# SHORT COMMUNICATION

# Effects of dietary supplementation with *Erythrina americana* on seminal characteristics of rams

## *Efectos de la suplementación dietaria con Erythrina americana sobre las características seminales de Carneros*

## *Efeitos da suplementação dietética com Erythrina americana nas características seminais de carneiros*

Rafael Nieto-Aquino[1]; Eleazar Altamirano-Mijangos[1]; Teódulo Salinas-Rios[1*]; Héctor M Rodríguez-Magadán[1]; Said Cadena-Villegas[2]; Cuauhtémoc Nava-Cuellar[3]; Jorge Hernández-Bautista[1].

[1]*Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma Benito Juárez de Oaxaca, México.*

[2]*Colegio de Postgraduados, Campus Tabasco, Tabasco, México.*

[3]*Departamento de Nutrición Animal y Bioquímica, Universidad Nacional Autónoma de México, Distrito Federal, México.*

***To cite this article:***

Nieto-Aquino R, Altamirano-Mijangos E, Salinas-Rios T, Rodríguez-Magadán HM, Cadena-Villegas S, Nava–Cuellar C, Hernández-Bautista J. Effects of dietary supplementation with *Erythrina americana* on seminal characteristics of rams. Rev Colomb Cienc Pecu 2024; 37(4):223–232. https://doi.org/10.17533/udea.rccp.v37n4a5

**Abstract**

**Background:** Antioxidants of natural origin can be used to improve cryopreservation of semen. **Objective:** To evaluate the effect of dietary supplementation with *Erythrina americana* on the seminal characteristics of rams. **Methods:** Ten rams were randomly distributed in two treatments: l) Group supplemented with *E. americana* (20% dry matter basis; AEA, n=5), and 2) Control group not supplemented (SEA, n=5). Both diets were iso-proteic and iso-energetic. The feeding period was eleven weeks. Ejaculates were obtained through an artificial vagina and were evaluated fresh (37 °C) and refrigerated (5 °C). Semen volume (VOL) was assessed by visual examination in a calibrated (mm) tube; sperm concentration (CON x106) with a photometer; and mass motility (MM, 1-5), individual motility (IM), normality (N), and live sperm (L) were assessed by microscopic observation. **Results:** Feeding *E. americana* did not affect ($p>0.05$) VOL, CON, MM, and N in fresh semen, but decreased ($p<0.05$) IM and L. The L and N variables did not differ ($p>0.05$) among treatment groups in the refrigerated semen; however, IM improved in the AEA group ($p<0.05$) compared to the SEA group. **Conclusion:** Dietary supplementation of rams with *E. americana* reduces sperm quality in fresh semen but is beneficial for the cooling process of refrigerated semen.

**Keywords**: *artificial insemination; antioxidants; Erythrina americana; fresh semen; rams; refrigerated semen; seminal quality; sheep; spermatozoa.*

*Received: July 20, 2023. Accepted: April 22, 2024*

***Corresponding author.** Facultad de Medicina Veterinaria y Zootecnia - Universidad Autónoma Benito Juárez de Oaxaca - Oaxaca, México. C.P. 68120. Tel.: (951) 438 59 88 / (951) 438 59 87. E-mail: salinas980@hotmail.com

**Resumen**

**Antecedentes:** La suplementación dietaria con antioxidantes de origen natural es una alternativa para mejorar la criopreservación del semen. **Objetivo:** Evaluar el efecto de la suplementación dietaria de carneros con *Erythrina americana* sobre algunas características seminales. **Métodos:** Diez carneros fueron distribuidos al azar en dos tratamientos: 1) Grupo suplementado con *E. americana* (20%, con base en materia seca; AEA, n=5); y 2) Grupo testigo, sin suplementación de *E. americana* (SEA, n=5). Ambas dietas fueron balanceadas isoprotéica e isoenergéticamente. El periodo de alimentación de los sementales fue de once semanas. Los eyaculados se obtuvieron mediante vagina artificial y se evaluaron en fresco (37 ºC) y refrigerado (5 ºC). El volumen (VOL) seminal se evaluó en un tubo graduado en mililitros; la concentración espermática (CON x106) con un fotómetro; y la motilidad masal (MM, 1-5), motilidad individual (IM), normalidad (N), y espermatozoides vivos (L) por observación microscópica. **Resultados:** La inclusión de *E. americana* en la dieta no afectó ($p>0.05$) los parámetros VOL, CON, MM, y N en semen fresco, pero disminuyó ($p<0.05$) la IM y L. No hubo diferencia ($p>0.05$) entre tratamientos en las variables L y N en semen refrigerado; sin embargo, la IM del grupo AEA mejoró ($p<0.05$) respecto al grupo SEA. **Conclusión:** La suplementación con *E. americana* en la dieta de carneros reduce la calidad espermática en semen fresco, pero coadyuva en el proceso de enfriamiento para el semen refrigerado.

