RESEARCH ARTICLE

# Correlations of Motion Characteristics and Kinematic Attributes of Fresh and Frozen-thawed Spermatozoa of Gir Bulls

PK Pathak[1], AJ Dhami[2], DV Chaudhari[3]


## Abstract

This investigation was carried out on semen of three healthy mature breeding bulls of Gir breed to evaluate the interrelationships among sperm quality attributes of fresh and frozen-thawed semen assessed by Biovis CASA. The ejaculates (n = 24) having >75% initial motility were diluted @80 million sperm/mL using TFYG extender, filled in French mini straws, and were frozen using a programmable bio freezer after 4 hours of equilibration. The straws were thawed in a water bath at 37°C for 30 sec. The freshly diluted and frozen-thawed samples were assessed for routine subjective tests and various motion characteristics/kinematics by Biovis CASA. The Pearson's correlations for sperm motility and velocity/kinematic parameters of total motile sperm as well as of progressively motile sperm were studied in freshly diluted and frozen-thawed semen. In fresh semen, total motile sperm assessed by CASA had significant (*p* <0.05, 01) correlations with rapid progressive motile sperm (r = 0.46), wobbling index (r = 0.52) and dancing frequency (r = -0.43) in fresh semen. In frozen-thawed semen, it was significantly correlated only with linearity (r = 0.46). The rapid progressive motile sperm in both fresh (r = 0.41 to 0.92) and frozen-thawed (r = 044 to 0.88) semen, however, had significant correlations with most of their velocity traits. Further, the average path velocity (VAP), curvilinear velocity (VCL), straight line velocity (VSL), linearity (LIN), straightness (STR), wobbling (WOB), beat-cross frequency (BCF), amplitude of lateral head displacement (ALH), and dancing mean (DNM) of sperm showed significant positive or negative interrelationships among each other in both fresh (r = 0.41 to 0.91) as well as post-thawed (r = 0.44 to 0.90) semen. Moreover, the correlations of motility and kinematics parameters of total motile sperm in both fresh and frozen-thawed semen were highly significant with velocity/kinematics traits of only progressively motile sperm, and the velocity traits among only progressively motile sperm were highly significantly interrelated in both fresh (r = 0.46 to 0.98) and frozen-thawed (r = 0.43 to 0.93) semen of Gir bulls, though the magnitudes of correlations were lower in frozen-thawed semen as compared to fresh semen. Thus, CASA analysis of fresh semen for motility and velocity traits could predict the post-thawed sperm motility and velocity/kinematics of bovine semen.


**Keywords:** CASA, Fresh and frozen-thawed sperm, Gir bull, Interrelationships, Kinematics, Motion characteristics.

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## Introduction

Semen analysis is commonly used to evaluate the quality of ejaculates and male fertility potential (Verstegen *et al.,* 2002; Patel *et al.*, 2012). However, no single test or combinations of tests have been proved to be totally reliable for accurate prediction of semen quality in relation to fertility. The conventional evaluation of bovine semen is relatively inaccurate, imprecise, time consuming (Christensen *et al*., 2005), and varies with experience and skill of the investigator (Patel and Dhami, 2016), while motility, velocity, and morphology assessed by CASA could improve precision and accuracy in less time and prove worth for assessing bull fertility (Ramachandran *et al.,* 2007). Hence recently more attention has been given to evaluating sperm motion characteristics using CASA (Amann and Waberski, 2014). The correlations of the physical characters with fertility are highly variable and relatively poor (Shelke and Dhami, 2001; Chaudhary *et al.,* 2017). The literature on the use of CASA in bovine semen laboratory, and the interrelationships of spermatozoal attributes of fresh and cryopreserved bovine semen assessed by CASA is meager (Anderson *et al.,* 1992; Mandal *et al.,* 2003; Patel and Dhami, 2013 and 2016; Pathak *et al.,* 2018). Hence, the objective of the present study was to know the interrelationships among the motility and kinematics attributes of fresh and frozen-thawed sperm of Gir bulls assessed using CASA.

