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The Efficacy of Slow-Release Copper Capsule Administration on Postpartum Anestrus in Dairy Cows

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Abstract

Deficiency of single or multiple minerals or their imbalances may cause various reproductive failures, such as infertility, poor conception, and anestrus. Copper deficiency is related to reproductive disorders. This study investigated the effects of slow-release copper bolus administration in postpartum anestrus cows. So, a hundred postpartum multiparous Holstein cows with a body condition score of 2.5-3 and an average milk yield of 30 kg, which did not show signs of estrus until at least the 45th day after parturition were selected, and two ultrasound examinations of uterus and ovaries were performed ten days apart. Cows with normal uterus in both examinations, but inactive ovaries and without active structure (s), such as corpus luteum or cyst, were selected and randomly divided into two groups 50. A 2.5-gram slow-release copper capsule (manufactured by Zofa Parnian Pars Company) was administered in the treatment group. In the control group, nothing was administered. Heat detection was done in both groups for 60 days after the copper bolus administration; on the 30th day, the pregnancy was checked by ultrasound. The average onset interval of estrus in the treatment group was 32 days, and in the control group was 43 days ($p<0.05$). The first service conception rate was 64% in the treatment group and 42% in the control group ($p=0.028$). The results showed that using a 2.5-gram slow-release copper capsule can effectively improve the estrus and increment of the first service conception rate in anestrus postpartum dairy cows.

Keywords: Copper; Anestrus; Slow-release capsule; Dairy cow.

Introduction

The postpartum period plays an essential role in cattle reproduction. The duration of anestrus after parturition substantially affects reproductive performance [1]. Some have suggested an increase in the incidence of anestrus in high-yielding dairy herds [2,3]. Increased energy allocation to milk production leads to anestrus by delaying the resumption of follicular activity. However, limited energy intake, low body stores, and postpartum diseases can delay the return to cyclicity. Normal parturition is prone to rapid resumption of ovarian activity after calving [4]. The appearance of follicular waves does not define the term postpartum anestrus but may be by follicular deviation or the appearance of the dominant follicle. Under favorable conditions, there is a deviation in follicular development, determination of the dominant follicle, development to maturity, ovulation, and luteolysis, which leads to the re-establishment of cyclic ovarian activity and an opportunity for conception. Failure of any of these processes drags out postpartum anestrus. So, failure to show estrus and poor heat detection efficiency can increase the rate of anestrus cows. Generally, anestrus was classified into physiologic and pathologic (clinical) types, with the following referring to the pathologic type: (a) Inactive ovaries (minimal follicular development, no ovulation, and no corpus luteum), (b) silent ovulation (ovulation without behavioral estrus), (c) ovarian dysfunction (persistent dominant follicle), (d) cystic ovarian disease (follicular or luteal cyst), and (e) persistent corpus luteum (lack of luteal regression) [5]. Recently, anestrus

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has been classified according to ovarian follicular and luteal dynamics [6]. The follicular wave dynamics consist of three main morphological events: emergence, deviation, and dominancy, culminating in ovulation or non-ovulation.

Lack of timely activity of the ovaries leads to substantial economic losses to the industrial farms. Dairy cows must show at least one estrus until 45 days after calving. Otherwise, every day of delay in the conception (which may even reach several months) makes a 2-5 dollar loss depending on the breed and country [7,8]. This loss occurs primarily due to increased days open, resulting in a drop in milk production and late parturition. On the other hand, due to the early drying of cows, disorders such as hypocalcemia, ketosis, and fatty liver syndrome will occur, and sometimes culling the affected cows is inevitable. Postpartum anestrus may be caused by poor nutrition, negative energy balance, milk yield, or imbalance of trace elements. After energy and proteins, minerals are dairy cows' essential nutrients for reproduction. Minerals like copper, cobalt, selenium, iodine, zinc, and iron can affect the reproductive performance of ruminants. Fertility disorders may be caused by the lack of single or multiple trace elements and their imbalance [9].

