PHYSICO-MORPHOLOGICAL AND FUNCTIONAL ATTRIBUTES OF SURTI BUFFALO SEMEN AND THEIR INTERRELATIONSHIPS

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Received 12-5-2014 Accepted 23-6-2014

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ABSTRACT

Forty eight semen ejaculates (8/bull) obtained at weekly interval during the favourable breeding season (November-February) from six mature Surti buffalo bulls of Central Sperm Station of the College under twice a week collection schedule in AV were evaluated for routine physico-morphological attributes including motility, viability, morphology (eosin-nigrosin), acrosomal integrity (Giemsa stain) and plasma membrane integrity (HOST 150 mOsm/L). The mean values of ejaculate volume, density (1-4 score), sperm concentration, mass activity (0-5 score), individual sperm motility, live sperm, abnormal sperm, intact acrosome and HOS reactive sperms observed in fresh semen were 3.43±0.10 ml, 2.14±0.11, 744.90± 36.38 million/ml, 3.45±0.07, 78.54±0.51 %, 90.48±0.33 %, 6.15±0.15 %, 94.40±0.20 % and 79.35±0.42 %, respectively. The variation between bulls was significant (P<0.05) for most of these traits. The overall segment-wise per cent abnormalities of sperm head, midpiece and tail region recorded in fresh semen were 1.83±0.09, 0.88±0.06 and 3.44±0.08, respectively. The mean percentages of spermatozoa having swollen, ruffled, detached and denuded acrosome in fresh semen were found to be 2.29±0.08, 1.60±0.10, 1.10±0.09 and 0.60±0.08, respectively. There were significant (P<0.01) interrelationships between sperm motility, viability, normal morphology, intact acrosome and plasma membrane integrity in fresh semen. The findings were within normal physiological limits of the breed, except poor sperm count.

KEY WORDS: Surti buffalo bull, Semen quality, HOS test, Interrelationships.

INTRODUCTION

An adequate evaluation of semen for breeding purposes has always been of great significance. Semen analysis is a valuable diagnostic tool to assess the fertility status of the male. However, the prediction of potential fertility of a male on the basis of a single assay is not reliable. There are numerous factors that may affect the motility, plasma membrane integrity, morphology and viability of semen. Acrosomal integrity is an important attribute of cell function, but it is not necessarily indicative of an intact plasma membrane (Amirat *et al.,* 2004). Therefore, more attention has been given recently to evaluating sperm plasma membrane integrity through HOS test as it is of fundamental importance in the fertilization process (Jayendran *et al.,* 1984; Lodhi *et al.,* 2008). The 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). Hence, this study was planned to know the physico-morphological and functional attributes of Surti buffalo semen and their interrelationships.

MATERIALS AND METHODS

The study was carried out at the Central Semen Station, Veterinary College, AAU, Anand, Gujarat during the favourable breeding season (Nov-Feb) of the year 2013-14. Six sexually mature buffalo bulls of Surti breed maintained in nearly identical nutritional and managerial conditions were included

in the study. They were in regular twice a week semen collection schedule in the morning hours using artificial vagina. A total of 48 ejaculates (8 per bull) were utilized for this study.

Each ejaculate soon after collection was evaluated through various macro- and micro-scopic tests. Mass activity (score 0-5) and individual sperm motility (%) were assessed in neat and diluted semen, respectively, under low and high magnifications of a phase contrast microscope fitted with a biotherm stage at 37°C (Tomar, 1986). Density of ejaculate was graded from 1 to 4 scale based on colour and thickness of semen. 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 including head, midpiece and tail were estimated by differential staining technique using eosin-nigrosin stain (Tomar, 1996). The percentages of sperms with intact acrosomes and those with different forms of acrosomal defects were determined by using Giemsa stain (Watson, 1975). The hypo-osmotic swelling test was performed using 150 mosm/l solution of sodium citrate-fructose (Jayendran *et al*., 1984). The data generated were analyzed statistically using CRD to know the variations between bull in their sperm parameters, and their interrelationships were worked out.

