SEASONAL VARIATION OF SPERM KINEMATICS IN MURRAH BULLS UNDER THE TROPICAL CLIMATIC CONDITION

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ABSTRACT

The present study was undertaken to identify the difference in sperm kinematics in Murrah buffalo bulls in different seasons. Eight Murrah buffalo bulls maintained at Artificial Breeding Research Centre, ICAR-NDRI Karnal. Frozen semen samples of the bulls were obtained and sperm kinematic parameters were measured by computer-assisted sperm analyzer. The sperm kinematic parameters included curvilinear velocity (VCL), linear velocity (VSL), average path velocity (VAP), distance of average path (DAP), distance straight line (DSL), straightness coefficient (STR), linearity (LIN), amplitude of lateral head displacement (ALH), and beat cross frequency (BCF). Morphological abnormality of sperm includes bent tail (BT), coiled tail (CT), distal droplets (DD) and distal mid-piece reflex (DMR). Analysis revealed that the proportion of BT was significantly (P<0.05) lower in winter and autumn season as compared to summer and rainy season. Percent of total motile and progressive motile sperm was significantly (P<0.05) higher in winter season as compared to summer and rainy season. The VAP, VSL and VCL of sperm motion were significantly (P<0.05) higher in winter and autumn season as compared to the summer season. The DAP and STR were significantly (P<0.05) higher in winter and autumn season as compared to summer and rainy season. DSL and LIN were significantly (P<0.05) lower in summer and rainy season followed by autumn and winter season. Post-thaw motility and motion parameter varied significantly between seasons, and higher sperm motility was maintained in semen cryopreserved in winter followed by autumn, rainy and summer season.

Keywords: *Bubalus bubalis*, buffaloes, buffalo bulls, CASA, season, sperm kinematics

INTRODUCTION

Worldwide, artificial insemination (AI) technique using cryopreserved semen of superior bulls has played an essential role in the

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improvement of milk production trait. Thus, in developing countries like India, the importance of AI in buffaloes is increasing at a faster rate and as a result demand of buffalo bull semen is also increasing. Therefore, AI with the quality frozen semen of genetically superior bulls is the only way through which a sizable population can be covered and genetically improved (Singh and Pant, 2000). However, AI coverage of buffaloes is less compared to cattle enduring to poor conception rate with frozen buffalo semen (Kumaresan and Ansari, 2001). Season influences the fertility of cryopreserved spermatozoa of water buffalo reported using AI (Ahmad *et al*., 2016). Semen freezability during the stressful summer season is unfavourable for buffaloes, and percent rejection rate of semen samples are relatively high (Singh *et al*., 2001). Seasonal variations of semen quality have been partially attributed to related changes in scrotal thermoregulation and heat dissipation mechanisms (Menegassi *et al.,* 2014; Bhakat *et al*., 2015). The testicle is more susceptible to increasing the environmental temperature which in turn leads to degenerative changes, characterised by a change in the testicular consistency and ultimately leads to the production of poor semen quality (McEntee, 1990). Moreover, the animal exposed to heat stress, decrease the synthesis of luteinizing hormone (LH), which affects the production of testosterone by the testes, LH is also playing an important role for quality sperm production in spermatogenesis process (Gilad *et al*., 1993). Individual variations among males have been reported in respect to fertility and cryotolerance of spermatozoa (Kumaresan *et al*., 2012), as compared to cattle spermatozoa the buffalo spermatozoa have less cryotolerance capacity and more susceptible to hazardous effect during the process of cryopreservation (Andrabi, 2009). The assessment of semen quality concerning motility, velocity, swimming pattern, sperm head displacement and sperm abnormalities, may help in the selection of bulls for semen cryopreservation (Perumal *et al*., 2014). Computer-assisted semen analyzers offer accurate information on different motion characteristics of spermatozoa (Amann and Waberski, 2014). CASA analysis proposed a potential indicator for accurate prediction of male fertility as compared to the traditional semen evaluation method (Broekhuijse *et al*., 2012). Motion characteristics of bull spermatozoa using CASA have shown correlation with oocyte penetration rate, *in-vitro* fertility, and field fertility (Kathiravan *et al*., 2008). Therefore, the study was planned to investigate morphological abnormalities of sperm, sperm motility and kinematics by CASA to understand the seasonal variation of Murrah bull semen.