**Palabras clave:** *antioxidantes; calidad seminal; carneros; Erythrina americana; espermatozoides; inseminación artificial; ovinos; ovejas; semen fresco; semen refrigerado.*

**Resumo**

**Antecedentes:** A utilização de antioxidantes de origem natural é uma alternativa para melhorar a criopreservação do sêmen ovino. **Objetivo:** Avaliar o efeito da suplementação de *Erythrina americana* na dieta sobre as características seminais de carneiros. **Métodos:** Dez carneiros foram distribuídos aleatoriamente em dois tratamentos: 1) grupo adicionado de *E. americana* (20%, com base na matéria seca) na dieta (AEA, n=5); e 2) o grupo controle sem adição de *E. americana* (SEA, n=5). Ambas as dietas foram isoprotetoras e isoenergeticamente balanceadas. O período de alimentação dos garanhões foi de onze semanas. Os ejaculados foram obtidos através de vagina artificial e avaliados frescos (37 ºC) e refrigerados (5 ºC). O volume (VOL) foi avaliado com tubo graduado em mililitros, a concentração espermática (CON x106) com fotômetro e motilidade de massa (MM, 1-5), motilidade individual (IM), normalidade (N) e espermatozoides vivos (L) por observação microscópica. **Resultados:** O sêmen fresco mostrou que a inclusão de *E. americana* na dieta de carneiros não modificou ($p>0,05$) as médias dos parâmetros VOL, CON, MM e N, mas diminuiu ($p<0,05$) o IM e L. No sêmen refrigerado as variáveis L e N não diferiram ($p>0,05$) entre os tratamentos; porém, no IM o grupo AEA melhorou ($p<0,05$) em comparação ao grupo SEA. **Conclusão:** A suplementação com *E. americana* na dieta de ovinos reduz a qualidade espermática no sêmen fresco, mas contribui para o processo de resfriamento para sêmen refrigerado.

**Palavras-chave:** *antioxidantes; carneiros; Erythrina americana; esperma; inseminação artificial; ovinos; ovelha; qualidade do sêmen; sêmen fresco, sêmen refrigerado.*

## Introduction

Using refrigerated semen has shown acceptable results in artificial insemination of sheep by laparoscope (Davendra and Krishnappa, 2019). Several dilutants are commonly used to preserve semen. Thy provide energy for the metabolism of spermatozoa and maintain osmotic pressure and pH of the medium (Salamon and Maxwell, 2000). Integrity of the spermatozoa membrane is compromised during the cooling process causing changes in sperm motility and viability due to increased production of reactive oxygen species (ROS). They cause oxidative damage to biomolecules by oxidation of proteins and DNA, thus compromising sperm viability (Rehman *et al.*, 2013).

Polyphenols are natural antioxidants derived from plants that can have a protective effect on sperm cells by neutralizing free radicals, thus improving total and progressive motility as well as functionality of the plasmatic and mitochondrial membrane of spermatozoa (Allai *et al.*, 2018; Abadjieva *et al.*, 2020; Abdi-Benemar *et al.*, 2020).

Few studies have supplemented animals with *Erythrina americana*; most are based on its properties and benefits within the pharmaceutical industry (Loizzo and Tundis, 2019; Cai *et al.*, 2020). A great number of secondary metabolites, such as alkaloids and flavonoids, have been isolated from the bark, seeds, roots, leaves and flowers of genus *Erythrina* (García-Mateos *et al.*, 2001). Pino *et al.* (2004) mentioned that the metabolites with highest presence in leaves are alkaloids (64%), flavonoids (27%), and triterpenoids (9%). Ibarra *et al.* (2011) reported the presence of erysodine in seeds, with antioxidant potential similar to that of ascorbic acid. Nevertheless, it is unknown if these compounds can influence seminal quality. Therefore, the objective of the present study was to evaluate the effect of dietary supplementation of rams with *E. americana* as a natural antioxidant on traits of fresh and refrigerated semen.

## Materials and Methods

### *Ethical considerations*

Animal handling complied with ethics and biosafety norms of the Council of International Organizations in Medical Science (CIOMS, 2016), and Mexican law 9NOM-062-ZOO-1999 for the use of animals in experimentation (DOF, 2001).

### *Study area*

The study was conducted from January to June, 2022 at Facultad de Medicina Veterinaria y Zootecnia of Universidad Autónoma Benito Juárez de Oaxaca, located in the Central Valleys of Oaxaca state, under semi dry climate, with 798 mm average precipitation and 22 °C annual temperature, at 17°05'00" latitude north and 96°45'00" longitude west, and 1,550 m.a.s.l. (García, 2004).

