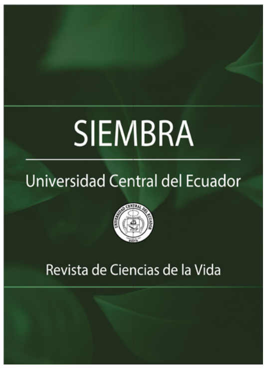
Micronutrient supplementation, health and efficiency of assisted reproduction in the female bovine

Suplementación de micronutrientes, salud y eficiencia de la reproducción asistida en la hembra bovina



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Abstract

Nutrition is a limiting factor in cattle productivity. Since information on micronutrient deficiencies and on the efficiency of the use of technologies applied in assisted reproduction technologies in pasture raised cows is scarce, it is important to know the influence of vitamin E, A, β-carotene and trace minerals such as selenium. The aim of this bibliographic review was to determine the influence of vitamin E, A, β-carotene and trace minerals such as selenium, copper, zinc, manganese and iron on the health and efficiency of assisted reproduction techniques in female cattle. These dietary components play an important role in the antioxidant mechanism and the responsiveness of the immune system in the body and are associated with parameters such as health status, reproductive performance and efficiency of assisted reproduction techniques. Deficiencies of some of these nutrients reduce the immune response, increase susceptibility to infections, reduce fertility and the efficacy of new reproductive biotechnologies, especially in pasture-raised cows in the tropics. Supplementation of these micronutrients can help improve the health, reproductive performance and technologies for estrus induction and synchronization, embryo transfer and *in vitro* embryo production in female cattle in areas where these nutritional deficiencies persist.

Keywords: micronutrients, health, reproduction, biotechnology.

Resumen

La nutrición es un factor que limita la productividad del ganado bovino. Dado que es escasa la información referente a las deficiencias de micronutrientes y su relación con la eficiencia en el uso de las tecnologías aplicadas en la reproducción asistida en vacas criadas en pastoreo, esta revisión bibliográfica tuvo como objetivo conocer la influencia de la vitamina E, A, el β-caroteno y los minerales trazas, tales como: el Selenio, el Cobre, el Zinc, el Manganeso y el Hierro sobre la salud y eficiencia en las técnicas de reproducción asistida de hembras bovinas. Estos componentes de la dieta juegan un importante papel en el mecanismo antioxidante y la capacidad de respuesta del sistema inmunológico en el organismo, y están relacionados con el estado de salud, el desempeño reproductivo y la eficiencia de las técnicas de reproducción asistida. Las carencias de

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algunos de estos nutrientes reducen la respuesta inmunológica, aumenta la sensibilidad a las infecciones, disminuye la fertilidad y la eficacia de las nuevas biotecnologías reproductivas, especialmente en vacas criadas en pastoreo en el trópico. La suplementación de estos micronutrientes puede contribuir a mejorar la salud, el desempeño reproductivo y las tecnologías de inducción y sincronización del celo, la transferencia y la producción *in vitro* de embriones en las hembras bovinas en aquellas áreas donde persistan estas deficiencias nutricionales.

Palabras clave: micronutrientes, salud, reproducción, biotecnología.

1. Introduction

Nutrition is an element that influences the state of health and reproduction. Notably, it is a factor that has a remarkable incidence on the correct functioning at the moment of incorporating assisted reproduction biotechnologies within the herd (Anchordoquy, 2012; Spears & Weiss, 2008).

Vitamin E, A, β-carotene and trace minerals such as Selenium, Copper, Iron, Manganese and Zinc are necessary for preserving reproductive health in cattle (Arthington & Ranches, 2021). Therefore, they are the subject of multiple studies in order to identify their deficiencies and the effect of their supplementation on health (Figueredo Rodríguez et al., 2017; Illek et al., 2021; Suttle, 2022), production and efficiency of estrus synchronization techniques (Pedroso Sosa & Roller Gutiérrez, 2024), as well as on the development of new biotechnological processes of reproduction (Rosa, 2015; Šmigoc et al., 2023).

These micronutrients play an important role in the exogenous and endogenous mechanisms of the antioxidant status of the organism and, therefore, how these contribute to mitigate oxidative stress, which is a term associated with the action of free radicals, and reactive oxygen species [ROS], affecting the functions of the cells that form the tissues, organs and systems of the body. In this sense, when there is not a correct relationship between the production of ROS, or different reactive species and the biological activity of exogenous (micronutrients) and enzymatic (endogenous) antioxidants of the organism, oxidative stress [OS] occurs, due to the lack of some of these micronutrients or as a consequence of certain physiopathological states that generate ROS (Coronado et al., 2015).

In this context, it is important to know the status of some micronutrients that are part of the body's antioxidant system given the role it plays in the maintenance of health and production and the efficiency of assisted reproduction techniques (Pedroso Sosa & Roller Gutiérrez, 2024).

This review aims to show the effect of supplementation of Vitamin E, Selenium [Se], Vitamin A, β-carotene, Copper [Cu], Iron [Fe], Manganese [Mn] and Zinc [Zn], on health and assisted reproductive efficiency in the bovine female.

2. Materials and Methods

For the development of the present study, a bibliographic information search was carried out in PubMed, Scopus, Web of Science and Web Animal Science electronic databases, from which different articles related to the use of micronutrients and their main impacts on health, reproduction and efficiency of assisted reproductive technologies were extracted. Key words used as search criteria were: oxidative stress, micronutrients, minerals, transition, health, fertilization, maturation, oocyte, supplementation, biotechnology and assisted reproduction.

