CHAPTER V

DISCUSSION

Because secretions in the female mammalian reproductive tract and in the blastocoel possess the high concentration of amino acids (Miller and Schultz, 1987; Van Winkle and Dickinson, 1995) as well as high osmolarity (Borland *et al.*, 1977), it can be assumed that these amino acids somehow acting as osmolytes in protecting embryos from harmful effect of the high osmolarity environment. To find out whether glycine, glutamine, and taurine play such a role, two-cell and eight-cell hamster embryos were cultured in eight treatment groups, either with or without these amino acids, singly or in combination, at 275, 325, and 375 mOsmol.

Before the protective effect of glutamine, glycine and taurine on the development of eight-cell embryos in high osmolarity medium (325 and 375 mOsmol) the effect of these amino acids on the development of eight-cell embryos at 275 mOsmol, the normal osmolarity for embryo culture *in vitro*, was tested. Results showed that glutamine and combination of glutamine with taurine or glycine seem to stimulate the development of eight-cell hamster embryos *in vitro*, since eight-cell embryos in medium supplemented with glutamine, taurine plus glutamine, and glutamine plus glycine significantly produced the high blastocyst formations at 48 hr. in culture (72.5%, 80.4%, and 84.0%) compared to the control group (46.5%). Percentages of blastocyst in medium containing taurine alone (48.8%), and glycine alone (57.8%) were not significantly different from that of the control group (46.5%). Results showed that the single amino acid being beneficial to the development of eight-cell hamster embryos was glutamine. This finding is in agreement with the previous studies (Carney and Bavister, 1985; Kane *et al.*, 1986; Kane and bavister, 1988). Carney and Bavister (1985) suggested that glutamine should be used as the energy source because it is degraded to α-ketoglutarate, a citric acid intermediate (Voet, Voet, and Pratt, 1999).

The requirement for glutamine in culture medium for the embryonic development was found in many mammalian species. Glutamine stimulated one-cell mouse embryos to develop to the four-cell stage (Chatot *et al.*, 1989). The one-cell rat embryos developed to blastocysts at high proportion (80%-90%) in medium (R1ECM) supplemented with glutamine alone as well as in medium with glutamine plus others essential and nonessential amino acids (Miyoshi *et al.*, 1995). The percentages of morula and blastocyst derived from two-cell human embryos in medium supplemented with glutamine (89%, 71%) were higher than those of the medium without glutamine (68%, 54%) (Devreker, Winston and Hardy, 1998).

In addition, glutamine is important for cultivating mammalian cells in tissue culture (Eagle, 1955). Most cell lines require glutamine such as HeLa cells (Reitzer, Wice, and Kennell, 1979), human diploid fibroblasts (Zielke *et al.,* 1976), heart muscle cells (Stanisz, Wice, Kennell, 1983) and kupffer and endothelial cells (Spolarics *et al.*, 1991). Hence glutamine is added in many culture media for culturing cell lines *in vitro* such as MEM, DMEM, M199, CMRL1066 (Freshney, 2000).

The other two amino acids, taurine and glycine, also influence on the development of eight-cell embryos when added together with glutamine. This result was in agreement with many previous experiments. Glycine was mentioned as one that stimulated one-cell hamster embryos developed to blastocyst when adding in basic medium contained glutamine (Bavister and Arlotto, 1990). Mckiernan and Clayton, and Bavister (1995) discovered that basic medium contained glutamine only (control group) produced the percentages of blastocyst derived from one-cell hamster embryos less than those of treatments contained either of glutamine plus taurine or glutamine plus glycine.