Copper is one of the essential components of superoxide dismutase, lysyl oxidase, and thiol oxidase, which play a vital role in antioxidant activity. Copper deficiency causes severe economic losses through symptoms such as pale coat, poor capillary integrity, myocardial atrophy, poor reproductive performance, and reduced resistance to infectious diseases [10]. Copper is an essential micronutrient for ruminants, but its excessive consumption can cause severe toxication [11]. Reproductive problems of copper deficiency include failure of embryo implantation, early embryonic dead, placental abruption and necrosis, and poor heat signs. Normal serum copper levels are related to a high conception rate, low service per conception, and short open days. Increasing the copper: molybdenum ratio was associated with improved fertility in silage-fed cows [12], and treatment of infertile cows with copper glycinate resulted in an improvement in pregnancy rates [11]. In bulls, copper improved the quality of semen, which was associated with an increase in sperm motility and a decrease in dead sperm [13]. Dairy cows with regular luteal activity have higher copper concentrations than cows with prolonged luteal, short luteal, and delayed ovulation [14]. During luteolysis, copper may play a role in the interaction between the corpus luteum and the endometrium. During the estrus cycle, the non-pregnant uterus synthesizes prostaglandins, leading to luteolysis and initiating the next follicular wave. A direct relationship between copper deficiency and reduced prostaglandin synthesis has been established in rats [15], although this relationship has not been proven in cattle.

There are several reports of copper effects on oocyte and embryo development. In vitro, copper supplementation reduced apoptosis and DNA damage of ovine cumulus-oophorus complex cells [16]. Cumulus cells are critical for oocyte survival, providing nutrient support and protection from damage during maturation. Copper supplementation during in vitro fertilization increased the rate of embryo cleavage, the number of embryos that reach the blastocyst stage, the number of total blastocyst cells and decreased the number of apoptotic blastomeres in bovine blastocysts [17]. Overall, copper may act to improve fertility rates in cows by affecting several reproductive processes (luteolysis and embryo formation) and several cell types (endometrial cells, luteal cells, cumulus cells) [18]. Copper deficiency in dairy cows causes reproductive abnormalities, especially early embryonic death [13], and in ewes, it causes implantation disorder, fertility reduction, and abortion [19]. So, this study aims to investigate the effects of using slow-release copper capsules on estrus behavior and also first service conception rate in dairy cows. This study hypothesizes that using copper capsules can increase the incidence of estrus and improve the first service conception rate in postpartum anestrus dairy cows.

Materials and methods

A hundred postpartum multiparous Holstein cows were obtained from March to December 2022 from industrial cattle farms around Tabriz, Iran. The reproductive and ovarian status in cows not expressed behavioral estrus till day 45 postpartum were assessed by per rectal ultrasonography of the genitalia on two occasions, at ten days intervals. The cows had a body condition score of 2.5-3 and an average milk yield of 30 kg daily. The cows with normal uterus in both examinations, but inactive ovaries and no active structure (s), such as corpus luteum or cysts, were selected and randomly divided into two groups of 50 cows. In the treatment group, a 2.5-gram slow-release copper capsule (manufactured by Zofa Parnian Pars Company) was administered by bolus gun, and in the control group, nothing was administered. The cows were fed up by an automatic feeder and maintained in the same conditions with no difference in keeping and comfort. During 60 days after copper capsule administration, heat detection was done in both groups by observation and closed-circuit television camera, and during this period, no drugs were administrated. After heat detection, the cows were inseminated, and after 30 days, the pregnancy status was checked by ultrasonography. The onset interval of estrus and pregnancy rate in each group was recorded. The statistical analysis done by SPSS version 22 software and the normal or abnormal distribution of the pregnancy rate were analyzed; if the frequency distribution was not normal, they were compared with the Mann-Whitney Test method.

Results

The average onset interval of estrus after administration of copper capsule in the treatment group, as well as in the control group at the same time, was recorded. The average onset interval of estrus in the treatment group was 32 days, and in the control group was 43 days ($p<0.05$) (Table 1).

Table 1: Statistical analysis of the mean interval of onset of estrus after administration of copper capsules in the treatment group and at the same time in the control group.

