The Indian Journal of Animal Reproduction; 24(2): 158-160; December 2003 **SHORT COMMUNICATION**

Studies on the effect of hydrogen ion concentration of extender on semen characters of Murrah buffalo bulls

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ABSTRACT

The effect of semen extender Hydrogen Ion Concentration (pH) on seminal characteristics of 27 Murrah buffalo bulls collected during the period from 1999 to 2001 were studied for Ejaculate volume, Initial motility, sperm concentration, pre-freezing and post thaw motility, livability and sperm head, midpiece and tail abnormalities. The mean ejaculate volume, initial motility, sperm concentration, pre-freezing and post-freezing motility was 6.35±0.09 ml, 87.66±0.65%, 1146.42±21.65 (10^6/ml), 86.57±0.15% and 64.80±0.36%, respectively. The ejaculate volume was higher in winter and initial, pre and post thaw motility in summer. Ejaculate volume and sperm concentration was lowest in summer. Effect of year and season of collection and the associated effect of the age of the bull and the sperm concentration were not important in pre-freezing but had significant effect on post thaw motility. The effect of bulls and extender pH groups and the associated effect of ejaculate volume and initial motility were needed to be considered for pre-freezing while sperm concentration in postthaw motility. The mean live and dead sperm % were observed to be 79.99±0.80 and 19.88±0.8% respectively. The head, midpiece and tail abnormalities were 1.70±0.09, 0.19±0.03 and 3.79±0.15%, respectively. Higher live sperms and lesser sperm abnormalities were observed when the pH of extender was between 6.7 to 6.8. The correlations between initial, pre and post thaw motility for different seasons were calculated and were generally significant. It was concluded that while freezing semen in Murrah buffaloe, the pH of extender should be between 6.7 to 6.8. The results also indicated necessity of further studies on microclimate of semen during freezing and for existing methods of estimation of sperm concentration to achieve higher post thaw motility.

Key words : Murrah bull, semen extender, pH, spermatozoa

Buffalo milk is still a major share in the milk production of the country. This animal thus plays an important role in the economy of village livestock industry. The A.I. is widely being accepted for improvement of buffalo, Murrah breed is preferred more in certain pockets of the country. The quality of semen and its delivery determines the success in A.I. Although, reports are available on the role of hydrogen ion concentration of semen extender in freezing semen, authors could not come across any report on breedwise variation in the effect of pH of extender on the semen characters. An attempt therefore was made to study some of the major seminal characteristics of Murrah bulls using Tris buffer extender with various pH values.

A total of 27 well-grown Murrah buffalo bulls ranging from 4.2 yrs to 7.3 years of age were selected for the present study. These bulls were healthy, free from reproductive and sexual disorders, housed in individual bull pens and were maintained at standard feeding and managerial conditions. Two collections per week per bull were taken regularly during the cooler hours of the morning period. Collections showing fresh sperm motility of less than 50% and sperm concentration of less than 600 millions/ml were rejected. The freshly collected semen was diluted using egg yolk tris dilutor. The pH of the extender was recorded before the semen was added to the diluter. Care was taken to ensure the even distribution of sperms in extended semen while the semen was being packed in the straws by automatic filling and sealing machine. The semen keeping thirty million sperms per dose was vapor frozen in automatic semen freezer in French mini straws and stored in liquid nitrogen. After 15 hours of storage the frozen semen was thawed in warm water (40°C) for 2 minutes for further evaluation. The seminal characters studied were initial motility (%) of the neat semen. Sperm concentration (estimated by photometer -(10^6/ml), pre-freeze motility (%) and post thawed motility (%) after freezing in the extended semen, live and dead sperms (%) and sperm abnormalities (head, midpiece and tail). The year was divided in three seasons (i.e. rainy (June-Sept.),

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Table 1. Year, season and extender pH groupwise means and standard errors of various characters in Murrah buffalo bull semen

Factors	Seminal characters	Ejaculate volume (ml)		Initial motility(%)		Sperm conc.(10^6/ml)		Pre-freeze motility(%)		Post-thaw motility(%)	
		N	Mean±SE	N	Mean±SE	N	Mean±SE	N	Mean±SE	N	Mean±SE
1. Year											
	1999	426	6.43±0.09^{bc}	426	87.8±0.23	426	1348.1±20.15^{bc}	426	87.2±0.23	426	62.1±0.38^{bc}
	2000	1023	6.58±0.05^{ca}	1023	88.2±0.15	1023	1149.6±16.17^{ac}	1023	87.5±0.13	1023	66.3±0.20^{a}
	2001	506	5.90±0.07^{ab}	506	87.7±0.20	506	970.6±11.50^{ab}	506	86.9±0.18	506	65.6±0.32^{a}
2. Season											
	Rainy	839	6.41±0.06	839	88.1±0.16	839	1194.7±13.49	839	87.3±0.16	839	64.1±0.27^{b}
	Winter	444	6.54±0.08	444	87.5±0.33	444	1150.5±31.70	444	87.1±0.18	444	65.7±0.26^{ab}
	Summer	672	6.22±0.07	672	88.3±0.11	672	1083.7±13.51	672	87.5±0.15	672	66.2±0.27^{b}
3. Extender pH groups x Pre-freeze motility groups											
	6.6 to 6.7x <= 80	92	6.20±0.21	92	84.8±0.45^{bcdegh}	92	1106.8±32.82	92	78.6±0.37^{bcdegh}	92	62.3±0.72^{bcegh}
	6.6 to 6.7x = 85	194	6.19±0.12	194	86.9±0.22^{acefh}	194	1105.8±33.73	194	85.0±0.00^{acefh}	194	64.6±0.55^{acefh}
	6.6 to 6.7x = 90	568	6.29±0.07	568	88.8±0.28^{abdefg}	568	1072.8±23.45	568	90.0±0.00^{abdfg}	568	66.9±0.21^{abdf}
	> 6.7 to 6.8x = 85	214	6.51±0.11	214	87.0±0.39^{abcefh}	214	1199.4±23.55	214	85.0±0.00^{acefh}	214	62.1±0.53^{cefgh}
	> 6.7 to 6.8x = 90	517	6.43±0.07	517	89.4±0.07^{abcdfg}	517	1228.2±17.15	517	90.0±0.00^{abdfg}	517	66.0±0.30^{abdfg}
	>6.8 <= 80	147	6.51±0.19	147	84.3±0.30^{bcdegh}	147	1259.7±49.12	147	78.1±0.53^{bcdegh}	147	60.4±0.80^{bcdegh}
	>6.8 = 85	93	6.51±0.17	93	87.0±0.30^{acefh}	93	1094.2±30.92	93	85.0±0.00^{acefh}	93	65.4±0.54^{adefh}
	>6.8 = 90	130	6.44±0.16	130	89.3±0.14^{abdfg}	130	1054.8±25.04	130	90.0±0.00^{abdfg}	130	67.7±0.59^{abdfg}
Total over all mean		1955	6.35±0.09	1955	87.66±0.65	1955	1146.42±21.65	1955	86.57±0.15	1955	64.80±0.36

