Review Report

Role of trace minerals in cow's reproductive function and performance: a clinical theriogenology perspective*

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Abstract

Trace minerals (TM) have a crucial role in cattle reproduction. Although microelements are required in small amounts, their bioavailability is essential for the cow reproductive physiology, and for adequate fertility and productivity. They are particularly important for antioxidant protection against cellular damage (e.g. gametes and embryonic cells), hormone synthesis, and pregnancy maintenance. Oral TM supplementation is a common and highly recommended management practice in cattle operations. However, there is substantial variability in TM bioavailability in animals receiving oral TM supplementation. The strategic use of injectable TM supplementation (without replacing traditional oral TM supplements) before episodes of marked stress (e.g. parturition), higher metabolic demand with TM depletion (e.g. last trimester of pregnancy), active immune response (e.g. uterine involution or vaccination), and before breeding helps to maintain adequate TM and oxidative status during critical points of the reproductive program. This manuscript reviews the research-based evidence regarding the effects of TM supplementation on bovine reproduction and its impact on beef and dairy cattle reproductive performance.

Keywords: Trace minerals, supplementation, cattle, reproduction

Introduction

Reproductive efficiency is essential to achieve the productive goals in dairy and beef cow-calf operations. Multiple factors such as subnutrition, stress, poor management of periparturient cows, along with inadequate breeding programs (e.g. failure of heat detection, inefficient artificial insemination [AI], and bull subfertility) are associated with low reproductive performance. Therefore, a successful reproductive program should be supported by the application of management practices directed to: 1. provide adequate nutrition; 2. minimize stress and control oxidative imbalance; 3. apply adequate breeding management tools represented by effective ovulation synchronization protocols and AI technique, and/or the use of reproductively sound bulls; 4. reduce the exposure to pathogens; and 5. generate protective immunity through vaccination against main pathogens that cause infertility.

Nutritional management of transition cows, comprising adequate supply of energy, protein, minerals, and vitamins, is one of the pillars to achieve adequate reproductive performance in cattle operations.[1] Reproductive function and particularly resumption of ovarian cyclicity and maintenance of pregnancy are lower-level priorities for nutrient distribution in cattle. Maintenance and lactation represent the main priorities for the use of carbohydrates, proteins, fatty acids, minerals and vitamins in the lactating cow. Moreover, energy and protein demands increase substantially after parturition; therefore, they are commonly the focus of any nutritional plan.[1] Nonetheless, the importance of mineral and vitamin supplementation on reproductive function and performance is commonly overlooked.

Mineral status and particularly trace minerals (TM; copper [Cu], zinc [Zn], selenium [Se], and manganese [Mn]) have important roles in cattle reproductive function and therefore in herd fertility. Although these elements are required in small amounts, their bioavailability is essential for many

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physiological processes, including several metabolic pathways, antioxidant protection against cellular damage, signal transduction, nucleic acid replication and transcription, among others.[2,3] Additionally, TM have specific functions in oocyte health, hormone production, fertilization, embryo and fetal development, and uterine environment to support pregnancy, parturition, and uterine involution.[4-8] Thus, appropriate TM supplementation is crucial for the achievement of optimal reproductive performance in beef and dairy cattle.

Several factors affect the TM status and requirements of cattle, including: the mineral composition of feedstuffs, forages, and soils; pregnancy and production stage; dry matter intake; the source TM formulations; and the presence of antagonists in water, forages and feed.[9] Assessment of the herd's TM status is a critical point of control before supplementing TM to attempt covering the needs for health and reproduction. Supplementation with TM may be provided by free-choice oral mix, salt blocks, drench, rumen boluses, and injectable formulations that vary in their TM bioavailability and absorption. Research evidence supports the strategic TM supplementation (combining both oral and injectable TM) as a promising aid to improve cattle reproductive performance.[10-15] However, other studies had no benefits of TM supplementation on cattle fertility.[16,17] This may be attributed to differences in baseline diets and TM formulations, TM deficit, animal type, and experimental designs, among others. This article summarizes the effects of TM supplementation on reproductive function in the bovine female and the impacts on dairy and beef cattle reproductive efficiency. Such effects will be discussed from a clinical theriogenology standpoint, based on clinically relevant aspects of reproductive physiology, pathology, and applied biotechnology.

Role of trace minerals in immunity and reproductive health of cattle

Various reproductive pathways, including uterine involution,[18,19] ovulation and CL development,[20] embryo-maternal interactions,[21] and vaginal-uterine mucosal defenses,[22] rely on the effective function of both branches of the immune system, namely, the innate and adaptive immunity.

A compromised immune response can have detrimental effects on reproductive function, including postpartum complications and delayed uterine regeneration, late resumption of ovarian cyclicity, failure of fertilization, and pregnancy loss.[23,24] It is well documented that TM (Cu, Se, Zn, and Mn) have a direct role in mucosal integrity and immunity, neutrophil and macrophage migration, phagocytosis and bacteria killing, antibody production, and cell mediated immunity.[25] Hence, maintaining an optimal balance of TM that supports a robust immunity is crucial for proper function of a healthy reproductive system.

Furthermore, prevalent reproductive disorders affecting dairy cattle productivity, such as retention of fetal membranes (RFM), puerperal metritis, endometritis, and ovarian cysts, along with other health issues like lameness and mastitis that indirectly affect cattle reproduction, are linked to oxidative stress.[23,24,26] This condition is defined as an imbalance in the generation and neutralization of prooxidant substances, specifically reactive oxygen species (ROS). These free radicals are normal byproducts of cellular oxidative metabolism and form when 1 atom of any molecule loses or gains 1 electron, making them unstable and extremely reactive with organic molecules like lipids.[27] The extent of the impact of oxidative stress on cow reproduction remains unclear. The excessive amount of these free radicals induces notable cell damage through lipid cell membrane peroxidation,[26,28] a process particularly impactful on leukocytes, gametes, and embryonic cells.[8] Consequently, the immune and reproductive functions are compromised during oxidative stress, resulting in increased susceptibility to diseases (e.g. mastitis, metritis, endometritis) and subfertility.[26,29,30] Antioxidants exert influence on the reproductive system in several ways. Particularly, Cu, Zn, Se, and Mn serve as integral components of enzymes responsible for ROS neutralization such as superoxide dismutase and glutathione peroxidase.[31-33] These structural and functional roles help mitigate the adverse impacts of stress on both the immune system and the reproductive function (Figure 1).

Zinc has an essential role as a structural element in the antioxidant enzyme superoxide dismutase (Zn-SOD), responsible for converting superoxide radicals into hydrogen peroxide.[34] preventing extensive cell membrane and DNA damage. Additionally, Zn has a crucial function in tissues that require active cell mitosis such as lymphoid tissue, and mucous membranes. Therefore, Zn contributes to integrity and healing of the epithelial lining of the endometrium and the mammary gland, particularly important during postpartum uterine involution and prevention of mastitis in dairy cows, respectively,[35] due to its involvement in regulatory processes of constant regeneration.[36,37] Its ability to affect endometrial cells proliferation and remodeling may be based on its direct function during nucleic acid replication, transcription, and translation.[38] Zinc is also essential for lymphocyte proliferation and differentiation before acquiring effector functions

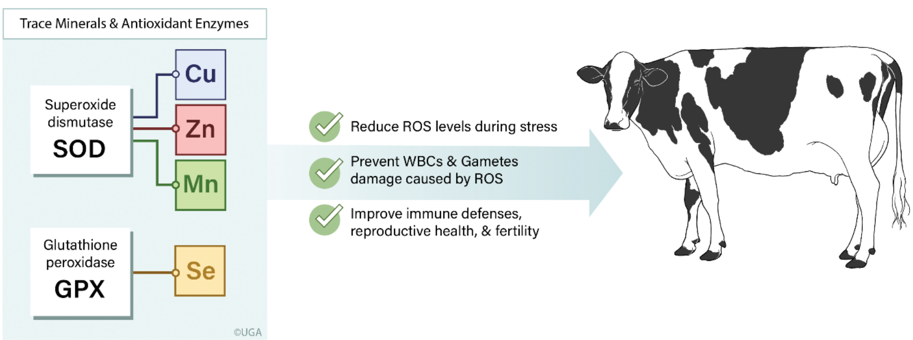

Figure 1. Role of trace minerals (Se, Cu, Zn, and Mn) as antioxidants in cattle

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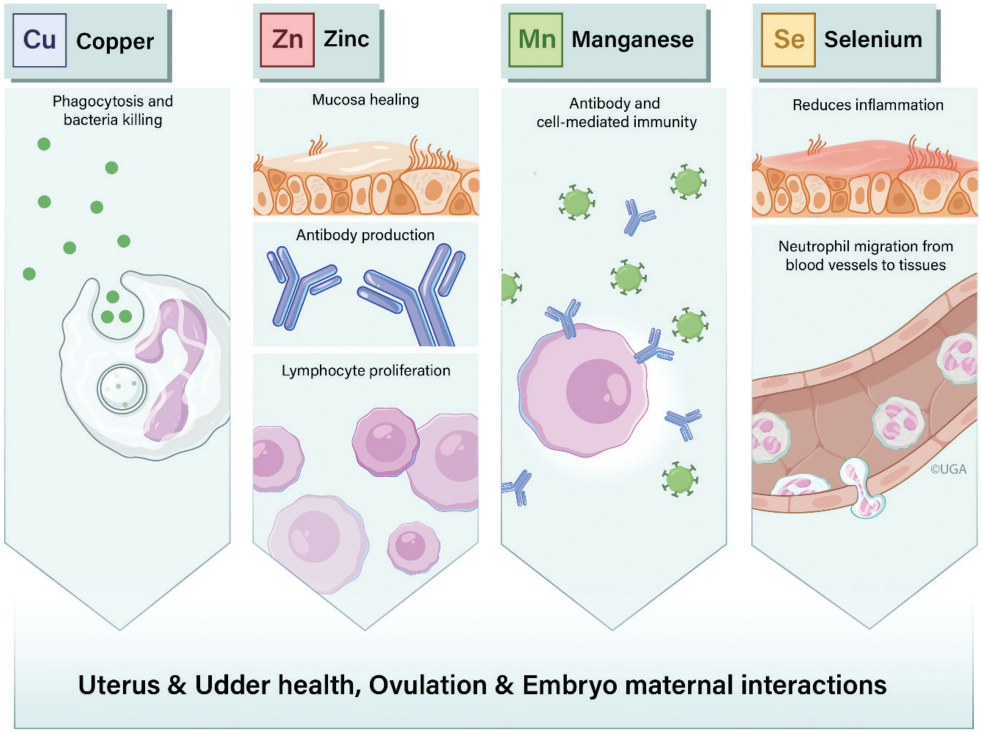

