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Research Article/Artículo de Investigación

CORRELATION BETWEEN CONVENTIONAL SPERM ASSAY PARAMETERS OF FREEZABLE AND NON-FREEZABLE BULL SEMEN

CORRELACIÓN ENTRE LOS PARÁMETROS ESPERMÁTICOS CONVENCIONALES EN SEMEN CONGELABLE Y NO CONGELABLE DE TOROS

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ABSTRACT

This study investigated correlation between various semen quality parameters for freezable and non-freezable ejaculates. Work was conducted at Livestock Development Board Sperm Station Palampur, Himachal Pradesh, India on semen from six Jersey and two H.F x Sahiwal crossbred bulls. Total 64 ejaculates (8 per bull), were analyzed at four stages of semen processing i.e., post dilution, post equilibration, post thaw and 1hr post thaw incubation at 37°C for progressive motility, livability, reaction to hypo-osmotic solution, acrosomal integrity and gross morphological abnormalities. Based on these parameters ejaculates were classified into freezable (n=49) and non-freezable (n=15). The percentages mean (±SE) values of each parameter were recorded at post dilution, post equilibration, post thaw and 1hr post thaw incubation for freezable and non-freezable ejaculates, respectively; live sperm (85.16±0.70, 80.51±0.76, 67.92±1.30, 53.33±1.56 vs. 80.80±1.97, 71.87±2.41 56.87±2.50, 42.13±2.17); progressive motility (80.31±0.72, 74.45±0.86, 57.33±1.76, 38.78±1.55 vs. 58.87±4.21, 53.20±4.42, 36.07±2.43, 24.27±1.90); HOST (78.51±1.00, 72.27±1.10, 60.00±1.60, 45.29±1.51 vs. 73.93±2.43, 64.00±2.81, 49.00±2.91, 37.40±1.89), intact acrosome (84.84±0.87, 78.82±1.03, 67.39±1.37, 54.12±1.38 vs. 87.60±1.45, 78.07±1.06, 66.40±2.90, 55.27±3.42) and morphological abnormalities (7.35±0.62, 7.61±0.59, 8.16±0.56, 9.14±0.63 vs. 9.27±1.18, 9.87±0.98, 10.00±1.00, 11.27±1.14). Statistical analysis of the data revealed that live sperm was positively correlated with motility (r= 0.94529, r= 0.77696, p<0.01), HOST (r= 0.88718, r= 0.90490, p<0.01), and intact acrosome (r= 0.61313, r= 0.65386, p<0.01) whereas, motility was negatively correlated with morphological abnormalities (r= -0.29514, r= -0.02852, p<0.01) in both freezable and non-freezable ejaculates, respectively.

Keywords: freezable, non-freezable, sperm quality, correlation.
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RESUMEN

Este estudio investigó la correlación entre varios parámetros de calidad espermática en eyaculados congelables y no congelables. El trabajo se llevó a cabo en Livestock Development Board Sperm Station Palampur, Himachal Pradesh, India, con el semen de seis toros Jersey y dos mestizos H.F. x Sahiwal. Se analizaron un total de 64 eyaculados (8 por toro) en cuatro etapas de procesamiento del semen, es decir, post dilución, post-equilibrio, post-descongelación y 1hr después de la descongelación a 37°C, para detectar motilidad progresiva, viabilidad, reacción a una solución hipoosmótica, integridad acrosomal y anormalidades morfológicas. En base a estos parámetros, los eyaculados se clasificaron en congelables (n = 49) y no congelables (n = 15). Los porcentajes medios (± SE) de cada parámetro en post-dilución, post-equilibración, post-descongelación y 1hr post-descongelación para los eyaculados congelables y no congelables, respectivamente, fueron los siguientes: espermatozoides vivos (85,16±0,70; 80,51±0,76; 67,92±1,30; y 53,33±1,56 vs. 80,80±1,97; 71,87±2,41 56,87 ± 2,50, 42,13 ± 2,17); motilidad progresiva (80,31 ± 0,72, 74,45 ± 0,86, 57,33 ± 1,76, 38,78 ± 1,55 vs. 58,87 ± 4,21, 53,20 ± 4,42, 36,07 ± 2,43, 24,27 ± 1,90); HOST (78,51 ± 1,00, 72,27 ± 1,10, 60,00 ± 1,60, 45,29 ± 1,51 vs. 73,93 ± 64,74, 64,00 ± 2,81, 49,00 ± 2,91, 37,40 ± 1,89), acrosoma intacto (84,84 ± 0,87, 78,82 ± 1,13, 67,39 ± 1,37 , 54,12 ± 1,38 vs. 87,60 ± 1,45, 78,07 ± 1,06, 66,40 ± 2,90, 55,27 ± 3,42) y anomalías morfológicas (7,35 ± 0,62, 7,61 ± 0,59, 8,16 ± 0,56, 9,14 ± 0,63 vs. 9,27 ± 1,18, 9,87 ± 0,98 , 10,00 ± 1,00, 11,27 ± 1,14). El análisis estadístico de los datos reveló que el porcentaje de espermatozoides vivo se correlaciono positivamente con la motilidad correlaciones positivas y negativas significativas (p<0,01) entre varios parámetros de calidad espermática. EL porcentaje de espermatozoides vivos tuvo una correlación positiva con la motilidad (r= 0,94529, r= 0,77696, p<0,01), HOST (r= 0,88718, r= 0,90490, p<0,01), y con el porcentaje de acrosomas intactos (r= 0,61313, r= 0,65386, p<0,01); mientras que la motilidad se correlacionó negativamente con las anormalidades morfológicas (r= -0,29514, r= -0,02852, p<0.01), tanto en los eyaculados congelables como no congelables, respectivamente.

