Effect of Multiple Showering and Vitamin Supplementation on Sexual Behaviour, Quality and Freezability of Buffalo Bull Semen

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ABSTRACT : In a summer study during May to July, involving 12 young Murrah buffalo bulls at forty months of average age, the effects of multiple shower vs single shower body cooling and vitamin A, D and E supplementation on the sexual behaviour, semen quality and freezability were investigated. The animals were divided into two groups (6 animals in each group) and housed in a half-walled shed with proper spacing, the feeding management being identical. The bulls in the control group were given a single shower at 1000 h, whereas the experimental bulls were given four showers at 10,12,14 and 16 h. In addition, the experimental bulls were given vitamin A, D and E injections at fifteen day intervals. The sexual behaviour of bulls was observed in terms of reaction time, sexual aggressiveness and ejaculatory thrust. Semen quality of all the bulls was assessed in terms of volume, mass activity, live-dead sperm and sperm concentration, sperm motility and morphology, and acrosomal abnormality. The sexual behaviour did not vary significantly between the groups, whereas semen quality differed significantly for volume, per cent live sperms, total sperms per ejaculate and total live sperm per ejaculate between groups. It can be concluded that sexual behaviour was not influenced by the thermal comfort treatment coupled with periodic vitamin A, D and E injections. But the treatments improved most of the seminal traits in the experimental group of bulls. However, benefit of treatment was not reflected in the freezability traits of the semen. *(Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 2 : 184-188)*

Key Words : Buffalo Semen, Vitamin, Season, Showering, Freezability

INTRODUCTION

Seasonal patterns of reproductive performance in both male and female buffaloes are well established (Roy et al., 1962, Sengupta et al., 1963, Qureshi et al., 1995, 1999). High heat stress during summer is known to depress the thyroid activity which consequently results in weak libido of breeding bulls, and poor semen quality, freezability and fertility. When buffalo bulls are not protected adequately from summer heat and solar radiation there is gradual decline in semen quality followed by total loss in libido and semen production, recovery from which takes six weeks after the onset of rainy seasons (Misra and Sengupta, 1965). Semen freezability in buffaloes during unfavorable season (hot-dry) is not of good quality and per cent rejections of semen samples at various stages viz., collection, processing and evaluation are relatively high. The extent of loss due to poor freezability during the hot-dry season might be minimised by providing thermal comfort and supplement feeding to the buffalo bulls during a stressful period. Therefore, this study investigated the effects of thermal comfort and supplementation of vitamins during a climatic and nutritionally stressful period on buffalo semen quality and freezability.

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MATERIALS AND METHODS

Selection of bulls

Twelve Murrah buffalo bulls were taken from the Central Institute for Research on Buffaloes herd for this study. These animals were divided into two similar groups of six on the bases of body weight and age. The experiment was conducted from May to mid July. The general health of the animals was good and the bulls were tested for the common diseases and vaccinated against them.

Management of bulls

All the bulls were housed in a half-walled shed throughout the experiment. They were fed on a concentrate mixture (18.5% CP and 70% TDN) daily as per ICAR recommendations (Ranjhan, 1991). Green and dry fodder was provided on the basis of dry matter requirement of the animal.

Plan of treatment

The experimental bulls were given vitamin A, D and E injections at fortnightly intervals throughout the period of trial. The experimental bulls were provided with *ad lib* water for drinking, and showering at 10, 12, 14 and 16 h, whereas bulls in the control group were provided with drinking water at above times but with one shower only at 10 h only daily. All the bulls were given exercise daily in the morning for 30 minutes. The physiological responses of both group in terms of respiration rate and rectal temperature were recorded in the morning and evening throughout the

experiment.

Semen collection - The bulls were given a washing before taking to the site of semen collection. The semen of all the bulls was collected in the early morning by artificial vagina technique (Walton, 1945).

Sexual behaviour - Sexual behaviour of all the bulls was observed at the time of collection as per the method described by Anzar et al. (1993). The reaction time, sexual aggressiveness and ejaculatory thrust were observed for every bull.

Physical attributes of semen - Immediately after collection the semen was assessed for physical attributes, viz: volume, mass activity, sperm concentration, sperm motility. Semen volume was measured by collecting the ejaculate in a graduated tube. Mass activity was assessed (Tomar et al., 1966) by putting a drop of ejaculated semen on a dry glass microscope slide on the basis of swirling current. Semen was rated into five categories and given a numerical grading; semen at +3 or a higher grade was used for further processing. Sperm motility was observed by putting a drop of semen on a dry glass microscope slide and taking the average of two persons' observations.

Freezing protocol - The semen was frozen as per the method described by Jindal (1994). Tris (hydroxy methyl amino methane pH 6.8) buffer was used with ten per cent egg yolk to prepare the extender. The composition of semen extender is given below-

Tris (hydroxymethyl amino methane)- 2.78 gm
Citric acid - 1.43 gm
Fructose - 1.13 gm
Double distilled water - 90 ml
Egg yolk - 10 ml

Pencillin sodium and Streptomycin were added at 1000 iu/ml and 1 mg/ml, respectively. The extension rate was fixed to keep the sperm concentration at 50 million sperm per inseminating dose with 20-25 million sperm motile. The extender was divided into two equal parts: A (non glycerolated) - semen was half diluted with extender; B (glycerolated) - extender plus 12 per cent glycerol was so adjusted that the final glycerol concentration was 6 per cent.

