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Parenteral Copper Administration at the Beginning of a Fixed-Time Artificial Insemination Protocol in Beef Cattle: Effect on Ovarian Function and Pregnancy Rates

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Abstract

The aim of this study was to evaluate the association between plasma copper (Cu) concentration and ovarian function during a fixed-time artificial insemination (FTAI) protocol and the effect of parenteral Cu administration (100 mg) at the start of such protocol (day 0) on area of preovulatory follicle (APF); area of corpus luteum (ACL), plasma estradiol (E2), and progesterone (P4) concentrations; CL blood flow (CLBF); and pregnancy rate in beef heifers and cows. In cows, plasma Cu concentration on days 0 and 7 correlated positively with APF. Copper administration increased plasma Cu concentration and decreased APF and plasma E2 concentration (day 9), without modifying ACL, plasma P4 concentration, and CLBF (day 16) in cows. Pregnancy rate was higher in Cu-supplemented cattle on day 41 after FTAI as compared with controls (58.76 and 45.28%, respectively). In conclusion, Cu administration at the beginning of the FTAI protocol increased pregnancy rate in beef heifers and cows, modifying APF and plasma E2 concentration in the latter.

Keywords Artificial insemination · Copper · Corpus luteum · Plasma estradiol · Preovulatory follicle

Introduction

In domestic animals, normal reproductive behavior is closely related to nutritional status [1]. Deficiency in a single mineral such as copper (Cu) may cause various reproductive failures in cattle, comprising delayed puberty, infertility, poor conception, and early embryonic loss [2]. Subfertility in cattle is a serious problem worldwide and a considerable proportion of animals respond positively to adequate Cu supplementation [3, 4]. Copper status in cattle is defined as deficient, marginal, and adequate for plasma Cu concentrations of < 30, 31–60, and > 60 μg/dL, respectively [5]. Studying the role of Cu in the bovine reproductive function is therefore crucial since Cu deficiency is the second most widespread mineral deficiency that affects grazing cattle globally [6, 7].

The effect of Cu on ovarian function has been known for a long time [8]. In rats, pigs and rabbits, Cu stimulated the release of hypothalamic-pituitary-ovarian axis sex hormones, such as gonadotropin-releasing hormone (GnRH), follicular-stimulating hormone (FSH), and luteinizing hormone (LH) [9–12]. An in vitro study has shown that Cu modifies the conformation of the GnRH receptor causing the release of gonadotropin granule content through calcium dependent mechanisms [10]. In rabbits, Fevold and colleagues [8] reported induction of ovulation by intravenous injection of Cu salts. These results reveal a possible pharmacological effect of Cu on ovarian function and ovulation [13]. In cattle, little is known about the physiological or pharmacological effect of Cu on reproductive hormones,

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ovarian function, or after applying biotechnologies where ovarian activity is intentionally manipulated. The fixed-time artificial insemination (FTAI) treatment consists of an intentional manipulation of the ovarian function employing reproductive hormones to allow cattle to be inseminated at a designated time without the need for estrus detection.

The aim of this study was to evaluate the association between plasma Cu concentration and ovarian function during an FTAI protocol and the effect of parenteral Cu administration at the start of such protocol on area of preovulatory follicle (APF), area of corpus luteum (ACL), plasma estradiol (E2) and progesterone (P4) concentrations, CL blood flow (CLBF), and pregnancy rate in beef cattle.

Materials and methods

The experimental procedures were approved by the School of Veterinary Sciences Institutional Animal Care and Use Committee (National University of La Plata, Buenos Aires, Argentina; Protocol No. 105–3-20P).

Experiment 1: Association Between Plasma Cu Concentration During FTAI Protocol and APF, ACL, and Plasma E2 and P4 Concentrations

Animals and Treatments

The trial was performed during the southern hemisphere breeding season (springtime, October–November in Buenos Aires, Argentina) in two commercial farms located in the Salado River Basin (farm 1: 34°59′54″S, 57°40′51″W; farm 2: 34°56′29″S, 59°08′23″W). Farm 1 provided 24-month-old Angus cycling heifers ($n=28$) with a body condition score (BCS) of 7.16 ± 0.05 on a 1–9 scale [14] and Angus cows ($n=26$) with a BCS of 7.02 ± 0.09 and more than 55 days post-partum at the start of the experiment. Farm 2 provided Angus cows ($n=15$) with a BCS of 7.13 ± 0.11 and more than 55 days post-partum at the start of the experiment. On day 0, at random stages of the estrous cycle, all animals received an intramuscular (IM) injection of 2 mg estradiol benzoate (EB, Syntex, Argentina) and an intravaginal P4-device (DIB, Syntex, Argentina) containing 0.5 g P4. On day 7, the device was removed and animals were administered 500 mg Cloprostenol (Ciclase DL, Syntex) and 0.5 mg estradiol cypionate (Cipiosyn, Syntex) by IM injection. On day 9, 52–56 h after DIB removal, AI was carried out by an experienced technician (Fig. 1).

