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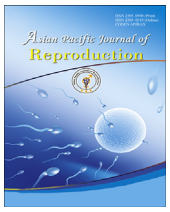

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Comparative blood and seminal plasma oxidant/antioxidant status of Arab stallions with different ages and their relation to semen quality

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ABSTRACT

Objective: To investigate the antioxidant/oxidant levels in blood serum and seminal plasma of Arab stallion with different ages and their relation to semen quality.

Methods: Healthy Arabian stallions ($n = 57$), were divided into three groups. Young (5–10years), Moderate (11–16 years) and Old stallions (>16 years) were subjected to semen evaluation. Seminal plasma and blood samples were collected and stored at −20 °C for measuring glutathione reduced, nitric oxide, Malondialdehyde, ascorbic acid, copper and zinc.

Results: Old stallions had significantly greater ($P < 0.05$) ejaculate volume, % live sperm, and total sperm number compared to young and moderate aged groups. The moderate age horses had significantly the lowest ($P < 0.05$) sperm concentration. Compared to young horses, serum zinc concentrations of moderate and old horses were significantly high ($P < 0.0001$), but NO concentrations were significantly ($P < 0.05$) low. Seminal plasma zinc, ascorbic acid and nitric oxide concentrations were significantly ($P < 0.05$ and 0.01) high in young stallion group. No significant correlations were observed between seminal zinc, copper, MDA and semen variables. Meanwhile, significant negative correlations were observed between seminal plasma ascorbic acid concentration and all semen variables except total sperm number and sperm abnormities %. Significant correlations were observed between reduced glutathione and both of sperm motility and % of live sperm. Nitric oxide concentrations correlated directly with individual sperm motility but adversely with total sperm number.

Conclusion: Stallion age has significant effect on some semen variables, antioxidant/oxidant status of either blood serum or seminal plasma.

1. Introduction

The sperm cell is one of the aerobic organisms that require oxygen. Spermatozoa are rich in targets for oxidative attack and the major source of ROS within the sperm is mitochondria [1]. Depleting Adenosine tri-phosphate within mitochondria causes loss of sperm motility, viability and capacity for fertilization [2,3]. Defective or immature sperms and semen leukocytes produces more ROS and causes sperm dysfunction in men [2] and stallions [4,5]. Physiological low levels of ROS are required for the capacitation process in bulls [6], and normal sperm function in fertile men [2]. Hydrogen peroxide is responsible for the acrosome reaction [7]. Sperm DNA damage of infertile men resulted from high levels of sperm-derived ROS [4,8,9]. The presence of little cytoplasm within the head of spermatozoa makes them deficient in antioxidants and DNA-repair systems [2]. In stallion semen, ROS damage cells by changing lipids, proteins and DNA. It was found that peroxidative stress triggers the mitogen activated protein kinase cascade and the extracellular signal-regulated protein kinase phosphorylation [5]. Spermatozoa are potentially susceptible to peroxidative damage caused by excess ROS due to high amounts of polyunsaturated fatty acids in membrane phospholipids and to sparse cytoplasm. High levels of ROS might be detrimental to stallion sperm survival during storage [10]. Spermatozoa plasma membrane contained high polyunsaturated fatty acids [11], so the deleterious effects of lipid

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peroxidation on spermatozoa are loss of motility and fertilizing capacity [12]. The epididymis and seminal plasma possesses a complex antioxidant system aiming to prevent oxidative damages on sperm lipids, proteins and DNA [13]. The main enzymatic scavenger in the stallion cauda epididymal fluid and seminal plasma is catalase [14]. Although equine spermatozoa have limited GPx and SOD-like activity but equine seminal plasma contains high activity of SOD [15]. Fertile stallion's seminal plasma contains lower concentration of ascorbic acid than infertile ones. From ascorbic acid antioxidant properties to diminish lipid peroxidation [15,16], to regenerate other antioxidant molecules, such as B-carotene, vitamin E and reduced glutathione from their relevant radical species [18]and top reserves membrane integrity of cooled equine sperm [19]. During equine semen storage partial removal of seminal plasma is required to improve sperm survival [20]. Partial seminal plasma removal during semen processing preservation results in removal of oxidative stress scavengers and in turn subjecting spermatozoa to the damaging effect of excessive reactive oxygen species [21].

Higher copper levels in blood serum [22], seminal plasma and sperm are conjugated with infertility [23], poor sperm maturation, motility and fertility in boar [24], ram and bull [25], water buffaloes [26]. In stallions, seminal plasma zinc deficiency not only affects testes development and spermatogenesis [22,27] but also plays a role in protecting sperms during freezing [28].

This study aimed to compare blood serum and seminal plasma copper, zinc, reduced glutathione, ascorbic acid and nitric oxide in Arab stallion with different ages and their relationship to sperm quality.

