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SEMEN CHARACTERISTICS AS INFLUENCED BY SEASONAL AND CLIMATIC VARIATIONS IN NILI-RAVI BUFFALO BREEDING BULLS

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ABSTRACT

The present study was conducted to determine the effect of seasonal and climatic variations on semen characteristics of Nili-Ravi buffalo breeding bulls in Sahiwal and Bahawalpur districts of Punjab, Pakistan. For this purpose, 14 adult Nili-Ravi buffalo breeding bulls (n=7 from each of Semen Production Unit (SPU) Qadirabad, Sahiwal and SPU Karaniwala, Bahawalpur), were used. Semen samples were collected fortnightly using artificial vagina. On each collection day, 2 ejaculates were collected from each bull. Results demonstrated significantly higher ($P<0.05$) ejaculatory volume in buffalo bulls of Sahiwal compared to Bahawalpur district, whereas seasonal influence on ejaculatory volume was non-significant ($P>0.05$). Semen color score was non-significantly different ($P>0.05$) between the districts with higher score in Sahiwal and it was higher in autumn and spring seasons but with non-significant ($P>0.05$) seasonal influence. Semen pH was significantly lower ($P<0.05$) in Sahiwal than Bahawalpur and significantly lower ($P<0.05$) pH was observed in autumn season. Mass motility score was non-significantly different ($P>0.05$) between the districts with higher score in Sahiwal and it was higher in autumn season but with non-significant influence of seasons. Significantly higher ($P<0.05$) individual sperm motility in Sahiwal was recorded as compared to Bahawalpur and it was higher in spring and autumn in Sahiwal. Sperm concentration was significantly increased ($P<0.05$) in Sahiwal than Bahawalpur. Similarly season influence was also significant with significantly higher ($P<0.05$) sperm concentration in autumn and spring. In conclusion, semen quality of Nili-Ravi buffalo breeding bulls is superior in autumn and spring and the bulls in Sahiwal district produce superior quality semen compared to Bahawalpur district.

Key words: Nili-Ravi, Buffalo, Bull, Semen, Season, Climate.

INTRODUCTION

The home tract of Nili-Ravi buffalo is canal irrigated areas of central Punjab province of Pakistan (Shah, 1991). Buffalo breeding bulls are used for semen collection for artificial insemination purpose in majority of buffalo breeding countries. Semen quality of breeding bulls reflects the degree of normality of the function of their testes, epididymis and genital tract (Barth and Waldner, 2002). The sexual activity of bulls is affected by the seasonal variations and may be due to the variations in environmental temperature (Fayemi and Adegbite 1982; Sekoni and Gustafsson 1987), which ultimately affect the semen quality. Any slight change in temperature of scrotum causes the ill effect on spermatogenesis (Januskauskas *et al.,* 1995).In general, of all seasons, summer exerts comparatively more adverse effects on semen quality. It affects the normal reproduction process by inhibiting the release or response of hormones like luteinizing hormone (LH) (Mandal *et al.,* 2000). LH is an important hormone responsible for the process of spermatogenesis. Increased environmental temperature during summer is an important limitation in animals' performance and the effects of high environmental temperature are aggravated when associated with increased humidity (Marai *et al.,* 2008).

It is well known that seasonality exists in buffalo reproduction (Qureshi *et al.,* 2000). The effect of season on reproductive behavior of buffalo bulls is both direct and indirect. The direct effect of season is through macro and micro climatic factors, like rainfall, humidity, temperature and photoperiod. On the other hand, indirectly, it acts by affecting vegetation, forage quality and soil-plant-animal interaction. The magnitude of variations differs from breeds, location, prevailing climatic conditions, feeding and general management (Mandal *et al.,* 2000). Buffalo bulls are comparatively more susceptible to heat stress due to their poor heat regulation mechanism than the females (Akhtar, 1988).

Morphological modifications occurring in the testes in response to the different factors brought about by seasonal variations modulate the hormonal fluctuations which, in turn, influence the spermatogenesis (AL-Sahaf and Ibrahim, 2012). Semen characteristics are generally affected by the seasonal variations. High summer temperatures generally decrease the semen volume and sperm concentration. Sperm motility is also

extremely susceptible to excessive heat or cold (Hafez and Hafez, 2000).