MATERIALS AND METHODS

Experimental animals

The present study was conducted on eight Murrah breeding bulls maintained at Artificial Breeding Research Centre, ICAR-NDRI, Karnal, Haryana, India. The Institute's Animal Ethics Committee approved all the experimental protocols. The bulls were maintained under individual housing and uniform management system. Vaccination, deworming, and herd-health programmes were followed as per the standard protocol.

The experimental period was subdivided into four seasons based on average mean monthly temperature and relative humidity as winter (December to February), summer (March to June), rainy (July to September) and autumn (October to December) season.

Post-thaw sperm kinematics parameters

Computer-assisted sperm analyser (HTM-version 12 IVos) was used to evaluate the kinetic parameters and morphological abnormality of sperm. CASA set-up for motility assessment of Murrah spermatozoa was Frame rate (60 Hz), Frames acquired (30), Minimum contrast (35), Minimum cell size (5 Pixels), Cell size (9 Pixels), Cell intensity (110 Pixels), Path velocity (VAP; 50 µm/s), Straightness (STR; 70%), VAP cut-off (30 µm/s), VSL cut-off (15 µm/s). Twenty four ejaculates from each season at different time intervals was diluted with Tris buffer. The frozen semen sample was thawed at 37°C and diluted with warm tris buffer to get a final concentration of 2 to 7 million spermatozoa per mL. Pre-warmed Leja 8-chambered slide having a depth of 20 µm was loaded with 1 µL semen sample. The sperms were automatically counted in 5 optical fields around the central reticulum of the chamber. The motion parameters like sperm motility (%), progressive motility (PM %), Average path velocity (VAP; µm/s), Straight line velocity (VSL; µm/s), Curvilinear velocity (VCL; µm/s), ALH (µm), BCF (Hz), STR (%), LIN (%) and sperm abnormalities like bent tail (BT %), coiled tail (CT %), distal droplets (DD %) and distal mid-piece reflex (DMR %) were observed and recorded (Kumar *et al*., 2014). CASA set up for motility assessment of frozen-thawed buffalo spermatozoa is presented below. Apart from the total motility and progressive motility, the kinetic parameters, as described by Muino *et al.* (2008), were recorded for each sample.

Statistical analysis

The data were analysed by one-way Analysis of Variance (ANOVA) using SPSS Statistics programme version 20 (IBM, Armonk, NY, USA) and means were compared using Duncan's multiple range test.

RESULTS

Post-thaw sperm kinematics parameters

The real-time assessment of frozen-thawed sperms of Murrah bull in winter, summer, rainy and autumn season was estimated in Computer Assisted Sperm Analyser (CASA), and results are presented in Table 1. Except for the bent tail (BT), percent coiled tail (CT), distal droplet (DD) and distal mid-piece reflex (DMR) were not affected by seasons, and the values were not statistically different. BT was significantly ($P<0.05$) higher in stressful summer and rainy season as compared to favourable winter and autumn season. In the summer (0.99±0.15) and rainy (0.96±0.14) season, the percent BT was increased by 2.06 fold as compared to winter (0.48±0.08) and autumn (0.51±0.05) season (Figure 1).

The real-time assessment of sperm kinematic parameters like total motile (%), Progressive motile (%), VAP (µm/sec), VSL (µm/sec), VCL (µm/sec), DAP (µm), DSL (µm), STR (%), LIN (%), ALH (µm), BCF (Hz) are very good indicator of sperm fertility (Figure 2 and 3). Frozen-thawed sperm kinematic parameters like VAP, VSL, VCL, ALH, and LIN were positively correlated with bull fertility. Results have shown that per cent total motile and progressively motile spermatozoa were significantly ($P<0.05$) higher in ejaculates of winter season as compared to summer and rainy season. However, there was no significant difference in total and progressively motile sperm between winter and autumn season. In kinematic parameters, VSL, VCL, DSL, LIN was found to be highest in the winter season. The values of these parameters were significantly (P<0.05) decreased in frozen-thawed sperms of bull ejaculates of stressful summer and rainy season as compared to the winter season. Whereas, non-significant between winter and autumn season. However, in the autumn season, VSL, VCL, DSL and LIN were significantly (P<0.05) higher than summer season, but remains statistically non-significant with the rainy season.

