



# PROCEEDING

*international seminar*

STRATEGY TO MANAGE BIO-ECO-HEALTH SYSTEM  
FOR STABILIZING ANIMAL HEALTH &  
PRODUCTIVITY TO SUPPORT PUBLIC HEALTH



Surabaya-Indonesia, 19-20 June 2012  
JW Marriott Hotel Surabaya

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FACULTY OF VETERINARY MEDICINE - UNIVERSITAS AIRLANGGA  
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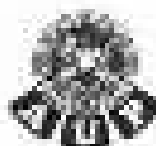
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# BIOACTIVITY OF INSULINE LIKE GROWTH FACTOR-I (IGF-I) DERIVED FROM THE HEPATOCTYE MONOLAYER CULTURE AGAINST CLEAVAGE AND DEVELOPMENT OF BOVINE EMBRYO IN VITRO

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## ABSTRACT

Liver cell (hepatocyte) have an ability to produce IGF-I through monolayer culture, but their concentration and the bioactivity against cleavage and development of embryos were not clear. The purpose of this study was to analyze and measure of IGF-I concentration derived from hepatocyte monolayer culture and to study bioactivity against cleavage and development of bovine embryos in vitro. Hepatocyte culture was prepared by growing liver tissues obtained by trepanation and repeatedly centrifugation in sterile heparin syringes. They were then cultured in TC1199 + FCS (10% + 94%) and then incubated in medium CPM/CO<sub>2</sub> at 38.5 for 2, 4, 8 and 12 days. IGF-I concentration in culture medium was measured by RIA technique. The results of hepatocyte bioactivity in culture showed that during incubation period resulted in the liver confluent liver culture and IGF-I in that medium was more than compared with other incubation periods. Bioactivity test of IGF-I in this research was carried out by adding the supplement media in the culture for 4 hours in an insemination media at bovine embryos by observing and recording a number of embryos which underwent cleavage (2-4 cells) on the day 2 after fertilization and embryos development (4-8 cells) on the day 5. The results of research showed media furnished with supplement medium taken from the hepatocyte monolayer culture containing IGF-I has produced the higher number of embryos undergoing cleavage and developing from two stages compared with the use of medium without supplementation. In conclusion, the IGF-I can be produced through the hepatocyte monolayer culture and it is found in the same cleavage rate and development rate as compared with the

**Keywords:** IGF-I, Liver cell, Hepatocyte, monolayer culture, cleavage

## INTRODUCTION

The application of biotechnology is an effort to improve the reproductive efficiency of livestock especially in order to get the cattle with good quality and quantity. In vivo embryo production is limited by the ability of female donor animals to produce embryos. Therefore, *in vitro* embryo production becomes the good alternative. With *in vitro* fertilization the reproductive sugar of the ovaries representing the donors from slaughterhouses can be used as a source of oocytes. Embryo transfer represents one of the methods, which is deemed efficient and effective in the field of reproductive biotechnology.

The liver is the main place to produce Insulin Like Growth Factor-I (IGF-I) as an endocrine hormone circulating in the blood. As a embryo factor, IGF-I acts as a regulator of postnatal growth by increasing skeletal growth through chondrocyte proliferation and increases extracellular tissue growth by increasing cell division and protein synthesis (MacCallum, 1985). IGF-I is not only produced by hepatocyte, but it is also produced by some tissues which act as endocrine, paracrine and autocrine, or follicle cells in the ovaries, endometrium epithelial cells, and endometrial epithelial cells (Blaker et al., 2000). Research about production of growth factor (IGF-I) through the monolayer culture of bovine liver cells has not been done yet. Thus far the liver is only used to meet consumption needs as a source of animal protein. Therefore, it is necessary to carry out a study to take advantage of the liver that has more important role as a major producer of IGF-I through the hepatocyte monolayer culture to produce IGF-I.