2. Materials and methods

2.1. Semen collection, evaluation and semen analysis

A total of 57 healthy Arabian stallions, between 6 and 26 aged years were used during this study. These stallions belonged to Police Academy Stud (Cairo), and several private horse studs (Giza) Egypt, and they have been used as sires in the regular natural breeding program of the stud. Stallions were divided according to age into young (5–10) years old; moderate (11–15) years old; and old (>15) years old. Three semen ejaculates were collected for each stallion at weekly interval. At the time of collection, early in the morning, a mare in estrus was used as a mount animal. Semen was collected using a lubricated and pre-warmed (45–50 °C) Colorado model artificial vagina with an inline filter to separate the gel fraction. Immediately after collection, semen samples were transferred to a well-equipped laboratory and the gel-free portion of the ejaculate was evaluated for volume, progressive motility, and concentration was determined by conventional methods [29]. Spermatozoa motility was examined and recorded using a pre-warmed stage of microscope (200×). Sperm concentration was determined with a hemocytometer. Total sperm count per ejaculate was calculated from volume and sperm concentration. The pH was determined with test strips (Merck, Darmstadt, Germany) and the morphological examination of semen samples was determined using nigrosin–eosin stain [30]. To obtain seminal plasma, an aliquot of semen was immediately centrifuged after semen collection at 1 000 ×g for 10 min. The procedure was repeated with the supernatant of the first centrifugation to insure sperm free seminal plasma and stored at −20 °C until they were analyzed.

2.2. Blood sampling and analytical methods

Blood for was collected from the external jugular vein into vacuum tubes. Immediately after collection, the blood samples were centrifuged at 1 000 ×g for 15 min, and sera was stored at −20 °C until they were analyzed.

2.3. Blood antioxidant and biochemical analysis

Serum and seminal plasma glutathione (GSH) reduced [31], nitric oxide (NO) [32], lipid peroxide product (Malondialdehyde, MDA) [33], Ascorbic acid [34], copper and zinc [35] were measured using commercial kits (Bio Diagnostic, Egypt).

2.4. Statistical analysis

All data were analyzed using the SPSS statistical software [36]. The data were expressed as mean ± standard error of means (SEM). Independent sample *t*-test, simple one way ANOVA and Pearson correlation test were used. Duncan Multiple Range test was used to differentiate between significant means at $P < 0.05$.

3. Results

Seminal plasma (Table 1) contained significantly high concentrations of zinc ($P < 0.0001$), NO ($P < 0.05$), reduced glutathione ($P < 0.0001$), ascorbic acid ($P < 0.001$) and MDA ($P < 0.05$) but nearly similar copper compared to their serum levels.

Semen characteristics of the three age groups (Table 2) showed a significant ($P < 0.05$) increase of ejaculate volume and % of live sperms ($P \leq 0.05$), but tendency to a decrease of abnormal sperm % for horses older than 15 y (O). Stallions of moderate age had significantly low sperm cell concentrations ($P < 0.05$) and total sperm count ($P < 0.01$) compared to young and old stallions (Table 2).

Blood serum of old stallions had significantly ($P < 0.05$) high zinc but significantly low NO ($P < 0.01$), compared to the other age groups (Table 3). Moderate aged stallions had significantly low zinc ($P = 0.03$), GHD and MDA ($P < 0.05$).

Seminal plasma of young stallions (Y, Table 4) had significantly the highest zinc ($P < 0.01$), NO ($P < 0.0001$) and ascorbic acid ($P < 0.01$) concentrations compared to the other age groups and tended to have high MDA and GHD ($P > 0.05$).

Seminal plasma zinc had only negative correlation with sperm individual motility ($r = -0.50$; $P < 0.05$) and tended to correlate with total sperm number ($r = 0.39$; $P > 0.05$), but copper had no significant correlations with semen evaluation parameters (Table 5). Ascorbic acid had significant negative correlation with sperm individual motility ($r = -0.69$; $P = 0.02$),

Table 1
Mean ± SEM of blood serum and seminal plasma antioxidant status, zinc and copper of Arab stallions. Q7

Parameter	Serum	Semen	*P*-value
Zinc (mg/dL)	0.39 ± 0.09	2.04 ± 0.37	**0.0001**
Copper (mg/dL)	0.368 ± 0.05	0.457 ± 0.03	**0.09**
MDA (nmol/mL)	4.26 ± 0.45	6.92 ± 2.09	**0.05**
NO (µmol/L)	23.04 ± 1.46	30.56 ± 2.34	**0.02**
Reduced glutathione (mg/L)	9.83 ± 0.66	23.33 ± 6.61	**0.0001**
Ascorbic acid (mg/L)	19.76 ± 4.49	61.59 ± 16.54	**0.001**

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