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Parenteral Zinc Supplementation Increases Pregnancy Rates in Beef Cows

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Abstract

Zinc (Zn) is required for normal reproductive performance in cattle. The aim of this study was to evaluate the effect of subcutaneous injection of 400 mg Zn at the beginning of fixed-time artificial insemination (FTAI) on preovulatory follicle and corpus luteum (CL) size, plasma estradiol (E2) and progesterone (P4) concentrations, and pregnancy rates in beef cows. Copper (Cu) concentration and alkaline phosphatase (ALP) activity in plasma were also evaluated. Zinc supplementation at the beginning of the FTAI protocol (day 0) increased the area of preovulatory follicle (APF, day 9; $P = 0.042$) and plasma P4 concentration (day 16; $P = 0.01$), whereas plasma E2 concentration (day 9) and area of CL (ACL; day 16) were not modified by Zn supplementation in cows with adequate plasma Zn concentration. Zinc supplementation in Zn-deficient cows increased ACL with respect to controls ($P = 0.048$) but did not modify plasma E2 concentration. Pregnancy rate on day 41 after FTAI was higher in cows supplemented with Zn compared with controls (80.95% and 51.61%, respectively; $P = 0.042$). Plasma Zn and Cu concentrations on days 7, 9, and 16 were not affected by Zn supplementation. In conclusion, the results obtained in the present study determined that parenteral Zn supplementation at the beginning of the FTAI protocol increased preovulatory follicle size, plasma P4 concentration, and pregnancy rates in beef cows.

Keywords Zinc · FTAI · Cattle · Corpus luteum · Progesterone

Introduction

Zinc (Zn) is involved in a wide range of biological processes, including cell proliferation, immune function, and defense against free radicals [1–4]. Also, a possible role of Zn in reproduction has been proposed [5–7] given that mild to severe Zn deficiencies produce atypical ovarian development, disruption of estrous cycle, delay in follicular growth, and absence of preovulatory Graafian follicles in mice, women, and monkeys [5, 6]. In fact, Zn deficiency has been suggested to be a causal factor for pregnancy loss in women, nonhuman primates, rodents, and sheep [8]. In humans and animals, several studies have investigated the relationship between maternal Zn status and pregnancy outcome with conflicting results [6, 9–18]. Therefore, the role of Zn in the reproductive function in cows remains to be elucidated.

Zinc homeostasis is complex and partially known so far. Although there are no reliable indicators of Zn status in bovines, plasma/serum Zn concentrations are the most frequently used [19]. However, plasma/serum Zn concentrations are variable indicators and must be considered carefully because many factors can modify them [20].

The aim of this study was to evaluate the effect of parenteral Zn supplementation at the beginning of the FTAI protocol on preovulatory follicle and CL size, plasma E2 and P4 concentrations, and pregnancy rates in beef cows. Copper concentration and ALP activity in plasma were also evaluated.

Materials and Methods

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(Protocol No. 78-2-18P), approved all procedures followed in this trial.

Location and Experimental Animals

The trial was performed with animals from two commercial cowherds located in Buenos Aires province, Argentina (farm 1: 35° 21′ 48″ S, 57° 13′ 37″ W; farm 2: 34° 56′ 29″ S, 59° 08′ 23″ W) in late spring 2017 (October–November).

A total of 62 Aberdeen Angus cows were used. Farm 1 provided 27 mature cows (4.74 ± 0.14 years old; range, 4–6) weighing 456.1 ± 4.7 kg, with a body condition score (BCS) of 3.32 ± 0.02 (range, 2.5–4) on 1–5 scale [21] and more than 62 days post-partum at the start of the experiment. Farm 2 provided 35 mature cows (4.85 ± 0.11 years old; range, 4–6) weighing 476.1 ± 6.2 kg, with 3.57 ± 0.03 BCS (range 2.5–4) and more than 65 days post-partum at the start of the experiment. Cows from both farms were managed under an extensive grazing system based on natural pastures.

Cows at a random stage of the estrous cycle received an intra-muscular (IM) injection of 2 mg estradiol benzoate (Syntex, Argentina) and an intra-vaginal P4 device containing 0.5 g P4 (DIB®, Syntex, Argentina) (day 0). Cows were randomly assigned to one of two treatments: (1) control group (farm 1, $n = 16$; farm 2, $n = 19$) received 4 mL of sterile NaCl solution (9 g/L), and (2) Zn group (farm 1, $n = 11$; farm 2, $n = 16$) was supplemented with a subcutaneous injection (4 mL) of 400 mg Zn sulfate ($ZnSO_4$). On day 7, DIB was removed, and cows were administered 500 mg cloprostenol (Ciclase DL, Syntex) and 0.5 mg estradiol cypionate (Cipiosyn, Syntex) by IM injection. Cows were AI 52–56 h after DIB removal by the same experienced technician (day 9). Semen from the same bull and batch was used for all cows.

