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Comparative Semen Evaluation of Malpura and Bharat Merino Rams by Computer-aided Sperm Analysis Technique Under Semi-Arid Tropical Environment

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Abstract: The present study was conducted to compare sperm motion characteristics of adult Malpura and Bharat Merino rams by the computer-aided sperm analysis (CASA) technique. Malpura is a hardy native sheep breed of the semi-arid tropical environment and Bharat Merino is a crossbred evolved in the same environment by crossing native sheep with exotic rams. Semen was collected from 8 donor rams of each breed at the onset of autumn season on 5 occasions at three days interval. The data were analyzed by analysis of variance using the general linear model repeated measures procedure. The CASA parameters which differed significantly ($p<0.05$) between the breeds were rapid motile sperm, medium motile sperm, slow motile sperm, linearity, straightness, curvilinear velocity, average path velocity, straight-line velocity, amplitude of lateral head displacement, beat frequency, sperm head area and sperm head elongation with higher values in all traits in Malpura breed. The semen volume and sperm concentration were higher in Bharat Merino breed but the differences were not significant. The body weight of rams had significant ($p<0.05$) effect on mass motility, curvilinear velocity and amplitude of lateral head displacement. The mass motility was higher in rams of more than 50 kg body weight while curvilinear velocity and amplitude of lateral head displacement was higher in rams of less than 50 kg body weight. A significant ($p<0.05$) influence of age of rams was observed on linearity and amplitude of lateral head displacement. The linearity was higher in rams of less than 3.5 years of age whereas amplitude of lateral head displacement was higher in rams of more than 3.5 years of age. In conclusion, CASA derived sperm motion characteristics revealed that the semen quality of native Malpura rams was better compared to crossbred Bharat Merino rams during major breeding season in a semi-arid tropical climate.

Key words: Bharat berino, breed, computer-aided sperm analysis, malpura, semi-arid climate and sheep

INTRODUCTION

Sheep farming plays an important role in agrarian economy by providing a major source of livelihood to large number of small, marginal and landless farmers in hilly, arid and semi-arid regions of India. Malpura is a hardy native sheep breed of the semi-arid tropical environment and is reared for wool and mutton production. Breed characteristics of Malpura sheep have been reported under farm (Arora *et al*., 1975; Acharya, 1982) and field conditions (Mehta *et al*., 1995). Mishra *et al.* (2005) have compiled in-depth information on genetic attributes, reproduction and production performance characteristics of this breed. Malpura ewes have been used for developing a prolific germplasm by introgression of fecundity gene (*FecB*) via artificial insemination (AI) using semen of small sized rams of prolific Garole sheep of West Bengal (Naqvi *et al.,* 2002; Sharma *et. al*., 2004). Bharat Merino sheep has been evolved for fine wool production by crossbreeding native ewes (Nali, Chokla, Malpura and Jaisalmeri) with exotic Rambouillet/Soviet Merino rams and stabilizing the population at 75% exotic inheritance (Singh *et al*., 2006). The Bharat Merino sheep has shown potential to perform well under semi-arid and sub-temperate agroclimatic conditions in India and act as a promising import substitute for exotic fine wool breeds expressed in terms of growth, reproduction, survivability and wool quality at par with exotics (Singh *et al*., 1999; Dixit *et al*., 2002). Genetically superior rams of these breeds are in great demand by the sheep owners and developmental agencies for improvement of farmer's flocks by natural mating. AI is an important management tool for optimizing production performance and maximizing the use of high value rams. One important element for selection of breeding rams for either natural mating or AI relies on semen quality evaluation. Subjective evaluation of semen quality attributes is simple but do not provide accurate estimates for correlating it with fertility (Rodriguez-Martinez, 2003). Computer-aided sperm analysis (CASA) quantifies wide range of parameters of sperm motility and provides a rapid and objective method for assessing the motility of ram spermatozoa (Joshi *et al*., 2003; Kumar *et al*., 2007; 2009). The aim of the present study was

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to compare sperm motion characteristics of adult Malpura and Bharat Merion rams during major breeding season under semi-arid tropical environment by automated CASA technique.

