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by Epy Muhammad Luqman

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Effectivity of Insulin Transferrin Selenium and Bovine Serum Albumin Addition on In Vitro Culture Medium on Fertilization and Blastocyst Rate of Mice (Mus musculus)

Widjiati[1], Epy Muhammad Luqman[1], Benjamin Christoffel Tehupuring[1]*

1. Veterinary Anatomy Department, Faculty of Veterinary Medicine, Airlangga University.

Abstract

The aim of this research was to know best composition of in vitro culture medium that can support the development of zygote and cleavage into blastocyst stage of embryo and ready to be transferred into recipient. Insulin Transferrin Selenium (ITS) and Bovine Serum Albumin (BSA) need to be added to optimize culture medium so it can produce embryo with high viability to support embryo transfer program.

The addition of Insulin Transferrin Selenium into in vitro culture medium bind free radical, trigger development of the cell, inhibit damage of the cell because of its antioxidant component so it can increase blastocyst viability. The addition of Bovine Serum Albumin can increase the competence of the embryo development to grow in the in vitro culture medium.

The research started with estrus synchronized, oocyte collection, in vitro fertilization, addition of Insulin Transferrin Selenium and Bovine Serum Albumin into culture medium and examine the number of fertilization and blastocyst. The result show combination of Insulin Transferrin Selenium and Bovine Serum Albumin supplementation increasing number of fertilization and blastocyst is better compare with group that added Insulin Transferrin Selenium only ($p>0,05$) but it is not different with group that added Bovine Serum Albumin only. The conclusion from this research is addition of Insulin Transferrin Selenium and Bovine Serum Albumincan increase the number of fertilization and support the development of embryo.

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Introduction

The successful of embryo transfer is depending its embryo quality that will be transferred and the condition of endometrium. Embryo stated as good quality if the embryo will develop and grow in the uterus of recipient. Good quality embryo can be obtained in vivo also in vitro. Embryo that obtained from in vitro has some advantages, the numerous of embryo produced and embryo produced is in the same stage[1].

Nowadays the provision of in vitro embryo as transfer embryo needs is not fulfilling the quality of embryo with high viability. It is based on the low number of pregnancy from the in vitro embryo recipient. It is necessary to review the low number of pregnancy in molecular reproduction because there are many factor that affect in vitro embryo culture like source of nutrition and stress during embryo culture[2].

Modifying condition of in vitro culture is one of technique to increase the number of fertilization and blastocyst viability due to the need of embryo transfer. Some growth factor is added into culture medium like Insulin Transferrin and Bovine Serum Albumin as maturation and culture medium to increase oocyte ability into meiosis II stage[3].

Insulin Transferrin Selenium is complex supplement medium that consist of insulin, transferrin and selenium if it is added into culture medium can decrease the binding of free radical[4,5]. Insulin Transferrin Selenium is complex protein that can increase development of the cell and inhibit cell damage because of its antioxidant so that in can maintain the viability of embryo. Insulin Transferrin Selenium can increase the number of fertilization, quality and viability of blastocyst from the result of in vitro culture[6,7].

Corresponding author:
Widjiati ,
Veterinary Anatomy Department,
Faculty of Veterinary Medicine, Airlangga University.
E-mail : widjiati@fkh.unair.ac.id

Bovine Serum Albumin as protein sources is composed many essential amino acids. BSA supplementation into culture medium can increase the competence of embryo development to grow into the in vitro culture medium, accelerate cleavage stage of embryo so that the embryo can grow and develop and maximally produce excellent blastocyst with high viability. Low viability of embryo will affect the implantation process on its attachment with endometrium. The decreasing of quality and viability of embryo also caused by the numerous of apoptotic trophoblast cell so the implantation and pregnancy is not occurred. Besides, endometriumthickness from recipient must be ready[8,9,10,11].

Viability of blastocyst from in vitro culture is affecting the successful number of embryo implantation and pregnancy after the blastocyst is being transferred. Because of that, the study to optimize culture medium is needed, so in vitro blastocyst can be produced as embryo bank and fulfill the embryo transfer needed and increase the number of pregnancy.

