Ensaios e Ciência
ISSN 1415-6938 e ISSN 2178-695X

DOI: 10.17921/1415-6938.2025v29n3p762-772

Antimicrobial Activity of Liposome-Entrapped Ozonated Sunflower Oil Against *Staphylococcus aureus* and *Pseudomonas aeruginosa* Isolated from Mares with Endometritis

Atividade Antimicrobiana do Óleo de Girassol Ozonizado Encapsulado em Lipossomas Contra Staphylococcus aureus e Pseudomonas aeruginosa Isolados de Éguas com Endometrite

Received: February 24, 2025
Accepted: August 18, 2025

Gabriel Souza dos Santos: Faculdade Anhanguera de Ipatinga. MG, Brazil.

Arthur Azevedo Perpétuo: Faculdade Anhanguera de Ipatinga. MG, Brazil.

Juliane Zacour Marinho: Universidade Estadual de Campinas. SP, Brazil.

Murillo Néia Thomaz da Silva: Universidade Federal de Uberlândia. MG, Brazil.

Geovane Marcos Guimarães de Souza: Hospital Sírio-Libanês, Brasilia, DF, Brazil.

Marcus Vinícius Dias-Souza: Faculdade Anhanguera de Ipatinga. MG, Brazil. E-mail: souzamvd@gmail.com

Abstract

Endometritis is a frequent cause of low fertility in mares dure to infection by bacterial pathogens in the endometrium. A critical issue in treating mares is the bacterial resistance of antimicrobial drugs. Natural products such as sunflower oil are of interest for the development of new antimicrobials, given that resistance to phytomolecules is rare, mostly due to the structural complexity of the molecules and their varied mechanisms of action. Liposomes are nanovesicles made of phospholipids that represent a strategic resource in drug therapy. They provide controlled release of entrapped substances and make it possible to used drugs in lower doses compared to non-entrapped counterparts. The aim of this study was to evaluate the antimicrobial activity of liposome-entrapped ozonated sunflower oil against 10 strains of *Staphylococcus aureus* and of *Pseudomonas aeruginosa* isolated from mares with endometritis, and to partially characterize them regarding size and loading capacity. Liposomes were produced by extrusion in PVDF membranes and partially characterized concerning size and loading capacity by spectrophotometry and by the Bligh-Dyer method, respectively. We performed minimal inhibitory concentration and minimal bactericidal concentration tests, as well as scanning electron microscopy. The vesicles presented an estimated size of 239.34 nm, an estimated loading capacity of 43.28%, and a MIC of 4 ug/mL for both species. The MBC was lower for *S. aureus* isolates. Liposome-entrapped ozonated sunflower oil was effective as antimicrobial, supporting the relevance of

liposome-based delivery for optimized pharmacological treatments.
Keywords: Liposomes. Sunflower oil. Ozonation. Endometritis. Mares.

Resumo

A endometrite é uma causa frequente de baixa fertilidade em éguas devido à infecção por patógenos bacterianos no endométrio. Um problema crítico no tratamento de éguas é a resistência bacteriana aos antimicrobianos. Produtos naturais como o óleo de girassol são de interesse para o desenvolvimento de novos antimicrobianos, posto que a resistência a fitomoléculas é rara, devido à complexidade estrutural e aos variados mecanismos de ação. Lipossomas são nanovesículas compostas por fosfolipídios que representam um recurso estratégico na farmacoterapia. Estas vesículas permitem a liberação controlada das substâncias e possibilitam o uso em doses menores que as formas não encapsuladas. Este estudo avaliou a atividade antimicrobiana do óleo de girassol ozonizado encapsulado em lipossomas contra 10 cepas de *Staphylococcus aureus* e de *Pseudomonas aeruginosa* isoladas de éguas com endometrite, além de caracterizá-los parcialmente quanto ao tamanho e capacidade de encapsulação. Os lipossomas foram produzidos por extrusão em membranas de PVDF e caracterizados parcialmente em relação ao tamanho e capacidade de carga por espectrofotometria e pelo método de Bligh-Dyer, respectivamente. Foram realizados testes de concentração inibitória mínima (CIM) e concentração bactericida mínima (CBM), além de microscopia eletrônica de varredura. As vesículas apresentaram um tamanho estimado de 239,34 nm e uma capacidade de carga estimada de 43,28%. Os lipossomas apresentaram CIM de 4 µg/mL para ambas as espécies, com valor de CBM menor para *S. aureus*. O óleo de girassol ozonizado encapsulado em lipossomas apresentou atividade antimicrobiana satisfatória, reforçando a relevância de substâncias encapsuladas em lipossomas para tratamentos farmacológicos otimizados.

