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A Comparative Study for Semen Quality Traits between Murrah and Nili Ravi Buffalo Breeding Bulls Maintained at an Organized Farm

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ABSTRACT

Background: The aim of the study was to compare the effect of season on the semen quality traits of Murrah and Nili Ravi buffalo breeding bulls.

Methods: For a period of 5 years, a total of 855 ejaculation records of 17 buffalo breeding bulls, including Murrah (11) and Nili Ravi (6) buffalo bulls maintained at an organised farm, were analysed for various semen traits using least squares analysis.

Result: Breed had a significant effect ($p<0.01$) on semen volume, consistency, mass activity (MA), total sperm count (TSC) and post thaw motility (PTM) and a non-significant effect on colour and initial motility (IM). In Murrah bulls, except for semen volume, the other parameters *viz*. consistency, MA, TSC and PTM were found to be significantly higher than Nili Ravi bulls. Season of semen collection had a significant effect on consistency, TSC and PTM whereas a non-significant effect on semen volume, colour, MA and IM was observed. During the summer season, overall consistency and TSC were higher, but PTM was found to be comparatively lower. Season and breed interaction had a significant effect ($p<0.01$) on consistency, TSC and IM while a non-significant effect on semen volume, colour, MA and PTM was observed. In Murrah buffalo bulls, a non-significant effect of season on consistency was observed whereas, in Nili Ravi bulls, a significantly higher consistency during summer and spring and; a significantly lower consistency during the rainy season was observed. In Murrah bulls, the highest TSC were observed during the rainy and summer seasons; whereas, in Nili Ravi bulls only during the summer season. In Murrah bulls, semen consistency, MA and IM were lower during the spring season; semen volume was lower during the prewinter season; total sperm count was lower during the winter season. In Nili Ravi bulls, semen volume was lower during summer; consistency, MA and TSC were lower during the rainy season; and IM was lower during the spring season. The PTM was lower during the summer season in both the breeds.

Key words: Buffalo, Murrah, Nili Ravi, Semen, Season.

INTRODUCTION

India is primarily an agricultural country and livestock is an integral part of the agriculture system. In terms of milk production India is at the top position with per capita milk availability of 444 grams/day/person (BAHS, 2022). The world's top buffalo breeds are found in India. The Murrah and Nili Ravi breeds of buffalo, whose average milk yields per lactation are about 1500-2500 kg and 1500-1800 kg, respectively, require special attention (Yadav et al., 2017). But, a significant loss in milk production and offspring results due to reproductive issues and lower fertility which reduces dairy farmers' income by decreasing milk production and increasing veterinary expenses (Singh *et al*., 2019). Therefore, reproductive traits seem to be more economically important.

A single bull is used to serve many female animals by artificial inseminations, therefore, bull fertility and semen-related parameters are crucial for maintaining overall herd fertility (Whiston *et al*., 2017). For artificial insemination to be successful, breeding bulls with superior genetics and high-quality semen is essential. The qualities of bull semen can be linked to the genetic parameters for fertility traits (Druet *et al*., 2009). Semen quality parameters such as sperm concentration, morphology, motility and freezeability can be used as bull fertility indicators (Morrell *et al*., 2017). Utilizing sub-fertile or infertile bulls might result in significant financial loss because it would extend calving intervals beyond the target of 365 days in dairy herds (Penny, 2009). India has

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superior germplasm of Murrah and Nili Ravi buffaloes, so by exporting frozen semen to other buffalo breeding nations such as Brazil, Indonesia, Malaysia, Thailand and Philippines, India has the chance to assist them in their efforts to enhance their breed improvement program.

At a breeding farm, the variations in semen quality depend on the breed, age, managemental conditions and reproductive health status of bulls during semen collection (Mukhopadhyay *et al*., 2010). Studies that address the effect of season on the semen quality parameters are available in different breeds and species of animals (Bhakat *et al*., 2015), but a very few studies are available that compare Murrah and Nili Ravi buffalo bulls during different seasons under the same conditions and no study is available that compares semen quality traits of Murrah and Nili Ravi buffalo bulls according to the climatic conditions of Punjab (India). Therefore, the current study analyses and compares semen quality parameters in buffalo breeding bulls during different seasons at Directorate of Livestock Farms, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (Punjab).