Blood Sample

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

Plasma Cu Concentrations

Plasma Cu concentrations were evaluated on days 0, 7, 9, and 16. Plasma samples were proportionally deproteinized with 10% (v/v) trichloroacetic acid. Copper concentrations were measured in supernatants using a double beam atomic absorption spectrophotometer (Perkin Elmer AAnalyst 200; AAS, International Equipment Trading Ltd., Mundelein, USA) with an internal quality control [15].

Hormone Assays

Plasma E2 concentration was determined on day 9 by chemiluminescence immunoassay (Elecsys®, Estradiol III, Roche, Mannheim, Germany) (9.4% intra-assay coefficient of variation; 2.6 pg/tube assay sensitivity). Plasma P4 concentration was determined by chemiluminescence immunoassay on day 16 (Elecsys®, Progesterone II, Roche, Mannheim, Germany) (9.6% intra-assay coefficient of variation; 0.1 ng/tube assay sensitivity).

Ultrasonography

The APF (day 9) and ACL (day 16) determinations were performed by transrectal ultrasonography using an Aloka 500 V equipped with a 7.5-MHz linear-array transducer (Aloka, Wallingford, CT). Images were taken to measure the maximum diameter of preovulatory follicle (PF), CL, and CL cavity, if present (Figs. 2a and b). The average of the horizontal and vertical diameters recorded was used for the statistical analysis. The location of PF that corresponded to the largest area was also recorded. The ACL was calculated by subtracting the luteal cavity area from the entire CL area.

Experiment 2: Effect of Parenteral Cu Administration on APF, ACL, and Plasma E2 and P4 Concentrations

Animals and Treatments

The trial was performed during November–December in a commercial farm located in the Salado River Basin, Buenos Aires, Argentina (35°21′48″S, 57°13′ 37″W). The animals used were 24-month-old Angus cycling heifers ($n=49$; BCS, 7.30 ± 0.06) and Angus cows ($n=19$; BCS, 7.10 ± 0.10) with more than 55 days post-partum at the start of the experiment. The same FTAI protocol as described above (*Experiment 1*) was applied to all animals. On day 0 of the protocol, heifers and cows were randomly assigned to one of two treatments: (1) the control group ($n=28$ heifers and $n=9$ cows) received 4 mL of sterile NaCl solution (9 g/L), and (2) the