Palavras-chave: Lipossomas. Óleo de girassol. Ozonização. Endometrite. Éguas.

1 Introduction

In reproductive veterinary medicine of large animals, especially mares and cows, low fertility is a complex health issue that, in most situations, leads to animal slaughter and several financial losses (Almeida *et al.*, 2025; Flores *et al.*, 2023). Among the clinical reasons for low fertility is endometritis, an uterine infectious disease caused by different bacterial pathogens in the endometrium, including *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus zooepidemicus*, followed by inflammatory process (Morrell; Rocha, 2022; Mouncey *et al.*, 2024). The disease is essentially polymicrobial, and yeasts might be also present in some animals (Nielsen *et al.*, 2021).

Concerning the breeding of mares, part of the available studies describe the general prevalence of endometritis ranging from 25 to 60%, based on data from the 1990 decade (Pycock; Newcombe, 1996; Traub-Dargatz *et al.*, 1991; Zent; Troedsson, 1998). More recent studies have found alarming rates of bacterial pathogens in the mares' uterus. A study in Italy conducted with 394 animal samples found 58% of infected uterus (Ravaioli *et al.*, 2022), and a study in Spain found 89% of infections in 363 animal samples (Díaz-Bertrana *et al.*, 2021). The treatment of the disease is dependent on the use of antimicrobials and good animal's husbandry practices, and the recent data on the prevalence of the disease and of bacterial resistance in equines indicates the need for new treatments (Kabir *et al.*, 2024).

Natural products are of interest for the development of new options to treat infectious diseases, as resistance to phytomolecules is rare. Sunflower oil is largely used in human and veterinary medicine for its wound healing and antimicrobial properties (Oliveira *et al.*, 2012; Silva *et al.*, 2021), however, studies with phytotherapics, especially fixed oils, in veterinary medicine context, are scarce. Although sunflower oil is routinely used in equine reproductive medicine (Ferreira *et al.*, 2021), proper evidence to support this practice is scarce.

Scientifically valid studies on the antimicrobial activity of sunflower oil against bacteria associated to endometritis in mares are relatively recent. A study of our group (Dos Santos *et al.*, 2023) alongside other works (Donato *et al.*, 2024; Smith *et al.*, 2023), provided contributions to this observation. However, the required effective concentrations of the oil for some microbial species can be somewhat high for natural products intended for clinical use. Therefore, we hypothesized that the use of nanotechnologies could improve the oil biological potentials by increasing its potency, as it happens for drugs.

Here we explored liposome-entrapped ozonated sunflower oil (LEOSO), against bacterial strains of *S. aureus* and *P. aeruginosa* isolated from mares with endometritis. Liposomes are phospholipid-based nanovesicles that have been used in drug delivery in human medicine (Gomes *et al.*, 2022) but are not as explored in veterinary medicine. We explored liposomes to increase the oil potency and overcome experimental limitations such as the poor water solubility. Given the scarcity of studies in this field and the clinical and economic significance of endometritis in mares, our work becomes even more relevant.

2 Material and Methods

2.1 Liposomes preparation

The steps of the liposomes preparation were carefully determined after sequential tests in different conditions, being partially based in a previous study of our group (Gomes *et al.*, 2022). When not under tests, the liposomes suspension was kept at 4 °C in sealed vials.

