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Effect of injectable copper, selenium, zinc and manganese on the pregnancy rate of crossbred heifers (*Bos indicus* × *Bos taurus*) synchronized for timed embryo transfer

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ABSTRACT

The success of synchronization protocols for timed embryo transfer (TET) depends on various factors such as breed, body condition scores and nutrition (including trace mineral status). In the present study, crossbred heifers were treated with subcutaneous injectable trace minerals (ITM — 100 mg zinc, 100 mg manganese, 50 mg copper and 25 mg selenium; Multimin®, Minerthal, Brazil) 17 days prior to embryo transfer. Estrus synchronization for TET, conception rate and pregnancy loss were evaluated. Subcutaneous administration of ITM did not increase the number of heifers successfully synchronized compared to non-injected controls [ITM group = 82.1% (308/375) versus control = 83.1% (375/451); $P = 0.76$]. However, heifers belonging to the ITM group (n = 219) had a 1.58 fold and 1.72-fold higher ($P = 0.005$) odds of being pregnant 23 and 48 days after TET compared to the control group (n = 276; not receiving ITM), respectively. In contrast, treatment with subcutaneous ITM did not significantly lower the pregnancy-loss rate [control group = 17.2% (17/99) versus ITM group = 10.6% (11/105); $P = 0.18$]. Even though subcutaneous administration of ITM 17 days prior to TET did not increase the number of heifers successfully synchronized, a significant increase in the conception rate (embryo survival) at 23 and 48 days after TET was noted in heifers of the ITM group.

1. Introduction

Timed embryo transfer (TET) protocols are well established in the dairy and beef industry. Several studies have been performed in order to reduce management problems that lessen the efficiency of TET, thus making the procedure financially viable (Baruselli et al., 2010; Binelli et al., 2001; Block et al., 2003; Bo et al., 2002). However, less effort has been directed toward investigating the effects of nutrition on TET, even though studies have clearly established a profound influence of nutrition on the reproductive performance of female cattle (Campanile et al., 2010; Robinson, 1996; Sales et al., 2008; Webb et al., 1999). Moreover, the effects of minerals in improving fertility have also been described (Wilde, 2006). Ballantine et al. (2002) observed that supplementation with Cu, Zn, Mn and Co (inorganic versus organic) reduced days open and tended to improve both the first-service conception rate and the percentage of cows pregnant at 150 days postpartum. Furthermore, the concept that certain essential trace minerals play crucial roles in enzymatic and metabolic pathways which are critical for embryo development during pregnancy in cattle has been described (Griffiths et al., 2007; Hostetler et al., 2003). The embryo relies entirely on the maternal system for its supply of trace minerals and other nutrients needed for normal development. Hostetler et al. (2003) observed that the levels of zinc, copper and manganese in the conceptus were severalfold greater than in the surrounding reproductive tissues, indicating preferential accumulation of these minerals by the conceptus. Trace minerals can be supplied to cattle by various means, including as free-choice minerals, drenches and pastes (Ahola et al., 2004). The

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recent development of injectable chelated minerals introduces a new option for supplementing these essential minerals.

The objective of this study was to evaluate the effect of injectable trace mineral (ITM) supplementation (100 mg zinc, 100 mg manganese, 50 mg copper and 25 mg selenium) on the number of heifers successfully synchronized, and on the conception and pregnancy-loss rates of embryo recipients submitted to TET protocols. The hypothesis of this study was that ITM supplementation increases both the number of heifers successfully synchronized and the conception rate, and reduces the pregnancy-loss rate of crossbred heifers submitted to TET protocols.

