

Syzygium cumini extract in Tris-yolk extender improves post-thaw bovine semen quality

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[*Extrato de Syzygium cumini no diluidor Tris-gema melhora a qualidade do sêmen bovino pós-descongelção*]

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ABSTRACT

The objective was to evaluate the supplementation of jambolan extract (*Syzygium cumini*) to the Tris-yolk extender used for bovine semen freezing. Concentrations of 1, 5, 10, and 20mM of the extract were tested in experimental extenders. The study was conducted using semen from four bulls, followed by the assessment of sperm kinetics via the CASA system, plasma and acrosomal membrane integrity, mitochondrial potential, and quantification of malondialdehyde (MDA) as a marker of lipid peroxidation. The concentrations of 5 and 10mM stood out for promoting higher total and progressive motility, as well as superior VCL, BCF, and hyperactivation indices. Although no statistical differences were observed for plasma and acrosomal membrane integrity, overall preservation of the evaluated parameters was noted. Mitochondrial potential was reduced to extreme concentrations (1 and 20mM), remaining stable at 5 and 10mM. Furthermore, all treatments with the extract significantly reduced MDA concentration compared to the control, suggesting effective antioxidant protection. Jambolan extract preserved sperm viability post-cryopreservation, with reduced oxidative stress and maintenance of cellular functions.

Keywords: Tris-yolk; sperm membranes; malondialdehyde

RESUMO

Objetivou-se avaliar a suplementação de extrato de jambolão (*Syzygium cumini*), ao diluidor Tris-gema utilizado na congelação do sêmen bovino. Foram testadas concentrações de 1, 5, 10 e 20mMol do extrato em diluidores experimentais. O estudo foi conduzido com sêmen de quatro touros, com posterior avaliação da cinética espermática via sistema CASA, integridade de membrana plasmática e acrossomal, potencial mitocondrial e quantificação de malondialdeído (MDA) como marcador de peroxidação lipídica. A concentração de 5 e 10mMol destacou-se por promover maior motilidade total e progressiva, além de índices superiores de VCL, BCF e hiperatividade. Apesar de não haver diferenças estatísticas para a integridade das membranas plasmática e acrossomal, observou-se preservação geral dos parâmetros avaliados. O potencial mitocondrial foi reduzido nas concentrações extremas (1 e 20mMol), mantendo-se estável com 5 e 10mMol. Além disso, todos os tratamentos com o extrato reduziram significativamente a concentração de MDA em comparação ao controle, sugerindo proteção antioxidante efetiva. O extrato de jambolão preservou a viabilidade espermática pós-criopreservação, com redução do estresse oxidativo e manutenção das funções celulares.

Palavras-chave: Tris-gema, membranas espermáticas, malondialdeído

INTRODUCTION

Cryopreservation of bovine semen is fundamental for animal reproduction, but it generates significant oxidative stress, resulting in

an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defenses of the cells. This stress can impair sperm physiology, leading to infertility problems in bulls. Although free radicals play important roles in reproductive processes, their excessive

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accumulation can be deleterious. In this sense, the addition of antioxidants to semen extenders is a promising strategy to preserve sperm quality and optimize reproductive capacity (Pintus *et al.*, 2021; Sapanidou and Ros-Santaella, 2023).

The inclusion of natural antioxidants, such as jambolan extract (*Syzygium cumini*), may be a viable alternative to currently used synthetic compounds. Jambolan is a plant native to India, adapted to Brazil, whose compounds, such as anthocyanins and flavonoids, possess recognized antioxidant properties. These compounds can neutralize free radicals and protecting germ cells against oxidative damage, potentially increasing sperm viability after cryopreservation. Thus, the use of jambolan extract in semen extenders can not only mitigate oxidative stress but also ensure the integrity and functionality of the genetic material (Silva *et al.*, 2012; Swami and Thakor, 2012; Duarte *et al.*, 2024).

