RESEARCH ARTICLE

Interrelationships Among Sperm Quality Parameters, Cryocapacitation Status, Oxidative Markers and CASA Traits of Fresh and Frozen-thawed Semen of Gir and Murrah Bulls

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Abstract

This investigation was carried out on three breeding bulls each of Gir and Murrah breeds to evaluate the interrelationships among sperm quality attributes, cryo-capacitation, oxidative markers and Biovis CASA traits of fresh and frozen thawed semen. The ejaculates (n=4/bull) having >75% initial motility were split-diluted @ 100 million sperm/mL in TFYG extender without (control) and with fortification of Mifepristone, Sericin and Taurine, filled in French mini straws, and were frozen using a programmable biofreezer. The straws were thawed in water bath at 37°C for 30 sec. The freshly diluted and frozen-thawed samples were assessed for various routine sperm quality parameters, cryo-capacitation status by CTC fluorescence, oxidative markers and for various motion characteristics/kinematics by Biovis CASA, the overall means of which are tabulated. The Pearson's correlations studied among all these traits in freshly diluted and frozen-thawed semen of both the species revealed that the sperm motility, HOS reactive sperm, live sperm and non-capacitated sperm were significantly ($p<0.01$) and positively interrelated (r= 0.29 to 0.88), while their correlations with capacitated & acrosome reacted sperm as well as seminal plasma GPx and MDA were negative (r= -0.25 to -0.99), but had no appreciable correlations with CASA traits of sperm, except initial motility with total and rapid progressive motile sperm, VCL, VAP and VSL of freshly diluted semen in Gir bulls (r= 0.28 to 0.41), and HOS reactive sperm with most of the CASA traits in Murrh bulls (r= 0.29 to 0.49). MDA activity of fresh semen and SOD of frozen-thawed semen in Murrah bulls also showed significant positive correlations with most of the CASA traits of respective stage. In post-thawed semen of both the species, all the sperm quality and capacitation traits including oxidative markers had significant positive or negative correlations with CASA derived total motile and rapid & slow progressive motile sperm (r= ± 0.29 to ± 0.70). Moreover, the sperm kinematics parameters of both fresh and frozen-thawed semen of both the species showed significant ($p<0.01$) positive interrelationship among themselves (r= 0.29 to 0.99), except slow progressive motile sperm and BCF in Gir bulls fresh semen and slow progressive motile sperm, ALH and DNC in Murrah bulls had negative correlations with other CASA traits, although no such specific trend was observed in post-thawed semen of either species. The first insemination conception rates also showed positive association with sperm motility, HOS reactive sperm, live sperm and non-capacitated sperm, SOD and GPx activity of plasma, but not with CASA traits. Thus, the initial and post-thawed sperm quality parameters including capacitation and oxidative status can predict the overall quality and fertility of bovine frozen-thawed semen.

Keywords: Bovine semen, Frozen-thawed sperm, Interrelationships, Kinematics, Oxidative markers, Sperm quality.

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Introduction

The sperm quality of frozen-thawed semen used in AI is significantly poor compared to freshly diluted semen since the cryopreservation leads to varying degrees of cryodamage to sperms. The reactive oxygen species (ROS) mediated oxidative stress is known to deteriorate the quality of frozen semen (Chhillar *et al.,* 2012), affecting its plasma membrane and DNA integrity and thereby reducing its potential fertility (Kadirvel *et al.,* 2009). During the cryopreservation process, sperm cells undergo precocious cryo-capacitation compromising *in vitro* and *in vivo* fertilization potential of frozen-thawed spermatozoa (Watson, 2000). The chlortetracycline (CTC) fluorescence assay is a direct, single-step staining method by which we can differentiate between acrosome reacted and non-reacted spermatozoa and between capacitated and non-capacitated spermatozoa. Different types of antioxidants and cryoprotectants have been incorporated into the semen extenders with varying degrees of success by many workers (Orin *et al.,* 2015; Dalal *et al.,* 2019; Patel *et al.,* 2019, Chaturvedi *et al.,* 2021[b]), indicating their crucial role in improving the preservability and fertility of the bull and buffalo semen.

