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Effect of Mineral Supplementation on Reproductive Efficiency of Beef Cows

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To the Graduate Council:

I am submitting herewith a thesis written by Felipe Guirado Dantas entitled "Effect of Mineral Supplementation on Reproductive Efficiency of Beef Cows." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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Effect of Mineral Supplementation on Reproductive Efficiency of Beef Cows

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DEDICATION

I dedicate my work in memoriam of dearest grandfather Jose Guirado Garcia.

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ABSTRACT

Reproductive efficiency is the major component for an economically efficient cow/calf operation and can be influenced by a number of factors such as breed, age, health and nutrition. Among the components of the diet, trace minerals are hypothesized to have a major impact reproductive efficiency in beef cattle. In order to test this specific hypothesis, a study directly evaluating the success of reproductive performance in cows fed complexed trace minerals versus inorganic trace mineral was designed. In this study, 68 cows where equally divided into treatment (cows fed with complexed trace minerals) and control (cows fed with inorganic trace minerals) groups. The cows started being fed 30 days prior to AI (d -30). Twenty-eight days after AI (d 28), all cows where diagnosed for pregnancy and non-pregnant cows where removed from the experiment. Twenty-four days after pregnancy diagnosis (d 52), pregnant cows where submitted to ovum pick-up (OPU) and a second OPU was performed on d 67. Although pregnancy rates did not differ (P = 0.33) between treatment and control, cows fed with complexed trace minerals had increased oocyte recovery (P = 0.03), *in vitro* embryo production (P = 0.06) and more efficient *in vitro* embryo production (P = 0.06). In summary, the results from this experiment demonstrated that supplementation of beef cows with a complexed source of trace minerals improves reproductive efficiency when compared to cows fed inorganic source of trace minerals.

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INTRODUCTION

Pregnancy failures account for $1.2 billion loss for the beef cattle industry in the US. In order to have an economically sound operation, it's imperative to have high reproductive efficiency. The major bottleneck of reproductive efficiency is nutrition. Short et al. (1990) proposed an order of nutrient priority where the principal priority is basal metabolism, and the estrous cycle and reproduction is one of the last priorities of the animal.

Mineral supplementation, one of the components of nutrition, is often overlooked in pasture or range based operation. Based on nutritional requirements, minerals can be divided in two: macro minerals, minerals that are need in large quantities, and micro or trace minerals, minerals that are required in low concentrations in the diet. Although trace minerals are needed in low concentrations, there impact on reproductive performance can be profound.

Trace mineral source is an important factor to be considered. Previous studies report greater bioavailability of organic trace mineral when compared to an inorganic form of the same mineral. This greater bioavailability leads to an increase blood and liver concentration of trace mineral. Understanding the mechanisms by which trace minerals improve reproductive performance in cattle is crucial to developing mineral supplementation programs that improve reproductive performance, and consequently enhance the economic sustainability of cow/calf operations.

CHAPTER I

KEYS TO MAXIMIZING REPRODUCTIVE EFFICIENCY IN A BEEF HERD

Publication statement

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Introduction

The most powerful and influential technology to improve genetic and reproductive management in a beef herd available to producers is the synchronization of estrus/ovulation associated with artificial insemination (AI) [1]. The need for estrus detection is eliminated with the adoption of Fixed-time AI (FTAI) or TAI, and allows insemination of heifers and cows at a predetermined time. In addition, timed embryo transfer, or TET, is another method, which allows for direct transfer of embryos at a predetermined time without the need for estrus detection. Resulting pregnancy rates from FTAI are similar to insemination following detection of estrus (see Figure 1.1). Furthermore, FTAI and estrous

synchronization protocols increase the proportion of heifers and cows that conceive early in the breeding season which, has important benefits for reproductive management and beef production [2].

An efficient synchronization protocol requires the following physiological processes: 1) synchronization of a follicular wave, 2) control of luteal lifespan, 3) induced ovulation of a physiologically mature dominant follicle, and 4) deposition of semen at the appropriate time relative to induction of ovulation [3]. The hormone injection sequence of GnRH-based protocols is based on the concept that synchronization of follicular waves will be initiated by ovulation of the dominant follicle following injection of GnRH. Seven days later, an injection of PGF to induce the luteolysis followed by a second injection of GnRH 48 to 66 hours after the PGF to induce ovulation of a new dominant follicle, and insemination occurs either simultaneously or 24 hours following to the second GnRH injection [4]. In the USA, basically all FTAI protocols are based around the GnRH-PGF-GnRH injection sequence with some variation on timing of injection and insemination and with addition of CIDR in almost all beef cows [2].

Alternatively, a treatment with estradiol and progesterone-releasing devices result in synchronous emergence of a new follicular wave by induction of dominant follicle turnover. On the first day of the protocol, cows receive a progesterone-releasing device, such as a CIDR, and an estradiol injection. Seven to nine days later, the device is removed and an injection of PGF is given. In addition, a second injection of estradiol is administered at a similar time to

induce ovulation of a physiologically mature dominant follicle. Approximately 30-56 hours following the second estradiol injection, the insemination is performed [5]. The estradiol and progesterone approach and its variations are the most frequently used synchronization protocols in Brazil and other countries that permit the use of estradiol.

In the last 20 years, many researchers have conducted studies to improve efficiency in both protocols [6-9]. Their research contributed to a better understanding of reproductive physiology in cows and led to the current FTAI and TET protocols. This review will outline the physiology behind synchronization protocols and how they have manipulated it to improve pregnancy rates.

Follicle size

Current FTAI protocols are 80-90% successful in synchronizing ovulation [10]. However, ovulatory follicle size at GnRH-induced or spontaneous ovulation is variable in beef cattle (Table 1.1) [3]. In postpartum beef cows, pregnancy rates were significantly reduced when follicles ≤ 12.0 mm where induced to ovulate by GnRH [11]. Bello, et al. [12] demonstrated a quadratic relationship between follicle size and pregnancy success in dairy cows these authors reported an increase in pregnancy rates as the dominant follicle size increases to a certain size, beyond which pregnancy rates decrease. Previous reports have shown reduction in pregnancy rates in both beef and dairy cattle when cows were induced to ovulate small physiologically immature follicles [7-9, 13-16].

Reduced concentration of estradiol at insemination is associated with ovulation of a physiologically immature follicle, as well as reduced progesterone secretion after insemination [2]. Perry, et al. [14] reported reduced reproductive performance of cows that ovulate small follicles. When GnRH-induced ovulation of a follicle ≤11 mm diameter, pregnancy rates were reduced and late embryonic mortality increased. However, follicle size seems to have no effect on fertility when spontaneous ovulation occurs [14].

It is important to note that it is not the size of the dominant follicle that affects the pregnancy rate but rather the physiological maturity of the dominant follicle in postpartum beef cows following FTAI. A dominant follicle that is physiologically mature may be defined as follows: 1) contains a competent oocyte, 2) secretes adequate amounts of estradiol during the preovulatory period, and 3) has the ability to form a corpus luteum capable of secreting adequate amounts of progesterone for establishment and maintenance of pregnancy.

There is evidence that follicle age can affect pregnancy rates in cattle. Cerri, et al. [17] reported greater embryo quality at d 6 in dairy cows that were induced to ovulate follicles approximately 1.5 days earlier than peak follicular dominance. Townson, et al. [18], demonstrated that cows experiencing two follicular waves during the estrous cycle ovulate an older follicle and have lower conception rates, when compared to cows experiencing three follicular waves during estrus cycle. Bridges, et al. [6] indirectly investigated the effect of

ovulatory follicle size by inducing ovulation of follicles approximately 1.5 d younger, resulting in increased pregnancy rates when cows with younger follicles experienced an extended proestrus. However, Abreu, et al. [13] reported no differences in pregnancy rates, follicle size at AI, and progesterone after AI in cows induced to ovulate a follicle approximately 3 days younger. Thus, it remains unclear as to what the exact effect age of the follicle has on pregnancy success. Recent evidence from Dias, et al. [19], suggests a potential effect of progesterone on LH receptors within the follicle that may play a role in some of these results.

Progesterone

The estrous cycle in cows can be divided in two phases: follicular and luteal. The follicular phase begins with pro-oestrus, followed by oestrus, and ends at ovulation. The luteal phase covers the metoestrus and diestrus and ends with luteolysis. During the luteal phase, progesterone is the predominant hormone. The concentration of progesterone during the estrous cycle regulates the secretion of GnRH from the hypothalamus, which regulates the secretion of gonadotropins (FSH and LH) from the anterior pituitary. FSH is responsible for follicle recruitment, while LH leads the final stages of follicular growth, oocyte maturation and promotes the production and secretion of estradiol from the dominant follicle [20-23]. Progesterone concentrations prior to ovulation have been found to suppress LH pulse frequency, which can affect oocyte maturation, follicular growth, and estradiol production [24]. Furthermore, the probability of conception is positively

associated with serum concentration of progesterone seven days after FTAI [25]. Martins, et al. [25] also reported higher serum concentrations of progesterone seven days after AI in heifers treated with low doses of progesterone during the estrous synchronization protocol. The authors also reported greater follicle diameter at FTAI, better ovulation rates, and higher estrus detection rates between CIDR removal (d9) and FTAI in heifers treated with low progesterone when compared to those treated with high progesterone during the estrous synchronization protocol. Moreover, cows induced to ovulate a larger follicle have greater serum concentration of progesterone after AI [14].