RESULTS AND DISCUSSION

Ejaculate Volume, Colour and Density

The mean ejaculate volume of semen recorded in 6 Surti buffalo bulls under study varied significantly (P<0.01) from 2.69 ± 0.13 to 4.00 ± 0.21 ml with an overall mean of 3.43 ± 0.10 ml (Table 1).

Table 1. Physico-morphological and functional characteristics of fresh semen of Surti buffalo bulls (Mean ± SE)

Seminal characteristics	Bull No.						Overall (n=48)
	1 (n=8)	2 (n=8)	3 (n=8)	4 (n=8)	5 (n=8)	6 (n=8)	
Ejaculate volume (ml)	3.44^{bc} ±0.18	3.56^{bc} ±0.22	3.69^{bc} ±0.25	2.69^{a} ±0.13	4.00^{c} ±0.21	3.19^{ab} ±0.19	3.43 ±0.10
Density score (1-4)	2.19^{b} ±0.19	3.06^{c} ±0.20	2.38^{b} ±0.21	2.06^{b} ±0.22	1.25^{a} ±0.09	1.88^{b} ±0.25	2.14 ±0.11
Sperm conc (million/ml)	841.38^{b} ±61.32	1035.88^{c} ±64.65	796.50^{b} ±67.28	702.38^{b} ±70.40	422.88^{a} ±36.57	670.38^{b} ±69.83	744.90 ±36.38
Mass activity score (0-5)	3.69^{cd} ±0.19	3.88^{d} ±0.13	3.19^{ab} ±0.09	3.63^{cd} ±0.16	2.88^{a} ±0.08	3.44^{bc} ±0.15	3.45 ±0.07
Individual sperm motility (%)	78.13^{abc} ±1.32	81.25^{c} ±1.25	77.50^{ab} ±0.94	80.63^{bc} ±1.13	75.63^{a} ±0.63	78.13^{abc} ±1.32	78.54 ±0.51
Live sperm (%)	90.63^{ab} ±0.98	89.25^{a} ±0.65	89.50^{a} ±1.00	92.50^{b} ±0.50	90.00^{a} ±0.65	91.00^{ab} ±0.65	90.48 ±0.33
Abnormal sperm (%)	6.38^{ab} ±0.50	6.00^{ab} ±0.27	6.88^{b} ±0.30	5.75^{a} ±0.25	5.63^{a} ±0.42	6.25^{ab} ±0.25	6.15 ±0.15
Acrosome intact sperm (%)	94.25 ±0.77	93.88 ±0.69	94.13 ±0.40	95.38 ±0.26	94.50 ±0.33	94.25 ±0.25	94.40 ±0.20
HOST reactive sperm (%)	80.25^{ab} ±0.70	77.38^{a} ±1.10	78.38^{a} ±1.10	82.00^{b} ±1.04	78.63^{a} ±0.68	79.50^{ab} ±0.82	79.35 ±0.42

Means bearing different superscripts within the row differ significantly between bulls (P<0.05).

Ejaculate volume together with sperm concentration and motility is of great importance in frozen semen production and wider application of artificial insemination in bovines. The colour of the Surti buffalo bull semen under investigation was found to be milky white, which is the normal colour of buffalo bull semen and the mean density score of semen in these bulls varied (P<0.0.01) from 1.25 ± 0.09 to 3.06 ± 0.20 with a mean of 2.14 ± 0.11 (Table 1). These findings are in accordance with many of the previous reports on buffalo bull semen. The normal colour of semen found in all bulls suggested that the genital tracts of all the bulls were normal healthy without any infection or trauma and the ejaculates were free from any contaminants. However, the Bull No. 5 consistently donated semen with less density score and it might be because of poor libido and senility. The density score of semen indirectly reflects the sperm concentration.

The findings obtained in present investigation were in accordance with those reported by Nema *et al.* (1983) and Khawaskar *et al.* (2012) in Surti; Tomar and Singh (1996) and Bhatt *et al.* (2004) in Murrah; and Bhavsar *et al.* (1989) and Patel *et al.* (2012) in Mehsana and Murrah buffalo bulls. However, comparatively higher ejaculate volume than the present one has been documented by Dubal (2007) and lower one by Dhami and Kodagali (1988) in Surti buffaloes. The differences in seminal volume and density score reported in different studies might be attributed to breed, age, health and genetic make-up of bulls, nutritional status, season/ climatic temperature and individual variation, apart from semen collection technique.

