



## Effect of heat stress on physico-morphological characteristics and sperm functions in Murrah buffalo semen

N KUMAR<sup>1</sup>, S K GHOSH<sup>2</sup>, T K MOHANTY<sup>3</sup>, J K PRASAD<sup>4</sup>, R K SINGH<sup>5</sup> and B PATEL<sup>6</sup>

ICAR-National Dairy Research Institute, Karnal, Haryana 132 001 India

Received: 24 April 2018; Accepted: 26 October 2018

### ABSTRACT

The objective of the present study was to study the effect of heat stress induced by summer in relation to winter on physico-morphological characteristics and sperm functions in Murrah buffaloes semen. The study was conducted at the Artificial Breeding Research Centre, NDRI, Karnal. Meteorological data were recorded during summer and winter, and THI was calculated. Six adult Murrah buffaloes were selected and two sample from each buffalo were collected every month by AV method. A total of 84 ejaculates were collected and assessed for various seminal attributes in summer and winter. Seminal parameters assessed included volume, pH, mass motility, progressive motility, livability, abnormality, membrane integrity, protamine deficiency and apoptosis assessment in fresh semen. Results of THI (80.92) during summer revealed moderate heat stress condition. There was no significant effect of heat stress on ejaculate volume and pH. Sperm motility was significantly higher during winter than summer. Similarly, significantly lower sperm livability and membrane integrity were observed during summer compared to winter. Abnormal and protamine deficient sperm per cent was significantly higher during summer. Lower apoptotic sperm per cent was found in winter. It can be concluded that seasonal variations had a significant effect on semen quality of Murrah buffalo. Heat stress induced by summer (hot humid) season had the most adverse effect on various physico-morphological and sperm functions in Murrah buffalo semen.

**Key words:** Buffalo, Heat stress, Murrah, Semen, Sperm function, THI

High ambient temperature compromise reproductive performance through reducing feed intake and decreasing nutrient utilisation, growth rate and feed efficiency which lead to economic losses in dairy animals especially buffaloes. Information on the effect of heat stress induced by summer on semen characteristics in bulls had been of conflicting nature. Though in many studies, summer stress significantly influenced semen production (Mandal *et al.* 2000, Bhagat *et al.* 2015) while some investigation (Brito *et al.* 2002) failed to detect any effect on semen quality. A better knowledge of the influence of seasons, ambient temperature and relative humidity during semen collection on semen characteristics would help to know the requirement of bulls to meet the demand of frozen semen and to provide any suitable additional managerial requirements time to time. Studies relating season with recent sperm function tests related to fertility like the status of protamine deficiency and apoptosis are almost lacking in buffaloes. Hence, the present study was undertaken to

investigate the effect of heat stress induced by summer on various characteristics of Murrah buffaloes semen.

### MATERIALS AND METHODS

The present study was conducted on 6 normal fertile Murrah buffalo bulls maintained at Artificial Breeding Research Centre of the institute. All bulls were in regular semen collection programme. Meteorological variables in terms of dry and wet bulb temperatures were recorded throughout the experimental period in morning and evening. Temperature humidity index (THI) was calculated using the formula given by Johnson *et al.* (1963).

Two ejaculates were collected from each bull per month during summer (July 2016 to September 2016) and winter (December 2016 to March 2017) using artificial vagina in morning. Immediately after semen collection, samples were placed in a water bath (37°C) to assess semen evaluation parameters. Ejaculates having initial progressive motility  $\geq 70\%$  were selected for the study. A total of 84 semen ejaculates were collected and assessed for different seminal attributes in both seasons. The experiment was approved by Institutional Animal Ethics Committee as per the article number 13 of the CPCSEA-rules, laid down by the Government of India.

