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

EDITORS:
Michael P. Ward (Australia)
Fauzi Rachrid (Africa)
Montip Gettayacamin (Thailand)
Fedik Abdul Rantam (Indonesia)
Suzanita Utama (Indonesia)

FACULTY OF VETERINARY MEDICINE - UNIVERSITAS AIRLANGGA
HMHERE SUB-COMPONENT B 2 C PERFORMANCE BASED CONTRACT

PROCEEDING
International Seminar

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

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

EDITORS:
Prof. Michael P. Ward, Ph.D., BVSc., FACVSc. (Australia)
Dr. Fouad Kasheid (Africa)
Montip Gettayacamin, DVM., DACLAM (Thailand)
Prof. Dr. Fedik Abdul Rantam, DVM. (Indonesia)
Suzanita Utama, M.Phil, DVM. (Indonesia)

FACULTY OF VETERINARY MEDICINE - UNIVERSITAS AIRLANGGA
IMHERE SUB-COMPONENT B.2.C PERFORMANCE BASED CONTRACT

First print — 2012

Publisher:
Center Publishing and Printing of Airlangga University (AUP)
Kampus C Unair, Mulyorejo Surabaya [illegible]
Phone [illegible] Fax [illegible]
E-mail: [illegible]

Printed by: **Center Publishing and Printing of Airlangga University (AUP)**
([illegible])

Library of National Catalogue-in-Publication Data

Pro: Proceeding International Seminar: Strategy to Manage Ecohealth System for Stabilizing Animal Health and Productivity to Support Public Health/
Ed.: Michael D. Ward ... [et al.] — First Print — Surabaya:
Center Publishing and Printing of Airlangga University, 2012.
[illegible]
Bibliography
ISBN 978-602-8967-[illegible]

I. Veterinary Public Health
I. Fauzi Kodra
II. Mustofa Helmi Effendi
III. Fedik Abdul Rantam
IV. Sucipto Hanto

636.089 2

12 13 14 15 16 / 9 8 7 6 5 4 3 2 1

MEMBER OF IKAPI: 001/JTI/95

First print — 2012

Publisher:
Center Publishing and Printing of Airlangga University (AUP)
Kampus C Unair, Mulyorejo Surabaya 60115
Phone. (62 31) 5992246, 5992247 Fax. (62 31) 5992248
E-mail: [illegible]

Printed by: Center Publishing and Printing of Airlangga University (AUP)
[illegible]

Library of National Cataloging in Publication Data

Pro Proceeding International Seminar: Strategy to Manage Bio-Eco-Health System for Stabilizing Animal Health and Productivity to Support Public Health
Ed. Michael P. Ward ... [et al.] — First Print — Surabaya:
Center Publishing and Printing of Airlangga University, 2012
[illegible] p.; 21 × 29.7 cm
Bibliography
ISBN [illegible]

1. Veterinary Public Health

I. [illegible]
II. [illegible]
III. Fedik Abdul Rantam
IV. [illegible]

[illegible]

12 13 14 15 16 / 9 8 7 6 5 4 3 2 1

MEMBER OF IKAPI: 001/JTI/95

CONTENTS

MESSAGES

INVITED SPEAKERS

FREE PAPER

ACKNOWLEDGEMENT

BIOACTIVITY OF INSULINE LIKE GROWTH FACTOR-1 (IGF-1) DERIVED FROM THE HEPATOCYTE MONOLAYER CULTURE AGAINST CLEAVAGE AND DEVELOPMENT OF BOVINE EMBRYO IN VITRO

Sri Mulyati and Lado Mahaputra

In Vitro Fertilization Laboratory, Department of Veterinary Reproduction
Faculty of Veterinary Medicine, Universitas Airlangga