Mass Activity and Individual Sperm Motility

The mean mass activity score of semen noted in 6 Surti buffalo bulls varied significantly (P<0.05) between 2.88 ± 0.08 and 3.88 ± 0.13 with an overall mean of 3.45 ± 0.07, whereas the mean individual sperm motility varied (P<0.01) from 75.63 ± 0.63 to 81.25 ± 1.25 % with an overall mean of 78.54 ± 0.51 % (Table 1). The mass motility of spermatozoa is a wholesome effect of sperm concentration and individual sperm motility. The present finding on mass activity concurred with reports of different workers quoted above for different breeds of buffaloes and Surti in particular. According to Dhami and Kodagali (1988) and Patel *et al.* (2012) mass activity score is more closely related with seminal traits rather than fertility. The finding on individual motility was in accordance with many of the earlier reports in different breeds of buffaloes Dhami and Kodagali, 1988; Dhami and Sahni, 1994; Dubal, 2007; Khawaskar *et al.,* 2012 and Patel *et al.,* 2012). However, the relatively lower individual sperm motility of fresh semen has been documented by Nema *et al.* (1983), Dhami *et al.* (2001), Bhatt *et al.* (2004) and Mahmoud *et al.* (2013) in various buffalo breeds.

The individual sperm motility is an important parameter for assessment of semen quality and can yield a credible picture of semen potency, because it gives idea regarding acceptance or rejection of the ejaculate for further processing, and it is positively correlated with keeping quality and freezability of semen sample (Shelke and Dhami, 2001; Patel *et al.,* 2012; Mahmoud *et al.,* 2013). Sperm motility is essential during their transportation in oviduct and oocyte penetration, which cannot be ruled out. However, a decrease in sperm motility is observed in disease condition, change of environment/season and temperature variations, and it fluctuates between breeds, individuals, age group and the evaluation technique employed. All Surti buffalo bulls under investigation donated acceptable ejaculates in terms of initial sperm motility.

Sperm Concentration

The mean sperm concentration of semen in bulls under study varied significantly (P<0.01) between 422.88 ± 36.57 and 1035.88 ± 64.65 million per ml with an overall mean of 744.90 ± 36.38 million (Table 1). Sperm concentration per unit volume is an important trait in semen processing, since along with the initial motility and viability the dilution rate depends on concentration of spermatozoa in given ejaculate. Better is the sperm count per unit volume of semen greater is the number of insemination doses that can be produced. The mean sperm concentration recorded in the study

corroborated with the values reported by Nema *et al.* (1983) and Khawaskar *et al.* (2012). However, Dubal (2007) found lower sperm concentration in Surti bulls, while many other workers reported much higher sperm concentration/ml of semen in different breeds of buffaloes. The sperm concentration in bovine species varies between breeds, age, libido, climate, testicular health, accessory sex glands function, frequency and method of semen collection etc, and in many cases there was also individual variation.

The poor sperm concentration found in Surti bulls under study relative to previous reports from the same station in the same breed could be attributed to lack of sufficient green fodder and availability of poor quality dry fodder during the study period on account of very long spell of continuous heavy rainfall and poor sunshine during entire monsoon season resulting into soil drain and stunted plant growth, and thereby malnutrition of animals. Moreover, bull no. 5 consistently donated watery semen, thus reducing the overall pooled average (Table 1).

Live and Abnormal Spermatozoa

The percentages of live and abnormal spermatozoa recorded in fresh semen of Surti buffalo bulls varied from 89.25±0.65 to 92.50±0.50 and 5.63±0.42 to 6.38±0.50 with the overall means of 90.48 ± 0.33 and 6.15 ± 0.15 per cent, respectively. The variation between bulls was significant ($P<0.05$) for both the traits (Table 1). The values of both the traits were very much in acceptable limit for use of semen in AI work. The present values of live and abnormal sperm per cent were comparable with the previous reports of Dhami and Kodagali (1988), Bhavsar *et al.* (1989) and Khawaskar *et al.* (2012). However, Dhami *et al.* (2001) and Mohmoud *et al.* (2013) reported relatively lower values of live sperm and higher values of abnormal sperm per cent in semen of different buffalo breeds.

