ECRONICON OPEN ACCESS
EC VETERINARY SCIENCE
Research Article

Source of Selenium and its Effect on Gestation and Response in Leucocytes, in Adult Sheep at the Time of Anestrus

Leonor Miranda Jiménez[1], Edith Ramírez Segura[1], Adrián Raymundo Quero Carrillo[1]* and Nazario Pescador Salas[2]

[1]*Postgraduate College-Montecillo Campus, Mexico-Texcoco Federal Highway, Montecillo, Texcoco, Mexico*

[2]*Faculty of Veterinary Medicine and Zootechnics, UAEMex. El Cerrillo Piedras Blancas, Toluca, Mexico*

***Corresponding Author:** Adrián Raymundo Quero Carrillo, Postgraduate College-Montecillo Campus, Mexico-Texcoco Federal Highway, Montecillo, Texcoco, Mexico.

Received: February 21, 2022; **Published:** March 31, 2022

Abstract

Selenium acts at the reproductive and immune system level. The toxicity, bioavailability and action depend on the type of selenium given. It was evaluated if the source of selenium affects the percentage of pregnant sheep and leukocytes (immune cells of the white blood cell package). Twenty adult sheep randomly distributed in: control (T_1), sodium selenite (T_2), encapsulated yeast enriched with selenium (T_3), and powdered yeast with selenium (T_4) were used for five days before artificial insemination (AI), 300mg day^{-1} animal^{-1}. Leukocytes were counted seven days before and up to 88 days after AI. A completely randomized design was used. For leukocyte data, a mixed model was used and, for gestation percentage, logistic regression. The powdered yeast with selenium (T_4) presented 100% of pregnancies and sodium selenite only 40%; while, the control sheep and those with encapsulated yeast enriched with selenium, 80% ($P < 0.05$). The cells that appeared in greater number were lymphocytes and neutrophils. Lymphocytes increased after day 13, with a maximum number on day 20 ($p < 0.05$). In T_2 the total number of lymphocytes decreased and that of monocytes increased. Basophils were increased at both T_3 and T_4. The selenium source is important in the presence of leukocytes and in the establishment and maintenance of pregnancy.

Keywords: *Immune Cells; Organic Selenium; Inorganic Selenium; Sodium Selenite; Gestational Immunity; Sheep*

Introduction

Selenium (Se) is an essential trace element for the physiological activity of the mammalian organism, it participates in processes as an antioxidant, as well as in the activation of granulosa cells, synthesis of thyroid hormones and immunity [1]. The nutritional deficiency of selenium causes alterations of systems and organs, both in females and males; in females, the growth, development and implantation of the embryo are affected [2]; in the case of the male, spermatogenesis. In both sexes, it causes depression of the antioxidant status, which leads to cell death; which is involved in defense systems, protects against oxidative stress and the elimination of reactive oxygen species.

In a generic way, selenium is found as an inorganic and organic form. The organic form of selenium (selenomethionine or selenocysteine) is less toxic, has greater bioavailability, is easily absorbed, and is immediately metabolized; the above, in contrast to the inorganic form [3].

The direct addition or in the diet as selenocysteine, in the organic form, facilitates its activity; which is incorporated as selenoproteins: glutathione peroxidase, deiodinases, iodothyronine, thioredoxin reductase, selenoprotein P, among other important proteins in normal pregnancy [4].

During early pregnancy, the mother's immune system is altered and, at the systemic level, cellular immunity decreases so as not to reject the developing embryo. During the third trimester of pregnancy, macrophages and neutrophils circulating in the blood increase phagocytosis and, therefore, the production of Reactive Oxygen Products (ROS); in addition, in this way, there is the possibility that cellular immunity decreases and the possibility of acquiring maternal intracellular infections increases [5]. It has been reported that as gestational age increases, there is an increase in antioxidants in the blood. It is at this time that selenium's antioxidant or immune-protective action becomes important. In female bovines, it has been observed that the application of yeast with selenium increases acute defense proteins against bacteria and fungi; similarly, to counteract systemic inflammation after childbirth [6]. The addition of vitamin E to selenium treatments helps to promote antioxidant activity, as well as the number of pregnancies after childbirth [7]. Many studies of the effect of selenium during pregnancy and immunity have been carried out; the above, in sheep and there are few studies on the role of selenium in immunity during pregnancy, especially in sheep.

