Heliyon 8 (2022) e11128

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Relation of seminal plasma trace mineral in the Arabian stallion's semen with the semen characteristics and subsequent fertility

Magdi M. Waheed [a,b,*], Ahmad Meligy [c,d], Abdulrahman K. Alhaider [a], Ibrahiem M. Ghoneim [a,b]

[a] Department of Clinical Sciences, College of Veterinary Medicine, King Faisal University, P.O. Box: 400, Al-Ahsa 31982, Saudi Arabia
[b] Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
[c] Central Diagnostic Lab., College of Veterinary Medicine, King Faisal University, P.O. Box: 400, Al-Ahsa 31982, Saudi Arabia
[d] Physiology Department, Plant Protection Research Institute (PPRI), Agricultural Research Centre (ARC), Giza, Egypt

ARTICLE INFO

Keywords:
Arabian horse
Fertility
Semen parameters
Trace elements

ABSTRACT

Background: Seminal plasma contains several microelements like Zn, Fe, Se, and Cu that affect sperm motility and male fertility. Biochemical evaluation of seminal plasma trace elements is important for assessing fertility and diagnosing male infertility.

Aims: The present study was designed to evaluate the effect of seminal fluid trace elements on sperm parameters and fertility in Arabian horses.

Methods: Ninety-four ejaculates from 25 Arabian stallions (4–27 years old) were used to investigate the effect of seminal fluid trace elements on semen parameters and fertility. Data divided according to season, stallion age, and fertility of stallions. The concentrations of Zn, Fe, Se, Cu, Cr and Mo were determined using an atomic absorption spectrophotometer. Percentage stallion fertility estimated by mares that conceived on their first cycle. Data were analyzed by ANOVA using SPSS statistical software program (2013), version 22.0.

Results: There was a significant effect of season on semen volume, pH, Fe, Se, Cu, Cr, and Mo. Stallion age had a significant effect on pH, sperm motility, concentration, total motile sperm count, sperm abnormalities, Zn, and Fe. Sperm motility was higher ($P < 0.05$) and sperm abnormalities were lower ($P < 0.05$) in group IV (>70% fertility) than in group I (infertile) and group II (<50% fertility). Sperm abnormalities were low in group IV and high in groups I and II. Seminal plasma Zn and Cu levels were higher ($P < 0.05$) in groups III (50_70% fertility) and IV than in group I. Fe levels were lower ($P < 0.05$) in group IV than in groups I, II, and III. Seminal plasma Mo concentrations were higher ($P < 0.05$) in group III than in group I.

Conclusions: High seminal plasma concentrations of Zn, Se, Cu, and Mo and low Fe concentrations are associated with improved stallions' semen parameters and fertility.

1. Introduction

Sperm dysfunction is the most prominent cause of infertility in males [1]. Infertility is complex and has many etiologies that include gene mutations, infectious diseases, erectile and ejaculatory dysfunctions, and environmental factors [2, 3]. Seminal plasma is a mixture of secretions produced in the testes, epididymis, and accessory sex glands that contain proteins, ions, and organic substances of a low molecular weight [4]. The seminal fluid can influence spermatozoa motility, morphology, acrosome reaction, and fertility [5, 6, 7]. Seminal plasma includes many microelements such as Zn, Fe, Se, and Cu in bound and free (ionic) forms [8, 9, 10]. Biochemical evaluation of seminal plasma is important for assessing fertility and diagnosing male infertility signs [11, 12]. Semen from mammals contains high levels of Zn, which found to be critical for spermatogenesis [13, 14, 15, 16]. Copper in seminal plasma is important for numerous enzymes and proteins that are involved in antioxidant metabolism [10, 17]. However, high levels of ionic Cu were toxic to spermatozoa [17, 18]. Abnormal levels of Zn [19, 20, 21] and Cu [21, 22] in seminal plasma correlated with human infertility [10]. Selenium has an important role in testicular development, spermatogenesis, and sperm motility and viability [23, 24]. In human, Fe in seminal plasma is involved in normal spermatogenesis [25] and its concentration negatively correlated with normal sperm morphology [26]. The normal Fe and Cu concentrations have a stimulating effect on the motility of semen [9]. Chromium insufficiency was associated with decreased sperm cell counts and fertility in rats [27]. In humans and animals, decreased Cr

* Corresponding author.
E-mail address: mmwaheed@kfu.edu.sa (M.M. Waheed).

https://doi.org/10.1016/j.heliyon.2022.e11128
Received 30 November 2021; Received in revised form 12 April 2022; Accepted 12 October 2022

concentrations in seminal plasma adversely affected sperm production, motility, and sperm cell concentrations [28, 29]. In mice, sperm motility, sperm count, and morphology improved by a moderate dose of Mo in the diet [30].

Therefore, this study was designed to evaluate the effect of seminal fluid trace elements (Zn, Fe, Se, Cu, Cr and Mo) on sperm parameters and fertility in Arabian horses.

2. Materials and methods

This study was approved ethically by the Ministry of Agriculture, Al-Ahsa, Kingdom of Saudi Arabia. Approval # MA 332/04/2020.

2.1. Study animals

Twenty-five healthy Arabian stallions (*Equus ferus caballus*), between 4 and 27 years old were selected for this study, which was conducted over a 12-month period. The stallions belonged to three Arabian horse farms (Al-Bushaier Mydod, Haleim Shah and Al-Hashem farms), located near Al-Ahsa (21.9113°N; 49.3653°E), Kingdom of Saudi Arabia. They used as sires in the studs' regular natural breeding program. The stallions housed in separate pens and fed barley and concentrates twice daily. Stallion fertility was estimated by the percentages of mares (18–28 mares were mated per stallion) that conceived in their first cycle of mating (two mattings performed one day apart/conception) with the stallion, retrospectively. The collection of fertility data occurred during the breeding season.

2.2. Semen collection and evaluation

Ninety-four ejaculates were collected from the stallions (four ejaculates were collected per stallion) while they mounted a mare in estrus using an Equine Artificial Vagina kit (Colorado State University Model, CSU, USA). The semen samples evaluated immediately after collection. The ejaculate volume and pH were recorded, and the percentage of sperm progressive motility, sperm abnormalities, and sperm cell concentration (10×10^6 sperm/mL) were determined objectively using Sperm Vision version 3.5 software (Minitube of America, Inc., Verona, USA). Semen aliquots (5 mL) centrifuged at 4000*g* for 10 min at room temperature, and seminal plasma was separated and stored at −80 °C. In order to prevent contamination with trace elements, all glassware and bottles used for isolating and analyzing the seminal plasma was soaked in diluted nitric acid (10%); then, the glassware and bottles thoroughly rinsed with double distilled water.

