Indian Journal of Animal Sciences **82** (7): 710–712, July 2012

Activity of antioxidant enzymes and malondialdehyde in seminal plasma and their relationship with semen characteristics in crossbred rams

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Received: 14 September 2011; Accepted: 24 March 2012

Key words: Antioxidant enzyme, Malondialdehyde, Crossbreed ram, Seminal plasma

Seminal plasma has an antioxidant system that seems to be very relevant to the protection of sperm. The sperm oxidative defense enzymes predominantly include superoxide dismutase (SOD), catalase (CAT) glutathione peroxidase (GPx) and glutathione reductase (Sikka 2004). These enzymes act as an antioxidant and inhibitor of lipid peroxidation. Since lipid peroxidation leads to loss of motility in spermatozoa, the possibility exists that sperm suffers from the lack of protection against lipid peroxidation due to lack of adequate or non-coordination between SOD, GPx and CAT activity in seminal plasma. Thus knowledge of the natural biochemical composition of seminal plasma is also important in improving genetic, management reproduction, and in order to determine the reproductive and productive potential of cross breed rams, a thorough knowledge of their reproductive physiology is essential. Therefore the objective of this study was, to determine the concentration of MDA, GPx, SOD and CAT in seminal plasma cross breed rams, and to determine their relationship with semen characteristics.

Animal and location: This trial was performed at the sheep breeding station, located in Tabriz, Iran. Sixteen crossbreed and fertile rams (4 from each breed Baluchi × Moghani, Ghezel × Baluchi, Ghezel × Merino, Merino × Moghani) 2–3 years-old and with a live weight of 60–65 kg used in the study.

Semen collection and quality evaluation: 48 ejaculates were collected using artificial vagina following prepucial washing. Semen was transported to labrotary and concentation, progressive motility, and viability was measured using standard procedure (Evans and Maxwell 1987).

Preparation of seminal plasma: The seminal plasma was separated immediately after collection. Fresh semen was centrifuged at 1500×g for 15 min at 5°C. The supernatants were transferred into 1.5 ml eppendoff tubes and recentrifuged at 14,000×g for 10 min at 5°C to eliminate the remaining sperm. Seminal plasma was stored at –20°C until used.

Measurement of Lipid peroxidation: The concentrations of malondialdhyde (MDA), as indices of the LPO in the sperm samples, were measured using the thiobarbituric acid reaction according to the method of Placer *et al*. (1966).

Measurement of CAT activity: The method described by Goth (1991) was used for the determination of CAT activity in the semen samples.

Measurement of GPx activity: Seminal plasma GPx was measured by a Ransel kit. The GPx assay was an indirect measure of the cellular GPx activity (Pagalia and Valentein 1967).

Measurement of SOD activity: SOD activity in spermatozoa was determined using a method proposed by Nishikimi *et al*. (1972) based on the principle that the reduction of nitro blue tetrazolium (NBT) with NADH mediated by phenazine methosulfate (PMS) is inhibited upon addition of SOD.

Statistical analysis: Data are expressed as mean and standard error mean (mean ±SEM). Analysis of variance (ANOVA) with Tukey's test was used subsequently for comparison of mean values at a significance level of $P < 0.05$. Co-efficients of correlation were calculated using non-parametric Spearman's coefficient(r). Database management and statistical analysis were performed using SPSS version 16.0 statistical software package.

A wide range of values has been previously reported for ram semen characteristics (Karagiannidis *et al.* 2000).The highest sperm viability, motility and sperm concentration were measured in the Merinos × Moghani ram, although these differences were not significant among breeds (Table1). The mean sperm motility and sperm viability in 4 crossbred rams ranged between (51–72% and 59–77%, respectively). This could be a reflection of various factors that may influence sperm production including genetics, environmental conditions and nutrition. This finding are in agreement with data on several sheep breeds in the temperate climates e.g. the Moghani rams (Zamiri *et al.* 2010).

