Level of nutrition affects semen characteristics and freezability of Malaysian bucks

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Abstract

Feeding is a major problem in goat production in Malaysia particularly it affects reproductive efficiency of goats. This research was aimed to confirm the effect of improper nutrition and management practice on the buck's fresh and frozen-thawed semen quality. Bucks were challenged nutritionally by the standard feeding (standard-nutrition) and underfeeding (under-nutrition), i.e. inadequate daily requirement of Napier grass (*Pennisetum purpureum*) and pelleted compound feed. Bucks from three different breeds (Jermasia n=4, Boer n=1 and Katjang n=1) were used for semen collection and semen freezing. The fresh semen quality was used as control. Fresh semen qualities, i.e. volume (ml/ejaculate), mass movement, concentration ($\times 10^9$/ml) and motility (%) were 0.48, 3.92, 3.97, 82.5 for Jermasia, 0.62, 4.00, 4.20, 86.00 for Boer, 0.55, 3.5, 5.35, 87.50 for Katjang, respectively while the values for frozen-thawed sperm motility for Jermasia, Boer and Katjang were 55.83, 66.00 and 67.50%, respectively. In comparison of the different nutritional regimes, for fresh semen qualities, i.e. volume (ml/ejaculate), mass movement, concentration ($\times 10^9$/ml) and motility (%) were 0.56, 4.00, 4.34 and 86.00 for standard-nutrition and 0.38, 3.50, 3.57 and 76.25 for under-nutrition, respectively, while the values for frozen-thawed sperm motility for standard-and under-nutrition were 63.33 and 46.25%, respectively. It is imperative that adequate nutrients in proper feeding be given serious attention to ensure the success of semen collection and semen freezing.

Keywords: breed, freezability, goat, nutrition, semen

Introduction

In small ruminants including caprine, modern Advanced Reproductive Techniques (ART) are being used for the improvement and preservation of livestock breeds and the enhancement of reproductive efficiency (Rahman *et al.*, 2008). Artificial insemination (AI) involves collection of semen from a buck and manually transferring the semen to the reproductive tract of the doe. The use of frozen semen for AI involving Jermasia and Katjang caprine breeds has been well documented in Malaysia by Nor Ashikin (1994). In order to ensure high quality semen is preserved for future use, it is crucial that each ejaculate be evaluated prior to freezing. Good progressive motility of sperm of more than 60%, reduced morphological abnormalities of less than 25% (Bearden and Fuquay, 1980) and a high concentration of sperm (Kalaimathee, 1988) will ensure high conception rate. In caprine, the infertility of the buck is one of the drawbacks that cause fluctuation over the quantity and quality of caprine production. Therefore, by performing AI technique combined with good quality frozen semen, the caprine production can be further improved.

Nutrition is an important factor which controls the production of sperm and

development of accessory fluids. Several studies on nutrition in rams have demonstrated that diet may have an effect on testis size and sperm production (Brown, 1994). A low plane of nutrition not only caused a decrease in libido, percentage live sperm and motility but also led to an increase in the percentage of abnormal sperm in caprines (Hiroe and Tomizuka, 1965). In most developing countries, there are problems with the quality and quantity of feed and forage due to natural calamities, poor soil fertility and lack of natural grasslands (Devendra and Leng, 2011), which may have impact on the semen quality with subsequent effects on ruminant production. In Malaysia, the AI technique in goats has yet to be fully commercialised up to this time. Improvement is still required for the technique to be practical enough for local farmers. The main objectives of this study were to determine the quality of fresh and frozen-thawed semen from three caprine breeds and to evaluate the effect of feeding regime on semen quality and freezability of semen.

Materials and Methods

Four mature Jermasia males, one Boer male and one Katjang male were used in this study. The average age and body weight of the six males were three years and 35 kg, respectively. The animals were kept in individual pens in wooden sheds with raised flooring, zinc roofing and slatted flooring at the Institute of Biological Sciences Mini Farm, University of Malaya, Kuala Lumpur, Malaysia. All bucks received either standard-nutrition or under-nutrition in two different phases. In phase 1 (September – December 2013), all the bucks received standard-nutrition. As for standard-nutrition diet, the bucks were fed pelleted compound feed and fresh Napier grass (*Pennisetum purpureum*) with respect to their energy requirement according to National Research Council (NRC, 2007). In the morning, before the grass was fed to the bucks, pellet was given at the rate of 500 g/animal/d. Fresh Napier grass was given twice a day; once in the morning and another in the afternoon at the rate of 1.5 kg fresh weight/animal/d. According to NRC (2007), daily approximate metabolisable energy (ME) requirement for 35-kg adult buck is 1670.6 kcal. Based on the theoretical calculation, if the bucks took all the feed given, they would have received 1314.5 kcal (from pelleted compound feed) and 537.8 kcal (from Napier grass). Hence, total ME for daily feeding of each buck was approximately 1852.3 kcal. This amount of ME was above the maintenance requirement of 35-kg buck's body weight.