MATERIALS AND METHODS

The study was conducted at the Institute farm, which is located in the semi-arid tropical area of the country at 75º-28′E longitude, 26º-26′N latitude and at an altitude of 320 m above mean sea level with yearly minimum and maximum temperature of 7 and 46 ºC, respectively. The annual rainfall ranges from 200 to 500 mm with an erratic distribution mainly concentrated during July to August.

The experiment was carried out at the onset of autumn, when major breeding activities commence at the farm. Eight adult Malpura rams of 2.3 to 4.8 years of age and weighing 41 to 57 kg with an average body weight of 50.58±1.91 kg, and eight adult Bharat Merino rams of 1.8 to 3.6 years of age and weighing 32 to 58 kg with an average body weight of 44.81±3.46 kg were used as semen donors. The rams were grazed for 8-10 h daily on natural vegetation interspersed with seasonal shrubs, grasses and forbs (*Achyranthes aspera, Commelina forskalaei, Eleusine aegypticae and Sorghum helepense).* In addition to grazing, the rams were provided a concentrate mixture of 300 g/day. After grazing, the rams were housed in a chain-linked fence enclosure having asbestos sheet roof open from all the sides.

For semen collection in artificial vagina, the rams were let out of the enclosure of the semen collection shed one by one and allowed to mount on the estrus ewe, which was restrained in a service crate. Each ram was scheduled in a random order for a single ejaculation, 5 times at alternate days. It was ensured that the evaluation of the previous ejaculate was first completed before the next semen sample was collected. After ejaculation, the semen samples were transferred immediately to the laboratory and assessed for: (i) volume: measured directly to the nearest 0.1 ml using a graduated glass collection cup, (ii) mass motility: graded on 0-5 point scale (iii) concentration: determined using a spectrophotometer, previously calibrated with a hemocytometer, and expressed as 10^6/ml and (iv) sperm motion characteristics: objectively evaluated by computer-assisted sperm analysis (CASA) technique using Hamilton-Thorn Biosciences HTM-IVOS version 12.1 M, Beverly, MA, U.S.A., motility analyzer (Kumar *et al*., 2007; 2009).

Prior to CASA analysis, each sample was diluted to approximately 25 x 10^6 sperms/ml with normal saline solution at 37ºC during the whole experiment so that the time elapse between semen dilution and CASA was very small and the sperm survive till the completion of analysis. The semen analyzer was set-up as follows: Image type: Phase contrast; Frames at frame rate: 30 at 60/sec; Minimum contrast: 60; Low and high static size gates: 0.8 to 6.25; Low and high static intensity gates: 0.25 to 1.50; Low and high static elongation gates: 20 and 70; Default cell size: 5 pixels; Default cell intensity: 55; Magnification: 1.89. Twenty µl of the diluted sample was placed in a prewarmed Makler counting chamber (10 µm deep, Sefi-Medical Instruments Ltd., Haifa, Israel) and 5 fields per chamber were examined at 37ºC in the analyzer (Kumar *et al.,* 2007; 2009).

The parameters measured with the analyzer were: curvilinear velocity (VCL, µm/sec), average path velocity (VAP, µm/sec), straight line velocity (VSL, µm/sec), % motility, % rapid motility (VAP>75 µm/sec), % medium motility (10<VAP<75 µm/sec), % slow motility (0<VAP<10 µm/sec), % linearity (LIN), % straightness (STR), % elongation (ratio of minor axis/major axis x 100), area μm^2 (major axis x minor axis), beat frequency (BF, Hz) and amplitude of lateral head displacement (ALH, µm) of the spermatozoa.

The data on ejaculate volume, mass motility, sperm concentration and the CASA estimates were analysed by analysis of variance using the general linear model repeated measures procedure of SPSS 13.0 (SPSS Inc. Headquarters, Chicago, IL, USA) after arc sin transformation of the values in percentage with five levels of replicates as within subject variable and breed (Malpura and Bharat Merino), body weight (< 50 and > 50 kg) and age of ram lamb (< 3.5 and > 3.5 yrs) as between subject variables for each measure. Values were considered to be statistically significant when $p< 0.05$.