### *Determination of antioxidants and crude protein*

Leaves and petioles from three *E. americana* trees in vegetative growth with 2 to 3 years of age were manually cut, dried at ambient temperature (in the shade), ground into 1-2 cm particles (Terramak, San Nicolás de los Garza, NL, México) and mixed in the experimental diet. The Kjeldahl method was used for the analysis of crude protein (AOAC, 2017).

To determine phenolic acids and flavonoids, a 100 g sample was kept in deep freeze (-180 °C) until analysis. A lyophilized sample (0.150 g) was weighed, and 3 ml 80% ethanol added, then placed in a double boiler with agitator for two periods of 5 minutes and one period of rest; and then centrifuged for 5 minutes at 2,500 rpm. A liquid gas chromatograph (Agilent model 1100) equipped with an automatic injector model 1200 and a diode array detector was used. The column was a Zorbax SB-C8, 4.6 × 75 mm; the mobile phase was: A) water with 0.1% trifluoracetic acid (TFA), and B) acetonitrile with 0.1% of TFA. The analysis was by T gradient (0.10 min) 85% A, 15% B; T (20 min) 65% A, 35% B and T (25 min) 65% A, 35% B. Flow velocity was 1.0 ml min-1 at 30 °C and the detector was adjusted to 254 nm.

**Table 1.** Phenolic components in *Erythrina americana* leaves.

| Compound | % |
|---|---|
| Crude protein | 17 |
| *Phenolic acids* | |
| Protocatechuic acid | 0.012 |
| Gallic acid | 0.023 |
| Chlorogenic acid | 0.290 |
| Vanillinic acid | 0.042 |
| P-Hydroxybenzoic acid | 0.051 |
| Ferulic acid | 0.008 |
| *Flavonoids* | |
| Quercetin | 0.090 |
| Naringenin | 0.047 |
| Floretin | 0.038 |
| Galangin | 0.007 |

**Table 2.** Proportion of ingredients in the diet with and without *Erythrina americana*.

| Ingredients | SEA (%) | AEA (%) |
|---|---|---|
| Soybean meal | 4.96 | 0 |
| Corn | 24.02 | 23.75 |
| Corn stubble | 63.86 | 49.09 |
| Molasses | 5.2 | 5.2 |
| Salt | 0.96 | 0.96 |
| Urea | 1 | 1 |
| *E. americana* | 0 | 20 |

SEA: Control group not fed *E. americana*. AEA: Group fed *E. americana* (20%, based on dry matter).

Semen collection was conducted with an artificial vagina once a week for 11 weeks. The fresh ejaculates were maintained in a thermostatic bath at 37 °C. Semen quality was assessed by microscopic observation (Zess primo star, Jena, Germany). The measurements taken from the ejaculate volume (VOL) were mass motility (MM) on a scale from 0 to 5 (microscopic observation 10X), individual motility (IM), percentage of live (L) and normal (N) sperm by means of eosin-nigrosin stain (40X; Zeiss primo star, Jena, Germany), and sperm concentration (CON) using a photometer (Minitube SDMI®, Tiefenbach, Rin-Hunsrück, Germany).

For refrigeration, the ejaculates were diluted with a mixture of 20% egg yolk, 60% distilled water, and 20% triladyl (Minitube®), and prepared at $50 \times 10^6$ sperm per 0.25 mL. For semen cooling, temperature was gradually lowered through from 1 to 2 °C every 2 min, from 36 °C until reaching 5 °C, where it was stabilized and semen stored for 24 hours (Purdy *et al.*, 2010).

Quality evaluation of the refrigerated semen was conducted at 24, 48, 72 and 96 hours during the 11 weeks, in which IM, N and L were determined in the same manner as for fresh semen.

*Statistical analysis*

A completely randomized design was used, with each ram considered as an experimental unit. Analysis of variance was performed to determine the effects of treatment on the days and weeks evaluated. For the analysis of fresh and refrigerated semen, treatment was nested within animal. Mean values were compared by the method of least square means using SAS version 9.0 software (SAS Institute Inc., Cary, NC, USA; 2002). Statistical difference was considered for $p<0.05$.

## Results

*Evaluation of fresh semen*

No interaction effects were observed for the comparison of seminal characteristics, so results are presented as main effects. Inclusion of 20% *E. americana* in the diet did not affect ($p>0.05$) VOL, CON, MM, and N, but decreased ($p<0.05$) IM and L (Table 3). No changes among weeks were observed for any of the variables ($p>0.05$; Table 4).