The information regarding the effect of seasons on semen characteristics in dairy bulls has been reported to be of a conflicting nature (Bhakat *et al.,* 2009). Some researcher have reported significant effect of season (Goswami *et al.,* 1991; Singh and Raina 2000), while others observed non-significant effect (Mathur *et al.,* 2002; Helbig *et al.,* 2007) of season on semen quality parameters. Hot-dry and hot-humid seasons have been reported to be unfavorable for reproduction (Bhakat *et al.,* 2011). Juinudeen and Hafez (2000) found that semen quality of bulls is influenced by the seasonal variations.

Semen quality has been found best during winter, poor during summer and intermediate during the rainy season (Bhakat *et al.,* (2014). Summer season deteriorates the quality of semen in Holstein-Friesian and Jersey bulls in sub-tropical environment of Pakistan (Fiaz *et al.,* 2010). In Cholistani breeding bulls, semen quality is found better during stressful seasons than stress free season (Farooq *et al.,* 2013). Younis *et al.* (1998) observed better various semen quality parameters in peek breeding season in Nili-Ravi buffalo bulls. Javed *et al.* (2000) also observed the influence of seasonal variations on various semen quality parameters in Nili-Ravi buffalo bulls.

In Nili-Ravi buffalo bulls, studies on semen quality parameters in different seasons are limited and no report is available regarding the climatic effect on semen quality, keeping the bulls under different climatic zones. Therefore studies in this connection are mandatory for selection of best climatic zone for optimum performance of Nili-Ravi buffalo bulls. This could be an important consideration while establishing location of semen production units for Nili-Ravi buffalo bulls. This paper describes the effect of seasonal and climatic variations on semen characteristics of Nili-Ravi buffalo breeding bulls in Sahiwal and Bahawalpur districts of Punjab Province of Pakistan.

MATERIALS AND M ETHODS

Geo-location and Climate of Study Areas: The current study was conducted in two districts viz. Sahiwal and Bahawalpur, located in different climatic zones of Punjab province of Pakistan districts. Sahiwal is located at latitudes 30^{o} and 31.15^{o}North and longitudes 73^{o} and 74^{o} East, and at an altitude of 564 feet above the sea level. This is a flat plane and an irrigated area of Central Punjab with very fertile soil. The average annual temperature is 31°C and rainfall is 183 mm. The average humidity level of the area is 76%.Bahawalpur is located at latitudes 27^{o} and 29^{o}North and longitudes 69^{o} and 75^{o} East, and at an altitude of 380 feet above the sea level. It is part of Southern Punjab and lies in Cholistan desert area. The climate of the district is hot and arid with average annual temperature 33°C and rainfall 78 mm. The average humidity of Bahawalpur district is 72% (Pakistan Meteorological Department).

Study Design: The study was conducted over a period of one year (2013-14) divided into four seasons viz. Autumn (September 16 to November 14), Winter (November 15 to February 15), Spring (February 16 to April 30) and Summer (May 1 to September 15). The summer season was further divide into Dry Summer (May 1 to June 30) and Humid Summer (July 1 to September 15) (Javed, 1998). A total of 14 adult Nili-Ravi buffalo breeding bulls (n=7 from Semen Production Unit (SPU) Qadirabad, Sahiwal district; n=7 from SPU Karaniwala, Bahawalpur district), aged 5-8 years, having clinically normal reproductive tract, were included in this study.

Health, Management and Feeding of Bulls: The Bulls were keenly observed for their health. They were also tested negative for tuberculosis and brucellosis through comparative tuberculin test and rose Bengal plate test, respectively (Radostits *et al.,* 2000). The bulls were routinely vaccinated against hemorrhagic septicemia and foot and mouth disease.

