SEASONAL VARIATION IN SEMEN QUALITY AND CONCEPTION RATE OF JAFFARABADI BUFFALO BULLS (*Bubalus bubalis*) IN INDIA

Parmar Kirankumar Hirabhai*, Tank Prafulchandra Hirjibhai, Savsani Harishkumar Hirjibhai and Vala Karsanbhai Bhagvanbhai

Received: 26 May 2020
Accepted: 19 September 2022

ABSTRACT

The study was conducted on four Jaffarabadi breeding bulls, 5 to 6 years old, to know the neat characteristics based on total of 192 semen ejaculates evaluated and cryopreserved during winter (10 to 25°C mean temperature) of November to February (n=96 ejaculates) and summer (38 to 45°C temperature) March to June (n=96 ejaculates). The neat seminal characteristics of Jaffarabadi bulls, *viz*., ejaculate volume (ml), colour/density (score), sperm concentration (million/ml), mass activity (score), initial motility (%), live sperm (%), abnormal sperm (%), HOST reactive sperm (%) and acrosomal integrity (%), first AI conception rate observed during winter season were 5.19±0.18, 2.38±0.10, 1253.36±24.75, 3.73±0.05, 80.31±0.05, 86.20±0.64, 5.00±0.40, 85.75±0.43, 93.56±0.56, 45.75±0.21 respectively, whereas the respective values in summer were 5.07±0.13, 2.00±0.00, 1186.60±24.78, 3.72±0.11, 78.75±0.46, 82.31±0.74, 7.13±0.52, 84.93±0.45, 90.56±0.23, 43.50±0.11. The semen quality of fresh samples was observed better during winter compared to summer season for use in breeding program with satisfactory first AI conception rate.

Keywords: *Bubalus bubalis*, buffaloes, Jaffarabadi bulls, neat semen, HOS reactive sperm, acrosomal integrity, conception rate

INTRODUCTION

Jaffarabadi buffalo is a one of world famous riverine buffalo in terms of kilo fat and very efficient in the conversion of roughage into milk that originated in Gujarat, India. India has 108.7 million buffaloes, which is approximately 55.7% of the total world buffalo population. India ranks first in milk production and buffalo contributes about 56% of country's milk pool which is much higher than the contribution of cattle (Singh, 2009). Owing to the high yielding buffalo breeds, India had achieved an annual milk output of 165.5 million ton during 2016 to 2017 with a growth of 6.26% than the previous year (NDDB, 2018).

The environmental changes significantly affect production and reproductive performance of animals. Among all climatic elements, temperature and season are the most important parameters affecting animal fertility (Kunavongkrit *et al*., 2005). The high ambient temperature increases the scrotal temperature and consequently a decline

Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Junagadh Agricultural University, Junagadh, India, *E-mail: parmarkiran16@yahoo.co.in

in the semen quality (Taylor and Bogart, 1988). Production and reproduction are impaired as a result of drastic changes in biological functions caused by heat stress (Marai *et al*., 1995). The seminal and biochemical parameters are significantly influenced by changes in seasonal climatic elements. The semen quality parameters like ejaculate volume, sperm concentration, mass-motility, progressive motility, live-dead count, sperm defect are significantly influenced by season. In riverine buffaloes (river-type), the ejaculate volume has been reported more in summer than other seasons (Bhattacharya *et al*., 1978), while Kapoor (1973) reported the ejaculate volume was largest in the months of moderate temperature, intermediate in the months with cooler temperatures and lowest in the months representing extremes of temperature. However, sperm concentration (per ml) has been reported to be higher during the rainy season and lower during summer season in riverine buffaloes (Bhattacharya *et al*., 1978) and swamp buffaloes (Sukhato *et al*., 1988). High ambient temperature during summer seems to affect sperm motility in both riverine and swamp buffalo bulls (Bahga and Khokhar, 1991) leading to the assumption that the proportion of live spermatozoa is lowest during summer (Kapoor, 1973). Similarly, hot-dry, and hot-humid seasons have been reported to be unfavorable for reproduction (Bhakat *et al*., 2011). In view of the above facts the present study was carried out to know the effect of winter and summer seasons on neat seminal attributes and conception rate from semen of Jaffarabadi buffalo bulls.