Increased concentrations of progesterone during the hormonal protocols has been shown to be inversely related to follicle growth, preovulatory estradiol concentrations and post ovulatory progesterone concentrations specifically in Nellore heifers [19]. In addition, as previously mentioned, high progesterone concentrations prior to ovulation have been found to suppress LH pulse frequency, which can affect oocyte maturation, follicular growth, and estradiol production [24]. Recent evidence suggests that LH can impact the expression of LH receptors in the granulosa cells, thus directly affecting fertility [26]. Preliminary data from Dias, et al. [19] indeed demonstrates that high progesterone concentrations during the synchronization period does decrease LH receptor expression in the granulosa cells of the dominant follicle in Nellore heifers. Future research is needed to confirm these observations, but it seems

that progesterone leading to changes in pulsatility of LH could be a major driver of fertility in these FTAI protocols.

Estradiol

Estrus expression occurs following a rise in serum concentrations of estradiol [27]. There have been multiple studies in different breeds of cattle and environments that have demonstrated an increase in estradiol has a direct correlation to an increase in fertility, fertilization rates, and pregnancy rates. Several physiological processes are controlled by preovulatory estradiol which contribute to establishment and maintenance of pregnancy. Follicle size positively affects circulating concentrations of estradiol [28], resulting in greater fertility to FTAI. Cows exhibiting estrus within 24 hours of TAI have also been reported to have increases in fertility [14, 29]. Estradiol's role during this period is multifaceted but the direct effect on follicular cells within the maturing follicle is critical. It has been reported that increased levels of estradiol have a positive effect on preparation of follicular cells through their ability to luteinize and secrete progesterone (progesterone's role previously reviewed).

Fertilization may be facilitated by increased circulating concentrations of estradiol by improvement in transportation of the ova and sperm [30, 31]. The effect of estradiol on uterine pH during estrus may be a potential explanation of how sperm transport is affected by circulating estradiol concentrations. Perry and Perry [32, 33] have conducted studies focused on the effect of exogenous estradiol administration and estrus expression on changes in uterine pH. Uterine

pH of cows in estrus or supplemented with estradiol were 0.3 units lower when compared to cows not displaying estrus. In addition, work from Roper [34] has provided preliminary data that uterine and vaginal pH at the time of AI or ET has a positive correlation with pregnancy success.

Prior to the LH surge and ovulation, serum concentration of estradiol seems to induce changes in the uterine environment. Circulating concentrations of estradiol have been shown to peak around 36 h before ovulation [35] and these increases in preovulatory estradiol have been reported by multiple groups to increase pregnancy success. Jinks, et al. [1] reported donor cows with greater circulating concentrations of estradiol were more likely to yield an embryo than an unfertilized oocyte. In the same study, recipient cows with greater estradiol at GnRH2 also had an increased pregnancy establishment [1]. In addition, when estradiol concentrations at GnRH2 were <8.4 pg/ml, a decrease in pregnancy rate was also observed.

It is clear that estradiol plays a critical role in the establishment and maintenance of pregnancy, however, that exact role remains unclear. Data reported above seems to primarily point to estradiol's ability to improve the maternal environment in the reproductive system but correlations with follicle size, embryo quality, and other factors cannot be discounted. Overall, increasing preovulatory estradiol or increasing the proportion of females exhibiting estrus leads to increased pregnancy success. Thus, increasing preovulatory estradiol

concentrations and estrus expression in a breeding program could help improve fertility rates in a cattle herd and reduce reproductive wastage.

Proestrus and estrus

Proestrus is generally defined as the period from initiation of luteolysis to the onset of estrus during which a dominant follicle and oocyte continue the maturation process. Several studies have demonstrated that proestrus length impacts pregnancy success in cattle. When cows were induced to ovulate following a short proestrus period, luteal function and embryo development were reduced [6, 36-38]. Reducing the length of proestrus resulted in inadequate luteal function following ovulation, and was independent of follicle diameter Mussard, et al. [38] reported inadequate luteal function, regardless of follicle diameter, when cows were exposed to a short proestrus period prior to ovulation. The author also reported reduced pregnancy rates following embryo transfer in cows induced to ovulate following a shorter proestrus period [38]. Collectively this data provides support to the hypothesis that the physiological maturity of the follicle has major influence in establishment and maintenance of pregnancy rather than follicle size alone. In the CO-Synch + CIDR protocol, removing a CIDR after 5 days instead of 7 days will increase the length of proestrus, and increases pregnancy rates in beef cows [6]. Meneghetti, et al. [7], reported an increase on pregnancy rates when length of proestrus is 2 days longer. In this experiment, the researchers increased the proestrous administering prostaglandin two days before CIDR withdrawal. In addition, recent evidence from Dias, et al. [19] has demonstrated

that increasing proestrus period in Nellore heifers mitigates the negative effects of high vs low progesterone by evaluating the LH receptor in the dominant follicle.

Estrus behavior has been positively correlated with pregnancy success for decades. Although as the industry has moved to more FTAI and timed embryo transfer (TET), the need for estrus detection and record keeping has decreased. Based on research data collected, it is clear that FTAI and TET work with a high degree of success. However, in all cases, cows exhibiting estrus prior to FTAI or TET tend to perform a few percentage points better, or have decreased pregnancy loss. Abreu, et al. [13] reported greater ovulatory follicle diameter at AI and higher pregnancy rates in cows that exhibit estrus behavior. Pohler et al., [39, 40] reported an increase in pregnancy rates and pregnancy associate glycoproteins (PAG) concentration on d 28 after FTAI in cows that expressed estrus prior to FTAI. In the same study, the pregnancy rates and PAG concentration increased as the intensity of estrus increased in Nellore beef cows. Similar data has also been observed in dairy cows undergoing TET. Pereira, et al. [41] reported that dairy cows receiving an embryo that had exhibited estrus prior to TET had decreased pregnancy loss when compared to those that did not. Therefore, even in timed AI or ET protocols, the added benefit of animals exhibiting estrus cannot be discounted.

Nutrition

Reproductive efficiency is a major contributor to the overall profitability and production efficiency of a cow/calf operation, and substantially influenced by the nutritional status of the dam. Short, et al. [42], proposed an approximate order of priority of nutrients which can be described as 1) basal metabolism, 2) activity, 3) growth, 4) basic energy reserves, 5) maintenance of pregnancy, 6) lactation, 7) additional energy reserves, 8) estrous cycles and initiation of pregnancy and 9) excessive reserves. This order of priority clearly illustrates how reproductive performance can be drastically affected by inadequate nutrients.

Mineral nutrition can be combined into overall nutrients, but often gets overlooked in the production system. Based on nutritional requirements, minerals can be divided into two categories: macro minerals, which are needed in higher quantities, and trace minerals, which are required in the diet at low concentrations [43]. An appropriate balance between trace minerals is essential for proper metabolic functions.

Based on their chemical structure, trace minerals can be divided into two forms organic or inorganic. In the organic form, the mineral molecule is attached to an organic molecule, such as an amino acid. For the inorganic forms the mineral molecule is bound to an inorganic molecule, for example, sulfate (SO_4). Mineral form can impact bioavailability to the animal, and thereafter the concentration in the liver, serum and others tissues. There is cumulative evidence that shows greater bioavailability of organic sources of trace minerals in comparison with

inorganic sources [44]. Marques, et al. [45], reported greater liver and serum trace mineral concentrations when cattle were fed with organic rather than inorganic sources.

Summary

In cattle, fertilization generally occurs > 90% of the time following insemination, but pregnancy rate at the earliest possible detection (day 27) is generally <70%. In this paper, we described some strategies to increase the pregnancy rates during synchronization protocol. The data provided in this paper demonstrates the numerous variables that contribute to successful establishment of pregnancy in beef cattle. It is also evident based on the data that the current FTAI protocols in beef cows are effective at generating pregnancies, but increasing success to a single ovulation is still an area that needs to be investigated.

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Apendix

Table 1.1 – Ovulatory follicle size and reproductive success (embryo development, conception and pregnancy) in beef cows and heifers.

