Shiraz University

IJVR
ISSN: 1728-1997 (Print)
ISSN: 2252-0589 (Online)

Vol. 19 No. 4 Ser. No. 65 2018

IRANIAN JOURNAL OF VETERINARY RESEARCH

Evaluation of semen characteristics, oxidative stress, and biochemical indices in Arabian horses of different ages during the hot summer season

Kandiel, M. M. M.* **and El Khawagah, A. R. M.**

Theriogenology Department, Faculty of Veterinary Medicine, Benha University, Benha, Egypt

****Correspondence:*** *M. M. M. Kandiel, Theriogenology Department, Faculty of Veterinary Medicine, Benha University, Benha, Egypt. E-mail: Mohamed.kandil@fvtm.bu.edu.eg*

(Received 20 Nov 2017; revised version 28 May 2018; accepted 7 Aug 2018)

Summary

Egypt is anticipated to be potentially influenced by the global climate warming. Therefore, the current study aimed to evaluate the influence of age on the fertility potential of Arabian stallions during summer breeding months. Arabian horses grouped according to their age into three groups, each involved six stallions: young (5-6 years), middle (11-12 years) and old (15-20 years) age groups, were weekly sampled during the months of July-August. Ejaculates were collected using artificial vagina, Missouri model, and examined for pH, volume, concentration, motility, livability and morphological abnormalities. Serum samples were harvested and assessed for testosterone, total antioxidant capacity (TAC), lipid profile, and copper and zinc levels. Semen pH ($P<0.005$), spermatozoa motility ($P=0.08$), sperm morphology ($P<0.001$), tail abnormalities ($P<0.001$), and sperm count per ejaculate differed noticeably between stallions' groups. Testosterone ($P=0.07$) and TAC ($P<0.05$) concentrations were markedly affected by stallions' age. Cholesterol correlated negatively with sperm normality, but serum copper and zinc levels correlated positively with semen volume, sperm cell count and spermatozoa livability. These results revealed that the fertility of stallions is age-dependent and is prominently influenced by lipid metabolism and oxidative stress during hot summer breeding season. It is highly advisable to provide animals' house (along with feed and drinking water) with the evaporative cooling system and allow morning or late afternoon outdoor activity to bypass the summer hot climates and sustain stallions' fertility.

Key words: Arabian stallion, Oxidative stress, Semen, Summer season, Testosterone

Introduction

Global warming of the earth is a universally accepted reality. Earth's atmospheric temperature has elevated by 0.74 ± 0.18°C in the 20th century and is expected to increase by 1.8 to 4.0°C by the end of the 21st century (IPCC, 2007). Egypt is assumed to belong to the potential countries that will be affected by the global warming (World Bank, 2009). Accordingly, there are upcoming expectations that the mean temperature in Egypt will increase by about 4°C in Cairo and 3.1 to 4.7°C in the other areas of Egypt by 2060 (Brauch, 2002). Global warming has complex influences in many natural, economic and social systems across the world, and these effects are expected to continue for prolonged periods in the future (IPCC, 2007). Although both domestic and wild animals have been documented to be affected by global climate alteration, information on the direct influence of climate change on animals is rare (Nardone *et al.*, 2010).

Horses, long-day seasonal breeders, exhibit annual cycles of breeding activity. Many physiological and ambient factors, such as the environmental temperature and photoperiod, affect stallion reproductive performance. Seasonal changes in testicular size, sperm production (Clay *et al.*, 1987), seminal pH and sex drive depicted by the reaction time, and mounts per ejaculate (Abou-Ahmed *et al.*, 1993) have been recorded. How the scrotum and its structures can relieve elevated temperatures brought about by heat stress is a focal point for some types of research (Mawyer *et al.*, 2012). Stallion's testicles are close to the abdominal wall, and the internal temperature of the scrotum stays several degrees cooler than the core body temperature, a necessity for normal spermatogenesis (Setchell *et al.*, 1994).

Body temperature is controlled by matching heat production with the heat loss (through convection, conduction, evaporation, and radiation) to the environment. The set-point temperature for body temperature control is not stable. It can fluctuate diurnally or in response to changes in ambient temperature (Heldmaier *et al.*, 2004). As endotherms, mammals typically function at high internal body temperatures, ranging from 35°C to 39°C (Prosser and Heath, 1991). The summer months bring many enjoyable horse-related activities, but they also bring heat and humidity. Many horse owners have questioned how summer heat stress may influence their horses' reproductive performance. Heat stress largely affects most aspects of mammalian reproductive functions, including spermatogenesis (Hansen, 2009). An exposure of the testes to elevated temperatures for prolonged periods may cause the development of spermatozoa to be damaged and die, resulting in a decrease in spermatozoa count per ejaculate or an increase in the percentage of morphologically abnormal sperm.

