Efectos de la suplementación con minerales y vitaminas sobre las tasas de preñez en ganado Nelore sometido a programas de IATF con diferentes puntuaciones de reactividad

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Resumen

Un temperamento de alta reactividad en el rodeo reduce la productividad y la rentabilidad en la ganadería bovina. En ganado Bos taurus indicus (Nellore), planteamos la hipótesis de que un puntaje de reactividad más alto está asociado con una menor tasa de preñez, y que la suplementación inyectable con minerales (Cu, Zn, Mn, Se) y vitaminas (A, E) aumentaría la tasa de preñez y reduciría la mortalidad embrionaria en vacas muy reactivas. Los tratamientos de suplemento inyectable o control se administraron 25 y 10 días antes de la inseminación artificial a tiempo fijo (IATF; Día 0: día de la IATF). Seleccionamos 1722 vacas 45 ± 7 días posparto, con diferentes niveles de reactividad conductual, y se midieron una serie de parámetros relacionados con la reproducción. Las vacas se agruparon aleatoriamente y recibieron 1 mL/50 kg de peso vivo del suplemento (n = 872) o placebo (n = 850), 25 y 10 días antes del protocolo de IATF (Día–25 y Día–10 respectivamente). En el Día –10, se insertaron dispositivos intravaginales con 1 g de progesterona y se aplicaron 2 mg de benzoato de estradiol por vía intramuscular. En el Día –2, se retiraron los dispositivos y se aplicaron 530 µg de D-cloprostenol sódico, 300 UI de gonadotropina coriónica equina y 1 mg de cipionato de estradiol. A las 48 horas después de la última inyección, las vacas fueron sometidas a IATF (Día 0). En el Día -25 (25 días antes de la IATF), el temperamento se calificó de 3 a 12 utilizando una puntuación de reactividad compuesta (RS), que evalúa el movimiento y la respiración mientras el animal está en el cepo, así como su velocidad de escape, y se utilizó para clasificar a las vacas como tranquilas (RS ≤ 4; n = 590), reactivas (RS 5 a 8; n = 573) y muy reactivas (RS ≥ 9; n = 559). En el Día 0, las vacas tranquilas tuvieron un mayor diámetro folicular en la IATF en comparación con las vacas reactivas y muy reactivas (14.4 ± 0.2 vs. 13.6 ± 0.4 y 13.2 ± 0.2 mm, respectivamente; P = 0.03). Se observaron interacciones, donde las vacas suplementadas en comparación con las no suplementadas tuvieron una mayor tasa de preñez en el Día 30 (P = 0.04; 60.00 vs. 54.10 % tranquilas y 58.00 vs. 52.50 % muy reactivas) y en el Día 60 (P = 0.03; 58.70 vs. 47.90 % tranquilas, 52.20 vs. 49.10 % reactivas y 48.40 vs. 41.30 % muy reactivas), y una menor pérdida embrionaria entre los días 30 y 60 (P = 0.04; 2.00 vs. 6.10 % tranquilas, 4.20 vs. 8.40 % reactivas y 9.60 vs. 11.20 % muy reactivas). La suplementación en los Días –25 y –10 mejoró las tasas de preñez y redujo la pérdida embrionaria en vacas tranquilas y muy reactivas.

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Effects of mineral and vitamin supplementation on pregnancy rates in Nelore cattle submitted to FTAI programs with different reactivity scores

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HIGHLIGHTS

- Mineral and vitamin injectable supplementation increase pregnancy rates.
- Injectable supplementation reduces embryonic loss in very reactive females.
- Very reactive non supplemented cows have the worst reproductive performance.
- Calm supplemented cows have better reproductive performance.

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ABSTRACT

A high-reactivity herd temperament reduces productivity and profitability in cattle farming. In *Bos taurus indicus* (Nellore) cattle, we hypothesized that a higher reactivity score is associated with a lower pregnancy rate, and that injectable supplementation with minerals (Cu, Zn, Mn, Se) and vitamins (A, E) would increase the pregnancy rate and reduce embryonic mortality in very reactive cows. Injectable supplement or control treatments were administered on days 25 and 10 before fixed-time artificial insemination (FTAI; Day 0 : day of FTAI). We selected 1722 cows 45 ± 7 days postpartum, with different behavioral reactivity levels, and a series of measures related to reproduction were measured. The cows were randomly grouped and received sc 1 mL/50 kg live weight of the supplement (n = 872) or placebo (n = 850), 25 and 10 days before the FTAI protocol (Day–25 and Day–10 respectively). On Day −10, devices containing 1 g progesterone were inserted into the vagina, and 2 mg estradiol benzoate was applied intramuscularly. On Day–2, the devices were removed and 530 μg of sodium D-cloprostenol, 300 IU of equine chorionic gonadotropin, and 1 mg of estradiol cypionate were applied. At 48 h after final injection, the cows were subjected to FTAI (Day 0). On Day - 25 (25 days before IATF), temperament was graded as 3 to 12 using composite reactivity score (RS), which evaluates movement and breathing while the animal is in the cattle chute, and their escape velocity, and was used to classify cows as calm (RS ≤ 4; n = 590), reactive (RS 5 to 8; n = 573), and very reactive (RS ≥ 9; n = 559). On Day 0, calm cows had a higher largest follicle diameter on the FTAI compared to reactive and very reactive (14.4 + 0.2 vs. 13.6 + 0.4 and 13.2 + 0.2 mm, respectively; P = 0.03). Interactions were observed, where supplemented cows compared to those no supplemented had higher pregnancy on Day 30 (P = 0.04; 60.00 vs. 54.10 % calm and 58.00 vs. 52.50 % very reactive) and Day 60 (P =

