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*Original article*

# Exploring the relationship between blood serum macro and micro minerals and sperm quality characteristics in fresh and frozen-thawed bulls' semen

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## Abstract

**Background:** One interesting topic in reproductive biology is to explore the correlations between minerals and male fertility. The aim of the present study was to investigate the relationships between Calcium (Ca), Magnesium (Mg), Zinc (Zn), Copper (Cu), and Iron (Fe) and their associations with semen quality in bulls.

**Methods:** Ejaculates were collected from Simmental bulls. Semen quality parameters including volume, concentration, motility, sperm viability and morphology were assessed. One step dilution method was utilized for semen freezing. Progressive motility of frozen-thawed semen (PMFT) was regarded as the main criterion for dividing data into three groups: group 1 (< 40% motility), group 2 (40% -50% motility), and group 3 (> 50% motility). Serum mineral concentrations were determined by atomic absorption. The Serum Fe was significantly lower in "> 50%" group compared with other groups.

**Results:** Semen volume was negatively correlated with PMFT ($r = -0.42$, $p \leq 0.05$ and serum Fe ($r = 0.44$, $p \leq 0.05$). Negative correlations were found between Zn to Cu ratio with frozen-thawed sperm abnormal morphology ($r = -0.43$, $p \leq 0.05$) and tail abnormality and fresh sperm mid-piece abnormality($r= 0.47$, $p \leq 0.05$). There was also a negative correlation between Zn and frozen-thawed sperm head abnormality ($r= -0.57$, $p \leq 0.05$).

**Conclusion:** With regard to the weak positive correlations between serum Cu and frozen-thawed sperm tail abnormal morphology, it can be concluded that the excess of serum Cu may have destructive effects on normal sperm morphology.

**Keywords:** Blood serum, Macro minerals, Micro minerals, Semen quality

## Introduction

It is generally assumed that macro- and micro-minerals have been proved to have an impact on sperm quality and that their levels could be regarded as important criteria for fresh and frozen-thawed sperm function and viability (1).

Calcium (Ca) is also an important macro-mineral, which is mainly associated with sperm acrosome reaction and motility, depending on the stage of sperm maturation, particularly sperm capacitation (2-4). Nevertheless, research findings demonstrate that the relationship between Ca and sperm characteristics is conflicting and that there are some inconsistent results (5, 6).

It is widely thought that Magnesium (Mg) is another important macro-mineral, which can indirectly influence the sperm quality and reproductive system. This could be due to hemostatic balances between calcium-phosphorus-magnesium (Ca-P-Mg). This is particularly the case for Mg deficiency due to appetite loss (7). Moreover, it is believed that Mg in seminal plasma can affect sperm quality through maintaining osmotic balance, which is important for sperm function. It can also cooperate with some enzymes such as Ca2+ and Mg2+- dependent ATPase (8).

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Among the micro minerals, it appears that zinc (Zn) has crucial effects on spermatogenesis and antioxidant status (9). Although it has been reported that the concentration of this trace element is extremely higher in seminal plasma than in blood serum (10), a significant correlation has already been found between serum and seminal plasma Zn levels (11). Furthermore, it has been indicated that both serum and seminal Zn concentrations are associated with sperm quality (12).

Copper (Cu), which is another important trace element, can also cooperate in many antioxidant actions, especially as a co-factor in superoxide dismutase (SOD) enzyme. Cu deficiency can lead to a decrease in libido and semen quality as well as testicular damage, even sterility, in rabbits (13). It can also result in a reduction in semen volume, sperm count, and motility as well as an increase in abnormal morphology in rams (14). On the other hand, it is stated that Cu could be a highly toxic element for sperm (15). It is speculated that excessive Cu accumulation in the testis could have destructive effects on spermatogenesis and sex hormone depletion (16).

It has been shown that Iron (Fe) has an important role in the enzymatic systems of sperm mid-piece, which are critical for the progressive motility of sperms (17). The positive correlations between sperm motility and Fe concentration were reported in previous in vivo and in vitro studies, which can declare the importance of this trace element in bull fertility (18-20). The destructive effects of Fe deficiency on semen quality might be due to its indirect effect, emanating from anemia, especially in combination with Cu deficiency (21). It was reported that blood Fe was positively correlated with seminal plasma Fe (22).