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The evaluation of the fertility potential of males generally involves analysis of fresh and frozen-thawed semen for its quality and conception rates in inseminated females (Verstegen *et al.,* 2002; Chaudhary *et al.,* 2017). However, no single test or combination of tests has been proven reliable for the accurate prediction of semen quality concerning fertility. The conventional evaluation of bovine semen is relatively inaccurate, imprecise, time-consuming (Christensen *et al.*, 2005). It varies with the experience and skill of the investigator (Patel and Dhami, 2016), while CASA could improve precision and accuracy in less time and prove worthy for assessing bull fertility (Ramachandran *et al.,* 2007; Amann and Waberski, 2014; Pathak *et al.,* 2020). The correlations of the physical attributes of semen with fertility are highly variable and relatively poor (Shelke and Dhami, 2001; Chaudhary *et al.,* 2017). The literature on the use of comprehensive evaluation of fresh and frozen-thawed bovine semen concerning sperm quality attributes, capacitation status by CTC assay, oxidative markers, and CASA including *in vivo* fertility trials, and their interrelationships is however meager, and most of the studies report only one or two of these aspects with different extender-additives (Anderson *et al.,* 1992; Lackey *et al.,* 1998; Mandal *et al.,* 2003; Perumal *et al.,* 2011; Zodinsanga *et al.,* 2015; Pathak *et al.,* 2018; Dalal *et al.,* 2019). Hence, the objective of present study was to evaluate all these aspects at a time comprehensively and to know their interrelationship in fresh and frozen-thawed semen of Gir and Murrah bulls.

Materials and Methods

The present study was carried out on three mature breeding bulls, each of Gir and Murrah breeds, aged 6-9 years, at the College of Veterinary Science, AAU, Anand, during the year 2019-20. All the bulls were in good health and under optimal veterinary care. They were maintained in nearly identical nutritional and managerial conditions throughout the study with twice a week semen collection schedule. Semen was collected using an artificial vagina from each bull in the morning hours over a dummy bull. Ejaculates collected at the weekly interval were used for this study.

Immediately after collection, the ejaculates were evaluated for various seminal attributes. Ejaculates (n = 4 per bull) with > 75 % initial motility were split into 4 aliquots and were extended at 34°C with Tris-citric acid-fructose-egg yolk-glycerol (TFYG) extender without (control) and with fortification of Mifepristone (10 µg/mL), Sericin (5 mg/mL) and Taurine (4 mg/mL), keeping 100 million sperm per mL. The extended semen was assessed subjectively for sperm motility, livability, HOST, cryo-capacitation status (F- non-

Table 1: Overall mean (± SE) values of various sperm quality parameters, CTC assay, oxidative markers and Biovis CASA traits of fresh and frozen-thawed semen of Gir and Murrah bulls (irrespective of additives)