Species	[1]Follicle Size	Range in follicle size	Source
Cows	≤ 12.0 mm	< 12 mm to > 18 mm	Lamb et al., 2001
Cows	≤ 11.3 mm	10 mm to 17 mm	Perry et al., 2005
Heifers	< 10.7 mm >15.7 mm	<10 mm to > 16 mm	Perry et al., 2007
Cows and Heifers	Linear	7.5 mm to 18.0 mm	Peres et al., 2009
Heifers	Linear	6 mm to 16 mm	Dias et al., 2009
Cows	Linear	< 9 mm to > 17 mm	Sa Filho et al., 2009
Cows	Linear	< 9 mm to > 16 mm	Meneghetti et al., 2009

[1]Follicle size at which reproductive success was significantly decreased. Linear refers to the significant line, which was fit to these data. As ovulatory follicle size increased there was an increase in pregnancy rates. Adapted from Pohler, et al. [3].

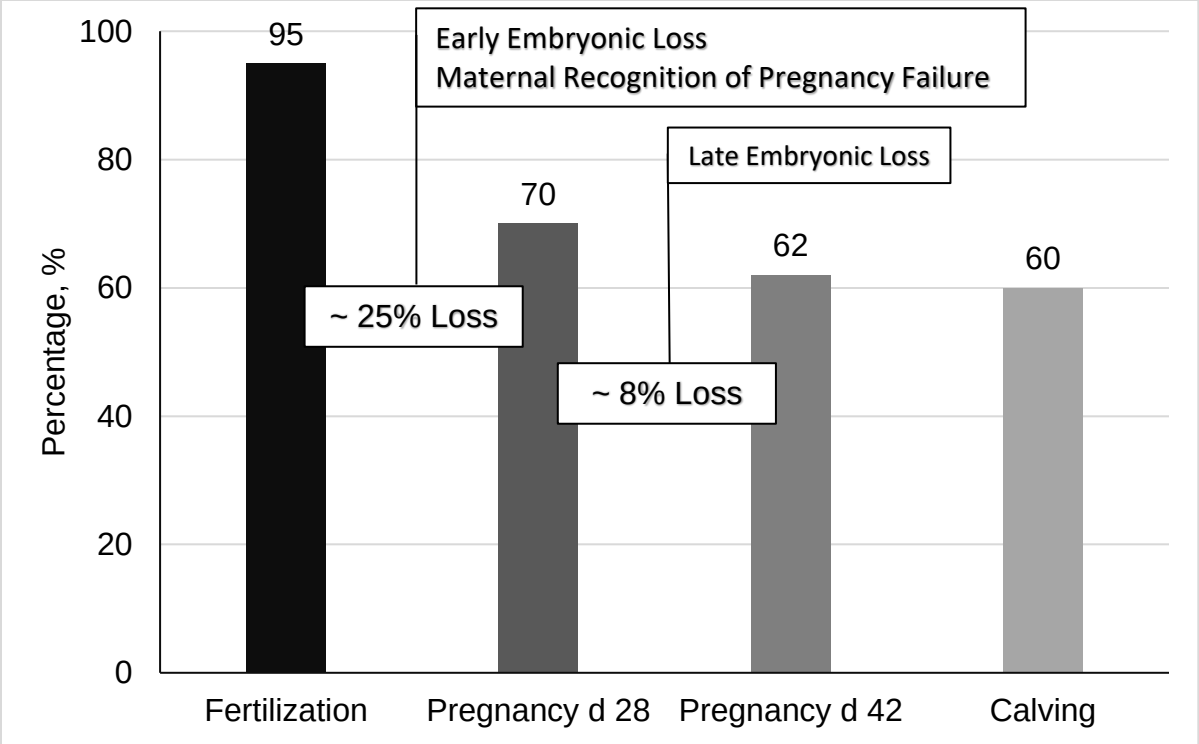

Figure 1.1 – Pregnancy rates during different times of gestation in beef cows (Bos Taurus and Bos indicus cattle may differ slightly following fertilization but overall trend is similar).

CHAPTER II

EFFECT OF COMPLEXED TRACE MINERALS ON OOCYTE AND EMBRYO PRODUCTION

Abstract

The objective of this experiment was to evaluate the impact of organic trace mineral supplementation on reproductive performance of lactating beef cows. Thirty days prior to FTAI (d −30), 68 postpartum cows were stratified by weight, body condition score, and parity before being randomly assigned to either a treatment (TRT) or a control (CNT) group. Each group received a weekly mineral supplement allotment of 1.2 kg/week per cow-calf pair for 14 weeks. Cows assigned to the TRT group received a mineral supplement that contained amino acid complexes of zinc, copper, and manganese, as well as cobalt glucoheptonate (Availa® Plus; Zinpro Corp., Eden Prairie, MN, USA), while cows assigned to the CNT group received a mineral supplement that contained the same concentrations these of trace minerals from inorganic sources. All cows were submitted to a 7-day CO-Synch + CIDR protocol on d −10 and bred using FTAI on d 0. Pregnancy diagnosis was performed on d 28, and nonpregnant cows were then removed from the experiment before being divided into 10 groups of 3 or 4 cows per group: 5 TRT groups (n = 20) and 5 CNT groups (n = 18). All pregnant cows were subjected to an ovum pick-up (OPU) on d 52 and 67. Collected oocytes were then evaluated prior to and following *in vitro* fertilization (IVF). Analysis of variance was conducted to determine effects of treatment on response variables, and group was considered the experimental unit. Mineral consumption did not differ (P = 0.48) between treatments (1.16 ± 0.1 vs. 1.07 ± 0.1 kg of DM/week per cow-calf pair, for TRT and CNT, respectively). While

pregnancy per AI was only numerically higher (P = 0.33) in cows assigned to the TRT group when compared to CNT cows (64.7% vs. 52.9%, respectively), total oocyte recovered was greater (P = 0.03) from TRT than CNT cows (22.4 ± 2.0 vs. 16.4 ± 1.4 oocytes/group for TRT and CNT, respectively). Moreover, culturable oocytes (grades A through C) recovery was increased in TRT cows (P = 0.05) when compared to CNT cows (15.9 ± 1.6 vs. 11.8 ± 1.0 oocytes/group, respectively). In addition, production of transferable embryos (grades 1 through 3) tended to be greater (P = 0.06) for TRT than CNT cows (4.7 ± 0.6 vs. 2.7 ± 0.7 embryos/group, respectively). In summary, supplementation of pregnant beef cows with zinc, manganese, and copper amino acid complexes, plus cobalt glucoheptonate, improved oocyte recovery and *in vitro* embryo development when compared to supplementation with inorganic forms of these trace minerals.

Introduction

Reproductive efficiency is a major contributor to the overall profitability and production efficiency of a cow/calf operation, and is substantially influenced by the nutritional status of the dam. Based on nutrient requirements, minerals are classified as belonging to one of two categories: macro minerals, which are required in the diet in relatively higher quantities, or micro minerals (also commonly referred to as trace minerals), which are required in the diet at relatively low concentrations [1]. An appropriate balance between trace minerals is essential for proper metabolic functions. Among the trace minerals, copper (Cu), iodine (I), manganese (Mn) selenium (Se), and Zinc (Zn) all play roles in

reproductive function of livestock species [2]. One way in which trace minerals play a role in reproduction is through their participation in the formation of metalloenzymes, which are important in several metabolic processes, including lipid metabolism, glucose utilization, DNA synthesis and transport, and free radical metabolism [2-6]. It has been hypothesized that embryonic and fetal development may be affected by trace mineral status through one or more of these mechanisms [2]; however, limited data exists in this area.

Bioavailability of trace minerals is a factor that influences the absorption of trace minerals by the animal, and thus may impact the animal's trace mineral status. Previous studies have reported improvement in liver and/or serum trace mineral concentrations when animals were fed with organic trace mineral forms [7-9]. Such forms are comprised of a metal ion bound with a carbon-based molecules with the objective of providing an alternative route of absorption and/or limiting their interaction with other nutrients in the digestive tract. Conversely, inorganic forms of trace minerals are subject to interaction that limits availability, more commonly referred to as antagonisms. However, other reports have reported no increase in bioavailability of organic sources of trace minerals [10-12].

Throughout pregnancy, the dam serves as the only source of nutrients, including trace minerals, for the fetus [13]. Therefore, it is essential that the requirements of the dam are met in order to avoid restricting supply to the developing fetus. Supplementation of gestating cows with a complexed form of

trace minerals has been shown to increase liver concentrations of Co, Cu, and Zn in the newborn calf when compared to un-supplemented contemporaries [13]. Additionally, trace mineral supplementation has been shown to increase concentrations of Co, Cu, and Zn in cotyledons [8].

Due to the potential impact of pregnancy outcomes on cow/calf production efficiency, and the lack of available information on the overall impact of trace mineral nutrition on reproductive performance in a beef herd, the objective of this study was to determine the effect of trace mineral form (organic vs. inorganic) on oocyte and IVF embryo production in lactating beef cows. Our hypothesis was that supplementation of lactating cows with an organic source of trace minerals would increase oocyte and embryo yield when compared to cows supplemented with an inorganic source of trace minerals.