Figure 2. Role of trace minerals (Se, Cu, Zn, and Mn) in bovine immunity and reproductive health

(Figure 2).[39,40,41] Additionally, Zn influences the function of macrophages by impacting toll-like receptor-triggered proinflammatory and type I interferon responses and nitric oxide synthesis.[34,42,43]

Copper has a vital role in mitochondrial energy production and various enzymatic reactions crucial for basal metabolism.[40] Similar to Zn, it is integral to structure and function of the enzyme superoxide dismutase (Cu-SOD; Figure 1). This is essential for regulating the production of free radicals by phagocytic cells to exert their bacterial-killing function in the uterine lumen (Figure 2). Additionally, this enzyme has a key role in mitigating oxidative stress by scavenging excess superoxide radicals, avoiding their accumulation and thereby preventing damage to cell membranes. Furthermore, ceruloplasmin, a copper-containing acute-phase protein, has anti-inflammatory properties.[44]

Selenium has a major role in migration of polymorphonuclear phagocytes from the blood stream into tissues and subsequent inflammation, via E-selectin and ICAM-1 molecules.[45] This is particularly important to control mammary gland and uterine inflammation during the early postpartum period (Figure 2). Additionally, Se functions as a fundamental component of the enzyme glutathione peroxidase; this enzyme contributes to reduction in hydrogen peroxide (H_2O_2) by producing H_2O and O_2, and preventing harmful effects to white blood cells (Figure 1). Both Se and glutathione peroxidase are crucial for normal detachment and expulsion of fetal membranes, uterine involution, and prevention of metritis and mastitis in cattle.[46]

Manganese has a role as a component in numerous enzymes participating in diverse metabolic pathways, including arginase, pyruvate carboxylase, and superoxide dismutase. Although evidence of manganese's function in the immune response is somewhat limited, it serves as a structural component in the Mn-SOD enzyme, also crucial in neutralizing ROS produced by phagocytic cells (Figure 1).[2]

Role of trace minerals in cow's reproductive function

Trace minerals including Zn, Se, Cu, and Mn are involved in several reproductive functions including granulosa cell proliferation and steroidogenesis,[47] corpus luteum (CL) function,[48] oocyte quality,[49-52] embryo development[51,53] and implantation, production of interferon tau, fetal development and organogenesis,[54] placentation, oxygen transportation, parturition, and uterine involution (Figure 3).[55]

Zinc governs appetite through the central nervous system, influencing dry matter intake, energy balance, and accumulation of fat reserves that are fundamental for resumption of postpartum ovarian cyclicity. As previously stated, Zn serves as an essential element in enzymes and transcription factors, contributing catalytic, structural, and regulatory functions.[56] Therefore, Zn is a substantial factor for the basal metabolism.

Zinc is an important element affecting pregnancy rate in cattle[57] due to its direct effects on hormone secretion and function (e.g. progesterone, insulin, $PGF_{2\alpha}$, among others), oocyte, and embryo viability[58] and fetal development,[59] contributing to maintain a viable pregnancy. Zinc also modulates IGF-1 secretion, bioavailability, and function.[8,60,61] This growth factor is involved in key reproductive functions including uterine involution, embryo implantation, and fetal development.[8] Zinc is critical for endometrium remodeling due to its action in nucleic acid replication, transcription, and translation during cell division. Zinc fingers are essential to binding of

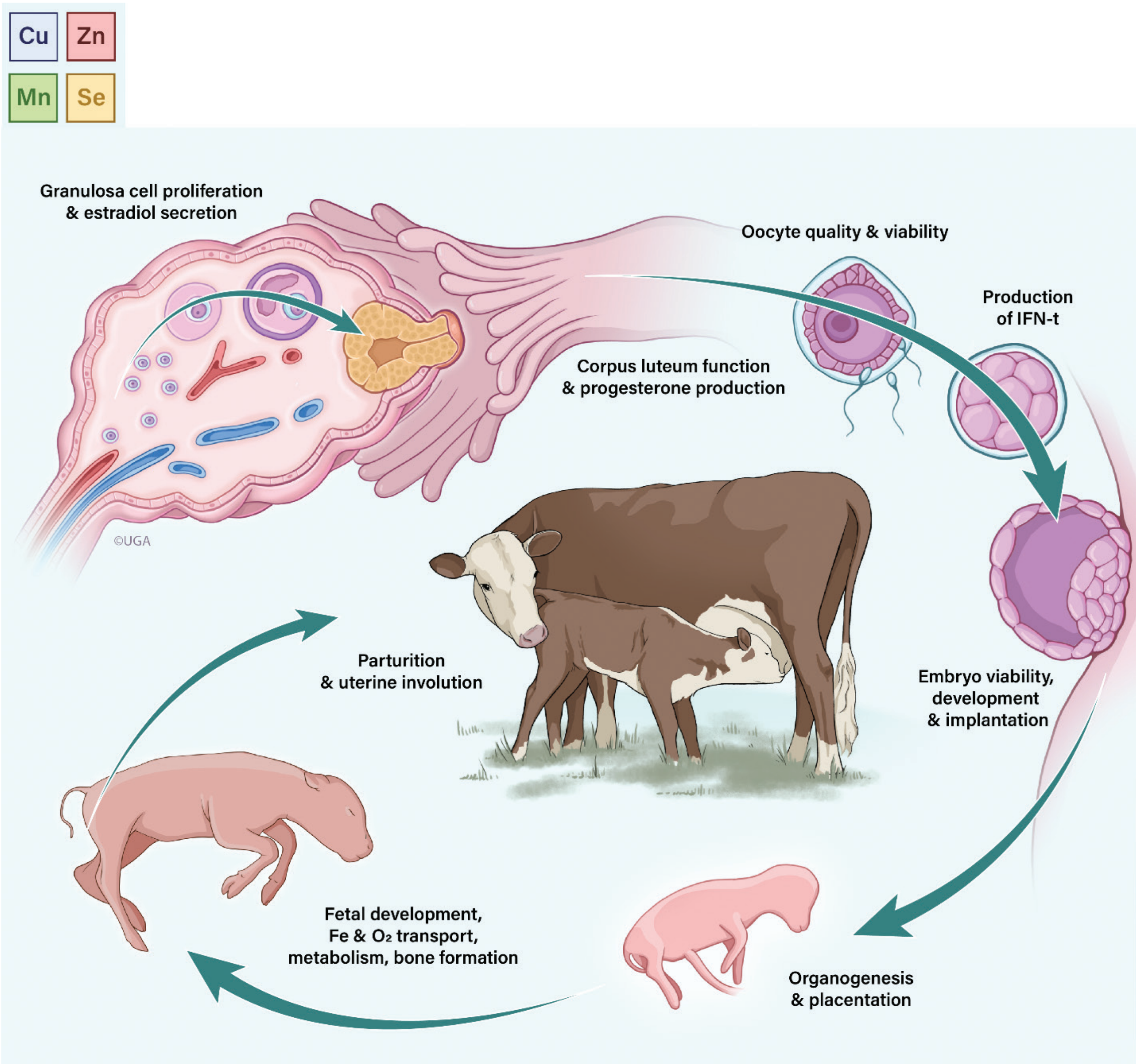

Figure 3. Role of trace minerals (Se, Cu, Zn, and Mn) in cattle reproductive functions

estrogens- and progesterone-receptor complexes to DNA to exert their actions.[62] The antioxidant properties of Zn as structural part of the Zn-SOD enzyme have a relevant role in protecting oocytes and embryonic cells from oxidative damage by superoxide free radicals.[63] Zinc also affects fetal growth and pregnancy due to its involvement in the metabolism of calcified bone matrix.[59]

A recent discovery about involvement of Zn in oocyte activation and early embryo development is a sequence of Zn exocytic events upon fertilization, known as 'zinc spark,' which leads to a reduction in the total amount of intracellular Zn that is necessary and sufficient to induce oocyte activation. Moreover, this Zn release has a role in preventing polyspermy by modifying zona pellucida. This is a remarkably conserved event across various mammalian species including cattle. In humans, it is being studied as a promising biomarker associated with embryo quality.[64,65]

Cows with adequate CL function, higher progesterone concentrations and pregnant status had higher Zn and Cu concentrations and SOD, GPX antioxidant capacity at TAI than cows with abnormal luteal phase length, delayed ovulation, and pregnancy failure.[57] There was a clear association between concentrations of antioxidant and Cu and Zn, and both CL function and fertility.[57]

Copper serves as a component in a variety of crucial metallo-enzymes and contributes to various physiologic pathways essential for reproduction, such as cellular respiration and mitochondrial energy production (e.g. via cytochrome oxidase), regulation of inflammation (e.g. ceruloplasmin), and antioxidant system (e.g. Cu-SOD). Copper exerts its influence to enhance pregnancy maintenance in cattle by affecting various reproductive processes, including enhancing oocyte viability and embryogenesis.[49,51,52,55] Additionally, Cu targets several other cell types such as endometrial cells, luteal cells, and cumulus cells that have substantial impact on early stages of embryogenesis.[55] Copper has a direct involvement in fetal development, particularly due to its participation in the immune system, bone and connective tissue formation, cardiac function, keratinization, and spinal cord myelination.[32] Fetal demands of Cu especially increase during the last trimester of pregnancy, when there is the most substantial fetal development.[55]

Selenium is critical for the proper function of numerous seleno-proteins (glutathione peroxidase, iodothyronine deiodinase, and thioredoxin reductase). By contributing to these enzymes, Se participates in neutralizing peroxides produced during lipid peroxidation.[33,66] Consequently, Se has a crucial function in maintaining the integrity of cell membranes and organelles. Through this mechanism, Se protects oocyte, early embryo, and fetus from mortality caused by oxidative damage.