*Semen evaluation:* The ejaculate volume of fresh semen was recorded directly from the graduated semen collection

Present address: <sup>1</sup>Scientist (drnishantvet@yahoo.com), <sup>3</sup>Principal Scientist (mohanty.tushar@gmail.com), Animal Reproduction Division; <sup>2,4</sup>Principal Scientist (skghoshivri@gmail.com, jkprasad2001@yahoo.com), Animal Reproduction, IVRI, Izatnagar; <sup>5</sup>Assistant Commandant (raushansingh704@gmail.com), BSF, Kishanganj; <sup>6</sup>Assistant Professor, College of Agriculture and Research Station, Raigarh.

tube. pH of fresh semen was measured by digital pH meter. The mass motility of fresh semen was recorded by placing a small drop of freshly collected semen on a clean grease free, pre-warmed glass slide at 37°C and examined without coverslip under a low power magnification (10×) of phase contrast microscope. The progressive motility of freshly diluted semen was assessed after covering a semen drop on a slide with a thin coverslip at 37°C, under high power magnification (40×). Livability status of fresh semen was assessed using Carboxy fluorescein diacetate-Propidium Iodide (CFDA-PI) staining as per method described by Harrison and Vickers (1990) with little modifications. Spermatozoa were observed under the fluorescent microscope in 100× using FITC filter (Emission 515–555 and excitation 465–495). Live spermatozoa appeared green, dead appeared red and moribund appeared dual stained. Sperm abnormality of fresh semen was assessed by Rose Bengal staining. Membrane integrity of sperm plasma membrane was assessed by hypo-osmotic swelling test (HOST) as per method described by Prasad *et al.* (1999) with slight modification.

*Statistical analysis:* The statistical analyses were performed using SAS Enterprise Guide (Version 5.1; SAS Inst. Inc., Cary, NC, USA) and data were analyzed by one way ANOVA and Tukey’s Studentized Range (HSD) test.

RESULTS AND DISCUSSION

*Meteorological variables:* Average values of meteorological variables recorded in summer and winter season have been presented in table 1. The temperature humidity index (THI) is a common indicator of heat stress used in cattle and buffaloes. Classification of THI for assessment of heat stress reported by Armstrong (1994) was used in this study. THI values were categorized into five different classes as no stress with THI value < 72, mild stress (72–78), moderate stress (79–88), severe stress (89–98) and dead cow with THI value more than 98. Average maximum and minimum temperature (°C) was 32.9 and 25.30 during summer and 23.0 and 8.8 during the winter season, respectively (Table 1). Mean THI from July to September (hot humid season) was 80.92 indicating moderate heat stress on all experimental animals and mean THI from December to March (62.08) fell under no stress category, indicating no stress. Therefore, during the summer

Table 1. Average values of meteorological variables for summer and winter season

Meteorological variables	Summer (July–Sep 2016)	Winter (Dec 2016–Mar 2017)
Maximum temperature (°C)	32.90	23.00
Minimum temperature (°C)	25.30	8.80
Dry bulb temperature	29.30	16.60
Wet bulb temperature	26.50	13.30
Mean temperature (°C)	29.10	15.90
Mean relative humidity	80.00	73.00
Temperature Humidity Index (THI)	80.92	62.08

season all experimental animals were under moderate heat stress.

*Ejaculate volume:* In our study, no significant difference was observed in ejaculate volume of summer and winter (Table 2). However, our findings of ejaculate volume were contrary to findings of Singh and Singh (1993) who reported highest semen volume in buffaloes during the spring season. Several factors such as the age of the animal, differences between species, number of specimens, level of nutrition, management practice and environmental conditions (Petrocelli *et al.* 2015) may be responsible for the differences in results.

*pH:* No significant difference was recorded in pH values of winter and summer (Table 2). Our results were in agreement with Koonjaenak *et al.* (2007) who did not found any signification seasonal influence on seminal pH in Swamp buffalo.

*Mass motility:* Mass motility in winter was significantly (P<0.01) higher than the summer (Table 2). The highest mass activity during the winter had been reported in buffaloes (Mandal *et al.* 2000, Sharma *et al.* 2014). However, Dhimi *et al.* (1998) observed its highest value during the rainy season, whereas Nazir (1988) obtained no significant seasonal variation in mass activity in Nili-Ravi bulls. Results are conflicting because mass motility was subjectively determined by microscopic examination of a drop of fresh semen; these data should be considered carefully.