Uniform management practices were done during the whole study span in both districts. The bulls were housed individually in separate bull pens. Each bull pen had both covered area and open space and the bulls were allowed to have free mobility with in the pen to avoid any stress. The dung removal frequency was twice a day to ensure the cleanliness and hygienic conditions within the bull pens. Bulls were showered twice daily in summer and once in other seasons. Uniform feeding practices were also done during the whole study span. Good quality seasonal fodder (In winter barseem and in summer maize, sorghum and barley) was offered daily at the rate of 10% of body weight. Concentrate (Anmol Wanda) at the rate of 2 kg per bull was offered daily. Anmol Wand contained cotton seed cake 18%, maize gluten-30 15%, canola meal 10%, maize grain broken 14%, wheat bran 25%, molasses 16% and mineral mixture 2%. Anmol Wanda contained crude protein (CP) 16% and total digestible nutrients (TDN) were 68%. Each 100 kg of mineral mixture used in Anmol Wanda contained Di-Calcium Phosphate (DCP) 70.81 kg, Common Salt 18.91 kg, Magnesium Sulfate 8.64 kg, Ferrous Sulfate 0.89 kg, Manganese Sulfate 0.49 kg, Zinc Sulfate 0.22 kg, Copper Sulfate 0.03 kg, Potassium Iodide 8.77 g, Cobalt Chloride 0.89 g and Sodium Selenite 1.50 g. Bulls were provided free access to drinking water round the clock in their bull pens throughout the experimental period.

Collection of Semen Samples: Throughout the study period of one year (2013-14),semen samples were collected from these bulls fortnightly during early morning (before sunrise) using artificial vagina (IMV,

France) connected to rubber cone and graduated glass collection tube at temperature of 42˚C. An intact teaser bull was used for mounting purpose during semen collection. On each collection day, 2 ejaculates were collected from each breeding bull (Javed *et al.,* 2000). After collection, semen was placed in water bath maintained at 37ºC (Ahmad *et al.,* 2005) and was processed for further analysis.

Semen Analysis: Semen volume and color were noted directly from graduated semen collection vial. The color of the semen was noted and assigned numerical score of 0 (watery), 1 (milky) and 2 (creamy) depending upon semen thickness (Javed *et al.,* 2000; Mulugeta *et al.,* 2006). The pH of each semen sample was determined by digital pH meter immediately after collection (Rehman *et al.,* 2012). To estimate the mass motility, a drop of undiluted semen was placed on pre-warmed microscopic glass slide at 37ºC and was examined under phase contrast microscope (×100) equipped with heated stage. Mass motility scoring of semen samples was performed on a scale of 0 to 5 according to wave's pattern (Nazir, 1988).For individual sperm motility, a drop of fresh semen diluted in sodium citrate solution (2.9%) was taken onto warm slide, covered with glass coverslip and examined under phase contrast microscope (×400). The individual sperm motility was scored on the basis of the percentage of spermatozoa with normal forward progressive movement, whereas the spermatozoa having circling movements or oscillating at one place were considered as immotile spermatozoa (Ahmad, 1994). Concentration of spermatozoa (million/mL) was determined at 560nm wavelength by photometer (Bovine photometer n˚ 1119, IMV, France). All the semen evaluation parameters were performed at respective semen production units.

Statistical Analysis: The MIXED procedure of SAS Institute, Inc. (2008) was used to test the significance level of fixed and random effects. The significance of fixed effects was tested using F statistics at a probability level of 0.05. The differences between various levels of fixed effects were tested using Scheffe adjustment in Proc mixed. The following statistical model was used:

$$Yijk = \mu + Ci + Sj(i) + eijk$$

where Yijk is a semen parameter from semen sample of the kth bull in the ith city and jth season nested within the ith city; μ is overall population mean; Ci is the fixed effect of ith city (2 levels); Sj(i) is the fixed effect of jth season nested within ith city (5 levels) and eijk is the random residual associated with each record.

RESULTS

Data on ejaculatory volume, semen color scoring, semen pH, mass motility, individual sperm motility and sperm concentration of Nili-Ravi buffalo breeding bulls of two districts is given in Table 1, whereas season wise data on these parameters in Sahiwal and Bahawalpur districts is given in Table 2 and 3, respectively.

