SEASONAL VARIATION IN PHYSICO-MORPHOLOGICAL AND FUNCTIONAL CHARACTERISTICS OF SEMEN OF SURTI BUFFALO BULLS AND THEIR INTERRELATIONSHIPS

Megha V. Khawaskar, M.T. Panchal, A.J. Dhami and J.A. Patel

Department of Animal Reproduction, Gynaecology & Obstetrics

College of Veterinary Science and Animal Husbandry

Anand Agricultural University, Anand - 388 110

Received 3-9-2012 Accepted 29-9-2012

ABSTRACT

This study was carried out using 60 semen ejaculates (10 ejaculates/bull/season) obtained from 2 sexually mature healthy Surti buffalo bulls over one year p eriod. The year was divided in to three seasons (monsoon, winter and summer). The bulls were managed identically and were under weekly twice semen collection schedule in AV. The overall mean ejaculate volume, consistency/ density score (0-4), initial pH, mass activity (score 0-5), individual sperm motility, sperm concentration, live sperm, total abnormal sperm and sperm with intact acrosome recorded were 3.16±0.76 ml, 3.05±0.92, 6.89±0.16, 2.96±0.18, 71.66±12.37 %, 885.42±242.90 millions/ml, 85.75± 4.65 %, 6.53±1.28 % and 90.73±3.16 %, respectively. The average values for cold shock resistant sperm, hypo-osmotic swelling reactive sperm, and methylene blue reduction time were 53.45±8.30 %, 59.28±6.10 % and 7.11±1.28 minutes, respectively. The bulls varied significantly only in their ejaculate volume (P=0.008), sperm concentration (P=0.002) and live sperm (P=0.014). Seasonal influence was significant for ejaculate volume (P=0.002), density score (P=0.05), mass activity (P=0.002), sperm concentration (P=0.047), live sperm (P=0.001), abnormal sperm (P=0.003) and intact acrosome (P=0.022), cold shock resistant (P=0.000) and hypo-osmotic swelling reactive sperm (P=0.000). The lowest values were observed during winter for ejaculate volume, sperm concentration and live sperm, whereas density, mass activity and individual motility was the lowest during monsoon, and abnormal sperm, cold shock resistant sperm, HOS reactive sperm and MBRT during summer. The bull x season interaction was significant only for cold shock resistant sperm. Significant correlations were observed for ejaculate volume with cold shock resistant sperm (0.35); sperm concentration with live sperm (0.30) and MBRT (-0.31); mass activity with abnormal sperm (-0.26) and cold shock resistant sperm (-0.39); initial motility with live sperm (-0.27) and HOS reactive sperm (0.25); live sperm with HOS reactive sperm (-0.49) and cold shock resistant sperm (-0.56); abnormal sperm with intact acrosome (0.25) and cold shock resistant sperm (0.32); intact acrosome with HOS reactive sperm (0.37); and HOS reactive sperm with live sperm (-0.49) and cold shock resistant sperm (0.29).

KEY WORDS: Buffalo semen, Seasonal Variation, Physical characteristics, Functional attributes.

INTRODUCTION

Artificial insemination is now a days a widely used technique for the enhancement of genetic potential of livestock species. But still the maximum benefit of this tool cannot be extracted with crossbred and buffalo bulls for certain inherent problems like poor semen quality and poor freezability. Correspondingly, there is a genetic and economic loss owing to disposal of these high genetic merit bulls. Apart from the inherent traits, season is one major factor which influences the reproductive performance of these animals and it exerts its effect through macro- and micro-climatic factors. Buffalo being seasonal breeder, there is vast difference in the quality of semen produced by the same bull during different seasons. Moreover, conventional semen analysis techniques are known to show limitations because they are unable to detect some functional impairment of sperm, which

are responsible for a decreased fertility (Aitken, 2006). Semen evaluation including sperm acrosomal integrity, HOS test and MBRT can be extremely helpful in predicting the quality and fertility of a given semen sample. Hence, this study was planned to know the seasonal variations in the physico-morphological and functional attributes and their interrelationships in Surti buffalo semen.

MATERIALS AND METHODS

The present study was carried out at the Semen Station, Veterinary College, AAU, Anand, Gujarat during the year 2011-12 over one year period. The year was divided into three seasons, viz., monsoon (July-Oct), winter (Nov-Feb) and summer (Mar-Jun) according to the prevailing agro-climatic conditions of the region. Two sexually mature buffalo bulls of Surti breed were included in the study. The bulls were maintained in nearly identical nutritional and managerial condition throughout the period of study. They were vaccinated regularly against foot and mouth disease and haemorrhagic septicaemia. The bulls were in regular twice a week semen collection schedule in the morning hours using artificial vagina. A total of 60 ejaculates (10 per bull/season) were utilized for this study.