To determine the protective effect of glutamine, glycine and taurine as osmoytes, the percentages of morula and blastocyst in all treatments were compared between 275, 325, and 375 mOsmol (figure13-14). The result showed that the increase of osmolarity (325 and 375 mOsmol) was detrimental to the development of eight-cell

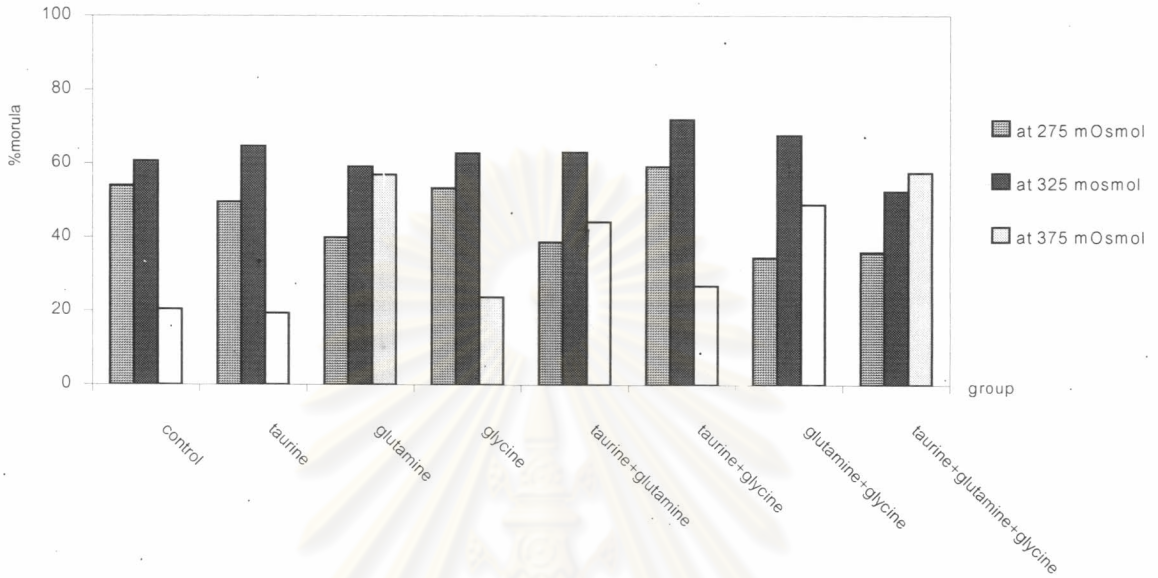
hamster embryos *in vitro*. However, when embryonic development was compared within groups at the same osmolarity, eight-cell embryos in medium containing glutamine or taurine, at 325 mOsmol, tend to develop to blastocyst better than the control. Glutamine seem to exert its effect during the first 24 hr. culture. Percentages of blastocyst obtained in medium containing glutamine alone, glutamine plus taurine, or glutamine plus taurine plus glycine (28.5%-34.7%) were higher than that of the control (20.3%), although the differences were not significant. Taurine probably started its effect when culture period was extended to 48 hr. since percentage of blastocyst in HECM-10 plus taurine (44.1%) was higher than that of the control (30.3%). This result was agreed with the earlier report of Dumoulin *et al.* (1997) in which [H^3] taurine transport in mouse embryos was found dependent on the osmolarity of culture medium. Moreover, Lawitts and Biggers (1992) reported that glutamine was able to protect two-cell mouse embryos when cultivated in high osmolarity medium. At 375 mOsmol, the eight-cell embryos in all treatment were still able to form morulae and a few embryos developed to blastocyst (some treatment) after 24 hr. culture, but all these embryos died and degenerated when the culture period was extended to 48 hr. Nevertheless, in the first 24 hr. culture, HECM-10 containing glutamine, either alone or in combination with the other two amino acids, taurine and glycine, supported the development of eight-cell embryos to morulae significantly better than control. It seems that glutamine protected eight-cell embryos from high osmolarity medium better than taurine and glycine because taurine and glycine expressed their effects only when added together with glutamine. This finding was agreed with the study of Dawson and Baltz (1997). They found that culture medium supplemented with glutamine supported mouse zygotes to develop to blastocyst stage significantly higher than that supplemented with taurine.

The overall results in this experiment seem to indicate that glycine, glutamine and taurine can possibly act as osmolytes, to a certain limit, in protecting embryos from the deleterious effect of the high osmolarity medium. Further experiment should

be conducted, employing radioactive glycine, glutamine and taurine, to find out whether these amino acids were accumulated within the embryos when they were cultured in such high osmolarity medium in order to confirm that they can really act as osmoprotectants.

