



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

NAAS Rating (2025): 4.61

VET 2026; 11(1): 380-384

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www.veterinarypaper.com

Received: 19-10-2025

Accepted: 23-11-2025

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Casa based assessment of post-thaw sperm motion traits as fertility indicators in Mehsana buffalo bulls

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DOI: <https://www.doi.org/10.22271/veterinary.2026.v11.i1f.2992>

Abstract

Computer Assisted Semen Analysis (CASA) enables efficient and objective evaluation of large numbers of spermatozoa, supporting semen quality assurance for artificial insemination. This study compared CASA-derived motility and kinematic parameters of frozen-thawed semen from 12 Mehsana buffalo bulls classified as high-fertile ($CR \geq 43\%$) or low-fertile ($CR \leq 35\%$) based on field conception rates. Motility was assessed at 0, 10, 60 and 120 minutes of post-thaw incubation at 37°C using the Hamilton Thorne IVOS II CASA system. Total and progressive motility declined significantly ($p < 0.05$) with incubation time in both groups and remained higher in high-fertile bulls. Velocity parameters (VAP, VSL and VCL) were significantly higher ($p < 0.05$) in high-fertile bulls at 0 minutes, increased at 10 minutes, and subsequently declined over time. Correlation analysis revealed positive associations of VAP, VSL and straightness with progressive motility, highlighting their potential as fertility indicators in Mehsana buffalo bulls.

Keywords: Buffalo bull, CASA, high fertile, low fertile, motility, kinematic

1. Introduction

The Indian dairy industry relies predominantly on cows and buffaloes, with domestic buffaloes contributing 91.82 million tonnes of milk and providing meat and draft power, thereby supporting rural economies. The use of high genetic merit bulls through artificial insemination (AI) is essential for improving future buffalo production performance (Vishwanath and Shannon, 2000) [1]. AI is the oldest and most widely used assisted reproductive technology in cattle and buffaloes; however, its adoption in buffaloes remains lower than in cattle, largely due to poor conception rates with frozen semen (Kumaresan and Ansari, 2001) [2]. Failure to achieve pregnancy and the need for repeated inseminations cause substantial frustration and economic losses for dairy farmers. Buffalo spermatozoa exhibit lower cryotolerance than cattle spermatozoa and are more susceptible to cryopreservation-induced damage (Andrabi, 2009) [3], which may contribute to reduced conception rates in buffaloes.

Most laboratories still rely on subjective assessment of sperm motion, concentration and morphology, which is prone to bias and inaccuracy, necessitating more objective evaluation methods (Rijsselaere *et al.*, 2012) [4]. Computer Assisted Semen Analysis (CASA) minimizes observer bias by providing reliable, standardized outputs for semen quality control and sample selection (Del Prete *et al.*, 2022) [5]. CASA enables automated and precise analysis of individual sperm motility patterns (Amann and Waberski, 2014) [6] using dark-field or negative phase-contrast imaging, in which sperm heads appear bright against a dark background, allowing rapid evaluation of large numbers of cells (Kathiravan *et al.*, 2011) [7]. Given the close association between sperm motility and fertility, CASA-based objective assessment of sperm motion and morphology supports reliable fertility prediction (Puglisi *et al.*, 2012) [8].

2. Materials and Methods

Twelve Mehsana buffalo bulls from the Dama semen production unit, Banas Dairy (Gujarat, India), were selected based on conception rates from the progeny testing program. Bulls were

classified as high-fertile ($CR \geq 43\%$; $n=6$) or low-fertile ($CR \leq 35\%$; $n=6$) using mean ± 1 SD of CR data from 98 bulls under NDP-I. Frozen semen straws (0.25 mL) from six batches per bull (72 samples) were randomly selected and stored in liquid nitrogen for semen analysis.