Palabras clave: congelable, no congelable, calidad espermática, correlación.
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INTRODUCTION

Livestock sector has undergone through a process of biotechnological incorporation with the goal of enhancing productivity and improving the genetic constitution. Artificial insemination (AI) is the first generation reproductive biotechnology that has made profound contribution to the genetic improvement, particularly in dairy cattle. With the dawn of cryopreservation of semen, transmission of superior quality germplasm has become extensive, crossing the geographical boundaries. Appropriate assessment of semen is of vital importance for MOET programs, in-vitro fertilization and for the production of sexed semen. Considering that sexed sperm has high cost of production and are subjected to a variety of adverse conditions during sorting, assessment of semen parameters will provide insight regarding its quality. Evaluation of sperm quality is linked with the desire for predicting fertility in a clinical setting or to enable maximum number of offspring from a valuable sire (Neild et al., 1999).

Conventionally semen is evaluated on the basis of motility, morphology and viability (Zubair et al., 2013).The traditional assessment of the quality of ejaculate has been mainly based on routine semen analyses which have limited capacity for the prediction of the potential fertility of an ejaculate (Jeyendran et al., 1984). Semen quality parameters (SQPs) are considered as vital indices of semen quality and are significantly correlated with freezability and/or fertility in bovine semen (Fiaz et al., 2010). Moreover, there is a correlation among SQPs and highly significant and positive correlation was observed among motility, livability and acrosomal integrity and thus these SQPs could be applied for practical utility in routine semen evaluation to predict freezability, preservability and fertility of spermatozoa (Bhoite et al., 2005).

In terms of prediction, if two variables were correlated perfectly, then knowing the value of one score permits a perfect prediction of the score on the second variable. Generally, when two variables are significantly correlated, the researchers may use the score on one variable to predict the score on the second (Ho, 2006). Thus, with establishment of correlation between routine sperm analysis parameters, would not be necessary to assay other related parameters and instead of them, other tests could be used for assessment of functional characteristics of sperm like zona free ova hamster test and Cervical mucus penetration test (Hafez, 1993).

The present study investigates the relationship between the various semen quality parameters of freezable and non-freezable ejaculates and drawing equations for the estimations of various seminal attributes on the basis of one parameter.

MATERIALS AND METHODS

Present study was conducted at Sperm Station Palampur, India (32.6°N, 76.3^{0}E, altitude 1290.8 m) on semen from six Jersey and two H.F x Sahiwal crossbred bulls.

Semen was collected twice a week by artificial vagina method. The collected semen was evaluated for volume, concentration and initial motility qualifying, after which they were processed as per the standard laboratory procedure. As per Central Monitoring Units (CMU) for sperm stations, the ejaculates with motility $\geq$ 70% and concentration $\geq$500 millions/ml were diluted in egg yolk tris buffer citrate and packed in 0.25 ml straws for cryopreservation. Total 64 ejaculates (8 per bull) were evaluated at four stages of the semen processing viz. post-dilution, post-equilibration, post thaw stage and 1hr post thaw incubation stage at 37°C for percentage of live sperm, progressive motility, reaction to 150 mOsmol hypo-osmotic solution, acrosomal integrity and morphological abnormalities. Based on the above mentioned parameters, ejaculates were classified into freezable (n=49) and non-freezable (n=15) ejaculates. Thawing of frozen semen was done at 37^{0}C for 30 seconds.

For measuring volume, each ejaculates was weighed using the integrated SMILE system which automatically got converted into the volume measure. The concentration of the spermatozoa (millions/ml) was determined by bovine photometer (IMV 7407). Progressive motility was estimated as per Sharma (2011). Percentage of live spermatozoa was estimated by differential staining technique using Eosin and Nigrosin stain and acrosome integrity was assessed by using Giemsa Stain as per Sharma (2011). HOST test was performed as described by Pant et al., (2002). Morphological abnormalities were assessed by Rose Bengal Stain as per Blom (1972).

Statistical analysis

Correlations between different SQPs were made with the combined data of all the four stages of semen processing. The data obtained was analysed using SAS statistical package version 9.2. Multivariate analysis was used to determine correlations and to frame regression equations and their significance as tested again by ANOVA.

