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RESEARCH ARTICLE

Assessment of Buffalo Bull Semen Quality Based on Sperm Motility Parameters, Motion Characteristics and *In Vitro* Fertilization Rate

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ABSTRACT

The objective of the present study was to determine the prognostic value of sperm motility parameters and motion characteristics to predict *in vitro* fertilization (IVF) rate in the buffalo. Sperm motility parameters (%; motile, progressive, rapid and medium) and motion characteristics (path velocity = VAP, µm/sec; progressive velocity = VSL, µm/sec; curvilinear velocity = VCL, µm/sec; amplitude of lateral head = ALH, µm and beat cross frequency = BCF, Hz) of 5 buffalo bulls (Tag # 2, 4, 6, 11 and 12) were determined by Cell Motion Analyzer (CASA) at post dilution (PD), pre freezing (PF) and post thaw (PT) stages. IVF ability of individual bull was determined by cleavage rate (CR, %). Multiple regression analysis was used to determine the prognostic values of sperm motility parameters and motion characteristics for CR as a dependent variable. At PT, Bull 2 showed the highest ($P<0.05$) progressive and rapid motility, and VAP, VSL and VCL values as compared to other bulls. Data of CR (%) were similar among the five buffalo bulls. However, the prognostic value to predict the cleavage rate by the equation of motility and motion characteristics accounted for 81% ($P=0.01$). In conclusion, although at PT, Bull 2 showed the highest sperm progressive and rapid motility parameters, and motion characteristics (VAP, VSL and VCL) as compared to other bulls, but the CR was similar. The prognostic value by the equation of motility and motion characteristics accounted significantly to predict the CR.

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INTRODUCTION

Artificial insemination (AI) is useful for the improvement of milk productivity in buffaloes by the propagation of animals with high genetic potential (Baruselli and Carvalho, 2005; Atiq *et al*., 2011; Ciptadi *et al*., 2012). Just one ejaculate can give us enough AI doses to increase the genetic potential of a herd. The fertility in the field through AI of the frozen thawed buffalo semen is less as compared to fresh semen samples. Because of this reason, proper evaluation of the frozen thawed semen is of great importance for the AI industry, since it can provide insights upon the fertilizing capacity of the cryopreserved spermatozoa. Sperm has a highly compartmentalized structure, with each compartment performing specific function. Therefore, a single test to check the whole spermatozoon intactness and to predict its potential in the field is required (Rodriguez-Martinez, 2003). In cattle, computer assisted sperm analysis (CASA) of *in vitro* capacitated bull sperm has been correlated with the fertility both *in vivo* and *in vitro* (Kathiravan *et al*., 2011).

In vitro fertilization (IVF) technology has given new insights for the evaluation of fertilizing capacity of a sperm, and we can predict field fertility of a semen donor bull. Such technique comprises the evaluation of field fertility with the zona binding or penetration, the fertilization of mature oocyte and at last to give up embryos post IVF. During IVF, spermatozoa have to mimic the functional changes that occur during *in vivo* gamete's interaction. The most common are the sperm capacitation, acrosomal reaction of the sperm, sperm binding to zona pellucida and subsequent events during fertilization (Graham and Moce, 2005). Among the spermatological and IVF parameters, cleavage rate was identified as being the most predictive of conception rate and accounted for 75% of variation in buffalo (Mehmood, 2007). To examine the semen quality by the IVF the cleavage rate at

48 hours might be the best parameter as compared to the blastocyst formation (7-8 days), which was found to be affected by the culture condition (Walters *et al*., 2004). Therefore, the present study is based on the hypothesis that prognostic value of sperm motility parameters and motion characteristics may predict IVF rate in buffaloes.

MATERIALS AND METHODS

Semen collection and processing: Semen was collected once in a week (for a period of 3 weeks) from five donor buffalo bulls (Tag # 2, 4, 6, 11 and 12) of Nili-Ravi breed, maintained at the Livestock Research Station, NARC, Islamabad. Two consecutive ejaculates were collected from each of the five bulls by artificial vagina (42^0C) on the day of experiment. The semen samples were kept at 37^0C for 15 min (holding time). The subjective motility was assessed with the phase contrast microscope (×400) connected with a closed circuit television. Ejaculates having more than 60% visual motility were selected for further processing (dilution, cooling, equilibration and freezing). At 37^0C semen was diluted with Tris–citric acid extender and finally cryopreserved in programmable cell freezer (KRYO 10 series III, UK) with the protocol described earlier (Rasul, 2000).