3. Results and Discussion

3.1. Effect of supplementation of vitamin E, Selenium, β-carotene, Copper, Iron, Manganese and Zinc on the health, performance and efficiency of assisted reproduction in the bovine female

In cattle farming, the main micronutrients that influence cattle productivity have been accurately determined (Palomares, 2022). However, data on their effect when applying assisted reproduction techniques are scarce (Gao et al., 2007; Kendall et al., 2006). In this context, identifying deficiencies and understanding the effect of applying these micronutrients is of great interest in crossbred cattle farming raised by grazing in tropical climates (Pedroso Sosa & Roller Gutiérrez, 2024).

3.2. Effect of Vitamin E and Selenium supplementation

Vitamin E and Se act synergistically and are one of the most important and essential micronutrients for the functioning of the antioxidant and immune defense mechanism of the organism (Lizarraga, 2021). This process constitutes one of the first stages of the peroxidation process of polyunsaturated fatty acids by transferring phenolic hydrogen to free peroxide radicals (Xiao et al., 2021).

Deficiencies of vitamin E and Se produce a significant decrease in the activity and life span of neutrophils, macrophages and leukocytes (Suttle, 2022) and cause increased sensitivity to infectious agents, deterioration of the health status of cows. Therefore, these nutritional deficiencies are related to increased uterine infections, placental retentions, abortion, embryonic mortality, lower quality of colostrum in cows, giving rise to increased morbidity and mortality, the presence of white muscle disease in calves and increased prevalence of clinical and subclinical mastitis (Ceballos-Marquez et al. 2010; Illek et al., 2021).

Oral or injectable supplementation of vitamin E and Se, before and after calving, improves the immune response of dairy cows and their offspring (LeBlanc et al., 2014). Therefore, supplementation of this vitamin and Se, during late gestation, decreased the incidence of retained placenta (Bourne et al., 2007; Gupta et al., 2005; Silvestre, 2008), and significantly reduced the prevalence of clinical and subclinical mastitis (Thatcher et al., 2010). While, in early lactation, it will strengthen the cow's immune system and the adequate delivery of immunoglobulins to the calf through colostrum, decreasing perinatal morbidity and mortality in calves (Salles et al., 2022). However, it should be noted that there are some divergent results where it has not been possible to clarify whether this action was caused by Selenium, or its synergic action with Vitamin E (Horn et al., 2010; Rutigliano et al., 2008).

Studies developed in the Czech Republic, in areas in which Se deficiency had been confirmed, showed a significant effect of Se supplementation with organic sources on the production of immunoglobulins, confirmed in their content in maternal blood and colostrum fed to their offspring. This significantly decreased calf morbidity and mortality (Andělová et al., 2024; Illek, 2021).

The impact of vitamin E and Se substitution on the efficacy of estrus synchronization in postpartum anestrus buffaloes was studied by Mujawar et al. (2019). The results of this experiment showed a higher pregnancy rate (12%) in the supplemented group of cows when compared to the unsupplemented control group.

Heat stress can change the physiological and productive conditions of mammals and is one of the causes of bovine infertility in the tropics. Thus, it has been postulated that given the contribution of vitamin E and Se in the production of progesterone at the level of the ovary and its role in the enzymatic system of the antioxidant mechanism of the organism, it is possible to affirm that supplementation can contribute to the increase of the conception rate and diminish the effect of oxidative stress that accompanies heat stress in hot climates. In this regard, it was demonstrated that the supply of Se and Vitamin E prior to estrus synchronization in cows during the period of higher environmental temperature improves the activity exerted by enzymes with antioxidant properties and manages to reduce the impact of oxidative stress. Also it increases the pregnancy rate during the hot season (Shaarawy et al., 2024).

Regarding the influence of Selenium supplementation on the efficiency of embryo transfer techniques, Moraes et al. (2012) developed a research focused on evaluating the effect of Selenium supplementation and demonstrated as results that the Oocyte Follicular Aspiration [OPU] and the *in vitro* production process in Jersey breed donor, when using the supplementation of 9.6 mg of selenium daily in the diet, produced the increase of the total number of collected Oocyte of grade I and II with respect to a control group ($p < 0.05$). This result may be attributed to the protective effect exerted by this microelement against oxidative damage. Based on these findings, they recommended to implement this supplementation strategy in order to improve the quantity and quality of the collected oocytes, and increase the rate of embryos obtained during the production process *in vitro* (Table 1).

In summary, it is evident that Vitamin E and Se supplementation during the transition period, and prior to the implementation of assisted reproductive technologies in females would be appropriate, emphasizing their importance in areas where they are found to be deficient and in herds under heat stress conditions. This can contribute to improve the health of cows, their offspring, post reproductive performance and the efficiency of assisted reproduction.

Table 1. Results of oocyte collection and quality of oocytes obtained by OPU and embryo production by *in vitro* fertilization process in Jersey cows supplemented with selenium 3.2 mg d^{-1} or 9.6 mg d^{-1} for 119 days.

Characteristics	Supplemented with 3.2 mg d^{-1} of Sodium selenite	Supplemented with 9.6 mg d^{-1} of Sodium selenite
Viable oocytes	23.10±2.16^{b}	35.11±2.65^{a}
Grade-I oocyte	4.75±0.97^{b}	11.61±1.58^{a}
Grade II oocyte	4.57±0.98	7.17±1.32
Grade III oocyte	8.90±1.32^{b}	16.40±2.10^{a}
Naked oocytes	////////////////	////////////////////////
Non-viable oocyte	5.48±1.16^{b}	11.23±1.65^{a}
Atretic oocyte	4.49±1.08^{b}	10.68±1.62^{a}

Source: Modified from Moraes et al. (2012).

