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Freezability of Cattle and Buffalo Semen and Association of Fresh and Frozen-Thawed Sperm Quality Parameters

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A B S T R A C T

This study was carried out on semen of three mature Gir cattle and three Surti buffalo bulls. The semen ejaculates (9/bull, total 54) collected in AV were evaluated for sperm motility, viability, morphology, acrosomal integrity, plasma membrane integrity (HOST) and enzyme GOT-GPT leakage pre- and post-freezing in TFYG diluent including post-thaw longevity till 3 h of incubation and their interrelationships. Most of the sperm parameters studied at initial, pre-freeze (PF) and post-thaw (PT) stages were highly significantly and positively interrelated in both the species (0.67 to 0.99). In Gir bulls, significant (P<0.01) correlations were observed for sperm concentration with PF and PT GOT (0.59, 0.57) as well as GPT (0.48, 0.48), and with PT HOS reactive sperm (-0.43); individual sperm motility in fresh semen with PF sperm motility and live sperm (0.94, 0.49) as well as PT motility at 0 and 60 min (0.46, 0.45) of incubation; initial HOS reactive sperm with PT abnormal sperm (-0.58); initial live sperm with PT motility and longevity (0.55, 0.54); pre-freeze motility with PF & PT live sperm (0.54, 0.55) and PT motility at 0 and 60 min (0.48, 0.45); pre-freeze HOS reactive sperm with PF abnormal sperm (-0.56); pre-freeze live sperm with PF intact acrosome (0.51); pre-freeze GOT with PT HOS reactive sperm (-0.45); post-thaw motility with PT live sperm (0.44) and longevity after 60 and 120 min of incubation (0.90, 0.81); post-thaw HOS reactive sperm with PT GOT (-0.47) and incubation motility (0.43); and post-thaw live sperm with PT intact acrosome (0.44). In Surti bulls, the initial sperm motility had significant (P<0.01) correlations with PF HOS reactive sperm (0.58) as well as PF & PT intact acrosome (0.55, 0.51) and GOT (0.39, 0.44); initial HOS reactive sperm with initial and PF live sperm (0.48, 0.42) and GOT (-0.42, -0.41); initial live sperm with HOS reactive sperm (-0.44); initial intact acrosome with PF motility (0.43) and live sperm (0.51); initial GOT with initial, PF and PT GPT (0.72, 0.53, 0.54) and with PT incubation motility (0.38); pre-freeze sperm motility with PF HOS reactive sperm and intact acrosome (0.64, 0.56) and with PT intact acrosome (0.46); pre-freeze HOS reactive sperm with PF & PT live sperm (0.57, 0.69) and intact acrosome (0.54, 0.66); pre-freeze live sperm with PF & PT intact acrosome (0.69, 0.70); pre-freeze intact acrosome with PT live sperm (0.46); pre-freeze GOT with PT motility (0.39); post-thaw motility with PT GOT (0.43) and PT longevity after 60 and 120 min (0.91, 0.65); post-thaw live sperm with PT intact acrosome (0.62) and incubation motility (0.54); and post-freeze GOT with PT GPT (0.60) and PT longevity of sperm (0.47).

Introduction

The literature on the ejaculate quality and freezability of bovine semen in different extenders is quite large (Dhami *et al.*, 1993, Lodhi *et al.*, 2008, Patel *et al.*, 2012, Mahmoud *et al.*, 2013, Chaudhari *et al.*, 2017), but the information about the interrelationships of various spermatozoal attributes of fresh and frozen-thawed bovine semen is meagre both in cattle and buffaloes (Rana and Dhami 2003, Raval and Dhami 2010, Chaudhari *et al.*, 2014). The evaluation of interrelationships of spermatozoa attributes of fresh and cryopreserved bovine semen would help to select a few most valid simple traits of fresh or diluted semen to predict future freezability and even fertility of such ejaculates, instead of going through a plethora of time consuming unpredictable cumbersome tests. Hence an attempt was made to study the freezability and the interrelationships of important spermatozoal attributes in fresh, pre-freeze and post-thawed samples of Gir cattle and Surti buffalo bulls semen to indicate easy, quick, simple, reliable and effective evaluation test to predict post-thaw sperm quality.