*Evaluation of refrigerated semen*

During refrigeration, variables L and N were similar ($p>0.05$) among treatments; nevertheless, the AEA group presented better IM results ($p<0.05$; Table 5).

**Table 3.** Characteristics (mean ± SE) of fresh semen from rams subjected to two dietary treatments.

| Treatment | VOL (mL) | CON (x106) | MM (1 - 5) | IM (%) | N (%) | L (%) |
|---|---|---|---|---|---|---|
| AEA | 0.83 ± 0.06 | 4,592.1 ± 222.4 | 3.9 ± 0.03 | 86.2 ± 0.5[b] | 94.8 ± 0.36 | 88.8 ± 0.91[b] |
| SEA | 0.80 ± 0.07 | 4,795.4 ± 210.4 | 3.8 ± 0.06 | 88.1 ± 0.6[a] | 95.3 ± 0.37 | 91.1 ± 0.93[a] |

VOL: volume; CON: concentration, x$10^6$: million sperm; MM: mass motility; IM: individual motility; N: normal sperm and L: live sperm. AEA: Group fed 20% DM of *E. americana*. SEA: Control group. Different superscript letters ([a, b]) within columns indicate statistical difference ($p<0.05$).

**Table 4.** Seminal characteristics (mean ± SE) of fresh ejaculates per week.

| Week | VOL (mL) | CON (x$10^6$) | MM (1-5) | IM (%) | N (%) | L (%) |
|---|---|---|---|---|---|---|
| 1 | 0.16 ± 0.16 | 5,589.50 ± 222.42 | 4.21 ± 0.09 | 84.98 ± 1.40 | 95.80 ± 0.84 | 83.20 ± 2.14 |
| 2 | 0.77 ± 0.16 | 5,476.60 ± 222.42 | 4.04 ± 0.09 | 88.59 ±1.61 | 88.80 ± 0.84 | 83.70 ± 2.14 |
| 3 | 0.69 ± 0.17 | 5,805.18 ± 237.22 | 3.68 ± 0.09 | 87.40 ± 1.40 | 94.64 ± 0.89 | 86.64 ± 2.29 |
| 4 | 0.87 ± 0.16 | 5,726.00 ± 222.42 | 3.93 ± 0.09 | 87.70 ± 1.31 | 95.40 ± 0.84 | 91.90 ± 2.14 |
| 5 | 0.77 ± 0.16 | 4,206.10 ± 222.42 | 3.83 ± 0.09 | 84.50 ± 1.31 | 94.30 ± 0.84 | 87.10 ± 2.14 |
| 6 | 0.90 ± 0.16 | 4,034.10 ± 222.42 | 4.00 ± 0.09 | 88.00 ± 1.31 | 96.10 ± 0.84 | 91.40 ± 2.14 |
| 7 | 1.00 ± 0.16 | 4,349.40 ± 222.42 | 3.93 ± 0.09 | 88.10 ± 1.31 | 96.00 ± 0.84 | 94.50 ± 2.14 |
| 8 | 0.86 ± 0.16 | 4,337.70 ± 222.42 | 3.87 ± 0.09 | 88.50 ± 1.31 | 96.00 ± 0.84 | 90.10 ± 2.14 |
| 9 | 0.80 ± 0.16 | 4,345.90 ± 222.42 | 3.91 ± 0.09 | 87.70 ± 1.31 | 97.00 ± 0.84 | 95.30 ± 2.14 |
| 10 | 0.98 ± 0.16 | 4,018.60 ± 222.42 | 3.92 ± 0.09 | 85.00 ± 1.31 | 95.79 ± 0.89 | 93.24 ± 2.29 |
| 11 | 0.74 ± 0.16 | 3,742.90 ± 222.42 | 3.98 ± 0.09 | 88.40 ± 1.31 | 96.20 ± 0.84 | 92.40 ± 2.14 |

VOL: volume; CON: concentration, x$10^6$: million sperm; MM: mass motility; IM: individual motility; N: normal sperm and L: live sperm.

**Table 5.** Refrigerated semen traits (mean ± SE) of rams subjected to two dietary treatments.

| Treatment | IM (%) | L (%) | N (%) |
|---|---|---|---|
| AEA | 71.90 ± 4.37[a] | 88.40 ± 0.194 | 95.30 ± 0.77 |
| SEA | 65.78 ± 4.42[b] | 89.36 ± 0.197 | 95.15 ± 0.78 |

IM: individual motility; L: live sperm; N: normal sperm; AEA: Group fed 20% DM of *E. americana*; SEA: Control group. Different superscript letters (a, b) within columns indicate statistical difference ($p<0.05$).

