Review Report

Role of trace minerals in bull reproductive physiology and semen quality*

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Abstract

Testicular dysfunction is often associated with an imbalance in antioxidant/oxidant homeostasis, leading to negative effects of oxidative stress on germ cell proliferation, steroidogenesis, and sperm function. Trace minerals (TM) are involved in basic homeostatic and enzymatic processes like free radical detoxification, cellular respiration, carbohydrates, lipids, nucleic acids, synthesis and metabolism of proteins, and stabilization of membranes and DNA. Dietary source of TM is necessary to support these processes. Supplementing cattle with TM is nowadays a common practice to help support growth, reproduction, and immunity. This review provides information on roles of TM in bulls testicular and sperm functions, and effects of TM mineral supplementation on bull reproductive parameters.

Keywords: Cattle, sperm, zinc, selenium, copper, manganese

Introduction

Every year, 30% of breeding bulls should be culled due to poor semen quality.[1] The most common cause of declining semen quality is testicular degeneration that is often associated with an imbalance in antioxidant/oxidant homeostasis; this leads to increased concentrations of reactive oxygen species (ROS) and oxidative stress that induces germ cell apoptosis and decreases germ cell proliferation.[2–4] In young bulls, sexual immaturity is also often associated with poor semen quality.

Trace minerals, e.g. selenium (Se), zinc (Zn), copper (Cu), and manganese (Mn), are cofactors for antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPX). Thus, trace minerals have an important role in preventing oxidative damage on cell membranes and nucleic acids in germ cells, and supporting spermatogenesis and steroidogenesis.[5,6] This review provides information on trace minerals' roles in bulls testicular and sperm function, and the effects of trace mineral supplementation on bull reproductive parameters.

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Oxidant-antioxidant balance in male reproductive tract and gametes

Oxygen-derived free radicals are molecules that contain unpaired electrons in their outermost orbits. These molecules are highly reactive and have a very short half-life since they tend to stabilize rapidly by yielding or accepting electrons from other molecules. Reactive oxygen species (ROS) include superoxide anions (O_2-), hydrogen peroxide (H_2O_2), proxyl ($\cdot ROO$) and hydroxyl ($\cdot OH$). Low concentrations of endogenous ROS are involved in spermatogenesis, sperm modification within the epididymis, and sperm function leading to fertilization.[7] Within testis, ROS can act as second messengers regulating cell-signaling pathways involved in spermatogonia self-renewal and proliferation.[7] They are also involved in regulating apoptosis that is fundamental to maintain an adequate germ cell to Sertoli cell ratio, and to eliminate defective sperm.[8] In epididymis, ROS participate in the formation of sperm nuclear disulphide bridges required for chromatin condensation, and in phosphorylation and dephosphorylation of proteins in the fibrous sheath of the flagellum, activating progressive motility.[7] In female tract, ROS are necessary for sperm capacitation, hyperactivation, acrosome reaction, and fertilization.

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Sperm-specific physiologic ROS production occurs mainly in the mitochondrial electron transport system.[9] Bull sperm contain ~ 72 mitochondria located within 3 spirals around midpiece.[10] Oxidative phosphorylation in these mitochondria is essential for sperm motility, but generates hydroxyl radicals, superoxide anion, H_2O_2, and nitric oxide.[8] Intracellular ROS are also produced through enzymes such as β-nicotinamide adenine dinucleotide phosphate (NADPH) oxidases in the cell membrane, and cytochrome P450-dependent oxygenases in the mitochondria and endoplasmic reticulum.[9] Although lower concentrations of ROS have a physiological role in male fertility, higher concentrations of ROS overwhelm the endogenous antioxidant system and cause oxidative stress.

