J. Appl. Environ. Biol. Sci., 1(11)552-556, 2011

ISSN: 2090-4215
Journal of Applied Environmental and Biological Sciences
www.textroad.com

The Capacitation Pattern of Bali Bull Sperms Filtrated by Sephadex G–200 Using Different Diluters during Freezing Process

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ABSTRACT

Mammalian sperm must undergo a series of controlled molecular processes in the female tract called capacitation before they are capable of penetrating and fertilizing the egg. Capacitation, as a complex biological process, is influenced by many factors, such as physical and biochemist. Sperm experiencing physical and chemical changes in the membrane during the process of gel filtration with Sephadex G-200 due to rubbing against the membrane of spermatozoa heads sperm others and Sephadex gel itself. This is the trigger of premature acrosome reaction. Use of appropriate diluents can prevent premature acrosome reaction during the freezing process.

Keywords: Bali bull sperm, capacitation, Sephadex G-200, acrosome reaction, freezing process.

INTRODUCTION

Improving genetic quality is the one aspect develops livestock productivity. Many efforts were done to widespread superior livestock trough reproduction biotechnology, such as Artificial Insemination (AI), Embryo Transfer (ET) and in vitro fertilization (IVF). Artificial Insemination is the most successful reproduction biotechnology and widely accepted by farmers, because it has cheap cost and effective tool to widespread superior cattle [1]. Mating system using AI is applied to improve Bali cattle genetics, quality and population rapidly. Bali Cattle are local beef having better reproduction characteristics than others [2]. The one effort to support it that is used X–Y sexing and produced calved suitable to our hopes [1].

Sexing sperms using Sephadex column (Sephadex G–200) produced 92% X sperms [3]. Based on this research, sexing using Sephadex G–200 filtrate X–Y sperms easily, cheap and high filtration affectivity. Filtrated sperms by Sephadex G–200 are better to be freezed, because they will be used widely and long time. During freezing process, physically and chemically conditions have to fulfill, in order to maintain their quality, especially capacitation ability. Capacitation is sperms physiological change (ion Ca^{2+} in sperms head membrane) for penetrating egg [4][5].

Acrosomal reaction is the acrosomal vesicle exocytosis process and intracellular Ca2+ increases on equatorial regions sperms that it causes unstable sperms and lyses acrosomal enzyme [6]. Giving plus value in AI programme, sexed sperms by Sephadex G–200 used suitable to need. For supporting it, diluters must guarantee sperms viability and motility during frozen storage in certain time. Egg yolk Tris aminomethane is common diluter and it is proved to produce good quality sexed and unsexed sperms. But, it was not supported by capacitation sperms researches. Based on it, for capacitation sperms testing were used alternative diluter, 10% bovine serum in egg yolk TCM 199, in order to maintain sperm quality and capacitation test.

MATERIALS AND METHODS

Semen Test and Filtration Process

Fresh Bali Bull semen from Singosari AI Centre, Malang, was collected by artificial vagina once per week. It was tested macroscopically and microscopically. After that, 2 ml semen placed on each Sephadex G–200 gel column for different diluters and added diluter (room temperature) ad libitum. Filtrate was colleted in 1 ml tube (tube 1 until 10) and sperm quality tested.

Cooling Process

Filtrate was placed in 15 ml tube and its temperature was decreased until 5^{o}C for 2 hours. And then, filtrate added by diluter + 14% glycerol gradually (glycerolization) and sperm quality tested. The next steps was filling and sealing sperms in straws using automatic filling and sealing machine. It was worked in cool top (5^{o}C). Straws were arranged on straw trays and let them to adapt with cryoprotectant (glycerol) for 9 minutes (equilibration time).

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Freezing and Post Thawing Motility Test

There are two freezing steps. First, straws on trays were steamed on liquid nitrogen (–140°C) in container storage. Second, straws had been dipped in liquid nitrogen (–196°C) for 24 to 48 hours. After that, sample straw had been thawed in 37°C water for 15 seconds. The middle part of straw was cut and semen was dropped on slide. Semen was observed using light microscope (400x).

Chlortetracycline (CTC) Staining

The method of CTC staining is 45 µl fresh or filtrated or cooled or frozen semen added by 45 µl CTC staining solution and mixed well by vortex machine for one minute. And then, it was added by 8 µl CTC fixative solution and mixed well by vortex machine for one minute. 10 µl solutions was put on slide and added by 10 µl DABCO solutions, mixed well by micropipette. After that, it was covered by cover slip and pressed using palm paper genteelly. Each side of cover slip was closed by nail polish [7]. It was observed using Epi–Fluorescence Microscope (Nikon Microscope OPTIPHOT–2 using Filter UV–2A consist of Excitation Filter EX330–338, Diachronic Mirror DM440 and Barrier Filter BA435) [8]. Determining of capacitated sperm based on 100 sperms on a slide space.

