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## Conception rates in ewes treated with organic selenium

Tasa de concepción en ovejas tratadas con selenio orgánico

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#### ABSTRACT

Selenium is a mineral that contributes to animal reproduction. The objective of this work was to evaluate conception rate and progesterone concentration in landrace ewes, during the reproductive season by organic selenium intake. 27 multiparous ewes were used with a body condition of 2.5; two groups were randomly designed: 11 ewes without selenium and 16 with selenium. Estrus was synchronized with intravaginal sponges containing fluorogestone acetate for 14 days, at the time of sponges removal, application of 400 UI of eCG was made. Artificial insemination (IA) was developed at a fixed time (55 hours after removal of sponges), consecutive application of 0.3 ppm day-1 of oral organic selenium was started seven days before IA. Progesterone concentration was evaluated in blood samples obtained along one estrus cycle (21 days), gestation diagnosis was developed at 35 days after the IA. Progesterone concentrations, between without selenium (5.28 ng mL-1) and treatment with organic selenium (6.58 ng mL-1), were not different between groups or between days ( $p \ge 0.05$ ), although in both groups the maximum progesterone concentration was observed on day 14 after the IA, in coincidence with a normal corpus luteum time. Also, conception rate was not different ( $p \ge 0.05$ ); in the group without selenium 81.8 % and with organic selenium 75 %. Based on the results, it is concluded that organic selenium application for seven days before IA did not influence progesterone concentration and conception rate in ewes.

Key words: Selenium, reproductive season, progesterone, artificial insemination.

#### RESUMEN

El selenio es un mineral que contribuye en la reproducción de los animales. El objetivo del trabajo fue evaluar la tasa de concepción y concentración de progesterona en ovejas locales, durante la época reproductiva y mediante la ingesta de selenio orgánico. Se utilizaron 27 ovejas, multíparas y con condición corporal de 2.5; se diseñaron dos grupos al azar: 11 ovejas sin selenio y 16 con selenio. Se sincronizó estro con esponjas intravaginales impregnadas con 20 mg de cronolona micronizada (acetato de fluorogestona) por 14 días y aplicación de 400 UI de eCG al retirar las esponjas. La inseminación artificial (IA) se realizó a tiempo fijo (55 horas después de retirar las esponjas), la aplicación oral de 0.3 ppm día<sup>-1</sup> de selenio orgánico se realizó siete días consecutivos antes de la IA. La concentración de progesterona se evaluó en muestras sanguíneas obtenidas en un período correspondiente a un ciclo estral de la oveja (21 días) y el diagnóstico de gestación se realizó 35 días después de la IA. Las concentraciones de progesterona de ovejas testigo (5.28 ng mL-1) y tratamiento con selenio orgánico (6.58 ng mL<sup>-1</sup>) no fueron diferentes ni entre grupos ni entre días ( $p \ge 0.05$ ); aunque en ambos grupos la máxima concentración de progesterona se registró el día 14 después de la IA lo que coincide con un cuerpo lúteo de vida normal. La tasa de concepción de testigo (81.8 %) y grupo con selenio orgánico (75 %) tampoco fue diferente ( $p \ge 0.05$ ). En base a los resultados se concluye que el selenio orgánico aplicado por siete días antes de la IA no influyó en concentración de progesterona y tasa de concepción en ovejas.

Palabras clave: Selenio, época reproductiva, progesterona, inseminación artificial.

### INTRODUCTION

In any species, including sheep and goats, reproduction is demanding in nutrients, hence the nutritional status is considered, an important modulator for it (Blache *et al.*, 2008). In the nutritional plane it is important to consider the addition of microminerals, such as selenium; this mineral is not only important for the normal functionality of the organism, but also to counteract or avoid pathologies, such as white or reproductive muscle disease. Despite the benefits that selenium can provide, toxicity is also reported due to high dose consumption. The mineral can be found as an "organic and inorganic" form; of them the one of greater toxicity is the inorganic one, whereas the organic one can be found in yeasts (Hall *et al.*, 2012), containing selenium linked to amino acids such as methionine and cysteine, being less toxic.