A solution of 100 mg/mL Zn was prepared by adding 191.19 mL of a commercial aqueous 2 M $ZnSO_4$ solution (Sigma-Aldrich, Cat. No. 83265) to 58.81 mL of double distilled and deionized water (Sigma-Aldrich, Cat. No. W4502). The solution was filtered and stored in a sterile multi-dose bottle until used.

Blood Samples

Samples of coccygeal blood (10 mL) were collected on days 0, 7, 9, and 16 in tubes containing EDTA, placed on ice immediately after collection, centrifuged at 350*g* for 10 min, and then plasma was separated and stored at − 20 °C.

Plasma Zn and Cu Concentrations

Plasma Zn and Cu concentrations were evaluated on days 0, 7, 9, and 16. Plasma samples were proportionally deproteinized with 10% (*v*/*v*) trichloroacetic acid. Zinc and Cu concentrations were measured in supernatants using a double beam flame atomic absorption spectrophotometer (Perkin Elmer AAnalyst 200; AAS, International Equipment Trading Ltd., Mundelein,USA) with an internal quality control [22]. Zinc and Cu standard solutions were purchased from Merck (Tokyo, Japan; Cat. No. 48096-2B and Cat. No. 08046-2B, respectively).

Hormone Assays

Plasma E2 concentration was determined on day 9 with a commercial RIA kit (Estradiol Double Antibody, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) (7.6% intra-assay coefficient of variation; 2.9 pg/tube assay sensitivity) [23]. Plasma P4 concentration was measured on day 16 with a commercial RIA kit (Coat A-Count®, Siemens Medical Solutions Diagnostics) (11.9% intra-assay coefficient of variation; 0.2 ng/tube assay sensitivity) [23].

Alkaline Phosphatase Activity Assay

Plasma ALP activity of cows from farm 2 was evaluated on days 0, 7, 9, and 16. The assay was performed in a commercial laboratory by using routine clinical laboratory tests (Clinical Chemistry Department, Iglesias-Haramburu Institute, La Plata, Argentina). The ALP was measured by a colorimetric method with an internal quality control [24]. This is based on the transformation of a substrate, p-Nitrophenyl phosphate, into p-Nitrophenol, a colored product whose absorbance was measured at 405 nm. Plasma ALP activity was expressed as units/L.

Ultrasonography

Area of preovulatory follicle (APF) at AI (day 9), area of CL (ACL, day 16), and pregnancy rate (day 50; 41 days after AI) were determined by transrectal ultrasonography (Aloka 500 V equipped with a 7.5-MHz linear-array transducer, Aloka, Wallingford, CT). Images were taken to measure the maximum diameter of PF, CL, and CL cavity, if present. The horizontal and vertical diameters were recorded, and the average was used in the statistical analysis. The location of PF that corresponded to the largest area was recorded. The ACL was calculated by subtracting the luteal cavity area from the entire CL area. Pregnancy diagnoses were performed by measuring the fetal heartbeat. All measurements were performed by the same operator.

Statistical Analysis

A completely randomized block design was used. Statistical models included the random effect of block (farm, $n = 2$), and the fixed effect of treatment and their second order interaction. The effect of Zn supplementation on response variables (Zn,

Cu, E2, and P4 concentration and, ALP activity, APF, and ACL) were analyzed with linear regression models by using the MIXED procedure of SAS (SAS Institute, Cary, NC, USA). The fixed effect of the age of cows on plasmatic Zn concentration was also evaluated. In the case of repeated measures (i.e., Zn and Cu concentration and ALP activity), a repeated measure linear model was used to account for autocorrelation. The APF was used as covariate in the analysis of ACL. The effect of Zn supplementation on pregnancy rate (41 days after FTAI) was analyzed by logistic regression analysis by using the GLIMMIX procedure (SAS Institute) with binomial distribution and logit link function. Results are expressed as least squared means ± SEM. Statistical significance was set at $P < 0.05$ and $P < 0.10$ for tendency and interactions.

Results

Animals

Plasma Zn concentration at the beginning of FTAI (day 0) was different between farms (farm 1, 91.5 ± 5.3; farm 2, 125.5 ± 4.6 μg/dL Zn; $P < 0.0001$). In farm 1, 66.6% of cows (18/27) were Zn-deficient (< 90 μg/dL) on day 0, whereas in farm 2, no cow was deficient (36/36 had plasma Zn concentration > 96 μg/dL). There were no significant differences in the age of cows between both farms ($P = 0.78$). The age of cows did not have effect on plasma Zn concentration on day 0 ($P = 0.98$), day 7 ($P = 0.65$), day 9 ($P = 0.76$), or day 16 ($P = 0.38$).

Effect of Parenteral Zn Supplementation on Plasma Zn and Cu Concentration

Time (day) had a quadratic effect ($P < 0.01$) on plasma Zn concentration (107.21 ± 3.9, 131.71 ± 4.5, 111.52 ± 3.9, and 103.68 ± 4.4 μg/dL Zn for days 0, 7, 9, and 16, respectively; $P < 0.01$; Fig. 1a). Zinc supplementation had no effect on plasma Zn concentration (116.11 ± 2.9 vs. 110.95 ± 3.2 μg/dL Zn for control vs. Zn, respectively; $P = 0.24$). The interaction of time by Zn supplementation had no effect on Zn concentration ($P = 0.55$).