	Gir bull semen		*Murrah bull semen*	
Parameters	*Fresh diluted*	*Post-thawed*	*Fresh diluted*	*Post-thawed*
Sperm motility (%)	84.15 ± 0.62	47.08 ± 0.88	83.85 ± 0.72	50.63 ± 1.14
HOST positivity (%)	76.35 ± 1.08	55.08 ± 1.10	75.88 ± 1.04	58.33 ± 0.85
Live sperm (%)	83.85 ± 0.75	65.71 ± 1.01	83.90 ± 0.67	67.83 ± 0.82
CTC 'F' pattern sperm (%)	81.98 ± 0.68	65.92 ± 1.17	81.06 ± 0.68	68.60 ± 1.12
Capacitated sperm (%)	15.92 ± 0.64	30.23 ± 1.06	16.69 ± 0.58	27.92 ± 1.02
CTC 'AR' sperm (%)	2.10 ± 0.12	3.85 ± 0.21	2.23 ± 0.14	2.50 ± 0.17
GPx (nmol/min/mL)	500.94 ± 10.65	448.73 ± 10.64	535.36 ± 9.14	474.34 ± 7.83
SOD (U/mL)	2.83 ± 0.06	2.40 ± 0.04	2.31 ± 0.08	1.93 ± 0.08
MDA (µmol//mL)	19.11 ± 0.35	20.82 ± 0.38	20.17 ± 0.24	22.89 ± 0.25
Total motile sperm (%)	85.50 ± 0.92	48.76 ± 1.69	85.03 ± 0.72	52.61 ± 1.46
Rapid Progr. mot sperm (%)	24.64 ± 1.07	6.66 ± 0.62	23.48 ± 1.59	8.74 ± 0.93
Slow Progr. mot sperm (%)	36.31 ± 0.83	20.68 ± 1.14	39.10 ± 1.16	25.67 ± 1.19
VCL (µm/s)	87.74 ± 0.88	73.68 ± 1.03	83.14 ± 1.22	72.26 ± 0.83
VAP (µm/s)	43.17 ± 0.65	30.89 ± 0.63	41.75 ± 0.92	33.35 ± 0.72
VSL (µm/s)	33.74 ± 0.65	23.98 ± 0.65	35.48 ± 1.00	27.29 ± 0.83
LIN, linearity (%)	40.86 ± 0.57	32.56 ± 0.76	42.59 ± 0.93	37.89 ± 0.98
STR, straightness (%)	77.89 ± 0.52	69.42 ± 0.84	79.93 ± 0.74	74.65 ± 1.00
BCF (hz)	11.71 ± 0.26	7.47 ± 0.23	11.70 ± 0.26	8.94 ± 0.33
ALH (µm)	2.21 ± 0.05	1.86 ± 0.04	2.13 ± 0.06	1.50 ± 0.04
DNC, dancing ($\mu m^2/s$)	199.33 ± 4.40	142.09 ± 4.05	178.54 ± 3.89	143.34 ± 3.51
1st AI conception (%)	(495 AI)	47.07%	(1446 AI)	50.76%

CTC 'F' pattern= Non-capacitated sperm, CTC AR= Acrosome reacted sperm.

capacitated, B- capacitated, and AR- acrosome reacted sperm pattern) using CTC fluorescence assay. The oxidative markers (MDA- malonaldehyde, SOD- superoxide dismutase, and GPx- glutathione peroxidase activity of seminal plasma) and motion characteristics/kinematics attributes of spermatozoa by Biovis CASA (Expert Vision Pvt. Ltd., Mumbai) were also studied adopting standard procedures (Chaturvedi *et al.,* 2021[a,b]).

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 programmable bio-freezer (IMV, France) employing standard freezing protocol. The straws were thawed in a water bath at 37°C for 30 seconds and assessed again (subjectively and objectively) for all traits studied in freshly extended semen. The CASA traits studied included total motile sperm, rapid progressive and slow progressive motile sperm, average path velocity (VAP), curvilinear velocity (VCL), straight-line velocity (VSL), straightness (STR), linearity (LIN), beat-cross frequency (BCF), the amplitude of lateral head displacement (ALH), and dancing velocity (DNC). *In vivo* fertility trials were also conducted under field conditions with frozen semen doses prepared without and with different additives. The generated data were analyzed to derive Mean ± SEs values, and Pearson's correlations were studied using a standard statistical package on SPSS software version 20.00 (Snedecor and Cochran, 1994).

Results and Discussion

The overall mean (± SE) values of various sperm quality parameters, capacitation status, oxidative markers, and CASA traits of total motile sperm observed in Freshly diluted and frozen-thawed semen of Gir and Murrah bulls are presented in Table 1. These parameters concerning the effect of additives Mifepristone, Sericin, and Taurine have been reported and discussed separately (Chaturvedi *et al.,* 2021[a,b]). The interrelationships among these attributes in fresh and frozen-thawed semen of Gir and Murrah bulls are presented in Tables 2 and 3, respectively.

Correlations of Freshly Diluted Gir Bull Semen:

The initial sperm motility of freshly diluted semen of Gir bulls, irrespective of additives used in the extender, showed significantly ($p<0.05$, $p<0.01$) positive correlations with initial live sperm, non-capacitated sperm, total motile & rapid progressive motile sperm, VCL, VAP, VSL, and negative correlations with capacitated & acrosome reacted (AR) sperm, oxidative markers, and ALH. The HOS reactive sperm in the freshly diluted semen had significant ($p<0.01$) positive correlations with live sperm, non-capacitated sperm, and seminal GPx activity, and negative correlations with capacitated & AR sperm and MDA activity. The live sperm revealed significant positive correlations with non-capacitated sperm and GPx activity and negative correlations with capacitated & AR sperm and MDA activity (Table 2).