Liposomes were prepared in aseptic conditions using Phosal 75 SA (Lipoid GmbH, Germany – containing phosphatidylcholine in ethanol and safflower oil, content ≥ 72.0 %). The ozonated sunflower oil (purchased locally at a compounding pharmacy, presenting 91.13 mEq peroxides/Kg) was added to Phosal (1:1 w/w) with vigorous agitation until a uniform mixture was obtained. Next, we added sterile deionized water to the system (7:3 v/w) and stirred at maximum speed for 30 min at home temperature. The resulting suspension contained liposomes in different sizes, with an aqueous core.

The liposomes suspension was then transferred to scintillation vials to be exposed to five

ultrasound cycles (25 KHz, 20 minutes each, 1 min of interval between each cycle). Following, we proceeded to the liposomes extrusion in sterile 450 nm PVDF membranes for 10 times, and then in sterile 220 nm PVDF membranes for 10 times (both from Merk Milipore, U.S.A.). Samples were checked for visual alterations in turbidity, to check the efficiency of the downsizing process preliminarily.

2.2 Liposomes partial characterization

We used the method described by Niu *et al.* (2003) to estimate the size of liposomes. The liposomes suspension was diluted in sterile deionized water (1:5 v/v) and was analyzed in a spectrophotometer at 436 nm. Four repetitions were performed, and the average of the readings was calculated. Liposomes size was defined in μm using the formula:

Log (Abs 436nm / Cp) = (1.8086 x Log D) – 4.0986

In which Abs 436nm is the average of the readings at 436 nm, Cp is the concentration of phospholipids in the suspension (in mM), and D is the size of the vesicles (in nm). The values 1.8086 and −4.0986 represent the slope coefficient and the intercept of the linear regression that underpins the equation, respectively.

We estimated the loading capacity (LC) using the Bligh-Dyer method, with slight adaptations. The experiment was conducted in duplicate, using liposomes prepared with the oil, and empty liposomes, both as aqueous suspensions. Samples of each formulation were added to absolute ethanol and chloroform (1:2:2 v/v/v). The blend was stirred at maximum speed for 1 minute and was left for complete separation of the layers. The ethanol layer was carefully removed by aspiration, and the chloroform layer was left for air-drying in home temperature, under continuous air flow. Finally, the lipid mass obtained with each sample was weighed, and the total of entrapped oil was calculated as the difference between them. LC was then calculated as follows:

LC = (total of entrapped oil / mass of empty liposomes) x 100%

2.3 Minimal inhibitory concentration (MIC) assay

MIC was determined in triplicate using untreated sterile 96-well polystyrene microtiter plates, following the Clinical and Laboratory Standards Institute guidelines (CLSI, 2018). Prior to the tests, the liposomes were centrifuged (20.000g, 1h, room temperature) and the pellets were resuspended to produce a stock solution of 2 mg/mL.

The bacterial strains used in this study were from the microorganisms collection of Anhanguera College (Ipatinga, Minas Gerais, Brazil). They were isolated from intrauterine lavage fluids of mares with endometritis in preparation for fixed-time artificial insemination. We used 10 strains of *S. aureus*

and 10 isolates of *P. aeruginosa*. They were cultivated in sterile brain-heart infusion broth (BHI, Difco) at 35±2 °C overnight prior to the experiments (Dos Santos *et al.*, 2023).

Following the bacterial growth, fresh cultures for MIC assays were prepared in Mueller Hinton broth (Himedia) in 0.5 McFarland scale and diluted to reach the concentration of $5x10^5$ CFU/mL. A total of 100 μL of each culture was dispensed in each well. The wells were prepared with bacterial cultures and liposomes (100 μL), reaching concentrations from 1024 μg/mL to 4 μg/mL. Plates were then incubated at 37 °C overnight (CLSI, 2018).

After the incubation period, 50 μL of a 0.1% resazurine solution (blue color) was added to the wells (Dias-Souza *et al.*, 2017). MIC was established as the lowest concentration that resulted in no color modification (blue to pink) for all the strains (Dos Santos *et al.*, 2023). Empty liposomes were used as a negative control.