2.1 Computer Assisted Semen Analysis (CASA)

Frozen semen straws were thawed at 37 °C for 30 seconds in a water bath (Mettler WNE 45, Germany) for post-thaw evaluation, with only one straw per bull processed at a time to avoid overcrowding. After thawing, 200 μ L of semen was transferred to a pre-warmed (37 °C) sterile 1.5 mL microcentrifuge tube and diluted with 800 μ L of pre-warmed TRIS buffer (1:4). Immediately, 3 μ L of diluted semen was loaded onto a pre-warmed 8-chamber Leja slide (45° angle) and analyzed using the Hamilton Thorne IVOS II CASA system. Ten microscopic fields per sample were examined to record total and progressive motility and kinematic parameters viz., average path velocity (VAP, μ m/s), straight-line velocity (VSL, μ m/s), curvilinear velocity (VCL, μ m/s), amplitude of lateral head displacement (ALH, μ m), beat cross frequency (BCF, Hz), straightness (STR, %), linearity (LIN, %), and the wobble coefficient (WOB, %) using standard manufacturer-recommended settings. To assess time-dependent changes, diluted semen samples were incubated at 37 °C for up to 120 min and re-evaluated at 10-, 60- and 120-min intervals following the same procedure.

2.2 Statistical Analysis

Data were tested for normality using the Shapiro-Wilk test, and percentage values were arcsine-transformed prior to

analysis. Repeated-measures ANOVA was used to evaluate the effects of fertility group, incubation time and their interaction. Group-wise Pearson correlation analysis was performed among sperm parameters. All analyses were conducted using SPSS software (IBM® SPSS® Statistics, version 20.0).

3. Results and Discussion

3.1 Sperm Motility Parameters

Mean total motility (TM, %) and progressive motility (PM, %) at different post-thaw incubation intervals in high- and low-fertile bulls are presented in Table 1. Total motility declined significantly ($p < 0.05$) from 0 to 10, 60 and 120 min in both groups, in agreement with Kumar *et al.* (2015) [9] and Swami *et al.* (2017) [10]. Both fertility group and incubation time significantly affected TM, whereas their interaction was non-significant. Total motility remained consistently higher ($p < 0.05$) in high-fertile bulls across all intervals, corroborating earlier reports (Kumar *et al.*, 2014 [11]; Singh *et al.*, 2017 [12]; El-Khawagah *et al.*, 2020 [13]; Turri *et al.*, 2021 [14]). Variations in TM values among studies have been reported, with higher values observed by Patel and Dhami (2016) [15], Kumar *et al.* (2016c) [16] and Pathak *et al.* (2019) [17], and lower values by Dalal *et al.* (2018) [18]. Higher TM in high-fertile bulls during early post-thaw incubation (10-15 min) has also been documented (Gliozzi *et al.*, 2017 [19]; Narud *et al.*, 2020 [20]), whereas Oliveira *et al.* (2012) [21] reported non-significant differences after 20 min. Compared with Swami *et al.* (2017) [10], TM at 60 and 120 min was lower in the present study, while Kumar *et al.* (2015) [9] observed higher TM at 60 min but lower values at 120 min.

Table 1: Percent sperm motility (Mean $\pm$ SE) of frozen-thawed semen incubated at 37 °C in high- and low-fertile Mehsana buffalo bulls

Parameters	Fertility Group	Incubation Time				p- value		
		0 Min	10 Min	60 Min	120 Min	Time (T)	Group (G)	T×G
Total motility- TM (%)	High	48.18 $\pm$ 0.05 ^{Bd}	40.74 $\pm$ 0.04 ^{Bc}	29.03 $\pm$ 0.04 ^{Bb}	23.03 $\pm$ 0.05 ^{Ab}	0.001	0.005	0.208
	Low	39.21 $\pm$ 0.05 ^{Ad}	34.40 $\pm$ 0.04 ^{Ac}	22.12 $\pm$ 0.04 ^{Ab}	15.44 $\pm$ 0.05 ^{Aa}			
	Overall	43.67 $\pm$ 0.03	37.54 $\pm$ 0.02	25.50 $\pm$ 0.02	19.09 $\pm$ 0.03			
Progressive Motility- PM (%)	High	30.63 $\pm$ 0.03 ^{Bd}	26.25 $\pm$ 0.03 ^{Bc}	10.08 $\pm$ 0.03 ^{Ab}	3.28 $\pm$ 0.02 ^{Aa}	0.001	0.011	0.001
	Low	22.30 $\pm$ 0.03 ^{Ac}	21.10 $\pm$ 0.03 ^{Ac}	7.80 $\pm$ 0.03 ^{Ab}	2.47 $\pm$ 0.02 ^{Aa}			
	Overall	26.36 $\pm$ 0.02	23.63 $\pm$ 0.02	8.91 $\pm$ 0.02	2.86 $\pm$ 0.01			