Employing x-ray fluorescence imaging, a study determined Se concentrates in large ovarian follicles' granulosa cells in cattle.[67] Additionally, the study indicated a significant increase in the expression of the selenoprotein gene GPx-1 in granulosa cells of the large healthy follicles. This particular positioning of Se in large follicles, coupled with its recognized role in antioxidant defense, implies a potential role in protecting oocyte from oxidative stress and cell damage throughout follicular development. Additionally, Se directly influences granulosa cells, promoting their proliferation and enhancing estradiol synthesis,[47] which is essential for oocyte maturation and LH surge, and ovulation. Therefore, Se has a dual role, stimulating folliculogenesis and estradiol secretion and shielding oocyte and embryo from cell damage due to its antioxidant function. Supplementation of selenium in dairy cows was associated with a healthy postpartum period, normal fetal membrane expulsion and greater pregnancy outcomes.[28,68] Moreover, Se supplementation has been associated to a potential decrease in the occurrence of postpartum metritis and ovarian cysts.[35] A strong correlation in Se concentrations between maternal and fetal liver was demonstrated[69] suggesting an efficient Se transfer from maternal circulation via placenta for supplying the higher fetal demands.

Manganese has a vital function in embryo development.[50,53,70] Bovine cumulus oocyte complexes (COCs) and preimplantation embryos actively transcribe and express Mn-SOD.[70] This enzyme promotes cellular differentiation and embryo viability via antioxidant protection against cell damage. Manganese might contribute to CL function, given that concentrations of Mn and Mn-SOD in the CL of ewes increased between 4 and 11 days.[71] Therefore, it is conceivable that Mn promotes pregnancy by enhancing progesterone secretion by the CL. Additionally, a study reported improved pregnancy rates in cattle following dietary Mn supplementation.[72] However, precise mechanisms underlying the positive impacts of Mn on reproductive performance remain unclear. A potential explanation is that manganese's involvement in cholesterol production could govern steroidogenesis throughout pregnancy.[73] Cholesterol is essential for synthesizing steroid hormones like progesterone that has a central role in maintaining an adequate uterine environment for embryo implantation and pregnancy. Suboptimal progesterone concentrations have been implicated as a potential factor in early embryonic loss.[74] Manganese also has a pivotal role in the formation of fetal bones during organogenesis, since it is crucial for the synthesis of chondroitin sulfate, a component of mucopolysaccharides within the organic bone matrix.[75] A study that investigated the impact of dietary Mn on the growth and reproductive performance of beef heifers suggested that 15.8 mg of Mn/kg of diet dry matter is deemed sufficient for supporting growth, cyclicity, and conception of beef heifers.[76] However, another report indicated that this diet may be inadequate for optimal fetal development in pregnant heifers, resulting in fetal structural deformities.[77,78]

Trace mineral status and requirements in cattle

Before defining a TM supplementation regime, it is highly recommended to determine the herd TM status, as well as the mineral content of forages and feedstuffs the animals will consume. This will help to determine the baselines to ensure that supplements provide optimal amounts of TM for animal production, health, and reproduction. This TM assessment is also important for preventing Se toxicity in some areas where Se concentrations in soil and forages are high, so that additional Se supplementation may not be needed.

The best indicator of the TM status in cattle is the hepatic concentration. Liver serves as the storage site, where TM are integrated into enzymes and released as needed. To determine the TM concentrations in liver, it is necessary to collect a liver biopsy sample. Liver biopsies (~ 10 mg of liver tissue) are collected after local anesthesia in the 10^{th} intercostal space (right side) using a sterile biopsy needle (14 G x 4 or 6″). Blood concentrations of TM may exhibit variability, inconsistency, and have limited predictive value. However, blood Se is very stable and is a useful tool to promptly diagnose Se toxicity (> 2.5 µg/ml). Copper concentrations in liver samples should exceed 75-90 ppm,[79] within a range of 50-600 µg/g on a dry matter basis.[80] A marginal deficiency of manganese (Mn) is indicated when liver concentrations are below 9 ppm, within a range of 5-15 µg/g.[80] Adequate selenium (Se) concentrations in the liver are between 0.8-1.0 ppm, with a range of 0.7-2.5 µg/g and concentrations below 0.2 ppm are deemed critically deficient.[79] For Zn, hepatic concentrations below 80-100 ppm are considered marginal or deficient,[79] with a range of 90-400 µg/g.[80] The TM status of cattle is influenced by various factors, encompassing the mineral composition of soils, forages, and feedstuffs, high variability in mineral intake, the existence of mineral antagonists in water, forages, and feedstuffs, and fluctuating TM requirements.

The TM requirements have been instituted as the principles that define animal necessities, and help assess the formulation of TM supplementation. These requirements are derived from research findings reported established by the National Academies of Sciences, Engineering, and Medicine (NASEM, 2016), Board on Agriculture Subcommittee on Beef Cattle Nutrition.[81] These TM requirements are not a 'one-size-fits-all' parameter, as they vary considerably and are affected by factors such as breed, physiological status (e.g. lactation and pregnancy), health status, environmental conditions, management practices, and level of stress. A rule of thumb for adequate nutritional management is to plan the TM supplementation regime based on information of TM requirements, status, and content in forage, feedstuffs, and soil.

A comprehensive study regarding the TM composition of forage samples collected from cow/calf operations across 18 states in the US indicated TM deficiencies in a substantial number of the samples.[79] Merely 2.5% of the forage samples exhibited adequate Zn concentrations. Copper concentrations were deemed sufficient in 36% of the samples.[79] Moreover, 50% of the samples were considered marginally deficient in copper, with 14% classified as critically deficient. Another report, encompassing 23 states in the US, yielded comparable findings of TM deficiencies. In Midwest US, only 4, 15, and 26% of fescue samples had adequate concentrations of Se, Zn, Cu, respectively.[82] Additionally, 18, 24, and 24% of native forage samples exhibited adequacy in Cu, Se, and Zn, respectively.[82] Notably, in both surveys, a substantial proportion of the samples had elevated or marginally high concentrations of iron (Fe), molybdenum (Mo), and sulfur (S).[79,82] The observed Cu deficiency in the tested forage samples is likely linked to antagonistic effects of Fe, Mo, and S that impede copper absorption through gastrointestinal tract. These antagonistic interactions could contribute to insufficient availability of Cu in the diet, further highlighting the complexity of TM dynamics in forage and its impact on cattle nutrition.

Based on the authors' experience, the observed variability in TM requirements and the difficulties to maintain an adequate herd TM status make it indispensable to establish a 'strategic TM supplementation plan' that supports herd's health and reproductive performance. This plan needs to be specifically adapted to each particular production system and should combine permanent traditional free-choice oral supplementation plus pulse-dose TM injections during critical points of the production system when TM demands are greater (e.g. 30 days before parturition and 30 days before breeding).

Trace mineral deficiencies on cattle health and reproduction

Deficiencies in TM have a gradual impact, progressively affecting various physiological and metabolic functions with multifaceted implications for overall health, reproduction, and performance. Commonly, the lack of specific clinical signs makes TM deficiency easily overlooked, with borderline concentrations possibly continuing to decrease, affecting first reproductive function, embryo viability, and immune response. This results in low reproductive performance (e.g. high incidence of early embryonic mortality) and increased susceptibility to health disorders, such as lameness, metritis, and mastitis (Figure 4).

Overall, TM deficiency impairs the immune response by affecting neutrophil and macrophage migration, phagocytic function, and killing activity, as well as the mechanisms of mucous membranes integrity. TM deficiency also affects lymphocyte activation, proliferation, and differentiation, cytokine secretion, and antibody production.[44,83,84]

When TM concentrations decrease to a severe extent, clinical deficiencies can manifest with distinct clinical signs, such as increased incidence of RFM and muscle degeneration, both associated with severe Se deficiency. Regularly assessing herd's mineral profile and animals' TM intake is crucial to identify and address their specific TM needs.