2.3. Digestion of samples

The seminal plasma samples (1ml) were dissolved and digested using a microwave digestion system (Model MARS 907511; CEM Cooperation, Mathews, North Carolina, USA). Briefly, 1 mL of seminal plasma added to a polytetrafluoroethylene (PTFE) digestion vessel with 5 mL (65%) nitric acid and 3 mL (30%) hydrogen peroxide. The starting temperature for the digestion process was ramped to 125 °C within 6 min. Then, the temperature was ramped to 185 °C within 15 min, followed by repeated time of 15 min. The samples held at 185 °C for another 15 min, and then cooled for 5 min. Samples diluted to 50 mL with Milli-Q water (Millipore, Bedford, MA) and filtered using Whitman filter paper 1. Then, the digested samples analyzed via atomic absorption spectrophotometry (AAS) [31].

2.4. Instruments

An atomic absorption spectrophotometer (AAS-6800 Shimadzu, Koyoto, Japan) coupled with flame atomic absorption spectrometry (FAAS), GFA-EX7 graphite furnace atomizer (GFA), and ASC-6100 auto sampler (Shimadzu, Koyoto, Japan) utilized for trace element analyses. A high-density graphite tube used for atomization of trace elements and. hollow cathode lamps used for irradiation. FAAS with employed with air/acetylene (10/1.5) and nitrous oxide acetylene flams for the determination of Mo with the burner position adjusted for maximum sensitivity.

2.5. Trace element analysis in stallion seminal plasma

The concentrations of six elements (Zn, Fe, Se, Cu, Cr, and Mo) determined by the digestion and analyses of the seminal plasma samples using AAS. Calibration curves obtained from stock solutions of Zn, Fe, Se, Cu, Cr, and Mo standards (1000 mg/L; Merck, Darmstadt, Germany). Standard solutions prepared by different serial dilutions of the stock (1000 mg/L) of all element standards in Milli-Q water. The determination of Zn, Fe, Cu, and Mo carried out using FAAS; the level of absorbance obtained by adjusting the hollow cathode lamps with the operating conditions. The FAAS wavelengths (nm) for Zn, Fe, Cu, and Mo were 213.9, 239.6, 324.8, and 390.3, respectively. The determination of Cr and Se performed using a GFA (Table 1). Full quantitative analysis mode applied for all measurements.

2.6. Quality control of the analytical process

The recovery of trace elements checked by spiking aliquots (three) of the seminal plasma with a standard solution that contained three µl/L of each element. Similar aliquots spiked with deionized water that used as the control. The aliquots subjected to the digestion procedure and analyzed for trace element concentrations to establish confidence in the accuracy and reliability of the data generated (Table 2). The recovery of certified elements varied between 85.5% and 97.5%, indicating that the analysis was reliable.

2.7. Statistical analysis

Data divided according to season, stallion age, and fertility of stallions. Seasons were autumn (September–November), winter (December–February), spring (March–May) and summer (June–August). Stallion ages were 4–10 (n = 10), 11–18 (n = 8) and >18 (n = 7) years old, separated into groups A, B, and C, respectively. The fertility of stallions in groups I, II, III, and IV was 0% (n = 9), <50% (n = 3), 50–70% (n = 5) and >70% (n = 8), respectively. Data were analyzed by three way ANOVA using SPSS statistical software program (2013), version 22.0 [32]. Associations between the parameters (seasons, age, and fertility) and seminal plasma trace elements (Zn, Fe, SE, Cu, Cr, and Mo), and their interactions were estimated using linear mixed effect model [33].

3. Results

Table 3 shows the effect of season on the semen parameters in the Arabian horse. Ejaculate volume was higher ($P < 0.05$) in winter than in spring. Semen pH was more alkaline ($P < 0.05$) in spring than in both winter and summer.

Table 1. The conditioning of heating program for selenium (Se) and chromium (Cr) in graphite furnace atomic absorption spectrophotometer (GFA-EX7).

Step	Temperature (°C)		Ramp (s)	Hold (s)	Argon flow rate (mL min^{-1})
	Selenium (Se)	Chromium (Cr)			
Drying 1	150	150	5	20	250
Drying 2	200	200	5	15	250
Pyrolysis	1200	1600	10	20	250
Atomization	2000	2300	0	5	0
Clean-out	2450	2500	1	3	250

Table 2. The recovery of trace elements from seminal plasma of Arabian horses.

Elements	Seminal plasma		
	Concentrations of metal added (mg/L)	Concentrations of metal recovered (mg/L)	Recovery (%)
Zinc (Zn)	3.0	2.75	91.5
Iron (Fe)	3.0	2.85	95.0
Selenium (Se)	3.0	2.68	89.5
Copper (Cu)	3.0	2.88	96.0
Chromium (Cr)	3.0	2.65	88.2
Molybdenum (Mo)	3.0	2.65	88.3

Concerning stallion age (Table 4), percentage sperm motility was lower ($P < 0.05$) in group A (4–10 years) than in group B (11–18 years). Meanwhile, both the sperm cell concentration and total motile sperm count (TMSC) were higher ($P < 0.05$) in group B (11–18 years) than in groups A (4–10 years) and C (>18 years). Percentage sperm abnormalities was higher ($P < 0.05$) in group A (4–10 years) than in both group B (11–18 years) and group C (>18 years; Table 4).

Table 5 shows the effect of season on the semen parameters in the Arabian horse. Semen volume was higher significantly ($P < 0.05$) in group II (<50% fertility) than in group I (infertile). Semen pH was higher ($P < 0.05$) in group I (infertile) than in fertile groups II (<50% fertility), III (50–70% fertility) and IV (>70% fertility). Percentage sperm motility was higher ($P < 0.05$) and the sperm abnormalities were lower ($P < 0.05$) in fertile group IV (>70% fertility) than in group I (infertile) and group II (<50% fertility). Total sperm motility in the ejaculates was greater ($P < 0.05$) in fertile group IV (>70% fertility) than in group I (infertile; Table 5). Percentage sperm abnormalities were low ($P < 0.05$) in group IV (>70% fertility) and high ($P < 0.05$) in both groups I (infertile) and II (<50% fertility; Table 5).

Figure 1 shows the effect of season on the concentrations of seminal plasma trace elements. The concentration of Fe was higher ($P < 0.05$) in spring compared to summer and winter. The concentrations of Se, Cu, and Cr were higher ($P < 0.05$) in the winter than in summer. The Mo concentration was elevated ($P < 0.05$) in winter when compared to the other seasons (Figure 1).