The overall segment-wise per cent abnormalities of fresh sperm head, midpiece and tail region recorded in bulls under study were 1.83 ± 0.09, 0.88 ± 0.06 and 3.44 ± 0.08, respectively with the total of 6.15 ± 0.15 %. Bhavsar *et al*. (1990) reported the comparable overall incidence of sperm head, midpiece, tail and total abnormalities in Mehsana bulls as 2.66 ± 0.02, 1.53 ± 0.03, 5.87 ± 0.10 and 9.93 ± 0.12 %, respectively. However, Shelke and Dhami (2001) recorded the corresponding mean values of 0.89 ± 0.16, 2.06 ± 0.35, 19.13 ± 3.22 and 22.18 ± 3.11 % in Jafarabadi buffalo semen, while Shukla and Mishra (2005) found the values as 1.27±0.09, 2.06±0.14, 9.22±0.22 and 12.57±0.25 % in Murrah buffalo bull semen, which are higher particularly in tail segment than the present findings.

It is now general consensus that for any semen sample to be accepted for use in AI should have more than 75 % initial motility and less than 20 % total sperm abnormalities. The segment-wise sperm abnormalities should not exceed 5 %. This is because only the live and morphologically normal sperm can migrate in the forward direction to reach the site of fertilization in the oviduct after being deposited in the reproductive tract of the female in estrus. The present findings are well within the acceptable limit for all these traits.

Acrosomal Integrity

In present study, the mean percentages of sperms with intact acrosome varied insignificantly between bulls from 93.88 ± 0.69 to 95.38 ± 0.26 with a mean of 94.40 ± 0.20 (Table 1). These findings concurred with the observations of Raval *et al.* (2005) and Khawaskar *et al.* (2012), but Ahmed *et al.* (2010) and Singh *et al.* (2014) found relatively lower values, while Singh *et al.* (2014) noted higher values of acrosomal integrity in fresh semen of bovines. The mean percentages of buffalo spermatozoa having swollen, ruffled, detached and denuded acrosome were found to be 2.29 ± 0.08, 1.60 ± 0.10, 1.10 ± 0.09 and 0.60 ± 0.08, respectively. These observations are to some extent in line with the report of Raval *et al.* (2005) in triplebred bulls. They recorded the mean percentage of sperms with swollen, ruffled, detached and denuded acrosome in fresh semen as 2.25 ± 0.06, 2.03 ± 0.08, 3.17 ± 0.12 and 2.00 ± 0.09 %, respectively, while many others have noted higher values of these forms of acrosomal defects in different breeds of bull and buffalo bull.

Mammalian spermatozoa are unable to fertilize the egg immediately after ejaculation. They require a period of incubation in the female reproductive tract in order to undergo capacitation and acrosome reaction, which are essential for the fertilization process. Spermatozoa which have lost their acrosome integrity spontaneously after ejaculation or induced by physical damages are devoid of acrosomal enzymes and are unable to bind to oocytes, and consequently they are unable to fertilize eggs. The evaluation of acrosome damage is therefore an important parameter to be considered for quality assessment of semen.

Hypo-Osmotic Swelling Test (HOST)

The mean percentages of hypo-osmotic swelling positive sperm in neat semen of 6 bulls varied significantly (P<0.05) between 77.38 ± 1.10 and 82.00 ± 1.04 with an overall mean of 79.35 ± 0.42 (Table 1). These findings were in accordance with the reports of Perumal *et al.* (2009) and Patel *et al.* (2012). Observations of Lodhi *et al.* (2008) and Shukla *et al.* (2011) show relatively higher results, while others (Ahmed *et al.,* 2010; Singh *et al.,* 2014) observed lower values of intact sperm plasma membrane in fresh buffalo semen. Moreover, significant (P<0.05) positive correlations between progressive motility, morphologically normal spermatozoa, sperm viability and percentage of HOS test positive spermatozoa have been found by some of these researchers, and have inferred that HOS test could be a valuable method for routine evaluation of semen for artificial insemination and as an indirect / *in vitro* fertility assessment test for a given sample.