Although it seems that the relationship between the seminal plasma elements with the semen quality parameters could be more logical, there are also several studies examining this relationship in blood serum (13, 22-26). On the other hand, Abou-skakra et al. (1989) suggested that the role of trace elements in infertility was more directly related to their sperm and serum levels than to the seminal plasma level (27). Therefore, the aim of our research was to evaluate the relationships between blood serum Ca, Mg, Zn, Cu, and Fe with sperm quality parameters in dual purpose simmental (Fleckvieh) bulls, a merit and economic cattle. It is also worth mentioning that there is little information about the physiological characteristics of this valuable breed.

## Materials & Methods

**Animals:** This research was done on eight healthy dual purpose simmental (Fleckvieh) bulls (2-6 years old) between winter and spring seasons over the period of three months. The semen samples were collected during the usual collection at the Iran Simmental Cattle Breeding Center (height above sea level: 47m, longitude: 520 23′57.76″E, latitude: 360 30′ 18.55″N) between 8 and 12 A.M. The sires were fed three times per day based on standard Simmental bulls' daily rations with the following formulae; silage 18kg, concentrate 9kg, alfalfa 3kg, straw and water ad libitum, Ca 0.74%, P 0.53%, Na 0.49%, Mg 0.29%, Zn 375 ppm, Mn 381/44 ppm, Cu 1•01 ppm, selenium (Se 2.75) ppm plus mineral and vitamin supplements.

**Semen and blood serum sample collection and processing:** Approximately, 10 mL of blood serum was collected via coccygeal venipuncture from each bull in the beginning, middle, and the end of the research. Collected samples were centrifuged at × 930 g for 15 minutes and moved into Eppendorf type micro-tubes and stored at -70˚C until further investigations.

Semen samples were collected by an artificial vagina, which was pre-warmed at 46 ˚C in oven. The sexual stimulation of the bulls was carried out by 10 min standing near the dummy (a restrained bull in the box) with three false jumps. Immediately after semen collection, the concentration (by photometer SDM [Minitube, Tiefenbach, Germany] calibrated for bull sperm cell counting) volume was recorded. In order to evaluate the fresh sperm motility, two small drops of extended semen were put on glass slide and analyzed using a binocular phase contrast microscope (Minitube, Tiefenbach, Germany) with the magnification of ×200, which was equipped with hot plate.

In addition, one step dilution method (room temperature semen packaging) was applied for semen freezing through the following procedure;

Pre-extender dilution was prepared by adding the extender (Steridyl CSS, Minitube, Tiefenbach, Germany) to the semen gently (with ratio of 1:1) and then placed in a water bath at 34 ˚C for 10 min. At this time, the volume of the finally- needed extender was calculated through the following formula; Number of



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doses = (semen volume × semen concentration × progressive motile sperm × morphologically normal sperm) ÷ (sperm per dose [15 million]). After 10 min, the final solution was prepared by adding the pre-extender to the final calculated extender volume and then left it at room temperature (20-24 ˚C) for 15 min. After that, the semen was packed into the 0.5 ml straws (Minitube, Slovakia) with MPP Uno automated filling and sealing machine (Minitube, Tiefenbach, Germany) and placed on the loading and counting tray. Then, the packed straws were kept at 4 ˚C for 3 3 hours in a refrigerator in order to reach in equilibration stage. Finally, the equilibrated semen was put in a Mount Freezer device Refrigerator (Minitube, Tiefenbach, Germany) at –120 ˚C for 10 min in order for the straws to freeze. The frozen straws were stored in separate goblets in the canisters of a liquid nitrogen container.

**Sperm viability and morphology evaluation:** The evaluation of sperm viability was carried out by eosin-nigrosin (Minitube, Tiefenbach, Germany) staining, and spermac (Minitube, Wellington, South Africa) staining was applied for sperm morphological abnormalities. Both methods were performed by the examination of 200 sperms per sample under 400 x magnifications.