To the best of the authors' knowledge, information about the interaction between age and stallions' reproductive characteristics during the hot summer breeding season is scarcely available. Therefore, the aim of the present study was to evaluate the changes in semen characteristics, testosterone hormone, metabolic profile, oxidative stress markers, and mineral levels in Arab stallions of different ages during the hot summer breeding season.

Materials and Methods

The present study was conducted at Al Zahraa stud, Ain Shams, Cairo (30°06' N and 31°25' E; Latitude 30.05), Egypt, during the hottest months, July-August, 2015.

All the procedures were accomplished according to the Ethics for Humane Treatment of Animal Use in Research Guidelines and complied with the relevant legislation of Faculty of Veterinary Medicine, Benha University, Egypt (Ref. No. 000R0105-2015).

Semen collection and evaluation

Arabian horses (n=18) were categorized into three groups according to their age (Neto *et al*., 2013), each included six stallions:
Group I (5-6 years)
Group II (11-12 years)
Group III (15-20 years)

Three ejaculates were collected from each stallion at a weekly interval, early in the morning, using Missouri model artificial vagina (IMV International Co., France) and behaviorally estrus mares as mount animals as described by Hanulakova *et al*. (2012).

Ejaculates were collected using a pre-warmed (45-48°C) lightly lubricated artificial vagina with an inline filter to separate the gel fraction. Semen, gel-free portion, was evaluated by conventional methods (Jasko, 1992; Juhász *et al*., 2000). Total sperm count per ejaculate (TSC/Ej) was calculated from the volume and sperm concentration (semen volume × concentration). The pH was determined with the waterproof pocket pH tester (HI98107, Hanna, USA).

Blood sampling and analytical methods

Jugular vein blood was sampled in the early morning prior to semen collection into vacutainer tubes containing EDTA and centrifuged at 1500 rpm for 15 min. The harvested plasma was kept at -20°C until analysis.

Hormonal analysis

Testosterone level was estimated in plasma as described by Marcus and Durnford (1985) with the use of the enzyme immunoassay test kit (Cat. No. BC-1115, BioCheck Inc., CA, USA) according to the manufacturer's instructions. In brief, 10 µL of plasma, 100 µL of Testosterone-HRP Conjugate Reagent, and 50 µL of rabbit anti-Testosterone reagent were mixed well (30 s) and incubated at 37°C for 90 min. The micro-wells were washed and 100 µL of TMB Reagent was added into each well before being incubated at room temperature for 20 min. The reaction was stopped, and the absorbance was assessed at 450 nm within 15 min.

Biochemical analysis

Lipid and protein profiles and oxidative stress markers were determined spectrophotometrically (UV-120-12, Shimadzu Corp., Kyoto, Japan).

Lipid and protein profile

Plasma level of cholesterol was measured according to Asadi *et al*. (2006) using cholesterol assay kit (Cholesterol Quantitation Kit, MAK043, Sigma-Aldrich, USA) as per the manufacturer's instructions. Briefly, plasma sample (5 µL) was added to Cholesterol Assay Buffer (45 µL), and reaction mix (50 µL), and mixed well by pipetting. The mixture was incubated for 60 min at 37°C and the absorbance was measured at 570 nm.

Triglyceride was measured according to Asadi *et al*. (2006) using triglyceride assay kit (Triglyceride Quantification Kit, MAK266, Sigma-Aldrich, USA) as per manufacturer instructions. Briefly, plasma sample (50 µL) was added to Lipase (2 µL), mixed and incubated for 20 min at room temperature (to convert triglyceride to glycerol). After that, the reaction mix (50 µL) was added, mixed, incubated for 60 min at room temperature, and the absorbance was measured at 570 nm.

The total protein level was measured according to Sharma *et al*. (2016) using protein quantification kit (protein quantification kit, 51254, Sigma-Aldrich, USA) and the protocol was done depending on the manufacturer's instructions. Sample (6 µL) was mixed with Coomassie Brilliant Blue G solution (300 µL), incubated at room temperature for 1 min, and the absorbance was measured at 570 nm.

Oxidative stress markers

Superoxide dismutase (SOD) was measured using SOD Assay Kit (K335, BioVision, Mountain View, CA, USA) as described by Kumar *et al*. (2016). Enzyme working solution was added at the rate of 20 µL to samples and the mixture was incubated at 37°C for 20 min. The SOD activity was measured after recording the absorbance at 450 nm.