As shown in Figure 2, Zn concentration was higher ($P < 0.05$) in group B (11–18 years) than in group A (4–10 years). However, Fe concentration was higher ($P < 0.05$) in group A (4–10 years) than in both groups B (11–18 years) and C (>18 years; Figure 2).

In Figure 3, the concentrations of Zn and Cu in stallion seminal plasma were higher ($P < 0.05$) in fertile groups III (50–70% fertility) and IV (>70% fertility) than in group I (infertile). Fe levels were lower ($P < 0.05$) in fertile group IV (>70% fertility) than in the other groups (infertile, <50% fertility, and 50–70% fertility). Seminal plasma Mo concentrations were higher ($P < 0.05$) in fertile group III (50–70% fertility) than in group I (infertile; Figure 3).

Table 3. The relation of seasons to semen parameters in stallions (mean ± SEM).

Seasons	Semen parameters					
	Volume (mL)	pH	Motility (%)	Sperm concentration ($\times 10^6$/mL)	Total motile ($\times 10^9$)	Sperm abnormalities (%)
Autumn ($N = 11$, $n = 18$)	$28.78^{ab} \pm 4.75$	$7.19^{ab} \pm 0.06$	67.50 ± 4.89	209.08 ± 34.37	3.69 ± 0.67	40.61 ± 3.87
Winter ($N = 14$, $n = 16$)	$37.31^{a} \pm 5.71$	$7.06^{a} \pm 0.05$	59.69 ± 4.62	193.25 ± 38.19	3.87 ± 0.73	41.00 ± 4.55
Spring ($N = 14$ & $n = 21$)	$27.21^{b} \pm 3.32$	$7.29^{b} \pm 0.06$	52.38 ± 7.15	301.33 ± 45.83	5.26 ± 1.49	31.33 ± 4.47
Summer ($N = 20$, $n = 39$)	$31.18^{ab} \pm 2.61$	$7.21^{a} \pm 0.06$	64.87 ± 4.34	210.56 ± 26.05	4.53 ± 0.66	43.00 ± 3.23

Means with dissimilar superscript letters in the same column of each variable are different at $P < 0.05$ N = the number of stallions, n = the number of ejaculates. N = the number of stallions, n = the number of ejaculates.

Table 4. The relation of stallion age to semen parameters in stallions (mean ± SEM).

Stallions' age	Semen parameters					
	Volume (mL)	pH	Motility (%)	Sperm concentration ($\times 10^6$/mL)	Total motile ($\times 10^9$)	Sperm abnormalities (%)
Group A 4–10 years ($N = 10$, $n = 22$)	28.14 ± 2.84	$7.23^{a} \pm 0.06$	$46.59^{a} \pm 6.93$	$147.77^{a} \pm 23.71$	$2.37^{a} \pm 0.58$	$48.45^{a} \pm 4.52$
Group B 11–18 years ($N = 8$, $n = 41$)	33.87 ± 3.30	$7.23^{b} \pm 0.04$	$69.27^{b} \pm 2.59$	$304.32^{b} \pm 25.62$	$6.01^{b} \pm 0.54$	$39.02^{b} \pm 2.86$
Group C >18 years ($N = 7$, $n = 31$)	28.87 ± 2.97	$7.14^{ab} \pm 0.08$	$62.42^{ab} \pm 5.11$	$182.82^{a} \pm 32.30$	$3.78^{a} \pm 1.05$	$34.06^{b} \pm 3.26$

Means with dissimilar superscript letters in the same column of each variable are different at $P < 0.05$ N = the number of stallions, n = the number of ejaculates. N = the number of stallions, n = the number of ejaculates.

Table 5. The relation of seasons, stallion age and fertility to semen parameters in stallions (mean ± SEM).

Stallions' fertility	Semen parameters					
	Volume (mL)	pH	Motility (%)	Sperm concentration ($\times 10^6$/mL)	Total motile ($\times 10^9$)	Sperm abnormalities (%)
Group I Infertile ($N = 9$, $n = 10$)	$22.80^{a} \pm 3.10$	$7.28^{a} \pm 0.08$	$16.00^{a} \pm 3.93$	109.70 ± 37.22	$0.27^{a} \pm 0.10$	$65.90^{a} \pm 4.40$
Group II <50 % ($N = 3$, $n = 10$)	$37.50^{b} \pm 3.96$	$7.01^{b} \pm 0.09$	$39.50^{b} \pm 7.73$	102.00 ± 35.23	$1.55^{ab} \pm 0.57$	$44.40^{b} \pm 6.06$
Group III 50–70 % ($N = 5$, $n = 33$)	$33.14^{ab} \pm 3.82$	$7.21^{b} \pm 0.07$	$70.61^{bc} \pm 3.50$	235.80 ± 28.46	$5.53^{ab} \pm 1.00$	$33.36^{bc} \pm 3.16$
Group IV >70% ($N = 8$, $n = 41$)	$29.41^{ab} \pm 2.57$	$7.21^{b} \pm 0.04$	$71.10^{c} \pm 2.82$	280.41 ± 27.55	$5.24^{b} \pm 0.53$	$37.02^{c} \pm 2.60$

Means with dissimilar superscript letters in the same column of each variable are different at $P < 0.05$ N = the number of stallions, n = the number of ejaculates. N = the number of stallions, n = the number of ejaculates.

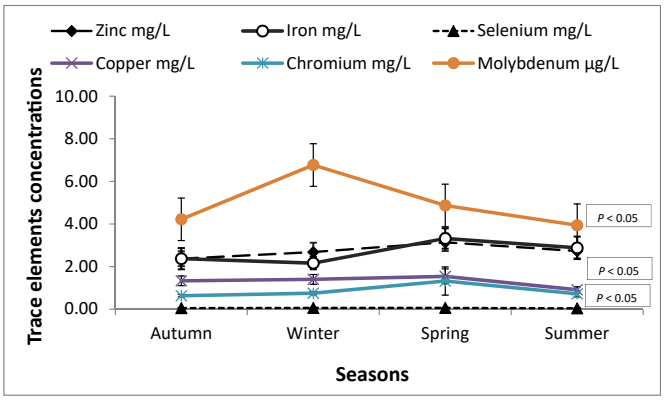

Figure 1. Effect of seasons on seminal plasma trace elements in stallions.