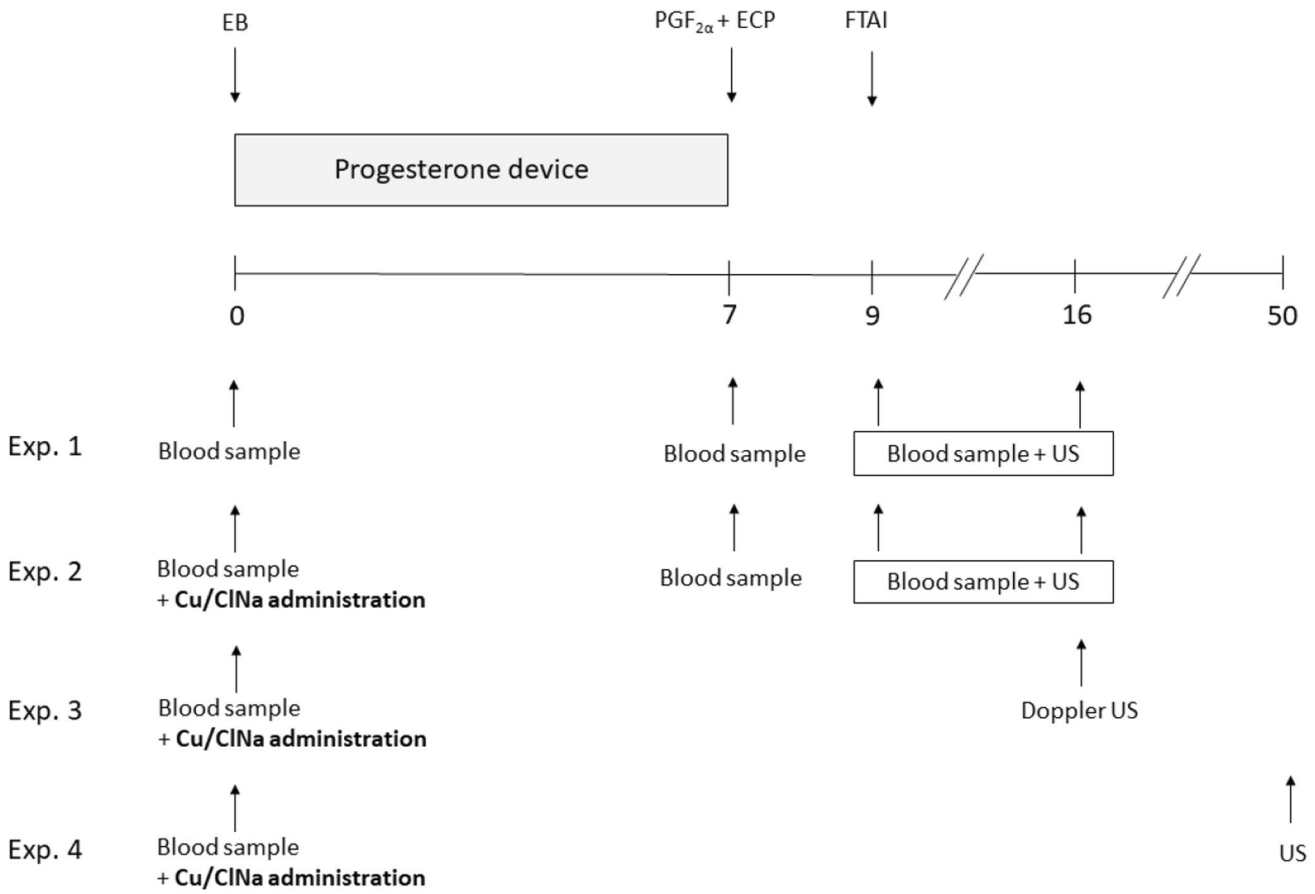

Fig. 1 Experimental design. The FTAI protocol consisted of an intravaginal progesterone device and 2 mg estradiol benzoate (EB) on day 0, followed by 500 mg cloprostenol ($PGF_{2\alpha}$) and 0.5 mg estradiol cypionate (ECP) on day 7 (at device removal). On day 9, AI was carried out. In Experiments 2, 3 and 4, animals were randomly assigned to one of two treatments on day 0: 1) the control group received NaCl solution, and 2) the copper (Cu) group received 100 mg Cu subcutaneously. Ultrasonographic (US) examinations of area of preovulatory follicle (APF), area of corpus luteum (ACL), and pregnancy diagnosis were performed on days 9, 16 and 50, respectively. Blood samples were used to evaluate plasma Cu (days 0, 7, 9 and 16), estradiol (day 9), and progesterone (day 16) concentrations. Doppler US was performed to evaluate CL blood flow (CLBF)

Cu group ($n=21$ heifers and $n=10$ cows) was supplemented with a subcutaneous injection (4 mL) of 100 mg Cu (dinitrile ethylene copper calcium acetate, Glypondin®, KÖNIG, Argentina). On day 9, AI was carried out by an experienced technician (Fig. 1).

Blood sample collection, determination of plasma Cu concentrations, hormone assays, and ultrasonography were performed as described above (*Experiment 1*). Blood samples were collected on day 0, before Cu and NaCl solution administration.

Experiment 3: Effect of Parenteral Cu Administration on CLBF

Animals and Treatments

This trial was performed in a commercial farm located in Buenos Aires, Argentina (35°20′18.6"S, 58°38′06.2"W) during spring (October). Angus cows ($n=27$, 6.27 ± 0.09 BCS and more than 55 days post-partum at the start of the experiment) were used. Cows were randomly assigned to either a control ($n=13$) or a Cu-supplemented group ($n=14$). The same FTAI protocol as described above (*Experiment 1*) was applied. On day 9 of the FTAI protocol, PF presence and location were determined by transrectal ultrasonography (Aloka, Wallingford, CT) (Fig. 1).