Cooling- Both A (glycerolated) and B (non-glycerolated) parts were kept at room temperature in a water beaker and then kept in cold handling cabinet for cooling to 5℃.

Glycerolisation and equilibration - Both A and B parts were mixed at 5℃ and then kept for 3-4 h at 5℃ for equilibration.

Filling and sealing of straws - Semen packaging was done in French mini (0.25 ml) straws. Before filling, the straws were kept under UV rays for 15 min and then cooled in the cold cabinet along with semen. The filling and sealing of the straws was done with the IMV automatic filling and sealing machine.

Freezing and thawing - After filling and sealing the straws were exposed to liquid N_2 vapours for 13 min. Thereafter straws were plunged into the liquid N_2 container for storage. The thawing of semen was done at 40℃ for 30 seconds for checking the post-thaw sperm motility.

Morphological attributes

Live-dead count - Live-dead count was determined according to procedure described by Blom (1950); in this method the Eosin-Nigrosin stain is used to prepare the slides. As per the procedure we placed one drop of semen and one to two drops of stain on a dry and clean glass slide at 37℃ using a warm stage. Thin smears were prepared from the mixture (semen and stain) on a clean and dry slide with the help of another slide. Care was taken to avoid cold shock in preparation of the smear, and the slide was allowed to dry in air. About 150 spermatozoa were counted in each slide in different microscope fields using oil immersion. The spermatozoa that had taken stain were considered to be dead, and the rest live. The composition of the Eosin-Nigrosin stain is given below-

Eosin - 100 mg
Nigrosin - 500 mg
Tris buffer - 10 ml
pH 6.8

Acrosomal damage - Giemsa's stain was used to prepare the slide for examination of acrosomal damage. The slides were examined under the microscope using the oil immersion by counting 150 sperm in different fields of the slide. The acrosomal cap of the sperm that had taken the stain was considered to have an intact acrosome, and the rest were considered as damaged acrosome (Watson, 1975). The composition of the Giemsa's stain is given below

Giemsa - 3 ml (Sigma Co., USA)
Phosphate buffer pH 7.2 - 2 ml
Glass distilled water (filtered in millipore) - 45 ml

Statistical analysis

The data were subjected to analysis of variance to study the effect of treatments on the physical and morphological attributes of semen at various stages of freeze preservation, as described by Snedecor and Cochran, 1967.

RESULTS AND DISCUSSION

Physiological reaction

The physiological reactions in terms of rectal temperature and respiration rate of all the bulls were recorded daily in the morning and evening; the

Table 1. Physiological responses of the bulls

	Control group		Experimental group	
	Mean ± SE	CV (%)	Mean ± SE	CV (%)
Respiration rate				
morning	24.60 ± 0.74[a]	29.62	26.00 ± 0.99[b]	18.35
evening	46.60 ± 3.76[a]	38.72	39.04 ± 2.21[b]	27.23
Rectal temp. (℃)				
morning	37.23 ± 0.12[a]	1.62	36.98 ± 0.12[a]	1.66
evening	38.15 ± 0.11[a]	1.44	38.27 ± 0.07[a]	0.95

Figures with different superscripts within the same row are significantly ($p<0.05$) different.

average values are presented in table 1. Statistical analysis revealed significant ($p<0.05$) difference in respiration rate between the groups, whereas rectal temperatures did not differ. The rise in respiration rate was marginal, and can be considered a routine physiological adjustment to combat the possible unfavourable effect of thermal stress. The non significant difference in rectal temperature between the groups shows that the animals in the control group could maintain their body temperature within the range of thermal comfort through the routine adjustment in the respiration rate.

The minimum and maximum temperatures and per cent relative humidity recorded inside and outside the shed did not differ significantly (table 2).

Sexual behaviour

The sexual behaviour of bulls in control and experimental groups was observed for reaction time, sexual aggressiveness and ejaculatory thrust but the these traits did not differ significantly between treatment (table 3).

Physical attributes of semen

The ejaculate volume of the treated group of bulls was significantly ($p<0.01$) higher than that of the control group (4.26 ± 0.38 vs 2.53 ± 0.20). The mass activity was not significantly different between groups. Live sperms percentage was significantly ($p<0.05$) higher in the treated group (88.09 ± 1.40 vs 80.81 ± 1.12) than control. The sperm concentration per ml did not differ significantly between groups whereas total sperms per ejaculate varied significantly ($p<0.05$) between groups (5124.86 ± 574.60 vs 2823.86 ± 387.06 million). The live sperms per ml did not differ significantly between groups but total live sperms per ejaculate varied significantly ($p<0.01$) between groups (4755.90 ± 618.05 vs 2332.55 ± 445.97 million) (table 4).