MATERIALS AND METHODS

Animal and data structure

For the present study, the data on semen quality parameters of Murrah (11) and Nili Ravi (6) buffalo bulls were collected over a duration of 5 years (2015-2019) from the records maintained at the Directorate of Livestock Farms, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana. A total of 855 ejaculation records from 17 buffalo breeding bulls were used for the present study.

All the bulls were maintained under similar management and feeding conditions and animals with incomplete records were excluded from the study. The various semen quality parameters to be incorporated in the current study were semen volume (ml), colour, consistency, mass activity, total sperm count (millions/ml), initial motility (%) and post thaw motility (%).

The semen from all the bulls was collected early in the morning, with the aid of an artificial vagina (37°C). The collected semen was brought to the laboratory immediately and placed in a water bath at 37°C. The volume of semen per ejaculate was measured directly from test tubes. For buffalo bulls, a cream colour was considered a normal colour. Code 1 was given to the normal semen colour and codes 2, 3 and 4 were given to milky, watery and bloody/pink colours, respectively. The mass activity of spermatozoa was recorded immediately after semen collection by examining a drop of semen on a clean, grease-free and dry slide maintained at a 30-35°C warm slide under a low power microscope with an attached stage warmer (at 37°C). The score was calculated on a 0-5 numerical scale. Initial motility, as a percentage of motile sperm cells, was estimated by placing a drop of diluted fresh semen on a clean and dry slide, covered by a cover slip and examined in a microscope. The total sperm count was measured using a Neubauer haemocytometer. The post thaw motility of the semen was measured after 24 hours of freezing.

Classification of season of semen collection

Based on the prevailing climatic conditions in Punjab, the months of the year were divided into five seasons *i.e*., spring, summer, rainy, pre-winter and winter (Table 1).

Statistical analysis

The data were subjected to least squares analysis to study the effect of breed, the season of semen collection and interaction between breed and season.

Statistical model:

$$Y_{ijklm} = \mu + B_i + S_j + (B * S)_{ij} + e_{ijlm}$$

Where,

Y_{ijklm} = Character variable of m^{th} observation of l^{th} bull under i^{th} breed, j^{th} season of semen collection.

μ = Overall population mean.

B_i = Fixed effect of i^{th} breed (i = 1, 2).

S_j = Fixed effect of season of semen collection (j = 1, 2...5).

$(B*S)_{ij}$ = Effect of interaction between breed and season.

e_{ijlm} = Random error for m^{th} observation of l^{th} bull under i^{th} breed, j^{th} season of semen collection ~NID (0, $\sigma^2 e$).

For statistical analysis of semen quality parameter data, SYSTAT software was used.

The percentage data generated for initial motility and post thaw motility were first transformed to an arcsine scale using (in Microsoft Excel) the following formula:

$$X = \text{asin } \{\text{sqrt } (Y/100)\} * 57.3$$

Where,

X = Arcsine value for initial motility and post thaw motility.

Y = Initial motility and post thaw motility in percentage.

The transformed data were subjected to an analysis of variance to study the effect of breed, season and interaction between breed and season of semen collection on semen quality traits.

RESULTS AND DISCUSSION

In the present study, it was observed that breed had a highly significant effect (P<0.01) on semen volume (Table 2). The least square means for semen volume in Murrah and Nili Ravi buffalo bulls were found to be 3.602±0.077 ml and 4.353±0.104 ml, respectively (Table 2). The similar findings were also reported for Nili Ravi (4.67±1.62 ml) buffalo bulls (Muhammad *et al*., 2000). A non-significant and lower estimate of per ejaculate semen volume in Murrah and Nili Ravi buffalo bulls (3.04±1.81 and 2.67±0.44 ml, respectively) were reported by Miraz *et al*. (2022). As compared to our study, higher estimates of semen volume were reported by Khatun *et al*. (2013) in Murrah (5.50±0.176 ml) and crossbred cattle (5.81±0.176 ml) bulls.

