Breed and blood level wise seasonal effect on semen quality of breeding bulls maintained under subtropical environment

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Abstract: The aim of the study was to investigate the effects of season on semen quality of breeding bulls and to find out the seasonal stress reduction measures. A total of 624 ejaculates were collected from 12 upgraded breeding bulls through the experimental year to study the effects of season on semen quality. The recorded data were analyzed using GraphPad Prism 5 software. Out of the 624 ejaculates, 554 (88.78%) were found to be creamy in color followed by 51 (8.17%) and 19 (3.05%) as yellowish and watery, respectively. Season had significant ($P<0.05$) effect on ejaculate volume, consistency, mass activity, sperm concentration, live sperm percentage, initial and post freezing motility. In case of upgraded Holstein Friesian bulls, the highest volume of semen was found during autumn season in HF_2×L bulls (8.077±0.33 ml) and the lowest volume was observed during winter season in HF×L bulls (4.692±0.36).Likely, in case of upgraded Sahiwal bulls, the highest volume of semen was found in the time of autumn in SL_2×L bulls (6.510±0.10) and the lowest amount was noticed during winter in SL×L bulls (4.788±0.21). The mass activity ranges from 3.308±0.07 to 4.365±0.09. Sperm concentration varied significantly with season and breed with blood level. There was not found significant differences in semen pH except SL×Land SL_1×L bulls. The live sperm ranges from 71.27% to 78.15%. Significant differences were observed in initial motility percent. Post freezing motility percent ranges from 42.88±0.49 to 55.96±0.73 and there was significant variation within the season and breed with blood level. It was concluded that the semen quality was optimal during winter, poor during summer and intermediate in rainy and autumn seasons hence, the bulls should be kept in a cool and comfortable environment and semen should be processed with great consciousness.

Key Words: Breeding bulls, Holstein Friesian, Sahiwal, Semen quality; Seasonal effect

INTRODUCTION

Animal productivity and quality production are greatly influenced by the climatic condition of the production system. Artificial Insemination (AI) is now widely used all over the world to improve the genetic potentiality of livestock species. The success and efficiency of AI program depends on several factors. Semen quality is top of them and seasonal variations largely influence the quality of semen. Good quality semen is obligatory for successful conception in cattle and therefore, a determinant of reproductive efficiency. Heat stress is the most important factor and has a great impact on libido and semen production of breeding bulls.

Among the different seasons hot dry and hot humid season recorded as unfavorable for animal production as well as reproduction [1].The environment has both direct and indirect impact on semen quality; directly through change in micro and macro climatic factors like temperature, humidity, rainfall and photoperiod, whereas indirectly by affecting feed intake, vegetation, forage quality, soil-plant-animal interaction [2].

Bangladesh is considered as a subtropical country and it has six seasons but mostly observed four (summer, rainy, autumn and winter). The highest temperature and relative humidity were recorded as 36.8°C and 92% and the lowest temperature and relative humidity were recorded as 11.6°C and 32% respectively, last year in the study location. Crossing of local cows with Holstein Friesian and Sahiwal is widely practiced in Bangladesh for dairy and beef purpose respectively. Two studies [3,4] reported that the qualities of semen i.e. ejaculate volume, sperm motility; viability and concentration etc. (et cetera) were affected by breeds and season. Therefore, the present study was planned to assess the seasonal effects on semen quality of upgraded Holstein Friesian (dairy) and Sahiwal (Beef) bulls maintained under subtropical environment.

MATERIALS AND METHOD

Place and time of study

The study was undertaken at a renowned research based animal breeding organization of Bangladesh , properly known as "Lal Teer Livestock Development Bangladesh Limited", located at Mymensingh district, 90 kilometers away from Dhaka City. The whole research activities considered in this study covered the period from November, 2018 to October 2019 (One year).

The year was subdivided into four seasons: Summer (mid-February to mid-May), rainy (mid-May to mid-August), autumn (mid-August to mid-November) and winter (mid-November to mid-February). The recorded average temperatures and relative humidity of aforesaid seasons are 32.6°C, 30.2°C, 27.7°C and 20.5°C and 72%, 77%, 58% and 49% respectively (Figure 1).