2.4 Minimum bactericidal concentration (MBC) assay

MBC was determined in triplicate using a method described by our group (Dias-Souza *et al.*, 2017), with slight modifications. A total of 30 μL of each well in which resazurine staining result was negative were spotted in Mueller-Hinton agar plates (Difco) spread- plated. The plates were incubated overnight at 37 °C and bacterial growth was observed. MBC was the lowest concentration that resulted in no bacterial growth in agar plates. Empty liposomes were used as a negative control.

2.5 Scanning electron microscopy (SEM)

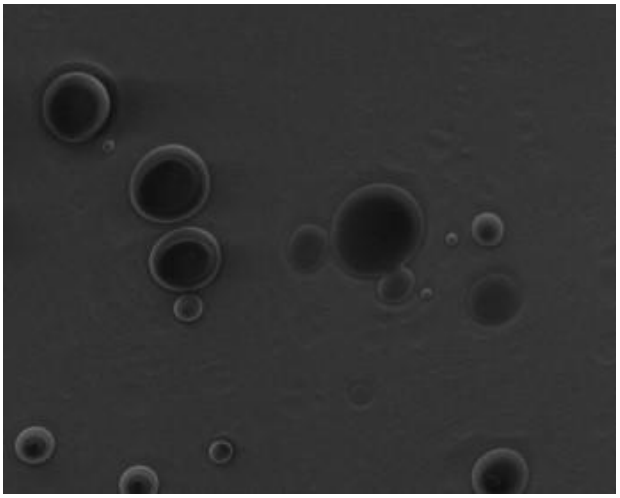
To obtain images of liposomes on the bacterial isolates, we used the liposomes at 1024 μg/mL and one of the *S. aureus* isolates at $5x10^5$ CFU/mL, prepared on sterile glass slides. After 2h incubation in room temperature, the slides were preserved with 5% formaldehyde at room temperature overnight. Following, the slides were coated with a gold layer under deep vacuum, placed over metallic supports and analyzed at 5 kV in a VEGA 3 LMU scanning electron microscope (Tescan, Czech Republic). Images were obtained using the software of the equipment, following the manufacturer's instructions.

3 Results and Discussion

3.1 Liposomes characteristics

The liposomes presented an estimated size of 239.34 nm, and an estimated loading capacity of 43.28%. However, as expected, some vesicles were not affected by the downsizing procedures, and the loading of the oil, which would be expected to happen at the membranes (and not the core, given the lipophilic nature of the oil) was also variable, as observed by SEM (Figure 1).

Figure 1 - SEM image of liposomes with variable size and loading efficiency



Source: the authors.

3.2 Antimicrobial activity of liposome-entrapped ozonated sunflower oil (LEOSO)

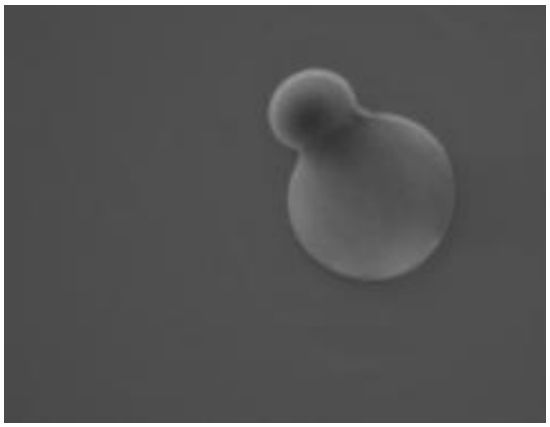
LEOSO were effective against the bacterial strains at low concentrations (Table 1). MBC for *P. aeruginosa* strains was higher than the one observed for *S. aureus*. Given the difference observed in MIC and MBC values, LEOSO presented a bacteriostatic profile. Interestingly, we could detect in SEM a liposome in partial fusion with a microbial cell in one of the slides (Figure 2).

