



Season Induced Changes in Seminal Characteristics of Sahiwal Breeding Bulls

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Abstract | The study was planned to determine the effect of seasonal variations on seminal characteristics of Sahiwal breeding bulls maintained at semen production unit, Qadirabad, district Sahiwal, Punjab, Pakistan. Semen ejaculates (N=1040) were collected weekly during the period of one year from Sahiwal bulls (N=10). Semen ejaculate volume was observed to be maximum with average 4.27 ± 0.16 ml in winter which differed significantly $p < 0.05$ with its lowest corresponding value in autumn (3.75 ± 0.21 ml). Mass activity (expressed in scale 1-5) differed considerably $p < 0.05$ throughout all seasons and was lowest (1.58 ± 0.05) in humid summer but spring and dry summer yielded best values (1.99 ± 0.05 and 2.00 ± 0.06 respectively). Highest mean values of individual motility and sperm concentration ($77.61 \pm 0.33\%$ and 954.4 ± 38.42 million/ml respectively) were also recorded in dry summer while lower mean values ($75.96 \pm 0.91\%$ and 845.7 ± 34.70 million/ml respectively) were observed in autumn season. Individual motility in humid summer ($74.91 \pm 0.65\%$) was significantly low ($p \leq 0.05$) as compared to all other seasons except autumn ($77.61 \pm 0.33\%$). It is concluded that Sahiwal bulls produced better quality semen in spring and dry summer while humid summer and autumn were negatively impacting the semen quality.

Keywords | Bovine, Seasonal changes, Semen quality, Sahiwal, Motility

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INTRODUCTION

The Sahiwal breed of cattle is native breed of Pakistan. Sahiwal breed is reputed to be known for its high tolerance against harsh climate, tropical disease resistance and having high production potential in least resource availability, making it one of the best cattle breeds of tropical regions (Ilatsia *et al.*, 2011). Originating from Pakistan, the breed has now spread to almost 29 other countries including 12 African countries (McSweeney and Mackie, 2012). The breed is also a part of many breeding plans to develop new breeds where tropical climate dominates e.g. Australian Friesian Sahiwal in Australia. Therefore, there has been ever increasing demand for the semen of Sahi-

wal cattle in Pakistan in general and globally in particular (Singh *et al.*, 2015).

Health and management of donor bulls is empirical for production of semen having good quality and quantity. Quality of semen is a function of various factors and season is most important among those. Season affects the reproductive performance of bulls through its major meteorological components like temperature, humidity, day-length and rainfall (Bhakat *et al.*, 2009). A recent study conducted on Friesian bulls in Libya (Alragubi, 2015) indicated that higher atmospheric temperature in summer season adversely affect different biological and physical characteristics of semen.

A similar study was planned to analyze the semen production performance of Friesland AI bulls in tropical climate which revealed that semen quality was significantly affected by season (Vilakazi and Webb, 2004). High temperature during the summer season specially when coupled with high humidity exerts pronounced negative effect on semen quality (Dombo, 2002). It is also reported that semen output increased when the humidity level was below 50% (Everett and Bean, 1982). The study on semen quality of Nili Ravi buffalo bulls revealed that the was better during the September–November as compared to the hot months of May to July (Younis et al., 1999) while better quality was reported in autumn and spring season in Nili-Ravi Buffalo (Hameed et al., 2017). Bhakat et al. (2014) reported that hot and dry season is the most deteriorating period for seminal characteristics while the good quality semen is yielded during winter days in breeding bulls of Karan Fries cattle breed. Similar results were reported in Murrah breed of buffalo (Mandal et al., 2003) where mass activity was found to be 2.27 in winter as compared to 1.91 and 1.90 in dry summer and rainy summer, respectively. Sperm concentration (million/ml) was also higher (1185.36) in winter than in summer (965.00).

Seasonal effects on seminal characteristics of Holstein bulls show low quality of semen in summer season as compared to spring (Salah et al., 1992). Higher index of temperature and humidity negatively affect the bovine semen characteristics (Sharma et al., 2017). Higher post thaw acrosomal damage was observed in bovine semen samples collected during summer as compared to winter (Orgal et al., 2012). The months of March and April remain most favorable for bovine seminal characteristics (Biniová et al., 2017).