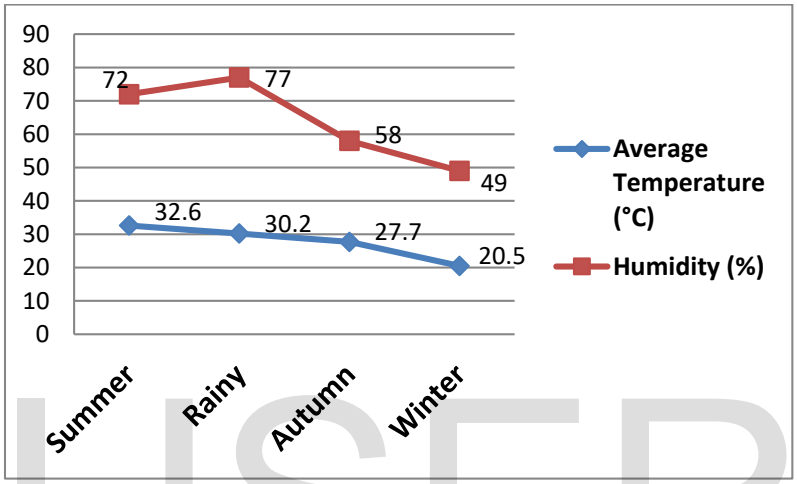

Figure-1: Season wise Average Temperature (°C) and Humidity (%)

Animals and their ration

12 (twelve) breeding bulls (nearly 30 to 50 months of age and body weight of 456.50 to 673.00 kg) were selected for this study. Out of 12 bulls, 2 were 50% Holstein Friesian +50% Local (HF ×L), 2 were 75% Holstein Friesian +25% Local (HF_1 ×L), 2 were 87.5% Holstein Friesian +12.5%% Local (HF_2×L), 2 were 50% Sahiwal+50% Local (SL×L), 2 were 75%Sahiwal+25% Local (SL_1×L), 2 were 87.5% Sahiwal+12.5%% Local (SL_2×L) bulls. The breeding bulls were maintained under optimal feeding and management during the whole period of the experiment. The bulls were physically fit, free from diseases, clinically normal and sound in breeding. All the bulls were vaccinated against Anthrax, FMD (Foot and Mouth Disease), BQ (Black Quarter), and HS (Hemorrhagic Septicemia) according to the schedule. The bulls were allowed ad libitum green grass supplemented with good quality concentrate mixture prepared with maize grain, rice polish, wheat bran, soybean meal, mustard oil cake, DCP, vitamin mineral premix and common salt (Table-1).

Table-1: Ration (Dry matter Basis) for breeding bulls at Lal Teer Livestock Development (BD) Limited

Ingredients	Amount (kg)	DM (kg)	TDN (kg)	DCP (kg)	Ca (kg)	P (kg)
Maize	32.00	28.80	25.40	3.12	0.0063	0.003
Rice Polish	20.00	17.70	16.02	2.98	0.0011	0.028
Wheat Bran	20.00	17.70	12.34	2.65	0.0027	0.020
Mastered Oil Cake	12.00	10.46	7.58	4.01	0.0080	0.002
Soybean Meal	10.00	8.90	7.62	4.32	0.0031	0.01
Lime stone powder	2.00	1.98	0.73	-	0.0740	-
D.C.P	2.00	1.98	0.81	-	0.0460	0.04
Common salt	1.00	0.99	-	-	-	-

Vita. Min premix	1.00	0.99	-	-	-	-
Total	100.00	89.50	70.50	17.08	0.14	0.10

Semen collection, evaluation and preservation

Semen was collected early in the morning twice a week from the bulls using sterilized bovine artificial vagina (IMV model-005417)maintaining proper temperature (42°-45°C),pressure and softness[5]. A male dummy was used for jumping the bulls and after 2 to 3 false jumps semen was collected from each bull by a skilled semen collector. Just after collection each ejaculate was placed into a hot water bath at 37°C and various standard laboratory tests for semen evaluation were recorded. Ejaculate volume of semen was measured directly in milliliter (ml) from the graduated centrifuge collection tube. Color and consistency of semen was observed with the naked eye. Semen pH was determined by indicator paper strips [6].Mass activity of semen was recorded by placing a small drop of fresh semen on the glass slide without cover slip under low magnification (10x) of a digital microscope and graded from 0 to 5 grades. Concentration of sperm per ml of semen was estimated through bovine sperm photometer (IMV technologies, France). Live and dead spermatozoa of the semen were counted by eosin nigrosin staining technique [7].

