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Physical Characteristics of Malabari Buck Semen and Sperm Characteristics of Semen with Good and Poor Freezability

Ambili John [1], Hiron M Harshan [2], Mayur Pawshe [3], K N Aravinda Ghosh [4], R Thirupathy V [5], T V Aravindakshan [6]

Department of Animal Reproduction, Gynaecology and Obstetrics
College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala 680 651

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ABSTRACT

The present study investigated for any difference in fresh semen characteristics in Malabari buck semen which could predict its freezability. Ninety-six ejaculates from eight Malabari bucks were collected; twice a week, at regular intervals using artificial vagina and cryopreservation was carried out by manual method. Creamy semen colour was the characteristic feature throughout the study. The mean values for physical constituents were; ejaculate volume 0.62 ± 0.04 ml, density DDDD, mass activity ++++, sperm concentration 2867.13 ± 203.29 million/ml, initial motility 88.75 ± 0.27 per cent, live sperm count 91.74 ± 0.3 per cent and abnormal sperm count 1.80 ± 0.10 per cent. Mean values for spermatozoa progressive motility, viability and abnormality after extension, at pre-freeze and post-thaw stages were recorded and analyzed. Out of the eight bucks subjected to freezability studies, four were identified as having good freezable quality semen and the semen of four animals was identified as not suitable for cryopreservation based on the post-thaw evaluation. Fertility studies proved that those animals with low semen freezability had lower fertility also.

Key Words: Malabari Buck, Semen, Freezability, Quality, Spermatozoa, Fertility.

INTRODUCTION

Goats have become popular due to their easy acclimatization, high prolificacy, low cost of maintenance and short gestational period. India ranks first among the countries of the world in goat population with 22 distinct breeds of goat. Rearing of goats has become popular in Kerala and artificial insemination (AI) using frozen semen is gaining importance in the state.

The success of AI depends on the management of semen collection, storage and use (Leboeuf *et al,* 2000). Frozen-thawed semen presents advantages over fresh or refrigerated semen, because of its longevity and transportability. Cryopreservation causes greater damage to spermatozoa resulting in lower fertilizing ability compared to fresh and refrigerated semen. The cryopreservation of mammalian spermatozoa is a complex process that involves balancing of many factors in order to ensure satisfactory results. Even though there are many similarities between sperm of goat and other domestic animals, goat sperm require unique attention to maximize the post-thaw quality (Purdy, 2006). Identification of physical traits of semen or markers which could predict freezability of semen is thus of great importance. Hence, this study was undertaken to evaluate the physical qualities and sperm properties of Malabari buck semen with high

*Corresponding Author: *ambili.john@kau.in

Assistant Professor, Krishi Vigyan Kendra Kannur, Kerala Agricultural University 9447913577

[2]Assistant Professor, Dept. of Animal Reproduction, Gynaecology and Obstetrics, KVASU, Pookod 9495661032

[3]Subject Matter Specialist (Animal Reproduction), WRTC, MAFSU, Nagpur 9747205002

[4]Retired Professor and Head, Dept. of Animal Reproduction, Gynaecology and Obstetrics, KVASU, Pookod 9447174765

[5]Professor, Dept. of Animal Genetics and Breeding, KVASU, Pookod 9747728625

[6] Professor and Head, Centre for Advanced studies in Animal Genetics and Breeding, KVASU, Pookod

Table 1. Malabari buck semen characteristics immediately after collection and after extension in Tris based extender.

Ejaculate Quality	Buck no.	Fresh Semen (n=12)		Sperm characteristics after extension (n=6, pooled)		
		Volume (ml)	Concentration (millions/ml)	Progressive Motility (in %)	Viability (in %)	Abnormality (in %)
High freezability	1	0.73 ± 0.09	2823.25 ± 221.76	87.5 ± 1.15	90.83 ± 0.86	1.42 ± 0.09
	2	0.54 ± 0.03	2227.67 ± 58.60	88.33 ± 1.12	90.92 ± 1.03	2.13 ± 0.19
	3	0.49 ± 0.02	4016.33 ± 183.77	90 ± 0.87	92.55 ± 0.64	1.71 ± 0.14
	4	0.69 ± 0.06	2514.5 ± 162.13	88.75 ± 1.25	91.63 ± 0.80	1.82 ±0.12
	Mean	0.61 ± 0.03	2895.44 ± 128.24	88.54 ± 0.55	91.48 ± 0.43	1.77 ± 0.08
Low freezability	5	0.6 ± 0.03	2568.75 ± 133.50	88.75 ± 1.25	91.3 ± 0.93	1.82 ± 0.11
	6	0.61 ± 0.05	2798.83 ± 100.49	88.33 ± 1.12	91.18 ± 0.66	2.07 ± 0.2
	7	0.73 ± 0.09	3144.33 ± 177.17	90.42 ± 0.74	93.23 ± 0.42	2.12 ± 0.15
	8	0.84 ± 0.80	2736 ± 129.18	90 ± 0.87	93.17 ± 0.68	1.6 ± 0.16
	Mean	0.69 ± 0.03	2811.98 ± 73.35	89.38 ± 0.51	92.22 ± 0.37	1.9 ± 0.08

Means did not differ significantly between rows.