Whatever the form of selenium contribution, this already in the organism is part of the enzyme glutathione peroxidase (GSH-Px), presenting antioxidant action (López and López, 2013). It has been reported that the administration of this mineral increases the total antioxidant capacity and activity of GSH-Px, and decreases levels of reactive oxygen species (ROS), thus improving the *in vitro* development of follicles (Abedelahi *et al.*, 2010). In the case of organic selenium, there has been an increase in the number of ovarian structures and size of the ovary, as well as an increase in the number of corpora lutea (Vázquez-Hernández et al., 2017), which will surely be reflected in the increase in fertility, because it is currently indicated that selenium contributes to maintain the function of the corpus luteum (Kamada *et al.*, 2014).

Another effect attributed to selenium is reduction of embryonic death during the first month of gestation (Mehdi and Dufrasne, 2016). In a study conducted by Sánchez et al. (2008), found that the supply of selenium in the form of selenomethionine (SeMet) to sheep; the reproductive parameters (conception rate, calving rate and prolificacy) were higher in comparison with those that did not receive the mineral. The positive effects that organic selenium produces are attributed to the greater bioavailability that this form of selenium presents, comparing it with the inorganic form (Hall et al., 2012).

Based on this, the following work was aimed developed; test if organic selenium influences serum progesterone concentration and conception rate of wool sheep in breeding season.

#### MATERIAL AND METHODS

The study was conducted in the Chiautla municipality of Mexico State, with small-scale producers of sheep. The production units are located at coordinates 19 ° 32'09 " and 19 ° 36'19 " north latitude, and 98 ° 51'40 " and 98 ° 54'38 " west longitude; with altitude of 2260 msnm. The climate is temperate semidry, with rains in summer and temperature 6 ° C minimum, maximum 32 ° C, and average annual temperature between 11 ° C and 19 ° C (Enciclopedia de los municipios y delegaciones de México, 2017).

Twenty-seven local sheep of wool, crossed by different breeds, in breeding season, multiparous and body condition (CC) of 2.5; the sheep were kept stabled and offered a diet based on corn stubble and reduced alfalfa (diet used by producers in the region). The distribution of the sheep was carried out in two groups at random; the control group or without selenium with 11, and group with organic selenium constituted by 16 sheep, which received yeast enriched with selenium (selenomethionine and selenocysteine). All the sheep were subjected to estrus synchronization protocol, by placing intravaginal sponges (Chronogest<sup>®</sup> CR), impregnated with 20 mg of micronized cronolone (fluorogestone acetate) for a period of 14 days.

The methodology for inserting sponges was as follows: disinfect the sponge with iodine and manually, introducing the sponge through the vulva; with a 45 ° inclination to deposit it in the vagina, keeping the cord towards the outside; (the person who applied the sponge protected his hand with a nitrile glove).

For seven consecutive days before artificial insemination (AI), sheep were given 0.3 ppm organic selenium [dose recommended by <u>NRC (2007)</u>; the organic selenium was granted as yeast enriched with selenium, to know the content of selenium in the yeast and to make the dose of 0.3 ppm; said content was evaluated with mass spectrometry]. Intrauterine-bicornuate AI was performed by means of fixed-time endoscopy; 55 hours after removing the sponges and the intramuscular application of 400 IU of eCG animal-1, the AI was performed with fresh semen obtained from Dorper males and provided by the Center for Genetic Improvement Ovine (CeMeGO).

Per period of a normal estrus cycle (21 days) of the sheep and to evaluate the concentration of progesterone in response to the administration of organic selenium, and which is a sign of corpus luteum functionality or possible pregnancy, blood samples were collected jugular vein on days: 2, 6, 10, 14 and 24. After the AI of these, the serum was obtained, which was kept at -20  $^{\circ}$  C until the progesterone concentration was evaluated by the chemiluminescent microparticle immunoassay technique.

A diagnosis of pregnancy was made 35 days after the AI, using a portable ultrasound scanner, Draminski brand, model 4Vet mini with abdominal transducer 7.5 MHz; the exploration was performed at the level of the iliac fossa, directing the transducer upwards and backwards of the coxal vertebrae.

