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Motion Characteristics and Kinematics of Fresh Spermatozoa of Gir, Surti and Murrah Bulls Assessed By Computer Assisted Semen Analyzer

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Abstract

A study was carried out on semen of nine breeding bulls, three each of Gir, Surti and Murrah breeds, to evaluate the comparative motion characteristics and kinematics of their fresh spermatozoa by CASA. The ejaculates (n=72, 24 of each breed) having >75% initial motility were diluted @ 80 million sperm/ml using TFYG extender and were assessed for motion characteristics by CASA. The overall mean values of rapid motile and immotile sperm per cent were observed significantly greater in Gir bulls semen, while total motile and slow motile sperms were apparently higher in buffalo semen. The mean values of sperm velocity/ kinematic parameters observed based on all motile sperms in Gir, Surti and Murrah bulls semen were: average path velocity 50.01±1.25, 48.51±1.03 & 49.14±1.30 µm/s; curvilinear velocity 88.62±1.66, 87.90 ±1.74 & 88.93±1.69 µm/s, straight line velocity 44.51±1.35, 43.14±1.12 & 41.73±2.24 µm/s; linearity 50.06±1.42, 48.86±1.32 & 48.49±1.84 %; straightness 85.17±0.92, 84.97±0.88 & 83.90±1.17 %; wobbling index 57.00±1.17, 55.32±1.05 & 55.30±1.48 %; beat-cross frequency 15.55±0.58, 16.14±0.43 & 14.97±0.54 hz; amplitude of lateral head displacement 2.39±0.18, 2.57±0.12 & 2.31±0.14 µm; dancing frequency 208.34±15.52, 225.00 ±10.74 & 211.29±13.03 $\mu m^2/s$, and dancing mean 5.70±0.46, 6.33±0.35 & 6.33±0.50 $\mu m^2/s$, respectively. Almost similar trend with little higher values were noted for sperm velocity/kinematics based on only progressively motile sperm, without breed/species variation. The semen of all three breeds behaved identically for sperm kinematics. The bull variation was insignificant for all the traits in all the three breeds.

Introduction

Semen analysis is the most valuable diagnostic tool to evaluate male fertility potential in all livestock species (Verstegen *et al.,* 2002; Patel *et al.,* 2012). The conventional subjective evaluation of bovine semen is relatively inaccurate, imprecise, time consuming (Christensen *et al*., 2005), and the motility judgment largely depends on the level of training and skills of the investigator (Knuth *et al.,* 1989). While computer assisted sperm analysis (CASA) is a quite faster, precise and useful objective tool for identifying differences in sperm parameters, related to motility, velocity and morphology, and it avoids subjective errors (Johnson *et al*., 1996). Hence now-a-days, there is an increasing trend and interest in evaluating sperm motion characteristics by CASA (Amann and Waberski,

2014; Rodriguez-Montaña and Roa-Guerrero, 2017). The kinematic values determined for each spermatozoon cover the velocity of movement, *viz*., average path velocity (VAP), curvilinear velocity (VCL) and straight line velocity (VSL), the width of the sperm head's trajectory and frequency of the change in direction of the sperm head (Mortimer *et al*., 1990) etc., and thus provide quantitative assessment of sperms. However, the literature on use and application of CASA in bovine semen laboratory is meager particularly in Indian context (Mandal *et al.,* 2003; Ramachandran *et al.,* 2007; Patel *et al.,* 2012; Patel and Dhami, 2016). Hence, this study was planned to objectively evaluate and compare the motion characteristics/kinematics of spermatozoa of fresh semen of cow bulls and buffalo bulls using CASA.

Materials and Methods

The study was carried out on semen of nine healthy mature breeding bulls (3 each of Gir, Surti and Murrah breed), aged 5-8 years, at the College of Veterinary Science, AAU, Anand-388 001 during the winter season of year 2017-18. All the bulls were in good health and under optimal veterinary care. They were maintained in nearly identical nutritional and managerial conditions throughout the period of study with twice a week semen collection schedule. Semen was collected using artificial vagina from each cattle and buffalo bull in the morning hours between 7.30 and 8.30 am over a dummy buffalo bull. Ejaculates collected at weekly interval were used for this study.

Immediately after collection, the ejaculates (n=72; 8 per bull) were evaluated for various seminal attributes. Samples with > 75 % initial motility were extended at 34ºC with Tris-citric acid-fructose-egg yolk-glycerol (TFYG) extender keeping 80 million sperm per ml. The extended semen was assessed for sperm motility subjectively and for sperm motion characteristics by Biovis CASA (Expert Vision Pvt. Ltd., Mumbai). The CASA traits studied included total motile sperm (*i.e.,* rapid, slow & non-progressive motile sperm), immotile sperm, average path velocity (VAP), curvilinear velocity (VCL), straight line velocity (VSL), straightness (STR), linearity (LIN), wobbling (WOB), beat-cross frequency (BCF), amplitude of lateral head displacement (ALH), dancing velocity (DNC) and dancing mean (DNM). The data so generated on sperm quality traits were analyzed statistically to derive mean ± SEs using standard statistical package on SPSS software version 20.00 and breed/bull differences were tested by critical difference test (Snedecor and Cochran, 1994).