Means bearing different superscripts (a,b,c,d) within row and (A,B) within column differ significantly ($p < 0.05$)

Progressive motility (PM) was significantly influenced by fertility group, incubation time and their interaction. PM was significantly higher ($p < 0.05$) in high-fertile bulls at 0 and 10 min, while differences at 60 and 120 min were non-significant (Table 1). The higher PM at 0 min and its subsequent decline during incubation are consistent with previous reports (Kumar *et al.*, 2016a,b,c [22,23,16]; Dalal *et al.*, 2018 [18]). Higher PM values have been reported by Patel and Dhami (2016) [15] and El-Khawagah *et al.* (2020) [13], whereas lower values were observed by Pathak *et al.* (2019) [17] in Surti and Murrah bulls. The significantly higher PM at 0 min in high-fertile bulls agrees with Turri *et al.* (2021) [14], while Kumar *et al.* (2014) [11] reported non-significant differences; Narud *et al.* (2020) [20] also observed higher PM after 15 min in high-fertile bulls. Compared with Swami *et al.* (2017) [10], PM at 60 and 120 min was lower in the present study, whereas Kumar *et al.* (2015) [9] reported lower values at both intervals. As sperm motility is critical for transport and fertilization (Kumar *et al.*, 2014) [11], its decline likely reflects metabolic exhaustion (Vianna *et al.*, 2009 [24]) and mitochondrial dysfunction (O'Connell *et al.*, 2002 [25]) during incubation.

3.2 Sperm Kinematic Parameters

The mean values of sperm kinematic parameters of post-thaw Mehsana buffaloes of high and fertile groups at different time intervals of incubation are presented in Table 2.

3.2.1 Velocity parameters

The mean values of velocity parameters (VAP, VSL and VCL; μ m/s) were significantly influenced by incubation time and the time $\times$ fertility group interaction, while the main effect of fertility group was non-significant. Velocity values were higher in high-fertile bulls up to 60 min and reversed at 120 min, with a significant difference ($p < 0.05$) observed only at 0 min. All three parameters increased significantly at 10 min compared to 0 min and then declined progressively at 60 and 120 min. Similar trends, including higher velocity parameters in high-fertile bulls, have been reported previously (Kumar *et al.*, 2014, 2015; Singh *et al.*, 2017; Swami *et al.*, 2017) [11,12,10], whereas Turri *et al.* (2021) [14] and Gliozzi *et al.* (2017) [19] observed non-significant group differences. Oliveira *et al.* (2012) [21] reported lower values, while Narud *et al.* (2020) [20] found higher VAP and VSL after 15-20 min

in high-fertile bulls. The overall mean velocity values in the present study were lower than those reported by Kumar *et al.* (2016a,b) [22, 23], but higher than those observed by Patel and Dharmi (2016) [15], Kumar *et al.* (2016c) [16], Swami *et al.* (2017) [10] and Pathak *et al.* (2019) [17]. Compared with Swami *et al.* (2017) [10], VSL and VAP at 60 and 120 min were lower, whereas Kumar *et al.* (2015) [9] reported lower VAP at 60 min and comparable values at 120 min. Variations in velocity traits may arise from intrinsic and extrinsic factors (Mortimer, 2000) [26], and the transient increase at 10 min likely reflects capacitation-like changes induced by freeze-thawing.