Ultrasonography and Color Doppler Imaging

The ultrasound evaluation was performed according to Siqueira and colleagues [16]. Briefly, on day 16, the ovaries of all cows were scanned for CL evaluation. Cows without CL or whose location did not match that of PF were removed from the study. A portable ultrasound machine (MyLab30 Vet Gold; Esaote SpA, Genova, Italy) equipped with a rectal linear-array transducer and capable of creating images in

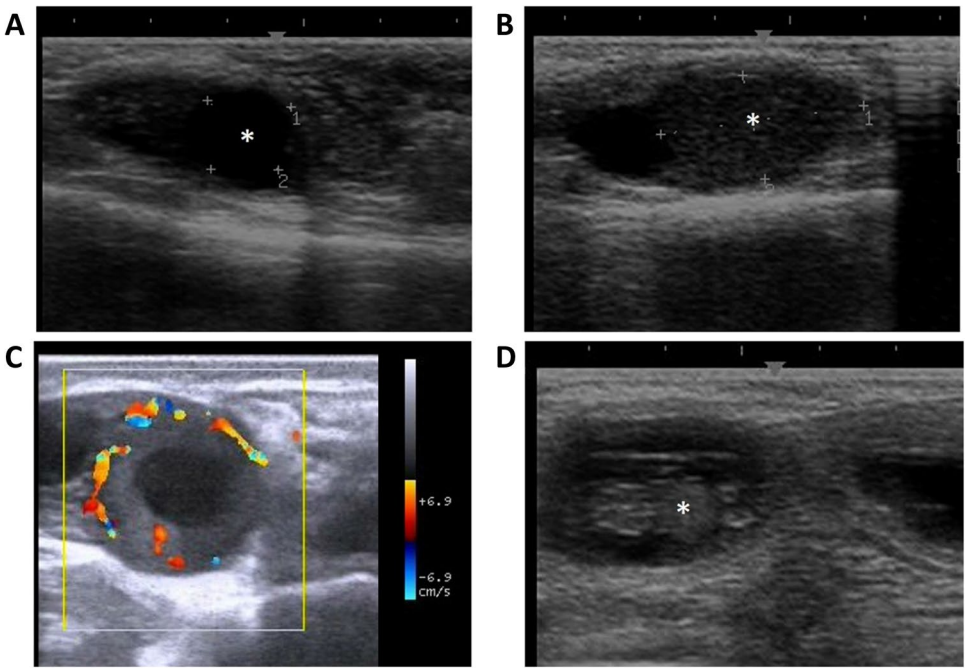

Fig. 2 Ultrasound images of ovaries and uterus. (**a**) Determination of area of preovulatory follicle (APF) on day 9 (*, PF). (**b**) Determination of area of corpus luteum (ACL) on day 16 (*, CL). (**c**) Color-Doppler mode (D-detection limit: 0.06 m/s) of a CL on day 16. (**d**) Ultrasound image of a 41-day pregnancy (*, embryo)

B-mode (grayscale imaging) and color-flow Doppler mapping mode (blood flow assessment) was used. In B-mode, transducer frequency was set to 8.0 MHz and 70% total gain. For color-flow mode, frequency was set to 6.6 MHz, 55% total gain, 750 Hz pulse repetition frequency, 23 frames/s frame rate, and 0.06 m/s minimum detectable velocity. After scanning the ovaries, B-mode and color flow-mode short video clips (7-s duration) of the ovary with CL were recorded (Fig. 2c). Later, ACL was calculated as described above (*Experiment 1*). ImageJ software (National Institutes of Health, Bethesda, MD) was used to evaluate CLBF and determine the area of colored pixels within the CL. An adjusted CLBF was calculated dividing CLBF by ACL (CLBF:ACL ratio) to determine the proportion of colored pixels relative to the ACL.

Experiment 4: Effect of Parenteral Cu Administration on Pregnancy Rates

Animals and Treatments

The trial was performed in spring (October–December) with animals from two commercial farms located in Buenos Aires, Argentina (farm 1: 35°38′25"S 58°24′13"W; farm 2: 35°15′37″S, 60°10′52″W). Farm 1 provided 85 24-month-old cycling Angus heifers (7.62 ± 0.10 BCS) and 31 Angus cows (7.17 ± 0.08 BCS and more than 55 days post-partum at the start of the experiment). Farm 2 provided 49 24-month-old cycling Angus heifers (7.21 ± 0.05 BCS) and 38 Angus cows (7.15 ± 0.05 BCS and more than 55 days post-partum at the start of the experiment). Animals were randomly assigned to either a control (heifers $n = 44$ and 28 from farms 1 and 2, respectively; and cows $n = 15$ and 19 from farms 1 and 2, respectively) or a Cu group (heifers $n = 41$ and 21 from farms 1 and 2, respectively; and cows $n = 16$ and 19 from farms 1 and 2, respectively). The same FTAI protocol as described above (*Experiments 1*) was applied by the same experienced technician. Pregnancy rate was determined by transrectal ultrasonography (Aloka, Wallingford, CT) on day 41 after FTAI (Fig. 1; Fig. 2d).