A non-significant effect of breed on semen colour was observed during the present study (Table 2). The least square means for semen colour in Murrah and Nili Ravi bulls were found to be 1.000±0.003 and 1.007±0.005, respectively. Muhammad *et al*. (2000) observed that in Nili Ravi buffalo bulls milky to creamy colour is an indication of good sperm concentration. Breed had a highly significant effect (P<0.01)

on consistency (Table 2). In Murrah and Nili Ravi bulls, the least square means for consistency of semen were found to be 2.737±0.029 and 2.496±0.038, respectively (Table 2).

It was observed that breed had a highly significant effect (P<0.01) on mass activity (Table 2). In Murrah and Nili Ravi bulls, the least square means for the mass activity of semen were found to be 3.159±0.064 and 2.732±0.127, respectively (Table 2). A similar result (2.65±1.14) was reported by Muhammad *et al*. (2000) in Nili Ravi buffalo bulls. However, lower estimates (1.88±0.07) of mass activity in Nili Ravi bulls were reported by Younis (1996). However, Saeed (1990) observed a higher mass activity (3.49) in adult buffalo bulls. On the other hand, Bhakat *et al.* (2015) reported that seasonal variations had a non-significant effect on semen volume.

Breed had a highly significant effect (P<0.01) on total sperm count. The least square means for total sperm counts in Murrah and Nili Ravi buffalo bulls were 1,482.176±32.211 million/ml and 1,152.543±37.793 million/ml, respectively. A non-significant effect of breed on initial motility was observed (Table 2). For initial motility, least square means were found to be 78.429±0.006% and 77.750±0.010% in Murrah and Nili Ravi buffalo bulls, respectively. Lower estimates of initial motility were reported by Younis (1996) and Muhammad *et al*. (2000) (60.45±0.48, 56.89±0.65%, respectively) in Nili Ravi buffalo bulls and by Suryaprakasam and Rao (1993) (69.00±0.86%) in Murrah buffalo bulls.

It was observed that breed had a highly significant effect (P<0.01) on post thaw motility (Table 2). The least square means for post thaw motility were found to be 47.465±0.016% and 42.052±0.019%, in Murrah and Nili Ravi buffalo bulls, respectively (Table 2). Miraz *et al*. (2022) also observed a significant effect (P<0.01) of breed on post thaw motility in buffalo (Indigenous, Murrah and Nili Ravi) bulls.

Table 1: Classification of season.

Month of semen collection	Season
March-Mid-April	Spring
Mid-April-June	Summer
July-September	Rainy
October-November	Prewinter
December-February	Winter

Effect of seasons on semen quality traits

The season has both direct and indirect effects on the semen quality traits. The temperature, humidity, rainfall and photoperiod are macro and microclimatic components that have a direct effect on the animal while the season affects vegetation and fodder quality indirectly. In this study, a non-significant effect of season of semen collection on overall per-ejaculate semen volume was observed. The overall least square means for semen volume during the spring, summer, rainy, prewinter and winter seasons were found to be 4.127±0.189, 3.988±0.158, 3.971±0.129, 3.920±0.125 and 3.881±0.107 ml, respectively (Table 3).

The season of semen collection had a non-significant effect on semen colour. For semen colour, the overall least square means were 1.000±0.008, 1.000±0.007, 1.017±0.006, 1.000±0.006 and 1.000±0.005 during the spring, summer, rainy, prewinter and winter seasons, respectively (Table 3). Statistical analysis showed that the season of semen collection had a highly significant effect (P<0.01) on consistency. The overall least square means for consistency of semen during the spring, summer, rainy, prewinter and winter seasons were observed as 2.667±0.070, 2.716±0.059, 2.437±0.048, 2.642±0.046 and 2.622±0.040 bulls, respectively (Table 3). So, it was observed that overall consistency was lowest during the rainy season compared to other seasons.