Prolonged exposure to temperature can drastically damage the spermatogenesis (Kastelic et al., 1996). On the other hand, reasons for better quality semen during cold season might be due to the natural climate condition which favored the testosterone action, spermatogenesis and secretion of accessory reproductive glands (Mandal et al., 2000). So, it is emphasized on finding the need-based nutritional and management approaches targeting the better quality of semen and minimizing the chances of unnecessary low quality ejaculates in deteriorating seasons (Dahiya and Singh, 2013). Some of the similar studies, on the other hand, observed no significant influence of temperature and humidity on semen parameters when the overall environment of the bulls was stable (Paldusova, et al., 2014).

Despite being the most important cattle breed of Pakistan, scarce information is documented on the influence of seasonal variability on semen quality in Sahiwal bulls. Moreover, climate change and human interventions into it have made it mandatory for the animal scientists to specifically analyze the effects of season on the health, performance

and welfare of domestic animals. Therefore current study has been proposed to analyze how the Sahiwal breeding bulls respond to different seasons of the year in terms of seminal characteristics.

MATERIALS AND METHODS

ANIMALS AND EXPERIMENTAL SITE

This study was conducted on healthy breeding bulls (age 6-8 years, N=10) of Sahiwal breed maintained at District Sahiwal in the Punjab Province of Pakistan.

HEALTH AND MANAGEMENT OF BREEDING BULLS

The health status of experimental bulls was kept under careful observation throughout the year. Health management practices like vaccination, deworming, dipping, hoof trimming and hair clipping were executed according to the prescribed schedule. Separate bull pens constructed according to the endemic climatic conditions and feeding and housing needs were made available to each bull.

Feeding management was also uniformly implemented for these bulls during the study period. Fresh green fodder of fine quality was offered to each bull at the rate of 10% of body weight daily. In order to meet other nutritional requirements, 2.5 kg wheat straw, 2 kg concentrate (Anmol Wanda®) and 100 g of mineral mixture (Anmol Mineral Mixture®) were also fed per bull on daily basis along with 250 g of stomach powder per bull per week.

SEMEN SAMPLING

Semen samples from each breeding bull were collected weekly in the early morning and 2 ejaculates were taken on each collection day, a method adapted from Javed et al. (2000). A total of 1040 ejaculates (in 52 weeks) were collected and analyzed for semen volume and other quality parameters.

SEMEN COLLECTION TECHNIQUE AND EQUIPMENT

Sterilized Artificial Vagina (IMV France), maintained at 42°C was used for semen collection. Each bull was sexually prepared and stimulated before collection according to the protocol. Immediately after the collection, neat semen in the graduated tube was transferred to laboratory where it was placed in water bath having a temperature of 37°C (Ahmad et al., 2005).

SEMEN ANALYSIS

Semen volume (in ml) was noted from graduated tube for each ejaculate. The mass motility of each ejaculate was estimated by observing a drop of neat (undiluted) semen launched at pre-warmed glass slide that was placed on stage-warmer at 37°C. Modern phase-contrast microscope (Olympus®) with 100X magnification was used for this

purpose. The mass motility score was given to each sample using a scale of 0 to 5 depending upon the wave pattern (density and speed) of semen (Ahmad et al., 2005).

For estimation of individual motility (in percentage), a small drop of neat semen was put on warm slide covered with glass cover-slip. Same microscope was used for this test but with magnification of 400X and percentage of progressively motile sperms was calculated. Sperm concentration was measured in units million per ml with Bovine Photometer (IMV, France) having a wavelength of 560 nm (Ahmad et al., 2005).

SEASONS OF THE YEAR

The study was conducted over a span of one year which was divided into five seasons: Autumn (16th September to 14th November), Winter (15th November to 15th February), Spring (16th February to 30th April), Dry Summer (1st May to 30th June) and Humid Summer (1st July to 15th September 15) (Hameed et al., 2016).

STATISTICAL ANALYSIS

The recorded data was subjected to statistical analysis using software package SPSS 16.0 (SPSS Inc. 2007). Correlations were calculated for different seminal characteristics. Multivariate analysis using ANOVA was also performed to determine the effect of each of the five seasons separately on semen variables.

RESULTS

The overall pattern of semen parameters under study (ejaculate volume, mass activity, individual motility and sperm concentration) throughout the year is shown in Table 1.