RESULTS

Semen evaluation was done at four stages to analyze the variation in the SQPs for freezable and non-freezable ejaculates. The parameters were statistically analyzed at all the stages of processing to determine correlation between them. The mean (±SE) of SQPs values recorded at post dilution, post equilibration, post thaw and 1hr post thaw incubation for freezable and non-freezable ejaculates are shown in Table 1.

Significant positive and negative correlations were found between the various semen quality parameters for freezable and non-freezable ejaculates (Table 2). However, only significant correlation of sperm morphological abnormalities was recorded in freezable ejaculates, with motility and HOST. The relationship between the various semen evaluation parameters along with their regression equations are shown in Table 1 and Figure 1 to 8.

Table 1. Means of sperm quality parameters in freezable and non-freezable ejaculates of bull semen

Sperm parameters	Semen quality	Semen processing stage			
		Post dilution	Post equilibration	Post thaw	1hr Post thaw
Livability	Freezable	85.16±0.70	80.51±0.76	67.92±1.30	53.33±1.56
	Non-freezable	80.80±1.97	71.87±2.41	56.87±2.50	42.13±2.17
Progressive motility	Freezable	80.31±0.72	74.45±0.86	57.33±1.76	38.78±1.55
	Non-freezable	58.87±4.21	53.20±4.42	36.07±2.43	24.27±1.90
HOST	Freezable	78.51±1.00	72.27±1.10	60.00±1.60	45.29±1.51
	Non-freezable	73.93±2.43	64.00±2.81	49.00±2.91	37.40±1.89
Intact acrosome	Freezable	84.84±0.87	78.82±1.03	67.39±1.37	54.12±1.38
	Non-freezable	87.60±1.45	78.07±1.06	66.40±2.90	55.27±3.42
Morphological abnormalities	Freezable	7.35±0.62	7.61±0.59	8.16±0.56	9.14±0.63
	Non-freezable	9.27±1.18	9.87±0.98	10.00±1.00	11.27±1.14

Table 2. Correlations between various semen quality parameters of freezable and non-freezable ejaculates of bull semen

Relationship between Parameters (n=64)		Quality	Correlation coefficient	Regression Estimate	Regression Equation
Livability	Motility	Freezable	0.94529**	1.194±0.02	y=1.194x-22.97
		Non-freezable	0.77696**	0.857±0.09	y=0.857x-10.82
	HOST	Freezable	0.88718**	0.948±0.03	y=0.948x-4.003
		Non-freezable	0.90490**	0.899±0.05	y=0.899x-0.484
	Morphology	Freezable	-0.32265^{NS}	-0.092±0.02	y=-0.092x+14.70
		Non-freezable	0.04913^{NS}	0.011±0.03	y=0.011x+9.355
	Acrosome	Freezable	0.61313**	0.899±0.05	y=0.598x+28.35
		Non-freezable	0.65386**	0.554±0.08	y=0.554x+37.42
Motility	HOST	Freezable	0.91411**	0.773±0.02	y=0.773x+15.53
		Non-freezable	0.69155**	0.622±0.08	y=0.622x+29.23
	Acrosome	Freezable	0.64070**	0.494±0.04	y=-0.494x+40.25
		Non-freezable	0.62801**	0.482±0.07	y=-0.482+51.49
	Morphology	Freezable	-0.29514**	-0.066±0.01	y=-0.066x+12.21
		Non-freezable	-0.02852^{NS}	-0.006±0.02	y=0.006x+10.36
HOST	Acrosome	Freezable	0.58178**	0.531±0.05	y= 0.531x+37.27
		Non-freezable	0.62609**	0.53±0.08	y= 0.534x+42.33
	Morphology	Freezable	-0.26161**	-0.070±0.01	y=-0.070x+12.56
		Non-freezable	0.06317^{NS}	0.015±0.03	y=0.015+9.24
Intact acrosome	Morphology	Freezable	0.06262^{NS}	0.018±0.02	y=0.018x+6.754
		Non-freezable	-0.01433^{NS}	-0.004±0.03	y=-0.004x+10.39