The addition of growth factors such as IGF-I in oocyte maturation media and embryo culture media has been widely reported. Masini et al. (2007) and Bhaskar et al. (2008) say that the IGF-I put into the embryo culture media can increase the rate of embryo cleavage. Furthermore, Lomson et al. (1995) and Margo et al. (2007) report that IGF-I can stimulate the process of mitosis and meiosis, increasing the occurrence of cumulus cells (granulosa cell mass that surrounds the ovum) in the de Graaf follicle; maturation of the oocyte nucleus and increases in embryonic cleavage rate. According to Datta et al. (2004) IGF-I possesses a synergistic effect in the process of stimulating oocyte maturation and embryo development *in vitro* in mice. Further, it is said that IGF-I is a mitogenic material which can cause cell proliferation and differentiation.

Growth factors commonly used in the *in vitro* fertilization, among others, are including epidermal growth factor (EGF) and insulin-like growth factor-I (IGF-I). The addition of growth factors (EGF, IGF, VEGF) in oocyte maturation media and embryo culture media has been reported to increase the oocyte maturation rate and embryo cleavage rate (Lomson et al., 1995; Li et al., 2002; Margo et al., 2007).

## MATERIALS AND METHODS

This study was bovine liver and ovaries where the cattle were killed at the slaughter (KPH) in Bogor, Sukoharjo. The liver is suitable material for liver cell culture (hepatocyte), whereas the ovaries used as a source of oocytes for *in vitro* fertilization using frozen semen from Madura cattle.

The research was carried out in several stages, preparation of hepatocyte monolayer culture in which the liver is removed from its membrane and connective tissues, cut and weighed at 1 gram. This liver was then crushed in a mortar, add 5 ml of physiological saline and 10 ml 0.25% trypsin, and allow to stand for 10 minutes, poured into the cell (flask) tubes, then centrifuge for 10 minutes at 3000 rpm. Supernatant was discarded, washed with 12 ml of TC medium and 1 ml FCS, centrifuged again for 10 minutes at 3000 rpm in which each washing was performed 2 times, then the supernatant was discarded and discarded by adding TCM 199 medium with a ratio of pellet TCM 199 = 1:1. It was stirred until homogeneous, and then the pellet was diluted again with 10 µl pellet + 990 µl TCM 199 (1:99). Then it was put into incubators, the number of liver cells were counted using Thoma technique. Concentration was made to  $1.9 \times 10^6$  cells/ml media. Then 100 ml of TCM 199 media is put into the 25 cm<sup>2</sup> flasks Petri dish, add the diluted pellet, and then 100 mm using Eppendorf micropipette, and then poured into TCM 199 media, incubated in 5% CO<sub>2</sub> incubator at temperature of 38.5°C. Monolayer cells were harvested on days 2, 5, 9, and 12. The concentration of insulin-like growth factor-I (IGF-I) of the culture was determined using immunoradiometric assay (IRMA) (Mahapatra, et al., 2000).

Bioreactivity test of IGF-I of the hepatocyte monolayer culture as media supplement for *in vitro* fertilization and embryo culture. Observation of cleavage and embryo development was done by preparing *in vitro* fertilization. Embryo cleavage was observed after 48 hours. Then the embryo that have undergone cleavage (3-4 cell stage) were transferred into Petri dish containing liquid from hepatocyte culture + TCM 199 + FCS 10%. Embryo cultures were incubated in 5% CO<sub>2</sub> incubator with relative humidity at temperature of 38.5°C. Embryo development was observed every day and media was replaced every 3 day. First observation of the embryo was performed once the embryo have reached morula stage or 6 days following the fertilization.

The data were tabulated. Before carrying out the statistical analysis, the normality test was done using one sample Kolmogorov-Smirnov test and homogeneity test was performed using Levene's test against the data obtained. Data analysis was tested by factorial (ANOVA) F-test, and the level of significance was tested with honestly significant different (HSD) test 5%. All statistical calculations were performed using SPSS 14.0 for Windows.



