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Trace Minerals and Antioxidant Profile of Normo, Oligo and Ashthenozoospermic Crossbred Frieswal Bulls

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ABSTRACT

The study was undertaken to assess the level of trace minerals, antioxidants and semen quality in normo, oligo and ashthenozoospermic breeding frieswal bulls. Semen samples were collected from breeding bulls (47) maintained at bull rearing unit of ICAR-Central Institute for Research on Cattle, Meerut. Trace minerals (Zn, Cu, Co and Fe) and oxidative stress parameters (SOD, Catalase and MDA) were determined in semen samples of breeding bulls. Sperm motility and concentration were measured in fresh ejaculates. The mean zinc and copper concentrations were significantly lower in blood and semen ($p<0.05$) in oligozoospermic and ashthenozoospermic bulls as compared to normozoospermic bulls. Cobalt and iron concentrations did not vary significantly in different group of breeding bulls. Significantly higher MDA and low SOD and catalase activities were present in seminal plasma of oligo and ashthenozoospermic bulls as compared to normozoospermic bulls. The MDA had significant negative correlation with motility ($p<0.05$, $r = -0.303$) and sperm concentration ($p<0.001$, $r = -0.473$) while SOD and Catalase had significant positive correlation with initial sperm motility ($p<0.05$, $r = 0.273$; $p<0.001$, $r = 0.435$) and sperm concentration ($p<0.001$, $r = 0.575$; $p<0.001$, $r = 0.631$). The study concluded that oligozoospermia and asthenozoospermia are associated with an increased MDA concentration and decreased activities of SOD and Catalase in the seminal plasma of breeding bulls. Present findings suggested that determination of antioxidant status of semen during infertility investigation seems to be useful.

HIGHLIGHTS

- Oligo and ashthenozoospermia are associated with increased oxidative stress in bulls.
- Estimation of semen antioxidants may be useful during fertility investigation in bulls.

Keywords: Bull, Oxidative stress, Semen, Zinc

Reactive oxygen species (ROS) are produced naturally in male reproductive system and play important role in fertility. Under physiological conditions, spermatozoa produces small amount of ROS, which are needed for capacitation, acrosome reaction and fertilization (Gil-Guzman *et al.,* 2001). Normally, equilibrium exists between ROS production and antioxidant scavenging activities in the male reproductive tract. Oxidative stress results from the imbalance between ROS and antioxidants and it is one of major causes of male infertility. High concentration of reactive oxygen species was detected in the semen of infertile men (Mehrotra *et al.,* 2013). Excessive amount of ROS produced by leukocytes and immature spermatozoa cause damage to the normal spermatozoa by inducing lipid peroxidation and DNA damage. The oxidative stress (OS) adversely affects sperm function by altering membrane fluidity, permeability and impairs sperm functional competence. Seminal plasma is considered as an important source of antioxidants which might be useful in the prediction of sperm fertilizing

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potential. SOD and catalase are important anti-oxidant enzymes scavenging both intracellular and extracellular free radicals and prevent the lipid per-oxidation of plasma membrane. Zinc (Zn) and Copper (Cu) are important antioxidant minerals which act directly and as a cofactor of Cu/Zn SOD against ROS (Abolbashari *et al.,* 2019). There are evidences that these trace minerals have important role in physiologic functions of sperm and their reduced levels result in low semen quality (Kumar *et al.,* 2006). The role of ROS and antioxidants in male fertility in human being is well established (Atig *et al.,* 2012; Mehrotra *et al.,* 2013; Hashemi *et al.,* 2018) however the information on the role of oxidative stress and antioxidants on the fertility of breeding bulls is meagre in the scientific literature. The present study was undertaken to assess the level of trace minerals, antioxidants and semen quality in normo, oligo and ashthenozoospermic breeding frieswal bulls.

MATERIALS AND METHODS

Sample collection and processing

Semen samples were collected from crossbred (Holstein Friesian X Sahiwal) breeding bulls (47) maintained at bull rearing unit of ICAR-Central Institute for Research on Cattle, Meerut. The semen samples from each bull were collected twice in a week using an artificial vagina. The bulls were maintained on standard feeding schedule and management conditions under a loose housing system in individual pens. 2 ml of neat semen was collected for trace mineral analysis. Seminal plasma was collected for measurement of oxidative stress parameters. The semen samples were centrifuged (10 min, 8000 × *g*, 4°C) and seminal plasma was separated and transferred into 1.5mL tubes and kept frozen (−20°C) until analysis.