MATERIALS AND METHODS

The present study was conducted on semen ejaculates (192) collected by artificial vagina (25 cm long and 6.5 cm in diameter) maintained at 42 to 45°C in incubator as per procedure described by Singh *et al*. (2000) in morning hours between 7.00 to 8.30 am (before feeding) from four Jaffarabadi breeding bulls and cryopreserved at Frozen Semen lab of Cattle Breeding Farm, JAU, Junagadh during winter (10 to 25°C temperature) of November to February (n=96 ejaculates) and summer (38 to 45°C) March to June (n=96 ejaculates). The Junagadh is located at on 21.29°N latitude and 70.27°E longitudes. It has an average elevation of 107 meters (351 ft.) from mean sea level. It has a tropical climate, with three distinct seasons observed, a mild winter from November to February, a hot summer from March to June and a monsoon from July to October. The animals were fed as per the Minimum Standard Protocol. The bulls were kept in sheltered paddocks with access to *ad libitum* water. The optimum health care was taken including clipping of preputial hair and vaccination against Haemorrhagic Septicaemia, Black Quarter and Foot and Mouth Disease. Besides this, screening for Tuberculosis, Johne's disease, Brucellosis, Campylobacteriosis and Trichomoniasis was also carried out. Immediately after collection, the ejaculates were assessed for Volume (ml), colour (1 = watery, 2 = milky, 3 = creamy), and sperm motility. A light microscope was used to determine mass activity (0 to 4 score) and the percentage of individual spermatozoa depicting a pattern of progressive, rectilinear movement. Sperm concentration as million per ml of semen was estimated using bovine photometer with diluter and printer (Accucel, IMV, France) against 530 nM wavelength. The morphological abnormalities of the spermatozoa were studied in the eosin-nigrosin stained slides. The acrosome integrity was evaluated by simplified nigrosine-eosin-Giemsa staining technique as described by Kutty

et al. (1996), whereas the hypo-osmotic swelling test (HOST) was done to evaluate the functional integrity of the sperm membrane, based on curled and swollen tails as described by Jayendran *et al.* (1984). For freezing of semen Andromed extender and French medium straws were used employing conventional wide mouth liquid nitrogen freezer. The trial on first AI conception rate was carried out on 800 Jafarabadi buffaloes inseminated with frozen-thawed semen of four bulls at Cattle Breeding Farm, JAU, Junagadh as well as in field during study period. The inseminated buffaloes were confirmed for pregnancy by per rectal palpation at 3 months post-insemination. The data was analyzed for calculating mean ± SE and significance by Statistical Packages for Social Sciences (IBM SPSS statistics, USA, version 20.0) software.

RESULTS AND DISCUSSIONS

Ejaculate volume (VOL)

The mean semen ejaculate volume (ml) was 5.19±0.18 and 5.07±0.13 during winter and summer seasons, respectively. The non-significant higher volume was recorded during winter than summer season (Table 1). The present findings of non-significant difference in ejaculate volume of semen during seasons corroborated with findings in Murrah bulls (Bhavsar *et al*., 1988; Ram *et al*., 2017), while De Castro *et al.* (2017) in Murrah bulls recorded higher semen volume in rainy compared to non-rainy season. Contrary to the present findings, larger ejaculate volume in summer than in the other seasons has been reported in Murrah bulls (Bhakat *et al*., 2015). The non-significantly higher variation of semen volume observed during winter compared to summer season might be due to the temperature during winter was favourable for production of semen in Jaffarabadi bulls. The production of semen as well as ejaculatory volume is influenced by many factors like breed-to-breed variation, body condition, scrotal size and weight, the reproductive health condition of bulls, the age of bulls, method and frequency of collection, pooled volume, nutrition, season, and management.