Table 1 - Antimicrobial activity of LEOSO

Species	MIC	MBC
S. aureus	4 µg/mL	16 µg/mL
P. aeruginosa	4 µg/mL	32 µg/mL

Source: research data.

Figure 2 - SEM image of liposome in contact with a bacterial cell of *S. aureus*



Source: the authors.

This study provides evidence on the antimicrobial activity of the ozonated sunflower oil when encapsulated in liposomes against bacterial strains isolated from mares with endometritis. Although our data is based on an *in vitro* model and the liposomes characterization was partial, this study still opens doors for other initiatives in exploring liposomes for infectious diseases in the context of veterinary medicine. Different benefits can be considered for animal health, and the main one is the possibility of using medication in lower doses compared to non-liposomal formulations (Almeida *et al.*, 2020; Liu *et al.*, 2022). Thus, a decreased risk of dose-related adverse reactions is likely (Al-Awsi *et al.*, 2023).

Liposomes represent an interesting nanotechnology for controlled drug delivery, and its versatility allows entrapping both lipophilic and hydrophilic substances in its membranes and core, respectively (Lombardo; Kiselev, 2022; Nsairat *et al.*, 2022). Few liposomal formulations are available for veterinary use worldwide, especially for infectious diseases. Beyond the elevated costs of the reagents, most techniques require using organic solvents in the preparation steps, and their removal is not always complete, what might impair the clinical use of the product (Lombardo; Kiselev, 2022).

To overcome this limitation, we explored Phosal, a pre-liposome product composed of phosphatidylcholine from soybeans, safflower seed oil, mono- and diglycerides, ascorbyl palmitate and tocopherol. Phosal does not require the use of organic solvents: we only added ozonated sunflower oil and water for the preparation of LEOSO, and according to the manufacturer, the only solvent used in the manufacturing of Phosal is ethanol. Phosal can be used as a food additive , indicating its safety for clinical purposes (Lippoid, 2024).

Previous studies of our group have provided evidence on the antimicrobial activity of sunflower oil – both ozonated and conventional forms. When the oils were tested against *S. aureus* and *E. coli* strains isolated from mares with endometritis, the conventional oil was not effective against both of them, whereas the ozonated form was effective against *S. aureus* at 512 μg/mL (Dos Santos *et al.*, 2023). Conversely, when tested against *P. aeruginosa* (also from mares with endometritis), the ozonated oil was not effective, and the conventional oil was effective (Perpétuo *et al.*, accepted for publication). Here, the MIC values of LEOSO were considerably lower than the non-liposomal forms of our previous studies (Table 2), and this is highly related to the remarkable ability of interaction with prokaryotic and eukaryotic membranes that liposomes present. The chemical similarity of phospholipids in cellular and liposome membranes makes it possible, as shown in figure 2, that a fusion takes place, and the active substances entrapped in it can be then released. A bursting effect (i.e., vesicle disruption) can also happen, and in some contexts, it may hamper pharmacotherapy (Billah *et al.*, 2024).

Table 2 - Comparison of the antimicrobial activity of free and liposome-entrapped ozonated sunflower oil

Tested Item	Pathogen	MIC	Source
LEOSO	*S. aureus*	4 μg/mL	Present study
Free ozonated oil	*S. aureus*	512 μg/mL	Dos Santos *et al.* (2023)
LEOSO	*P. aeruginosa*	4 μg/mL	Present study
Free ozonated oil	*P. aeruginosa*	Not active	Perpétuo *et al.* (2025)[Δ]
Free ozonated oil	*E. coli*	Not active	Dos Santos *et al.* (2023)

LEOSO: liposome-entrapped ozonated sunflower oil. [Δ]Accepted for publication in 2025.
Source: research data.

