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Maturation of Rams Spermatozoa on Lamb Granulose Cell Culture (LGC) with Supplementation of Fetal Bovine Serum (Fbs) *In Vitro*

Heru Nurcahyo[a)], Ciptono and Himmatul Hasanah

Department of Biology Education, FMIPA, Yogyakarta State University, Indonesia

[a)]corresponding author: herunurcahyo62@gmail.com

Abstract. The objectives of this study were to investigate the maturation of rams spermatozoa from cauda epididymis on the lamb granulose cell culture (LGC) with supplementation of various concentration of fetal bovine serum (FBS) in vitro. This experimental research was conducted in the cell culture laboratory of Department of Biology Education of FMIPA Yogyakarta State University (YSU) and Ilmu Faal Laboratory of Medicine Faculty of Gadjah Mada University (GMU) Yogyakarta. Rams testis were collected from a local slaughter house in Yogyakarta, and transported to the laboratory in Phosphate Buffered Saline (PBS). Epididymal spermatozoa was collected from cauda of epididymis. In vitro maturation (IVM) of spermatozoa was performed in the LGC with supplementation of FBS 2,5%, 5%, and 10% and incubated at 37ºC with 5% CO_2 for 5 hours. Evaluation of spermatozoa maturation was determined based on motility and viability of spermatozoa. Based on the research result showed that the motility and viability of rams spermatozoa were significantly longer in LGC with addition FBS in various concentration. It was concluded that addition of 10% FBS showed higest spermatozoa motility and viability. It was concluded that rams spermatozoa from cauda epididymis were capable maturation on cultured LGC with addition FBS in vitro.

INTRODUCTION

Recently, the reproductive biology techniques (RBT) for example artificial insemination (AI), embryo transfer (ET), and in vitro fertilization (IVF) in livestocks have developed rapidly and have many benefit effects for optimize livestock productivities. Some programs of RBT in livestocks and wild animal were useful to solve fertility problems, increasing genetic quality and conservation of wild animal [5]. One of the main prerequisite to be successful RBT are maturation and quality of spermatozoa. The most famous accelerated maturating spermatozoa programs was in vitro maturation (IVM). The main problems to carried out IVM were medium for maturation. The alternative solution was used medium originated from primer cell culture because in the late of log phase, primer cell culture produce substances like a tissue origin [3]. Poor maturation tends to create a unsuccesful IVM. In the livestocks studied, ejaculated spermatozoa cannot immediately fertilize an egg. Additionally, we propose a logical approach to evaluate spermatozoa maturation in the medium lamb granulose cell culture (LGC) as a medium for preparing spermatozoa maturation to successful IVM outcomes. In this research was used rams spermatozoa originated from cauda of epididymis as a object of research due to cauda epididymal spermatozoa has mature and naturally motile [5]. In addition, epididymis rams as a waste in slaughter house and such as availability [4]. Spermatozoa motility and viability using as a parameter to be successful *in vitro Fertilization* (IVF). Measuring semen quality very effective to increase result of IVF [8]. Factors that was very important in measuring semen quality was motility and viability of spermatozoa [6]. Maturation process of spermatozoa in cultur medium was needed substances that crucial for motility and viability of spermatozoa.

Lamb granulose cell culture (LGC) from folicle ovary in late of log phase can secreted many substance like hormone for example estrogen dan progesterone [4]. Based on the above rationale, this research was conducted

to evaluate the effect of LGC from lamb's ovary follicle with supplementation of various consentration of FBS as a media maturation of motility and viability of ram spermatozoa *in vitro*.

EXPERIMENTAL

Cell culture

Culture of granulose cell (LGC) made of from lamb ovary granulose cell were plated at a density of $5x10^5$ cells/ml in 48 microwells plate and incubated for 24 hrs at 37°C. Addition with FBS 2,5%, 5%, and 10%

Spermatozoa Preparation

Rams testis were collected from a local slaughter house in Giwangan Yogyakarta. Ram was local strain, sexual maturity, 1.5-2 years old were used in this study. Testis were transported to the laboratory in Phosphate Buffered Saline (PBS) solution on ice box.

