Indian Journal of Animal Sciences **87** (1): 64–69, January 2017/Article

Effect of season on semen quality parameters in Murrah buffalo

K NITHARWAL[1], P SINGH[2], M BHAKAT[3], A CHAURASIA[4], M ABDULLAH[5] and C PRABHA[6]

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

Received: 7 May 2016; Accepted: 6 June 2016

ABSTRACT

Seasonal influence on frozen semen quality in Murrah buffalo breeding bulls was determined. Frozen semen samples of 6 Murrah buffalo bulls were collected and semen frozen in 4 different seasons, viz. winter (Dec-Feb), spring (mid Feb-Apr), summer (May-Jun) and rainy (Jul-Aug) were assessed. Samples (12) of each bull, in a season, were evaluated for sperm motility, viability and acrosome integrity. Motility and other kinematics of spermatozoa during incubation (37°C) at 0, 30, 60, 90 and 120 min of thawing were assessed with computer assisted semen analyzer. Post-thaw sperm total motility and viability differed significantly among the seasons, the highest was in winter. Sperm plasma membrane integrity, acrosome integrity, progressive motility, rapid motility and other CASA evaluated parameters did not differ significantly among the seasons. Higher values of plasma membrane integrity (PMI), progressive motility, rapid motility, average path velocity (VAP), straight line velocity (VSL), curvilinear velocity (VCL), beat cross frequency (BCF), linearity (LIN) and straightness (STR) were obtained in winter season as compared to other seasons. Post-thaw motility at 0 min and 60 min of post-thaw incubation varied significantly between seasons and higher sperm motility was sustained for a longer period in semen cryopreserved in winter followed by rainy season, summer and spring. It can be concluded from this study that buffalo bull semen produced and frozen during winter season resulted in higher sperm motility, viability and post-thaw longer survivability in comparison to other seasons.

Key words: Incubation test, Murrah buffalo, Season, Semen quality, Sperm kinematics

Artificial insemination is an important tool for the improvement of milk productivity in buffaloes by the propagation of animals with high genetic potential (Baruselli and Carvalho 2005). Season influences the quality of semen significantly (Snoj *et al.* 2013, Bhakat *et al.* 2014). Post-thaw plasma membrane integrity, stability and DNA fragmentation index (DFI) were significantly better in ejaculates processed during winter than other seasons (Koonjaenak *et al.* 2007a). In bull, the basis of variation in semen quality was due to the change in the seasonal expression of low density lipid receptors on the surface of sperm cells which influence the utilization seminal plasma constituents in between seasons (Argov *et al.* 2007). Therefore, there is need to study more sperm functional attributes and also to evaluate the post-thaw *in-vitro* survivability of the semen frozen during different seasons. Therefore, this study was undertaken to assess the effect of season on certain functional attributes and post-thaw *in-vitro* survivability of frozen spermatozoa of Murrah buffalo bull.

Present address: [1]Veterinary Officer (kuldeep.ndri @gmail.com), Rajasthan; [4]Veterinary Officer (ajit@gmail.com), Uttar Pradesh; [3]Senior Scientist (bhakat.mukesh@gmail.com); [2]Principal Scientist (pawansing@scientist.com); [5]Ph D Scholar (mabhatndri@gmail.com), [6]Govt. Teacher (dr.prabha@ gmail.com), Rajasthan.

MATERIALS AND METHODS

The present investigation was conducted on frozen semen of 6 healthy Murrah buffalo bulls (age, 3 to 7 year; body weight, 585 to769 kg), maintained at Artificial Breeding Research Centre, ICAR-National Dairy Research Institute, Karnal, Haryana. Bulls were kept in individual bull pens (30′×10′) under loose housing system on concrete floor with the orientation of east-west direction through its long axis. The bulls were fed according to farm schedule. The bulls were provided daily 1 h exercise in a week, and 1 h prior to the semen collection in a rotatory exerciser. Vaccination for common diseases; deworming and other herd-health programmes were followed as per the farm schedule, to maintain the proper health of animals. These bulls were in regular semen collection and 2 ejaculates on each collection twice in a week were taken. Semen of these bulls frozen in winter (Dec to Mid-Feb), spring (Mid-Feb to Apr), summer (May to Jun) and rainy season (Jul to Aug) were used for this study.