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0.03; 58.70 vs. 47.90 % calm, 52.20 vs. 49.10 % reactive and 48.40 vs. 41.30 % very reactive) and reduced embryonic loss at 30 to 60 d ($P = 0.04$; 2.00 vs. 6.10 % calm, 4.20 vs. 8.40 % reactive and 9.60 vs. 11.20 % very reactive). Supplementation on Days–25 and–10 improved pregnancy rates and reduced embryonic in calm and very reactive cows.

1. Introduction

In animal husbandry, "temperament" describes a herd's behavioral response to fear when exposed to human manipulation (Fordyce et al., 1988). Poor temperament is associated with a more excitable response to human contact and handling. A very reactive (VR) temperament substantially reduces productivity and profit in the meat industry. *Bos taurus indicus* (i.e.: Nelore) females are typically more reactive than *Bos taurus* females (i.e.: Angus).

Temperament and reproductive performance in cattle are strongly related, with more reactive cows showing lower reproductive performance (Cooke et al., 2009, 2011; Rueda et al., 2015; Brandão e Cooke et al., 2021; Fernandez-Novo et al., 2020) due to physiological changes in ovulation, conception, and pregnancy establishment (Curley et al., 2008; Dobson et al., 2001). More reactive cows have higher plasma cortisol (Cooke et al., 2014; Mello et al., 2020) and lower luteinizing hormone (LH) levels (Echternkamp, 1984), possibly owing to reduced gonadotropin-releasing hormone (GnRH) pulses (Dobson and Smith, 2000). In beef cows, excitability results in reduced LH levels, possibly contributing to a reduction in the dominant follicle diameter and compromising reproductive performance. VR heifers (Kasimanickam et al., 2014) and cows (Cooke et al., 2011, 2012; Rueda et al., 2015; Mello et al., 2020) have lower pregnancy rates than less-reactive females. In addition, artificially inseminating more reactive cows is harder and more time-consuming, thus affecting animal handling (Rueda et al., 2015).

Oral supplementation of trace minerals in the diet of cattle reduces the number of days to first estrus, and increases conception rates, enhancing beef cow production (Muehlenbein et al., 2001). However, dietary mineral supplements may not be fully absorbed due to interactions with other nutrients at the ruminal level, posing a major disadvantage to their use (Stake, 1977; Suttle, 1986; Goonerate et al., 1989, King, 1971). Therefore, there is substantial interest in the use of injectable trace minerals that avoid dietary antagonists and generates a rapid increase in systemic mineral status (Hartman et al., 2018). Parenteral supplementation studies have traditionally involved the use of vitamins A and E, as well as Cu, Zn, Mn, and Se, with increases in fertilization and pregnancy rates and reductions in the calving to conception interval (Harrison et al., 1984; Graham, 1991). In cows and heifers, deficiencies in minerals like Cu led to reproductive disorders such as reduced conception rates, infertility, anestrus, and embryo loss (Aderman, 1963; Baldwin et al., 1981; Phillippo et al., 1987; Corah and Ives, 1991). Most of these studies have evaluated dietary mineral supplementation; few have evaluated the effects of injectable supplementation on reproductive performance.

Several studies found that very reactive animals have high plasma cortisol (Echternkamp, 1984; Cooke et al., 2014; Mello et al., 2020) and less antioxidant activity (Khan et al., 2016). Cows supplemented with injectable minerals (Ca, P, Na, Mg, S, F, Co, I, Fe, Zn, Mn, Se, Cu) and vitamins (A, D3, and E) 30 d before FTAI exhibited high antioxidant enzyme levels following supplementation; for instance, levels of superoxide dismutase [SOD] were high 13 d post-administration, and those of glutathione peroxidase [GSH-Px] were high 4 d post-administration (Vedovatto et al., 2019). The minerals Zn, Mn, Se, and Cu participate in enzymes such as Cu/Zn-SOD and Mn-SOD (Markclund, 1980), metalloenzymes that contribute to catalyzing the dismutation of the superoxide anion into molecular oxygen and hydrogen peroxide (Sordillo and Aitken, 2009). Selenium, a component of GSH-Px, is required to catalyze hydroperoxide reduction (Rotruck et al., 1973). Therefore, injectable minerals and vitamins before the breeding season could improve reproductive performance, especially in more reactive cows with higher cortisol concentrations and less antioxidant activity.

