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Influence of microclimate modification on sexual behaviour and semen characteristics of Murrah buffalo bull during hot humid period in India

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Among various factors of seasonal variation, change in ambient temperature and humidity affects bulls the most. *Bos taurus* bulls have minimum sperm output during mid winter and late summer, concomitantly with the presence of the highest percentages of abnormal spermatozoa. Furthermore, the ability of their spermatozoa to survive freezing is lowest in summer (Koonjaenak *et al*. 2007). Murrah buffalo breed is native to Haryana, however, during extreme temperature and humidity, semen quality and sexual behaviour of bulls are adversely affected resulting in very poor freezibility of semen ejaculate taken from healthy bulls. At certain time even the bulls possessing good quality semen with good freezibility and as well as good libido also tend to produce low grade ejaculates under such stressful conditions (Koonjaenak *et al*. 2007). This degradation of semen quality results in depreciation of artificial insemination (AI) success rate. Such seasonal effects may cause early disposal of genetically superior quality bulls before their actual time period thus resulting in a huge economical loss. Therefore, the trend now is to develop strategies or refine methodologies so as to maximize the production of good quality semen without discarding too many poor quality ejaculates. Mist cooling and fanning treatment is found to be the easiest and low cost effective methodology, which reduces higher ambient temperature stress from animals and help them to regain their normal status. In the present study, Murrah buffalo bulls were subjected to mist cooling and fanning treatment to reduce effect of heat stress during elevated ambient temperature and humidity. It would, therefore have positive effect on sexual behavior as well as semen characteristics of bulls.

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Healthy Murrah buffalo bulls (12), 3–4 year-old, maintained in ABRC (Artificial Breeding Research Center) at the institute were housed individually under loose housing system and fed *ad lib*. Water sprinkling and fanning system were installed to reduce the general body stress during hot and humid months. Experimental animals were classified into 2 groups:

Group 1: Control group in which bulls were not exposed to cooling treatment.

Group 2: Bulls were exposed to mist cooling and fanning thrice/ a day, 15 min for each time.

The sexual behaviour was recorded at the time of semen collection by using different score cards. Temperament score (0–5) was given according to Tullah (1961). Libido score assessment was performed as per Chenoweth *et al*. (1977). Bulls were given score 1–9 on the basis of their sexual responses and mounting during semen collection. Erection score during seeking was observed and recorded as per Joshi and Kharche (1992). In brief, bull were given score from 0–4 on the basis of their erection during semen collection.The extent of protruding penis during seeking of vaginal orifice was observed and recorded as per by Joshi and Kharche (1992). In brief, the protrusion score was classified from 0–4, on the basis of their protrusion. Score 0 was give for the absence of protrusion and 4 score was for very good protrusion. The intensity of thrust score (ITS) was observed and recorded as per Joshi and Kharche (1992). The scores were given from 0–4 from no thrust and up to the excellent thrust. Reaction time (RT), dismounting time (MT) and total time taken in mounts (TTTM) were recorded by using stop watch.The frequency of Flehman's response was recorded for each bull separately.

Semen collection was performed with the aid of artificial vagina (AV), maintained at temperature 47°C with sufficient pressure and lubrication. Two consecutive ejaculates were taken once in a week with a minimum time interval of 20 min for every bull. Immediately after ejaculation semen samples were kept in a water bath incubator maintained at 37°C. Fresh ejaculated semen was evaluated for physical

Table 1. Mean value (mean value±SEM) of semen volume, colour and mass activity in (group 2) cooling treatment group and control (group 1). Percentage value of semen quality parameters, i.e., initial progressive motility, viability (Non-eosinophilic spermatozoa), membrane integrity (HOST) and post thaw motility of control and cool treatment received group of bulls. Different letters indicate significant (P< 0.05) difference.

Treatments	Semen volume	Semen colour	Mass activity	Initial Progressive Motility (%)	Non Sperm (%) eosinophlic	Post thawMotility (%)	Host (%)
Group1	2.85±0.23	2.13±0.10	2.45±0.16[a]	74.00±4.27[a]	81.33±3.80[b]	46.50±3.08[a]	59.6±5[a]
Group2	2.98±0.37	2.37±0.17	2.84±0.16[b]	85.00±3.07[b]	87.50±4.69[a]	30.17±2.50[b]	71.2±8[b]
Overall mean	2.913±0.31	2.25±0.135	2.64±0.16	79.5±3.67	84.42±0.42	38.33±2.79	58.3±6

attributes (volume and colour) and quality parameters (i.e., mass activity, individual motility, viability and acrosome integrity) within 1h after collection. Semen samples with minimum +3.5 mass activity or more than 90% individual motility were considered for cryopreservation. The final motility score was estimated as per Tomar (1997) and Reddy *et al.* (2010). Method of Therien and Manjunath (2003) was used for estimation of viability. The stained spermatozoa were spread on a grease free slides and allowed to dry for 10 to 15 min. Randomly 400 sperms were counted in different fields in the slide and unstained spermatozoa (non-eosinophilic) and stained spermatozoa (eosinophilic) were counted under compound microscope at 400× magnification. Minimum 2 slides were examined per ejaculate per bull (Bloom 1950). Percentage acrosome integrity spermatozoa were assessed according to Hancock (1952). Integrity of acrosomal region in 200 spermatozoa were recorded from minimum 5 different fields under compound microscope (1000× magnification) (Sanchez *et al.* 1995).