The catalase activity was estimated according to Vu and Acosta (2014) using the catalase activity assay kit (K773, BioVision, Mountain View, CA, USA) as per the manufacturer's instruction. In brief, sample (50 µL) was adjusted to volume of total 78 µL with assay buffer, and 12 µL of fresh 1 mM H_2O_2 was added and incubated at 25°C for 30 min, before adding of 10 µL of stop solution. 50 µL of the developer mix was added, mixed well and incubated at 25°C for 10 min before being assessed spectrophotometrically at 570 nm.

The total antioxidant capacity (TAC) was estimated by colorimetric assay kit (K274, BioVision, Mountain View, CA, USA) as described by Kumar *et al*. (2016).

Sample (0.1 µL) was adjusted to 100 µL with distilled H_2O, and 100 µL of Cu^{2+} working solution was added before being incubated at room temperature for 90 min. The TAC was calculated after recording the absorbance at 570 nm.

Malondialdehyde (MDA) concentration was determined according to Kumar *et al*. (2016) using the TABARS assay kit (Cayman Chemical Company). Briefly, to each tube 100 µL of sample, 100 µL of SDS solution and 4 ml color reagent were added. The mixture was boiled in water bath for 1 h. After that, the samples were placed in an ice bath for 10 min to stop the reaction. After cooling, the suspension was centrifuged for 10 min at 1600 × g in cooling centrifuge at 4°C. The 150 µL suspensions were loaded into the colorimetric plate and absorbance was measured at 535 nm.

The reduced glutathione (GSH) level was measured according to Moron *et al*. (1979) as follows: 100 µL of plasma was deproteinized by 3 ml of 5% TCA. After mixing, tubes were kept for 5 min at room temperature and then centrifuged. To 1 ml of supernatant 4 ml of 0.3 M Na_2HPO_4 (pH = 8.0) and 0.5 ml of 0.6 mM DTNB was added. The contents were mixed by vortexing and absorbance was recorded within 10 min at 412 nm.

Micro-elements

Copper and zinc levels in plasma were measured according to Kurz *et al*. (1972) by atomic absorption spectrophotometry (Perkin-Elmer 2380, USA) at 324.80 and 213.90 nm wavelengths, respectively.

Statistical analysis

The data (presented as mean±SEM) were analyzed with one-way analysis of variance and post-hoc least significant difference test using SPSS (Statistical Package for Social Sciences Inc., 2007, Version 16). The correlations between various semen attributes and biochemical parameters were evaluated with Pearson's correlation coefficient test. P<0.05 was set to delineate statistical significance.

Results

Semen characteristics and hormonal profile

Spermiogram of stallions displayed significant differences in semen pH (P<0.005), normal sperm % (P<0.001), tail abnormalities (P<0.001), and TSC/Ej (P<0.05) of different age groups during the hot summer season (Table 1). The semen of young stallions (5-6 years) had high individual motility (62.50 ± 5.20%), but low pH (6.57 ± 0.08), tail abnormalities (28.00 ± 1.15%), and TSC/Ej (5.18 ± 1.71 × 10^9). Middle age group showed high semen volume (58.33 ± 0.88 ml) and TSC/Ej (17.59 ± 0.71 × 10^9). Old age stallions showed slightly alkaline semen pH (7.63 ± 0.12), low normality rate (36.67 ± 1.45%), but comparatively high semen volume (35.00 ± 2.89 ml), spermatozoa tail abnormalities (58.67 ± 1.76%), and TSC/Ej (16.32 ± 1.01 × 10^9).

The stallions of middle age showed testosterone level (1.74 ± 0.28 nmol/L) higher (P<0.05) than that of young group (0.80 ± 0.24 nmol/L), but insignificantly varied from that recorded in the aged group (1.15 ± 0.24 nmol/L).

Biochemical indices

Lipid and protein profile

Lipid profile, particularly cholesterol, demonstrated significant (P<0.05) increase in middle- and old-age stallions as compared to young-age group (2.25 ± 0.07 and 2.40 ± 0.20 vs. 1.52 ± 0.12 mmol/L, respectively) during hot weather (Table 2).

Oxidative stress biomarkers

The evaluation of oxidative stress indicators, principally TAC, revealed a significant (P<0.05) increase in its levels in old-age stallions than young- and middle-age groups (0.25 ± 0.07 vs. 0.10 ± 0.02 and 0.10 ± 0.03 mmol/L, respectively) (Table 2).

