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Original Article

Influence of multiple showering on quality of buffalo semen during hot-humid season

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ABSTRACT

Objectives: This experiment was conducted on buffaloes to see effect of showering on maintaining good quality of buffalo semen in hot season.
Materials and methods: This study was conducted on 6(six) indigenous buffalo bulls in hot summer of March and April 2017. The effect of multiple showering *vs* single shower alone on fresh and equilibrated semen quality was observed. The buffalos were divided into similar two groups (according to age and weight) and housed in half-walled openshed with adequate spacing and the feeding management being identical. The bulls in the control group were allowed to a single shower at 10.00 am and experimental bulls were allowed to four showers at 07.00, 10.00, 13.00 and 16.00. Temperature and humidity were recorded during this time. The average temperature was around 35°C along with 72% relative humidity. The quality of experimental bull's semen was evaluated in terms of volume, live sperm, sperm concentration, sperm motility, motion parameters and morphology for normal and abnormal sperm. For this analysis Computer Assisted Semen Analysis (CASA) system was used.
Results: Between experimental group and control group semen quality differs significantly for volume (2.04±0.13 mL; 2.53±0.27 mL) (P<0.01); live sperm (81.38±1.22%; 90.28±1.53%) (P<0.01), normal fraction (36.87±6.38%; 47.87±12.01%) (P<0.05); DMR (6.89±5.86%, 5.86±1.45%) (P<0.05) and for proximal droplet (57.86±4.30, 45.26±10.96) (P<0.05). Motility and motion parameters for fresh semen were not significantly different.
Conclusion: In short, showering showed significant effect on different parameters of fresh semen quality of buffalo but not in case of further processing.

KEYWORDS

CASA; Equilibration; Semen quality; Showering

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INTRODUCTION

Buffalo (*Bubalus bubalis*, belongs to the sub-family Bovinae of family Bovidae) has promising potential resource for livestock development because of contributions to milk, meat, hides and draft power for agricultural operations to improve socioeconomic status of farmers particularly in Asia. The primary limited factor that affects the productivity of buffalos is introduction of superior germplasm. Indigenous buffalo breeds are upgraded though progeny testing program in many Asian countries, where, highly genetic potential of local buffalo breed is essential. Faruque et al. (1995) reported that buffalo reproductive efficiency is very low with 2 calves in 3 years is usual and they are seasonal breeder. This shows the productivity of indigenous buffaloes should be improved by changing of genotypes that includes production of good quality semen and use of artificial insemination (AI). Seasonality in reproductive performance in both sexes of buffaloes is evident in most countries (Bhosrekar et al., 1992; Perera, 2008).

During summer, high heat stress suppresses the thyroid activity that results in weak libido of breeding bulls, and poor semen quality, freez abilty and fertility (Falvey and Chantalakhana, 1999; Bhakat and Mohanty, 2009). Continuous exposure of summer heat and solar radiation causes gradual decline in semen quality followed by total loss in libido and semen production, recovery from which may take six weeks after the onset of rainy season (Sengupta et al., 1963). The effect of heat stress is more aggravated with high ambient humidity (Marai and Habeeb, 2010). So, heat stress during summer months is one of the major factor for low productive and reproductive performance of buffaloes. Extended exposureof high ambient temperature along with high relative humidity hamper to dairy animals in dissipation of excess body heat (Marai et al., 2009).

Buffaloes are more thermal tolerant due to dark coatcolor, thick skin epidermal layer and less sweat glands per unit skin area (Marai and Habeeb, 2010). The modification of genetics, physical environment and nutrition can help to reduce the summer heat stress to dairy animals (Beede and Collier, 1986). Different methods (shade, water splashing, sprinkling, showering, fanning, forced ventilation and wallowing, etc.) have been tried with varied success to alleviate the effect of heat stress in buffalo. Through this study, we tried to reduce the effect of summer heat and humidity on quality of buffalo semen. For this we focused on management like multiple showering while feeding and other managements were kept constant.