In summary, it is evident that Vitamin E and Se supplementation during the transition period, and prior to the implementation of assisted reproductive technologies in females would be appropriate, emphasizing their importance in areas where they are found to be deficient and in herds under heat stress conditions. This can contribute to improve the health of cows, their offspring, post reproductive performance and the efficiency of assisted reproduction.

3.3. Effect of vitamin A and β-carotene supplementation

Vitamin A and β-carotene are substances known for their antioxidant activity, attributable to their role on the elimination of simple oxygen radicals and on the presence of thiol radicals, considering that vitamin A is essential in the integrity of epithelia, the functionality of the immune system and reproductive functions. Meanwhile, β-carotene is important during the process of vitamin A synthesis, in reproduction and thyroid functions (Ikeda et al., 2005). Deficiency of these micronutrients has been associated with delayed ovulation and a proportional increase in abortions, embryonic mortality and the risk of ovarian cysts.

These dietary components are involved in the follicular growth of steroids generated by granulosa and theca cells, and are part of the composition of the environment of the oviduct and uterus. They protect ovarian and uterine cells from the effects of oxidative stress. In addition, they have a beneficial effect on the function of corpus luteum.

Within the productive parameters, the presence of vitamin A in cattle ranges from 25 to 85 µg dl^{-1} while those of β-carotenes range from 300 to 1,200 µg dl^{-1}, respectively. On the other hand, values lower than 7 µg dl^{-1} of vitamin A or 100 µg dl^{-1} of β-carotene would imply a deficiency. Diagnosis can be established by determination of their values in blood serum, or liver reserves.

In different studies it has been shown that supplementation of Vitamin A and β-carotene for more than 40 days can reduce the negative effect of heat stress, improve conception rate, embryo survival and the resumption of ovarian activity after calving. This result is probably associated with the role of these compounds in the control mechanism of oxidative stress, especially in dairy cows reared in hot climates.

In some studies, vitamin A supplementation of cows prior to super ovulatory treatment has been reported to achieve a significant increase in the total number of quality embryos (Rani & Panneerselvam, 2001; Sales et al., 2008). Similarly, a positive correlation has been described between the level of β-carotene in the blood of dairy cows with the number of transferable embryos obtained after super ovulatory treatment. In this context, it has been suggested that these micronutrients have a marked influence on the competence of the oocytes and the uterine environment (Ikeda et al., 2005; Livingston et al., 2004).

Trojačanec et al. (2012) found a high correlation in β-carotene values, on the day of insemination and seven days after service, with progesterone concentration ($r = 0.33$, $p < 0.05$), and conception rate ($r = 0.51$, $p < 0.01$). The data suggest that the treatment improved the ability to synthesize and secrete progesterone by the corpus luteum. These findings were later corroborated by Aguiar-Zalzano and Rojas-Bourrillon (2015), who found a positive correlation between β-carotene levels and the establishment of early pregnancy. Cows with β-carotene levels above 587 mg dl^{-1} had a higher pregnancy rate.

In this same sense it would be beneficial and helpful to understand the status and quality of these specific elements that make up the diet of dual-purpose crossbred cows raised in grazing systems, especially considering that there is a remarkable paucity of information available on this particular topic in dry tropical regions.

3.4. Effect of Copper, Iron, Manganese and Zinc supplementation

Recent studies indicate that metabolic and endocrine changes in the blood affect the content of follicular fluid and uterine fluids, influencing fertilization and embryo development (De Bie, 2017; LeBlanc, 2014; Leroy et al., 2015). In that direction, it is important to highlight that there is little scientific information on grazing cows in areas with micronutrient deficiencies (Kor et al., 2013; Pedroso & Roller, 2009). Consequently, it can be appreciated that deficiencies of these nutrients affect the efficiency of new breeding biotechnologies and that this effect can be minimized by supplementation of these micronutrients.

Supplementation of microelements in deficient bovine females improves calving and pregnancy rates, reduces calving intervals and reduces abortions (García Díaz et al., 2013; Pedroso Sosa & Roller Gutiérrez, 2024). Similar results have been obtained by oral or parenteral supplementation of copper, zinc, iron and manganese (García et al., 2007). However, not all the research carried out has been able to demonstrate the beneficial effect of this supplementation on the dynamics of growth and development of the ovarian follicle, pregnancy rate and embryo quality (Hackbart et al., 2010; Vanegas et al., 2004), when the basic diet provides adequate levels of these components and there are no factors that lead to the manifestation of these deficiencies. This suggests that prior diagnosis of these deficiencies is a priority to obtain satisfactory results in the different supplementation strategies.

Experimental evidence supports that deficiencies of these micronutrients in blood and reserve tissues are reflected in the composition of follicular fluid and the uterine environment (Nocek et al., 2006; Rabiee et al., 2010). It is likely that its deficiency may affect oocyte and uterine competence (Leroy et al., 2015).

One of the most significant studies carried out for this purpose was the one developed by Sales et al., (2011), who demonstrated that the inclusion of microelements (Cu, Fe, Mn and Zn) improves the reproductive performance of dairy cows and directly on the results of assisted reproduction. According to the data from this experiment, parenteral supplementation of these microelements prior to embryo transfer in crossbred cows (*Bos indicus* x *Bos taurus*) in Brazil resulted in a significant increase in embryo survival rate (Table 2).

Table 2. Effect of subcutaneous administration of 5 ml of an injectable microelement preparation on the embryo survival rate (%) in crossbred heifers (*Bos indicus* x *Bos taurus*) at 23 and 48 days post embryo transfer.

