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Concurrence of HOST to some conventional sperm quality parameters and seminal enzymes of Jersey bulls semen

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Artificial insemination is an important assisted reproductive technique that facilitates the extensive dissemination of genetics from elite sires. Cryopreservation, is known to cause damage to the spermatozoa (Kumar *et al*. 2016). A number of laboratory evaluation tests measuring the physical and functional integrity of spermatozoa have been devised over the past few decades, although no single measurement is regarded as reliable indicator for predicting fertility (Faulkner and Pinedia 1980). One such quick and easy tool of evaluating sperm membrane integrity by Jeyendran *et al.* (1984) is HOS test, proved to be radically important in fertilization process (Lodhi *et al.* 2008). Endeavors have been made to correlate sperm plasma membrane integrity to fertility, though a great disparity is seen between studies and methods used, still positive correlation had been reported between HOS test and non-return rate (Correa *et al*. 1997) which prospectively makes it one of the most suitable and effortless method for assessment of semen quality. Biochemical estimation of various enzymes, considered as an indicator for determining the semen quality as they play a key role in functional integrity and function of sperm cell membranes (Macanovic *et al*. 2015). In order to maintain the conformance quality, a minimum standards protocols (MSP) for production of semen had evolved in consultation with experts from Bharatiya Agro-Industries Foundation (BAIF), National Dairy Development Board (NDDB), National Dairy Research Institute (NDRI) and Central Frozen Semen Production and Training institute (CFSP&TI) and has been made effective since 2004. In order to analyze the above facts, a study was undertaken to determine the relationship of HOS test to other semen quality parameters and seminal enzymes in semen of Jersey bulls and to validate viability of HOS test to evaluate sperm quality.

Work was conducted from December 2017-March 2018. Semen from the 8 Jersey bulls maintained at Himachal Pradesh Livestock Development Board Sperm Station, Palampur. Total 64 ejaculates were collected (8 from each bull) and were classified into the freezable and non-freezable Ejaculates depending upon their initial parameters (volume, concentration, mass motility and progressive motility). According to Central Monitoring units for semen stations, ejaculates with motility ≥70% and concentrations ≥500 million/ml were processed after dilution in egg yolk trisbuffer citrate and packed in 0.25 ml straws for further processing. After dilution, progressive motility, morphological abnormalities, acrosomal integrity and HOST were assessed as per the standard methods. In order to estimate progressive motility, a drop of fresh diluted semen was placed on a slide with a cover slip on a warm stage and assessed using phase contrast microscope. Acrosomal integrity based on the acrosomal damage was studied by Giemsa stain (Watson 1975) and morphological abnormalities were assessed using Rose Bengal Stain. Hypo-osmotic swelling test was performed as per Jeyendran *et al*. (1984). A drop of incubated semen in test and control solution was placed on a clean sterilized dry glass slide and covered with a cover slip. A total of 200 spermatozoa were counted in different fields at 400× under phase contrast microscope. For estimation of enzymes, viz. alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), seminal plasma was separated by centrifuging the semen at 4,000 rpm for 15 min. Estimation of these enzymes was done using the

Table 1. Correlation between hypo-osmotic swelling test (HOST) with acrosome integrity, morphology, ALP, AST and ALT

	Parameter	Quantity	Correlation coefficient
HOST	Acrosome	Freezable	0.58178**
		Nonfreezable	0.62609**
	Morphology	Freezable	–0.26161**
		Nonfreezable	0.06317
	ALP	Freezable	–0.66682**
		Nonfreezable	–0.79098**
	AST	Freezable	0.08898
		Nonfreezable	–0.11490
	ALT	Freezable	–0.63720**
		Nonfreezable	–0.74058**

**P<0.01.

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Table 2. Quality semen parameters in freezable and nonfreezable semen of Jersey bulls

Parameter	HOST (%)**	Acrosome integrity (%)**	Morphological abnormalities (%)*	ALP (U/l)**	AST (U/l)**	ALT (U/l)**
Freezable (n=49)	78.51±1.00	84.84±0.87	7.35±0.62	759.77±18.78	156.81±11.35	14.16±0.73
Nonfreezable (n=15)	73.93±2.43	87.60±1.45	9.27±1.18	523.00±21.5	164.00±15.40	24.86±1.23