Season of semen collection had a non-significant effect on overall mass activity. For mass activity, the overall least square means during the spring, summer, rainy, prewinter and winter seasons were 2.786±0.292, 3.217±0.132, 2.855±0.098, 2.980±0.080 and 2.889±0.083, respectively (Table 3). Gill *et al*. (1974) observed lower mass activity in buffalo bulls during the winter season (3.06), while, Muhammad *et al*. (2000) reported that water buffalo bull possesses better mass activity during autumn and lower during the winter season. Overall, least square means for total sperm count during the spring, summer, rainy, prewinter and winter seasons were 1,314.223±73.298, 1,505.904±60.791, 1,263.823±49.800, 1,255.968±46.896 and 1,246.879±40.796 million/ml respectively (Table 3). During the summer season, significantly (P<0.05) the higher sperm count was observed.

The season had a significant effect (P<0.01) on post thaw motility in buffalo bulls. The post thaw motility during the

Table 2: Least squares mean±S.E. for semen volume, colour, consistency, mass activity, total sperm count, initial motility and post thaw motility for Murrah and Nili Ravi bulls.

	Murrah	Nili ravi
Semen volume (ml)	3.602±0.077[a] (519)	4.353±0.104[b] (284)
Colour	1.000±0.003 (500)	1.007±0.005 (274)
Consistency	2.737±0.029[a] (436)	2.496±0.038[b] (269)
Mass activity	3.159±0.064[a] (296)	2.732±0.127[b] (178)
Total sperm count (million/ml)	1,482.176±32.211[a] (387)	1,152.543±37.793[b] (274)
Initial motility (%)	78.429±0.006 (413)	77.750±0.010 (267)
Post thaw motility (%)	47.465±0.016[a] (294)	42.052±0.019[b] (228)

Data in parentheses indicate the number of observations.

[a,b] Different superscripts indicate significant differences between two levels (P<0.05).

spring, summer, rainy, prewinter and winter seasons were found to be 47.134±0.077, 38.459±0.059, 44.994±0.036, 45.689±0.031 and 47.552±0.022%, respectively (Table 3). From the above results, it can be concluded that post thaw motility is higher at lower temperatures and lower when the environmental temperature is higher.

Effect of breed and season interaction on semen quality traits

The effect of breed and season interaction was estimated in the analysis to know the semen quality traits during different seasons in Murrah and Nili Ravi buffalo bulls. The comparison has been made row-wise, since the columns represent different seasons if there is no common symbol between the levels of sub-effects then it indicates a significant difference at a 5% level of significance (Table 4).

Season and breed interaction had a non-significant effect ($p<0.05$) on semen volume. In Murrah bulls, semen volume during the spring, summer, rainy, prewinter and winter seasons were found to be 3.882±0.229, 3.825±0.176, 3.508±0.153, 3.387±0.166 and 3.406±0.025 ml, respectively (Table 4). In Nili Ravi bulls, semen volume during the spring, summer, rainy, prewinter and winter seasons were 4.372±0.300, 4.150±0.262, 4.435±0.209, 4.453±0.188 and 4.355±0.177, respectively (Table 4). Muhammad *et al.* (2000) reported that higher per ejaculate semen volume was observed in Nili Ravi bulls during the autumn season and low during the humid summer. Similarly, Bhakat *et al.* (2015) observed that in Murrah buffalo bulls, seasonal variations had a non-significant effect on semen volume.

Non-significant effect ($p<0.05$) of season and breed interaction on semen colour was observed. The least square means for semen colour during the spring, summer, rainy, prewinter and winter seasons were 1.000±0.010, 1.000±0.008, 1.000±0.007, 1.000±0.007 and 1.000±0.005, in Murrah bulls, respectively and 1.000±0.014, 1.000±0.012, 1.033±0.009, 1.000±0.009 and 1.000±0.008, respectively, in Nili Ravi bulls (Table 4). Muhammad *et al*. (2000) observed a less abnormalities ($P<0.05$) in semen colour in Nili Ravi buffalo bulls during autumn.