This research was conducted at the cell culture laboratory of Department of Biology Education FMIPA Yogyakarta State University (YSU) to prepare the spermatozoa from epididymis. To examined the motility and viability of spermatozoa carried out in the Laboratory of Ilmu Faal Faculty of Medicine Gadjah Mada University (GMU). Testes washing with sterile water and then rinse with PBS sterile with NaCl 0.9% (b/v) three times. Spermatozoa was collected from cauda epididymis. After that the spermatozoa were placed in the microwell plate which cultured lambs granulose cell (LGC). Then the spermatozoa put in the LGC with addition of FBS 2,5%, 5%, and 10% then was incubated in incubator 5% CO_2 at temperature of 37° C for 5 hours.

Evaluation of spermatozoa maturation was determined by motility and viability under phase contrast microscope then convert to percentage of sperm motility and sperm viability. Spermatozoa viability was determinated by staining using tripan blue stain (TBS) to know live sperm or died, based on spermatozoa morfology observation, dark its mean dead sperm and vice versa. Microscopic examination, one drop of each sperm suspension was immediately placed on an hemocytometer chamber (dept 0.1 mm) then were examined by phase contrast at room temperature. According Lindsay [6], grade of spermatozoa motility: (0) if spermatozoa not motile, (1) if spermatozoa move around, (2) if 50% spermatozoa has progressive movement, (3) if 50-80% spermatozoa has progressive movement, (4) if spermatozoa has progressive movement 90%, (5) if very progressive moving 100% spermatozoa motile.

Statistical Analysis

The data were statistically analyzed by one-way ANOVA. When there is significant difference data analyzed continously by *Duncan Multiple Range Test* (DMRT) for comparison for each group (Steel & Torri). A value of $p<0.05$ was considered statistically significant.

RESULT AND DISCUSSION

Sperm Motility

The rams spermatozoa after collected from cauda epididymis and maintain in LGC showed that life sperm observed according characteristics of spermatozoa as shown in figure 1.

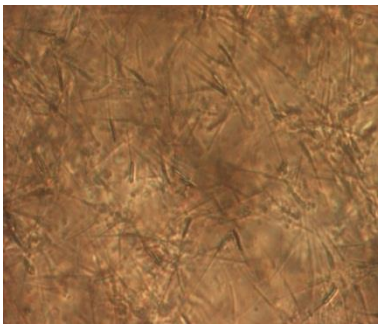

FIGURE 1. Photo micrograph of rams spermatozoa after collected from cauda epididymis (magnifying 200x)

The research result showed that the average of spermatozoa motility of spermatozoa after maintain in LGC with supplementation various concentration of FBS medium as shown in figure 2.

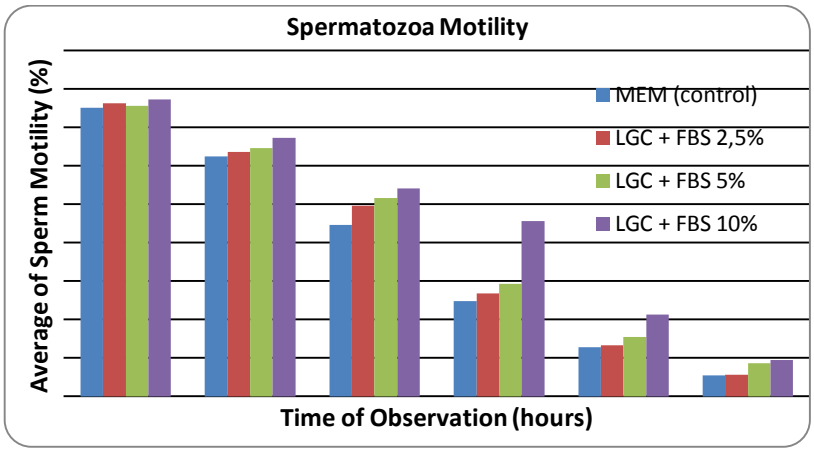

FIGURE 2. Average of percentage spermatozoa motility (%) after maintain in LGC with supplementation of various concentration of FBS

Analized data by ANOVA showed that any significant differences between group treatment. Data analized by DMRT showed that sperm viability has significant differences between R0, R1, R2 dan R3. Based on data viability of rams spermatozoa on final observation show that longer average on the treatment group R3 (dose 10% FBS) sperm viability 83%, and lower average on the control group R0 (without add FBS) viability 79%. Based on the result we can interpretated that addition of FBS started 2.5% ml until 10 % to the LGC can influence to significantly improve viability of rams spermatozoa. This is proved that spermatozoa collected from cauda epididymis can mature *in vitro* in LGC add with FBS. The addition of FBS 2.5% - 10% induced progressive movement.

