Research Article

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STUDY OF SEMEN QUALITY IN RELATION TO SEMINAL PLASMA PROTEIN AND OXIDATIVE STATUS IN FRIESWAL CROSS-BRED BULLS

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ABSTRACT

Harvesting a good quality frozen semen from the crossbred bulls is the major shortfall owing to poor freezability and post-thaw survival of their spermatozoa. The present study was envisaged with the objective to find out the correlation among seminal proteins, oxidative status and post-thaw semen quality traits in Frieswal cross-bred bulls.Semen samples (n=24) were collected from six apparently healthy Frieswal crossbred bulls and evaluated for different initial as well as post-thaw qualityattributes and oxidative status. Aliquots (1ml) of fresh semen were also collected from each ejaculate for protein isolation and characterization. Post-thaw semen quality had significant positive correlation with the level of antioxidants and 28-kDa HBP. Whereas, significant negative correlation was noticed between post-thaw semen quality and levels of Malondialdehyde (MDA), Protein Carbonyl Content (PCC) and Seminal Plasma- Heparin Binding Proteins (SP-HBP).

Key words: Frieswal, Heparin Binding Proteins, Oxidative Status, Semen, SOD

During recent years, despite a lot of knowledge gain and understanding regarding bovine semen cryobiology and cryopreservation protocols, the rejection rate of frozen semen especially from crossbred bulls is still very high. It is reported to be 58.88% in Frieswal (Holstein Friesian x Sahiwal) bulls (Tyagi *et al*., 2006). Therefore, harvesting the good quality semen from them is of major concern (Mandal *et al*., 2015). It is also evident that functional, structural or biochemical deterioration of sperm during cryopreservation significantly affects its post-thaw quality and hence the fertilizing ability. Mammalian seminal plasma contains several proteins and majority of them are the secretory products of epididymis and seminal vesicles of male reproductive tract (Chandonnet *et al*., 1990) which modulates the sperm functions in many ways (Nass *et al*., 1990; Calvete *et al*., 1995). Oxidative stress (OS) is also one of the culprit for poor post-thaw survival of the sperm due to excessive production of reactive oxygen species (ROS) by the moribund and dead spermatozoa (Ghosh *et al*., 2018). Although, semen is well endowed with variety of antioxidants such as superoxide dismutase (SOD), glutathione and catalase (CAT) that provides a powerful defence system to the spermatozoa against ROS attack. However, *in-vitro* absence of endogenous defence mechanism and exposure of sperms to various manipulation techniques as well as environment may expose them to OS (Agarwal *et al*., 2005). Therefore, the present study was envisaged with the objective to find out the correlation among seminal proteins, oxidative status and post-thaw semen quality traits in Frieswal cross-bred bulls.

MATERIALS AND METHODS

The present study was conducted at ICAR-Central Institute for Research on Cattle, Meerut Cantt. (UP). The institute is located at latitude of 29.00° North, longitude of 77.67° East and an altitude of 230 m above the mean sea level and has a subtropical climate characterized by hot summer and cooler winter with humidity ranging from 30 to 100 per cent during different months of the year. Total 24 semen samples were collected from six apparently healthy Frieswal crossbred bulls, which were then assessed for initial as well as post-thaw semen quality parameters like individual progressive motility (IPM), viability, morphological abnormality, acrosome integrity and hypo-osmotic swelling test (HOST) response as per the standard methods. The oxidative status of post-thaw semen was assessed through malondialdehyde (MDA; Suleiman *et al*., 1996), total antioxidant capacity (TAC; Benzie and Strain, 1996), CAT (Bergmeyer, 1983), SOD (Madesh and Balasubramanian, 1998) and protein carbonyl content (PCC; Levine *et al*., 1990). Aliquots (1ml) of fresh semen were also collected from each ejaculate for the isolation of seminal plasma and sperm membrane proteins. The extracted proteins were subjected to Heparin-sepharose affinity column chromatography for the isolation of Heparin Binding Proteins (HBP) as per Singh *et al.* (2014). Further, seminal plasma as well as sperm membrane HBP were fractionated using high performance liquid chromatography (b-RPLC, C5) according to McCauley *et al.* (1999) with some modifications. The eluted fractions corresponding to different protein peaks were lyophilized and characterized by SDS-PAGE (Laemmli, 1970). The protein quantitation was done as per the method of Lowry *et al.* (1951). The statistical analysis of the data was done using SPSS 16.0, software for windows. The data recorded were subjected to angular transformation before analysis.The correlation between different variables was established using two-tailed Pearson's correlation coefficient.