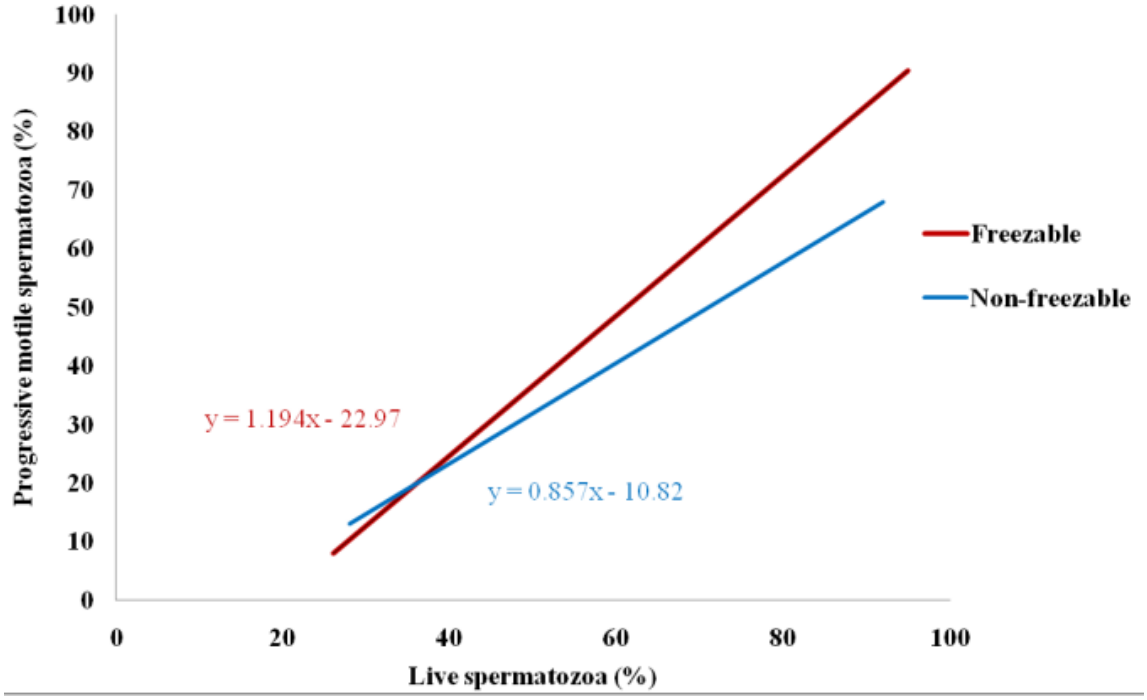

Figure 1. Relationship between sperm livability and progressively motile spermatozoa of freezable and non-freezable bull semen

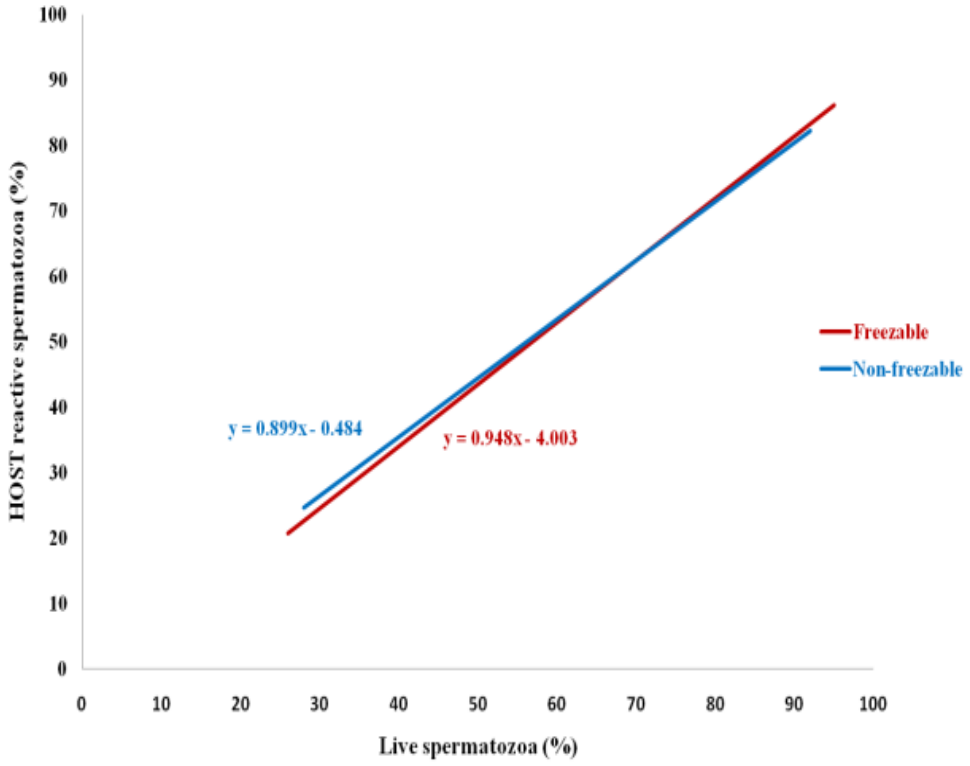

Figure 2. Relationship between sperm livability and HOST reactive spermatozoa of freezable and non-freezable bull semen

