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*Original article*

# Evaluating the potential relationship between the semen quality parameters and serum catalase, superoxide dismutase, and glutathione peroxidase in Bulls

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## Abstract

**Background:** It is evident that catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) are the first line defense antioxidant enzymes. This study was conducted to elucidate the relationships between the serum levels of these enzymes and the semen quality parameters in Bulls.

**Methods:** Semen samples were collected from eight healthy dual-purpose Simmental (Fleckvieh) Bulls by artificial vagina. The semen quality parameters such as volume, concentration, sperm motility, viability, and abnormal morphology were analyzed. One step dilution method was performed for semen freezing procedure. The progressive motility of frozen-thawed semen was considered as the main criterion for data classification, which is as follows: <40%, 40 – 50% and >50%. The Bulls' blood samples were collected via coccygeal venipuncture. Serum CAT, SOD, and GPx were determined through enzyme-linked immunosorbent assay (ELISA) method.

**Results:** The serum Gpx activity was higher in the <40% group. Surprisingly, significant negative correlations were found between the gross and progressive motility of frozen-thawed semen with serum Gpx status. Moreover, positive correlations were found between Gpx and CAT in blood serum.

**Conclusion:** Our results illustrated some valuable information about the serum levels of antioxidant enzymes and their relationships with semen quality in Fleckvieh Bulls. It can also be concluded that measuring these enzymes only cannot be deemed as a sufficient reflection of reproductive performance, especially the semen quality of Bulls.

**Keywords:** Catalase, Glutathione peroxidase, Semen quality, Superoxide dismutase

## Introduction

It is generally assumed that the evaluation of sperm quality characteristics such as progressive motility and semen concentration is a pivotal step before semen banking in human and animal artificial insemination centers (1, 2). It is also thought that severe damage to the membrane and acrosome of the sperm may occur during the freezing step, which could be due to temperature changes, the induction of osmotic stress, and the formation of ice crystals (3, 4).

Although it has been proven that low physiological concentrations of reactive oxygen species (ROS) are essential for sperm capacitation, hyperactivation, and sperm-oocyte fusion (5, 6), excessive ROS production can reduce both sperm quality and function, which is due to the generation of detrimental chemicals and structural alterations to sperm DNA and membrane lipids (7, 8). Three major antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) have been reported as the first line defense antioxidants, which can play a fundamental role in the entire antioxidant defense network in the body (9). It is presumed that these enzymatic antioxidants are recognized as the cell and body oxidant status regulators (10) and are, as a

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result, regarded as important markers for semen quality assessment in animals such as bulls and buffalos, and in human (11-14). It is widely believed that CAT can reduce the hydrogen peroxide in spermatozoa and seminal plasma (15). It is also assumed that SOD can catalyze the dismutation of superoxide (O2-) radical into either ordinary molecular oxygen (O2) or hydrogen peroxide (H2O2) (16). Glutathione peroxidase (GPx) is a critical enzyme in sperm development and maturation, especially in nuclear compaction and mid-piece structural function (17).

Although there are several studies reporting associations between seminal plasma antioxidant enzymes and semen quality parameters (11, 13, 14, 18-20), there are some other studies revealing significant associations between serum antioxidant enzymes and semen quality (21-25).The aim of the present study was to determine serum CAT, SOD, and GPx activities in dual purpose Simmental (Fleckvieh) Bulls and their relationships with semen quality parameters.

## Materials & Methods

Animals: A total of eight healthy dual-purpose Simmental (Fleckvieh) Bulls (age range, 2-6 years) were included in this study. The semen samples were collected during routine weekly collection at 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 over a three-month period and during winter season. The animals were fed three times per day based on Fleckvieh Bulls' daily diet (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, Co 1/01 ppm, Se 2.75 ppm plus mineral and vitamin supplements).

Samples collection and processing: Blood serum (approximately 10 mL) was collected via coccygeal venipuncture from each bull in day 1, day 45, and day 90 of study (three samples from each sire). Blood serums were centrifuged at × 930 g for 15 minutes; serum samples were collected and stored at -70˚C for biochemical analysis.

