

# 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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# BIOACTIVITY OF INSULINE-LIKE GROWTH FACTOR-I (IGF-I) DERIVED FROM THE HEPATOCELL MONOLAYER CULTURE AGAINST CLIVAGAGE AND DEVELOPMENT OF BOVINE EMBRYO IN VITRO

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## ABSTRACT

Liver cells hepatocytes have capability to produce IGF-I. It is not known yet, how their concentration and the bioactivity against cleavage and development of embryo were not clear. The purpose of this study was to analyze the number and IGF-I concentration derived from hepatocyte monolayer culture and to get a knowledge the growth cleavage and development of bovine embryos. In this, hepatocyte cultures were prepared by grinding their tissues followed by centrifugation and repeatedly wash hepatocytes in a short time series. They were then cultured in TC154W+PCG 10% + 10% FBS, until they obtained a monolayer U-35<sup>14</sup>CO, 0.125<sup>3</sup>U for 3, 6, 8 and 12 days. Concentration of IGF-I was measured by ELISA technique. The results of hepatocyte number, in culture showed that 6 days incubation period resulted in the more confluent liver cells. IGF-I production was higher compared with other incubation periods. Bioactivity test of IGF-I in this research was carried out by adding the supplement at different times after fertilization in a monolayer culture of bovine embryos by observing and counting a number of embryos which underwent cleavage (2-cell) on the day 2 after fertilization and until you developings, in a monolayer on the day 3. The results of research showed that IGF-I produced in this culture system has the ability to increase the hepatocyte monolayer culture containing IGF-I and it produced cleavage, and an embryonic development cleavage and cleavage from the all stages compared with those of control without supplementation. In conclusion, the IGF-I can be produced by hepatocyte monolayer culture and it is found in the same cleavage rate and development of bovine embryo.

**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 vitro 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 organs of the female representing the uterus from slaughtered can be used as a source of oocytes. Embryo transfer represents one of the methods, which is deemed efficient and efficient 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 growth 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 (MacLennan, 1985). IGF-I is not only produced by hepatocyte, but it is also produced by some tissues which act as endocrine, paracrine and autocrine, epithelial cells in the ovaries, Fallopian tube epithelial cells, and endometrial epithelial cells (Makrilia et al., 2006). 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-1 to oocyte maturation media and embryo culture media has been widely reported. Maccini et al. (2007) and Blaschke et al. (2008) say that the IGF-1 potentiates the embryo culture medium to increase the rate of embryo cleavage. Furthermore, Lomax et al. (1995) and Margalit et al. (2007) report that IGF-1 can stimulate the process of maturation and increase the recruitment of cumulus cells (granulosa cell layer that surrounds the ova) in the denuded egg. Moreover, maturation of the oocyte nucleolus increases the embryonic cleavage rate. According to Darszon et al. (2004) IGF-1 possesses a synergistic effect in the process of stimulating oocyte maturation and embryo development *in vivo*. Furthermore, it is the IGF-1 a regulatory material which can cause cell proliferation and differentiation.

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

## MATERIALS AND METHODS

This study uses bovine live and ovaries where the eggs were killed at the slaughter (AHL) in Belgian Standard. The live ovary material for the cell cultures (hypothysis), whereas the ovaries used as a source of oocytes for *in-vitro* fertilization using frozen semen from Masella cattle.

The research was carried out in several stages, preparation of hypothysis monolayer culture in which the live is removed from its mesothelial and connective tissues, cut and weighed at 1 gram. This live was then crushed in a mortar, add 5 ml of physiological saline and 10 µl 0.2% trypsin, and allow to stand for 30 minutes, poured into the centrifuge tubes, then centrifuge the 10 minutes at 3000 rpm. Supernatant was discarded, washed with 10 ml of DM medium and 1 ml FBS, centrifuge again for 10 minutes at 3000 rpm in which each washing was performed 2 times, then the supernatant was discarded and diluted by adding 1 CM 199 medium with a ratio of pellet: TCM 199 = 1:1, FBS were added until homogenization, and then the pellet was diluted again with 10 µl pellet: 1990 µl TCM 199 (1:99). Then it was put into incubator, the number of live cells were counted using Thoma microscope. Concentration was made to 19x10<sup>6</sup> cellular cells. Then 100 ml of TCM 199 media is put into the 26 mm-dia Petri dish, add the diluted pellet, and take 100 nm using Biocoat micropipette, and then performed 11 CM 199 media, maintained in 5% CO<sub>2</sub>, incubator at temperature of 38.5°C. Monolayer cells were harvested on days 3, 6, 9, and 12. The concentration of insulin-like growth factor-I (IGF-I) of the culture was determined using immunoradiometric assay (IRMA) (Maharajay, et al. 2009).

Bioreactivity test of IGF-I of the hypothysis monolayer culture as media supplement for *in-vitro* fertilization and embryo culture. Once 90% of cleavage and embryo development was done by performing *in-vitro* fertilization, Embryo cleavage was observed after 48 hours. Then, the embryos that have undergone cleavage (2-4 cell stage) were transferred into Petri dish containing liquid from hypothysis culture + TCM 199 + FCS 10%. Embryo cultures were transferred to 5% CO<sub>2</sub> incubator with relative humidity at temperature of 38.5°C. Every one day operation was observed every day and media was replaced every 7 day. First observation of the embryo was performed once the embryo have reached mean a mass of 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 collected. Data analysis was tested by factorial (ANOVA) F-test, and the level of significance was tested with Tukey's significant different (P<0.05) and 5%. All statistical calculations were performed using SPSS 14.0 for Windows.