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## Materials and methods

The present study was carried out on semen of three healthy mature breeding bulls of Gir breed, aged 5–6 years, at the College of Veterinary Science, AAU, Anand-388 001 during the winter season of the year 2017–2018. 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 bull in the morning hours between 7.30 and 8.30 hours over a dummy buffalo bull. Ejaculates collected at the weekly interval were used for this study.

Immediately after collection, the ejaculates (n = 24; 8 per bull) were evaluated for various seminal attributes. Samples with >75% initial motility were extended at 34°C with Tris-citric acid-fructose-egg yolk-glycerol (TFYG) extender keeping 80 million sperm per ml. The extended semen was assessed subjectively for sperm motility and by Biovis CASA (Expert Vision Pvt. Ltd., Mumbai) for all motion characteristics and velocity/kinematics parameters of spermatozoa. For this 50 µL of diluted semen was placed on grease-free clean sterile ordinary glass-slide, covered with a coverslip, and was examined under 40 X of a phase contrast microscope with Biotherm connected to a CCTV screen. The analysis set up was selected as per the manufacturer's instruction.

The extended semen was soon filled and sealed in French mini straws by IS4 machine (IMV, France), cooled to 4°C, equilibrated for 4 hours and frozen in liquid nitrogen vapor using a bio-freezer (IMV, France) employing standard freezing protocol. The straws were thawed in a water bath at 37°C for 30 seconds and were assessed again subjectively and objectively for all the traits studied in freshly extended semen. The CASA traits studied included total motile sperm, rapid progressive, slow progressive and 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 kinematic traits were studied among total motile sperm as well as among only progressively motile sperm. The data so generated on sperm quality traits were analyzed for Pearson's correlations using a standard statistical package on SPSS software version 20.00 (Snedecor and Cochran, 1994).

## Results and discussion

The interrelationships of sperm motility and velocity parameters of fresh semen, as well as post-thawed semen of Gir bulls assessed by Biovis CASA, are presented in Table 1.

Amongst CASA traits of fresh Gir bulls semen, the percentage of total motile sperm had significant ($p$ <0.05) positive correlations with rapid progressively motile sperm (0.46) and WOB (0.52), and negative correlation with DNC (–0.43) of total motile sperm. It was also positively correlated with LIN (0.47) and WOB (0.46), and negatively with ALH (–0.41), DNC (–0.46) and DNM (–0.46) of progressively motile sperm. In post-thawed semen, the percentage of total motile sperm had significant ($p$ <0.05) negative correlation only with LIN (–0.46) of total motile sperm.

The percentage of rapid progressive motile sperm in fresh semen had highly significant ($p$ <0.01) positive correlations with almost all velocity and kinematics attributes of total motile as well as progressively motile sperm (0.41 to 0.93) and negative correlation with slow progressive motile sperm (–0.61). The rapid progressive motile sperm in frozen-thawed semen had significant ($p$ <0.01) positive correlations with VCL, VAP, VSL, ALH, and BCF of total motile and progressively motile sperm (0.44 to 0.88), and negative correlation with slow progressive motile sperm (–0.62). The percentage of slow progressive motile sperm in fresh semen and frozen-thawed semen had almost similar but inverse relationships to those of rapid progressive motile sperm with other traits (Table 1).

The VCL of total motile sperm in fresh semen had significant positive correlations ($p$ <0.01) with VAP (0.68), VSL (0.56), BCF (0.71) and DNC (0.48) of all motile sperm, and with VCL (0.73), VAP (0.56), VSL (0.56), BCF (0.56) of progressive motile sperm. The VCL of total motile sperm in post-thawed semen had highly significant ($p$ <0.01) positive correlations with VAP, BCF, ALH, DNC and DNM (0.49 to 0.72) of total motile sperm, and with VCL, VAP, VSL, BCF, ALH, DNC, DNM (0.57 to 0.86) of progressive motile sperm. It had negative correlations with WOB index (–0.47, –0.50) of both total motile and progressively motile sperm.