Zn deficiency is associated with various conditions including anorexia, reduced IGF-1 concentrations, CL insufficiency, low progesterone concentrations, higher risk of abortion, fetal teratogenesis, prolonged pregnancy, calving difficulties, fetal growth retardation, low birth weight, weak offspring, skin parakeratosis, and neonatal calf diarrhea (Figures 4 and 5).[85,86]

Deficiencies of Cu in cattle may occur due to low dietary intake or consumption of antagonists (e.g. Mo, Fe, and S), and

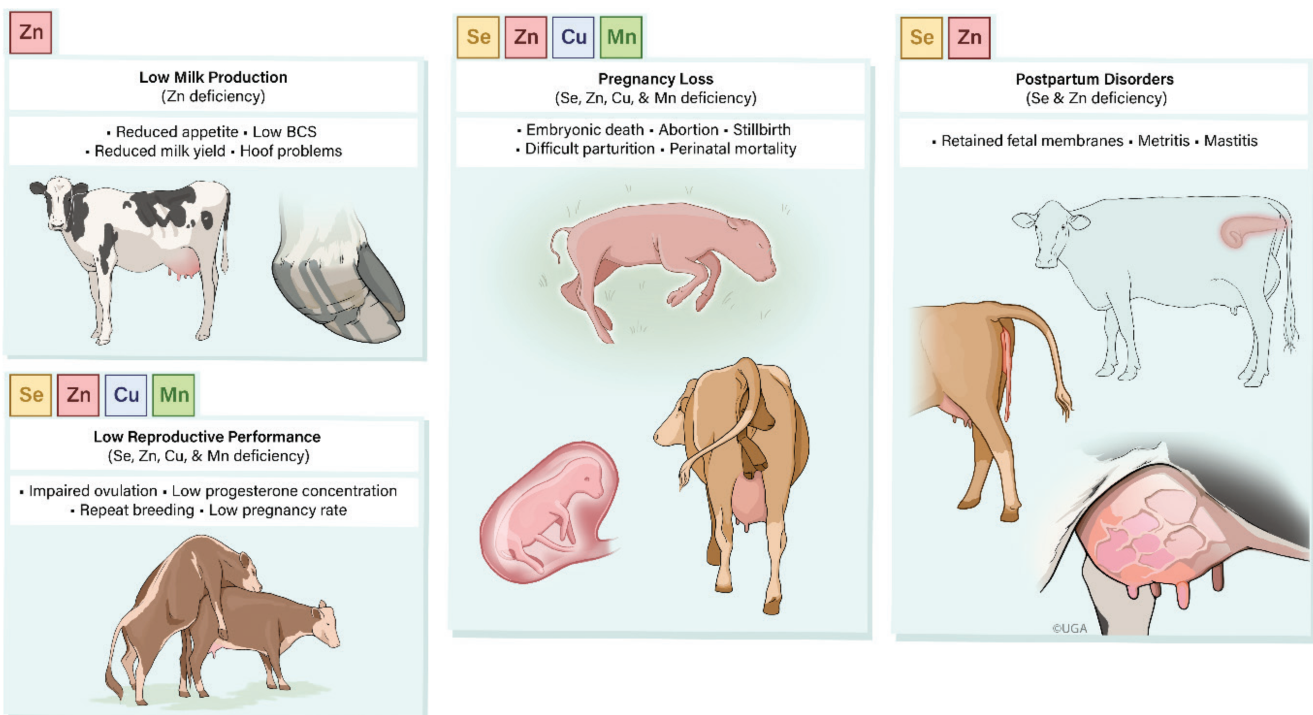

Figure 4. Effects of trace mineral deficiencies on cow production and reproduction

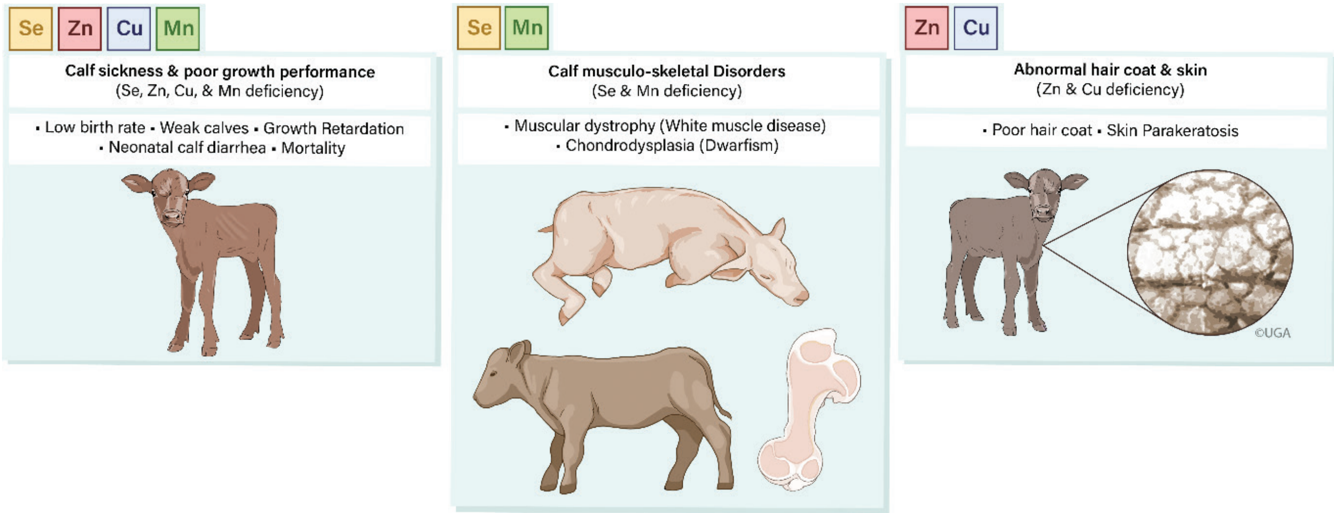

Figure 5. Effects of trace mineral deficiencies on calves' health and performance

are associated with low pregnancy rate, embryonic and fetal death, anestrus and repeat breeding, stillbirth, immunosuppression, and poor calf health and performance (mainly diarrhea, growth retardation, and mortality).[85]

Reproductive disorders related to Se deficiency encompass low fertility[87] and abortion occurrences.[88,89] Low concentrations of Se and glutathione peroxidase (GSHPx) in plasma have been associated with a higher incidence of RFM (Figure 4).[46] Further studies supported this, revealing that animals supplemented with Se experienced none or few cases of RFM, whereas the control group receiving a Se deficient basal diet had a RFM incidence between 17 and 32%.[90-92] Moreover, Se deficiency has also been identified as a factor that increases the likelihood of puerperal metritis in dairy cattle.[28,93] As mentioned above, Se deficient calves may have muscular degeneration known as nutritional muscular dystrophy or white muscle disease (Figure 5) that results in heart degeneration and death, the predominant pathology in neonatal calves. Marginally Se deficient calves may have weakness and low vigor.

Insufficient Mn leads to suboptimal growth and compromised reproduction, characterized by impaired ovulation, silent estrus, diminished conception rates, occurrences of abortions, and decreased birth weight.[94] A deficiency in dietary manganese generally manifests with relatively mild effects on immunity, except for a notable impact on the humoral immune response.[95] Manganese deficiency has been linked to adverse outcomes such as abortion and congenital deformities in long bones among newborn calves. These deformities include enlarged joints, stiffness, twisted legs, shorter bones, dwarfism, brachygnathism, chondrodysplasia, and overall physical weakness after birth.[77,78,96,97]

A retrospective study to determine the association between plasma TM (Cu, Zn, and Se glutathione peroxidase) status on reproductive performance and health of commercial dairy and beef herds (n = 2,080) in France revealed that Cu, Zn, and Se deficiencies may be important risk factors for impaired production, reproduction, and health in both beef and dairy herds.[85] Copper deficiency had negative effects on health and performance of calves and cows, whereas, Zn and Se deficiencies substantially affected adults. Selenium deficient status was associated with increased risk of infectious abortion, poor immune response, and higher calf morbidity and mortality. Zinc deficiency was highly correlated with low milk yield and lameness in dairy herds, as well as neonatal calf diarrhea and poor growth. Further, in that study, Se deficiency in cows was associated with increased incidence of RFM.[85]

Oral trace minerals supplementation: effects on cows' reproductive performance

Oral mineral supplements can be provided in various forms, including free-choice blends of mineralized salt, blocks, or alongside energy and/or protein supplements within the total mixed ration. There are various TM sources for cattle supplementation, categorized into organic or inorganic compositions. Organic sources, including chelates, amino acid or polysaccharide complexes, proteinates, propionates, and yeast derivatives, are more biologically available for absorption and utilization. This makes them particularly advantageous for improving health and reproductive performance compared to inorganic forms,[98,99] such as sulfates, oxides, carbonates, and chlorides that are structured with sulfur, oxygen, or chloride molecules. Sulfate forms of Zn, Cu, and Mn are the most bioavailable inorganic sources. Although inorganic TM may prevent severe deficiencies, supplementation with organic forms such as Se yeast could offer additional benefits by providing enhanced TM status and antioxidant activity during periods of heightened stress, such as after parturition.

Over the past decades, numerous studies have highlighted the value of oral TM supplementation in enhancing reproductive function and efficiency in cows.[98-100]

A recent meta-analysis considering 20 research articles assessed the impacts of organic TM supplementation on milk yield and composition, and reproductive performance in dairy cows.[100] The results indicated that organic TM supplementation increased daily milk production, milk fat, and milk protein, with no effects on milk somatic cell count. Organic TM supplementation reduced days open, number of services per conception, and increased the risk of pregnancy on day 150 of lactation. This study suggested that organic TM supplementation could enhance production and reproduction in lactating dairy cows.[100] Other researchers have reported similar improvements in conception rates and days to first service in dairy cows when substituting inorganic sulfates with organic sources of Cu, Zn, Mn, and Co, although the precise mode of action remains unclear.[99] An additional effect of TM supplementation (particularly the use of organic forms) on dairy herds is the reduction in the occurrence of clinical mastitis, somatic cell counts,[35] and increase in milk yield.[101]

Another study was performed using crossbred, multiparous grazing beef cows to assess the impacts of Cu, Zn, and Mn supplementation and the mineral source on reproduction and performance over a 2-year period.[102] The cows received either organic (50% organic and 50% inorganic Cu, Zn, and Mn); inorganic (100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$) or no supplemental Cu, Zn, or Mn from 82 days (year 1) and 81 days (year 2) before calving through 110 days (year 1) and 135 days (year 2) after calving. In year 1, pregnancy rate to AI in control cows did not differ from supplemented cows, but there was a trend for higher pregnancy rate in organic compared to inorganic TM supplementation cows. In year 2, supplemented cows had a higher pregnancy rate to AI than controls. In both years, when animals were inseminated after observed estrus, supplemented cows had a higher pregnancy rate than controls. Overall, 60-day pregnancy rate tended to be higher for supplemented cows. This study indicated that TM supplementation and the TM source can improve the pregnancy rate to AI.[102]

Contrasting findings have emerged from other studies, indicating marginal or negligible benefits of oral TM supplementation on reproduction of dairy[103] and beef cattle.[104–106] Effects of organic or inorganic TM supplementation on ovarian follicular dynamics, ovulation, and embryo production in Angus heifers submitted to hormonal protocols for superovulation were determined.[106] In that study, dietary TM supplementation had no influence on the number of ova/embryos recovered from beef heifers or the CL counts, indicating no difference between control, organic or inorganic TM supplementation.[106] This suggested that the source of TM supplementation did not produce a substantial impact on the number or quality of embryos in Angus heifers and it is unlikely to have a direct impact on follicular dynamics and ovulation.[106] However, it is important to notice in that study that TM supplementation tended to reduce the number of degenerated embryos and unfertilized ova, supporting the role of TM on enhancing fertilization and embryo quality.[106]

The inconsistency in results across studies on TM supplementation can be attributed to various factors, including differences in the forms of minerals supplemented (organic versus inorganic), high variability in TM concentration in control diets, variations in animals' TM requirements and initial TM status, differences in intake and absorption, negative mineral interactions and antagonisms, varying levels of stress, and discrepancies in experimental models and endpoints. These diverse factors make the interpretation and comparison of data from several studies particularly challenging.