The non-capacitated sperm percent in fresh semen showed significantly ($p<0.01$) negative correlations with capacitated & AR sperm, SOD, and MDA activity. In comparison, capacitated sperm percent had significant ($p<0.05$) positive correlations with AR sperm, SOD, MDA, and LIN of sperm motion, and acrosome reacted (AR) sperm with MDA activity of seminal plasma (+0.33) and BCF of sperm (-0.32). Further, the correlations of the above sperm quality traits and oxidative markers with all the CASA traits were negligible or very low in freshly diluted Gir bull semen.

Among the CASA traits of fresh Gir bull sperm, the total motile sperm had significant ($p<0.05$) positive correlations with initial subjective motility score and rapid progressive motile sperm, VCL, VAP, and VSL. At the same time, rapid progressive motile sperm showed negative correlations with slow progressive motile sperm and positive correlations with VCL, VAP, VSL, LIN, STR, BCF, and DNC of CASA traits. The slow progressive motile sperm revealed inverse correlations with these traits. The VCL of freshly diluted Gir bull sperm revealed significant ($p<0.05$) positive correlations with VAP, VSL, BCF, and DNC, while VAP of fresh sperm showed significant ($p<0.05$) positive correlations with VSL, LIN, STR, BCF, and DNC, and the VSL had significant ($p<0.05$) positive correlations with LIN, STR, BCF, and DNC. The LIN of fresh sperm showed significant ($p<0.05$) positive correlations with STR and BCF, while STR had a positive correlation with BCF. The BCF, in turn, showed significant ($p<0.05$) positive correlations with ALH and DNC of sperm (Table 2).

Correlations of Post-thawed Gir Bull Semen:

The post-thawed percent sperm motility had significant ($p<0.01$) positive correlations with post-thawed live sperm, HOS reactive sperm, non-capacitated sperm, and LIN, and negative correlations with capacitated sperm and MDA activity. The post-thawed HOS reactive sperm showed significant ($p<0.01$) positive correlations with post-thawed live sperm and non-capacitated sperm and negative correlations with capacitated & AR sperm and MDA activity. The post-thawed live sperm percent revealed significant ($p<0.01$) positive correlations with post-thawed non-capacitated sperm, total motile, rapid and slow progressive motile sperm, LIN, STR, and BCF, and negative correlations with capacitated & AR sperm, SOD, and MDA activity.

The post-thawed non-capacitated sperm percent in Gir bull semen was significantly ($p<0.01$) and positively correlated with post-thawed total motile, rapid and slow progressive motile sperm, LIN, STR, and negatively with capacitated & AR sperm, SOD and MDA activity while post-thawed capacitated sperm percent had significant ($p<0.01$) positive correlations with AR sperm and SOD activity, and negative correlations with total motile, rapid and slow motile sperm, LIN, and STR. The post-thawed acrosome reacted sperm was significantly ($p<0.01$) and positively correlated with SOD and MDA activity of seminal plasma and negatively

Table 2: Interrelationships between sperm quality, cryocapacitation, oxidative markers and CASA traits of freshly diluted and frozen-thawed semen of Gir bulls