Three semen samples from each Bull (total of 24 samples), collected simultaneously with the blood samples, were routinely collected by pre-warmed artificial vagina (46 ˚C in oven). The sexual preparation of bulls was accomplished through three false mounts by standing for 10 min in the collection area. Semen concentration was measured by SDM photometer (Minitube, Tiefenbach, Germany) calibrated for bull sperm cell counting. To estimate fresh sperm motility, two small drops from diluted semen were put on a glass slide and analyzed using a binocular phase contrast microscope (Minitube, Tiefenbach, Germany) at magnification of ×200 equipped with warm stage. For semen freezing, one step dilution method (room temperature semen packaging) was performed, which is briefly described below:

- Preparation of Pre-extender dilution: gently add the extender (Steridyl CSS, Minitube, Tiefenbach, Germany) to the semen (with ratio of 1:1) and place it in a water bath at 34 ˚C for 10 min.
- Calculation of final extender volume as following formula; Number of doses = (semen volume × semen concentration × progressive motile sperm × morphologically normal sperm) ÷ (sperm per dose [15 million]).
- Preparation of final solution by adding the pre-extender to the final calculated extender volume and then leave it at room temperature (20-24 ˚C) for 15 min.
- Packaging the 0.5 ml straws (Minitube, Slovakia) with MPP Uno automated filling and sealing machine (Minitube, Tiefenbach, Germany).
- Equilibration stage: carrying out the packed straws at 4 ˚C for 3 h in refrigerator.
- Freezing stage: putting the equilibrated straws at –120 ˚C for 10 min in MT freezer freezing device (Minitube, Tiefenbach, Germany).
- Storing the frozen semen in liquid nitrogen containers.

Sperm viability and morphology evaluation:

To evaluate spermatozoa viability and morphology, semen samples were stained using eosin-nigrosin (Minitube, Tiefenbach, Germany) and Spermac (Minitube, Wellington, South Africa) staining, respectively. 200 sperms per sample were also examined under 400x magnification.

Computer assisted sperm analysis (CASA):

The motility of frozen thawed sperms was analyzed by CASA software (HooshmandFanavar, Tehran, Iran) after thawing at 37 ˚C for 35-45 second, and the following parameters were evaluated: progressive motility (PM), curvilinear velocity (VCL), straight line

velocity (VSL), average path velocity (VAP), lateral head displacement (ALH), beat cross frequency (BCF), the degrees of deviation (MAD) and linearity (LIN [VSL/VCL]). All analyses were performed by a light microscope equipped with a warm stage for maintaining samples at 37 ˚C and a chamber (Sperm meter, Depth 10 micron, Surface Graticule, 100x 0.1 sqmm) to avoid the reduction of sperm quality during the assessment.

Determination of enzymes status:

Serum Catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities were determined using commercial kits (ZellBio GmbH, Germany) based on the manufacturer's instructions.

**Statistical analysis:**

All data were expressed as mean ± SEM. The correlation interaction was analyzed by Spearman's correlation coefficient test. To better illustrate the interaction between motility with enzymes status and semen quality parameters, the progressive motility of frozen thawed semen (PMFT) was chosen as the main criterion. It is worth mentioning that this was accomplished through a study by Li et al. (2016), which showed that progressive motility was the main predictive factor for semen evaluation (26). Accordingly, samples were divided into three groups: <40%, 40 – 50% and >50%. The differences, between-subject effects of PMFT various groups compared to sperm characteristics and enzyme status, were determined by the analysis of variance (Welch ANOVA) test.