Each ejaculate soon after collection was evaluated for various macro- and micro-scopic tests. Sperm motility was assessed using phase contrast microscope fitted with a biotherm. Initial pH was estimated by Welltronix Digital pH meter using single glass rod combined electrodes. The concentration of spermatozoa (million/ml) was determined by the Digital-Photometer (Accucell Photometer, IMV Technology, France) at 530 nm wavelength. The percentages of live and morphologically abnormal spermatozoa were estimated by differential staining technique using eosin-nigrosin stain. The percentage of intact acrosomes was determined by using Giemsa stain. The hypo-osmotic swelling test was performed using 150 mosm/l solution of sodium citrate-fructose (Jayendran et al., 1984). The cold shock resistance and methylne blue reduction tests were performed as per Tomar (1970). The data generated were analyzed statistically using 2 factors factorial CRD on SAS system to know the influence of bull, season and bull x season interaction, and the interrelationships between various parameters studied were worked out statistically.

RESULTS AND DISCUSSION

Season-wise and overall mean (±SE) values of various physico-morphological and functional attributes observed in Surti buffalo semen and their interrelationships are presented in Table 1 and 2, respectively.

The overall mean ejaculate volume, consistency/density score (0-4), initial pH, mass activity (score 0-5), individual sperm motility, sperm concentration, live sperm and total abnormal sperm recorded were 3.16±0.76 ml, 3.05±0.92, 6.89±0.16, 2.96±0.18, 71.66±12.37 %, 885.42±242.90 millions/ml, 85.75±4.65 %,and 6.53±1.28 % respectively (Table 1). The bulls varied significantly only in their ejaculate volume (P=0.008), sperm concentration (P=0.002) and live sperm (P=0.014). Seasonal influence was significant for ejaculate volume (P=0.002), density score (P=0.05), mass activity (P=0.002), sperm concentration (P=0.047), live sperm (P=0.001) and abnormal sperm (P=0.003). The lowest values were observed during winter for ejaculate volume, sperm concentration and live sperm, whereas density, mass activity and individual motility was the lowest during monsoon, and abnormal sperm during summer. The bull x season interaction was non-significant for all these attributes.

The average values for intact acrosome, cold shock resistant sperm, hypo-osmotic swelling reactive sperm and methylene blue reduction time were 90.73±3.16 %, 53.45±8.30 %, 59.28±6.10 % and 7.11±1.28 minutes, respectively. The variation between bulls was non-significant for all these tests. However, seasonal influence was significant for intact acrosome (P=0.022), cold shock resistant sperm (P=0.000) and hypo-osmotic swelling reactive sperm (P=0.000). The lowest values were observed during summer for cold shock resistant sperm, HOS reactive sperm and MBRT.

Table 1: Seasonal influence on physico-morphological and functional attributes of Surti buffalo semen (Mean ± SE)

Seminal attribute	Seasons			Overall Average
	Monsoon	Winter	Summer	
Ejaculate volume (ml)	3.50±0.51^{Ba}	2.75±0.71^{a}	3.25±0.85^{b}	3.16±0.76
Consistency (Score 0-4)	2.75±0.85^{a}	2.95±0.88^{a}	3.45±0.94^{b}	3.05±0.92
Seminal pH	6.91±0.17	6.85±0.16	6.92±0.14	6.89±0.16
Mass activity (score 0-4)	2.65±0.74^{a}	3.05±0.68^{ab}	3.45±0.60^{b}	3.05±0.74
Individual motility (%)	71.00±12.09	75.00±8.88	69.00±15.18	71.66±12.37
Sperm concentration (million/ml)	860.75 ±130.77^{ab}	772.78 ±227.16^{a}	1022.73 ±284.03^{b}	885.42 ±242.90
Live sperm (%)	86.00±3.38^{ab}	81.10±2.84^{a}	90.15±2.10^{b}	85.75±4.65
Abnormal sperm (%)	7.15±1.38^{b}	6.65±1.03^{ab}	5.80±1.05^{a}	6.53±1.28
Intact acrosome (%)	89.70±3.38^{a}	92.30±3.51^{b}	92.20±1.82^{b}	90.73±3.16
Cold shock resistant sperm (%)	59.10±4.75^{b}	57.50±4.69^{b}	43.75±4.43^{a}	53.45±8.30
HOS resistant sperm (%)	56.35±3.62^{a}	65.62±4.27^{b}	55.87±4.90^{a}	59.28±6.10
Methylene blue reduction time (min)	8.20±1.38	6.75±1.03	6.40±1.05	7.11±1.28

Means bearing different superscripts in differ significantly between seasons ($P<0.05$).