Results and Discussion

Sperm Motility Profile

The mean values of initial sperm motility assessed subjectively under microscope for semen of Gir, Surti and Murrah bulls were 80.21±0.88, 84.58±0.60 and 84.38±0.76 % ($p<0.05$), respectively, being significantly lower in Gir than buffalo bulls (Table 1). These values were lower (by 3-4%) than the objective assessment by CASA (84.30±1.46, 86.55±1.12 and 88.20±1.08%, respectively, Table 1). The CASA results reflected positive association between total motile sperm and subjectively assessed sperm motility, and concurred with the report of Patel *et al.* (2012).

The CASA analysis of fresh semen revealed the overall mean motility parameters like total motile sperm and non-progressive/static motile sperm per cent to be significantly ($p<0.05$) higher in Murrah bulls than Gir bulls, and the values for Surti bulls were intermediate. The rapid progressive and slow progressive motile sperm per cent did not vary significantly between breeds, but the per cent rapidly motile sperms were apparently greater in cattle, while slow motile sperms were greater in buffalo semen. On the contrary, the immotile sperm per cent was significantly higher in Gir bulls than Murrah bulls (Table 1). Moreover, the bull variation was statistically insignificant for all these traits in all three breeds.

Our results on motility rating of bull and buffalo bull spermatozoa using Biovis CASA and ordinary plain slides were in accordance with the findings of Kumar *et al.* (2018) using same version of CASA on buffalo semen, and with reports of Karthikeya (2003), Kathiravan *et al.* (2008), Hoflack *et al.* (2007), Patel *et al.* (2012) and Patel and Dhami (2016), who employed Leja slides and Hamilton Thorne CASA for such analysis. The percentages of total motile and progressive motile sperms varied among different

Table 1: Average (±SE) sperm motility parameters of fresh semen of Gir cattle and Surti and Murrah buffalo bulls assessed by Biovis CASA

Seminal attributes	Breeds of bull		
	Gir (n=24)	Surti (n=24)	Murrah (n=24)
Total motile sperm (%)	84.30^{x}±1.46	86.55^{xy}±1.12	88.20^{y}±1.08
Rapid progressive motile sperm (%)	40.01±2.28	38.66±2.11	38.05±2.30
Slow progressive motile sperm (%)	30.94±1.76	34.05±2.50	33.53±2.29
Non-progressive motile sperm (%)	13.36^{x}±1.19	13.83^{x}±1.24	16.70^{y}±1.73
Immotile sperm (%)	15.07^{y}±1.46	13.45^{xy}±1.12	11.72^{x}±1.07

Means bearing uncommon superscripts within the row differ significantly ($p<0.05$). Bull variation was not significant for any of the traits in any of the breeds.

studies due to variation in the bull, breed and initial semen quality, as well as different software and make of CASA machines used. The present results were fairly accurate and repeatable.

Sperm Velocity / Kinematics

The CASA analysis of freshly diluted semen depicted various velocity and kinematics parameters of sperms based on total motile sperms and progressively motile sperms. The mean ± SE values of these traits are presented in Table 2 and 3, respectively. None of these traits differed significantly between breeds/species or between bulls in any of the breed.

For the CASA analysis based on total motile sperms, the average path velocity (VAP, µm/s) of spermatozoa was apparently higher in Gir than Murrah and Surti bull's semen, curvilinear velocity (VCL, µm/s) was little lower in Surti than Murrah and Gir bulls, straight line velocity (VSL, µm/s) was higher in Gir than Surti and Murrah bulls, and linearity (LIN, %), straightness (STR, %) and wobbling index (WOB, %) were higher in Gir compared to Surti and Murrah bull's semen. Moreover, the BCF, ALH and DNC were non-significantly higher in Surti than the Murrah and Gir bulls.

The sperm velocity parameters recorded based on the progressively motile sperms of bulls under study revealed almost similar pattern and values to those found based on total motile sperms (Table 3). The values of velocity parameters, linearity and straightness were almost similar in cattle and buffalo semen. However, the values of BCF, ALH, and DNC were little higher in Surti bulls than in Gir and Murrah bulls for progressive motile sperms, while DNM was lower in cow bulls than buffalo bulls. Interestingly, the values of all kinematic traits recoded were somewhat higher based on progressive motile sperms as compared to total motile sperms, particularly for VAP, VSL, LIN, STR, WOB, and BCF with lower DNM, but no differences were noted for values of ALH and DNC in all three breeds. In general, the curvilinear velocity (µm/s) and straightness (%) were higher (88-92) as compared to average path velocity (µm/s), straight line velocity (µm/s), linearity (%) and wobbling index (all around 48-60%) in sperms of all three breeds (Table 3). The ratio of BCF to ALH and DNC to DNM were around 7:1 and 45:1, respectively.

