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The quality of frozen semen of Etawah crossbreed buck after washing by centrifugation

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Abstract. This study aimed to determine the effect of washing sperms using the centrifugation process on the semen quality of Etawah crossbreed (*Peranakan Etawah*, PE) buck. This study used one PE buck, in which the semen was collected 4 times by using an artificial vagina. The parameters measured in the study were the percentage of motility and viability of the sperms using Computer-Assisted Sperm Analysis (CASA). The semen was washed using Andromed and centrifuged at 3000 rpm for 20 minutes. Andromed was used as an extender to increase the volume of semen based on sperms concentration in the straw. The concentration of sperms was divided into three treatment groups; group 1, the concentration of sperms was 100×10^6 cells/straw, group 2 was 150×10^6 cells/straw, and group 3 was 200×10^6 cells/straw. The study was arranged based on a completely randomized design with three treatments and four replications and analyzed using ANOVA. The results of this study showed that the percentage of individual motility of the sperms after thawing on the three treatments did not differ significantly ($P > 0.05$), with the values were 79.2%, 77.5%, 84.4%, respectively. The progressive motility was 63.2%, 64.4%, and 73.9%, respectively, and the viability was 84.6%, 84.6%, and 84.6%, respectively. It can be concluded that the quality of Etawah crossbreed frozen semen after washing using centrifugation and at different concentrations in the straw was able to maintain the motility and viability of the semen after thawing.

1. Introduction

The Etawah crossbreed (*Peranakan Etawah*, known as PE in Indonesia) goat is the result of crossing an Etawah goat and Kacang goat which has good adaptation in the tropics. The result of Etawah crosses is potential livestock that can be developed into a milk producer. The development of PE goats can be used as a potential for increasing other local goats by crossing through reproductive technology, such as artificial insemination (AI) [1].

One way to increase the genetic value of goats at the moment is by using artificial insemination which is expected to increase productivity with the quality of sperms. However, the application has not been as good as expected. Some of the problems faced in the supply of semen, especially when handling semen for PE goats. Generally, the main problem in handling goat semen is it containing phospholipase



An enzymes [1–4], when mixed with animal protein it will coagulate and can damage the quality of the semen.

One solution that can be done is washing and separating the sperms from the semen plasma using the centrifugation method. The washing of sperms by centrifugation is carried out by conventional methods used to overcome the harmful effects resulting from the interaction between semen plasma and egg yolk or milk protein, thereby reducing the viability of spermatozoa. The process of washing sperm is done by separating the spermatozoa from the sperm plasma. Separation of semen plasma is expected to minimize coagulation in semen. Therefore, this study aimed to determine the effect of washing sperms using the centrifugation process on the semen quality of PE buck.

2. Materials and methods

2.1. Materials

This study was conducted at the Animal Centre and Laboratory of Animal Reproduction, Faculty of Animal Science, Hasanuddin University. The equipment used in this study were artificial vagina, scale tube, measuring cup, cover glass, glass object, oven, centrifuge, refrigerator, water bath, warm flat, Styrofoam, straw, Bunsen, macro and micropipette, cuvettes, Photometer SDM 6, Computer-Assisted Sperm Analysis (CASA). The materials used in this study were 6 years old male PE goat, Vaseline, liquid N₂, gel ice, pH paper, physiological NaCl, parafilm, Andromed, 0.2% eosin aquabidest, and 70% alcohol.

2.2. Methods

This study used one PE goat. The semen was collected by holding the semen 4 times using an artificial vagina. The parameters measured were the percentage of motility and viability of PE semen using Computer-Assisted Semen Analysis (CASA). The semen was washed using Andromed and centrifuged at a speed of 3000 rpm for 20 minutes. Semen was diluted with Andromed and divided into 3 groups of sperms concentration; 100×10^6 cells/straw, 150×10^6 cells/straw, and 200×10^6 cells/straw. Furthermore, the semen was frozen and stored for 3 days then thawed using warm water with a temperature of 37°C for 30 seconds then evaluated under a microscope to evaluate the motility and viability of the sperm.

2.3. Parameters

The parameters measured in this study were sperms motility (individual and progressive motility) and sperms viability. Each parameter was carried out in three stages; fresh semen, after washing, and after thawing.

2.4. Data analysis

The data obtained in the study were tabulated in the Microsoft Office Excel program. The treatment groups were arranged and compared using a completely randomized design and ANOVA with the parameters measured (fresh semen, after washing, and after thawing).