Ejaculatory Volume: The ejaculatory volume was 4.63±0.10mL and 2.60±0.10mL in buffalo bulls of Sahiwal and Bahawalpur districts, respectively. In buffalo bulls of Sahiwal district, a non-significant (P>0.05) difference in ejaculatory volume was recorded in all the seasons with maximum volume in spring season followed by dry summer, humid summer, autumn and winter seasons. Similarly, in buffalo bulls of Bahawalpur district the seasonal influence on the ejaculatory volume was also non-significant (P>0.05) and maximum ejaculatory volume was recorded in humid summer followed by autumn, spring, winter and dry summer. On district wise comparison, ejaculatory volume was significantly higher (P<0.05) in buffalo bulls of Sahiwal district compared to Bahawalpur district.

Semen Color: Semen color was creamy (score 1.06±0.07) and milky (score 0.89±0.07) in buffalo bulls of Sahiwal and Bahawalpur districts, respectively. In Nili-Ravi buffalo bulls of Sahiwal district, a non-significant difference (P>0.05) in semen color score was recorded with maximum score in autumn followed in order by spring, dry summer, humid summer and winter. Similarly in Bahawalpur district the non-significant effect of season on semen color score was observed in same pattern as in Sahiwal district. Semen color score was also non-significantly different (P>0.05), when compared between two districts. However it was higher in Sahiwal.

Semen pH: Semen pH of Nili-Ravi buffalo bulls of Sahiwal and Bahawalpur districts was 6.47±0.03 and 6.67±0.03, respectively. In Sahiwal district, significantly lower (P<0.05) semen pH was observed in autumn and dry summer than winter, spring and humid summer. In Bahawalpur district, significantly lower (P<0.05) pH was observed in autumn than all other seasons. When semen pH was compared district wise, it was found significantly lower (P<0.05) in buffalo bulls of Sahiwal district than Bahawalpur district.

Mass Motility: Mass motility score was 2.06±0.03 and 1.86±0.03in buffalo bulls in Sahiwal and Bahawalpur district, respectively. Mass motility score of buffalo bulls in Sahiwal district was non-significantly increased (P>0.05) in autumn followed by spring, dry summer, humid summer and winter. In the buffalo bulls of Bahawalpur district highest mass motility score was also observed in autumn followed by dry summer, spring, humid summer and winter and the difference was also non-significant (P>0.05) among all the seasons as in Sahiwal district. When compared between districts, also a non-significant difference (P>0.05) in mass motility score

of buffalo bulls was observed with higher score in Sahiwal as compared to Bahawalpur district.

Individual Sperm Motility: Individual sperm motility in buffalo bulls was 66.02±2.02% and 49.72±2.02% in Sahiwal and Bahawalpur districts, respectively. Seasonal influence on individual sperm motility was observed non-significant (P>0.05) in Sahiwal district. Lowest individual sperm motility was recorded during winter season and highest was observed during spring season. Buffalo bulls of Bahawalpur district also exhibited non-significant difference (P>0.05) in individual sperm motility in different seasons with maximum individual sperm motility in dry summer followed in order by autumn, spring, humid summer and winter seasons. On district wise comparison, overall individual sperm motility was significantly higher (P<0.05) in buffalo bulls of Sahiwal district than Bahawalpur district.

Sperm Concentration: The sperm concentration of Nili-Ravi buffalo breeding bulls was 1023.27±24.36 million/mL and 854.27±24.36 million/mL in Sahiwal and Bahawalpur districts, respectively. The buffalo bulls of Sahiwal district showed significantly increased (P<0.05) sperm concentration in autumn and spring compared to other seasons. The sperm concentration was the lowest in winter. Buffalo bulls in Bahawalpur district also demonstrated significant influence (P<0.05) of seasons on sperm concentration with significantly higher (P<0.05) sperm concentration in autumn followed in order by spring, dry summer, humid summer and winter. District wise comparison demonstrated significantly higher (P<0.05) sperm concentration in semen of buffalo bulls of Sahiwal district compared to Bahawalpur district.

Table 1. Semen characteristics of Nili-Ravi buffalo bulls in Sahiwal and Bahawalpur districts†.