Group	Number	Mean (days)	Std. Deviation	Std. Error Mean	p-value (2-tailed)
Treatment	50	32.0000	7.01020	.99139	.000
Control	50	43.0000	8.24374	1.16584	.000

Pregnancy was diagnosed using ultrasound 30 days after insemination, and the first service conception rate was calculated. The first service conception rate was 64% in the treatment group, and 42% in the control group (Figure 1). The distribution of conception rate data in the control and treatment groups was analyzed with the one-sample Kolmogorov-Smirnov test, but the data distribution was not normal ($p<0.05$) (Table 2). So, the Mann-Whitney test was used to compare the mean conception rate between the control and treatment groups, and the difference was significant ($p=0.028$) (Table 3).

Table 2: Normality or abnormality analysis of the treatment and control group's data distribution to compare the first service conception rate using the one-sample Kolmogorov-Smirnov Test.

		Conception
Number		100
Normal Parameters	Mean	1.47
	Std. Deviation	0.502
Most Extreme Differences	Absolute	0.356
	Positive	0.356
	Negative	-0.325
Kolmogorov-Smirnov Z		3.556
Asymp. Sig. (2-tailed)		0.000

Table 3: The Mann-Whitney test compared the mean conception rate in the control and treatment groups.

	Conception
Mann-Whitney U	975.000
Wilcoxon W	2250.000
Z	-2.193
Asymp. Sig. (2- tailed)	.028

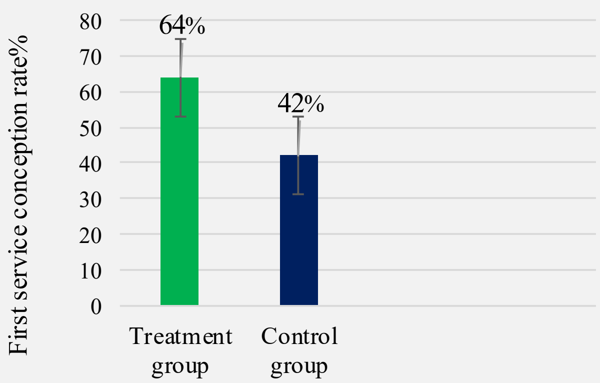

Figure 1: Details of the polyethylene terephthalate (PET) shield. **(A)**. The blue print of the PET shield. All measurements are in mm.

Discussion

Copper plays a critical role in the growth and development of follicles and the maturation of oocytes, and its deficiency has adverse effects on the reproduction process in animals and humans [17]. Like females, its deficiency in male animals also harms the process of spermatogenesis [20]. In addition to the role of copper in the reproductive process, it plays a more critical role in improving the immune system and especially in preventing mastitis [21]. Lack of minerals, like copper, can cause reproductive problems such as failure to show estrus signs, repeat breeding, early embryonic death, and insufficiency of gametes [22]. The distance between the therapeutic dose and the toxic dose of copper is very close; therefore, in this experiment, we decided to use its slow-release forms to prevent toxic effects.

As shown in Table 1, the use of 2.5 grams of the slow-release copper capsule in the treatment group significantly reduced the interval of onset of estrus compared to the control group (32 days vs. 43 days) ($p<0.05$). In a similar study, by adding a supplement containing copper to the diet of postpartum cows, 70% of the cows showed estrus, but none of the cows in the control group [23]. The serum concentration of copper is vital in dairy cow's anestrus complications. Tabrizi et al. reported that the mean copper serum of cyclic cows was higher than anestrus cows (124.12 vs. 99.59 µg/dl, $p<0.05$) [24]. Mudgal et al. supplemented anestrus cows with a deficiency of copper with copper sulfate pentahydrate (inorganic salt of copper) once a week for two months, and finally, 42% of cows showed estrus in the first month of supplementation, while 35% showed estrus in the second month. Hence, just two months of copper supplementation dramatically responded, and estrus was recorded in about 80% of the cows [25]. A study on buffalo cows with hypocuprosis showed that 21.84% of these cases suffered from ovarian inactivity, and progesterone levels of the follicular and luteal phases were significantly lower than in the control group. It has been mentioned that copper deficiency causes oxidative stress in buffalo cows resulting in cessation of ovarian activity and failure to show estrus [26]. Du Plessis et al. showed that ovaries were decreased in measure and had a decreased response to the FSH-induced superovulation regimen in hypocuprosis. The last mentioned may have central impacts on the hypothalamus-pituitary axis on LH secretion leading to decreased ovarian oestradiol discharge and absence of estrus signs [27]. Copper deficiency also has a vital role in women's reproductive disorders. Sun et al. studied 348 patients with Polycystic Ovary Syndrome (PCOS). They concluded that the concentration of copper in cystic ovarian fluids was much higher, and the number of good quality oocytes was much lower than in normal ones. There was a relation between the concentration of copper by testosterone and progesterone hormones in follicular fluids. Granulosa cells cultured in a high copper medium in the same experiment had high estrogen and low testosterone, so increasing the amount of copper in follicular fluids can cause disruption of steroidogenesis and the formation of ovarian cysts [28].