Semen collection and cryopreservation: The bulls were examined and showed good libido based on the sexual behavior observations. The bulls were in regular semen collection throughout the year. Their ejaculate quality and freezability were above the acceptable level. The bulls were washed 1 h prior to taking them to the site of collection, which was adjacent to semen processing laboratory in the

morning hours beginning from 6:30 AM. Semen was collected using bovine artificial vagina with smooth neoprene liner, over a male dummy buffalo bull twice a week. The temperature of AV was maintained between 42°C–45°C with sufficient pressure and proper lubrication with sterilized KY jelly if required. Two successive ejaculates were collected at an interval of 20 to 30 min between 2 ejaculates, and each ejaculate was preceded by a period of sexual preparation consisting of at least 2 false mounts separated by about 1 min restraint. Immediately after collection, each ejaculate was placed in a water bath at 30°C. Every semen sample was evaluated for its suitability for further processing and cryopreservation. Each semen sample was diluted using tris-egg yolk-glycerol extender so that each straw (0.25 ml, French mini) should have 20 million sperms. Each semen sample was further processed for cryopreservation as per standard procedure followed in the Artificial Breeding Research Centre.

Frozen semen evaluation: Two frozen semen samples of every bull from each season, during mid to late part of the season, were taken and thawed at 37°C for 30 sec in a thawing unit for evaluation of sperm kinematics, viability, plasma membrane and acrosome integrity. For incubation test, after thawing semen samples were kept at 37°C in a water bath for evaluation of motility parameters at 0, 30, 60, 90 and 120 min of thawing with computer assisted semen analyzer, Integrated Visual Optical System. Following thawing, an aliquot of the semen was further diluted for making the sperm concentration to 10×10^6/ml per sample using tris buffer for CASA analysis. One microlitre of the prepared sample was loaded in the CASA slide (8-chambered Leza slide) for assessment of sperm kinematics, 6 microscopic fields were analyzed for each semen sample. Per cent sperm motility (SM), progressive motility (PSM), average path velocity (VAP) (µm/s), straight line velocity (VSL) (µm/s), curvilinear velocity (VCL) (µm/s), lateral amplitude of head displacement (ALH) (µm), beat cross frequency (BCF) (Hz), straightness (STR) (%), and linearity (LIN) (%) were analyzed as per Partyka *et al.* (2012).

Semen quality tests

Hypo-osmotic swelling test: It was done by evaluating the integrity of sperms in sodium citrate solution with concentrations of 150 milliosmoles and 300 milliosmoles about 1 h of incubation at 37°C. The integrity of sperms was evaluvated by calculating the percentage of curved/ straight sperms from about 200 sperms at 40×.

Live and dead and acrosome integrity: This was done immediately after thawing of frozen semen straws. The live dead count of sperms of the semen sample was evaluated by using Eosin-Nigrosine stain in ratio of 1:4. The membrane of dead sperms gets impaired so they are stained by eosin (pinkish red) while live sperms with intact membrane don't take any colour and remain white. The live dead count of about 200 sperms was done under oil immersion microscope at 100×.

Acrosome integrity: This was done by immersing the slide with fixed smear of semen in Gemsa- Sorenson's solution (3 ml Gemsa, 2 ml Sorenson's buffer plus 35 ml water in a couplin jar for 2 h), then after washing the percentage of acrosome integrity is calculated out of 200 sperms under oil immersion at 100×.

All the experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC).

Statistical analyses: To assess the effect of season of cryopreservation on frozen semen attributes, data were analyzed by one way Analysis of variance (ANOVA) using SAS (9.3) and means were compared with Duncan's new multiple range test (DMRT).

RESULTS AND DISCUSSION

Quality semen production is the main aim of the semen stations, but various factors affect semen quantity and quality, among them, season is playing a major role through direct and indirect effect on semen production of breeding bulls. Buffalo bulls are vulnerable in quality semen production due to their inherent constraints during summer season. The results pertaining to seasonal influence on the viability, plasma membrane integrity and acrosome integrity are presented in Table 1.