Semen quality analysis

The fresh ejaculates were evaluated for volume, sperm concentration and initial motility as per standard procedures. The concentration of spermatozoa was measured with Accucell bovine photometer (IMV, France). The sperm motility was assessed using an Olympus BX40 phase contrast microscope (Olympus, Tokyo, Japan).

Trace mineral analysis

Blood and semen samples were digested with nitric and perchloric acid mixture. The trace elements zinc, copper, cobalt and iron concentrations were estimated using atomic absorption spectrophotometer (GBC Scientific) at the wave length of 213.9, 324.7, 240.7, 248.3 with 7, 6, 7, 5 mA current, respectively. The standards procured (Sisco Research Laboratory, Mumbai, India) for each element were used to calibrate the equipment.

Measurements of oxidative stress parameters

Oxidative stress parameters were measured colorimetrically based on reaction of the target substance and a subsequent UV/VIS spectrophotometric detection at certain wavelength. The lipid peroxidation (LPO) was determined by measuring malondialdehyde (MDA) concentration which is an end product of lipid peroxidation. Superoxide dismutase (SOD) and Catalase activities were estimated as per the method of Marklund (1976) and Bergmeyer and Grabl method (1983) respectively.

Statistical analysis

The data were analyzed using analysis of variance (ANOVA) to find out the statistical difference between the mean values of various parameters of different group of animals using SPSS 16 (SPSS Incorporated Chicago, IL, USA). The bulls were categorized into three groups as oligozoospermic (sperm concentration < 500 million/ml, sperm motility- > 70%), ashthenozoospermic (sperm motility- < 40%, sperm concentration > 500 million/ml) and Normozoospermic (sperm concentration > 500 million/ml, sperm motility- > 70%) A parametric (Pearson) correlation between oxidative stress markers and semen quality parameters was analyzed using standard statistical methods (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Trace mineral profile

The mean Zn and Cu concentrations were significantly lower ($p<0.05$) in oligozoospermic and ashthenozoospermic bulls as compared to normozoospermic bulls. As regards Co and Fe concentrations, no significant difference was observed among different category of bulls (Table 1). Zn and Cu concentrations were found significantly($p<0.05$)

lower in seminal plasma of oligozoospermic bulls as compared to normozoospermic bulls however no significant difference was observed in seminal Zn and Cu concentrations of ashtheno and oligozoospermic bulls (Fig. 1). Seminal plasma Co and Fe concentrations did not vary significantly in different group of breeding bulls.

Table 1: Blood trace mineral profile of crossbred Frieswal bulls

Parameters	Oligozoo-spermic bulls (n=11)	Asthenozoosper-mic bulls (n=17)	Normozoosper-mic bulls (n=19)
Zn(µg/ml)	1.85± 0.13^{a}	1.93± 0.07^{ac}	2.34± 0.13^{b}
Cu(µg/ml)	1.35± 0.11^{a}	1.26± 0.06^{ac}	1.71± 0.11^{b}
Co(µg/ml)	2.78± 0.27^{a}	2.62± 0.16^{a}	3.11± 0.20^{a}
Fe(µg/ml)	133.20± 12.22^{a}	144.93± 8.91^{a}	145.66± 7.89^{a}

Values with different superscripts in a row differ significantly ($p<0.05$).

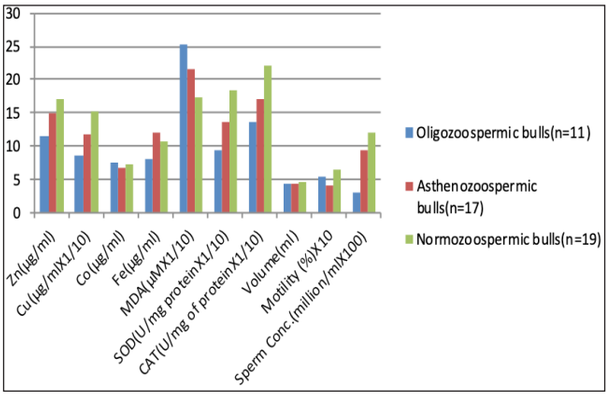

Fig. 1: Semen trace minerals and antioxidant profile and semen quality parameters of crossbred Frieswal bulls