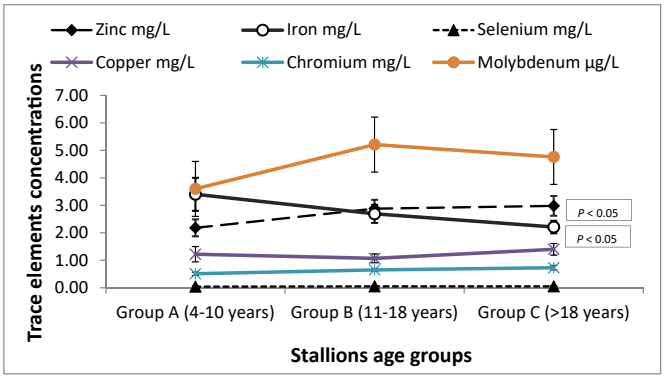

Figure 2. Effect of stallion age on seminal plasma trace elements.

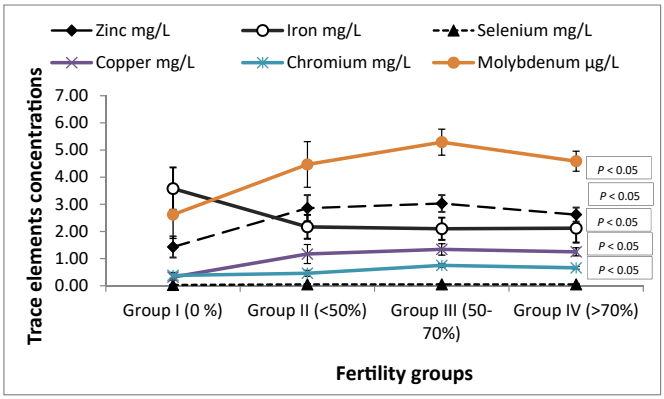

Figure 3. Seminal plasma trace element concentrations in relation to stallion fertility.

Table 6 shows significant ($P < 0.05$) positive correlations between seminal plasma Se and sperm motility (r = 0.37), sperm concentration (r = 0.38), TMSC (r = 0.41), and stallion fertility (r = 0.32). Significant ($P < 0.05$) negative (r = −0.39 and r = −0.33) correlations were found between sperm abnormalities and both seminal plasma Se and Mo, respectively. There were significant ($P < 0.05$) positive correlations between semen pH and Fe (r = 0.34), sperm motility and Zn (r = 0.39), and TMSC and Mo (r = 0.33). Stallion fertility was negatively correlated (r = −0.32; $P < 0.05$) with seminal plasma Cr. Significant positive correlations (r = 0.47, r = 0.57, and r = 0.89; $P < 0.05$) were recorded between seminal plasma Zn and Se, Se and Mo, and Cu and Cr, respectively (Table 6).

Table 6. Correlations coefficients (r^2) between semen parameters and seminal plasma trace elements in stallions.

Semen parameters and trace elements (n = 94)	Correlations coefficients (r^2)
pH X iron (Fe) mg/L	0.24*
Motility (%) × zinc (Zn) mg/L	0.29*
Motility (%) × selenium (Se) mg/L	0.27*
Sperm concentration ($\times 10^6$/mL) × selenium (Se) mg/L	0.28*
Total motile ($\times 10^9$) × selenium (Se) mg/L	0.31*
Total motile ($\times 10^9$) × molybdenum (Mo) µg/L	0.23*
Sperm abnormalities (%) × selenium (Se) mg/L	−0.29*
Sperm abnormalities (%) × molybdenum (Mo) µg/L	−0.23*
Stallion'' fertility × selenium (Se) mg/L	0.22*
Stallions' fertility × chromium (Cr) mg/L	−0.22*
Zinc (Zn) mg/L × selenium (Se) mg/L	0.37*
Selenium (Se) mg/L × molybdenum (Mo) µg/L	0.47*
copper (Cu) mg/L × Chromium (Cr) mg/L	0.89*

Values with astral superscript are significantly different at P < 0.05.
n = the number of ejaculates.

4. Discussion

The purpose of this study was to evaluate the relationships between sperm parameters and seminal fluid trace elements and season, age, and fertility in Arabian stallions. Our analysis showed that ejaculate volume was higher in winter than in spring. This might be attributed to the availability of green fodder during winter that improved some semen parameters. Similar findings reported in humans [34, 35]. Semen pH was more alkaline in spring than in both winter and summer. Most human semen samples with a normal pH [35] and semen samples associated with high pregnancy rates in mares [36] are recorded during spring.

In the present study, both the sperm cell concentrations and TMSC were higher in group B (11–18 years) than in groups A (4–10 years) and C (>18 years). Similar findings are reported in another study, where higher sperm cell concentrations were found in stallions in the middle age group (11–15 years) than in the young (5–10 years) and old (>15 years) age groups [37]. In the present study, the percentage of sperm abnormalities and Fe concentration were higher in group A (4–10 years) than in both group B (11–18 years) and C (>18 years). Sperm abnormalities were high in young stallions under 3 years of age [38]. However, there was no difference in sperm abnormalities and Fe between young (22–28 years) and old (65–80 years) men [39].

In the present study, the stallions in group II (<50% fertility) had a larger semen volume than those in group I (infertile). Ejaculate volume is an important parameter in the semen evaluation of infertile men [40]. Nevertheless, semen volume is not suitable for assessing fertility in men [41]. In the present study, semen pH was higher in group I (infertile) than in fertile groups II (<50% fertility), III (50–70% fertility), and IV (>70% fertility). The determination of seminal pH is an essential parameter in the evaluation of stallion semen [42]. The normal pH of raw stallion semen ranges from 7.2 to 7.7. Significant alterations in pH may cause a reduction in equine fertility [43]. In the present study, percentage sperm motility and total sperm motility and sperm abnormalities were lower in fertile group IV (>70% fertility) than in group I (infertile) and group II (<50% fertility). Stallion fertility was found to be highly correlated with percent total sperm motility and percent morphologically normal sperm [44, 45].

In the present study, Fe concentration in stallion seminal plasma was higher in spring than in summer and winter. However, Fe concentrations in stallion blood plasma were higher during spring and summer than during autumn [46]. In the current study, stallion seminal plasma concentrations of Se, Cu, and Cr were higher in winter than in summer. In bovine semen, Se concentrations were high during autumn [47]. Moreover, Cu concentrations in stallion blood plasma were higher during

spring and summer than during autumn [46]. In the present study, Mo concentrations in stallion seminal plasma were higher in winter than in the other seasons. Nevertheless, Mo concentrations in the seminal plasma of Marino rams were lower during October than during April [48].