Objective of the Study

The objective was to evaluate if the source of selenium affects the percentage of pregnant ewes and the type of white blood cells (cells that act in immunity) present in this period. It is suggested that the number of ewes that become pregnant, when adding selenium, depends on the type of selenium used and, similarly, its influence on the type of leukocytes.

Materials and Methods

This study was carried out from January to May (seasonal reproductive anoestrus period in sheep); in a sheep production unit (ejido) in the municipality of San Andrés Chiautla, State of Mexico: 19° 32" 09" and 19° 36' 19" N latitude, 98° 51' 40" and 98° 54' 38" longitude W. For the research, 20 adult sheep of undefined breed were used; being the local Creole breed, the genetic basis. The average live weight of the sheep was 40 ± 3.26 kg, with 3.05 ± 1.02 years of age and body condition (CC) from 2.5 to 3, on a scale of 1 to 5. Before starting the treatments, the sheep were dewormed with Febendazole (10%) orally at a dose of 1 mL per 20 kg of LW.

The sheep were stabled, with food and water *ad libitum*. Feed based on shrunken alfalfa and corn stubble (routine in the area) was provided. The animals were randomly distributed in four treatments (Table 1). Selenium was given orally at a dose of 300 mg day^{-1} animal^{-1} [(requirements according to NRC [8])], for five days, starting seven days before artificial insemination (AI).

Treatment	Selenium type	Selenium dose	Sheep per treatment (n)
T_1:	Witness	0	5
T_2:	Sodium selenite (Na_2SeO_3)	0.3 ppm	5
T_3:	Yeast encapsulation selenium enriched	0.3 ppm	5
T_4:	Selenium enriched yeast	0.3 ppm	5

Table 1: *Treatments of different sources of selenium applied to adult sheep, during the season of seasonal anoestrus, to determine the number of pregnancies and differential leukocyte count.*

n: Number of animals per treatment, ppm: Parts per million and T_1, T_2, T_3, T_4: Treatments.

To determine the concentration of selenium contained in the yeast, it was subjected to a mass spectrophotometric analysis (Hydrosciences Laboratory, Postgraduate College, Montecillo *Campus*); the reported concentration was 1363.12 mg of selenium kg^{-1}. Selenium was weighed in individual daily doses on a Bel Engineering model HPB-105i analytical balance with a resolution of +0.01 mg.

The selenium-enriched yeast encapsulate was made based on the determined concentration of selenium in the yeast. This package is in the process of applying for a patent, which is why its description is limited.

Experimental sheep were synchronized using Cronolone intravaginal sponges (Chronogest® CR, Intervet Laboratories); which contained 20 mg of synthetic progestogen per female, for 14 days, vaginally. Upon removal of the sponges, 400 IU of eCG per female (Folligon®, Intervet) were applied. Artificial insemination (AI) was performed by endoscopy at a fixed time (55 hours after removing the sponges).

The diagnosis of pregnancy was made at the level of the iliac fossa, a first diagnosis 26 days after AI and the second diagnosis 35 days after AI, using a microconvex electronic probe of the Draminski 4Vet ultrasonography.

