

Improvement quality of Bligon goat sperm trough separation by albumen

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ABSTRACT: This experiments were conducted to evaluate the influence of different concentration of albumen gradient in sperm separation on sperm quality of Bligon goat. Semen from 8 heads male Bligon goats aged around 2 years was collected by artificial vagina with frequency of once in four days. Sperm separation was conducted with different albumen gradient. Medium I: 5% upper fraction and 10% lower fraction, medium II: 10% upper fraction and 30% lower fraction, and medium III: 15% upper fraction and 45% lower fraction. The results showed sperm separation with different albumen gradient was significantly difference ($P < 0.01$) on motility, viability, and abnormality of sperm, respectively. Sperm motility before separation and after separation on medium I, II and III upper fraction was 80.5; 82.1; 83.5 and 85.2%, lower fraction was 80.5; 86.1; 87.2 and 88.3% respectively. Sperm viability before separation and after separation on medium I, II and III upper fraction was 90.4; 93.6; 93.1 and 93.6%, lower fraction was 90.4; 86.1; 87.2 and 88.3% respectively. Sperm abnormality before separation and after separation on medium I, II and III upper fraction was 13.1; 11.2; 9.2 and 7.1%, lower fraction was 13.1; 6.1; 5.3 and 4.2% respectively. The conclusion of this study was that there was albumen gradient may be used for improve sperm quality by increase motility and viability and decrease abnormality.

Key words: Bligon Goat, sperm quality, sperm, separation, albumen

INTRODUCTION

Small ruminant animal especially Bligon goats have very important role in providing animal protein. Bligon goats are good meat provider and they are suitable to be raised in villages due to the fact that it is not necessary to provide big capital. Demand for goats has been increasing from year to year, including demand for export (Anonym, 2007). The increased demand for goats may cause decreased in their population if there is no attempt to increase their population. On one side, this is a good opportunity for the farmers or breeders to improve their life, but on the other side, this situation threatens the population of the goats. For this reason, attempt is needed to increase the population of Bligon goats without disturbing the population for fulfilling the demand for export. One of the efforts to expand population is by improving animal reproduction. One of the efforts to improve goat productivity and reproductivity is by adopting artificial insemination (AI). The worth of AI technology can be improve by improvement of sperm quality. Sperm quality improvement can be done by sperm separation. Sperm separation could be done with several ways, sephadex filtration, density percoll sentrifugation gradient (Susilawati dan Sumitro, 1998), albumen gradient (Rasyid *et al.*, 2006; Pratiwi *et al.*, 2006), *swim-up* (Palomo *et al.*, 1999).

Albumen is a name usually refers to transparent liquid contained in an egg. Naturally, albumen has function to protect yolk and to provide additional nutrition for the development of an embryo. Studies on albumen for medium of sperm cell separation have been conducted on sperm of Ongole-bred cows (Afiati, 2004), Boer goats (Sonjaya, *et al.*, 2005).

Albumen is not toxic to the sperm and consists of protein, mineral, fat, and glucose as energy source for sperm. Several globular protein for example *ovalbumin*, *ovotransferin*, *ovomucoid*, *globulin*, *lysozyme*, *ovomucin*, dan *avidin*. Ovalbumin is the highest protein percentage in eggs, about 54%, molecule weight 45.000 with protein globular structure (Gossett *et al.*, 1984; Raikos *et al.*, 2006). It could be use as sperm medium separation, first tried in Ongole cross bred sperm (Pratiwi *et al.*, 2006; Rasyid *et al.*, 2006; and Afiati, 2004) and Boer goats (Sonjaya *et al.*, 2005).

Sperm separation influenced, motility, viability, and abnormality (Palomo, 1999). Sperm with low motility, dead, and abnormal decreased moreover lost their strength to migrate into separation medium (White, *et al.*, 1984; Silverman *et al.*, 2002). Separation used centrifuge could be harmful to the sperm (Lopez *et al.*, 1996; Carvajal *et al.*, 2004; Padila and Foote, 1991; Susilowati, 2007). The

damage caused by centrifugation could break the sperm plasma membrane. Centrifugation process did not allow too fast and long time (Rijsselaere *et al.*, 2002).

The aims of this research are to evaluate the effect of different concentration of albumen gradient on motility, viability and abnormality of Bligon goat sperm in the process of sperm separation.

MATERIALS AND METHODS

Sperm from 8 heads male Bligon goats aged around 2 years was collected by artificial vagina with frequency of once in four days. Yolk citrate extender, albumen, *aquabidest*, larutan eosin, *penicillin* dan *streptomycin*.