## RESULTS AND DISCUSSION

Observation of monolayer cell number and concentration of IGF-1 from the culture results obtained at different incubation times is shown in Table 1.

**Table 1.** Averages and standard deviation of monolayer cells (%) and IGF-1 concentration after the culture in different incubation times

Culture duration	Monolayer cell number (%)	Concentration of IGF-1 (µg/ml)
3 days	60.0P ± 15.73	76.38 ± 26.531
6 days	71.25P ± 10.876	123.44 ± 63.123
9 days	62.5P ± 8.580	122.81P ± 75.381
12 days	40.10P ± 1.100	15.31P ± 11.943

P=0.05 or lower (p-values in the same column show significant difference ( $p < 0.05$ )).

The table above shows that the culture duration on day 6 produces a higher cell number than the days 3, 9 and 12. This is consistent with the research conducted by Trilokavani (2008), suggesting that the culture on the day 6 represents an optimal time for cell culture because the cells have grown to form confluent cells. On day 3 of cell culture, the cells do not attach and grow perfect in the bottom of a petri dish, so the number of cells produced is still small. Conversely, on day 9 and 12, since the nutrients existing in the DM 199 culture medium have begun to diminish, so many cells do not grow well in the end and many are floating on the surface of the media. This caused the IGF-1 derived from the hepatocyte monolayer culture on day 6 of incubation has the highest concentration.

In associated with the observation of the bioactivity test of Growth Factor (GF-1) of liver cell as media supplement, in vitro fertilization and embryo culture can be determined by counting the number of embryos which were cleaved into 2-8 cell (day 3) and developed into morula (day-6) as shown in Table 2 below.

**Table 2.** Averages and Standard Deviation of the Embryo Developing into 2-8 Cell Stage and Morula Stage (%) with culture containing IGF-1 of the hepatocyte monolayer culture.

Culture Media	Average Embryo Number - Standard Deviation (%)			
	1-4 cell stage (%)	Transformation (%)	Morula Stage (%)	Transformation (%)
Fertilization Media	26.907 ± 5.553	3.136 ± 0.571	5.619 ± 1.875	2.481 ± 0.153
Fertilization Media + Media Supplement of the Culture	40.870P ± 10.561	5.327 ± 1.751	28.667 ± 9.415	5.348 ± 0.643

P=0.05 or lower (p-values in the same column show significant difference ( $p < 0.05$ )).

The average percentage of embryos cultured in fertilization media with supplement media containing IGF-1 derived from hepatocyte monolayer culture on the day 3 (2-8 cells) amounted to 40.870% ± 10.561, showing a highly significant difference ( $p < 0.01$ ) compared to the fertilization media.

The average percentage of embryos that developed in fertilization media culture with supplement media containing IGF-1 on the day 5 (morula) amounted to 28.667 ± 9.415%, showing a highly significant difference ( $p < 0.01$ ) compared with the number of embryos cultured in fertilization medium.

There is become the role of IGF-I in influencing the development of cells as a mitogenic remedy (Hafas et al., 2003). The IGF-I, derived from the hypoxanthine medium culture as a protein factor is shown to promote the development of cells up to 4th, 5th, 6th and 2-3 cell stage is even become normal. It is evident that the IGF-I as a growth factor serves as a mitogenic remedy that acts by increasing cell proliferation and differentiation, so that any blockade in embryonic development is able to be easily occurs at 8-cell stage can be prevented (Norton et al., 2002) and Block et al. (2003).

## CONCLUSION

Concentration of growth factors (IGF-I) derived from the hypoxanthine medium culture was the highest in the 6-day culture, while the lowest concentration was obtained in the 12-day culture. The percentage of embryos that developed into 2-3 cell stage and morula stage which were cultured in vitro in the hypoxanthine medium culture media showed very significant differences compared with the percentage of embryos cultured in the fertilization medium.

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