Haryana Vet. (April, 2019) 58 (S.I), 46-49

Research Article

EFFECT OF SEASON ON FREEZABILITY OF MURRAH BUFFALO BULL SEMEN

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Received : 25.02.2019; Accepted : 28.02.2019

ABSTRACT

The present study was carried out to assess the effect of seasons on freezability of buffalo semen. A total of 48 semen ejaculates from six Murrah bulls were collected through artificial vagina method during winter (15-25°C temperature) of October-November (n=48 ejaculates) and heat stress (42-48°C temperature) periods of May-June (n=48 ejaculates) to study the effect of heat stress on semen freezability. The semen straws were cryopreserved in liquid nitrogen (-196°C) after fresh semen evaluation and post-thaw semen characteristics were assessed 24 hrs after cryopreservation. Overall mean values of post thaw motility (43.08±2.36 vs 38.75±2.86%), live sperm (55.91±1.73 vs 55.85±3.11%), abnormal sperm (14.58±0.87 vs 17.79±0.69%; P<0.01), intact acrosome (70.62±3.13 vs 65.50±4.29%), cervical mucus penetration (11.89±0.68 vs 11.27±0.85 mm) and hypo osmotic swelling reactive sperm (49.44±0.75 vs 38.98±2.91%; p<0.05) were in frozen semen during winter and heat stress periods, respectively. Significant higher functional integrity of sperm plasma membrane and lower percent abnormal sperm was observed during winter than in heat stress. Similarly, higher numbers of semen doses were produced during winter than heat stress period. It is concluded from above study that winter period is more favorable for semen production in Murrah buffalo bulls.

Key words : Heat stress, Murrah bulls, Semen freezability

Accurate prediction of fertility is a useful means for successful exploitation of production potential of sires. Artificial insemination (AI) with frozen thawed semen in buffalo has been reported to have low success rate than in cattle because of poor freezability and fertility (Kumaresan *et al.,* 2005). The quality of the frozen thawed semen is one of the most influential factors affecting the conception (Saacke, 1984). Environmental variables like photoperiod or day length, temperature, relative humidity and rainfall, either alone or in combination play significant roles in controlling reproductive behaviour. High ambient temperature during summer adversely affects testicular size, libido and semen quality (Soderquist *et al.,* 1996). The existence of a biological mechanism which makes them sensitive to the breeding stimulus during a particular season of the year is well established, but the exact nature of the internal mechanism that controls seasonal responses is not known. Most of the tests that are used for evaluation of semen are based on physical characters of spermatozoa. However, very little information is available on effect of environmental variables like temperature, humidity and day length in a particular season (Tiwari *et al*., 2011). Season may have it's effect on seminal attributes, freezability and fertility. Therefore, the present research work was carried out to study the effect of heat stress on frozen semen attributes and semen production in Murrah buffalo bulls.

MATERIALS AND METHODS

The study was conducted on six Murrah buffalo bulls 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 42-48°C) of May-June (n=48 ejaculates) and winter (temperature ranged between 15-25°C) of October-November (n=48 ejaculates) 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 to 47.4°C and relative humidity between 20 to 75%. 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 42-45°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. The sample of neat semen was processed as per mandate of minimum standard protocol of bovine semen in Accucell Bovine Photometer (IMV technologies France), so as to pack 20 million sperms per 0.25 ml per straw following 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 4ºC for 4 hrs and then to a programmable bio-freezer (IMV technologies, France) for 8-10 min. so as to reach the temperature to -140ºC. 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). Post-thaw semen straws were assessed at least 24

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hrs after cryopreservation. Evaluation for progressive motility, per cent live sperm (Campbell *et al.,* 1953), per cent total abnormal sperm, per cent intact acrosome (Watson, 1975), cervical mucus penetration test (Prasad *et al*., 1999) and Hypo-osmotic swelling test (Jeyendran *et al.*,1984) were carried out. Neat semen ejaculates with less volume (<1 ml), poor initial progressive motility (<70 percent) or poor sperm concentration (<500 millions spermatozoa/ml) were discarded. Frozen semen ejaculates were discarded with poor post thaw motility (<50 percent). 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

Season wise fresh and frozen semen characteristics (Mean±SE) of six Murrah bulls during winter and heat stress are presented in Table 1.

Progressive motility

No significant difference was observed on progressive motility between winter period and heats stress and between bulls (Table 2 and 3). Sagdeo *et al.* (1991) reported higher post thaw motility in winter followed by summer and monsoon season, and with a significant individual variation between Surti buffalo bulls. However, significant difference in (P<0.05) post thaw motility between seasons in Murrah buffalo bulls was observed by Tiwari *et al.* (2011). Similarly, post-thaw semen motility was significantly affected by season of collection, being lowest in summer and highest in winter in buffalo semen which may be due to spermatozoa are fragile and unable to withstand the stress of freezing during summer (Bagha and Khokar, 1991).