HOST (hypo-osmotic swelling test) has been proved to be a good tool for evaluating the functional integrity of sperm membrane based on curled and swollen tails. The number of twisted or curled spermatozoa observed in control solution was deducted from twisted spermatozoa in HOST solution (Revell and Mrode 1994).

Results were expressed as the means±SEM. A difference with value P<0.05 was considered statistically significant. Data were analyzed by One-way ANOVA and t- test. The statistical differences between the various treatment groups were determined by using the Statistical Product and Service Solutions, Version 17.0.1 software.

The entire data were analyzed for assessing the effect of the cooling treatment on sexual behavior characteristics and semen parameters.

Sexual behavior traits

Reaction time (sec): RT was not significantly influenced by the cooling treatment using mist cooling and fanning. The bulls in control group were found active in spite of the fact that those were not exposed to cooling treatment. This might be due to adaptation of Murrah bulls to the stress of heat, with individual variation of reaction time. The RT observed in the experiment (40.00±1.89 sec) was slightly higher than the value reported by Kumar (1995) (24.61sec). Similar records of reaction time (29.80±2.97sec) were obtained by Mandal and Tyagi (2004) in Murrah bulls, whereas higher values were found in summer (2.78±0.41 min) and winter (2.75±0.52 min) by Tomar and Singh (1998) in Murrah bulls.

Dismounting time: A significant effect of cooling (mist cooling + fanning) on DMT was observed. The animals receiving cooling treatment recorded lesser DMT (6.24±0.306) than those of control (7.32±0.28). However, the estimates reported earlier were slightly lower (3.22±0.09) in Sahiwal bulls (Mandal and Tyagi 2004) and still lower (2.1, 2.8, 2.4 sec) in Ongole, Jersey and Jersey × Ongole bulls (Rao *et al.* 1996). Purohit *et al*. (2000) reported similar values (5.63±0.36) in Surti buffalo bulls.

Total time taken in mounts: TTTM was significantly influenced (P<0.01) by the effect of the cooling using mist cooling and fanning. The TTTM in control group was 47.32±1.8sec, which was significantly higher to bulls receiving cooling treatments (40.48±1.83sec). The values in the present study were lower than the estimates in crossbred bulls (3.39±0.089 min; Pathkar *et al*. 1990), Sahiwal bulls (59.67±5.10sec; Mandal and Tyagi 2004), Tharparkar bulls (5.14 min; Mishra *et al.* 1972), and in Murrah bulls (47.48 and 40.50 sec; Kumar 1995). Our results indicated that all bulls reacted quickly and took little time in ejaculation than values reported by other worker, which might be due to genetic and management factors.

Flehmen's response: There was no significant difference observed between groups of cooling treatment and control. The overall mean of FR for different types of cooling treatment was 6.95±1.61. Higher values were reported by Panwar (1989), Kumar (1993), Panwar and Nagpaul (1994) and Kumar (1995) in Murrah bulls. Similar findings were reported by Salvador *et al*. (2003).

Erection score: It was significantly (P<0.01) influenced by cooling treatment using mist cooling and fanning in dry summer. Bulls exposed to the cooling treatment by mist cooling and fanning showed significantly higher values of ES (3.16±0.187) than control group which was not exposed to cooling with value (3.04±0.175). A slightly lower value was reported by Mandal and Tyagi (2004) (2.49±0.03) and Kumar (1993) (2.91±0.089) in Murrah bulls. However, higher score for erection was reported by Joshi and Kharche (1992) in crossbreds. The observation clearly showed negative effect

of heat stress during dry hot summer on ES.

Protrusion score: PS was found significantly (P<0.01) influenced by cooling using mist cooling and fanning in dry summer season. Bulls exposed to the cooling treatment by mist cooling and fanning showed significantly higher values of PS as 2.95±0.164 than control group which was not exposed to cooling with PS value as 2.79±0.19. Similar values (2.52±0.03) were estimated by Mandal and Tyagi (2004). However, lower values and were observed in Sahiwal (1.83±0.133) and Murrah bulls (1.24±0.068) by Kumar (1993).

Intensity of thrust: Intensity of thrust was found significantly (P<0.01) influenced by cooling using mist cooling and fanning in dry summer season. Bulls exposed to the cooling treatment by mist cooling and fanning showed significantly higher values of ITS (2.73±0.08) than control group which was not exposed to cooling with value (1.80±0.19). Similar findings were obtained by Kumar (1993) in Sahiwal (2.22±0.115) and Murrah bulls (1.68±0.071); and by Mandal and Tyagi (2004) in Sahiwal bulls (2.38±0.04). Higher values were reported by Mathur and Vyas (1969) in Nagauri bulls (3.98) and by Joshi and Kharche (1992) in crossbred (3.867±0.044).