Different superscript in the same column differ significantly ($P < 0.05$)

winter (Oct-Jan.) and summer (Feb-May). Based on the initial motility and the pH of extender, eight pH extender groups ranging from pH 6.6 to 6.8 and motility 80% to 90% compared for effect of pH. The data collected during three years i.e. July, 1999 to July, 2001 was utilized for this study and were analysed using standard statistical procedures described by Snedecor and Cochran (1966).

Year, season and extender pH group wise means and standard errors for ejaculate volume, initial motility, sperm concentration. Pre and post thaw motility of Murrah buffalo bull semen are presented in Table 1. The mean ejaculate volume, initial motility, sperm concentrations, pre-freezing and post freezing motility was 6.35±0.09 ml, 87.66±0.65%, 1146.42±21.65 (10^6/ml), 86.57±0.15% and 64.80±0.36%, respectively. The semen characters studied showed better performance in the year 2000 than in other years studied. The ejaculate volume was highest in winter while the initial, pre and post thaw motility were highest in summer. In summer season, however, semen volume and the sperm concentration was lowest.

The semen extender pH groups with initial motility of 80% and lower showed lower values of semen characters, while in a group ranging from pH above 6.7 to 6.8 showed highest values of semen characters when the initial motility was 90%. Lower pre and post freezing motility in Murrah breed has been reported by Dutta *et al.* (1994). The sperm concentration and seasonal variation in pre-freeze and post-thawed semen samples of Murrah buffaloe was in partial agreement with findings of A.K. Sharma *et al.* (1979) and Sall *et al.* (1980).

Analysis of variance was carried out to study the effect of year, season of semen collection, bull and extender pH groups on pre and post freezing motility. Since the age of the bull, ejaculate volume, initial motility and sperm concentration can have effect on pre and post thaw motility, these characters were included as covariate in the analysis. The differences in prefreezing motility due to year and season of collection and the associated effect of the age of the bull and the sperm concentration were not important but the bulls and extender pH groups and the associated effect of ejaculate volume and initial motility significantly contributed to the variation. In case of post freezing motility all the factors studied significantly affected the character. The associated effect of sperm concentration and initial motility was also significant on the post thaw motility. The effect of secretion of accessory glands thus increasing semen plasma, method of packing of semen dose (like German, French Mini or French Midi) can lead to expression of significant effect of initial sperm concentration and ejaculate volume on the post thaw motility. The results thus indicated need of further investigation in this respect.

The mean live and dead sperm% wee observed to be 79.99±0.80 and 19.88±0.8%, respectively. The Head, midpiece and tail abnormalities were 1.70±0.09, 0.19±0.03 and 3.79±0.15%, respectively. The analysis of variance indicated that pH group of semen sample showing initial motility of 90% showed higher live sperms and lesser sperm abnormalities when the pH of extender was above 6.7 to 6.8. The ejaculate volume, sperm concentration and extender pH groups was found significantly variable in different seasons of the years in Murrah bull semen and was in total agreement with the findings of Madhukar Bhosrekar (1980). Oloufa *et al.* (1959) and El Sawal *et al.* (1971) recorded highly significant seasonal variation in ejaculate volume of Egyptian buffalo bulls, which are in close agreement with present study. Also the present experiments findings were in close agreement with those of Oloufa *et al.* (loc. cit.), Van Denmark (1961) and Salisbury and Kodagali (1972).

Season wise correlations between initial motility and pre-freezing motility (0.3215), between pre-freezing and post thaw motility (0.3401) and between initial and post thaw motility (0.1596) were generally highly significant. Considering the significant effect of environmental factors (like year, season and extender pH groups), and the genetic factors (bulls) and the significant associated effet of ejaculate volume, sperm concentration and pre-freezing motility on the post-thaw motility, it was concluded that while freezing semen in Murrah buffaloe, the pH of extender should be above 6.7 and upto 6.8. The results also indicated necessity of further study on microclimate of semen during freezing for standardization of sperm concentration to achieve higher post thaw motility.

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