Individual motility of semen was assessed by placing a small drop of semen on the glass slide and covering by cover slip under high magnification (40x) using phase contrast microscope. Semen with motility of more than or equal 70% was diluted with egg yolk-citrate-glycerol semen extender. The diluted semen was subsequently loaded in 0.25 ml/straw (IMV technologies, France), cooled at 4°C and equilibrated for 3.5 to 5 hours. Semen straw was then frozen using IMV bio freezer following the standard procedure of IMV technologies. After that, frozen straws were transferred into liquid nitrogen until used for insemination. Post freezing motility of semen was assessed as individual motility was assessed.

Statistical Analysis

The recorded data was compiled by Microsoft Excel 2010. Compiled data was then analyzed using GraphPad Prism 5 software. Column statistics were done for mean and standard error. Turkey test was performed for multiple comparison and level of significance.

RESULTS AND DISCUSSION

In this study, the experimental year (November,2018 to October,2019) was divided in to four seasons (summer, rainy, autumn and winter) and the animals were six type {50% Holstein Friesian +50% Local (HF ×L), 75% Holstein Friesian +25% Local (HF_1 ×L), 87.5% Holstein Friesian +12.5% Local (HF_2 ×L), 50% Sahiwal+50% Local (SL×L), 75%Sahiwal+25% Local (SL_1×L), 87.5% Sahiwal+12.5%% Local (SL_2×L)}.26 ejaculates from each type of bull per season were studied, hence, a total of 624 (26×6×4) ejaculates were evaluated.

Color and Consistency

Out of the 624 ejaculates 554 (88.78%) were found to be creamy in color followed by 51 (8.17%) and 19 (3.05%) as yellowish and watery, respectively. A previous study reported out of 181 seminal ejaculates 82.3% were creamy, 8.8% were yellowish and 2.2% were watery, which are more or less similar to the present study [8]. There is a great seasonal effect on semen consistency of crossbred breeding bulls. In our study, we divided the consistency of semen into three categories (Thick, moderate thick and thin). We observed consistency of semen with significant differences among seasons as well as breeds(Table-2).In the study, the highest percentage (92.3%) of thick category of semen was found in autumn in SL-F1,followed by, the highest percentage of moderate thick and thin category semen was found in autumn and winter in HF-F1 and SL-F3 bulls, respectively (Table-2).

Table-2: Seasonal Effects on Semen Consistency of Upgraded (Holstein Friesian and Sahiwal) Bulls

Breed with Blood level	Season	Thick (No.)	(%)	Moderate thick (No.)	(%)	Thin (No.)	(%)	Total (No.)	Total (%)
50% HF+50% L (HF×L)	Summer	11	42.31	12	46.15	3	11.54	26	100
	Rainy	14	53.85	10	38.46	2	7.69	26	100
	Autumn	7	26.92	18	69.23	1	3.85	26	100
	Winter	16	61.54	5	19.23	5	19.23	26	100

75% HF+25%L (HF_1×L)	Summer	16	61.54	6	23.08	4	15.38	26	100
	Rainy	11	42.31	12	46.15	3	11.54	26	100
	Autumn	19	73.08	4	15.38	3	11.54	26	100
	Winter	18	69.23	6	23.08	2	7.69	26	100
87.5% HF+12.5%L (HF_2×L)	Summer	20	76.92	3	11.54	3	11.54	26	100
	Rainy	20	76.92	4	15.38	2	7.69	26	100
	Autumn	15	57.69	9	34.62	2	7.69	26	100
	Winter	16	61.54	9	34.62	1	3.85	26	100
50% SL+50% L (SL×L)	Summer	14	53.85	11	42.31	1	3.85	26	100
	Rainy	19	73.08	5	19.23	2	7.69	26	100
	Autumn	19	73.08	6	23.08	1	3.85	26	100
	Winter	8	30.77	15	57.69	3	11.54	26	100
75% SL+25% L (SL_1×L)	Summer	21	80.77	3	11.54	2	7.69	26	100
	Rainy	19	73.08	5	19.23	2	7.69	26	100
	Autumn	22	84.62	3	11.54	1	3.85	26	100
	Winter	20	76.92	4	15.38	2	7.69	26	100
87.5% SL+12.5% L (SL_2×L)	Summer	22	84.62	1	3.85	3	11.54	26	100
	Rainy	21	80.77	3	11.54	2	7.69	26	100
	Autumn	24	92.31	1	3.85	1	3.85	26	100
	Winter	15	57.69	5	19.23	6	23.08	26	100