Micro-elements

The analysis of minerals in stallions' plasma is presented in Table 2. The level of copper was 16.97 ± 0.87, 17.94 ± 2.70 and 14.76 ± 0.50 µmol/L in group I, II and III, respectively. Meanwhile, zinc levels these groups were 2.79 ± 0.44, 2.81 ± 0.52 and 2.88 ± 0.52 µmol/L, respectively. The variation in copper and zinc levels in the stallion groups was not statistically verified (P=0.55 and 0.50, respectively).

Table 1: The effects of animal age on semen characteristics in Arabian stallions during the hot summer breeding season

Parameter	Stallion groups			P-value
	Group I (Age 5-6 Y)	Group II (Age 11-12 Y)	Group III (Age 15-20 Y)	
Volume (ml)	25.00 ± 5.77^b	58.33 ± 0.88^a	35.00 ± 2.89^{ab}	<0.01
Semen pH	6.57 ± 0.08^c	7.10 ± 0.06^b	7.63 ± 0.12^a	0.005
Individual motility (%)	62.50 ± 5.20^a	55.00 ± 5.00^{ab}	45.00 ± 2.89^b	0.08
Livability (%)	45.00 ± 5.77	50.00 ± 2.89	50.67 ± 2.60	0.58
Normal sperm (%)	63.50 ± 2.02^a	45.00 ± 2.89^b	36.67 ± 1.45^c	<0.001
Abnormal tail (%)	28.00 ± 1.15^c	37.50 ± 1.44^b	58.67 ± 1.76^a	<0.001
Abnormal head (%)	8.00 ± 1.15	9.00 ± 0.58	5.67 ± 1.76	0.24
Concentration (×10^6)	285.83 ± 57.31	267.00 ± 16.50	250.00 ± 11.55	0.78
TSC/Ej (×10^9)	5.18 ± 1.71^b	17.59 ± 0.71^a	16.32 ± 1.01^a	<0.05

Volume estimated was gel-free semen volume. Y: Year, and TSC/Ej: Total sperm count per ejaculate. Values presented as mean (±SEM, n=18) with different superscript letters ($^{a, b, c}$) within the same raw were significantly different

Table 2: Age-related changes in the biochemical indices of Arabian stallions during the hot summer breeding season

Assessment category	Item	Stallion groups			P-value
		Group I (Age 5-6 Y)	Group II (Age 11-12 Y)	Group III (Age 15-20 Y)	
Hormonal level	Testosterone (nmol/L)	0.80 ± 0.24^{b}	1.74 ± 0.28^{a}	1.15 ± 0.24^{ab}	0.07
Lipid profile	Cholesterol (mmol/L)	1.52 ± 0.12^{b}	2.25 ± 0.07^{a}	2.40 ± 0.20^{a}	<0.05
	TG (mmol/L)	0.49 ± 0.07	0.46 ± 0.10	0.49 ± 0.04	0.94
Protein profile	Total protein (g/L)	44.7 ± 1.4	38.2 ± 5.4	41.0 ± 4.7	0.56
Oxidative stress biomarkers	Catalase (U/L)	0.29 ± 0.05	0.24 ± 0.05	0.21 ± 0.02	0.35
	SOD (U/L)	376.63 ± 7.48	343.31 ± 27.1	379.10 ± 3.81	0.26
	GSH (mmol/L)	0.82 ± 0.32	1.60 ± 0.69	0.73 ± 0.10	0.33
	MDA (mmol/L)	0.0029 ± 0.0006	0.0025 ± 0.0003	0.0028 ± 0.0007	0.85
	TAC (mmol/L)	0.10 ± 0.02^{b}	0.07 ± 0.01^{b}	0.25 ± 0.07^{a}	<0.05
Micro-elements	Copper (µmol/L)	16.97 ± 0.87	17.94 ± 2.70	14.76 ± 0.50	0.55
	Zinc (µmol/L)	2.79 ± 0.44	2.81 ± 0.52	2.88 ± 0.52	0.50

Y: Years, TG: Triglycerides, SOD: Superoxide dismutase, GSH: Reduced glutathione, MDA: Malondialdehyde, and TAC: Total antioxidant capacity. Values presented as mean (±SE, n=18) with different superscript letters ($^{a, b, c}$) within the same row were significantly different

Table 3: Correlation (Pearson-coefficient) between biochemical indices and semen characteristics in Arabian stallions during their breeding season