Variables	N	Control*	Treated group*
Embryonic survival at 23 days	276	36^a	48^a
Embryonic survival at 48 days.	219	30^b	43^b

Source: Adapted from Sales et al. (2011).

* Different letters per line differ from each other at $p < 0.005$. The treatment was administered 17 days prior to embryo transfer; the injectable microelement compound contained 100 mg Zn, 100 mg Mn, 50 mg Cu and 25 mg Se (Multimin®,Minerthal, Brazil).

Copper deficiency is a common problem in ruminants, being the second most frequent problem in grazing cattle after phosphorus (Picco et al., 2005), in several cattle producing countries: Great Britain, Holland, Scotland, New Zealand, Canada, Australia and USA and in the Czech Republic. In Latin America, cases have been reported in Argentina, Brazil and Mexico (Picco et al., 2005).

In Cuba, copper deficiency has been found in cattle herds throughout the country (García Díaz et al; 2013). These studies found low values in serum and liver tissue, associated with anestrus, repeat service, retained placentas, abortion, delayed puberty and higher prevalence of mastitis (Torres et al., 2016) and low efficiency in the application of assisted reproduction techniques (Rosa, 2015; Pedroso Sosa & Roller Gutiérrez, 2024). In these territories, Cu deficiency was associated with brackish water consumption, low availability in the combination of minerals and pasture content. In addition, to a state of metabolic acidosis (Pedroso & Roller, 2009). These findings were more common during the rainy season, in the transition period of the cows in the crossbred genotypes *Bos indicus* x *Bos Taurus*.

The diagnosis of hypocuprosis is approached by identifying the deficiency in animals and investigating its causes. The former reveals the Cu balance in animals and the latter includes environmental studies that identify risk zones and the epidemiological behavior of the disease. However, no clear parameter has been determined to indicate when animals suffer alterations due to hypocuprosis (Nicastro et al., 2021).

In several experiments developed based on oral or parenteral copper supplementation in grazing crossbred cows (Table 3, Figures 1 and 2), it was demonstrated that different procedures of adequate supply of this microelement contributed to improve reproductive efficiency (García Díaz et al., 2013), the immunological response capacity, decreased the prevalence of mastitis and significantly improved the fertility of induction treatments and better estrus synchronization in dairy cows, hypocupremic cows in the climatic conditions of the humid tropics kept in pasture (Torres et al., 2016; Pedroso Sosa & Roller Gutiérrez, 2024).

Table 3. Effect of prenatal copper supplementation (50 mg) before and after calving on the risk index of contracting mastitis in 5/8 Holstein x 3/8 Brahman cows under tropical climatic conditions.

Risk	Estimation	CI (95%)	
Risk in exposed	0.37	-	-
Risk in unexposed	0.62	-	-
Relative risk	0.59	0.35	0.97
Difference in risks	-0.25	-0.48	-0.03
Prevented fraction in exposed individuals	0.40	0.02	0.64
Population prevented fraction	0.20	0.01	0.32

Source: Figueredo Rodríguez et al. (2017).