In phase 2 (January – April 2014), all of the bucks received under-nutrition diet. As for under-nutrition, the bucks were fed with inadequate compound pelleted feed and fresh Napier grass with respect to their energy requirement. In the morning, before the grass was fed to the bucks, pelleted compound feed was given at the rate of 100 g/animal/d. Napier grass was given the same amount as in standard-nutrition. Based on theoretical calculation, if the bucks took the feed completely, they would have received 262.9 kcal (from pelleted compound feed) and 537.8 kcal (from Napier grass). Hence, total ME for daily feeding (under-nutrition) of each buck was approximately 800.7 kcal. This amount of ME was less than daily energy requirement of the bucks as the essential amount of ME for maintenance was 1670.6 kcal. In both phases, a salt lick was provided for each pen and fresh water was provided *ad libitum*.

Semen collection was performed using an artificial vagina. The presence of oestrus does as teasers was required. Collection of semen was carried out in the morning between 0800 to 1100 hours. The semen was collected at least five times from

each individual buck. Semen was diluted with tris-citric acid egg yolk glycerol extender at 37 °C to a final concentration of 100 million sperms/ml. Diluted semen was cooled 4 °C and packed into 0.5 ml French straws. The straws were kept 2-3 cm above the liquid nitrogen and plunged into liquid nitrogen. Finally, the straws were stored in liquid nitrogen before evaluated to determine freezability of corresponding semen. At least 100 straws were prepared from each individual buck. The measurements of parameters included the volume per ejaculation, mass movement, sperm concentration and sperm motility. The volume of ejaculates was evaluated directly from calibrated tubes used for the semen collection. Motility of sperm was evaluated by visual estimation using naked eyes. The concentration of sperm in a sample was determined using a haemocytometer. Semen was frozen in straws as described by Nor Ashikin (1994) before evaluated to determine frezzability of corresponding semen. Evaluation of mass movement was performed by placing fresh semen on a pre-warmed slide under a light microscope using a scoring system from 0-5 as shown in Table 1. In semen thawing, the frozen straws taken out from the liquid nitrogen tank were immediately immersed in a water bath at 37 °C for 2 min. The straws were thoroughly wiped dry before cutting off the end to place a drop of frozen-thawed semen on the slide for evaluation. Frozen semen were thawed a wk after freezing date for second evaluation to determine sperm freezability.

Table 1: Rating system for mass movement (Nelson and Lin, 1983)

Score	Class	Description
5	Very good	Dense, very rapid wave motion. 90% or more of the spermatozoa are active.
4	Good	Vigorous wave motion but not as rapid as score 5. 70-85% of sperm cells are active.
3	Fair	Slow wave motion. About 45-65% of sperm cells are active.
2	Poor	No wave motion, but some movement of sperm is visible. About 20-40% of sperm cells are alive but poor motility.
1	Very poor	Only about 10% of sperm show signs of live. Only weak movement.
0	Dead	All sperm cells are motionless.

Statistical Analysis

The data presented for effect of breeds on semen quality were analysed using one-way ANOVA and significant differences between the means were analysed using Duncan Multiple Range Test (DMRT). The t-test was used to estimate the significance difference of means between standard- and under-nutrition feeding. Experimental data were expressed as mean ± SEM. A probability of $P<0.05$ was used to be significantly different. The analysis was carried out using SPSS (version 20).

Results and Discussion

Table 2 shows the effects of buck breeds on fresh semen characteristics and semen freezability regardless of nutrition level. There was no significant ($P>0.05$) difference between breeds on semen characteristics and their freezability. For Jermasia and Boer, percent motility of fresh semen was higher than frozen-thawed semen.

Katjang breed showed the highest fresh semen quality in terms of sperm concentration and percent motility while Boer breed showed the highest fresh semen quality in terms of semen volume and mass movement of sperm.