In Murrah bulls, the season had a non-significant effect on the consistency of semen. The least square means for consistency of semen during the spring, summer, rainy, prewinter and winter seasons were 2.622±0.085, 2.709±0.070, 2.796±0.059, 2.854±0.063 and 2.705±0.047, respectively (Table 4). But, in Nili Ravi bulls, the season had a significant effect ($p<0.05$) on the consistency of semen. In Nili Ravi bulls the least square means were found to be 2.712±0.112, 2.722±0.095, 2.079±0.076, 2.431±0.067 and 2.538±0.065, during spring, summer, rainy, prewinter and winter seasons, respectively (Table 4).

Season and breed interaction had a non-significant effect on mass activity. During the spring, summer, rainy, prewinter and winter seasons the mass activity were observed as 3.071±0.207, 3.208±0.158, 3.226±0.021 and 3.179±0.026 and 3.109±0.020, respectively in Murrah bulls and 2.500±0.547, 3.225±0.212, 2.485±0.165, 2.782±0.112 and

Table 3: Least squares mean±S.E. for semen volume, colour, consistency, mass activity, total sperm count, initial motility and post thaw motility for buffalo bulls during different seasons.

Season	Semen volume (ml)	Colour	Consistency	Mass activity	Total sperm count (million/ml)	IM (%)	PTM (%)
Spring	4.127±0.189 (79)	1.000±0.008 (77)	2.667 ± 0.070^{a} (71)	2.786±0.292 (24)	$1,314.223\pm73.298^{bc}$ (62)	79.903±0.035 (70)	47.134 ± 0.077^{a} (49)
Summer	3.988±0.158 (122)	1.000±0.007 (114)	2.716 ± 0.059^{a} (103)	3.217±0.132 (56)	$1,505.904\pm60.791^{a}$ (100)	78.054±0.027 (91)	38.459 ± 0.059^{b} (67)
Rainy	3.971±0.129 (172)	1.017±0.006 (169)	2.437 ± 0.048^{b} (150)	2.855±0.098 (115)	$1,263.823\pm49.800^{b}$ (139)	78.342±0.016 (151)	44.994 ± 0.036^{a} (107)
Prewinter	3.920±0.125 (169)	1.000±0.006 (164)	2.642 ± 0.046^{a} (154)	2.980±0.080 (141)	$1,255.968\pm46.896^{bc}$ (151)	75.954±0.015 (149)	45.689 ± 0.031^{a} (119)
Winter	3.881±0.107 (261)	1.000±0.005 (250)	2.622 ± 0.040^{a} (227)	2.889±0.083 (138)	1,246.879±40.796c (209)	78.126±0.011 (219)	47.552 ± 0.022^{a} (180)

Data in parentheses indicate the number of observations.

^{a,b,c} Different superscripts indicate significant differences between two levels ($P<0.05$).

2.669±0.133, respectively in Nili Ravi bulls (Table 4). Bhakat *et al*. (2011) analysed that in Murrah buffalo bull season had a significant effect on mass activity. Bajwa *et al*. (1982) observed lower mass activity during the winter season (2.23), whereas Heuer *et al*. (1987), Nazir (1988) and Bhakat *et al.* (2015) reported a non-significant effect of seasons on mass activity in Nili-Ravi and Murrah buffalo bulls.