Here, we hypothesized that Nelore cows with a higher reactivity score (RS) and subjected to FTAI, would have a lower pregnancy rate and that injectable supplementation with minerals (Cu, Zn, Mn, Se) and vitamins (A, E) would increase the pregnancy rate and reduce embryonic mortality in VR females.

2. Materials and methods

2.1. Animals and location

The study was conducted at the Fernando Costa Campus, University of São Paulo (Pirassununga, São Paulo, Brazil) and on commercial farms during 2013 to 2016 breeding seasons. In total, 1722 Nellore multiparous cows were included. Cows were maintained on *Brachiaria decumbens* pasture with free access to water and mineral supplementation and were handled only occasionally for veterinary or reproductive procedures. This study was approved by the Ethics in Animal Use Committee of the University of São Paulo (study no. 7,822,020,818).

2.2. Assessment of body weight and body condition score (BCS)

Twenty-five days before the FTAI (on Day −25), body weight was measured to estimate the volume of treatment (injectable supplement or control) to be administered to each animal. In addition, BCS was evaluated several times during the experiment Day −25, Day 0, and Day 30 (Day 0 : day of FTAI) according to the scale proposed by Nicholson and Butterworth (1986), which ranges from 1 (very thin) to 9 (very fat). The animals were classified by a single evaluator.

2.3. Treatments

Cows (45 ± 7 days postpartum) were randomly divided into two groups: control group, received a subcutaneous injection (SC) of saline solution ($n = 857$) and, injectable supplementation with minerals (Cu, Zn, Mn, Se) and vitamins (A, E) 1 mL/50 kg live weight of Kit Adaptador® supplement; Biogénesis Bagó; $n = 865$). Each 100 ml of injectable supplement contains 1.0 g Cu, 4.0 g Zn, 1.0 g Mn, 0.5 g Se, 3.5 g vitamin A, and 5.0 g vitamin E. Both treatments were administrated twice: 25 and 10 days before the FTAI protocol (Day − 25 and Day −10 respectively).

2.4. Fixed-time artificial insemination protocol

All cows were subjected to the same hormonal protocol. On Day −10, the animals received an intravaginal progesterone device (Cronipress®, Biogénesis Bagó) and intramuscular (im) administration of 2 mg of estradiol benzoate (Bioestrogen®, Biogénesis Bagó). On Day −2, the devices were removed, and cows were treated with im injection of 530 μg cloprostenol sodium (Croniben®, Biogénesis Bagó), 300 IU eCG (Ecegon®, Biogénesis Bagó), and 1 mg estradiol cypionate (Cronicip®, Biogénesis Bagó). After 48 h, FTAI was performed (Day 0). Semen from 14 Nellore bulls was used for the FTAI. Bulls were randomly distributed among all reactivity scores and treatment groups. All semen batches from all used bulls were previously evaluated by microscopy and computer analysis and considered adequate for FTAI.

2.5. Determination of estrus presentation

On Day−2, the base of the tail was painted using a marker stick (WalMur, Porto Alegre, Brazil) suitable for estrus detection. On Day 0, at the time of the FTAI, observations were made to determine estrus presentation. Animals no longer showing these markings were classified as presenting estrus; those with partial or total markings were classified as not presenting estrus.

2.6. Reactivity score assessment

Reactivity score (RS) was evaluated during animal handling on Day −25, using a composite RS methodology adapted from proven temperament assessment methodologies (Burrow et al., 1888; Grandin 1993; Cooke et al., 2011; Sant'Anna et al., 2013). This methodology evaluates movement, breathing, and exit velocity from the cattle chute and scores each cow from 1 to 4 as described on Table 1. For all animals, RS was assessed by two observers, and the final score was the average of both observers. The sum of the three scores (movement, breathing, and exit velocity) defined the RS and ranges from 3 to 12. The animals were classified as calm (RS $\leq$ 4; $n = 590$), reactive (RS 5 to 8; $n = 575$), and very reactive (RS > 8; $n = 557$).