*Progressive motility:* Progressive motility of spermatozoa during winter was significantly (P<0.01) higher than the summer (Table 2). A significant (P<0.01) seasonal difference in per cent progressive motility recorded corresponds to observations in Murrah (Ravimurugan

Table 2. Mean (±SE) of physico-morphological and functional characteristics of fresh semen of Murrah Buffalo bulls in summer and winter

Seminal parameter	Summer (July–Sep 2016)	Winter (Dec 2016–Mar 2017)
Ejaculate volume (ml)	3.44±0.31	3.72±0.27
pH	6.79±0.01	6.76±0.01
Mass motility	2.81±0.04 <sup>a</sup>	3.12±0.04 <sup>b</sup>
Progressive motility (%)	71.63±0.34 <sup>a</sup>	80.54±0.42 <sup>b</sup>
Live sperm (%)	72.50±0.45 <sup>a</sup>	83.77±0.30 <sup>b</sup>
Moribund sperm (%)	9.47±0.28 <sup>a</sup>	8.29±0.22 <sup>b</sup>
Dead sperm (%)	17.50±0.74 <sup>a</sup>	7.93±0.36 <sup>b</sup>
Abnormal sperm (%)	12.86±0.43 <sup>a</sup>	7.89±0.27 <sup>b</sup>
Membrane integrity (HOST %)	59.27±0.37 <sup>a</sup>	68.70±0.29 <sup>b</sup>
Protamine deficiency (%)	2.16±0.18 <sup>a</sup>	0.60±0.10 <sup>b</sup>
Non-apoptotic	69.72±0.49 <sup>a</sup>	80.33±0.35 <sup>b</sup>
Early apoptotic	10.97±0.29 <sup>a</sup>	5.37±0.28 <sup>b</sup>
Late apoptotic	9.69±0.30 <sup>a</sup>	4.95±0.21 <sup>b</sup>
Necrotic	9.61±0.25	9.33±0.26

<sup>a,b</sup>Means bearing different superscripts in a row differ significantly (P<0.01).

*et al.* 2003) and Surti (Bhosrekar *et al.* 1988). However, our findings were contrary with few past reports on buffaloes (Mandal *et al.* 2000, Koonjaenak *et al.* 2007, Das *et al.* 2017) where no significant seasonal variation in progressive motility was reported. This discrepancy may be because the months comprising seasons in this study were not exactly similar in the season of their study and also the difference of temperature between seasons of this study and their study was not equal. Lower heat resistance of Murrah buffaloes became apparent by the significantly decreased sperm motility in summer compared with the winter. Low sperm motility in summer is a clear indication of heat stress and high temperatures. The elevated environmental temperature during the summer can increase testicular temperatures, metabolic rate and oxygen requirements. If intensified metabolism is not followed by enhanced blood flow, testicle tissue becomes hypoxic, which results in excessive production of reactive oxygen species and lipid peroxidation, oxidative stress and decline in spermatozoa motility (Nichi *et al.* 2006).

*Live, moribund and dead sperm per cent:* Live sperm per cent in winter was significantly ( $P<0.01$ ) higher in Murrah buffalo bulls compared to summer (Table 2). Our results of highest live sperm per cent during winter corresponds to observations of Mandal *et al.* (2000) and Bhagat *et al.* (2015) in buffalo bulls. Singh and Singh (1993) observed the lowest value of live sperm per cent in Murrah bulls during winter and Prajapati (1995) reported the lowest value during the rainy season in Mehsana bulls. Such differences could be attributed to sheltering, temperature or humidity. Moribund spermatozoa per cent was significantly ( $P<0.01$ ) higher during heat stress condition of summer (9.47) than winter (8.29). No literature could be traced out regarding seasonal influence on moribund sperm per cent for comparison of results. Moribund sperm per cent may be affected by the elevated temperature during summer due to increased production of reactive oxygen species (ROS) by increased oxidative stress. Dead sperm per cent was maximum during summer (17.50) followed by winter (7.93). However, no significant difference among bulls was observed during any season.

*Abnormal sperm per cent:* In present study, abnormal sperm per cent of summer was significantly ( $P<0.01$ ) higher than obtained in winter (Table 2). Highest abnormal sperm per cent in the summer and lowest in winter observed in present study corresponds to observations of Koonjaenak *et al.* (2007), Bhagat *et al.* (2015) and Ram *et al.* (2017) in Buffalo bulls. The increase in the total number of defective sperm during the heat stress was consistent with the well known effects of increased temperature on semen quality which impair testicular functions leading to sperm production with abnormal morphology. Spermatogenesis may be interrupted partially or entirely by factors such as oxidative stress and increased scrotal temperature which produces defective spermatozoa (Durairajanayagam *et al.* 2015).