Computer assisted semen analysis: Cell Motion Analyzer (CEROS, Hamilton Thorne Biosciences, Beverly, USA) was used to evaluate sperm motility parameters and motion characteristics of buffalo bulls (n = 5) at Post dilution (PD), Pre freezing (PF) and Post thaw (PT) stages of cryopreservation. The motility parameters were percentage (%) of motile, progressive motile, rapid motile and medium motile spermatozoa. The sperm motion characteristics included path velocity (VAP; µm/sec), progressive velocity (VSL; µm/sec), track speed (VCL; µm/sec) amplitude of lateral head (ALH; µm) and beat cross frequency (BCF; Hz).

***In vitro* maturation and *in vitro* fertilization:** A total of 100 ovaries were collected from the carcasses of buffaloes at Sihala Abattoir, Islamabad. Ovarian collection was done once in a week (for a period of 3 weeks) and brought to the Animal Reproduction Laboratory, NARC, Islamabad, in a thermos (37°C, normal saline).

Methods of cumulus oocyte complex (COC) recovery, *in vitro* maturation (IVM) and IVF were the same as reported earlier for buffalo (Mehmood *et al*., 2007) with a slight modification of using fetal calf serum (FCS) and epidermal growth factor in the culture media (TCM 199). Briefly, COCs were collected by aspiration in culture medium with 2% FCS. A total of 130 good quality COCs (oocytes with homogenous ooplasm and surrounded with compact multiple layers of cumulus cells) were used for IVM. Following IVF, presumptive zygotes were examined under stereomicroscope to record the number of oocytes cleaved (2-cell or >2 cell stage). *In vitro* fertilization ability of individual bull was determined by cleavage rate and calculated as:

Cleavage rate = No of oocytes cleaved x 100 / total oocytes inseminated.

Statistical analysis: Data of sperm motility parameters and motion characteristics at PD, PF and PT stage were analyzed by ANOVA (analysis of variance) to observe the bull difference and effect of freezing stage. Least significant difference (LSD) was used to compare the means. Cleavage rate was analyzed with Chi-square statistics. Multiple regression analysis was used to determine the prognostic values of sperm motility parameters and motion characteristics for cleavage rate as a dependent variable. The level of significance was $P<0.05$. Minitab (MINITAB® Release 12.22, 1998) statistical package was used for data analysis.

RESULTS

Motility parameters (%) among the 5 buffalo bulls at PD, PF and PT stages of cryopreservation are presented in Table 1. The progressive and rapid motility parameters differed ($P<0.05$) among the 5 buffalo bulls at all the three stages, whereas percent motile sperm and medium motility parameter remained similar. At PT, Bull 2 showed the highest ($P<0.05$) progressive and rapid motility as compared to other bulls.

Sperm motion characteristics among buffalo bulls at PD, PF and PT stages of cryopreservation are presented in Table 2. The mean values of VAP, VSL and VCL sperm motion characteristics differed ($P<0.05$) among the 5 buffalo bulls at all the three stages, whereas ALH and BCF parameters remained similar. At PT, Bull 2 showed the highest ($P<0.05$) VAP, VSL and VCL values as compared to other bulls.

Pooled data (5 buffalo bulls) of motility parameters (%) at PD, PF and PT stages of cryopreservation are shown in Fig. 1. At PT, percent motile sperm, progressive and rapid motility parameters decreased significantly, whereas medium motility parameter increased significantly.

Pooled data (5 buffalo bulls) of sperm motion characteristics at PD, PF and PT stages of cryopreservation are shown in Fig. 2. At PT stage, VAP, VSL, VCL and ALH values were decreased significantly, whereas BCF was increased significantly. Data of cleavage rate (%) were similar ($P>0.05$) among the five buffalo bulls (Table 3). However, the prognostic value to predict the cleavage rate by the equation of motility and motion characteristics accounted for 81% ($P=0.01$).