MATERIALS AND METHODS

Ethics statement: All animal procedures and treatment in this experiment were used according to welfare recommendations of code of practice for the care and use of animals for scientific purposes of Bangladesh Livestock Research Institute (BLRI). This experimental healthy buffaloes were provided by BLRI maintaining its rights and welfare.

Duration and site of the experiment: This experiment was conducted in buffalo farm of BLRI, Savar, Dhaka, Bangladesh. The duration of the trial period was March, April and May of 2017.

Selection of animal: Six (6) buffalo of indigenous type were taken from the BLRI Buffalo Farm. These were divided into two similar groups based on body weight and age (in between 2.5 to 3.5 years). The general health of the animals was good and bulls were vaccinated against common diseases.

Management of bulls: All the bulls were housed in a shed comprised of roofed stall and unroofed paddock area. The space allowed for each bull was 142 square meters and the space was separated with 1.3 meter high steel pipe. They were fed the similar amount of fodder 20 kg (fresh) and concentrate of 3 kg/day.

Plan of treatment: The control group was given only one showering at 10.00 for two (2) min. The treatment group was given 4 times showering per day at times 0f 07.00, 10.00, 13.00 and 16.00. All bulls were given exercise daily in the morning for 30 min. The surrounding temperature, humidity inside the shed was collected in the morning and evening and THI (temperature humidity index) was calculated.

Collection of semen: The bulls were given a washing before taking to the site of semen collection. Semen collection was done once a week by using artificial vagina where the temperature was maintained around 40°C. The collection was done in the early morning by artificial vagina technique (Walton, 1945).

Physical, morphological and motion attributes of semen: Immediately after collection, the semen was assessed for physical and morphological attributes, like as volume, motility (static, progressive, slow); sperm concentration and tail abnormality (bent tail, coiled tail), head abnormality (DMR, distal droplet, proximal droplet). For this, Computer Assisted Semen Analysis system (Hamilton Throne Ivos) was used. Fresh semen drop was diluted with normal saline solution (NaCl) in a

ratio of 1:100 and then put into 20 micron standard count 4 chamber slide and input in the analyzer. Sperm kinematics was also recorded.

Live and dead sperm: Live dead count was determined according to procedure described by Blom (1950); in this method Eosin Nigrosin stain (Eosin- 100 mg, Nigrosin-500 mg, Tris buffer- 10 mL, pH 6.8) was used to prepare the slides. According to procedure, we placed 1 (one) drop of semen and 1 or 2 drops of stain on a dry and clean glass slide at 37°C using a warm stage. Thin smears were prepared by using another slide. The slide was allowed to dry in air. About 150 spermatozoa were counted in each slide in different microscopic fields using oil immersion. The spermatozoa that had absorbed stain were considered to be dead and the rest live.

Cooling protocol: The semen was frozen by using commercial extender Andromed (Andromed-200 mg, Distilled Water-800 mL). The extension rate was fixed to keep the sperm concentration at 20 M/dose. Sperm dose was of 0.25 mL. The extended mixture of semen and extender was kept at room temperature, and then kept in cold handling cabinet for 4 h. In the cold cabinet the temperature was maintained at 5°C. After equilibration quality was estimated again.

Statistical analysis: The data were subjected to analysis of variance (ANOVA) to study the effect of treatments on the physical, morphological and motion attributes of buffalo semen. It was also subjected to analysis of variance to determine the changes in motility before and after freezing. This analysis was subject of paired *t*-test using Statistical Package software (SPSS) software.

RESULTS AND DISCUSSION

Temperature and Humidity Record: Temperature Humidity Index (THI) has been used to determine stress condition of animal. Heat stress starts when THI is more than 72. Animal get seriously affected when THI is more than 78 and THI more than 82 affects production and reproduction of animal severely. In this experiment period THI was noticeably high to keep animal in heat stress (**Table 1**).

