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Original Research

Influence of Seminal Plasma Antioxidants and Osteopontin on Fertility of the Arabian Horse

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ABSTRACT

This study was designed to investigate enzymatic antioxidants' activity and nonenzymatic antioxidants' levels in seminal plasma of stallions and to relate them with season, age, and fertility of stallions. Fifty ejaculates were collected from six healthy Arabian stallions, 4-22 years old. Ejaculates were evaluated by conventional methods. Five milliliters of each semen sample was centrifuged, and the supernatant seminal plasma was stored at −20°C. Five antioxidants, in addition to osteopontin (OPN) and testosterone, were determined in stallion seminal plasma by using commercial enzyme-linked immunosorbent assay kits. Results revealed that uric acid, ascorbic acid, OPN, and testosterone concentrations and glutathione peroxidase (GPx) activity in stallions' seminal plasma were high ($P < .05$) during spring. GPx activity was higher ($P < .05$) in age group B (11-18 years old) than in age group A (4-10 years old). The effect of stallions' age on GPx activity in the fertility groups was highly significant ($P < .01$). OPN concentration was highest ($P < .05$) in age group A. Uric acid and OPN concentrations and GPx activity in stallions' seminal plasma and percent of sperm motility were higher ($P < .05$) in fertility group III (>70%) than in fertility group I (<50%). However, ascorbic acid concentration, catalase activity and percentage of sperm abnormalities were lower ($P < .05$) in fertility group III than in fertility group I. It was concluded that season and stallion age may affect antioxidant defense systems in stallions' seminal plasma. The impairment of seminal antioxidants and OPN could lead to low fertility.

1. Introduction

Reactive oxygen species (ROS) are known to be involved in lipid peroxidation, as well as DNA and protein damage that may lead to cell death [1,2]. The balance between ROS production and their detoxification may be an important factor in sperm survival and function [3]. Oxidative stress has been associated with perturbation of normal sperm function, including damage to chromatin, proteins, and membrane lipids resulted in a decrease in sperm motility, a decreased capacity for acrosome reaction, and loss of fertility [4-6]. ROS are found in all tissues in the body and also in semen, where they are produced by damaged and dead spermatozoa [7], from cytoplasmic remnants in immature spermatozoa [7], and also by leukocytes [8] and cellular debris [9]. High levels of ROS are associated with infertility in humans [10] but were previously thought not to cause sperm damage in equine semen, on the grounds that the antioxidative systems present in stallion seminal plasma (glutathione peroxidase [GPx], superoxide dismutase [SOD], and catalase [CAT]) would neutralize ROS [9,11]. Seminal plasma is a rich source of enzyme scavengers of ROS, such as SOD, CAT, and GPx. These act to degrade superoxide anion, hydrogen peroxide or lipid peroxides [6], and nonenzymatic low-molecular-weight factors such as lactate, urate, taurine, hypotaurine, pyruvate, ascorbic acid, tocopherol, ergothioneine, and albumin are capable of removing certain ROS [12,13], whereas the sperm cell has limited antioxidant capacity, related to its small

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Table 1
Stallion's age, fertility index, and number of mares used per group

Age group	Stallion age (yr)	Fertility % (group)	Number of mares bred by each stallion
A	4	46.67 (I)	15
	10	65.85 (II)	41
B	13	78.57 (III)	56
	17	72.73 (III)	22
C	19	56.25 (II)	16
	22	36.36 (I)	11

cytoplasmic volume [6]. In rams, seminal plasma has a very effective antioxidant system that can provide spermatozoa with a protective environment against oxidative stress [14]. However, low seminal total antioxidant capacity has been shown to be related to human and stallion infertility [15,16]. In horse seminal plasma, the osteopontin (OPN) protein is positively associated with fertility (72 kDa) [17]. Male infertility is significantly associated with raised seminal plasma testosterone [18,19].

This investigation was designed to find out whether stallion semen contains high activities and levels of antioxidants. If so, what is the relationship between the presence of these antioxidants in the seminal plasma and the quality of freshly ejaculated semen as well as fertility.

2. Materials and Methods

2.1. Animals

A total of six healthy Arabian stallions, between 4 and 22 years old were involved in this study during a period of 11 months. These stallions belonged to three Arabian horse farms (Al-Bushaier Mydod, Haleim Shah, and Al-Hashem farms) at Al-Ahsa, Kingdom of Saudi Arabia, and they have been used as sires in the regular breeding program of their farms. The fertility of stallions was determined by the percentages of mares that conceived on one mating by the stallion, retrospectively.