## RESULTS AND DISCUSSION

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

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

Culture duration	Monolayer cell number (%)	Concentration of KGF-1 (ng/ml)
3 days	64.08 ± 16.76	76.58 ± 20.53
6 days	71.29* ± 11.67%	223.44 ± 63.11
9 days	62.50 ± 16.90	122.31* ± 75.38
12 days	40.00 ± 1.00	15.30* ± 11.54

\*Differs significantly in all three columns from significant difference ( $p < 0.01$ )

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 Tribblestone (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 the day 3 of cell culture, the cells do not attach and grow parallel 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 11.8% FBS culture medium have begun to diminish, many cells do not grow well in the, and many are floating on the surface of the media. This caused the KGF-1 derived from the hepatocyte monolayer culture on day 6 of incubation has the highest concentration.

In association with the observation of the biactivity test of Growth Factor (KGF-1) of liver cell in media supplementation, in vitro teratocarcin and embryo culture can be determined by counting the number of embryos which were cleaved into 2-8 cell (day 2) and developed into morula (day 6) as shown in Table 2 below.

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

Culture Media	Average Embryo Number - Standard Deviation (%)			
	2-8 cell stage 1y%	Transformation fr%	Morula Stage 1y%	Transformation fr%
Fertilization Media	26.90% ± 5.99	5.156 ± 0.521	5.84% ± 1.87	2.421 ± 0.193
Bioterilization	40.870* ± 10.526	6.321 ± 1.751	28.667 ± 9.415	3.346 ± 0.943
Media + Media Supplement of the Extract				

\*Differs significantly in all three columns from significant difference ( $p < 0.01$ )

The average percentage of embryos cultured in fertilization media with supplement media containing KGF-1 derived from hepatocyte monolayer culture on the day 2 (2-8 cells) amounted to 26.90% ± 5.99%, showing a highly significant difference ( $p < 0.01$ ) compared to the fr. Bioterilization media.

The average percentage of embryos that developed in fertilization media culture with supplement media containing KGF-1 on the day 6 amounted to 28.667 ± 9.415%, showing a highly significant difference ( $p < 0.01$ ) compared with the number of embryos cultured in Bioterilization media.



This is because the role of EGF-like in influencing the development of embryos at a mitogenic capacity (Baker et al., 2002). The EGF-like derived from the hypoxia-induced culture medium is a growth factor that is able to promote the development of embryos in vitro, either a 2-3 cell stage or zygoblastula. It is evident that the EGF-like was derived from secreted mitogenic proteins that are by increasing cell proliferation and differentiation, so that any blockade in embryonic development, probably mainly occurs at 8-cell stage can be prevented (Nordström et al., 2002) and Block et al. (2002).

## CONCLUSION

Concentration of growth factors (EGF-like) derived from the hypoxic culture medium 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 zygoblastula which were defined as above in the hypoxia-treated layer culture media showed very significant differences compared with the percentage of embryos cultured in the fertilization medium.

## REFERENCES

- Baker, J., Chen, M., Morrissey, R.L., St. John, H., Sato, K.M., Park, J., Lee, H.H., Kim, C.M., Kang, J., Cho, J., and Kim, S.H., 2002. Use of Activin-N-Like-Signaling-Activating Proteins (ASPs) to Improve Embryo Rates Following In Vitro Culture of Freshly Picked Primate Oocytes. *J. Assisted Reprod. Sci.*, 19, 199-207.
- Baker, J., Gross, C., Givens, J., Dickey, F., Fugiet, V., and Williams, A., 2003. Effect of Activin-A on Human Factor-Beta-Subunit and Follicle-Colestrol Biomarkers. In V. V. Vaidya (Ed.), *Advances in Biotechnology: A Micro Biology of Reproduction Anthology*. 1601-1630.
- Baker, J.S.F., Johnson, M.J., and Rowson, J.V., 2001. Human Growth Factors and Reproduction. In *Human Reproduction in Early Years*, K. T. Telfer (Ed.), pp. 103-116. WILEY & Sons, Chichester.
- GYH., L., RH., Lin, H., and Wang, W.H., 2002. Serological effects of gamma-irradiation on cattle and its application in sterilization of bovine oocytes. *Zoologica Sinica*, 48, 3-5.
- Gross, C., Baker, J., and Williams, A., 1997. Influence of growth factors on the time dependent growth progression of human oocytes during the first 12 hours of culture. *Tissue Eng.*, 3, 51-52, 77-87.
- Melgarejo, I., Fernández, D., and Barrios, R., 2000. *Primeras Técnicas de la Biología Celular Aplicadas a la Producción de Clones y a la Creación de Nuevas Especies de Animales*. Ediciones Universidad de Valencia, Valencia.
- McGraw, S., 1991. *Dictionary of Gestational Development*, 1st edn, P. J. T. Blamey and J. D. Edwards (Eds.), Academic Press, New York, McGraw-Hill, Inc., pp. 1619-1639.
- Moore, K.R., Verlinsky, Y.Y., Ivakh, M.I., Reznikoff, S., and Verlinsky, T., 2002. Growth Factors and Growth Hormone Transfected Embryos and a novel protocol of purified bovine blastocysts. *Transplantation*, 73(7), 745-751, 745-751.
- Shibata, T., and H., 2002. *Practical Guide to Stem Cell Culture and Cell Lines*. 1st edn, K. Shioya (Ed.), Springer-Verlag Berlin Heidelberg New York, Springer-Verlag Berlin Heidelberg New York.

