ABSTRACT

IDENTIFICATION AND BIOACTIVITY TEST OF GROWTH FACTOR AND SEX STEROID HORMONE PRODUCED BY BOVINE LIVER AND CUMULUS CELL MONOLAYER CULTURE

Cumulus cells and liver cells (hepatocyte) have an ability to produce IGF-1 and estrogen through monolayer culture, but the concentration and the bioactivity in embryo was not clear.The aim of this study was to monitor, analysis and identify bioactivity of IGF-1 and estrogen there found in product cell. Liver cell culture of cattle was prepared by grinding liver tissue followed by trypsination and repeatedly centrifuged were obtained hepatocyte. Cumulus cells were obtained from aspirating ovarian follicles with diameter 2-6 mm. Liver and cumulus cells were cultured in TCM 199 + FCS 10% + BSA 10%. Cell concentration was 1.9 x 10^6 /ml medium then culture was incubated in incubator at 38.5^{o}C 5% CO_2 for 3,6,9,12 days. IGF-1 concentration media after culture was measured by IRMA, while estrogen and progesterone using RIA technique. To minimize progesterone concentration in product cell it was used binding technique using antiprogesterone (antiP4) coated polyesterene tubes. Bioactivity test of IGF-1 and estrogen obtained from this research as supplementation media in in vitro fertilization and as embryo culture media was performed at 2 and 6 days after fertilization by observating embryo cleavage 2-8 cells

and embryo development up to morula stage. The mitogenic and antiapoptotic of IGF-1 and estrogen were examined by the reduction of apoptosis in embryo resulted. The result of research showed that 6 days incubation period resulted the most confluent monolayer compared to the other incubation period, either in liver or cumulus cells, therefore resulting the highest concentration of IGF-1 as well as estrogen. In liver cell culture IGF-1 concentration was higher compared to these of the cumulus cells, meanwhile in cumulus cells the estrogen was found higher than IGF-1. AntiP4 could be used to absorb progesterone resulted from monolayer culture. IGF-1 and estrogen from monolayer culture before and after progesterone absorbtion resulted in cleavage rate were 27.48% and 53.61%, respectively stage morula embryo rate were 5.73% and 27.59% , and could also reduce the incidence apoptosis in the embryos cultured in liver cell were 23.71% and in cumulus cells were 31.01%, respectively while without supplemented apoptotic rate in embryo cell was 70.58%. The conclusion of this research was that monolayer culture of bovine liver and cumulus cells could produce IGF-1 and estrogen growth factor which acted as mitogenic substances that could be used as supplemen media in in vitro fertilization and embryo development as well as able to reduce the incidence of apoptosis in embryo resulted.

Keywords : IGF-1, estrogen, progesterone, monolayer culture, liver cell (hepatocyte), cumulus cell.