Semen Parameters	Districts		Overall P value
	Sahiwal	Bahawalpur	
Ejaculatory volume (mL)	4.63 ±0.10^{a}	2.60 ±0.10^{b}	<0.0001
Semen color (score 0-2)	1.06±0.07^{a}	0.89±0.07^{a}	0.0856
Semen pH	6.47 ±0.03^{a}	6.66 ±0.03^{b}	<0.0001
Mass motility (score 0-5)	2.06±0.08^{a}	1.86±0.08^{a}	0.07
Individual sperm motility (%)	66.02 ±2.02^{a}	49.72^{b} ±2.02^{b}	<0.0001
Sperm concentration (million/mL)	1023.27^{a} ±24.36^{a}	854.27^{b} ±24.36^{b}	<0.0001

†Values are least square mean±SE.
Values in each row with different superscript letters differ significantly (P<0.05).

Table 2. Semen characteristics of Nili-Ravi buffalo bulls in Sahiwal district during different seasons†.

Semen Parameters	Seasons					Overall P value
	Autumn	Winter	Spring	Dry Summer	Humid Summer	
Ejaculatory volume (mL)	4.55±0.22^{a}	4.29±0.22^{a}	4.87±0.22^{a}	4.84±0.22^{a}	4.6±0.22^{a}	0.5211
Semen color (score 0-2)	1.36±0.15^{a}	0.75±0.15^{a}	1.11±0.15^{a}	1.09±0.15^{a}	0.96±0.15^{a}	0.1921
Semen pH	6.22±0.07^{a}	6.71±0.07^{b}	6.44±0.07^{c}	6.37±0.07^{acd}	6.63±0.07^{bce}	<0.0001
Mass motility (score 0-5)	2.38±0.17^{a}	1.72±0.17^{a}	2.13±0.17^{a}	2.07±0.17^{a}	1.99±0.17^{a}	0.2503
Individual Sperm motility (%)	67.41±4.51^{a}	61.17±4.51^{a}	70.21±4.51^{a}	66.16±4.51^{a}	65.12±4.51^{a}	0.692
Sperm Concentration (million/mL)	1267.16±54.48^{a}	857.93±54.48^{b}	1080.67±54.48^{ac}	1017.54±54.48^{cd}	893.06±54.48^{bde}	<0.0001

†Values are least square mean±SE.
Values in each row with different superscript letters differ significantly (P<0.05).

Table 3. Semen characteristics of Nili-Ravi buffalo bulls in Bahawalpur district during different seasons†.

Semen Parameters	Seasons					Overall P value
	Autumn	Winter	Spring	Dry Summer	Humid Summer	
Ejaculatory volume (mL)	2.67±0.22^{a}	2.52±0.22^{a}	2.6±0.22^{a}	2.34±0.22^{a}	2.85±0.22^{a}	0.5211
Semen color (score 0-2)	1.07±0.15^{a}	0.74±0.15^{a}	0.96±0.15^{a}	0.91±0.15^{a}	0.75±0.15^{a}	0.1921
Semen pH	6.44±0.07^{a}	6.79±0.07^{b}	6.67±0.07^{bc}	6.68±0.07^{bcd}	6.73±0.07^{bcde}	<0.0001
Mass motility (score 0-5)	2.09±0.17^{a}	1.73±0.17^{a}	1.84±0.17^{a}	1.88±0.17^{a}	1.76±0.17^{a}	0.2503
Individual Sperm motility (%)	53.13±4.51^{a}	45.00±4.51^{a}	49.93±4.51^{a}	54.47±4.51^{a}	46.07±4.51^{a}	0.692
Sperm Concentration (million/mL)	920.38±54.48^{a}	761.62±54.48^{b}	884.50±54.48^{abc}	881.61±54.48^{abcd}	823.23±54.48^{abcde}	<0.0001

†Values are least square mean±SE.
Values in each row with different superscript letters differ significantly (P<0.05).