The bull x season interaction was significant only for cold shock resistant sperm.

The values of ejaculate volume, density and pH obtained in the present study were in accordance with those reported by Rehman et al. (2012), Tiwari et al. (2009) and Dhami and Kodagali (1987). However, Sajjad et al. (2007), Dhami et al. (2001) and Sagdev et al. (1991) reported comparatively lower values than the present ones. Further, the seasonal variation in ejaculate volume and density has also been documented by previous workers (Dhami et al., 1998; Sagdev et al., 1991; Pangawkar et al., 1978), though such variation was insignificant in other studies (Tiwari et al., 2010; Koonjaenak et al., 2007). The ejaculate volume, sperm concentration/density and semen pH have direct relevance with the testicular and accessory sex glands functions.

The results of mass activity/initial sperm motility obtained compared well with those of Shelke and Dhami et al. (2001) and Saxena and Tripathi (1983). However, the present mass activity score was higher than that reported by Sajjad et al. (2007) and Dhami et al. (2001), and the mean individual sperm motility was lower than that reported by Tiwari et al. (2009). The present insignificant seasonal variation found in the initial sperm motility is in accordance with the report of Koonjaenak et al. (2007) in Swamp buffalo, but Tiwari et al. (2010), Dhami et al. (1998) and Dhami and Kodagali (1987) recorded significant seasonal variation in sperm motility of Surti and Murrah bulls. Initial sperm motility is an important attribute for acceptance or rejection of the ejaculate for further processing and use in AI, and it is positively correlated with keeping quality, freezability and fertility of that sample (Shelke and Dhami, 2001; Tiwari et al., 2010). Relatively lower mass activity score observed in the ejaculates during monsoon could be attributed to very high temperature-humidity index that prevailed during this season, which is known to adversely affect the semen. The variation

observed in the sperm motility is attributed to age and breed of bull, season/climate, degree of sexual excitement, method and frequency of semen collection, and such other factors.

The values of sperm concentration obtained in this study corroborated with those reported by Tiwari et al. (2009) and Kumar et al. (1993). However, Tiwari et al. (2010) and Rana et al. (2001) reported comparatively higher sperm concentration/ml, while Saxena and Tripathi (1983) reported lower value. Significant seasonal and bull variations noted in the sperm concentration corroborated with the reports of Tiwari et al. (2010), Dhami et al. (1998) and Dhami and Kodagali (1987) in Surti and Murrah bulls. However, Koonjaenak et al. (2007) did not find significant seasonal variation in sperm concentration of Swamp buffalo bulls. Buffalo being seasonal breeder, such variation in semen quality is conceivable. The wide variation in the sperm concentration has been attributed to factors like season, individuality, age and breed of bull, sexual excitement, frequency of collection etc.

Sperm viability and abnormalities also reflect the functional and health status of the gonads and epididymes as well as handling of the ejaculate. The values of live and abnormal sperm percentages obtained in the study were agreeable with those reported by Tiwari et al. (2009) and Dhami and Sahni (1994). However, Dhami et al. (2001) and Pangawkar et al. (1978) reported comparatively higher live and abnormal sperm percentages. Koonjaenak et al. (2007) reported highest morphologically normal sperm during summer, which is comparable with present lowest abnormal sperm recorded in that season. Tiwari et al. (2009), Dhami et al. (1998), Dhami and Kodagali (1987) and Pangawkar et al. (1978) also reported highly significant seasonal variation in live and/or abnormal sperm per cent in buffalo semen.

The intact acrosome obtained in the study compared with Rana and Dhami (2002) and Prasad et al. (1999). However, Sharma et al. (1992) reported comparatively lower percentage of intact acrosome. The lowest percentage of intact acrosome recorded during monsoon season is conceivable, as the high temperature-humidity prevailed during this season is known to adversely affect the semen production, its quality as well as sperm motility and morphology in buffalo bulls. Spermatozoal motility is the most frequently examined characteristic, however, spermatozoa could be highly motile but not fertile, owing to the acrosomal damage. Therefore, the acrosomal integrity is a major part of assessment of sperm quality as the acrosomal enzymes play a key role during sperm penetration in zona pellucida.

