EFFECT OF HEAT STRESS ON SEMINAL CHARACTERISTICS OF MURRAH BUFFALO BULL SEMEN

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ABSTRACT

A total of 48 semen ejaculates from 6 Murrah bulls were collected during winter period (15°C to 25°C) and heat stress (42°C to 48°C) through artificial vagina method to study the effect of heat stress on seminal characteristics of Murrah buffalo bull semen. Overall mean values of ejaculated volume (3.95±0.33 Vs 3.43±0.21 ml), sperm concentration (1324.80±67.84 Vs 1133.11±74.27 millions/ml), total number of sperm per ejaculate (6575.74±1306.37 Vs 4785.31±619.18), mass motility (3.63±0.09 Vs 3.33±0.10), initial progressive motility (72.50±0.89 Vs 73.33±0.87%), live sperm (85.82±0.77 Vs 84.12±0.94%), abnormal sperm (8.71±0.47 Vs 12.25±0.72%), intact acrosome (83.54±0.30 Vs 82.92±0.32%), cervical mucus penetration (21.10±0.32 Vs 24.31±0.48 mm) and hypo osmotic swelling reactive sperm (55.88±2.65 Vs 52.08±0.34%) were in fresh semen during winter period (15°C to 25°C) and heat stress (42°C to 48°C), respectively. The overall mass activity ($P<0.05$) and percent HOS positive sperm ($P<0.01$) were found significantly lower during heat stress whereas percent abnormal sperm and the mean distance travelled by vanguard spermatozoa (CMPT) was found significantly higher ($P<0.01$) during heat stress. There is variation in sensitivity among individual bulls to heat stress for sperm concentration, initial progressive motility, abnormal sperm, intact acrosome, cervical mucus penetration and hypo osmotic swelling reactive sperm.

Keywords: buffaloes, *Bubalus bubalis*, fresh semen, heat stress, Murrah bulls, physical characteristics

INTRODUCTION

Artificial insemination using frozen semen is now worldwide tool employed for improving the genetic potential of livestock. Attempts are being intensified to increase the coverage of AI so as to exploit the full potential of the technology. The use of the best bulls is often restricted by the limited number of doses of semen produced as there are several inherent and functional constraints in realizing the breeding goals through AI. Moreover, the quality of frozen thawed semen is one of the most influential factors affecting the likelihood of conception (Saacke, 1984). Application of AI with frozen thawed semen has been reported on a limited scale in buffalo, because of poor freezability and fertility of buffalo spermatozoa when compared

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with cattle spermatozoa (Kakar and Anand, 1981; Muer *et al*., 1988; Raizada *et al*., 1990; Singh and Pant, 2000; Andrabi *et al*., 2001; Ahmad *et al.*, 2003; Senatore *et al*., 2004; Kumaresan *et al*., 2005). Semen quality and quantity of breeding bulls is influenced by several non-genetic factors including age of the bulls and season of the year. High ambient temperature during summer adversely affects testicular size, libido and semen quality (Soderquist *et al*., 1996).

Similarly, epididymal spermatozoa are adversely affected by elevated testicular temperature resulting in a decreased ability of spermatozoa to maintain motility and acrosomal integrity after freezing. 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*., 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. However, very little information is available on environmental factor variables like temperature, humidity and day length in a particular season as during recent past years, the temperature is rising very high to 42°C to 48°C during summer. Therefore, the present research work was carried out to study the effect of heat stress on seminal characteristics in Murrah buffalo bull.

MATERIALS AND METHODS

The study was conducted on six Murrah buffalo bulls aged between of 5 to 6 years of age maintained in identical feeding and management regimes according to minimum standard protocol (MSP) of Government of India at Central Semen Station (CSS), Anjora, Durg (Chhattisgarh). A total of 48 semen ejaculates from 6 bulls (8 ejaculates from each bull) were collected during period of heat stress (temperature ranged between 42°C to 48°C) and winter (temperature ranged between 15°C to 25°C) during year 2013. Durg is located at an altitude of 951 feet above the mean sea level at a latitude of 21°10' North and a longitude of 81°16' East. The climate of this place is of tropical region with temperature ranging between 7.3°C to 47.4°C and relative humidity between 20 to 75%. All the bulls were maintained in identical feeding and management regimen according to minimum standard protocol (MSP) of Government of India. Semen from experimental bulls was collected twice a week, in morning hours between 7.00 to 8.30 A.M. before feeding by using Artificial Vagina (25 cm long and 6.5 cm in diameter) maintained at 42°C to 45°C in incubator as per procedure described by Singh *et al*., 2000. A male partner of the same species was used as a dummy for semen collection. Two false mounts were provided to each bull before collection. Immediately after collection, the semen was kept at 37°C in a water bath placed inside the passbox.