Time (day) had a quadratic effect ($P < 0.01$) on plasma Cu concentration (51.29 ± 1.1, 51.81 ± 1.3, 42.84 ± 1.8, and 55.07 ± 1.3 μg/dL Cu for days 0, 7, 9, and 16, respectively; $P < 0.01$; Fig. 1b). Zn supplementation had no effect on plasma Cu concentration (51.35 ± 1.3 vs. 49.15 ± 1.5 μg/dL Cu for control vs. Zn, respectively; $P = 0.27$). The interaction of time by Zn supplementation had no effect on Cu concentration ($P = 0.96$).

Effect of Parenteral Zn Supplementation on ALP Activity

Time (day) had no effect on ALP activity ($P < 0.20$). Parenteral Zn supplementation tended to increase ALP activity on day 7 ($P = 0.09$) (days 0, 7, 9, and 16; control, 62.4 ± 14.7; 46.1 ± 5.8; 57.8 ± 2.5; 45.4 ± 2.2 U/L; Zn, 44.3 ± 16; 62.3 ± 7.1; 61.2 ± 2.7; 44.7 ± 2.3 U/L, respectively; Fig. 2). The interaction of time by Zn supplementation had no effect on ALP activity ($P < 0.19$).

Effect of Parenteral Zn Supplementation on APF, ACL, E2, and P4 Concentrations and Pregnancy Rates

The effect of parenteral Zn supplementation on APF, ACL, and plasma E2 and P4 concentrations is shown in Table 1. Zn supplementation increased APF ($P = 0.042$) and plasma P4 concentration ($P = 0.01$). Conversely, it had no effect on plasma E2 concentration and ACL. In Zn-deficient cows, Zn supplementation increased ACL (3.40 ± 0.30 vs. 4.40 ± 0.39 cm^2 for control vs. Zn, respectively; $P = 0.048$), tended to increase APF (0.97 ± 0.12 vs. 1.36 ± 0.15 cm^2 for control vs. Zn, respectively; $P = 0.09$), and increased plasma P4 concentration only numerically (4.1 ± 0.6 vs. 5.4 ± 0.8 ng/mL for control vs. Zn, respectively; $P = 0.25$). Conversely, it had no effect on plasma E2 concentration (18.1 ± 1.4 vs. 15.7 ± 1.7 pg/mL for control vs. Zn, respectively; $P = 0.31$). Finally, Zn supplementation increased the odds for pregnancy (OR = 3.98; 95% CI = 1.06–15.05; $P = 0.042$) given that the percentage of pregnant cows was 80.95% (17/21) for Zn-supplemented cows vs. 51.61% (16/31) for control cows (Table 2). The interaction of farm by Zn supplementation had no effect on any of the variables assessed ($P > 0.30$).

Discussion

We found that Zn supplementation, at the beginning of the FTAI protocol, increased plasma P4 concentration and pregnancy rate, regardless of the Zn status of beef cows.

In the present study, Zn supplementation not only increased ACL in Zn-deficient cows but also increased plasma P4 concentration in "normal" cows. Plasma P4 concentration in deficient cows was increased only numerically with Zn treatment, probably due to the scarce number of animals with this condition. It has been shown that Zn inhibits apoptosis in luteal cells of rats [7] and dietary Zn deficiency causes a shrunken CL in mature female mice [5]. Moreover, different studies support the key role of Cu/Zn superoxide dismutase (Cu/Zn-SOD) to maintain CL size and P4 production [25–28]. The enzyme Cu/Zn-SOD is one of the main mechanisms for cellular defense from reactive oxygen species [29]. It scavenges the harmful superoxide radicals and protects cells from