Table 2: Effect of different buck breeds on fresh semen characteristics and sperm freezability regardless of standard-and under-nutrition

Semen characteristics	Breed		
	Jermasia	Boer	Katjang
Mass movement*	3.92±0.13[a]	4.00±0.21[a]	3.50±0.33[a]
Semen volume/ejaculation (ml)	0.48±0.05[a]	0.62±0.07[a]	0.55±0.12[a]
Sperm concentration/ml ($\times 10^9$)	3.97±0.40[a]	4.20±0.62[a]	5.35±0.98[a]
Sperm motility of fresh semen (%)	82.50±2.23[a]	86.00±3.46[a]	87.50±5.47[a]
Sperm motility of frozen-thawed semen (%)	55.83±3.48[a]	66.00±5.39[a]	67.50±8.52[a]

*Mass movement was evaluated based on Rating System of Nelson and Lin (1983).
[a]Mean values within a row with same superscripts were not significantly different (P>0.05).

Table 3 shows the effect of nutrition on semen characteristics and semen freezability regardless of buck's breed. All semen characteristics, except sperm concentration were significantly (P<0.05) higher in standard-nutrition than under-nutrition. For both standard- and under-nutrition of all the breeds studied, the percent motility of fresh semen were significantly (P<0.05) higher than frozen-thawed semen. Percent motility of fresh and frozen-thawed semen of standard-nutrition was significantly (P<0.05) higher than under-nutrition. For standard-nutrition, the fresh semen quality was higher in terms of all characteristics compared to the under-nutrition.

Table 3: Effect of nutrition on fresh semen characteristics and sperm freezability in bucks regardless of buck's breed

Semen characteristics	Standard-nutrition	Under-nutrition
Mass movement*	4.00±0.12[b]	3.50±0.21[a]
Semen volume/ejaculation (ml)	0.56±0.04[b]	0.38±0.08[a]
Sperm concentration/ml ($\times 10^9$)	4.34±0.36[a]	3.57±0.69[a]
Sperm motility of fresh semen (%)	86.00±1.69[b]	76.25±3.27[a]
Sperm motility of frozen-thawed semen (%)	63.33±2.73[b]	46.25±5.29[a]

* Mass movement was evaluated based on Rating System of Nelson and Lin (1983).
[ab]Means within a row with different superscripts were significantly different (P<0.05).

The findings of this study showed that there were differences in freezability (percent motility of sperm) between fresh and frozen thawed spermatozoa in all breeds. This was considered normal in cryopreservation process whereby it decreased the motility of sperm in livestock animals including caprine species (Dorado *et*

al., 2005; Martinez-Pastor *et al.*, 2005; Thurston *et al.*, 2011). The reason for Katjang breed had better freezability than Jermasia and Boer was not known, however, it could be due to the small sample size of the former leading to large standard error for the mean. Nor Ashikin (1994) concluded that freezability of Jermana buck was better compared to Katjang and Jermasia. However, the high sperm freezability of Jermasia also followed with high post-thawed abnormality in this breed. In previous research, some semen were more affected by cryoinjury than others (Corteel *et al.*, 1987) which was probably attributable to their membrane biochemical and biophysical properties (Arav *et al.*, 2000). This gave an idea that the even if Katjang breed had better freezability in this study, it might actually have been caused by individual variation rather than the breed itself.

In this study, Katjang breed showed the highest fresh semen quality in terms of sperm concentration and percent motility. The mean value of sperm concentration/ml and percent motility was 5.35×10^9 and 87.5%, respectively. The concentration recorded was lower than those reported by Noran *et al.* (1998), with concentration 6.19×10^9 and 5.82×10^9 for first and second ejaculates, respectively. Meanwhile, the percent motility reported in their research was lower compared to this study, with percent motility 77.61 and 81.97% for first and second ejaculates, respectively. Boer breed showed the highest fresh semen quality in terms of semen volume and mass movement of sperm. The higher semen volume in Boer bucks may have been caused by larger body size compared to small native Zambian goats (Igboeli, 1974).

In standard and under-nutrition, there was a significant difference in freezabilty of the sperm. This indicated that both nutritional regimes affected the freezability of sperm significantly. Previous researchers have reported that cryopreservation induced injuries in sperm cells (Watson, 2000) and consequently reduced the sperm motility, membrane integrity and freezability (Purdy, 2006). In fact, under the best experimental condition only one half of the population of motile spermatozoa could survive the freeze-thaw process (Watson, 1995).

Conclusion

Katjang breed showed the highest sperm concentration and percent motility, whereas Boer breed showed the highest semen volume and mass movement of sperm, although no significant difference was observed among the breeds. The nutritional regime is therefore imperative in determining successful collection and production of good quality fresh semen.

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