Sperm viability: The mean per cent sperm viability was 66.54±1.90, 55.69±1.79, 49.00±3.82 and 52.24±1.82 in winter, spring, summer and rainy season, respectively. Sperm viability showed significant ($P<0.01$) variation among the seasons and higher per cent sperm viability was obtained in winter as compared to other seasons. The results were in consonance with previous studies in riverine buffaloes, which confirmed better survival of spermatozoa frozen during winter season as compared to summer (Bhakat *et al.* 2015). Whereas Sarder (2007) reported no differences in sperm viability between seasons. This might be due to different geographical location, breed differences, experimental design and protective measure provided during experimental period. Poor semen quality during summer season similar to present findings were reported by Valeanu *et al.* (2015).

Sperm plasma membrane integrity: Sperm membrane integrity was marginally higher in winter season as compared to other seasons. The results are in agreement with the findings of Shukla and Misra (2007) in Murrah bulls, but

Table 1. LSM ± SE (%) post-thaw sperm functional attributes

Season	Viability	Plasma membrane integrity	Acrosome damaged
Winter	66.54 [a] ± 1.90	92.17 ± 0.75	26.71 ± 3.06
Spring	55.69 [b] ± 1.79	91.33 ± 0.51	32.06 ± 5.04
Summer	49.00 [b] ± 3.82	91.76 ± 0.68	31.26 ± 0.31
Rainy	52.24 [b] ± 1.82	91.61 ± 1.82	31.93 ± 4.62

Values with different superscripts in same column differ significantly ($P<0.01$).

lower values were reported by Rasul *et al*. (2001) in Nili-Ravi buffalo, which may be due to differences in the breed, freezing methods, extender used, thawing rate and procedure of evaluation. PMI is more accurate in predicting fertility than sperm motility (Fraser *et al.* 2001) as it is essential for cell viability and the ability of the spermatozoa to interact with the female reproductive environment. Therefore, any modification of the intactness of the plasma membrane caused by handling, including cryopreservation, would impair or at least limit the fertilizing ability of the spermatozoa (Rodriguez-Martinez 2003).

Sperm acrosome damage: Mean per cent value of sperm acrosome damaged was 26.71±3.06, 32.06±5.04, 31.26±0.31 and 31.93±4.62 in winter, spring, summer and rainy season, respectively. Acrosome integrity and acrosome damage may have a predictive role in the field fertility (Birck *et al.* 2010). Sperm acrosome membrane damage was lower in winter as compared to other seasons. On similar line, Bhakat *et al.* (2014) also reported a significant effect of season on acrosome integrity. The better status of acrosome during the winter could be due to the fact that there was less capacitation like changes occurred during colder months of the year in buffaloes (Albero *et al.* 2014) and it is further emphasizing on a better fertilization potential of spermatozoa frozen during winter. Whereas Valeanu *et al*. (2015) reported no significant seasonal differences in acrosome status.

Post-thaw in-vitro *survivability*: Incubation was done at 0, 30, 60, 90 and 120 min for assessment of duration of sustainability of sperm motility and sperm velocity parameters evaluated by CASA, after thawing and the results of post thaw semen quality at different hours of incubation are shown in Tables 2, 3.

Post-thaw sperm motility: The results showed that immediately post-thaw (at 0 min) sperm motility (%) of semen frozen in winter (61.00±4.70) was significantly (P<0.01) higher than the semen frozen in summer (45.71±2.76) and rainy (50.64±1.61) season but not than spring season (54.00±1.65). A significant difference (P<0.05) in sperm motility was found at 60 min of incubation among the seasons, but not at 30, 90 and 120 min of incubation (Table 2). Maurya *et al*. (2003) emphasized that the success of AI depends upon effective prolongation of fertile life of spermatozoa under *in-vitro* condition and he reported that the acceptable percentage (40%) of post-thaw sperm motility considered fit for artificial insemination in buffaloes was maintained up to 60 min of incubation in semen, which was frozen in winter and rainy seasons, whereas semen frozen in spring and summer seasons could maintain it only up to less than 30 min of post-thaw incubation (Table 2). No significant difference between progressive and rapid sperm motility was observed at different time interval of incubation across the seasons.