	ISM	*HOST*	*LSP*	*C-FP*	*C-BP*	*C-ARP*	*GPX*	*SOD*	*MDA*	*T.Mot*	*Rp pr*	*Sl pr*	*VCL*	*VAP*	*VSL*	*LIN*	*STR*	*BCF*	*ALH*	*DNC*
									Correlations : Diluted Semen											
ISM	1	0.19	0.29*	0.63**	-0.63**	-0.27	-0.29*	-0.25	-0.25	0.41**	0.29*	0.08	0.47**	0.33*	0.28	0.08	0.13	.121	-0.29*	0.04
HOST	0.79**	1	0.87**	0.53**	-0.44**	-0.60**	0.37**	-0.27	-0.45**	0.02	-0.01	0.01	0.12	0.03	0.04	0.01	0.07	0.14	0.15	0.23
LSP	0.81**	0.81**	1	0.70**	-0.63**	-0.62**	0.35*	-0.24	-0.34*	0.03	-0.01	-0.09	0.12	0.03	-0.02	-0.12	-0.12	0.14	0.17	0.22
C-FP	0.56**	0.42**	0.55**	1	-0.99**	-0.45**	-0.16	-0.37**	-0.37*	0.06	-0.02	-0.05	0.24	0.04	-0.02	-0.23	-0.13	0.03	0.01	0.11
C-BP	-0.58**	-0.40**	-0.51**	-0.99**	1	0.29*	0.21	0.35*	0.33*	-0.09	0.03	0.06	-0.27	-0.03	0.05	0.28	0.18	0.03	-0.01	-0.10
C-ARP	-0.18	-0.30*	-0.45**	-0.57**	0.43**	1	-0.19	0.26	0.33*	0.07	-0.07	-0.04	0.03	-0.07	-0.11	-0.19	-0.24	-0.32*	-0.02	-0.06
GPX	-0.05	0.07	0.07	-0.13	0.17	-0.14	1	0.08	0.18	-0.11	0.15	-0.17	-0.02	0.18	0.21	0.28	0.16	0.27	0.24	0.22
SOD	-0.07	-0.09	-0.24	-0.44**	0.38*	0.57**	-0.08	1	-0.03	-0.12	-0.01	-0.10	-0.02	-0.11	-0.09	-0.14	-0.12	-0.05	-0.08	-0.11
MDA	-0.29*	-0.35*	-0.25	-0.25	0.21	0.30*	-0.04	0.18	1	-0.09	-0.12	0.15	-0.30*	-0.13	-0.09	0.06	0.03	-0.22	-0.10	-0.31*
T.Mot	0.21	0.23	0.31*	0.41**	-0.37**	-0.38**	-0.28	-0.33*	-0.56**	1	0.43**	0.18	0.36*	0.31*	0.26	0.07	0.04	0.24	-0.01	0.21
Rp pr	0.22	0.18	0.30*	0.26	-0.22	-0.29*	-0.03	-0.39**	-0.26	0.50**	1	-0.41**	0.71**	0.89**	0.90**	0.65**	0.50**	0.49**	-0.06	0.34*
Sl pr	0.20	0.21	0.29*	0.44**	-0.43**	-0.29*	-0.70*	-0.23	-0.50**	0.86**	0.25	1	-0.37*	-0.42**	-0.29*	-0.02	0.32*	-0.25	-0.33*	-0.44**
VCL	-0.02	0.07	0.08	0.04	-0.01	-0.17	-0.09	-0.33*	-0.40**	0.58**	0.59**	0.33*	1	0.77**	0.72**	0.23	0.26	0.39**	-0.03	0.50**
VAP	0.15	0.09	0.23	0.10	-0.08	-0.16	-0.03	-0.33*	-0.19	0.39**	0.91**	0.19	0.58**	1	0.97**	0.72**	0.51**	0.61**	0.10	0.53**
VSL	0.18	0.12	0.27	0.19	-0.17	-0.19	-0.13	-0.33*	-0.19	0.42**	0.89**	0.32*	0.52**	0.97**	1	0.82**	0.69**	0.56**	-0.02	0.40**
LIN	0.28	0.11	0.30*	0.31*	-0.32*	-0.12	-0.11	-0.21	0.02	0.20	0.61**	0.29*	-0.04	0.69**	0.78**	1	0.82**	0.45**	-0.07	0.15
STR	0.21	0.19	0.33*	0.37**	-0.37**	-0.19	-0.33*	-0.20	-0.19	0.51**	0.54**	0.68**	0.29*	0.59**	0.75**	0.77**	1	0.31*	-0.26	-0.02
BCF	0.15	0.12	0.27	0.10	-0.07	-0.19	-0.02	-0.32*	-0.24	0.42**	0.72**	0.19	0.53**	0.79**	0.74**	0.47**	0.41**	1	0.45**	0.63**
ALH	0.10	0.07	0.09	-0.08	0.11	-0.07	0.29*	0.05	-0.15	-0.01	0.35*	-0.19	0.04	0.38**	0.27	0.20	-0.09	0.46**	1	0.82**
DNC	0.08	0.16	0.15	-0.05	0.09	-0.17	0.26	-0.14	-0.36*	0.27	0.58**	0.01	0.49**	0.60**	0.48**	0.18	0.11	0.65**	0.85**	1
									Correlations : Frozen-Thawed Semen											

n=48; *Correlations significant at p<0.05 level; **Correlation significant at p<0.01 level (2-tailed).