3. Results and discussion

3.1. Individual motility of PE buck sperms after washing

Individual motility of PE buck sperms of fresh semen, after washing and post thawing at different sperms concentrations in the straw are presented in figure 1. Figure 1 shows that there is a decline in the individual motility of the PE buck sperms from fresh semen to after washing and after thawing at each concentration. Individual motility of sperms at fresh semen was an average of 94.5%, and it decreased after washing to 98.3%, 86.5%, and 88.1%, respectively for groups 1, 2 and 3. Furthermore, the individual motility of the sperms decreased again after thawing at each group. The decrease in the motility of sperms in each group was likely in a linear pattern and the decrease did not show any significant difference among the groups. A previous study [5] stated that the percentage of motile sperms

in PE buck was an average of 72.79% and another study [6] also shown the value of good motility; 60% – 80%. This means that the individual motility of the sperms in the present study is in line with the previous studies [5,6].

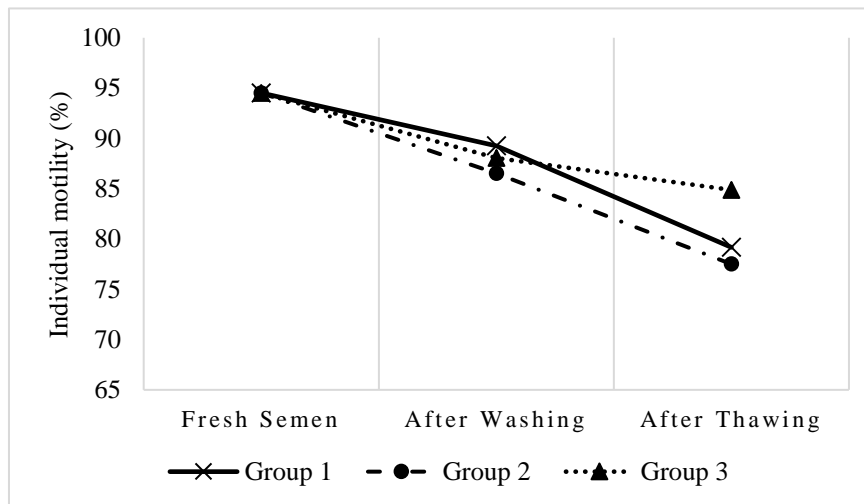


Figure 1. Individual motility of PE buck sperms of fresh semen, after washing and after thawing at different sperms concentrations. Sperm concentration group ($\times 10^6$ cells/straw) i.e., 100 (group 1); 150 (group 2); 200 (group 3).

The decrease in sperms motility is caused by several factors, one of them is the occurrence of cold shock during the freezing process. The use of high velocity during centrifugation and separation of semen plasma can impair the sperms [7,8]. During the sperm freezing process, cold shock results in loss of selective permeability and plasma membrane integrity, release of intracellular enzymes and lipids, redistribution of ions and resulting in permanent changes to the acrosome and mitochondrial membranes, loss of motility and decreased metabolism [9]. The cold shock incident that causes the formation of ice crystals is one of the main biophysical factors that result in cell death during cryopreservation [10]. The clotting process can also induce lipid peroxidation, which causes structural damage to spermatozoa accompanied by decreased motility, membrane integrity, and spermatozoa fertilization capacity [11,12].

3.2. Progressive motility of PE buck sperms after washing

Progressive motility of PE buck sperms of fresh semen, after washing and after thawing at different sperms concentrations in the straw are presented in figure 2. It is expected that there is a decrease in the progressive motility of the PE buck sperms from fresh semen to after washing and after thawing at each concentration (figure 2). The progressive motility of the sperms at fresh semen was 85.9%, it decreases to 76.9%, 74.0%, and 75.4%, respectively in each group. Likewise, a decrease of progressive motility also occurs in each group after thawing to 63.2%, 64.4%, and 73.9%. A previous study [13] stated that fertile males have progressive motility values ranging from 50-80%, this indicates that the progressive motility shown in figure 2 is still in the normal range. Progressive motility has an important role in successful fertilization. Sperm that have good progressive motility can be a reference for the ability of sperm to fertilize the ovum [14]. One of the factors that influence the decline in the progressive value is that when the freezing process occurs a metabolic change occurs causing the medium to become acidic. Acidic conditions are toxic and cause death in spermatozoa [15]. When handling the semen, particularly during cooling, freezing, and thawing, it affects the membrane and the stability of the function of spermatozoa cells, and it can interfere with spermatozoa metabolism which is a factor in decreasing the percentage of progressive motility of spermatozoa [16].