The VAP of total motile sperm in fresh semen had significant ($p$ <0.01) positive correlations with VSL, LIN, STR, WOB and BCF (0.46 to 0.98) of total motile sperm, and with VAP, VSL, VCL, LIN, STR and WOB (0.50 to 0.91) of progressive motile sperm. The VAP of total motile sperm in frozen-thawed semen showed highly significant ($p$ <0.01) positive correlations with VSL, BCF, WOB (0.93, 0.76, 0.44) of all motile sperm, and with VCL, VAP, VSL, WOB, BCF (0.41 to 0.88) of progressively motile sperm.

The VSL of total motile sperm of fresh semen had significant ($p$ <0.01) positive correlations with LIN (0.76), STR (0.76), WOB (0.71) of all motile sperm, and with VAP, VSL, VCL, LIN, STR and WOB (0.41 to 0.91) of progressive motile sperm. It had a negative correlation with DNM of both total motile (–0.52) and progressively motile (–0.48) sperm, while VSL of all motile sperm in post-thawed semen showed highly significant ($p$ <0.01) positive correlations with LIN, STR, WOB, BCF (0.55 to 0.60) of total motile sperm, and with VAP, VSL, LIN, WOB, BCF (0.41 to 0.83) of progressive motile sperm.

The LIN percent of total motile sperm in fresh semen had highly significant ($p$ <0.01) positive correlations with STR (0.86) and WOB (0.93) of total motile sperm, and with VAP,

**Table 1:** Correlation coefficients (r) of sperm quality parameters of fresh and frozen-thawed semen of Gir bulls assessed by Biovis CASA