Semen colour (COL)

The mean values for colour score recorded during winter and summer were 2.38±0.10 and 2.00±0.00, respectively. The season showed significant effect on semen color of Jaffarabadi bulls, the score being higher during winter than in summer season, but there was no significant variation among bulls (Table 1). The effect of season on mean colour score was significantly ($P<0.05$) better during winter season as compared to that of the summer. Similarly, Javed *et al*. (2000) reported milky white to creamy colour in Nili-Ravi buffalo bulls during autumn compared to winter and summer, while non-significant seasonal variation was reported by Alavi and Babazadeh (2006) in Azarbaijani buffalo bull semen. Contrary to the present findings, Bhakat *et al.* (2014) reported that the semen colour was not affected by season. The variation in colour during different season might be due to variation in sperm concentration, hygienic condition of external genitalia of bull, environmental contamination of collection area of semen and status of genital tracts.

Sperm concentration (CON)

The average sperm concentrations were 1253.36±24.75 and 1186.60±24.78 million/ml during winter and summer, respectively. The concentration was significantly ($P<0.05$) higher during winter as compared to summer season (Table

1). Similar, significant seasonal variations in mean sperm concentration have also been reported in Murrah bulls (Bhakat *et al*., 2015; Ram *et al*., 2017) and Nili-Ravi buffalo bulls (Javed *et al*., 2000). Whereas significantly higher sperm concentration has been reported during summer (hot) than that of the winter (cold) season in Simmental and Nellore bulls (Chacur *et al*., 2013). Too high temperature and long summer adversely affect the vigour and testosterone production of bulls thereby suppress the testicular function and accessory sex gland secretion. The seasonal influence on the functional activity of accessory sex glands and testis may not be to the extent that can affect the sperm concentration in Jaffarabadi bulls as evidenced in the present study. Moreover, the Jaffarabadi breed has evolved as a sturdy animal over hundreds of years of adaptation to the hot and arid location of the area.

Mass activity (MA)

The mean values for mass activity score were 3.73±0.05 and 3.72±0.11 during winter and summer, respectively. The non-significantly higher mass activity of spermatozoa was observed in winter than summer (Table 1). The present study corroborated well with the reports in Murrah buffalo bulls (Bhakat *et al*., 2015; Ram *et al*., 2017) and swamp buffalo bulls (Das *et al*., 2017). Contrary to the present findings, Javed *et al*. (2000) reported significantly (P<0.05) higher mass activity during summer as compared to that of the winter in Nili-Ravi buffalo bulls, whereas De Castro *et al*. (2017) reported significant difference between the two periods (rainy season and non-rainy season). The mass motility is affected by many factors like season, age of the bull, the frequency of collection, the degree of stimulus provided and type of thrust (Tomar, and Gupta, 1984). The variation in result may also be attributed to difference in judgment of mass activity, total number of observations made and climatic condition.

Initial motility (IM)

The per cent initial motility during winter and summer were 80.31±0.50 and 78.75±0.46, respectively. There was a significant (P<0.05) seasonal effect on initial motility, being higher during winter season (Table 1). In the present study, significant higher initial motility percentage of sperm was observed during winter than summer. Similar observation of significant seasonal effects on initial motility in Murrah (Dhami *et al*., 1998; Tiwari *et al*., 2011) has been reported previously. However, non-significant seasonal difference Murrah bulls (Sahu and Pandit, 1997) and swamp buffalo bull (Koonjaenak *et al*., 2007) have also been reported. The initial sperm motility is an important attribute for acceptance or rejection of the ejaculate for further processing and use in AI. The variation observed in the initial sperm motility is attributed to degree of sexual excitement, method of semen collection, frequency of semen collection and such other factors.

Live sperm count (LND)

The average percent live spermatozoa during winter and summer were 86.20±0.64 and 82.31±0.74, respectively. It was significantly (P<0.05) higher during winter as compared to that of the summer season (Table 1). In the present study percent live sperm were found to be significantly (P<0.05) higher during the winter as compared to that of the summer season in the Jaffarabadi bull which is corroborated with the study of Ramadan *et al*. (2009) who reported lowest (P<0.05) live sperm percentages during the spring season, followed by winter, summer and autumn seasons.

While non-significant difference in Swamp (Das *et al*., 2017) and Murrah bulls (Ram *et al*., 2017) have been reported. Jaffarabadi bull had a very high percentage of live spermatozoa throughout the study. That might be due to use of bulls of known good fertility and maintenance of identical and optimal conditions of feeding and management throughout the study period.