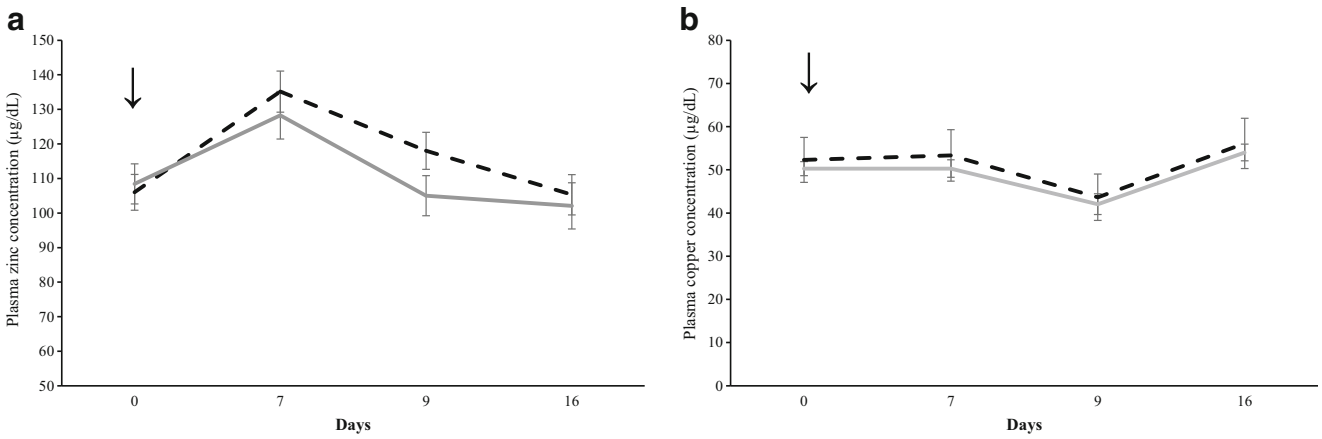

Fig. 1 Effect of parenteral Zn supplementation at the beginning of fixed-time artificial insemination protocol on plasma Zn and Cu concentrations in grazing Aberdeen Angus cows. **a** Plasma Zn concentrations on days 0, 7, 9, and 16. **b** Plasma Cu concentrations on days 0, 7, 9, and 16. Cows were injected at the beginning of FTAI protocol (day 0; arrow) with saline solution (control group; dotted line) or 400 mg zinc sulfate (zinc group; solid line). Results are expressed as means ± SEM. Time (day) had a quadratic effect on plasma Zn and Cu concentration ($P < 0.01$). The effect of treatment was not significant ($P > 0.05$). Interaction of time by Zn supplementation had no effect on Zn and Cu concentration ($P > 0.05$)

the related damaging effects. Thus, binding of Zn to SOD is essential for its adequate biological function [30, 31]. In Zn-deficient animals, decreased Cu/Zn-SOD activity may damage luteal cells, diminish CL size, and reduce P4 production.

Progesterone is essential for the establishment and maintenance of pregnancy. Decreased plasma P4 concentrations during early embryo development reduce embryo interferon-tau (IFN-t) secretion and alter the expression of genes associated with specific transporters, cell proliferation/migration, and remodeling proteins, and of genes contributing to embryo elongation [32, 33]. Different studies have reported a positive association between P4 concentration during early pregnancy and bovine conceptus growth [34, 35]. Furthermore, P4 supplementation during the early stages of pregnancy has been shown to favor embryo development and uterine secretion [36, 37]. In dairy cattle, P4 concentration was associated with embryo survival [38, 39]. Additionally, plasma P4 concentrations below 2.8 ng/mL resulted in up to 50% losses in pregnancy rates in these cows [40]. In Holstein cattle, P4 concentration of recipient cows during early pregnancy (days 0 to 7) was associated with pregnancy rates (days 28, 42, and 63) obtained by embryo transfer [41]. In suckled beef cows, reduction of serum P4 increased late embryonic/early fetal loss [40]. These results confirm that plasma P4 concentration is an important embryo/fetal survival indicator during early pregnancy. In our study, the increase in P4 concentration elicited by Zn supplementation was accompanied by an increase in pregnancy rate.

Although the literature about the effect of Zn supplementation on pregnancy rates in cows is scarce, several studies have evaluated trace mineral mixtures containing Zn [42–47]. Ahola and colleagues [43] observed an increase in pregnancy rate

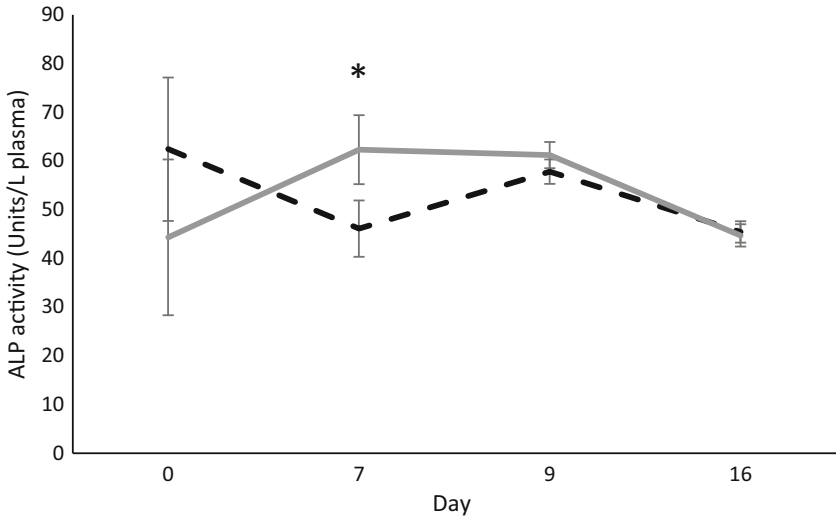

Fig. 2 Effect of parenteral Zn supplementation at the beginning of fixed-time artificial insemination protocol on plasma alkaline phosphatase (ALP) activity in grazing Aberdeen Angus cows. Plasma ALP activity of cows from farm 2 was evaluated on day 0, 7, 9, and 16. Cows were subcutaneously injected at the beginning of FTAI protocol with saline solution (control group; dotted line) or zinc sulfate (zinc group; solid line). Results are expressed as means ± SEM. Parenteral Zn supplementation tended to increase ALP activity on day 7 (*, $P = 0.09$)