Strategic injectable trace minerals supplementation on reproductive performance

As mentioned above, offering TM through free-choice oral supplementation may often result in TM deficiencies due to considerable variability in mineral composition and intake, binding of minerals to undigested feed particles, negative interactions and diminished absorption in the digestive tract, and antagonistic effects.

There is a rising trend in cattle operations to adopt strategic extra supplementation using single treatment, pulse-dose trace mineral products. Whether given orally or through injections, this approach aims to prevent TM deficiencies or enhance the overall TM status. It ensures that the necessary TM amounts are provided during crucial stages of the production cycle when animals experience heightened TM demands. Good examples of such critical times may be: 1. the last trimester of pregnancy, when there is substantial TM transfer from the dam to the fetus due to increased fetal growth and its TM demand; and 2. at parturition, which is accompanied by substantial oxidative stress and a decrease in plasma Zn concentrations,[107,108] or before the breeding season in preparation for the upcoming reproductive cycle and pregnancy. It is important to note that although this strategic supplementation is valuable, it is not meant to replace the regular supply of oral minerals.

Injectable TM (ITM) treatment in Angus-cross steers with sufficient TM status led to a quicker and more substantial increase in plasma concentrations of Zn, Se, and Mn through the first 24 hours, compared to other single-dose supplementation methods including drench, paste, or boluses.[109] Additionally, hepatic Se concentrations remained higher through day 29 in steers treated with ITM in comparison to the groups receiving TM paste or drench.[109] This study demonstrated that ITM is the most effective way to provide additional TM to be available during specific times when the animal's needs are greater.

Utilization of ITM ensures the delivery of a known and well-controlled quantity of TM that is rapidly and efficiently absorbed and stored after injection.[110] This becomes particularly crucial for cattle experiencing reduced dry matter intake and mineral consumption, such as after vaccination or before parturition. It is also beneficial in situations where cattle have limited access to free-choice mineral supplements, as is common in extensive rangeland systems or flooded pastures. Treatment of ITM reduces the variability in TM status observed in animals with free-choice mineral intake.[111]

A commercially available ITM product, Multimin® 90 from Axiota Animal Health® that contains Zn (60 mg/ml), Cu (15 mg/ml), Mn (10 mg/ml), and Se (5 mg/ml) has demonstrated rapid absorption, leading to an increase in blood TM concentrations within 8 hours after injection, maintaining elevated circulating concentrations for 24 hours, after which they are stored in liver.[110,112]

Multiple studies have been performed to assess the impact of supplementation with ITM on production and reproduction of dairy cows. In a trial using 1,416 multiparous dairy cows that received ITM or not at 230 and 260 days of pregnancy, as well as 35 days postpartum, ITM supplementation led to a decrease in the incidence of periparturient fetal deaths and cases of endometritis.[10] However, it is noteworthy that this treatment did not have any marked effects on reproductive performance, milk yield, or other health variables. Furthermore, treatment of ITM yielded positive outcomes for udder health with a reduction in linear somatic cell count scores, and the occurrence of mastitis.[10] In another report in dairy cows, TM supplementation increased serum SOD activity, without affecting leukocyte function.[11]

A subsequent trial using 923 multiparous cows from 2 commercial dairy farms treated with ITM or not, revealed that ITM supplementation had a tendency to lower the incidence of metritis and stillbirths. Additionally, it exhibited improvements in polymorphonuclear leukocyte function and contributed to a better inflammatory status in dairy cows undergoing the transition period, under high temperature-humidity conditions.[12]

Treatment of ITM 30 days before calving and 30 days before breeding in beef cows synchronized using a 5-day CO-Synch + CIDR protocol, and TAI at 72 hours after CIDR removal resulted in higher P/TAI (60.2%) compared to control cows (51.2%). Final pregnancy rate was not different but the calving distribution for ITM-treated cows improved (12.5% more early calving), with a greater proportion of cows becoming pregnant (77.5%) during the first 20 days of the breeding season compared to control cows (65.0%).[13]

Effects of ITM treatment at the beginning of the TAI protocols (11 days before AI) have been evaluated in several trials. A study in *Bos indicus* cows[14] demonstrated that ITM improved CL function, resulting in decreased CL diameter, although progesterone production was more efficient, having similar concentrations to control group.[14] This might be due to the observed increase in antioxidant enzymes SOD and GPX, reduced ROS, and improved steroidogenesis. In the same study, ITM improved overall pregnancy rate ~ 5% points higher (particularly in cows with low BCS; 53.7 versus 48.1% for ITM and control cows, respectively), probably by enhancing oocyte quality and CL function.[14] Moreover, in another trial, Angus heifers treated with ITM at the initiation of a 14-day CIDR protocol tended to have an increased AI pregnancy rate (62 versus 45%) compared to control heifers, despite no difference in overall pregnancy rate.[17]

The strategic supplementation with ITM at ovulation synchronization in dual purpose and beef cows under tropical grazing conditions in Mexico confirmed these findings, consistently reaching 5-8% greater (p = 0.05) pregnancy rate at TAI in treated versus control cattle (58.1 versus 50.9%).[113] In a trial performed in Brazil, crossbred heifers underwent treatment with ITM (ITM 100 mg Zn, 100 mg Mn, 50 mg Cu, and 25 mg Se; Multimin®, Minerthal, Brazil) 17 days before timed embryo transfer (TET). Subcutaneous treatment of ITM (n = 219) resulted in 1.58-fold and 1.72-fold higher odds of being pregnant 23 and 48 days after TET compared to control group (n = 276), respectively. Therefore, ITM treatment 17 days before TET did not enhance the synchronization success, but it did result in increased conception rates (embryo survival) at both 23 and 48 days after TET.[15]

In contrast, other studies suggested that supplementation beyond an animal's nutritional requirements may not provide benefits to reproductive performance.[16,114] A research trial[17] stated that repeated treatment with ITM before AI did not have substantial impact on pregnancy rates in beef heifers. Another study in dairy cattle reported that animals undergoing a 2-dose ITM protocol, first given before calving and second before breeding, exhibited lower conception rates at the first service.[16]

Trace mineral supplementation and in vitro production of bovine embryos

Supplementation with TM has been used to improve in vitro embryo production (IVP), during both in vivo oocyte collection (ovum pick-up, OPU) and in vitro maturation (IVM) and culture (IVC). Benefits and impacts of oral organic (amino acid complex) TM supplementation on OPU and in vitro embryo production in lactating beef cows submitted to OPU on days 52 and 67 of pregnancy were demonstrated.[115] Oral supplementation with organic TM increased total COCs recovery and IVM compared to unsupplemented cows. The production of transferable embryos tended to be greater for TM supplemented cows. Additionally, the ratio of recovered COCs meeting maturation per transferable embryo was enhanced (lower) for cows receiving TM supplementation. Therefore, in TM supplemented cows, IVP was more efficient, needing fewer mature COCs to produce a transferable embryo. Replacing inorganic trace minerals with an organic source improved not only the number of recovered COCs via OPU, and the ability to reach maturation, but also the in vitro embryo production in beef cows.[115]

Addition of TM to IVM and IVC media have provided benefits to the production of bovine embryos. Adding Cu during oocyte maturation (4-6 µg/ml of IVM medium) notably increased both intracellular glutathione content and DNA integrity of cumulus cells,[49] decreased apoptosis, and improved subsequent embryo development up to blastocyst stage.[49,51,116] In a similar fashion, supplementation of the IVM medium with Zn (1.1-1.5 µg/ml) decreased DNA damage and apoptosis in cumulus cells. This supplementation also led to increased cleavage and blastocyst rates, along with an increase in the inner cell mass and total cell numbers.[49,58,117] Manganese supplementation in the IVM medium (6 ng/ml), regardless of cumulus cells presence, also augmented the COCs quality (reduced % of apoptosis, and improved SOD activity), enhancing subsequent embryo development.[53] Similar findings were observed when bovine oocytes were matured in IVM medium containing Se (10 ng/ml); there was a reduction in the number of apoptotic cells and increased embryo hatching rates compared to the control oocytes.[118] In a more recent study, supplementation of IVM medium with a combination of Mn, Se, Cu, and Zn, (6, 100, 600, and 400 ng/ml, respectively) had increased blastocyst rates and cell number, and decreased ROS and lipid content.[119] These studies demonstrated that addition of TM to the IVM and/or IVC may be a promising strategy to enhance the efficiency of the bovine IVP systems.