| Sperm traits | Motile sperm | | | Sperm velocity / kinematics (based on total motile sperm) | | | | | | | | | | Sperm velocity / kinematics (based on progressive motile sperm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total motile | Rapid progre | Slow progre | VCLt | VAPt | VSLt | LINt | STRt | WOBt | BCFt | ALHt | DNCt | DNMt | VCLp | VAPp | VSLp | LINp | STRp | WOBp | BCFp | ALH | DNCp | DNMp |
| Fresh Semen | | | | | | | | | | | | | | | | | | | | | | | |
| Motile | --- | 0.46* | 0.21 | 0.04 | 0.34 | 0.37 | 0.37 | 0.27 | 0.52** | -0.09 | -0.31 | -0.43* | -0.38 | -0.18 | 0.19 | 0.25 | 0.47* | 0.40 | 0.46* | -0.31 | -0.41* | -0.46* | -0.46* |
| Rapid | 0.35 | --- | -0.61** | 0.55** | 0.93** | 0.92** | 0.67** | 0.60** | 0.67** | 0.50* | -0.23 | -0.04 | -0.41* | 0.41* | 0.84** | 0.86** | 0.63** | 0.51* | 0.62** | 0.35 | -0.29 | -0.14 | -0.38 |
| Slow | 0.23 | -0.62** | --- | -0.65** | -0.61** | -0.49* | -0.08 | 0.06 | -0.10 | -0.61** | -0.12 | -0.39 | -0.10 | -0.65** | -0.71** | -0.64** | -0.14 | 0.04 | -0.18 | -0.66** | -0.13 | -0.34 | -0.115 |
| VCLt | 0.40 | 0.58** | -0.45* | --- | 0.68** | 0.56** | -0.09 | 0.07 | -0.06 | 0.71** | 0.31 | 0.48* | 0.19 | 0.73** | 0.56** | 0.56** | -0.04 | 0.13 | -0.07 | 0.56** | 0.22 | 0.38 | 0.20 |
| VAPt | 0.01 | 0.88** | -0.60** | 0.49* | --- | 0.98** | 0.64** | 0.62** | 0.63** | 0.46* | -0.25 | -0.01 | -0.41* | 0.50* | 0.90** | 0.91** | 0.60** | 0.52** | 0.59** | 0.32 | -0.31 | -0.14 | -0.39 |
| VSLt | -0.07 | 0.76** | -0.37 | 0.38 | 0.93** | --- | 0.76** | 0.76** | 0.71** | 0.37 | -0.35 | -0.12 | -0.52** | 0.41* | 0.86** | 0.91** | 0.69** | 0.66** | 0.66** | 0.23 | -0.50 | -0.25 | -0.48* |
| LINt | -0.46* | 0.11 | 0.10 | -0.52** | 0.40 | 0.58** | --- | 0.86** | 0.93** | -0.15 | -0.72** | -0.57** | -0.84** | -0.13 | 0.54** | 0.63** | 0.90** | 0.73** | 0.88** | -0.23 | -0.72** | -0.66** | -0.80** |
| STRt | -0.32 | -0.04 | 0.40 | -0.26 | 0.26 | 0.56** | 0.80** | --- | 0.73** | 0.07 | -0.45* | -0.32 | -0.67** | -0.03 | 0.42* | 0.52** | 0.66** | 0.78** | 0.59** | -0.09 | -0.47* | -0.43* | -0.55** |
| WOBt | -0.27 | 0.22 | 0.00 | -0.47* | 0.44* | 0.55** | 0.90** | 0.59** | --- | -0.20 | -0.75** | -0.68** | -0.89** | -0.28 | 0.45* | 0.54** | 0.96** | 0.67** | 0.96** | -0.37 | -0.79** | -0.75** | -0.87** |
| BCF t | 0.26 | 0.80** | -0.51* | 0.64** | 0.76** | 0.60** | -0.07 | -0.04 | 0.03 | --- | 0.67** | 0.77** | 0.46* | 0.68** | 0.43* | 0.36 | -0.27 | -0.09 | -0.28 | 0.88** | 0.62** | 0.71** | 0.55** |
| ALHt | 0.29 | 0.44* | -0.40 | 0.63** | 0.28 | 0.06 | -0.55** | -0.42* | -0.48* | 0.71** | --- | 0.92** | 0.91** | 0.42* | -0.21 | -0.31 | -0.83** | -0.59** | -0.82** | 0.66** | 0.98** | 0.94** | 0.96** |
| DNCt | 0.11 | 0.38 | -0.44* | 0.72** | 0.33 | 0.15 | -0.50* | -0.27 | -0.52** | 0.68** | 0.90** | --- | 0.87** | 0.71** | 0.10 | -0.01 | -0.74** | -0.53** | -0.74** | 0.85** | 0.93** | 0.99** | 0.91** |
| DNMt | 0.16 | 0.09 | -0.36 | 0.55** | -0.11 | -0.33 | -0.79** | -0.65** | -0.84** | 0.31 | 0.77** | 0.77** | --- | 0.46* | -0.23 | -0.34 | -0.91** | -0.71** | -0.89** | 0.60** | 0.94** | 0.92** | 0.96** |
| VCLp | 0.14 | 0.47* | -0.51* | 0.86** | 0.42* | 0.33 | -0.44* | -0.19 | -0.52** | 0.59** | 0.59** | 0.80** | 0.60** | --- | 0.70** | 0.62** | -0.27 | -0.12 | -0.29 | 0.84** | 0.47* | 0.63** | 0.44* |
| VAPp | -0.01 | 0.84** | -0.76** | 0.59** | 0.88** | 0.78** | 0.17 | 0.05 | 0.18 | 0.70** | 0.31 | 0.45* | 0.10 | 0.69** | --- | 0.99** | 0.48* | 0.37 | 0.48* | 0.49* | -0.19 | -0.00 | -0.27 |
| VSLp | 0.01 | 0.78** | -0.62** | 0.57** | 0.84** | 0.83** | 0.26 | 0.22 | 0.24 | 0.57** | 0.13 | 0.30 | -0.06 | 0.65** | 0.95** | --- | 0.58** | 0.51* | 0.56** | 0.40 | -0.30 | -0.12 | -0.37 |
| LINp | -0.28 | 0.19 | -0.05 | -0.49* | 0.35 | 0.48* | 0.86** | 0.50* | 0.93** | -0.17 | -0.64** | -0.68** | -0.83** | -0.56** | 0.15 | 0.25 | --- | 0.76** | 0.99** | -0.39 | -0.86** | -0.80** | -0.92** |
| STRp | 0.03 | -0.18 | 0.43* | -0.04 | -0.12 | 0.20 | 0.30 | 0.59** | 0.19 | -0.41* | -0.56** | -0.45* | -0.51* | -0.12 | -0.16 | 0.13 | 0.35 | --- | 0.66** | -0.25 | -0.64** | -0.61** | -0.68** |
| WOBp | -0.28 | 0.27 | -0.18 | -0.50* | 0.41* | 0.44* | 0.81** | 0.34 | 0.92** | -0.05 | -0.49* | -0.59** | -0.72** | -0.56** | 0.21 | 0.22 | 0.95** | 0.04 | --- | -0.39 | -0.85** | -0.79** | -0.91** |
| BCFp | 0.18 | 0.70** | -0.55** | 0.65** | 0.65** | 0.50* | -0.16 | -0.10 | -0.12 | 0.92** | 0.68** | 0.74** | 0.40 | 0.76** | 0.77** | 0.65** | -0.29 | -0.40 | -0.19 | --- | 0.70** | 0.81** | 0.64** |
| ALHp | 0.20 | 0.37 | -0.41* | 0.58** | 0.23 | 0.01 | -0.54** | -0.42* | -0.53** | 0.67** | 0.98** | 0.93** | 0.80** | 0.62** | 0.32 | 0.12 | -0.68** | -0.60** | -0.53** | 0.68** | --- | 0.97** | 0.98** |
| DNCp | 0.12 | 0.37 | -0.48* | 0.72** | 0.28 | 0.08 | -0.57** | -0.38 | -0.60** | 0.65** | 0.91** | 0.99** | 0.83** | 0.80** | 0.43* | 0.27 | -0.72** | -0.50* | -0.61** | 0.71** | 0.95** | --- | 0.96** |
| DNMp | 0.18 | 0.17 | -0.32 | 0.62** | 0.03 | -0.17 | -0.71** | -0.48* | -0.75** | 0.50* | 0.90** | 0.93** | 0.92** | 0.69** | 0.19 | 0.03 | -085** | -0.50* | -0.75** | 0.57** | 0.94** | 0.95** | --- |
| Post-thaw semen | | | | | | | | | | | | | | | | | | | | | | | |