A significant effect ($p<0.05$) of season and breed interaction on total sperm count has been observed. In Murrah buffalo bulls, total sperm count during the spring, summer, rainy, prewinter and winter seasons was 1,378.515±100.260, 1,564.955±70.894, 1,668.181±63.219, 1,444.141±65.213 and 1,355.087±51.107 million/ml, respectively (Table 4). Total sperm count was higher in Murrah bulls during the rainy and summer followed by prewinter, spring and winter seasons. Total sperm count in Nili Ravi buffalo bulls during the spring, summer, rainy, prewinter and winter seasons were 1,249.931±106.951, 1,446.853±98.774, 859.464±76.964, 1,067.795±67.410 and 1,138.671±63.603 million/ml, respectively (Table 4). As far as the effect of the season is concerned, it has been observed that in Nili Ravi buffalo bulls, the total sperm count was significantly ($P<0.05$) higher during the summer, spring, and winter seasons, followed by the prewinter and rainy seasons, while Muhammad *et al*. (2000) reported that in Nili Ravi buffalo bulls, the total sperm count was higher in milder (spring and autumn) seasons. A non-significant effect of season of semen collection on total sperm count was reported by Bhakat *et al.* (2015) in Murrah buffalo bulls.

The least square analysis of variance revealed that season and breed interaction had a highly significant effect ($P<0.01$) on initial motility. In Murrah buffalo bulls during the spring, summer, rainy, prewinter and winter seasons least square means for Initial motility were 74.081±0.054, 78.299±0.040, 80.735±0.025, 79.903±0.029 and 78.943±0.017%, respectively (Table 4). Initial motility was higher during the rainy, prewinter, winter and summer but comparatively lower during the spring season. In Nili Ravi buffalo bull's the initial motility during the spring, summer, rainy, prewinter and winter seasons were found to be 85.213±0.086, 77.823±0.069, 75.850±0.040, 71.755±3.383 and 61.343±0.029%, respectively (Table 4). Further by Fisher's least significant difference test, it has been observed that in Nili Ravi bulls initial motility was significantly ($P<0.01$) higher during the spring followed by summer, rainy, prewinter and winter seasons (Table 4). Mohan *et al*. (1977) and Gill *et al*. (1974) found that buffalo bulls had higher motility during the winter season (75 and 65%, respectively), whereas Muhammad *et al*. (2000) and Singh *et al*. (1992) reported that the Murrah breed had less initial motility during the winter. While Saeed *et al*. (1990) found no relationship between the seasons and initial motility.

The non-significant effect of season and breed interaction on post thaw motility was observed. In Murrah bulls, the lowest post thaw motility was observed during the summer (37.679±0.091%) season, followed by rainy (47.186±0.060%), prewinter (47.866±0.064%), winter (51.233±0.034%) and spring (53.464±0.170%) seasons (Table 4). In Nili Ravi bulls,

Table 4: Least squares mean±S.E. of semen volume, colour, consistency, mass activity, total sperm count, initial motility and post thaw motility for Murrah and Nili Ravi bulls during. different seasons.

Semen quality parameter	Breed	Spring	Summer	Rainy	Prewinter	Winter
Semen volume(ml)	Murrah	3.882±0.229 (50)	3.825±0.176 (84)	3.508±0.153 (112)	3.387±0.166 (95)	3.406±0.025 (178)
	Nili Ravi	4.372±0.300 (29)	4.150±0.262 (38)	4.435±0.209 (60)	4.453±0.188 (74)	4.355±0.177 (83)
Colour	Murrah	1.000±0.010 (49)	1.000±0.008 (77)	1.000±0.007 (109)	1.000±0.007 (94)	1.000±0.005 (171)
	Nili Ravi	1.000±0.014 (28)	1.000±0.012 (37)	1.033±0.009 (60)	1.000±0.009 (70)	1.000±0.008 (79)
Consistency	Murrah	2.622±0.085 ^{bde} ^{f}(45)	2.709±0.070 ^{abde} (67)	2.796±0.059 ^{abd} (93)	2.854±0.063^{ad} (82)	2.705±0.047^{abd} (149)
	Nili Ravi	2.712±0.112^{cd} (26)	2.722±0.095^{cd} (36)	2.079±0.076^{h} (57)	2.431±0.067^{fg} (72)	2.538±0.065^{ceg} (78)
Mass activity	Murrah	3.071±0.207 (21)	3.208±0.158 (36)	3.226±0.021 (82)	3.179±0.026 (70)	3.109±0.020(87)
	Nili Ravi	2.500±0.547 (3)	3.225±0.212 (20)	2.485±0.165 (33)	2.782±0.112 (71)	2.669±0.133 (51)
Total sperm count (million/ml)	Murrah	1,378.515±100.260^{bd} (33)	1,564.955±70.894^{ab} (66)	1,668.181±63.219^{a} (83)	1,444.141±65.213^{bc} (78)	1,355.087±51.107^{cd} (127)
	Nili Ravi	1,249.931±106.951^{cde} (29)	1,446.853±98.774^{abc} (34)	859.464±76.964^{f} (56)	1,067.795±67.410^{e} (73)	1,138.671±63.603^{e} (82)
Initial motility(%)	Murrah	74.081±0.054^{ce} (43)	78.299±0.040^{bc} (57)	80.735±0.025^{ab} (93)	79.903±0.029^{bd} (81)	78.943±0.017^{bd} (139)
	Nili Ravi	85.213±0.086^{a} (27)	77.823±0.069^{bc} (34)	75.850±0.040^{cde} (58)	71.755±3.383^{e} (68)	61.343±0.029^{bc} (80)
Post thaw motility(%)	Murrah	53.464±0.170 (22)	37.679±0.091 (41)	47.186±0.060 (62)	47.866±0.064 (58)	51.233±0.034 (111)
	Nili Ravi	40.849±0.138 (27)	39.241±0.143 (26)	42.812±0.083 (45)	43.538±0.061 (61)	43.884±0.054 (69)