ISM-Individual sperm motility, HOST-Hypoosmotic swelling test, Lsp-Live sperm, C-FP/BP/ARP-Capacitation 'F' 'B' & 'AR' pattern, GPx-Glutathione peroxidase, SOD-Superoxide dismutase, MDA-Malondialdehyde, T.Mot-Total motile sperm by CASA, Rp pr-Rapid progressive motile sperm, Sl pr-Slow progressive motile sperm, VCL-Curvilinear velocity, VAP-Average path velocity, VSL-Straight line velocity, LIN-Linearity, STR-Straightness, BCF-Beat cross frequency, ALH-Lateral head displacement, DNC-dancing frequency in CASA.

Table 3: Interrelationships between sperm quality, cryocapacitation, oxidative markers and CASA traits of fresh and frozen-thawed semen of Murrah bulls

	ISM	*HOST*	*LSP*	*C-FP*	*C-BP*	*C-ARP*	*GPX*	*SOD*	*MDA*	*T.Mot*	*Rp pr*	*Sl pr*	*VCL*	*VAP*	*VSL*	*LIN*	*STR*	*BCF*	*ALH*	*DNC*
									Correlations : Fresh Diluted Semen											
ISM	1	0.58**	0.71**	0.52**	-0.50**	-0.43**	-0.11	-0.11	-0.09	0.17	0.03	-0.01	0.02	-0.01	-0.01	-0.05	-0.06	-0.04	0.01	0.01
HOST	0.80**	1	0.88**	0.53**	-0.48**	-0.56**	0.04	-0.18	-0.54**	0.01	-0.42**	0.14	-0.29*	-0.49**	-0.49**	-0.42**	-0.42**	-0.45**	0.29*	0.04
LSP	0.82**	0.81**	1	0.51**	-0.47**	-0.50**	0.09	-0.22	-0.30*	0.09	-0.14	0.05	-0.06	-0.20	-0.21	-0.20	-0.18	-0.19	0.07	0.07
C-FP	0.39**	0.58**	0.56**	1	-0.99**	-0.75**	-0.16	-0.14	-0.42**	0.24	-0.08	0.09	-0.06	-0.15	-0.15	-0.17	-0.14	-0.14	0.17	0.09
C-BP	-0.40**	-0.57**	-0.58**	-0.99**	1	0.64**	0.17	0.11	0.37*	-0.24	0.05	-0.08	0.04	0.11	0.11	0.13	0.11	0.11	-0.16	-0.11
C-ARP	-0.19	-0.38**	-0.20	-0.63**	0.52**	1	0.11	0.19	0.49**	-0.21	0.20	-0.13	0.12	0.26	0.27	0.27	0.25	0.23	-0.22	-0.06
GPX	-0.19	-0.33*	-0.39**	-0.27	0.26	0.17	1	-0.46**	-0.10	0.10	-0.22	0.19	0.01	-0.20	-0.20	-0.22	-0.12	-0.25	0.01	-0.04
SOD	-0.02	-0.07	-0.05	-0.29*	0.32*	0.10	-0.31*	1	0.35*	-0.46**	0.02	-0.32*	-0.06	0.09	0.07	0.07	-0.07	0.02	-0.07	-0.05
MDA	0.19	-0.06	0.04	-0.62**	0.59**	0.51**	0.01	0.35*	1	0.04	0.48**	-0.27	0.41**	0.54**	0.51**	0.44**	0.36*	0.26	-0.43**	0.01
T.Mot	0.49**	0.36*	0.51**	0.45**	-0.43**	-0.37**	0.19	-0.15	-0.12	1	0.12	0.42**	0.11	-0.01	-0.01	-0.03	0.06	-0.13	-0.05	0.03
Rp pr	0.16	0.17	0.19	0.18	-0.19	-0.14	-0.03	0.36*	0.03	0.47**	1	-0.55**	0.66**	0.96**	0.96**	0.78**	0.74**	0.71**	-0.54**	0.06
Sl pr	0.43**	0.31*	0.49**	0.39**	-0.37**	-0.36*	0.14	-0.20	-0.16	0.80**	0.09	1	-0.62**	-0.58**	-0.49**	-0.14	0.02	-0.45**	0.05	-0.43**
VCL	0.11	-0.01	0.01	0.03	-0.01	-0.15	-0.01	0.18	0.15	0.29*	0.47**	-0.07	1	0.72**	0.64**	0.14	0.23	0.47**	-0.34*	0.45**
VAP	0.19	0.19	0.18	0.15	-0.13	-0.20	-0.04	0.42**	0.05	0.45**	0.94**	0.16	0.49**	1	0.99**	0.76**	0.72**	0.78**	-0.49**	0.16
VSL	0.18	0.18	0.19	0.15	-0.13	-0.19	-0.05	0.41**	0.05	0.44**	0.93**	0.23	0.40**	0.99**	1	0.84**	0.81**	0.77**	-0.57**	0.02
LIN	0.16	0.21	0.25	0.21	-0.19	-0.19	-0.02	0.29*	-0.08	0.40**	0.72**	0.42**	-0.07	0.79**	0.86**	1	0.92**	0.63**	-0.57**	-0.32*
STR	0.19	0.16	0.25	0.21	-0.18	-0.26	-0.01	0.22	-0.09	0.49**	0.69**	0.58**	0.10	0.74**	0.83**	0.92**	1	0.56**	-0.71**	-0.43**
BCF	0.18	0.07	0.16	0.03	-0.02	-0.08	-0.06	0.38**	0.08	0.42**	0.85**	0.18	0.41**	0.90**	0.90**	0.73**	0.72**	1	-0.17	0.21
ALH	0.20	0.16	0.12	0.01	0.02	-0.12	0.04	0.32*	0.11	0.31*	0.54**	0.09	0.22	0.62**	0.58**	0.43**	0.36*	0.61**	1	0.62**
DNC	0.25	0.17	0.13	0.02	-0.01	-0.12	0.05	0.31*	0.16	0.41**	0.62**	0.10	0.61**	0.70**	0.62**	0.33*	0.34*	0.65**	0.87**	1
									Correlations : Frozen-Thawed Semen											