n = 24, *Significant at $p$<0.05 level, **Significant at $p$ <0.01 level; t, Total motile sperm; p, Progressive motile sperm

VSL, LIN, STR, and WOB (0.54 to 0.88) of progressively motile sperm. It had negative correlations with ALH, DNC and DNM (–0.57 to –0.80) of both total motile as well as progressive motile sperm. The LIN of total motile sperm in post-thawed semen showed significant ($p$ <0.01) negative correlations with ALH, DNC and DNM (–0.50 to –0.79) of total motile and progressive motile sperm, while it had positive correlations with STR (0.80), WOB (0.90) of total motile sperm, and with LIN (0.86) and WOB (0.81) of progressive motile sperm.

The STR of total motile sperm had significant ($p$<0.01) negative correlations with ALH, DNC and NDM (–0.43 to –0.67) of both total motile as well as progressive motile sperm. It had significant ($p$ <0.01) positive correlation with WOB (0.73) of total motile sperm, and with VAP, VSL, LIN, STR and WOB (0.42 to 0.78) of progressively motile sperm. The STR of total motile sperm in post-thawed semen revealed significant ($p$ <0.05) positive correlation with WOB (0.59) of total motile sperm, and with LIN (0.50) and STR (0.59) of progressive motile sperm, while it had negative correlations with ALH and DNM (–0.42 to –0.65) of both total motile and progressively motile sperm.