Table 1 Effect of parenteral Zn supplementation at the beginning of fixed-time artificial insemination protocol on area of preovulatory follicle, area of corpus luteum and plasma estradiol, and progesterone concentration

Treatment	APF (cm^2)	E2 (pg/mL)	ACL (cm^2)	P4 (ng/mL)	
Control	0.88 ± 0.05[a]	15.5 ± 0.7	3.35 ± 0.19	3.7 ± 0.3[a]	
Zinc		1.09 ± 0.08[b]	15.8 ± 0.8	3.64 ± 0.22	5.0 ± 0.3[b]

Cows were subcutaneously injected whit 400 mg zinc sulfate at the beginning of fixed-time artificial insemination protocol (day 0 of the trial) or acted as negative controls

APF area of preovulatory follicle at the time of insemination (day 9), *E2* plasma estradiol concentration at the time of insemination (day 9), *ACL* area of corpus luteum on day 16, *P4* plasma progesterone concentration on day 16. Results are expressed as means ± SEM

Different superscripts within each column had $P < 0.05$

post-AI in cows supplemented orally with Zn, Cu, and manganese (Mn) for 2 years. Moreover, in a meta-analysis of 20 research papers and reports, Rabiee and colleagues [48] found that supplementation with oral organic Zn, Cu, Mn, and cobalt mixture reduced the number of services per conception and increased the risk of pregnancy in lactating dairy cows.

In our study, the injection of Zn at the beginning of the FTAI protocol increased APF. On the other hand, subcutaneous injection of a trace mineral complex containing Cu, Mn, selenium, and Zn to over-conditioned cows 14 days before follicular wave synchronization failed to find an association between mineral supplementation and diameter of the preovulatory follicle [47]. Numerous studies have demonstrated nutritional effects on follicular development. Retardation of ovarian follicular growth and lack of preovulatory Graafian follicles have been observed in Zn-deficient monkeys and mice [5, 49]. Using X-ray fluorescence, Ceko and colleagues [50] described Zn distribution in bovine ovaries and found higher Zn concentration in healthy than in regressed follicles (the healthy cohort showed three times more Zn than regressed ones).

Table 2 Effect of parenteral Zn supplementation at the beginning of fixed-time artificial insemination protocol on pregnancy rate in grazing Aberdeen Angus cows

			Odds ratio of pregnancy		
		%	OR	95% CI	*P*
Farm	1	55.56	1		0.39
	2	67.65	1.67	0.50–5.58	
Zn	No	51.61	1		0.04
	Yes	80.95	3.98	1.05–15.05	

OR odds ratio, *CI* confidence interval. The trial was performed with animals from two commercial farms. Cows from zinc-supplemented group were subcutaneously injected with 400 mg zinc sulfate at the beginning of FTAI protocol. Pregnancy rate was determined by transrectal ultrasonography 41 days after artificial insemination. The interaction of farm by Zn supplementation had no effect on pregnancy rate ($P = 0.45$)

Even though a direct effect of Zn on follicles and CL would be possible [7, 50], another probable cause for increased APF, increased CL size (only in Zn-deficient cows), and elevated P4 concentration in Zn-supplemented cows might be that Zn participates in follicle-stimulating hormone (FSH) and luteinizing hormone (LH) synthesis and/or secretion in females [6]. While FSH is the principal regulator of follicular growth, LH stimulates both large follicles and CL growth and increases progesterone secretion by CL in cattle [51–54]. Although we did not examine plasma FSH and LH concentrations in this study, they could have been increased by Zn supplementation.

Our results show that Zn supplementation did not induce an increase in plasma Zn concentrations, even in Zn-deficient animals. The same was described by González-Maldonado and colleagues [47] in over-conditioned cows supplemented with a trace mineral mix including Zn. These authors speculated that these minerals could be absorbed and stored rapidly in selected tissues such as muscle, fat tissue, and the small intestine, in the case of Zn [47]. Interestingly in our study, plasma Zn concentration increased on day 7 not only in the Zn group but also in the control group. The increase observed in the untreated group (control) confirms the variability in plasma Zn concentration values. This could be due to the FTAI treatment, where several hormones are administered. It has been shown that steroid hormones in many forms such as adrenal corticosteroids and gonadal steroids modify circulating Zn concentrations [20]. In addition, Deuster and colleagues [55] demonstrated that plasma Zn concentration is high during the follicular phase, and then declines during the ovulation and luteal phases in women.

While Zn and Cu are known to have a strong mutual antagonism, we found no effect of Zn supplementation on plasma Cu concentrations. Zinc is capable of competing for Cu binding site because of their similar chemistry organization [2]. High concentrations of dietary Zn reduced Cu body stores and protected against Cu toxicity in sheep [56]. Moreover, maternal high-Zn diet induced Cu deficiency in neonatal pigs [57].