Conclusion

Trace minerals supplementation is a key support for the success of any dairy or beef herd breeding program. Oral TM supplementation is a common and highly recommended practice in cattle operations. However, evidence indicated substantial variability in TM bioavailability in animals receiving oral TM supplementation. Research and clinical evidence support the strategic use of ITM supplementation (without replacing traditional oral TM supplements) before episodes of: substantial stress (e.g. parturition), higher metabolic demand and TM depletion (e.g. the last trimester of pregnancy), active immune response (e.g. uterine involution or vaccination) and before breeding. An effective protocol has suggested treatment of ITM 30 days before parturition and 30 days postpartum or ~ 10 or 14 days before breeding (e.g. at starting ovulation synchronization protocol). This approach has enhanced herd's oxidative balance, protective immunity, and fertility; reduced the occurrence of postpartum disorders in dairy cows (RFM, metritis, and mastitis) and enhanced pregnancy outcomes (5% greater pregnancy risk and 12% more calvings at the beginning of the season) with the final benefit of producing more uniform and heavier calf crops. Implementation of TM supplementation plan should be preceded by assessment of the herd's TM status by collecting liver biopsies in a representative number of various animal groups (e.g. calves, heifers, pregnant, and nonpregnant cows) and determination of the TM content of forages and feedstuffs.

Conflict of interest

None to report.

References

1. Lean IJ, Van Saun R, DeGaris PJ: Energy and protein nutrition management of transition dairy cows. Vet Clin Food Anim 2013;29:337-366. doi: 10.1016/j.cvfa.2013.03.005
2. Tomlinson DJ, Socha MT, DeFrain JM: Role of trace minerals in the immune system. In: Proceedings of the 2008 Penn State Dairy Cattle Nutrition Workshop, November 12-13. Grantville, PA. p. 39-52.
3. Haase H, Rink L: Multiple impacts of zinc on immune function. Metallomics 2014;6:1175-1180. doi: 10.1039/c3mt00353a
4. Davis GK, Mertz W: Copper. In: Trace Elements in Human and Animal Nutrition, Mertz W: editor. 5th edition, San Diego; Academic Press: 1987. p. 301-364.
5. Hambidge KM, Casey CE, Krebs NF: Zinc. In: Trace Elements in Human and Animal Nutrition, Mertz W: editor. 1986; 4th edition, San Diego; Academic Press: 1986. p. 1-37.
6. Hidiroglou M: Manganese in ruminant nutrition. Can J Anim Sci 1979;59:217-236. doi: 10.4141/cjas79-028
7. Hurley LS, Keen CL: Manganese. In: Mertz W: editor. Trace Elements in Human and Animal Nutrition. 5th edition, San Diego, CA; Academic Press: 1987. p. 185-223.
8. Hostetler CE, Kincaid RL, Mirando MA: The role of essential trace elements in embryonic and fetal development in livestock. Vet J 2003;166:125-139. doi: 10.1016/s1090-0233(02)00310-6
9. Sager B, Van Saun RJ: Trace mineral supplementation for beef cows: dry range environment. Vet Clin Food Anim 2023;39:471-489. doi: 10.1016/j.cvfa.2023.08.012
10. Machado VS, Bicalho ML, Pereira RV, et al: Effect of an injectable trace mineral supplement containing selenium, copper, zinc, and manganese on the health and production of lactating Holstein cows. Vet J 2013;197:451-456. doi: 10.1016/j.tvjl.2013.02.022
11. Machado VS, Oikonomou G, Lima SF, et al: The effect of injectable trace minerals (selenium, copper, zinc, and manganese) on peripheral blood leukocyte activity and serum superoxide dismutase activity of lactating Holstein cows. Vet J 2014;200:299-304. doi: 10.1016/j.tvjl.2014.02.026
12. Silva TH, Guimaraes I, Menta PR, et al: Effect of injectable trace mineral supplementation on peripheral polymorphonuclear leukocyte function, antioxidant enzymes, health, and performance in dairy cows in semi-arid conditions. J Dairy Sci 2022;105:1649-1660. doi: 10.3168/jds.2021-20624
13. Mundell LR, Jaeger JR, Waggoner JW, et al: Effects of prepartum and postpartum bolus injections of trace minerals on performance of beef cows and calves grazing native range. Appl Anim Sci 2012;28:82-88. doi: 10.15232/S1080-7446(15)30318-1
14. Vedovatto M, Moriel P, Cooke RF, et al: Effects of a single trace mineral injection at beginning of fixed-time AI treatment regimen on reproductive function and antioxidant response of grazing Nellore cows. Anim Reprod Sci 2019;211:106234:1-8. doi: 10.1016/j.anireprosci.2019.106234
15. Sales JN, Pereira RV, Bicalho RC, et al: Effect of injectable copper, selenium, zinc and manganese on the pregnancy rate of crossbred heifers (Bos indicus × Bos taurus) synchronized for timed embryo transfer. Livest Sci 2011;142:59-62. doi: 10.1016/j.livsci.2011.06.014
16. Vanegas JA, Reynolds J, Atwill1 ER: Effects of an injectable trace mineral supplement on first-service conception rate of dairy cows J Dairy Sci 2004;87:3665-3671. doi: 10.3168/jds.S0022-0302(04)73505-5
17. Stokes RS, Ralph AR, Mickna AJ, et al: Effect of an injectable trace mineral at the initiation of a 14 day CIDR protocol on heifer performance and reproduction. Anim Sci 2017;1:458-466. doi: 10.2527//tas2017.0050
18. Sheldon IM: The postpartum uterus. Vet Clin North Am Food Anim Pract 2004;20:569-591. doi: 10.1016/j.cvfa.2004.06.008
19. Dadarwal D, Palmer C, Griebel P: Mucosal immunity of the postpartum bovine genital tract. Theriogenology 2017;104:62-71. doi: 10.1016/j.theriogenelogy.2017.08.010
20. Walusimbi SS, Pate JL: Physiology and endocrinology symposium: role of immune cells in the corpus luteum. J Anim Sci 2013;91:1650-1659. doi: 10.2527/jas.2012-6179
21. Farias-Fiorenza M, Amaral CS, Anunciação ARA, et al: Possible impact of neutrophils on immune responses during early pregnancy in ruminants. Anim Reprod 2021;18:e20210048. doi: 10.1590/1984-3143-AR2021-0048
22. Corbeil LB, Bon Durant RH: Immunity to bovine reproductive infections. Vet Clin North Am Food Anim Pract 2001;17:567-583. doi: 10.1016/s0749-0720(15)30007-4
23. Mordak R, Stewart PA: Periparturient stress and immune suppression as a potential cause of retained placenta in highly productive dairy cows: examples of prevention. Acta Vet Scand 2015;57:84. doi: 10.1186/s13028-015-0175-2
24. Klaus L Ingvartsen KL, Moyes KM: Factors contributing to immunosuppression in the dairy cow during the periparturient period. Jpn J Vet Res 2015;63:15-24. PMID: 25872323
25. Palomares RA: Trace minerals supplementation with great impact on beef cattle immunity and health. Animals 2022;12:2839:1-21. doi: 10.3390/ani12202839
26. Sordillo LM, Aitken SL: Impact of oxidative stress on the health and immune function of dairy cattle. Vet Immunol Immunopathol 2009;128:104-109. doi: 10.1016/j.vetimm.2008.10.305
27. Aurousseau B, Gruffat D, Durand D: Gestation linked radical oxygen species fluxes and vitamins and trace mineral deficiencies. Reprod Nutr Dev 2006;46:601-620. doi: 10.1051/rnd:2006045
28. Spears JW, Weiss WP: Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet J 2008;176:70-76. doi: 10.1016/j.tvjl.2007.12.015
29. Bernabucci U, Ronchi B, Lacetera N, et al: Influence of body condition score on relationships between metabolic status and