Endogenous sources of pathologically increased ROS production in the ejaculate include immature germ cells, leucocytes, dead or abnormal sperm, and epithelial cells.[9] Immature germ cells in the ejaculate contain excess cytoplasm. Additional cytoplasm contains glucose-6-phosphate dehydrogenase enzyme that controls intracellular NADPH production. The NADPH fuels the production of ROS via NADPH oxidase.[11] Leukocytes contain peroxidase and can produce 1,000 times more ROS than sperm. Activation of the myeloperoxidase system from neutrophils and macrophages leads to respiratory burst (the increase in cell metabolism and oxygen consumption during phagocytosis, coupled with the release of reactive oxygen species [ROS], and increased ROS products). Increased ROS production in the ejaculate is present in cases of inflammation, heat stress or pollutants such as phthalates and heavy metals.[11] A low antioxidant activity in seminal plasma can also lead to imbalance and accumulation of seminal ROS.[7]

Sperm damage by ROS results in alterations in nuclear and mitochondrial DNA, plasma membrane lipid peroxidation, loss of ATP, and axonemal injury.[11] Reactive oxygen species can also affect Leydig cells and decrease testosterone production.[11] Oxidation of DNA bases occurs more commonly in areas with less protamine. Guanine base in DNA is oxidized by free radicals and converted to 8-hydroxyguanine (8-OHG). Sperm have 8-oxoguanine DNA glycosylase enzymes that can remove 8OHdG. However, sperm lack the downstream components of the repair pathways and are unable to repair the DNA at the sites of the base excisions. Result is fragmentation of DNA, altered gene expression, and activation of apoptosis and autophagy.[7,11] Epididymal or seminal ROS can oxidize free thiol groups, inhibiting formation of disulphide bridges in sperm proteins within the flagellum that causes sperm morphological defects, such as bent tails.[7,9] Oxidation of sulfhydryl groups decreases the phosphorylation of axonemal proteins, decreasing sperm motility.[8] In addition, sperm plasma membrane is rich in polyunsaturated fatty acids with double bonds separated by methylene groups that render them particularly vulnerable to oxidation. Lipid peroxidation generates lipid radicals that propagate oxidation across plasma membrane; this process leads to generation of by-products such as malondialdehyde (MDA), 4hydroxynonenal (4HNE) and acrolein. Malondialdehyde is mutagenic, whereas 4NHE and acrolein have a direct genotoxic effect on DNA, and increase mitochondrial ROS production.[8] Membrane lipid peroxidation reduces plasma membrane integrity and fluidity, with alterations in ion transport, receptors and enzymes; this affects membrane potential, sperm motility, viability and capacitation, and induces a premature acrosome reaction.[7,9] Higher concentrations of ROS can also alter mitochondrial membrane, resulting in caspase activation and apoptosis.[11]

To keep the amount of ROS under control, sperm have endogenous intracellular antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase, and catalase (CAT). Seminal plasma is also rich in enzymatic and nonenzymatic antioxidants.[8,12] In addition, compartmentalization of mitochondria in midpiece provides a physical separation from nuclear DNA, thus preventing possible damage in case of ROS overproduction.[8]

Superoxide dismutase spontaneously dismutates O_2– to form oxygen (O_2) and H_2O_2. Superoxide dismutase is the most important antioxidant enzyme in bovine sperm and seminal plasma, and controls mitochondrial and plasma membrane O_2- production. Intracellular SOD-1 or CuZnSOD, contains copper and zinc in its active center, and is present in sperm cytoplasm. Intracellular SOD-2 or MnSOD is located in the mitochondrial matrix, and contains manganese in the active center.[13,14] Extracellular form of SOD or SOD-3 also contains zinc and copper in its active center and originates in prostate.[12–13] Due to SOD activity, H_2O_2 is produced that is eliminated by CAT or GPX. Glutathione peroxidase in sperm and semen has various isoforms, with most of them containing selenium. The GPX4 is located on the postacrosomal and apical head region, and within sperm nucleus, whereas GPX5 is expressed in the accessory sex glands, testis, and epididymal epithelium, and is present in seminal plasma.[12] Glutathione reductase is present in the flagellum and seminal plasma. Catalase catalyzes the decomposition of H_2O_2 into O_2 and H_2O. Bull sperm may acquire CAT from extracellular milieu, and this membrane-bound CAT may have an important role in protecting these cells during maturation and transport in the female reproductive tract. Although some investigators have not detected CAT activity in bull sperm, CAT activity remains higher in bovine seminal plasma that originate from prostate.[12] Peroxiredoxins are nonselenium enzymes capable of scavenging H_2O_2, organic hydroperoxides, and ONOO–, and are widely distributed in all compartments of bovine sperm.[14] Nonenzymatic antioxidants in bovine seminal plasma include glutathione, melatonin, ergothionine, carnitines, ascorbic acid, α-tocopherol, pyruvate, taurine/hypotaurine, retinol, and urate.[12] Oxidative damage can also occur due to a deficiency in enzymatic antioxidants, generated by a genetic mutation, incorrect posttranslational modification, posttranslational deactivation, nutritional deficiency, disease states, or toxicities.[12]