During refrigeration, IM and L percentages remained stable until 72 h; however, they decreased after 96 h ($p<0.05$). Normality remained constant (Table 6).

**Table 6.** Seminal traits (mean ± SE) of rams during several refrigeration periods.

| Hours | IM (%) | N (%) | L(%) |
|---|---|---|---|
| 24 | 76.00 ± 6.18[a] | 96.40 ± 0.27 | 93.70 ± 1.08[a] |
| 48 | 71.28 ± 5.97[a] | 94.86 ± 0.26 | 91.95 ± 1.05[a] |
| 72 | 71.57 ± 6.65[a] | 94.95 ± 0.29 | 92.06 ± 1.17[a] |
| 96 | 56.50 ± 6.18[b] | 94.70 ± 0.27 | 77.80 ± 1.08[b] |

IM: individual motility; L: live sperm; N: normal sperm; AEA: Group fed 20% DM of *E. americana*; SEA: Control group. Different superscript letters (a, b) within columns indicate statistical difference ($p<0.05$).

## Discussion

Our results feeding *E. americana* as a source of natural antioxidants on sperm quality of rams showed that IM and V decreased in fresh semen, while IM improved in refrigerated semen. However, the values reached in fresh semen are within the acceptable range for sperm viability (Quintero *et al.*, 2016).

The cell membrane of ram sperm is rich in polyunsaturated fatty acids. This makes it susceptible to peroxidation (Ezazi *et al.*, 2019), which begins with ROS production as a natural result of metabolism (Kaeoket *et al.*, 2010; Allai *et al.*, 2018). Incorporation of antioxidants improves sperm function in terms of motility, membrane integrity, and reduced effects of ROS (Forouzanfar *et al.*, 2010; Sapanidou *et al.*, 2016).

Natural antioxidants can reduce oxidative stress and improve reproductive performance (Vizzardi *et al.*, 2021). Plants with antioxidant properties have been evaluated in animal diets; for example, rosemary seeds and leaves on seminal quality of rabbits (Attia *et al.*, 2017), and oregano oil plus fish oil upon antioxidant capacity and productive parameters in boar semen (Liu *et al.*, 2016). In rams, inclusion of 10% grape pomace for 74 days decreased ROS concentration and increased catalase and glutathione peroxidase, thus improving epididymal sperm quality (Zhao *et al.*, 2017). Al-Najar *et al.* (2022) supplemented the diet of Awasian sheep with marjoram leaves (*Origanum vulgare*) for 49 days, finding increased superoxide dismutase and glutathione peroxidase concentrations in seminal plasma, as well as increased LH, testosterone, and estrogens in blood serum. Ezazi *et al.* (2019) reported that the addition of sunflower oil with vitamin C to the diet of lambs over 84 days improved progressive motility and increased the proportion of spermatozoa with normal acrosomes, leading to higher fertility of inseminated ewes. Therefore, dietary inclusion of plants with antioxidant properties can improve ram fertility. In the present study, inclusion of 20% *E. americana* in the diet decreased individual motility of fresh semen and, in contrast, increased it in refrigerated semen. Most of these plants are rich in phenolic acids and flavonoids that can reach intra and extracellularly into the spermatozoon blocking hydroperoxide formation or scavenging free radicals (Córdoba *et al.*, 2009) helping to restore testicular cells and protecting sperm against oxidative factors (Al-Najar *et al.*, 2022).

Dietary inclusion of herbal extracts rich in carotenoids, flavonoids and phenolic compounds represents an alternative for semen cryopreservation (Embuscado, 2015). According to literature reports, rosemary (*Rosmarinus officinalis*), clove (*Syzgium aromaticum*), green tea (*Camellia sinensis*), and oregano (*Origanum vulgare*) can eliminate free radicals and improve total and progressive motility, as well as functionality of plasma membranes and fertility rate of frozen semen (Del Valle *et al.*, 2013; Baghshahi *et al.*, 2014; Abadjieva *et al.*, 2020). However, not all results are favorable. Motlagh *et al.* (2014) used a 1% soy-lecithin base with 0, 2, 4, 6 and 8% rosemary, reporting that treatments with 4 and 6% rosemary had higher total and progressive motility and membrane integrity, but the 8% group resulted in lower live sperm. Similarly, Vahedi *et al.* (2018) evaluated the effects of thyme (*Thymus vulgaris*) as a natural antioxidant for ram semen cryopreservation, adding 0, 2, 4, 8, 12, and 16 mL of thyme per dL of dilutant, finding better sperm viability with 4 and 8 mL dL-1, but these values decreased as the addition increased to 12 and 16 mL dL-1 of thyme.