Materials and Methods

The present investigation was undertaken during the favourable breeding season from September 2016 to February 2017 on three mature Gir cattle and three Surti buffalo bulls at Sperm Station of Gynaecology Department of the College at AAU, Anand. The bulls were maintained under identical nutritional and managerial conditions under regular veterinary health care. The semen was collected twice a week from all the bulls in the morning hours using artificial vagina. Immediately after collection, the ejaculates (9/bull; total 54) were evaluated for routine physico-morphological attributes. The ejaculates were soon extended at the concentration of 100 $\times 10^6$ spermatozoa ml^{-1} at 34°C with the standard TFYG extender. The extended semen samples were evaluated for sperm quality parameters, viz., motility, viability, morphology, acrosomal integrity, plasma membrane integrity (HOST) including enzyme ALT/AST activity in seminal plasma through standard procedures.

Soon after sampling for above evaluation, the extended semen samples were filled in French mini straws using IS4 system (IMV, France), and gradual cooled 4-5°C over 60-90 minutes, equilibrated for 4 hrs in cold handling cabinet (IMV, France), and then straws were frozen in liquid nitrogen vapour using a programmable bio-freezer (Digitcool 5300 CE ZH 350, IMV, France). The straws were again evaluated at pre-freezing (after equilibration) and after 18-24 hrs of freezing and storage (post-thaw stage) for the same quality parameters including enzyme leakage. Thawing of straws was done in water bath at 37°C for 30 seconds. Post-thaw longevity of sperms was also assessed by evaluating sperm progressive motility at 0, 30, 60, 120 and 180 min of post-thaw incubation (37ºC) in water bath. The sperm progressive motility was determined at 37°C temperature under high power magnification (40 X) of phase contrast microscope (Longshou, USA) fitted with a biotherm stage. The viability and morphology of spermatozoa were assessed in eosin-nigrosin stained semen smears. The percentages of sperm with intact acrosome were assessed using Giemsa stain (Watson 1975). The plasma membrane integrity of spermatozoa was assessed using a hypo-osmotic swelling (HOS) test employing 150 mOs/L solution of sodium citrate and fructose with one hr of incubation at 37°C (Jeyendran *et al.*, 1984; Rasul *et al.*, 2000). The enzymes GOT-GPT were estimated in seminal plasma using standard procedures and assay kits procured from Coral Clinical System, Goa, India (Bergmeyer *et al.*, 1986).

The data were analyzed statistically to work out the means ± standard errors, and the correlations between fresh, pre-freeze and post-thawed sperm quality parameters were estimated breed wise for Gir and Surti bulls (Snedecor and Cochran 1994).

Results and Discussion

The findings on overall Mean ± SE values and the interrelationships found through correlation matrix analysis between various sperm parameters in fresh, pre-freeze and post-thawed semen samples of Gir cattle and Surti buffalo bulls are presented in Tables 1 to 3.

Quality of fresh and cryopreserved spermatozoa

The mean ejaculate volume, sperm concentration and mass activity score for Gir cattle and Surti buffalo semen examined averaged 6.20±0.42 and 3.34±0.23 ml (P<0.01), 1169.44±61.71 and 846.30±54.82 million (P<0.01), 3.44±0.09 and 3.42±0.08, respectively.

Further, the initial, pre-freeze and poat-thaw motility, live sperm, abnormal sperm, intact acrosome. HOS reactive sperm as well as seminal plasma enzyme GOT-GPT activity noted at three stages of cryopreservation process usibng TFYG extender for Gir and Surti buffalo bulls semen are shown in Table 1.

The values and trend of observations of various attributes found corroborated well with several of the earlier reports on bovine semen (Sharma *et al.,* 1992; Dhami *et al.,* 1993; Shelke and Dhami 2001; Taraphder *et al.,* 2001; Rana *et al.,* 2003; Lodhi *et al.,* 2008; Tiwari *et al.,* 2009; Khawaskar *et al.,* 2012; Mahmoud *et al.,* 2013) and were within normal physiological acceptable limits.