In this study, the Zn concentration was higher in group B (11–18 years) than in group A (4–10 years). Similarly, Zn concentrations in stallion seminal plasma were higher in the old age group (>15 years) than in the young groups (5–10 and 11–15 years) [40]. In humans, Zn concentrations in seminal plasma were higher in the old (65–80 years) than in the young (22–28 years) age group [39].

In the present study, the concentrations of Zn and Cu in stallion seminal plasma were higher in fertile groups III (50–70% fertility) and IV (>70% fertility) than in-group I (infertile). Other studies found is a significant correlation between fertility and the concentrations of Zn and Cu in stallion seminal plasma [4, 9, 17, 18, 49]. Seminal plasma concentrations of Zn have an influence on stability of sperm membranes and chromatin [50, 51]. In humans, fertile males had higher seminal plasma Zn levels than infertile males [52, 53, 54]. Zn maintains testicular development and increases sperm concentration and sperm motility [55, 56]. A reduction in oxidative stress and an improvement in spermatogenesis are associated with Zn concentrations in the male genital tract [57]. Seminal plasma Cu concentration is highly correlated with spermatogenesis and fertility in humans [10, 58]. In the present study, seminal plasma Mo concentrations were higher in fertile group III (50–70% fertility) than in-group I (infertile). Molybdenum plays an essential role in normal testicular development, spermatogenesis [59], and maintenance of cell membrane integrity [60]. In the present study, seminal plasma Fe levels were lower in fertile group IV (>70% fertility) than in the other groups (infertile, <50% fertility, and 50–70% fertility). In humans, seminal plasma Fe concentrations were higher in infertile than in fertile males and were associated with oxidative stress in male gametes [58, 61]. Iron plays an essential role in spermatogenesis and sperm metabolism [62, 63]. Excess levels of Fe bring about an adverse effect on spermatogenesis and are associated with degeneration of the seminiferous tubules [61, 63, 64, 65].

In the present study, seminal plasma Se correlated positively with sperm motility, sperm concentration, TMSC, and fertility of stallions. Similarly, better sperm quality and improved fertility were associated with Se levels in both stallion spermatozoa [66] and human seminal plasma [53, 54, 67]. The present study found negative correlations between stallion sperm abnormalities and both Se and Mo levels in seminal plasma. Improved sperm morphology was associated with Se levels in both stallion spermatozoa [66] and human seminal plasma [54]. In adult mice, increased sperm morphology correlated with a moderate dose of Mo (25 mg/L), which regulates testicular oxidative stress [30]. In the present study, positive correlations found between semen pH and Fe, sperm motility and Zn, and TMSC and Mo. In humans, Fe is involved in the regulation of sperm pH and plays an essential role in normal spermatogenesis and sperm metabolism [25, 62, 63]. In humans, Zn is important for normal testicular development and sperm motility [21, 52, 54, 55, 56]. In moderate doses (25 mg/L), Mo increased sperm motility in adult mice [30]. Our analysis revealed a negative correlation between seminal plasma Cr and stallion fertility. Other studies have shown that Cr deficiency adversely affects semen quality and fertility in animals and humans [27, 29]. The present study showed positive correlations between seminal plasma Zn and Se, Se and Mo, and Cu and Cr. In infertile men, there were reduced levels of Zn and Se in their seminal plasma [53, 54]. In fertile men, positive correlations found between Zn and Se concentrations in the seminal plasma [68, 69, 70, 71]. In men, there was a positive correlation between seminal plasma Se and Mo levels [72]. Moreover, positive correlations found between seminal plasma Cu and Cr concentrations in humans [72, 73].

5. Conclusions

Our findings showed that high concentrations of seminal plasma trace elements Zn, Se, Cu, and Mo low concentrations of Fe are related to elevated semen parameters and fertility of the Arabian horse. Relatively, high seminal plasma concentrations of Zn, Se, Cu, and Mo were associated with improved semen quality and high fertility in Arab stallions.

Declarations

Author contribution statement

Magdi M. Waheed, Ph.D: Conceived and designed the experiment; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ahmad M.A. Meligy, Ph.D and Ibrahiem M. Ghoneim, Ph.D: Performed the experiments.

Abdulrahman K. Alhaider, Ph.D: Contributed reagents, materials, analysis tools or data.

Funding statement

Magdi, M. Waheed was supported by Deanship of Scientific Research, King Faisal University (568).

Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors thank the Deanship of Scientific Research, King Faisal University, Saudi Arabia for the support of this study (Grant 568; 30000 SR).

References

[1] A.J. Hamada, S.C. Esteves, A. Agarwal, A Comprehensive Review of genetics and genetic testing in azoospermia, Clinics 68 (Suppl 1) (2013) 39–60. https://www.ncbi.nlm.nih.gov.

[2] M. Ollero, E. Gil-Guzman, M.C. Lopez, R.K. Sharma, A. Agarwal, K. Larson, D. Evenson, A.J. Thomas Jr., J.G. Alvarez, Characterization of subsets of human spermatozoa at different stages of maturation: implications in the diagnosis and treatment of male infertility, Hum. Reprod. 16 (2001) 1912–1921. https://pubmed.ncbi.nlm.

[3] C.A. Burtis, E.R. Ashwood, D.E. Bruns, Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 2006. https://www.ncbi.nlm.nih.gov.

[4] T. Katila, M. Kareskoski, Components of stallion seminal plasma and their influence on spermatozoa, Pferdeheilkunde 22 (2) (2006) 193–200.

[5] E. Töpfer-Petersen, D. Waberski, O. Hess, S. Bellair, A. Schambony, M. Ekhlasi-Hundrieser, M. Gentzel, A. Reineke, The role of seminal plasma in fertilization - a short overview, Tierärztliche Umsch. 53 (1998) 447–454. https://www.researchgate.net/publication/286037821.

[6] M.B. Sørensen, I.A. Bergdahl, N.H. Hjøllund, J.P. Bonde, M. Stoltenberg, E. Ernst, Zinc, magnesium and calcium in human seminal fluid: relations to other semen parameters and fertility, Mol. Hum. Reprod. 5 (1999) 331–337. https://academic.oup.com/molehr/article/5/4/331/1141041.

[7] R. Asadpour, Relationship between mineral composition of seminal plasma and semen quality in various ram breeds, Acta Sci. Vet. 40 (2) (2012) 1027–1034. https://www.redalyc.org/pdf/2890/289023567001.