The WOB index of total motile sperm in fresh semen showed significantly ($p$ <0.01) positive correlations with VAP, VSL, LIN, STR and WOB (0.45 to 0.96) of progressive motile sperm, and negative correlations with ALH, DNC and DNM (0.68 to –0.89) of both total motile and progressive motile sperm. The WOB of total motile sperm in frozen-thawed semen had significant ($p$ <0.01) negative correlations with ALH, DNC and DNM (–0.48 to –0.84), and positive correlations with LIN (0.93) and WOB (0.92) of total motile sperm, and negative correlation with VCL, ALH, DNC and DNM (–0.52 to –0.75) of progressive motile sperm.

The BCF of total motile sperm in fresh semen was significantly ($p$ <0.01) and positively correlated with ALH, DNC, DNM (0.46 to 0.77) of total motile sperm, and with VCL, VAP, VSL, BCF, ALH, DNC and DNM (0.36 to 0.88) of progressively motile sperm. The BCF of total motile sperm in post-thawed semen showed highly significant ($p$ <0.01) positive correlations with ALH, DNC, and DNM (0.50 to 0.71) of total motile sperm, and with VCL, VAP, VSL, BCF, ALH, DNC (0.59 to 0.92) of progressively motile sperm.

The ALH of total motile sperm in fresh semen had significant ($p$ <0.01) positive correlations with DNC and DNM (0.92, 0.91) of total motile sperm, and with VCL, BCF, ALH, DNC, DNM (0.42 to 0.98) of progressive motile sperm, while its correlations with LIN, STR, WOB (–0.59 to –0.83) of progressively motile sperm were negative. The ALH of all motile sperm in post-thawed semen had significant ($p$ <0.01) positive correlations with DNC (0.90), DNM (0.77) of total motile sperm, and with VCL, BCF, ALH, DNC, DNM (0.59 to 0.98) of progressively motile sperm.

The DNC of total motile sperm in fresh semen showed significant ($p$ <0.01) positive correlation with DNM (0.87) of total motile sperm, and with VCL, BCF, ALH, DNC and DNM (0.71 to 0.99) of progressively motile sperm. It had negative correlations with LIN, STR, WOB (–0.74, –0.53, –0.74) of progressively motile sperm. The DNC of total motile sperm in frozen-thawed semen revealed highly significant ($p$ <0.01) positive correlation with DNM (0.77) of total motile sperm, and with VAP, VCL, BCF, ALH, DNC, DNM (0.45 to 0.99) and with LIN, STR and WOB (–0.45 to –0.68) of progressive motile sperm.

The DNM of total motile sperm in fresh semen had significant ($p$ <0.01) positive correlations with VCL, BCF, ALH, DNC and DNM (0.46 to 0.96), and negative correlations with LIN, STR, WOB (–0.91, –0.71, –0.89) of progressive motile sperm, while DNM of total motile sperm in frozen-thawed semen showed highly significant ($p$ <0.01) positive correlations with VCL, ALH, DNC, DNM (0.60 to 0.92) and negative correlations with LIN, STR and WOB (–0.51 to –0.83) of progressively motile sperm.

Further, among the progressively motile sperm in fresh semen, the VCL had significant ($p$ <0.01) positive correlations with VAP, VSL, ALH, BCF, DNC, and DNM (0.44 to 0.84). The VAP revealed significant ($p$ <0.05) positive correlations with VSL, LIN, WOB, BCF (0.48 to 0.99), while VSL showed significant ($p$ <0.01) positive correlations with STR, LIN and WOB (0.51 to 0.58). The LIN of progressively motile sperm had significant (p <0.01) positive correlations with STR and WOB (0.76, 0.99), and negative correlations with ALH, DNC and DNM (–0.80 to –0.92), while STR showed significant ($p$ <0.01) positive correlation with WOB (0.66) and negative correlations with ALH, DNC, and DNM (–0.61 to –0.68). The WOB of progressive motile sperm had significant ($p$ <0.01) negative correlations with ALH, DNC and DNM (–0.85, –0.79, –0.91), while BCF showed positive ($p$ <0.01) correlations with ALH, DNC and DNM (0.70, 0.81, 0.64). The ALH of progressively motile sperm had highly significant ($p$ <0.01) positive correlations with DNC and DNM (0.97, 0.98), while the DNC revealed a positive correlation with DNM (0.96).