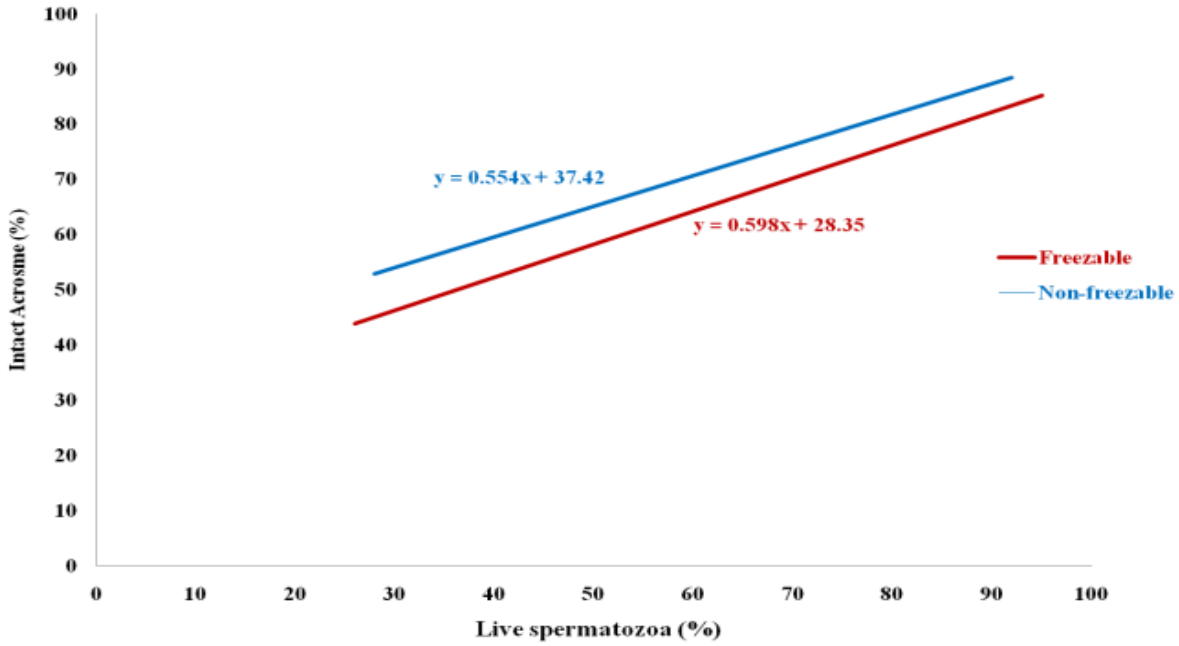

Figure 3. Relationship between live and intact acrosome spermatozoa of freezable and non-freezable bull

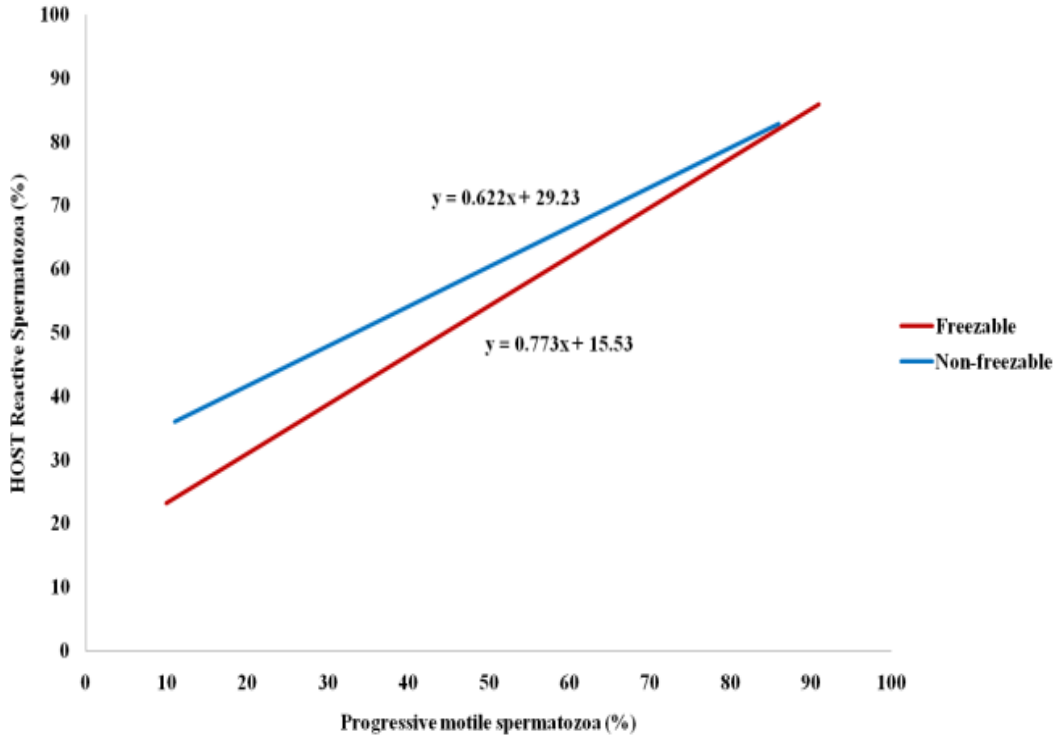

Figure 4. Relationship between progressively motile spermatozoa and HOST reactive (%) spermatozoa of freezable and non-freezable bull semen

