International Journal of Bio-resource and Stress Management 2016, 7(6):1356-1360 HTTPS://DOI.ORG/10.23910/IJBSM/2016.7.6.1723

Short Research Article

Effect of Winter and Summer Season on Lipid Peroxidation and Total Seminal Plasma Protein of Tharparkar Bull Semen

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Article History

Manuscript No. AR1723
Received in 28th October, 2016
Received in revised form 29th November, 2016
Accepted in final form 5th December, 2016

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Keywords

Tharparkar, lipid peroxidation, season, seminal plasma protein, spermatozoa

Abstract

The present study was undertaken to comparatively study the various particulars in fresh, pre-freeze and post-thaw stage across the season in indigenous breed Tharparkar. A total of 60 ejaculates, 30 each of winter and summer (mass motility ≥3+, initial motility ≥70%) were collected from three bulls (20 from each bull). Part of fresh ejaculate was centrifuged and seminal plasma was used for estimation of TSPP while the sperm pellet was used for the estimation of extent of LPO. Rest of the sample was diluted with Tris-fructose-egg yolk-citrate, equilibrated and frozen by the standard procedure. The total semen volume was significantly (p<0.01) higher in summer (4.45±0.18 ml) as compared to winter season (3.56±0.22 ml). However the levels of total seminal plasma proteins (TSPP) in fresh semen were similar in both winter (10.02±0.66 g dl^{-1}) and summer (9.75±0.19 g dl^{-1}) seasons. TSPP were also found to be negatively correlated with concentration (r=-0.39), mass activity (r=-0.47), initial progressive motility (r=-0.29) and livability of sperm (r=-0.29) in winter season and positively correlated with LPO (r=0.39). Mean pre-freeze and post-thaw motility in winter and summer season did not differ significantly. A significantly (p<0.01) lower values of LPO were estimated in winter (4.26±0.07 n mol 10^{8-1} spermatozoa) than in summer (4.48±0.07 n mol 10^{8-1} spermatozoa) season at post-thaw stage. The levels of LPO in winter and summer were similar at fresh and pre-freeze stages but differed significantly at the post thaw stage suggesting that this parameter may act as an indicator for assessment of damages rendered to spermatozoa by the freeze-thaw cycle. LPO levels were found to be negatively correlated with concentration (r=-0.57), mass activity (r=-0.6), initial progressive motility (r=-0.79) and live per cent (r=-0.79) at fresh stage in both seasons

1. Introduction

Tharparkar is one of the most important dual purpose breed of India. Population of Tharparkar in India is about 5.5 lacs with maximum population in the Rajasthan followed by Uttar Pradesh (18th livestock census, 2007). They are well adopted to harsh environmental conditions and highly resistant to many tropical diseases with good heat tolerance ability. Artificial insemination has remained the main vehicle for the rapid dissemination of valuable germplasm and is the method of choice for dairy farmers worldwide to improve the genetic quality of their livestock. During processing and freezing, semen is exposed to atmospheric oxygen and cold shock enhancing their susceptibility to lipid peroxidation (LPO) due to production of reactive oxygen species. Its effect on spermatozoa are numerous such as membrane damage, irreversible loss of motility, inactivation of enzyme and a high rate of leakage of intracellular sperm constituents like adenine, pyridine and enzymes, protein and DNA damage (Perumal et al., 2016a; Perumal et al., 2016b).

Seminal plasma proteins constitute the major part of seminal biochemical constituents, which originate by exudation from blood plasma and partly are also synthesized by various reproductive organs. These proteins are involved in regulation of osmotic pressure, pH of seminal plasma, transport of ions,

lipids and hormones, maturation of spermatozoa, development of forward progressive motility and fertilizing capacity (Kulkarni, 2003). Several studies have shown that seasonal variations in bull semen quality under varying environmental conditions such as environmental temperature, humidity, photo period and quality of basal diet (Quereshi et al., 1995). Effect of season on seminal attributes has been reported by various workers in crossbred bulls (Verma, 1997; Rafiq, 2009) however, no such information was traced out for Tharparkar bull semen. Therefore present study was undertaken to study comparatively the LPO in fresh, pre-freeze and post-thaw stage and total seminal plasma protein (TSPP) in the winter and summer semen sample to understand seasonal effect.

2. Materials and Methods

The study was conducted at Germ-Plasm Centre (Animal Reproduction Division, Indian Veterinary Research Institute, Bareilly). The climate touches both the extremes of cold and hot weather and the relative humidity ranges between 15 and 85%. Three Tharparkar bulls aged between 4−6 years were housed under natural light and maintained under a uniform constant nutritional regime.