RESULTS AND DISCUSSION

Fresh Semen Condition

Table 1 Characteristic of fresh semen used in the experiment

Parameter	Mean ± SD
Color	Creamy
Consistency	Less Opaque
pH	6,4±0,19
Volume (ml)	6,75±2,73
Concentration (x 10^6/ml)	1189±114,15
Mass motility	2+
Individual motility (%)	70
Capacitated Sperm (%)	15,77±2,49

Fresh semen test showed that some sperms undergone capacitation (15,77%) and acrosomal reaction (6,92%) process after collecting semen (Figure 1). It was estimated that materials in seminal plasma triggered capacitation and acrosomal reaction process. Another process was capacitated sperms undergone metabolism and membrane structure change, in order to be acrosomal reaction and likely to snap. More enzymes in sperms head released and it caused sperms have short life [9].

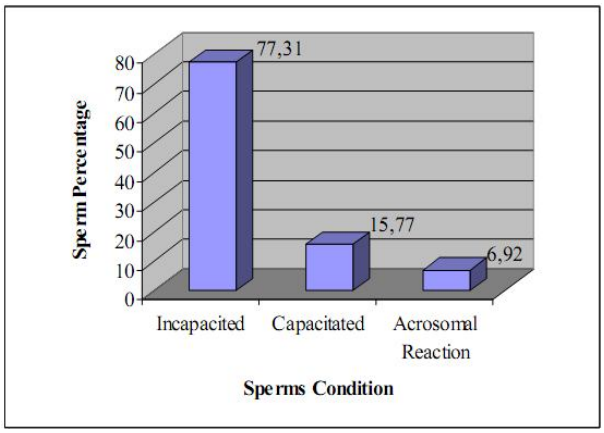

Figure 1 Fresh semen condition (before filtrating)

Sperms Condition after Filtration using Different Diluters

Filtration process using Sephadex G–200 gel influenced capacitation and acrosomal reaction condition. It caused friction between sperms head and gel, in order to influence membrane structure membrane.

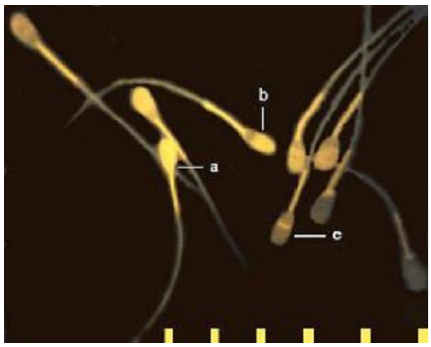

Figure 2 Sperms physiologic condition after CTC staining observed by Epi–fluorescence microscope (400X)
a: incapacitated sperm
b: capacitated sperm
c: acrosomal reaction sperm

Based on statistical analysis, capacitated (23,58%) and acrosomal reaction (17,11%) sperms using Egg yolk Tris aminomethane diluter (P<0,01) were higher than capacitated (22,32%) and acrosomal reaction (16,3%) sperms using 10% bovine serum in egg yolk TCM 199 diluter (P<0,05) (Figure 2). It caused by 10% bovine serum in egg yolk TCM 199 diluter was able to protect early capacitated and acrosomal reaction sperms process. In fact, it contains essential and non-essential amino acids influencing cells physiological and growth [10]. Adding serum maintains cells osmotic pressure, in order to be isotonic. Besides that, it contains protein (to protect cold shock), growth and hydrocortisone hormones (to stimulate cells growth and development), amino acids and glucose (as energy and mineral source to maintain osmotic pressure). Most plasma seminal was blocked through Sephadex gel during filtration process caused sperms head membrane protection decreased, and then they fused between outer sperms head and acrosomal membrane [11].

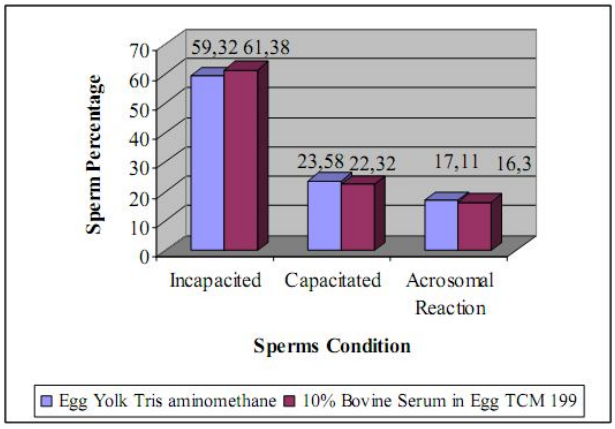

Figure 3 Semen condition after filtrating using different diluters

Sperms Condition on Before Freezing Using Different Diluters

Capacitated (25,17%) and acrosomal reaction (20,03%) sperms using Egg yolk Tris aminomethane diluter (P<0,01) were lower than capacitated (25,37%) and acrosomal reaction (22,24%) sperms using 10% bovine serum in egg yolk TCM 199 diluter (P<0,05) (Figure 3). It means that Egg yolk Tris aminomethane diluter was able to maintain sperms condition, because it gave sperms head membrane protection during cooling and before freezing process. Materials in it, especially cryoprotectant (glycerol) and egg yolk protect sperms head membrane from damaging caused by temperature changing during cooling and before freezing process. During before freezing, sperms had been prepared to maintain their condition on freezing process. Besides that, sperms undergone very low metabolism to maintain their condition. They must adapt with diluters temperature 37^{o}C (filtration process) to 5^{o}C. If they could not adapt, they would be cold shock and their membrane were damaged [12].