DISCUSSION

In present study, ejaculatory volume was 4.63±0.10mL and 2.60±0.10mL in buffalo bulls of Sahiwal and Bahawalpur districts, respectively. Similar results were reported by Kiani *et al.* (2014) and Younis *et al.* (1998) in adult Nili-Ravi buffalo bulls. On the other hand, Rehman *et al.* (2012) recorded decreased ejaculatory volume (1.70±0.09mL) in Kundi buffalo breed. The difference in ejaculatory volume might be due to the effect of breed. In the present study, season did not influence the ejaculatory volume significantly in both districts which is congruent with the findings of Nazir (1988). Contrary to this, Javed *et al.* (2000) recorded increased semen volume in autumn and *vise-versa* in humid summer. Likewise, Younis *et al.* (1998) recorded increased ejaculatory volume in low breeding season than peak breeding season. Increased ambient temperature in Bahawalpur district resulted in decreased ejaculatory volume of buffalo bulls than Sahiwal district. In present study, semen color was also considered a parameter of semen quality. Our results demonstrated that semen color was creamy (score 1.06±0.07) and milky (score 0.89±0.07) in Sahiwal and Bahawalpur districts, respectively. The semen color is indicative of semen thickness together with pigment color and also an important indicator of sperm concentration. These findings are congruent with the results of previous studies (Nazir, 1988; Javed *et al.,* 2000) in Nili-Ravi buffalo bulls. Likewise, Kiani *et al.* (2014) recorded milky white to creamy white semen color in buffalo bulls aged between 6 to 8 years. Similar results were observed in Swamp buffalo (Koonjaenak *et al.,* 2007). Although our results indicated that season had non-significant impact on semen color score in buffalo bulls of Sahiwal district, but exhibited better semen color score in autumn than other seasons. Similarly season did not show significant impact on semen color of buffalo bulls in Bahawalpur district. Nili-Ravi buffalo bulls demonstrated better semen color score in autumn compared to other seasons (Javed *et al.,* 2000). Koonjaenak *et al.* (2007) documented non-significant effect of season on semen color score similar to results of this study in both the districts. Better semen color score observed in buffalo bulls in Sahiwal district than Bahawalpur district is indicative of favorable ambient conditions for enhanced spermatogenesis in Sahiwal district. In present study, semen pH of buffalo bulls of Sahiwal and Bahawalpur districts was 6.47±0.03 and 6.67±0.03, respectively. Likewise, Javed *et al.,* (2000) documented semen pH 6.45±0.04 in Nili-Ravi buffalo bulls while semen pH was observed as 5.81±0.06 in Kundhi buffalo bulls and 6.16±0.07 in Nili-Ravi buffalo bulls by Rehman *et al.* (2012) and Younis *et al.* (1998) respectively. Semen pH of Kundi buffalo breeding bulls aged 6 to 8 years was 6.71±0.035 (Kiani *et al.*, 2014). In present study, season had significant impact on semen pH in buffalo bulls in both districts. Semen pH was decreased in autumn compared to the other seasons in both the districts while increased semen pH was determined in winter season. These findings are in close agreement with the findings of Javed *et al.* (2000) and Younis *et al.* (1998) who reported low semen pH in autumn and an increase in semen pH in winter season. According to Bhakat *et al.* (2015) different seasons like hot dry-summer, hot humid-rainy and cold humid-winter also significantly influenced the semen pH in buffalo breeding bulls. Contrary to this, seasons did not influence semen pH in Swamp buffalo breeding bulls (Koonjaenak *et al.,* 2007). Increased semen pH in Bahawalpur district than Sahiwal district in our study may be attributed to inferior semen quality in Bahawalpur, because semen pH is negatively correlated

(r= -0.39) with sperm concentration (Younis, 1996). Moreover, dense and thick semen samples have low pH than thin samples (Shalash, 1972).