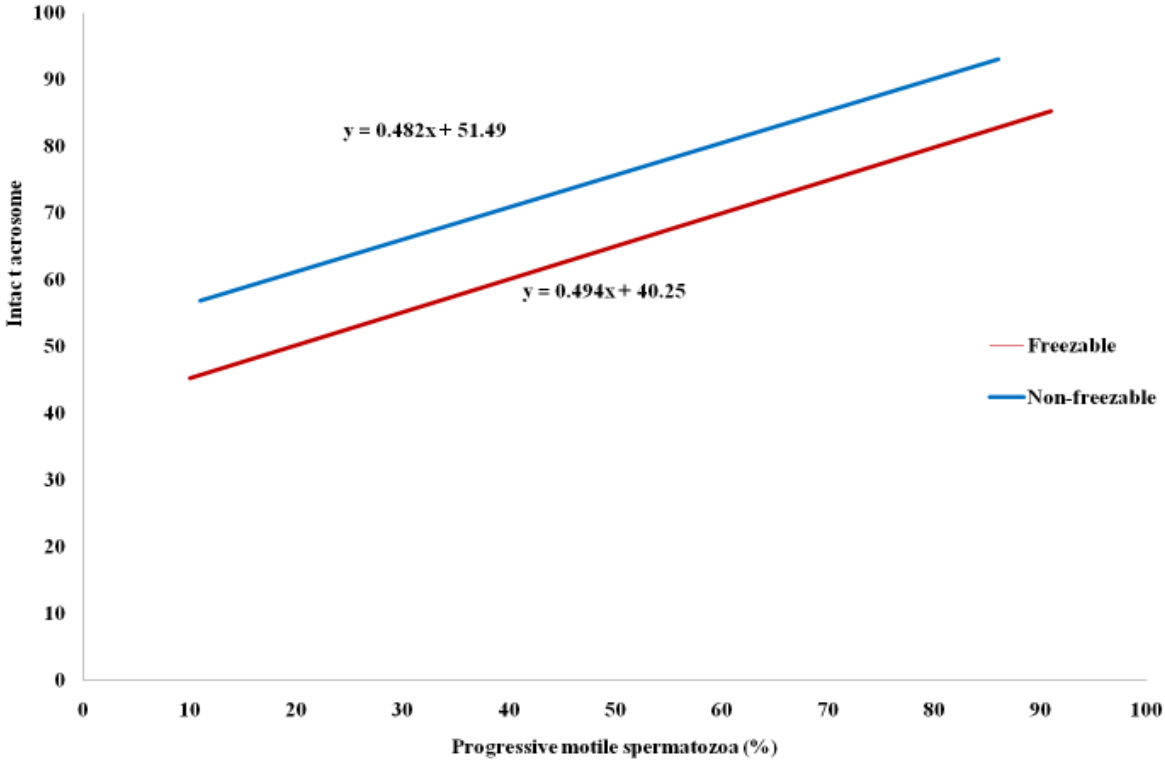

Figure 5. Relationship between progressively motile spermatozoa and intact acrosome spermatozoa of freezable and non-freezable bull semen