For the experiment, the animals were distributed randomly for each treatment group, the progesterone concentration data were analyzed with a linear mixed model, using the MIXED procedure with  $p \le 0.05$ ; while the data of pregnant sheep were by logistic regression, and  $p \le 0.05$  was considered for significance; the statistical program used was SAS, version 9.0.

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## RESULTS

The average concentrations of progesterone per day and per group did not present a significant difference ( $p \ge 0.05$ , figure 1); however, there is a difference in the sampling day interaction ( $p \le 0.05$ ); the day in which low or basal concentrations were presented was one day after AI and high concentrations on day 14; day that coincides with the moment of embryonic implantation. By day 24 the concentration dropped to not less than 4.07 ng ml<sup>-1</sup> day<sup>-1</sup> for the control group and 3.46 ng ml<sup>-1</sup> day<sup>-1</sup> for the organic selenium group; without descending to basal levels, which can be considered as indicative of the beginning of gestation.

In the case of a certain conception rate with a diagnosis of gestation at 35 days after AI, there was no significant difference ( $p \ge 0.05$ ) between the groups of sheep with organic selenium compared to the control group. The conception rate for the organic selenium group was 75%, while for the control it was 81.8% (Figure 2).

The results in conception rate are related to those of progesterone concentration, since there is no significant difference between groups on the last day of sampling; the number of pregnant ewes was not different either. The evaluation of progesterone in blood and ultrasound are methods that help determine pregnant ewes.

<u>Figure 3</u> shows ultrasound images of the diagnosis of gestation at 35 days after the AI, where fluid was visualized in the gestational sac, inflammation of the uterus, gestational sac and even the embryo. <u>Figure A and B</u> show pregnancy-positive uteri.



Figure 1. Progesterone concentration in sheep with and without organic selenium. G1 (control), G2 (organic selenium).



Figure 2. Pregnancy rate in sheep with and without organic selenium. G1 (control), G2 (organic selenium).



Figure 3. Ultrasound image of sheep treated with and without organic selenium at 35 days after Al. a) organic selenium), b) control. DISCUSSION

The values in progesterone concentration per day found in this research are similar to that reported by Rahman (2006) and DeNicolo *et al.* (2009), who indicate that in reproductive age the concentration of progesterone increases between days two or three after ovulation, when a new corpus luteum begins its activity; observing maximum concentrations (5 ng ml<sup>-1</sup>) between days 10 and 14 proestrus. Results similar to what was found in this study, where the highest concentration was found between days 10 and 14 (eight and 12 days proestrus); for control (5.28 ng ml<sup>-1</sup>) and group with organic selenium (6.58 ng ml<sup>-1</sup>). These results coincide with that found by Khanum *et al.* (2008), who worked with dwarf goats and recorded progesterone concentrations from baseline values of  $0.1 \pm 0.03$  ng ml<sup>-1</sup> on day zero to  $3.0 \pm 0.09$  ng ml<sup>-1</sup> on day six with respect to their estrous cycle, reaching concentrations maximum values of  $7.7 \pm 0.6$  ng ml<sup>-1</sup> on day 12.

The results on early gestation obtained in this study were not significant; however, authors such as <u>Fraire-Cordero *et al.* (2013)</u> found selenium and vitamin E (Se-VE) in Pelibuey sheeps, a gestation percentage of 75%, a result similar to those found in this research with G2 = organic selenium (75%) and G1 = control (81.8%). However, these results differ with

the work of <u>Gabryszuk and Klewiec (2002</u>), where they evaluated the effect of sodium selenite in Polish Merino sheep, and found that when injecting selenium before mating significantly increases fertility (100%), compared to animals that did not receive selenite.

In this study no difference was found, which is attributed to the body condition of the sheep; given that in studies conducted by this same research group with application of equal doses of selenium to sheep in non-breeding season; resulted in greater number of pregnant ewes (unpublished data), which coincides with that reported by <u>Gabryszuk and Klewiec (2002)</u>, who reported fertility in 100% of sheep treated with sodium selenite; this can also be taken as an indication that the response of this species to selenium depends on the reproductive season.

## CONCLUSION

The organic selenium administered for seven days before AI did not influence progesterone concentration and conception rate in sheep. It is recommended not to apply selenium in the way it was carried out in this research, since it could be carried out in another concentration and for another period.

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