Our results are consistent with the observation of others that nanotechnological systems involving lipophilic phytoextracts can be effective against microorganisms. However, most of the studies considered essential oils, whereas fixed oils are poorly explored. An industrialized eye drops formulation of liposomal ozonated sunflower oil combined to hypromellose was successfully used to treat conjunctivitis in animals (horse, cat and dog) and humans, being more effective than iodine to *in vivo* control bacterial growth (Grassi *et al.*, 2024; Spadea *et al.*, 2018). This formulation was also effective to prevent biofilm formation of *S. aureus* and *P. aeruginosa* strains (Zerillo *et al.,* 2022). Lemongrass essential oil was entrapped in liposomes prepared in different combinations of chitosan, pectin, gum arabic and carrageenan. The vesicles were effective bacteriostatic systems and were explored as antimicrobial food additives (Gan *et al.*, 2024).

The chemical composition of the technology used in nanoencapsulation is critical for its effects. A recent study compared the encapsulation of the essential oil of *Lippia origanoides* combined with curcumin in distearoyl-glycero-phosphocholine (DSPC) and egg yolk phosphatidylcholine (EYPC). EYPC liposomes presented higher entrapment efficiency and were effective against *S. aureus* ATCC 25923 (Bedoya-Agudelo *et al.*, 2024). Another study compared the encapsulation of thyme essential oil in β and γ-cyclodextrin. The latter presented higher encapsulation efficiency and antioxidant activity, being more effective against *Listeria monocytogenes* and *Salmonella enterica* sv. Typhimurium (Ahmed *et al.*, 2022).

4 Conclusion

This study highlights the potential of liposome-entrapped ozonated sunflower oil as an effective antimicrobial against bacterial strains relevant to veterinary medicine, particularly in cases of endometritis in mares. LEOSO offers a promising approach for safer and more efficient therapeutic applications, following the advantages of liposomal encapsulation such as enhancing interaction with microbial membranes and reducing effective doses. The use of solvent-free liposome preparations is relevant concerning large-scale production for clinical use. Nevertheless, our study is preliminary and not without limitations. To proceed for *in vivo* testing of LEOSO, it is necessary to determine more

accurately the size, entrapment and loading efficiencies of the liposomes, as well as conduct stability studies under different temperature and storage conditions. Other relevant bacterial pathogens may also be considered in future tests. Such limitations, however, do not impair our measuring of the pharmacological properties of the liposomes developed by our group.

References

AHMED, J. *et al.* Thermal, structural, antimicrobial, and physicochemical characterisation of thyme essential oil encapsulated in β- and γ-cyclodextrin. *J. Microencapsul.*, v.39, n.4, p.364-379, 2022. doi: https://doi.org/10.1080/02652048.2022.2090626

AL-AWSI, G.R.L. *et al.* Application of nano-antibiotics in the diagnosis and treatment of infectious diseases. *Braz. J. Biol.*, v.84, e264946, 2023. doi: https://doi.org/10.1590/1519-6984.264946.

ALMEIDA, B. *et al.* Recent Progress in Bioconjugation Strategies for Liposome-Mediated Drug Delivery. *Molecules*, v.25, n.23, p.5672, 2020. doi: https://doi.org/10.3390/molecules25235672.

ALMEIDA, R.G.H. *et al.* Revisão Sistemática de Septicemia Neonatal Equina: Aspectos Microbianos e Terapêuticos. *Ens. Ciênc.*, v.26, n.4, p.390-397, 2023. doi: https://doi.org/10.17921/1415-6938.2022v26n4p390-397.

BEDOYA-AGUDELO, J.P. *et al.* Assessment of Antimicrobial and Cytotoxic Activities of Liposomes Loaded with Curcumin and *Lippia origanoides* Essential Oil. *Biomolecules*, v.14, n.7, p.851, 2024. doi: https://doi.org/10.3390/biom14070851.

BILLAH, M.M. *et al.* Processes and mechanisms underlying burst of giant unilamellar vesicles induced by antimicrobial peptides and compounds. Biochimica et Biophysica Acta (BBA) - *Biomembranes*, v.1866, n.5, p.184330, 2024. doi: https://doi.org/10.1016/j.bbamem.2024.184330.