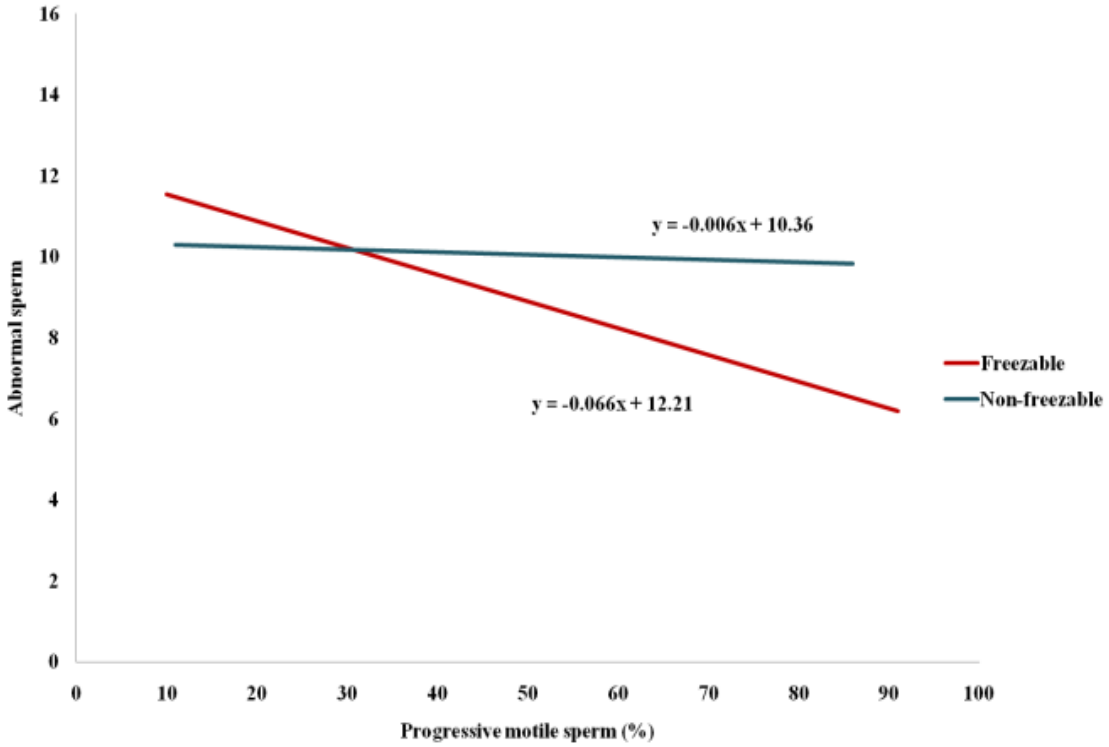

Figure 6. Relationship between progressive motility and abnormal sperm morphology of freezable and non-freezable bull semen

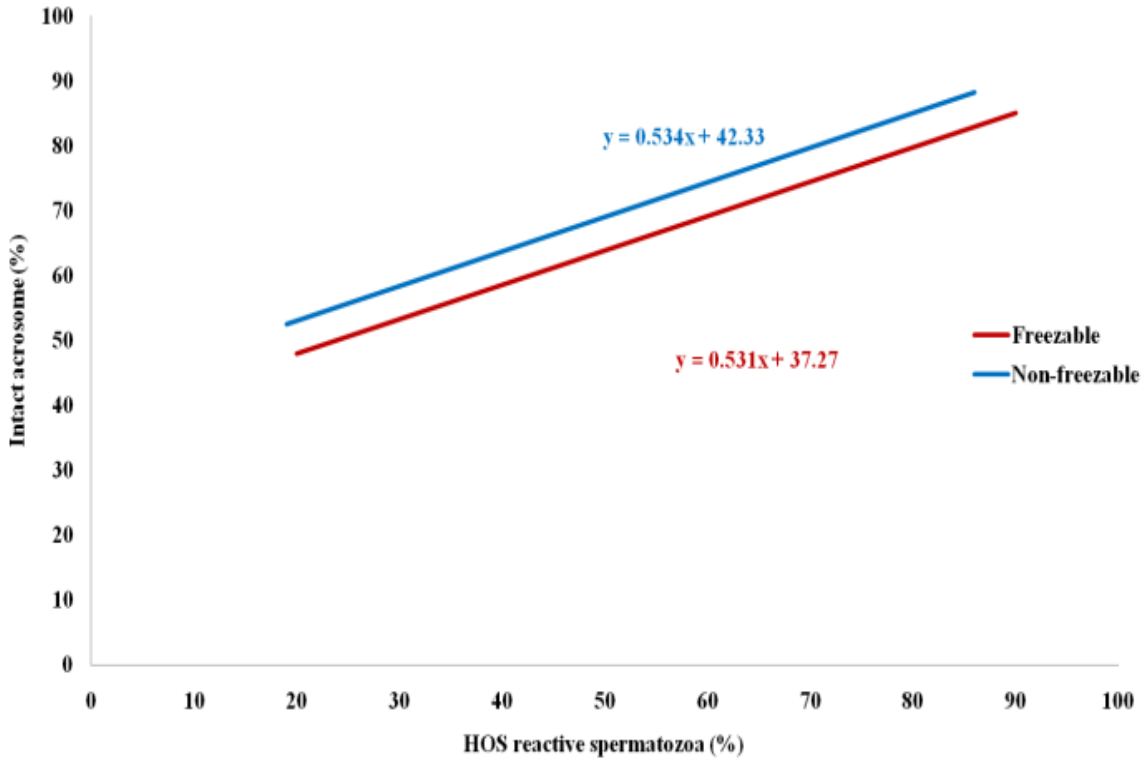

Figure 7. Relationship between HOST reactive and intact acrosome spermatozoa of freezable and non-freezable bull semen