**Computer-assisted sperm analysis (CASA):**

Frozen thawed sperms motility was determined by CASA (Hooshmand Fanavar, Tehran, Iran) after thawing the semen at 37 ˚C for 40 second in water bath. Parameters such as progressive motility (PM), curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), lateral head displacement (ALH), beat cross frequency (BCF), degrees of deviation (MAD), and linearity (LIN [VSL/VCL]) were evaluated. All analyses were performed by a light microscope, which was equipped with hot plate and maintained samples at 37 ˚C in a chamber (Sperm meter, Depth 10 micron, Surface Graticule, 100x 0.1 SQMM) to prevent sperm motility decrease during the analyses.

**Determination of blood serum macro and micro minerals:** The concentration of Ca, Mg, Zn, Cu, and Fe, was measured by flame atomic absorption spectrometry (Varian AA240FS, USA). Briefly, 1 ml of the whole blood was taken and digested with 5 ml 69 % of pure concentrated nitric acid (Merck, Germany). The wavelengths for the elements were: Zn (213.9 nm), Cu (324.7 nm), Fe (248.3 nm) (13), Ca (422.8 nm), and Mg (285.2) (28). The calibration curves were made using the standard solutions of various concentrations for each trace element.

**Statistical analysis:** All data were expressed as mean ± SEM. The correlation interaction was analyzed by Spearman's correlation coefficient test. In order to lucidly illustrate the interaction between the motility with enzymes status and semen quality parameters, the progressive motility of the frozen thawed semen (PMFT) was chosen as the main criterion, which was based on the study of Li et al. (2016). The results depicted that progressive motility was the main predictive factor for semen evaluation (29). Consider "normal" sperm motility to be 60% or greater. Our study, consistent with many other studies, regarded men with 40% or greater sperm motility as "normal".

Motility characteristics: Samples, based on progressive motility of frozen-thawed semen (PMFT), were divided into the following three groups: group 1 (<40% motility), group 2 (40 – 50% motility), and group 3 (>50%). The differences between-subject effects of PMFT in various groups, compared to sperm characteristics and enzymes status, were determined through the analysis of variance (Welch's ANOVA) test.

## Results

**Effect of sperm motility alteration and blood mineral concentration:** All values such as scrotal circumferences (SC), semen quality parameters, and blood serum mineral concentration of the bulls are represented in Table 1. As expected, the viability and motility of fresh and frozen-thawed spermatozoa as well as all CASA parameters were significantly different in all three groups. It is worth noting that the Fe concentration in blood serum was found to be lower in group 3 than in other groups.

**Correlations between semen quality parameters and blood serum mineral status:** All correlations were shown in Table 2. No correlation was detected between SC with all semen parameters. The semen volume was negatively correlated with PMFT and serum Fe. The total abnormal morphology and the sperm tail abnormal morphology in both fresh and frozen-thawed conditions were correlated with CASA parameters, which indicated that sperm morphological abnormalities were directly associated with frozen thawed sperm quality. Also, there was a negative



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correlation found between serum zinc and sperm head abnormality after freezing. Interestingly, there was a significant negative correlation detected between Zn to Cu ratio with total sperm abnormal morphology (AMFT) and sperm tail abnormal morphology (Tail FT) in frozen-thawed conditions and fresh sperm mid-piece abnormality (Mid-piece BF).

Table 1. Scrotal circumferences, semen quality parameters, and blood serum minerals concentrations of dual-purpose Simmental (*Fleckvieh*) bulls (24 blood and 32 semen samples) in three* groups study

