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Pregnancy Rate in Ewes Injected with Zinc Oxide during an Estrus Synchronization Protocol

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ABSTRACT

Objective: To measure the effect of zinc injection, during an estrus synchronization protocol, on pregnancy rate in sheep from Valle de Mexicali.

Design/Methodology/Approach: The experimental units were 157 ewes, which were allocated in five farms (F): F1 (n=19), F2 (n=27), F3 (n=20), F4 (n=71), and F5 (n=21). In each farm, the ewes were randomly assigned to one of three treatments: control, z-100 and z-200. The ewes from control groups were subcutaneously injected with 4 mL of olive oil as placebo. The ewes from groups z-100 and z-200 were subcutaneously injected with 100 and 200 mg of zinc oxide. The response variables were the preovulatory diameter of the largest follicle and pregnancy rate.

Results: The differences between experimental groups in diameter of the largest preovulatory follicle and pregnancy rates were not significant ($p>0.05$).

Study Limitations/Implications: The ewe's reproductive response to zinc injection might be affected by the animal mineral status, it is recommended to carry on supplementation based on mineral blood concentrations.

Findings/Conclusions: The subcutaneous injection with 100 or 200 mg of zinc oxide did not affect the size of the largest preovulatory follicle and pregnancy rate in ewes.

Keywords: Minerals, reproduction, sheep.

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INTRODUCTION

The increasing world population and demand for foods of animal origin (Turk, 2016) urbanization and rising incomes are driving greater demand for animal source food in most parts of the world but especially in developing countries. The United Nations Food and Agriculture Organization (UN-FAO) makes it necessary to apply strategies in



livestock production systems to improve their productivity. In Mexico, sheep production systems are diversified, regarding their technological advance and the breeds used to produce mutton (Partida de la Peña *et al.*, 2017). There are regions such as Valle de Mexicali where sheep production is considered as a subsistence activity, with a low degree of technification (Martínez-Partida *et al.*, 2011). Therefore, there is a need to manage and promote the development of this activity through research studies.

The profitability of sheep production systems can be improved by applying reproductive biotechnologies and nutritional strategies (Gifford & Gifford, 2013) such as supplying specific nutrients during the occurrence of relevant reproductive events (Delgadillo & Martin, 2015). The list of these nutrients includes zinc and other trace minerals, which are essential for a variety of physiological functions in the animal (López-Alonso, 2012).

Zinc is an essential mineral to sustain life and one of the most abundant trace minerals inside the animal's organism, but its endogenous storage is low and it must be provided to animals by feed or mineral supplementation (Mir *et al.*, 2020; Swain *et al.*, 2016). In the organism, zinc participates as enzymatic cofactor, regulates cell growth, immunity and reproductive function in mammals (Hill & Shannon, 2019; Nasiadek *et al.*, 2020) whereas zinc body deficiency causes low productive (Masters *et al.*, 1985) and reproductive performance (Tian & Diaz, 2012; Tian *et al.*, 2014). Zinc can be administrated to animals orally or by injection. The oral administration of zinc has been effective in improving the reproductive performance in ewes (Monem & El-Shahat, 2011) and goats (Kundu *et al.*, 2014), while a similar effect of zinc injection has been observed in cows (Anchordoquy *et al.*, 2019). The latter is considered as more effective in increasing endogenous zinc concentrations in sheep (Lamand *et al.*, 1983). However, we are unaware of the effects of zinc injection on reproductive variables in sheep.

Therefore, the objective of this study was to measure reproductive variables in ewes injected with zinc, during an estrus synchronization protocol, in Valle de Mexicali.

MATERIALS AND METHODS

Location

The study was undertaken in five sheep farms located in Valle de Mexicali during the increasing photoperiod season (March-April). The climate in the region is dry arid (BWh), the average rainfall is 85 mm and the temperature range throughout the year is 0 to 52 °C (Garcia, 1988). The animals were managed following the Canadian Council on Animal Care guidelines (CCAC, 2009).

Animals and experimental design

The experimental units were 157 crossbred ewes (Dorper×Pelibuer×Katahdin) with at least one lambing and non-lactating. The ewes were distributed in five different sheep farms (F): F1 (n=19), F2 (n=27), F3 (n=20), F4 (n=71), and F5 (n=21). In each farm, the ewes were randomly assigned to one of three treatments: Control (0 mg of zinc oxide), z-100 (100 mg) and z-200 (200 mg). The zinc oxide (Zinc Óxido, Jalmek) was dissolved in 4 mL of olive oil and subcutaneously injected to ewes in z-100 and z-200 treatments on day nine of the estrus synchronization protocol (day 0 is the day of intravaginal device (CIDR®

ovis, Zoetis) insertion). The number of ewes assigned to each treatment within each farm was as follows: Control: 6, 9, 7, 24 and 7; z-100: 6, 9, 6, 24 and 7; z-200: 7, 9, 6, 23 and 7; for F1 to F5, respectively.

