

## CASA-BASED EVALUATION OF SPERM MOTILITY AT FROZEN-THAWED STAGE IN HARIANA BULLS

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### ABSTRACT

Thawed frozen semen of Hariana bulls (n=4, one straw from each of 15 ejaculates per bull) was evaluated by CASA for sperm motion and velocity parameters. One of the bulls had higher (p<0.05) average path velocity of sperm (VAP), curvilinear velocity (VCL) and amplitude of lateral head displacement (ALH) and, had lower (p<0.05) slow velocity when compared to other bulls. This suggested that CASA can be used to indirectly assess the fertility potential of Hariana bulls.

**Keywords:** CASA, Fertility, Frozen semen, Hariana bull, Thawing

The population of Hariana cattle, a prominent dual purpose cattle breed of North India, is following a declining trend (Joshi *et al.*, 2012). However, the superior germplasm of Hariana bulls can be used for grading up the non-descript cattle. Computer Assisted Semen Analysis (CASA) is suggested to provide prediction about fertility potential of semen ejaculates by measuring not only the proportion of motile spermatozoa but also other sperm motion parameters derived from individual sperm cells (Smith *et al.*, 2001). Therefore, the present study was conducted to determine the sperm motion parameters in frozen-thawed semen of Hariana bulls by using CASA.

Semen was collected from four healthy Hariana bulls, twice a week, using artificial vagina (at 42°-45°C). The semen was diluted with Tris egg yolk-glycerol. The freezing of straws was carried out by computerized programmable bio-freezer. After 24 h of cryopreservation, sixty frozen semen straws (one straw from each of 15 ejaculates of each bull) were thawed in water bath maintained at 37°C for 30 seconds and were evaluated by CASA (Hamilton

Thorne Biosciences, IVOS Version 12.3; Najjar *et al.*, 2013). The frozen-thawed samples were assessed for sperm motility and velocity parameters like total motility (TM, %), progressive motility (PM, %), average path velocity of sperm (VAP, m/s), straight line velocity of sperm (VSL, m/s), curvilinear velocity (VCL, m/s), amplitude of lateral head displacement (ALH, m), beat cross frequency (BCF, Hz), straightness (STR, %), linearity (LIN, %), elongation (%), area (m sq) as well as rapid, medium, slow and static velocities (%). Statistical analysis between different parameters was conducted by ANOVA and *post-hoc* analysis using SPSS statistics software version 20.

The results revealed that H-674 Bull had higher (p<0.05) VAP, VCL, ALH and lower (p<0.05) slow velocity when compared to other three bulls (Table 1). However, other velocity and kinetic parameters were similar (p>0.05) among the bulls (Table 1). As the literature was not available regarding CASA parameters in Hariana bulls, therefore, the comparisons were made with other breeds of cattle bulls including buffaloes. The observed values of VAP, VCL and ALH in frozen semen of Sahiwal bull (after 24 h of freezing) were lower (Galmessa *et al.*, 2014) than the present results in Hariana bulls. In HF X Kankrej bull (F1), VCL and

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**Table 1: CASA parameters of frozen-thawed semen of Haryana bulls**

Parameters	Bull number			
	H-761	H-674	H-617	H-624
Total motility, %	89.0±1.5	84.7±4.2	93.6±1.1	85.3±3.5
PM, %	55.1±3.5	57.0±5.1	55.2±4.9	58.9±3.0
VAP, m/s	73.2±3.7 <sup>ab</sup>	83.2±6.5 <sup>b</sup>	73.4±3.7 <sup>ab</sup>	67.5±3.9 <sup>a</sup>
VSL, m/s	55.1±3.5	63.9±6.5	54.0±2.3	52.3±3.3
VCL, m/s	130.1±5.2 <sup>ab</sup>	142.3±8.5 <sup>b</sup>	128.8±7.2 <sup>ab</sup>	111.5±3.9 <sup>a</sup>
ALH, m	7.0±0.5 <sup>ab</sup>	7.4±0.5 <sup>b</sup>	7.4±0.4 <sup>b</sup>	6.1±0.2 <sup>a</sup>
BCF, Hz	24.9±1.7	22.9±1.7	23.0±1.6	25.0±1.2
Straightness, %	71.3±2.6	72.9±2.8	71.7±2.0	75.6±2.0
Linearity, %	41.6±2.7	43.9±3.2	42.1±1.8	46.1± 1.9
Elongation, %	48.3±1.8	46.7±2.0	46.1±1.8	44.2±1.0
Area, m sq	6.6±0.8	6.4±0.1	6.8±0.8	7.7±1.0
Rapid velocity, %	64.6±2.9	68.3±4.0	71.5±1.7	67.5±3.3
Medium velocity, %	23.4±2.6	17.0±3.1	20.6±2.0	17.6±1.9
Slow velocity, %	7.8±0.9 <sup>b</sup>	3.7±1.1 <sup>a</sup>	3.6±0.6 <sup>a</sup>	6.1±1.0 <sup>ab</sup>
Static velocity, %	3.1±1.2	10.2±3.5	2.6±0.7	8.6±3.2

Figure having different superscript within row (a, b) differ significantly ( $p < 0.05$ ); PM, Progressive motility; VAP, Average path velocity of sperm; VSL, Straight line velocity of sperm; VCL, Curvilinear velocity; ALH, Amplitude of lateral head displacement; BCF, Beat cross frequency

ALH were similar to present observations, whereas, VAP was lower than the present findings (Patel and Dhama, 2013).

The average path velocity of sperm (VAP), curvilinear velocity (VCL) indicating major bending of the mid piece and the amplitude of lateral head displacement (ALH) were linked with fertilization rates *in vivo* and could be bioindicators of the fertilizing ability of sperm (Hirano *et al.*, 2001; Perumal *et al.*, 2011). These parameters signify the hyperactivation of the spermatozoa due to its high-energy state that is essential for sperm penetration through the cervical mucus, zona *pellucida*, fusion with oocytes, and subsequently resulting in a successful fertilization (Aitken *et al.*, 1985). Moreover, the spermatozoa motility and velocity parameters reflect their mitochondrial function and energy status that is indirectly correlated to fertility of sperm (Farrell *et al.*, 1996). In brief, most of the CASA parameters of Haryana bulls were at acceptable level. Certain velocity parameters were

better in a bull compared to other bulls that indirectly indicated their better fertility rate.

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