Materials and methods

This study was conducted at the East Tennessee AgResearch and Education Center (ETREC) in accordance with IACUC-approved procedures. Cows utilized in this experiment were maintained on fescue (*Festuca arundinacea*) and mixed clover (*Trifolium repens*) pastures, and provided with *ad libitum* access to water. Prior to the initiation of the experiment (d -30 = initiation of experiment), all cows were provided with *ad libitum* access to a common complete mineral supplement that contained only inorganic forms of trace minerals for a 90-d washout period. Thirty days prior to artificial insemination (d -30), 68 postpartum cows were stratified by weight, body condition score,

ultrasound-estimated rump fat, ultrasound-estimated rib fat, days post-partum, and age before being equally and randomly assigned to either a treatment (TRT) or a control (CNT) group (Table 2.1). Each group then received a weekly mineral supplement (Table 2.2) allotment of 1.16 kg of DM/week/cow-calf pair for 14 weeks. Consumption was assessed weekly, upon which time all remaining mineral supplement was removed and weighed prior to offering the next week's allotment. Weekly samples of mineral supplement offerings and refusals were obtained for nutrient analysis. Cows assigned to the TRT group received a mineral supplement that contained amino acid complexes of zinc, copper, and manganese, along with cobalt glucoheptonate (Availa®Plus; Zinpro Corp., Eden Prairie, MN; Table 2.3), while cows assigned to the CNT group received a mineral supplement that contained the same concentrations of these trace minerals from inorganic sources (Table 2.3).

All cows were submitted to a 7-day CO-Synch + CIDR protocol on d -10 and FTAI on d 0 to two different sires by two FTAI technicians. Pregnancy diagnosis was performed via transrectal ultrasound on d 28, and pregnancy was determined based on the presence of an embryonic/fetal heartbeat. Cows diagnosed as non-pregnant were then removed from the experiment and not included in the OPU or IVF section. Immediately following pregnancy diagnosis, all remaining pregnant cows were subdivided into 10 groups of 3 or 4 cows per group: 5 treatment groups (20 total cows) and 5 control groups (18 total cows; Table 2.4) within their original treatment or control designation. All pregnant

cows were then subjected to an ovum pick-up (OPU) and oocytes submitted forward for *in vitro* embryo production on d 52 and d 67.

Liver biopsy

Liver biopsies were collected from all pregnant cows on days 30 and 67 using procedures previously described by Chapman Jr, et al. [14]. In brief, 10 mL of 2% lidocaine was administered subcutaneously between the 11th and 12th ribs on the animal's right side prior to biopsy collection in order to provide local anesthesia. Once anesthetized, the biopsy site was disinfected prior to making a stab incision with a scalpel. A biopsy needle (Tru-Cut biopsy needle; CareFusion Corporation, San Diego, CA) was placed in the incision, and progressed cranially and ventrally toward the opposite elbow until the needle had advanced into the liver. Three biopsy samples were collected per cow and immediately placed on ice and stored at -20 ºC until further analysis. Only cows that were confirmed pregnant at day 28 of gestation were used for liver mineral analysis.

Ovum pick-up procedure

Ovum pick-up was performed on all cows on d -30 (baseline measurement), and on all pregnant cows on d 52 and d 67, as previously described by Seneda, et al. [15]. In brief, 5 ml of 2% lidocaine was administered via epidural injection prior to OPU. An ultrasound-guided OPU device (Aloka 500V, Aloka, Wallingford, CT), equipped with a 5-MHz curved probe coupling and an 18-gauge needle was used to conduct the OPU procedure. The OPU

device was placed in the vagina and connected to a 50-ml collection tube, loaded with 20 ml of collection medium [Tissue Culture Medium 199 with Hank's Salts (MP Biomedical) supplemented with 150 cmM HEPES, 4.2 mM NaHCO3, 2% fetal bovine serum, 2 mM L-glutamine, 0.5X pen/strep, and 10,000 U/L heparin], which was connected to a vacuum pump set at 90-100 mmHg to collect the cumulus oocyte complex (COC). All follicles greater than 5 mm were ruptured and collected.

In vitro production of embryos

Oocytes were washed extensively to remove blood cells and debris before being evaluated and graded (A-D) by a single technician using the procedures previously described by De Loos, et al. [16]. In brief, category A COCs had an oocyte with homogenous, evenly granulated cytoplasm and numerous layers of compact, non-expanded cumulus cells. Category B COCs were similar to A, but with fewer layers of cumulus cells. Category C COCs had ooplasmic irregularities (i.e., "salt and pepper", large vacuoles, etc.), very few cumulus cells, or expanding cumulus cells. Category D COCs were atretic (expanded and dark cumulus vestment), too small (noticeably less than the expected oocyte diameter of ~100 µm), denuded, lysed, and/or otherwise morphologically abnormal. All COC graded A through C were pooled within the cows in the same treatment experimental group, and grade D COC discarded. *In vitro* maturation, fertilization, and embryo culture was performed as previously described by Rispoli, et al. [17]. In brief, fertilization was performed using a pool of semen from two Angus sires,

and all oocytes from the cows within the same experimental group were fertilized together. Embryos were evaluated, and cleavage rates were measured three days following fertilization. Embryos were then evaluated to determine stage of development and quality (1-4) using the procedures outlined in the IETS guidelines [18] eight days following fertilization. Immediately following evaluation, all embryos graded 1 through 3 were place into ethylene glycol for 5 to 10 minutes at room temperature and then loaded, individually, into 0.25-ml straws. Each straw was then placed in a cryo-chamber for 2 minutes before being seeded by touching the wall of the embryo straw with a cold cotton swab until an ice crystal formed. Embryo straws were then held at -6 °C for 10 minutes, and then cooled from -6 to -32°C at a rate of 0.5 °C/minute. Once the temperature reached -32 °C, straws were transferred into liquid nitrogen.

Mineral analysis

Mineral and liver samples were sectioned and digested overnight in a 95°C oven, using approximately 10x the dry tissue mass of nitric acid. Separate sections were dried overnight in a 75°C oven to determine the dry matter fraction and calculate the dried sample mass. If there was not enough sample to determine the dry matter fraction separately, the section of sample used for the mineral analysis was dried prior to digestion. The digested samples were diluted with water to 100x the dried tissue mass.

Elemental analysis followed the methods previously described by Wahlen, et al. [19], using an Agilent 7900 Inductively Coupled Plasma – Mass

Spectrometer (ICP/MS; Agilent Technologies Inc, Santa Clara CA). Briefly, a fraction of each diluted tissue digest and calibration standard were diluted 20-fold with a solution containing 0.5% EDTA and Triton X-100, 1% ammonium hydroxide, 2% propanol and 5 ppb of scandium and 7.5ppb of germanium, rhodium, indium and bismuth as internal standards. The ICP/MS was tuned to yield a minimum of 7500 cps sensitivity for 1 ppb yttrium (mass 89), less than 1.0% oxide level as determined by the 156/140 mass ratio and less than 2.0% double charged ions as determined by the 70/140 mass ratio.
Elemental concentrations were calibrated using a 5-point linear curve of the analyte-internal standard response ratio. Standards were from Inorganic Ventures (Inorganic Ventures, Christainsburg, VA). National Institute of Standards and Technology (NIST, Gaithersburg MD) Bovine Liver and Muscle standards were used as controls.

Statistical analysis

One-way ANOVA was generated using the GLIMMIX procedure of SAS (SAS v.9.4, SAS Inst. Inc., Cary, NC) to test the differences among cow-related quantitative and binary data and calf-related quantitative data. Main effects of treatment on oocyte production, cleavage rates, and embryo production were determined using group as the experimental unit. Main effects of treatment on pregnancy rates were determined using cow as the experimental unit. Main effects of treatment on calf-related data were determined using calf as the experimental unit, and the model included group as a blocking factor, as well as

calf gender and the interaction of treatment x gender as covariates. Results are reported as LSM ± SEM, covariate adjusted to calf gender and separated using PDIFF. In addition, relationship between liver trace mineral concentration and number of culturable oocytes recovered per group, independent of treatment, was analyzed using PROC GENMOD. The model includes the effect of mineral concentration on number of oocytes per group and overdispertion was corrected. To generate odds ratio analysis, PROC GENMOD was used, and the model included the effect of treatment on oocyte and embryo production. Significant difference was considered when $P \leq 0.05$, tendency when $P > 0.05$ and $P \leq 0.1$, and no significant difference when $P < 0.1$

Results

Cow and calf performance

Although pregnancy rate was numerically higher for TRT when compared to CNT cows, there was no significant differences ($P = 0.33$; Table 1). At the beginning of the experiment (d -30), no differences were observed between TRT and CNT cows for body weight ($P = 0.75$; Table 1), BCS ($P = 0.94$), ultrasound-estimated rib fat ($P = 0.71$), rump fat ($P = 0.34$), age ($P = 0.54$) or days post-partum at AI ($P = 0.60$). Measurements of cow and calf performance for all animals that were not removed from the experiment due to being diagnosed as non-pregnant are shown in Table 4. Throughout the experiment, cow and calf

performance was not different ($P \geq 0.12$) for any measurement collected (Table 4).