Despite the knowledge about the beneficial properties of jambolan, there is a gap in information about its specific effect on the cryopreservation of bovine semen, especially regarding the formation of ROS and the protection of sperm cells. Conducting studies that evaluate the addition of jambolan extract to the semen extender may provide valuable insights into its efficacy as a natural antioxidant and its ability to improve sperm viability and fertility after cryopreservation (Baldi *et al.*, 2020).

Despite the antioxidant properties of jambolan (*Syzygium cumini*), there is no data on the influence of this natural antioxidant on ROS formation during the cryopreservation of bovine semen. Thus, by investigating the application of jambolan extract (*Syzygium cumini*) in sperm cryopreservation, this study aims to fill a gap in scientific literature, as well as propose a viable alternative for improving reproductive efficiency in bulls, highlighting the convergence between reproductive biotechnology and the use of natural resources with antioxidant properties.

ETHICAL ASPECTS

The research was submitted to the Ethics Committee on Animal Use of the University Federal of Maranhão, and approved under the number No.23115.005617/2022-43.

MATERIAL AND METHODS

The prediction of pharmacokinetic, physicochemical, and especially toxicological (safety) properties of jambolan constituents (Table 1) was determined through online platforms using 3D structures obtained from PubChem. Among the platforms used are: In Silico Toxicology, ProToxII, PreADMET, Molinspiration, and PASS online.

Jambolan was harvested in October 2022, on the campus of the Federal University of Maranhão, Chapadinha Science Center, located in the municipality of Chapadinha, Maranhão (3°44'26"S, 43°21'33"W), considering those with a completely dark purple color and soft texture as mature. After harvesting, at the Governador Alberto Silva school plant of the Federal University of Piauí, the jambolan fruits were washed in running water, manually depulped with the aid of a spatula, and PA ethanol was added. The mixture was stirred for 30 minutes, then filtered and placed in a rotary evaporator at a temperature of 55°C until complete evaporation of the solvent.

The Tris-egg yolk extender composed of 3.605g of Tris, 2.024g of citric acid, 1.488g of fructose, 25mg of gentamicin, 50,000IU of penicillin, 100mL of distilled water, 20% egg yolk, and 5% glycerol, with osmolality ~350mOsm and pH 6.8, was prepared at the Laboratory of Animal Reproduction Biotechnology of the Center for Agricultural Sciences, Federal University of Piauí, and used to dilute and freeze the semen. Five different experimental extenders were prepared: 0; 1; 5; 10 and 20mM of jambolan extract added to the Tris-yolk extender.

Four bulls from Fazenda Nova (3°44'39.3"S; 43°28'54.3"W), located in the city of Chapadinha, Maranhão, Brazil, were used. They had an average age of 5 years, weighed between 510 and 750kg, and had a body condition score of 3 to 4 (scale of 1 to 5). The bulls had a history of proven fertility and were evaluated for general health, reproductive organ integrity, and semen quality. During the experiment, the bulls were kept under an extensive system, with free access to *Panicum maximum* paddocks, water, and mineral salt.

Table 1. Constituents of jambolan (*Syzygium cumini*)