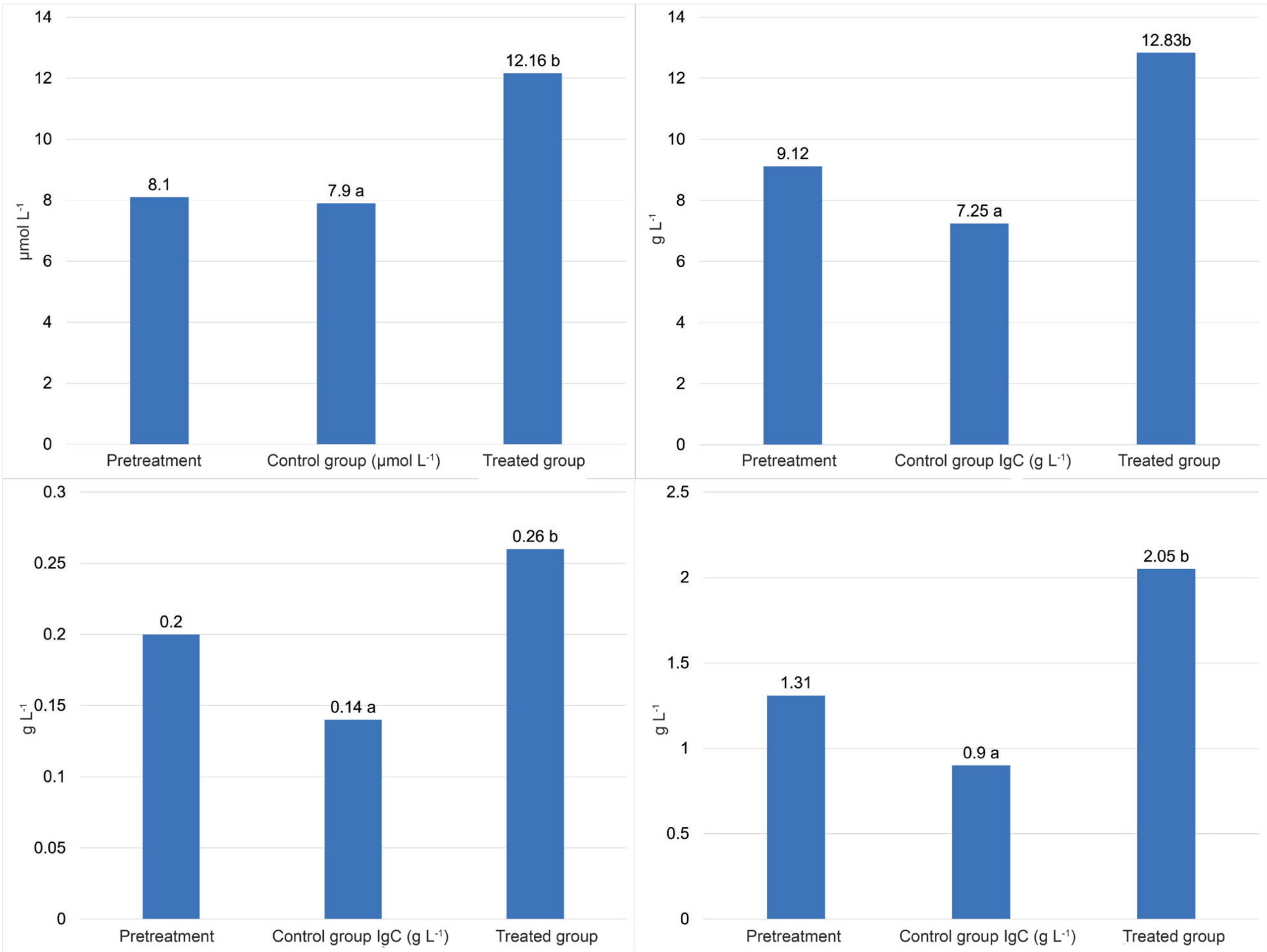

Figure 1. Effect of parenteral copper supplementation (50 mg) in hypocupraemic (mild) cows on immunoglobulin and copper levels in the blood serum of 5/8 Holstein x 3/8 Brahman cows in the tropics.*

Source: Figueredo Rodríguez et al. (2017).

* Different letters per column are significantly different at $p < 0.05$.

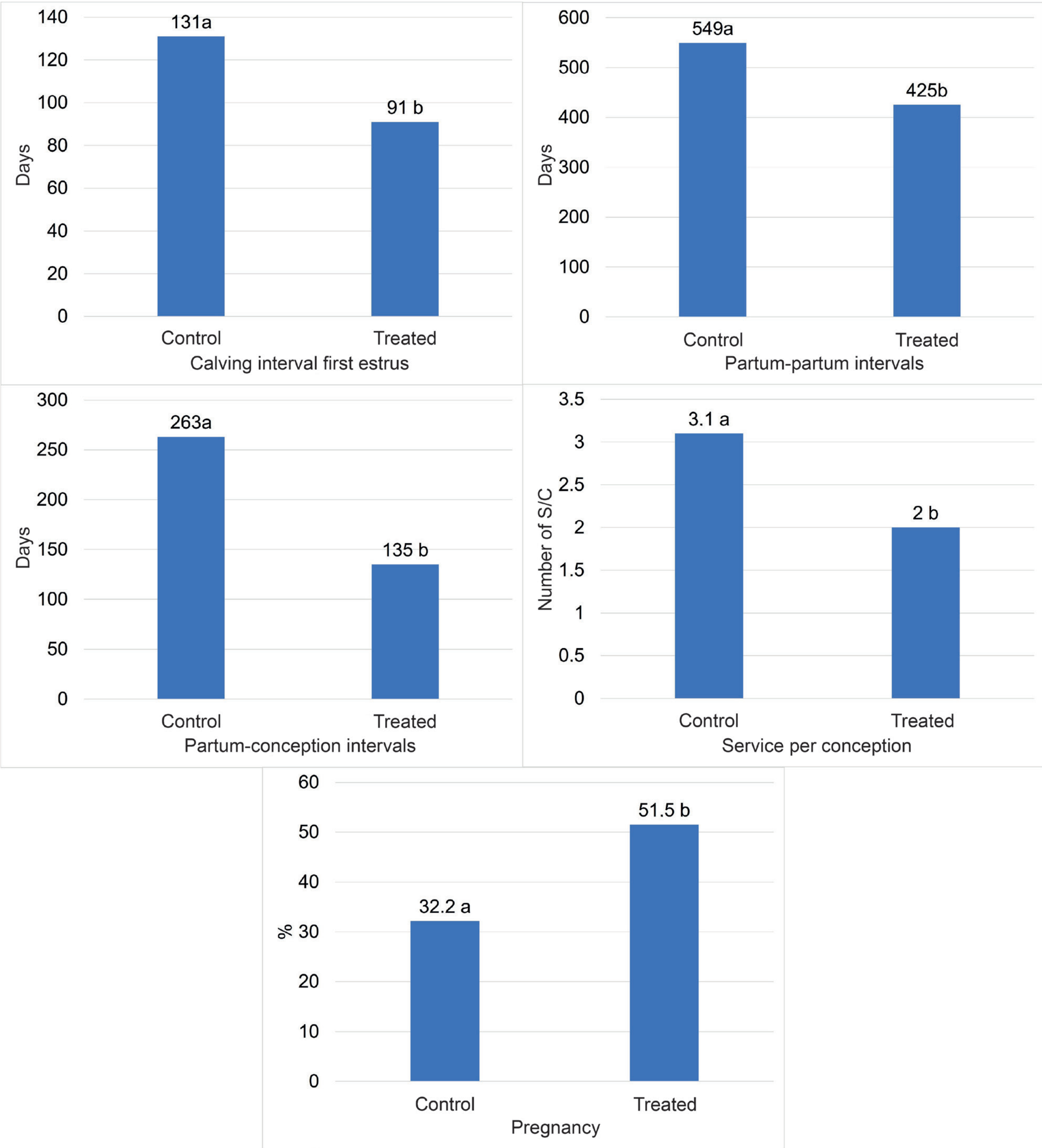

Figure 2. Reproductive response of 5/8 Holstein x 3/8 Brahman hypocupraemic cows supplemented with parenteral copper before and after calving in the humid tropics.*

Source: Figueredo Rodríguez et al. (2017).