Table 1: Mean and Standard deviation of seminal quality parameters of Sahiwal bulls

Semen Parameters	N	Mean ± SD	Range	
			Minimum	Maximum
Ejaculate Volume (ml)	1040	4.07±0.58	2.7	5.6
Mass Activity (1-5)	1040	1.84±0.24	1.33	2.42
Individual Motility (%)	1040	76.6±1.93	69.75	80.00
Sperm Concentration (million/ml)	1040	907.2±117.65	667.90	1219.50

N= Number of total ejaculates studied; SD= Standard Deviation

EJACULATE VOLUME

The ejaculate volume (EV) was 3.75±0.21 ml in autumn that increased in next three seasons i.e. 4.27±0.16 ml,

4.25±0.09 ml and 4.21±0.14 ml in winter, spring and dry summer respectively (Figure 1). The value was again decreased to 3.78±0.21 ml in humid summer. The maximum value of ejaculate volume (5.55 ml) was observed in winter while the minimum (3.78 ml) was in humid summer.

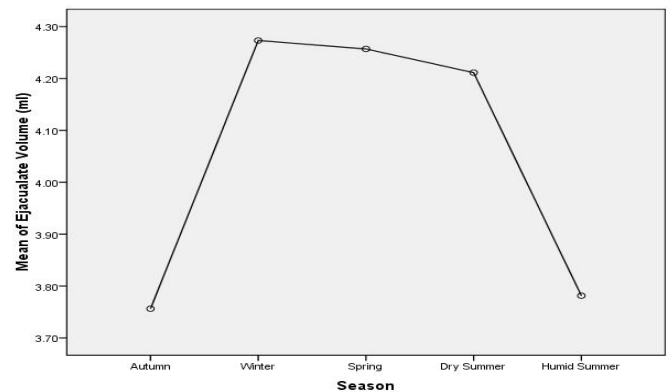


Figure 1: Overall variations in ejaculate volume of Sahiwal bulls semen in different seasons of the year.

MASS ACTIVITY

The mass activity (MA) (expressed in scale 1-5) was 1.72±0.07 in autumn, 1.88±0.05 in winter, 1.99±0.05 in spring and 2.00±0.06 in dry summer (Figure 2). Then, it decreased to 1.84±0.03 in humid summer.

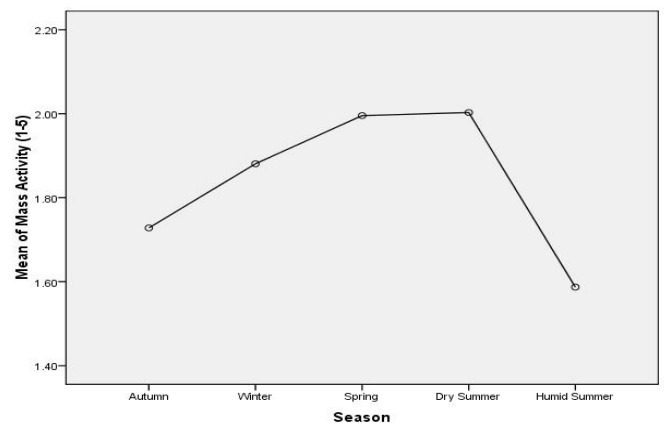


Figure 2: Overall variation in mass activity of Sahiwal bulls semen in different seasons of the year

INDIVIDUAL MOTILITY

The individual motility (IM) gradually increased from autumn (75.96±0.91%) to dry summer (77.61±0.33%) while it decreased to 74.91±0.65% in humid summer. The maximum value of 80.0% was observed in winter while the minimum of 69.7% was in autumn.