The values of VAP, DAP and STR were found to be significantly (P<0.05) higher in winter and autumn seasons as compared to summer and rainy season. However, these parameters did not differ significantly between winter and autumn season, but values in both of these seasons were significantly (P<0.05) higher than summer and rainy season. Beside this, no statistically significant difference was observed in the ALH and BCF in buffalo frozen-thawed sperms of ejaculates of any of the tested seasons (Table 1).

DISCUSSIONS

The method of Assisted Reproductive Technologies (ART) using artificial insemination technique can only be implemented with the use of cryopreserved semen (Thibier and Wagner, 2002). It is well documented that, the ability of fresh ejaculate sperm to maintain its functional status after post-thaw is greatly affected due to the seasonal variation at the time of ejaculate collection (Orgal *et al*., 2012). Thus, there is a need of strong indication to assess the fertility of bovine sperm to achieve best semen freezability and during comfortable and stressful seasons in tropical climatic conditions. Now a days, CASA is widely used to explore the sperm fertility by generating useful information on a wide array of functional and structural attributes of sperm, which are associated to its fertilizing potential (Graham and Moce, 2005; Sallem *et al*., 2015). Ahmed *et al.* (2016) predicted fertility in buffalo bull during low-breeding season using *in-vivo* fertility and post-thaw kinematic parameters and found a significantly positive correlation for *in-vivo* fertility with sperm progressive motility (r=0.64), rapid velocity (r=0.57), VAP (r=0.52), VSL (r=0.56), plasma membrane integrity (r=0.74) and spermatozoa with intact acrosome (r=0.88). Sperm kinematic parameter like sperm motility, VAP, VSL, VCL, ALH and LIN was positively correlated with fertility in Murrah buffalo bulls (Singh *et al*., 2017).

Thus, in the present study, fresh ejaculates of bulls during different seasons were collected, extended and cryopreserved to assess actual sperm freezability and fertility by CASA. The results depicted that kinematic sperm parameters such as total motility, progressive motility, VAP, VSL, VCL, DAP, DSL, STR and LIN were significantly (P<0.05) decreased during stressful summer and rainy season as compared to the comfortable winter season. In the similar line, Perumal *et al.* (2017) assessed the effect of season on frozen-thawed Mithun bull sperm kinematic parameters and reported that total sperm motility, progressive motility, VSL, LIN and STR were significantly (P<0.05) lower in summer as compared to the winter season. However, ALH, BCF and sperm abnormalities except bent tail percent were statistically independent of seasonal variations. In a similar line, Nitharwal *et al.* (2017) reported a non-significant effect of the season in ALH and

BCF and a significant effect on sperm motility in Murrah bull. In the similar line of our study, Wang *et al.* (2015) investigated the effect of season (spring, summer, autumn and winter) on CASA based pre-freeze and post-thaw seminal quality parameters in Xinong Saanen bucks. Authors reported a distinct effect of season of ejaculate collection on quality parameters and it was highest in autumn. Sperm kinetic parameters (VSL, VAP, ALH, BCF, LIM and STR) reported significant variation between winter and summer parameters except for VCL in Khuzestan buffalo bulls (Mayahi *et al*., 2014). Various authors (Heuer *et al*., 1987; Koonjaenak *et al*., 2007; Andrabi, 2009) reported that lower cryodamage of spermatozoa and higher bull fertility in the favourable season as compared to the stressful summer season.