RESULTS AND DISCUSSION

Semen evaluation is an important aspect that must be accurately done to ensure the use of breeding rams with good fertility. *In vitro* evaluation of semen is of high diagnostics importance for assessing testicular and epididymal function of the male (Rodriguez-Martinez, 2003). CASA provides rapid, precise and validated objective sperm motion characteristics (Holt and Palomo, 1996) and has been applied for short-term (Joshi *et al*., 2001) and long-term preservation of ram spermatozoa (Edward *et al.,* 1995; Bag *et al.,* 2002a, b; 2004; Joshi *et al*., 2005, 2008). Centola (1996) demonstrated that CASA gives much more detailed results that are less changed by errors then the manual microscopic observation. CASA measurements are more closely related to fertility than are subjective motility measurements, even if the technicians estimating subjective motility are highly trained (Farrell *et al*., 1998). Recent developments in CASA technique have shown that it provides powerful insights into sperm function and semen heterogeneity (Holt *et al*., 2007).

The effects of breed, body weight and age of rams on semen attributes and track dimensions of spermatozoa are depicted in Table 1 and on sperm motion characteristics in Table 2. Breed had significant ($p<0.05$) effect on VCL, VAP, VSL, ALH, BF, sperm head area, rapid motile sperm, medium motile sperm, slow motile sperm, LIN,

Table 1: Effect of breed, age and body weight of rams on semen attributes and track dimensions of spermatozoa

Factors	N	Volume (ml)	Mass motility	Concentration (10^6/ml)	VCL (µm/s)	VAP (µm/s)	VSL (µm/s)	ALH (µm)	BF (Hz)	Area (μm^2)
Breed										
Bharat Merino	8	1.14±0.13	4.56±0.08	3186.8±200.3	228.6±6.48 [a]	119.4±4.68 [a]	83.8±4.06 [a]	9.2±0.11 [a]	24.5±0.70 [a]	7.3±0.19 [a]
Malpura	8	1.09±0.13	4.79±0.08	2549.8±200.3	253.3±6.48 [b]	179.6±4.68 [b]	155.6±4.06 [b]	7.0±0.11 [b]	32.3±0.70 [b]	6.2±0.19 [b]
Body weight										
< 50 kg	8	1.14±0.13	4.46±0.08 [a]	2750.6±200.3	252.7±6.48 [a]	154.1±4.68	123.2±4.06	8.5±0.11 [a]	29.1±0.70	6.6±0.19
> 50 kg	8	1.10±0.13	4.89±0.08 [b]	2986.0±200.3	229.2±6.48 [b]	145.0±4.68	116.1±4.06	7.7±0.11 [b]	27.7±0.70	6.8±0.19
Age										
< 3.5 yr	7	0.93±0.14	4.79±0.08	2604.8±217.7	233.0±7.02	147.3±5.09	119.1±4.41	7.8±0.12 [a]	28.2±0.76	6.8±0.20
> 3.5 yr	9	1.30±0.12	4.56±0.07	3131.8±181.2	248.8±5.84	151.7±4.24	120.3±3.67	8.4±0.10 [b]	28.6±0.64	6.7±0.17
Overall	16	1.12±0.09	4.68±0.05	2868.3±141.6	240.9±4.56	149.5±3.31	119.7±2.87	8.1±0.08	28.4±0.50	6.7±0.13

Values are mean ± SE and means with different superscripts (a and b) within columns differ significantly (p<0.05)

Table 2: Effect of breed, age and body weight on the motion characteristics of ram spermatozoa

