Semen Quality Assessment of Local Katjang and Cross-Bred (Katjang × German) Bucks

A. M. Noran[1], T. K. Mukherjee[2] and R. Abdullah[3]
Department of Zoology, Faculty of Life Sciences, UKM, 43600 Bangi, Malaysia

ABSTRACT : Semen quality was compared between the local Katjang and the cross-bred (local Katjang ♀ × German Fawn ♂) bucks. There were no significant genotypic differences in semen characteristics of concentration (*first ejaculate*: 6.19 ± 1.30 - versus 6.33 ± 1.40 × 10^9/ml; *second ejaculate*: 5.82 ± 1.10 - versus 5.68 ± 1.45 × 10^9/ml, for Katjang and the cross-breds, respectively), percentage live (*first ejaculate*: 77.61 ± 1.33% versus 77.81 ± 0.53%; *second ejaculate*: 81.97 ± 1.59% versus 82.74 ± 0.96%, for Katjang and cross-breds, respectively) and percentage of normal sperms (*first ejaculate*: 12.54 ± 3.88% versus 26.45 ± 3.83%; *second ejaculate*: 38.68 ± 3.65% versus 28.54 ± 4.38%, for Katjang and cross-breds, respectively), with the exception of seminal volume and sperm motility. Means of all variables were within the values reported for other goat breeds. In contrast, the differences in semen characteristics between the first and second ejaculations of both genotypes were more distinct, the second ejaculations always had more volume, more normal sperms and better sperm motility but less sperm concentrations. Removing the seminal plasma and replacing it with tris-citrate buffer greatly prolonged the viability of sperms of both genotypes when stored at 5℃. Sperm motility seems to be a good indicator of sperm viability. However, the sperms of the cross-bred bucks withstood the washing process better and their swimming abilities were superior (8.12 ± 0.46 mm/min) when compared to those of the local Katjang breed (5.42 ± 0.49 mm/min). The higher content of calcium ions in their seminal plasma (*first ejaculate*: 10.5 ± 0.8 versus 10.6 ± 0.8 mg/100 ml; *second ejaculate*: 15.3 ± 0.8 versus 16.1 ± 0.8 mg/100 ml, for Katjang and cross-breds, respectively) means that in natural matings the sperms of the cross-breds would be at an advantage compared to those of the local Katjang, since calcium ions reportedly initiate acrosomal reactions.
(Key Words: Local Katjang, Cross-Bred, Semen Quality)

INTRODUCTION

Common indicators used to evaluate semen quality included sperm concentration, percentages of sperm abnormalities and live-dead ratio, semen volume and pH, sperm initial motility and mass movements (Berndtson and Pickett, 1980). The criteria is based on the general observation that a decline in semen quality is invariably accompanied by gross reduction in the value of initial motility, sperm numbers and an increase in the proportion of abnormal sperms because seminal attributes move in one direction. However, it is too time consuming to carry out all the tests mentioned when dealing with large stock populations. Selection of one or two of these characteristics which are reliable measure of sperm viability, would greatly aid the routine screening of large numbers of samples.

Under present farm practices in the tropics, the problems of getting males of good stock for natural mating purposes have led to efforts to collect and conserve semen of good breeders for artificial inseminations. Problems arose in the case of the bucks because some component of the seminal plasma was deleterious to the sperms on exposure to egg-containing extenders (Roy, 1957) which led to various efforts to remove the seminal plasma, including washing, prior to preservation attempts at low temperatures in various extenders (Ritar and Salamon, 1982).

In this study, the quality of fresh semen of the local Katjang and cross-bred (local Katjang ♀ × German Fawn ♂) bucks from the first and second ejaculations was assessed on the basis of semen characteristics such as sperm concentration (× 10^9/ml), seminal volume (ml),

[1] Address reprint requests to A. M. Noran.
[2] Institute of Postgraduate Studies and Research (IPSP), University of Malaya, 50603 Kuala Lumpur, Malaysia.
[3] Institute of Biological Sciences, University of Malaya, 50603 K. Lumpur, Malaysia.
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percentage of live sperms (% live), percentage of normal sperms (% normal), percentage of progressive forward motility (% PFM) and mass movements (MM). This was followed by analysing the effects of washing with tris-citrate buffer followed by storage at 5℃ for 0, 2, 4, 6 and 24 hours, compared to fresh semen. Finally, the swimming rates of washed sperms in the vaginal discharge of oestral does as well as the ionic contents of calcium, magnesium, potassium and sodium in the seminal plasma were measured.