The adverse effect of semen quality in summer may be directly due to the increase in core body temperature and change in physiological responses followed by testicular temperature. The buffalo bulls cannot cope up with the environmental stress due to compromised physiological responses and thermo-regulatory mechanism due to blackish skin colour and less sweat gland (Shafie and El-Khair, 1970). Sperm ejaculated in summer season showed three-fold lower expressions of very low-density lipoprotein receptors (VLDLr) as compared to the winter season, which altered fatty-acid composition and utilization of extracellular lipid, to reduced semen quality during summer (Argov-Argaman *et al*., 2007; Argov-Argaman *et al*., 2013). Under the stressful climatic condition, ejaculates collected during high heat stress have lower mitochondrial membrane potential (MMP), loss of plasma membrane integrity results in impaired mitochondrial function (Rahman *et al*., 2014).

During a stressful condition is associated with abnormal sperm quality due to higher ROS production and their highly reactive, may result in cellular damage through structural and functional changes (Valeanu *et al*., 2015). This results in the generation of reactive oxygen species which negatively affect spermatogenesis and semen quality traits (Kastelic *et al*., 2001; Setchell, 2006). Regarding the poor CASA parameter in Holstein-Friesian bull's ejaculate collected during the summer season, Malama *et al.* (2017) reported that season affects sperm mitochondrial function and sperm ability to retain low intracellular (Ca^{2+}) and undergo acrosome reaction. Thus, for this defect, a relation between the functional status of individual sperm organelles and seasonal climatic conditions can be assumed. However, the very little scientific evidence is available regarding the physiological mechanisms underlying seasonal variations of sperm kinematics.

CONCLUSION

It can be concluded that ejaculates collected under stressful summer and rainy season showed poor post-thaw survivability in comparison to winter seasons in Murrah bulls. Semen produced during winter and autumn season have better post-thaw sperm kinematics as compared to summer and rainy seasons. Summer season is the stressful condition for Murrah bulls. Better management decision can be taken to reduce thermal stress to minimise the effect of climatic conditions on male fertility in the bull.

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Table 1. Means±SEM of Post thaw CASA parameters of Murrah buffalo bull in different seasons.

Parameters	Winter	Summer	Rainy	Autumn
Morphological abnormalities				
BT (%)	0.48[a]±0.08	0.99[b]±0.15	0.96[b]±0.14	0.51[a]±0.05
CT (%)	0.05±0.03	0.09±0.04	0.08±0.04	0.06±0.03
DD (%)	0.11±0.04	0.15±0.05	0.14±0.05	0.13±0.04
DMR (%)	3.34±0.15	3.87±0.17	3.88±0.25	3.41±0.24
Kinematic parameters				
Motile (%)	54.83[a]±5.38	38.68[bc]±5.51	41.32[bc]±6.91	48.89[ac]±6.49
Progressive motile (%)	36.04[a]±2.82	23.12[b]±4.50	25.28[bc]±3.98	32.69[ac]±4.59
VAP (µm/sec)	102.01[a]±3.01	92.15[b]±3.30	93.61[b]±2.97	99.30[ab]±2.53
VSL (µm/sec)	80.58[a]±2.38	66.92[b]±4.15	70.48[bc]±3.38	75.08[ac]±2.16
VCL (µm/sec)	187.39[a]±4.82	164.42[b]±2.59	173.63[bc]±5.96	181.32[ac]±7.20
DAP (µm)	32.39[a]±0.53	24.73[b]±0.56	27.17[b]±1.33	31.60[a]±0.74
DSL (µm)	25.32[a]±0.91	19.26[b]±1.19	22.50[bc]±0.93	24.55[ac]±1.59
STR (%)	81.06[a]±1.32	74.03[b]±0.45	76.59[b]±1.41	80.55[a]±0.48
LIN (%)	46.44[a]±1.03	39.41[b]±1.15	42.18[bc]±1.44	44.47[ac]±0.96
ALH (µm)	8.05±0.19	8.32±0.35	8.26±0.23	8.17±0.24
BCF (Hz)	24.73±0.64	26.12±0.91	25.17±0.43	25.26±0.69

Mean bearing different superscripts a, b, c in a row differ significantly ($P<0.05$).