oxidative stress in periparturient dairy cows. Dairy Sci 2005;88:2017-2026. doi: 10.3168/jds.S0022-0302(05)72878-2
30. Castillo C, Hernandez J, Bravo A, et al: Oxidative status during late pregnancy and early lactation in dairy cows. Vet J 2005;169:286-292. doi: 10.1016/j.tvjl.2004.02.001
31. Michiels C, Raes M, Toussaint O, et al: Importance of Se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress. Free Radic Biol Med 1994;17:235-248. doi: 10.1016/0891-5849(94)90079-5
32. Andrieu S: Is there a role for organic trace element supplements in transition cow health? Vet J 2008;176:77-83. doi: 10.1016/j.tvjl.2007.12.022
33. Abuelo A, Hernández J, Benedito JL, et al: Immunity to bovine reproductive infections. The importance of the oxidative status of dairy cattle in the periparturient period: revisiting antioxidant supplementation. Anim Physiol Anim Nutr 2015;99:1003-1016. doi: 10.1111/jpn.12273
34. Bonaventura P, Benedetti G, Albarède F, et al: Zinc and its role in immunity and inflammation. Autoimmun Rev 2015;14:277-285. doi: 10.1016/j.autrev.2014.11.008
35. Wilde D: Influence of macro and micro minerals in the peri-parturient period on fertility in dairy cattle. Anim Reprod Sci 2006;96:240-249. doi: 10.1016/j.anireprosci.2006.08.004
36. Tomlinson DJ, Mülling CH, Fakler TM: Formation of keratins in the bovine claw: roles of hormones, minerals, and vitamins in functional claw integrity. J Dairy Sci 2004;87:797-809. doi: 10.3168/jds.S0022-0302(04)73223-3
37. Capuco AV, Wood D, Bright SA, et al: Regeneration of teat canal keratin in lactating dairy cows. J Dairy Sci 1990;73:1745-1750. doi: 10.3168/jds.S0022-0302(90)78851-0
38. Zhang X, Hou Y, Huang Y, et al: Interplay between zinc and cell proliferation and implications for the growth of livestock. Anim Physiol Anim Nutr 2023;107:1402-1418. doi: 10.1111/jpn.13851
39. Spears JW: Micronutrients and immune function in cattle. Proc Nutr Soc 2000;59:587-594. doi: 10.1017/s0029665100000835
40. Failla ML: Trace elements and host defense: recent advances and continuing challenges. J Nutr 2003;133:1443S-1447S. doi: 10.1093/jn/133.5.1443S
41. Puertollano MA, Puertollano E, de Cienfuegos GÁ, et al: Dietary antioxidants: immunity and host defense. Curr Top Med Chem 2011;11:1752-1766. doi: 10.2174/156802611796235107
42. Wang C, Liu X, Liu Y, et al: Zinc finger protein 64 promotes Toll-like receptor-triggered proinflammatory and type I interferon production in macrophages by enhancing p65 subunit activation. J Biol Chem 2013;288:24600-24608. doi: 10.1074/jbc.M113.473397
43. Abbas AK, Lichtman AH: Innate immunity. In: Cellular and Molecular Immunology. 9th edition, Philadelphia, PA; Saunders Elsevier: 2018. p. 275-297.
44. Percival SS: Copper and immunity. Am J Clin Nutr 1998;67:1064S-1068S. doi: 10.1093/ajcn/67.5.1064S
45. Maddox JF, Aherne KM, Reddy CC, et al: Increased neutrophil adherence and adhesion molecule mRNA expression in endothelial cells during selenium deficiency. J Leukoc Biol 1999;65:658-664. doi: 10.1002/jlb.65.5.658
46. Brzezinska-Slebodzinska E, Miller JK, Quigley JD, et al: Antioxidant status of dairy cows supplemented prepartum with vitamin E and selenium. J Dairy Sci 1994;77:3087-3095. doi: 10.3168/jds.S0022-0302(94)77251-9
47. Basini G, Tamanini C: Selenium stimulates estradiol production in bovine granulosa cells: possible involvement of nitric oxide. Domest Anim Endocrinol 2000;18:1-17. doi: 10.1016/s0739-7240(99)00059-4
48. Fujii J, Iuchi Y, Okada F: Fundamental roles of reactive oxygen species and protective mechanisms in the female reproductive system. Reprod Biol Endocrinol 2005;3:43.1-10 doi: 10.1186/1477-7827-3-43
49. Picco SJ, Rosa DE, Anchordoquy JP, et al: Effects of copper sulphate concentrations during in vitro maturation of bovine oocytes. Theriogenology 2012;77:373-381. doi: 10.1016/j.theriogenology.2011.08.009
50. Anchordoquy JP, Anchordoquy JM, Sirini MA, et al: Effect of different manganese concentrations during in vitro maturation of bovine oocytes on DNA integrity of cumulus cells and subsequent embryo development. Reprod Domest Anim 2013;48:905-911. doi: 10.1111/rda.12184
51. Rosa DE, Anchordoquy JM, Anchordoquy JP, et al: Analyses of apoptosis and DNA damage in bovine cumulus cells after in vitro maturation with different copper concentrations: Consequences on early embryo development. Zygote 2016;24:869-879. doi: 10.1017/S0967199416000204
52. Anchordoquy JM, Anchordoquy JP, Nikolo N, et al: High copper concentrations produce genotoxicity and cytotoxicity in bovine cumulus cells. Environ Sci Pollut Res Int 2017;24:20041-20049. doi: 10.1007/s11356-017-9683-0
53. Anchordoquy JP, Anchordoquy JM, Sirini MA, et al: The importance of manganese in the cytoplasmic maturation of cattle oocytes: blastocyst production improvement regardless of cumulus cells presence during in vitro maturation. Zygote 2016;24:139-148. doi: 10.1017/S0967199414000823
54. Rojas MA, Dyer IA, Cassatt WA: Manganese deficiency in the bovine. J Anim Sci 1965;24:664-667. doi: 10.2527/jas1965.243664x
55. Van Emon M, Sanford C, McCoski S: Impacts of bovine trace mineral supplementation on maternal and offspring production and health. Animals 2020;10:2404. doi: 10.3390/ani10122404
56. Maywald M, Wessels I, Rink L: Zinc signals and immunity. Int J Mol Sci 2017;18:2222. doi: 10.3390/ijms18102222
57. Nazari A, Dirandeh E, Ansari-Pirsaraei Z, et al: Antioxidant levels, copper and zinc concentrations were associated with postpartum luteal activity, pregnancy loss and pregnancy status in Holstein dairy cows. Theriogenology 2019;133:97-103. doi: 10.1016/j.theriogenology.2019.04.034
58. Wooldridge LK, Nardi ME, Ealy AD: Zinc supplementation during in vitro embryo culture increases inner cell mass and total cell numbers in bovine blastocysts. J Anim Sci 2019;97:4946-4950. doi: 10.1093/jas/skz351
59. Hansard SL, Mohammed AS, Turner JW: Gestation age effects upon maternal-fetal zinc utilization in the bovine. J Anim Sci 1968;27:1097-1102. doi: 10.2527/jas1968.2741097x
60. Sackett R, McCusker RH: Multivalent cations depress ligand affinity of insulin-like growth factor-binding proteins-3 and -5 on human GM-10 fibroblast cell surfaces. J Cel Biochem 1998;69:364-375. doi: 10.1002/(SICI)1097-4644(19980601)69:3<364::AID-JCB13>3.0.CO;2-C

61. McCusker RH, Kaleko M, Sackett RL: Multivalent cations and ligand affinity of the Type 1 insulin-like growth factor receptor on P2A2-LISN muscle cells. J Cell Phys 1998;176:392-401. doi: 10.1002/(SICI)1097-4652(199808)176:2<392::AID-JCP18>3.0.CO;2-5
62. Freedman LP: Anatomy of the steroid receptor zinc finger region. Endocr Rev 1992;13:129-145. doi: 10.1210/edrv-13-2-129
63. Picco SJ, Anchordoquy JM, de Matos DG, et al: Effect of increasing zinc sulphate concentration during in vitro maturation of bovine oocytes. Theriogenology 2010;74:1141-1148. doi: 10.1016/j.theriogenology.2010.05.015
64. Zhang N, Duncan FE, Que EL, et al: The fertilization-induced zinc spark is a novel biomarker of mouse embryo quality and early development. Sci Rep 2016;6:22772. doi: 10.1038/srep22772. PMID: 26987302
65. Que EL, Duncan FE, Lee HC, et al: Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation. Theriogenology 2019;127:41-48. doi: 10.1016/j.theriogenology.2018.12.031
66. Burk RF: Molecular biology of selenium with implications for its metabolism. FASEB J 1991;5:2274-2279. doi: 10.1096/fasebj.5.9.1830557
67. Ceko MJ, Hummitzsch K, Hatzirodos N, et al: X-Ray fluorescence imaging and other analyses identify selenium and GPX1 as important in female reproductive function. Metallomics 2015;7:66-77. doi: 10.1039/c4mt00228h
68. Kommisrud E, Osterås O, Vatn T: Blood selenium associated with health and fertility in Norwegian dairy herds. Acta Vet Scand 2005;46:229-240. doi: 10.1186/1751-0147-46-229
69. Van Saun RJ, Herdt TH, Stowe HD: Maternal and fetal selenium concentrations and their interrelationships in dairy cattle. J Nutr 1989;119:1128-1137. doi: 10.1093/jn/119.8.1128
70. Lequarré AS, Feugang JM, Malhomme O, et al: Expression of Cu/Zn and Mn superoxide dismutase during bovine embryo development: influence of in vitro culture. Mol Reprod Dev 2001;58:45-53. doi: 10.1002/1098-2795(200101)58:1<45::AID-MRD7>3.0.CO;2-J
71. Hidiroglou M, Shearer DA: Concentration of manganese in the tissues of cycling and anestrous ewes. Can J Comp Med 1976;40:306-309. PMCID: PMC1277770
72. Wilson JG: Bovine functional infertility in Devon and Cornwall–response to manganese therapy. Vet Rec 1966;79:562-566. doi: 10.1039/c4mt00228h
73. Trumbo P, Yates AA, Schlicker S, et al: Dietary reference intakes: vitamin a, vitamin k, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. J Am Diet Assoc 2001;101:294-301. doi: 10.17226/10026
74. Rhinehart JD, Starbuck-Clemmer MJ, Flores JA, et al: Low peripheral progesterone and late embryonic/early fetal loss in suckled beef and lactating dairy cows. Theriogenology 2009;71:480-490. doi: 10.1016/j.theriogenology.2008.07.031
75. Leach Jr, RM: Role of manganese in the synthesis of mucopolysaccharides. Fed Proc 1967;26:118-120.
76. Hansen SL, Spears JW, Lloyd KE, et al: Growth, reproductive performance, and manganese status of heifers fed varying concentrations of manganese. J Anim Sci 2006;84:3375-3380. doi: 10.2527/jas.2005-667
77. Hansen SL, Spears JW, Lloyd KE, et al: Feeding a low manganese diet to heifers during gestation impairs fetal growth and development. J Dairy Sci 2006;89:4305-4311. doi: 10.3168/jds.S0022-0302(06)72477-8
78. Schwertz CI, Bianchi RM, Vielmo A, et al: Nutritional chondrodysplasia in cattle in Brazil. Trop Anim Health Prod 2022;55:26. doi: 10.1007/s11250-022-03438-7
79. Corah LR, Dargatz D: Forage Analyses from Cow/Calf Herds in 18 States. Beef. Chapa. Cow Calf Health and Productivity Audit. Fort Collins CO: USDA: APHIS: VS, National Animal Health Monitoring System: 1996.
80. Herdt TH, Hoff B: The use of blood analysis to evaluate trace mineral status in ruminant livestock. Vet Clin North Am Food Anim Pract 2011:27:255-283. doi: 10.1016/j.cvfa.2011.02.004
81. National Academies of Sciences, Engineering, and Medicine: Nutrient Requirements of Beef Cattle. 8th edition, Washington, DC, USA; Subcommittee on Beef Cattle Nutrition, Committee on Animal Nutrition, Board on Agriculture, National Research Council. National Academy Press: 2016. doi: 10.17226/19398
82. Mortimer RG, Dargatz DA, Corah LR: Forage Analyses from Cow/Calf Herds in 23 States. Report: NAHMS Beef 1997. Fort Collins CO: USDA; 1999.
83. Rink L, Gabriel P: Zinc and the immune system. Proc Nutr Soc 2000;59:541-552. doi: 10.1017/s0029665100000781
84. Reffett JK, Spears JW, Brown TT: Effect of dietary selenium on the primary and secondary immune response in calves challenged with infectious bovine rhinotracheitis virus. J Nutr 1988;118:229-235. doi: 10.1093/jn/118.2.229
85. Enjalbert F, Lebreton P, Salat OJ: Effects of copper, zinc and selenium status on performance and health in commercial dairy and beef herds: Retrospective study. Anim Physiol Anim Nutr 2006;90:459-466. doi: 10.1111/j.1439-0396.2006.00627.x
86. Graham TW, Thurmond MC, Gershwin ME, et al: Serum zinc and copper concentrations in relation to spontaneous abortion in cows: implications for human fetal loss. J Reprod Fert 1994;102:253-262. doi: 10.1530/jrf.0.1020253
87. Segerson Jr, EC, Murray FA, Moxon AL, et al: Selenium/vitamin E: role in fertilization of bovine ova. J Dairy Sci 1977;60:1001-1005. doi: 10.3168/jds.S0022-0302(77)83978-7
88. Hedstrom OR, Maas JP, Hultgren BD, et al: Selenium deficiency in bovine, equine, and ovine with emphasis on its association with chronic diseases. In: Proceedings of the 29th Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians, 1986; p. 101-126. doi: 10.1016/S0749-0720(15)30034-7
89. Yamini B, Mullaney TP: Vitamin E and selenium deficiency as a possible cause of abortion in food animals. In: Proceedings of the 28th Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians, p. 131-144. doi: 10.1016/S0749-0720(15)30034-7
90. D'Aleo J, Shelford A, Fisher LJ: Selenium-sulphur interactions and their influence on fertility in dairy cattle. Can J Anim Sci 1983;63:999. In: Proceedings of the Annual Meeting of the Canadian Society of Animal Science. Nova Scotia Agricultural College, Truro, N.S., B2N 5E3 July 1983. doi: 10.4141/cjas83-116
91. Harrison JH, Conrad HR: Effect of selenium intake on selenium utilization by the nonlactating dairy cow. J Dairy Sci 1984;67:219-223. doi: 10.3168/jds.S0022-0302(84)81288-6