Nutritional sources of Zn, Cu, Mn, and Se are required for sufficient SOD and GPX production and activity.[12] Deficiencies in these trace minerals can result in inadequate production of these enzymes because they are integral parts of their active core. Sperm from Zn and Cu deficient animals have reduced longevity, viability, acrosome integrity, and motility.[12] Similarly, GPX deficiency has been associated with Se deficiency, and these sperm have incomplete DNA condensation, DNA fragmentation, and abnormal morphology.[12]

Role of copper in male reproductive function

Copper is mainly in the highly soluble cupric (Cu^{2+}) oxidized state. Dietary Cu absorption is reduced in the rumen due to interactions with other minerals, mostly Molybdenum and Zn. Organic Cu has better absorption than the inorganic form. Absorbed dietary Cu is bound to albumin and is carried to liver. Small amount of Cu is excreted in the bile, whereas the majority binds to ceruloplasmin and is released into bloodstream. Within testes, ~ 80% of Cu is bound to

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ceruloplasmin in Sertoli cells. The remaining Cu is bound to metallothioneins in Sertoli and spermatogenic cells.[15] Copper is an essential element since it participates in electron transfer reactions within a large number of proteins involved in basic homeostatic processes, such as cellular respiration (cytochrome c oxidase), free radical detoxification (SOD), iron transport (ceruloplasmin), neuropeptide synthesis (peptidylglycine α-amidating monooxygenase), connective tissue formation (lysyl oxidase), and pigment synthesis (tyrosinase). Dietary sources of Cu are needed for proper synthesis and function of these enzymes.

Sperm from Cu-deficient ruminants and rodents have reduced concentrations, longevity, viability, acrosome integrity, and motility as a result of reduced SOD activity and oxidative stress.[12,15] In addition, Cu is a cofactor in the mitochondrial cytochrome c oxidase, and is required for aerobic ATP production by sperm. Copper is also a cofactor for the enzymes involved in prostaglandin and steroid synthesis.[15] Daily dietary supplementation in nondeficient bucks with 12.5 or 25 mg/kg of Cu proteinate increased sperm concentration, motility, membrane integrity, acrosome integrity, and cryosurvival.[16–19] This was associated with increased expression and activity of SOD-1, CAT, and GPX4 in sperm and decreased lipid peroxidation.[17,18] Supplementation of bucks with Cu also increased plasma testosterone concentrations, sexual behavior, scrotal circumference, and advanced puberty ~ 30 days.[16,18,20]

Although Cu is essential for spermatogenesis and sperm function, hypercuprosis can be toxic, especially in sheep. Highly reactive 'free' cuprous ions (Cu+) react with H_2O_2, generating hydroxyl radicals via Fenton and Haber-Weiss reactions.[15] Copper can also bind directly to free thiols of cysteines, leading to oxidation and crosslinks between proteins, thus inactivating enzymes or impairing structural proteins. Dietary supplementation of higher concentrations of Cu to rodents and pigs decreased spermatogenesis, testicular weight, SOD, and GPX expression and increased germ cell apoptosis and autophagy, and testicular ROS production and lipid peroxidation.[15,21–23] Sperm count, motility, viability, and morphology, as well as testosterone production, were negatively affected with hypercuprosis.[21,23]

Role of zinc in male reproduction

Zinc can be supplemented in inorganic forms, such as sulphate ($ZnSO_4$) or oxide (ZnO), or in organic forms, such as propionate or proteinate. Bioavailability of organic forms is higher than that of inorganic Zn. Tryptophan content in the diet is important for absorption of dietary Zn. Tryptophan is metabolized to picolinic acid by pancreatic exocrine cells of that is secreted into the intestinal lumen, where it combines with Zn, with the picolinic acid–Zn complex absorbed by the intestine.[24]