Interrelationships of fresh, and cryopreserved Gir bulls spermatozoa

The sperm concentration had significant positive correlations with SGOT (0.48, P<0.05) at initial stage and with SGOT (0.59, 0.57, P<0.01) as well as SGPT (0.48, 0.48) at pre- and post-thaw stages. It had significant negative correlation with post-thaw HOS reactive sperm (-0.43). Mass activity had highly significant (P<0.01) positive correlations with individual sperm motility (0.62, 0.53) at initial and pre-freeze stage as well as with SGPT (0.41) at initial stage and with post-thaw motility at 0 and 60 min of incubation (0.40, 0.41).

The initial sperm motility had highly significant (P<0.01) positive correlations with pre-freeze and post-thaw sperm motility (0.94, 0.46), live sperm (0.49, 0.49) as well as with post-thaw incubation motility at 60 min (0.45).

The initial HOS reactive sperm showed significant (P<0.01) positive correlations with pre-freeze HOS reactive sperm (0.86), and negative correlation with post-thaw abnormal sperm (-0.58). The initial live sperm had significant (P<0.05) negative correlation with abnormal sperm in fresh semen (-0.41), positive correlations with pre-freeze live sperm (0.48) and with post-thaw incubation motility at 0, 60 and 120 min (0.55, 0.56, 0.54). Intact acrosome at initial stage had highly significant (P<0.01) positive correlations with pre- and post-freeze intact acrosome (0.99, 0.91), while SGOT activity had highly significant (P<0.01) positive correlations with pre- and post-freeze SGOT (0.96, 0.79) and SGPT (0.78, 0.72) activity.

SGPT activity at initial stage had highly significant (P<0.01) positive correlations with pre- and post-freeze SGOT (0.68, 0.60) and SGPT (0.87, 0.74) activity.

Table.1 Overall mean (± SE) initial, pre-freeze and post-thaw sperm quality in semen of Gir cattle and Surti buffalo bulls cryopreserved in TFYG extender (100 x 10^6 sperm/ml)

Spermatozoal Traits	Gir bull semen			Surti buffalo semen		
	Initial quality	Freezability (at -196°C) / Step		Initial quality	Freezability (at -196°C) / Step	
		Pre-freeze	Post-thaw		Pre-freeze	Post-thaw
Motile sperm (%)	75.00±0.95	69.38±0.92	40.42±1.50	80.42±0.73	74.38±0.81	39.58±1.85
HOST reactive sperm (%)	76.92±1.27	71.79±1.44	28.67±1.29	82.25±0.62	77.04±0.56	28.04±1.31
Live sperm (%)	76.50±1.30	69.92±1.74	48.92±1.94	81.75±0.70	77.04±0.63	52.38±1.49
Abnormal sperm (%)	5.25±0.36	7.26±0.42	11.06±0.34	4.67±0.37	6.00±0.39	10.13±0.33
Head abnormality (%)	1.29±0.27	1.76±0.30	2.77±0.22	1.25±0.27	1.47±0.26	2.60±0.20
Mid-piece abnormality (%)	0.79±0.23	1.25±0.28	2.03±0.35	0.88±0.30	1.03±0.26	1.73±0.24
Tail abnormality (%)	3.17±0.23	4.25±0.31	6.26±0.22	2.54±0.18	3.50±0.22	5.80±0.21
Intact acrosome (%)	94.50±0.40	90.50±0.45	79.50±0.59	94.04±0.36	90.04±0.39	79.04±0.44
Seminal plasma GOT (U/L)	1382.8±32.0	1451.4±33.6	1564.1±37.9	1367.8±27.8	1436.6±28.5	1543.2±28.8
Seminal plasma GPT (U/L)	1388.3±37.8	1501.7±31.4	1592.6±32.3	1425.2±26.3	1520.4±23.7	1625.1±21.5

