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Study on sperm motility and velocity parameters of freshly collected mithun semen through computer-assisted sperm analyser (CASA)

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ABSTRACT

The objective of this study was to evaluate the quality of mithun semen by computer assisted sperm analysis (CASA). Ejaculates (50) were analysed by CASA. Semen motility was evaluated for kinetics parameters. The percentage of total motile and progressive motile spermatozoa were determined. Several velocities parameters were also determined viz., average path velocity (VAP, µm/s), track speed (VCL, µm/s), progressive velocity (VSL, µm/s), lateral amplitude (ALH), beat frequency (BCF, Hz), straightness (%), elongation (%), linearity (%), and area (µm sq). The result revealed a strong positive correlation between various CASA parameters (PMOT-VAP, PMOT- VSL, VAP-VSL, VAP-ALH, VSL-ALH, STR-LIN).The present study confirmed the usefulness of CASA for a quick and objective analysis of sperm concentration, motility and other velocity parameters.

Key words: CASA, Mithun, Sperm motility, Velocity parameters

Till now, assessment of semen quality (i.e. sperm concentration, motility, and morphology) was based on subjective microscopic techniques (Rijselaere *et al.* 2005). Yet, the inconsistency of results obtained with the use of conventional methods of semen evaluation is high (Iguer–ouada *et al*. 2001). Computer-assisted sperm analysis (CASA) system allows an accurate, repeatable, reliable and objective assessment of different semen parameters such as concentration, total and progressive motility, and different velocity parameters (Rijselaere *et al.* 2012) based on the measurement of individual sperm cells. Recent reports suggested that CASA does not only measure the proportion of motile spermatozoa but also measures other sperm motion parameters derived from individual sperm cells and it has more predictive power on fertility potential of semen ejaculates (Mortimer 1994). Spermatozoa kinematic parameters such as forward progressive motility (PFM), straight line velocity (VSL), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), and linearity (LIN) were positively correlated with bull fertility (Perumal *et al.* 2011). A spermatozoon has significantly higher VCL and ALH, indicating that there is major bending of the mid piece and large amplitude of lateral head displacement. This signifies the hyperactivation of the spermatozoa. Hyperactivation in turn implies high energy state of the spermatozoa, which is essential for sperm penetration through cervical mucus, *zona pellucida*, fuse with the oocytes, and successful fertilization (Aitken *et al.* 1985). Study of CASA parameters was reported in domestic animal species such as cattle (Perumal *et al.* 2011). Hence, the aim of this study was to determine the sperm motile parameters of mithun semen using a computer assisted sperm analyser.

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MATERIALS AND METHODS

Mithun semen ejaculates (50) were collected during March 2015 to March 2016. Semen samples were collected through rectal message method from adult healthy mithun, maintained in the mithun farm of National Research Centre on Mithun (ICAR), Jharnapani, Nagaland, India with age ranging between 3–5 years and body weight approximately 300–450 kg. During the study, the animals were handled as per regulations of Institutional Animals Ethics Committee. Immediately after collection, the semen samples were kept in a water bath at 37°C and transported to the laboratory in an insulated flask maintaining 37°C for routine analysis (volume, consistency, colour and pH) within an hour. After the preliminary evaluations, ejaculates were subjected to the initial dilution with pre-warmed (37°C) tris-citrate extender. Semen samples were assayed using CASA (Hamilton Thorne Sperm Analyser, IVOS 11; HTM-IVOS, Version 10.8, Hamilton Thorne Research, Beverly, MA, USA) equipment. This CASA system is equipped with a phase-contrast microscope, camera, minitherm heating stage and image digitizer attached with a computer. The settings for CASA system are shown in Table 1. Diluted semen (2 µl) was mounted in a disposable Leja counting chamber

Table 1. Software application settings for CASA system used in this study

Parameter	Value
Chamber type	Leja 4
Temperature of analysis (°C)	37.0
Fields acquired	10
Frame rate (Hz)	60
Number of frames	30
Minimum static contrast	35
Minimum cell size (pixels)	5
Straightness (STR), thresholds (%)	70
VAP cut-off (µm/s)	30
Progressive minimum VAP (µm/s)	50
VSL cut-off (µm/s)	15
Cell intensity	80
Magnification	1.89

and was allowed to settle on the minitherm heating stage (37°C) before the analysis and sperm concentration and parameters of sperm motility determined.