Parameter	Test.	Semen volume	Semen pH	Sperm motility	Livability	Normality	Tail Ab.	Head Ab.	SCC	TSC/Ej
Test.		0.63*	0.23^{NS}	-0.18^{NS}	0.09^{NS}	-0.39^{NS}	0.28^{NS}	0.49^{NS}	-0.14^{NS}	0.33*
Chol.	0.11^{NS}	-0.57^{NS}	0.70*	-0.47^{NS}	-0.02^{NS}	-0.60*	0.73*	-0.30^{NS}	-0.18^{NS}	-0.58
TG	-0.51*	0.39^{NS}	-0.20^{NS}	-0.06^{NS}	0.15^{NS}	0.24^{NS}	-0.04^{NS}	-0.23^{NS}	0.15^{NS}	0.43^{NS}
Protein	-0.51*	0.39^{NS}	-0.04^{NS}	0.28^{NS}	0.23^{NS}	0.11^{NS}	-0.12^{NS}	-0.49^{NS}	0.07^{NS}	0.38^{NS}
CAT	0.01^{NS}	0.54^{NS}	-0.35^{NS}	0.83**	-0.48^{NS}	0.47^{NS}	-0.49^{NS}	-0.11^{NS}	-0.16^{NS}	0.37^{NS}
SOD	0.17^{NS}	0.16^{NS}	0.07^{NS}	0.12^{NS}	0.22^{NS}	-0.07^{NS}	0.18^{NS}	-0.47^{NS}	-0.35^{NS}	-0.01^{NS}
GSH	0.25^{NS}	-0.34^{NS}	0.24^{NS}	-0.29^{NS}	0.57^{NS}	-0.40^{NS}	0.11^{NS}	-0.01^{NS}	0.03^{NS}	-0.25^{NS}
MDA	-0.20^{NS}	0.48^{NS}	-0.78*	0.27^{NS}	-0.06^{NS}	-0.69*	0.68*	0.68*	0.53^{NS}	-0.71**
TAC	-0.08^{NS}	-0.52^{NS}	0.77*	-0.41^{NS}	0.43^{NS}	-0.66*	0.67*	-0.57^{NS}	-0.09^{NS}	-0.47^{NS}
CU	0.01^{NS}	0.10^{NS}	-0.32^{NS}	-0.24^{NS}	0.10^{NS}	0.28^{NS}	-0.32^{NS}	0.46^{NS}	0.69*	0.42^{NS}
Zn	0.06^{NS}	0.52^{NS}	-0.17^{NS}	-0.50^{NS}	0.77*	0.38^{NS}	-0.33^{NS}	-0.33^{NS}	-0.33^{NS}	0.22^{NS}

Test.: Testosterone, Chol.: Cholesterol, TG: Triglycerides, CAT: Catalase enzyme, SOD: Superoxide dismutase, GSH: Reduced glutathione, MDA: Malondialdehyde, TAC: Total antioxidant capacity, Ab.: Abnormality, SCC: Sperm cell concentration, TSC/Ej: Total sperm count per ejaculate, and NS: Non-significant. * Correlation is significant at the 0.05 level (2-tailed), and ** Correlation is significant at the 0.01 level (2-tailed)

Relationship between semen and biochemical indices

Table 3 shows the spermiogram and biochemical indices' inter-relationship in Arabian horses. Testosterone levels were correlated positively to semen volume and total sperm count per ejaculate (P<0.05). Cholesterol correlated positively (P<0.05) with semen pH and tail abnormalities (r=0.07 and r=0.73, respectively), and negatively (P<0.05) with sperm normality (r=-0.60).

Catalase was positively correlated with spermatozoa motility (r=0.83, P<0.001). Malondialdehyde correlated negatively with semen pH, sperm normality and TSC/Ej (r=-0.78, -0.69 and -0.71, P<0.05, respectively), and positively with sperm head/tail abnormalities (r=0.68, P<0.05).

Total antioxidant capacity correlated positively (P<0.05) with semen pH and tail abnormalities (r=0.77 and r=0.67, respectively), and negatively (P<0.05) with normal sperm rate (r=-0.66).

Copper level correlated positively (P<0.05) with semen volume and sperm count (r=0.62 and r=0.69, respectively). Zinc level correlated positively (P<0.05) with semen volume (r=0.63) and sperm livability (r=0.77).

Discussion

Many ailments related to physiologic, pathologic, and management processes distresses stallions' fertility. In this study, hot weather aggravated age-related alternations in stallions' semen in association with lipid disorders and oxidative stress.