* Different letters per column are significantly different at $p < 0.05$.

Regarding the supplementation of these microelements in *in vitro* embryo production media, several studies have been developed to evaluate the role of this microelement in the competence of the oocyte for fertilization maturation and embryo development.

In this regard, Kendall et al. (2006) conducted a study to determine the effect of Cu supplementation to culture media on the functions of theca cells. According to the research data, Cu supplementation in serum-free cultures was shown to enhance the differentiation of teak cells *in vitro*. These findings may explain cases of infertility related to functional disorders of the hypothalamic-pituitary-ovarian axis. These are clinically manifested as anestrus, irregular and anovulatory cycles, influenced by the presence of ovarian hormones and their effect on the release of GnRH and the hormones FSH and LH.

Later, Gao et al. (2007) evaluated the effect of copper on the maturation and growth of *in vitro* fertilized ewes, where the content of these minerals in follicular liquor of various diameters was determined before and after treatment. According to the results of this research, the addition of Cu to the maturation medium improved the maturation rate and the number of embryos in the process of *in vitro* embryo production [IVP]. According to the results, it is likely that subfertility originating from Cu deficiencies is a consequence of the alteration in the growth and development patterns of ovarian follicles, as well as in the process of steroidogenesis (Kendall et al., 2006). It is evident that the incorporation of Cu, in amounts that are comparable to those that have been reported as normal for a healthy animal, when used in different IVP media, could have a positive and beneficial impact on the maturation process, and on the development of the embryo that has been produced by *in vitro* techniques.

In this context, Rosa (2015) pointed out, that the Cu status in blood plasma determines the content of follicular fluid in the ovary in the bovine female. Consequently, in animals deficient in this microelement may decrease the efficiency of *in vitro* embryo culture. Studies indicate that the addition of Cu in the maturation medium increased the concentration of GSH-GSSG, reduced DNA damage apoptosis, and improved blastocyst rate and quality. (Santiago Hernández, 2020).

This means that the addition of Cu to the *in vitro* culture media in a range of normal blood plasma values, on the one hand, contributed to improve the antioxidant response capacity of the cells and, on the other hand, favored embryo development (Figure 3). This fact could explain why the embryos obtained during the summer season, where heat and oxidative stress in the tropics is manifested with greater magnitude, have a lower competence for implantation and maintenance of pregnancy and.

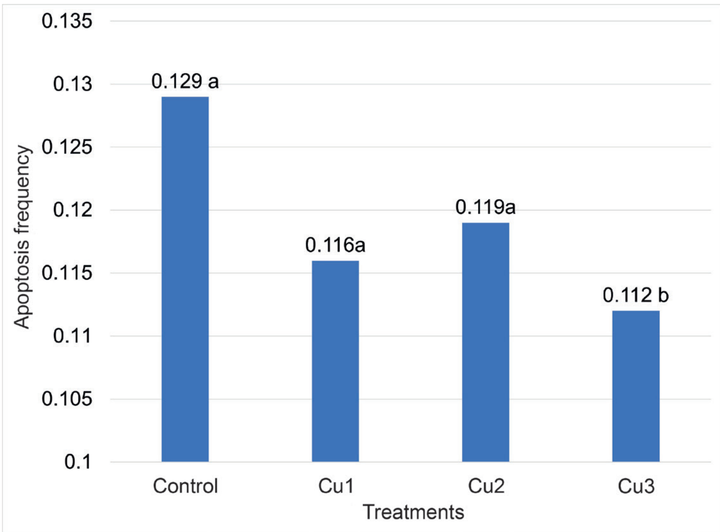

Figure 3. Effect of different copper concentrations during PIE on the apoptosis rate of cumulus cells.*

Source: Rosa (2015).

* Bovine COCs were incubated in IVM medium unsupplemented (Control) or supplemented with Cu: Cu1) 20 µg dl^{-1}; Cu2) 40 µg dl^{-1} and Cu3) 60 µg dl^{-1}. 800 COCs distributed in four replicates were matured on different days (200 COCs per replicate, 50 COCs per treatment). A total of 500 COCs per treatment were analysed.

Iron is a vital component in animal metabolism, especially in cellular respiration processes. This microelement exists in the organism in a complex form linked to proteins and enzymes. Iron requirements range from 30 to 60 ppm. Nevertheless, the requirements in calves less than three months of age, fed exclusively on milk, can reach the figure of 100 ppm, since the Fe content in milk is low (Suttle, 2022).

Iron deficiency is rare in grazing cattle, except when the incidence of parasitic infestations is high. This is because the main source of Fe is forage, which contains approximately 200 mg kg^{-1} of Fe. This figure can amply cover the requirements of the organism in this species. However, it is important to note that the iron contained in the soil may be poorly bioavailable.

In general, iron deficiency causes microcytic hypochromic anemia and decreases tranferrin saturation capacity, although there is not much information regarding the effect of iron deficiency and reproductive behavior in ruminants. However, low hemoglobin and hematocrit levels have been associated with high incidence of repeated estrus and increased incidence of tick parasitosis (Pedroso, 2003).

For the diagnosis of Fe deficiencies, blood serum concentration, hemoglobin values and hematocrit can be used, although these indicators do not change until the hepatic reserves are affected. Therefore, the determination of the transferritin saturation value is the most appropriate means to establish a prophylactic diagnosis (McDowell y Arthington, 2005).