2.7. Ultrasound assessment

Ultrasonographic evaluation was performed on Days −10, 0, 30 and 60, using a Mindray DP-2200 Vet ultrasound device with a linear rectal transducer. The ovaries were evaluated on Day –10 to determine the presence or absence of a corpus luteum (CL) and identify the cyclicity rate of cows. On Day 0, the largest follicle diameter was measured at its widest point and at a right angle to the first measurement. The follicular diameter was calculated as the average of these two measurements. On Day 30 and Day 60, pregnancy diagnosis was performed, classifying females as pregnant (presence of an amniotic vesicle containing a live embryo [day 30] or live fetus [day 60]), or not pregnant. On day 60, the females were again evaluated for the presence or absence of a CL.

2.8. Statistical analysis

All statistical procedures were performed using Statistical Analysis Systems 2012 (SAS/STAT®, SAS Inst. Inc., Cary, NC). Data were checked for normality (Shapiro-Wilk test) and homogeneity of variance, and outliers were excluded or transformed when necessary. Two statistical models were used to assess pregnancy rates 30 and 60 d after FTAI, and pregnancy loss from 30 to 60 d post-FTAI.

The statistical model included RS (calm, reactive, or very reactive), treatment (no supplemented vs. supplemented), year of the breeding season (2013 to 2016), and all possible interactions as fixed effects. Treatment and year were also included as random effects based on reactivity. The following model was considered: $yijkl = m + aj + bi + (ab)\,ij + eijkl$, where *yijkl* is the observed value, *m* is an inherent constant for all observations, *aj* is the fixed effect of year *j* of the breeding season, *bi* is the effect of group *i* (very reactive, reactive, or calm), *l* is the effect of the treatment (no supplemented vs. supplemented group), *(ab) ijl* is the effect of the interaction between group *i* and period *j* and group *l*, and *eijkl* is the random error associated with observation *yijkl*. Because the year of the breeding season has no effect on any of the response variables, a new model excluding only the year of the breeding season and all its interactions was constructed. We set the significance level for rejecting the null hypothesis to $P < 0.05$.

Table 1
Behavioral characteristics were used to classify cows as calm, reactive, or very reactive according to the reactivity score (RS). Three different temperament assessment methodologies (movement score, breathing score, and exit velocity) were assessed when cows were handled 25 days before FTAI (Day – 25; Day 0: day of FTAI).

Movement score	Behavioral characteristics for classification
1	Little or no displacement most of the time, occasional and relaxed tail movements
2	Animal calm but does not remain in the same position for a long time, movements occasional with vigorous tail movements
3	Frequent and vigorous body and tail movements
4	Continuous, vigorous, and abrupt movements, jumping, forcing out, frequent tail movements
Breathing score	
1	Not audible breathing
2	Audible and rhythmic breathing, similar to physiological
3	Audible, very deep breathing, and different from physiological
4	Puffing and/or snoring
Exit velocity	
1	Animal not leaving the holding trunk and needing to be touched
2	Animal leaving the holding trunk walking
3	Animal leaving the holding trunk at a median speed
4	Animal leaving the holding trunk at a high running speed

3. Results

There was no effect of treatment or the treatment by RS interaction ($P > 0.05$) when analyzing movement score, breathing score, and escape velocity on Day −25. For these variables, data presenting the RS effect are presented in Table 2. As expected, movement score ($P = 0.02$), breathing score ($P = 0.04$), and escape velocity ($P = 0.02$) were lower in calm cows compared to reactive cows and presented the highest values in very reactive cows. In addition, largest-follicle diameter at FTAI was also affected by the RS ($P = 0.03$; Table 2) but not by treatment or the treatment by RS interaction. On Day 0, largest-follicle diameter at FTAI was greater for the calm (14.4 + 0.2 mm) than reactive (13.6 + 0.4 mm) and very reactive groups (13.2 + 0.2 mm, $P = 0.03$).

RS, treatments and interactions effect are presented in Table 3. No significant differences were observed for BCS at FTAI (BCS Day 0; $P = 0.35$) and at 30 days post-FTAI (BCS Day 30; $P = 0.51$) and proportion of females that manifesting estrus from Day −2 to Day 0, observed the time of the FTAI ($P = 0.7$). In addition, none of the variables analyzed herein were affected only by treatment however, several variables were affected or tended to be affected by the treatment by RS interaction (Table 3).

Significant differences between the temperament groups were observed for proportion of females with a CL at Day–10 d ($P = 0.03$), when observed interaction between treatment and RS ($P = 0.03$), where supplementation of very reactive cows higher cyclicity rate on Day −10

Table 2
Association between the reactivity score (RS) groups (Calm, Reactive, or Very Reactive) evaluated 25 days before FTAI (Day–25) with movement score, breathing score, escape velocity, and largest-follicle diameter (mm) on the day of FTAI (Day 0) in Nellore multiparous cows ($n = 1722$) subjected to FTAI. Data are shown as means ± SEM.

