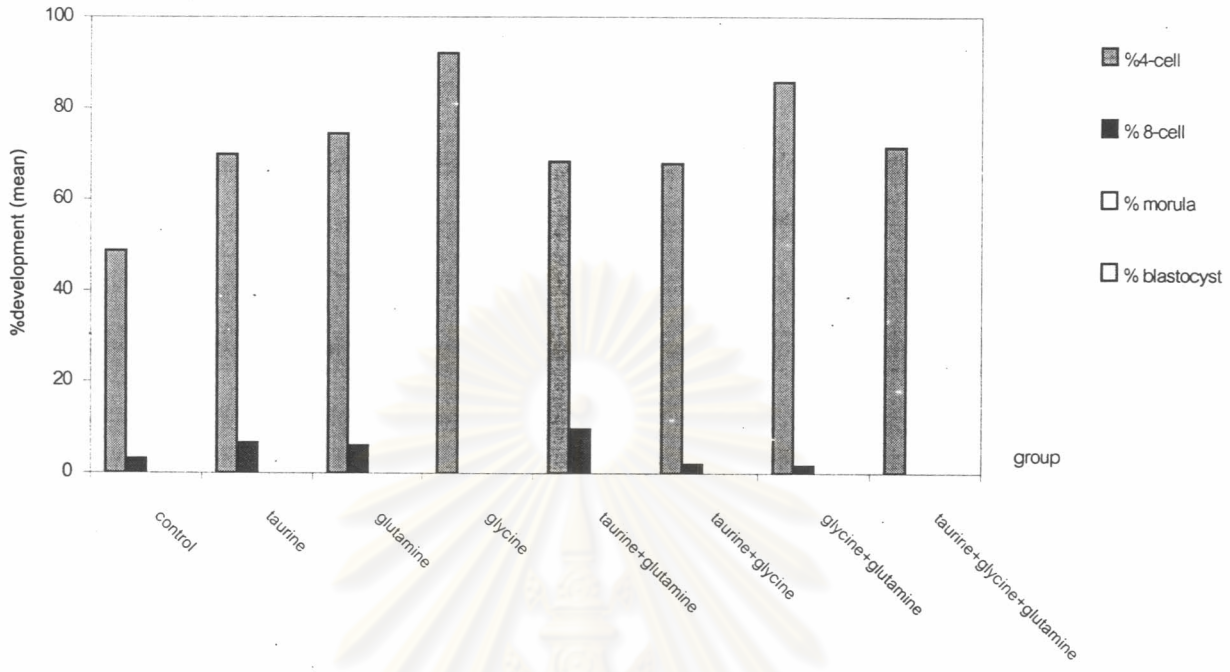

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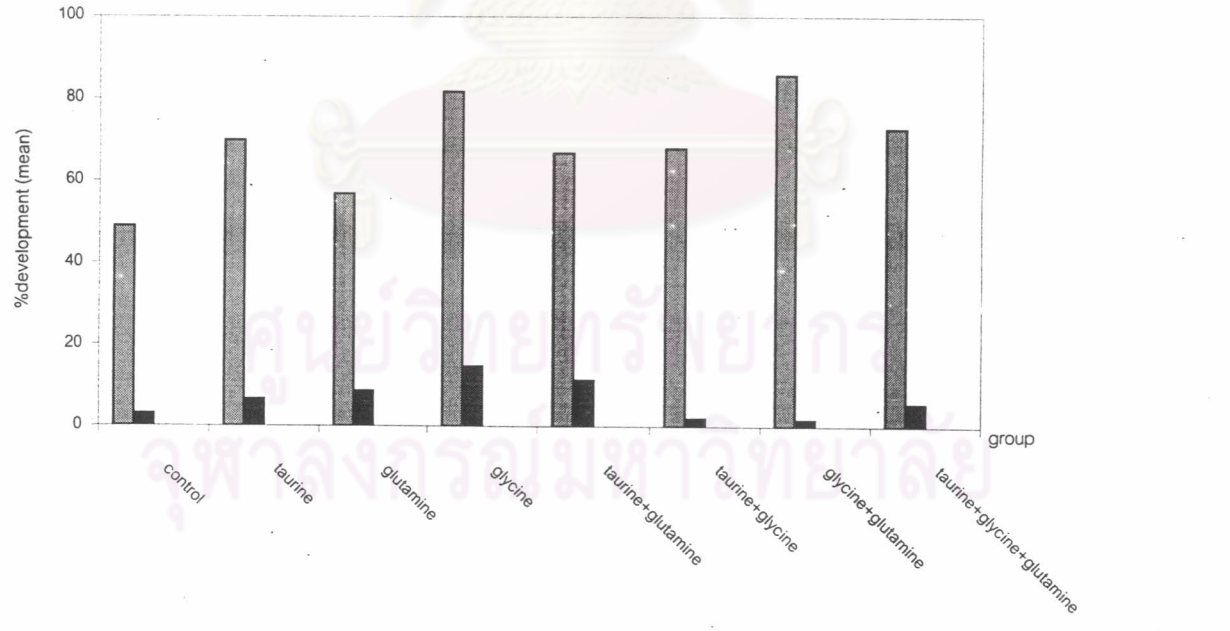