Experiment was sperm cell separation of Bligon goat. The separation was conducted on medium of three different concentration of albumen gradient; medium I: 5% upper fraction and 10% lower fraction, medium II: 10% upper fraction and 30% lower fraction, and medium III: 15% upper fraction and 45% lower fraction. Before separation sperm was evaluated the motility, viability, and abnormality.

Two (2) ml sperm diluted to $300 \times 10^6/\text{ml}$ into the albumen which divided in two (upper and lower) fraction (2 ml each fraction) with concentration as the treatment. Sperm left in 28°C for 30 minutes and each fraction of albumen filled into the tube which contained 2 ml yolk citrate diluter then centrifuged 2.500 rpm ($r=14,5$ cm) for 10 minutes. Supernatan through out 3 ml and the separation resulted 1 ml.

After separation sperm motility, viability, and abnormality was evaluated to compare before and after separation.

Data of motility, abnormality, and motile sperm percentage were analyzed using nested completely randomized design, and if there was difference then followed by DMRT (Astuti, 1980).

RESULTS AND DISCUSSION

Sperm Motility

The average motility of separated sperm of Bligon goat with different concentration of albumen gradient is shown in Table 1.

Table 1. The average motility, viability and abnormality of separated sperm of Bligon goat with different concentration of albumen gradient

Variable	Before Separation	Medium I		Medium II		Medium III	
		Upper Fraction	Lower Fraction	Upper Fraction	Lower Fraction	Upper Fraction	Lower Fraction
Motility, %	80.5 ^a ± 1.2	82.1 ^b ± 1.4	86.1 ^c ± 1.1	83.5 ^d ± 1.2	87.2 ^e ± 1.1	85.2 ^f ± 1.3	88.3 ^g ± 1.3
Viability, %	90.4 ^a ± 2.5	93.6 ^b ± 2.9	93.4 ^b ± 2.4	93.1 ^b ± 3.3	93.1 ^b ± 2.4	93.6 ^b ± 2.4	93.4 ^b ± 2.2
Abnormality, %	13.1 ^a ± 0.4	11.2 ^c ± 0.4	6.1 ^d ± 0.3	9.2 ^e ± 0.2	5.3 ^f ± 0.4	7.1 ^g ± 0.3	4.2 ^h ± 0.3

^{a,b,c,d,f,g} Within a row, means without common superscript difference ($P<0.01$).

Results of variance analysis showed that sperma separation with different concentration of albumen gradient affected ($P<0.01$) on sperm motility compared to before the separation. Sperm separation may affect sperm motility. Immotile sperm will have difficulties to migrate to the separation medium (White, *et al.*, 1984). Only sperm with high motility, therefore, which was able to migrate to the separation medium, thus the final result of sperm separation was better motility than before separation.

Upper Fraction. Comparing medium I, II and III on the upper fraction, it indicated that the sperm motility was increased. The higher the concentration of albumen the higher the sperm motility. This was because only sperm with high motility which might migrate to higher concentration of separation

medium . Sperm motility on the upper fraction medium for before separation groups, medium I, II and III were 80.5; 82.1; 83.5 and 85.2% respectively.

Lower Fraction. Comparing medium I, II and III on the lower fraction, it showed that sperm motility was increased. The higher the concentration of albumen the higher the sperm motility. This was due to the higher the concentration of the medium the more difficult the sperm to migrate. Therefore, only sperm with high motility which might migrate to higher concentration of separation medium. Sperm motility on the lower fraction medium for before separation group, medium I, II and III were 80.5; 86.1; 87.2 and 88.3% respectively. Comparison between results of upper and lower fraction. Comparison between upper and lower fraction on each medium was as follows:

Medium I, Sperm Motility of The Upper Fraction was Lower than The Lower Fraction. The motility of the upper fraction was 82.1% and the lower was 86.1%. This was because sperm with higher motility could migrate to the medium with high concentration and therefore, on medium with higher concentration, it was produced sperm with higher motility too.

Medium II, Sperm Motility of The Upper Fraction was Lower than The Lower Fraction. The motility of the upper fraction was 86.1% and the lower was 83.5%. This was because sperm with high motility could migrate to the medium with high concentration and thus, on medium with higher concentration, it was produced sperm with higher motility.

Medium III, Sperm Motility of the Upper Fraction was Lower than The Lower Fraction. The motility of the upper fraction was 85.2% and the lower was 88.3%. This was due to sperm with high motility could migrate to the medium with high concentration and thus, on medium with higher concentration, sperm motility would be higher too.

The percentage of motility of sperm produced from sperm separation in this study was good. Sperm motility of goat and sheep is 75% and good sperm motility is higher than 60% (Gomes, 1997). Factors affecting motility after sperm comes out of testes are sun rays, oxygen, sperm metabolism, osmotic pressure, microbes, temperature and thinning (Toelihere, 1985), pigment concentration, sperm washing, thinner and color mixture (Salisbury and VanDemark, 1985).