ABSTRACT

Liver cells (hepatocyte) have an ability to produce IGF-1 through monolayer culture, but their concentration and the bioactivity against cleavage and development of embryo were not clear. The purpose of this study was to analyze the concentration of IGF-1 derived from hepatocyte monolayer culture and to [illegible] bioactivity against cleavage and development of bovine embryos in vitro. Hepatocyte culture was prepared by grinding liver tissues followed by trypsinization and repeatedly centrifugation [illegible]. They were then cultured in TCM199 + FCS 10% + BSA 10%, and then incubated in incubator 5% CO_2 at 38.5°C for 3, 6, 9 and 12 days. IGF-1 concentration [illegible] was measured by ELISA technique. The results of hepatocyte monolayer culture showed that [illegible] incubation period resulted in the most confluent liver cells and IGF-1 [illegible] compared with other incubation periods. Bioactivity test of IGF-1 in this research was carried out by adding the supplement [illegible] in vitro [illegible] and development media of bovine embryos by observing and counting a number of embryos which underwent cleavage (2-8 cell) in the day 2 after fertilization and embryos developing [illegible] on the day 5. The results of research showed media furnished with [illegible] from the hepatocyte monolayer culture containing IGF-1 [illegible] produced the higher number of embryos undergoing cleavage and developing [illegible] compared with those of media without supplementation. In conclusion, the IGF-1 can be produced through bovine hepatocyte monolayer culture and it is found to increase cleavage rate and [illegible] development in vitro.

Keywords: IGF-1, 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, the *in vitro* embryo production becomes the good alternative. With *in vitro* fertilization the reproductive organ of the ovaries representing the wastes 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-1* (IGF-1) as an endocrine hormone circulating in the blood. As a growth factor, IGF-1 acts as a regulator of postnatal growth by increasing skeletal growth through chondrocyte proliferation and increases extraskeletal tissue growth by increasing cell division and protein synthesis (MacGillivray, 1995). IGF-1 is not only produced by hepatocyte, but it is also produced by some tissues which act as endocrine, paracrine and autocrine, such as cells in the ovaries, Fallopian tube epithelial cells, and endometrial epithelial cells (Hafez et al., 2000). Research about production of growth factor (IGF-1) 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 main important role as a major producer of IGF-1 through the hepatocyte monolayer cultures to produce IGF-1.

The addition of growth factors such as IGF-1 in oocyte maturation media and embryo culture media has been widely reported. Machaty et al. (2000) and Block et al. (2003) say that the IGF-1 put into the embryo culture medium can increase the rate of embryo cleavage. Furthermore Lorenzo et al. (1994) and Margawati (2007) report that IGF-1 can stimulate the process of embryo and maternal recognition, the occurrence of cumulus cells (granulosa cell mass that surrounds the ovum in the de Graaf follicle), maturation of the oocyte nucleus and increase in embryonic development rate. According to Donnaloia et al. (2004) IGF-1 possesses a synergistic effect in the process of steroidogenesis, oocyte maturation and embryo development rate in mice. Further it is said that IGF-1 is an antigonadotropic material which can cause cell proliferation and differentiation.

Growth factors commonly used in the in vitro fertilization among others, are including epidermis 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 embryonic cleavage rate (Lorenzo et al., 1995; Li et al., 2002; Maurer et al., 2002).

MATERIALS AND METHODS

The study uses bovine liver and ovaries where the cattle were killed at the slaughterhouse (RPH) in Pegirian Surabaya. The liver is used as material for hepatocyte (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 vitro. In vitro liver is removed from its membranes and connective tissue, cut and weighed at 1 gram. The 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 centrifuge tube, then centrifuge the 10 minutes at 3000 rpm. Supernatant was discarded, washed with 10 ml of TC medium and 1 ml FCS, centrifuge again for 10 minutes at 3000 rpm in which each washing was performed 2 times. Liver cell suspension was counted and diluted by adding TCM 199 medium with a ratio of pellet: TCM 199 = 1:1. It was diluted until homogeneous, and then the pellet was diluted again with 10 µl pellet : 990 µL TCM 199 (1:99). Then it was put into a counting, the number of liver cells were counted using Trypan technique. Concentration was made to 1×10⁵ cells/ml media. Then 100 ml of TCM 199 media is put into the 96 microplate. Petri dish, add the diluted pellet, and then 100 mm using hemocytometer, and then poured into TCM 199 media, incubated in 5% CO_2, 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) (Maharaja, et al., 2000).