In this study, young-age stallions showed a reasonable semen quality characterized by higher sperm motility and normality rates, and lower tail abnormalities. In the meantime, middle-age stallions (11-12 years) had voluminous semen with high TSC/Ej. These findings agreed with former studies claimed that the stallions between 3 and 11 years of age demonstrated the best semen characteristics (Dowsett and Knott, 1996). Also, in partial agreement with El Sisy *et al.* (2016), who showed that old stallions (>16 years) had semen of greater volume, spermatozoa livability, and sperm count compared to young and moderate aged groups. We assume that the variations of semen characteristics in stallions in this study are age-dependent that influences the testicular function (either exocrine or endocrine). The interplay between season and stallions' reproductive physiology is deemed controversial. While some authors have pointed to the lack of seasonal influence on testicular volume and semen parameters in tropical stallions (Leme *et al*., 2012), others have argued for the variation in fresh semen volume, spermatozoa motility and count between summer and winter seasons (Janett *et al*., 2003). At latitudes higher than 30°, photoperiod is the most important cue regulating seasonal reproduction (Bronson and Heideman, 1994). Therefore, photoperiod might be implemented in the age-associated alterations in stallions' semen under summer conditions recorded in our study.

Regarding the androgens levels, the elder stallions (middle and old-age groups) showed higher testosterone level than that of young group. Moreover, the testosterone levels were correlated with semen volume and TSC/Ej. In former study, Harem stallions were recorded to have higher testosterone levels than those in bachelor stallions (McDonnell and Murray, 1995). There is a strong correlation between serum testosterone level and stallions fertility (Inoue *et al*., 1993). Stallions with azoospermic showed lower testosterone and total estrogen levels as compared with normal mature horses (Inoue *et al*., 1993). Therefore, serum levels of testosterone could be a good indicator of the testicular endocrine function and libido intensity in stallions.

Studies on cholesterol, triglycerides, and total proteins referred to the existence of clear dissimilarities among animals' species and individuals. Samples from clinically normal and healthy stallions during summer in this work showed that the cholesterol differed notably with age. These data are in accordance with former studies in horses, which declared a significant increase in lipid profile parameters with age (Nazifi *et al*., 2003). Nevertheless, our data disagree with Mayer Valor *et al*. (1984), who did not verify a significant effect of age on total lipids and cholesterol. The variance in lipid profile with age might be interrelated to the thyroid gland activity. This gland greatly influences all aspects of fat metabolism (synthesis, mobilization and degradation), and the degradation is greatly affected than the synthesis. Thyroid hormones' depletion elevates serum cholesterol levels in mammals (Gueorguieva and Gueorguiev, 1997). Horses' feeding programs as well as exercises, which need glucose consumption for energy, could explain to these differences (Hambleton *et al*., 1980).

The role of oxidative stress in aging and chronic pathological conditions' progression has been verified. An ambient temperature and humidity, judged by TAC or key antioxidants estimation, noticeably influence oxidative stress. Data herein showed an age-related statistical variation in TAC between stallions during the summer. Low TAC could be indicative of an increased-susceptibility to oxidative stress (Young, 1999). High liability to oxidative stress occurs due to an imbalance in antioxidants, increased exposure to oxidants from the environment or increased oxygen metabolism during exercise within the body (McBride and Kraemer, 1999). Sharma *et al*. (2016) showed that the oxidative stress marked with seminal plasma super oxide dismutase activity is influenced by season and this depicts some semen traits in buffalo species. These findings could deduce the drop in young stallions' fertility during summer breeding season to the disorder of oxidant status.

There are some studies that have demonstrated the correlation between trace elements and male fertility. High blood or semen metal ions' levels appear to be positively correlated with male infertility. In this study, while serum copper and zinc levels did not vary between stallions of different ages, copper and zinc were correlated with semen volume, sperm cell count and spermatozoa livability. These results are in agreement with Wong *et al*. (2001), who showed that zinc and copper critically participate in spermatogenesis and fertility, though these elements levels (in blood and seminal plasma) cannot distinguish between fertile and sub-fertile males. Massányi *et al*. (2004) studied copper, zinc and others in the semen of bull and ram and declared that these elements directly influence spermatozoa quality. In the summer period, copper reached highest levels (Vranković *et al*., 2015) and this is associated with greater muscle activity and feed intake in the horses.

From the presented results, it could be concluded that hot summer climate markedly impacts stallion fertility indicated by hormonal secretion and semen quality, and these effects are age-dependent. Obesity, along with oxidative stress mediates the heat stress effects. If stallions, especially young or old, are going to be used for breeding during the hottest months of the summer, the owner is greatly recommended to consider cooling systems, ration modification with antioxidants supplements and morning or late afternoon outdoor activities.