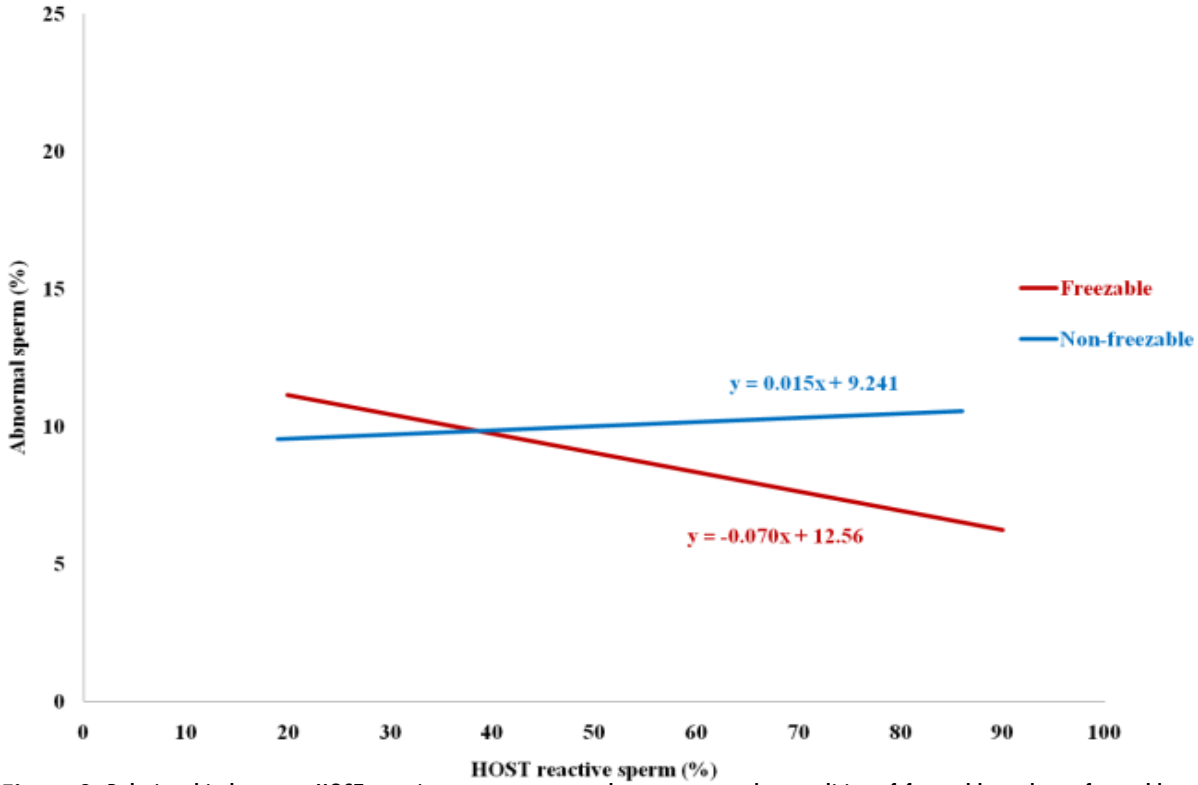

Figure 8. Relationship between HOST reactive spermatozoa and gross sperm abnormalities of freezable and non-freezable bull semen

DISCUSSION

Various conventional semen quality parameters have been used to adjudge the quality of semen. Establishing relationship between semen quality parameters is important, since on the basis of one parameter a fair opinion about other can be contrived. Results of present study corroborated with some previous work on cattle (Lodhi et al., 2008), ram (Bohlooli et al., 2012), goat (Nur et al., 2005) and mithun (Perumal et al., 2015).

Significant correlation that was recorded between livability, motility, HOST and acrosome reaction was in accordance with (Brito et al., 2003) and this was expected since, these all parameters are related to plasma membrane integrity and possibly because sperm plasma membrane is a continuous structure covering the head, mid-piece and tail (Karp, 2009).

Plasma membrane integrity and its stability are two important features required for viability of spermatozoon because if plasmalemma is intact but functionally unstable, the spermatozoon is not capable of interacting with its environment and thus, is unable to fertilize the ovum (Rodriguez-Martinez, 2007).

Correlation between HOST and motility is attributed to the fact that spermatozoon motility partly depends on transports of compounds across membrane of spermatozoa (Jeyendran et al., 1984). Therefore, damage to plasma membrane due to death or anisosmotic conditions causes a rapid leakage of intracellular adenosine triphosphate (ATP), which is required to maintain sperm motility (Bohlooli et al., 2012).

Higher correlation between motility and membrane integrity was attributed to the fact that both are determinants of the integrity of the tail membrane. Similar to the present findings Kirk et al., (2005) reported correlation between acrosome intact sperms with percentage of motile sperms.

Similarly in earlier studies correlation between livability, motility, HOST and acrosomal integrity has been reported by various workers (Singh et al., 2015; Bansal & Cheema, 2014; Zubair et al., 2013; Lodhi et al., 2008). Contrarily, Ray & Ghosh, (2013) reported weak correlation between HOST and progressive motility/Livability. Martins et al., (2013) found no correlation between HOST and other semen attributes.

Negative correlations of morphological abnormalities with other semen quality parameters have been reported (Vyas et al., 1992).However, in present study, no significant correlation of morphological abnormalities with other parameters were recorded except motility and HOST, where significant negative correlations was recorded in freezable ejaculates.

CONCLUSIONS

It was concluded from study that strong positive and negative correlation exists between various semen quality parameters in freezable and non-freezable ejaculates. Therefore, we can evaluate one of them and if necessary other parameters can be evaluated for the establishment of correlation.

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