Spermatozoa Viability

The research result showed that the average of spermatozoa viability of spermatozoa after maintain in LGC with supplementation various concentration of FBS medium as shown in figure 3.

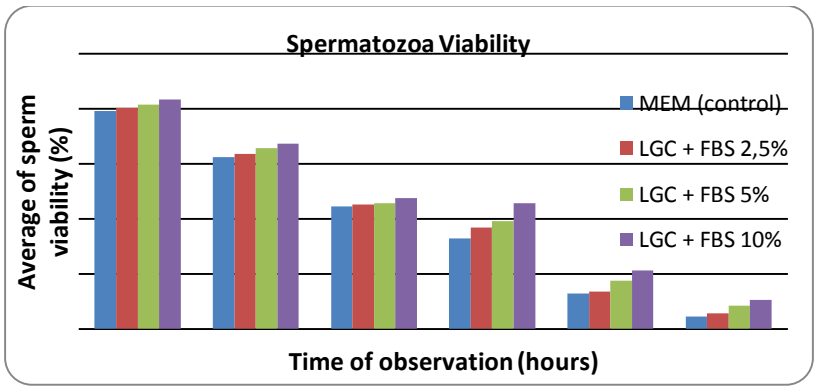

FIGURE 3: Average of percentage spermatozoa viability (%) after maintain in LGC with supplementation of various concentration of FBS

Analized data by ANOVA showed that any significant differences between group treatment. Data analized by DMRT showed that sperm viability has significant differences between R0, R1, R2 dan R3. Based on data viability of rams spermatozoa on final observation show that longer average on the treatment group R3 (dose 10% FBS) sperm viability 75%, and lower average on the control group R0 (without add FBS) viability 51%. Based on the result we can interpretated that addition of FBS started 2.5% ml until 10 % to the LGC can influence to significantly improve viability of rams spermatozoa. This is proved that spermatozoa collected from

cauda epididymis can mature *in vitro* in LGC add with FBS. The addition of FBS 2.5% - 10% induced progressive movement.

The result of this study showed that spermatozoa that collected from cauda epididymis has motility and viability. Maintain the spermatozoa in LGC with addition of fetal bovine serum (FBS) in various consentration 2,5%, 5%, and 10% increase significantly compared with control group (P<0,05). This study demonstrates that addition of FBS to the LGC could induced longer motility and viability of spermatozoa. Reported by Hunter [9], special nature of spermatozoa that isolated from rams cauda epididymis testes was motile spermatozoa. Sperm acquired forward motility during epididymal maturation [8]. Ejaculated spermatozoa cannot immediately fertilize an egg. They require a certain period of residence in the female genital tract to become functionally competent cells. As spermatozoa traverse through the female genital tract, they undergo multiple biochemical and physiological changes collectively referred to as capacitation. Only capacitated spermatozoa interact with the extracellular egg coat, the zona pellucida. The tight irreversible binding of the opposite gametes triggers a $Ca(^{2+})$-dependent signal transduction cascade [9]. In vivo substance like glucosa produced by vesicula seminalis as a source of energy sperm cell to alive and fertilization egg [7]. The role of LGC as a medium can produce some substanes and also has a crucial role to maintain condition to keep carried out metabolism that useful for the sperm viability to still alive. In addition, Heru Nurcahyo [5], reported that LGC contain glucose as a energy source and promote motility of spermatozoa. Number of amino acid increase as high as sperm motility. According Freshney [9], FBS contain various amino acid and protein. FBS contain many material that useful for motility and viability of spermatozoa in culture. Heru Nurcahyo [2], said that granulosa cell in culture secreting amino acid and some hormones such as estrogen and progesterone which are usefull for sperm cell metabolisme and motility and viability. Chian [2], have shown that a protein present in epididymal plasma induces forward motion in washed bovine caput spermatozoa in the presence of phosphodiesterase inhibitor. The results indicate that LGC supplementation with FBS was also significantly improved motility of spermatozoa. Ombelet et al. [10], said that motility of spermatozoa is characteristics and the behavior of caput spermatozoa. Many factors that influences viability spermatozoa in the medium maturation such as; pH, osmotic pressure, electrolit and non electrolit. Hunter [7], said that lactosa is very improtant substances for sperm capasitation. According Ombelet et al. [10], many variables may influence on success rates after IVF, including sperm quality in the native and washed semen sample. The sperm parameters which used as an IVF outcome is significantly improved performance most frequently examined were total motility in the native sperm sample: threshold value of 30% [6, 7], spermatozoa motility more than 40%. It seems logical that sperm quality has to be one of the main determinants to predict IVF success. According Hunter [5], investigating the predictive value of semen quality persentage of spermatozoa motility in good condition about 40-70%, and excellent if persentage of spermatozoa motility more than 80%. The evaluation of sperm morphology an essential component of semen analysis indicate that spermatozoa fertile have persentage of spermatozoa motility about 50-80% and forward movement [11].