n=48; *Correlations significant at p<0.05 level; **Correlation significant at p<0.01 level (2-tailed).

ISM-Individual sperm motility, HOST-Hypoosmotic swelling test, Lsp-Live sperm, C-FP/BP/ARP-Capacitation 'F' 'B' & 'AR' pattern, GPx-Glutathione peroxidase, SOD-Superoxide dismutase, MDA-Malondialdehyde, T.Mot-Total motile sperm by CASA, Rp pr-Rapid progressive motile sperm, Sl pr-Slow progressive motile sperm, VCL-Curvilinear velocity, VAP-Average path velocity, VSL-Straight line velocity, LIN-Linearity, STR-Straightness, BCF-Beat cross frequency, ALH-Lateral head displacement, DNC-dancing frequency in CASA.

with total motile, rapid, and slow progressive motile sperm (Table 2).

The post-thawed seminal plasma GPx activity showed significant ($p<0.01$) positive correlations with ALH and DNC of sperm and negative correlations with slow progressive motile sperm and STR. In comparison, SOD activity had significant ($p<0.01$) negative correlations with post-thawed total motile and rapid progressive motile sperm, VCL, VAP, VSL, and BCF. The seminal MDA activity revealed significant ($p<0.01$) negative correlations with total motile and slow progressive motile sperm, VCL, and DNC. However, the correlations of all above sperm quality traits and oxidative markers with all the CASA traits were negligible or very low in post-thawed semen.

