Assiut Vet. Med. J. Vol. 49 No. 99. October 2003

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LEVEL OF ANTIOXIDANT ACTIVITIES IN SEMINAL PLASMA AND ITS RELATION TO RAM BREED

(With 3 Tables)

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(Received at 20/8/2003)

مستوى انشطة مضادات الاكسدة في البلازما المنوية وعلاقتها بجنس الكبش

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اجريت هذه الدراسة بهدف معرفة مستوى مضادات الاكسدة في البلازما المنوية وعلاقتها بجنس الكبش . استخدم في هذه الدراسة خمسة عشر كبشا بالغا (تسعه أكباش عواسي وستة أكباش شارولیه). تم جمع السائل المنوي من هذه الاكباش (مرتين كل اسبوع لمدة شهر) خلال فترة الانتقال الى موسم التناسل باستخدام التنبيه الكهربائي لجمع السائل المنوي. وقد اظهرت نتائج هذه الدراسة أن جنس الكبش له تأثير معنوي على المستوى الكلي لمضادات الاكسدة ($P<0.05$) وعلى مستوى نشاط انزيم GPx ومستوى هرمون التيستوستيرون ($P<0.01$). وكما اظهرت النتائج ان الكبش ضمن نفس الجنس أظهر تأثير معنوي ($P<0.05$) على مستوى نشاط انزيم GPx. كما وجد ان مستوى نشاط انزيم GPx كان اعلى معنوياً في كباش الشارولیه (374.28 ±46.38) مليجرام بروتين مقارنة مع كباش العواسي (285.58 ±24.96) مليجرام بروتين. كما لوحظ ان مستوى نشاط انزيم SOD اعلى في البلازما المنوية لأكباش الشارولیه مقارنة بكباش العواسي ، ولكن هذا الفرق غير معنوي . وقد اظهرت النتائج ايضاً ان مستوى النشاط الكلي لمضادات الاكسدة كان اعلى معنوياً ($P<0.05$) في البلازما المنوية لكباش العواسي (0.78 ± 0.08) مقارنة مع أكباش الشارولیه (0.43 ± 0.13) مليجرام بروتين. وقد دلت هذه النتائج انه نظراً لوجود اختلافات بين جنس الاكباش وكذلك بين الاكباش من نفس الجنس في مستوى مضادات الاكسدة في البلازما المنوية فإنه من الضروري اخذ ذلك في الاعتبارعند اختيار نوع مضادات الاكسدة المضافة الى مخففات السائل المنوي لتحسين كفاءته خلال الحفظ.

SUMMARY

A study was conducted to determine the level of some antioxidant activities in seminal plasma (SP) and their relation to ram breed. A total of 15 mature intact rams (9 Awassi and 6 Charollais) were involved in the study. Semen samples were collected (twice a week for one month during the transitional period to the breeding season) from each ram by means of an electro-ejaculator. There were significant effect of ram breed on total antioxidant status (TAS) ($P < 0.05$), glutathione peroxidase (GPx) activity ($P < 0.01$) in SP and plasma testosterone (T) level ($P < 0.01$). There was no significant effect of breed on superoxide dismutase (SOD) activity. However, rams within same breed had highly significant ($P< 0.001$) effect on GPx activities. There was a significant ($P < 0.05$) higher level of GPx activities (347.28 ± 46.38 U/mg protein) in SP from Charollais rams in comparison with Awassi rams (285.58 ± 24.96 U/mg protein). SOD activity tended to be higher in SP from Charollais rams in comparison with Awassi rams but statistically not significant. Moreover, TAS in SP from Awassi rams was significantly ($P< 0.05$) higher than in SP from Charollais rams (0.78 ± 0.08 mmol/mg versus 0.43 ± 0.13 mmol/mg protein). The differences between ram breeds and among individual rams within each breed regarding the antioxidant activities in SP make it necessary to select the proper antioxidant to be used as additives to semen extenders to improve semen quality during semen preservation.

Key words: *Ram, Breed, Antioxidants, Seminal plasma*

INTRODUCTION

Fertility of male and successful reproduction is important to efficient livestock production (Duguma *et al.*, 2002). In ovine species, moderate heritability has been summarized for testicular traits and semen quality (Fogarty, 1995). Besides, there is a difference in the biochemical constituents of seminal plasma (SP) between different ovine breeds (Laudate *et al.*, 1997; Abdel-Rahman *et al.*, 2000). Many environmental, physiological and genetic factors have been implicated in the normality of sperm function. Like all cells living under aerobic conditions, spermatozoa are normally exposed to a background level of oxidative stress (OS) which is induced by reactive oxygen species (ROS) (Sikka *et*

al., 1995; De Lamirande *et al.*, 1997). The polyunsaturated fatty acids (PUFA) are one of main targets of free radicals damage (Aitken and Clarkson, 1987). Mammalian spermatozoa membranes are rich in PUFA and sensitive to oxygen induced damage mediated by lipid peroxidation (LPO) (Sikka, 1996). This damage results in a decrease in sperm motility, presumably by a rapid loss of intracellular ATP causing axonemal damage (De Lamirande and Gagnon, 1992; Griveau *et al.*, 1995; Sikka, 1996). Limited endogenous antioxidant mechanisms exist to reverse these damages (De Lamirande *et al.*, 1997). In addition, testosterone (T) levels were found to influence the constituents of SP of rams (Borque and Vazquez, 1999). Antioxidants are compounds and reactions which dispose, scavenge and suppress the formation of ROS or opposite their actions (Droge, 2001). A variety of biological and chemical antioxidants that attack ROS and LPO are still under investigation (Sies, 1993; Speake *et al.*, 1996).