Blood smears were made to determine leukocytes by treatment; the above, with a drop of blood from each female per sampling day; which was placed in the center of the slide, obtaining a uniform and thin scan, which was fixed to the slide with 100% ethanol. Blood sampling began two days before selenium supplementation and on days -7 to 0 and from 0 to +4 to continue on days 13, 20, 27, 60 and 90 after AI i.e. day zero was considered as AI day. The smears were identified and transported to the nanotechnology module of the Colegio de Postgraduados, Campus Montecillo, where they were stained with Hematoxylin (Gill's No.3; Sigma-Aldrich, Inc.) for five minutes and then washed with distilled water. The observation and differential count of leukocytes was performed with an optical microscope at 40X resolution, the cells present in four fields per sample were counted. The protocol used for the development of this part of the research is routinely used in hematology laboratories and is used with modifications according to the availability of equipment, such as flow cytometry, droplet, among others; although, by not having equipment suitable for counting blood cells; still a valid option.

For each treatment the sheep were randomly distributed. A completely randomized design was used, with a sheep as the experimental unit and repetition i.e. five sheep per treatment. For the evaluation of data on the percentage of pregnant females, multivariate statistics were used; while, for the number of pregnancies, a logistic regression analysis with $p \leq 0.05$ [9] because it is a binary variable (pregnant or non-pregnant); and, for the differential leukocyte count, a linear mixed model with $p \leq 0.05$ was used for the means test [10].

Results and Discussion

The results for pregnant ewes show that there is no difference between the control group and the group that consumed yeast enriched with encapsulated selenium. Both groups presented 80% of pregnancies ($p \geq 0.05$). Although there was a difference with the supply of sodium selenite ($p \leq 0.05$), this treatment presented the least number of pregnant ewes (40%); while 100% of pregnancies were reached, in those who consumed selenium-enriched yeast (Figure 1; $p \leq 0.05$). It is important to highlight the importance of these results, given that they were obtained during the season of the year when sheep gestate with difficulty, due to their racial condition mixed with seasonal and non-seasonal breeds i.e. in the season of seasonal anoestrus, normal in the Central Altiplano region of Mexico (Central High Plateau).

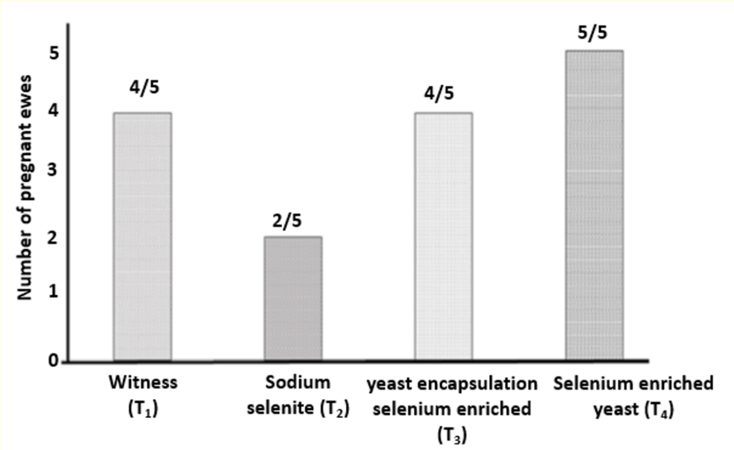

Figure 1: *Number of pregnant ewes with different source of selenium, provided to adult ewes seven days before AI and during seasonal anoestrus.*

The percentage (100%) of pregnant females in the group with selenium-enriched yeast coincides with that reported by [11], who mention that the effect of supplying selenium in the diet of pregnant females helps the proper development of the embryo and ensures the correct implantation of this, an effect that they attribute to the bioavailability and to the lower toxicity that this type of selenium presents. In addition [3], they mention that organic selenium (yeast enriched with selenium bound to amino acids) is easily absorbed and metabolized immediately. It has also been documented that components such as methionine in the diet (selenomethionine) alter the intrauterine environment and at the same time can serve as donors of the methyl group necessary for DNA methylation and cause alterations in it, which will be reflected during adult development [12] and possibly from embryonic and fetal life; the above, in contrast to inorganic forms such as selenite and sodium selenate. Likewise, angiogenesis is favored; which stimulates the supply of nutrients to both the follicle and the oocyte [13]. In 1999 [14] and [15], report that the quality of the follicle, oocyte and oviduct is improved by the action of selenium-dependent glutathione peroxidase which traps reactive oxygen species. On the other hand, oxidative stress influences early embryonic development by modifying transcription factors and, therefore, gene expression, all of which is reflected in the number of pregnant females reported in this study. However, [16] did not find l differences in pregnancies of sheep during the reproductive season and treated with organic selenium, compared to control sheep, since they did not show statistical differences ($P > 0.05$). The foregoing is controversial given that ewes in the non-reproductive season, as is the case in this study, did increase the number of pregnancies ($P < 0.05$). On the other hand, the reason why the females treated with sodium selenite (inorganic Se) presented a lower pregnancy rate, possibly due to a toxicity effect presented when using this source of selenium [3].