Physical parameters: Buffalo has fewer sweat glands and farm raised buffalo isn't provided with wallowing facility. It causes heat stress for buffalo. Because of heat stress, in summer season spermatogenesis is inversely affected. The maximum temperature for optimum spermatogenesis is 29.4°C whereas minimum temperatures is 15.5°C (Singh et. al., 2013). It affects the physical attributes of semen quality of buffalo. In this experiment ejaculated volume, live sperm was significantly increased in 4 times showered group of buffaloes. As buffalo is affected by high temperature due to lack of sweat glands, multiple shower may have reduced the negative effect of high temperature in the experimental group.

Ejaculated volume: Ejaculation volume (mL) was significantly different ($P<0.01$) between control (single shower) and treatment (4 times shower) group in this experiment which were 2.04±0.13 and 2.53±0.27 respectively (**Table 2**). This result has similarity with the findings of Singh et al. (2001). That experiment used multiple showering and vitamin supplement to reduce heat stress on buffalo. Similar significant difference was found by Mayahi et al. (2014) in case of seasonal variation.

Table 1: Temperature, humidity and temperature humidity index of animal shed during the experiment

Parameter	Values
Lowest Temperature	20.7°C
Average minimum temperature	23.48°C
Highest Temperature	37.2°C
Average maximum temperature	35.62°C
Average Relative humidity	72.79%
Average THI in Morning	72.66±2.30
Average THI in Afternoon	81.66±2.51

Sperm concentration: There was no significant difference for sperm concentration of semen (million/mL) between control (single shower) and treatment (multiple showers) group of buffalo (**Table 2**). The findings of Singh et al. (2001) showed the similar result of non-significance. SRS de Castro et al. (2017) also found non-significant difference of sperm concentration between seasons. The mean values were so different but not significant is may be because of animal age. Age range was 2.5-3.5 years that some animals just came into maturity those were lacking in sperm concentration. On the other hand, Mayahi et al. (2014) and Bhakat and Mohanty (2015) found significance difference in semen according to seasonal variation.

Live sperm: There was significant difference ($P<0.01$) for live sperm (%) between control (single shower) and treatment (multiple showers) groups of buffalo which valued 81.38±1.22 and 90.28±1.53, respectively (**Table 2**). Singh et al. (2001) found similar result in his experiment which is 80.81±1.7 and 88.09±1.40. That means we can say that multiple showering has effect on live sperm of buffalo semen.

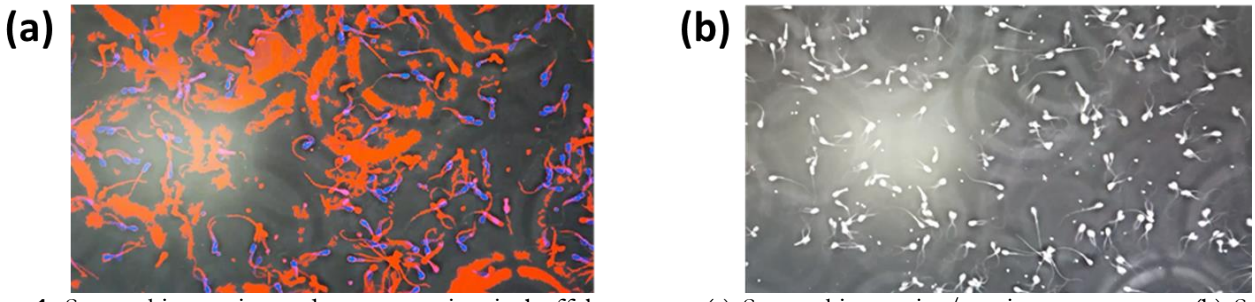

Figure 1. Sperm kinematics and concentration in buffalo semen. (a) Sperm kinematics/motion parameters, (b) Sperm concentration/motility. Objective name: Zeiss 10X NH IVOS-ii 160 mm. Objective mag x=1.21 (pixel/µm), Mag y=1.2 (pixel/µm).