Zinc is involved in several enzymatic reactions, and is closely associated with carbohydrate, lipid, nucleic acid, and protein metabolism. Apart from its involvement in the antioxidant system, Zn directly stabilizes membranes, lysosomes, ribosomes, RNA and DNA; it is also involved in DNA replication, transcription, and translation into proteins.[16,18] Within testis, Zn is critical for germ cell proliferation, spermiogenesis and histone substitution by protamine, and regulates testosterone synthesis.[25] In post-testicular sperm, Zn is essential for production and function of numerous enzymes that are important in normal sperm function and prevention of sperm damage, and has a regulatory role in sperm capacitation and acrosome reaction. Zinc controls sperm motility by controlling energy utilization through Zn-containing enzymes like sorbitol dehydrogenase and lactate dehydrogenase.[6] In addition, proteins in the outer dense fiber (ODF) incorporate and release Zn depending on the external environment.[26] Zinc in chromatin and the ODF forms salt bridges with thiols, thus preventing oxidation of thiols. Prostatic fluid is rich in Zn that likely contributes to protecting sperm chromatin and ODF from shear forces at ejaculation.[26,27] After ejaculation, Zn from chromatin and ODF is rapidly lost, probably after the contribution of chelating agents from vesicular glands. Removal of Zn from chromatin and ODF allows for formation of disulfide bridges and stiffening of these structures. Stiffening of the ODF results in small flagellar wave amplitudes characteristic of progressive motility. In contrast, incorporation of Zn causes softening, leading to hyperactivated motility. Therefore, chelation of Zn has a role in inducing progressive motility and preventing premature hyperactivation. Zinc is also important in preventing sperm DNA damage since it inhibits DNAase.[26,27]

Zinc deficiency resulted in spermatogenic arrest, germ cell apoptosis and degenerative changes in seminiferous tubules, extracellular matrix, blood-testis barrier, and Leydig cells.[28] Testicular changes resulted in decreased sperm concentration and motility, with an increase in sperm acrosome, and head and mitochondrial abnormalities.[28] Supplementation of Zn proteinate in nondeficient bucks increased sperm concentration, motility, and acrosome and plasma membrane integrity.[16,18–20] This was associated with decreased oxidation of sperm lipids and proteins due to increased total antioxidant capacity and activity of seminal plasma SOD, CAT, and GPX.[16,19,29] However, the beneficial effects of supplementation were only observed when Zn was supplemented at low (20 mg/kg of dry matter-DM) or medium (40 mg/kg DM) levels. Feeding a higher level of Zn proteinate (60 mg/kg DM) had detrimental effects on antioxidant capacity, oxidative stress and sperm parameters.[16,18–20,29] Feeding 50 - 200 mg of $ZnSO_4$ to goats also improved semen volume, sperm motility, and SOD and GPX activity in seminal plasma.[30] In 2-year old normospermic beef bulls, feeding organic or inorganic forms of Zn for 6 months increased sperm count, motility, viability, acrosome integrity, membrane functionality, and mucous penetration.[6] Zn supplementation also increased blood testosterone concentrations.[6]

Role of manganese in male reproduction

Manganese is a component of several enzymes, particularly MnSOD, and it scavenges free oxygen radicals and stabilizes cell membrane.[31] Manganese is also a potent stimulator of sperm motility through the stimulation of adenylate cyclase activity. Supplementation with lower concentrations of Mn in rats increased LH, FSH, and testosterone production, increased daily sperm production and efficiency of spermatogenesis, and accelerated puberty.[32] Lower concentrations of Mn also prevented negative effects of formalin on testicular weight, and sperm motility, viability, morphology, and count in mice.[31] Oral supplementation in mature dairy bulls with $MnSO_4$ (30 ppm), $CoClo_2$ (0.22 ppm) and $CrCl_2$ (2 ppm) for 120 days increased sperm motility, viability, membrane functionality, and acrosome integrity in fresh and frozen semen. This was associated with increased SOD activity in blood and decreased lipid peroxidation in semen.[33]