In bovines, plasma or serum Zn concentrations are widely used indicators to establish Zn status [19], probably because of its relatively easy accessibility with respect to other tissue samples [20, 58]. It has been agreed that plasma Zn concentrations above 90 μg/dL should be considered normal, between 80 and 90 μg/dL as marginal, and below 80 μg/dL as indicative of Zn deficiency [59]. However, considering that plasma Zn concentration is a variable indicator [20, 60, 61], some authors proposed other indices in addition to plasma Zn concentration to estimate Zn status, including the determination of Zn-dependent enzyme activities such as ALP [6]. Alkaline phosphatase is an enzyme which has Zn as an important co-factor. Changes in Zn status may reflect in changes in ALP activity [62–65]. To investigate if Zn supplementation improved Zn-dependent metabolism, we evaluated the effect of Zn injection on plasma ALP activity. The present results show that while plasma Zn concentration was not modified by Zn supplementation, ALP activity tended to be increased ($P = 0.09$) in Zn-supplemented cows (only on day 7 of FTAI). These results suggest that plasma ALP activity is a more sensitive indicator of Zn supplementation than plasma Zn concentration itself.

Conclusions

The results of the present study provide evidence that parenteral Zn supplementation, at the beginning of the FTAI protocol, increased preovulatory follicle size, plasma P4 concentration, and pregnancy rates in beef cows without modifying plasma Zn levels.

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Author Contributions J.M.A., J.P.A., N.N, E.M.G., and C.C.F. conceived and designed the experiments; E.M.G, N.A.F., and J.M.A. conducted the experiments; M.J.G. analyzed the data; C.F., J.M.A., and L.E.F. critically wrote and revised the paper. All authors read and approved the final manuscript.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that there are no conflicts of interest.

References

1. McCall KA, Huang CC, Fierke CA (2000) Function and mechanism of zinc metalloenzymes. J Nutr 130:1437–1446
2. Powell SR (2000) The antioxidant properties of zinc. J Nutr 130(5S Suppl):1447S–1454S
3. Cousins RJ, Liuzzi JP, Lichten LA (2006) Mammalian zinc transport, trafficking, and signals. J Biol Chem 281(34):24085–24089
4. Sheikh AA, Aggarwal A, B I, Aarif O (2017) Inorganic zinc supplementation modulates heat shock and immune response in heat stressed peripheral blood mononuclear cells of periparturient dairy cows. Theriogenology 95:75–82
5. Taneja SK, Kaur R (1990) Pathology of ovary, uterus, vagina and gonadotrophs of female mice fed on Zn-deficient diet. Indian J Exp Biol 28(11):1058–1065
6. Bedwal RS, Bahuguna A (1994) Zinc, copper and selenium in reproduction. Experientia 50(7):626–640
7. Zhang JH, Yu J, Li WX, Cheng CP (1998) Evaluation of Mn2+ stimulated and Zn2+ inhibited apoptosis in rat corpus luteal cells by flow cytometry and fluorochromes staining. Chin J Physiol 41(2): 121–126
8. Graham TW, Thurmond MC, Gershwin ME, Picanso JP, Garvey JS, Keen CL (1994) Serum zinc and copper concentrations in relation to spontaneous abortion in cows: implications for human fetal loss. J Reprod Fertil 102(1):253–262
9. Kynast G, Saling E (1980) The relevance of zinc in pregnancy. J Perinat Med 8:171–182
10. Apgar J (1985) Zinc and reproduction. Annu Rev Nutr 5:43–68
11. Apgar J (1992) Zinc and reproduction: an update. J Nutr Biochem 3:266–278
12. Simmer K, Thompson RPH (1985) Zinc in the fetus and newborn. Acta Paediatr Scand Suppl 319:158–163
13. Villa Elizaga I, da Cunha Ferreira RMC (1985) Zinc, pregnancy and parturition. Acta Paediatr Scand Suppl 319:150–157
14. Swanson CA, King JC (1987) Zinc and pregnancy outcome. Am J Clin Nutr 46:763–771
15. Campbell DM (1988) Trace element needs in human pregnancy. Proc Nutr Sot 47:45–53
16. Valdes-Ramos R (1992) Zinc: a perinatal point of view. Prog Food Nutr Sci 16:279–306
17. Tamura T, Goldenberg RL (1996) Zinc nutriture and pregnancy outcome. Nutr Res 6(1):139–181
18. Swain PS, Rao SBN, Rajendran D, Dominic G, Selvaraju S (2016) Nano zinc, an alternative to conventional zinc as animal feed supplement: a review. Anim Nutr 2(3):134–141
19. Underwood EJ, Suttle NF (1999) The mineral nutrition of livestock. CABI Publishing, London
20. Solomons NW (1979) On the assessment of zinc and copper nutriture in man. Am J Clin Nutr 32(4):856–871
21. Houghton PL, Lemenager RP, Horstman LA, Hendrix KS, Moss GE (1990) Effects of body composition, pre- and postpartum energy level and early weaning on reproductive performance of beef cows and preweaning calf gain. J Anim Sci 68:438–446
22. Piper HG, Higgins G (1967) Estimation of trace metals in biological material by atomic absorption spectrophotometry. Proc Assoc Clin Biochem 7:190–195
23. Aller JF, Callejas SS, Alberio RH (2013) Biochemical and steroid concentrations in follicular fluid and blood plasma in different follicular waves of the estrous cycle from normal and superovulated beef cows. Anim Reprod Sci 142(3–4):113–120