Table 2: Physical attributes of semen from control and treatment groups of buffalo.

Parameter	Control (Mean±SD)	Treatment (Mean±SD)	Level of Signifinance
Ejaculated Volume (mL)	2.04±0.13	2.53±0.27	**
Sperm Concentration (Million/mL)	1374.31±611.29	2111.60±951.97	NS
Live Sperm (%)	81.38±1.22	90.28±1.53	**
Motility (%)	84.64±9.36	89.86±6.75	NS
Progressive Motility (%)	64.41±14.91	69.34±11.85	NS

***=Significant at 1% level, *=Significant at 5% level, NS= not significant*

Table 3: Morphological parameters of semen from control and treatment groups.

Parameter	Control (Mean±SD)	Treatment (Mean±SD)	Level of Significance
Normal Fraction(%)	36.87±6.38	47.87±12.01	*
Bent Tail(%)	1.631.81	1.18±0.73	NS
Coiled Tail(%)	0.13±0.15	0.13±0.11	NS
DMR(%)	6.89±5.86	1.69±1.45	*
Proximal Droplet(%)	57.86±4.30	45.26±10.96	*
Distal droplet(%)	0.4±0.22	0.23±0.05	NS

***=Significant at 1% level, *=Significant at 5% level, NS= not significant*

Table 4: Freezability parameters of semen from control and treatment groups of buffalo.

Parameters	Control (Mean±SD)	Treatment (Mean±SD)	Level of Significance
Motility after equilibration (%)	73.23±3.96	76.40±6.07	NS
Normal fraction after equilibration (%)	36.25±0.91	43.5±1.55	*

**=Significant at 5% level, NS= not significant*

Table 5: Motion parameters of semen from control and treatment groups of buffalo.

Parameters	Control	Treatment (Mean±SD)	Level of Significance
VAP=Average path Velocity (microm/sec)	136.21±3.65	134.24±4.40	NS
VSL=Straight Line Velocity (microm/sec)	120.44±2.61	119.36±3.30	NS
VCL=Curvilinear Velocity (microm/sec)	222.56±10.16	214.76±10.14	NS
LIN=Linearity (%)	55.82±1.58	56.64±1.22	NS
ALH=Amplitude of Lateral Head Displacement	8.46±0.37	8.27±0.47	NS

NS= not significant

Motility: There was no significant difference between control and treatment (multiple showers) groups of buffalo in case of motility. The result was 84.64±9.36 and 89.86±6.75 for single shower and multiple showers, respectively (**Table 2; Figure 1a-b**). This result is similar to findings of Singh and Singh (2000) and de Castro et al. (2017). They didn't find any significant difference of motility while using multiple showering to reduce heat stress on buffalo.Another study on seasonal variation by Mayahi et al. (2014) and Bhakat and Mohanty (2015) showed similar result in buffalo.

Progressive motility (%): There was no significant difference between control (single shower) and treatment (4 shower) groups of buffalo in case of progressive motility. The mean value were different and they were 64.41±14.91 and 69.34±11.85, respectively (**Table 2**), but this difference was non-significant. This motility value

was closer to the result of Koonjaenak et al. (2007) which was 72.8±1.4 in summer. Mayahi et al. (2014) found similar value of progressive motility but that showed significantdifference between winter and summer season.So, we can say that summer season doesn't affect progressive motility of buffalo semen.

Morphological parameters: Heat stress may cause metamorphosis of sperm which cause semen degeneration (Coser et al., 1979). So, abnormalities were seen higher in summer season for buffalo semen quality. In this experiment abnormalities were significantly lower in multiple showered group of buffaloes as they were showered multiple times to reduce negative effect of high temperature and high humidity.