Abnormal sperm count (ABN)

The percent abnormal sperm count during winter and summer was 5.00±0.40 and 7.13±0.52, respectively, with a significantly ($P<0.01$) higher abnormal sperm count during summer as compared to winter (Table 1). The significantly ($P<0.05$) higher abnormal sperm count observed during summer as compared to winter in the present study corroborated well with previous reports on Murrah bulls (Ram *et al*., 2017), while Alavi and Babazadeh (2006) reported significantly higher mean values of sperm abnormalities in winter than other seasons in Azarbaijani buffalo bull semen. However, non-significant difference in sperm abnormalities in buffalo bull (Koonjaenak *et al*., 2007) has been reported. The sperm abnormalities may either be hereditary or arise because of defects caused by infectious diseases or environmental factors. While significantly higher abnormalities in summer compared to winter might be due to the elevated environmental temperature during summer which impairs testicular functions and lead to increased sperm production with abnormal morphology (Rao and Rao, 1978).

HOS reactive spermatozoa (HOST)

The percent HOS reactive spermatozoa were 85.75±0.43 and 84.93±0.45% during winter and summer season, respectively. Statistical analysis revealed that the percent HOS reactive spermatozoa differed non-significantly between the winter and summer seasons (Table 1). The percent HOS reactive spermatozoa differed non-significantly between seasons with higher winter (cold) than summer (hot) season in the present study, which corroborated with the findings in Murrah (Bhakat *et al*., 2015) while significantly higher HOS reactive spermatozoa was reported in Murrah bulls by Ram *et al*. (2017). However, De Castro *et al.* (2017) reported higher plasma membrane integrity (HOST %) in rainy season compared to non-rainy season in Murrah bulls. This variation could be due to the difference in the freezing methods, extender, thawing rate, and method of measurement.

Acrosome integrity of the neat semen

The values of acrosome integrity were 93.56±56 and 90.56±0.23% during winter and summer season, respectively. Significant higher acrosome integrity was revealed in winter compared to summer season (Table 1). The present findings corroborated well with the findings of Bhakat *et al.* (2015); Soren *et al.* (2016); Ram *et al.* (2017) in different breeds of buffalo. The lowest percentage of sperm with intact acrosome or otherwise highest percentage of sperm with damaged acrosome recorded during summer season is conceivable, as the high temperature prevailed during this season is known to adversely affect the semen production, its quality as well as sperm motility and morphology in buffalo bulls.

First AI conception rate (FCR)

The mean first AI conception rate was observed to be 45.75±0.21 (183/400) and 43.50±0.11 (174/400) during winter and summer season, respectively (Table 1). The mean first AI conception rate of Jaffarabadi bull semen was

Table 1. Seasonal variation in neat seminal characteristic (Mean ± SE) of Jaffarabadi bulls during summer and winter seasons.

Characteristics	Winter (n=96)	Summer(n=96)	t-value	P-value
Volume (VOL) (ml)	5.19±0.18	5.07±0.13	0.57	0.57
Colour (COL) (1-4 scale)	2.38±0.10[a]	2.00±0.00[b]	3.57	0.00
Mass activity (MA) (0-5 scale)	3.73±0.05	3.72±0.11	0.161	0.87
Initial motility (IM) (%)	80.31±0.50[a]	78.75±0.46[b]	2.30	0.02
Concentration (CON) million/ml	1253.36±24.75[a]	1186.60±24.78[b]	1.91	0.05
Live sperm (LND) (%)	86.20±0.64[a]	82.31±0.74[b]	0.15	0.00
Abnormal sperm (ABN) (%)	5.00±0.40[a]	7.12±0.52[b]	0.17	0.00
Host reactive sperm (HOST) (%)	85.75±0.43	84.93±0.45	0.66	0.36
Acrosomal integrity (ACRM) (%)	93.56±0.56[a]	90.56±0.23[b]	0.15	0.00
First AI conception rate (FCR) (%)	45.75±0.21[a] (n=183/400)	43.50±0.11[b] (n=174/400)	7.41	0.000