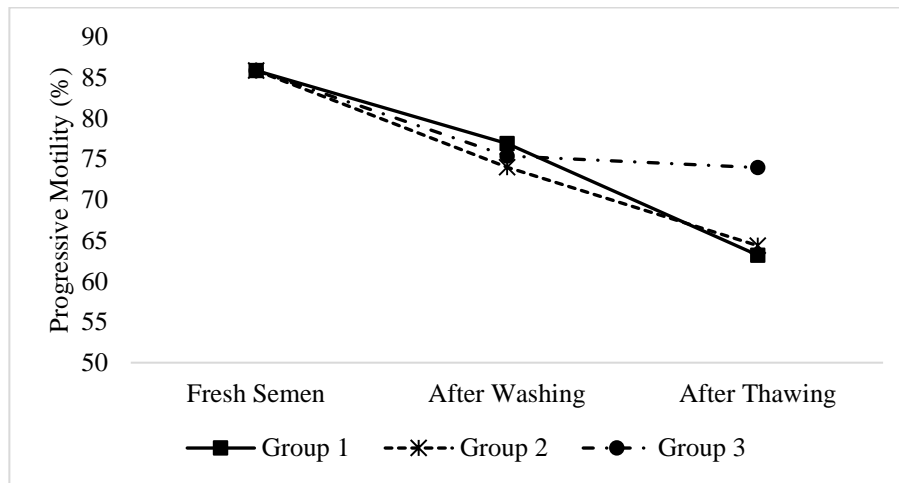


Figure 2. Progressive motility of PE buck sperms of fresh semen, after washing and after thawing at different sperms concentrations. Sperm concentration group ($\times 10^6$ cells/straw) i.e., 100 (group 1); 150 (group 2); 200 (group 3).

3.3. Viability of PE buck sperms after washing

The viability of PE buck sperms of fresh semen, after washing and after thawing at different sperms concentrations in the straw are presented in figure 3.

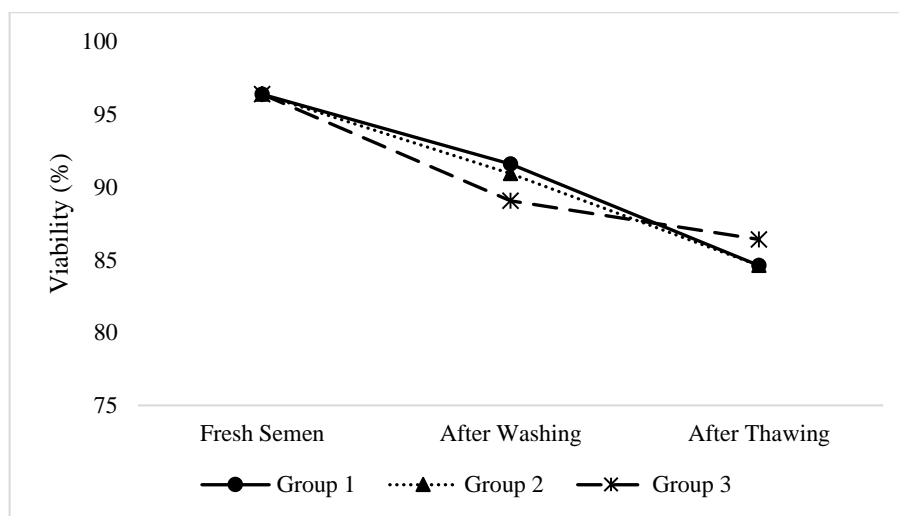


Figure 3. Viability of PE buck sperms of fresh semen, after washing and after thawing at different sperms concentrations. Sperm concentration group ($\cdot 10^6$ cells/straw) i.e., 100 (group 1); 150 (group 2); 200 (group 3).

Figure 3 shows that there is a decrease in the viability of the PE buck sperms from fresh semen to after washing and after thawing at each concentration. The viability of sperms at fresh semen was an average value of 96.4%, and it decreased after washing to 91.6%, 90.9%, and 89.05%, respectively at each group. The decrease in the viability after thawing was also occurred to 84.6%, 84.6%, and 86.4%, respectively in each group. However, the decrease in viability seems to be linear in each group and did not show any significant difference in each group. A study [17] stated that the percentage value of good spermatozoa viability is above 50%. For PE goats, a study [5] stated that the percentage of goat spermatozoa is 82.54% on average. This suggests that the viability of the sperms shown in figure 3 is

still in the normal range. The integrity of the cell membrane is closely related to the viability of spermatozoa. The permeability of the plasma membrane selectively regulates intracellular molecular activity, pH, and ion composition. The plasma membrane has an important role in the entry and exit of substances and ions that are required in the process of metabolism thus maintain electrolyte balance intracellular and extracellular [18].

4. Conclusion

This study concluded that the quality of Etawah crossbreed frozen semen after washing using centrifugation and at different concentration in the straw were able to maintain the motility and viability of the semen after thawing.

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