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

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A B S T R A C T

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
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 were 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 matings 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 (1 mL) 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 and 3 mL (30%) hydrogen peroxide. The starting temperature for the digestion process was ramped to 125 °C and 3 mL (30%) hydrogen peroxide. The starting temperature for the digestion process was ramped to 125 °C and 3 mL (30%) hydrogen peroxide. The starting temperature for the digestion process was ramped to 125 °C and 3 mL (30%) hydrogen peroxide. The starting temperature for the digestion process was ramped to 125 °C and 3 mL (30%) hydrogen peroxide.

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 = 8), <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) |
|----------|------------------|----------|----------|---------------------------|
| Drying 1 | 150              | 5        | 20       | 250                       |
| Drying 2 | 200              | 5        | 15       | 250                       |
| Pyrolysis| 1200             | 10       | 20       | 250                       |
| Atomization | 2300          | 0        | 5        | 0                         |
| Clean-out| 2450             | 1        | 3        | 250                       |
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 (TMSMC) 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 stallions (mean ± SEM).

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

| Elements          | 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         |

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 ($\times 10^7$/mL) | Total motile ($\times 10^6$) | Sperm abnormalities (%) |
|-----------|------------------|-------------|--------|--------------|--------------------------------------|-----------------------------|------------------------|
| Autumn    |                  | 28.78±b     | 4.75   | 7.19±b       | 0.06                                 | 67.50±4.89                  | 3.69±0.67              | 40.61±3.87            |
| Winter    |                  | 37.31±5.71  | 4.05   | 7.06±0.05    | 0.06                                 | 59.69±4.62                  | 3.87±0.73              | 41.00±4.55            |
| Spring    |                  | 27.21±3.32  | 4.05   | 7.29±0.06    | 0.06                                 | 52.36±7.15                  | 5.26±1.49              | 31.33±4.47            |
| Summer    |                  | 31.18±b     | 2.61   | 7.21±a       | 0.06                                 | 64.87±4.34                  | 4.53±0.66              | 43.00±3.23            |

Means with dissimilar superscript letters in the same column of each variable are different at $P < 0.05$, $N_1$ = the number of stallions, $n_1$ = the number of ejaculates. $N_2$ = the number of stallions, $n_2$ = 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 ($\times 10^7$/mL) | Total motile ($\times 10^6$) | Sperm abnormalities (%) |
|----------------|------------------|-------------|--------|--------------|--------------------------------------|-----------------------------|------------------------|
| Group A 4–10 years (N = 10, n = 22) |                  | 28.14±2.84  | 7.23±0.06 | 46.59±6.93 | 147.77±23.71 | 2.37±0.58 | 48.45±4.52 |
| Group B 11–18 years (N = 8, n = 41) |                  | 33.67±3.30  | 7.23±0.04 | 69.27±2.59 | 304.32±25.62 | 6.01±0.54 | 39.02±2.86 |
| Group C >18 years (N = 7, n = 31) |                  | 28.87±2.97  | 7.14±0.08 | 62.42±5.11 | 182.82±32.30 | 3.78±1.05 | 34.08±3.26 |

Means with dissimilar superscript letters in the same column of each variable are different at $P < 0.05$, $N_1$ = the number of stallions, $n_1$ = the number of ejaculates. $N_2$ = the number of stallions, $n_2$ = 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 ($\times 10^7$/mL) | Total motile ($\times 10^6$) | Sperm abnormalities (%) |
|----------------------|------------------|-------------|--------|--------------|--------------------------------------|-----------------------------|------------------------|
| Group I Infertile (N = 9, n = 10) |                  | 22.80±3.10  | 7.28±0.08 | 16.00±3.93 | 109.70±37.22 | 0.27±0.10 | 65.90±4.40 |
| Group II <50% (N = 3, n = 10) |                  | 37.50±3.96  | 7.01±0.09 | 39.50±7.73 | 102.00±35.23 | 1.55±0.57 | 44.40±6.06 |
| Group III 50–70% (N = 5, n = 33) |                  | 33.14±3.82  | 7.21±0.07 | 70.61±3.50 | 235.80±28.46 | 5.53±1.00 | 33.36±3.16 |
| Group IV >70% (N = 8, n = 41) |                  | 29.41±2.57  | 7.21±0.04 | 71.10±2.82 | 280.41±27.55 | 5.24±0.53 | 37.02±2.60 |

Means with dissimilar superscript letters in the same column of each variable are different at $P < 0.05$, $N_1$ = the number of stallions, $n_1$ = the number of ejaculates. $N_2$ = the number of stallions, $n_2$ = the number of ejaculates.
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 ($\times 10^9$/mL) × selenium (Se) mg/L | 0.28* |
| Total motile ($\times 10^6$) × selenium (Se) mg/L | 0.31* |
| Total motile ($\times 10^6$) × 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*                            |
| Stallion 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 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 Ibrahim M. Ghoneim, Ph.D: Performed the experiments.

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

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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.

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