Table.2 Interrelationships of sperm quality attributes in fresh (on dilution), pre-freeze and post-thawed semen of Gir bulls processed with standard TFYG extender

Stage	Traits	Initial										Pre-freeze							Post-thaw							
		Vol	Conc	MA	IM	HOST	LSP	AbSP	IA	SGOT	SGPT	IM	HOST	LSP	AbSP	IA	SGOT	SGPT	PTM0	HOST	LSP	AbSP	IA	SGOT	SGPT	PTM60
Initial	Conc	-0.08																								
	MA	-0.19	0.22																							
	IM	-0.11	-0.01	.62**																						
	HOST	0.07	0.04	0.14	0.11																					
	LSP	-0.11	-0.19	0.34	0.34	0.37																				
	AbSP	0.02	0.02	-0.04	-0.14	0.21	-.41*																			
	IA	-0.18	-0.10	0.25	0.21	-0.08	0.01	-0.07																		
	SGOT	-0.24	0.48*	0.37	0.27	-0.13	0.03	-0.06	-0.06																	
	SGPT	0.02	0.37	0.41*	0.23	-0.19	-0.10	0.12	-0.22	.71**																
Pre-freeze	IM	0.04	-0.08	.53**	.94**	0.13	0.37	-0.07	0.13	0.19	0.18															
	HOST	0.15	-0.09	0.11	0.05	.86**	0.30	0.18	0.21	-0.23	-0.31	0.13														
	LSP	-0.01	-0.09	0.27	.49**	0.19	0.48*	-0.23	0.30	-0.03	-0.02	.54**	0.17													
	AbSP	-0.12	-0.19	0.14	-0.03	-0.12	0.10	0.48*	0.05	-0.07	0.06	0.01	-0.16	0.01												
	IA	-0.17	-0.07	0.24	0.24	-0.05	0.01	-0.08	.99**	-0.01	-0.20	0.16	0.23	0.35	-0.02											
	SGOT	-0.24	.59**	0.37	0.31	-0.13	-0.03	-0.05	0.01	.96**	.68**	0.25	-0.19	0.01	-0.16	0.05										
	SGPT	-0.07	0.48*	0.29	0.21	-0.32	-0.2	0.08	-0.02	.78**	.87**	0.12	-0.37	-0.08	-0.01	0.01	.79**									
Post-thaw	PTM0	-0.15	-0.17	0.40*	0.46*	0.25	.55**	-0.01	0.09	0.29	0.30	0.48*	0.22	0.26	0.06	0.12	0.26	0.18								
	HOST	0.16	-.43*	-0.11	-0.12	0.37	0.33	0.14	0.03	-0.35	-0.21	-0.14	0.34	-0.11	0.12	0.03	-.45*	-0.34	.33							
	LSP	0.10	-0.16	0.33	.49**	0.13	0.34	-0.17	0.21	0.05	0.28	.55**	0.24	.64**	-0.18	0.26	0.08	0.12	.44*	.11						
	AbSP	0.12	-0.18	0.08	-0.05	-.58**	-0.18	0.23	-0.11	0.02	0.28	-0.01	-.56**	-.39*	.56**	-0.17	-0.03	0.19	.14	.16	-.13					
	IA	-0.1	-0.08	0.23	0.30	-0.07	0.14	-0.24	.91**	0.03	-0.08	0.26	0.23	.51**	-0.12	.93**	0.12	0.07	.22	-.06	.44*	-.25				
	SGOT	-0.03	.57**	0.18	0.13	-0.07	-0.33	0.13	-0.07	.79**	.60**	0.09	-0.18	-0.1	-0.21	-0.01	.84**	.70**	.14	-.47*	-.08	-.01	.02			
	SGPT	-0.01	0.48*	0.21	0.11	-0.21	-0.20	0.11	0.14	.72**	.74**	0.02	-0.25	0.02	0.02	0.18	.72**	.90**	.17	-.27	.10	.07	.24	.74**		
	PTM60	-0.08	-0.20	0.41*	0.45*	0.28	.56**	-0.10	0.14	0.28	0.33	0.45*	0.25	0.31	0.06	0.16	0.23	0.13	.90**	.39*	.44*	.12	.27	.09	.13	
	PTM120	0.01	-0.32	0.29	0.22	0.18	.54**	-0.12	-0.06	0.25	0.29	0.26	0.12	0.15	0.12	-0.04	0.14	0.05	.81**	.43*	.25	.22	.07	.05	.11	.82**