Our results in terms of values were in accordance with the reports of Mandal *et al.* (2003), Patel *et al.* (2012) and Patel and Dhami (2016). However, the ratings of velocity and kinematics traits varied among different studies due to variation in the breed/species of bulls, initial semen quality, and software and model of CASA machines used with or without Leja slides in the assessment.

Karthikeya (2003), however, recorded comparatively higher values of all CASA traits of Jersey bull than our findings in Gir or buffalo bulls. Goovaerts *et al.* (2006) studied quality of fresh bull sperm conventionally and by Hamilton-Thorne CASA and reported the values of motility (total & progressive), straight line velocity,

Table 2: Average sperm velocity and kinematics attributes of total motile sperms of fresh semen of Gir cattle and Surti and Murrah buffalo bulls assessed by Biovis CASA

Seminal attributes	Breeds of bull		
	Gir (n=24)	Surti (n=24)	Murrah (n=24)
Average path velocity (VAP, µm/s)	50.01±1.25	48.51±1.03	49.14±1.30
Curvilinear velocity (VCL, µm/s)	88.62±1.66	87.90±1.74	88.93±1.69
Straight line velocity (VSL, µm/s)	44.51±1.35	43.14±1.12	41.73±2.24
Linearity (LIN, %)	50.06±1.42	48.86±1.32	48.49±1.84
Straightness (STR, %)	85.17±0.92	84.97±0.88	83.90±1.17
Wobbling index (WOB, %)	57.00±1.17	55.32±1.05	55.30±1.48
Beat cross frequency (BCF, hz)	15.55±0.58	16.14±0.43	14.97±0.54
Amplitude of lateral head displacement (ALH, µm)	2.39±0.18	2.57±0.12	2.31±0.14
Dancing velocity (DNC, $\mu m^2/s$)	208±15.52	225±10.74	211±13.03
Dancing mean (DNM, $\mu m^2/s$)	5.70±0.46	6.33±0.35	6.33±0.50

No significant difference was found between breeds or between bulls within the breed for any of the traits ($p>0.05$).

Table 3: Average sperm velocity and kinematics attributes of only progressively motile sperms of fresh semen of Gir, Surti and Murrah bulls assessed by Biovis CASA

Seminal attributes	Breeds of bull		
	Gir (n=24)	Surti (n=24)	Murrah (n=24)
Average path velocity (VAP, µm/s)	53.14±1.24	52.27±1.18	53.30±1.44
Curvilinear velocity (VCL, µm/s)	88.95±1.72	89.64±1.95	90.92±2.10
Straight line velocity (VSL, µm/s)	49.39±1.28	48.55±1.23	49.47±1.50
Linearity (LIN, %)	56.05±1.21	54.51±1.10	54.86±1.43
Straightness (STR, %)	92.05±0.39	92.06±0.46	91.86±0.45
Wobbling index (WOB, %)	60.25±1.08	58.65±0.95	59.06±1.29
Beat cross frequency (BCF, hz)	16.81±0.66	17.83±0.51	16.80±0.67
Amplitude of lateral head displacement (ALH, µm)	2.35±0.19	2.58±0.12	2.28±0.15
Dancing velocity (DNC, $\mu m^2/s$)	210±16.67	229±11.68	212±14.54
Dancing mean (DNM, $\mu m^2/s$)	4.48±0.37	4.86±0.26	4.57±0.33

No significant variation was seen between breeds or between bulls within the breed for any of the traits ($p>0.05$).

curvilinear velocity, straightness and lateral head displacement as 79.9 & 58.4 per cent, 98.3 µm/sec, 156.4 ìm/s, 84.5 per cent, and 5.0 µ, respectively. Hoflack *et al.* (2007) using CASA found lower quality of Blue bull sperms as compared to those of HF bulls. Patel *et al.* (2012) reported significantly ($p<0.05$) higher mean values of motile and progressively motile sperm, average path velocity and BCF in fresh semen of Jafarabadi and Mehsana buffalo bulls than in crossbred bulls, but the other Hamilton CASA traits did not differ between them. Our findings in terms of all motion characteristics of freshly diluted buffalo sperms concurred well with the recent report of Kumar *et al.* (2018) using similar Biovis CASA, suggesting that this simple CASA can give reliable and useful information more accurately than visual assessment and may serve the purpose of economically assessing the sperm kinematics on bovine semen stations.