Based on the mentioned reports, adding antioxidant-rich plants can have variable results, probably due to cell needs and antioxidant concentration in plants. Mata-Campuzano *et al.* (2014; 2015), working with rams, reported that increasing the level of inclusion of soy lecithin from 1.5 to 3.5% had a negative effect on sperm motility and mitochondrial activity, and combination with cysteamine is not recommended for freezing ram semen.

Enzymatic antioxidants perform an important function during the conservation process and the relationship can be positive or negative either

because the enzymes are used in excess or they do not have the capacity to maintain sperm quality (Kasimanickam *et al.*, 2006; Bucak *et al.*, 2008; Marti *et al.*, 2008). In this regard, inclusion of 50, 100 and 200 µg/mL of catalase in the diluents helps reducing cell membrane damage during conservation at 5 °C, but catalase is toxic for ovine semen at concentrations higher than 200 µg/mL (Câmara *et al.*, 2011; Allai *et al.*, 2018).

Inclusion of 20% *E. americana* implies a high concentation of phenolic acids and flavonoids as sources of natural antioxidants that should be further studied given that they can have detrimental effects on seminal quality during the conservation process. Additionally, although it has not been determined, there may be effects from other compounds of the same plant.

In conclusion, supplementation with *E. americana* in the diet of rams reduces sperm quality in fresh semen; however, it helps in the cooling process of refrigerated semen, so it should be considered in future studies.

## Declarations

*Acknowledgements*

The authors would like to thank the people at Consejo Nacional de Humanidades Ciencia y Tecnologia (CONAHCyT) for supporting the *Programa de Estancias Posdoctorales in Mexico*, and their scientific and technological research programs to benefit society.

*Funding*

This study was entirely funded by the authors.

*Conflicts of interest*

The authors declare they have no conflicts of interest regarding the work presented in this report.

*Author contributions*

Rafael Nieto-Aquino: analysis, synthesis and writing; Eleazar Altamirano-Mijangos: planning, writing and experimental work; Teodulo Salinas-Rios: design, planning, financing and writing review; Héctor Maximino Rodríguez-Magadán: support in laboratory analysis, planning and writing review; Said Cadena-Villegas: planning and review of the development of the experiment; Cuahutémoc Nava-Cuellar: Support in laboratory analysis and planning; Jorge Hernández-Bautista: planning and logistical support.

*Use of artificial intelligence (AI)*

No AI or AI-assisted technologies were used during the preparation of this work.

## References

Abadjieva D, Yotov S, Mladenova V, Lauberte L, Kalvanov I, Krasilnikova J, Telesheva G, Kistanova E. Positive effect of natural antioxidant oregonin from *Alnus incana* bark on ram semen quality stored at 5 °C for 48 h. Res Vet Sci 2020; 131: 153–158.https://doi.org/10.1016/j.rvsc.2020.04.021

Abdi-Benemar H, Khalili B, Zamiri MJ, Ezazi H, Sotoudeh, AG, Hamidzadeh S, Simanoorf N. Effects of astaxanthin supplementation on the freezability, lipid peroxidation, antioxidant enzyme activities and post-thawing fertility of ram semen. Small Rum Res 2020; 192:106213. https://doi.org/10.1016/j.smallrumres.2020.106213

Allai L, Benmoula A, Maia MD, Nasser B, Amiri BE. Supplementation of ram semen extender to improve seminal quality and fertility rate. Anim Reprod Sci 2018; 192:6–17. https://doi.org/10.1016/j.anireprosci.2018.03.019

Al-Najar EM, Abdullah AM, Al-Rubaye TA, Hadi, SM. Evaluation of marjoram leaves (*Oregano vulgar*) as feed supplement on quality of semen in Awasian Pollination rams. Arch Razi Inst 2022; 77(5):1831–1835. https://doi.org/10.22092/ari.2022.358366.2211

Association of Official Analytical Chemists (AOAC). Official methods of analysis. Association Official Analytical Chemists. 20th Edition. Arlington, VA. 2017.

Attia Y, Hamed R, Bovera F. Semen quality, antioxidant status and reproductive performance of rabbit's bucks fed milk thistle seeds and rosemary leaves. Anim Repro Scie 2017; 184:178–186. https://doi.org/10.1016/j.anireprosci.2017.07.014

Baghshahi H, Riasi A, Mahdavi AH, Shirazi A. Antioxidant effects of clove bud (*Syzygium aromaticum*) extract used with different extenders on ram spermatozoa during cryopreservation. Cryobiology 2014; 69:482–487. https://doi.org/10.1016/j.cryobiol.2014.10.009

Bucak MN, Ateşşahin A, Yüce A. Effect of anti-oxidants and oxidative stress parameters on ram semen after the freeze–thawing process. Small Rumin Res 2008; 75:128–134. https://doi.org/10.1016/j.smallrumres.2007.09.002