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

Vol ejaculate volume; MA mass activity; IM individual sperm motility; HOST hypoosmotic swelling test; LSP live sperm; AbSP abnormal sperm; IA intact acrosome; SGOT seminal glutamic oxaloacetate transaminase; SGPT seminal alanine amino transaminase.

Table.3 Interrelationships of sperm quality attributes in fresh, pre-freeze and post-thawed semen of Surti bulls processed with standard TFYG extender

Stage	Traits	Initial										Pre-freeze							Post-thaw							
		Vol	Conc	MA	IM	HOST	LSP	AbSP	IA	SGOT	SGPT	IM	HOST	LSP	AbSP	IA	SGOT	SGPT	PTM0	HOST	LSP	AbSP	IA	SGOT	SGPT	PTM60
Initial	Conc	-.56**																								
	MA	-.15	.34																							
	IM	.04	.08	.19																						
	HOST	.25	.17	.11	-.29																					
	LSP	-.20	.31	-.01	-.23	.48*																				
	AbSP	.45*	-.31	.32	-.18	.16	-.15																			
	IA	.28	-.12	-.01	.37	.23	-.06	.09																		
	SGOT	-.22	-.20	-.10	.36	-.42*	-.17	-.09	-.14																	
	SGPT	-.08	-.16	-.15	.01	-.02	-.10	.11	-.26	.72**																
Pre-freeze	IM	.19	.14	.07	.87**	-.08	-.15	-.06	.43*	.28	.08															
	HOST	.32	-.07	-.19	.58**	.01	-.44*	-.01	.58**	.16	.15	.64**														
	LSP	.14	.33	.12	.36	.42*	-.01	-.17	.51**	-.10	-.06	.42*	.57**													
	AbSP	.31	-.13	.37	-.05	.17	.00	.45*	-.16	-.01	.21	.01	-.16	-.11												
	IA	.24	-.01	.08	.55**	.10	-.16	-.03	.91**	-.01	-.18	.56**	.69**	.69**	-.15											
	SGOT	-.24	-.16	-.11	.39*	-.41*	-.17	-.17	-.10	.98**	.74**	.28	.21	-.05	-.02	.05										
	SGPT	-.17	-.10	-.02	-.03	.03	-.11	.11	-.22	.53**	.90**	-.01	.15	-.06	.22	-.15	.59**									
Post-thaw	PTM0	-.21	.18	.16	.34	.08	.26	.05	.01	.38	.28	.29	.15	-.01	.20	.05	.39*	.25								
	HOST	-.11	.02	.14	.10	-.36	-.15	.03	-.01	-.04	-.06	-.08	.03	-.05	.05	.06	.03	.19	.05							
	LSP	-.07	.23	.21	.27	.33	-.17	.02	.37	-.04	.13	.23	.54**	.54**	-.01	.46*	.06	.34	.37	.32						
	AbSP	.39*	-.12	.25	-.07	.18	-.09	.45*	-.11	-.09	.23	-.09	.04	-.13	.55**	-.13	-.09	.23	.05	.15	.02					
	IA	.18	-.02	.16	.51**	.13	-.33	-.10	.79**	-.09	-.25	.46*	.66**	.70**	-.19	.89**	-.03	-.19	.03	.11	.62**	-.19				
	SGOT	-.22	-.09	-.06	.44*	-.37	-.15	-.19	-.08	.94**	.68**	.33	.25	-.03	-.02	.09	.97**	.56**	.43*	.07	.12	-.07	.01			
	SGPT	-.17	.01	-.10	.08	-.08	-.19	-.01	-.15	.54**	.84**	.17	.29	-.01	.10	.00	.60**	.90**	.31	.11	.33	.07	-.09	.60**		
	PTM60	-.19	.19	.12	.36	.17	.25	-.07	.13	.38*	.33	.26	.26	.23	.12	.23	.43*	.33	.91**	.18	.54**	.11	.19	.47*	.37	
	PTM120	-.37	.22	-.06	.11	.12	.33	-.33	.14	.30	.18	.00	.01	.23	-.06	.21	.33	.12	.65**	.14	.30	-.05	.17	.32	.15	.80**