DISCUSSION

Bull effect and stage of freezing were the main sources of variation when data on motility parameters and sperm motion characteristics were analyzed. The progressive and rapid motility parameters and the motion characteristics (VAP, VSL and VCL) differed among the 5 buffalo bulls at all the three stages. In an earlier study, Muino *et al*. (2008) reported that semen samples having more rapid and progressive subpopulations of spermatozoa were comparatively more cryo-resistant.

At PT stage, VAP, VSL, VCL and ALH were decreased significantly, whereas BCF was increased significantly. These results showed that the holding time before semen dilution and equilibration time before freezing are directly affecting the motion characteristics of buffalo spermatozoa (Lessard *et al*., 2000). During freezing and thawing, the most sensitive kinematics to monitor both the physical and biochemical changes in buffalo spermatozoa are the VCL and ALH (Rasul, 2000). In the present study, all the sperm motion characteristics

Table 1: Motility parameters (%) among the 5 buffalo bulls at post dilution, pre freezing and post thaw stages of cryopreservation

Bull No.	Post dilution stage				Pre-freezing stage				Post thaw stage			
	Mot	Prog	Rapid	Med	Mot	Prog	Rapid	Med	Mot	Prog	Rapid	Med
2	98.3±0.6	45.0±11.5 ^{a}	77.3±11.0^{a}	20.7±10.0	98.3±1.2	38.3±13.9^{a}	74.3±17.2^{a}	23.7±16.3	93.0±6.6	31.3±6 5^{a}	44.3±7.1^{a}	48.3±6.8
4	98.0±1.0	45.3±8.6^{ab}	71.7±14.4 ^{b}	26.7±13.3	96.7±3.2	36.0±7.8^{ab}	59.3±10.^{b}	37.0±11.5	81.0±11.0	20.3±7.6^{b}	26.0±5.0^{c}	54.3±5.5
6	99.0±0.0	40.7±5.0^{bc}	63.3±5.0 ^{b}	35.3±5.5	97.3±0.6	28.3±5.9^{bc}	48.3±4.6 ^{c}	49.0±5.2	89.0±4.6	15.3±8.0^{c}	31.3±9.5^{b}	54.7±14.4
11	98.0±1.0	31.7±13.3^{c}	49.0±13.9^{c}	49.0±14.7	95.3±0.6	27.7±3.1^{bc}	51.0±14.5^{bc}	44.3±14.0	72.0±14.2	17.3±2.5^{bc}	23.0±1.0^{c}	48.7±12.7
12	98.3±0.6	47.0±8.7^{ab}	74.3±10.2^{ab}	24.3±9.9	96.7±2.5	24.3±1.5^{c}	49.7±9.2 ^{c}	47.3±11.9	82.7±12.1	20.3±7.2^{b}	32.3 ±6.4^{b}	49.7±6.1

Values are Mean±SD of 3 replicates (repeated 3 times with different semen batch); Values in a column with different superscripts differ (P<0.05).
Mot= Motile; Prog= Progressive; Med=Medium.

Table 2: Sperm motion characteristics among the 5 buffalo bulls at post dilution, pre freezing and post thaw stages of cryopreservation.