Cai L, Li YP, Wei ZX, Li XL, Jian XR. Effects of dietary gallic acid on growth performance, diarrhea incidence, intestinal morphology, plasma antioxidant indices, and immune response in weaned piglets. Anim Feed Sci Tec 2020; 261:114391. https://doi.org/10.1016/j.anifeedsci.2020.114391

Câmara DR, Mello-Pinto MMC, Pinto LC, Brasil OO, Nunes JF, Guerra MMP. Effects of reduced glutathione and catalase on the kinematics and membrane functionality of sperm during liquid storage of ram semen. Small Rumin Res 2011; 100:44–49. https://doi.org/10.1016/j.smallrumres.2011.05.010

CIOMS (Council for international Organizations of Medical Sciences). "International Guiding Principles for Biomedical Research Involving Animals". 2016. Geneva, Switzerland.

Córdova IA, Lang CR, Córdova C, Córdova M, Guerra J, Rodríguez B, Arancibia K. Estrés oxidativo y antioxidantes en la conservación espermática. Rev Comp Cien Vet 2009; 3(1): 01–38.

Davendra K, Krishnappa B. Recent advances in cryopreservation of semen and insemination in sheep: A review. Indian J Small Rumin 2019; 25(2):134–137. https://doi.org/10.5958/0973-9718.2019.00043.6

Del Valle I, Souter A, Maxwell WM, Muiño-Blanco T, Cebrián-Pérez JA. Function of ram spermatozoa frozen in diluents supplemented with casein and vegetable oils. Anim Rep Sci 2013; 138:213–219. https://doi.org/10.1016/j.anireprosci.2013.02.022

DOF (Diario Oficial de la Federación). "Norma Oficial Mexicana NOM-062-ZOO-1999: Especificaciones técnicas para la producción, cuidado y uso de animales de laboratorio". 2001. México, D.F.

Embuscado ME. Spices and herbs: Natural sources of antioxidants–a mini review. J Fun Foods 2015; 18:811–819. https://doi.org/10.1016/j.jff.2015.03.005

Ezazi H, Abdi-Benemar H, Taghizadeh A, Khalili B, Seifdavati J, Jafaroghli M, Elghandour M, Salem AZ. The influence of dietary sunflower oil, rich in n-6 polyunsaturated fatty acids, in combination with vitamin C on ram semen parameters, sperm lipids and fertility. J Sci Food Agric 2019; 99(8): 3803–3810. https://doi.org/10.1002/jsfa.9602

Forouzanfar M, Sharafi M, Hosseini SM, Ostadhosseini S, Hajian M, Hosseini L, Abedi P, Nili N, Rahmani HR, Nasr-Esfahani MH. *In vitro* comparison of egg yolk-based and soybean lecithin-based extenders for cryopreservation of ram semen. Theriogenology 2010; 73:480–487. https://doi.org/10.1016/j.theriogenology.2009.10.005

García, E. Modificaciones al Sistema de Clasificación Climática de Köppen (para adaptarlo a las condiciones de la República Mexicana) 5° ed. México: Instituto de Geografía, Universidad Nacional Autónoma de México (UNAM); 2004. p. 50–56.

García-Mateos R, Soto-Hernández M, Vibrans H. *Erythrina americana* Miller "Colorín": Fabaceae, a versatile resource from Mexico: A review. Economic Botany 2001; 55(3):391–400. https://doi.org/10.1007/BF02866562

Ibarra EE, Pacheco SM, García MR, San Miguel CR, Ramírez VG, Soto HR. Actividad antioxidante de alcaloides de *Erythrina americana* Miller. Rev Fit Mex 2011; 34(4):241–246. https://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0187-73802011000400005

Kaeoket K, Sang-urai P, Thamniyom A, Chanapiwat P, Techakumphu, M. Effect of docosahexaenoic acid on quality of cryopreserved boar semen in different breeds. Reprod Dom Anim 2010; 45: 458–463. https://doi.org/10.1111/j.1439-0531.2008.01239.x

Kasimanickam R, Pelzer KD, Kasimanickam V, Swecker WS, Thatcher CD. Association of classical semen parameters, sperm DNA fragmentation index, lipid peroxidation and antioxidant enzymatic activity of semen in ram-lambs. Theriogenology 2006; 65:1407–1421. https://doi.org/10.1016/j.theriogenology.2005.05.056

Loizzo MR, y Tundis R. Plant antioxidant for application in food and nutraceutical industries. antioxidants (Basel, Switzerland) 2019; 8(10):453. https://doi.org/10.3390/antiox8100453