Mean values having different superscripts within a row differ significantly from each other at P<0.01 and/or at P<0.05.

found to be 44.63±0.14% in the present study, which is comparable with findings in Murrah and local buffaloes (Guangsheng *et al*., 2013; Ghuman and Dhami, 2017; Abdullah *et al*., 2017), while lower conception rate in Surti buffaloes (Dhami and Kodagali, 1986), Mehasana buffaloes (Bhavsar *et al*., 1990), Murrah buffaloes (Gokhale and Bhagat, 2000) have been reported, whereas, higher conception rate in Nili-Ravi buffalo (Guangsheng *et al*., 2013) have been reported. The non-significantly higher conception rate obtained in winter compared with summer in the present study very well corroborated with different reports in buffaloes (Das *et al*., 2017; Abdullah *et al*., 2017). The conception rates in artificially inseminated bovines varied considerably across species, breeds, and geographical locations. These could have been due to multiple animals, environmental factors, expression and detection of heat symptoms, artificial insemination worker, semen handling from collection, processing to cryopreservation and thawing, individual dam effect and embryonic mortality.

The seasonal variations of conception rate might be due to differences in stress levels during different seasons as well as availably of grass. Heat stress directly affects fertility by reducing oocyte quality and indirectly by reducing feed intake thereby causing negative energy balance in animals and negatively affects reproductive performance by reducing quality of oocyte and embryo. Buffaloes have black body coat and less efficient evaporative cooling owing to poor sweating ability as they possess less number of sweat glands (Marai and Haeeb, 2010), which might contribute more heat load during summer season. Our findings are in line with Dash *et al*. (2015), who found depression in conception rate in Murrah buffaloes during the

period of heat stress (summer).

CONCLUSION

The semen quality of fresh samples was observed better during winter compared to summer season for use in breeding program with satisfactory first AI conception rate.

ACKNOWLEDGEMENT

The authors are thankful to the authorities of Junagadh Agricultural University, Junagadh for granting permission to perform the research and providing necessary facilities.

REFERENCES

Abdullah, M., T.K. Mohanty, T.K. Patbandha, T.K. Bhakat, M. Madkar, A. Kumaresan and A.K. Mohanty. 2017. Pregnancy diagnosis-positive rate and conception rate as indicator of farm reproductive performance. *Indian J. Anim. Res.*, **51**: 170-174. DOI: 10.18805/ijar.5708

Alavi, S.S. and H.B. Babazadeh. 2006. Seasonal variation in the characteristics of the Azarbaijani buffalo (*Bubalus bubalis*) semen. *Iran. J. Vet. Res.*, **7**(14): 49-54.

Bahga, C.S. and B.S. Khokar. 1991. Effect of different seasons on concentration of plasma luteinizing hormone and seminal quality *vis-a-vis* freezability of buffalo bulls (*Bubalus bubalis*). *International Journal of Biometeorolgy*, **35**: 222-224. DOI: 10.1007/BF01047289

Bhakat, M., T.K. Mohanty, A.K. Gupta and M. Abdullah. 2014. Effect of season on semen quality of crossbred (Karan Fries) bulls. *Advances in Animal and Veterinary Sciences*, **2**(11): 632-637. DOI: 10.14737/journal.aavs/2014/2.11.632.637

Bhakat, M., T.K. Mohanty, A.K. Gupta, S. Prasad, A.K. Chakravarty and H.M. Khan. 2015. Effect of season on semen quality parameters in Murrah buffalo bulls. *Buffalo Bull.*, **34**(1): 100-112. Available on: https://kukrdb.lib.ku.ac.th/journal/BuffaloBulletin/search_detail/result/288696

Bhakat, M., T.K. Mohanty, A.K. Gupta, V.S. Raina, H.M. Khan, R.K. Mahapatra and M. Sarkar. 2011. Effect of age and season on semen quality parameters in Sahiwal bulls. *Trop. Anim. Health Pro.*, **43**(6): 1161-1168. DOI: 10.1007/s11250-011-9817-1

Bhattacharya, M.K., G.J. King and J.R. Behra. 1978. Buffalo semen quality in various seasons. *Indian Vet. J.*, **55**(8): 591-594.