Animal nutrition

The ewes in F1 were fed with 2 kg day⁻¹ of a total mixed ratio (48% alfalfa, 28.84% Sudan silage and 23.07% oat straw as feed). The ewes in F2 to F4 were fed *ad libitum* with mixed forage hay (*Cynodon dactylon*, *Echinochloa colona*, *Echinochloa crus-galli*, *Sorghum halepense* and *Medicago sativa*). The ewes in F5 had free access to *Lolium multiflorum* Lam pastures during the entire experimental period. The ewes in the five farms did not receive any mineral supplementation before, during or after the experimental period.

Reproductive management

The estrus cycle of the ewes was synchronized by intravaginal CIDR insertion for 12 days. The ewes were injected with 350 IU of equine chorionic gonadotropin (eCG, Novormon® 5000, Virbac) at day 10 of the estrus synchronization protocol. The intravaginal device was removed at day 12 and the ewes were injected with 12.5 mg of dinoprost (Lutayse®, Zoetis). The ewes were inseminated 28-30 h after CIDR removal by cervical insemination method. The semen was provided by the same ram at all times. The semen was collected by artificial vagina just before the artificial insemination; its concentration was determined by Neubauer chamber and diluted with a commercial medium (Triladyl®) to the desired concentration (300×10^6 sperm cells mL⁻¹). The diluted semen was placed in a plastic tube surrounded by ice during artificial insemination.

Response variables

The response variables to the zinc oxide injection were the diameter of the largest preovulatory follicle before insemination and the pregnancy rate. The diameter of the largest preovulatory follicle was measured in five ewes from each experimental group in every farm by transrectal ultrasonography (Handscan V8, Sunway Medical). The two ovaries were observed and the position of the largest preovulatory follicle was recorded. The follicle diameter was calculated by the average of the horizontal and vertical measurements of the largest follicle. The pregnancy diagnose was carried out by transrectal ultrasonography between 30-35 days after artificial insemination.

Statistical analysis

The residual normality for the diameter of the largest preovulatory follicle variable was tested by the Shapiro-Wilk test. The data from this variable was analyzed using ANOVA and considering the farms as blocks. The means comparison was carried out by Tukey's test. The pregnancy rate was analyzed by Fisher's exact test. However, the data for pregnancy rate from F5 were not considered in the statistical analysis because there was an accidental loss of the semen sample and a lower dose of sperm (100×10^6 sperm cells) was used to inseminate the ewes. A value of $p \leq 0.05$ was considered as significant. The SAS University Edition statistical package was used at all times.

RESULTS AND DISCUSSION

Mineral supplementation is essential to sustain adequate animal reproductive activity (López-Alonso, 2012). The minerals can be supplied to animals in feed or by injection. There is evidence that pregnancy rate in ewes is increased by multiple injections of selenium and vitamin E during a synchronized estrus protocol (Awawdeh *et al.*, 2019). Similarly, oral supplementation of zinc is effective to improve pregnancy rate (Ali *et al.*, 1998) and prolificacy in ewes, possibly from reduced embryo mortality (Masters & Fels, 1980). These results show that mineral supplementation both by oral administration and by injection is effective to improve the ewe's reproductive performance. However, zinc supplementation by injection has been reported to be a more effective way to restore zinc blood concentrations in deficient animals than by oral administration (Lamand *et al.*, 1983). A high zinc blood concentration is reached after three days of mineral injection (Lamand, 1978) and it remains elevated for up to 28 days (Lamand *et al.*, 1980). Therefore, it is suggested that supplementation by injection ensures a good supply of trace minerals to achieve optimum reproductive performance (Stokes *et al.*, 2017).

The effect of subcutaneous injection of zinc oxide on the means of the largest preovulatory follicle diameter, measured in the different experimental groups, is shown in Table 1. In general, the effect of the treatment, block and their interaction were not significant ($p>0.05$). The latter is contradictory to published results in cows, where it was observed that the diameter of the preovulatory follicle increased with the injection of 400 mg of zinc oxide (Anchordoquy *et al.*, 2019). This contradiction can be due to the day when the zinc oxide injection was applied, since in the case of the study in cows by Anchordoquy *et al.*, supplementation was carried out at the beginning of the estrus synchronization protocol, which exposed the animal organism to the effects of zinc for a longer time. It is suggested that the zinc function at the follicular level is directed more to the oocyte (Ménézo *et al.*, 2011), which is particular sensitive to zinc deficiency during the last stages of maturation (Tian & Diaz, 2012). Delayed development of embryos and pregnancy loss have been observed when zinc dietary deficiencies are created four to five days before ovulation (Tian *et al.*, 2014). This agrees with the fact that intrafollicular zinc concentrations increase as the follicle's size increases (Kor *et al.*, 2013).