In the area of reproduction, the role of OS and its therapy with antioxidants still in their initial stages and thereby warrant additional exploration. However, numerous studies have been conducted to determine antioxidant activities in SP and their relation to sperm physiology in human (Sikka *et al.*, 1995; De Lamirande *et al.*, 1997; Ivanova and Ivanov, 2000). Little information about the levels of antioxidant activities is available for farm animals' especially ovine species (Abu-Erreish *et al.*, 1978; Mann and lutwak-Mann, 1981), however, no literatures are available on Awassi breed. The objective of the present study was to assess activities of some antioxidants in SP and their relation to ram breeds.

MATERIAL and METHODS

Animals

Fifteen mature intact rams (9 Awassi and 6 Charollais), 1.5-2.0 years old with an average body weight of 55 - 64 Kg were used in this study. The animals were allocated in the Agriculture Research and Production Center at Jordan University of Science & Technology. The rams were housed under conditions of natural day-length and temperature. The animals were fed on crop residues available during summer season and green forage. Adequate concentrate were added according to the NRC requirements. Fresh water was available ad

libitum. After trough examination, all rams were judged to be free from physical defects and had normal external genitalia.

Experimental procedures

At the beginning of March 2002 (the transitory period from non-breeding to breeding season), the rams were weighed. Semen was collected from all rams by means of electro-ejaculator twice a week for one month. After semen collection, the ejaculate was evaluated immediately to assess color, volume and percentage of sperm exhibiting forward motility. Each semen sample was centrifuged at 9000 g for 5 min; SP was separated and kept frozen in duplicate cryogenic vials until assayed. Protein concentration content in SP was determined using specific kit (BioMereux, France). Total antioxidant status (TAS); superoxide dismutase (SOD) activity and glutathione peroxidase activity (GPx) were determined using specific kits (Randox Lab. LTD, USA).

Blood samples (10 ml) were collected at about 10.00 AM by jugular venipuncture using vacutainer tubes containing EDTA before semen collection. Immediately after collection, the samples were centrifuged at 3000 g for 10 min., the plasma was separated and kept frozen at – 20 °C until assayed. Plasma T level was determined by EIA method (DRG, Diagnostics USA).

Statistical analysis

The data were analyzed statistically by analysis of variance (ANOVA). Analysis of variance for repeated measurements was used to test overall effect of breed on TAS, SOD and GPx activities in SP, as will as plasma T concentration. Comparison between means of variables at different breed was carried out by using t-Test.

RESULTS

The overall-means, standard errors (S.E), minimum and maximum values for some biochemical constituents in SP of tested rams are presented in table (1). The activities of GPx and SOD in SP were 319.81± 24.03 and 12.47 ± 1.00 U/mg protein, respectively.

Analysis of variance (Tables. 2&3) showed that the breed and rams within breeds had no effect on SOD activities in SP. In contrast, the breed affects significantly TAS values ($P < 0.05$), GPx activity ($P < 0.01$), and plasma T concentration ($P < 0.01$) (Table 2). The higher value of GPx activity (374.28 ± 46.38 U/mg protein) was observed in SP from

Charollais rams, while the lowest value of GPx activity (285.58 ± 24.96 U/mg protein) was noticed for Awassi rams (Table 3). Also, the mean plasma T concentration was significantly higher ($P< 0.05$) in Charollais rams (3.78±0.45 ng/mL) than for Awassi rams (2.25±0.29 ng/mL). However, the mean values of TAS (0.43 ± 0.13 mmol/mg protein) was significantly lower ($P <0.05$) in SP from Charollais rams in comparison with Awassi rams (0.78± 0.08 mmol/mg protein). SOD activity in SP tended to be higher for Charollais rams (13.95±1.51 U/mg protein) than for Awassi rams (11.44 ±1.63 U/mg protein) but the difference was no significant (Table 3).

DISCUSSION

The somatic cells cytoplasm contains several antioxidant enzyme systems, Glutathione and SOD (Droge, 2001). However, sperm cells are devoid of most of this cytoplasm, so sperm cells have little protection against ROS (Li, 1975). The oxidative modification of cell components via ROS is one of the most potentially damaging processes for proper cell function (Kim and Parthasarathy, 1998). Therefore, sperms of different mammalian species are weak and they can ready undergo lipoperoxidation (Sikka, 1996). The SP has efficient antioxidants systems that can attenuate the effect of OS by scavenging ROS, balance LPO and prevent excessive peroxide formation (Alvarez and Storey, 1989; De Lamirande and Gagnon, 1992; Foote and Hare, 1997).