However, high doses of Mn can be detrimental for fertility due to a decrease in sperm concentration, motility and viability.[34] These changes were associated with oxidative stress within testis. Even though the activity of SOD increased, higher Mn concentrations decreased CAT activity. Increased SOD activity can eliminate superoxide radicals by dismuting them to H_2O_2; however, reduction in CAT activity affected the detoxification of H_2O_2.[34] In bulls, Mn supplementation at ≥ 1,300 mg/kg of mineral mix reduced sperm membrane and acrosome integrity, and even the small amount of Mn contained in the control diet (540 mg/kg of mineral mix) had a negative effect on sperm mitochondrial membrane potential.[35] Thus, supplementation effect with Mn on fertility is not entirely clear.

Role of selenium in male reproduction

Selenium is a component of several enzymes and has a role in many biological processes including antioxidant defense, fertility, immunity, thyroid metabolism, endocrine function, carcinogenesis, and muscle function. Inorganic Se is present in 3 oxidation states: selenite (Se^{4+}), selenate (Se^{6+}), and selenide (Se^{2-}). Plants obtain Se^{4+}or Se^{6+} from soil to synthesize Se-containing amino acids. Sodium selenite is the inorganic form most commonly used in animal diets, whereas organic Se is supplemented as selenized yeast or selenomethionine. Organic Se was more effective at improving sperm morphology and viability in buffaloes than inorganic Se.[36]

Dietary Se is transported to testis by plasma selenoprotein P. In testis, Sertoli cells Se uptake is by the apolipoprotein E receptor-2 (ApoER2), and is utilized for the synthesis of selenoproteins. The main testicular selenoprotein is GPX4, expressed primarily in sperm. The GPX4 is present in 3 isoforms: mitochondrial, nuclear, and cytosolic, each carrying out separate functions but encoded by a single gene. Mitochondrial GPX4 is involved in enzymatic defense against oxidative damage to mitochondria, and becomes active in spermatocytes and spermatids. Mitochondrial GPx4 also maintains the form and structure of the mitochondrial capsule by constructing cross-linkages with itself and other proteins. Nuclear GPx4 stabilizes condensed chromatin during sperm maturation. Lastly, both GPX1 and cytosolic GPX4 are expressed in the epididymal epithelium.[37]

Fate of Se after injection was studied in bulls using radioactive ^{75}Se. Injection of ^{75}Se into bulls resulted in an increase in ^{75}Se concentrations in the blood within 6 hours, followed by a decline within 48 hours.[38,39] Thereafter, ^{75}Se distributed primarily to the liver, kidneys, and reproductive tract, main storage sites for this element. Concentrations of ^{75}Se in seminal plasma exceeded the concentrations in blood due to accumulation and secretion from prostate and seminal vesicles. ^{75}Se in seminal plasma reached peak concentrations 5 days after injection and declined after 12 days. Seminal plasma was the only source of ^{75}Se in semen during the first 2 weeks after injection.[38,39] Starting from 12 to 15 days after injection, ^{75}Se appeared in ejaculated sperm. Sperm ^{75}Se accumulation peaked ~ on day 20 and, after plateauing, declined rapidly after 40 days.[38,39] Timing of ^{75}Se appearance in sperm is due to the fact that Se is incorporated by the spermatid but not during passage through epididymis.[38]

Effect of Se supplementation on reproductive parameters were studied in Se-deficient bulls, buffaloes, boars, rams, and mice.[36,40–43] Although supplementation increased concentrations of Se and GPX activity in reproductive tissues and semen, effect on semen quality was variable. Effects ranged from no changes[40,42] to increased ejaculate volume, sperm concentration, motility, morphology, and viability.[36,41,43] Se supplementation also increased blood testosterone concentrations, and the number of round and elongated spermatids.[36,40,43] Effects of supplementing nondeficient males with Se are less well characterized. Although providing an excessive amount of Se to deficient mice improved semen quality compared to control animals, it did not provide any additional benefits of feeding above an adequate dose.[43]