Acknowledgements

The authors would like to express deep appreciation for the General Manager and Veterinarian at Al Zahraa stud, for animals' allowance and great support during the practical part of the study. Special thanks to Prof. Hanem F. Khater, Professor of Parasitology, Faculty of Veterinary Medicine, Benha University, for her suggestions and linguistic revision of the manuscript.

Conflict of interest

There is no conflict of interest.

References

Abou-Ahmed, MM; El-Belely, MS; Ismail, ST; El-Baghdad, YRM and Hemeida, NA (1993). Influence of age and season on certain biochemical constituents of seminal plasma of Arabian horses. Anim. Reprod. Sci., 32: 237-244.

Asadi, F; Mohri, M; Adibmoradi, M and Pourkabir, M (2006). Serum lipid and lipoprotein parameters of Turkman horses. Vet. Clin. Pathol., 35: 332-334.

Brauch, GH (2002). Climate change, environmental stress and conflict cases of Bangladesh and Egypt. International Conference on Climate Change and Disaster Preparedness, The Hague, 26 June.

Bronson, FH and Heideman, PD (1994). Seasonal regulation of reproduction in mammals. In: Knobil, E and Neill, JD (Eds.), *Physiology of reproduction*. (2nd Edn.), Vol. 2, New York, NY, Raven Press. PP: 541-584.

Clay, CM; Squires, EL; Amann, RP and Pickett, BW (1987). Influences of season and artificial photoperiod on stallions: testicular size, seminal characteristics and sexual behavior. J. Anim. Sci., 64: 517-525.

Dowsett, KF and Knott, LM (1996). The influence of age and breed on stallion semen. Theriogenology. 46: 397-412.

El Sisy, GA; Abo El-Maaty, AM and Rawash, ZM (2016). Comparative blood and seminal plasma oxidant/antioxidant status of Arab stallions with different ages and their relation to semen quality. Asian Pac. J. Reprod., 5: 428-433.

Gueorguieva, TM and Gueorguiev, IP (1997). Serum cholesterol concentration around parturition and in early lactation in dairy cows. Rev. Med. Vet., 148: 241-244.

Hambleton, PL; Slade, LM; Hamar, DW; Kienholz, EW and Lewis, LD (1980). Dietary fat and exercise conditioning effect on metabolic parameters in the horse. J. Anim. Sci., 51: 1330-1339.

Hansen, PJ (2009). Effects of heat stress on mammalian reproduction. Philos. T. Roy. Soc. B., 364: 3341-3350.

Hanulakova, S; Mamica, O; Machal, L; Křivanek, I; Filipčik, R; Hošek, M and Chladek, G (2012). Physical properties of stallion semen in relation to some qualitative and quantitative characteristics. Acta Univ. Agric. Silvic. Mendel. Brun., 12: 97-101.

Heldmaier, G; Ortmann, S and Elvert, R (2004). Natural hypometabolism during hibernation and daily torpor in mammals. Respir. Physiol. Neurobiol., 141: 317-329.

Inoue, J; Cerbito, WA; Oguri, N; Matsuzawa, T and Sato, K (1993). Serum levels of testosterone and oestrogens in normal and infertile stallions. Int. J. Androl., 16: 155-158.

IPCC (2007). WG1 4th Assessment Report: Climate Change 2007: The Physical Science Basis: Summary for Policymakers. Paris.

Janett, F; Thun, R; Bettschen, S; Burger, D and Hassig, M (2003). Seasonal changes of semen quality and freezability in Franches-Montagnes stallions. Anim. Reprod. Sci., 77: 213-221.

Jasko, DJ (1992). Evaluation of stallion semen. Vet. Clin. North Am. Equine Pract., 8: 129-148.

Juhász, J; Nagy, P; Kulcsár, M and Huszenicza, GY (2000). Methods for semen and endocrinological evaluation of the stallion: a review. Acta Vet. Brno, 69: 247-259.

Kumar, P; Saini, M; Kumar, D; Jan, MH; Swami, DS and Sharma, RK (2016). Quantification of leptin in seminal plasma of buffalo bulls and its correlation with antioxidant status, conventional and computer-assisted sperm analysis (CASA) semen variables. Anim. Reprod. Sci., 166: 122-127.

Kurz, D; Roach, J and Eyring, EJ (1972). Direct determination of serum zinc and copper by atomic absorption spectrophotometry. Biochem. Med., 6: 274-281.