BT: Bent Tail, CT: Coiled Tail, DD: Distal Droplets, DMR: Distal Mid-piece Reflex,

VAP: Average Path Velocity, VCL: Curvilinear Velocity, VSL: Straight-Line Velocity,

DAP: Distance of Average Path, DSL: Distance Straight Line, STR: Percentage of Straightness,

LIN: Linearity, ALH: Amplitude of Lateral Head Displacement, BCF: Beat Cross Frequency

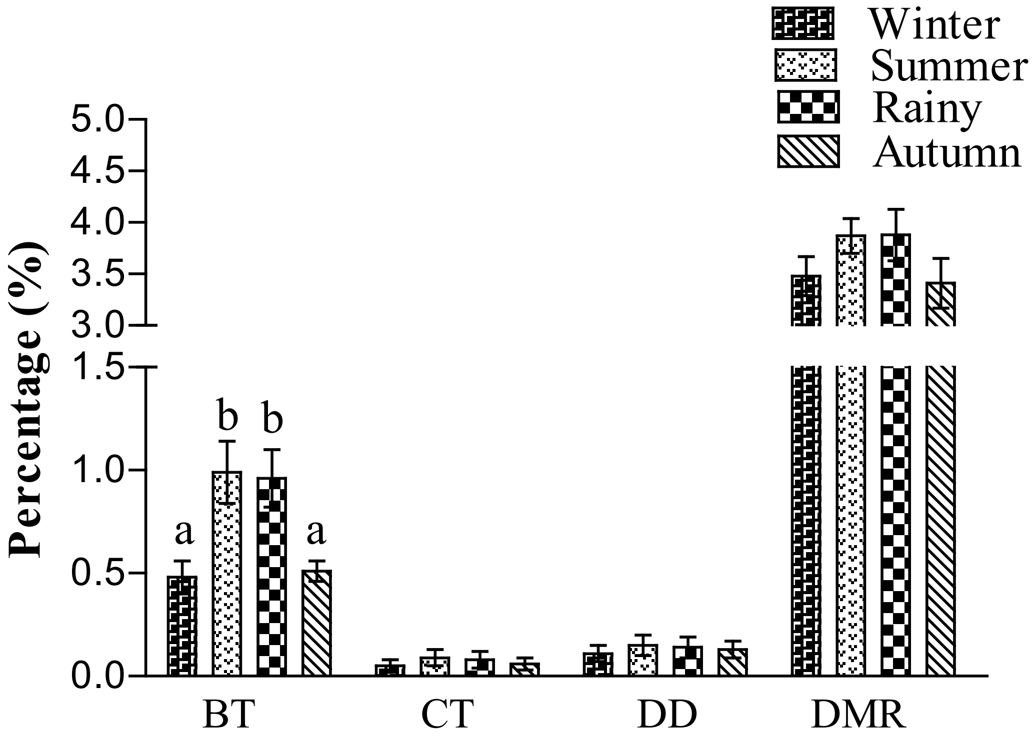

Figure 1. Morphological abnormalities (Mean ± SEM) of Murrah buffalo bulls in different seasons.