[8] D. Zambelli, R. Raccagni, M. Cunto, G. Andreani, G. Isani, Sperm evaluation and biochemical characterization of cat seminal plasma collected by electro ejaculation and urethral catheterization, Theriogenology 74 (8) (2010) 1396–1402. https://daneshyari.com/article/preview/2097816.

[9] E. Tvrda, N. Lukac, M. Schneidgenova, J. Lukacova, C. Szabo, Z. Goc, A. Gren, P. Massanyi, Impact of seminal chemical elements on the oxidative balance in bovine seminal plasma and spermatozoa, J. Vet. Med. 8 (2013), e125096.

[10] A.R. Hamad, H.I. Al-Daghistani, W.D. Shquirat, M. Abdel-Dayem, M. Al-Swaifi, Sodium, potassium, calcium and copper levels in seminal plasma are associated

with sperm quality in fertile and infertile men, Biochem. Pharmacol. 3 (2014) 141–147. https://www.longdom.org/open-access.
[11] I. Barrier-Battut, H. Delajarraud, E. Legrand, J.F. Buyas, F. Fieni, D. Tainturier, C. Thorin, H. Pouliquen, Calcium, magnesium, copper and zinc in seminal plasma of fertile stallions and their relationship with semen freezability, Theriogenology 58 (2002) 229–232. https://www.researchgate.net/publication/282719219.
[12] P. Massanyi, R. Toman, J. Trandzik, P. Nad, M. Skalicka, B. Korenekova, Concentration of copper, zinc, iron, cadmium, lead and nickel in bull, ram, boar, stallion and fox semen, Trace Elem. Electrolytes 21 (1) (2004) 45–49. https://www.researchgate.net/publication/288147781.
[13] R. Henkel, J. Bittner, R. Weber, F. Hüther, W. Miska, Relevance of zinc in human sperm flagella and its relation to motility, Fertil. Steril. 71 (6) (1999) 1138–1143. https://pubmed.ncbi.nlm.nih.gov/10360924/.
[14] S. Leonhard-Marek, Influence of drugs, pollution and trace elements on male fertility, in: W. Busch, A. Holzmann (Eds.), Andrology in Veterinary Medicine, Schattauer, Stuttgart, 2001, pp. 474–481.
[15] C. Baldauf, W. Miska, W. Weidner, W.B. Schill, R. Henkel, Principal cells in the cauda epididymidis resorb zinc eliminated from spermatozoa, Andrologia 35 (1) (2003) 3. https://onlinelibrary.wiley.com/doi/epdf/10.1046/j.1439-0272.2003.00531_3.x.
[16] W.D. Shquirat, H.I.A. Daghistani, A.W.R. Hamad, M.A. Dayem, M.A. Swaifi, Zinc, Manganese, and Magnesium in seminal fluid and their relationship to male infertility in Jordan, Int. J. Pure Math. Sci. 3 (2013) 1–10. https://www.semanticscholar.org/paper/.
[17] S. Pesch, M. Bergmann, H. Bostedt, Determination of some enzymes and macro- and microelements in stallion seminal plasma and their correlations to semen quality, Theriogenology 66 (2) (2006) 307–313. https://pubmed.ncbi.nlm.nih.gov/16413936/.
[18] W.Y. Wong, G. Flik, P.M. Groenen, D.W. Swinkels, C.M. Thomas, J.H. Copius-Peereboom, H.M. Merkus, R.P. Steegers-Theunissen, The impact of calcium, magnesium, zinc, and copper in blood and seminal plasma on semen parameters in men, Reprod. Toxicol. 15 (2) (2001) 131–136. https://pubmed.ncbi.nlm.nih.gov/11297872/.
[19] V.K. Pandy, M. Parmeshwaran, S.D. Soman, Concentrations of morphologically normal, motile spermatozoa: Mg, Ca and Zn in the semen of infertile men, Sci. Total Environ. 27 (1) (1983) 49–52. https://pubmed.ncbi.nlm.nih.gov/6867707/.
[20] S.E. Chia, C.N. Ong, L.H. Chua, L.M. Ho, S.K. Tay, Comparison of zinc concentrations in blood and seminal plasma and the various sperm parameters between fertile and infertile men, J. Androl. 21 (2000) 53–57. https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.319.4442&rep=rep1.
[21] Y.L. Huang, W.C. Tsen, S.Y. Cheng, T.H. Lin, Trace elements and lipid peroxidation in human seminal plasma, Biol. Trace Elem. Res. 76 (3) (2000) 207–215. https://link.springer.com/article/10.1385/BTER:76:3:207.
[22] R. Stanwell-Smith, S.G. Thompson, A.P. Haines, R.J. Ward, G. Cashmore, J. Stedronska, W.F. Hendry, A comparative study of zinc, copper, cadmium, and lead levels in fertile and infertile men, Fertil. Steril. 40 (5) (1983) 670–677. https://www.fertstert.org/article/S0015-0282(16)47429-7/fulltext.
[23] M.K. Moslemi, S. Tavanbakhsh, Selenium–vitamin E supplementation in infertile men: effects on semen parameters and pregnancy rate, Int. J. Gen. Med. 4 (2011) 99–104. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3048346/.
[24] U. Ahsan, Z. Kamran, I. Raza, S. Ahmad, W. Babar, M.H. Riaz, Z. Iqbal, Role of selenium in male reproduction – a review, Anim. Reprod. Sci. 146 (2014) 55–62. https://europepmc.org/article/med/24613013.
[25] A.A. Nikolaev, D.L. Lutskiĭ, N.N. Nikolaeva, L.V. Lozhkina, Iron and nonheme iron protein metabolism in ejaculates with varying degrees of fertility, Urol. Nefrol. 5 (1998) 27–31. https://pubmed.ncbi.nlm.nih.gov/9820043/.
[26] U. Marzec-Wróblewska, P. Kamiński, P. Lakota, M. Szymański, K. Wasilow, G. Ludwikowski, M. Kuligowska-Prusińska, G. Odrowąż-Sypniewska, T. Stuczyński, J. Michałkiewicz, Zinc and iron concentration and SOD activity in human semen and seminal plasma, Biol. Trace Elem. Res. 143 (1) (2011) 167–177. https://link.springer.com/article/10.1007%2Fs12011-010-8868-x.
[27] R.A. Anderson, M.M. Polansky, Dietary chromium deficiency effect on sperm count and fertility in rats, Biol. Trace Elem. Res. 3 (1) (1981) 1–5. https://europepmc.org/article/med/24271557.
[28] I.E. Bassey, O.E. Essien, I.K.P. Isong, A.E. Udoh, G.S. Agbara, Seminal plasma chromium, cadmium and lead levels in infertile men, J. Med. Sci. 13 (6) (2013) 497–500. https://scialert.net/fulltext/?doi=jms.2013.497.500&org=11.
[29] K. Jurkowska, E.M. Kratz, E. Sawicka, A. Piwowar, The impact of metalloestrogens on the physiology of male reproductive health as a current problem of the XXI century, J. Physiol. Pharmcol. 70 (3) (2019) 337–355. https://www.researchgate.net/publication/335965759.
[30] X.W. Zhai, Y.L. Zhang, Q. Qi, Y. Bai, X.L. Chen, L.J. Jin, X.G. Ma, R.Z. Shu, Z.J. Yang, F.J. Liu, Effects of molybdenum on sperm quality and testis oxidative stress, Syst. Biol. Reprod. Med. 59 (5) (2013) 251–255. https://www.tandfonline.com/doi/full/10.3109/19396368.2013.791347.
[31] F. Li, X. Zeng, C. Wu, Z. Duan, Y. Wen, G. Huang, X. Long, M. Li, M. Li, J. Xu, Ecological risks assessment and pollution source identification of trace elements in contaminated sediments from the pearl river delta, China, Biol. Trace Elem. Res. 155 (2) (2013) 301–313. https://www.researchgate.net/publication/256102009.