Ejaculate volume and Mass activity

In case of upgraded Holstein Friesian, the highest volume of semen was found during autumn season in HF_2×L bulls (8.077±0.33 ml) and the lowest volume was observed during winter season in HF×L bulls (4.692±0.36). Likely, in case of upgraded Sahiwal, the highest volume of semen was found in the time of autumn in SL_2×L bulls(6.510±0.10) and the lowest amount was noticed during winter in SL×L bulls(4.788±0.21).The values were varied significantly with season and breed and blood level(Table-3). This may be due to the higher level of exotic blood of breed and the optimum temperature and humidity in autumn for maximum secretion of semen from bulls. Several researchers showed significant effect of season on semen volume in upgraded bulls [3, 9, and 10] and the present study disagrees with the result of another study [11], where they noticed the highest volume in summer lowest in winter. It was observed in another experiment that one group significant and another group insignificant variation in semen volume between seasons [12], which partially agrees with the present study.

The highest mass activity was found to be 4.365±0.09 during autumn in SL_2×L bulls, and 4.058±0.12 in HF_2×L bulls and the lowest value of mass activity was found in summer in SL×L bulls (3.308±0.07) and in HF×L bulls (3.519±0.08). Few seasons have a significant effect on mass activity and few have notable differences (Table-3). It was also found in some studies that the highest mass activity in winter, followed by rainy and summer in Karan Fries bulls[13], whereas, in Frieswal bulls and in American bison bulls obtained no significant seasonal differences in mass activity[14,15].

Table-3: Mean ± SE of Seasonal Effects on Ejaculate Volume and Mass Activity of Semen

Seminal Parameter	Breed with Blood level	Summer (Mid-February to Mid-May) (N=26)	Rainy (Mid-May to Mid-August) (N=26)	Autumn (Mid-August to Mid-November) (N=26)	Winter (Mid-November to Mid-February) (N=26)
Ejaculate	50% HF+50% L(HF×L)	$5.096±0.37^{ab}$	$5.173±0.37^{ab}$	$6.115±0.41^{a}$	$4.692±0.36^{b}$

Volume (ml)	75% HF+25%L(HF_1×L)	6.481±0.24^{ab}	5.904±0.11^{bc}	7.212±0.27^{a}	5.308±0.16^{bc}
	87.5%HF+12.5%L(HF_2×L)	6.365±0.32^{b}	7.942±0.41^{a}	8.077±0.33^{a}	6.904±0.40^{ab}
	50% SL+50% L(SL×L)	4.942±0.13^{ab}	5.365±0.18^{ab}	5.712±0.33^{a}	4.788±0.21^{b}
	75% SL+25%L(SL_1×L)	5.365±0.19^{b}	6.000±0.25^{ab}	6.173±0.19^{a}	5.462±0.20^{ab}
	87.5%SL+12.5%L(SL_2×L)	5.442±0.14^{c}	6.115±0.13^{ab}	6.510±0.10^{a}	5.750±0.23^{bc}
Mass Activity (0-5)	50% HF+50% L(HF×L)	3.519±0.08^{b}	3.769±0.09^{ab}	3.827±0.06^{a}	3.865±0.06^{a}
	75% HF+25%L(HF_1×L)	3.654±0.09^{b}	3.865±0.08^{ab}	3.846±0.07^{ab}	3.942±0.04^{a}
	87.5%HF+12.5%L(HF_2×L)	3.615±0.09^{b}	3.731±0.14^{ab}	4.058±0.12^{a}	3.865±0.07^{ab}
	50% SL+50% L(SL×L)	3.308±0.07^{b}	3.346±0.09^{b}	3.923±0.07^{a}	3.500±0.17^{ab}
	75% SL+25%L(SL_1×L)	3.577±0.09^{b}	3.731±0.09^{ab}	3.942±0.11^{a}	4.019±0.04^{a}
	87.5%SL+12.5%L(SL_2×L)	3.654±0.09^{b}	4.154±0.11^{a}	4.365±0.09^{a}	4.115±0.06^{a}