Evaluation for volume (Sardar, 2007), mass motility (Salisbury *et al*., 1978), initial progressive motility (Ahmad, 1994), sperm concentration (Mishra *et al*., 2012), total number of spermatozoa per ejaculate (Bane, 1954), percent live sperm (Campbell *et al*., 1953), percent total abnormal sperm (Kedia, 2011) and percent intact acrosome (Watson, 1975), Cervical mucus penetration test (Kremer, 1965; Matouseket *et al*., 1989; Prasad *et al*., 1999) and Hypo-osmotic swelling test (Jeyendran *et al.*, 1984) were carried out. The sample of neat semen was processed in Accucell Bovine Photometer (IMV technologies France), so as to pack 20 million sperms per 0.25 ml per straw

following the initial evaluation and finally diluted with a calculated quantity of Tris diluent (Rasbech, 1975). Filling and sealing of straws was done in integrated system-4 (IS-4, IMV technologies France) under laminar air flow cabinet. Then straws were transferred to the cold handling cabinet (IMV technologies, France) for equilibration at 4°C for 4 h and then to a programmable bio-freezer (IMV technologies, France) for 8 to 10 minutes. so as to reach the temperature to -140°C. The straws were then collected in the pre-cooled goblet and were immersed directly into the liquid nitrogen (-196°C) to ensure the proper freezing after equilibration under standard conditions (Graham *et al*., 1985).

The means of the seminal characteristics were calculated as per procedures outlined by Snedecor and Cochran (1994) and the data was analyzed statistically using standard procedure of ANOVA with the help of SPSS computer software.

RESULTS AND DISCUSSION

Fresh seminal characteristics

Seminal characteristics of fresh semen of six Murrah bulls during winter and heat stress are presented in Table 1.

Volume

There was no significant difference in ejaculate volume during winter period and heat stress. However between bulls, significant (P<0.05) variation was recorded during winter period only (Table 2). The seminal volume of Murrah bulls in present study is in close agreement with Bhavsar *et al*. (1986); Hussain *et al*. (1985); Hardin *et al*. (1982); Igboeli and Rakha (1971); Sardar *et al*. (2000) between season. Similar findings between seasons in Murrah bulls were observed by Tiwari *et al*. (2011) but differed significantly (P<0.05) between bulls. Significant variance in ejaculate volume between bulls was also reported by Bhavsar *et al*. (1986) in Murrah bulls.

Sperm concentration

There was no significant difference in sperm concentration during winter period and heat stress. Between bulls, there was significant difference (P<0.05) in sperm concentration during heat stress. However, no significant difference between bulls was observed during winter period (Table 2). Similar observations in sperm concentration in semen of Murrah bulls were reported by Tomar *et al*. (1966). Sperm concentration of present study is in close agreement between Murrah bulls as well as seasons (Tiwari *et al*., 2011).

Total number of spermatozoa per ejaculate

There was no significant difference in total spermatozoa per ejaculate between winter period and heat stress. Significant (P<0.05) bull to bull variation was observed during winter, whereas during heat stress, there was no significant difference between bulls (Table 2). Tiwari *et al*. (2011) reported comparable seasonal variation in total number of spermatozoa per ejaculate in Murrah bulls. However, the finding of Mishra *et al*. (2012) revealed that season had significant (P<0.05) effect on sperm out per ejaculate in Jersey bulls and recorded highest during winter and lowest during summer seasons, respectively.

Mass motility

The overall mass motility at winter period was found to be significantly (P<0.05) higher than heat stress. However, no significant bull to bull variation was observed during winter period and heat stress. The findings in the present study

were comparable to that reported by Dugwekar (1968) and Tuli (1984) in Murrah buffalo bulls. The variation in result may be attributed due to subjective assessment of nature of mass motility.

Initial progressive motility

The average initial progressive motility did not differ significantly between winter period and heat stress but differed significantly (P<0.05) between bulls (Table 2) during winter and heat stress. The findings of the present study were contrary to that reported by Tiwari *et al*. (2011) with significant (P<0.05) difference between season. A range of comparable individual spermatozoal motility (60.00 to 83.60±0.83%) in Murrah buffalo was reported by Nath *et al*. (1991); Tuli (1984); Singh *et al*. (1983); Gopalkrishna and Rao (1978); Eusebio (1977); Bhosrekar and Nagarcenkar (1973); Dugwekar (1968); Pangawkar (1968). The variation in the present initial motility might be due to attributed factor like degree of sexual excitement, method of semen collection and frequency of semen collection (Tomar, 1986).