Parameters measured included percentage of motile spermatozoa (MOT), percentage of spermatozoa with a progressive motility (PMOT), velocity average pathway (VAP), the mean trajectory of the spermatozoa per unit of time; velocity straight line (VSL), the straight trajectory of the spermatozoa per unit of time; velocity curvilinear (VCL), the instantaneously recorded sequential progression along the whole trajectory of the spermatozoon per unit of time; amplitude of lateral head displacement (ALH), the mean width of sperm head oscillation (Schäfer-Somi and Aurich 2007); beat cross frequency (BCF), the frequency with which the sperm crosses the smoothed path; straightness (STR), and linearity (LIN), the ratio of the straight displacement in the sum of elementary displacements during the time of the measurement which is defined as (VSL/VCL) × 100. According to the low VAP cut-off and medium VAP cut-off, the sperm population was additionally divided into four categories viz. rapid, medium, slow and static. A minimum of 200 spermatozoa from at least two different drops of each sample were analyzed from each specimen.

The results were analysed statistically and expressed as the mean±SEM. Correlation between the motility and velocity parameters was established with correlation coefficient being done as per Pearson's method. Differences at P<0.01 were considered to be statistically significant by using SPSS 16.

RESULTS AND DISCUSSION

The result of sperm motility showing mean values for each sperm parameter after selection of motile sperms are depicted in Table 2. The sperm motility recorded in this study was same as earlier report (50 to 86%), but the progressive sperm motility is higher marginally (20 to 38%).This result was in agreement with Alapati *et al.* (2009) who reported that the percentage of motile sperm must be above 33%. For the percentage of progressive sperm, our observation was also consistent with Galli *et al.* (1991) who said that this should be above 22%. These motile parameters are important because they were highly correlated with sperm viability and fertility (Kommisrud *et al.* 1996).

Table 2. Mean (±SEM) motility and velocity parameters of mithun semen

CASA parameter	Mean ± SEM (n=50)
Cell count	
Motility (%)	61.74±1.30
Progressive motility (%)	27.14±0.94
Velocity distribution	
Rapid (%)	51.76±1.87
Medium (%)	10.08±1.45
Slow (%)	9.40±0.79
Static (%)	28.72±1.46
Mean value	
Path velocity (VAP) (µm/s)	104.76±3.03
Track speed (VCL) (µm/s)	219.96±19.62
Progressive velocity (VSL) (µm/s)	74.58±2.43
Lateral amplitude (ALH) (µm/s)	8.24±0.25
Beat frequency (BCF) (Hz)	25.77±0.59
Straightness (STR) (%)	69.18±0.77
Linearity (LIN) (%)	38.98±0.94
Elongation (%)	48.18±1.17
Area (µm sq)	11.31±0.61

In our study, the velocity parameters of mithun bulls were highly varied as compared with earlier report (Farrell *et al.* 1996). This may be due to some factors such as age, time of collection, time between ejaculations, energy stores of sperm, presence of surface acting agents in the cell membrane such as agglutinins and detergents, viscosity, osmolarity, pH, temperature, ionic concentration of seminal plasma, and presence of the mineral elements (Cu, Zn, Mn), hormones and prostaglandins, that affect sperm motility (Blasco 1984).

The motility parameters are essential for the fertilisation process in other species (Donnely *et al.* 1998). Fertilisation rates of human oocytes *in-vitro* have been shown to correlate positively with sperm velocity (Sallam *et al.* 2003). Thus, not only assessment of the percentage of motile spermatozoa is important, but also velocity parameters are different depending on the fertility of the male.

Table 3. Pearson Correlation coefficients between several CASA parameters of mithun sperm

	MOT	PMOT	VAP	VSL	VCL	ALH	BCF	STR	LIN
MOT	1	.477**	.036	-.077	.065	-.111	-.177	-.077	.095
PMOT		1	.662**	.661**	.117	.298	-.177	.209	.160
VAP			1	.951**	.083	.723**	-.164	-.063	-.149
VSL				1	.080	.666**	-.197	.170	.015
VCL					1	.168	.052	.017	-.041
ALH						1	.067	-.239	-.358
BCF							1	-.297	.371**
STR								1	.901**
LIN									1

**P< 0.01.

The average path velocity was significantly and positively correlated with progressive velocity (VSL) and lateral amplitude (ALH).The high positive correlation observed between PMOT and VSL, between VAP and VSL indicated that the velocity parameters were correlated and interrelated among them and with VSL. Likewise, there was a strong positive correlation between VAP and ALH, between VSL and ALH revealing their interrelation with ALH.There was a strong positive correlation observed between LIN and STR (Table 3). Similar result was reported by Perumal *et al.* (2014). There was a negligible correlation observed between BCF and ALH which is in contradiction with the work reported by Perumal *et al.* (2011) and Kumar *et al.* (2011) though ALH and BCF representing the head behaviour of the sperm are highly variable and mean values of these parameters were within the range and corroborated with the studies of Perumal *et al.* (2011) and Kumar *et al.* (2011). Our study is in agreement with the work of Oliveira *et al.* (2013) and in contradiction with the studies of Gillan *et al.* (2008) who showed that merely VSL, VCL, ALH, BCF parameters were involved in certain combinations with other specific *in vitro* assessed parameters which had high