Two-cell embryos did not successfully develop as did the eight-cell embryos. This might due to the different requirements of amino acids between two-cell and eight-cell stages embryos. Seshagiri and Bavister (1991) compared the ability of the development of hamster two-cell and eight-cell embryos in two versions of hamster embryo culture medium (HECM-1 and HECM-2). These media differed in the number of amino acids present. HECM-1 composed of twenty amino acids (alanine, arginine, asparagine, asprartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleuscine, leucine, lysine, methionine, phenylalanine, proline, serine, taurine, threonine, tryptophan, valine), while HECM-2 composed of four amino acids (glutamine, isoleucine, phenylalanine, methionine). The results showed that twenty amino acids in HECM-1 were benefit for the development of two-cell embryos to blastocyst stage while the four amino acids in HECM-2 were sufficient for blastocyst formation of eight-cell embryos. Due to the difficulty to obtain development of two-cell hamster embryos beyond eight-cell stage in HECM-10 at 275 mOsmol *in vitro*, therefore the studies on the protective effect of these amino acids on the development of two-cell hamster embryos at 325 and 375 mOsmol were not performed.

a



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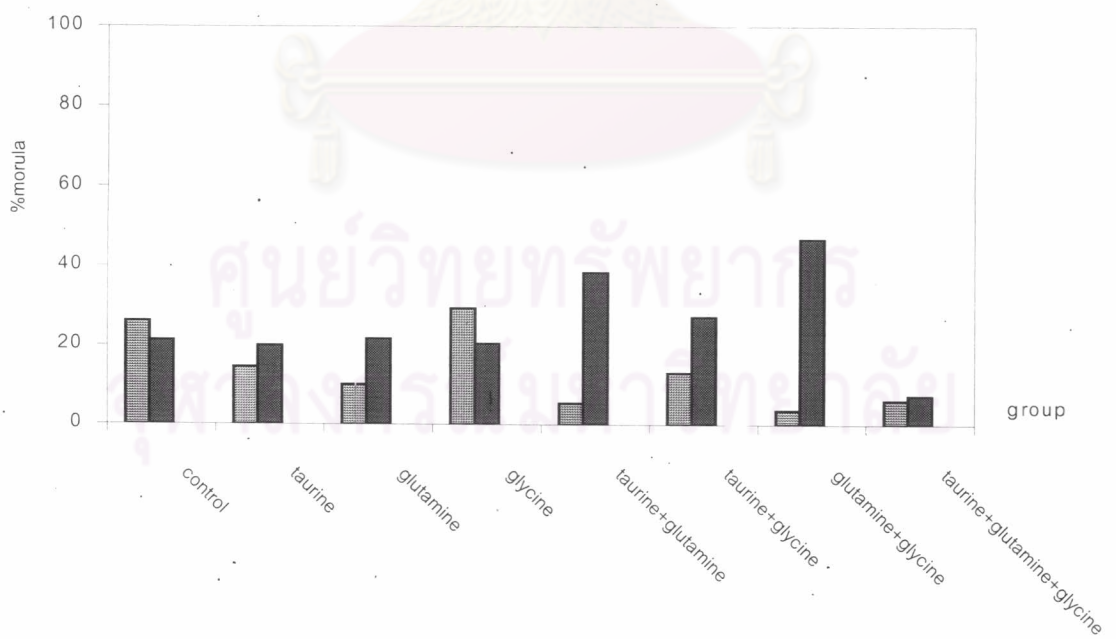
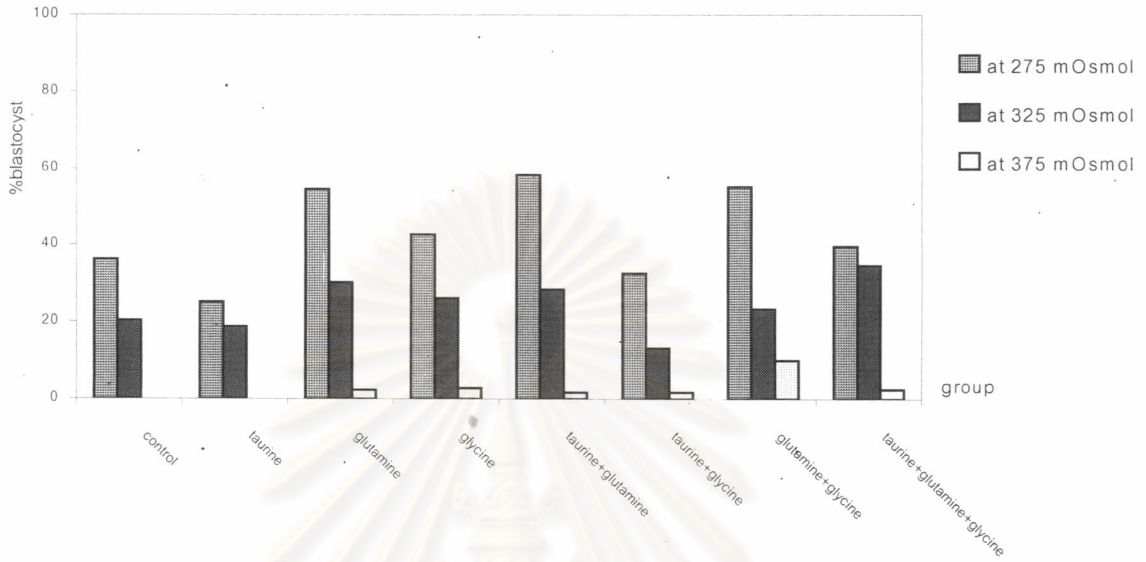