Bhavsar, B.K., A.J. Dhami and S.B. Kodagali. 1990. Abnormal sperm content in Mehsana buffalo semen with regard to freezabiity, seasonality and fertility. *Indian Vet. J.*, **67**: 233-237.

Bhavsar, B.K., A.J. Dhami and S.B. Kodagali. 1988. Monthly variations in seminal characteristics of Mehsana buffalo bulls and their relationships with fertility. *Artificial Insemination News*, **3**: 93-94.

Chacur, M.G.M., K.T. Mizusaki, L.R.A.G. Filho, E. Oba and A.A. Ramos. 2013. Seasonal effects on semen and testosterone in Zebu and Taurine bulls. *Acta Sci. Vet.*, **41**(1): 111-120.

Das, G.C., P.K. Das, S. Deori, H. Mazumdar, B.N. Bhattacharyya and P. Arundhati. 2017.

Seasonal variation in the characteristics of the swamp buffalo semen of northeast India. *Buffalo Bull.*, **36**(1): 215-219. Available on: https://kukrdb.lib.ku.ac.th/journal/BuffaloBulletin/search_detail/result/366256

Dash., A.K. Chakravarty, V. Sah, V. Jamuna, A. Behera, N. Kashyap and B. Deshmukh. 2015. Influence of temperature and humidity on pregnancy rate of Murrah buffaloes under subtropical climate. *J. Anim. Sci.*, **28**(7): 943-950. DOI: 10.5713/ajas.14.0825

De Castro, S.R.S., A.C. Morini, A.D.S. Da silva, K.A.L. Neves, A.H.H. Minervino, A.A. De mourameneses and W.G. Vale. 2017. Influence of seasonality on buffalo semen. *Animal and Veterinary Sciences*, **5**(3): 45-51. DOI: 10.11648/j.avs.20170503.12

Dhami, A.J. and S.B. Kodagali. 1986. Frozen semen and fertility in Surti buffaloes. *Indian J. Anim. Reprod.*, **7**: 14-21.

Dhami, A.J. and S.B. Kodagali. 1988. Seminal characteristics and their interrelationships in Surti buffalo. *Indian Vet. J.*, **65**: 61-64.

Ghuman, S.P.S. and D.S. Dhami. 2017. Seasonal variation in AI and pregnancy rate in buffalo and improving their fertility status following application of FTAI during non-breeding season. *Indian J. Anim. Reprod.*, **38**(1): 4-8.

Gokhale, S. and R.L. Bhagat. 2000. Status of reproductive performance in rural buffaloes artificially inseminated using deep frozen semen. *Indian J. Anim. Sci.*, **70**(4): 366-368.

Guangsheng, Q., Y. Bingzhuang, T. Zhengzhun, L.I. Hui, H. Jian and L. Xianwei. 2013. Effect of frozen semen from Italian Mediterranean buffalo on some reproductive parameters and conception rate performed to different species water buffalo in southern China Guangsheng. *Buffalo Bull.*, **32**(Sppl.2): 577-581. Available on: https://kukrdb.lib.ku.ac.th/journal/BuffaloBulletin/search_detail/result/286594

Javed, M.T., A. Khan and R. Kausar. 2000. Effect of age and season on some semen parameters of Nili-Ravi buffalo (*Bubalus bubalis*) bulls. *Vet. Arhiv*, **70**(2): 83-94. Available on: https://hrcak.srce.hr/file/141654

Jeyendran, R.S., H.H. Vandervent, Z.M. Perz-Pelaez, B.G. Crabo and L.J.D. Zanevald. 1984. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to their semen characteristics. *J. Reprod. Fertil.*, **70**(1): 219-228. DOI: 10.1530/jrf.0.0700219

Kapoor, P.D. 1973. Monthly variations in the semen quality of buffalo bulls. *Indian J. Anim. Sci.*, **43**(7): 573-578.