Liu Q, Duan RJ, Zhou YF, Wei HK, Peng J, Li JL. Supplementing oregano essential oil to boar diet with strengthened fish oil: Effects on semen antioxidant status and semen quality parameters. Andrologia 2016; 49(10):1–8. https://doi.org/10.1111/and.12764

Marti E, Marti JI, Muiño-Blanco T, Cebrián-Pérez JA. Effect of the cryopreservation process on the activity and immunolocalization of antioxidant enzymes in ram spermatozoa. J. Androl 2008; 29:459–467. https://doi.10.2164/jandrol.107.003459

Mata-Campuzano M, Álvarez-Rodríguez M, Tamayo-Canul J, López-Urueña E, de Paz P, Anel L, Martínez-Pastor F, Álvarez M. Refrigerated storage of ram sperm in presence of Trolox and GSH antioxidants: Effect of temperature, extender and storage time. Anim Reprod Sci 2014; 151:137–147. https://doi.org/10.1016/j.anireprosci.2014.10.006

Mata-Campuzano M, Soleilhavoup C, Tsikis G, Martinez-Pastor F, de Graaf SP, Druart X. Motility of liquid stored ram spermatozoa is altered by dilution rate independent of seminal plasma concentration. Anim Reprod Sci 2015; 162:31–36. https://doi: 10.1016/j.anireprosci.2015.09.004

Motlagh MK, Sharafi M, Zhandi M, Mohammadi-Sangcheshmeh A, Shakeri M, Soleimani M, Zeinoaldini S. Antioxidant effect of rosemary (*Rosmarinus officinalis* L.) extract in soybean lecithin-based semen extender following freeze-thawing process of ram sperm. Cryobiology 2014; 69:217–222. https://doi.org/10.1016/j.cryobiol.2014.07.007

Pino RS, Prieto G, Pérez M, Molina, TJ. Género *Erythrina*: Fuente de metabolitos secundarios con actividad biológica. Acta Farm Bonaer 2004; 23(2):252–258.

Purdy PH, Stobart EM, Murdoch WJ, Moss GE, Larson B, Ramsey S, Graham JK, Blackburn HD. The fertility of ram sperm held for 24h at 5°C prior to cryopreservation. Anim Repro Sci 2010; 118(2):231–235. https://doi.org/10.1016/j.anireprosci.2009.06.014

Quintero EJ, Sánchez FC, Olguín AH. Contribución en el desarrollo de un índice de calidad del semen para la valoración de sementales ovinos. Veterinaria 2016; 58(1):105–109. https://erevistas.uacj.mx/ojs/index.php/culcyt/article/view/1395

Salamon S, Maxwell WMC. Storage of ram semen. Anim Reprod Sci 2000; 62:77–111. https://doi.org/10.1016/S0378-4320(00)00155-X

Rehman F, Zhao C, Shah MA, Qureshi MS, Wang X. Semen extenders and artificial insemination in Ruminants. Veterinaria 2013; 1:1–8.

Russel AJF, Doney JM, Gunn RG. Subjective assessment of fat in live sheep. J Agricult Sci 1969; 72: 451–454.

Sapanidou V, Taitzoglou I, Tsakmakidis I, Kourtzelis I, Fletouris D, Theodoridis A, Lavrentiadou S, Tsantarliotou M. Protective effect of crocetin on bovine spermatozoa against oxidative stress during *in vitro* fertilization. Andrology 2016; 4:1138–1149. https://doi.org/10.1111/andr.12248

Statistical Analysis System Institute (SAS). SAS User's Guide: Statistics (Version 5). Cary, N.C. USA. 2002. Inst. Inc. 584 pp.

Vahedi V, Hedayat EN, Behroozlak M, Dirandeh E. Antioxidant effects of thyme (*Thymus vulgaris*) extract on ram sperm quality during cryopreservation. Iran Jour Appl Anim Sci 2018; 8(2):263–269. https://ijas.rasht.iau.ir/article_540373.html

Vizzari F, Massányi M, Knízatová N, Corino C, Rossi R, Ondruška L, Tirpák F, Halo M, Massányi P. Effects of dietary plant polyphenols and seaweed extract mixture on male-rabbit semen: Quality traits and antioxidant markers. Saudi Jour Biol Sci 2021; 28(1):1017–1025. https://doi.org/10.1016/j.sjbs.2020.11.043

Zhao J, Jin Y, Du M, Liu W, Ren Y, Zhang C, Zhang J. The effect of dietary grape pomace supplementation on epididymal sperm quality and testicular antioxidant ability in ram lambs, Theriogenology 2017; 97:50–56. https://doi.org/10.1016/j.theriogenology.2017.04.010