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and low freezability and their fertility post AI.

MATERIALS AND METHODS

Twelve pooled duplicate semen ejaculates were collected from each of eight adult healthy Malabari bucks stationed at the AI centre of College of Veterinary and Animal Sciences, Mannuthy. The collections were taken twice a week, at regular intervals using Danish type artificial vagina maintained at 43ºC under adequate pressure. Two false mounts were given for stewing the bucks before semen collection. Immediately after collection, the semen was transferred to a water bath maintained at 37º C. The ejaculate was subjected to macroscopic and microscopic evaluation for assessing volume, colour, density, mass activity and concentration. After assessing the sperm concentration, the semen sample was extended with a Tris based extender (Tris - 3.028 g, Citric acid – 1.675 g, Fructose – 1.25 g, Egg yolk – 5 ml, Glycerol – 6 ml, Benzyl penicillin – 1000IU/ml, Streptomycin - 1000µg/ml, Millipore water to make up to 100 ml) maintained at 37ºC so as to contain 300 million progressively motile spermatozoa per ml. Spermatozoa progressive motility, viability and abnormality were assessed after extension. The rest of the extended semen was packaged in 0.5 ml French straws and subjected to equilibration in a cold handling cabinet. The straws were allowed to equilibrate for a period of 3 h after attaining a temperature of 5ºC. Spermatozoa progressive motility, viability and abnormality were assessed after equilibration as pre-freeze evaluation. The equilibrated straws were subsequently subjected to manual freezing in a Styrofoam box by exposing to nitrogen vapour at ultra low temperatures. The frozen straws were subsequently stored in a canister with goblet maintained under adequate liquid nitrogen in a cryocan.

Thawing of semen was done after at least 24 h of cryopreservation at 37º C for one min. Post-thaw spermatozoa progressive motility, viability and abnormality were assessed. Based on the post-thaw motility, the bucks were classified as having either high or low semen freezability. Semen having post-thaw motility above 35 per cent were considered as

Table 2. Malbari buck Semen characteristics at pre-freeze and post-thaw stages of cryopreservation

Ejaculate Quality	Buck no.	Pre-freeze Sperm characteristics (n=6, pooled)			Post-thaw Sperm characteristics (n=6, pooled)		
		Progressive Motility (in %)	Viability (in %)	Abnormality (in %)	Progressive Motility (in %)	Viability (in %)	Abnormality (in %)
High freezability	1	76.67 ± 1.42	80.53 ± 0.94	2.63 ± 0.22	41.67 ± 1.42	44.86 ± 1.42	4.042 ± 0.26
	2	78.33 ± 1.12	81.68 ± 1.11	3.52 ± 0.22	40 ± 0.87	42.7 ± 0.97	4.72 ± 0.28
	3	80.83 ± 1.04	84.17 ± 0.70	3.43 ± 0.54	43.75 ± 1.09	48.58 ± 1.44	5.34 ± 0.57
	4	79.17 ± 1.35	81.87 ± 1.32	2.67 ± 0.14	44.17 ± 1.20	48.67 ± 1.19	4.29 ± 0.23
	Mean	78.75 ± 0.64	82.06 ± 0.54	3.06 ± 0.17	42.39 ± 0.61	46.20 ± 0.72	4.60 ± 0.19
Low freezability	5	78.33 ± 1.28	80.83 ± 1.07	3.18 ± 0.11	18.33 ± 1.42	23.63 ± 1.39	6.1 ± 0.46
	6	78.33 ± 1.28	80.97 ± 1.09	3.63 ± 0.25	19.16 ± 1.35	21.48 ± 0.84	5.52 ± 0.22
	7	80 ± 0.87	83.37 ± 0.57	3.27 ± 0.20	20 ± 0.87	23.25 ± 1.25	6.1 ± 0.27
	8	78.33 ± 1.42	81.35 ± 1.73	2.35 ± 0.24	13.33 ± 1.12	17.08 ± 1.52	5.13 ± 0.31
	Mean	78.75 ± 0.60	81.63 ± 0.59	3.10 ± 0.12	17.71 ± 0.70	21.36 ± 0.73	5.71 ± 0.17

Means with different superscripts between rows differed significantly ($p<0.01$)

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having high semen freezabiltiy and those with less than 30 per cent post-thaw motility were classified as having poor semen freezability (Hidalgo *et al,* 2007). Cryopreserved semen of bucks with low and high semen freezability were assessed for their first service conception rate. A total of 91 (minimum 10 does each with each buck) adult healthy Malabari does of proven fertility were inseminated employing a double intra cervical insemination protocol at 24 h interval.

RESULTS AND DISCUSSION

The mean values of different parameters of fresh semen and after extension are presented in Table 1. Pre-freeze and post-thaw semen parameters are given in Table 2.