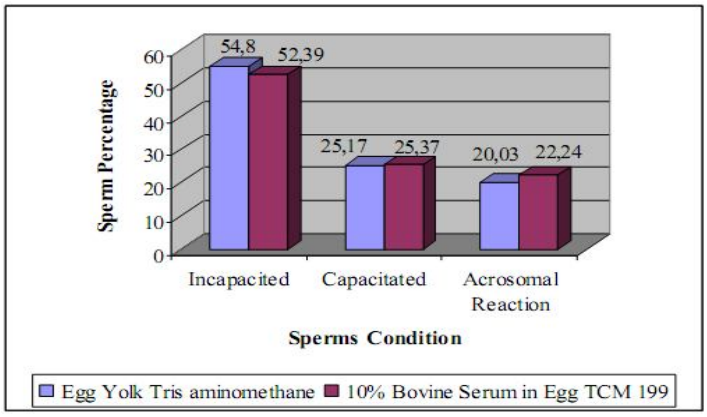

Figure 4 Semen condition on before freezing using different diluters

Sperms Condition after Post Thawing Using Different Diluters

Capacitated sperms (27.82%) using 10% bovine serum in egg yolk TCM 199 diluter was lower than capacitated sperms (28,73%) using Egg yolk Tris aminomethane diluter (P<0,05). And then, acrosomal reaction sperms (26,31%) using 10% bovine serum in egg yolk TCM 199 diluter was higher than acrosomal reaction sperms (21,28%) using Egg yolk Tris aminomethane diluter (P<0,01) (Figure 5). Result showed that Egg yolk Tris aminomethane diluter able to maintain sperms condition during freezing. In fact, materials in it have function as buffer for pH change protection caused by sperms metabolism, maintain osmotic pressure and electrolytes balance [13].

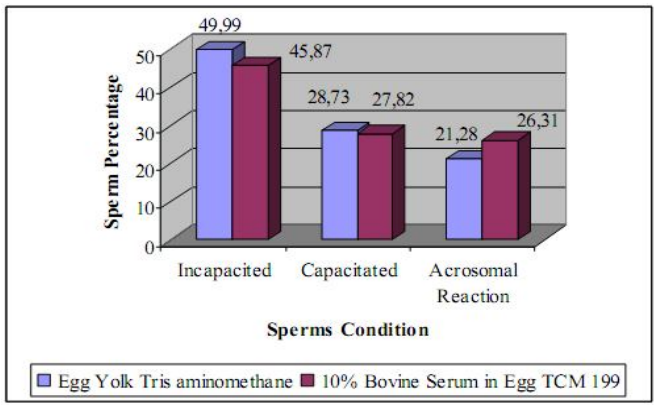

Figure 4 Semen condition after post thawing using different diluters

Acrosomal reacted sperms caused by omnipresence of fluorescence on sperms head membrane. And then, it was estimated that capacitated process causes impermeable or damaging membrane by physical process. Yanagimachi [11] and Bazer *et al.*[14] said there are two types acrosomal reaction, true and false acrosomal reaction. True acrosomal reaction is fusion involving sperms plasma and outer acrosomal membrane followed by enlarging anterior vesicular region. False acrosomal reaction undergoes during aging process or generative factors sperms head membrane [5]. Acrosomal reaction during filtration and freezing process were estimated as false acrosomal reaction, because their membranes damaged by physically process. And then, it is necessary to examine deeply about acrosomal reaction caused by physically process or generative membrane. Capacitated and acrosomal reaction sperms pattern from after filtration to post thawing using different diluter show exactly increasing. Based on that condition, after post thawing sexed sperms it must be inseminated as soon as possible to cow.

CONCLUSION

In conclusion, in this study the author addressed several important questions concerning the effect of sephadex G-200 filtration and freezing process on Bali bull sperm capacitation pattern. This effect depends strongly

on the stage of the capacitation progress, filtration time, diluters and semen quality during freezing process. These observations have a significant impact on my understanding of the previous results concerning filtration effects in sperm and should be helpful to uncover the specific mechanisms of the physical and biochemist effects in sperm physiology**.**

Acknowledgement

The authors would like to thank to General Director of High Education for providing the research funding by Competitive Grant Project, Singosari AI Centre, Prof. Dr. Ir. Trinil Susilawati, MS and Prof. Sutiman B. Sumitro for the research facilities.

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