## Results

Effect of sperm characteristics and blood enzymatic status:

The level of semen quality parameters and serum enzyme activities in 24 blood and semen samples of 8 Fleckvieh Bulls are represented in Table 1. There were significant differences in sperm viability before freezing (VBF), sperm Mid-piece abnormality before freezing (Mid-piece BF), Tail abnormality of frozen-thawed spermatozoa (Tail FT), Frozen-thawed sperm viability (VFT) and serum Gpx in different motility groups, which are all shown in Table.1. The viability before and after freezing was considerably higher in ">50%" and "40%-50%" groups, respectively. The morphological abnormality evaluation of the parameters also revealed a meaningful increase at mid-piece BF and tail FT in "<40%" group. Moreover, Gross motility before freezing (GMBF), Progressive motility before freezing (PMBF), the gross motility of frozen-thawed semen (GMFT), the progressive motility of frozen-thawed semen (PMFT), the average path velocity of frozen-thawed semen (VAPFT), the curvilinear velocity of frozen-thawed semen (VCLFT), the straight line velocity of frozen-thawed semen (VSLFT), the linearity of frozen-thawed semen (LINFT), the lateral head displacement of frozen-thawed semen (ALHFT), the beat cross frequency of frozen-thawed semen (BCFFT), the degrees of deviation of frozen-thawed semen (MADFT), and the frozen-thawed sperm viability (VFT) of the group "<40%" were all significantly lower than those of the other two groups. GMFT, VAPFT, VCLFT, VSLFT, and Tail FT in group "40% - 50%" were significantly lower than ">50%". VBF of group "<40%" was significantly lower than the "40% - 50%" group. Mid-piece BF of ">50%" group was significantly lower than "<40%" group.

The level of Gpx activity in serum was significantly higher in less than 40% group, compared with other groups. Although there were no statistically significant differences between the activities of serum antioxidant enzymes and three PMFT groups, the levels of serum CAT and SOD activities were slightly higher in <40%" group, compared with 40-50% and more than 50% groups.

Correlation between, scrotal circumference, sperm parameters, and serum enzymatic status:

The results of the correlations between sperm quality characteristics and serum antioxidant enzymes in 8 Fleckvieh bulls are represented in Table 2. It was found that fresh sperm motility was associated with frozen-thawed sperm motility parameters and Gpx, the abnormal morphology of frozen-thawed spermatozoa (AMFT), the head abnormality of frozen-thawed spermatozoa (Head FT), and the mid-piece abnormality of frozen-thawed spermatozoa (Mid-piece FT). Surprisingly, significant negative correlations were detected between PMFT, GMFT, VAPFT and ALHFT with Gpx status. There were, nonetheless, positive correlations between Gpx and CAT activities in blood serum.

Table 1. Semen quality parameters and serum enzyme levels in 24 blood and semen samples of 8 dual-purpose Simmental (Fleckvieh) Bulls (data are presented as the mean ± standard error).