	Reactivity Score – RS			
	Calm ($n = 590$)	Reactive ($n = 575$)	Very reactive ($n = 557$)	*P value*
Movement Score	**1.14 + 0.06^{c}**	**2.0 + 0.03^{b}**	**2.7 + 0.02^{a}**	**0.02**
Breathing Score	**1.1 + 0.02^{c}**	**1.5 + 0.02^{b}**	**1.8 + 0.06^{a}**	**0.04**
Escape velocity	**1.15 + 0.01^{c}**	**1.8 + 0.03^{b}**	**2.7 + 0.03^{a}**	**0.02**
Largest-follicle diameter (mm) on the day of FTAI (D 0)	**14.4 + 0.2^{a}**	**13.6 + 0.4^{b}**	**13.2 + 0.2^{b}**	**0.03**

[a,b,c]Different letters overwritten on the same line represent significant statistical differences ($P < 0.05$).

Table 3
Body condition score (BCS) on Day 0 and Day 30, cyclicity rate on Day 0, manifested estrus from Day −2 to Day 0 evaluated at the time of the FTAI, pregnancy per fixed-timed AI (P/FTAI) on Day 30 and Day 60, embryonic losses observed between Day 30 and Day 60, cyclicity rate in non-pregnant cows on the Day 30 for Calm, Reactive, and Very Reactive in Nellore cows subjected to fixed-timed AI (FTAI; Day 0) and treated or not with injectable vitamins and trace minerals 25 (Day - 25) and 10 days (Day - 10) before the FTAI protocol.

	Reactivity Score – RS									
	Calm control (n = 290)	Calm Supplemented (n = 300)	Reactive Control (n = 289)	Reactive Supplemented (n = 286)	Very Reactive Control (n = 278)	Very Reactive Supplemented (n = 279)	Overall mean	P Value Treatment	P Value RS	Interaction (Treatment x RS)
BCS Day 0	5.60 + 0.26	5.70 + 0.21	5.60 + 0.15	5.40 + 0.24	5.40 + 0.17	5.40 + 0.18	5.50 + 0.20	0.88	0.06	0.35
BCS Day 30	5.90 + 0.22	5.70 + 0.24	5.90 + 0.17	5.80 + 0.19	5.80 + 0.21	5.70 + 0.18	5.80 + 0.21	0.63	0.09	0.51
Cyclicity rate on Day −10 (%)	60.20 [a]	58.70 [a]	**59.70 [a]**	**55.00 [b]**	**53.80 [b]**	**56.80 [a]**	57.40	0.03	0.02	**0.03**
Manifested estrus from Day −2 to Day 0 (%)	81.10	79.90	81.00	79.70	80.1	80.00	80.30	0.53	0.80	0.70
P/FTAI Day 30 (%)	**54.10 [b]**	**60.00 [a]**	**57.50 [a]**	**56.40 [b]**	**52.50 [b]**	**58.00 [a]**	56.50	0.01	0.01	**0.04**
P/FTAI Day 60 (%)	**47.90 [c]**	**58.70 [a]**	**49.10 [c]**	**52.20 [b]**	**41.30 [d]**	**48.40 [c]**	49.60	0.01	0.02	**0.03**
Embryonic losses (Day 30 to Day 60) (%)	**6.10** [c]	**2.00 [d]**	**8.40 [b]**	**4.20 [c]**	**11.20 [a]**	**9.60 [b]**	6.9	0.01	0.02	**0.04**
Cyclicity rate in non-pregnant cows Day 30 (%)	**73.70 [a]**	**61.10 [c]**	73.90 [a]	76.60 [a]	**68.00 [b]**	**76.00 [a]**	71.55	0.03	0.02	**0.03**

[a,b,c]Different letters overwritten on the same line represent significant statistical differences ($P < 0.05$).

when compared to no supplemented (56.80 vs 53.80 %; respectively; Table 3).

Supplemented cows had higher pregnancy rates than the no supplemented on Day 30 for ($P = 0.04$) very reactive (58.00 vs. 52.50 % respectively) and calm group (60.00 vs. 54.10 % respectively) on Day 60 ($P = 0.03$) for very reactive (48.40 vs. 41.30 % respectively), reactive (52.20 vs. 49.10 % respectively) and calm group (58.70 vs. 47.90 % respectively; Table 3). Supplemented cows had lower ($P = 0.04$) rate of embryonic loss at Day 30 to Day 60 of pregnancy for very reactive (9.60 vs. 11.20 % respectively), reactive (4.20 vs. 8.40 % respectively), and calm group (2.00 vs. 6.10 % respectively; Table 3). Cows supplemented had a cyclicity rate ($P = 0.03$) in non-pregnant cows on Day 30, a very reactive group (76.00 vs. 68.00 % respectively; Table 3).