SPERM CONCENTRATION

Variation in sperm concentration (SC) with respect to season (Figure 4) shows the maximum mean value of 954.4±38.42 million/ml in dry summer and minimum mean value of 845.7±34.70 million/ml in autumn. Overall m-

Table 2: Variation among different seasons in terms of ejaculate volume

Dependent Variable	(I) Season	(J) Season	Mean Difference (I-J)	SE	Sig.
Ejaculate Volume	Autumn	Winter	-.51683*	.24821	.043
		Humid Summer	.49176*	.22629	.035
	Humid Summer	Spring	.47551*	.23553	.049
Mass Activity	Autumn	Spring	-.26733*	.08609	.003
		Dry Summer	-.27465*	.09003	.004
	Humid Summer	Winter	-.29365*	.07590	.001
		Spring	-.40833*	.07900	.001
Individual Motility	Humid Summer	Winter	-2.12179*	.69398	.004
		Spring	-2.60606*	.72232	.001
	Dry Summer	-2.69444*	.76139	.001	
Sperm Concentration	Humid Summer	Dry Summer	-103.57475*	50.72962	.047

*Significant; **highly significant; Level of Significance p=0.05

aximum and minimum values were observed in winter and humid summer respectively.

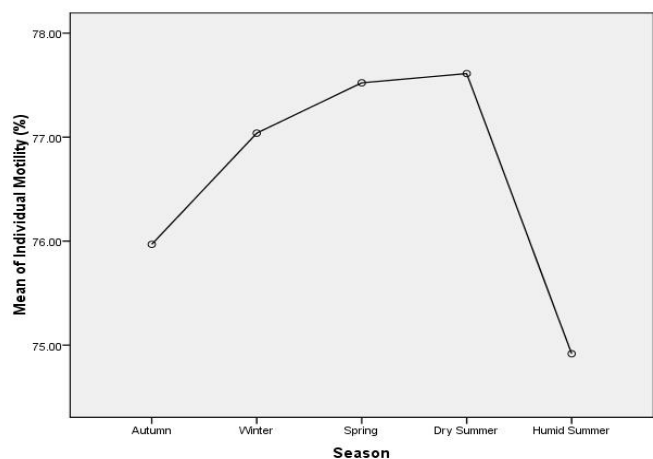


Figure 3: Overall variation in individual motility of Sahiwal bulls semen in different seasons of the year.

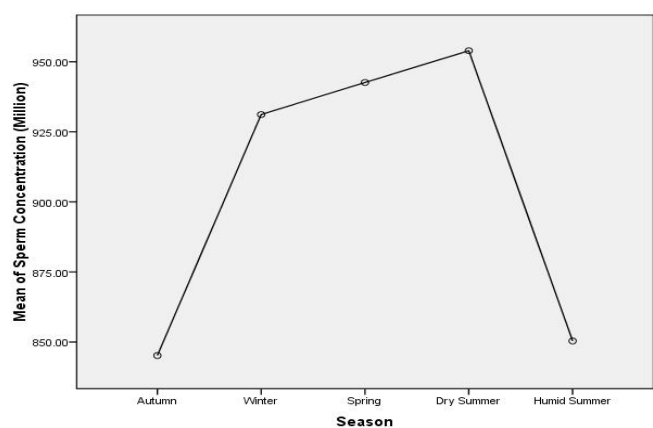


Figure 4: Overall variation in sperm concentration of Sahiwal bulls semen in different seasons of the year.

VARIATION IN SEMEN PARAMETERS AMONG DIFFERENT SEASONS (CORRELATION COMPARISON)

The multiple correlation analysis described whether any two weathers significantly differed from each other in terms of semen parameters under study (Table 2).

EFFECT OF SEASON ON EJACULATE VOLUME

The values indicate that ejaculate volume differed significantly in winter and humid summer ($p \leq 0.05$). Similar but less significant correlation existed between autumn and winter and also between spring and humid summer. Overall it showed that humid summer was the most significantly negatively correlated to ejaculate volume.

EFFECT OF SEASON ON MASS ACTIVITY

The multiple correlation analysis (Table 2) defined whether any two weathers significantly differed from each other in terms of mass activity. The values indicate that mass activity differed highly ($p \leq 0.01$) in humid summer in comparison to winter, spring and even dry summer. Autumn season was also significantly different ($p \leq 0.05$) from spring and dry summer in terms of mass activity.

EFFECT OF SEASON ON INDIVIDUAL MOTILITY

The results of effects of different seasons on individual motility of sperms (Table 2) show that individual motility was only significantly ($p \leq 0.05$) hampered in humid summer as compared to rest of the four seasons. So as compared to semen volume and mass activity, this semen parameter seems to be less affected by major seasons other than humid summer. It was also noted that humid summer differed highly significantly ($p \leq 0.01$) from spring and dry summer.