24. Bowers GN, McComb RB (1975) Measurement of total alkaline phosphatase activity in human serum. Clin Chem 21(13):1988–1995
25. Nishimura R, Komiyama J, Tasaki Y, Acosta TJ, Okuda K (2008) Hypoxia promotes luteal cell death in bovine corpus luteum. Biol Reprod 78(3):529–536
26. Noda Y, Ota K, Shirasawa T, Shimizu T (2012) Copper/zinc superoxide dismutase insufficiency impairs progesterone secretion and fertility in female mice. Biol Reprod 86(16):1–8
27. Al-Gubory KH, Garrel C, Faure P, Sugino N (2012) Roles of antioxidant enzymes in corpus luteum rescue from reactive oxygen species-induced oxidative stress. Reprod BioMed Online 25(6):551–560
28. Kawaguchi S, Sakumoto R, Okuda K (2013) Induction of the expressions of antioxidant enzymes by luteinizing hormone in the bovine corpus luteum. J Reprod Dev 59(3):219–224
29. Ho E (2004) Zinc deficiency, DNA damage and cancer risk. J Nutr Biochem 15(10):572–578
30. Borchelt DR, Lee MK, Slunt HS, Guarnieri M, Xu ZS, Wong PC, Brown RH Jr, Price DL, Sisodia SS, Cleveland DW (1994) Superoxide dismutase 1 with mutations linked to familial amyotrophic lateral sclerosis possesses significant activity. Proc Natl Acad Sci U S A 91(17):8292–8296
31. Sahawneh MA, Ricart KC, Roberts BR, Bomben VC, Basso M, Ye Y, Sahawneh J, Franco MC, Beckman JS, Estévez AG (2010) Cu, Zn-superoxide dismutase increases toxicity of mutant and zinc-deficient superoxide dismutase by enhancing protein stability. J Biol Chem 285(44):33885–33897
32. Forde N, Beltman ME, Duffy GB, Duffy P, Mehta JP, O'Gaora P, Roche JF, Lonergan P, Crowe MA (2011) Changes in the endometrial transcriptome during the bovine estrous cycle: effect of low circulating concentrations of progesterone and consequences for conceptus elongation. Biol Reprod 84:266–278
33. Forde N, Mehta JP, Minten M, Crowe MA, Roche JF, Spencer TE, Lonergan P (2012) Effects of low progesterone on the endometrial transcriptome in cattle. Biol Reprod 87:124
34. Mann GE, Lamming GE (2001) Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. Reproduction 121:175–180
35. Green MP, Hunter MG, Mann GE (2005) Relationships between maternal hormone secretion and embryo development on day 5 of pregnancy in dairy cows. Anim Reprod Sci 88:179–189
36. Garrett JE, Geisert RD, Zavy MT, Morgan GL (1988) Evidence for maternal regulation of early conceptus growth and development in beef cattle. J Reprod Fertil 84:437–446
37. Mann GE, Lamming GE, Fray MD (1995) Plasma oestradiol and progesterone during early pregnancy in the cow and the effects of treatment with buserelin. Anim Reprod Sci 37:121–131
38. McNeill RE, Diskin MG, Sreenan JM, Morris DG (2006) Associations between milk progesterone concentrations on different days and with embryo survival during the early luteal phase in dairy cows. Theriogenology 65:1435–1441
39. Stronge AJ, Sreenan JM, Diskin ME, Mee JF, Kenny DA, Morris DG (2005) Post-insemination milk progesterone concentrations and embryo survival in dairy cows. Theriogenology 64:1212–1224
40. Rhinehart JD, Starbuck-Clemmer MJ, Flores JA, Milvae RA, Yao J, Poole DH, Inskeep EK (2009) Low peripheral progesterone and late embryonic/early fetal loss in suckled beef and lactating dairy cows. Theriogenology 71(3):480–490
41. Kenyon AG, Mendonca LGD, Lopes G, Lima JR, Santos JEP, Chebel RC (2012) Minimal progesterone concentration required for embryo survival after embryo transfer in lactating Holstein cows. Anim Reprod Sci 136:223–230
42. Olson PA, Brink DR, Hickok DT, Carlson MP, Schneider NR, Deutscher GH, Adams DC, Colburn DJ, Johnson AB (1999) Effects of supplementation of organic and inorganic combinations of copper, cobalt, manganese, and zinc above nutrient requirement levels on postpartum two-year-old cows. J Anim Sci 77(3):522–532
43. Ahola JK, Baker DS, Burns PD, Mortimer RG, Enns RM, Whittier JC, Geary TW, Engle TE (2004) Effect of copper, zinc, and manganese supplementation and source on reproduction, mineral status, and performance in grazing beef cattle over a two-year period. J Anim Sci 82(8):2375–2383
44. Black DH, French NP (2004) Effects of three types of trace element supplementation on the fertility of three commercial dairy herds. Vet Rec 154(21):652–658
45. Vanegas JA, Reynolds J, Atwill ER (2004) Effects of an injectable trace mineral supplement on first-service conception rate of dairy cows. J Dairy Sci 87(11):3665–3671
46. Siciliano-Jones JL, Socha MT, Tomlinson DJ, DeFrain JM (2008) Effect of trace mineral source on lactation performance, claw integrity, and fertility of dairy cattle. J Dairy Sci 91(5):1985–1995
47. González-Maldonado J, Rangel-Santos R, Rodríguez-de Lara R, García-Peña O (2017) Effect of injectable trace mineral complex supplementation on development of ovarian structures and serum copper and zinc concentrations in over-conditioned Holstein cows. Anim Reprod Sci 181:57–62
48. Rabiee AR, Lean IJ, Stevenson MA, Socha MT (2010) Effects of feeding organic trace minerals on milk production and reproductive performance in lactating dairy cows: a meta-analysis. J Dairy Sci 93(9):4239–4251
49. Swenerton H, Hurley LS (1980) Zinc deficiency in rhesus and bonnet monkeys, including effects on reproduction. J Nutr 110(3):575–583
50. Ceko MJ, Hummitzsch K, Bonner WM, Aitken JB, Spiers KM, Rodgers RJ, Harris HH (2015) Localization of the trace elements iron, zinc and selenium in relation to anatomical structures in bovine ovaries by X-Ray fluorescence imaging. Microsc Microanal 21(3):695–705
51. Snook RB, Brunner MA, Saatman RR, Hansel W (1969) The effect of antisera to bovine LH in hysterectomized and intact heifers. Biol Reprod 1(1):49–58
52. McNeilly AS, Fraser HM (1987) Effect of gonadotrophin-releasing hormone agonist-induced suppression of LH and FSH on follicle growth and corpus luteum function in the ewe. J Endocrinol 115(2):273–282
53. Weems YS, Lammoglia MA, Vera-Avila HR, Randel RD, King C, Sasser RG, Weems CW (1998) Effect of luteinizing hormone (LH), PGE2, 8-EPI-PGE1, 8-EPI-PGE2, trichosanthin, and pregnancy specific protein B (PSPB) on secretion of progesterone in vitro by corpora lutea (CL) from nonpregnant and pregnant cows. Prostaglandins Other Lipid Mediat 55(1):27–42
54. Kumar P, Sait SF (2011) Luteinizing hormone and its dilemma in ovulation induction. J Hum Reprod Sci 4(1):2–7
55. Deuster PA, Dolev E, Bernier LL, Trostmann UH (1987) Magnesium and zinc status during the menstrual cycle. Am J Obstet Gynecol 154:964–968
56. Bremner I, Young BW, Mills CF (1976) Protective effect of zinc supplementation against copper toxicosis in sheep. Br J Nutr 36(3):551–561
57. Hill GM, Ku PK, Miller ER, Ullrey DE, Losty TA, O'Dell BL (1983) A copper deficiency in neonatal pigs induced by a high zinc maternal diet. J Nutr 113(4):867–872
58. Hurley LS (1981) The roles of trace elements in foetal and neonatal development. Philos Trans R Soc Lond Ser B Biol Sci 294(1071):145–152
59. Enjalbert F, Lebreton P, Salat O (2006) Effects of copper, zinc and selenium status on performance and health in commercial dairy and