| Parameters | Group 1 Mean ± SE | Group 2 Mean ± SE | Group3 Mean ± SE |
|---|---|---|---|
| Scrotal circumference (Cm) | 42.6 ± 1.2[a] | 42.3 ± 0.9[a] | 42.3 ± 1.1[a] |
| Semen volume (ml) | 8.9 ± 1.2[a] | 8.4 ± 1.4[a] | 6.7 ± 0.9[a] |
| Semen concentration ($\times 10^6$ /ml) | 1319.8 ± 78.2[a] | 1495.3 ± 119.8[a] | 1441.5 ± 90.1[a] |
| Total motility before freezing (%) | 70.1 ± 2.2[b] | 79.5 ± 0.2[ab] | 82.2 ± 1.0[a] |
| Progressive motility before freezing (%) | 62.6 ± 2.3[b] | 74.5 ± 0.2[ab] | 77.2 ± 1.0[a] |
| Viability before freezing (%) | 74.9 ± 4.7[b] | 87.3 ± 1.4[a] | 85.1 ± 1.1[ab] |
| Abnormal morphology before freezing (%) | 11.0 ± 2.5[a] | 7.8 ± 1.8[a] | 7.8 ± 1.2[a] |
| Head abnormality before freezing (%) | 3.1 ± 1.0[a] | 3.4 ± 1.9[a] | 1.9 ± 0.5[a] |
| Mid piece before freezing (%) | 1.9 ± 0.3[a] | 1.4 ± 0.3[a] | 1.3 ± 0.4[a] |
| Tail abnormality before freezing (%) | 5.1 ± 1.3[a] | 2.6 ± 0.5[a] | 4.1 ± 0.8[a] |
| Cytoplasmic droplet before freezing (%) | 0.5 ± 0.2[a] | 0.5 ± 0.1[a] | 0.5 ± 0.1[a] |
| Gross motility of frozen-thawed semen (%) | 50.5 ± 4.5[c] | 70.5 ± 0.9[b] | 80.8 ± 1.2[a] |
| Progressive motility of frozen-thawed semen (%) | 26.8 ± 3.3[c] | 46.3 ± 1.4[b] | 57.4 ± 1.7[a] |
| Average path velocity of frozen-thawed semen (µm/s) | 25.7 ± 3.2[c] | 40.8 ± 1.1[b] | 53.3 ± 1.6[a] |
| Curvilinear velocity of frozen-thawed semen (µm/s) | 38.6± 4.5[c] | 57.0 ± 2.1[b] | 74.0± 2.7[a] |
| Straight line velocity of frozen-thawed semen (µm/s) | 21.4 ± 2.9[c] | 35.9 ± 1.0[b] | 47.1 ± 1.6[a] |
| Linearity of frozen-thawed semen (%) | 32.2 ± 3.1[c] | 47.7 ± 1.2[b] | 55.9 ± 1.3[a] |
| Lateral head displacement of frozen-thawed semen (µm) | 1.8 ± 0.2[c] | 2.34 ± 0.09[b] | 3.0 ± 0.14[a] |
| Beat cross frequency of frozen-thawed semen (Hz) | 0.6 ± 0.1[c] | 0.9 ± 0.0[b] | 1.2 ± 0.1[a] |
| Degrees of deviation of frozen-thawed semen | 17.9 ± 2.4[b] | 25.5 ± 1.8[a] | 30.9 ± 1.7[a] |
| Frozen-thawed sperm viability (%) | 43.2 ± 4.0[b] | 70.0 ± 2.9[a] | 71.6 ± 2.2[a] |
| Abnormal morphology of frozen-thawed spermatozoa (%) | 16.8 ± 4.6[a] | 8.3 ± 2.0[a] | 9.2 ± 1.5[a] |
| Head abnormality of frozen-thawed spermatozoa (%) | 5.3 ± 1.8[a] | 2.7 ± 0.9[a] | 2.1 ± 0.7[a] |
| Mid-piece abnormality of frozen-thawed spermatozoa (%) | 2.1 ± 0.5[a] | 1.8 ± 0.5[a] | 1.9 ± 0.4[a] |
| Tail abnormality of frozen-thawed spermatozoa (%) | 8.9 ± 2.4[a] | 3.5 ± 1.1[a] | 4.7 ± 0.9[a] |
| Cytoplasmic droplet of frozen-thawed spermatozoa (%) | 0.6 ± 0.2[a] | 0.3 ± 0.2[a] | 0.5 ± 0.1[a] |
| Serum Zinc (µg/dl) | 126.7 ± 18.3[a] | 124.6 ± 26.5[a] | 131.9 ± 16.4[a] |
| Serum Copper (µg/dl) | 96.6 ± 13.1[a] | 63.1 ± 10.8[a] | 81.3 ± 9.9[a] |
| Serum Iron (mg/dl) | 2.3 ± 0.1[ab] | 2.5 ± 0.2[a] | 1.9 ± 0.1[b] |
| Serum Calcium (mg/dl) | 8.7 ± 0.4[a] | 9.4 ± 0.7[a] | 9.6 ± 0.5[a] |
| Serum Magnesium (mg/dl) | 2.4 ± 0.2[a] | 2.4 ± 0.2[a] | 2.7 ± 0.1[a] |
| Serum Zinc to Copper ratio | 1.6 ± 0.5[a] | 2.5 ± 0.7[a] | 2.0 ± 0.4[a] |
| Serum calcium to magnesium ratio | 3.7 ± 0.3[a] | 4.1 ± 0.6[a] | 3.6 ± 0.2[a] |