Zn and Cu are important trace elements and act as cofactors in proteins, hormones and numerous enzymatic reactions. Activity of some antioxidant enzymes is enhanced by these minerals to counteract the oxidative stress. The catalytic reaction of Cu/Zn SOD is performed by the cyclic reduction and oxidation of the copper ion (Abolbashari *et al.,* 2019). They are structural ions of SOD and reduce oxidative stress by induction of metallothionein synthesis (Li *et al*., 2006). Because of their pivotal role in the redox mechanisms, their imbalanced status may lead to an increased susceptibility to oxidative damage (Abolbashari *et al.,* 2019). Our study showed significantly low concentrations of seminal zinc and copper concentrations in oligo and ashthenozoospermic bulls as compared to the normozoospermic bulls. Increased MDA and low SOD acitvity in the seminal plasma of oligo and ashthenozoospermic bulls of present study may explain the decrease of the effective concentration of Zn and Cu, increasing the harmful effects of ROS to sperm cells consequently leading to abnormal semen quality parameters. Studies carried out in human beings mentioned that decrease in Zn and Cu concentration leads to an increase in oxidation of DNA, proteins, and lipids causing reduced semen quality (Hashemi *et al.,* 2018). High concentration of seminal Zn was found to be associated with enhanced sperm count and sperm motility (Marzony and Chaichi, 2009). Zhao and Xiong *et al.* (2005) also observed a positive relationship between poor production of sperm and poor sperm motility with a lower seminal content of Zn. These findings supported the contribution of seminal Zn and Cu to maintain the physiologic functions of sperm.

Correlation between oxidative markers and semen quality

The MDA had significant negative correlation with motility ($p<0.05$, r = -0.303) and sperm concentration ($p<0.001$, r = -0.473) while SOD and Catalase had significant positive correlation with sperm motility ($p<0.05$, r = 0.273; $p<0.001$, r = 0.435) and sperm concentration ($p<0.001$, r = 0.575; $p<0.001$, r = 0.631).

Table 2: Correlation between oxidative markers and semen quality parameters in Frieswal bulls

	Volume	Motility	Sperm Conc.
MDA	-.159	-.303*	-.473**
SOD	.031	.273*	.575**
Catalase	.153	.435**	.631**

* P<0.05, ** p<0.001.

Oxidative stress markers and semen quality

Significantly higher MDA and low SOD and catalase activities were present in seminal plasma of oligo and ashthenozoospermic bulls as compared to normozoospermic bulls. It is well-known that spermatozoa

themselves contain negligible levels of antioxidants, and thus protected against oxidative stress by antioxidants present in seminal plasma (Gil-Guzman *et al.,* 2001). Seminal plasma contains several antioxidants comprising enzymatic and non-enzymatic systems that play an important role in the normal function of sperm. Studies carried out in past suggested that decreased levels of antioxidants in seminal plasma might be a potential cause of infertility (Mehrotra *et al.,* 2013). Impaired antioxidant status was observed in seminal plasma of oligo and ashtenozoospermic bulls in present study which may be an important risk factor of oxidative damage making the sperm susceptible to lipid per-oxidation.

Increased MDA levels in seminal plasma of oligo and ashthenozoospermic bulls of present study explained the pathologic lipid per-oxidation effects on spermatozoa membrane and consequently on sperm motility and concentration. MDA production reflects the peroxidation of membrane polyunsaturated phospholipids (Colagar *et al.,* 2013). Lipid peroxidative degradation of sperm membrane may be responsible for abnormal sperm motility and concentration. Another study showed that decreasing seminal plasma antioxidants status especially total antioxidant capacity might have significant effect on impaired sperm function. Higher level of ROS was correlated with a decreased number of motile sperm while greater sperm motility was observed in samples with lesser amount of ROS (Colagar *et al.,* 2013).

The findings of the present study are in accordance with previously published observations of the other authors in studies conducted on human beings (Ben Abdallah *et al.,* 2009; Hashemi *et al.,* 2018). Ben Abdallah *et al.* (2009) reported that MDA content was elevated in oligozoospermic and asthenozoospermic groups. Murawski *et al.* (2007) also showed significant and positive correlations between seminal SOD activity and sperm concentration and motility, which are regarded as the important criteria for normal fertilizing ability of the spermatozoa. The increased activities of seminal SOD and catalase in normozoospermic bulls of present study and the noted associations between these enzymes and sperm quality proved their ability to remove free radicals and important biological role in controlling the fertilizing potential of spermatozoa.

CONCLUSION

The study concluded that oligozoospermia and asthenozoospermia are associated with increased MDA concentration, decreased concentration of Zn and Cu and reduced activities of SOD and Catalase in the seminal plasma of breeding bulls. Present findings indicated usefulness of routine determination of antioxidant status of semen during infertility investigation.

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