Iron deficiencies are not common in grazing cattle. However, recent studies conducted by Gao et al. (2007) to determine the role of iron in the IVF medium are of interest. In this experiment, it was found that the demand for this micronutrient was highest 48 hours after the start of the process, and that the addition of a Fe concentration of 1.96 mg l^{-1} to IVF media improves the rate of blastocyst development and reduces the incidence of apoptosis in bovine embryos. (Table 4).

Table 4. Effect of Fe concentration *in vitro* embryo culture medium on the rate of maturation and subsequent embryonic development.

Number of matured oocytes			**Percentage of developed embryos ***			
Concentration of (Fe mg l^{-1}) added to the Médium	**Oocytes**	**Number (%) of matured oocytes**	**2 cells**	**8 cells**	**Mora**	**Blastocysts**
3,26	254	208	122 (58,7)	108(51,9)[a]	101(48,0)[a]	65 (31,2)[a]
1,96	243	185	123(66,5)	114(61,6)[a]	94(50,8)[a]	78(42,2)[b]
0,81	271	209	128 (61,1)	118 (56,5)[a]	73(34,9)[b]	55(26,3)[a]
0,45	240	193	122 (63,4)	102(52,3)[a]	65(33,7)[b]	47(24,3)[a]
Control	201	158	101(63,9)	60 (38,0)[b]	35(22,2)[c]	22(13,9)[c]

Source: Adapted from Gao et al. (2007).

* Different letters per column are significantly different at $p < 0.05$.

Manganese deficiencies are not frequent in the tropics (McDowell & Arthington, 2005). But, this microelement is necessary for the conformation of bone structure, reproduction and normal function of the immune system, nervous system and the enzymatic mechanism of the antioxidant system (Wang et al., 2017).

Manganese acts mainly as an activator of enzymes such as hydrolases, kinases, decarboxylases and transferases; these, are activated by binding the metal directly to the protein or substrate. Many activations are nonspecific, so Mn can be substituted by magnesium, an exception being glycosyltransferase, which is only activated by Mn. Mn deficiency reduces glycosyltransferase activity, decreasing the synthesis of mucopolysaccharides in epiphyseal cartilage and affecting bone growth; one of the most common clinical signs in young people with a lack of this mineral is skeletal deformation (Anchordoquy, 2012).

Mn activates several enzymes, such as pyruvate carboxylase, arginase and glutamine synthetase, which are firmly associated with the protein portion and cannot be removed without losing enzymatic activity (Vásquez Loaiza & Molina Coto, 2021). Pyruvate carboxylase catalyzes the first step of gluconeogenesis and regulates carbohydrate metabolism. Arginase is an enzyme that converts L-arginine to L-ornithine and urea, which is fundamental in the urea cycle and eliminates nitrogenous residues from protein catabolism (di Costanzo et al., 2010). In this sense, the lack of Mn leads to a reduction of the enzyme activity, increasing blood ammonium and decreasing plasma urea.

Recently, it is considered that the most important role of Mn in bovine reproduction is through its antioxidant capacity by forming a structural part of the enzyme Superoxide Dismutase manganese-dependent [Mn-SOD], located in the intracellular liquor and mitochondria of ovarian cells, protecting them from OS. Given that the processes of follicle growth and development, ovulation, steroid synthesis and production involve inflammatory events that generate ROS, it is important to note that these processes are not only a consequence of oxidative stress but also of the development of follicle growth and development, ovulation, steroid synthesis and production (Bidne et al., 2018)

This enzyme is an important factor contributing to the establishment between ROS production and the antioxidant mechanism, contributing to the proper growth and development of the preovulatory follicle, oocyte maturation, steroid production and synthesis by ovarian cells, with emphasis on corpus luteum progesterone production and embryo survival (Wang et al., 2017; Rodriguez-Campos et al., 2020).

Some studies were directed to evaluate the effect of Mn supplementation to *in vitro* embryo production media. In this regard, Anchordoquy (2012) conducted a research in order to determine the levels of Mn in plasma and follicular liquor and to evaluate the effect of Mn supplementation to *in vitro* cultured oocyte maturation media. According to the results, no significant differences in Mn concentration were found between blood plasma and those found in follicular liquor. However, in both cases these were higher than those in the *in vitro* culture media. The addition of Mn to the maturation medium (2.3, 4.3 and 6.3 µmol L^{-1}), increased the concentration of glutathione [GSH], and SOD activity in the oocyte and cumulus cells, decreased DNA damage and the rate of apoptosis. But, although the cleavage rate was not changed, embryo quality and developmental speed was higher in the medium where Mn was supplemented. These experimental findings have provided insights that partly explain one of the possible mechanisms by which manganese deficiencies affect reproduction, as well as the efficiency of *in vitro* fertilization technology in cattle.

In an experiment where supplementation of the basal diet with 80 mg Mn kg^{-1} DM -using manganese glycine-, increased serum glucose concentrations and decreased BCS losses, supplementation of the basal diet with 80 mg Mn kg^{-1} DM increased the presence of CL in the ovaries, and improved the pregnancy rate in the second service (Qashqai et al., 2020).

Zinc is an essential dietary component for the maintenance of health and development in ruminants. Its primary function appears to be related to its association with the activity of multiple enzymes, and the stabilization of RNA, DNA and ribosome structures. In addition, it has many interactions with the production, storage and secretion of several hormones involved in all female and male reproductive processes in the bovine species (Suttle, 2022).