In our study, the use of slow-release copper capsules significantly increased the first service conception rate in the treatment group compared to the control group (64% versus 42%) (Figure 1). Similarly, Phillippo et al. found that the copper deficient heifers had a decreased pregnancy rate [29]. Also, copper supplementation positively affected the 30th day conception rate in cows with copper deficiency before and during the breeding season [30]. Reduced levels of serum copper (0.31 ppm) correlated with the reproductive problems of dairy animals [31]. In a study, crossbred heifers were treated with subcutaneous injectable trace minerals, including copper, 17 days before embryo transfer. Heifers in the treatment group had a 1.58 fold, and 1.72 fold higher ($P=0.005$) odds of conception rate 23 and 48 days after embryo transfer compared to the control group [32]. In the study by Ingraham et al., Holstein cows and heifers were given mineral supplements containing copper and magnesium 30 days before parturition, and the results showed that the first service conception rates were 57% in the magnesium and copper treatment group compared to 33% in the control group. The average pregnancy on the 210 days after parturition was 92% in the treatment group and 75% in the control group, so cows supplemented with copper and magnesium showed improved fertility [33]. The use of mineral supplementation containing copper, zinc, and magnesium increased the liver concentration of these compounds in the treated group ($P<0.01$), and supplemented cows had a higher ($P<0.02$) pregnancy rate for artificial insemination than controls. When cows were inseminated after an observed estrus, supplemented cows had a higher ($P<0.04$) pregnancy rate than controls. Also, the overall 60-day pregnancy rate tended (P=0.10) to be higher for supplemented cows than for controls [34]. In contrast to the results of our study, injection of 400 mg of copper glycinate into alternate cows during the first week after parturition resulted in no significant differences in the average interval between parturition and first observed heat, services per conception, or first service conception rate [35], however, this contrast can be caused by the level of minerals in the ration, the conditions of maintenance, the breed, the age, and parity.

Roshanzamir et al. showed that supplementing the diet of Holstein cows before and after calving with magnesium, zinc, and copper, such as methionine, glycine, or sulfate salts, can improve digestion, colostrum performance, milk SCC and maintenance of pregnancy, which is consistent with the results of our studies. In addition, it was shown that the increase of IgA and IgM in the blood of newborn calves is possible by supplementing their mothers with manganese, zinc, and copper [36]. Plasma concentrations of copper and zinc were associated with pregnancy rates. Nazari et al. investigated the relationship between antioxidant status and copper, iron, and zinc minerals concentration with postpartum luteal activity and fertility. Mean plasma SOD and GPX activities and TAC levels were higher in cows with regular luteal activity than in prolonged luteal phase and anovulation cows, as well as in ovulation compared to anovulation cows (P=0.03). Pregnant cows had greater SOD, GPX, and TAC levels at fixed-time artificial insemination than non-pregnant cows (P=0.01). Plasma copper and zinc concentrations increased in pregnant compared to non-pregnant cows at fixed-time artificial insemination [14]. Therefore, copper is a crucial element in many important biological activities, and using it in various routes, including the administration of slow-release copper capsules, can benefit dairy cows.

Conclusion

Finally, according to the results of the present study, we can conclude that copper deficiency is one of the critical factors related to reproductive disorders of postpartum dairy cows. The results also showed that using a 2.5-gram slow-release copper capsule can effectively improve the estrus and the first service conception rate in anestrus postpartum dairy cows.

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