3.2.2 Amplitude of lateral head displacement

Time and the time $\times$ fertility group interaction had a significant effect on ALH (μm), whereas the main effect of fertility group was not significant. No significant differences in ALH were observed between fertility groups at 0, 10, or 60

min; however, at 120 min, ALH was significantly higher ($p < 0.05$) in the low-fertile group. Overall, ALH showed a tendency to be higher in the low-fertile group.

Similar non-significant differences between fertility groups have been reported by Kumar *et al.* (2014) [11], Turri *et al.* (2021) [14], Oliveira *et al.* (2012) [21], and Gliozzi *et al.* (2017) [19], whereas Singh *et al.* (2017) [12] observed higher ALH values in high-fertile bulls. The ALH values at 0 min were comparable to those reported by Kumar *et al.* (2016a,b) [22, 23], while lower values were reported by Swami *et al.* (2017) [10], Pathak *et al.* (2019) [17] and Abdelli *et al.* (2025) [27]. At 60 and 120 min, ALH values were lower than those reported by Swami *et al.* (2017) [10], whereas Kumar *et al.* (2015) [9] reported higher ALH at 60 min and lower values at 120 min. In the present study, ALH peaked at 10 min, consistent with the fluctuating patterns described by Kumar *et al.* (2015) [9] and Swami *et al.* (2017) [10].

Table 2: Sperm kinematic parameters (Mean $\pm$ SE) of frozen-thawed semen incubated at 37 °C in high- and low-fertile Mehsana buffalo bulls