The results related to highest sperm motility during winter and lowest during summer season were in agreement with the findings of Bhakat *et al.* (2014), Majic-Balic *et al.* (2012) and Ghasemi *et al.* (2014). On the contrary, the lower motility in winter in Murrah bulls reported by Javed *et al.* (2000) may be due to study on fresh semen, breed difference, more subjective assessment of motility, different managemental practices and micro environment of bull shed. Argov-Argaman *et al*. (2007) also reported a significant reduction in sperm motility during summer season. It may be due to more oxidative stress during summer season to seminal plasma and spermatozoa that lead to decrease in sperm progressive motility, which further leads to semen quality deterioration (Majic-Balic *et al.* 2012).

Post-thaw sperm kinematics VAP, VSL, VCL, ALH, BCF, straightness and linearity

Our results showed that there was no significant

Table 2. LSM ± SE (%) sperm total motility, progressive motility and rapid motility on different time interval of post-thaw incubation

Season	Incubation time interval				
	0 min	30 min	60 min	90 min	120 min
			Total motility (%)		
Winter	61.00 ^{a} ± 4.70	49.50 ± 6.91	43.00 ^{a} ± 5.50	32.20 ± 2.91	20.20 ± 3.43
Spring	54.00 $^{a\,b}$ ± 1.65	37.00 ± 5.25	25.17 ^{b} ± 4.91	20.00± 4.51	13.50 ± 3.57
Summer	45.71 ^{b} ± 2.76	35.40 ± 1.72	31.83 $^{a\,b}$ ± 1.83	26.67± 2.19	20.80 ± 2.60
Rainy	50.64 ^{b} ± 1.61	43.17 ± 2.87	40.17 $^{a\,b}$±3.18	25.50± 4.09	21.13 ± 4.06
			Progressive motility (%)		
Winter	28.71 ± 4.96	23.00 ± 3.34	22.00 ± 6.15	11.00 ± 2.68	8.00 ± 1.67
Spring	20.17 ± 3.65	10.25 ± 2.46	7.67 ± 3.28	3.40 ± 1.17	1.25 ± 0.48
Summer	16.71 ± 3.34	10.00 ± 2.05	12.17 ± 2.23	7.83 ± 1.49	5.20 ± 1.53
Rainy	26.55 ± 2.15	18.00 ± 4.24	17.17 ± 4.24	9.00 ± 3.88	6.38 ± 2.56
			Rapid motility (%)		
Winter	36.43 ± 5.79	27.25 ± 9.42	25.5 ± 6.39	15.00 ± 3.89	9.80 ±1.24
Spring	30.17 ± 4.87	14.75 ± 3.75	11.17 ± 4.02	5.40 ± 0.81	1.50 ±0.29
Summer	23.86 ± 3.99	14.00 ± 1.92	16.17 ± 2.27	12.17 ± 2.06	8.00 ±2.17
Rainy	33.73 ± 2.15	23.00 ± 4.60	20.67 ± 3.97	10.63 ± 4.33	8.38 ±3.11

Values with different superscripts in same column differ significantly (P <0.01) at 0 min andat 60 min (P<0.05) among seasons.

Table 3. LSM±SE (%) sperm kinematics on different time interval of post-thaw incubation