92. Harrison JH, Hancock DD: The role of selenium and Vitamin E deficiency in postpartum reproductive disease of the bovine. In: Hogan, J: editor. Proceedings of the Alvin Lloyd Moxon Honorary Lectures on Selenium and Vitamin E. Wooster, OH; The Ohio State University, Ohio Agricultural Research and Development Center: 1999.
93. Sordillo LM: Selenium-dependent regulation of oxidative stress and immunity in periparturient dairy cattle. Vet Med Int 2013;2013:154045. doi: 10.1155/2013/154045
94. Milatovic D, Gupta RC: Manganese In: Gupta RC: editor. Veterinary Toxicology: Basic and Clinical Principles, 3rd edition. Cambridge; Academic Press: 2018. p. 445-454.
95. Bazhora II, Shtefan EE, Timoshevskiĭ VN: The effect of microelements-copper, manganese and cobalt--on the antibody forming function of lymphoid tissue. Mikrobiol Zh 1974;36:771-776. PMID: 4475360
96. Dyer IA, Cassatt Jr, WA, Rao RR: Manganese deficiency in the etiology of deformed calves. BioScience 1964;14:31-32. doi: 10.2307/1293356
97. Hidiroglou M, Ivan M, Bryan MK, et al: Assessment of the role of manganese in congenital joint laxity and dwarfism in calves. Ann Rech Vet 1990;21:281-284. PMID: 2288454.
98. Boland MP, O'Donnell G, O'Callaghan D: The contribution of mineral proteinates to production and reproduction in dairy cattle. Biotechnology in the Feed Industry. In: Lyons TP, Jaques K: editors. Proceedings of the 12th Annual Symposium. Nottingham; University Press: 1996. p. 95-103.
99. Ballantine HT, Socha MT, Tomlinson DJ, et al: Effects of feeding complexed zinc, manganese, copper and cobalt to late gestation and lactating dairy cows on claw integrity, reproduction and lactation performance. Prof Anim Sci 2002;18:211-218. doi: 10.15232/S1080-7446(15)31524-2
100. Rabiee AR, Lean IJ, Stevenson MA, et al: Effects of feeding organic trace minerals on milk production and reproductive performance in lactating dairy cows: a meta-analysis. J Dairy Sci 2010;93:4239-4251. doi: 10.3168/jds.2010-3058
101. Kinal S, Korniewicz A, Jamroz D, et al: Dietary effects of zinc, copper and manganese chelates and sulphates on dairy cows. J Food Agric Environ 2005;3:168-172.
102. Ahola JK, Baker DS, Burns PD, et al: Effect of copper, zinc, and manganese supplementation and source on reproduction, mineral status, and performance in grazing beef cattle over a two-year period. Affiliations expand. J Anim Sci 2004;82:2375-2383. doi: 10.2527/2004.8282375x
103. Campbell MH, Miller JK, Schrick FN: Effect of additional cobalt, copper, manganese and zinc on reproduction and milk yield of lactating dairy cows receiving bovine somatotropin. J Dairy Sci 1999;2:1019-1025. doi: 10.3168/jds.S0022-0302(99)75322-1
104. Arthington JD, Larson RL, Corah LR: The effects of slow-release copper boluses on cow reproductive performance and calf growth. Prof Anim Sci 1995;11:219-222. doi: 10.15232/S1080-7446(15)31907-0
105. Muehlenbein EL, Brink DR, Deutscher GH, et al: Effect of inorganic and organic copper supplemented to first-calf cows on cow reproduction and calf health and performance. J Anim Sci 2001;79:1650-1659. doi: 10.2527/2001.7971650x
106. Lamb GC, Brown DR, Larson JE, et al: Effect of organic or inorganic trace mineral supplementation on follicular response, ovulation, and embryo production in super ovulated Angus heifers. Anim Reprod Sci 2008;106:221-231. doi: 10.1016/j.anireprosci.2007.04.007
107. Dufty JH, Bingley JB, Cove LY: The plasma zinc concentration of nonpregnant, pregnant and parturient Hereford cattle. Aust Vet J 1977;53:519-522. doi: 10.1111/j.1751-0813.1977.tb07935.x
108. Pryor WJ: Plasma zinc status of dairy cattle in the periparturient period. N Z Vet J 1976;24:57-58. doi: 10.1080/00480169.1976.34283
109. Jackson TD, Carmichael RN, Deters EL, et al: Comparison of multiple single-use, pulse-dose trace mineral products provided as injectable, oral drench, oral paste, or bolus on circulating and liver trace mineral concentrations of beef steers. Appl Anim Sci 2019;36:26-35. doi: 10.15232/aas.2019-01856
110. Pogge DJ, Richter EL, Drewnoski ME, et al: Mineral concentrations of plasma and liver after injection with a trace mineral complex differ among Angus and Simmental cattle. J Anim Sci 2012;90:2692-2698. doi: 10.2527/jas.2012-4482
111. Arthington JD, Swenson CK: Effects of trace mineral source and feeding method on the productivity of grazing Braford cows. Prof Anim Sci 2004;20:155-161. doi: 10.15232/S1080-7446(15)31290-0
112. Genther ON, Hansen SL: A multielement trace mineral injection improves liver copper and selenium concentrations and manganese superoxide dismutase activity in beef steers. J Anim Sci 2014;92:695-704. doi: 10.2527/jas.2013-7066
113. Renteria I, Bo G, Prado J, et al: Effects of trace mineral administration on pregnancy rate of multiparous cows submitted to fixed-time artificial insemination. In: Maraña D: editors. 7th International Symposium of Advances on Bovine Reproduction. Jalisco; Ovusem Guadalajara: July 2022. p. 267.
114. Perry GA, Perkins SD, Northrop EJ, et al: Impact of trace mineral source on beef replacement heifer growth, reproductive development, and biomarkers of maternal recognition of pregnancy and embryo survival. J Anim Sci 2021;99:160. doi: 10.1093/jas/skab160
115. Dantas FG, Reese ST, Filho RV, et al: Effect of complexed trace minerals on cumulus-oocyte complex recovery and in vitro embryo production in beef cattle. J Anim Sci 2019;97:1478-1490. doi: 10.1093/jas/skz005
116. Gao G, Yi J, Zhang M, et al: Effects of iron and copper in culture medium on bovine oocyte maturation, preimplantation embryo development, and apoptosis of blastocysts in vitro. J Reprod Dev 2007;53:777-784. doi: 10.1262/jrd.18109
117. Anchordoquy JM, Picco SJ, Seoane A, et al: Analysis of apoptosis and DNA damage in bovine cumulus cells after exposure in vitro to different zinc concentrations. Cell Biol Int 2011;35:593-597. doi: 10.1042/CBI20100507
118. Lizarraga RM, Anchordoquy JM, Galarza EM, et al: Sodium selenite improves in vitro maturation of Bos primigenius taurus Oocytes. Biol Trace Elem Res 2020;197:149-158. doi: 10.1007/s12011-019-01966-2
119. Anchordoquy JP, Balbi M, Farnetano MA, et al: Trace mineral mixture supplemented to in vitro maturation medium improves subsequent embryo development and embryo quality in cattle. Vet Res Com 2022;46:1111-1119. doi: 10.1007/s11259-022-09982-9