Table 2. Climatic observations indoor and outdoor recorded during experiment

	Minimum temp. (℃)	Maximum temp. (℃)	Relative humidity (%)
Indoor	30.26	38.29	32.50
Outdoor	26.45	39.50	35.10

Table 3. Mean values of sexual behaviour

Parameter	Control group		Experimental group	
	Mean ± SE	CV (%)	Mean ± SE	CV (%)
Reaction time (sec)	99.00 ± 15.54[a]	60.80	129.41 ± 25.47[a]	81.17
Sexual aggressiveness	2.73 ± 0.1[a]	16.17	2.64 ± 0.11[a]	18.05
Ejaculatory thrust	2.70 ± 0.11[a]	16.17	2.60 ± 0.14[a]	22.22

Figures with similar superscripts with in the same row are not significantly different from each other.

Sperm motility and morphology

The sperm motility after dilution did not differ significantly between groups, whereas sperm motility pre-freezing was significantly ($p<0.05$) higher in the treated group than the control (63.82 ± 1.97 vs 59.58 ± 2.53 per cent) whereas post thaw sperm motility and acrosomal damage did not vary significantly between the groups (table 5).

These results are in agreement with the earlier studies that showed an unprotected group of buffalo bulls during summer in the semi-arid environment of north west India drastically lost libido and reduced semen quality (Misra and Sengupta, 1965; Heuer, 1987). Chalapathy and Ramamohan (1981) observed only marginal improvement in the semen quality of protected Murrah buffalo bulls reared under the warm-humid climate of Andra Pradesh. The seminal profile in terms of all physical attributes, namely volume, live sperms concentration, total sperm per ejaculate and total live sperm per ejaculate, was significantly higher in the treated group than the control. As is evident from the physiological responses recorded during the experiment, there was a small rise in the respiration rate of the control compared with the experimental group at both morning and evening. This rise in the respiration rate in the control group was considered a routine physiological adjustment by the animal during unfavourable conditions. Provision of extra showers to the experimental bulls did not produce any tangible effect on the physiological comfort of the animals as reflected in the thermoregulatory responses, yet they did produce a better seminal profile as compared to control. Such improvement in semen quality was perhaps more due to the fortnightly injections of vitamin A, D and E

Table 4. Mean values of physical attributes of semen

Attributes	Control group		Experimental group	
	Mean ± SE	CV (%)	Mean ± SE	CV (%)
Volume (ml)	2.53 ± 0.20[a]	31.73	4.26 ± 0.38[b]	37.55
Mass activity	2.50 ± 0.30[a]	51.30	2.97 ± 0.06[a]	9.07
Live sperm (%)	80.81 ± 1.72[a]	7.07	88.09 ± 1.40[b]	5.29
Sperm conc., 10^6/ml	1,020.66 ± 115.78[a]	48.21	1,329.33 ± 107.90[a]	31.43
Total sperm, 10^6/eja	2,823.86 ± 387.06[a]	58.53	5,124.86 ± 574.60[b]	43.42
Live sperm, 10^6/ml	877.09 ± 104.00[a]	50.72	1,216.63 ± 104.79[a]	28.56
Total live sperm, 10^6/eja	2,332.35 ± 445.97[a]	70.27	4,755.90 ± 618.05[b]	43.10

Figures with same superscripts within the same row are not significantly different from each other ($p<0.05$).

than to the temporary cooling effect of extra showers.

The present findings draw support from earlier studies on bulls and buffalo bulls (Afiefy et al., 1984; Sty-Ko et al., 1985) where injections of vitamins produced improved quality of semen. Similar beneficial effects of feeding vitamin A, D and E in terms of volume, and sperm concentration in breeding rams and boars, have also been reported. When rams were given a supplemental diet with vitamin E and selenium, semen characteristics and acrosomal morphology were found to have significantly improved (Gokcon et al., 1990). It is possible that in the present study the administration of vitamins in purified injectable forms produced a favourable response in the experimental buffalo bulls during summer when the dietary availability of these vitamins are likely to be restricted (Rranjhan and Pathak , 1983).

On the basis of the above findings it can be concluded that the thermal comfort treatment coupled with periodic vitamin A, D and E injections improved most of the seminal traits. However, benefit of treatment was not reflected in the freezability traits of the semen.

Table 5. Mean values of sperm motility and morphology

Parameter	Control group		Experimental group	
	Mean ± SE	CV (%)	Mean ± SE	CV (%)
Sperm motility after dilution (%)	70.83+2.41[a]	11.82	74.11+1.56[a]	8.73
Sperm motility pre-freezing (%)	59.58+2.53[a]	14.71	63.82+1.97[b]	12.75
Post-thaw motility (%)	32.91+1.09[a]	11.53	34.41+2.59[a]	31.07
Acrosomal damage pre-freezing (%)	23.37+1.45[a]	30.81	20.84+1.06[a]	35.40
Acrosomal damage post-freezing (%)	29.37+1.83[a]	26.81	27.46+1.40[a]	28.99

Figures with different superscripts within the same row are significantly different from each other ($p<0.05$).

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