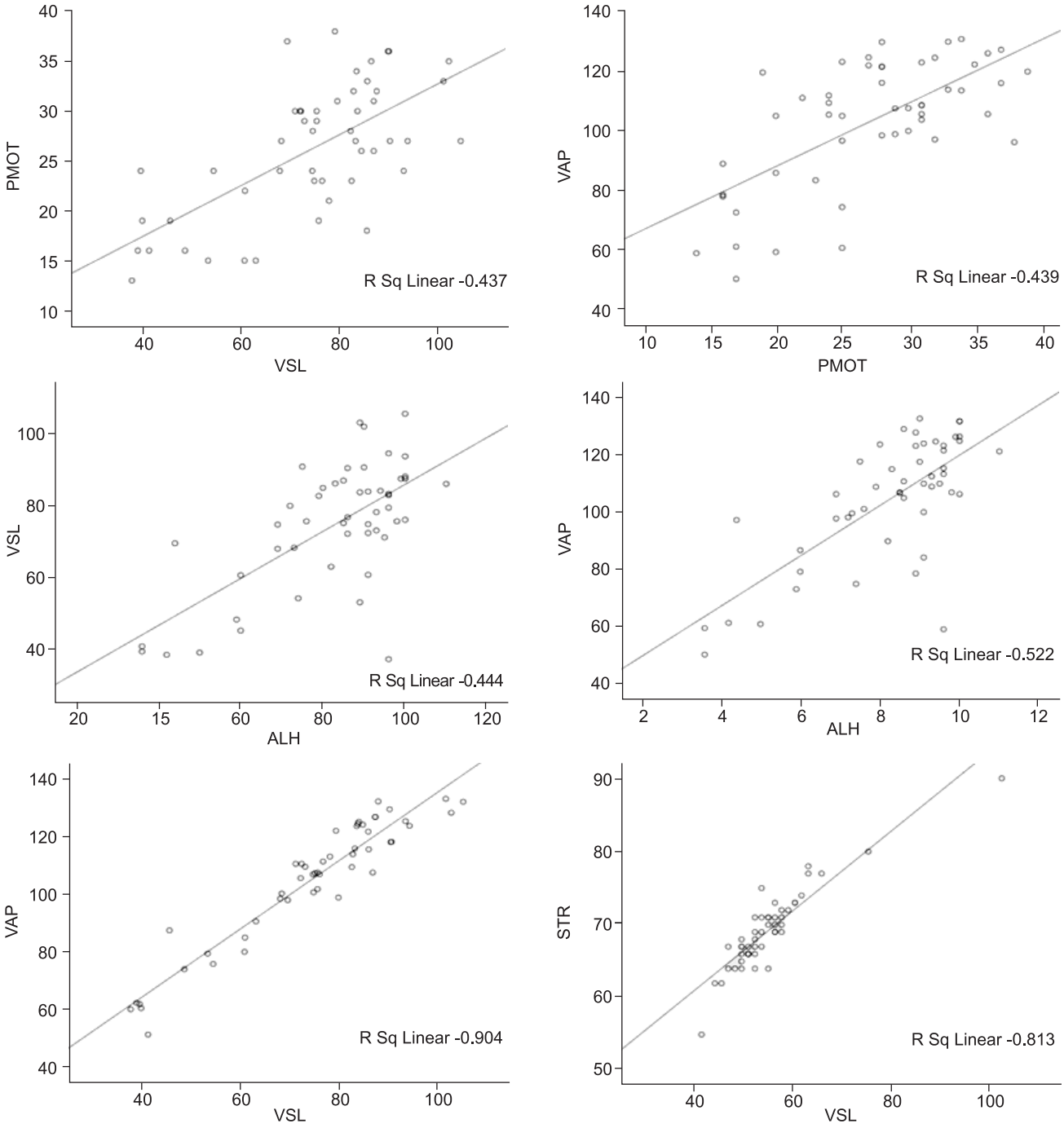

Fig. 1. Scatter diagram showing correlation between Computer-aided sperm analysis (CASA) parameters of mithun semen ejaculates. Data points represent individual ejaculates (n=50).

correlation values.

The CASA variable such as linearity or linear motility is higher indicating that spermatozoa have higher rate of fertilization potential in comparison to the total motility percentage (Cremades *et al.* 2005) and semen samples containing such spermatozoa have higher fertility rates and pregnancy rates after artificial insemination (Farrell *et al.* 1998).

In summary, the assessment of sperm motility using the conventional microscopical methods is difficult and subjective. There is always a chance of high variations for the estimation of motility parameters of the same ejaculates. The Hamilton-Thorne IVOS sperm analyzer is a reliable method for determining sperm parameters, because of its ability to sense even the least changes in sperm motion and to produce unfailing results without any bias.

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