*Sample based on progressive motility of frozen-thawed semen (PMFT): Group 1 (<40% motility), Group 2 (40 – 50% motility), and Group 3 (>50%)
Different letters in the same line indicate a significant difference between the groups ($p$<0.05).

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Table 2. Correlation of scrotal circumferences, semen quality parameters, and blood serum minerals concentrations of dual-purpose Simmental

| | SC | Vol | Con | TMBF | PMBF | VBF | AMF | Head BF | Mid.piece BF | Tail BF | Cy.Dr BF | TMFT | PMFT | VCL | VSL | VAP | MAD | ALH | BCF | LIN | VFT | AMFT | Head FT | Mid.piece FT | Tail FT | Cy.DrF T | Zn | Cu | Fe | Ca | Mg | Zn.Cu ratio | Ca.Mg ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SC | 1 | .34 | .10 | -.20 | -.21 | .18 | .84 | -.06 | .31 | .87 | -.39 | .03 | -.02 | .03 | -.03 | -.02 | .11 | .08 | -.00 | -.02 | -.35 | -.09 | .01 | .06 | -.06 | .16 | .00 | .22 | .06 | -.13 | -.00 | -.17 | -.06 |
| Vol | | 1 | -.07 | -.36 | -.42* | .05 | -.25 | -.16 | .08 | -.34 | .03 | -.24 | -.27 | -.22 | -.24 | -.24 | -.22 | -.22 | -.18 | -.25 | -.28 | -.13 | -.12 | .02 | -.15 | .07 | .30 | -.31 | .44* | .05 | -.18 | .26 | .17 |
| Conc | | | 1 | .16 | .12 | -.05 | .16 | .34 | -.00 | -.00 | -.00 | .20 | .20 | .12 | .17 | .18 | .18 | .23 | .21 | .17 | -.05 | -.12 | .10 | -.12 | -.22 | .13 | -.40 | -.16 | .17 | .19 | .09 | .10 | .02 | .12 |
| TMBF | | | | 1 | .96** | .55** | .44* | -.10 | -.48* | -.32 | .01 | .86** | .88** | .86** | .86** | .88** | .72** | .76** | .88** | .85** | .87** | .78** | -.06* | -.26 | -.56** | -.09 | .34 | -.39 | -.28 | .17 | .32 | .34 | -.09 |
| PMBF | | | | | 1 | .42* | -.26 | -.28 | -.43* | -.25 | -.01 | .86** | .88** | .82** | .86** | .88** | .88** | .72** | .86** | .88** | .83** | .78** | -.09 | -.29 | -.48* | -.04 | .11 | -.28 | -.29 | .19 | .31 | .32 | -.02 |
| VBF | | | | | | 1 | -.66** | -.39 | -.57** | -.90** | -.40 | .91** | .95** | .84* | .91* | .85* | .85* | .35 | .93** | .88** | .95** | .95** | -.07* | -.66** | -.63* | -.60** | .11 | -.36 | .20 | -.15 | -.07 | -.15 | .15 | .16 | -.17 |
| AMBF | | | | | | | 1 | .39* | -.38* | .82** | .17 | -.69* | -.68* | -.69* | -.66* | -.67* | -.52** | -.66* | -.92** | -.68* | -.65* | -.76* | .89** | .82** | .78* | .37 | -.29 | .22 | .06 | -.09 | -.18 | -.19 | .09 |
| Head | | | | | | | | 1 | .12 | .48* | -.10 | -.24 | -.29 | -.28 | -.35 | -.36 | -.28 | -.40 | -.24 | -.31 | -.34 | .44* | .87* | .12 | .39 | .11 | -.17 | .17 | .08 | .08 | -.08 | -.09 | .15 |
| Mid.p | | | | | | | | | 1 | .48* | .18 | -.26 | -.35 | -.26 | -.34 | -.32 | -.39 | -.15 | -.26 | -.40 | -.40 | .86** | .86** | .78** | .86** | .20 | -.24 | .22 | .07 | -.20 | -.17 | .62* | -.86 |
| Tail | | | | | | | | | | 1 | .01 | -.39 | -.30 | -.37 | -.34 | -.35 | -.42* | -.34 | -.41* | -.35 | -.37 | .85** | .68* | .74* | .86** | .20 | -.29 | .28 | -.18 | -.01 | -.11 | -.22 | -.21 |