Factors	n	Motility (%)	Rapid (%)	Medium (%)	Slow (%)	Linearity (%)	Straightness (%)	Elongation (%)
Breed								
Bharat Merino	8	73.55±1.75	55.92±1.46 [a]	23.78±0.90 [a]	12.71±0.87 [a]	38.40±0.40 [a]	57.06±0.64 [a]	47.71±0.46 [a]
		(91.0)	(67.6)	(15.2)	(3.8)	(37.6)	(69.4)	(53.7)
Malpura	8	76.59±1.75	69.49±1.46 [b]	13.40±0.90 [b]	7.74±0.87 [b]	52.76±0.40 [b]	67.80±0.64 [b]	45.09±0.46 [b]
		(93.6)	(86.7)	(4.4)	(0.98)	(62.4)	(84.7)	(49.1)
Body weight								
< 50 kg	8	73.72±1.75	62.99±1.46	17.54±0.90	9.53±0.87	45.02±0.40	62.49±0.64	46.41±0.46
		(91.1)	(78.4)	(8.1)	(1.7)	(49.0)	(77.7)	(51.5)
> 50 kg	8	76.42±1.75	62.42±1.46	19.64±0.90	10.92±0.87	46.15±0.40	62.37±0.64	46.40±0.46
		(93.5)	(77.6)	(10.3)	(2.6)	(51.0)	(77.5)	(51.4)
Age								
< 3.5 yr	7	74.69±1.91	61.06±1.59	19.89±0.98	10.87±0.95	46.33±0.44 [a]	62.81±0.69	46.13±0.50
		(92.0)	(75.6)	(10.6)	(2.6)	(51.3)	(78.1)	(51.0)
> 3.5 yr	9	75.46±1.59	64.36±1.32	17.29±0.81	9.58±0.79	44.83±0.37 [b]	62.05±0.58	46.67±0.41
		(92.7)	(80.3)	(7.8)	(1.8)	(48.7)	(77.0)	(51.9)
Overall		75.07±1.24	62.71±1.03	18.59±0.64	10.23±0.62	45.58±0.29	62.43±0.45	46.40±0.32
		(92.4)	(83.4)	(9.2)	(2.1)	(50.0)	(77.6)	(51.4)

Values are mean ± SE of the arc sin transformed values in percentage, whereas values in parenthesis are actual means of data. Means within the same column followed by dissimilar superscripts (a and b) are significantly (p< 0.05) different.

STR and sperm head elongation, with higher values of almost all the sperm motion characteristics in Malpura breed. The semen volume and sperm concentration were higher in Bharat Merino breed but the differences were not significant. The average ejaculate volume of both the breeds were in the range of average values of 1.0 - 1.5 ml reported for other sheep breeds (Chemineau *et al.*, 1991). The body weight of rams had significant (p<0.05) effect on mass motility, VCL and ALH, with higher mass motility in rams of more than 50 kg body weight and higher VCL and ALH in rams of less than 50 kg body weight.

A significant (p<0.05) influence of age of rams on LIN and ALH of spermatozoa was observed with higher LIN in rams of less than 3.5 years of age and higher ALH in rams of more than 3.5 years of age. Similar significant effect of age of ram on LIN has been reported in small size Garole (Joshi *et. al.,* 2003) and Garole x Malpura sheep (Kumar *et al.,* 2007). The interaction between breed and age was significant (P<0.05) for medium motile sperm; between breed and body weight for mass motility, medium motile sperm and LIN; between age and body weight for LIN; and between breed and age and body weight for LIN. The interactions for other traits were however not significant.

The majority of the spermatozoa were rapidly motile with very low population of medium and slow motile sperm reflecting optimum semen quality of high vigour in samples of both the breeds during major breeding season. Sperm velocity and motility are among the most important essential parameters in the examination of sperm quality and establishment of a correlation between sperm quality and fertility (Aitken, 1990; Vestergen *et al.*, 2002). Most of the evidences suggest a strong correlation between CASA measurements and fertility (Budworth *et al.*, 1988; Lavara *et al.*, 2005; Sutkevicience *et al.*, 2005). The higher values of sperm motility, LIN, STR, VCL, VAP, VSL and BF obtained in Malpura than Barat Merino rams might be attributed to breed differences as Malpura is a native sheep of semi-arid tropical environment.

CONCLUSION

In conclusion, CASA derived sperm motion characteristics revealed that the semen quality of native Malpura rams is better compared to crossbred Bharat Merino rams during major breeding season in a semi-arid tropical climate. Further studies are required to correlate motility parameters with fertility of ram semen.

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