Percent live sperm

No significant difference was observed in percent live sperm between winter period and heat stress, as well as between bulls during heat stress (Table 2 and 3). However, significant difference was recorded between bulls during winter period. Our present findings are contrary to findings of Nath *et al*. (1991). These differences in observations may be attributed to age and season (Thongtip *et al*., 2008), breed and genetic reasons (Singh and Pangaonkar, 1990).

Table 1:

Fresh and frozen semen characteristics (Mean± SE) of Murrah bulls during winter period (15-25°C) and heat stress (42-48°C) period

Parameters	Season	Fresh semen	Frozen semen
		Mean± SE	Mean± SE
Progressive Motility (%)	Winter	72.5±0.89	43 .08±2.36
	Heat stress	73.33±0.87	38.75±2.86
Live Sperm (%)	Winter	85.82±0.77	55.91±1.73
	Heat stress	84.12±0.94	55.85±3.11
Abnormal Sperm (%)	Winter	8.71±0.47^{a}	14.58±0.87^{a}
	Heat stress	12.25±0.72^{b}	17.79±0.69^{b}
Intact Acrosome (%)	Winter	83.54±0.30	70. 62±3.13
	Heat stress	82.92±0.32	65.50±4.29
CMPT (mm)	Winter	21.10±0.3^{a}	11.89±0.68
	Heat stress	24.31±0.48^{b}	11.27±0.85
HOST (%)	Winter	55.88±2.65^{a}	49.44±0.75^{a}
	Heat stress	52.08±0.34^{b}	38.98±2.91^{b}

Progressive motility (%) refers to initial progressive motility in fresh and post thaw motility in frozen semen, respectively. Different superscript between season for various parameter differ significantly (P<0.01, except HOST P<0.05)

Sperm abnormalities

There was significant difference (p<0.01) in sperm abnormalities between winter and heat stress period in post thawed frozen semen samples. A significant individual differences (p<0.05) between bulls during winter period as well as during heat stress was observed (Table 2 and 3). The elevated environmental temperature

Table 2:

Bull wise fresh 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
Initial Progressive Motility (%)	winter period	70.63±1.75^{ab}	74.38±1.13^{a}	75.00±1.34^{a}	75.00±1.34^{a}	72.50±1.34^{ab}	67.50±4.00^{a}
	Heat stress	70.62±3.71^{b}	73.12±0.91^{b}	73.12±2.48^{ab}	77.5±0.94^{a}	74.37±0.62^{ab}	71.25±2.05^{ab}
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
Abnormal Sperm (%)	winter period	10.63±0.82^{ab}	6.50±1.25^{c}	6.63±0.50^{c}	12.00±0.65^{a}	7.63±0.50^{c}	8.88±1.42^{bc}
	Heat stress	18.25±1.55^{a}	11.00±2.01^{bc}	14.20±1.22^{b}	9.50±0.86^{c}	11.06±1.37^{bc}	9.50±1.42^{c}
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±0.95^{a}	82.12±0.54^{ab}	83.62±0.67^{ab}	81.25±0.49^{b}	83.00±0.90^{ab}	83.62±0.82^{ab}
CMPT (mm)	winter period	23.00±1.00^{a}	20.63±75^{ab}	20.25±0.49^{b}	20.88±0.64^{ab}	20.50±0.78^{b}	21.38±0.80^{ab}
	Heat stress	25.87±1.12^{a}	25.25±0.99^{a}	25.75±1.12^{a}	25.12±0.97^{a}	22.00±1.16^{b}	21.87±0.91^{b}
HOST (%)	winter period	69.00±2.53^{a}	59.67±4.46^{b}	59.50±2.17^{ab}	58.80±0.93^{ab}	61.60±1.41^{ab}	58.60±2.31^{b}
	Heat stress	53.78±0.83^{a}	52.31±0.60^{abc}	51.75 ±0.56^{abc}	51.00±0.65^{bc}	50.25±0.88^{c}	53.37±0.82^{ab}

Bull wise values with different superscript (a,b,c,d) in same row differ significantly (P<0.05)

Table 3:
Bull wise frozen semen characteristics (Mean± SE) of Murrah bulls during winter period (15-25°C) and heat stress (42-48°C) period