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Table 1. Seminal plasma levels of superoxide dismutase (SOD) glutathione peroxidase (GPx) catalase (CAT) and malondialdehyde in seminal plasma and semen characteristics in fresh ejaculates of the crossbreed rams

Merino× Moghani	Ghezel× Merino	Ghezel× Baluchi	Baluchi× Moghani	Parameter
73±4.0[a]	72±5[a]	71±3[a]	64±5[a]	Spermatozoa viability (%)
68±4.0[a]	67±5[a]	66±3[a]	59 ±6[a]	Spermatozoa Motility (%)
4.20±0.29[a]	3.6±0.24[a]	3.71±0.31[a]	3.79±0.22[a]	Spermatozoa Concentration($\times 10^9$)
1.4±0.08[a]	1.1±0.10[a]	1.3±0.17[a]	1±0.11[a]	Semen volume (mL)
10.63±1.92[a]	10.63±1.92[a]	11.95±1.3[a]	15.16±2.80[a]	Spermatozoa abnormality (%)
6.83±0.08[a]	6.45±0.10[a]	7.07±0.30[a]	6.4±0.23[a]	Semen pH
18.65±1.66[a]	20.16±1.72[a]	18.72±1.69[a]	17.28±2.07[a]	SOD(U/mL)
10.97±.097[a]	3.88±2.08[a]	1.76±0.67[a]	3.92 ±0.73[a]	GPx(U/mL)
6.50±0.40[a]	2.83±1.97[a]	2.18±1.21[a]	6.50±0.50[a]	CAT(U/mL)
3.32±0.13[a]	3.17±0.44[a]	3.61±0.41[a]	4.13±0.18[a]	MDA(nomL/mL)

Means within each rows within each parameter without the letter did not differ significantly from each other ($P < 0.05$)

Our result showed that a negative significant correlation existed between MDA levels and sperm concentration($r = -0.533$, $P<0.05$). In this study MDA levels were negative correlated with SOD enzyme activity ($r = -0.479$, $P<0.05$). While, there were no significant correlation between CAT and GPx activity with MDA seminal plasma. Their interpretation was that higher concentration of spermatozoa might produce higher levels of SOD and that decreased production MDA level in seminal plasma.

In this study, it was showed that there are positive significant correlation between seminal plasma, GPx with motility and viability of spermatozoa ($r = 0.580$, $r = 0.452$, $P<0.05$ respectively). Moreover we observed negative correlation between GPx and sperm abnormality ($r = -0.454$, $P < 0.05$). The current study was in agreement with results of Calamera *et al*. (2000). They were reported that good semen quality due to balanced ROS and GPx. SOD and CAT activities in seminal plasma 4 breed weren't correlated to motility and viability of spermatozoa. This finding weren't supported by other studies (Alvarez and Storey 1982), who observed that SOD activity in seminal plasma was positively correlated to spermatozoa motility. The Ghezel × Merinos rams had a higher SOD level. Perhaps the SOD was not utilized to protect spermatozoa from the toxic effects of ROS, even though the spermatozoa underwent higher oxidative stress (Dandekar *et al.* 2002) utilized enzymes not sufficient to protect sperm quality. (Bilodeau *et al*. 2000).The Baluchi × Moghani ram had a higher CAT level but semen quality in breed is low. Possible explanation for high CAT and low semen quality is that available enzymes not utilized for protection and not able to protect sperm quality. The results of the present study demonstrate that ram semen quality can be maintained by the effects of GPx.

SUMMARY

Semen samples (48) collected from 16 fertile crossbreed rams and activity of the 3 antioxidant enzymes included by superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and lipid peroxidation (LPO) was evaluated. The highest sperm viability, motility, sperm concentration, Gpx and CAT activity were recorded in the Merinos × Moghani rams. Significant correlation was observed between GPx enzyme activity with progressive motility and viability. In conclusion, the results were showed that the semen quality of crossbreed rams can be maintained in proper values by the glutathione peroxidase influence.

ACKNOWLEDGMENTS

This research was supported by grant number 27/1770–9 from University of Tabriz. The authors wish to thanks the collaboration from the veterinary staff of university of Tabriz.

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