*Means with different superscripts within a row differ significantly at 5% level

Sperm concentration and pH

Sperm concentration is considered to be one of the most important semen attributes in relation to variation of semen quality due to season. The results for Sperm concentration of the present study summarized in table 4 indicated that sperm concentration varied significantly with seasonal variation and blood levels of the studied breed. The highest sperm concentration was found in HF_2×L bulls during winter and SL_1×L bulls in autumn and the lowest sperm concentration was found during the rainy season in HF_1×L bulls and in summer in SL×L bulls (Table-4). It was also shown in an experiment that maximum sperm concentration in winter followed by rainy and summer [1]. However, another previous study reported that the highest sperm concentration during cold-dry in zebu-exotic half breeds [16] and the lowest value during the rainy season [17]. On the other hand, another study reported insignificant seasonal variation in sperm concentration in Frieswal bulls [14]. In the present study there was not found significant differences in semen pH except SL×LandSL₁×L bulls. The highest value of semen pH was observed in SL_2×Lbulls during the rainy season and in SL_1×L bulls in summer. It was also observed the insignificant seasonal differences in semen pH in crossbred (Karan Fries) bulls [1].

Table-4: Mean ± SE of Seasonal Effects on Sperm Concentration and pH of Semen

Seminal Parameter	Breed with Blood level	Summer (Mid-February to Mid-May) (N=26)	Rainy (Mid-May to Mid-August) (N-26)	Autumn (Mid-August to Mid-November) (N=26)	Winter (Mid-November to Mid-February) (N=26)
Sperm Concentration (million/ml)	50% HF+50% L(HF×L)	1188±80.13^{b}	1238±59.39^{ab}	1216±40.03^{b}	1455±47.93^{a}
	75% HF+25%L(HF_1×L)	1324±46.02^{ab}	1161±64.38^{b}	1360±44.14^{ab}	1491±70.41^{a}
	87.5%HF+12.5%L(HF_2×L)	1261±66.79^{b}	1376±80.54^{ab}	1433±57.20^{ab}	1511±56.34^{a}
	50% SL+50% L(SL×L)	1183±54.59^{b}	1270±51.31^{ab}	1452±57.59^{a}	1317±80.49^{ab}
	75% SL+25%L(SL_1×L)	2053±60.67^{a}	2209±54.69^{a}	2210±47.99^{a}	1787±51.01^{b}
	87.5%SL+12.5%L(SL_2×L)	1498±83.23^{b}	1793±52.37^{a}	1789±64.57^{a}	1069±64.93^{c}
PH	50% HF+50% L(HF×L)	6.415±0.02^{a}	6.430±0.02^{a}	6.442±0.02^{a}	6.426±0.02^{a}
	75% HF+25%L(HF_1×L)	6.485±0.01^{a}	6.477±0.01^{a}	6.500±0.01^{a}	6.515±0.01^{a}
	87.5%HF+12.5%L(HF_2×L)	6.759±0.02^{a}	6.772±0.02^{a}	6.763±0.02^{a}	6.762±0.02^{a}
	50% SL+50% L(SL×L)	6.508±0.01^{a}	6.377±0.01^{bc}	6.410±0.01^{b}	6.452±0.01^{b}

75% SL+25%L(SL_1×L)	6.619±0.01[a]	6.481±0.01[b]	6.465±0.01[b]	6.590±0.01[a]
87.5%SL+12.5%L(SL_2×L)	6.418±0.01[a]	6.398±0.01[a]	6.381±0.01[a]	6.391±0.01[a]

*Means with different superscripts within a row differ significantly at 5% level

Live sperm percentage

Present study also revealed the significant effect on live sperm percentage in semen in studied breeding bulls. In case of upgraded Holstein Friesian bulls, the maximum percentage of live sperm observed during winter season in HF×L bulls (77.92%) and minimum percentage of live sperm was found in summer in HF×L bulls (71.27%) whereas, in upgraded Shaiwal bulls, the highest value was recorded during winter in SL_1×L bulls (78.15%) and the lowest value was found during summer in SL_1×L bulls (71.85%) [Figure 2 & 3].

It was reported in a study that the live sperm during hot-dry; 61.73%, hot-humid; 64.79%, autumn; 66.00%, winter; 67.00% and summer; 76.11% [18] , which is slightly lower than the results of the present study. On the contrary, in another experiment it was observed a slightly higher value then the present study as spring; 82.24% and winter; 83.70% in Hariana bulls [19].