| **Parameters** | **More than 50** | **40-50** | **Less than 40** |
|---|---|---|---|
| Scrotal circumference (Cm) | 42.8 ± 1.4[a] | 42.3 ± 1.0[a] | 42.3 ± 1.5[a] |
| Semen volume (ml) | 6.7 ± 1.29[a] | 8.4 ± 1.4[a] | 9.2 ± 1.6[a] |
| Semen concentration ($\times 10^6$ /ml) | 1420.1 ± 130.3[a] | 1495.3 ± 119.8[a] | 1342.3 ± 99.9[a] |
| Gross motility before freezing (%) | 82.6 ± 1.3[a] | 79.8 ± 0.2[a] | 69.3 ± 2.9[b] |
| Progressive motility before freezing (%) | 77.6 ± 1.3[a] | 74.8 ± 0.3[a] | 61.0 ± 2.8[b] |
| Viability before freezing (%) | 86.0± 1.3[ab] | 87.3 ± 1.4[a] | 71.6 ± 5.7[b] |
| Abnormal morphology before freezing (%) | 7.6 ± 1.5[a] | 7.8 ± 1.8[a] | 12.9± 2.8[a] |
| Head abnormality before freezing (%) | 1.9 ± 0.6[a] | 3.4 ± 1.8[a] | 3.8 ± 1.2[a] |
| Mid-piece abnormality before freezing (%) | 0.9 ± 0.3[b] | 1.4 ± 0.3[ab] | 2.2 ± 0.4[a] |
| Tail abnormality before freezing (%) | 4.3 ± 1.0[a] | 2.6 ± 0.5[a] | 5.7 ± 1.6[a] |
| Cytoplasmic droplet before freezing (%) | 0.4 ± 0.2[a] | 0.5 ± 0.1[a] | 0.7 ± 0.2[a] |
| Gross motility of frozen-thawed semen (%) | 80.6 ± 1.8[a] | 70.5 ± 0.9[b] | 46.9 ± 5.3[c] |
| Progressive motility of frozen-thawed semen (%) | 56.6 ± 2.4[a] | 46.3 ± 1.4[a] | 25.1 ± 4.2[b] |
| Average path velocity of frozen-thawed semen (µm/s) | 52.1 ± 2.1[a] | 40.8 ± 1.1[b] | 24.1 ± 3.9[c] |
| Curvilinear velocity of frozen-thawed semen (µm/s) | 72.4 ± 3.5[a] | 56.9 ± 2.1[b] | 36.1 ± 5.8[c] |
| Straight line velocity of frozen-thawed semen (µm/s) | 46.0 ± 1.9[a] | 35.9 ± 1.0[b] | 19.9 ± 3.6[c] |
| Linearity of frozen-thawed semen (%) | 55.7 ± 1.7[a] | 47.7 ± 1.2[a] | 30.2 ± 3.7[b] |
| Lateral head displacement of frozen-thawed semen (µm) | 2.9 ± 0.2[a] | 2.3 ± 0.1[a] | 1.7 ± 0.2[b] |
| Beat cross frequency of frozen-thawed semen (Hz) | 1.2 ± 0.1[a] | 0.9 ± 0.0[a] | 0.5 ± 0.1[b] |
| Degrees of deviation of frozen-thawed semen | 29.9 ± 2.1[a] | 25.5 ± 1.8[a] | 16.6 ± 3.1[b] |
| Frozen-thawed sperm viability (%) | 70.4 ± 3.0[a] | 70.0 ± 2.9[a] | 39.5 ± 3.8[b] |
| Abnormal morphology of frozen-thawed spermatozoa (%) | 7.8 ± 1.5[a] | 8.3 ± 2.0[a] | 20.1 ± 5.5[a] |
| Head abnormality of frozen-thawed spermatozoa (%) | 1.5 ± 0.4[a] | 2.7 ± 0.9[a] | 6.47 ± 2.14[a] |
| Mid-piece abnormality of frozen-thawed spermatozoa (%) | 1.5 ± 0.3[a] | 1.79 ± 0.5[a] | 2.65 ± 0.49[a] |
| Tail abnormality of frozen-thawed spermatozoa (%) | 4.3 ± 1.1[a] | 3.5 ± 1.1[b] | 10.43 ± 2.89[ab] |
| Cytoplasmic droplet of frozen-thawed spermatozoa (%) | 0.6 ± 0.2[a] | 0.3 ± 0.2[a] | 0.71 ± 0.25[a] |
| Serum catalase (U/ml) | 15.9 ± 3.0[a] | 17.9 ± 4.5[a] | 20.23 ± 6.07[a] |
| Serum superoxide dismutase (U/ml) | 8.85 ± 0.5[a] | 8.5 ± 0.7[a] | 10 ± 1.07[a] |
| Serum glutathione peroxidase (U/ml) | 1.69 ± 0.5[a] | 2.60 ± 1.0[ab] | 4.86 ± 0.95[b] |

Different letters in the same line indicate a significant difference between the groups ($p < 0.05$).