Bull No.	VAP	VSL	VCL	ALH	BCF
Post dilution stage					
2	132.2±24.1^{a}	104.5±18.7^{a}	193.3±36.4^{a}	6.0±1.0	6.0±1.0
4	114.5±17.2^{b}	92.3±14.1^{b}	170.9±28.6^{b}	6.0±0.8	5.5±0.9
6	101.8±0.4^{b}	82.9±2.5^{bc}	148.4 ±2.8^{b}	5.3±0.3	5.5±0.8
11	91.5±12.9^{b}	74.6±12.0^{c}	129.5±13.1^{c}	5.3±0.3	5.5±0.5
12	105.7±13.9^{b}	84.4±11.8^{bc}	159.4±23.2^{bc}	5.9±0.7	3.9±1.9
Pre freezing stage					
2	128. ±15.0^{a}	98.9±14.1^{a}	196.2±25.3^{a}	6.5±0.8	6.5±0.8
4	100.6±11.2^{b}	79.6±8.5^{b}	147.9±18.6^{b}	5.5±0.4	4.7±2.6
6	92.5±6.2^{b}	73.2±6.9^{b}	135.4±9.1^{b}	5.2±0.6	4.4±1.2
11	96.4±13.9^{b}	74.8±7.1^{b}	136±19.8.^{b}	5.2±0.3	4.5±0.7
12	95.7±10.0^{b}	72.3±5.1^{b}	139.3±15.2^{b}	5.5±0.5	3.8±1.6
Post thaw stage					
2	85.5±5.7^{a}	71.4±2.8^{a}	125.1^{a} ±9.0	4.8±0.5	4.8±0.5
4	70.2±1.8^{b}	59.8±4.4^{b}	99.1±6.6^{b}	4.8±0.4	9.7±5.9
6	75.4±9.1^{b}	61.2±5.8^{b}	109.6±14.9^{b}	5.0±0.9	8.2±7.1
11	69.3±3.0^{b}	58.6±1.0^{b}	98.9±3.9^{b}	4.4±0.6	8.8±1.5
12	75.5±3.2^{b}	59.9±4.7^{b}	112.0±4.4^{b}	4.9±0.1	7.1±2.1

Values are Mean ± SD of 3 replicates (repeated 3 times with different semen batch); Values in a column with different superscripts differ (P<0.05); VAP = Path velocity (μm/sec), VSL = Progressive velocity (μm/sec), VCL = Curvilinear velocity (μm/sec), ALH = Lateral head displacement (μm), BCF = Beat cross frequency (Hz).

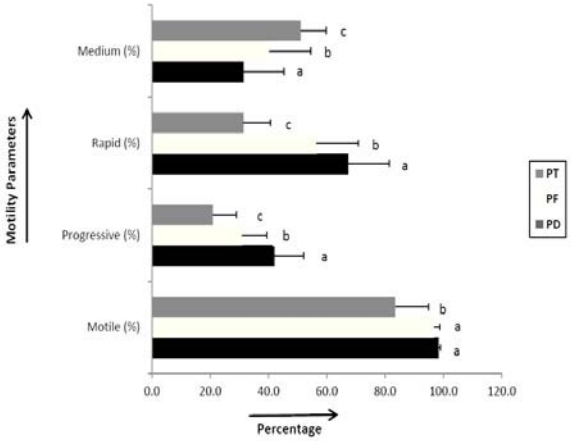

Fig. 1: Pooled data (5 buffalo bulls) of motility parameters (motile, progressive, rapid and medium) at Post dilution (PD), Pre freezing (PF) and Post thaw (PT) stages of cryopreservation. Bars with different superscripts differ (P<0.05).

except BCF were decreased due to cryopreservation. This shows that there is a change in the motility pattern of spermatozoa, which becomes less linear or progressive, and this state is called as a ''hyperactivated movement'' (Mortimer, 1997). Pena *et al*. (2003) suggested that after freeze-thaw process, capacitation like events occur in sperm motility. The temperature (5°C) during cooling and equilibration also initiates capacitation like changes, as reported in boar spermatozoa (Green and Watson, 2001).

In the present study, data of cleavage rate were similar among the five buffalo bulls. Suzuki *et al*. (2003)

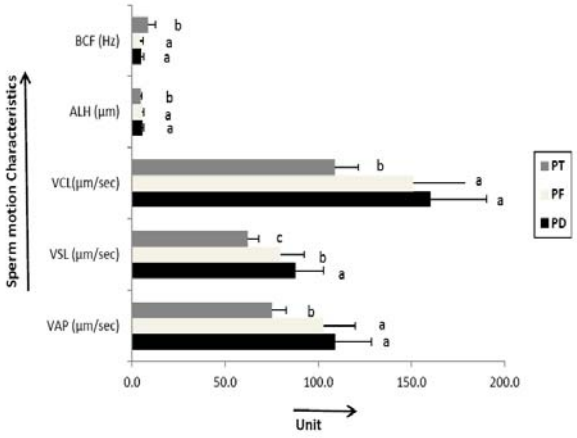

Fig. 2: Pooled data (5 buffalo bulls) of sperm motion characteristics (VAP = Path velocity, VSL = Progressive velocity, VCL = Curvilinear velocity, ALH = Lateral head displacement, BCF = Beat cross frequency) at Post dilution (PD), Pre freezing (PF) and Post thaw (PT) stages of cryopreservation. Bars with different superscripts differ (P<0.05).