Total of 60 ejaculates, 30 each in both winter and summer season from three bulls (20 from each bulls) were collected during entire period of study (Table 1). Semen was collected twice weekly during morning hours following standard practice using artificial vagina (AV). Ejaculates having mass motility ≥+3 and progressive motility ≥70% were used in semen processing. Fresh semen samples were evaluated for volume, mass activity, concentration, per cent initial motility, per cent livability and abnormalities. Immediately after collection, each ejaculate was divided into three aliquots; one aliquot was used for seminal parameters. Second aliquot was centrifuged at 4000 rpm for 20 min for separation of seminal plasma and was stored at -20 °C till further use while third aliquot was cryopreserved as per standard protocol (-196 °C).

Immediately after collection, concentration of spermatozoa (m ml^{-1}) in neat semen was determined by the Sperm Quality Analyser (SQA-Vb). Per cent live and abnormal spermatozoa were evaluated using Eosin-Nigrosin stain. Separated seminal plasma obtained after centrifugation was used for the estimation of TSPP while the sperm pellet was used for estimation of extent of LPO. After extension semen was filled in 0.5 ml straws and kept in refrigerator at 5 °C for three and half hours for equilibration. At this stage pre-freeze motility, per cent livability and abnormality were evaluated and after this semen were frozen by using Biological Cell Freezer (IMV, France).

Estimation of TSPP was done by Lowry's method (Lowry et al., 1951). Extent of LPO in fresh and frozen-thawed semen was assessed using colorimetric assay method to detect malonaldehyde (MDA), the end product of LPO which react with thiobarbituric acid (TBA) to give a red species absorbing at 535 nm (Suleiman et al., 1996). MDA concentration was calculated by specific absorbance coefficient (1.56×10^5 mol^{-1} cm^{3-1}). MDA level produced by 10^9 sperm (in 3.0 ml volume) in nmol was calculated by applying simplified formula.

MDA produced (n mol 10^9 $sperm^{-1}$)=(OD×10)/1.56×F

Where, F is the pre-treatment dilution factor i.e. 5.

Thawing of frozen semen was done at 37 °C for 30 sec in water bath. The data were analyzed using students 't' test to

Table 1: Physico-morphological seminal attributes of fresh ejaculates during winter and summer season in Tharparkar bulls

Seminal attributes	Season	
	Winter	Summer
Volume (ml)	3.56±0.22^B (2 to 4.5)	4.45±0.18^A (2.4 to 6.5)
Mass activity	4.03±0.11 (3 to 5)	3.97±0.13 (3 to 5)
Concentration (×106 ml^{-1})	1093.73±71.26 (635.5 to 1895.7)	936.51±67.52 (478.1 to 1804.4)
Initial progressive motility (%)	82.83±3.41 (70 to 90)	82.17±3.06 (70 to 90)
Livability (%)	85.10±1.22 (75 to 94)	86.73±1.21 (75 to 94)
Abnormality (%)	9.73±0.29 (6 to 14)	9.50±0.25 (6 to 12)
TSPP (g %)	10.02±0.66 (7.61 to 12.79)	9.75±0.25 (4.5 to 12.85)
Pre-freeze semen parameters		
Motility (%)	73.33±1.50 (65 to 80)	72.33±1.24 (60 to85)
Livability (%)	79.87±2.50 (72 to 88)	77.73±3.95 (66 to 88)
Abnormality (%)	11.80±0.49 (6 to 16)	13.17±0.46 (8 to 17)
Post-thaw semen parameters		
Motility (%)	52.16±1.06 (45 to 65)	50.53±1.09 (45 to 60)
Livability (%)	68.57±1.98 (62 to 78)	66.30±0.96 (58 to 75)
Abnormality (%)	14.67±0.54^B (8 to 18)	16.20±0.41^A (13 to 20)

Means with dissimilar superscript in a row differ significantly $p<0.01$; Values with different superscripts in a row differ significantly [AB ($p<0.01$)]; Figures in parenthesis indicates range;

find out the significance of difference in the mean values and the mean of parameters were subjected to Pearson's correlation coefficient "r" (Snedecor and Cochran, 1994) analysis to using SPSS 16 software.

3. Results and Discussion

A significantly (p<0.01) higher ejaculate volume in summer (4.45±0.18 ml) in comparison to winter season (3.56±0.22 ml) was observed. Similar finding with high volume of ejaculate was reported during summer season followed by winter season by Mostari et al. (2005). Ejaculate volume varies from breed to breed and within a breed from bull to bull (Rao et al., 1996) and might be influenced by various factors like body weight, scrotal size, age, pre-coital stimulation, frequency and season of semen collection (Swain and Singh, 2004; Bhoite et al., 2008).