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

Vol ejaculate volume; MA mass activity; IM individual sperm motility; HOST hypoosmotic swelling test; LSP live sperm; AbSP abnormal sperm; IA intact acrosome; SGOT seminal glutamic oxaloacetate transaminase; SGPT seminal alanine amino transaminase.

The pre-freeze sperm motility had highly significant (P<0.01) positive correlations with pre- and post-freeze live sperm (0.54, 0.55) and with post-thaw motility at 0 and 60 min of incubation (0.48, 0.45). The pre-freeze HOS reactive sperm had highly significant (P<0.01) negative correlation with post-freeze abnormal sperm (-0.56). The pre-freeze live sperm was significantly and positively correlated with post-freeze intact acrosome (0.51), and negatively with post-freeze abnormal sperm (-0.39). Intact acrosome at pre-freeze and post-thaw were significantly interrelated (0.93, P<0.01). Pre-freeze SGOT activity had highly significant (P<0.01) positive correlations with pre- and post-freeze SGPT activity (0.79, 0.72) and negative correlations with post-freeze HOS reactive sperm (-0.45). Pre-freeze SGPT activity had highly significant (P<0.01) positive correlations with post-freeze SGOT activity (0.70). Post-thaw motility had significant (P<0.05) positive correlations with post-thaw live sperm (0.44) and post-thaw motility after 60 and 120 min of incubation (0.90, 0.81). The post-thaw HOS reactive sperm showed significant (P<0.05) positive correlations with 60 and 120 min incubation motility (0.39, 0.43), and negative correlation with post-thaw SGOT activity (-0.47). Post-thaw live sperm had significant (P<0.05) positive correlation with post-thaw intact acrosome (0.44) and 60 min incubation motility (0.44). The post-thaw incubation motility at different intervals were highly significantly (P<0.01) interrelated (0.81 to 0.90).

Interrelationships of fresh, and cryopreserved Surti bulls spermatozoa

The ejaculate volume in Surti bulls had highly significant (P<0.01) negative correlation with sperm concentration/ml (-0.56) and positive correlations with abnormal sperm at initial and post-thaw stage (0.45, 0.39). The initial sperm motility had highly significant (P<0.01) positive correlations with pre-freeze sperm motility and HOS reactive sperm (0.87, 0.58), and pre- and post-freeze intact acrosome (0.55, 0.51) and SGOT activity (0.39, 0.44). The initial HOS reactive sperm showed significant (P<0.05) positive correlations with initial and pre-freeze live sperm (0.48, 0.42) and negative correlation with SGOT activity (-0.42,-0.41). Initial live sperm had significant (P<0.05) negative correlation with HOS reactive sperm (-0.44). The initial intact acrosome had highly significant (P<0.01) positive correlations with pre-freeze motile, HOS reactive and live sperm (0.43, 0.58, 0.51) as well as pre- and post-freeze intact acrosome (0.91, 0.79). SGOT activity at initial stage had highly significant (P<0.01) positive correlations pre- and post-freeze SGPT activity (0.53, 0.54) and with 60 min post-thaw incubation motility (0.38). SGPT activity at initial stage had highly significant (P<0.01) positive correlations with pre- and post-freeze SGOT (0.74, 0.68) and SGPT (0.90, 0.84) activity.