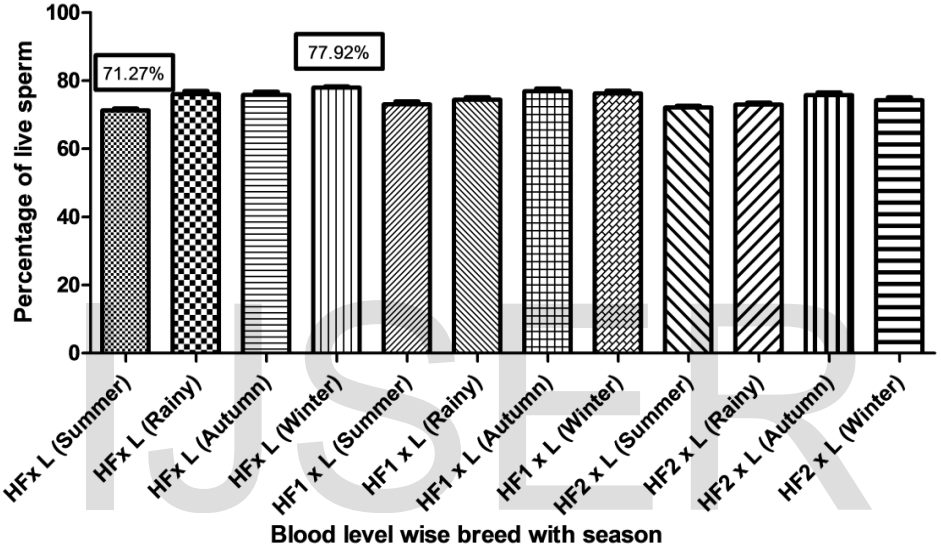

Figure-2: Season wise live sperm (%) of semen of upgraded (HF) bulls

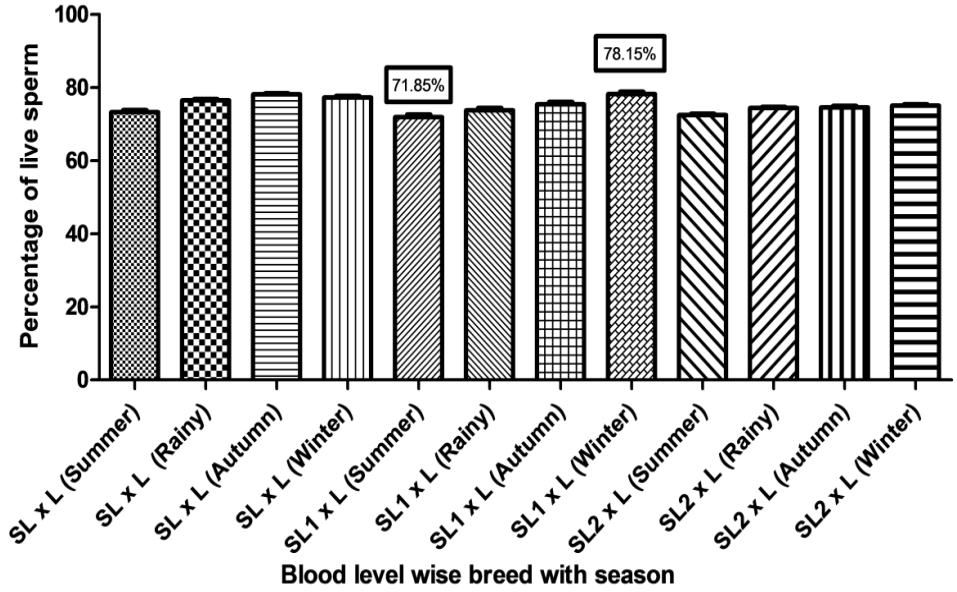

Figure-3: Season wise live sperm (%) of semen of upgraded (SL) bulls

Initial motility and Post freezing motility

Significant differences were observed in initial motility percent of semen of both upgraded Holstein Friesian and Sahiwal bulls which is in agreement with the findings of other studies in Zebu-taurus [18] and in *Bos brachycerus* bulls[20] . On the other hand, in Frieswal bulls and in Exotic and crossbred bulls there was no significant variation in initial motility percent [14,17]. In the present study the highest value of initial motility percent was recorded as 81.35±0.76in SL_1×L bulls during the rainy season and lowest value was found as 70.58±0.32 in SL×L bulls during winter (Table-5).