*Membrane integrity:* HOST reactive sperm per cent was

significantly ( $P<0.01$ ) higher during winter ( $68.70\pm 0.29$ ) than summer ( $59.27\pm 0.37$ ) (Table 2). Results of the present investigation were in agreement with Mandal *et al.* (2000), Bhagat *et al.* (2015) and Ram *et al.* (2017) who obtained lowest membrane integrity per cent in summer and highest during the winter in buffaloes. Contrary observation on membrane integrity per cent was observed by Koonjaenak *et al.* (2007) who reported highest membrane integrity during summer and lowest in winter in swamp buffaloes. It may be due to the difference in the breed and geographical conditions. Sperm membrane integrity may be adversely affected by the elevated temperature during the summer season due to increased production of reactive oxygen species (ROS) by increased oxidative stress. It may account for the reduction in plasma membrane integrity during the period of heat stress.

*Protamine deficiency:* The protamine deficient sperm per cent was significantly ( $P<0.01$ ) higher in summer ( $2.16\pm 0.18$ ) compared to winter (Table 2). There was no traceable literature available relating to heat stress with sperm protamine deficiency of bovine. However, findings of this study were indirectly supported by Rahman *et al.* (2011) who reported that bulls, when exposed to a testicular heat insult through scrotal insulation, showed decreased protamine content in spermatozoa. Heat stress on bulls during germ cell development, especially at the spermiogenesis stage, altered sperm chromatin condensation. Moreover, it has been reported that high testicular temperature adversely affects sperm cell acrosome status and sperm chromatin protamination (Rahman *et al.* 2011). The higher temperature of summer may have accounted for increased protamine deficient sperm during the summer season.

*Sperm apoptosis per cent:* Annexin V and propidium iodide (PI) stain were used to identify apoptotic spermatozoa. During the assessment of sperm apoptosis per cent, four different types of sperm populations were observed (Table 2). They were non-apoptotic (viable), early apoptotic, late apoptotic and necrotic sperm. Significantly ( $P<0.01$ ) higher non-apoptotic (viable) sperm per cent was observed in winter than summer. Significantly ( $P<0.01$ ) lower early apoptotic sperm per cent was observed in winter than summer.

Similarly, significantly ( $P<0.01$ ) lower late apoptotic sperm per cent was observed in winter compared to summer. Necrotic sperm per cent was significantly ( $P<0.01$ ) higher during summer than winter. No literature could be traced out regarding heat stress and apoptotic sperm per cent of Murrah buffalo. Our findings of non-apoptotic sperm per cent are supported by the observation of Koonjaenak *et al.* (2007) who performed Annexin assay and found highest per cent of viable/non-apoptotic sperm per cent in the winter. However, they recorded the highest late apoptotic and necrotic sperm per cent during summer and highest fresh apoptotic sperm per cent in winter. It had been reported that when germ cell apoptosis occurs, it is also influenced by the severity and duration of heat stress. Spermatozoa

resulting from sperm cells exposed to hyperthermia in mice undergo apoptosis and contain damaged DNA, leading to poor fertilizing capacity *in vivo* and *in vitro*. Oxidative stress is a significant cause for thermal damage of spermatogenic cells and leads to apoptosis and DNA strand breaks.

It can be concluded that seasonal variations had a significant effect on semen quality of Murrah buffaloes. Summer (hot humid) had the most adverse effect on various physico-morphological, functional and post-thaw quality of sperm. Winter was the most favourable for quality semen production. Heat stress as expressed by high THI values during summer (80.92) adversely affected semen quality.

#### ACKNOWLEDGEMENTS

The authors are thankful to Incharge, Artificial Breeding Research Centre and Director, ICAR-National Dairy Research Institute for providing necessary facilities and financial assistance during the research work.