Table 2. Correlation coefficients of sperm quality characteristics and serum antioxidants in 24 blood and semen samples of 8 dual-purpose simmental (Fleckvieh) Bulls.

| | S.C | Vol | Con | TMBF | PMBF | VBF | AMBF | Head | Mid_p | Tail | Cy.Dr | TMFT | PMFT | VCL | VSL | VAP | MAD | ALH | BCF | LIN | VFT | AMFT | Head | Mid_p | Tail | Cy.D | CAT | SOD | GPx |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S.C | 1 | .26 | .37* | -.06 | -.12 | .34 | .17 | -.05 | .27 | .030 | -.27 | .12 | .10 | .15 | .11 | .14 | .19 | .15 | .12 | .11 | -.24 | .03 | -.08 | .09 | .15 | .23 | .43 | -.30* | .06 |
| Vol. | | 1 | -.02 | -.49* | -.56** | .25 | -.23 | -.29 | .15 | -.29 | .12 | -.37 | -.36 | -.30 | -.31 | -.31 | -.17 | -.25 | -.25 | -.35 | -.23 | .004 | -.12 | .22 | .03 | .20 | .40 | -.00 | .36 |
| Conc. | | | 1 | .11 | .08 | -.09 | .11 | .25 | -.09 | -.12 | -.07 | .05 | .07 | .14 | .02 | .05 | .27 | .18 | .07 | .02 | -.26 | -.033 | .28 | -.24 | -.14 | .11 | -.12 | -.409 | -.05 |
| TMBF | | | | 1 | .95** | .37* | -.42 | -.28 | -.72** | -.24 | -.13 | .89** | .89** | .85** | .89** | .88** | .74** | .80** | .83** | .85** | .72** | -.575** | -.43 | -.66** | -.60** | -.22 | -.23 | .01 | -.47* |
| PMBF | | | | | 1 | .26 | -.26 | -.18 | -.65** | -.17 | -.16 | .87** | .88** | .78** | .85** | .85** | .62** | .71** | .80** | .84** | .74** | -.488* | -.30 | -.63** | -.57** | -.14 | -.22 | -.03 | -.46* |
| VBF | | | | | | 1 | -.48* | -.44 | -.39 | -.45 | -.43 | .39 | .39 | .39 | .43 | .43 | .50* | .34 | .52* | .44 | .60** | -.537* | -.66** | -.23 | -.44 | -.42 | .32 | -.03 | .14 |
| AMBF | | | | | | | 1 | .79** | .57** | .76* | -.07 | -.34 | -.36 | -.47* | -.41 | -.39 | -.54* | -.51* | -.44 | -.32 | -.51* | .616** | .69** | .45 | .54* | .48* | -.09 | -.51* | -.07 |
| Head | | | | | | | | 1 | .30 | .54* | -.14 | -.34 | -.36 | -.46* | -.42 | -.41 | -.41 | -.52* | -.38 | -.30 | -.47* | .398 | .50* | .19 | .34 | .25 | -.33 | -.43 | -.00 |
| Mid_p | | | | | | | | | 1 | .46* | .11 | -.61** | -.63** | -.53* | -.58** | -.56* | -.54* | -.52* | -.59** | -.63** | -.65** | .754** | .56* | .73** | .77** | .38 | .20 | -.12 | .12 |
| Tail | | | | | | | | | | 1 | -.01 | -.12 | -.11 | -.20 | -.12 | -.12 | -.38 | -.22 | -.24 | -.10 | -.30 | .493* | .42 | .44 | .52* | .31 | -.06 | -.35 | -.20 |
| Cy.Dr | | | | | | | | | | | 1 | -.15 | -.17 | -.05 | -.10 | -.11 | -.07 | .02 | -.17 | -.28 | -.25 | .205 | .18 | .27 | .13 | .34 | -.07 | .11 | .09 |
| TMFT | | | | | | | | | | | | 1 | .99** | .93** | .97** | .97** | .77** | .89** | .93** | .96** | .70** | -.530* | -.53* | -.55* | -.48* | -.13 | -.05 | -.13 | -.50* |
| PMFT | | | | | | | | | | | | | 1 | .93** | .97** | .97** | .75** | .88** | .93** | .96** | .72** | -.540* | -.51* | -.55* | -.52* | -.14 | -.07 | -.11 | -.48* |
| VCL | | | | | | | | | | | | | | 1 | .96** | .96** | .89** | .97** | .95** | .84** | .62** | -.446 | -.48* | -.44 | -.38 | -.10 | -.01 | .00 | -.42 |
| VSL | | | | | | | | | | | | | | | 1 | .99** | .79** | .90** | .95** | .93** | .73** | -.532* | -.53* | -.48* | -.49* | -.10 | 0.00 | -.04 | -.44 |
| VAP | | | | | | | | | | | | | | | | 1 | .81** | .92** | .95** | .92** | .70** | -.507* | -.51* | -.48* | -.46* | -.08 | -.01 | -.07 | -.46* |
| MAD | | | | | | | | | | | | | | | | | 1 | .91** | .88** | .64** | .49* | -.488* | -.50* | -.48* | -.36 | -.20 | .00 | .07 | -.26 |
| ALH | | | | | | | | | | | | | | | | | | 1 | .89** | .77** | .54* | -.423 | -.46* | -.44 | -.34 | -.12 | -.03 | .03 | -.46* |
| BCF | | | | | | | | | | | | | | | | | | | 1 | .87** | .72** | -.540* | -.57** | -.49* | -.47* | -.17 | .02 | .00 | -.34 |
| LIN | | | | | | | | | | | | | | | | | | | | 1 | .75** | -.591** | -.57* | -.54* | -.57* | -.23 | -.03 | -.16 | -.43 |
| VFT | | | | | | | | | | | | | | | | | | | | | 1 | -.653** | -.61** | -.43 | -.71** | -.46* | .08 | .18 | -.21 |
| AMFT | | | | | | | | | | | | | | | | | | | | | | 1 | .77** | .77** | .91** | .52* | -.14 | -.16 | -.04 |
| Head | | | | | | | | | | | | | | | | | | | | | | | 1 | .45 | .57** | .54* | -.29 | -.13 | -.05 |
| Mid_p | | | | | | | | | | | | | | | | | | | | | | | | 1 | .79** | .34 | .23 | -.01 | .27 |
| Tail | | | | | | | | | | | | | | | | | | | | | | | | | 1 | .44 | -.03 | -.11 | .02 |
| Cy.Dr | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | .05 | -.29 | -.14 |
| CAT | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | -.03 | .53* |
| SOD | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | .19 |
| GPx | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |

*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 catalase (CAT), serum superoxide dismutase (SOD), serum glutathione peroxides (GPx) Spearman correlation analysis, $^{*}p \leq 0.05$ and $^{**}p \leq 0.01$.



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cells contain minimal amounts of cytoplasmic antioxidant enzymes and, as a result, have limited capability for protecting the sperm tail plasma membrane from oxidative damage (28). It is also thought that sperm protection against oxidative stress heavily depends on seminal plasma antioxidant scavengers, which are themselves dependent on the entire body antioxidant capacity (29). Although some of the seminal plasma constituents are specified to it, most of its components are derived from blood (30). The determination of both semen and blood oxidative status has recently been recommended as a suitable method for the evaluation of the sperm function and fertility ability (24).

In human, it has been shown that serum TAC was significantly lower in infertile men compared to fertile ones (31). It is assumed that low serum antioxidant levels could reduce seminal plasma antioxidant capacity and could, therefore, result in increased abnormalities in the tails of spermatozoa in infertile men (31). The results of our study showed that the bulls' serum Gpx levels in the group of "<40%" were higher than those of the other two groups. Also, according to the results, GPx had a negative correlation with gross and progressive motility as well as some CASA parameters such as VAP and ALH in frozen-thawed semen. To the best of our knowledge, this is the first report about the correlation between serum GPx activity and semen quality parameters in Fleckvieh Bulls.