The cold shock resistant sperm percentages obtained in the study were in accordance with those reported by Fayez et al. (1987). However, Patel et al. (1988) and Pangawkar (1982) reported comparatively higher cold shock resistant sperm percentages. Significantly poor cold shock resistant sperm found during summer corroborated with the report of Pangawkar (1982) and Mohanty et al. (1985). The cold shock induces irreversible damage to the spermatozoa, hence this test is used to predict cryopreservaility of the ejaculate. Greater the cold shock resistance index better is the keeping quality and freezability of that ejaculate. The ejaculates obtained during summer appeared to be more fragile and showed poor cold shock resistance compared to other seasons.

The mean values of HOS reactive sperm obtained corroborated with those reported by Rana and Dhami (2002). However, Lodhi et al. (2008) and Patel et al. (2012) reported comparatively higher HOS positive sperms. Like our findings, Rajoria et al. (2011) also reported significantly higher HOS reactive sperm during winter than summer. Koonjaenak et al. (2007), however, reported highest plasma membrane integrity during summer in Swamp buffalo spermatozoa. The findings proved that winter is the ideal season for Surti buffalo bulls to produce semen with higher proportion of sperm with intact plasma membrane. Assessment of sperm membrane function appears to be a significant marker for the fertilizing capacity of spermatozoa, since it is involved in metabolic changes with the surrounding medium and in the process of capacitation, acrosome reaction and fusion with the oocyte membrane. The HOS test is more conclusive in evaluating biochemically active sperm

membrane and thereby fertilizing ability of spermatozoa.

The values of average methylene blue reduction time recorded compared with those reported by Foote (1999). Omar and Younes (1995) reported that reduction of methylene blue in Egyptian buffalo semen had their best value in summer season as compared to other seasons, which to some extent corroborates with the present findings. Reduction of methylene blue depends upon the dehydrogenase activity of spermatozoa. The semen sample which has higher concentration of active spermatozoa will reduce methylene blue in less time.

The ejaculate volume was significantly (P<0.05) and positively correlated with cold shock resistant sperm (0.35) and MBRT (0.26). Its' correlations with all other attributes were negligible. Sperm concentration per ml was significantly and positively correlated with live sperm (0.30), and negatively with MBRT (-0.31). Its' correlations with intact acrosome, HOS reactive sperm and cold shock resistant sperm were high and negative. Mass activity was significantly and negatively correlated with abnormal sperm (0.26) and cold shock resistant sperm (-0.39). It had high positive correlation with live sperm. Initial motility had significant positive correlation with HOS reactive sperm (0.25), and negative correlation with live sperm (-0.27), while pH had high negative correlation with intact acrosome. Live sperm per cent was highly significantly (P=0.01) and negatively correlated with HOS reactive sperm (-0.49) and cold shock resistant sperm (-0.56). The abnormal sperm was significantly and positively correlated with intact acrosome (0.25) and cold shock test (0.32). The intact acrosome showed significant positive correlation with HOS reactive sperm (0.37), whereas HOS reactive sperm was significantly and positively correlated with cold shock resistant sperm (0.29), and negatively with live sperm (-0.49). The cold shock resistant sperm had significant correlations with ejaculate volume, mass activity, live sperm, abnormal sperm and HOS reactive sperm. The correlations of MBRT with all attributes were negligible (Table 2).

The present correlation findings among the physico-morphological attributes are in accordance with the previous reports of Dhami and Sahni (1994) and Dhami and Kodagali (1987) in Surti and Murrah bulls, and of Kaker and Arora (1976) in crossbred bulls. Fayez et al. (1987) found significant

Table 2. Correlation coefficients (r) among physico-morphological and functional attributes of semen in Surti buffalo bulls

Character	Ejacul Volume	Conc/ ml	MA	IM	pH	Live sperm	Ab Sperm	Intact Acros	HOS test	Cold shock
Sperm Conc/ml	0.06	--								
Mass activity	0.02	0.08	--							
Initial motility	0.09	0.10	-0.17	--						
Seminal pH	-0.14	0.10	-0.03	-0.19	--					
Live sperm	-0.16	0.30*	0.24	-0.27*	0.04	--				
Abnormal sperm	0.06	-0.09	-0.26*	-0.14	0.04	-0.14	--			
Intact acrosome	-0.01	-0.17	-0.04	-0.01	-0.22	-0.15	0.25	--		
HOS +Ve sperm	0.07	-0.21	-0.05	0.25*	-0.01	-0.49**	0.01	0.37**	--	
Cold Shock test	0.35**	-0.22	-0.39**	0.09	-0.05	-0.56**	0.32**	0.07	0.29*	--
MBRT	0.26*	-0.31*	-0.19	-0.06	0.02	-0.07	0.05	-0.13	-0.04	0.12