#### REFERENCES

- Bhakat M, Mohanty T K, Gupta A K, Prasad S, Chakravarty A K and Khan H M. 2015. Effect of season on semen quality parameters in Murrah buffalo bulls. *Buffalo Bulletin* **34**(1): 100–112.
- Bhosrekar M R, Purohit J R, Pande A B and Mangurkar B R. 1988. Effect of different seasons on frozen semen production in Murrah buffalo bulls. *Indian Journal of Animal Sciences* **58**(9): 1073–74.
- Brito L F C, Silva A E D F, Rodrigues L H, Vieira F V, Deragon L A G and Kastelic J P. 2002. Effect of age and genetic group on characteristics of scrotum, testes and testicular vascular cones, and on sperm production and semen quality in AI bulls in Brazil. *Theriogenology* **58**: 1175–86.
- Das G C, Das P K, Deori S, Mazumdar H, Bhattacharyya B N and Phookan A. 2017. Seasonal variation in the characteristics of the swamp buffalo semen of northeast India. *Buffalo Bulletin* **36**(1): 215–19.
- Dhami A J, Greesh M and Sahni K L. 1998. Seasonal influences on the quality and freezability of semen of Friesian and Murrah buffalo bulls. *Indian Journal of Animal Reproduction* **19**(1): 55–58.
- Durairajanayagam D, Agarwal A and Ong C. 2015. Causes, effects and molecular mechanisms of testicular heat stress. *Reproductive BioMedicine Online* **30**: 14–27.
- Harrison R A P and Vickers S E. 1990. Use of fluorescent probes to assess membrane integrity in mammalian spermatozoa. *Journal of Reproduction and Fertility* **88**: 343–52.
- Koonjaenak S, Chanatinart V, Aiumlamai S, Pinyopumimintr T and Rodriguez-Martinez H. 2007. Seasonal variation in semen quality of swamp buffalo bulls (*Bubalus bubalis*) in Thailand. *Asian Journal of Andrology* **9**(1): 92–101.
- Mandal D K, Nagpaul P K and Gupta A K. 2000. Seasonal variation in seminal attributes and sexual behaviour of Murrah buffalo bulls. *Indian Journal of Dairy Science* **53**: 278–83.
- Nazir M. 1988. Semen evaluation and sperm morphology. Monograph on Reproductive Pattern of Riverine Buffaloes and Recommendations to Improve their Reproductive Performance at Small Farmer Level. PARC, Islamabad.
- Nichi M, Bols P E, Züge R M, Barnabe V H, Goovaerts I G, Barnabe R C and Cortada C N. 2006. Seasonal variation in semen quality in *Bos indicus* and *Bos taurus* bulls raised under tropical conditions. *Theriogenology* **66**: 822–28.
- Petrocelli H, Batista C and Gosalvez J. 2015. Seasonal variation in sperm characteristics of boars in southern Uruguay. *Revista Brasileira de Zootecnia* **44**(1): 1–7.
- Prajapati K B. 1995. 'The effect of exercise and feeding bypass protein on sexual behaviour and seminal attribute in Mehsana buffalo.' PhD Thesis, NDRI, Karnal, India.
- Prasad J K, Kumar S, Mohan G, Shankar U and Agarwal S K. 1999. Hypo-osmotic swelling test (HOST) and its response in fresh and freeze thawed semen. *Indian Journal of Animal Sciences* **69**(10): 766–69.
- Rahman M B, Vandaele L, Rijsselaere T, Maes D, Hoogewijs M and Frijters A. 2011. Scrotal insulation and its relationship to abnormal morphology, chromatin protamination and nuclear shape of spermatozoa in Holstein-Friesian and Belgian Blue bulls. *Theriogenology* **76**: 1246–57.
- Ram K L, Tiwari R P, Mishra G K, Sahasrabudhe S A and Nair A K. 2017. Effect of heat stress on seminal characteristics of Murrah buffalo bull semen. *Buffalo Bulletin* **36**(2): 369–78.
- Ravimurgan T, Raman K S and Thangaraju P. 2003. Seasonal influences on the semen production traits of Murrah buffalo bulls. *Indian Buffalo Journal* **1**(2): 85–87.
- Sharma L, Pandey V, Nigam R, Singh P, Saxena A and Swain D K. 2014. Seasonal variations in seminal plasma proteins of Buffalo. *Reproduction in Domestic Animal* **49**(3): 387–391.
- Singh B and Singh D S. 1993. Effect of season and quantity of semen in Jersey and Murrah buffalo. *Indian Journal of Animal Research* **27**(2): 81–87.
- Storey B T. 2008. Mammalian sperm metabolism: oxygen and sugar, friend and foe. *International Journal of Developmental Biology* **52**: 427–37.