beef herds: retrospective study. J Anim Physiol Anim Nutr 90:459–466
60. Rosa DE, Fazzio LE, Picco SJ, Furnus CC, Mattioli GA (2008) Metabolismo y deficiencia de zinc en bovinos. Analecta Vet 28: 34–44
61. Pieper L, Schmidt F, Müller AE, Staufenbiel R (2017) Zinc concentrations in different sample media from dairy cows and establishment of reference values. Tierarztl Prax Ausg G Grosstiere Nutztiere 45(4):213–218
62. Weismann K, Høyer H (1985) Serum alkaline phosphatase and serum zinc levels in the diagnosis and exclusion of zinc deficiency in man. Am J Clin Nutr 41(6):1214–1219
63. Naber TH, Baadenhuysen H, Jansen JB, van den Hamer CJ, van den Broek W (1996) Serum alkaline phosphatase activity during zinc deficiency and long-term inflammatory stress. Clin Chim Acta 249(1–2):109–127
64. Sheikh G, Masood Q, Majeed S, Hassan I (2015) Comparison of levels of serum copper, zinc, albumin, globulin and alkaline phosphatase in psoriatic patients and controls: a hospital based case control study. Indian Dermatol Online J 6(2):81–83
65. Ray C. S, Singh B, Jena I, Behera S, Ray S (2017) Low alkaline phosphatase (ALP) in adult population an indicator of zinc (Zn) and magnesium (mg) deficiency. Curr Res Nutr Food Sci 5(3)