ID	PubChem CID	Ligand	IUPAC	FM ^a	PM ^c
j1	128861	Cyanidin	2-(3,4-dihydroxyphenyl)chromenylium-3,5,7-triol	C ₁₅ H ₁₁ O ₆ ⁺	287.24
j2	441773	Peonidin	2-(4-hydroxy-3-methoxyphenyl)chromenylium-3,5,7-triol	C ₁₆ H ₁₃ O ₆ ⁺	301.27
j3	441774	Petunidin	2-(3,4-dihydroxy-5-methoxyphenyl)chromenylium-3,5,7-triol	C ₁₆ H ₁₃ O ₇ ⁺	317.27
j4	159287	Malvidin	2-(4-hydroxy-3,5-dimethoxyphenyl)chromenylium-3,5,7-triol	C ₁₇ H ₁₅ O ₇ ⁺	331.30
j5	197081	Cyanidin 3-O-glucoside	(2S,3R,4S,5S,6R)-2-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol;chloride	C ₂₁ H ₂₁ ClO ₁₁	484.83
j6	14311151	Peonidin 3-O-glucoside	2-[5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)chromenylium-3-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol	C ₂₂ H ₂₃ O ₁₁ ⁺	463.41
j7	443651	Petunidin 3-O-glucoside	(2S,3R,4S,5S,6R)-2-[2-(3,4-dihydroxy-5-methoxyphenyl)-5,7-dihydroxychromenylium-3-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol	C ₂₂ H ₂₃ O ₁₂ ⁺	479.41
j8	443652	Malvidin 3-O-glucoside	(2S,3R,4S,5S,6R)-2-[5,7-dihydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)chromenylium-3-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol	C ₂₃ H ₂₅ O ₁₂ ⁺	493.43
j9	10100906	Delphinidin 3,5-diglucoside	(2R,3S,4S,5R,6S)-2-(hydroxymethyl)-6-[7-hydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-2-(3,4,5-trihydroxyphenyl)chromenylium-5-yl]oxyoxane-3,4,5-triol	C ₂₇ H ₃₁ O ₁₇ ⁺	627.52
j10	71587075	Petunidin 3,5-diglucoside	(2S,3R,4S,5S,6R)-2-[2-(3,4-dihydroxy-5-methoxyphenyl)-7-hydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromenylium-5-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol;chloride	C ₂₈ H ₃₃ ClO ₁₇	677
j11	441765	Malvidin 3,5-diglucoside	(2S,3R,4S,5S,6R)-2-[7-hydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromenylium-5-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol	C ₂₉ H ₃₅ O ₁₇ ⁺	655.6

^a Molecular Formula; ^b PubChem; ^c Molecular Weight (g/mol)

Semen samples were collected once a week for six weeks, totaling 24 ejaculates, via electroejaculation (Biocon®, Uberaba, Minas Gerais, Brazil), using a sterile graduated 15 mL test tube, duly protected with aluminum foil to prevent semen exposure to light. Immediately after collection, the semen samples were evaluated and processed until freezing in a laboratory present on the farm.

Semen samples from each animal were placed in a water bath at 37°C and evaluated separately for color, appearance, volume, total motility, and vigor, under an optical microscope (Olympus Ltd., Tokyo, Japan), following recommendations described in the Manual for Andrological Examination and Animal Semen Evaluation of the Brazilian College of Animal Reproduction.

Sperm concentration was obtained in a Neubauer chamber at a 1:200 dilution in a 4%

formaldehyde sodium citrate solution. The wet chamber method was used to analyze sperm morphology following recommendations described in the Manual for Andrological Examination and Animal Semen Evaluation of the Brazilian College of Animal Reproduction. Only ejaculates with total motility $\geq 80\%$; vigor ≥ 3 ; sperm concentration $\geq 3.5 \times 10^9$ spermatozoa/mL and sperm pathologies $\leq 20\%$ were used in the study. When approved, samples from the four ejaculates were mixed to form a pool to increase semen volume and eliminate variability among the studied samples. Immediately after forming the pool, it was divided into five aliquots, kept at 37°C in a water bath before dilution in the experimental extenders.

Five previously evaluated and approved semen aliquots were diluted in Tris-yolk medium (37°C) containing jambolan extract (1; 5; 10 and 20mM), while one aliquot without any supplementation was kept as a control. The diluted semen was placed in 0.25mL straws, with a final concentration of 160×10^6 spermatozoa/mL, and frozen in a TK3000® machine (TK Tecnologia em Congelação Ltda, Uberaba, MG, Brazil). The curve was programmed to cool the semen at $0.25^\circ\text{C}/\text{min}$ down to 5°C , the temperature at which the straws were kept for 4 hours. For freezing, a curve with a speed of $-20^\circ\text{C}/\text{min}$ was used until reaching a temperature of -120°C .