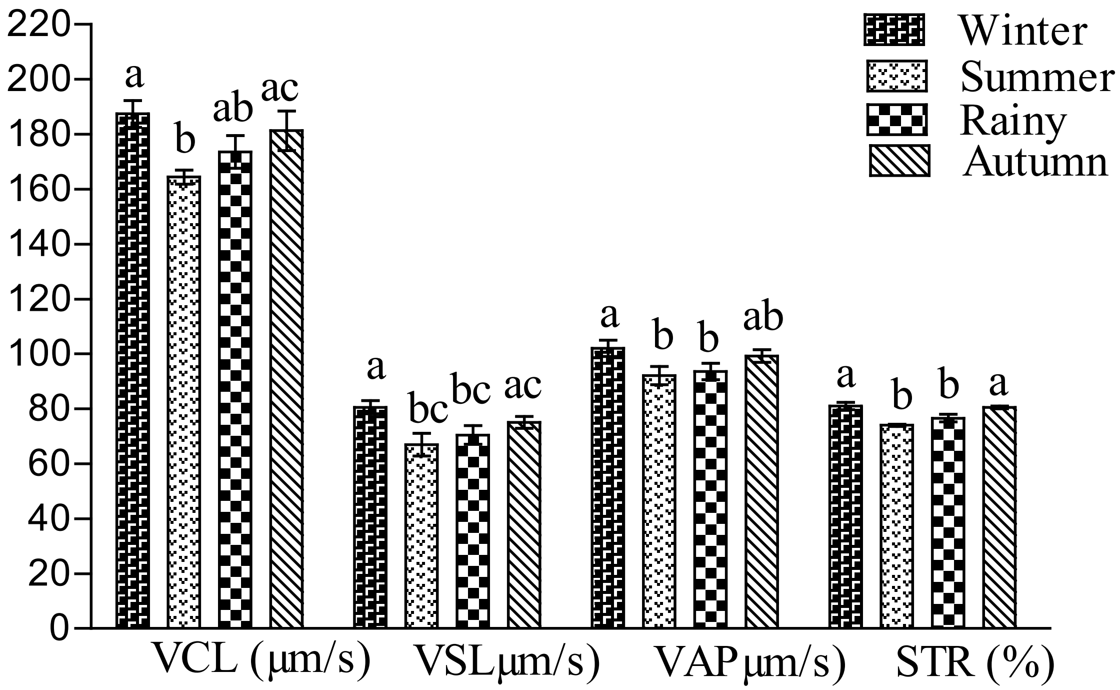

Figure 2. Kinematics of frozen-thawed spermatozoa (Mean ± SEM) of Murrah buffalo bulls in different seasons.

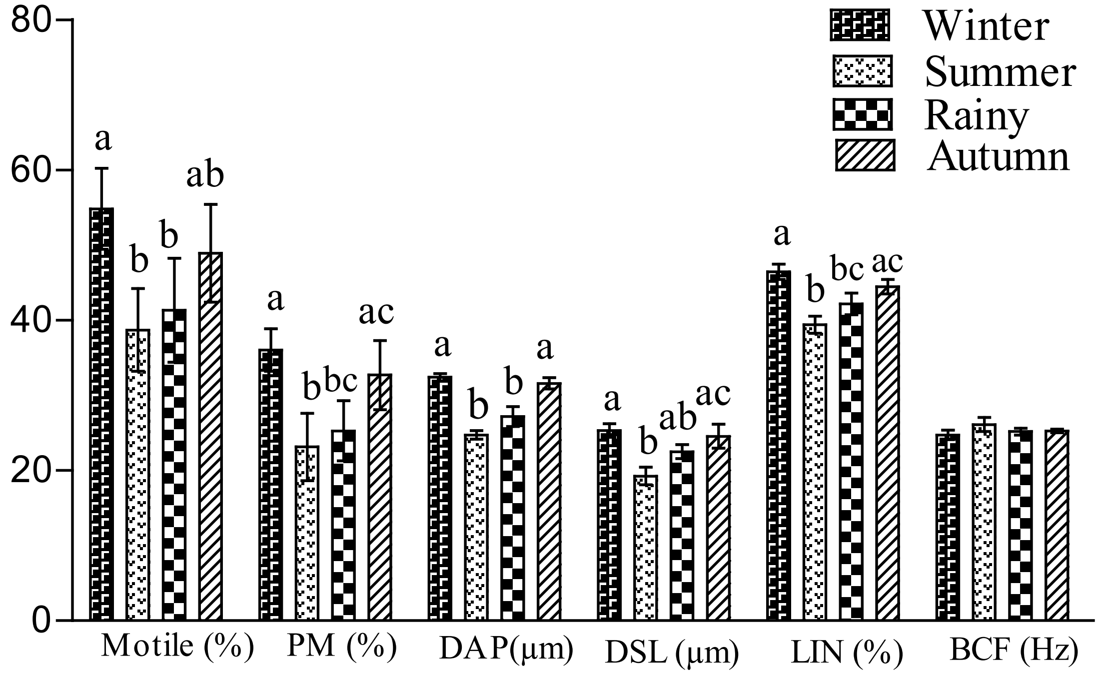

Figure 3. Kinematics of frozen-thawed spermatozoa (Mean ± SEM) of Murrah buffalo bulls in different seasons.

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