In our study, post freezing motility percent ranges from 42.88±0.49 to 55.96±0.73 and there was significant variation within the season and breed. It was reported that the motility of sperm after freezing varied from 62.2-63.6% in crossbred bulls [21], which is slightly higher than the results of the present study. Lower post freezing motility than initial motility indicated that freezing of semen reduced sperm motility.

It might be assumed that the consequences of sperm cryo-injury are caused by cryopreservation [22]. The plasma membrane of sperm is the primary site of damage induced by cryopreservation [23]. Both freezing and thawing implicate tremendous alteration in volume of cell water, which result in considerable mechanical stress on the sperm membrane and consequently reduce sperm motility [23].

Table-5: Mean ± SE of Seasonal Effects on Initial Motility % and Post Freezing Motility % of Semen

Seminal Parameter	Breed with Blood level	Summer (Mid-February to Mid-May) (N=26)	Rainy (Mid-May to Mid-August) (N=26)	Autumn (Mid-August to Mid-November) (N=26)	Winter (Mid-November to Mid-February) (N=26)
Initial Motility (%)	50% HF+50% L(HF×L)	73.27±0.73^{b}	76.54±0.61^{a}	76.35±0.59^{a}	75.77±0.53^{a}
	75% HF+25%L(HF_1×L)	76.73±0.55^{b}	77.50±0.80^{b}	81.12±0.81^{a}	77.88±0.69^{b}
	87.5%HF+12.5%L(HF_2×L)	75.19±0.52^{b}	76.35±0.44^{ab}	77.31±0.57^{a}	75.58±0.58^{ab}
	50% SL+50% L(SL×L)	73.65±0.52^{a}	71.15±0.42^{b}	70.58±0.32^{b}	70.58±0.32^{b}
	75% SL+25%L(SL_1×L)	78.46±0.87^{ab}	81.35±0.76^{a}	78.08±0.74^{b}	77.69±0.89^{b}
	87.5%SL+12.5%L(SL_2×L)	73.08±0.49^{b}	79.04±0.73^{a}	75.58±0.75^{b}	79.04±0.74^{a}
Post Freezing Motility (%)	50% HF+50% L(HF×L)	46.15±0.97^{b}	47.69±0.79^{ab}	46.54±0.67^{ab}	49.23±0.82^{a}
	75% HF+25%L(HF_1×L)	50.19±0.65^{b}	49.62±0.73^{b}	54.81±0.89^{a}	54.04±0.68^{a}
	87.5%HF+12.5%L(HF_2×L)	51.15±0.69^{b}	52.12±0.79^{ab}	54.81±0.89^{a}	49.23±0.91^{b}
	50% SL+50% L(SL×L)	45.58±0.75^{a}	44.23±0.60^{ab}	42.88±0.49^{b}	43.27±0.55^{b}
	75% SL+25%L(SL_1×L)	52.88±0.49^{b}	53.46±0.61^{b}	55.96±0.73^{a}	51.54±0.72^{b}
	87.5%SL+12.5%L(SL_2×L)	47.69±0.63^{b}	53.08±0.49^{a}	52.31±0.50^{a}	53.08±0.49^{a}

*Means with different superscripts within a row differ significantly at 5% level

CONCLUSION

It can be concluded that there is a strong significant effect of season on semen quality and the summer season adversely affects the bio-physical characteristics of semen in upgraded breeding bulls. Winter is the most favourable season for excellent quality semen production and the autumn and rainy seasons might be considered as the intermediate among the four studied seasons. Semen quality was also influenced by freezing, generation and type of breed and blood level, hence it is suggested to freeze the

semen with great consciousness. It is advised that during summer, breeding bulls should be kept cool and comfortable by running ceiling fan, splashing water at least 3-4 times a day. It is also suggested that bulls should be protected from direct wind blast, housed in a place with favorable micro-environment with least humidity, fed during cool hours and have free access to cool drinking water.

CONFLICT OF INTEREST

Authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

ACKNOWLEDGMENTS

The authors are grateful to the Chief Executive Officer (CEO) and Board of Directors of Lal Teer Livestock Development Bangladesh Limited for providing the research facilities as well as funding for this manuscript publication.

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