Only capacitated spermatozoa interact with the extracellular egg coat, the zona pellucida (ZP). Successful fertilization in the lamb and several other species, involves several sequential steps that are sperm capacitation in the female genital tract. The sperm capacitation and the induction of the acrosomal reaction are $Ca(^{2+})$-dependent signaling events that have been of wide interest to reproductive biologists for over half a century [9]. The most important aspects of the sperm acrosome, from its formation during sperm maturation in the testis (spermatogenesis) to its modification in the epididymis [10]. It is important to mention that only present on the surface of capacitated spermatozoa are capable of binding to their complementary glycan chains on the zona pellucida. Mammalian fertilization is the net result of a complex set of molecular events which allow the capacitated spermatozoa to recognise and bind to the egg's extracellular coat, the zona pellucid (ZP), undergo the acrosome reaction, and fuse with the egg plasma membrane.

CONCLUSION

Based on the research result showed that the motility and viability of rams spermatozoa were significantly longer in LGC with addition FBS in various concentration. It was concluded that rams spermatozoa from cauda epididymis were capable maturated on cultured LGC with addition 10% FBS in vitro

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REFERENCES

1. C. Bedetti, and A. Cantafora. Regulatory Mechanism of Ecosanoids Biosynthesis in Processes Related to Human Reproduction Comparison Between Studies with Tissue and Primary Cell Cultures. *J. of Biotech*. Vol. 15. (1990) pp. 47-56.
2. Chian, Ri-Cheng., Buckett, William M., Tan, Seang-Lin. Review: "*In-Vitro* Maturation of Human Oocytes". *Reproductive BioMedicine Online.* Vol. 8. No. 2 (2003) pp. 148-166.
3. R.I. Freshney. *Culture of Animal Cells. A Manual of Basic Technique*. 2nd.ed. New York: John Wiley & Sons, Inc. Publication, 1990.
4. Heru Nurcahyo. Uji Toksisitas Kurkumin pada Kultur Sel Granulosa Folikel Ovarium. *Laporan Penelitian*. FMIPA Universitas Negeri Yogyakarta. 2003
5. R.H.F.Hunter. Fisiologi dan Teknologi Reproduksi Hewan Betina Domestik. Bandung: ITB. 1995.
6. D.R. Lindsay, K.W. Entwistle, & A. Winantea. *Reproduction in Domestic Livestock in Indonesia*. Published by the Australian Universities International Development Program (AUIDP). 1982.
7. A.V. Nalbandov. *Fisiologi Reproduksi pada Mamalia dan Unggas. Edisi Ketiga.* Jakarta: UI Press. 1990.
8. M.R. Toelihere. *Fisiologi Reproduksi pada Ternak*. Bandung: Angkasa. 1981
9. D.R. Tulsiani, and A. Abou-Haila. Biological Processes that Prepare Mammalian Spermatozoa to Interact with an Egg and Fertilize It. Academic Press Copyright. 2000.
10. W. Ombelet, N. Dhont, A. Thijssen, E. Bosmans, and T. Kruger. Semen quality and prediction of IUI success in male subfertility: a systematic review. Reproductive Healthcare Ltd. Published by Elsevier Ltd. Copyright © 2013.
11. S.A. Shabtaie, S.A. Gerkowicz, T.P. Kohn, and R. Role of Abnormal Sperm Morphology in Predicting Pregnancy Outcomes. Reproductive Healthcare Ltd. Published by Elsevier Ltd. 2013.