Role of iron in male reproduction

Iron (Fe) is essential for oxygen transport and exchange, intracellular enzyme function and mitochondrial electron transport chain, and has a dual role in regulating redox reactions. Iron is the bioactive center of catalase and superoxide dismutase. Within testis, Fe is essential to support mitosis and spermatogenesis since it is needed for DNA synthesis, protein synthesis, cellular respiration, proliferation, and differentiation.[44] Circulating Fe is transported through the blood-testis barrier by Sertoli cells bound to testicular transferrin to be taken up by germ cells. Mitochondria of spermatids are also engulfed by Sertoli cells that recycle Fe back to primary spermatocytes in the basal compartment. Thus, Sertoli cells modulate Fe homeostasis within the seminiferous tubule, protecting germ cells from iron fluctuations.[44] Ferritin is an important source of iron during sperm development and provides an additional layer of protection for testicular tissues. Peritubular myoid cells and Sertoli cells are the main locations of testicular cytosolic ferritin storage, whereas mitochondrial ferritin is mainly expressed in germ cells and Leydig cells. Mitochondrial ferritin insulates excessive iron, making it less susceptible to Fenton and Haber-Weiss reactions and protecting mitochondria from oxidative damage.[44]

Iron deficiency decreased testosterone synthesis, and glutathione peroxidase and catalase activity, whereas it increased cleaved-caspase 8 and caspase 3 expression in Fe-deficient rats.[45] Anemia caused by severe Fe deficiency creates a hypoxic environment for testis, impairing spermatogenesis.[44] Men with sickle cell disease had impaired sperm production, motility, and morphology associated with testicular failure and lower testosterone concentrations.[15]

Iron aids in sperm motility and energy metabolism. Concentrations of Fe in bovine seminal plasma were positively correlated with sperm motility, membrane integrity and DNA integrity.[46] Supplementation of the medium with lower concentrations of Fe improved motility of bovine sperm.[47] However, supplementation with higher concentrations were toxic and increased ROS production.[47] Other studies have reported increased intracellular Fe concentrations in sperm from bulls with low fertility, associated with DNA damage and decreased motility.[48] This dual action is due to excess Fe ability to aggravate the generation of ROS via the Fenton and Haber–Weiss reactions. Thus, iron homeostasis is critical for testicular and sperm function. Iron overload was associated with hypogonadism in boars, bulls, and men.[15] Exposure of sperm to higher Fe concentrations reduced motility and viability, decreased mitochondrial membrane potential, increased intracellular ROS generation, and impaired sperm fertilizing ability.[15]

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Associations between the concentrations of trace minerals in bull semen and semen quality

Most abundant TMs in semen samples from fertile normospermic bulls are Mg, Zn, Se, and Fe.[49] However, the literature reports contradictory findings regarding the association between concentrations of TMs in semen samples and semen quality. In bovine seminal plasma, the concentrations of Zn and Fe were positively correlated with sperm motility, viability, acrosome integrity and DNA integrity in fresh and frozen semen.[46,50,51] Some authors reported no correlation between Cu concentrations in seminal plasma and sperm motility, whereas others reported a positive correlation between these 2 parameters.[50,51] Conversely, concentrations of Se in seminal plasma were negatively correlated with sperm motility, viability, acrosome integrity, and DNA integrity.[46]

Intracellular concentrations of Zn and Fe in bovine sperm were negatively correlated with sperm DNA integrity and motility parameters, and were lower in bulls with high fertility.[46] However, another study reported a positive correlation between bovine sperm motility and intracellular concentrations of Zn, Fe, and Cu.[47] Intracellular concentrations of Mn was negatively correlated with sperm motility, acrosome integrity, ATP content, and DNA integrity.[46]

Supplementing bulls with trace mineral mixes

Trace minerals supplementation in cattle is nowadays a common practice to help support growth, reproduction, and immunity. Primary source of trace minerals for grazing cattle is forage, grain, and feed. Forage uptakes trace minerals from the soil. In some geographical areas or conditions, the soil is deficient in some trace minerals, and the trace minerals provided during grazing are not enough, requiring supplementation. Oral supplements are commonly provided in the form of loose mineral mixes, fortified salt blocks, and fortified energy or protein supplements. In addition, trace minerals can be given in injectable forms that bypass gut-level antagonisms and overcome poor intestinal absorption and the associated variation in trace-mineral intake.