Mineral analysis

Mineral consumption did not differ ($P = 0.48$) between TRT and CNT cows (1.12 ± 0.07 vs. 1.06 ± 0.07 kg of DM/week/cow-calf pair, respectively). Results from liver biopsy are shown in table 5. Baseline liver mineral concentration measured at d -30 (start of the experiment) did not differ between TRT and CNT cows for Cu (226.78 ± 21.26 vs. 202.40 ± 10.57 µg/g dry weight, respectively; $P = 0.30$), Zn (91.41 ± 4.21 vs. 98.10 ± 4.27 µg/g dry weight, respectively; $P = 0.49$), Mn (9.70 ± 0.74 vs. 8.69 ± 0.41 µg/g dry weight, respectively; $P = 0.37$), and Co (0.20 ± 0.01 vs. 0.20 ± 0.01 µg/g dry weight, respectively; $P = 0.99$). Following 97 days of mineral supplementation, TRT cows had increased Cu (226.78 ± 21.26 vs. 179.66 ± 10.57 µg/g dry weight, respectively; $P = 0.03$) and Co (0.18 ± 0.01 vs. 0.45 ± 0.02 µg/g dry weight, respectively; $P < 0.0001$) relative to d -30 liver concentrations. Cows treated with control mineral only significantly differed in liver concentrations of Co at the end of the treatment period (0.20 ± 0.01 vs. 0.31 ± 0.02 µg/g dry weight, respectively; $P = 0.0001$). While liver concentrations did not differ between TRT and CNT cows at d 67 for Cu (179.66 ± 9.40 vs. 186.68 ± 8.43 µg/g dry weight, respectively; $P = 0.61$), Zn (103.13 ± 6.39 vs. 94.51 ± 2.73 µg/g dry weight, respectively; $P = 0.90$) and Mn (10.26 ± 0.74 vs. 8.92 ± 0.25 µg/g dry weight, respectively; $P = 0.18$), they differed for Co (0.45 ± 0.02 vs. 0.31 ± 0.01 µg/g dry weight, respectively; $P < 0.0001$).

Ovum pick up and oocyte collection

Prior to the initiation of the study, all cows were submitted for a baseline OPU on d -30. No differences were observed between TRT and CNT cows for total oocytes recovered (3.90 ± 0.94 vs. 4.06 ± 0.87 oocytes/cow, respectively; *P* = 0.90) or culturable oocytes (2.40 ± 0.45 vs. 3.17 ± 0.75 oocytes classified A through C/cow, respectively; *P* = 0.41). Following treatment administration, total oocyte yield (Fig. 1a) was increased for TRT when compared to CNT cows (22.39 ± 2.04 vs. 16.36 ± 1.44 oocytes classified A through D/group, respectively; *P* = 0.03). Moreover, culturable oocytes (oocytes classified A through C; Fig 1b) was increased for TRT when compared to CNT cows (15.9 ± 1.6 vs. 11.8 ± 1.01 oocytes classified A through C/group, respectively; *P* = 0.05). Additionally, odds ratio analysis demonstrated that TRT cows were 53% (*P* = 0.003) more likely to lead to recovery of an oocyte and 43% (*P* = 0.03) more likely to recover a culturable oocyte than CNT cows. Interestingly, the liver concentration of Zn and Mn did not differ between treatment and control supplemented cows at d 67. However, when analyzed in regression, including both d -30 and d 67 liver concentrations of Zn and Mn, there was a significant positive relationship between liver concentration and number of culturable oocytes recovered. As liver concentration of Zn increased from 20 to 230 µg/g dry weight, the number of culturable oocytes recovered increased from 2.07 to 8.00 culturable oocytes per cow (*P* = 0.04; Fig. 2). In addition, as liver concentration of Mn increased from 4.5 to 29 µg/g dry weight, the number of culturable oocytes recovered increased

from 1.96 to 2.26 per cow (P = 0.003; Fig. 3). Furthermore, Co had a tendency to be associated with number of culturable oocytes recovered. As liver concentration of Co increased from 0.05 to 0.62 µg/g dry weight, the number of culturable oocytes increased from 2.46 to 5.20 per cow (P = 0.07; Fig. 4).

In vitro embryo production

Although cleavage rates (79.74 ± 3.63 vs. 79.95 ± 5.36 % for TRT and CNT cows, respectively; P = 0.97) and percentage of 8-16 cell embryos (56.14 ± 5.48 vs. 64.94 ± 6.10 % for TRT and CNT cows, respectively; P = 0.44) were unaffected by trace mineral form, oocytes from TRT cows resulted in greater transferable embryo production (4.70 ± 0.64 vs. 2.70 ± 0.65 embryos classified 1 through 3/group, respectively; P = 0.06; Fig. 5a). Additionally, the percentage of 8-16 cell embryos that progressed to the blastocyst stage tended to increase (66.57 ± 11.50 vs. 40.83 ± 5.54 % for TRT and CNT cows, respectively; P = 0.08). Furthermore, the ratio of culturable oocytes that were required to produce a transferable embryo was decreased for TRT when compared to CNT cows (3.10 ± 0.93 vs. 7.02 ± 1.60, respectively; P = 0.06; Fig. 5b), which is a measure of efficiency in an *in vitro* embryo production system. Moreover, odds ratio analysis demonstrate that TRT cows were 75% (P = 0.02) more likely to produce a transferable embryo when compared to CNT cows.

Discussion

Trace mineral deficiency can impact several metabolic processes which can compromise immune function, production, and reproductive performance. Common reproductive symptoms of a trace mineral deficiency include increased number of services per conception, prolonged anestrus, delayed estrus, impaired ovulation, decreased pregnancy rates, and increased embryonic mortality and pregnancy loss [2, 20, 21]. In this specific study, there were clear increases in reproductive performance, measured by increased oocyte and embryo yield, as well as a numerical increase in pregnancy rate. Different sources of trace minerals have distinct bioavailability which affects absorption and, consequently, tissue concentration. Previous studies have reported greater serum or liver trace mineral concentrations when animals were fed an organic form of trace minerals when compared to inorganic forms [7-9]. In contrast, there are conflicting reports that demonstrate no effect of organic trace mineral supplementation on reproductive outcomes in beef cattle [20]. One possible explanation for the lack of effect in the previous report may be the source of the organic mineral used. In that study [20], the organic trace mineral was a proteinate form, which differs from the organic trace mineral used in the present study (amino acid complex). Lamb, et al. [22] found no differences in number of total or transferable embryos recovered from super-ovulated heifers fed with either organic trace mineral, inorganic trace mineral (positive control), or not receiving trace minerals (negative control). However, the heifers reported in that experiment that received

organic trace minerals yielded fewer unfertilized oocytes when compared to positive or negative controls.

In the present study, with exception to Co, we were unable to detect any difference in liver mineral concentration in animals fed an organic form of trace minerals when compared to those fed an inorganic source of trace minerals. It is also worth noting that the cows used for these experiments have been provided access to a free-choice mineral supplement and been on a mineral nutrition program for their entire production lifespan. Perhaps cattle that have never been part of a mineral program would yield more profound differences in liver mineral concentrations. Interestingly, liver Co concentrations were significantly higher in cattle fed the organic form of trace minerals when compared to those fed an inorganic form of trace minerals. In ruminants, vitamin B12 synthesis by rumen microorganisms requires Co as a necessary substrate. Average daily feed intake, average daily gain and nutrient digestibility has been shown to be influenced by dietary Co content [23, 24]. In the present study, dietary Co levels were equal across treatment groups. Nonetheless, Schwarz, et al. [25] reported maximum daily gain when animals were fed 118 µg/kg of DM and maximum feed intake when animals were fed diets containing between 160 and 180 µg/kg of DM. For growing cattle, the minimum Co intake necessary to maximize the production of vitamin B12 in the liver is 236 µg/kg of DM [26]. In the literature, there is limited data in regard to Co playing major roles in reproductive performance in ruminants. Particularly, in regard to local mechanisms or effects on oocyte

