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

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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 H2O2 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 H₂O, and 100 μL of Cu²⁺ 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 μL 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 supernatants 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 μL 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 Na₂HPO₄ (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 × 10⁹). Middle age group showed high semen volume (58.33 ± 0.88 ml) and TSC/Ej (17.59 ± 0.71 × 10⁹). 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 × 10⁹).

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).

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (Age 5-6 Y)</th>
<th>Group II (Age 11-12 Y)</th>
<th>Group III (Age 15-20 Y)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>25.00 ± 5.77b</td>
<td>58.33 ± 0.88a</td>
<td>35.00 ± 2.89c</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Semen pH</td>
<td>6.57 ± 0.08c</td>
<td>7.10 ± 0.06b</td>
<td>7.63 ± 0.12a</td>
<td>0.005</td>
</tr>
<tr>
<td>Individual motility (%)</td>
<td>62.50 ± 5.20a</td>
<td>55.00 ± 5.00a</td>
<td>45.00 ± 2.89b</td>
<td>0.08</td>
</tr>
<tr>
<td>Livability (%)</td>
<td>45.00 ± 5.77</td>
<td>50.00 ± 2.89</td>
<td>50.67 ± 2.60</td>
<td>0.58</td>
</tr>
<tr>
<td>Normal sperm (%)</td>
<td>63.50 ± 2.02b</td>
<td>45.00 ± 2.89b</td>
<td>36.67 ± 1.45c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abnormal tail (%)</td>
<td>28.00 ± 1.15b</td>
<td>37.50 ± 1.44b</td>
<td>58.67 ± 1.76b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abnormal head (%)</td>
<td>8.00 ± 1.15</td>
<td>9.00 ± 0.58</td>
<td>5.67 ± 1.76</td>
<td>0.24</td>
</tr>
<tr>
<td>Concentration (x10⁹)</td>
<td>285.83 ± 57.31</td>
<td>267.00 ± 16.50</td>
<td>250.00 ± 11.55</td>
<td>0.78</td>
</tr>
<tr>
<td>TSC/Ej (x10⁹)</td>
<td>5.18 ± 1.71b</td>
<td>17.59 ± 0.71a</td>
<td>16.32 ± 1.01b</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

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 and semen characteristics of Arabian stallions during their breeding season