BLIGH, E.G.; DYER, W.J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.*, v.37, n.8, p.911-917, 1959. doi: https://doi.org/10.1139/o59-099.

CLSI - Clinical and Laboratory Standards Institute -. Performance standards for antimicrobial susceptibility testing; 27th informational supplement. M100-S28. Clinical and Laboratory Standards Institute, Wayne, PA, 2018.

DIAS-SOUZA, M.V. *et al.* Antibiofilm activity of cashew juice pulp against *Staphylococcus aureus*, high performance liquid chromatography/diode array detection and gas chromatography-mass spectrometry analyses, and interference on antimicrobial drugs. *J. Food Drug Anal.*, v.25, n.3, p.1-10, 2017. doi: https://doi.org/10.1016/j.jfda.2016.07.009

DÍAZ-BERTRANA, M.L. *et al.* Microbial Prevalence and Antimicrobial Sensitivity in Equine Endometritis in Field Conditions. *Animals*, v.11, n.5, p.1476, 2021. doi: https://doi.org/10.3390/ani11051476

DONATO, G.G. *et al.* In vitro effects of different ozone preparations on microorganisms responsible for endometritis in the mare. *Theriogenology,* v.219, p.132–137, 2024. doi: https://doi.org/10.1016/j.theriogenology.2024.02.011

DOS SANTOS, G.S. *et al.* Susceptibility of bacterial species isolated from mares to ozonated sunflower oil. *Int. J. Secon. Metab.*, v.10, n.1, p.119-123, 2023. doi: https://doi.org/10.1016/10.21448/ijsm.1167867

FERREIRA, J.C. *et al.* Ozonioterapia no tratamento auxiliar de éguas inférteis: potencial angiogênico e antimicrobiano. *Rev. Bras. Reprod. Anim.*, v.45, n.4, p.482-488, 2021.

FLORES, A.M. *et al.* Transferência de embriões em equinos: receptoras acíclicas. *Ens. Ciênc.*, v.26, n.4, p.402-406, 2023. doi: https://doi.org/10.1016/10.17921/1415-6938.2022v26n4p402-406

GAN, N. *et al.* Encapsulation of lemongrass essential oil by bilayer liposomes based on pectin, gum Arabic, and carrageenan: Characterization and application in chicken meat preservation. *Int. J. Biol. Macromol.*, v.281, p.135706, 2024. doi: https://doi.org/10.1016/j.ijbiomac.2024.135706

GOMES, A.S. *et al.* Effectiveness of free and liposome-entrapped antitumoral drugs against hepatocellular carcinoma: A comparative in vitro study. *Biointerface Res. Appl. Chem.*, v.13, n.2, p.122-130, 2022. doi: https://doi.org/10.33263/BRIAC132.122

GRASSI, M.O. *et al.* Liposomal ozonated oil ensures a further reduction in the microbial load before intravitreal injection: the "OPERA" Study. *Ophthalmol. Ther.*, v.13, n.10, p.2771-2788, 2024. doi: https://doi.org/10.1007/s40123-024-01006-w

KABIR, A. *et al.* Antimicrobial resistance in equines: a growing threat to horse health and Beyond-a comprehensive review. *Antibiotics*, v.13, n.8, p.713, 2024. doi: https://doi.org/10.3390/antibiotics13080713

LIPPOID. Phosal. *Lipoid.* Disponível em: https://lipoid.com/en/products/phospholipid-systems/phosal/. Acesso em: 20 dez. 2024.