Regarding the differential number of leukocytes for each sampling day, the cells that were observed in greater quantity included lymphocytes and neutrophils ($P < 0.05$; Table 2); the above, in all treatments. That is, this variable was not influenced by the type of selenium applied to animal consumption.

	Lym (%)	Mo (%)	Eos (%)	Bas (%)	Neu (%)
Witness	80.91^a	2.07^b	0.5^b	0.82^a	15.73^a
Sodium selenite	61.41^b	23.76^a	0.5^b	0.72^a	13.63^a
Encapsulated yeast enriched with selenium	81.70^a	1.49^b	1.01^a	0.87^a	14.93^a
Selenium enriched yeast	80.48^a	1.66^b	1.04^a	0.14^b	16.68^a

Table 2: *Leukocytes observed in adult sheep that received different sources of selenium, during the period of seasonal anoestrus. Lym= Lymphocytes, Mo= Monocytes, Eos= Eosinophils, Bas= Basophils and Neu= Neutrophils.*

The source of selenium does seem to be important in the case of lymphocytes, the sheep that received sodium selenite presented a lower number ($p \leq 0.05$), compared to the rest of the treatments; the foregoing, possibly due to the toxic effect that occurs with the application of this type of selenium (UN Classification: Class 6.1 Toxic; data obtained from the product's safety data sheet). In addition, with sodium selenite, monocytes increased cells that are generally activated by inflammatory reactions, since they are cells rich in histamine granules. The effect of lymphocyte reduction, caused by sodium selenite, was also reflected in the reduction of pregnancies observed in the sheep of this investigation.

Regarding the elevation of lymphocytes and neutrophils, in all treatments, this is also recorded in normal pregnancy conditions, where they are similarly elevated. Although in this investigation and in the case of lymphocytes, the number was higher than that reported as normal in pregnant sheep [17]. In 2006 [18], he mentions that the values reported for lymphocytes and neutrophils in normal physiological status in sheep can be modified when the animal presents illness, stress, or in the case of various physiological events: suckling, pregnancy and weaning, among others. Also, under normal conditions, the number of these two types of cells in circulation is greater, compared to the rest of the cells involved in immunity (leukocytes or white blood cells). This behavior is attributed to its function as a second defense barrier involving phagocytosis, in the case of neutrophils, and the production of substances such as type II interferon, in the case of lymphocytes [18]. This indicates that the elevation of immune cells such as lymphocytes and neutrophils is required to protect the surrogate mother from pathogenic organisms and allow a successful pregnancy.

On the other hand, from day +1 to +13, a decrease in lymphocytes was recorded in sheep that received sodium selenite, to decrease again after day 20 and remain in this way until day 88 of gestation [last day of sampling; Figure 2 ($p \leq 0.05$)]. Decrease that may be the cause of the lower number of pregnancies in sheep that received sodium selenite.