The mean ejaculate volume in bucks with high semen freezability was 0.61 ± 0.03 ml and in bucks with low semen freezability it was 0.69 ± 0.03 ml while the mean sperm concentration was 2895.44 ± 128.24 million/ml and 2811.98 ± 73.35 million/ml respectively. All the ejaculates were observed to have creamy colour, DDDD density and ++++ mass activity. No significant difference was observed in these parameters between semen of high and low freezability. In high and low semen freezability animals, the mean spermatozoa progressive motility, viability and abnormality assessed after extension

Table 3. Conception rate after AI using cryopreserved semen of high and low semen freezability Malabari bucks

Animal No	Freezability status of the ejaculate	No. of inseminations	No.of animals conceived	Conception rate
1	High freezability	12	6	50
2		11	6	55.55
3		11	5	45.45
4		13	6	46.15
Mean		47	23	49.04 ± 3.22[a]
1	Low freezability	10	2	20
2		12	4	33.33
3		11	3	27.27
4		11	2	18.18
Mean		44	11	24.69 ± 3.48[b]

Means with different superscripts between columns differed significantly (p<0.01)

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were 88.54 ± 0.55, 91.48 ± 0.43, 1.77 ± 0.08 per cent and 89.38 ± 0.51, 92.22 ± 0.37, 1.9 ± 0.08 per cent, respectively. Mean pre-freeze spermatozoa progressive motility, viability and abnormality in high and low semen freezability animals were respectively 78.75 ± 0.64, 82.06 ± 0.54, 3.06 ± 0.17 and 78.75 ± 0.60, 81.63 ± 0.59, 3.10 ± 0.12 per cent. In high and low semen freezability animals, the mean post-thaw spermatozoa progressive motility, viability and abnormality in high and low semen freezability animals were 42.39 ± 0.61, 46.20 ± 0.72, 4.60 ± 0.19 per cent and 17.71 ± 0.70, 21.36 ± 0.73, 5.71 ± 0.17 per cent respectively. An overall conception rate of 49.04 ± 3.22 and 24.69 ± 3.48 per cent was observed respectively in high and low semen freezability animals, which differed significantly (p<0.01).

The present study identified four bucks as having semen with good freezability and four having semen with poor freezability based on post-thaw semen characteristics. Spermatozoa progressive motility, viability and abnormality assessed after extension or at pre-freeze stage did not differ significantly between high and low semen freezability animals whereas the post-thaw values were significantly lower (p<0.01) in semen of low semen freezabiltiy animals when compared to high semen freezabiltiy animals. Thus no differences were noted in fresh semen or pre-freeze semen characteristics of Malabari bucks with high or low semen freezability.

Semen parameters recorded in the present study were similar to those recorded in previous studies with Malabari breed of goat (Bhai, 2012; Behra, 2012). Cryopreservation induces detrimental effects in sperm cells, resulting in a reduction of motility, membrane integrity and fertilizing ability (Purdy, 2006). In the present study, cryopreservation provoked a reduction in motility and viability of frozen spermatozoa; however, the mean values obtained fall within the ranges obtained by other authors (Ritar and Ball, 1993; Aboagla and Terada, 2004 and Dorado *et al,* 2009). Verstegen *et al* (2002) suggested that because sperm survival, motility and fecundity are decreased after thawing of frozen semen, the characteristics of fresh semen correlate poorly with quality of the inseminating post-thaw specimen. Dorado *et al* (2009) reported that total motile spermatozoa, abnormal sperm morphology, acrosome membrane integrity, lateral head

displacement, mean path velocity and curvilinear velocity before freezing could be used as predictors of sperm freezability.

Dorado *et al* (2010) reported no significant difference between ejaculates of Florida bucks having good semen freezability or poor semen with respect to their fresh or pre-freeze spermatozoa motility while the abnormal sperm morphology percentage differed significantly between these groups at fresh, pre-freeze and at post-thaw stages. Inter-male variability in sperm freezability has been recognized a source of variation during freeze-thawing (Holt, 2000), and it is necessary to assess the semen quality after freezing-thawing to define the donor bucks as good or bad freezers (Lebouf *et al,* 2000).

Conception rate after AI using cryopreserved semen of high and low semen freezability Malabari bucks is given in Table 3. The conception rates obtained with cryopreserved semen of low semen freezability bucks was significantly ($p < 0.01$) lower than the conception rate with cryopreserved semen of bucks with high semen freezability. This observation was expected as all the parameters of semen quality assayed at post thaw for the semen of bucks with low semen freezability were significantly lower ($p < 0.01$) than those of semen from bucks with high semen freezability.

Hidalgo *et al* (2007) reported similar conception rate (47.6 %) using frozen semen after a double intra cervical insemination. Dorado *et al* (2009) reported 42.86 per cent conception rate following double intra cervical insemination of frozen thawed semen. Rajan (2010) obtained a lower conception rate following single insemination with cryopreserved semen (27.27%) in Malabari does. Pawshe (2016) recorded a lower conception rate of 40 per cent in Malabari does inseminated at os cervix on two consecutive days.

CONCLUSION

It was concluded that assessment of fresh semen characteristics or pre freeze semen characteristics could not be used as a measure of the freezability of Malabari buck semen. Additionally, it was also be inferred that as the fresh semen and prefreeze semen of bucks with low semen freezability had similar quality as those with better semen freezability, they could probably be used for natural mating or AI with chilled semen.

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