recovery, follicular development and embryo quality, the authors are unaware of any specific reports. There are reports that supplementation of grazing lactating dairy cows with Co glucoheptonate and amino acid complexes of Zn, Mn and Cu via water increased lactation performance, along with fertility, as well as increased Cu and Vitamin B12 stores [27]. Most of the work in this area has been conducted evaluating Co-deficient diets. In ewes, Co deficiency reduces the number of lambs produced and increases the number of stillbirths and neonatal mortality. Moreover, lambs from Cobalt-deficient ewes had reduced serum concentrations of immunoglobulin and vitamin B12, and increased concentrations of methylmalonic acid when compared with lambs born to non-Co deficient ewes [28]. Supplementation of cows with Co resulted in higher liver concentrations of vitamin B12 [26, 29], and higher pregnancy rates when compared to un-treated cows [29]. Although a numerical increase in pregnancy rates was observed for cows fed an organic form of trace minerals in the present study, this difference was not statistically significant. However, we speculate that the lack of statistical significance is due to a low number of animals, which was confirmed when a retrospective power analysis revealed a power of 0.16. Although we were unable to detect a statistically significant difference in pregnancy rates, we observed an improvement in reproductive performance of cows fed an organic form of trace minerals, as indicated by IVF embryo production. Furthermore, there was a positive association between liver concentrations of Co and recoverable/culturable oocytes. The numer of

culturable oocyte recovered more than doubled when the Co concentration increased from 0.05 to 0.62 µg/g dry weight. Thus, even though the mechanism by which Co drives this increase in follicular development and oocyte recovery remains poorly understood, supplementation seems to be critical for proper function. One possible explanation for the observed increase in oocyte recovery and embryo yield is that increased levels of dietary Co increase feed intake and average daily gain as mentioned previously, although that was not measured in this study. Greater feed intake and daily gain will led to a better overall nutritional status which potentially leads to a more favorable environment for follicular and oocyte development [30, 31]. Moreover, the follicles produced in a more favorable nutritional environment have better capacity to develop and generate a viable embryo after fertilization [30, 31].

Surprisingly, liver concentrations of Zn and Mn did not differ between treatments in this specific study; however, when analyzed using poisson regression, independent of treatment, liver concentrations of both mineral were associated with increases in number of culturable oocytes recovered. As the liver concentration of both minerals increased, the number of culturable oocytes increased. Tian and Diaz [32] reported epigenetic defects in oocytes and decreased *in vitro* fertilization efficiency resulting from a Zn deficiency; however, no differences were noticed when fertilization was performed *in vivo* in Zn-deficient mice. Spears [33] reported an increase in reproductive performance during the first 21 days of the breeding season when beef cows were fed an

organic form of Zn. Although we did not observe differences in liver Zn concentration between TRT and CNT treated cow, a positive relationship between Zn concentration and reproductive performance was observed, which is similar to previous findings [34].

Conclusion

Although we did not observed differences in liver trace mineral concentrations, with exception to Co, the results from this experiment support our hypothesis that organic trace mineral supplementation improves the reproduction efficiency of lactating beef cows submitted to OPU and IVF. However, further research is necessary to elucidate the specific mechanisms by which organic trace minerals improve reproductive efficiency.

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Apendix

Table 2.1 – Measurements of body composition, reproductive parameters, and age for cows assigned to supplement groups at study initiation (d -30) and d 28.

Item	**TRT**	**CNT**	***P*-value**	***SEM***
Initial measurements (d -30)				
n	34	34	--	--
Body Weight, kg	622.18	628.91	0.75	16.29
BCS	5.85	5.83	0.94	0.16
Rib Fat, cm	0.98	0.93	0.71	0.05
Rump Fat, cm	1.49	1.32	0.34	0.13
Day post-partum	93.97	94.73	0.60	1.02
Age, years	4.47	4.88	0.54	0.47
Pregnancy rate (d 30), %				
Tech1	64.71 (11/17)	43.75 (7/16)	0.23	12.40
Tech2	64.71 (11/17)	61.11 (11/18)	0.83	11.59
Overall	64.71 (22/34)	52.9 (18/34)	0.33	8.6

Table 2.2 – Nutrient composition of mineral supplements.

Nutrient[a]	TRT	CNT	Units, of DM
Calcium	16.79	16.88	%
Chlorine	15.87	15.97	%
Sodium	10.42	10.53	%
Phosphorus	6.49	6.53	%
Magnesium	1.75	1.62	%
Sulfur	0.25	0.92	%
Potassium	0.02	0.06	%
Zinc	8127	8166	mg/Kg
Manganese	2792	2809	mg/Kg
Copper	1349	1359	mg/Kg
Iron	1100	1131	mg/Kg
Iodine	175	177	mg/Kg
Cobalt	138	138	mg/Kg
Selenium	32	33	mg/Kg
Vitamin A	216.47	217.62	kIU/kg
Vitamin D	21.71	21.82	kIU/kg
Vitamin E	1.08	1.09	kIU/kg

[a] Total supplement concentrations calculated from nutrient content of individual ingredients included in supplement.

Table 2.3 – Mineral supplement ingredient composition (% of DM).

Name	TRT	CNT
Monocalcium phosphate	28.27	28.01
Salt	24.5	24.5
Limestone	23.65	23.75
Availa Plus	12.50	--
Dried distillers grains with solubles	--	9.35
Selenium premix[1]	5.00	5.00
Magnesium oxide	2.60	2.35
Zinc sulfate	--	2.08
Soybean oil	2.00	2.00
Vitamin ADE premix[2]	1.14	1.14
Manganese sulfate	--	0.91
Copper sulfate	--	0.5
Vitamin AD 60-15 Premix	0.3	0.3
Vitamin A 650 kIU/g	0.04	0.04
Calcium iodate	--	0.04
Cobalt carbonate	--	0.03

[1]Selenium premix contained 0.06 % selenium

[2]Vitamin ADE premix contained 57.5 kIU/kg vitamin A, 8.7 kIU/kg vitamin D, and 193.0 kIU/kg vitamin E

Table 2.4 – Pregnant cow/calf pair stratification between TRT and CNT group submitted to OPU.

Parameter	TRT	CNT	*P*-value	*SEM*
Cow				
n	20	18	--	--
Initial Weight, Kg	613.64	638.91	0.40	21.02
Final Weight, Kg	628.48	643.50	0.54	16.98
Weight Gain, Kg	14.83	4.90	0.46	7.95
Initial BCS	5.75	5.80	0.83	0.19
Final BCS	6.00	6.12	0.71	0.28
Initial Rib Fat, cm	1.00	0.95	0.61	0.07
Final Rib Fat, cm	0.72	0.75	0.67	0.05
Initial Rump Fat, cm	1.50	1.52	0.94	0.19
Final Rump Fat, cm	1.55	1.64	0.68	0.16
Calf				
Overall				
n	20	18		--
Initial Weight, Kg	99.22	100.50	0.80	3.93
Final Weight, Kg	217.16	217.03	0.99	7.23
Weight Gain, Kg	117.93	117.11	0.91	4.05
Male				
n	12	10		--
Initial Weight, Kg	97.26	107.18	0.12	5.05
Final Weight, Kg	216.42	231.92	0.15	9.42
Weight Gain, Kg	121.38	126.22	0.54	5.53
Female				
n	8	8		--
Initial Weight, Kg	97.26	92.14	0.17	5.34
Final Weight, Kg	218.26	198.54	0.13	7.09
Weight Gain, Kg	112.75	105.71	0.41	3.57

Table 2.5 – Liver mineral concentrations[1] on day d -30 and d 67 for cows submitted to OPU.

	d-30	**d67**	***P-value***	***SEM***
TRT				
Cu	226.76^{x}	179.66^{y}	0.03	21.26
Zn	91.41	103.13	0.15	9.04
Mn	9.7	10.26	0.58	1.05
Co	0.2^{x}	0.45^{a,y}	<0.0001	0.02
CNT				
Cu	202.4	185.53	0.46	11.92
Zn	98.1	93.66	0.61	4.27
Mn	8.69	8.85	0.87	0.41
Co	0.2^{x}	0.31^{b,y}	<0.0001	0.01