Trace mineral mixes supplementation were used in beef bull calves in an attempt to hasten puberty and to improve reproductive parameters in peripubertal animals.[48–55] Trace mineral-depleted 9-month old calves were fed a supplement containing various concentrations of complexed or sulfated Zn, Mn, Cu, and Co for 100 days.[52] Trace minerals supplementation did not improve scrotal circumference, sperm motility, morphology, or concentration. It also did not increase blood concentrations of luteinizing hormone, follicular stimulating hormone, testosterone, or seminal plasma concentrations of trace minerals.[52] However, a higher percentage of bulls reached puberty after 42 days of treatment when fed complexed trace minerals (79%) compared to those fed sulfated forms (47%).[52] Moreover, feeding high concentrations of sulfated trace minerals delayed puberty. This was presumably because of the higher sulphur content that may affect Se and Cu metabolism.[52] Similarly, feeding trace mineral-depleted 9-month old calves with 641 mg/day of Zn hydroxychloride, 193 mg/day of Cu chloride, or their combination for 84 days did not improve body weight, time to puberty, breeding soundness examination (BSE) classification, scrotal circumference, or sperm motility, morphology, and concentration.[53] However, feeding Zn and Cu combination improved sperm viability after 3 hours of incubation, and mitochondrial membrane potential and acrosome integrity.[53]

Reproductive benefit of supplementing nondeficient calves with trace minerals is less clear. Treatment of nondeficient 9-month old calves with a single dose of 1 ml/45 kg of injectable trace minerals (Cu 15 mg/ml, Mn 10 mg/ml, Se 5 mg/ml, and Zn 60 mg/ml) did not improve semen quality, scrotal circumference or the percentage of bulls classified as satisfactory breeders as yearlings 91 days after injection.[54] However, 2 doses of injectable trace minerals at 7 months (1 ml/54 kg) and 10 months of age (1 ml/68 kg) resulted in mild increases in sperm motility 2 months after second injection.[55] Injectable trace minerals did not improve the percentage of bulls classified as satisfactory breeders, scrotal circumference, or sperm morphology, but sperm motility was slightly higher during the second BSE at 12 months of age in trace mineral-treated bulls (42%) compared to control bulls (40%).[55] Of the bulls classified as nonsatisfactory breeders during the first BSE at 10 months of age, there was a tendency ($p = 0.1$) for a higher percentage of bulls to be reclassified as satisfactory breeders 2 months later when treated with trace minerals (98%) compared to control bulls (94%).[55]

Mature trace mineral-depleted bulls benefited from supplementation with Cu (83.3 ppm), Zn (233.3 ppm), and Mg (300 ppm) sulfate or chloride for 71 days.[53] Although scrotal circumference and sperm morphology did not differ from bulls fed a trace mineral-deficient control diet, sperm motility and concentration were higher in trace mineral-supplemented bulls. Moreover, bulls fed trace minerals in chloride form had higher sperm motility and concentration than bulls fed trace minerals in sulfate form. This was likely due to a higher bioavailability of chloride forms, resulting in higher liver concentrations of Zn and Cu.[53] Nondeficient mature normospermic bulls fed organic amino acid complexed Zn, Cu, Co, and Mn for 123 days had higher total and progressive sperm motility than bulls fed inorganic sulfated forms.[56] However, the benefit of feeding trace minerals to these bulls cannot be assessed since there was no control group without trace mineral supplementation. In addition, although the improvement in sperm motility was significantly different, the effect of a small numerical difference on fertility of normospermic bulls has not been critically evaluated.

Conclusion

Trace minerals have an important role in male reproductive function. Deficiencies in trace minerals can impact testicular and sperm function, affecting semen quality and fertility. Nutritional supplementation of trace mineral-deficient males can improve semen quality. Benefits of supplementing nondeficient normospermic bulls on fertility are poorly understood. Additionally, effects of trace mineral supplementation on testicular function and semen quality have not been evaluated in bulls with heat stress, testicular dysfunction, or reproductive disease. Any recommendations for this purpose remain empirical.

Conflict of interest

None to declare.

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