| Cy.Dr | | | | | | | | | | | 1 | -.12 | -.11 | .03 | -.05 | -.03 | .06 | .11 | -.11 | -.26 | -.17 | .34 | .26 | .32* | .18 | .35 | -.05 | -.33 | .07 | .06 | -.02 | .18 | .03 |
| TMFT | | | | | | | | | | | | 1 | .96** | .84* | .97** | .96** | .94** | .97** | .96** | .91* | .92* | -.67* | -.44* | -.30 | -.69* | -.28 | .15 | -.19 | -.26 | .12 | .19 | .19 | .02 |
| PMFT | | | | | | | | | | | | | 1 | .83* | .98** | .98** | .98** | .97** | .98** | .98** | .97** | -.65* | -.37 | -.26 | -.56* | -.24 | .10 | -.25 | -.24 | .20 | .19 | .19 | .01 |
| VCL | | | | | | | | | | | | | | 1 | .96** | .94** | .92** | .96** | .97** | .96** | .97** | -.62* | -.36 | -.19 | -.52* | -.16 | .05 | -.20 | -.31 | .23 | .28 | .23 | -.06 |
| VSL | | | | | | | | | | | | | | | 1 | .99** | .92** | .95** | .98** | .95** | .98** | -.64* | -.35 | -.25 | -.55* | -.18 | .08 | -.23 | -.26 | .20 | .25 | .16 | -.01 |
| VAP | | | | | | | | | | | | | | | | 1 | .96** | .96** | .97** | .96** | .98** | -.64* | -.35 | -.24 | -.56* | -.18 | .08 | -.23 | -.28 | .21 | .22 | .16 | -.02 |
| MAD | | | | | | | | | | | | | | | | | 1 | .92** | .97** | .92** | .97** | -.62** | -.38 | -.15 | -.52** | -.19 | -.02 | -.16 | -.28 | .24 | .32 | .03 | -.15 |
| ALH | | | | | | | | | | | | | | | | | | 1 | .97** | .96** | .97** | -.46* | -.34 | -.12 | -.46* | -.12 | .04 | -.16 | -.36 | .26 | .32 | .07 | -.08 |
| BCF | | | | | | | | | | | | | | | | | | | 1 | .96** | .98** | -.68* | -.40 | -.28 | -.59* | -.25 | .06 | -.23 | -.23 | .24 | .31 | .13 | -.05 |
| LIN | | | | | | | | | | | | | | | | | | | | 1 | .92** | -.68* | -.41* | -.32 | -.60* | -.21 | .16 | -.23 | -.24 | .21 | .24 | .03 | .01 |
| VFT | | | | | | | | | | | | | | | | | | | | | 1 | -.58* | -.43* | -.24 | -.58* | -.40 | .12 | -.25 | -.23 | .12 | .15 | .12 | -.01 |
| AMFT | | | | | | | | | | | | | | | | | | | | | | 1 | .71** | .71** | .97** | .39 | -.36 | .33 | .09 | -.08 | -.08 | .62* | -.05 |
| Head | | | | | | | | | | | | | | | | | | | | | | | 1 | .85** | .80 | .55** | .34 | .35 | .08 | -.07 | -.35 | .10 |
| Mid.p T | | | | | | | | | | | | | | | | | | | | | | | | 1 | .87* | .23 | -.22 | .11 | -.25 | -.26 | -.04 | -.21 | -.39 |
| Tail FT | | | | | | | | | | | | | | | | | | | | | | | | | 1 | .36 | -.25 | .43* | -.00 | -.13 | -.07 | .44* | -.10 |
| Cy.Dr FT | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | -.06 | .00 | .05 | .39 | -.04 | -.11 | .21 |
| Zn | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | -.20 | -.23 | .16 | -.16 | .83** | .23 |
| Cu | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | -.29 | -.17 | -.07 | -.76** | -.13 |
| Fe | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | .18 | -.29 | -.02 | .28 |
| Ca | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | .00 | .21 | .88*** |
| Mg | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | -.01 | -.71** |
| Zn.Cu ratio | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | .28 |
| Ca.Mg ratio | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |

*Correlation is significant at ≤ 0.05 level, ** Correlation is significant at ≤ 0.01 level

*Abbreviations*: Scrotal circumference (SC), Semen volume (Vol), Semen concentration (Con), Gross motility before freezing (GMBF), Progressive motility before freezing (PMBF), Viability before freezing (VBF), Abnormal morphology before freezing (AMBF), Head abnormality before freezing (Head BF), Mid-piece abnormality before freezing (Mid-piece BF), Tail abnormality before freezing (Tail BF), Cytoplasmic droplet before freezing (Cy.D BF), Gross motility of frozen-thawed semen (GMFT), Progressive motility of frozen-thawed semen (PMFT), Average path velocity of frozen-thawed semen (VAPFT), Curvilinear velocity of frozen-thawed semen (VCLFT), Straight line velocity of frozen-thawed semen (VSLFT), linearity of frozen-thawed semen (LINFT), Lateral head displacement of frozen-thawed semen (ALHFT), Beat cross frequency of frozen-thawed semen (BCFFT), Degrees of deviation of frozen-thawed semen (MADFT), Frozen-thawed sperm viability (VFT), Abnormal morphology of frozen-thawed spermatozoa (AMFT), Head abnormality of frozen-thawed spermatozoa (Head FT), Mid-piece abnormality of frozen-thawed spermatozoa (Mid-piece FT), Tail abnormality of frozen-thawed spermatozoa (Tail FT), Cytoplasmic droplet of frozen-thawed spermatozoa (Cy.D FT), Serum Zinc (Zn), Serum Copper (Cu), Serum Iron (Fe), Serum Calcium (Ca), Serum Magnesium (Mg), serum Zinc to Copper ratio (Zn.Cu ratio), serum Calcium to Magnesium ratio (Ca.Mg ratio).



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## Discussion

A quick look at the existing literature indicates that the differences in blood serum and seminal plasma Ca concentrations were not statistically significant in both normal fertile and infertile men in Wong's et al. study (24). There was a significant positive correlation found between blood Ca and sperm motility in bulls, suggesting that Ca blood concentrations were responsible for concentrations in seminal plasma (25). Although the serum concentration of Ca was greater than those of the other two groups in our study, this difference was not significant. The inconsistency of the results obtained from our research, compared with Machal's et al. (2002), could be due to the classification of data based on PMFT in our study. It was also reported that blood and seminal plasma Mg concentrations were not different between fertile and infertile men (24), which is in agreement with the results of our study. Liang et al. (2016), nevertheless, reported that seminal Cu to Mg ratio was negatively correlated with sperm concentrations (30). Our findings demonstrated that serum Ca to Mg ratio was not correlated with semen quality parameters.

