CHAPTER I

INTRODUCTINON

Cell volume is perturbed from osmotic imbalance environment. Cells swell in the hypotonic fluid and shrink in the hypertonic fluid. Cells regulate their volumes by adding the amount of osmotically active solutes in their cytoplasm. Cell swelling is countered by release of solutes while the shrinkage is reversed by import of solutes. The principle solutes involved in osmotic regulation are K^+ and Cl^- but the increased intracellular concentration of inorganic ions destabilizes proteins and disrupts biochemical reactions and metabolism. The organic compounds that are used for adjusting osmotic pressure are termed the compatible osmolytes because they stabilize proteins. These osmolytes are divided into three groups. There are small zwitterionic amino acids such as taurine, methylammonium compounds such as betaine. The last group is sugars and polyols such as myo- inositol.(Lang *et al.*, 1998.)

The osmolarity of mouse oviduct fluid is at least 360 mOsmol and a concentration of Na^+ is 140 mM. (Borland *et al.*, 1977). It is similar to the concentration of Na^+ in human fluid. (Borland *et al.*, 1980.) Besides, alanine, glutamine, glutamate, glycine and taurine are abundant in the mouse oviductal lumen (Van Winkle and Dickinson, 1995.). The development of one–cell and two–cell mouse embryos to blastocyst in medium which its osmolarity is at 310–340 mOsmol were decrease, however, the blastocyst formation increased when taurine, glycine and glutamine were added in medium (Biggers, Lawitts, and Leechene, 1993; Dawson and Baltz, 1997.). Furthermore, there were many reports to support that some amino acids such as taurine and glycine function as the osmolytes. Domoulin *et al.* (1997) reported that two-cell and eight–cell human embryos and two-cell mouse embryos released taurine from their cell when they were incubated in hypotonic medium (200 and 240 mOsmol). Moreover, Dawson, Collins, and Baltz (1998) found that glycine are accumulated by embryos during *in vitro* culture from the zygotes to two-cell stage. The amount of glycine are accumulated at 310 -340 mOsmol more significantly than at 250 mOsmol. Also, Hammer *et al.* (2000) proposed that early cleavage-stage embryos

accumulated glycine when they were in hyperosmotic medium by transporting glycine via Gly transporter which is similar to mouse embryos.

Hamster embryos at preimplantation stage developed *in vitro* to blastocyst within limited osmolarity range like mouse embryos. For example, Bavister *et al.* (1983) reported that eight–cell hamster embryos developed to blastocyst *in vitro* in media between 225 and 300 mOsmol. Mckiernan and Bavister (1990) found that two– cell embryos developed to blastocyst in media between 250 and 325 mOsmol. The role of taurine, glycine and glutamine on development of one-cell hamster embryos to blastocyst were studied. The blastocyst formation increased in the group which amino acids were added when compare to the group without amino acid. (Bavister and Arlotto, 1990; Mckiernan, Clayton, and Bavister, 1995). Thus, Bavister (1995) suggested that amino acids which act as osmolytes should be added in the culture medium. However, there are no reports to confirm that taurine, glycine and glutamine can work as the osmolytes for the hamster embryos. Consequently, the role of taurine, glycine and glutamine on preimplantation hamster embryo development in abnormally high osmolarity medium should be investigated.

Objective

To find out the protective effects of glycine, glutamine and taurine on the development of two-cell and eight-cell hamster embryos in abnormally high osmolarity medium (Hamster Embryo Culture Medium-10 at 275, 325, and 375 mOsmol).

Experimental design

To study the development of two-cell and eight-cell hamster embryos in medium without any amino acid (control group) compared to those in the medium containing either single amino acids (glycine, glutamine, taurine) or combination of these amino acids, i.e., glycine plus glutamine, glycine plus taurine, and glutamine plus taurine or all three amino acids (glycine plus glutamine plus taurine). All four treatments were done at 275, 325, and 375 mOsmol.