Based on the background the research is needed to prove the Effectivity of Insulin Transferrin Selenium and Bovine Serum Albumin Addition on In vitro Culture Medium on Apoptotic Trophoblast Cell, Blastocyst Number and Successful of Embryo Transfer.

Materials and methods

This research were using mice oocyte and embryo as the sample. This research consist of 3 treatment groups, Treatment Group 1 (T1) : Minimum Essential Medium Eagle Minimum + Insulin Transferrin Selenium 5% + Bovine Serum Albumin 5%, Treatment Group 2 (T2): Minimum Essential Medium Eagle + Insulin Transferrin Selenium 5% and Treatment Group 3 (T3): Minimum Essential Medium Eagle + Bovine Serum Albumin 5%.

Research Materials and Equipments

Materials that used in this research include male mice aged 5 month, female mice aged 3 month, Insulin Transferrin Selenium (ITS), Bovine Serum Albumin (BSA), *Pregnant Mare Serum Gonodotropin* (PMSG) (Folligon®, Intervet, Boxmeer, Holland), *Human Chorionic Gonadotropin* (HCG) (Chorulon®, Intervet, Boxmeer, Holland), *Phosphate Buffer Saline* (PBS), Medium Eagle Minimum (Sigma®, St. Louis, USA), ethilen glikol (Sigma®, St. Louis, USA), propanediol (Sigma®, St. Louis, USA), mineral oil (Sigma®, St. Louis, USA), $C0_2$. Equipments that used in this research include CO_2 incubator (Thermo), mikroskop inverted (Meiji), program image raster 3.0, syringe (Terumo), pipet pasteur (Thermo), Hemi straw, petridish dispossible (Thermo), millipore Tthermo),

Research Variables

The independent variable of this research are Insulin Transferrin Selenium, Bovine Serum Albumin.The dependent variable of this research are the number of fertilization and blastocyst.

Research Method

1. Superovulatation and oocyte collection

Female mice were being injected with Pregnant Mare Serum Gonadotropin (PMSG or Foligon) with dosage 5 IU. Ethical clearance received from Faculty of Veterinary Medicine Airlangga University with number 717-KE. Forty-eight hours later continued by injecting Human Chorionic Gonadotropin (HCG or Chorulon) and mated with vasectomy male mice used mono-matting method. Seventeen hours after mated then the vaginal plug was checked. The female mice with vaginal plug was decapitated and the fallopian tube was collected. The fallopian tube was washed in Phosphate Buffer Saline and moved into petri dish and rip fertilization sac under the inverted microscope. The oocyte collected was washed.

2. In Vitro Fertilization

The oocyte collected was washed three times using PBS and MEM medium then moved into the fertilization medium while waiting for the separation of spermatozoa. Spermatozoa was collected from cauda of epididymis from male mice and after that the spermatozoa was put on the same fertilization medium with oocyte before. Oocyte with spermatozoa then incubated on CO_2 5% incubator with temperature 37° C during 7 hours and the granulosa cell was fallen out to examine zygote or 2 cells.

3. Embryo Culture until Blastocyst Stage

The zygote was moved into culture medium and incubated on CO_2 5% incubator with temperature 37°C. Culture medium was changed twice a day until reaching the blastocyst stage.

Results

Result of this research were the number of fertilization from in vitro fertilization and the

number of blastocyst obtained from in vitro culture. Supplementation of Insulin Transferrin and Bovine Serum Albumin show the number of fertilization increased better than fertilization medium that only added with Insulin Transferrin Selenium or Bovine Serum Albumin. Analytic result from the number of fertilization were showed in the table 1 and table 2 below.

Group	Mean ± SD	p
T1	98,3340± 3,72529	0,0036
T2	90,9100± 6,42760	
T3	93,3360± 3,72529	

Table 1. Mean and standard deviation of fertilization number from BSA, ITS and Combination of BSA and ITS on in vitro fertilization medium group.

Fertility number	Group	P
	T1-T2	0,032*
	T1-T3	0,072
	T2-T3	0,080

Table 2. Mann Whitney Test result for determining the difference between treatment group to fertility number.

*The difference between each treatment group.

T1 : Minimum Essential Medium Eagle Minimum + Insulin Transferrin Selenium 5% + Bovine Serum Albumin 5%.