Season	0 min	30 min	60 min	90 min	120 min
			Average path velocity (VAP) µm/sec		
Winter	118.50 ± 5.46	88.73 ± 9.76	66.43 ± 6.08	59.20 ± 6.73	60.54 ± 4.66
Spring	108.92 ± 5.25	85.28 ± 9.55	72.78 ± 7.52	55.46 ± 5.64	47.68 ± 3.26
Summer	108.14 ± 8.53	95.48 ±10.31	87.30 ± 2.78	73.13 ± 9.69	66.54 ± 8.18
Rainy	110.45 ± 5.15	91.83 ± 9.30	82.58 ± 7.61	61.40 ± 6.43	56.13 ± 5.19
			Straight line velocity (VSL) µm/sec		
Winter	98.41 ± 5.97	77.23 ± 8.48	55.43 ± 4.49	48.24 ± 4.23	50.16 ± 4.65
Spring	81.92 ± 3.84	66.65 ± 4.98	57.07 ± 6.43	43.68 ± 4.39	33.65 ± 5.55
Summer	82.57 ± 8.03	74.50 ± 7.91	67.25 ± 1.82	55.20 ± 5.95	53.14 ± 6.87
Rainy	91.12 ± 4.82	73.30 ± 8.44	66.10 ± 5.57	50.38 ± 4.97	44.39 ± 3.59
			Curvilinear velocity (VCL) µm/sec		
Winter	224.54±11.69	157.68±18.81	120.92±11.77	112.18±15.70	115.84±4.56
Spring	212.70±10.10	158.73±23.87	140.47±10.07	111.76±8.24	106.45±9.86
Summer	209.70±18.35	178.68±19.92	178.88±11.28	144.10±20.87	132.48±19.43
Rainy	209.75±11.73	169.77±16.80	149.52±14.27	115.73±11.76	112.04±9.59
			ALH (µm)		
Winter	8.30 ± 0.28	6.45 ± 0.51	5.45 ± 0.39	5.86 ± 0.47	6.08 ± 0.42
Spring	8.38 ± 0.33	6.73 ± 0.55	7.12 ± 0.19	5.04 ± 1.28	6.55 ± 2.85
Summer	8.79 ± 0.73	7.92 ± 0.92	7.87 ± 0.59	6.40 ± 0.64	6.48 ± 0.76
Rainy	8.30 ± 0.38	7.03 ± 0.37	6.77 ± 0.29	5.01 ± 0.80	5.87 ± 0.60
			BCF (Hz)		
Winter	34.70 ± 1.50	34.76 ± 1.94	33.34 ± 1.65	31.44 ± 2.11	30.16 ± 1.98
Spring	30.58 ± 1.19	31.72 ± 1.35	31.13 ± 0.73	30.46 ± 1.81	23.35 ± 7.94
Summer	30.89 ± 1.00	29.92 ± 1.65	29.82 ± 1.55	30.70 ± 0.88	29.28 ± 0.64
Rainy	33.32 ± 0.93	33.93 ± 1.04	32.60 ± 0.96	31.64 ± 1.12	31.00 ± 0.92
			STR (%)		
Winter	82.00 ± 2.21	86.50 ± 1.94	84.67 ± 2.22	83.00 ± 2.53	83.20 ± 1.83
Spring	75.83 ± 0.87	79.25 ± 3.33	78.17 ± 1.01	78.20 ± 2.63	70.00 ± 6.56
Summer	76.14 ± 1.96	77.20 ± 4.00	78.50 ± 1.91	78.00 ± 2.32	80.00 ± 2.39
Rainy	81.55 ± 1.21	79.00 ± 2.29	80.67 ± 2.06	82.62 ± 1.59	79.62 ± 1.39
			LIN (%)		
Winter	46.00 ± 2.21	51.50 ± 2.53	48.17 ± 2.21	45.40 ± 3.26	44.00 ± 3.21
Spring	40.83 ± 0.87	45.25 ± 3.57	41.33 ± 2.11	39.60 ± 2.14	32.00 ± 4.32
Summer	41.29 ± 1.96	43.40 ± 3.20	40.83 ± 1.70	41.50 ± 2.10	42.20 ± 2.27
Rainy	44.73 ± 1.20	44.17 ± 1.92	46.17 ± 1.58	45.38 ± 1.28	41.00 ± 1.00

ALH, lateral amplitude of head displacement; BCF, beat cross frequency; STR, straightness; LIN, linearity.