Figure 13 Effect of taurine, glutamine, and glycine on the development of eight-cell hamster embryos in HECM-10 to morulae at 275, 325, and 375 mOsmol (a) after 24 hr. in culture (b) after 48 hr. in culture

a



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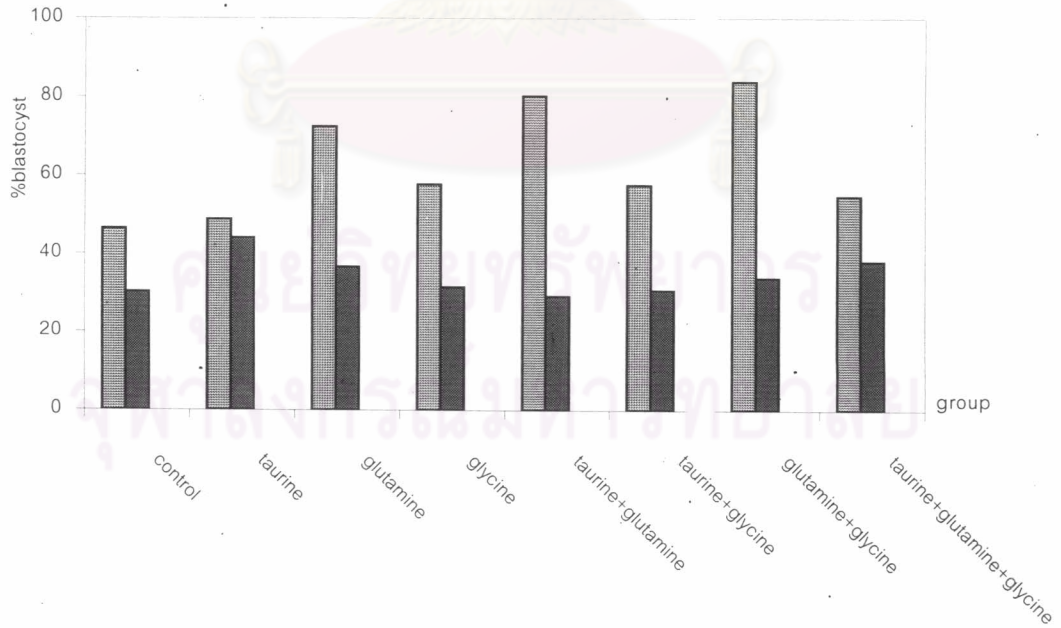

Figure 14 Effect of taurine, glutamine, and glycine on the development of eight-cell hamster embryos in HECM-10 at 275, 325, and 375 mOsmol (a) after 24 hr. in culture (b) after 48 hr. in culture