The present study indicated the presence of appreciable amounts of GPx and SOD activities in SP of ram irrespective to ram breeds. Such results are partially in agreement with previous work by Abu-Erreish *et al.* (1978) who reported the presence of appreciable amount of SOD activities (15 - 22 U/mg) and much lower concentration of GPx (10 – 25 U/mg) in semen collected from mature ram during breeding season. This difference could be partially attributed to stage of breeding season. Recently, Kelso *et al.* (1997) reported the relationship between reproductive period of bull and changes in major antioxidant enzyme systems including GPx and SOD activities in SP. Moreover, the presence of high levels of GPx in seminal plasma indicated immaturity of spermatozoa. Foresta *et al.*, (2002) reported that the GPx levels and its activity (reduced or oxidized form) are related to sperm maturity which were abundantly expressed as active peroxide in semen samples

contained immature spermatozoa. Recently, the function of GPx, an essential selenium-containing antioxidant enzyme has been linked with nitric oxide (Hou *et al*., 1996).

During the transition period to the breeding season, there is a gradual improvement in semen parameters (EL-Din Zain and Mousa, 1998). The authors reported progressive increase in basal T level during the transition period as the breeding season approached. Moreover, the plasma T level has a strong effect on biochemical constituents of SP (Borque and Vazquez, 1999) and control the radox status of both spermatozoa and SP (Purohit *et al*., 2000). This could partially explain the variability in some antioxidant activities as well as TAS in SP of rams within breed in the present study.

Previous studies measured different biochemical constituents (proteins, enzymes, hormones, lipids) of SP in different ram breeds (Rekkas *et al*., 1993; Laudate *et al*., 1997; Abdel-Rahman *et al*., 2000). However, there is no detailed data on the effect of breed on antioxidant activities in SP. Therefore, one important finding from the present work was the clear differences between Awassi and Charollais rams in some antioxidant activities of SP during the transitory period to breeding season. Abdel-Rahman *et al*., (2000) and Karagiannidis *et al*., (2000) reported the importance of assessment of the biochemical constituents of SP in the interpretation of results obtained in the fertility evaluation of various ram breeds as well as among rams within each breed. This support the present results about the significant effect of ram within breed on antioxidant status in SP. Moreover, this can explain the inconstant benefits from addition of different antioxidants to semen of different ram breeds (Maxwell and Stojanov, 1996; Upreti, *et al*. 1998). Therefore, it is of value to determine the level of antioxidants in semen samples when adding any antioxidants to diluents for improving semen quality.

ACKNOWLEDGEMENTS

The authors wish to thank the Deanship of Scientific Research in Jordan University of Science and Technology for financial support, and Dr. K.Zhadi Dept. Anim. Prod., Fac. Agric. for helping in statistical analysis. The thanks are also extended to staff of Jordan University Research Farm for caring the animals.

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Table (1): Some Biochemical constituents in seminal plasma and plasma testosterone level of rams[1].

	Mean	S.E	Range	
			Minimum	Maximum
GPx (U/mg)[2]	319.81	24.03	44.31	781.90
SOD (U/mg)[2]	12.47	1.00	3.30	42.63
TAS (mmol/mg)[2]	0.68	0.07	0.06	1.35
Testosterone (ng/ml)	2.60	0.26	0.32	7.20

[1]: n = 15 rams' [2]: mg protein. GPx= Glutathione Peroxidase.
SOD= Superoxide Dismutase. TAS = Total Antioxidant Status.

Table (2): Analysis of variance for some Biochemical constituents of seminal plasma and plasma testosterone level of rams[1].

Source of variations	df	GPx		SOD		TAS		Testosterone	
		MS	*P*	MS	*P*	MS	*P*	MS	*P*
Breed	1	137389.93	0.01	58.58	0.25	0.59	0.05	20.69	0.01
Animal (Breed)	13	68626.43	0.001	75.33	0.09	0.18	0.31	3.94	0.18
Model	14	71315.41	0.001	75.48	0.08	0.24	0.14	5.09	0.06
Error		20092.39		42.44		0.14		2.70	

[1]: n= 15 rams Df. : degree of freedom MS: Mean square *P*: probability

Table (3): Effect of breed on some Biochemical constituents of seminal plasma and plasma testosterone level of rams.

Breed of rams	GPx (U/mg)[1]	SOD (U/mg)[1]	TAS (mmol/mg)[1]	Testosterone (ng/mL)
Awassi (n = 9)	285.58± 24.96[a]	11.44± 1.63	0.78± 0.08[a]	2.25± 0.29[a]
Charollias (n = 6)	374.28± 46.38[b]	13.95± 1.51	0.43± 0.13[b]	3.78± 0.45[b]

[a,b]: Different Superscripts in the same column differ significantly (P < 0.05).