There are controversial reports in the literature with regard to GPx activity in seminal plasma in human. Several studies have shown that seminal activity of GPx does not significantly correlate with semen quality parameters, and that there is no difference in the levels of GPx activity between normal fertile and oligozoospermicor asthenozoospermic men (32, 33). Others have, nevertheless, reported that GPx activity in healthy men is 10 times higher than that of infertile ones (34). It is worth noting that, in the case of Holstein and Jersey bulls, there was no relationship found between GPx and SOD with regard to frozen-thawed sperm progressive motility (20).

In our study, no significant differences were found in the activity of SOD in three different groups under study. Several human studies have also reported that there were no statistically significant differences between the semen SOD levels of asthenozoospermic or oligozoospermic patients and those of normal fertile men (32, 35). Furthermore, no correlations between SOD and sperm motility and semen concentration have ever been found (36). On the other hand, it has been reported that the seminal activity of SOD in oligozoospermic and infertile patients is more than that of normozoospermic and fertile men (37, 38). Although there were no significant differences in serum SOD in the three PMFT groups in our study, the levels of this enzyme were slightly higher in "<40%" group than the other two groups.

Contrary to our findings, Shamsi et al. reported that there was a positive correlation between serum SOD and CAT with sperm count and a negative correlation of serum CAT with abnormal morphology and the percentage of dead spermatozoa in both fertile and infertile men. On the other hand, they also reported that there was a significant difference between the levels of antioxidants in the blood of patients compared with that of the control group (24). The contradiction between our findings and those of Shamsi's study may be due to the difference in the nature of the experimental model (bulls versus men). From a different angle, it could be attributed to the fact that they worked on both fertile and infertile men, but our study was conducted just on normal healthy Bulls, which were divided into three groups based on sperm progressive motility after thawing.

It is worth mentioning that we found no correlation between serum CAT activities and semen quality parameters in Fleckvieh Bulls. To the best of our knowledge, this is the first report on the relationship between Fleckvieh Bulls' serum CAT and semen quality parameters. Although a positive correlation between ROS production and catalase activity in human seminal plasma has been reported (15), Zini et al. (2002) claimed that there was no correlation between catalase activity and semen volume (39).

Benedetti et al. (2012) also demonstrated that the blood plasma total antioxidant capacity (TAC) levels of infertile men were significantly lower than those of normal fertile men and that there was a strong positive correlation between blood and seminal plasma antioxidants (21). These findings had already been proven by semen antioxidant analysis, indicating that seminal excessive ROS could disrupt both motility (40) and the normal morphology of spermatozoa (30). Although these correlations were not observed in our

study, it is important to note that the current study was conducted on normal healthy sires with a similar reproductive performance and the conception rates of inseminated cows with their frozen thawed semen. On account of the fact that a sub-fertile bull is culled very soon from the herd, the chance of performing the research in this case is practically low. But in line with our study, Eroglu et al. (2014) found no significant differences between serum TAC in normal fertile men and those with idiopathic infertility. Moreover, there was no relationship between serum and semen levels of TAC in their research as well as serum levels of TAC and sperm parameters (41). Also, no significant differences were observed in blood serum TAC concentrations between fertile and infertile men in another study (42).

## Conclusion

Our study revealed considerable information about serum antioxidant enzymes and their differences in three PMFT groups as well as their correlations with semen quality parameters. We found that the Fleckviehbulls' serum Gpx levels were higher in the low progressive motility of frozen-thawed semen group than the moderate and good progressive motility groups. We also found that serum GPx had a negative correlation with gross and progressive motility as well as with VAP and ALH in frozen-thawed semen. In conclusion, the results of our study demonstrated that utilizing serum antioxidant enzymes measurement only is not sufficient for evaluating the breeding soundness or semen quality of the bulls. It is imperative that both the serum and seminal plasma oxidants as well as antioxidant agents be investigated so as to better understand the relationships between them and the quality of spermatozoa.

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## Conflicts of Interest

The authors have no conflicts of interest to declare.

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