Among the CASA traits of post-thawed Gir bull sperm, the total motile sperm and rapid progressive motile sperm had significant ($p<0.05$) positive correlations with all other CASA traits, while slow progressive motile sperm revealed significant ($p<0.05$) positive correlations with VCL, VSL, LIN and STR of post-thawed sperm. The post-thawed VCL of Gir bull sperm showed significant ($p<0.05$) positive correlations with VAP, VSL, LIN, and STR. The post-thawed VCL of sperm showed significant ($p<0.05$) positive correlations with VAP, VSL, STR, BCF, and DNC, while the VAP and VSL had significant ($p<0.05$) positive correlations with all CASA traits. The post-thawed LIN of sperm revealed significant ($p<0.05$) positive correlations with STR and BCF, while STR had a positive correlation with BCF. The post-thawed BCF and ALH had significant ($p<0.05$) positive correlations with all CASA traits (Table 2).

Correlations of Fresh and Post-thawed Semen of Murrah Bulls:

In Murrah bulls also the freshly diluted as well as post-thawed sperm parameters showed more or less similar patterns, but with a little higher magnitude of significant correlations than Gir bull semen, with few exceptions (Table 3). The initial subjectively assessed sperm motility in buffalo semen was significantly ($p<0.01$) and positively correlated with HOS reactive sperm, live sperm, non-capacitated sperm, and negatively with capacitated & acrosome reacted sperm but had no significant correlations with oxidative markers and CASA traits. Similarly, HOS reactive sperm of fresh semen revealed significant ($p<0.01$) positive correlations with live sperm, non-capacitated sperm, and ALH, and negative correlations with capacitated & AR sperm, MDA activity of plasma, and most of the CASA traits. Further, the freshly diluted seminal plasma GPx and SOD activity also did not reveal significant correlations with sperm quality and capacitation traits, and the MDA activity and ALH of sperm had significant ($p<0.01$) positive correlations with most of the CASA traits when compared with Gir bull semen (Table 2, 3).

Furthermore, in post-thawed buffalo semen, the sperm quality and capacitation traits had correlations were similar to those found in Gir bull semen, except that capacitated sperm percent also had significant ($p<0.01$) positive correlations with oxidative markers, and the MDA activity showed negligible correlations with CASA traits. However, like Gir bull semen, most of the post-thawed CASA traits were also significantly interrelated in Murrah bull semen (Table 3).

A very few studies in the literature showed evaluation of the interrelationship of fresh and frozen-thawed sperm quality assessed by routine tests, CTC assay, oxidative markers, and CASA. A significant correlation for post-thaw motility of bulls sperm assessed by subjective means and by CASA, but not with non-return rates, was reported by Anderson *et al.* (1992), while Ferrell *et al.* (1998) observed bull fertility to be positively correlated with motile spermatozoa, progressive motility, VCL, VAP and VSL values. Lackey *et al.* (1998) recorded improved CASA traits with insulin-like growth factors I and II in frozen semen extenders. Perumal *et al.* (2011) observed improved post-thaw quality and CASA traits of bull sperm with the inclusion of glutathione and cysteine in extender. They recorded a moderate non-significant correlation of the post-thaw sperm progressive motility with conception rate ($r=0.4$) and a significant ($p<0.05$) correlation of post-thaw VSL with field fertility ($r=0.7$). However, the present correlation findings for CASA traits were in close agreement with those reported by Patel and Dhami (2013, 2016) and Pathak *et al.* (2020) in fresh and frozen-thawed semen of cattle and buffalo bulls. Moreover, there were significant correlations of initial motility and live sperm assessed subjectively with CASA traits. Kathiravan *et al.* (2008) observed that among different CASA variables, 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 of HOS reactive sperm with different sperm kinematics attributes in Murrah bulls ($r = 0.25$ to 0.60). 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. However, Pathak *et al.* (2018) 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. They suggested velocity traits of fresh semen to be predictive of freezability of semen. The relationships of sperm quality and functional parameters with the fertility of frozen-thawed semen have also been reported by Zodinsanga *et al.* (2015). Similarly, Dalal *et al.* (2019) and Patel *et al.* (2020) showed a positive association with sperm quality parameters, including CASA traits and oxidative markers in bovine semen fortified with Mifepristone and Secicin.

Thus, the present correlation findings suggested that sperm quality parameters, CTC assay, oxidative markers together with CASA analysis of frozen-thawed semen could predict the sperm quality and fertility of cryopreserved bovine semen.

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