EFFECT OF SEASON ON SPERM CONCENTRATION

Effect of different seasons of year on sperm concentration was also analyzed (Table 2). Findings were very similar

as two seasons were different from each other in terms of this semen parameter, humid summer that slightly differed from dry summer ($p \leq 0.05$).

DISCUSSION

Results of all similar previous research works are not so agreeable with each other probably because of different climatic regions and dissimilar breeds of bulls used. In current study, the overall effect of varying seasons on semen parameters particularly mass activity and individual motility was found significant ($p < 0.05$). As reported, winter and spring are the advantageous seasons for the semen production while hot and hot-humid are the unfavorable seasons in local bulls (Dahiya and Singh, 2013). In current study, similar findings are observed as far as semen volume is concerned. Results for mass activity and individual motility were different as the dry summer was found favorable for both of these parameters. However, this finding was contrasting with a study that found that season change had no highly significant influence on semen production giving the reason that overall environment of bulls remained stable throughout the year (Mandal et al., 2000).

The current study is describing the winter as best season for semen volume yielding the value of 4.27 ± 0.01 ml while values in dry summer and humid summer were 4.21 ± 0.01 ml and 3.78 ± 0.04 ml respectively. Autumn was found to be the most deteriorating season of year in terms of semen volume which is supported by a study which ranked winter, summer and autumn as first, second and third respectively (Bhakat et al., 2009). The overall effect of seasons on ejaculate volume in this study revealed that humid summer is having significantly negative effect on semen volume as compared to other seasons of the year. In a similar study on Tharparker cattle bulls of India it was concluded that ejaculate volume was higher in summer season than in winter season (Rajoriya et al., 2013).

The overall effect of changing seasons on mass activity in current study was highly significant ($p < 0.01$) and declared that autumn and humid summer both in combination differed significantly from other three seasons of the year. The explanation of this finding is also in line with the biological process of spermatogenesis (Fuerst-Waltl et al., 2006). The lower values of mass activity in autumn are due to the fact that autumn comes after humid summer and seasonal stress experienced by bulls in humid summer persisted till autumn collections.

The overall average individual motility for whole study year was calculated as $76.6 \pm 1.93\%$ which is significantly greater than the value of $66.02 \pm 2.02\%$ observed in buffalo bulls of same region (Hameed et al., 2016). Regarding the seasonal effect on individual motility, current study determined that

it was highest in dry summer and lowest in humid summer which is exactly in line with the previous research work done in Red Sindhi (Mustafa et al., 2003). Similar study conducted in Pakistan on Holstein and Jersey bulls revealed that overall summer was deteriorating season for semen production (Fiaz et al., 2010). All these results explain the fact that our indigenous breeds are much tolerant to higher environmental temperatures.

The average sperm concentration in semen of experimental bulls for one year was found to be 907.2 ± 11.7 million/ml that is significantly greater than 766.69 ± 5.50 million/ml observed in same breed maintained in India (Bhakat et al., 2011). This variation can be explained by the climatic difference between the two regions and also due to different gene pool of the same breed. The results of seasonal effects on sperm concentration in current study have shown that it was maximum in dry summer, moderate in spring and winter and minimum in autumn and humid summer. These findings slightly differ from a previous study which declared winter as the most negatively affecting season for sperm concentration giving reason that testis get close to body of bull in winter that increases the testicular temperature and hampers the spermatogenesis (Koivisto et al., 2009).

CONCLUSION

The current study concluded that overall semen parameters such as ejaculate volume, mass activity, individual motility and sperm concentration varied significantly with changing seasons of the year. Winter and spring are the best months for ejaculate volume while dry summer proved to be most favorable season for other three parameters (mass activity, individual motility and sperm concentration). Humid summer was overall found the most significantly deteriorating season for semen parameters followed by autumn. It is recommended that, if the managers of semen production units desire to have quality control over their semen production, they should be capable of predicting the potential deleterious effects of coming seasons on semen quality.

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CONFLICT OF INTEREST

No conflict of interest.

Muhammad Fahad Bhutta worked as key researcher, Muhammad Tariq and Muhammad Tarique Tunio helped in planning and conduct of the experiment as supervisor, Hafiz Abdul Rauf and Moazzam Javed helped to execute research at experimental site, Abubakar Sufyan and Safdar Imran helped in data analysis, interpretation and writing the manuscript.