**P<0.01, *P<0.05.

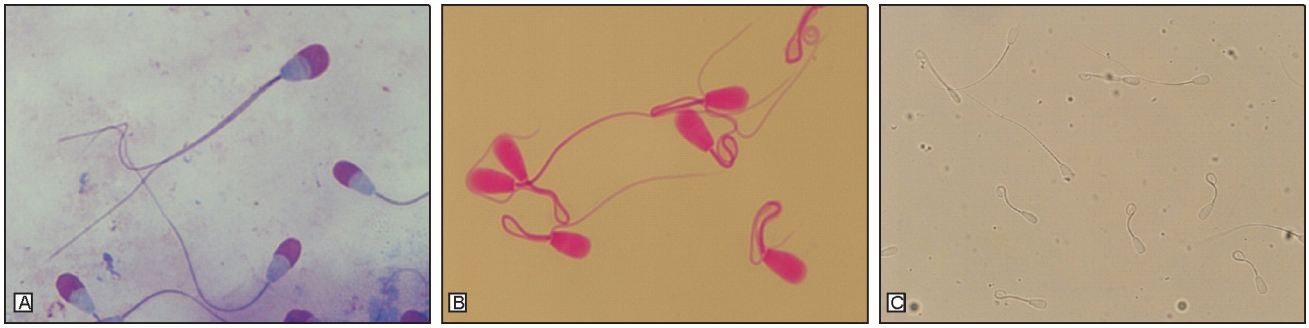

Fig. 1. Acrosome intact, morphological abnormal sperm and HOS positive cells seen under microscope. **(A)** Acrosome intact sperm cells, **(B)** Morphological abnormal, **(C)** HOS positive (coiled tail).

Agappe diagnostic kit as per manufacturer's instructions. The wavelength used for estimation of AST and ALT was 340 nm, whereas for ALP it was 405 nm, respectively.

The Pearson linear correlation method was used to correlate HOS test to other sperm parameter (acrosome integrity, morphological abnormalities, ALP, AST and ALT) and t-test was applied for testing the significance. Difference was considered significant at P≤0.01.

The ejaculates of bulls that were classified as freezable (49) and subsequently used for cryopreservation, had the overall semen quality parameters as per the MSP Guidelines (volume, mass motility and concentration were 5.62±0.25 ml, 3.0±0.08 and 1064.96±61.77 millions/ml, respectively). Whereas, non-freezable ejaculates (n=15) were having mass motility less than 3.0 (volume, mass motility and concentration were 5.68±0.33 ml, 2.5±0.09 and 860.46±64.76 millions/ml, respectively). The results (Tables 1, 2) showed significant positive (P<0.01) correlation between HOST and motility (0.91411; 0.69155) and acrosomal integrity (0.58178; 0.62609) in both freezable and non-freezable ejaculates, respectively. Similar findings were corroborated in semen of buffalo bull (EI-Sisy *et al.* 2010), Sahiwal bull and Nilli-Ravi buffaloes (Lodhi *et al.* 2008), humans (Jeyendran *et al.* 1984), equines (Mantovani *et al.* 2002) and fresh goat semen (Fonseca *et al.* 2005). In our study, significant (P<0.01) negative correlation between HOST and enzyme ALP (–0.66682; –0.79098) and ALT (–0.63720; –0.74058) was recorded in semen of Jersey bulls for both freezable and nonfreezable ejaculates, respectively. However, no significant correlation was found between HOST and AST. Whereas, in present study, significant correlation was found between HOS test and acrosome integrity, which is considered as a stable parameter of sperm function (Henkel *et al.* 1994) and fertility in males (Suri 2005). HOS test has the ability to predict the fertility potential of semen and intactness of sperm plasmalemma (Zubair *et al.* 2013).

Thus, HOS-test can be considered as a useful tool for assessment of semen quality on daily basis to acquire a quick quality result since it is easy, simple, frugal and quick technique and also correlated with other evaluation parameters.

SUMMARY

In this study, hypoosmotic swelling test (HOST) was correlated with sperm quality such as motility, acrosome integrity, morphological abnormality and seminal enzymes including AST, ALT and ALP. Results revealed positive concurrence of HOS test to motility and acrosomal integrity and negative correlation between ALP and ALT in both the freezable and nonfreezable ejaculates. Since the HOS test is quick, easy and inexpensive method so it can be deployed as routine semen assessment procedure in semen stations to differentiate between the freezable and non-freezable bull semen.

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