Parameters	Fertility Group	Incubation Time				Time (T)	Group (G)	T $\times$ G
		0 Min	10 Min	60 Min	120 Min			
VSL ($\mu\text{m/s}$)	High	78.04 $\pm$ 1.78 ^{Bc}	82.74 $\pm$ 1.78 ^{A^d}	42.18 $\pm$ 1.47 ^{A^b}	31.13 $\pm$ 0.98 ^{A^a}	0.001	0.058	0.001
	Low	69.34 $\pm$ 1.78 ^{A^c}	79.74 $\pm$ 1.78 ^{A^d}	40.03 $\pm$ 1.47 ^{A^b}	32.61 $\pm$ 0.98 ^{A^a}			
	Overall	73.69 $\pm$ 1.35	81.24 $\pm$ 1.26	41.10 $\pm$ 1.04	31.87 $\pm$ 0.69			
VCL ($\mu\text{m/s}$)	High	172.35 $\pm$ 4.3 ^{Bc}	203.46 $\pm$ 3.95 ^{A^d}	121.88 $\pm$ 2.86 ^{A^b}	103.01 $\pm$ 2.19 ^{A^a}	0.001	0.061	0.065
	Low	158.17 $\pm$ 4.3 ^{A^c}	196.88 $\pm$ 3.95 ^{A^d}	116.08 $\pm$ 2.86 ^{A^b}	104.73 $\pm$ 2.19 ^{A^a}			
	Overall	165.26 $\pm$ 2.80	200.17 $\pm$ 2.80	118.98 $\pm$ 2.04	103.87 $\pm$ 1.54			
VAP ($\mu\text{m/s}$)	High	97.69 $\pm$ 1.95 ^{Bc}	106.51 $\pm$ 1.92 ^{A^d}	58.92 $\pm$ 1.64 ^{A^b}	45.52 $\pm$ 1.05 ^{A^a}	0.001	0.051	0.032
	Low	90.28 $\pm$ 1.95 ^{A^c}	104.96 $\pm$ 1.92 ^{A^d}	54.44 $\pm$ 1.64 ^{A^b}	46.08 $\pm$ 1.05 ^{A^a}			
	Overall	93.99 $\pm$ 1.44	105.74 $\pm$ 1.35	56.68 $\pm$ 1.18	45.80 $\pm$ 0.74			
ALH (μm)	High	6.79 $\pm$ 0.20 ^{A^c}	8.31 $\pm$ 0.16 ^{A^d}	5.60 $\pm$ 0.11 ^{A^b}	5.36 $\pm$ 0.10 ^{A^a}	0.001	0.539	0.002
	Low	6.35 $\pm$ 0.20 ^{A^b}	8.32 $\pm$ 0.16 ^{A^c}	5.80 $\pm$ 0.11 ^{A^a}	5.93 $\pm$ 0.10 ^{B^{ab}}			
	Overall	6.57 $\pm$ 0.14	8.31 $\pm$ 0.11	5.70 $\pm$ 0.08	5.65 $\pm$ 0.08			
LIN (%)	High	48.68 $\pm$ 0.02 ^{A^d}	42.86 $\pm$ 0.00 ^{A^c}	36.07 $\pm$ 0.01 ^{A^b}	31.62 $\pm$ 0.00 ^{A^a}	0.001	0.843	0.665
	Low	48.28 $\pm$ 0.02 ^{A^d}	43.33 $\pm$ 0.00 ^{A^c}	35.86 $\pm$ 0.01 ^{A^b}	32.50 $\pm$ 0.00 ^{A^a}			
	Overall	48.48 $\pm$ 0.61	43.09 $\pm$ 0.00	35.97 $\pm$ 0.00	32.06 $\pm$ 0.00			
STR (%)	High	79.10 $\pm$ 0.02 ^{A^d}	77.12 $\pm$ 0.01 ^{A^c}	71.62 $\pm$ 0.01 ^{A^b}	68.51 $\pm$ 0.01 ^{A^a}	0.001	0.236	0.064
	Low	78.94 $\pm$ 0.02 ^{A^c}	77.54 $\pm$ 0.01 ^{A^c}	73.58 $\pm$ 0.01 ^{A^b}	71.26 $\pm$ 0.01 ^{B^a}			
	Overall	79.05 $\pm$ 0.01	77.33 $\pm$ 0.00	72.61 $\pm$ 0.00	69.88 $\pm$ 0.00			
BCF (Hz)	High	35.04 $\pm$ 0.36 ^{A^d}	32.33 $\pm$ 0.36 ^{A^c}	28.16 $\pm$ 0.47 ^{A^b}	24.80 $\pm$ 0.55 ^{B^a}	0.001	0.153	0.002
	Low	36.13 $\pm$ 0.36 ^{B^d}	32.24 $\pm$ 0.36 ^{A^c}	26.96 $\pm$ 0.47 ^{A^b}	22.87 $\pm$ 0.55 ^{A^a}			
	Overall	35.58 $\pm$ 0.26	32.28 $\pm$ 0.25	27.56 $\pm$ 0.34	23.84 $\pm$ 0.30			
WOB (%)	High	59.40 $\pm$ 0.01 ^{A^d}	54.20 $\pm$ 0.00 ^{A^c}	49.33 $\pm$ 0.00 ^{A^b}	45.34 $\pm$ 0.00 ^{A^a}	0.001	0.88	0.079
	Low	59.72 $\pm$ 0.01 ^{A^d}	55.00 $\pm$ 0.00 ^{A^c}	47.95 $\pm$ 0.00 ^{A^b}	45.17 $\pm$ 0.00 ^{A^a}			
	Overall	59.56 $\pm$ 0.00	54.60 $\pm$ 0.00	48.64 $\pm$ 0.00	45.26 $\pm$ 0.00			

Means bearing different superscripts (a,b,c,d) within row and (A,B) within column differ significantly ($p < 0.05$)