reported differences among the individual parameters of sperm motion characteristics and IVF rate. Similarly, Mehmood *et al*. (2009) reported differences among the buffalo bulls in the cleavage rate following IVF with spermatozoa harvested by swim up method. Whereas, Alomar *et al*. (2008) reported that there was no difference in the bulls at the early cleavage stage. Selvaraju *et al*. (2008) reported that the cleavage rate differed between bulls, and the sire used in IVF had great effect on the fertilization of oocytes. This shows that in the IVF procedure the large number of sperm masks the bull effect on *in vitro* embryo development, because very few of sperm reach oocytes during in vivo fertilization (Saacke *et al*., 2000).

Table 3: Cleavage rate of semen from 5 buffalo bulls

Bull No.	Oocytes inseminated	Oocytes cleaved	Cleavage rate (%)
2	24	10	41.7
4	25	13	52.0
6	24	15	62.5
11	30	12	40.0
12	27	15	55.6

In our study, the prognostic value to predict the cleavage rate by the equation of motility parameters and motion characteristics accounted for 81% (P=0.01). Farrell *et al.* (1998) conducted a study on the relationship of sperm motion characteristics to fertility (non return rate of 59-day) and found higher r^2 values (0.68 to 0.98) for ALH, VCL, VSL, LIN and BCF parameters. Similarly, Elia *et al*. (2010) concluded that differences between the sperm motility parameters were the necessary parameters

Table 4: Prognostic value to predict the cleavage rate (CR, %) by the predictive equation of motility parameters (%) and motion characteristics

Response	Predictive equations	R^2	P-value
Cleavage rate	CR% = 27.4 + 1.52 Motility – 0.854 Progressive – 1.18 Rapid - 0.95 Medium.	49.3%	0.12
	CR% = 44.6 + 3.89 VAP – 3.63 VSL – 0.96 VCL + 5.34 ALH + 2.23 BCF.	42.6%	0.33
	CR% = 89.9 + 0.894 Motility + 6.27 VAP – 6.36 VSL – 1.52 VCL – 8.46 ALH + 1.91 BCF.	81.0%	0.01

VAP = Path velocity (μm/sec), VSL = Progressive velocity (μm/sec), VCL = Curvilinear velocity (μm/sec), ALH = Lateral head displacement (μm), BCF = Beat cross frequency (Hz)

for the evaluation of sperm fertilization ability. It was reported earlier, that among the sperm motion characteristics, progressive motility and velocity parameters (VCL, VSL and VAP) could be used to predict the fertility of the bull spermatozoa. However, the ALH and BCF had no correlation with fertility of the bull (Kathiravan *et al*., 2011).

In conclusion, although at PT, Bull 2 showed the highest sperm progressive and rapid motility parameters, and motion characteristics (VAP, VSL and VCL) as compared to other bulls, but the cleavage rate was similar. The prognostic value by the equation of motility and motion characteristics accounted significantly to predict the cleavage rate.

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REFERENCES

Alomar M, I Donnay, H Tasiaux, S Remacle, F George and D Paul, 2008. Kinetics of fertilization and development, and sex ratio of bovine embryos produced using the semen of different bulls. Anim Reprod Sci, 107: 48-61.

Atiq N, N Ullah, SMH Andrabi and S Akhter, 2011. Comparison of photometer with improved neubauer hemocytometer and makler counting chamber for sperm concentration measurement in cattle. Pak Vet J, 31: 83-84.

Baruselli PS and NAT Carvalho, 2005. Biotechnology of reproduction in buffaloes *(Bubalus bubalis)*. Brazilian J Anim Breed, 29: 4-17.