Parameters	Season	Bull No 44816	Bull No 116627	Bull No 114161	Bull No 70501	Bull No 43712	Bull No 81027
Post thaw Motility (%)	Winter	48.57±1.33	43.75±4.20	51.00±0.68	40.00±6.54	47.50±5.90	43.33±16.33
	Heat stress	37.50± 8.18	33.75±7.99	38.75±7.42	40.00±6.54	38.75±7.42	43.75±6.25
Live Sperm (%)	Winter	59.71±0.63 ^{a}	56.50±1.12 ^{a}	58.75±0.96 ^{a}	59.50±0.78 ^{a}	57.12±0.55 ^{a}	58.75±0.70 ^{b}
	Heat stress	46.75±10.21	53.75±7.69	56.12±8.05	62.87±0.64	55.00±7.87	60.62±8.67
Abnormal Sperm (%)	Winter	11.12±2.48 ^{b}	13.50±1.99 ^{ab}	13.87±2.20 ^{ab}	19.00±0.80 ^{a}	15.37±2.27 ^{ab}	14.62±2.23 ^{ab}
	Heat stress	16.57±0.09 ^{bc}	20.00±0.88 ^{a}	17.50±0.86 ^{abc}	19.88±0.72 ^{ab}	17.75±0.88 ^{abc}	17.75±0.88 ^{c}
Intact Acrosome (%)	Winter	77.17±0.90	76.50±0.73	76.62±0.68	76.66±0.83	78.57±0.6 7	79.40±0.40
	Heat stress	59.62±13.00	70.00±10.00	49.75±14.56	78.25±0.59	69.37±9.93	66.00±9.49
CMPT (mm)	Winter	15.33±0.48 ^{a}	11.33±0.73 ^{cd}	11.38±0.82 ^{bc}	13.17±1.17 ^{ab}	15.57±1.24 ^{a}	9.20±0.46 ^{d}
	Heat stress	9.75±2.18	12.62±2.08	10.37±3.14	12.5±0.57	14.00±2.16	8.37±1.27
HOST (%)	Winter	52.75±0.65 ^{ab}	41.00±0.46 ^{d}	45.38±0.86 ^{c}	52.25±0.0.59 ^{ab}	51.00±0.80 ^{b}	54.25±1.21 ^{a}
	Heat stress	41.12±9.08 ^{a}	40.00±6.1 ^{ab}	19.25±5.95 ^{b}	38.25±3.12 ^{ab}	44.62±6.42 ^{a}	50.62±7.37 ^{a}

Bull wise values with different superscript (a,b,c,d) in same row differ significantly (P<0.05)

during summer impairs testicular functions and leads to decreased sperm production with abnormal morphology which may be due to testicular hyperthermia (Rao *et al.*, 1996).

Percent intact acrosome

No significant difference was observed in percent intact acrosome between winter period and heat stress and in between various bulls (Table 2 and 3). Acrosomal integrity is significantly correlated with fertility of frozen thawed semen (Saacke and White, 1972) and refrigerated semen (Singh *et al*., 1992). Slightly lesser percent intact acrosome during heat stress (65.50±4.29) than winter period (70.62±3.13) may occur due to damage during dilution, cooling, freezing and thawing processes (Tasseron *et al.,* 1977).

Cervical mucus penetration test (CMPT)

There was no significant difference between winter and heat stress period and between bulls (Table 2 and 3) during heat stress, but differed significantly ($p<0.05$) between bulls in winter period. Prasad *et al*. (1999) found that sperm penetration distance (SPD) was greatly influenced by cryopreservation of semen and decreasing trend was observed in frozen semen than fresh semen in bovines.

Hypo-osmotic swelling test (HOST)

Functional integrity of sperm plasma membrane differed significantly ($p<0.05$) between winter period and heat stress as well as between the bulls during winter period and heat stress (Table 2 and 3). Our findings are in line with the findings of Colenbrander and Kemp (1990). Semen freezability in buffaloes during hot-dry season was lower (abnormal sperm % and HOST) and percent rejection semen samples was relatively high. Freezing affect not only post thaw motility but may cause functional and structural changes on sperm which might affect the fertility of semen (Mishra and Sengupta, 1965).

Semen Production

Total 48 semen ejaculates collected during heat stress and winter period from six Murrah bulls. Two and seven neat semen ejaculates were discarded during winter period and heat stress after initial evaluation, respectively. However, seven semen ejaculates were discarded during winter and heat stress having low progressive motility (less than 50%). A total of 10721 (39 ejaculates) and 8246 (34 ejaculates) semen doses were frozen during winter period and heat stress. Our present findings are in line with the report of Tiwari *et al*. (2011).

From the present study, it can be concluded that winter period is more favorable for semen production in Murrah bull and the extent of loss due to poor freezability during the hot-dry season could be minimized by providing thermal comfort during a stressful period. There is significant variation in freezability on production of abnormal spermatozoa and HOST between seasons particularly between bulls.

ACKNOWLEDGEMENTS

Authors are highly thankful to Dean, College of Veterinary Science and Animal Husbandry, Anjora, Durg and Director Veterinary Services (C.G.) for permitting to carry out the research project at Central Semen Station, Anjora, Durg and providing necessary facilities.

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