[32] SPSS, Statistical Package for Social Sciences, Copyright© for Windows, Chicago, IL, USA, 2013. https://www.ibm.com/support/pages/spss-statistics-220-available-download.
[33] R.C. Littell, P.R. Henry, C.B. Ammerman, Statistical analysis of repeated measures data using SAS procedures, J. Anim. Sci. 76 (1998) 1216–1231. https://academic.oup.com/jas/article-abstract/76/4/1216/4625238.
[34] A.J. Gaskins, D.S. Colaci, J. Mendiola, S.H. Swan, J.E. Chavarro, Dietary patterns and semen quality in young men, Hum. Reprod. 27 (10) (2012) 2899–2907. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3442634/.
[35] A. De Giorgi, R. Volpi, R. Tiseo, M. Pala, R. Manfredini, F. Fabbian, Seasonal variation of human semen parameters: a retrospective study in Italy, Chronobiol. Int. 32 (5) (2015) 711–716.
[36] F. Crespo, C. Quiñones-Pérez, I. Ortiz, M. Diaz-Jimenez, C. Consuegra, B. Pereira, J. Dorado, M. Hidalgo, Seasonal variations in sperm DNA fragmentation and pregnancy rates obtained after artificial insemination with cooled-stored stallion sperm throughout the breeding season (spring and summer), Theriogenology 148 (2020) 89–94. https://europepmc.org/article/med/32169626.
[37] G.A. El Sisy, A.M. Abo El-Maaty, Z.M. Rawash, Comparative blood and seminal plasma oxidant/antioxidant status of Arab stallions with differentages and their relation to semen quality, Asian Pac. J. Reprod. 5 (2016) 428–433. https://www.cabdirect.org/cabdirect/abstract/20163354601.
[38] K.F. Dowsett, L.M. Knott, The influence of age and breed on stallion semen, Theriogenology 46 (1996) 397–412. https://www.sciencedirect.com/science/article/abs/pii/0093691X96001628.
[39] T.E. Schmid, P.G. Grant, F. Marchetti, R.H. Weldon, B. Eskenazi, A.J. Wyrobek, Elemental composition of human semen is associated with motility and genomic sperm defects among older men, Hum. Reprod. 28 (1) (2013) 274–282. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3619967/.
[40] M. Roberts, K. Jarvi, Steps in the investigation and management of low semen volume in the infertile man, Can. Urol. Assoc. J. 3 (6) (2009) 479–785. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2792416/.
[41] E. Bostofte, J. Serup, H. Rebbe, Relation between sperm count and semen volume, and pregnancies obtained during a twenty-year follow-up period, Int. J. Androl. 5 (3) (1982) 267–275.
[42] D.J. Jasko, Evaluation of stallion semen, Vet. Clin. N. Am. Equine Pract. 8 (1) (1992) 129–148. https://europepmc.org/article/MED/1576546.
[43] P.M. McCue, Evaluation of pH and osmolarity of semen, in: J.J. Dascanio, P.M. McCue (Eds.), Equine Reproductive Procedures, 2014, pp. 399–400. Chapter 122.
[44] C.C. Love, Relationship between sperm motility, morphology and the fertility of stallions, Theriogenology 76 (3) (2011) 547–557. https://europepmc.org/article/med/21497893.
[45] D.D. Varner, Z. Gibb, R.J. Aitken, Stallion fertility: a focus on the spermatozoon, Equine Vet. J. 47 (2015) 16–24.
[46] L. Vranković, J. Aladrović, B. Beer-Ljubić, M. Zdelar-Tuk, Z. Stojević, Seasonal changes in enzyme activities and mineral concentrations in Holstein stallions blood plasma, Vet. Arhiv. 85 (3) (2015) 235–246. https://www.researchgate.net/publication/282791966.
[47] R. Sławeta, T. Laskowska, E. Szymańska, Seasonal changes in total and selenium-dependent glutathione peroxidase activity in bovine semen in relation to lipid peroxide, Anim. Reprod. Sci. 17 (3–4) (1988) 303–307. https://www.sciencedirect.com/science/article/abs/pii/037843208890067X#.
[48] B. Bulbul, P.P. Akalin, N. Başpinar, M.N. Bucak, A comparative study of seminal plasma and blood serum macro and trace elements in the breeding (October) and the non-breeding (April) seasons in merino ram, J. Hellenic Vet. Med. Soc. 70 (1) (2019) 1365–1372. https://ejournals.epublishing.ekt.gr/index.php/jhvms/article/view/20341/17777.
[49] P. Massányi, J. Trandzik, P. Nad, R. Toman, M. Skalicka, B. Korenekova, Seminal concentrations of trace elements in various animals and their correlations, Asian J. Androl. 5 (2003) 101–104. https://www.researchgate.net/publication/10733421.
[50] M. Chvapil, New aspects in the biological role of zinc: a stabilizer of macromolecules and biological membranes, Life Sci. 13 (8) (1973) 1041–1049. https://www.sciencedirect.com/science/article/abs/pii/002432057390372X?via%3Dihub.
[51] L. Björndahl, U. Kvist, Human sperm chromatin stabilization: a proposed model including zinc bridges, Mol. Hum. Reprod. 16 (2010) 23–29. https://academic.oup.com/molehr/article/16/1/23/1059189.
[52] A.H. Colagar, E.T. Marzony, M.J. Chaichi, Zinc levels in seminal plasma are associated with sperm quality in fertile and infertile men, Nutr. Res. 29 (2) (2009) 82–88. https://www.researchgate.net/publication/24200203.
[53] S. Türk, R. Mändar, R. Mahlapuu, A. Viitak, M. Punab, T. Tiiu Kullisaar, Male infertility: decreased levels of selenium, zinc and antioxidants, J. Trace Elem. Med. Biol. 28 (2) (2014) 179–185. https://www.sciencedirect.com/science/article/abs/pii/S0946672X1300206X?via%3Dihub.
[54] D. Chyra-Jach, Z. Kaletka, M. Dobrakowski, A. Machoń-Grecka, Levels of macro- and trace elements and select cytokines in the semen of infertile men, Biol. Trace Elem. Res. 197 (2020) 431–439. https://link.springer.com/article/10.1007%2Fs12011-019-02022-9.
[55] M.S. Khan, S. Zaman, M. Sajjad, M. Shoaib, G. Gilani, Assessment of the level of trace element zinc in seminal plasma of males and evaluated of its role in male infertility, Int. J. Appl. Basic Med. Res. 1 (2011) 93–96. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3657968/.
[56] M. Raigani, B. Yaghmaei, N. Amirjannti, N. Lakpour, M.M. Akhondi, H. Zeraati, M. Hajihosseinal, M.R. Sadeghi, The micronutrient supplements, zinc sulphate and folic acid, did not ameliorate sperm functional parameters in oligoasthenoteratozoospermic men, Andrologia 46 (9) (2014) 956–962.
[57] J.S. Gabrielsen, C. Tanrikut, Chronic exposures and male fertility: the impacts of environment, diet, and drug use on spermatogenesis, Andrology 4 (2016) 648–661.
[58] B. Aydemir, A.R. Kiziler, I. Onaran, B. Alici, H. Ozkara, M.C. Akyolcu, Impact of Cu and Fe concentrations on oxidative damage in male infertility, Biol. Trace Elem. Res. 112 (3) (2006) 193–203.