Figure 11 Effect of taurine, glutamine, and glycine on the development of two-cell hamster embryos in HECM-10 at 275 mOsmol (a) after 24 hr. in culture (b) after 48 hr. in culture

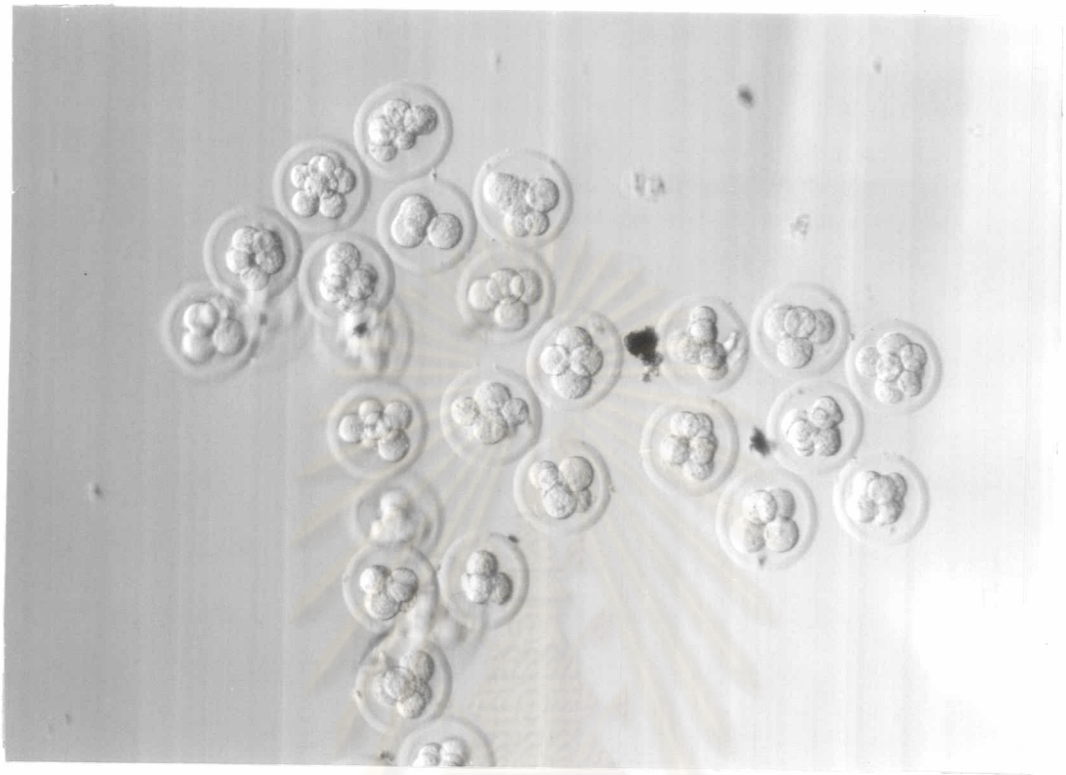

Figure 12 The development of two-cell hamster embryos in HECM –10 supplemented with glycine at 275 mOsmol after 24 hr. in culture, X10