Normal fraction: There was significant difference (P<0.05) between control (single shower) and treatment (4 shower) groups of animal in respect of normal fraction which is 36.87±6.38 and 47.87±12.01, respectively (**Table 3**). This difference seems to be due to difference in proximal droplet and DMR. This result is similar to lower defects in rainy season and higher defects during summer season in buffalo semen (de Castro et al., 2017).

Bent tail: There was no significant difference for bent tail (%) between control (single shower) and treatment (4 shower) groups of animals. The mean value were 1.63±1.81 and 1.18±0.73 respectively (**Table 3**) which is closer to the findings of Koonjaenak et al. (2007) that is 2.0±0.2 in summer.

Coiled tail: There was no significant difference between control and treatment groups for coiled tail (%). The mean values were 0.13±0.15 and 0.13±0.11 for control and treatment group respectively. Koonjaenak et al. (2007) has reported similar results in case of seasonal variation of coiled tail of buffalo season. That means summer heat didn't affect the bent tail (%) of buffalo semen.

DMR: There was significant difference (P<0.05) between control (single shower) and treatment (4 shower) groups of animals for DMR (distal midpiece reflex). The value is 6.89±5.86 and 1.69±1.45 respectively (**Table 3**). The value is lower in treatment group. In experiment of Koonjaenak et al. (2007) mid-piece abnormality did not differ significantly due to season.

Proximal droplet: There was significant difference (P<0.05) for proximal droplet in between control (single shower) and treatment (4 shower) groups. The mean values were 57.86±4.30 and 45.26±10.96 respectively (**Table 3**). Proximal droplet is lower in older animal, but as I have younger animals in both groups these values were high (Roy Lewis, 2014).

Distal droplet: There was no significant difference for distal droplet between treatment and control group. Their mean values were 0.4±0.22 and 0.23±0.05 for control and treatment group respectively. Koonjaenak et al. (2007) reported no significant difference of distal droplet in buffalo semen due to season.

Post equilibration parameters: Motility of semen after equilibration was not significantly different between control (single shower) and treatment (4 shower) groups. The mean values were 73.23±3.96 and 76.40±6.07 respectively (**Table 4**) which is similar to the findings of Singh et al. (2001). Their results were 70.83±2.41 and 74.11±1.56. As semen equilibration was done to keep motility of sperm in the doses at least 40% and each dose should contain 20 million sperm, motility came similar for both control and treatment groups. Normal fraction for both groups were significantly different as the fresh semen had the significantdifference.

Motion parameters: There was no significant difference for motion parameters between multiple showered and single showered groups of buffaloes. There was no significant difference for motion parameters between multiple showered and single showered groups of buffaloes.

The VAP, VSL, VCL, LIN, ALH mean values were 136.21±3.65 and 134.24±4.40; 120.40±2.61 and 119.36±3.30; 222.56±10.16 and 214.76±10.14; 55.82±1.58 and 56.64±1.22; 8.46±0.37 and 8.27±0.47, respectively (**Table 5**) for control and treatment group. Mandal et al. (2003) has reported significant difference of sperm kinematics due to season with exception for LIN mean higher in summer season. Mayahi et al. (2014) reported significant difference in kinematics except VCL between winter and summer season. In this experiment mean values were higher in control group than the treatment group that is similar to Mandal et al. (2003) findings, but they are not significantly different.

CONCLUSION

From the result of the experiment it may be concluded that multiple showering may increase value of many parameters of buffalo semen quality. These higher parameters will allow us to make more doses from single ejaculation of sperm for cryopreservation and AI use. In short, a little more showering as housing management may increase semen quality in an economic way.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHORS' CONTRIBUTION

MRH and MSRS designed the study. MSRS supervised the overall research work and provided valuable suggestions throughout the experiment. MFHM and GKD assist help in the collection of semen from bull. MRH, MSR and SBN conducted laboratory analysis of semen. All the authors contributed in writing and reviewing the manuscript, and approved the final manuscript.

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