Immediately after, the straws were immersed in liquid nitrogen, organized in racks, and stored in a cryogenic cylinder at -196°C until thawing. The samples were thawed in a water bath at 37°C for 30 seconds and evaluated for sperm kinetics, plasma membrane integrity, acrosomal membrane integrity, mitochondrial membrane potential, and the thiobarbituric acid test.

Sperm kinetics were evaluated by a computer-assisted semen analysis (CASA) system. The CASA is a phase-contrast optical microscopy system (Nikon™ H5505, Eclipse 50i, Japan), with stroboscopic illumination and a warm stage at 37°C , a video camera (Basler Vision Technologies™ A312FC, Ahrensburg, Germany), and a PC with Sperm Class Analyzer™ software (Microptics, S.L., version 3.2.0, Barcelona, Spain).

Sperm kinetic variables were evaluated after diluting the sample in 2.94% sodium citrate (v/v) and then incubating in a water bath at 37°C for 5 minutes. The following variables were evaluated: total motility (TM-%), progressive motility (PM - $\mu\text{m}/\text{s}$), curvilinear velocity (VCL- $\mu\text{m}/\text{s}$), straight-line velocity (VSL- $\mu\text{m}/\text{s}$), average path velocity (VAP- $\mu\text{m}/\text{s}$), linearity (LIN-%), straightness (STR-%), lateral head displacement (ALH- μm), wobble (WOB-%), beat cross frequency (BCF-Hz), and hyperactivation (HYPER-%), for each analyzed spermatozoon.

To evaluate plasma membrane integrity, the double staining method with carboxyl fluorescein diacetate (CFDA; Sigma-Aldrich®, St. Louis, MO, USA) and propidium iodide (PI; Sigma-Aldrich®, St. Louis, MO, USA), modified by Coletto *et al.* (2002), was used. Aliquots (50 μL) of post-thaw semen were diluted in 150 μL of Tris (3.605g Tris, 2.024g citric acid, 1.488g fructose, 100mL distilled water) containing 5 μL of CFDA (0.46mg/mL in DMSO) and 20 μL of PI (0.5mg/mL in PBS) and incubated for 10 minutes at 37°C . A total of 200 spermatozoa were evaluated under epifluorescence microscopy (Olympus Optical Co., Ltd., Tokyo, Japan) at 400X magnification, using the DBP 580-630nm emission filter and DBP 485/20nm excitation. Spermatozoa were classified as having an intact membrane when stained green and a damaged membrane when stained red.

Acrosome integrity was evaluated using fluorescein isothiocyanate conjugated with peanut agglutinin (FITC-PNA; Sigma-Aldrich®, St. Louis, MO, USA) staining, according to the technique described by Roth *et al.* (1998). An aliquot of 20 μL of the FITC-PNA stock solution (1mg/mL) was thawed and added to 480 μL of phosphate-buffered saline (PBS) solution to obtain a final concentration of 100 $\mu\text{g}/\text{mL}$. Aliquots (20 μL) of this solution were placed on smear slides containing spermatozoa, which were incubated for 20 minutes in a humid chamber at 4°C , without light.