4. Discussion

As reproductive biotechnology, FTAI that helps to reduce the calving interval and promotes genetic gain (Reichenbach et al., 2008). However, TAI protocols require intensification of animal handling, where large numbers of cows are managed within a short time interval, exacerbating their stress. Very reactive cows have higher stress levels, which can reduce the FTAI pregnancy rate (Cooke et al., 2009, 2011; Mello et al., 2020). Our findings indicate that temperament scoring via the RS can be efficient in evaluating female reactivity. Movement, breathing score, and exit velocity were lower in calm females than in reactive females (Table 2), and were highest in very reactive females. Similar scores have been used to determine animal reactivity, and we have previously applied this RS methodology (Mello et al., 2020). Our findings indicate that female temperament affects reproductive performance in Nellore cattle. The calm-group individuals had higher largest-follicle diameters at FTAI, consistent with findings for been cows that excitability reduces LH levels, contributing to a reduction in dominant-follicle diameter (Echternkamp, 1984).

Similarly, we have previously shown that more reactive cows have higher plasma cortisol, which can affect follicle development (Mello et al., 2020). Greater reactivity may reduce GnRH pulses (Dobson and Smith, 2000), possibly explaining the lower pregnancy rates observed here at 30 and 60 d post-FTAI in the very reactive cows. In this study observed that on day FTAI, largest-follicle diameter at FTAI was greater for the calm (14.4 + 0.2 mm) than reactive (13.6 + 0.4 mm) and very reactive groups (13.2 + 0.2 mm, $P = 0.03$).

The RS categories of movement score, breathing score, and exit velocity did not differ between the control and supplemented groups, confirming that the cows were randomly assigned to the groups. BCS on the day of FTAI at 30 d post-FTAI did not differ between the control and supplemented groups, consistent with prior findings (Vedovatto et al., 2019) that injectable trace-mineral supplementation did not significantly affect BCS or body weight in Nellore females. This suggests that these animals can freely obtain dietary trace minerals, meeting their requirements for optimal body condition.

No significant differences between the supplemented and control groups were observed for largest-follicle diameter at Day 0, consistent with prior findings (Vedovatto et al., 2019). These groups did not differ in the proportion manifesting estrus from Day–2 to Day 0, as the follicle diameter did not differ; this consistency was expected, given that follicle diameter is positively correlated with estradiol production and with estrus (Vasconcelos et al., 2001; Sá Filho et al., 2010; Torres-Júnior et al., 2011). The higher pregnancy rates following supplementation do not appear to be due to changes in ovarian structures.

Supplemented very reactive females had the highest cyclicity rate on Day–10 and cyclicity rate in non-pregnant cows at Day 30, compared control group. Our findings may reflect the fact that very reactive cattle have high oxidative-stress levels, which induce cellular damage. Mineral and vitamin supplementation can increase antioxidant action by increasing antioxidant enzyme levels (Vedovatto et al., 2019). Very reactive cows had high pregnancy rates at 30 and 60 d, and lower rates of embryonic loss than the very reactive control, which had the lowest pregnancy rate at 60 d The very reactive control had the worst reproductive performance, due to the high levels of oxidative stress. This remains to be verified.

Unexpectedly, for reactive animals, the proportion presenting a CL at −10 d was lower in the supplemented group than in the control, possibly explaining why supplementation did not affect the pregnancy rates at Day 30 in this group. The proportion of cycling animals (presenting a CL) at the start of FTAI increases the pregnancy rate (Sa Filho et al., 2011). For very reactive animals, the proportion presenting a CL at −10 d was higher in the supplemented group than in the control, possibly favoring the pregnancy rate at 30 and 60 days.

For all RS, supplemented cows had a higher pregnancy rate at 60 days and lower embryonic mortality between 30 and 60 days of pregnancy. The oxidative stress can cause damage to the embryo damage, resulting in embryonic death in many species including cattle and humans (Agarwal et al., 2005; Guerin et al., 2001; Bielecka et al., 2021). Higher levels of oxidative stress products, and lower levels of antioxidant proteins, have been reported in cows after embryonic mortality (Celi et al., 2012; Bielecka et al., 2021), explaining why embryonic loss was highest in all of the control groups. This provides evidence that supplementation effectively reduces embryonic loss at 30 to 60 d of pregnancy.

For the calm group, supplementation effectively improved the pregnancy rate at both 30 and 60 d following FTAI; at 60 d, the pregnancy rate was highest in the calm supplemented group. This is consistent with our finding that calmer cows have better reproductive performance, and indicates that supplementation can further improve performance, probably by reducing oxidative stress.