[59] Y. Zhou, X.M. Fu, D.L. He, X.M. Zou, C.Q. Wu, W.Z. Guo, W. Feng, Evaluation of urinary metal concentrations and sperm DNA damage in infertile men from an infertility clinic, Environ. Toxicol. Pharmacol. 45 (2016) 68–73. https://pubmed.ncbi.nlm.nih.gov/27262988/.

[60] W. Guzikowski, M.I. Szynkowska, H. Motak-Pochrzęst, A. Pawlaczyk, S. Sypniewski, Trace elements in seminal plasma of men from infertile couples, Arch. Med. Sci. 11 (3) (2015) 591–598. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4495155/.

[61] E. Tvrda, R. Peer, S.C. Sikka, A. Agarwal, Iron and copper in male reproduction: a double-edged sword, J. Assist. Reprod. Genet. 32 (1) (2015) 3–16. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4294866/.

[62] P.T. Lieu, M. Heiskala, P.A. Peterson, Y. Yang, The roles of iron in health and disease, Mol. Aspect. Med. 22 (1–2) (2001) 1–87. https://pubmed.ncbi.nlm.nih.gov/11207374/.

[63] T. Wise, D.D. Lunstra, G.A. Rohrer, J.J. Ford, Relationships of testicular iron and ferritin concentrations with testicular weight and sperm production in boars, J. Anim. Sci. 81 (2003) 503–511. https://pubmed.ncbi.nlm.nih.gov/12643495/.

[64] M.L. Pereira, F.G. Costa, Spermatogenesis recovery in the mouse after iron injury, Hum. Exp. Toxicol. 22 (5) (2003) 275–279.

[65] J. Wang, K. Pantopoulos, Regulation of cellular iron metabolism, Biochem. J. 434 (2011) 365–381. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3048577/.

[66] H. Bertelsmann, S. Keppler, M. Höltershinken, H. Bollwein, D. Behne, D. Alber, G. Bukalis, A. Kyriakopoulos, H. Sieme, Selenium in blood, semen, seminal plasma and spermatozoa of stallions and its relationship to sperm quality, Reprod. Fertil. Dev. 22 (5) (2010) 886–891. https://pubmed.ncbi.nlm.nih.gov/20450841/.

[67] M. Eroglu, S. Sahin, B. Durukan, O.B. Ozakpinar, N. Erdinc, L. Turkgeldi, K. Sofuoglu, A. Karateke, Blood serum and seminal plasma selenium, total antioxidant capacity and coenzyme Q10 levels in relation to semen parameters in men with idiopathic infertility, Biol. Trace Elem. Res. 159 (2014) 46–51.

[68] B. Xu, S.E. Chia, M. Tsakok, C.N. Ong, Trace elements in blood and seminal plasma and their relationship to sperm quality, Reprod. Toxicol. 7 (6) (1993) 613–618. https://pubmed.ncbi.nlm.nih.gov/8118112/.

[69] B. Xu, S.E. Chia, C.N. Ong, Concentrations of cadmium, lead, selenium, and zinc in human blood and seminal plasma, Biol. Trace Elem. Res. 40 (1994) 49–57.

[70] N.B. Oldereid, Y. Thomassen, K. Purvis, Selenium in human male reproductive organs, Hum. Reprod. 13 (8) (1998) 2172–2176. https://www.researchgate.net/publication/13530289.

[71] M.I. Camejo, L. Abdala, G. Vivas-Acevedo, R. Lozano-Hernández, M. Angeli-Greaves, E.D. Greaves, Selenium, copper and zinc in seminal plasma of men with varicocele, relationship with seminal parameters, Biol. Trace Elem. Res. 143 (3) (2011) 1247–1254. https://www.academia.edu/11755503/.

[72] A. Heidari, An experimental biospectroscopic study on seminal plasma in determination of semen quality for evaluation of male infertility, Int. J. Adv. Technol. 7 (2) (2016) 1000e007. https://www.longdom.org/open-access/an-experimental-biospectroscopic-study-on-seminal-plasma-in-determination-of-semen-quality-for-evaluation-of-male-infertility-0976-4860-1000e007.pdf.

[73] L.I. Yan, G.A.O. Qiaoyan, L.I. Mingcai, L.I. Mengyang, G.A.O. Xueming, Cadmium, chromium, and copper concentration plus semen quality in environmental pollution site, China, Iran. J. Public Health 43 (1) (2014) 35–41. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4454041/.