REFERENCES

- Ahmad M, Asmat MT, Rehman N (2005). Relationship of testicular size and season in Sahiwal bulls. *Pak. Vet. J.* 25(2): 67 - 70.
- Alragubi SM (2015). Factors affecting on semen production in Friesian bulls in Libya. *Int. J. Pharmaceut.* 6(1): 32 - 36.
- Bhakat M, Mohanty TK, Gupta AK, Abdullah M (2014). Effect of season on semen quality of crossbred (Karan Fries) bulls. *Adv. Anim. Vet. Sci.* 2(11): 632 - 637. <https://doi.org/10.14737/journal.aavs/2014/2.11.632.637>
- Bhakat M, Mohanty TK, Gupta AK, Raina VS (2009). Effect of season and management on semen quality of breeding bulls-A review. *Agric. Rev.* 30 (2): 79 - 93.
- Bhakat M, Mohanty TK, Raina VS, Gupta AK, Khan HM, Mahapatra RK, Sarkar M (2011). Effect of age and season on semen quality parameters in Sahiwal bulls. *Trop. Anim. Health. Pro.* 43(6): 1161 - 1168. <https://doi.org/10.1007/s11250-011-9817-1>
- Biniová Z, Ducháček J, Stádník L, Doležalová M, Hájek R (2017). Effects of climatic conditions on bovine semen characteristics. *Acta Univ. Agric. Silv. Mendel. Brun.* 65(1): 0017 - 0023. <https://doi.org/10.11118/actaun201765010017>
- Dahiya SS, Singh P (2013). Nutritional and other management practices for optimum semen production in buffalo bulls. *Buffalo Bull.* 32(1): 277 - 284.
- Dombo HM (2002). Seasonal effect on semen quality of Gomo Altai and South African indigenous goats. M. Inst. Agrar. (Thesis). University of Pretoria, South Africa.
- Everett RW, Bean B (1982). Environmental influences on semen output. *J. Dairy Sci.* 65(7): 1303 - 1310. [https://doi.org/10.3168/jds.S0022-0302\(82\)82344-8](https://doi.org/10.3168/jds.S0022-0302(82)82344-8)
- Fiaz M, Usmani RH, Abdullah M, Ahmad T (2010). Evaluation of semen quality of Holstein Friesian and Jersey bulls maintained under subtropical environment. *Pak. Vet. J.* 30(2): 75 - 78.
- Fuerst-Waltl B, Schwarzenbacher H, Perner C, Sölkner J (2006). Effects of age and environmental factors on semen production and semen quality of Austrian Simmental bulls. *Anim. Reprod. Sci.* 95(1): 27 - 37. <https://doi.org/10.1016/j.anireprosci.2005.09.002>
- Goswami SC, Mehta SN, Georgie GC, Dixit VP, Lohan IS, Kaker ML (1991). Effect of meteorological factors and seasons on luteinizing-hormone, testosterone and semen of Zebu-Taurus bulls. *Indian. J. Anim. Sci.* 61(6): 579 - 583.
- Hameed S, Masood S, Zaneb H, Khan MS, Younis M, Avais M, Khan MUR (2016). Dimensional characteristics of spermatozoa of Nili-Ravi buffalo bulls: seasonal and climatic influences. *Turk. J. Vet. Anim. Sci.* 40(2): 207 - 213.
- Hameed S, Masood S, Zaneb H, Khan MS, Younis M,

Habib-ur-Rehman, Avais M, Khan MUR (2017). Semen characteristics as influenced by seasonal and climatic variations in Nili-Ravi buffalo breeding bulls. *J. Anim. Plant. Sci.* 27(6): 1750 - 1757.