Conclusion

The subjective sperm motility rating and total motile sperm obtained using Biovis CASA were almost same in both cattle and buffalo semen. In sperms of all three breeds, curvilinear velocity (µm/s) and straightness (%) were higher (88-92) as compared to average path velocity (µm/s), straight line velocity (µm/s), linearity (%) and wobbling index (all around 48-60). The ratio of BCF to ALH and DNC to DNM were roughly 7:1 and 45:1, respectively.

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Conflict of Interest:

The authors have no conflict of interest.

References:

Amann, R.P. and Waberski, D. (2014). Computer-assisted sperm analysis (CASA): Capabilities and potential developments. *Theriogenology*, **81**: 5-17.

Christensen, P., Boelling, D., Pedersen, K.M., Korsgaard, I.R. and Jensen, J. (2005). Relationship between sperm viability as determined by flow cytometry and nonreturn rate of dairy bulls. *J. Androl.,* **26**: 98-106.

Goovaerts, I.G., Hoflack, G.G., Van Soom, A., Dewulf, J., Nichi, M., de Kruif, A. and Bols, P.E. (2006). Evaluation of epididymal semen quality using the Hamilton-Thorne analyser indicates variation between the two caudae epididymides of the same bull. *Theriogenology*, **66**: 323-330.

Hoûack, G., Opsomer, G., Rijsselaere, T., Van Soom, A., Maes, D., Kruif de, A. and Duchateau, L. (2007). Comparison of computer assisted sperm motility analysis parameters in semen from Belgian Blue and Holstein-Friesian bulls. *Reprod. Dom. Anim.,* **42**: 153-161.

Johnson, J., Boone, W. and Blackhurst, D. (1996). Manual versus computer-automated semen analysis. Part I. Comparison of counting chambers. *Fertil. Steril.,* **65**: 150-155.

Karthikeya, G. (2003). *Computer automated motility and morphometric analysis of bull and buck spermatozoa.* MVSc Thesis, Tamil Nadu Veterinary & Anim. Sci. Univ., Chennai, India.

Kathiravan, P., Kalatharan, J., Edwin, M.J. and Veerapandian, C. (2008). Computer automated motion analysis of crossbred bull spermatozoa and its relationship with *in vitro* fertility in zona-free hamster oocytes: *Anim. Reprod. Sci.,* **104**: 9-17.

Knuth, U.A., Neuwinger, J. and Nieschlag, E. (1989). Bias of routine semen analysis by uncontrolled changes is laboratory environment – detection by long term sampling of monthly means for quality control. *Int. J. Androl.,* **12**: 373-383.

Kumar, S., Kumar, A., Singh, A. K., Honparkhe, M., Singh, P. and Malhotra, P. (2018). Improvement in post-thaw semen quality by minimizing the lipid peroxidation following herbal treatment in sub fertile buffalo bulls. *The Pharma Innovation*, **7**(5, Part D): 240.

Mandal, D.K., Nagpaul, P.K. and Gupta, A.K. (2003). Motion characteristics of Murrah buffalo bull spermatozoa in various seasons and its relationship with functional integrity of the plasmallema. *Theriogenology,* **60**(2): 349-358.

Mortimer, D., Keel, B.A. and Webster, B.W. (1990). Objective analysis of sperm motility and kinematics. Eds. *Handbook of the Laboratory Diagnosis and Treatment of Infertility.* Boca Raton, Fla. CRC Press, pp. 97-133.

Patel, J.B. and Dhami, A.J. (2016). Computer assisted sperm analysis of fresh and frozen-thawed buffalo semen and their interrelationship. *Indian J. Anim. Res.*, **50**(1): 8-13.

Patel, J.B., Dhami, A.J., Patel, P.A. and Savalia, F.P. (2012). Assessment of motility and velocity parameters of freshly ejaculated spermatozoa of *Bubalus bubalis* and *Bos indicus* bulls through computer assisted semen analyzer. *Indian J. Field Vets*., **8**(1): 18-22.

Ramachandran, N., Verma G.S., Gupta, A.K., Raina, V.S., Mohanty, T.K. and Singh, M.K. (2007). Prediction of fertility based on frozen-thawed seminal traits in Sahiwal bulls. *Indian J. Anim. Sci.,* **77**(8): 884-887.

Rodriguez-Montaña, D. and Roa-Guerrero, E. (2017). Objective assessment of bull sperm motility parameters using computer vision algorithms. *African J. Biotech.,* **16**(37): 1871-1881.

Snedecor, G.W. and Cochran, W.G. (1994). *Statistical Methods*. 14th edn. Oxford and IBH Publishing House, New Delhi, India.

Verstegen, J., Iguer-Ouada, M. and Onclin, K. (2002). Computer assisted semen analyzers in andrology research and veterinary practice. *Theriogenology,* **57**(1): 149-179.

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