In present study, mass motility score was 2.06±0.03 and 1.86±0.03 in buffalo bulls in Sahiwal and Bahawalpur district, respectively. Mass motility (score 2.18 ±0.11) documented in adult Nili-Ravi buffalo breeding bulls previously at Sahiwal district (Younis *et al.,* 1998) is comparable with the result of present study in Sahiwal district. An increased mass motility was noted in adult Nili-Ravi buffalo breeding bulls (Javed *et al.,* 2000). Similarly, increased mass motility is reported in Kundhi and Murrah buffalo breeding bulls (Kiani *et al.,* 2014; Bhakat *et al.,* 2015). In present study, although non-significant impact of seasons on mass motility was observed, but it was increased in autumn and decreased in winter season in buffalo bulls in Sahiwal district. Similar pattern of mass motility was observed in Bahawalpur district with non-significant differences among the seasons. These findings in both the district are supported by previous findings in adult Nili-Ravi buffalo bulls, Swamp buffalo bulls and Murrah buffalo bulls (Javed *et al.,* 2000; Koonjaenak *et al.,* 2007; Bhakat *et al.,* 2015). However, results of seasonal influence on mass motility of buffalo bulls in present study are contrary with the findings of Younis *et al.* (1998). Mass motility was observed better in Sahiwal district compared to Bahawalpur district showing the better semen produced in Sahiwal district in present study. Individual sperm motility in buffalo bulls was 66.02±2.02% and 49.72±2.02% in Sahiwal and Bahawalpur districts, respectively. Rehman *et al.* (2012) and Younis *et al.* (1998) documented individual sperm motility in buffalo bulls congruent with the findings of present study in Sahiwal district. Contrarily, Javed *et al.* (2000) recorded individual sperm motility (59.50±1.09 %) in adult Nili-Ravi buffalo bulls. Increased individual sperm motility was reported by Kiani *et al.* (2014) in Kundhi buffalo breeding bulls and in Swamp buffalo bulls by Koonjaenak *et al.* (2007). In present study although non-significantly, but decreased individual sperm motility was observed in winter compared to autumn, spring, dry summer and humid summer in Sahiwal district. Similarly seasonal variations did not influence individual sperm motility in Bahawalpur district. Javed *et al.* (2000) recorded low individual sperm motility in winter. Decreased individual sperm motility in winter was also found by Singh *et al.* (1992) in Murrah buffalo bulls. Younis *et al.* (1998) and Heuer *et al.* (1987) observed increased individual sperm motility in peek breeding season (autumn) in Nili-Ravi buffalo breeding bulls. According to Koonjaenak *et al.* (2007) seasonal variations do not influence individual sperm motility. Individual sperm motility was observed higher in Sahiwal district compared to Bahawalpur district demonstrating the better quality semen produced in Sahiwal district than Bahawalpur district in the present study. This may be attributed to the more favorable climatic conditions of Sahiwal district than Bahawalpur district for better quality semen production. In the present investigation, sperm concentration of 1023.27±24.36 million/mL and 854.27±24.36 million/mL was observed in Sahiwal and Bahawalpur district respectively. Javed *et al.* (2000) published the sperm concentration of 1050±40 million/mL in adult Nili-Ravi buffalo breeding bulls in Sahiwal district which is in close agreement with the finding of present study in Sahiwal district. Similar results (1016.68±21.25 million/mL) were observed by Bhakat *et al.* (2015) in Murrah buffalo bulls. However Younis *et al.* (1998) observed the higher value of sperm concentration (1160±60 million/mL) and similar results were found by Koonjaenak *et al.* (2007). Seasonal effect on the sperm concentration was found significant in both the district. Significantly low sperm concentration was observed in winter. Sperm concentration was highest in autumn in both the districts and is supported by the findings of Javed *et al.* (2000) and Younis *et al.* (1998) in Nili-Ravi buffalo bulls. Higher sperm concentration during the milder seasons is also documented by Zafar *et al.* (1988) and Dumitrescu *et al.* (1988) supporting our findings. Bhosrekar *et al.* (1992) and Koonjaenak *et al.* (2007) reported the non-significant effect of season on sperm concentration in Swamp buffalo is contrary to the findings of present study in both districts. However, Bhakat *et al.* (2015) documented the significant effect of season on sperm concentration supporting the results of present study. In the present study overall higher sperm concentration in Sahiwal district compared to the Bahawalpur district might be due to enhanced spermatogenesis in Sahiwal district due to favorable climatic conditions which also lead to increase the diameter and thickness of seminiferous tubules (Ibrahim *et al.,* 2013).In conclusion, semen quality of Nili-Ravi buffalo breeding bulls is superior in autumn and spring and bulls in Sahiwal district produce superior quality semen compared to Bahawalpur district.

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