The hypo-osmotic swelling (HOS) test with osmolarity of 75 to 150 mOsm per litre is a predictor of an intact plasma membrane (Jeyendran *et al.*, 1984). Spermatozoa with damaged plasma membranes do not inflate and no swelling or curling of the tails occur. In the human species, a close correlation between the percentage of swollen sperm cells and the percentage of denuded hamster oocytes is found suggesting that HOS could be an indicator of human fertility. However, the suitability of the HOS test as a predictive tool for *in vitro* fertility of bulls has not yet been established (Rota *et al.,* 2000). These species specific differences could be related to different membrane elasticity, maximal swell volumes, water permeability and cell geometry (Courtens *et al.,* 2001).

Interrelationships of Spermatozoal Attributes

The interrelationships observed among various spermatozoal attributes studied are presented in Table 2.

Table 2. Interrelationships of seminal attributes of fresh ejaculates in Surti buffalo bulls

Traits	Ejaculate volume	Sperm Conc	Mass activity	Initial motility	HOS test	Live Sperm	Abnormal sperm
Sp Conc	-0.26	---					
MA	-0.28*	0.40**	--				
IM	-0.22	0.36*	0.77**	--			
HOST	-0.16	-0.29*	0.17	0.19	--		
Live Sp	-0.12	-0.33*	0.24	0.24	0.49**	--	
Abn Sp	0.09	0.26	-0.28*	-0.32*	-0.24	-0.36*	--
Intact acrosome	-0.05	-0.34*	0.15	0.20	0.40**	0.65**	-0.50**

N = 48, * P<0.05, ** P<0.01.

Ejaculate volume revealed high negative correlations with motility and sperm concentration (-0.26, -0.28). Sperm concentration had significant (P<0.05) positive correlations with mass activity and individual motility (0.44, 0.36) and negative correlations with HOS reactive sperm, live sperm and intact acrosome percentage (-0.29, -0.33, -0.34). Mass activity was significantly (P<0.01) and positively correlated with individual sperm motility (0.77). The individual sperm motility had significant (P<0.05) negative correlation with abnormal sperm (-0.32). Live sperm showed significant (P<0.01) positive correlations with intact acrosome (0.65) and negative correlation with abnormal sperm (-0.36). Abnormal sperm had significant (P<0.01) negative correlations with per cent intact acrosome (-0.50). The HOS reactive sperm per cent in fresh semen showed significant (P<0.01) positive correlations with per cent live sperm and intact acrosome (0.49, 0.40). These correlation findings corroborated well with many of the earlier reports, particularly of Dhami and Sahni (1994), Shelke and Dhami (2001), Raval *et al* (2005), Lodhi *et al.* (2008), Patel *et al.* (2012) and Mahmoud *et al.* (2013) in bovine semen. Further, the results showed that motility may be a candidate marker for semen quality, considering that significant correlations were found between motility and both sperm abnormalities and acrosome as well as plasma membrane integrity, which is in accordance with the opinion of Mahmoud *et al.* (2013).

Under the conditions of the present study, it was inferred that the semen quality donated by Surti bulls was in general within acceptable physiological limits and that the HOS test in addition to motility could be a valuable and practical tool to know the functional capacity of fresh buffalo spermatozoa, hence could be added in the routine analysis of semen samples.

ACKNOWLEDGEMENTS

We are grateful to the Dean, Faculty of Veterinary Science, AAU, Anand for providing necessary laboratory facilities to carry out this research work.

REFERENCES :

Ahmed E., Ahmad, N., Naseer, Z., Aleem, M., Khan, M.S., Ashiq, M. and Younis, M. (2010). *Trop. Anim. Health Prod.,* DOI 10.1007/s11250-010-9668-1.

Aitken, R.J. (2006). *Int. J. Androl.,* **29**: 69-75.