Based on the above explanation, medium III was able to increase sperm motility with the highest percentage. The percentage of sperm motility on the upper fraction was 85.2% and the lower was 88.3%.

Sperm Viability

The average percentage of viability of separated sperm of Bligon goat with different concentration of albumen gradient is shown in Table 1. Results of variance analysis showed that sperm separation with albumen gradient on different concentration affected ($P<0.01$) on sperm viability compared to before separation. However, on medium I, II and III, there was no significant difference on the upper and lower fraction. Immotile sperm could not move and migrate to the separation medium both on the lowest (medium I) and the highest concentration (medium III) and thus on the medium I, II and III there was no significant difference, both on the upper and the lower fraction. Immotile sperm could not migrate to the separation medium and remained in the medium. Sperm in the separation medium was alive sperm, thus on the final results of separation, sperm viability would be higher than before separation. This study also showed that medium used for sperm separation was in compliance with the requirements of sperm medium (Bearden *et al.*, 2004).

Factors affecting sperm viability are direct sunrays exposure, osmotic pressure, electrolyte and non electrolyte effect, microbes, temperature of storage and thinning would affect sperm viability (Toelihere, 1985). Good sperm viability of goat is 90%, but if this cannot be achieved, viability not less than 50% may produce good fertility.

Sperm Abnormality

The average percentage of abnormality of separated sperm of Bligon goat with different concentration of albumen gradient is shown in Table 1. Results of variance analysis showed that sperm separation with albumen gradient on different concentration affected ($P<0.01$) on sperm abnormality compared to before separation.

Sperm with abnormal morphology would be blocked by separation medium and it had difficulties to migrate in the separation medium. Sperm succeeding to enter separation medium was abnormal sperm, and thus the percentage of sperm abnormality after separation was better than before separation.

Upper fraction. Comparing medium I, II and III on the upper fraction, it showed that sperm abnormality was decreasing. This was due to the higher the concentration of the medium the more difficult the sperm to enter the medium. Only the sperm with normal morphology and motile which could enter the separation medium and therefore the percentage of sperm abnormality in the separation medium was lower. Sperm abnormality in the upper fraction medium for before separation group, medium I, II and III were 13.1; 11.2; 9.2; and 7.1 respectively.

Lower fraction. Comparing medium I, II and III on the lower fraction, it showed that sperm abnormality was decreasing. The higher the concentration of the albumen the lower the sperm abnormality. This was due to the higher the concentration of the medium the more difficult the sperm to enter the medium. Only the sperm with normal morphology and motile which could enter the separation medium. Sperm abnormality in the lower fraction medium for before separation group, medium I, II and III were 13.1; 6.1; 5.3; and 4.2 respectively.

Comparison between results of upper and lower fraction. Comparison between upper and lower fraction on each medium was as follows:

Medium I, sperm abnormality in the upper fraction was higher than the lower. Sperm abnormality in the upper fraction was 11.2% and the lower was 6.1%. This was because many sperm with abnormal morphology would be difficult to enter the medium, thus in higher concentration, the abnormality was decreasing.

Medium II, sperm abnormality in the upper fraction was higher than the lower. Sperm abnormality in the upper fraction was 9.2% and the lower was 5.3%. This was due to many sperm with abnormal morphology would be difficult to enter the medium, thus in higher concentration, the abnormality was decreasing.

Medium III, sperm abnormality in the upper fraction was higher than the lower. Sperm abnormality in the upper fraction was 7.1% and the lower was 4.2%. This was because many sperm with abnormal morphology would be difficult to enter the medium, thus in higher concentration, the abnormality was decreasing.

Based on the above description, medium III would decrease the percentage of sperm abnormality with the lowest percentage. The percentage of sperm abnormality in the upper fraction was 7.1% and the lower was 4.2%.

Sperm abnormality is categorized into primary and secondary abnormality. Primary abnormality is caused by spermatogenesis disorders in the tubulus semineferus, while secondary abnormality occurs after sperm leaves tubulus seminiferus (Bearden *et al.*, 2004). Abnormality after sperm ejaculated is affected by temperature, direct sunrays, excess warming, quick cooling, contaminated by water, urine or antiseptic (Toelihere, 1985). Proper treatments of sperm after collecting will minimize the rate of sperm abnormality. According to Hernawan (2005), sperm abnormality of goat is 14.71% and according to Wasiyatun (2000) is 15.34%.

CONCLUSION

The conclusion of this study was that sperm separation with albumen could increase the percentage of motility, viability and decrease the percentage of sperm abnormality. Separation in medium III (albumen concentration of upper fraction 15% and the lower fraction 45%) offered the best result on the percentage of motility, viability and abnormality of sperm.

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