Plasma Cu Concentrations

Blood samples of animals from the two farms were collected on day 0 before Cu and NaCl solution administration. Blood sample collection and plasma Cu determinations were performed as described in *Experiment 1*.

Statistical Analysis

Associations and coefficients of determination between plasma Cu concentration and plasma E2 and P4 concentration, APF and ACL were assessed using PROC CORR and PROC REG of SAS (SAS Institute, Cary, NC, USA), respectively (*Experiment 1*). Completely randomized designs were used, with the following statistical models: *Experiment 2* included the fixed effect of treatment (Control vs. Cu), category (heifer vs. cow) and their interaction (treatment*category). *Experiment 3* included the fixed effect of treatment (Control vs. Cu). *Experiment 4* included the random effect of block (farm, $n = 2$), the fixed effect of treatment (Control vs. Cu), category (heifer vs. cow) and their

interaction (treatment*category). The effect of Cu administration on continuous response variables such as plasma E2 and P4 concentration and APF, ACL, and CLBF was analyzed with linear regression models by using the MIXED procedure of SAS. In the case of repeated measures (Cu concentration), a repeated measure linear model was used to account for autocorrelation. The effect of Cu administration on pregnancy rate was analyzed by logistic regression analysis using the GENMOD procedure (SAS Institute). Results are expressed as least squared means ± SEM (continuous variables) and percentages (discrete variables). Statistical significance was set at $P < 0.05$ and $P < 0.10$ for tendency and interactions. $P \leq 0.15$ was considered to indicate tendency for interactions.

Results

Experiment 1: Association Between Plasma Cu Concentration During FTAI Protocol and APF, ACL, and Plasma E2 and P4 Concentrations

In heifers, no association was found between plasma Cu concentration and any of the evaluated parameters ($P > 0.05$; Table 1). In cows, plasma Cu concentration on days 0 and 7 correlated positively with APF ($P < 0.05$; Table 2), whereas a negative correlation between plasma Cu concentration on day 9 and ACL was found ($P < 00.05$; Table 2).

Experiment 2: Effect of Parenteral Cu Administration on APF, ACL, and Plasma E2 and P4 Concentrations

Time (day) had effect on plasma Cu concentration (90.88 ± 1.62, 93.37 ± 1.63, 89.74 ± 1.70 and 89.15 ± 1.65 µg/dL Cu for days 0, 7, 9, and 16, respectively, ($P < 0.05$), whereas such concentration tended to be different in heifers and cows (93.15 ± 1.49 and 88.42 ± 2.31 µg/dL Cu, respectively; $P < 0.1$). Although Cu administration had no effect on plasma Cu concentration (control vs. Cu group, 89.34 ± 1.88 vs. 92.22 ± 1.84 µg/dL Cu; $P > 0.15$), time*Cu administration did and was category-dependent ($P < 0.05$; Fig. 3). In heifers, plasma Cu concentration tended to be higher on days 7 and 9 in Cu-supplemented animals ($P < 0.1$), whereas in cows it was higher on day 7 ($P < 0.05$; Fig. 3).

The effect of parenteral Cu administration on APF, ACL and plasma E2 and P4 concentrations is shown in Table 3. The APF was lower in Cu-supplemented animals ($P < 0.05$). However, the effect of Cu tended to differ by category ($P = 0.15$), namely, Cu decreased APF in cows but not in heifers (Fig. 4a). Plasma E2 concentration was decreased with Cu administration ($P < 0.05$). However, the effect of Cu differed according to category, since addition of Cu decreased plasma E2 in cows but not in heifers (Cu administration*category = $P < 0.1$; Fig. 4b). Conversely, Cu administration did not affect plasma P4 concentration and ACL ($P > 0.15$).