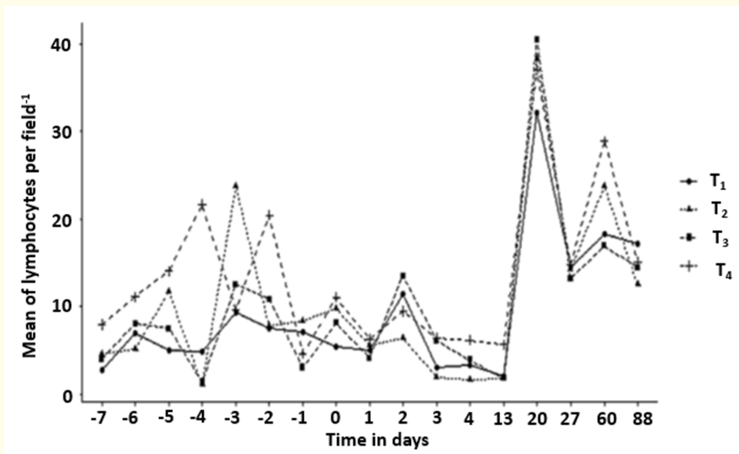

Figure 2: *Average lymphocytes field-1 day-1 of sampling in adult sheep, at the time of seasonal anoestrus, treated with different sources of selenium, days before AI (day zero) and sampled up to 88 days of gestation.*

T1, Witness; T_2, Encapsulated yeast; T_3, Encapsulated yeast enriched with selenium; T4, Selenium enriched yeast.

Similarly, an increase in the number of lymphocytes was observed; which was recorded after day +13 with a maximum value for day +20 ($p \leq 0.05$). This fact coincides with the moment of arrival of the embryo in the uterus and the beginning of embryonic elongation and implantation, whose contact (uterus: embryo) triggers the release of interferon Tau [Interferon T (type I)] from the trophoblast. This molecule is important for the prolongation of the functionality of the corpus luteum and maternal recognition [19]. In a study carried out by [20], they found similar behavior in this type of cells; however, the elevation of lymphocytes was recorded from day 3 after artificial insemination to be maintained until day 23, which may be due to timing of selenium application; which, in the specific case of this investigation, was five days; however, this aspect is not mentioned by these authors. Similarly, it may be the effect of the time of year (seasonal anoestrus) in which the study was conducted; however, it is not mentioned in the reference study, similarly carried out in the Central Highlands of Mexico [20].

The elevation of immunity granted by lymphocytes-macrophages-immunoglobulins, a normal immunological route when circulating lymphocytes are activated, as in the present investigation and recorded from day 13 may not harm the embryo, since the placental barrier is protecting it; however [5], has indicated that during pregnancy, the decrease in the activity of the mother's immune system occurs until the third trimester of pregnancy. This is contrary to what was found in this study, where this decrease was observed in the first days of pregnancy; which may be the cause of fewer successful pregnancies. According to [21], throughout pregnancy, the fetus is protected by immunomodulatory molecules such as progesterone, enzyme 2-3-dioxygenase, free radicals, glycodelin and interferon T, where progesterone participates in the maintenance of pregnancy in mammals and increases considerably during gestation; which works through its intracellular receptor and regulates cellular and molecular aspects involved in the implantation process, being important to reduce the reactive capacity of the mother against fetal antigens, promoting the success of effective implantation and gestation.

The last point to consider is the registered elevation of eosinophils in sheep both with encapsulated organic selenium-enriched yeast treatment (T_3) and in powdered organic selenium-enriched yeast (T_4), and considering that eosinophils are immune cells whose action is directed against parasites, the elevation observed in this investigation may indicate that the yeast used in it could be contaminated by this type of microorganisms; therefore, it is recommended to treat the yeast with organic selenium to avoid the presence of contaminating microorganisms, before being used.

Conclusion

The source or type of selenium is important to increase or decrease the percentage of pregnant ewes: powdered yeast enriched with selenium (T_4; organic) resulted in more pregnancies compared to sodium selenite and in the season of seasonal anoestrus.

Observed pregnancy reduction matched with lymphocytes reduction in response to the use of sodium selenite (T_2, inorganic), indicating that the immune response in ewes, granted through leucocytes is important to pregnancy establishment, maintenance and success.

The decrease in pregnancies coincides with the decrease in lymphocytes produced by the application of sodium selenite. This indicates that, in sheep, the immune response conferred through leukocytes is important for the establishment, maintenance and success of pregnancy.