The pre-freeze sperm motility had highly significant (P<0.01) positive correlations with pre-freeze HOST reactive and live sperm (0.64, 0.42) and with pre- and post-freeze intact acrosome (0.56, 0.46). The pre-freeze HOS reactive sperm had highly significant (P<0.01) positive correlations with pre- and post-freeze live sperm (0.57, 0.69) and intact acrosome (0.54, 0.66). The pre-freeze live sperm was significantly (P<0.01) and positively correlated with pre- and post-freeze intact acrosome (0.69, 0.70) as well as post-freeze live sperm (0.54). Pre-freeze intact acrosome had significant (P<0.05) positive correlation with post-freeze live sperm (0.46) and intact acrosome (0.89). Pre-freeze SGOT activity had highly significant (P<0.01) positive correlations with pre- and post-freeze SGPT (0.59, 0.60), post-freeze SGOT (0.97) and post-thaw motility (0.39). Pre-freeze SGPT activity had highly significant (P<0.01)

positive correlations with post-freeze SGOT (0.56) and SGPT (0.90) activity. Post-thaw motility immediately after thawing had significant (P<0.05) positive correlations with post-thaw SGOT (0.43) and post-thaw motility after 60 and 120 min of incubation (0.91, 0.65). Post-thaw live sperm had highly significant (P<0.01) positive correlation with post-thaw intact acrosome (0.62) and 60 min incubation motility (0.54). Post-freeze SGOT activity had significant (P<0.01) positive correlations with post-thaw SGPT (0.60) and post-thaw incubation motility after 60 min (0.47). Like Gir bulls, the post-thaw incubation motility at different intervals in Surti bulls was also highly significantly (P<0.01) interrelated (0.65 to 0.91).

These findings on correlation coefficients observed in Gir and Surti buffalo semen corroborated well with many of the earlier reports, particularly of Dhami *et al.,* (1993), Dhami and Sahni (1994), Shelke and Dhami (2001), Rana and Dhami (2003), Lodhi *et al.*, (2008), Tiwari *et al.,* (2009), Patel *et al.*, (2012) and Mahmoud *et al.,* (2013) in bovine semen. Raval and Dhami (2010) recorded highly significant (P<0.01) correlation for initial motility with mass activity and negative correlations for total abnormal sperm with initial motility and live sperm in triple crossbred bulls. Patel *et al.,* (2012) found significant (P<0.01) positive correlation for individual sperm motility and hypo-osmotic swelling test.

Rana and Dhami (2003) and Raval and Dhami (2010) also found significant (P<0.01) and positive interrelationships for the percentages of motile spermatozoa in fresh, post-thawed semen of bovine and bubaline species. Similar were the findings for the percentages of live sperms, abnormal sperms, intact acrosome (0.17 to 0.90). Further, the results found in this study showed that motility may be a contender marker for semen quality, considering that significant correlations were found between motility and both sperm abnormalities and acrosome as well as plasma membrane integrity, which is in agreement with the opinion of Mahmoud *et a.* (2013). Dhami *et al.,* (1993) and Chaudhari *et al.,* (2014) recorded highly significant positive correlations (0.68 to 0.98) for the sperm motility traits of refrigerated and frozen-thawed semen of HF, Murrah and Surti bulls at various storage intervals/processing steps, and concluded that freezability of semen could be predicted based on its keeping quality at 5°C.

The results of the present study showed significant positive correlations between mass activity and progressive motility (%) as well as negative between sperm viability (%) and abnormal spermatozoa (%) for Gir cattle bull semen as well as significant positive correlation between HOST score and progressive motility in Surti buffalo semen.

These findings, thus in general, suggest that motility estimation in fresh, pre-freeze and post-thawed semen is a legitimately good indicator of live and abnormal sperm and acrosomal integrity of spermatozoa at various steps of semen processing / freezing, and hence, this trait alone can be adopted in routine assessment of semen quality, rather than going into the time consuming clumsy staining procedures for evaluation of viability, morphology and acrosomal integrity, which in fact are not always correlated with *in vivo* fertility. The sperm motility was also correlated with sperm abnormalities and membrane integrity (HOST). Thus, it was concluded that HOS test in addition to motility could be a valuable and practical tool to know the functional capacity of fresh and preserved cattle and buffalo spermatozoa, hence could be added in the routine analysis of semen samples to be selected for use in AI programme.

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