Bioactivity 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 embryos 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_2 incubator with relative humidity at temperature of 38.5°C. Development and maturation observed every day and media was replaced every 3 day. Final observation of the embryo was performed once the embryos 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 the sample Kolmogorov-Smirnov test and homogeneity test was performed using Levene's test system for data collected. Data analysis was treated by factorial (ANAVA) F-test, and the level of significance was tested with the very significant difference (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 time 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 (pg/ml)
3 days	84.00^{a} ± 10.770	76.36^{a} ± 20.523
6 days	71.25^{a} ± 10.078	223.44^{a} ± 68.123
9 days	62.50^{a} ± 8.960	122.81^{a} ± 75.281
12 days	40.00^{a} ± 3.000	53.31^{a} ± 11.943

^{a} Different superscripts in the same column show significant difference ($p < 0.05$)

The table above shows that the culture system on day 6 produces a higher cell number than the days 3, 9 and 12. This is consistent with the research conducted by Iritkhowo (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 reach 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 TCM 199 culture media have begun to diminish, so many cells do not grow well or die, 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 (IGF-1) of liver cell as media supplements, in vitro fertilization and embryo culture can be examined 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 Embryos 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 (%)			
	2-4 cell stage (%)	Transformation (%)	Morula Stage (%)	Transformation (%)
Fertilization Media	26.90^{a} ± 5.556	3.136 ± 0.651	5.60^{a} ± 1.875	2.441 ± 0.983
Fertilization Media + Media Supplement of the Embryo	40.870^{b} ± 10.546	4.220 ± 1.591	28.667^{b} ± 9.415	5.346 ± 1.943

^{a} Different superscripts 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 2 (2-8 cells) amounted to 40.870% ± 10.546, showing a highly significant difference ($p < 0.001$) 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 6 (morula) amounted to 28.667 ± 9.415%, showing a highly significant difference ($p < 0.05$) compared with the number of embryos cultured in fertilization medium.

This is because the role of IGF-I in influencing the development of cells as a mitogenic remedy (Hafez et al., 2000). The IGF-I derived from the hepatocyte monolayer culture as a growth factor is shown to promote the development of embryos in vitro, either at 2-8 cell stage or even become morula. 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 in cattle that usually occurs at 8-cell stage can be prevented (Mtango et al. 2002) and Block et al. (2003).

CONCLUSION

Concentration of growth factors (IGF-I) derived from the hepatocyte monolayer 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 2-8 cell stage and morula stage which were cultured in vitro in the hepatocyte monolayer culture media showed very significant differences compared with the percentage of embryos cultured in the fertilization medium.

REFERENCES

Block J, Drost M, Monson RL, Rutledge JJ, Rivera RM, Paula-Lopes FF, Ocon OM, Krininger CE, Liu J, Hansen PJ, 2003. Use of insulin-like growth factor-I during embryo culture and treatment of recipients with gonadotropin-releasing hormone to increase pregnancy rates following the transfer of in vitro produced embryos to heat-stressed, lactating cows. J Anim Sci. 81: 1590–1602.

Demeestere I, Gervy C, Centner J, Devreker F, Englert Y, and Delbaere A. 2004. Effect of Insulin-Like Growth Factor-I During Preantral Follicular Culture on Steroidogenesis, In Vitro Oocyte Maturation, and Embryo Development in Mice. Biology of Reproduction. 70: 1664–1669.

Hafez ESE, Jainudeen MR and Rosnina Y. 2000. Hormones, Growth Factors, and Reproduction. In: Hafez Reproduction in Farm Animals. 7th Edition. Lippincott Williams & Wilkins. Philadelphia.

Li YH, [illegible] RH, [illegible] and Wang WH. [illegible]. Synergistic effects of epidermal growth factor and estradiol on cytoplasmic maturation of porcine oocytes. Zygote [illegible].

Lorenzo PL, Illera MJ, Illera JC, and Illera M. 1995. Influence of growth factors on the time-dependent meiotic progression of rabbit oocytes during in vitro maturation. Rev Esp Fisiol. 1995; 51(2): 77–[illegible].

Mahaputra L, [illegible] 2000. [illegible]

McGilvery RW. [illegible]. Dynamics of Growth and Development. [illegible] Endocrinology and Metabolism. [illegible] New York: McGraw-Hill, Inc. pp. [illegible].

Mtango NR, Varisanga MD, Dong YJ, Rajamahendran R, and Suzuki T. 2003. Growth Factors and Growth Hormone Enhance In Vitro Embryo Production and post-thaw survival of vitrified bovine blastocysts. Theriogenology 2003 Mar; 59(5-6): [illegible].

[illegible] 2006. [illegible] Universitas Airlangga Surabaya.