- Ilatsia ED, Migose SA, Muhuyi WB, Kahi AK (2011). Sahiwal cattle in semi-arid Kenya: genetic aspects of growth and survival traits and their relationship to milk production and fertility. *Trop. Anim. Health. Pro.* 43(8): 1575 - 1582. <https://doi.org/10.1007/s11250-011-9845-x>
- Javed MT, Khan A, Kausar R (2000). Effect of age and season on some semen parameters of Nili-Ravi buffalo (*Bubalus bubalis*) bulls. *Vet. Arh.* 70(2): 83 - 94.
- Kastelic JP, Cook RB, Coulter GH, Saacke RG (1996). Insulating the scrotal neck affects semen quality and scrotal/testicular temperatures in the bull. *Theriogenology.* 45(5): 935 - 942. [https://doi.org/10.1016/0093-691X\(96\)00023-4](https://doi.org/10.1016/0093-691X(96)00023-4)
- Koivisto MB, Costa MTA, Perri SHV, Vicente WRR (2009). The effect of season on semen characteristics and freezability in *Bos indicus* and *Bos taurus* bulls in the southeastern region of Brazil. *Reprod. Domest. Anim.* 44(4): 587 - 592. <https://doi.org/10.1111/j.1439-0531.2008.01023.x>
- Mandal DK, Nagpaul PK, Gupta AK (2000). Seasonal variation in seminal attributes and sexual behaviour of Murrah buffalo bulls. *Indian J. dairy Sci.* 53(4): 278 - 283.
- Mandal DK, Nagpaul PK, Gupta AK (2003). Motion characteristics of Murrah buffalo bull spermatozoa in various seasons and its relationship with functional integrity of the plasmallema. *Theriogenology.* 60(2): 349 - 358. [https://doi.org/10.1016/S0093-691X\(03\)00026-8](https://doi.org/10.1016/S0093-691X(03)00026-8)
- McSweeney C, Mackie R (2012). Commission on Genetic Resources for Food and Agriculture. Micro-organisms and ruminant digestion: State of knowledge, trends and future prospects. Background Study Paper (FAO). 61: 1 - 62.
- Mustafa MI, Latif M, Bashir MK, Ahmad B (2003). Reproductive performance of Red Sindhi cattle under hot and humid environment of Balochistan province of Pakistan. *Pak. Vet. J.* 23(2): 66 - 72.
- Orgal S, Zeron Y, Elior N, Biran D, Friedman E, Druker S, Roth Z (2012). Season-induced changes in bovine sperm motility following a freeze-thaw procedure. *J. Reprod. Develop.* 58(2): 212 - 218. <https://doi.org/10.1262/jrd.10-149N>
- Paldusova M, Kopec T, Chladek G, Hosek M, Machal L, Falta D (2014). The effect of the stable environment and age on the semen production in the Czech Fleckvieh bulls. *Mendel Net.* : 178 - 182.
- Rajoriya JS, Prasad JK, Ghosh SK, Perumal P, Kumar A, Kaushal S, Singh M (2013). Effects of seasons on enzymatic changes and cholesterol efflux in relation to freezability in Tharparkar bull semen. *Asian Pac. J. Reprod.* 2(4): 280 - 288. [https://doi.org/10.1016/S2305-0500\(13\)60163-3](https://doi.org/10.1016/S2305-0500(13)60163-3)
- Salah MS, El-Nouty FD, Al-hajri MR, Mogawer HH (1992). Effect of season on seminal characteristics of Holstein bull under semi-arid environment I. Biophysical characteristics. *Asian-australas. J. Anim. Sci.* 5(3): 439 - 447. <https://doi.org/10.5713/ajas.1992.439>
- Sharma M, Yaqoob B, Singh A, Sharma N, Rawat S (2017). Effect of Temperature Humidity Index on Semen Quality of Bovine Bull. *Int. J. Curr. Microbiol. Appl. Sci.* 6(12): 1822 - 1830. <https://doi.org/10.20546/ijcmas.2017.612.206>
- Singh S, Bhakat M, Mohanty TK, Kumar A, Gupta AK, Chakravarty AK, Singh P (2015). Sexual behavior and its relationship with semen quality parameters in Sahiwal breeding bulls. *Vet. World.* 8(6): 745 - 749. <https://doi.org/10.5455/vetworld.2015.080607>

- Vilakazi DM, Webb EC (2004). Effect of age and season on sperm morphology of Friesland bulls at an artificial insemination centre in South Africa. *S. Afr. J. Anim. Sci.* 34(1): 62 - 69. <https://doi.org/10.4314/sajas.v34i1.4041>
- Younis M, Samad HA, Ahmad N, Ahmad I (1999). Fertility of frozen-thawed semen collected from young, adult and old buffalo bulls during low and peak breeding seasons. *Pak. Vet. J.* 19(2): 78 - 80.