* P=0.05, ** P=0.01.

relationships for cold shock resistant sperm with the percentages of live/abnormal sperm and progressively motile spermatozoa, while Prasad et al. (1999) found significant positive correlations of HOS positive sperm with mass activity, initial motility, live sperm count, intact acrosome and sperm

concentration in crossbred bulls. Sajjad et al. (2007) did not find significant correlation of ejaculate volume with any other traits, but the sperm motility had positive correlation with sperm concentration and negative correlation with semen pH, which in turn was negatively correlated with sperm concentration. Lodhi et al. (2008) recorded significant (P=0.05) positive correlation for HOS test with progressive motility, sperm viability and morphologically normal spermatozoa in both Nili-Ravi and Sahiwal bulls. Thus, the present findings are one or the other way corroborate with most of these reports, suggesting that the sperm motility, morphology and viability assessment is sufficient to grade semen under routine laboratory procedures.

REFERENCES

Aitken, R.J. (2006). Sperm function tests and fertility. Int. J. Androl., **29**: 69-75.

Dhami, A.J. and Kodagali, S.B. (1987). Correlation between biochemical and enzymatic constituents of semen of Surti buffalo bulls. Indian J. Anim. Sci., **57**(12): 1283-1286.

Dhami, A.J. and Sahni, K.L. (1994). Comparative appraisal of physico-morphological and enzymatic attributes of semen and their interrelationships in ox and buffalo. J. Applied Anim. Res., **5**(1): 13-20.

Dhami, A.J., Mohan, G. and Sahni, K.L. (1998). Seasonal influence on the quality and freezability of semen of Friesian and Murrah buffalo bulls. Indian J. Anim. Reprod., **19**(1) : 55-58.

Dhami, A.J., Shelke, V.B., Dave, R.R. and Kavani, F.S. (2001). Scrotal biometry, sexual behaviour and semen production in breeding bulls of different breeds - A comparative study. Indian J. Dairy Sci., **54**: 255-260.

Dhami, A.J., Shelke, V.B., Virani, A.C., Kavani, F.S. and Dave, R.R. (2002). Transaminases and phosphatase activity of blood and seminal plasma in breeding bulls of different breeds. Indian J. Ainm. Reprod., **23**(1): 35-37.

Fayez, I., Marai, M., Daader, A.H. and Nasir, E. (1987). Some physical and biochemical attributes of buffalo semen. Egyptian J. Anim. Prod., **25**(1): 99-104 (ABA 56: 2659).

Foote, R.H. (1999). Resazurin reduction and other tests of semen quality and fertility of bulls. Asian J. Androl., **1**(3): 109-114.

Jayendran, R.S., Van der Ven, H.H., Perez-Pelaez, M., Crabo, B.G. and Zaneveld, L.J.D. (1984). Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J. Reprod. Fertil., **70**: 219-228.

Kaker, M.L. and Arora, K.L. (1976). Relationships among various physical and biochemical characteristics of semen from young crossbred bulls. HAU Res. J., **6**(3-4): 251-253.

Koonjaenak, S., Chanatinart, V., Aiumlamai, S., Pinyopumimintr, T. and Rodriguez-Martinez, H (2007). Seasonal variation in semen quality of swamp buffalo bulls (Bubalus bubalis) in Thailand. Asian J. Androl., **9**(1): 92-101.

Kumar, S., Sahni, K.L. and Bishta, G.S. (1993). Cytomorphological characteristics of motile and static semen of buffalo bulls. Buffalo J., **9**(2): 117-127.

Lodhi, L.A., Zubair, M., Qureshi, Z. I., Ahmad, I. and Jamil, H. (2008). Correlation between hypo-osmotic swelling test and various conventional semen evaluation parameters in fresh Nili-Ravi buffalo and Sahiwal cow bull semen. Pakistan Vet. J., **28**(4): 186-188.