Koonjaenak, S., P. Pongpeng and S. Wirojwuthikul. 2007. Seasonality affects post-thaw plasma membrane intactness and sperm velocities in spermatozoa from Thai AI swamp buffaloes (*Bubalus bubalis*). *Theriogenology*, **67**(9): 1424-1435. DOI: 10.1016/j.theriogenology.2007.02.010

Kunavongkrit, A., A. Suriyasomboon, N. Lundeheim, T.W. Heard and S. Einarsson. 2005. Management and sperm production of boars under differing environmental conditions. *Theriogenology*, **63**(2): 657-667. DOI: 10.1016/j.theriogenology.2004.09.039

Kutty, I.C., M. Joseph and C.P.N. Iyer. 1996. A simplified nigrosine-eosin-geimsa staining technique to distinguish acrosome damaged live and dead spermatozoa. *Indian J. Anim. Res.*, **17**(2): 122-123.

Marai, I.F.M., A.A.M. Habeeb, A.H. Daader and

H.M. Yousef. 1995. Effects of Egyptian sub-tropical conditions and the heat stress alleviation techniques of water spray and diaphoretics on the growth and physiological functions of Friesian calves. *J. Arid Envir.*, **30**(2): 219-225.

Marai, I.F. and A.A.M. Habeeb. 2010. Buffaloes reproductive and productive traits as affected by heat stress. *Tropical and Subtropical Agroecosystems*, **12**(2): 193-217.

NDDB. 2018. *NDDB Annual report 2018-19.* National Dairy Development Board, Gujarat, India.

Ram, K.L., R.P. Tiwari, R.P. Mishra, S.A. Sahasrabudhe and A.K. Nair. 2017. Effect of heat stress on seminal characteristics of Murrah buffalo bull semen. *Buffalo Bull.*, **36**(2): 369-378. Available on: https://kukrdb.lib.ku.ac.th/journal/BuffaloBulletin/search_detail/result/368842

Ramadan, T.A., T.A. Taha, M.A. Samak and A. Hassan. 2009. Seasonal and monthly variations in semen characteristics of egyptian buffalo bulls. *Alexandria Journal of Agricultural Research*, **54**(2): 13-23.

Rao, T.L.N. and A.R. Rao. 1978. Semen characteristics of crossbred bulls. *Indian Vet. J.*, **55**: 692-700.

Sahu, S.B. and R.K. Pandit. 1997. Grading of breeding Murrah bulls on sexual behavior and seminal characteristics including cryopreservation. *Indian Vet. J.*, **74**(7): 557-569.

Singh, I. 2009. Buffalo reproduction: An Indian perspective. *Revista Brasileira de Reprodução Animal*, **2009**(6): 91-97.

Singh, S.P., R.K. Pandit and H.B.S. Bhadoria. 2000. Sexual behavior and seminal characteristics in Jersey, Sahiwal and half-bred bulls. *Indian J. Anim. Sci.*, **70**(3): 279-280.

Soren, S., V.S. Sohan and A. Kumar. 2016. Influence of season on semen quality in Karan fries (Tharparkar X Holstein Friesian) bulls. *J. Anim. Res.*, **6**(2): 121-125. DOI: 10.5958/2277-940X.2015.00179.5

Sukhato, P., M. Amornruji, V. Chanatinart, B. Sanithvong and R. Uangvachanitgul. 1988. Swamp buffaloes semen characteristics during different seasons of the years: A sperm morphology study. Department of Livestock Development (DLD), Ministry of Agriculture and Cooperatives, Bangkok, Thailand. p. 194-203.

Taylor, R.E. and R. Bogart. 1988. *Scientific Farm Animal Production: An Introduction to Animal Science*, 3rd ed. Macmillan, New York, USA.

Tomar, S.S. and H.C.L. Gupta. 1984. Effect of season on sex desire and semen quality of Hariana bulls. *Indian J. Anim. Health*, **19**: 37-40.

Tiwari, R., G.K. Mishra, M.K. Shukla, R.B. Singh, S.K. Saxena and M.U. Siddiqui. 2011. Seasonal variations in semen production of Murrah buffalo bulls. *Indian J. Anim. Reprod.*, **32**(2): 5-7.