T2: Minimum Essential Medium Eagle Minimum + Insulin Transferrin Selenium 5%.

T3 : Minimum Essential Medium Eagle Minimum + Bovine Serum Albumin 5%.

Figure 1. Embryo that have been cleavage into 2 cells.

Analytic result from the development of embryo from zygote to 2 cells, 4 cells, 8 cells, morula and blastocyst were showed in table 3 and table 4 below. Supplementation Insulin Transferrin Selenium and Bovine Serum Albumin into in vitro culture medium can increase the number of embryo that develop become blastocyst stage and has significant different with group that only added with Insulin Transferrin Selenium or Bovine Serum Albumin.

Group	Zygote-2 Cells (Mean ± SD)	P	2 Cells-4 Cells (Mean ± SD)	P	4 Cells – 8 Cells (Mean ± SD)	P	8 Cells-Morula (Mean ± SD)	P	MORULA – Blastocyst (Mean ± SD)	p
T1	100,00 ± 0,0	0,008	74,55 ± 8,4	0,977	80,00 ± 11,7	0,041	100,00 ± 0,0	1,000	91,31 ± 8,1	0,037
T2	87,27 ± 5,0		77,11± 4,1		70,00± 7,4		100,00 ± 0,0		72,33± 11,8	
T3	91,21± 5,9		74,73± 7,6		84,28± 5,3		100,00 ± 0,0		87,14± 13,8	

Table 3. Mean and standard deviation of blastocyst number from BSA, ITS and Combination of BSA and ITS on in vitro culture medium group.

Embryo Development	Group	P
Zygote-2cells	T1-T2	0,005*
	T1-T3	0,017*
	T2-T3	0,238
4cells-8cells	T1-T2	0,160
	T1-T3	0,242
	T2-T3	0,014*
Morula-Blastocyst	T1-T2	0,014*
	T1-T3	0,588
	T2-T3	0,070

Table 4. Mann Whitney Test result to determine the difference between each treatment group to blastocyst number.

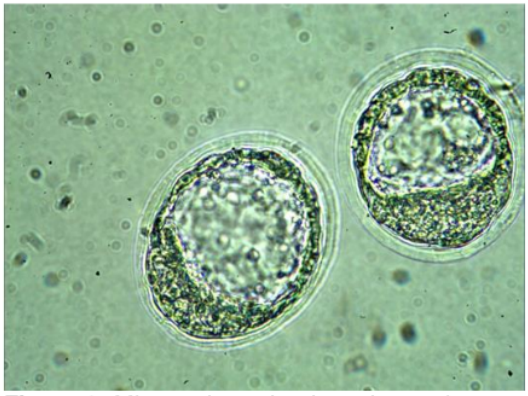

Figure 2. Mice embryo that have been cleavage into blastocyst stage from in vitro fertilization.

Discussion

Culture medium is one of the most important component on producing in vitro embryo, many culture medium have been

develop for embryo culture importance. Generally, culture medium is containing serum or BSA. Medium that has serum or BSA will increase the rate of in vitro embryo development. BSA can bind free radical, metal, toxin, regulate redox potential, pH and osmolality and finally increase embryo development[12].

Insulin is polypeptide hormone that can affect glucose absorption and amino acid and also has mitogenic effect[13] . Addition of Insulin and Insulin Growth Factor on IVC and IVM medium can increase oocyte and embryo quality of pig. Selenium (Se) is trace elementthat important for some physiological activity[14]. Selenium on culture medium will create sodium selenite that has function to protect the cell from oxidative damage by decreasing the production of free radical and inhibit lipid peroxidation[15]. ITS is the best supplement to increase oocyte development and generally used in various in vitro culture[16]. ITS supplementation can support the development of follicle and in vitro oocyte maturation[17,18].

Conclusions

The conclution from this research is supplementation using Insulin Transferrin and Bovine Selenium Albumin can increase the number of fertility compared with treatment group only added Insulin Transferrin or Bovine Selenium Albumin. The addition of Insulin Transferrin and Bovine Selenium Albumin can increase the number of embryo that developed into blastocyst compared with treatment group added with Insulin Transferrin but not with treatment group added Bovine Selenium Albumin.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

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PAGE 1

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