Mohanty, D.N., Biswas, R.K. and Dugwekar, Y.G. (1985). Cold shock resistance test as an indicator of spermatozoan freezability in HF bulls. Indian J. Anim. Sci., **55**(2): 107-109.

Omar, M.A. and Younes, A.A. (1995). Seasonal variation in semen quality and some blood constituents of Egyptian buffaloes. Acta Medica Veterinaria, **41**(3): 207-213.

Pangawkar, G.R., Dugwekar, Y.G., Khan, A.G. and Mittal, V.P. (1978). Seasonal variations in semen quality of buffalo bulls. J. Remount Vet. Corps, **17**: 201-204.

Pangawkar, G.R. (1982). The cold shock resistance of buffalo bull spermatozoa during breeding and non-breeding season. Anim. Breed. Abstr., **50**(12): 7142 (abstr).

Patel, J.B., Dhami, A.J. and Patel, P.A. (2012). Comparative evaluation of physico-morphological attributes of semen of Jafarabadi, Mehsana (Bubalus bubalis) and crossbred (HF x Kankrej, Bos indicus) bulls. Indian J. Field Vets., **7**(3): 1-7.

Patel, K.V., Dhami, A.J. and Kodagali, S.B. (1988). Comparison of physico-biochemical semen characteristics in normal and problem bulls. Indian J. Anim. Sci., **58**(10): 1169-1172.

Prasad, J.K., Satish Kumar, Greesh Mohan, Agarwal, S.K. and Uma Shanker (1999). Simple modified method for cervical mucus penetration test for quality assessment of bull semen. Indian J. Anim. Sci., **69**(2): 103-105.

Rajoria, J.S., Prasad, J.K., Ghosh, S.K., Kumar, S., Singh, M., Ramteke, S. Kumar, A. and Kumar A. (2011). Effect of season on physico-morphological and enzymatic profiles of Tharparkar bull semen. XVII annual convention of ISSAR & national symposium, Mizoram, 27-29 Sept., p 84.

Rana, C.M. and Dhami, A.J. (2002). Physical attributes, intact acrosome, HOS test and freezability of semen of Gir and Jafarabadi bulls. XVIII annual convention of ISSAR and national symposium, Nov. 14-16., IVRI, Izatnagar, India.

Rehman, Z.U., Samo, M.U., Qureshi, T.A., Khan, S., Qureshi, M.S. Khan, F.A. and Bahadadr, S. (2012). Studies on the freezability of Kundhi buffalo bull semen. J. Anim. and Plant Sci., **22**(2): 18-23.

Sagdev, L.R., Chitnis, A.B. and Kaikimi, A.S. (1991). Effect of seasonal variations on freezability of Surti buffalo bull semen. Indian J. Anim. Reprod., **12**(1): 1-3.

Sajjad, M., Ali, S., Ullah, N., Anwar, M., Akhter, S. and Andrabi, S.M.H. (2007). Blood serum testosterone level and its relationship with scrotal circumference and semen characteristics in Nili-Ravi buffalo bulls. Pakistan Vet. J., **27**(2): 63-66.

Saxena, V.B. and Tripathi, S.S. (1983). Seasonal variation in quality and preservability of semen of Murrah bulls. Cheiron, **12**: 193-199.

Sharma, M.L., Mohan, G. and Sahni, K.L. (1992). Study on acrosomal damage on cryopreservation of crossbred bull semen. Indian Vet. J., **69**: 962-964.

Shelke, V.B. and Dhami, A.J. (2001). Comparative evaluation of physico-morphological attributes and freezability of semen of Gir cattle (Bos indicus) and Jafarabadi buffalo (Bubalus bubalis) bulls. Indian J. Anim. Sci., **71**: 319-324.

Tiwari, M., Prasad, R.B. and Gupta, H.P. (2009). Physio-morphology and in-vitro fertility of semen/ spermatozoa of Tarai buffalo semen. Indian J. Anim. Physiol., **1**: 11-14.

Tiwari, R., Mishra, G.K., Shukla, M.K., Singh, R.B., Saxena, S.K. and Siddiqui, M.U. (2010). Seasonal variations in seminal attributes and reproductive losses of Murrah buffalo bulls. Proc. XXVI Annual Convention of ISSAR and International Symposium, GBPUA & T, Pantnagar (UP), India, 10-12 Nov., p.1.

Tomar, N.S. (1970). Semen evaluation. In: Artificial Insemination and Reproduction of Cattle and buffalo. 3rd edn, Saroj Prakashan, Allahabad, India, p. 64-65.

❑