For the very reactive group, the proportion of females presenting a CL after negative pregnancy at 60 d post-FTAI was higher in the supplemented group than in the control. the supplementation used herein likely alters factors such as oxidative stress responses in very reactive cows, which could increae their ovulation rate and cause them to present a CL. For the calm group, in contrast, this proportion was higher in the control than in the supplemented group: the calm control group had the highest pregnancy rate at Day 60, among all of the groups. Therefore, factors other than temperament and supplementation may reduce the proportion of ovulatingcows and presenting a CL after negative pregnancy at 60 d post-FTAI. To elucidate this, evaluating the pregnancy rates of cows subjected to resynchronization would be necessary.

5. Conclusion

Our findings support our hypothesis that more reactive female Nelore cattle would have lower pregnancy rates than lower-reactivity individuals. In addition, injectable supplementation with Cu, Zn, Mn, Se, and vitamins A and E increased pregnancy rates and reduce embryonic mortality in very reactive females.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Agarwal, A., Gupta, S., Sharma, R., 2005. Role of oxidative stress in female reproduction. Reprod. Biol. Endocrinol. 3, 28.
Baldwin, W.K., Hamar, D.W., Berlach, M.L., 1981. Copper-molybdenum imbalance in range cattle. Bov. Prac. 2, 9.
Bielecka, A., Jamioł, M., Kankofer, M., 2021. Antioxidative and Oxidative Profiles in Plasma and Saliva in Dairy Cows during. Pregnancy. Animals. 11, 3204.
Brandão, A.P., Cooke, E.F., 2021. Effects of temperament on reproduction of beef cattle. Anim. 11, 3325.
Burrow, H.M., Seifert, G.W., Corbet, N.J., 1988. A new technique for measuring temperament in cattle. Proc. Austr. Soc. Anim. Prod. 17, 154–157.
Celi, P., Merlo, M., Barbato, O., Gabai, G., 2012. Relationship between oxidative stress and the success of artificial insemination in dairy cows in a pasture-based system. Vet. J. 193, 498–502.
Cooke, R., Bill, E., 2014. Kunkle Interdisciplinary Beef Symposium: temperament and acclimation to human handling influence growth, health, and reproductive responses in Bos taurus and Bos indicus cattle. J. Anim. Sci. 92, 5325–5333.
Cooke, R.F., Arthington, J.D., Austin, B.R., Yelich, J.V., 2009. Effects of acclimation to handling on performance, reproductive, and physiological responses of Brahman-crossbred heifers. J. Anim. Sci. 87, 3403–3412.
Cooke, R.F., Bohnert, D.W., Meneghetti, M., Losi, T.C., Vasconcelos, J.L.M., 2011. Effects of temperament on pregnancy rates to fixed-timed AI in Bos indicus beef cows. Livest. Sci. 142, 108–113.
Corah, L.R., Ives, S., 1991. The effects of essential trace minerals on reproductions in beef cattle. Beef Cattle Nutrition 0749, 0720–0791.
Curley Jr., K.O., Neuendorff, D.A., Lewis, A.W., Cleere, J.J., Welsh Jr., T.H., Randel, R.D., 2008. Functional characteristics of the bovine hypothalamic-pituitary-adrenal axis vary with temperament. Horm. Behav. 53, 20–27.
Dobson, H., Smith, R.F., 2000. What is stress, and how does it affect reproduction? Anim. Reprod. Sci. 60, 743–752.
Dobson, H., Tebble, J.E., Smith, R.F., Ward, W.R., 2001. Is stress really all that important? Theriogenology 55, 65–73.
Echternkamp, S.E., 1984. Relationship between LH and cortisol in acutely stressed beef cows. Theriogenology 22, 305–311.
Fordyce, G.E.R., Dodt, R.M., Wythes, J.R., 1988. Cattle temperaments in extensive beef herds in northern Queensland. Factors affecting temperament. Aust. J. Exp. Agric. 28, 683–687.
Graham, T.W., 1991. Trace element deficiencies in cattle. Vet. Clin. North Am. Food Anim. Pract. 7, 153–215.
Grandin, T., 1993. Behavioral agitation during handling of cattle is persistent over time. Appl. Anim. Behav. Sci. 36, 1–9.
Guerin, P., El Mouatassim, S., Menezo, Y., 2001. Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. Hum. Reprod. Update 7, 175–189.
Harrison, J.H., Hancock, D.D., Conrad, H.R., 1984. Vitamin E and selenium for reproduction of dairy cow. J. Dairy. Sci. 67, 123–132.
Hartman, S.J., Genther-Schroeder, O.N., Hansen, S.L., 2018. Comparison of trace mineral repletion strategies in feedlot steers to overcome diets containing high concentrations of sulfur and molybdenum. J. Anim. Sci. 96, 2504–2515.