Table 1 Association between plasma Cu concentration during the FTAI protocol (days 0, 7, 9 and 16) and area of preovulatory follicle (APF), plasma estradiol (E2), area of corpus luteum (ACL), and progesterone (P4) concentrations in heifers

	APF		E2		ACL		P4	
Plasma Cu concentration	r	*P* value	r	*P* value	r	*P* value	r	*P* value
Day 0	0.22	NS	0.13	NS	0.23	NS	−0.15	NS
Day 7	0.20	NS	0.04	NS	0.16	NS	−0.29	NS
Day 9	0.20	NS	0.10	NS	0.25	NS	−0.22	NS
Day 16	-	-	-	-	0.09	NS	−0.01	NS

The APF and E2 determinations were performed on day 9; ACL and P4 were evaluated on day 16. NS: Not significant. Statistical significance was set at $P < 0.05$

Table 2 Association between plasma Cu concentration during the FTAI protocol (days 0, 7, 9 and 16) and area of preovulatory follicle (APF), plasma estradiol (E2), area of corpus luteum (ACL), and progesterone (P4) concentrations in cows

	APF			E2			ACL			P4		
Plasma Cu concentration	r	R^2	*P* value	r	R^2	*P* value	r	R^2	*P* value	r	R^2	*P* value
Day 0	0.42	0.17	0.01	0.45	-	NS	−0.10	-	NS	0.20	-	NS
Day 7	0.47	0.22	0.01	0.03	-	NS	−0.24	-	NS	0.22	-	NS
Day 9	0.16	-	NS	−0.02	-	NS	−0.44	0.19	0.02	0.08	-	NS
Day 16	-	-	-	-	-	-	−0.06	-	NS	0.25	-	NS

The APF and E2 determinations were performed on day 9; ACL and P4 were evaluated on day 16. Coefficients of determination (R^2) were assessed when the correlation coefficient (r) between two variables was significant ($P < 0.05$). *NS* not significant

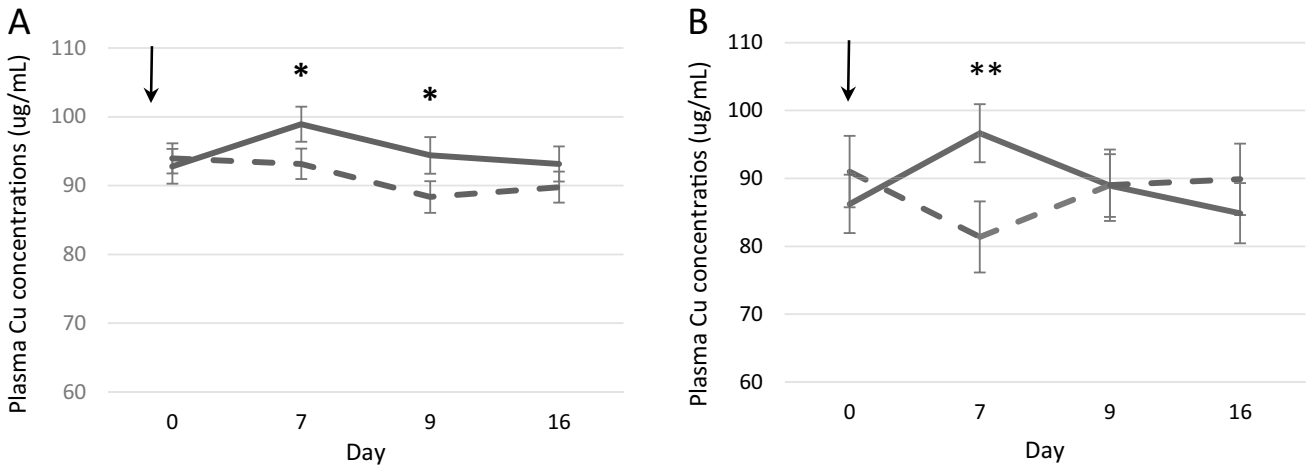

Fig. 3 Effect of parenteral Cu administration at the beginning of the FTAI protocol on plasma Cu concentrations in beef cattle. Plasma Cu concentrations were evaluated on days 0, 7, 9 and 16. Heifers (**a**) and cows (**b**) were injected at the beginning of FTAI (day 0; arrow) with saline solution (control group; dotted line) or 100 mg copper (Cu group; solid line). Results are expressed as means ± SEM. Time*Cu administration had effect on plasma Cu concentration and depended on category ($P < 0.05$). *, $P < 0.1$.; **, $P < 0.05$

Table 3 Effect of parenteral Cu administration at the beginning of the FTAI protocol on plasma estradiol (E2) and progesterone (P4) concentrations, area of preovulatory follicle (APF), and area of corpus luteum (ACL)