Mineral supplementation was not part of the feeding program for ewes in any of the farms in the present study. Therefore, there was speculation that sheep had zinc deficiency and that a positive effect from zinc supplementation could impact follicular size, oocyte quality, and pregnancy rate. This study is limited regarding the unknown mineral balance of the ewes before and after zinc injection. In addition, oocyte quality was not evaluated, but pregnancy rate results and the available scientific evidence allow speculating about the results from this study.

The effect of zinc injection on the pregnancy rate in ewes is depicted in Figure 1. In general, no dependency was observed between zinc injection and pregnancy rate ($p>0.05$), which is similar to the results found in ewes supplemented orally with 50-150 ppm of zinc oxide (Monem & El-Shahat, 2011). This is contrary to the results reported in cows (Anchordoquy *et al.*, 2019) and goats (Kundu *et al.*, 2014), where an increase in pregnancy

rate was observed by injecting 400 mg of zinc sulfate or by oral administration of 100 ppm of zinc oxide to goats.

Table 1. Diameter of the largest preovulatory follicle (mm, mean \pm EE) in ewes injected with 0 (Control), 100 (z-100) and 200 (z-200) mg of zinc oxide in five farms (F1-5).

Group	Preovulatory follicle diameter
Control	6.66 \pm 0.23
z-100	6.24 \pm 0.23
z-200	6.14 \pm 0.23
Block	
F1	6.56 \pm 0.30
F2	6.00 \pm 0.30
F3	6.46 \pm 0.30
F4	6.26 \pm 0.30
F5	6.43 \pm 0.30

Means with different superscript letter are significantly different ($p \leq 0.05$)

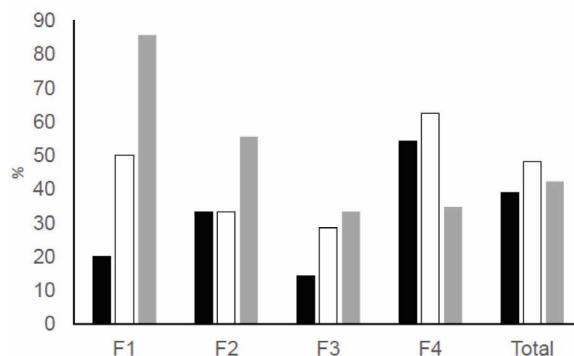


Figure 1. Pregnancy rate of ewes injected with 0 (black bars), 100 (white bars) and 200 (gray bars) mg of zinc oxide on four farms (F1-4).

The beneficial effect of zinc supplementation on pregnancy rate is sustained, at least partly, on the blood concentrations of this mineral being higher in pregnant females than in empty ones (Nazari *et al.*, 2019). In addition, zinc supplementation in laboratory conditions reduces the fragmentation of genetic material in the oocyte (Abdel-Halim *et al.*, 2018), increasing the number of oocytes that reach the blastocyst stage and their quality (Choi *et al.*, 2016; Jeon *et al.*, 2014). In this regard, zinc supplementation to the culture medium increases the size of the embryo inner cell mass, which might be associated to a higher pregnancy rate in cows (Wooldridge *et al.*, 2019). On the other hand, supplementing bulls (Kumar *et al.*, 2006) and adding this mineral to the fertilization medium improves sperm

activity and quality (Galarza *et al.*, 2020), which suggests that the fertilization process could be improved under *in vivo* conditions.

The lack of statical significant differences between experimental groups in pregnancy rates overrides the beneficial effect of zinc on female reproductive performance. However, it can be suggested that the lack of significant difference between treatments could be due to the small sample size in some sheep farms, such as F1 and F2. In addition, it has been suggested that the intrinsic characteristics of each farm, such as the initial mineral balance of the animals, affects differently the pregnancy rate in response to the zing levels supplemented, since the low levels of pregnancy are only observed in females with a zinc status considered as deficient, but not in those with a marginal or adequate zinc status (Galarza *et al.*, 2020).

CONCLUSIONS

The subcutaneous injection of 100 or 200 mg of zinc oxide during an estrus synchronization protocol did not affect the diameter of the largest preovulatory follicle or the pregnancy rate in ewes. It is advisable to perform mineral supplementation according to the female's mineral status.

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