Kasimanickam, R., Schroeder, S., Assay, M., Kasimanickam, V., Moore, D.A., Gay, J.M., Whittier, W.D., 2014. Influence of temperament score and handling facility on stress, reproductive hormone concentrations, and fixed time AI pregnancy rates in beef heifers. Reprod. Dom. Anim. 49, 775–782.
Khan, I., Qureshi, M.S., Akhtar, S., Ali, L., Ghufranullah, 2016. Fertility Improvement in Cross-Bred Dairy Cows Through Supplementation of Vitamin E as Antioxidant Pakistan. J. Zool. 48 (4), 923–930.
King, J.O.L., 1971. Nutrition and fertility in dairy cows. Vet. Rec. 89, 320.
Markclund, S., 1980. Distribution of CuZn superoxide dismutase and Mn superoxide dismutase in human tissues and extracelular fuids. Acta. Physiol. Scand. Suppl. 492, 19–23.
Mello, B.P., Maturana Filho, M., Lemes, K.M., Gonçalvez, R.L., Lollato, J.P.M., Zanella, A. J., Ferreira, T.F.V., Pugliesi, G., Madureira, E.H., Gonella-Diaza, A., Membrive, C.M. B., 2020. Importance of temperament in the pregnancy by timed insemination in bovine females Bos taurus indicus. Livest. Sci. 240, 104104.
Muehlenbein, E.L., Brink, D.R., Deutscher, D.H., Carlson, M.P., Johnson, A.B., 2001. Effects of inorganic and organic copper supplemented to first-calf cows on cow reproduction and calf health and performance. J. Anim. Sci. 79, 1650–1659.
Nicholson, M.J., Butterworth, M.H., 1986. A guide to condition scoring of zebu cattle. Local: Addis Ababa, Ethiopia.
Phillippo, M., Humphries, W.R., Atkinson, T., et al., 1987. The effect of dietary molybdenum and iron on copper status, puberty, fertility and oestrous cycles in cattle. J. Agric. Sci. 109, 321.
Reichenbach, H.D., Moraes, J.C.F., Neves, J.P., 2008. Tecnologia de sêmen e inseminação artificial em bovinos. In: Gonçalves PBD, Figueiredo JR, Freitas VJF. Biotécnicas Aplicadas à Reprodução Animal. 4th ed. São Paulo, Roca. p. 57–82.
Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G., Hoekstra, W.G., 1973. Selenium: biochemical role as a component of glutathione peroxidase. Sci. 179, 588–590.
Rueda, P.M., Sant'Anna, A.C., Valente, T.S., Paranhos da Costa, M.J.R., 2015. Impact of the temperament of Nellore cows on the quality of handling and pregnancy rates in fixed-time artificial insemination. Livest. Sci. 177, 189–195.
Sá Filho, M.F., Crespilho, A.M., Santos, J.E.P., Perry, G.A., Baruselli, P.S., 2010. Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled Bos indicus cows. Anim. Reprod. Sci. 120, 23–30.
Sá Filho, M.F., Santos, J.E.P., Ferreira, R.M., Sales, J.N.S., Baruselli, P.S., 2011. Importance of estrus on pregnancy per insemination in suckled Bos indicus cows submitted to estradiol/progesterone-based timed insemination protocols. Theriogenology 76, 455–463.
Sant'Anna, A.C., Paranhos da Costa, M.J.R., Baldi, F., Albuquerque, L.G., 2013. Genetic variability for temperament indicators of Nellore cattle. J. Anim. Sci 91, 3532–3537.
Sordillo, L.M., Aitken, S.L., 2009. Impact of oxidative stress on the health and immune function of dairy cattle. Vet. Immunol. Immunopathol 128, 104–109.
Stake, P.E., 1977. Trace element absorption factors in animals. 38th Minnesota Nutrition Conference. 137.
Suttle, N.F., 1986. Problems in the diagnosis and anticipation of trace element deficiencies in grazing livestock. Vet. Rec. 119, 148.
Torres-Júnior, J.R.S., Aguiar, H.M.V.B., Cavalcanti, P.H., 2011. Reproductive ultrasonography for use of high cost semen in FTAI programs. Annual Meeting SBTE, 25th, Cambuco, CE, Brazil. Acta Scientiae Veterinariae. v.39(Suppl1). Abstract.
Vasconcelos, J.L.M., Sartori, R., Olievira, H.N., Guenther, J.G., Wiltbank, M.C., 2001. Reduction in size of the ovulatpry follicle reduces subsequente luteal size and pregnancy rate. Theriogenology 6, 307–314.
Vedovatto, M., Moriel, P., Cooke, R.F., Costa, D.S., Faria, F.J.C., Neto, I.M.C., Pereira, C. S., Bento, A.L.D.L., Almeida, R.G., Santos, S.A., Franco, G.L., 2019. Effects of a single

trace mineral injection on body parameters, ovarian structures, pregnancy rate and components of the innate immune system of grazing Nellore cows synchronized to a fixed-time AI protocol. Li-vestock Sci.. 225, 123–128.