	Treatment (Trt)		Category (Cat)		*P* value		
	Control	Copper	Heifer	Cow	Trt	Cat	Trt*Cat
E2 (pg/mL)	56.82 ± 1.10	38.86 ± 1.09	40.04 ± 1.07	54.59 ± 1.12	0.01	0.03	0.07
APF (mm2)	96.83 ± 5.53	76.77 ± 5.53	88.29 ± 4.20	85.31 ± 6.24	0.01	NS	0.15
P4 (ng/mL)	3.15 ± 1.15	3.16 ± 1.12	3.26 ± 1.16	3.06 ± 1.09	NS	NS	NS
ACL (mm2)	209.73 ± 26.40	223.54 ± 27.97	213.79 ± 21.81	219.48 ± 30.59	NS	NS	NS

The APF and E2 determinations were performed on day 9; ACL and P4 were evaluated on day 16. Data are expressed as least square means ± SEM. NS: Not significant. Statistical significance was set at $P < 0.05$ and $P < 0.10$ for tendency and interactions. $P \leq 0.15$ was considered to indicate tendency for interactions

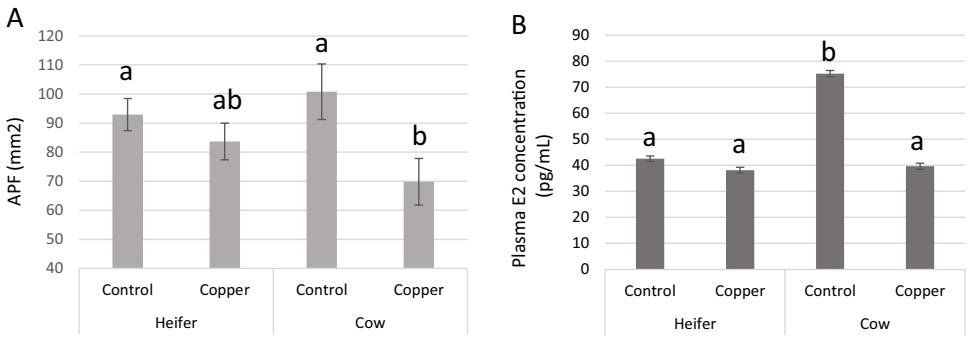

Fig. 4 Interaction effects of Cu administration with category on plasma estradiol (E2) and area of preovulatory follicle (APF). Cows and heifers were subcutaneously injected with 100 mg Cu at the beginning of the FTAI protocol (day 0 of the trial) or acted as negative controls. (**a**) Cu*Category effect on APF. (**b**) Cu*Category effect on E2 concentration. ^{a,b}Bars with different letters differed statistically ($P < 0.05$). Results are expressed as least squared means ± SEM

Experiment 3: Effect of Parenteral Cu Administration on CLBF

The effect of parenteral Cu administration on CLBF and adjusted CLBF is shown in Table 4. As can be seen, Cu administration did not modify CLBF in cows ($P > 0.15$).

Experiment 4: Effect of Parenteral Cu Administration on Pregnancy Rate

Plasma Cu concentration at the beginning of the FTAI protocol (day 0) was higher in farm 2 than farm 1 (86.0 ± 1.9 and 69.7 ± 3.9 µg/dL Cu, respectively; $P < 0.05$).

Table 4 Effect of parenteral Cu administration at the beginning of the FTAI protocol on area of corpus luteum (ACL), corpus luteum blood flow (CLBF) and adjusted CLBF

	Treatment		
	Control	Copper	*P* value
ACL (mm^2)	233.89 ± 18.68	220.23 ± 22.88	NS
CLBF (mm^2)	19.12 ± 1.23	24.23 ± 1.29	NS
Adj. CLBF (%)	8.29 ± 1.22	11.29 ± 1.28	NS

The ACL, CLBF and adjusted CLBF were evaluated on day 16. Data are expressed as least square means ± SEM. NS: Not significant. Statistical significance was set at $P < 0.05$

Copper administration increased the odds for pregnancy (OR = 1.78; 95% CI = 1.02–3.22; $P < 0.05$); the percentage of pregnant cattle was 58.76% in Cu-supplemented animals vs. 45.28% in controls. Farm, category, and category*Cu administration had no effect on pregnancy rate ($P > 0.15$).