difference in VAP, VSL, VCL, ALH, BCF, straightness and linearity among seasons. The results were in consonance with the findings of Valeanu *et al*. (2015). Post-thaw VAP was higher at 0 min incubation interval in winter and at 30, 60, 90 and 120 min interval in summer season. VSL and VCL also showed the almost similar trend at various post-thaw incubation intervals. Summer season could sustain sperm velocities better than other three seasons at various post thaw incubation stages. However, the values of the above explained velocity were higher in summer across the incubation stages except at 0 min of incubation. There was no significant difference in post-thaw amplitude of lateral head displacement (ALH), beat cross frequency (BCF), straightness and linearity, across incubation intervals among seasons. However, BCF, straightness and linearity were maximum in semen frozen during winter and minimum in spring season at 0 min post-thaw. ALH did not show any fix pattern at different time interval during incubation across the seasons, although it was higher in summer at the initial stage of incubation.

There is only a little information available on the CASA evaluated sperm velocity parameters at different stages of incubation of semen frozen in various seasons. However, a Koonjaenak *et al*. (2007b) studied sperm velocities of Thai swamp buffalo semen, frozen during different seasons, and assessed it during incubation up to 60 min of thawing. In their findings, they obtained a significant difference for VAP, VSL, VCL and ALH at 0 min of incubation except linearity, whereas, at 60 min VAP, VSL and ALH differ significantly except VCL and linearity among the seasons. However, in the present study, no sperm velocity parameter differed significantly across the incubation intervals among the seasons. But there is no sufficient literature available on buffalo sperm parameters assessed with CASA to compare the present findings on sperm velocity parameters of semen frozen during various seasons, other than only subjective sperm motility evaluated with a microscope, a traditional method. The variation in the result may be due to

geographical and extender composition, freezing method and the breed difference.

On the contrary, a significant seasonal variation was observed in sperm kinetics except linearity; the mean values of sperm dynamic were significantly higher during summer and rainy season and lower in winter in fresh Murrah buffalo semen (Mandal *et al.* 2003). In this study, though these parameters did not differ significantly between the seasons, but their values were higher in winter season. The proportion of linearly motile spermatozoa is of higher relevance for fertilization *in vitro* and among the attributes that link to *in vivo* fertility (Zhang *et al.* 1998), in the present study also, the linearity was higher in winter, which further proved that the winter is more conducive for quality frozen semen production. In our findings, winter was found to be better than summer as the values of VSL, STR, LIN were higher at 0 h of incubation, which is supported by the findings of Mortimer *et al*. (1999).

The results portrayed superiority of the winter for post thaw sperm motility (motility, progressive motility and rapid motility), viability, plasma membrane integrity and acrosomal integrity as compared to other seasons. This may be due to congenial weather condition during winter to maintain the required temperature for successful spermatogenesis as compared to other seasons (Mandal *et al.* 2005). The spermatogenesis process is dependent on testosterone hormone, which is better liberated from interstitial cells during winter may probably influence the spermatogenesis for better quality semen production, besides that performance and secretion from accessory sex gland also improves during this season (Mandal *et al.* 2005). It is apparent from the climatic table that winter in this part can be considered neither extreme nor severe to the extent to become detrimental for quality semen production (Bhakat *et al.* 2014) and reflecting better adaptability of Murrah buffalo bulls during winter.

It is well known that adverse effects of the summer on semen quality parameters are more as compared to other seasons (Bhakat *et al.* 2014) which is also true in our findings, which may be due to more fragileness of summer spermatozoa. The adverse effect of summer may be directly due to increase in core body temperature followed by increase in testicular temperature as well as the inability of the buffalo bulls to cope up with the environmental odds due to compromised thermo-regulatory mechanism which is attributed to its black body colour and presence of less number of sweat glands. Lower semen quality in summer than other seasons may be due to a higher ambient temperature during summer, which may result in a reduction in scrotal circumference, hormonal changes (Ghasemi *et al.* 2014). The increase in environmental temperature affect the gonadal axis by increasing the release of ACTH, which inhibits the effect of LH + (Clarke and Tilbrook 1992), an important hormone responsible for spermatogenesis, on the other side, heat stress reduces the release of GnRH, which in turn affects the release of hormones responsible for spermatogenesis. During summer, libido of the breeding bulls get affected due to physical exhaustion leads to less interest and eagerness of serving the artificial vagina resulted in higher reaction time and total time for successful ejaculation, thus having an ultimate effect on production of quality sperms (Mandal *et al.* 2000). Argov-Argaman *et al*. (2007) reported that the semen samples collected during the summer showed three folds lower expression of very low-density lipoprotein receptors (VLDLr) in the spermatozoa as compared to winter, which affects extracellular lipid utilization, and altered fatty-acid composition leading to reduced semen quality during summer (Argov-Argaman *et al*. 2013). It is evident that for better sperm functionality, reactive oxygen species (ROS) level needs to be maintained adequately (Goncalves *et al.* 2010), but during summer, higher ROS production and their highly reactive nature leads to reaction with other molecules, may result in cellular damage through structural and functional changes (Valeanu *et al*. 2015).