<table>
<thead>
<tr>
<th>Assessment category</th>
<th>Item</th>
<th>Stallion groups</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormonal level</td>
<td>Testosterone (nmol/L)</td>
<td>Group I: 2.79 ± 0.44 Group II: 2.81 ± 0.44 Group III: 2.88 ± 0.52</td>
<td>0.0001</td>
</tr>
<tr>
<td>Lipid profile</td>
<td>Cholesterol (nmol/L)</td>
<td>Group I: 17.94 ± 0.50 Group II: 14.76 ± 0.50 Group III: 11.36 ± 0.50</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Protein profile</td>
<td>Total protein (g/L)</td>
<td>Group I: 41.0 ± 4.7 Group II: 41.0 ± 5.6 Group III: 41.0 ± 4.7</td>
<td>0.76</td>
</tr>
<tr>
<td>Oxidative stress biomarkers</td>
<td>Catalase (U/L)</td>
<td>Group I: 0.29 ± 0.05 Group II: 0.24 ± 0.05 Group III: 0.21 ± 0.02</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>SOD (U/L)</td>
<td>Group I: 376.63 ± 1.4 Group II: 343.31 ± 2.71 Group III: 379.40 ± 3.81</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>GSH (nmol/L)</td>
<td>Group I: 0.82 ± 0.32 Group II: 1.60 ± 0.69 Group III: 0.73 ± 0.10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>MDA (nmol/L)</td>
<td>Group I: 0.0029 ± 0.0006 Group II: 0.0025 ± 0.0003 Group III: 0.0028 ± 0.0007</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>TAC (nmol/L)</td>
<td>Group I: 0.10 ± 0.02a Group II: 0.07 ± 0.01b Group III: 0.25 ± 0.07c</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Micro-elements</td>
<td>Copper (μmol/L)</td>
<td>Group I: 16.97 ± 0.87 Group II: 17.94 ± 2.07 Group III: 14.76 ± 0.50</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Zinc (μmol/L)</td>
<td>Group I: 0.29 ± 0.05 Group II: 1.60 ± 0.69 Group III: 0.73 ± 0.10</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test.</th>
<th>Semen volume</th>
<th>Semen pH</th>
<th>Sperm motility</th>
<th>Livability</th>
<th>Normality</th>
<th>Tail Ab.</th>
<th>Head Ab.</th>
<th>SCC</th>
<th>TSC/Ej</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test.</td>
<td>0.11</td>
<td>0.23NS</td>
<td>0.18</td>
<td>-0.18</td>
<td>-0.05</td>
<td>0.39</td>
<td>0.28</td>
<td>0.49</td>
<td>-0.14</td>
<td>0.33</td>
</tr>
<tr>
<td>Chol.</td>
<td>-0.57NS</td>
<td>-0.20NS</td>
<td>0.07</td>
<td>-0.47</td>
<td>-0.04</td>
<td>0.23</td>
<td>0.11</td>
<td>-0.49</td>
<td>0.07</td>
<td>0.38</td>
</tr>
<tr>
<td>TG</td>
<td>0.39NS</td>
<td>0.06NS</td>
<td>-0.47</td>
<td>-0.06</td>
<td>0.23</td>
<td>0.11</td>
<td>-0.12</td>
<td>-0.48</td>
<td>0.07</td>
<td>0.38</td>
</tr>
<tr>
<td>Protein</td>
<td>0.51NS</td>
<td>0.28NS</td>
<td>-0.45</td>
<td>-0.28</td>
<td>-0.48</td>
<td>0.11</td>
<td>-0.12</td>
<td>-0.49</td>
<td>0.16</td>
<td>0.37</td>
</tr>
<tr>
<td>CAT</td>
<td>0.54NS</td>
<td>0.12NS</td>
<td>0.83</td>
<td>0.22</td>
<td>0.22</td>
<td>0.18</td>
<td>-0.47</td>
<td>0.16</td>
<td>-0.16</td>
<td>0.37</td>
</tr>
<tr>
<td>SOD</td>
<td>0.16NS</td>
<td>0.12NS</td>
<td>0.28</td>
<td>0.22</td>
<td>0.22</td>
<td>0.18</td>
<td>-0.47</td>
<td>0.16</td>
<td>-0.16</td>
<td>0.37</td>
</tr>
<tr>
<td>GSH</td>
<td>0.28NS</td>
<td>0.09NS</td>
<td>-0.45</td>
<td>-0.09</td>
<td>0.22</td>
<td>0.18</td>
<td>-0.47</td>
<td>0.16</td>
<td>-0.16</td>
<td>0.37</td>
</tr>
<tr>
<td>MDA</td>
<td>0.48NS</td>
<td>-0.07NS</td>
<td>0.27</td>
<td>0.43</td>
<td>0.43</td>
<td>0.22</td>
<td>-0.47</td>
<td>0.16</td>
<td>-0.16</td>
<td>0.37</td>
</tr>
<tr>
<td>TAC</td>
<td>0.08NS</td>
<td>0.03NS</td>
<td>-0.14</td>
<td>-0.04</td>
<td>0.07</td>
<td>0.03</td>
<td>0.22</td>
<td>0.16</td>
<td>-0.16</td>
<td>0.37</td>
</tr>
<tr>
<td>Zn</td>
<td>0.06NS</td>
<td>0.01NS</td>
<td>0.17</td>
<td>0.35</td>
<td>0.77</td>
<td>0.35</td>
<td>-0.33</td>
<td>-0.33</td>
<td>0.22</td>
<td></td>
</tr>
</tbody>
</table>

Relationship between semen and biochemical indices

Table 3 shows the spermogram and biochemical indices’ inter-relationship in Arabian horses. Testosterone levels were correlated positively with 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 distress stallions’ fertility. In this study, hot weather aggravates 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 (Vrankovič 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.

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Conflict of interest

There is no conflict of interest.

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