Leme, DP; Papa, FO and Roser, JF (2012). Reproductive characteristics of stallions during the breeding and non-breeding season in a tropical region. Trop. Anim. Health Prod., 44: 1703-1707.

Marcus, GJ and Durnford, R (1985). Simple enzyme-linked immunoassay for testosterone. Steroids. 46: 975-986.

Massányi, P; Trandzik, J; Nad, P; Koreneková, B; Skalická, M; Toman, R; Lukac, N; Halo, M and Strapak, P (2004). Concentration of copper, iron, zinc, cadmium, lead, and nickel in bull and ram semen and relation to the occurrence of pathological spermatozoa. J. Environ. Sci. Health A Tox Hazard Subst Environ. Eng., 39: 3005-3014.

Mawyer, JD; Gordon, RK; Cavinder, CA; Vogelsang, MM; Sigler, DH; Love, CC; Brinsko, SP; Blanchard, TL and Teague, S (2012). Thermoregulation of the testicle in response to exercise and subsequent effects on semen characteristics of stallions. J. Anim. Sci., 90: 2532-2539.

Mayer Valor, R; Gómez Cárdenas, G and Fernández Gómez, M (1984). Total lipids and cholesterol in blood serum of the Spanish race of andalusian type horses. Arch. Zootec., 33: 43-48.

McBride, JM and Kraemer, WJ (1999). Free radicals, exercise, and antioxidants. J. Strength Cond. Res., 13: 175-183.

McDonnell, SM and Murray, SC (1995). Bachelor and harem stallion behavior and endocrinology. Biol. Reprod. Mono., 1: 577-590.

Moron, MS; Depierre, JW and Mannervik, B (1979). Levels of glutathione, glutathione reductase and glutathione-s-transferase activities in rat lung and liver. Biochim. Biophys. Acta. 582: 67-78.

Nardone, A; Ronchi, B; Lacetera, N; Ranieri, MS and Bernabucci, U (2010). Effects of climate changes on animal production and sustainability of livestock systems. Livest. Sci., 130: 57-69.

Nazifi, S; Saeb, M and Abedi, M (2003). Serum lipid profiles and their correlation with thyroid hormones in clinically healthy Turkoman horses. Comp. Clin. Path., 12: 49-52.

Neto, CR; Monteiro, GA; Delfiol, DJZ; Farras, MC; Dell'aqua Jr, JA; Papa, FO and Alvarenga, MA (2013). The relationships between scrotal surface temperature, age and sperm quality in stallions. Livest. Sci., 157: 358-363.

Prosser, CL and Heath, JE (1991). Temperature. In: Prosser, CL (Ed.), *Environmental and metabolic animal physiology*. (4th Edn.), New York, John Wiley and Sons, Inc., PP: 109-165.

Setchell, BP; Maddocks, S and Brooks, DE (1994). Anatomy, vasculature, innervation and fluids of the male reproductive tract. In: Knobil, E and Neill, JD (Eds.), *Physiology of reproduction*. (2nd Edn.), Vol. 1, New York, Raven Press. PP: 771-825.

Sharma, L; Pandey, V; Nigam, R; Saxena, A; Swain, DK and Yadav, B (2016). Association of oxidative status and semen characteristics with seminal plasma proteins of buffalo semen. Iran. J. Vet. Res., 17: 226-230.

SPSS (2007). SPSS for Windows. Version 16.0. Chicago, SPSS Inc.

Vranković, L; Aladrović, J; Beer-Ljubić, B; Zdelar-Tuk, M and Stojević, Z (2015). Seasonal changes in enzyme activities and mineral concentrations in Holstein stallion blood plasma. Vet. Arh., 85: 235-246.

Vu, HV and Acosta, TJ (2014). Catalase and glutathione peroxidase expression in bovine corpus luteum during the estrous. Anim. Reprod., 11: 74-84.

Wong, WY; Flik, G; Groenen, PM; Swinkels, DW; Thomas, CM; Copius-Peereboom, JH; Merkus, HM and Steegers-Theunissen, RP (2001). The impact of calcium, magnesium, zinc, and copper in blood and seminal plasma on semen parameters in men. Reprod. Toxicol. (Elmsford, NY). 15: 131-136.

World Bank (2009). Water and climate change: understanding the risks and making climate-smart investment decisions. Washington, DC: World Bank, 52911.

Young, IS (1999). Measurement of total antioxidant capacity. J. Clin. Pathol., 54: 339.