Gratitude

Ample thanks are extended to M.C. Alfredo Garrido Arcos for his valuable help in data collection. This work was carried out as part of the activities of the LGAC-Technological Innovation and Food Safety in Livestock.

Bibliography

1. Ruggeri RM., *et al*. "Selenium exerts protective effects against oxidative stress and cell damage in human thyrocytes and fibroblasts". *Endocrine* 68.1 (2019): 151-162.

2. Sharma RK and Agarwal A. "Role of reactive oxygen species in gynecologic diseases". *Reproductive Medicine and Biology* 3.4 (2004): 177-199.

3. Yue W., *et al*. "Effect of supplemental selenomethionine on growth performance and serum antioxidant status in taihang black goats". *Asian-Australasian Journal of Animal Sciences* 22.3 (2009): 365-370.

4. Guerin P., *et al*. "Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings". *Human Reproduction Update* 7.2 (2001): 175-189.

5. Draca S. "Peroxidación lipídica en gestaciones con pre eclampsia y diabetes". *Autoimmune* 35 (2002): 307-312.

6. Ren ZH., *et al*. "Comparative iTRAQ proteomics reveals multiple effects of selenium yeast on dairy cows in parturition". *Human Reproduction Update* 197.2 (2019): 464-474.

7. Khatti A., *et al*. "Supplementation of vitamin E, selenium and increased energy allowance mitigates the transition stress and improves postpartum reproductive performance in the crossbred cow". *Theriogenology* 104 (2017): 142-148.

8. NRC. National Research Council. "Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids". Washington, DC, USA. National Academy Press (2007): 362.

9. SAS. SAS Institute Inc. Cary, NC, USA. Version 9 for Windows edition (2000).

10. SAS. SAS Institute Inc. Cary, NC, USA. Version 9.4 for Windows edition (2004).

11. Pappas AC., *et al*. "Selenoproteins and maternal nutrition". *Comparative Biochemistry and Physiology - Part B: Biochemistry and Molecular Biology* 151.4 (2008): 361-372.

12. García RR., *et al*. "Epigenética: definición, bases moleculares e implicaciones en la salud y en la evolución humana". *Revista Ciencias de la Salud* 10.1 (2012): 59-71.

13. Vázquez HSD., *et al*. "Desarrollo de folículos y cuerpo lúteo en cabras, como respuesta al suministro de Selenio". *Agro Productividad* 10.2 (2017): 15-18.

14. Mouatassim S., *et al*. "Expression of genes encoding antioxidant enzymes in human and mouse oocytes during the final stages of maturation". *Molecular Human Reproduction* 5.8 (1999): 720-725.

15. Dennery PA. "Role of redox in fetal development and neonatal diseases". *Antioxidants and Redox Signaling* 6.1 (2004): 147-53.

16. Cabrera MJ., *et al*. "Tasa de concepción en ovejas tratadas con selenio orgánico". *Abanico Veterinario* 9 (2019): e93.

17. Plaza CA., *et al*. "Tasa de gestación en ovinos de pelo criollos en el departamento de Córdoba, Colombia, Unidad de Colombia". *Revista Colombiana De Ciencia Animal - RECIA* 11.1 (2019): 1-9.

18. Ramírez L. "Los leucocitos en mamíferos domésticos". *Mundo Pecuario* 2.2 (2006): 37-39.

19. Spencer TE and Bazer FW. "Conceptus signals for establishment and maintenance of pregnancy. review". *Animal Reproduction Science* 82-83 (2004): 537-550.

20. López VMM., *et al*. "Leucocitos en el desarrollo embrionario de ovejas como respuesta a selenio". *Abanico Veterinario* 9 (2019): 1-10.

21. Barrera D., *et al*. "Immunological role of progesterone in the maintenance of pregnancy". *Revista de Investigación Clínica* 59.2 (2007): 139-145.

Volume 7 Issue 4 April 2022