Data in parentheses indicate the number of observations.
^{a,b,c,d,e} Different superscripts indicate non-significant differences between two sub-effects ($P<0.05$).

lowest estimates of post thaw motility were found to observed during the summer (39.241±0.143%) season, followed by spring (40.849±0.138%), rainy (42.812±0.083%), prewinter (43.538±0.061%) and winter (43.884±0.054%) seasons. Post thaw motility in both Murrah and Nili Ravi bulls was found to be lower during the hot season of the year (summer season).

CONCLUSION

From the current study, it has been observed that semen volume per ejaculate is higher in Nili Ravi bulls (4.353 ml) than in Murrah buffalo bulls (3.602 ml). Moreover, it has been observed that sperm consistency, mass activity and total sperm count in Murrah buffalo bulls were 2.737±0.029, 3.159±0.064 and 1,482.176±32.211 million/ml, respectively and in Nili Ravi buffalo bulls were 2.496±0.038, 2.732±0.127 and 1,152.543±37.793 million/ml, respectively. In buffalo bulls' consistency and total sperm count were comparatively higher during the summer season. At the same time, it has been concluded that in buffalo bulls post thaw motility is significantly higher during the cold season and lowest during the summer season. The overall performance of buffalo bulls is good in all seasons. But in Murrah buffalo bulls, consistency, mass activity, total sperm count and initial motility were lower during the spring season. In Nili Ravi buffalo bulls, semen colour abnormalities were more prevalent in rainy season; similarly, consistency, mass activity and total sperm count were also lower during the rainy season.

Conflict of interest: None.

REFERENCES

BAHS, (2022). Basic Animal Husbandry Statistics. Department of Animal Husbandry and Dairying, Government of India.

Bajwa, M.A., Shah, I.H., Tahir, M. and Ahmad, N. (1982). Seasonal effect on seminal attributes in Nili-Ravi buffalo bulls. Pakistan Journal of Animal Science. 4: 35-44.

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.

Bhakat, M., Mohanty, T.K., Raina, V.S., Gupta, A.K., Khan, H.M., Mahapatra, R.K. and Sarkar, M. (2011). Effect of age and season on semen quality parameters in Sahiwal bulls. Tropical Animal Health and Production. 43(6): 1161-1168.

Druet, T., Fritz, S., Sellem, E., Basso, B., Gerard, O., Salas Cortes, L. Humblot, P., Druart, X. and Eggen, A. (2009). Estimation of genetic parameters and genome scan for 15 semen characteristics traits of Holstein bulls. Journal of Animal Breeding and Genetics. 126(4): 269-277.