LIU, P. *et al.* A review of Liposomes as a drug delivery system: current status of approved products, regulatory environments, and future perspectives. *Molecules*, v.27, n.4, p.1372, 2022. doi: https://doi.org/10.3390/molecules27041372

LOMBARDO, D.; KISELEV, M.A. Methods of Liposomes Preparation: formation and control factors of versatile nanocarriers for biomedical and nanomedicine application. *Pharmaceutics*, v. 14, n. 3, p. 543, 2022. doi: https://doi.org/10.3390/pharmaceutics14030543

MORRELL, J.M.; ROCHA, A. A Novel Approach to Minimising Acute Equine Endometritis That May Help Prevent the Development of the Chronic State. *Front. Vet. Sci.*, v. 8, p.799619, 2022. doi: https://doi.org/10.3389/fvets.2021.799619

MOUNCEY, R. *et al.* Prevalence of Microbial Isolates Cultured from Endometrial Swab Samples Collected from United Kingdom Thoroughbred Mares from 2014 to 2020. *Vet. Sci.*, v.11, n.2, p.82, 2024. doi: https://doi.org/10.3390/vetsci11020082

NIELSEN, J.M. *et al.* Impact of Yeast Endometritis on Fertility in the Mare. *J. Equine Vet. Sci.*, v.99, p.103409, 2021. doi: https://doi.org/10.1016/j.jevs.2021.103409

NIU, G.Q. *et al.* Spectrophotometry on measuring the size of liposomes. *Yao Xue Xue Bao = Acta Pharm. Sinica*, v.38, n.7, p.547–551, 2003.

NSAIRAT, H. *et al.* Liposomes: structure, composition, types, and clinical applications. *Heliyon*, v.8, n.5, p. e09394, 2022. doi: https://doi.org/10.1016/j.heliyon.2022.e09394

OLIVEIRA, L.A. *et al.* Effects of Topical Application of Sunflower-Seed Oil on Experimentally Induced Wounds in Horses. *J. Equine Vet. Sci.*, v.32, p.139-145, 2012. doi: https://doi.org/10.1016/j.jevs.2011.08.006

PERPÉTUO, A.A.; DOS SANTOS, G.S.; DIAS-SOUZA, M.V. Antimicrobial activity of ozonated sunflower oil against *Pseudomonas aeruginosa* strains isolated from mares, and interactions with antimicrobial drugs. *Int. J.f Secondary Metabol.*, v.12, n.4, p.755-762, 2025. doi: https://doi.org/10.21448/ijsm.1606326

PYCOCK, J.F.; NEWCOMBE, J.R. Assessment of the effect of three treatments to remove intrauterine fluid on pregnancy rate in the mare. *Vet. Record*, v.138, n.14, p.320-323, 1996. doi: https://doi.org/10.1136/vr.138.14.320

RAVAIOLI, V. *et al.* Infectious Endometritis in Mares: microbiological findings in field Samples. *J. Equine Vet. Sci.*, v.112, p.103913, 2022. doi: https://doi.org/10.1016/j.jevs.2022.103913

SILVA, J.S. *et al.* Evaluation of antibacterial activity of sunflower oil: Support for nursing. *Res., Soc. Develop.*, v.10, n.9, p.e8710917941, 2021. doi: https://doi.org/10.33448/rsd-v10i9.17941

SMITH, J. *et al.* Advances in equine reproductive medicine. *Journal of Equine Veterinary Science*, v. 45, n. 3, p. 123–130, 2023. doi: https://doi.org/10.1016/j.jevs.2023.03.015

SPADEA, L. *et al.* Use of Ozone-Based Eye Drops: A Series of Cases in Veterinary and Human Spontaneous Ocular Pathologies. *Case Rep. Ophthalmol.*, v.9, n.2, p.287-298, 2018. doi: https://doi.org/10.1159/000488846

TRAUB-DARGATZ, J.L. *et al.* Medical problems of adult horses, as ranked by equine practitioners. *J. Am. Vet. Med. Assoc.*, v.198, n.10, p.1745-1747, 1991.

ZENT, W.; TROEDSSON, M. H. T. Postbreeding uterine fluid accumulation in a normal population of thoroughbred mares: a field study. *Proc. Am. Assoc. Equine Practitioners*, v.44, p.64-65, 1998.

ZERILLO, L. *et al.* Antibiofilm and repair activity of ozonated oil in liposome. Microbial Biotechnology, v.15, n.5, p.1422–1433, 2022. doi: https://doi.org/10.1111/1751-7915.13949