^{a,b} Different letter in the same column differ $P < 0.0001$

^{x,y} Different letter in the same row differ $P < 0.05$

[1] Liver concentrations are reported in µg/g of dry weight

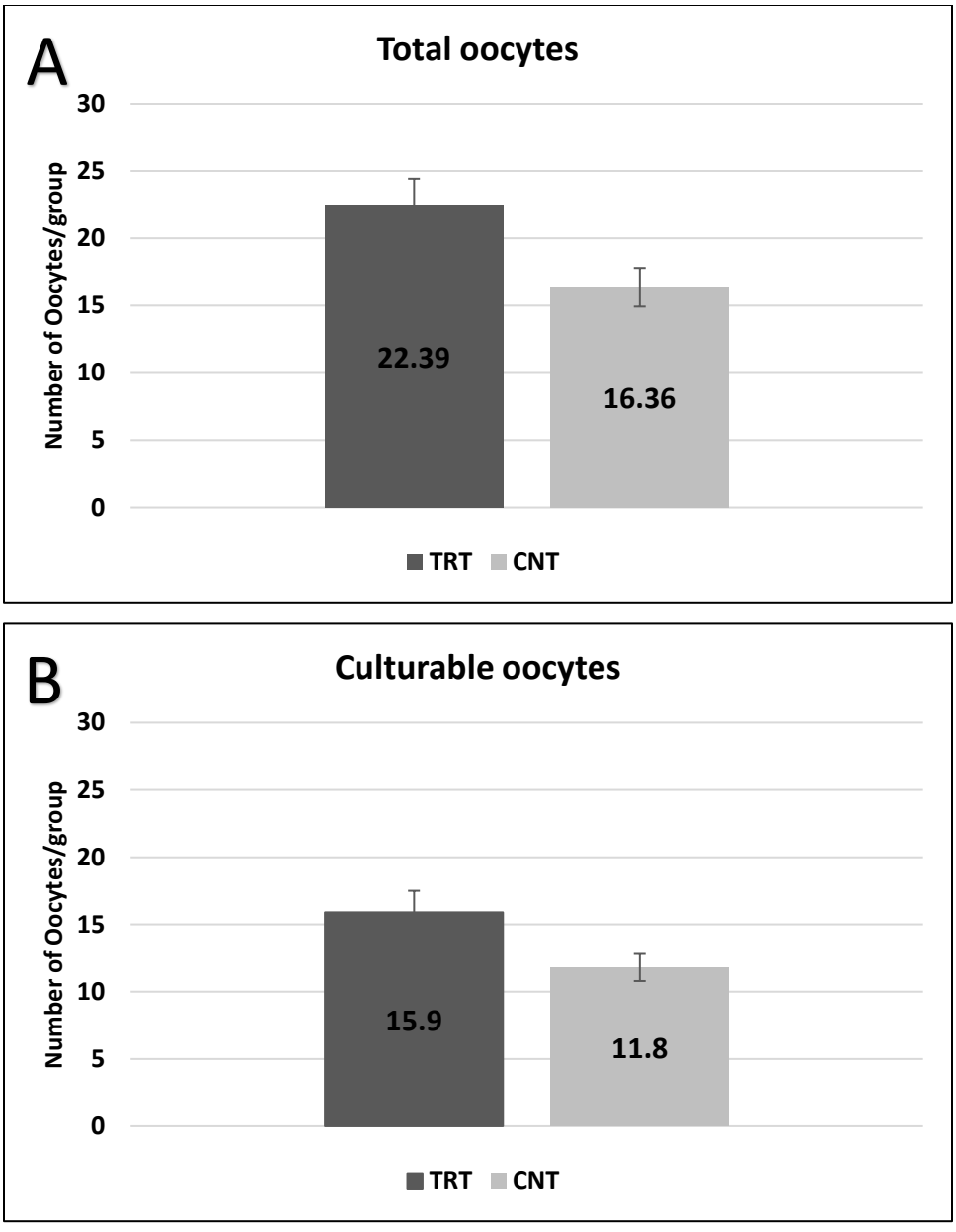

Figure 2.1 – Total and culturable oocyte recovered.
Cows receiving treatment mineral are shown in dark bar and cows receiving control mineral are shown in light bar. (A) Treated cow had higher total production of oocytes (P = 0.03) and (B) higher production of culturable oocyte (A through C; P=0.05).

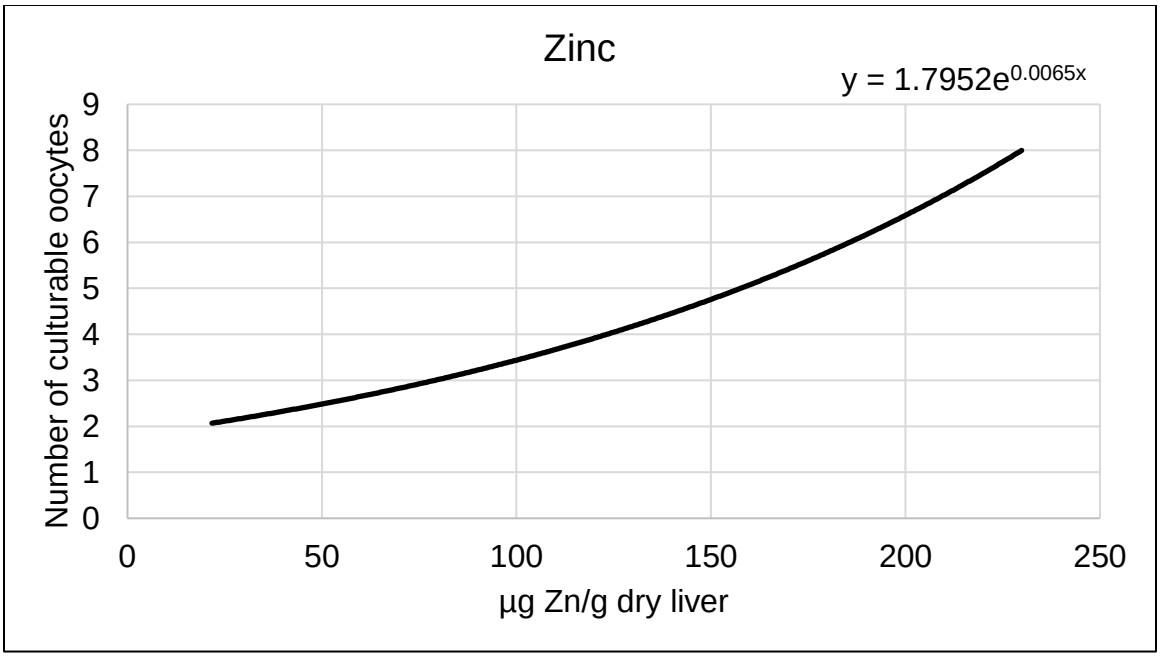

Figure 2.2 – Relationship of liver Zn concentration on the number of culturable oocytes recovered by OPU.
As liver concentration of Zn increases, the number of culturable oocytes recovered increases (*P* = 0.04).

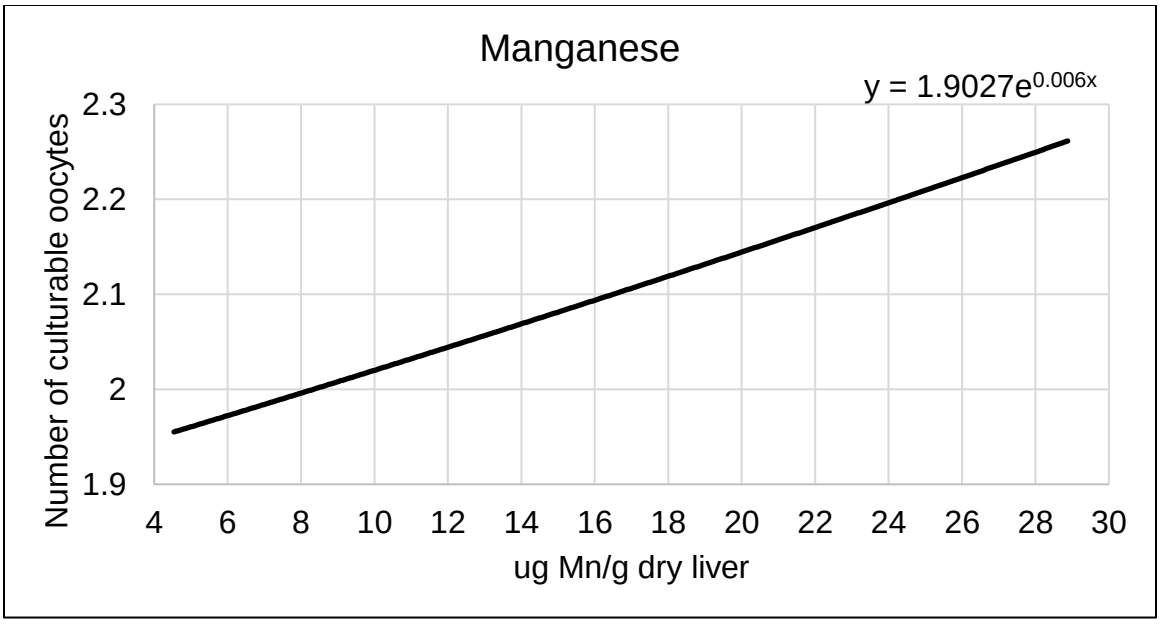

Figure 2.3 – Relationship of liver Mn concentration on the number of culturable oocytes recovered by OPU.
As liver concentration of Mn increases, the number of culturable oocytes recovered increases (*P* = 0.003).