RESULTS AND DISCUSSION

Correlation between post-thaw semen quality and seminal proteins

Post-thaw motility (PTM), viability, HOST and acrosome integrity (Acr-Int) of spermatozoa had significant ($P<0.01$) negative correlation with seminal plasma-heparin binding proteins (SP-HBP) level, whereas

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Table 1 :
Pearson correlation coefficients between post-thaw semen quality and seminal proteins

Attributes	SPHBP	SMHBP	SP-28kDa	SM-28kDa
PTM	-.536**	-.013	.199	.414*
Viability	-.549**	-.029	.228	.435*
Abnormality	.561**	-.037	-.107	-.423*
HOST	-.560**	.007	.224	.443*
Acr-Int	-.579**	-.008	.236	.447*

significant (P<0.05) positive correlation was observed between above mentioned semen attributes and sperm membrane-28 kDa HBP (SM-28 kDa) level. Sperm abnormality had significant (P<0.01) positive correlation with SP-HBP, while it was significantly (P<0.05) negatively correlated with SM-28 kDa level (Table 1).

The extended association of spermatozoa and HBP *in-vitro* causes efflux of phospholipid and cholesterol from the sperm membrane in a dose and time-dependent manner (Therien *et al*., 1998). This destabilizes the sperm membrane and renders it vulnerable to stress and damage (Bergeron and Manjunath, 2006). However, the presence of 28-31 kDa HBP on the sperm membrane had positive influence on the *in-vitro* sperm characteristics in terms of high proportion of sperm cells with intact structural and functional membrane, DNA and acrosome integrity (Karunakaran and Devanathan, 2017). All these reports are in accordance to our present findings.

Post-thaw semen quality and oxidative status

TAC, SOD and CAT had significant (P<0.01) positive correlation with PTM, viability, HOST and Acr-Int. Morphological abnormality of spermatozoa had significant (P<0.01) negative correlation with TAC, SOD and CAT. MDA and PCC levels had significant (P<0.01) negative correlation with PTM, viability, HOST andAcr-Int. Sperm abnormality had significant positive correlation with MDA (P<0.01) and PCC (P<0.05) levels (Table 2).

Table 2 :
Pearson correlation coefficients between post-thaw semen quality and oxidative status

Attributes	PTM	Viability	Abnormality	HOST	Acr-Int
MDA	-.709**	-.737**	.749**	-.727**	-.731**
TAC	.723**	.731**	-.770**	.744**	.735**
PCC	-.518**	-.533**	.495*	-.519**	-.515**
SOD	.756**	.773**	-.808**	.768**	.766**
CAT	.618**	.634**	-.584**	.614**	.597**

The plasma membrane of mammalian spermatozoa is rich in polyunsaturated fatty acids and is susceptible to lipid peroxidation during freezing and thawing processes (Andrabi, 2009). Lipid peroxidation produces MDA which damages structure and function of the plasma membrane of spermatozoa (Cocuzza *et al*., 2008). The production of MDA in semen is directly related to the amount of polyunsaturated fatty acids in the cell membrane and ROS in semen (Ayala *et al*., 2014). Recently, Ahmed *et al*. (2018) reported strong negative association of lipid peroxidation with sperm viability and DNA integrity. Increase in lipid peroxidation of spermatozoa during cryopreservation has been attributed to the reduction of antioxidant enzymes after semen extension (Baumber *et al*., 2003). All the above reports are in agreement with the present study.

Seminal proteins and oxidative status in post-thaw semen

SP-HBP level had significant positive correlation with MDA (P<0.01) and PCC (P<0.05) level, whereas it had significant (P<0.01) negative correlation with TAC, SOD and CAT activities. SM-28 kDa levels had significant (P<0.05) negative correlation with MDA and significant (P<0.05) positive correlation with TAC, SOD and CAT. (Table 3)

As mentioned earlier that detrimental effect of higher SP-HBP might have adversely affected the sperm viability as extended association of seminal plasma HBP causes efflux of phospholipid and cholesterol from the sperm membrane (Therien *et al*., 1998) making them vulnerable to death. Furthermore, as the proportion of dead and moribund spermatozoa increases the level of ROS also increases (Ghosh *et al*., 2018) which in turn increased the level of OS. In contrast, SM-28 kDa might have exerted its anti-oxidative action in protecting the sperm membrane (Karunakaran and Devanathan, 2017). Further more, the treatment of crossbred cattle bull semen

Table 3 :
Pearson correlation coefficients between seminal proteins and oxidative status in post-thaw semen

Attributes	MDA	TAC	PCC	SOD	CAT
SPHBP	.557**	-.695**	.416*	-.578**	-.338
SMHBP	.023	-.074	.183	.205	.000
SP-28kDa	-.193	.103	.014	.289	.303
SM-28kDa	-.410*	.467*	-.343	.484*	.405*
MDA		-.867**	.804**	-.768**	-.689**
TAC			-.746**	.614**	.575**
PCC				-.459*	-.604**
SOD					.737**

with 31-kDa HBP protects the spermatozoa from cold shock effect by coating the sperm surface (Patel *et al*., 2016). In another study, it was opined that the presence of 28-kDa protein on the sperm membrane imparted better post-thaw semen characteristics (Pande *et al*., 2018).

Therefore, it can be inferred that increase in the level of 28-kDa HBP, TAC, SOD and CAT in the semen improves the freezabilityas well as post-thaw survivability of the spermatozoa. Whereas higher level of SP-HBP, MDA and PCC promotes damage to the spermatozoa.

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