Post-thawing sperm quality of Boer buck semen diluted in phosphate buffer saline supplemented with bovine serum albumin

A R I Putri, G Ciptadi, A Budiarto and I Santoso
Faculty of Animal Science, Brawijaya University, Malang

Corresponding author: ciptadi@ub.ac.id

Abstract. This study aimed to determine post-thawing sperm quality of Boer buck which diluted in Phosphate Buffer Saline (PBS) medium supplemented with Bovine Serum Albumin (BSA) for optimizing its quality in addressing In Vitro Fertilization (IVF) purposes. In this research thawed semen was diluted in PBS with the addition of three different BSA concentration namely T0 (BSA 0%), T1 (BSA 0.1%), and T2 (BSA 1%). Semen and sperm quality such as motility, viability and abnormality (all in %) were observed at initial thawing and after diluted with PBS and BSA before and after incubation. At initial thawing, semen characteristic shows motility at 62.67±7.03%. Meanwhile, in all sperm quality after dilution at T0, T1 and T3 shows decreased after incubation time, although no differences found between BSA concentration (P>0.05). From this study, it is concluded that adding BSA as complementary nutrition in PBS medium could maintain sperm quality at affordable level for further IVF purpose.

1. Introduction
Reproductive biotechnologies such as embryo transfer, in vitro fertilization (IVF), and especially artificial Insemination (AI), are essential tools to improve the rate of genetic and breeding efficiency, allowing the selection of high-production, fertile, and healthy livestock. Understanding the sources of semen quality variation and identification of genetic markers for early detection of highly fertile bucks and with good quality thawed frozen semen would be of great interest to all livestock breeders [1]. In this regard, semen evaluation could be used as an indicator of fertility in male animals.

Cold storage or cryopreservation in semen is used to reduce metabolism and to maintain sperm quality over an extended period of time. There are problems often found when using frozen semen, such as the presence of cold shock and intracellular changes due to the formation of crystals during freezing [2]. In IVF, spermatozoa must have optimal quality, a decrease in the quality of sperm from a frozen straw can be minimized by improving the quality of the diluents as supplementation, besides the incubation period in the IVF preparation process. Both could affect to the spermatozoa quality.

Bovine serum albumin (BSA) is known to eliminate the free radicals generated by oxidative stress (ROS) and protects the sperm cells membrane integrity from cold shock. The addition of BSA to goat sperm has been shown to improve sperm motility and membrane integrity during sperm cryopreservation or in the liquid and fresh state. But in the thawing process, the quality of sperm will decrease when compared with fresh semen and thawed sperm cannot be re-freeze. From some other research, the addition of BSA as serum may improve the motility, plasma membrane integrity,
viability, and DNA integrity of spermatozoa [3]. For that, the objective of this study was to determine the quality of spermatozoa after post-thawing in PBS medium and supplemented with BSA.

2. Material and methods
Fifteen straws of Boer frozen semen were obtained from Artificial Insemination Center Singosari Malang East Java Indonesia. Thawing was done in water bath at 38°C in 15 second. The semen was evaluated and accepted for evaluation if it has minimum Post Thawing Motility (PTM) 40%. Next, semen was centrifuged in glass tubes at 1500 rpm for 5 minutes, then diluted with PBS and BSA namely T0 100% PBS (3 ml) + 0% BSA, T1 99.9% PBS + 0.1 % BSA, and T2 99% PBS + 1% BSA. After dilution with specific BSA concentration, semen was incubated at 5% CO2 for 30 minutes and then analyzed on the percentage of sperm motility, viability, and total sperm abnormality.

Sperm motility was analyzed using microscope with x40 magnification and examine the progressive motility defined as a sperm with active forward motion. The motility calculated to find the percentage using formula: (total progressive sperm: (progressive sperm + non-progressive sperm)) x 100%. Sperm viability determined using eosin-nigrosine staining, live sperm shown as non-eosinophilic or did not absorb the staining. Same staining method also applied to analyze the sperm abnormality [4]. Data were then analyzed by ANOVA and significant value was at P<0.05.

3. Result and discussion
3.1. Semen preliminary evaluation
The quality of post thawing semen showed at Table 1. The individual motility value shows 62.67 ± 7.03% with minimum threshold 40%. Viability shows 80.5 ± 4.6% which meets the minimum standard of 50%. The abnormality value shows 1.4±0.95 which is meets the standard less than 5%, and for concentration reach 50 million/ml.

Table 1. Post thawing semen characteristics of Boer buck

| Semen Quality | Mean ± S.E  |
|---------------|------------|
| Individual motility (%) | 62.67 ± 7.03 |
| Viability (%) | 80.5 ± 4.6 |
| Abnormality (%) | 1.4 ± 0.95 |
| Concentration (million/ml) | 50 |

3.2. Sperm evaluation before incubation
Addition of BSA to PBS before incubation showed good quality of sperm (Table 3). Motility showed values above 60%, average viability was above 79%, and abnormalities were below 2%. Various concentrations of BSA can improve post-thawing sperm motility and viability, and protect acrosome and membrane integrity. The right concentrations of BSA is able to protect sperm cells from lipid peroxidation [5].

Table 2. Sperm quality post thawing before incubation

| Treatment | Motility | Viability | Abnormality |
|-----------|----------|-----------|-------------|
| T0        | 64 ± 8.9 | 79.9 ± 4.76 | 1.9 ± 1.14 |
| T1        | 60 ± 0.0 | 81.3 ± 2.79 | 1.1 ± 0.54 |
| T2        | 64 ± 8.9 | 80.3 ± 6.6 | 1.4 ± 1.08 |

3.3. Sperm evaluation after incubation
The percentage of motility and viability of spermatozoa after BSA supplementation was decreased when compared to before incubation (Table 3) even though not significantly different (P> 0.05). Bovine serum albumin (BSA) is known as the agent for elimination of free radicals generated by
oxidative stress. It is also able to protect the sperm cells from heat shock during freezing-thawing of semen [6]. Commonly, BSA can be substituted by using egg-yolk in the ram semen diluent, and it would enhance the motility and viability of ram sperm following the freezing-thawing process [7].

Table 3. Sperm quality post thawing after incubation

| Treatment | Semen Quality (%) |
|-----------|-------------------|
|           | Motility | Viability | Abnormality |
| T0        | 36 ± 5.47 | 58.5 ± 12.33 | 1.9 ± 0.65 |
| T1        | 38 ± 4.47 | 57.7 ± 14.35 | 1.3 ± 0.75 |
| T2        | 40 ± 0.0  | 60.4 ± 19.79 | 1.8 ± 1.25 |

Free radical compounds in semen can produce various oxidation processes, cell metabolism, and others. Supplementation of PBS which combined with BSA can serve the incidence of oxidation as a result of free compounds [8]. In addition to chemical processes, centrifuge and incubation also affect the quality of spermatozoa. Phosphate Buffer Saline medium supplemented with BSA can be safely used if insemination is to be performed soon after sperm preparation [9].

Sperm abnormality showed no significant effect (P>0.05) due to combination of PBS and BSA. Sperm abnormality could occur naturally during spermatogenesis, and also during handling process [10]. The percentage of sperm abnormality in this study is above minimum standard for use in IVF.

4. Conclusions
It is concluded that BSA as supplementation in PBS Medium could maintain sperm quality at the acceptable level for IVF. According to the result, adding BSA at 1% give better result compared to others concentration.

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