Percent live sperm

There was no significant difference in live sperm percent between winter period and heat stress as well as between bulls (Table 2). Percent live sperm between Murrah bulls is in close agreement with the findings of Sahu (1996); Mandal *et al*. (2000) was observed decrease in live sperm percent in high temperature in Murrah buffalo bulls.

Sperm abnormalities

There was significant difference (P<0.01) in mean sperm abnormalities between winter period and heat stress. However, bull to bull significant (P<0.05) variation of sperm abnormalities was also observed during winter and heat stress period (Table 2). Rao *et al.* (1996) were also reported comparable percent sperm abnormalities in Ongole bulls. Elevated environmental temperature during

Table 1. Seminal characteristics of fresh semen of Murrah bulls during winter period (15-25^{o}C) and heat stress (42-48^{o}C).

Semen Parameters	Overall mean during period	
	Winter period	Heat stress
Ejaculate volume (ml)	3.95±0.33	3.43±0.21
Sperm concentration (Million/ml)	1324.80±67.84	1133.11±74.27
Total number of sperm per ejaculate (Million)	6575.74±1306.37	4785.31±619.18
Mass motility (0-5 scale)	3.63±0.09	3.33±0.10*
Initial progressive motility (%)	72.50±0.89	73.33±0.87
Live sperm (%)	85.82±0.77	84.12±0.94
Abnormal sperm (%)	8.71±0.47	12.25±0.72**
Intact acrosome (%)	83.54±0.30	82.92±0.32
CMPT (mm)	21.10±0.32	24.31±0.48**
HOST (%)	55.88±2.65	52.08±0.34**

*Differed significantly (P<0.05); **Differed significantly (P<0.01).

Table 2. Bullwise seminal characteristics of fresh semen of Murrah bulls during winter period (15-25°C) and heat stress (42-48°C).

Parameters	Season	Bull No 44816	Bull No 116627	Bull No 114161	Bull No 70501	Bull No 43712	Bull No 81027
Ejaculate volume (ml)	Winter period	3.20^{bcd} ± 0.28	2.95^{cd} ± 0.44	4.61^{ab}c ± 0.78	5.51^{a} ± 1.00	5.28^{ab} ± 1.03	2.18^{d} ± 0.46
	Heat stress	3.47 ± 0.34	3.17 ± 0.47	2.90 ± 0.47	3.57 ± 0.72	4.22 ± 0.71	3.29 ± 0.26
Sperm concentration (Million/ml)	Winter period	1168.13 ± 186.21	1570.00 ± 132.88	1318.80 ± 144.97	1182.91 ± 91.16	1236.23 ± 193.08	1471.99 ± 215.83
	Heat stress	1198.47^{ab} ± 173.4	823.17^{b} ± 119.86	1085.85^{ab} ± 112.98	1071.18^{ab} ± 114.48	1422.32^{a} ± 292.46	1197.66^{ab} ± 194.40
Total no. of sperm/ejaculate (Million)	Winter period	3735.36^{b} ± 739.85	4753.00^{b} ± 877.11	6653.56^{ab} ± 1527.03	6370.64^{b} ± 1582.36	14525.05^{a} ± 7039.96	3416.07 ^{b}± 852.10
	Heat stress	4567.81 ± 1121.10	2868.35 ± 670.70	5810.50 ± 2301.66	4111.48 ± 1051.45	6900.46 ± 2242.84	4426.08 ± 835.53
Mass Activity (0-5 scale)	Winter period	3.50 ± 0.19	3.88 ± 0.23	3.88 ± 0.23	3.63 ± 0.18	3.50 ± 0.27	3.38 ± 0.18
	Heat stress	3.37 ± 0.182	3.12 ± 0.22	3.37 ± 0.18	3.12 ± 0.48	3.62 ± 0.18	3.37 ± 0.18
Initial Progressive Motility (%)	Winter period	70.63^{ab} ± 1.75	74.38^{a} ± 1.13	75.00^{a} ± 1.34	75.00^{a} ± 1.34	72.50^{ab} ± 1.34	67.50^{b} ± 4.01
	Heat stress	70.62^{b} ± 3.71	73.12^{b} ± 0.91	73.12^{ab} ± 2.48	77.50^{a} ± 0.94	74.37^{ab} ± 0.62	71.25^{ab} ± 2.05
Live Sperm (%)	Winter period	84.95 ± 2.38	85.59 ± 2.82	86.25 ± 1.15	85.25 ± 1.78	88.00 ± 1.31	84.88 ± 1.68
	Heat stress	81.25 ± 4.28	85.62 ± 1.08	86.87 ± 1.80	87.37 ± 1.61	80.12 ± 1.07	83.87 ± 1.52

Table 2. Bullwise seminal characteristics of fresh semen of Murrah bulls during winter period (15-25°C) and heat stress (42-48°C). (Cont.)