Gill, R.S., Gangwar, P.C. and Takkar, O.P. (1974). Seminal attributes in buffalo bulls as affected by different seasons. Indian Journal of Animal Sciences. 44: 415-418.

Heuer, C., Tahir, M.N. and Amjad, H. (1987). Effect of season on fertility of frozen buffalo semen. Animal Reproduction Science. 13: 15-21.

Khatun, M., Kaur, S., Kanchan and Mukhopadhyay, C.S. (2013). Subfertility problems leading to disposal of breeding bulls. Asian-Australasian Journal of Animal Sciences. 26(3): 303-308. https://doi.org/10.5713/ajas.2012.12413.

Miraz, M.F., Hossain, S.M.J., Akter, S. and Deb, G. (2022). Sperm quality and fertility of Murrah and Nili-Ravi bulls compared with indigenous buffalo bulls in Bangladesh. Bangladesh Livestock Research Institute. 10.21203/rs.3.rs-2075224/v1.

Mohan, G., Madan, M.L. and Razdan, M. N. (1977). Composition of Murrah buffalo bull semen during winter and summer months in India. Tropical Agriculture. 54: 21-28.

Morrell, J., Nongbua, T., Valeanu, S., Lima-Verde I, Lundstedt-Enkel, K., Edman, A. and Johannisson, A. (2017). Sperm quality variables as indicators of bull fertility may be breed dependent. Animal Reproduction Science. 185: 42-52.

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

Mukhopadhyay, C.S., Gupta, A.K., Yadav, B.R., Khate, K., Raina, V.S., Mohanty T.K. and Dubey, P.P. (2010). Subfertility in males: An important cause of bull disposal in bovines. Asian-Australian Journal of Animal Science. 23: 450-455. https://doi.org/10.5713/ajas.2010.90298 .

Nazir, M. (1988). Semen evaluation and sperm morphology-monography on reproductive pattern of Riverine buffaloes and recommendations to improve their reproductive performance at small farmer level. PARC, Islamabad.

Penny, C.D. (2009). The importance of the bull in herd fertility. National Animal Disease Information System. https://www.nadis.org.uk/disease-a-z/cattle/beef-herd-fertility/bull-fertility/.

Saeed, A., Chaudhry, R.A., Khan, I.H. and Khan, N.U. (1990). Morphology of semen of buffalo bulls of different age groups. Proc. II World Buffalo Congress. 3: 12-16.

Singh, P., Sodhi, S.S., Dash, S.K., Kaur, S., Malhotra, P. and Dhindsa, S.S. (2019). Study of incidence of reproductive disorders in murrah buffaloes in relation to non- genetic factors through an animal model. Journal of Animal Research. 9: 633-644. DOI: 10.30954/2277-940X.05.2019.2.

Singh, R.S., Tomar, N.S., Sharma K.C. and Sharma K.B. (1992). Studies on acrosomal abnormalities of cattle and buffalo spermatozoa in relation to other semen characteristics and fertility. Indian Veterinary Journal. 69: 267-268.

Suryaprakasam, T.B. and Rao, A.V.N. (1993): Studies on seminal characteristics of multiple breed A. I. bull. Indian Veterinary Journal. 70: 629-632.

Whiston, R., Finlay, E.K. and McCabe M.S. (2017). A dual targeted β-defensin and exome sequencing approach to identify, validate and functionally characterise genes associated with bull fertility. Scientific Reports. 7: 12287. https://doi.org/10.1038/s41598-017-12498-x.

Yadav, A.K., Singh, J. and Yadav, S.K. (2017). Characteristic features of registered indigenous buffalo breeds of India: A review. International Journal of Pure and Applied Bioscience. 5: 825-831.

Younis, M. (1996). Studies on semen quality, freezability and fertility of buffalo bulls during the low and the peak breeding seasons. Ph.D. Thesis. Department of Animal Reproduction, University of Agriculture. Faisalabad, Pakistan.