D-3. The long road to a representative in vitro model of bovine lactation(Abstracts of the International Symposium on Recent Advances in Animal Science(IS-RAAS),Joint meeting of 2^<nd> IS-AS and 3^<rd> IS-IFS)

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Lactational physiologists would find a truly representative *in vitro* model of bovine lactation of great use for gaining a greater understanding of mammary gland biology in the cow. A number of attempts have been made to replicate the function of the bovine mammary gland *in vitro*. Many of these attempts may be criticised because the epithelial cells have been in a relatively undifferentiated state, the cells have been unnaturally immortal, the other cell types normally found in the mammary gland have been absent, or the culture conditions have been undefined. Here we discuss an alternative methodology that avoids some of these criticisms. We have used an untransformed clonal cell line isolated from lactating bovine mammary tissue. The cells are plated onto two-dimensional, porous, culture-well inserts, which allow repeated access to products secreted by the cells. The membranes can be coated with specific extracellular matrix proteins. In this system, we have shown that bovine mammary epithelial cells respond to lactogenic hormones (prolactin, dexamethasone and insulin) in terms of milk protein synthesis. We have shown that these cells are also able to secrete their own extracellular matrix. Additionally, the cells secrete milk proteins to a greater extent when the lactogenic hormones are added to the basolateral side of the cells only. This methodology is open to further improvement, in particular with regard to the use of foetal calf serum. It nevertheless represents an improvement over some of the alternative methodologies discussed. Further research will concentrate on eliminating FCS from the culture media.

D-4. Development of a new cell death inhibitor-Bax Inhibiting Peptides (BIPs) derived from Ku70-

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Bax is a proapototic member of Bcl-2 family protein that mediate mitochondria-dependent cell death pathway. Apoptotic stimuli can induce translocation of Bax from the cytosol to mitochondria. Previously, we reported a new cell death inhibitor, named Bax Inhibiting Peptide (BIP) (Nature Cell Biol, 2003; BBRC, 2004). BIP is designed from the amino acid sequence of Ku70 protein that protects cells from apoptosis by inhibiting Bax. We found that Ku70 binds Bax and inhibits the mitochondrial translocation of Bax, and that BIP directly binds Bax and inhibits cytotoxic activity of Bax. In this symposium, we will introduce the effects of BIP on trophic factor deprivation-induced cell death in primary cumulus cells and in myeloid cell line (32D cells) *in vitro* culture. We used three versions of BIP: VPMLK (derived from human Ku70), VPTLK (from mouse Ku70) and VPALR (from rat Ku70). Each BIP showed cell permeability in cumulus and myeloid cells. Biotin-labeled BIPs were added to the cell lysates of human kidney endothelial cells (HEK293T cells), and biotin-BIPs were precipitated by streptavidin beads. Specific interaction of each BIP with Bax was confirmed by analysis with Western blotting of the precipitated samples. BIPs suppressed hormone-deprivation induced apoptosis in cumulus cells of three species (mouse, rat, and porcine), and also suppressed IL-3 deprivation-induced apoptosis of 32D cells in a dose dependent manner (50-400 μM). These results suggest that BIP may become a useful tool to prevent degenerative condition induced by trophic factor deprivation that triggers Bax-mediated apoptosis.