The season of semen cryopreservation had influenced significantly post-thaw sperm motility and viability, which was higher in winter followed by spring, rainy seasons and summer. The values of progressive, rapid motility, sperm membrane integrity and sperm velocities (VAP, VSL and VCL) were higher in semen frozen in the winter as compared to other 3 seasons, whereas, sperm acrosome damage and average of sperm lateral head displacement (ALH) was lowest in winter than other seasons. Post-thaw sperm motility, progressive motility and rapid motility was higher across the incubation intervals in semen frozen during winter than other seasons. An overview of the results suggested that semen frozen in winter was of superior quality than summer; however, wider study is warranted over a longer period of time taking more bulls and evaluation of seminal plasma and spermatozoa during different seasons to establish the association between the seasonal variations and semen quality in buffalo bulls.

ACKNOWLEDGEMENT

The authors are thankful to Incharge of Artificial Breeding Research Centre and Semen Bank, Central Institute for Research on Buffaloes for providing necessary information. The authors also thankful to the Director (National Dairy Research Institute) for financial assistance provided during the research work.

REFERENCES

Albero G, Zullo G, Salzano A, Brun R, Longobardi V, Bifulco G and Gasparrini B. 2014. Effect of season on cryocapacitation of buffalo (*Bubalus bubalis*) semen. *Reproduction Fertility and Development* **27**(1): 221–21.

Argov-Argaman N, Mahgrefthe K, Zeron Y and Roth Z. 2013. Season-induced variation in lipid composition is associated with semen quality in Holstein bulls. *Reproduction* **145**: 479–89.

Argov-Argaman N, Sklan D, Zeron Y and Roth Z. 2007. Association between seasonal changes in fatty-acid composition, expression of VLDL receptor and bovine sperm quality. *Theriogenology* **67**: 878–85.

Baruselli P S and Carvalho N A T. 2005. Biotechnology of reproduction in buffaloes *(Bubalus bubalis)*. *Brazilian Journal of Animal Breeding* **29**(2): 4–17.

Bhakat M, Mohanty T K, Gupta A K and Abdullah M. 2014. Effect of season on semen quality of crossbred (Karan Fries) bulls. *Advances in Animal and Veterinary Science* **2**(11): 632–37.

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.

Birck A, Christensen P, Labouriau R, Pedersen J and Borchersen S. 2010. *In-vitro* induction of the acrosome reaction in bull sperm and the relationship to field fertility. *Theriogenology* **73**(9): 1180–91.

Clarke I J and Tilbrook A J. 1992. Influence of non-photoperiodic environmental factor on reproduction in domestic animals. *Animal Reproduction Science* **28**: 219–28.

Fraser L, Gorszczaruk K and Strzezek J. 2001. Relationship between motility and membrane integrity of boar spermatozoa in media varying in osmolality. *Reproduction of Domestic Animals* **36**: 325–29.

Ghasemi M V and Ghorbani A. 2014. Environmental and genetic factors affecting on semen quality in Iranian Holstein bulls. *Iranian Journal of Applied Animal Science* **4**(1): 33–37.