MATERIALS AND METHODS

Animals and semen collection

Ten local Katjang and 10 cross-bred (local Katjang ♀ × German Fawn ♂) adult bucks of nearly the same age were selected and kept in individual pens under one shed. The animals were fed with fresh Napier grass twice daily, supplemented with pellets comprising of molasses, brewer's waste, palm oil sludge, and poultry waste. A salt lick was provided together with *ad libitum* supply of water. Two ejaculations were collected from each buck in the morning prior to feeding, using a teaser female and the artificial vagina (AV). Semen was collected from only 5 bucks each day so as to facilitate semen processing.

Semen processing

Each tube of collected semen was centrifuged at 30,000 rpm for 10 minutes and the separated seminal plasma was placed in another tube which was labelled and stored in the freezer for mineral content analyses. Tris-citrate buffer (37℃), at 10 times the original seminal volume, was immediately added to the solid residue of sperms left after centrifugation and mixed thoroughly.

Vaginal discharge collection

All goats at the IPSP farm were detected for heat twice daily with a buck, once in the morning and again in the afternoon. Only the vaginal discharges on the first day of heat that were watery and clear in appearance were collected in microhaematocrit tubes (B.S. 4316) placed in the vagina. The tubes were then sealed with plasticine and stored in the freezer.

Experimental designs

Four experiments were conducted. In the first experiment semen characteristics of concentration, volume, percentage live (% live), percentage normal (% N) and percentage of progressive forward motility (% PFM) scores for the first and second ejaculations from each buck were recorded. In the second experiment, effects of a single washing procedure with tris-citrate buffer on semen characteristics of % live, % normal, % of primary (% 1°), secondary (% 2°) and tertiary (% 3°) abnormalities, % PFM and mass movements (MM) on storage at 5℃ were compared to that of fresh semen. In the third experiment, the swimming rates of sperms were tested by thawing the required number of micro-haematocrit tubes containing vaginal discharges in a water bath (37℃) for about 5 minutes before placing them vertically in testtubes containing the washed sperm samples to be tested. After 5 minutes the tubes were checked under a light microscope and the distance travelled by the furthest sperm marked and then measured (mm). The fourth experiment was conducted to determine the mineral contents of the seminal plasma by preparing 1 ppm concentrations of the seminal plasma samples in 100 ml volumetric flasks and the contents of calcium, magnesium, potassium and sodium ions were determined with an Atomic Ion Analyser.

RESULTS

Experiment 1

Comparisons of the mean semen concentration, % live sperms and % normal sperms (table 1), of the first as well as the second ejaculations, between the local Katjang and cross-bred bucks were not significantly different. However, for each genotype, the difference in seminal motility (% PFM) and volume between the first and second ejaculations were significant ($p < 0.05$), being more motile as the volume increased for the second ejaculations. Furthermore, the volume was always significantly less for the local Katjang compared to the cross-bred ($p < 0.05$), for both the first and second ejaculations.

Experiment 2

For both genotypes, the semen characteristics of % live, % PFM and MM scores of fresh semen changed significantly ($p < 0.05$) after just 2 hours of storage at 5℃ (table 2). In contrast, the % normal, % 1° (head), % 2° (midpiece) and % 3° (tail) abnormalities remained unchanged even after 8 hours storage. For washed semen, the % live, % normal, % 1°, % 2° and % 3° abnormalities did not change significantly even after 24 hours of storage. However, the washing process resulted in the mean values of percentage of primary abnormalities to be higher ($p < 0,01$) at zero storage hour compared to those of fresh semen, with the sperms of the local Katjang being more vulnerable ($p < 0.001$) compared to those of the cross-breds. The % PFM and MM scores did not change significantly up to 6 hours of storage and only

Table 1. Mean values of some semen characteristics of local katjang and cross-bred bucks

Variables	Local Katjang		Cross-bred	
Ejaculate number	First	Second	First	Second
Sperm Concentration ($\times 10^9$/ml)	6.19±1.30 (194)	5.82±1.10 (154)	6.33±1.40 (200)	5.68±1.45 (193)
Seminal Volume (ml)	0.60±0.02 (245)	0.78±0.02 (159)	0.90±0.02 (329)	1.02±0.01 (154)
Live Sperms (%)	77.61±1.33 (161)	81.97±1.59 (96)	77.81±0.53 (217)	82.74±0.96 (154)
Motility (%)	80 (49)	86 (56)	75 (60)	77 (70)
Normal Sperms (%)	12.54±3.88 (161)	38.68±3.65 (96)	26.45±3.83 (217)	28.54±4.38 (154)

Values in parentheses are the number of samples.
Means of all variables differed between 1st and 2nd ejaculates ($p < 0.05$).
Means of seminal volume and sperm motility also differed between Katjang and cross-bred bucks ($p < 0.05$).

