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COEFFICIENT OF VARIATION ASSESSMENT FOR SEMINAL TRAITS EVALUATED BY COMPUTER ASSISTED SEMEN ANALYSIS (CASA)

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Abstract: Objective assessment of qualitative and quantitative sperm traits were analysed by Computer assisted semen analysis (CASA). The reliability of the CASA results is questionable because precision of the results depends on many parameters. The present study was focussed on the attainment of most reliable or consistent variable among the CASA parameters by deriving Coeffienct of Variation (CV).

Keywords: sperm traits, Computer assisted semen analysis, reliability, precision, Coefficient of variation.

Introduction

Computer assisted semen analysis (CASA) is considered as an efficient method for the prediction of male fertility due to its efficiency in objective measurement of semen quality rather than conventional semen analysis. It is primarily used to obtain accurate and objective kinetic sperm measurements but the reliability of data is often limited (Ehlers et al., 2011) due to its variability. The variability is expected to adversely impact patient care when results for semen analysis parameters (ie, sperm concentration, motility, and vitality) on the same semen sample are reported as normal by one laboratory and as consistent with infertility by another laboratory (Auger et al., 2000). The repeatability of CASA parameters indicates high precision of the data which can be enhanced by standard operating procedure, dilution factor, optimal mixing of semen and dilution buffer, and optimal training of technicians (Broekhuijse et al., 2011). Thus the coefficient of variation (CV) indicates the precision of the data by giving the percentage variation in the mean. In the present study, the less variable seminal parameters were assessed among CASA parameters to know the consistent seminal trait which gives accurate information about the sperm characteristics.

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Material and methods

In the present study, semen (108 ejaculates) was collected from Eight Deccani Rams stationed at Instructional Livestock Farm Complex (ILFC), located at college of Veterinary science, Rajendranagar, Hyderabad. The collected semen was diluted in the buffer (1:4 ratio) and the semen characteristics (motility, velocity and morphological traits) were assessed objectively using a computer assisted sperm analysis (CASA; HTMIVOS v. 10.6; Hamilton–Thorne, Beverly MA, USA). Semen samples (5 µl of diluted semen containing 20×10^6 spermatozoa/ml) were placed on pre-warmed slides at 37°C and covered by coverslip before immediate transfer to the CASA. Semen characteristics were determined by assessment of at least six randomly selected microscopic fields (>200 spermatozoa/sample). Description of CASA parameters measured in this study was listed in Table No 1. (Boshoff, 2014). Among the motility parameters, Wobble percentage was derived by the formula: (VAP/VCL) x 100 (Mircu et al., 2008) and expressed in percentage.

After assessing the mean and the standard deviation of the seminal traits by Analysis of Variance (ANOVA), the coefficient of variation (CV) was derived by the following formula:

Coefficient of Variation = (Standard Deviation / Mean) * 100.

Results and Discussion

The CV values for total motility, progressive motility, rapid motility, averge path velocity, straightline velocity, curvilinear velocity, straightness, linearity, wobble, amplitude of lateral head displacement, beat cross frequency, area and elongation were represented in the Table No.2.

Oliveira et al., (2013) also reported that total motility, progressive motility or BCF, were found to be predictors of bovine in vivo fertility. In the present study, the lower CV values of total motility (%) and progressive motility (%) indicates its precision and were regarded as more useful for assesment of semen quality followed by sperm elongation (%), area (µm), ALH (µm), BCF (Hz). Motility (%) had lowest CV (12%) which was comparable to bull seminal studies (16%) while CV of progressive motility, VAP, VSL, VCL ALH, BCF, STR and LIN was lower compared to that of bull semen (21-44%) (Yowell, 2011).

Davis *et al.* (1992) analysed human semen samples reported that the coefficient of variation (CV) for repeated measures was between 1% and 8% for each variable on all CASA

instruments. In the present study, the CV ranged between 12.5 to 31.74 % which is comparable to the CV of semen parameters of boar stud (4.7% to 34.7%) (Reicks, 2012). The difference in CV in between the human and animal semen samples can be attributed to the threshold setting which is particular for each CASA machine and for ram semen samples, the precise threshold setting for each machine was not standardised which caused a higher CV compared to other species. Thus there was a need for standardisation of threshold settings pertaining to ram semen samples to increase the precision of the data.

Conclusion

The coefficient of variation was least for total motility (%) and progressive motility (%) indicating it as the most useful parameters among the other CASA motility parameters due to its high precision.

There is no conflict of interest regarding the present study.

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Table No. 1: Description of motility parameters measured in Computer assisted semen analysis (CASA)

Motility parameter	Description
Motility (%)	Percentage of sperm on field showing motility, classified into fast, medium, or slow motility, and thus also the amount of static/immotile sperm.
Progressive motility (%)	Percentage of sperm moving progressively forward.
Curvilinear velocity (VCL)	Measured in μm/s. It is the time/average velocity of a sperm head along its actual curvilinear trajectory.
Average path velocity (VAP)	Measured in μm/s. It is the time/average of a sperm head along the spatial average trajectory of sperm.
Straight/line velocity (VSL)	Measured in μm/s. It is the time/average velocity of a sperm head along the straight line between the first detected position and the last detected position.
Amplitude of the lateral head displacement (ALH)	Measured in μm. It is the magnitude of the lateral displacement of a sperm head about its spatial average trajectory. It can be expressed as either a maximum, or an average of these displacements.
Straightness (STR)	This measures the linearity of the spatial average path, and is calculated by dividing the VSL with the VAP.
Linearity (LIN)	It measures the linearity of the curvilinear trajectory, calculated as the VSL divided by the VCL.
Wobble (WOB)	This measures the oscillation of the actual trajectory.
Beat cross frequency (BCF)	Measured in Hz. This is the time average-average rate at which the curvilinear sperm trajectory crosses its average path trajectory.

Table No.2: Coefficient of variation (CV) for CASA parameters

CASA PARAMETERS	**CV**
Total motility (%)	12.50
Progressive motility (%)	13.10
Rapid motility (%)	14.62
Average path velocity (µm/sec)	28.67
Straight line velocity (µm/sec)	31.74
Curvilinear velocity (µm/sec)	24.93
Straightness (%)	19.33
Linearity (%)	28.70
Wobble (%)	24.77
Amplitude of lateral head displacement (µm)	18.44
Beat cross frequency (Hz)	18.76
Elongation (%)	14.85
Area (μm^2)	14.86