Zinc deficiencies result in decreased feed intake, growth rate and feed efficiency. Zinc plays an important role in the immune system (Spears & Weiss, 2008), and is required for the proper functioning of the enzyme superoxide dismutase of the antioxidant system. It is essential in the integrity of epithelial tissue and the formation of keratin thereby providing a powerful physiological barrier to infection. This microelement is a component of transcription factors including DNA-binding proteins and over 300 enzymes including Superoxide Dismutase and plays an important role in protecting the cell from oxidative and DNA damage. Consequently, it is involved in gene function and stability, increased risk to the prevalence of anestrus, repeated estrus, spontaneous abortion (Ceylan et al., 2008), and embryonic development. At present, there are few studies aimed at determining the effect of zinc deficiency on the efficacy of *in vitro* fertilization technology. It is likely that females destined for these purposes require prior supplementation with this micronutrient, due to the role it plays in cell function and embryonic development.

Insufficiencies of this microelement cause a rapid decrease of its content in blood, tissues (liver, bone, hair), and are accompanied by a significant decrease in the activity of the enzyme phosphatase in blood plasma. The diagnosis of this deficiency can be established by determining Zinc in blood, liver, bone or hair samples. Zn is forming a binary, and tertiary complex with Glutathione. Zn deficiencies can cause a significant decrease in reduced glutathione. Zn status in cattle is defined as marginal and normal when plasma concentrations are between 0.2 to 0.4 and 0.5 to 0.8 g ml^{-1}, respectively.

The influence of Zinc deficiencies on the efficacy of new reproductive biotechnologies was addressed by Picco et al. (2012), who studied the effect of Zinc supplementation in bovine oocyte maturation media *in vitro*, and evaluated the consequences on DNA and cumulus cell integrity, intracellular concentration of zinc-dependent Glutathione-Peroxidase in oocytes, cumulus cells and furthermore, the developmental capacity of mature oocytes. According to the results of this experiment, supplementation of 0.7, 1.1 and 1.5 µg ml^{-1} of Zn to the maturation media induced a significant increase in cell division and Glutathione [GSH] concentration and decreased DNA damage in oocytes and cumulus cells. This indicates that providing an appropriate value of Zn to bovine oocyte maturation and *in vitro* fertilization media is likely to enhance the intracellular activity of the GSH enzyme, and enhance its role in the antioxidant defense mechanism, contributing to the maintenance of DNA integrity during the *in vitro* oocyte maturation process, and contributing to embryo development (Table 5).

Based on these reports, it would be beneficial to conduct research to evaluate the impact of supplementation of these micronutrients on the response to estrus induction and synchronization treatments, the *in vitro* fertilization process, and embryo transfer under tropical conditions. These studies could contribute to the development of new strategies to improve the efficiency of these reproductive control procedures, and the efficacy of new biotechnologies, especially in regions where animals are fed on pasture and deficiencies of these micronutrients are frequent.

Table 5. Development capacity of the bovine oocyte fertilized and matured *in vitro* with various concentrations of Zinc.

	Zn concentrations (μg ml^{-1})*			
	0	**0.7**	**1.1**	**1.5**
Number of Oocyte	313	339	309	304
Divided Oocytes	67.16± 1.17 a	73.15 ±1.15b	74.0±1.23b	72.76± 0.74b
Oocyte/Blastocyst Ratio	17.83 ± 2. 15^{a}	21.95± 0. 95^{a}	27.05±1.61b	30.33 ± 2.78c
Divided Oocytes/Blastocysts Ratio	26.62 ±3.24b	29.99 ± 1.07^{a}	36.48±3.09^{a}	41.82± 4.09b

Source: Adapted from Picco et al. (2012).
* Different letters per column are significantly different at $p < 0.05$.

Studies aimed at modifying the content of vitamins and micronutrients in the media for maturation, oocyte fertilization and embryo development during the IVF process can help to partially correct the consequences of deficiencies of these micronutrients in the diet on the structure and expression of some genes (Picco et al., 2005), and provide new insights into the pathophysiological mechanisms by which oxidative stress and micronutrient deficiencies affect health, reproduction and the efficiency of assisted reproductive technologies in the tropics (Picco et al., 2005).

Within this context, it is essential to point out that the current trend is to supplement micronutrients through mineral and vitamin complexes. These are supplied parenterally, or enriched mineral mixtures or nutritional blocks. The purpose of this supplementation is to stimulate the immune response, mitigate negative energy balance, OS, avoid epigenetic changes and improve the efficiency of assisted reproduction (De et al., 2014; Pedroso Sosa & Roller Gutierrez, 2024). Generally, these compounds contain vitamin A, E, Se, Cu, Zn, Fe and Mn; and are supplied during the transition period. However, many times it is not precisely known whether or not there is a deficiency of these micronutrients in the diet. As a consequence, it is common to find divergences in the efficacy of micronutrient supplementation (Rodríguez-Campos, 2020; Studer et al., 2022).

4. Conclusions

Vitamin E, Se, Cu, Fe, Mn and Zn deficiencies are related to the antioxidant capacity of the organism. Their deficiency is associated with the immunological response capacity, and with the efficiency of assisted reproduction techniques. *In vivo* corrected supplementation, prior to estrus induction treatments or their addition to *in vitro* bovine embryo production means, can contribute to improve the efficiency of this technology. Therefore, it would be useful to identify the deficiencies of these micronutrients in cows reared on pasture under tropical conditions, and then to evaluate the effect of supplementation on reproduction and the efficiency of assisted reproductive technology.

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Contributor roles

- Junior German Campozano Zambrano: conceptualization, visualization, writing – original draft.
- Rodolfo Pedroso Sosa: conceptualization, visualization, writing – original draft.
- Felicia Roller Gutiérrez: conceptualization, visualization, writing – original draft.

Ethical implications

Ethics approval not applicable.

Conflict of interest

The authors declare that they have no affiliation with any organization with a direct or indirect financial interest that could have appeared to influence the work reported.

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