Ciptadi G, M Nasich, A Budiarto, Nuryadi and VMA Nurgiartiningsih, 2012. The estrus synchronization response following PGF2α treatment in Indonesian Madura cattle with different body condition scores. Pak Vet J, 32: 624-626.

Elia J, F Mazzilli, N Imbrogno, M Delfino, R Mazzilli and T Rossi, 2010. The importance of the sperm motility classes-Future directions. Androl J, 2: 42-43.

Farrell PB, GA Presicce, CC Brockett and RH Foote, 1998. Quantification of bull sperm characteristics measured by computer-assisted sperm analysis (CASA) and the relationship to fertility. Theriogenology, 49: 871-879.

Graham JK and E Moce, 2005. Fertility evaluation of frozen-thawed semen. Theriogenology 64: 492-504.

Green CE and PF Watson, 2001. Comparison of the capacitation-like state of cooled boar spermatozoa with true capacitation. Reproduction, 122: 889-898.

Kathiravan P, J Kalatharan, G Karthikeya, K Rengarajan and G Kadirvel, 2011. Objective sperm motion analysis to assess dairy bull fertility using computer-aided system: A review. Reprod Dom Anim, 46: 165-172.

Lessard C, S Parent, P Leclerc, JL Bailey and R Sullivan, 2000. Cryopreservation alters the levels of the bull sperm surface protein P25b. J Androl, 21: 700-707.

Mehmood A, 2007. Development of a homologous *in vitro* fertilization test to predict fertility of buffalo semen. PhD Thesis, University of Arid Agriculture, Rawalpindi, Pakistan.

Mehmood A, M Anwar and SMS Naqvi, 2007. Capacitation of frozen thawed buffalo bull (*Bubalus bubalis*) spermatozoa with higher heparin concentrations. Reprod Dom Anim, 42: 376-379.

Mehmood A, M Anwar and SMS Naqvi, 2009. Motility, acrosome integrity, membrane integrity and oocyte cleavage rate of sperm separated by swim-up or Percoll gradient method from frozen–thawed buffalo semen. Anim Reprod Sci, 111: 141-148.

Mortimer ST, 1997. A critical review of the physiological importance and analysis of sperm movement in mammals. Hum Reprod Update, 3: 403-439.

Muino R, AI Pena, C Tamargo and CO Hidalgo, 2008. Identification of sperm subpopulations with defined motility characteristics in ejaculates from Holstein bulls: Effects of cryopreservation and between-bull variation. Anim Reprod Sci, 109: 27-39.

Pena FJ, A Johannisson, M Wallgren and H Rodriguez-Martinez, 2003. Assessment of fresh and frozen–thawed boar semen using an annexin-v assay: a new method of evaluating sperm membrane integrity. Theriogenology, 60: 677-689.

Rasul Z, 2000. Cryopreservation of buffalo semen. PhD Thesis, Quaid-i-Azam University, Islamabad, Pakistan.

Rodriguez-Martinez H, 2003. Laboratory semen assessment and prediction of fertility: still utopia? Reprod Dom Anim, 38: 312-318.

Saacke RG, JC Dalton, S Nadir, RL Nebel and JH Bame, 2000. Relationship of seminal traits and insemination time to fertilization rate and embryo quality. Anim Reprod Sci, 60-61: 663-677.

Selvaraju S, JP Ravindra, J Ghosh, PSP Gupta and KP Suresh, 2008. Evaluation of sperm functional attributes in relation to *in vitro* sperm-zona pellucida binding ability and cleavage rate in assessing frozen thawed buffalo (*Bubalus bubalis*) semen quality. Anim Reprod Sci, 106: 311-321.

Suzuki K, M Geshi, N Yamauchi and T Nagai, 2003. Functional changes and motility characteristics of Japanese Black bull spermatozoa separated by percoll. Anim Reprod Sci, 77: 157-172.

Walters AH, WE Eyestone, RG Saacke, RE Pearson and FC Gwazdauskas, 2004. Sperm morphology and preparation method affect bovine embryonic development. J Androl, 25: 554-563.