2. Materials and methods

2.1. Study animals, facilities and diets

The study was performed from December 2007 to November 2009 at five different farms in Brazil. At each farm the heifers received free-choice oral mineral supplementation offered according to nutritional requirements of the specific animal category (NRC, 2001). In total, 826 crossbred heifers (*Bos indicus*×*Bos taurus*) cycling (presence of the corpus luteum; CL), presenting a body condition score (BCS) of 2.75 to 3.5 (1 to 5 scale, Wildman et al., 1982) and a body weight of 300–320 kg were enrolled in the study. During the summer, the animals were maintained in *Brachiaria brizantha* grass paddocks in a rotational grazing system. In winter, the heifers were supplemented with sugarcane and a protein and mineral supplement to compensate for the low nutritional value of the forage. During both seasons (summer and winter), the animals had ad libitum access to water and mineral salts (Minerthal 160 Corte®, Minerthal, Brazil; Table 1). The protein and mineral supplement was composed of 10% mineral salts, 10% urea, 17.5% salt, 47.5% cotton seed and 15% citrus pulp and was provided every 2 days. The expected daily intake was 75 g of mineral salt (summer) or 400 g of protein and mineral supplement (winter). All minerals were supplied at NRC recommended levels (NRC, 2001).

2.2. Estrus synchronization for timed embryo transfer

At unknown stages of the estrous cycle (D0), all animals received 2 mg of estradiol benzoate (Sincrodiol®, Ouro Fino, Brazil) and one progesterone-releasing intravaginal device (Sincrogest®, Ouro Fino, Brazil). Eight days later (D8), the intravaginal device was removed and 500 μg of cloprostenol (Sincrocio®, Ouro Fino, Brazil), 300 IU of equine chorionic gonadotropin (eCG) (Novormon®, Syntex, Argentina) and 1.0 mg of estradiol cypionate (SincroCP ®, Ouro Fino, Brazil) were injected in all animals. Embryo transfer was performed on day 17. Only grade 1 in vitro-derived *B. indicus* embryos (Stringfellow and Seidel, 1998) were used. Pregnancy diagnosis was performed using ultrasonography at 23 and 48 days after TET. An ultrasound machine with an 8 MHz crystal array vet linear transducer Aquila Probe (Pie Medical, ESAOTE, The Netherlands) was used.

Table 1
Mineral salt composition. Levels of guarantee per kg of product.

Element	Mineral salt
Phosphorus (g)	90
Calcium (g)	160
Magnesium (g)	15
Sulfur (g)	15
Sodium (g)	141
Zinc (mg)	3600
Cupper (mg)	960
Manganese (mg)	1020
Cobalt (mg)	100
Iodine (mg)	63
Selenium (mg)	13
Fluorine (mg)	900

2.3. Treatment groups

The heifers were randomly allocated to one of two treatments with an allocation ratio of 1:1 in a blinded fashion. The randomization was stratified by farm and BCS. The experimental groups were: control (n = 276), where animals did not receive any kind of ITM supplementation, and ITM (n = 219), where animals received 5 ml subcutaneous injection of copper, manganese, zinc and selenium (Multimin®, Minerthal, Brazil). The injection was administered at the onset of the TET synchronization protocol (D0).

This trial was conducted in agreement with the ethical principles in animal research adopted by the Bioethics Commission of the School of Veterinary Medicine and Animal Science of the University of São Paulo, Brazil.

2.4. Statistical analysis

Synchronization and pregnancy rates were summarized in tabular format using frequencies and percentages. The chi-square test was used to compare synchronization rates between groups. Pregnancy rates were analyzed using the intent-to-treat population, which was defined as all animals which were successfully synchronized and received an embryo. Logistic regression analysis was performed to compare all study outcomes (pregnant at day 23 after TET, pregnant at day 48 after TET, pregnancy loss) between the two study arms. In order to adjust the analysis for *farm site* and *year effect*, those variables were included as covariates in the logistic regression analysis models. All *P*-values are two-sided, with $P<0.05$ indicating a statistically significant difference. Data analysis was performed using SAS® version 9.2 software (SAS Corp. Cary, NC).

3. Results

Of the 826 heifers enrolled for synchronization, only 495 (control n = 276 and ITM n = 219) received an embryo. Despite the fact that approximately 80% of heifers had been successfully synchronized (presence of CL on D17), the number of embryos produced in vitro was insufficient to allow enough embryos to be transferred to all synchronized animals.

There was no interaction ($P = 0.94$) between the treatment, BCS, year and farm. Furthermore, no statistical difference between the number of heifers successfully synchronized and ready for TET was observed between animals in the control