Xu et al. (1993) reported that there was a significant positive correlation between the blood and seminal plasma concentrations of zinc. They concluded that essential trace elements may contribute to maintain the physiological equilibrium between blood and seminal plasma (10). Several studies failed to find any correlations between semen quality and blood zinc concentrations (22, 23, 31, 32). In the same vein, our findings did not show any significant correlations, either. We only found negative correlations between serum zinc and sperm head abnormality after freezing. Wong et al. reported that although zinc concentrations in both blood and seminal plasma were not different between fertile and infertile men, there were significant correlations found between blood Zn level and sperm concentrations, and motility and morphology (24). Moreover, Eggert et al. (2002) reported that both serum and seminal zinc levels of infertile patients were lower than those of normal fertile men (33). Although the Zn serum levels (which represent the excellent sperm quality) were highest in group 3 among the three groups in our study, this difference is not significant.

Nonetheless, Yuyan et al. (2008) reported that by increasing serum zinc levels, sperm viability decreased and sperm abnormal morphology increased. Furthermore, they concluded that when the concentration of zinc becomes less than 870 µm/l, the risk of asthenozoospermia will increase (34). Akinloye et al. (2011) also reported that the serum concentration of zinc was significantly higher in oligozoospermic patients compared to the normal fertile and azospermic men. They reported that there was a significant inverse correlation between serum Zn and sperm count, which is inconsistent with the results of some studies (35, 36) and consistent with those of others (37-39), including ours.

There are two studies reporting that serum Cu concentration was higher than seminal plasma (24, 26). There was a significant positive correlation found between blood Cu concentrations with sperm count and motility in bulls (Machal et al., 2002), suggesting that the blood concentrations of Cu corresponded with its concentrations in seminal plasma (25). There were also significant correlations between Cu concentrations and sperm count, and motility and abnormal morphology (40).

Another study also reported that Cu blood concentration was correlated with sperm motility (24). Some studies mentioned that the blood Cu concentrations had no correlations with semen quality (22). On the other hand, the adverse effect of high liver Cu concentrations on sperm morphology was shown in impala (41). We also found a weak positive correlation between serum Cu and tail abnormal morphology after freezing. Moreover, Yuyan et al. (2008) reported that sperm progressive motility was significantly reduced by increasing the serum Cu concentrations (34). In addition, there was a significant correlation found between serum Cu and a decrease in sperm motility and viability (42), which do not support our results. Our findings showed that although the highest serum Cu levels were assigned to group 1, this difference was not significant among the three groups. On the other hand, the serum Zn to Cu ratio in our study was negatively correlated with sperm mid-piece abnormality in fresh semen, the total abnormal morphology of frozen thawed semen and the frozen-thawed sperm tail abnormality. Regarding these results and those of Ackerman et al. (1999) research (41), it can be interpreted that excessive Cu level could be destructive for sperm normal morphology.

Akalin et al. (2015) reported that the blood concentrations of Fe had no correlations with any of



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the sperm characteristics in rams (22). Interestingly, our results showed that the serum Fe concentrations were significantly higher in the group 1 (and positively correlated with semen volume). Although the semen volumes were not significantly different among the three PMFT groups in our study, the mean volumes of the groups were as follows: group 1, group 2, and group 3, respectively. Therefore, we presumed that the excessive serum Fe could directly correlate with low sperm motility and indirectly with low semen concentrations due to the increasing volume of the semen. It is worth noting that the results firmly established the notion that progressive motility and semen concentration are two most important parameters in male fertility potential evaluation.

According to the results of our study, it appears that the sole measuring of the serum levels of Ca, Mg, Cu, Zn, and Fe, which can play a pivotal role in male reproductive physiology, is not a sufficient indicator for predicting the breeding soundness of a bull or the fertility evaluation of men.

## Conclusion

In conclusion, we can say that among these minerals, serum Zn and Fe may have positive and negative influences on sperm motility, respectively. We recommend that in order to exactly evaluate the semen quality, it is imperative to determine serum and seminal plasma minerals and some important antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase or the total antioxidant capacity. In addition, their relationships with sperm quality parameters could also reveal some important evidence, which may lead to a deeper understanding of male reproductive physiology.

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## Conflicts of Interest

The authors declare that they have no competing interests.

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