Table 2. Means of several variables of fresh and washed semen of both buck types combined over storage time intervals at 5℃

Storage hours		Parameters					
	% Live	% Normal	%1° Abnormality	%2° Abnormality	%3° Abnormality	% PFM	MM
(i)	76.51±1.47^a	25.04±0.09^b	0.98±0.06^b	21.63±0.06^a	51.99±0.03^a	61.64±4.70^{bc}	4.36
(ii)	72.49±1.92^{ab}	49.78±1.93^a	6.65±2.90^{ab}	9.21±0.54^c	34.78±4.89^b	76.41±1.23^a	4.85
(i)	61.59±2.68^{bc}	31.70±0.41^b	0.78±0.05^b	20.09±0.36^a	48.27±0.21^a	37.18±1.23^d	3.10
(ii)	–	–	–	–	–	73.60±0.19^{ab}	4.83
(i)	58.46±1.50^c	28.38±2.36^b	0.79±0.32^b	19.38±1.45^{ab}	51.30±0.91^a	28.71±4.05^d	2.94
(ii)	–	–	–	–	–	71.24±0.40^{ab}	4.80
(i)	57.18±3.66	28.66±1.50^b	1.39±0.12^b	20.08±1.06^a	49.86±1.07^a	29.90±3.03^d	2.66
(ii)	–	–	–	–	–	68.53±0.02^{ab}	4.70
(i)	54.79±1.56^c	29.68±1.85^b	1.16±0.64^b	17.95±1.41^{ab}	51.20±0.78^a	28.98±4.77^d	2.78
(ii)	–	–	–	–	–	–	
(i)	–	–	–	–	–	–	
(ii)	65.99±3.00^{abc}	43.39±2.68^a	11.49±3.97^{bc}	14.18±1.69^{bc}	30.95±3.00^b	55.42±0.84^c	4.16

(i) Fresh semen.
(ii) Washed semen.
Different superscripts within a column indicate significant difference ($p < 0.05$) with Duncan's Multiple Range Test.

declined after 24 hours. Hence, a single wash appeared to be sufficient in enhancing the viability period.

Experiment 3

There was significant variation between the two genotypes ($p < 0.001$) with regard to swimming rates of sperms. The mean swimming rate was higher for the cross-breds (8.12 ± 0.46 mm/min) than the local Katjang (5.42 ± 0.49 mm/min) (table 3). Effect of individual buck of both genotypes was also significant ($p < 0.05$).

Experiment 4

The mean values of the concentrations of the four ions, namely calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) in the seminal plasma are presented in table 4. Statistical analyses showed concentrations of Ca ($p < 0.001$) and Mg ($p < 0.05$) were significantly different between the 2 genotypes, being higher for the cross-breds. There was no effect of ejaculation number on the concentration of any of the four ions studied.

Table 3. Swimming rates (mm/min) of sperms of local katjang and cross-bred bucks

Local Katjang			Cross-bred (Local Katjang × German Fawn)		
Indiv.	n	x ± S.E.	Indiv.	n	x ± S.E.
1	4	5.20 ± 1.50^{abc}	1	5	7.06 ± 1.34^{abc}
2	4	7.40 ± 1.50^{abc}	2	5	8.40 ± 1.34^{abc}
3	4	7.35 ± 0.50^{abc}	3	5	6.96 ± 2.22^{abc}
4	4	6.85 ± 1.50^{abc}	4	4	9.68 ± 1.50^{ac}
5	4	3.8 ± 1.50^{ab}	5	–	–
6	4	2.95 ± 2.13^{a}	6	4	7.45 ± 1.50^{abc}
7	4	4.58 ± 1.50^{abc}	7	5	8.74 ± 1.34^{abc}
8	3	3.80 ± 1.74^{abc}	8	5	7.70 ± 1.34^{abc}
9	3	5.53 ± 1.74^{abc}	9	4	9.48 ± 1.50^{ac}
10	4	5.53 ± 1.50^{abc}	10	4	8.03 ± 1.50^{abc}
10	36	5.42±0.49	9	41	8.12±0.46

Significant differences exist between individual bucks ($p < 0.05$) and both genotypes in Duncan's Multiple Range Test.