Discussion

In the present study, a positive correlation between plasma Cu concentration and APF during the FTAI protocol was observed in cows but not in heifers. Copper administration at the beginning of the FTAI protocol increased pregnancy rate without modifying CL characteristics in heifers and cows and decreased APF and plasma E2 concentration in the latter. Interestingly, Cu administration had a beneficial effect on pregnancy rate even when the average plasma Cu concentrations in each farm were adequate (> 60 µg/dL Cu [5],). We suggest at least two possible explanations for that result: (a) on the one hand, the plasma Cu concentration necessary for normal reproductive function could be higher than that defined as adequate by Kincaid [5]. Recently, García-Díaz and colleagues [17] reported a positive effect of Cu administration on the percentage of heat detection and gestation in cattle with plasma Cu concentration ≤ 88.9 µg/dL Cu, suggesting 89 µg/dL Cu as the lower limit of adequate Cu level for reproductive efficiency in cattle. In the present study, the plasma Cu concentration of the animals used to evaluate pregnancy rate was below 89 µg/dL Cu (*Experiment 4*). (b) On the other hand, Cu could have a pharmacological effect on ovarian function and ovulation when applied parenterally, as suggested by Phillippo [18] and Underwood and Suttle [13]. In ruminants, FSH is the principal regulator of follicular growth, whereas LH stimulates large follicles [19, 20]. It has been demonstrated that Cu and GnRH complexes are more effective than native GnRH for LH release, and more potent in inducing in vivo FSH release [11, 21, 22]. In addition, Hazum [10] found that cupric ions affect the pituitary, resulting in a high release of LH. Even more, ovulation has been induced by intravenous injection of Cu salts in female rabbits [9, 23, 24]. The Cu-induced APF reduction observed in cows was probably due to the advancement of ovulation. Preliminary data from our group indicate that Cu administration to cows at the beginning of the FTAI protocol tends to advance the ovulation time.

It has been demonstrated that parenteral Cu injection 1 month before the mating season increased plasma Cu levels but did not modify the reproductive performance [18]. In the present study, Cu administration 9 days before ovulation increased the pregnancy rate, suggesting that the beneficial effect of parenteral Cu injection could be related to the time of administration. The effect of Cu on pregnancy observed in the present study would be more related to follicular than luteal changes (at least with the 7-day-old CL), since (a) Cu administration reduced APF and plasma E2 concentration in cows, without modifying ACL or plasma P4 concentration, and (b) Cu administration only increased plasma Cu levels during follicular development (first 7–9 days of the FTAI protocol; Fig. 3). It has been demonstrated that Cu plays an important role in oocyte and cumulus and granulosa cell function. In vitro, Cu supplementation improves bovine oocyte maturation [25], decreases DNA damage and apoptosis in bovine cumulus cells [26], and stimulates insulin like growth factor I (IGF-I) release by porcine granulosa cells [27]. Thus, the increased pregnancy rate in heifers and cows induced by Cu administration could be partly explained by other variables not assessed in this study, such as improvement in oocyte quality and follicular cell function.

In the early CL, volume increases in parallel with plasma P4 concentration and blood flow [28]. While subcutaneous Cu injection did not modify ACL, a negative correlation between plasma Cu concentration on day 9 and ACL was found, suggesting a certain effect of Cu on this variable. The CL is one of the most highly vascularized organs in the body [29]. Many vascular functions are dependent on Cu, which is necessary for several microvascular control mechanisms affecting regulation of peripheral blood flow [30]. Vascular function can be directly or indirectly affected by defects in Cu-dependent enzymes, such as dopamine β-hydroxylase, soluble guanylate cyclase, and Cu/Zn SOD [30]. It has been shown that P4 levels depend primarily on the CLBF [28]. In the present study, neither P4 nor CLBF were modified by Cu administration. This could be due, at least in part, to the fact that plasma Cu levels in supplemented animals were similar to those of the control group during CL development (Fig. 3).

In conclusion, the results of the present study show that Cu administration at the beginning of the FTAI protocol increased pregnancy rate in beef cattle without modifying ACL, plasma P4 concentration, and CLBF and reduced APF and plasma E2 concentration in cows.

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Authors' contributions JPA and JMA: designed the study, coordinated the experiment, and wrote the manuscript. SNL, GSP, NAF, DER and MCF: assisted whit data collection. ACC-M, and NN: analyzed the data. CCF: Funding acquisition, reviewed and edited the manuscript.

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Data availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Code Availability Not applicable.

Declarations

Ethics Approval The experimental procedures were approved by the School of Veterinary Sciences Institutional Animal Care and Use Committee (National University of La Plata, Buenos Aires, Argentina; Protocol No. 105–3-20P).

Consent for Publication Not applicable.

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

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