Parameters	Season	Bull No 44816	Bull No 116627	Bull No 114161	Bull No 70501	Bull No 43712	Bull No 81027
Abnormal Sperm (%)	Winter period	10.63^{ab} ± 0.82	6.50^{c} ± 1.25	6.63^{c} ± 0.50	12.00^{a} ± 0.65	7.63^{c} ± 0.50	8.88^{bc} ± 1.42
	Heat stress	18.25^{a} ± 1.55	11.00^{bc} ± 2.01	14.20^{b} ± 1.22	9.50^{c} ± 0.86	11.06^{bc} ± 1.37	9.50^{c} ± 1.42
Intact acrosome (%)	Winter period	83.00 ± 0.65	83.75 ± 0.49	84.00 ± 0.65	82.75 ± 0.65	84.25 ± 0.75	83.50 ± 1.13
	Heat stress	83.87^{a} ± 0.95	82.12^{ab} ± 0.54	83.62^{ab} ± 0.67	81.25^{b} ± 0.49	83.00^{ab} ± 0.90	83.62^{ab} ± 0.82
CMPT (mm)	Winter period	23.00^{a} ± 1.02	20.63^{ab} ± 0.75	20.25^{b} ± 0.49	20.88^{ab} ± 0.64	20.50^{b} ± 0.78	21.38^{ab} ± 0.80
	Heat stress	25.87^{a} ± 1.12	25.25^{a} ± 0.99	25.75^{a} ± 1.12	25.12^{a} ± 0.97	22.00^{b} ± 1.16	21.87^{b} ± 0.92
HOST (%)	Winter period	69.00^{a} ± 2.53	59.67^{b} ± 4.46	59.50^{ab} ± 2.17	58.80^{ab} ± 0.93	61.60^{ab} ± 1.41	58.60^{b} ± 2.31
	Heat stress	53.78^{a} ± 0.83	52.31^{abc} ± 0.60	51.75^{abc} ± 0.56	51.00^{bc} ± 0.65	50.25^{c} ± 0.88	53.375^{ab} ± 0.82

Values with different superscript (a,b,c, d) in same row differ significantly ($P<0.05$).

summer may be one of possible reason to impair the testicular functions which leads to decreased sperm production with abnormal morphology.

Percent intact acrosome

There was no significant difference in acrosomal integrity during winter period and heat stress. Acrosomal integrity differed significantly ($P<0.05$) between bulls during heat stress whereas no significant difference between bulls during winter period (Table 2). The findings of present study was corresponding to the study undertaken by McNitt and First (1970); Heitman and Cockrell (1984); Larsson and Einarsson (1984); Colenbrander and Kemp (1990). This may be due to the disturbed spermatogenesis caused by high ambient temperature.

Cervical mucus penetration test (CMPT)

Cervical mucus penetration distance differed significantly ($P<0.01$) between winter period and heat stress. Cervical mucus penetration

distance differed significantly (P<0.05) between bulls during winter period as well as during heat stress (Table 2). *In vitro* cervical mucus penetration test it was showed that cervical mucus (CM) acted as a barrier which eliminates the spermatozoa with abnormal morphology and allows only the spermatozoa with normal morphology to pass and the spermatozoa which cannot penetrate in to the mucus lack the ability to fertilize the ovum (Robayo *et al*., 2008). Only limited information is available on the effective usage of this method for prediction of Murrah bull fertility (Dev *et al*., 1996).

Hypo-osmotic swelling test (HOST)

There was significant difference in HOS positive sperms between winter period and heat stress (P<0.01) as well as between bulls (P<0.05). Observations of present study were similar to Mandal *et al*. (2000); McNitt and First (1970); Heitman and Cockrell (1984); Larsson and Einarsson (1984) and Colenbrander and Kemp (1990).

The present study indicates that heat stress result in significant decrease in mass motility (P<0.05), increase in abnormal spermatozoa percent (P<0.01). The significant effect of season is observed between bulls for semen volume, sperm concentration, total number of sperms per ejaculate, initial motility, abnormal sperm percent, cervical mucus penetration and hypoosmotic swelling of spermatozoa.

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