Table 4. Mineral contents of seminal plasma (mg /100 ml) of first and second ejaculates of local katjang (LK) and cross-bred (CB) bucks.

Mineral	First Ejaculate (x ± S.E.)		Second Ejaculate (x ± S.E.)	
	LK	CB	LK	CB
1. Calcium	10.5 ± 0.8 (53)^{a}	15.3 ± 0.8 (51)^{b}	10.6 ± 0.8 (49)^{a}	16.1 ± 0.8 (51)^{b}
2. Magnesium	9.2 ± 0.5 (51)	10.0 ± 0.5 (51)	8.9 ± 0.5 (51)^{a}	10.3 ± 0.5 (51)^{b}
3. Potessium	174.1±15.2 (53)	192.2±15.5 (51)	164.4±15.8 (49)	196.4±15.5 (51)
4. Sodium	235 ±33 (43)	184 ±35 (38)	188 ±35 (39)	219 ±35 (39)

Values in parentheses are the number of samples.
^{ab} Values within the same row (within ejaculate) with different superscripts differ at $p < 0.05$.

DISCUSSION

When the semen of the two genotypes under study were compared, the only significant differences were for the seminal volume and sperm motility (% PFM), the former being higher in the cross-breds and the latter in the local Katjangs. Both variables together with other sperm characteristics of concentration, % live and % normal sperms varied significantly between the first and second ejaculates of each genotype and their values were always higher for the second ejaculate with the exception of sperm concentration. It, therefore, appears that ejaculation number, rather than genotypic differences, is the primary factor affecting semen quality. In addition, semen from the second ejaculation had more volume but sperm count did not differ, as reflected by the lower concentration, thus allowing more sperms to be normal and an increase in sperm motility. Overall, the mean values obtained for the various semen characteristics in both genotypes fell well within the ranges reported for goat breeds (Blokhuis, 1962).

When the storage abilities (at 5℃) of fresh and washed semen were compared, the mean values for % live, % PFM and MM at the commencement of the storage process for the former fell significantly after 2 hours for both genotypes while for the latter, even after 24 hours of storage the mean values for all 3 seminal characteristics did not differ significantly from those at the commencement of storage. Furthermore, the mean values of % normal, % PFM and MM significantly fell and % abnormalities rose only after 24 hours storage for washed semen compared to 8 hours for fresh semen. The sperms of the local Katjang appeared to be more vulnerable to the washing process, indicative by the higher percentage of primary abnormalities compared to those of fresh semen at zero storage hour, than those of the cross-breds. It can, therefore, be concluded that generally a single wash is sufficient in improving storage abilities of goat sperms at low temperature and supports earlier reports that the presence of the seminal plasma in

fresh semen affected the viability of sperms over a period of time (Iritani and Nishikawa, 1961; Ritar and Salamon, 1982). Furthermore, the sperms of the cross-breds are more hardy and can withstand the washing process better than those of the local Katjangs.

The mean values of the swimming rates of sperms between the two genotypes were significantly different, being faster for the cross-breds. Thus, swimming abilities alone gave an advantage to the cross-bred sperms as it meant less exposure to the hostile acidic environment of the oestrus mucus, since sperms reportedly required alkaline conditions (Tampion and Gibbons, 1963) for maximum viability.

The observations that there were no differences in concentrations of sodium and potassium ions in the seminal plasma of the first and second ejaculations and between the two genotypes, were in accordance with the observations reported for the Zambian and Boer (Igboeli, 1974) as well as the Cheghu and Changthang goat breeds (Bhat and Singh, 1987). However, the magnesium and calcium ions concentrations were observed to be significantly higher in the cross-breds, in contrast to Igboeli's (1974) assertions that there were no breed differences in their concentrations. Potassium ions in the medium has been reported to increase the duration of sperm motility and survival at 37℃ (Shannon and Curson, 1987) while calcium ions initiated acrosomal reactions that led to successful fertilizations (Memon and Ott, 1981). Hence, in natural matings the cross-bred bucks would be at a slight advantage compared to the local Katjangs.

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