2.2. Semen Collection and Evaluation

Fifty ejaculate samples were collected from the stallions using Equine Artificial Vagina kit (Colorado State University Model, CSU, USA). Immediately after collection, semen samples were transferred to a well-equipped laboratory and evaluated by conventional methods. The percentages of progressively motile sperm and sperm abnormalities, as well as sperm cell concentration ($\times 10^6$ sperm/mL) were determined objectively using Sperm Vision version 3.5 software (Minitube of America, Inc.). An aliquot of the semen sample (5 mL) was centrifuged at 1500 $\times g$ for 15 minutes, and seminal plasma was collected and stored at -20°C pending analysis.

2.3. Determination of Enzymatic Antioxidants Activity, Nonenzymatic Antioxidants, OPN, and Testosterone Levels in Seminal Plasma

Five antioxidants in stallion seminal plasma were determined by enzyme-linked immunosorbent assay (ELISA; Absorbance microplate reader ELx 800, BioTek; microplate strip washer ELx 50, BioTek) using commercial kits. The antioxidants were uric acid (measured in concentrations of mg/dL; catalog no. K608−100; BioVision); ascorbic acid (in nmol/mL; catalog no. K671-100; BioVision); CAT (in nmol/min/mL; catalog no. 707002; Cayman Chemical Company); SOD (in U/mL; catalog no. 706002; Cayman Chemical Company); and GPx (in nmol/min/mL; catalog no. 703102; Cayman Chemical Company). In addition, OPN (in pg/mL; catalog no. E90899Bo; Uscn, China) was determined in the seminal plasma by using an ELISA kit. Testosterone was measured in the seminal plasma of stallions by using a horse testosterone ELISA kit (in ng/mL; catalog no. CSB-E13193Hs; Cusabio Biotech Co., China).

2.4. Statistical Analysis

Data were divided according to season, age, and fertility of stallions. Seasons were autumn (September-November), winter (December-February), spring (March-May), and summer (June-August). Stallions in group A were 4-10 years old; 11-18 years old in group B; and >18 years old in group C. The fertility of stallions in group I was <50%, 50%-70% in group II, and >70% in group III (Table 1). Data are presented as means ± SEM for seasons and stallions' age and fertility were compared by analysis of variance (ANOVA) using SPSS version 16.0 software [20].

3. Results

As presented in Table 2, uric acid concentrations in stallions' seminal plasma were higher ($P < .05$) during spring and summer than during autumn and winter. Moreover, ascorbic acid concentrations were higher

Table 2
Mean values obtained for antioxidant activity, OPN, and testosterone concentration in stallions' seminal plasma according to season

Antioxidant (concentration)	Mean ± SEM value in:			
	Autumn (n = 7)	Winter (n = 14)	Spring (n = 6)	Summer (n = 23)
Uric acid (mg/dL)	$2.20^{a} \pm 0.25$	$3.89^{b} \pm 0.31$	$6.08^{c} \pm 0.56$	$5.50^{c} \pm 0.32$
Ascorbic acid (nmol/mL)	$4.41^{a} \pm 0.80$	$2.71^{b} \pm 0.48$	$5.13^{a} \pm 0.86$	$2.12^{b} \pm 0.27$
CAT (nmol/min/mL)	9.58 ± 0.40	9.56 ± 0.49	8.31 ± 0.58	10.14 ± 0.71
SOD (U/mL)	11.74 ± 0.46	9.73 ± 0.47	11.11 ± 0.61	9.73 ± 0.58
GPx (nmol/min/mL)	$104.02^{a} \pm 14.04$	$287.05^{b} \pm 52.50$	$316.48^{b} \pm 54.97$	$325.45^{b} \pm 57.1$
OPN (pg/mL)	$37.00^{a} \pm 4.21$	$35.93^{a} \pm 2.88$	$62.17^{b} \pm 11.36$	$31.55^{a} \pm 2.16$
Testosterone (ng/mL)	$1.04^{ab} \pm 0.57$	$0.67^{a} \pm 0.17$	$1.14^{b} \pm 0.12$	$0.92^{ab} \pm 0.19$

Mean values with dissimilar superscript letters in the same row are significantly different at $P < .05$.