Amirat, L., Tainturier, D., Jeanneau, L., Thorin, C., Gerard, O., Courtens, J. and Anton, M. (2004). *Theriogenology,* **61**: 895-907.

Bhatt, V., Honnappa, T.G. and Dubey, B.M. (2004). *Indian J. Anim. Reprod.,* **25**(1): 23-24.

Bhavsar, B.K., Dhami, A.J. and Kodagali, S.B. (1989). *Indian J. Dairy Sci.,* **42**(2): 246-250.

Bhavsar, B.K., Dhami, A.J. and Kodagali, S.B. (1990). *Indian Vet. J.,* **67**(3): 233-237.

Courtens, J.L. and Rety, J.M. (2001). *Genet Sel. Evol.,* **33**(1): 85-104.

Dhami, A.J. and Kodagali, S.B. (1988). *Indian Vet. J.,* **65**(1): 61-64.

Dhami, A.J. and Sahni, K.L. (1994). *J. Applied Anim. Res.,* **5**(1): 13-20.

Dhami, A.J., Shelke, V.B., Dave, R.R. and Kavani, F.S. (2001). *Indian J. Dairy Sci.,* **54**: 255-260.

Dubal, P.B. (2007). Effect of preservation of spermatozoa at 5°C for 24 hrs and 48 hrs on DNA integrity. M. V. Sc. Thesis, Anand Agricultural University, Anand, Gujarat, India.

Jayendran, R.S., Van der Ven, H.H., Perez-Pelaez, M., Crabo, B.G. and Zaneveld, L.J.D. (1984). *J. Reprod. Fertil.,* **70**: 219-228.

Khawaskar, M.V., Panchal, M.T., Dhami, A.J. and Patel, J.A. (2012). *Indian J. Field Vets.,* **8**(2): 55-61.

Lodhi, L.A., Zubair, M., Qureshi, Z. I., Ahmad, I. and Jamil, H. (2008). *Pakistan Vet. J.,* **28**(4): 186-188.

Mahmoud, K.G.M., Sokary, A.A.E., Roos, M.E.A., Ghaffar, A.D.A. and M. Nawito, M. (2013). *Iranian J. Applied Anim. Sci.,* **3**(4): 777-783.

Nema, S.P., Kodagali, S.B., Jankiraman, K. and Prabhu, G.A. (1983). *Ind. J. Anim. Reprod.,* **3**(1): 5-8.

Patel, J.B., Dhami, A.J. and Patel, P.A. (2012). *Indian J. Field Vets*., 7(3): 1-7.

Perumal, P., Barik, A.K., Mohanty, D.N., Das, R.K. and Mishra, P.C. (2009). *Proc. XXV Annual Convention of ISSAR and International Symposium,* Namakkal, India, 10-12 Nov., pp. 196.

Raval, R.J., Dhami, A.J. and Savaliya, F.P. (2005).. *GAU Res. J.,* **30**(1-2): 111-118.

Rota, A., Penzo, N., Vincenti, L. and Mantovani, R. (2000). *Theriogenology,* **53**: 1415-20.

Shelke, V.B. and Dhami, A.J. (2001). *Indian J. Anim. Sci.,* **71**: 319-324.

Shukla, M.K. and Mishra, A.K. (2005). *Indian J. Anim. Sci.,* **75**(3): 263-266.

Shukla, M.K., Saha, S., Mishra, G.K. and Saxena, S.K. (2011). *Indian J. Anim. Reprod.,* **32**(1): 52-54.

Singh, D., Singh, V. and Saxena, A. (2014). Studies on semen quality, freezability and fertility performance of Murrah buffalo bulls. IInd *annual convention of SVSBT and National Seminar on Biotechnological Approaches*, DUVASU, Mathura, India, 6-7 March, p. 93.

Tomar, N.S. (1986). *Artificial Insemination and Reproduction of cattle and buffaloes*. 3rd edn. Saroj Prakashan, Allahabad, India.

Tomar, S.S. and Singh, S.P. (1996). *Indian J. Anim. Res.,* **30**(1): 49-54.

Watson, P.F. (1975). *Vet. Rec.,* **97**: 12-15.

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