3.2.3 Linearity, Straightness and Wobbling

LIN, STR and WOB (%) were significantly influenced by time, while the effects of fertility group and the time $\times$ group interaction were non-significant (Table 2). No group differences were observed up to 60 min; however, STR was significantly higher in the low-fertile group at 120 min. All three parameters declined significantly ($p < 0.05$) with incubation. The 0-min values were comparable to those reported by Kumar *et al.* (2016c) [16], whereas higher values were reported by Kumar *et al.* (2016a) [22] and Swami *et al.* (2017) [10], and lower LIN by Patel and Dharmi (2016) [15] and Pathak *et al.* (2019) [17]. The 60- and 120-min values agreed with Swami *et al.* (2017) [10], while Kumar *et al.* (2015) [9] reported higher values. Consistent with the present findings, Kumar *et al.* (2014) [11] and Turri *et al.* (2021) [14] observed non-significant group differences, whereas Singh *et al.* (2017) [12] reported higher values in low-fertile bulls. In contrast, Gliozzi *et al.* (2017) [19] observed higher LIN in high-fertile

bulls after 10 min, Narud *et al.* (2020) [20] reported non-significant differences after 15 min, and Oliveira *et al.* (2012) [21] found higher values in low-conception bulls after 20 min.

3.2.4 Beat cross frequency

BCF (Hz) was significantly influenced by time and the time $\times$ fertility group interaction, while the main effect of fertility group was non-significant. BCF was significantly higher ($p < 0.05$) in the low-fertile group at 0 min, with the reverse trend at 120 min (Table 2), and declined progressively from 0 to 120 min in both groups. The overall mean values were comparable to those reported by Kumar *et al.* (2016a,b) [22, 23], whereas lower values were reported by Patel and Dharmi (2016) [15], Swami *et al.* (2017) [10], Pathak *et al.* (2019) [17] and El-Khawagah *et al.* (2020) [13]. The 60-min value agreed with Swami *et al.* (2017) [10], although they reported higher values at 120 min, while Kumar *et al.* (2015) [9] observed higher BCF at both 60 and 120 min. Higher BCF in low-

fertile bulls was also reported by Singh *et al.* (2017) ^[12], whereas Kumar *et al.* (2014) ^[11] and Turri *et al.* (2021) ^[14] found no group differences. Non-significant differences at later incubation times were reported by Gliozzi *et al.* (2017) ^[19], Narud *et al.* (2020) ^[20] and Oliveira *et al.* (2012) ^[21].

3.3 Correlation among CASA traits in high- and low-fertile Mehsana buffalo bulls

Pearson's correlation coefficients among CASA traits of frozen-thawed semen in high- and low-fertile Mehsana buffalo bulls are presented in Table 3. In both groups, progressive motility (PM) showed significant positive correlations with total motility (TM) and STR. VSL was positively correlated with VAP, and significant positive interrelationships were observed among VCL, VAP and ALH. In contrast, VCL showed significant negative correlations with LIN, STR and WOB, while VAP and ALH were negatively correlated with LIN; ALH was also negatively correlated with STR and WOB. LIN was positively correlated with STR and WOB, and STR with WOB, in both groups. Marked differences between fertility groups were evident:

TM-VAP correlations were positive and non-significant in high-fertile bulls but negative and significant in low-fertile bulls; PM-VAP correlations were positive and significant in high-fertile bulls but negative and non-significant in low-fertile bulls; PM-ALH correlations were negative and significant in low-fertile bulls but positive and non-significant in high-fertile bulls; VSL-ALH correlations were positive and significant in high-fertile bulls but negative and non-significant in low-fertile bulls; and VSL-LIN correlations were positive and significant in low-fertile bulls but negative and non-significant in high-fertile bulls.

Among sperm motility patterns, progressive motility represents the most linear and forward-moving trajectory, enabling spermatozoa to traverse the female reproductive tract efficiently and reach the site of fertilization earlier than non-progressive sperm. The significant positive correlations of straight-line velocity, average path velocity and straightness with progressive motility in the present study suggest that these parameters may serve as reliable indicators of bull fertility.