The levels of TSPP in fresh semen were similar in both winter (10.02±0.66 g dl^{-1}) and summer (9.75±0.19 g dl^{-1}) seasons. Therefore, this parameter would not be an indicator for seasonal variation in semen of Tharparkar bull. Overall mean of total seminal plasma protein concentration in present study were well within the normal ranges as reported by many workers in crossbred bull semen (Loyi et al., 2012). Dhami et al. (1992) reported low mean protein value of static ejaculate than motile ejaculate which supports higher numerical value of TSPP in present study. Similarly, Singh et al. (1989) reported a positive association of protein values in semen with its freezability. Reason could be the great importance of protein for motility and the survival of spermatozoa during storage (Singh et al., 1989; Moura et al., 2007).

TSPP were found to be negatively correlated with concentration (r=-0.39), mass activity (r=-0.47), initial progressive motility (r=-0.29) and livability of sperm (r=-0.29) in winter season. Pangawkar et al. (1988) related higher seminal plasma protein content with lowered freezability of ejaculates in Holstein bulls, which could support our findings. TSPP was positively correlated with LPO (r=0.39) in winter season, which could further support the present study.

Method to judge quality of semen is assessment of its motility in cryopreserved semen and is the main criteria to assess the freezability (Sagdeo et al., 1990). Mean pre-freeze and post-thaw motility in winter and summer season did not differ significantly. Post-thaw motility in Tharparkar bull semen was higher in comparision to report of Rafiq (2009).

Though per cent abnormal spermatozoa had no significant correlation between summer and winter season but it was well within the permissible range (Sharma et al., 1992; Dhanju et al., 2006). This relationship between the sperm abnormalities and freezability has been presented by Dhanju et al. (2006); Perumal et al. (2012a). Abnormality of the spermatozoa is influenced by the age (Rao and Rao, 1996) and season (Patel et al., 1989).

A significantly (p<0.01) lower values of LPO were estimated in winter (4.26±0.07 n mol 10^8 $spermatozoa^{-1}$) than in summer (4.48±0.07 n mol 10^8 $spermatozoa^{-1}$) season at post-thaw stage. These values are in agreement with our concurrent findings in the form of slightly higher values for PTM, livability and lower values for abnormalities, at post thaw stage of evaluation, in winter season. LPO in spermatozoa was negatively correlated with per cent progressive motile and morphologically normal spermatozoa, and positively correlated to percentage of primary sperm defects as have been demonstrated by other workers also (Kasimanickam et al., 2006; Nair et al., 2006; Baruah et al., 2016). However, such a comparative study on LPO levels has not been reported earlier for Tharparkar bulls. Levels of LPO were reported in various species at fresh as well as frozen spermatozoa (Cassani et al., 2005; Kasimanickam et al., 2006; Perumal et al., 2012b) (Table 2).

Table 2: LPO levels in sperm pack during winter and summer season in Tharparkar bulls at fresh, pre-freeze and post thaw stage

Season	LPO (n mole 10^8 $sperm^{-1}$)		
	Fresh	pre-freeze	post-thaw
Winter	$1.13±0.05^{A}$ (0.6 to 1.5)	$1.6±0.04^{B}$ (1 to 2.1)	$4.26±0.07^{Cb}$ (3.5 to 4.8)
Summer	$1.22±0.05^{A}$ (0.7 to 1.8)	$1.69±0.05^{B}$ (1.1 to 2.6)	$4.48±0.07^{Ca}$ (3.7 to 5.3)

A-C: means with dissimilar superscript in a row differ significantly (p<0.01); a-b: means with dissimilar superscript in a column differ significantly (p<0.01)

In present study, levels of LPO in winter and summer were similar at fresh and pre-freeze stages. This could be due to the typical character of Tharparkar bull semen, but differed significantly at the post thaw stage and therefore, this parameter could be an indicator for assessment of damages rendered to spermatozoa by the freeze-thaw cycle. Also there are reports regarding association of lipid peroxidation with enhanced damages to DNA of frozen semen. Hence, this parameter can be employed for evaluating semen before being used in assisted reproductive technologies like *in-vitro* fertilization and in tracytoplasmic sperm injections.

LPO levels were found to be negatively correlated with concentration (r=-0.57), mass activity (r=-0.6), initial progressive motility (r=-0.79) and live per cent (r=-0.79) at fresh stage in both seasons. Similar findings were obtained at both the pre-freeze and the post thaw stages in two seasons. LPO was found to be positively (r=0.53) correlated with abnormal spermatozoa in winter season (Perumal et al., 2011).

4. Conclusion

The result revealed significantly low LPO was observed in winter than in summer ejaculates at post-thaw indicated less cryo damages to the sperm that collected during winter season. It was concluded winter season has higher beneficial effects on cryopreservation of Tharparkar bull semen in the present location.

5. Acknowledgement

The authors thank to the Director and Jt. Director (Res.), Indian Veterinary Research Institute, Izatnagar, U.P., India for providing the necessary facilities.

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