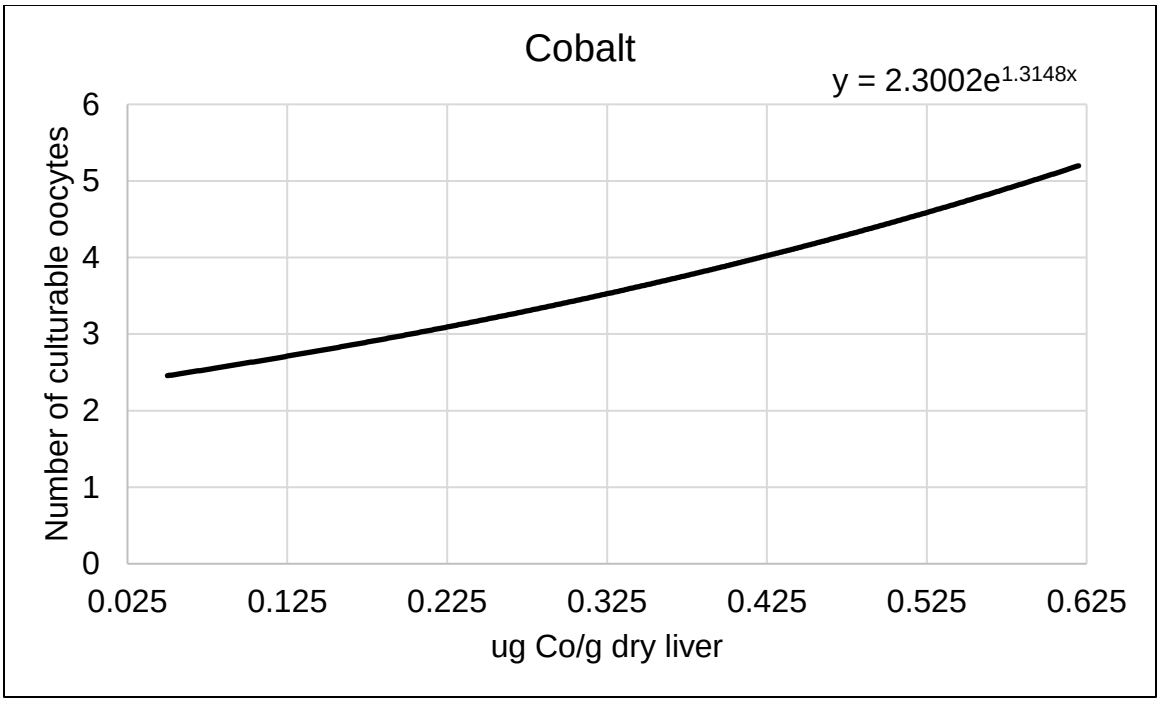

Figure 2.4 – Relationship of liver Co concentration on the number of culturable oocytes recovered by OPU.
As liver concentration of Co increases, the number of culturable oocytes recovered increases (*P* = 0.07).

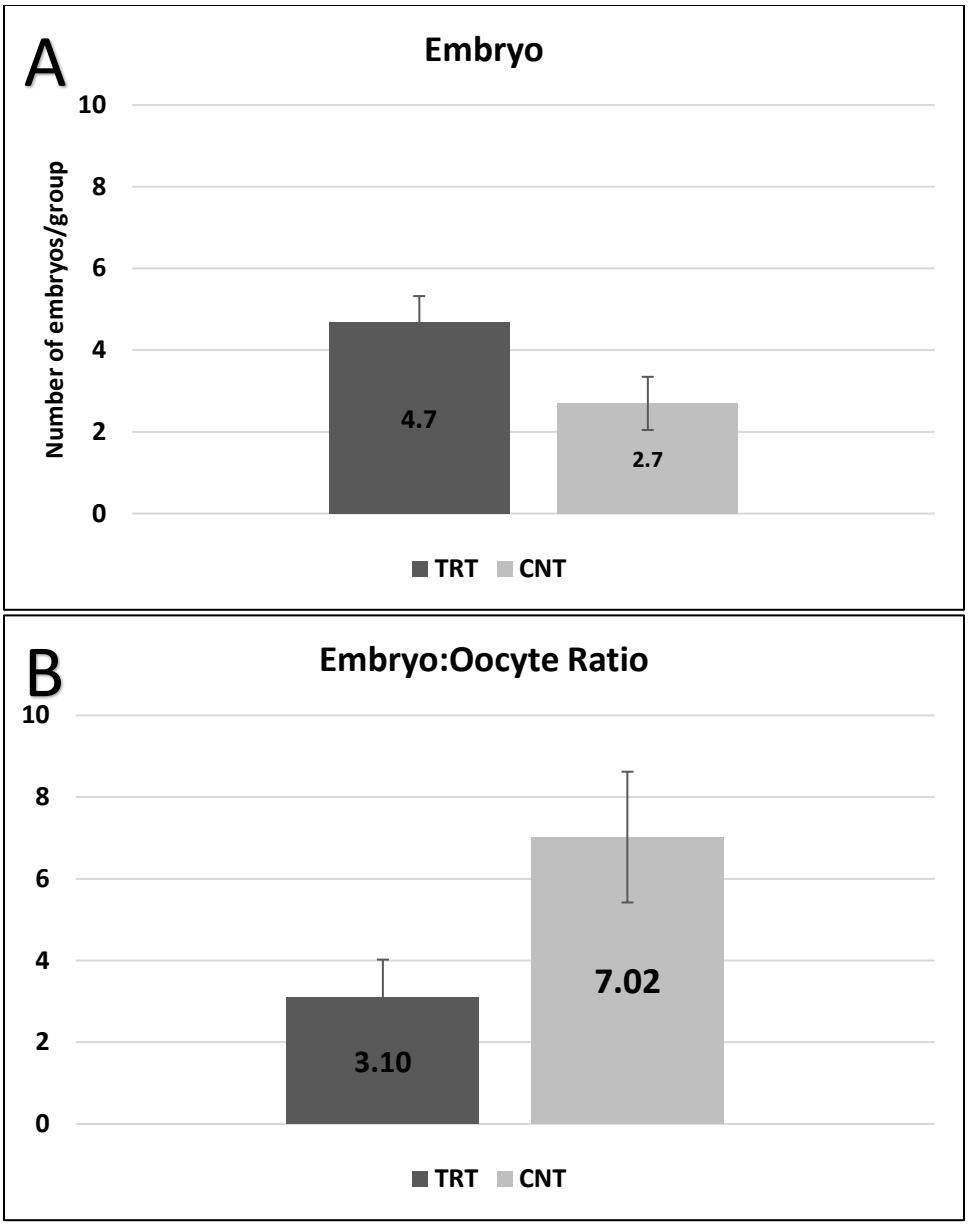

Figure 2.5 – Embryo production of TRT and CNT cows**.**
(A) Production of embryos/group was greater for treatment when compared with control groups cows (*P* = 0.06). (B) Ratio of oocytes:embryo for cows receiving complexed trace mineral was significantly lower than the control groups (P < 0.05); 1 transferable embryo was formed for every 3.10 culturable oocytes subjected to fertilization. For cows receiving control mineral 1 transferable embryo was formed for every 7.02 culturable oocytes subjected to fertilization (*P* = 0.06).

CONCLUSION

Reproductive efficiency is crucial for production efficiency and sustainability of cow/calf operations and largely affected by the nutritional status of the dam. Trace mineral nutrition is often overlooked but has a great impact on reproductive efficiency. Collectively, the results presented in this study combined with previous studies demonstrated that reproductive efficiency of lactating beef cow can be increased by supplementation with organic trace minerals.

VITA

Felipe Guirado Dantas was born in Sao Jose do Rio Preto, SP, Brazil to Vanderlei Dantas and Marines Martinez Guirado Dantas. He grew up helping his grandfather and uncle farm and raise beef cattle on his family's farm which developed a strong interest for the care and management of cattle. In March of 2008, he was accepted at Universidade Estadual Paulista, Julio de Mesquita Filho in Botucatu, SP, Brazil. During the last year of his undergraduate career, he had the opportunity to do an internship at the University of Minnesota under the mentorship of Dr. Allen Bridges and in December of 2014, he received his BS in Veterinary Medicine. During his undergraduate degree, he worked, under the orientation of Dr. Jose Luiz Moraes Vasconcelos, toward gaining experience in research, especially reproductive physiology of cows. In 2015, he was invited by Dr. Ky Pohler to do an internship at University of Tennessee – Knoxville, TN and the fall of 2016 he was accepted as a graduate research assistantship at the University of Tennessee - Knoxville, TN where he conducted research focused on reproductive physiology, specifically the impact of nutrition on reproduction, which is the topic of his thesis. Felipe graduated with a Master of Science degree in Animal Science in May 2018. Following completion of his degree, Felipe pursued a career in the beef cattle industry.