Table 3: Pearson correlation coefficients between the various seminal attributes of frozen thawed semen of high and low fertile Mehsana buffalo bulls

Parameters	TM	PM	VSL	VCL	VAP	ALH	LIN	STR	BCF	WOB
Correlations: High Fertile Bulls										
TM	1	0.883** (0.001)	0.267 (0.116)	0.004 (0.980)	0.141 (0.412)	-0.100 (0.560)	0.351* (0.036)	0.395* (0.017)	-0.003 (0.985)	0.309 (0.067)
PM	0.798** (0.001)	1	0.614** (0.001)	0.233 (0.171)	0.444** (0.007)	0.026 (0.882)	0.325 (0.053)	0.488** (0.003)	0.167 (0.331)	0.226 (0.186)
VSL	-0.252 (0.138)	0.277 (0.102)	1	0.748** (0.001)	0.926** (0.001)	0.494** (0.002)	-0.045 (0.793)	0.234 (0.169)	0.115 (0.115)	-0.160 (0.351)
VCL	-0.285 (0.092)	-0.299 (0.077)	0.177 (0.302)	1	0.910** (0.001)	0.915** (0.001)	-0.654** (0.001)	-0.351* (0.036)	-0.173 (0.313)	-0.705** (0.001)
VAP	-0.401* (0.015)	-0.143 (0.407)	0.628** (0.001)	0.820** (0.001)	1	0.731** (0.001)	-0.352* (0.035)	-0.124 (0.471)	-0.001 (0.995)	-0.389* (0.019)
ALH	-0.331* (0.049)	-0.448** (0.006)	-0.51 (0.769)	0.939** (0.001)	0.663** (0.001)	1	-0.831** (0.001)	-0.588** (0.001)	-0.420* (0.11)	-0.835** (0.001)
LIN	0.200 (0.243)	0.414* (0.012)	0.405* (0.014)	-0.744** (0.001)	-0.348* (0.038)	-0.865** (0.001)	1	0.856** (0.001)	0.295 (0.080)	0.951** (0.001)
STR	0.065 (0.707)	0.343* (0.041)	0.417* (0.011)	-0.710** (0.001)	-0.294 (0.082)	-0.759** (0.001)	0.903** (0.001)	1	0.245 (0.149)	0.662** (0.001)
BCF	-0.212 (0.216)	0.039 (0.821)	0.259 (0.128)	0.045 (0.792)	0.278 (0.101)	-0.116 (0.500)	0.027 (0.874)	-0.016 (0.926)	1	0.282 (0.095)
WOB	0.223 (0.192)	0.379* (0.23)	0.360* (0.31)	-0.716** (0.001)	-0.326 (0.052)	-0.852** (0.001)	0.980** (0.001)	0.821** (0.001)	0.064 (0.711)	1
Correlations: Low Fertile Bulls										

** Correlations at $p < 0.01$, *Correlations at $p < 0.05$.

4. Conclusion

High-fertile buffalo bulls showed superior early post-thaw motility and velocity traits compared to low-fertile bulls. Total and progressive motility and velocity parameters (VAP, VSL, VCL) declined significantly with incubation time in both groups, with a transient increase at 10 minutes. Linear motion traits (LIN, STR, WOB) and flagellar activity indicators (ALH, BCF) decreased over time with minimal group differences. Distinct correlation patterns among CASA traits between fertility groups indicate functional differences in sperm motion, suggesting that early post-thaw motility and velocity characteristics may serve as reliable indicators of buffalo bull fertility.

5. Acknowledgement

Authors are thankful to the Principal, College of Veterinary Science and AH, Kamdhenu University, Sardarkrushinagar; management, Banaskantha District Co-Operative Milk

Producers' Union Ltd, Palanpur and staff members of Dama Semen Production Unit, Banas Dairy for providing necessary help and facilities.

Conflict of Interest

Not available

Financial Support

Not available

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How to Cite This Article

Patel MP, Chaudhari CF, Sutaria TV, Chaudhari RK, Joshi RD, Patel KG, *et al*. Casa based assessment of post-thaw sperm motion traits as fertility indicators in Mehsana buffalo bulls. *International Journal of Veterinary Sciences and Animal Husbandry*. 2026;11(1):380-384.

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