Temperament score: Cooling did not significantly influence TS. Murrah buffalo bulls in both cooling treatment and control groups were showing quite low TS (0.24±0.14 and 0.18±0.05). The low temperament score indicated that Murrah bulls were well adapted to the controlled management conditions. Different values of behavioral temperament were observed by Panwar (1989) in Sahiwal bulls and Kumar (1993) in Sahiwal and Murrah bulls.

Libido score (LS): Cooling treatment significantly influenced libido score (P<0.01). Bulls exposed to the mist cooling and fanning showed significantly higher values of LS (7.05±0.133) than the control group which was not exposed to cooling (6.75±0.20). The present results were in conformity with the findings of Panwar (1989) and Kumar (1993).

Semen characteristics

Volume (ml): The results showed no significant effect of cooling treatment by mist cooling and fanning on ejaculated volume. The overall mean of semen volume was 2.91±0.31 ml. The present estimate of semen volume was within the range of average semen volume reported in Sahiwal bulls (3.36±0.14 to 4.57±0.32) by various workers (Tomar 1964, Bosrekar and Nagpaul 1972, Kumar 1993, Keshava 1996, Gupta *et al.* 1990, and Panwar and Nagpaul 1994).

Physical appearance: Colour of semen was not significantly (P<0.01) influenced by the cooling treatment using mist cooling and fanning. The estimate of present study was similar to the findings of Panwar (1989), Kumar (1993) in Sahiwal bulls.

Mass activity: The results revealed significant (P<0.01) variation in mass activity due to the cooling treatment. Bulls exposed to the cooling treatment by mist cooling and fanning showed significantly higher values of semen mass activity (2.84±0.16) than those of control group which was not exposed to cooling (2.45±0.16). The results indicated that mist cooling and fanning improved the semen quality of Murrah bulls in dry summer. The overall mean of mass activity was 2.64±0.16 of which was lower than the value (2.80±0.06) reported by Singh and Pangawkar (1990) and the value (2.70) reported by Keshava (1996). Slightly lower average mass activity of the ejaculates in the present study could be due to heat stress in dry and hot summer when the experiment was conducted.

Initial progressive motility: IPM (%) was found significantly (P<0.01) influenced by mist cooling and fanning. Bulls exposed to the cooling treatment by mist cooling and fanning showed significantly higher values of semen IPM (85.00±3.07) than control group which was not exposed to cooling (74.00±4.27), which indicated that cooling improved the quality of Murrah bulls in dry summer.The estimated IPM values in ourt study were higher than the estimates, reported by Kumar (1995) and Mandal and Tyagi (2004).

Non eosinophilic spermatozoa count (NESC): Analysis revealed significant (P<0.01) influence of the cooling treatment using mist cooling and fanning on NESC (%). Bulls exposed to the cooling treatment showed significantly higher values of NESC (87.50±4.69) than control group not exposed to cooling (81.33±3.80), which indicated that cooling improved semen quality of Murrah bulls in dry summer. The estimated NESC values of the present study were found higher than the observations reported in Sahiwal bulls (72.44±0.51%) and similar to the value reported by Rao *et al.* (1996) in Ongle bulls (84.6).

Post thaw motility (PTM): The results showed significant (P<0.01) influence of the cooling treatment by mist cooling and fanning on PTM (%). Bulls exposed to the mist cooling and fanning showed significantly higher values of semen PTM (46.50±3.08) than those of control group which was not exposed to cooling (30.17±2.50), thereby indicating that mist cooling and fanning improved the semen quality of Sahiwal bulls in dry summer.

Hypo osmotic swelling test (HOST): The results showed significant (P<0.01) influence of the cooling treatment by mist cooling and fanning on HOST (%). Bulls exposed to the mist cooling and fanning showed significantly higher values of semen HOST (71.2±8.0) than control group which was not exposed to cooling (59.6±5), thereby indicating that mist cooling and fanning improved the semen quality of Murrah bulls in dry summer.

From the present finings it could be concluded that keeping Murrah bull comfortable during peak summer months through managemental intervention viz. mist cooling coupled by fanning would improve quality and quantity of semen.

SUMMARY

Healthy breeding bulls (12) were selected to find out the effect of mist cooling and fanning treatment in reducing the higher ambient temperature stress from animals and help them to regain their normal status. All bulls were uniformly divided into 2 groups. Sexual behaviour and semen characteristics (ejaculate volume, sperm viability, membrane integrity, individual motility) were recorded. A significant improvement was observed in sexual behaviour (DMT, ES, PS, ITS and libido) of bulls under mist cooling and fanning. However, no significant effect was observed in their TS, RS and Flehmen's Reaction. Mist cooling treatment also exerted positive effects on their semen characteristics by improving significantly their motility, viability, membrane integrity and post thaw motility whereas no significant effect on semen volume and colour.

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