Similarly among the progressively motile sperm in frozen-thawed semen, the VCL had highly significant ($p$ <0.01) positive correlations with VAP, VSL, BCF, ALH, DNC, DNM (0.62 to 0.80) and negative correlations with LIN (-0.56) and WOB (–0.56), while VAP had significant ($p$ <0.01) positive correlations with VSL, BCF and DNC (0.43 to 0.95). The VSL had highly significant ($p$ <0.01) positive correlation with BCF (0.65), while LIN had significant ($p$ <0.01) positive correlation with WOB (0.95) and negative correlations with ALH, DNC, DNM (–0.68 to –0.85). The STR of progressively motile sperm had significant ($p$ <0.05) negative correlations with ALH, DNC, and DNM (–0.50 to –0.60), while WOB showed highly significant ($p$ <0.01) negative correlations with ALH, DNC, DNM (–0.53 to –0.75). The BCF had highly significant ($p$ <0.01) positive correlations with ALH, DNC, DNM (0.68, 0.71, 0.57), while ALH had positive correlations with DNC (0.95) and DNM (0.94), and the DNC of progressively motile sperm was positively correlated with DNM (0.95). The interrelationships of other traits of fresh and frozen-thawed semen were not significant.

In the literature reviewed, very few studies showed the evaluation of the interrelationship of fresh and frozen-thawed sperm quality assessed particularly by CASA. Anderson *et al.* (1992) found a significant correlation for post-thaw motility of bulls assessed by subjective means and by CASA, but not between post-thaw motility and non-return rates. Ferrell *et al.* (1998) observed bull fertility to be positively correlated with motile spermatozoa, progressive motility, VCL, VAP, and VSL values. The present correlation findings for CASA traits were however in close agreement with the reports of Patel and Dhami (2013, 2016) in fresh and frozen-thawed semen of crossbred and buffalo bulls. Kathiravan *et al.* (2008) observed that among different CASA variables, progressive motility alone contributed to 62.60 % variation in the *in vitro* fertilization percentage. The VAP and VSL, together with progressive motility and HOS spermatozoa contributed to 66.10% of the variation ($p$ <0.05) in fertilization percentage.

Mandal *et al.* (2003) found significant ($p$ <0.01) positive correlations (r = 0.25 to 0.60) of different sperm kinematics with plasmalemma integrity in Murrah bulls. Patel and Dhami (2016) reported all the sperm motility and velocity traits of fresh and frozen-thawed semen assessed by CASA to be significantly interrelated in fresh and frozen-thawed semen of Jafarabadi and Mehsana buffalo bulls; moreover, there were significant correlations of initial motility and live sperm assessed subjectively with CASA traits. The present correlations were in line with these reports of motion and kinematics attributes of fresh and frozen semen assessed by CASA and its correlations. Anand and Yadav (2016) reported the motion and kinematics characteristics of frozen-thawed sperm of Sirohi goat, while Kumar *et al.* (2018) evaluated the motion and kinematics of pre-freeze and post-thawed buffalo semen using CASA, but the correlations as we studied have not been documented by them. Pathak *et al.* (2018) however recorded significant correlations of subjective and objective assessment traits of fresh cattle and buffalo sperm assessed by Biovis CASA with those of post-thawed samples and suggested velocity traits of fresh semen to be predictive of freezability of semen.

Thus, the present correlation findings suggested that CASA analysis of fresh semen for motility and velocity traits could predict the post-thawed sperm motility and velocity/kinematics of bovine semen.

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