Goncalves F, Barretto L S S, Arruda R P, Perri S H V and Mingoti G Z. 2010. Effect of antioxidants during bovine *in vitro* fertilization procedures on spermatozoa and embryo development. *Reproduction of Domestic Animals* **45**:129–35.

Javed M T, Khan A and Kausar R. 2000. Effect of season on some semen parameters of Nili-Ravi buffalo (*Bubalus bubalis*) bulls. *Veterinarski Arhiv* **70**(2): 83–94.

Koonjaenak S, Chanatinart V, Aiumlamai S, Pinyopumimintr T and Rodriguez-Martinez H. 2007a. Seasonal variation in semen quality of swamp buffalo bulls (*Bubalus bubalis*) in Thailand. *Asian Journal of Andrology* **9**(1): 92–101.

Koonjaenak S, Pongpeng P, Wirojwuthikul S, Johannisson A, Kunavongkrit A and Rodriguez- Martinez H. 2007b. Seasonality affects post-thaw plasma membrane intactness and sperm velocities in spermatozoa from Thai AI swamp buffaloes (*Bubalus bubalis*). *Theriogenology* **67**: 1424–35.

Majic-Balic I, Milinkoviæ-Tur S, Samardzija M and Vince S. 2012. Effect of age and environmental factors on semen quality, glutathione peroxidase activity and oxidative parameters in simmental bulls. *Theriogenology* **78**(2): 423–43.

Mandal D K, Nagpaul P K and Gupta A K. 2000. Seasonal variation in seminal attributes and sexual behavior of Murrah buffalo bulls. *Indian Journal of Dairy Science* **53**: 278–83.

Mandal D K, Nagpaul P K and Gupta A K. 2003. Motion characteristics of Muraah buffalo bull spermatozoa in various seasons and its relationship with functional integrity of the plasmallema. *Theriogenology* **60**: 349–58.

Mandal D K, Tyagi S K and Mathur A K. 2005. Semen production performance of Sahiwal bulls. *Indian Journal Animal Sciences* **75**(1): 17–19.

Maurya V P and Tuli R K. 2003. Post-thaw thermal resistance test on motility and acrosomal integrity of filtered and non-filtered frozen semen of Murrah buffalo bulls. *Asian Australasian Journal Animal Science* **16**(10): 1424–28.

Mortimer S T and Maxwell W M. 1999. Kinematic definition of ram sperm hyperactivation. *Reproduction Fertility and Development* **11**(1): 25–30.

Partyka A, Nizanski W and Ochota M. 2012. Methods of assessment of cryopreserved semen. *Current Frontiers in Cryobiology*. (Ed.) Katkov Igor. ISBN: 978-953–51–0191–8, In Tech.

Rasul Z, Ahmad N and Anzar M. 2001. Changes in motion characteristics, plasma membrane integrity, and acrosome morphology during cryopreservation of buffalo spermatozoa. *Journal of Andrology* **22**: 278–83.

Rodriguez-Martinez H. 2003. Laboratory semen assessment and prediction of fertility: still utopia? *Reproduction of Domestic Animals* **38**: 312–18.

Sarder M J U. 2007. Environment related variations in the semen characteristics of bulls used for artificial insemination (AI) programme in Bangladesh. *University Journal of Zoology, Rajshahi University* **26**: 81–88.

Shukla M K and Misra A K. 2007. Effect of bradykinin on Murrah buffalo (*Bubalus bubalis*) semen cryopreservation. *Animal Reproduction Science* **97**: 175–79.

Snoj T, Kobal S and Majdic G. 2013. Effects of season, age, and breed on semen characteristics in different *Bos taurus* breeds in a 31-year retrospective study. *Theriogenology* **79**(5): 847–52.

Valeanu S, Johannisson A, Lundeheim N and Morrell J M. 2015. Seasonal variation in sperm quality parameters in Swedish Red dairy bulls used for artificial insemination. *Livestock Science* **173**: 111–18.

Zhang B R, Larsson B, Lundeheim N and Rodriguez-Martinez H. 1998. Sperm characteristics and zona pellucida binding in relation to field fertility of frozen-thawed semen from dairy AI bulls. *International Journal of Andrology* **21**: 207–16.