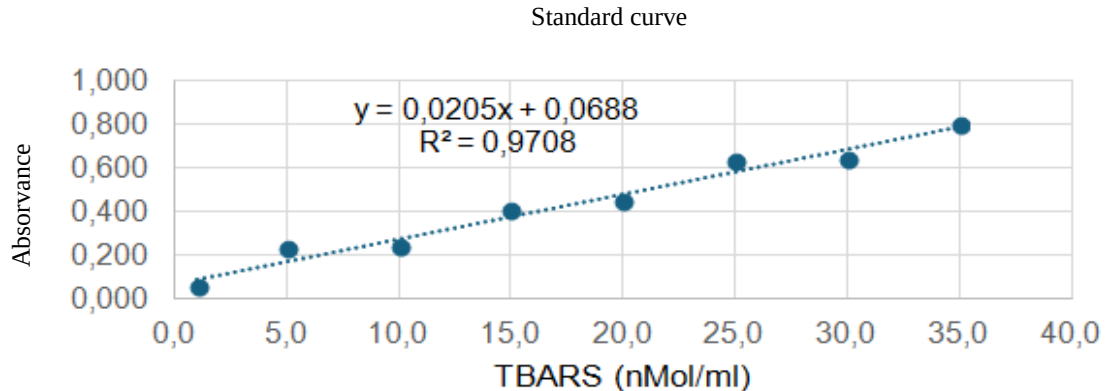
After incubation, these slides were washed twice in cooled PBS (4°C) and allowed to dry in the dark. Immediately before evaluation, 5 μL of UCD mounting medium (4.5mL glycerol, 0.5mL PBS, 5mg sodium azide, and 5mg p-phenylenediamine) were placed on the slide and

covered with a coverslip. Two hundred spermatozoa were evaluated per slide, at 1000x magnification under immersion oil, on an epifluorescence microscope (Olympus Optical Co. Ltd., Tokyo, Japan), using an LP 515nm emission filter and BP 450-490nm for excitation. Spermatozoa were classified as having an intact acrosome when the acrosomal region was stained with green fluorescence or as acrosome-reacted when they showed a green, fluorescent band in the equatorial region of the sperm head or showed no green fluorescence in any region of the head.

Mitochondrial function was determined using a lipophilic cationic fluorochrome JC-1 (Guthrie and Welch, 2006). For this, 50µl aliquots of post-thawed semen were diluted in 150µL of Tris containing 5µL of JC-1 (0.15mM in DMSO) and incubated for 10 minutes at 38°C. A total of 200 spermatozoa were evaluated under epifluorescence microscopy (Olympus Optical Co., Ltd., Tokyo, Japan) at 1000x magnification with immersion oil, using an LP 515nm emission filter and BP 450-490nm for excitation. Cells with orange staining were classified as having

high mitochondrial membrane potential, and those with green staining were classified as having low potential.

Malondialdehyde (MDA) quantification was estimated by measuring the MDA level using thiobarbituric acid (TBA), based on the method described by Maia (2006). Malondialdehyde quantification was measured after supplementing 500µL of post-cryopreserved semen, plus Tris-citric acid buffer, pH 7.4, added to 1mL of TBA reagent (15% trichloroacetic acid; 0.25N hydrochloric acid and 0.375% thiobarbituric acid) and 1% (v/v) of 50mM BHT. The mixture was treated in boiling water (100°C) for 15min. Subsequently, the samples were cooled and centrifuged at 1,200g for 15min. The supernatant was removed, and the absorbance was measured at 532nm in a UV-VIS spectrophotometer (Perkin Elmer - Lambda 25). The MDA concentration was determined by the calibration curve made with malondialdehyde as standard (Figure 1), at concentrations from 1 to 20µMol. The MDA produced was expressed in nmol of TBARS/mL of extender.



Source: Author (2025).

Figure 1. Standard curve of Thiobarbituric Acid Reactive Substances.

A randomized block experimental design was used with five treatments: control, 1, 5, 10, and 20mM of jambolan extract (*Syzygium cumini*), over six collections. The studied variables were subjected to analysis of variance (ANOVA). Means were compared using the parametric Tukey test at 5% probability; some tests were subjected to regression analysis. Polynomial contrasts were used to determine the linear and quadratic effects of the treatments. Analyses

were performed using the Statistical Analysis System program (SAS Institute Inc., 2013).

RESULTS AND DISCUSSION

The post-thaw kinetic parameters of semen cryopreserved under different concentrations of jambolan extract (*S. cumini*) are shown in Table 2.

Table 2. Post-thaw kinetic parameters of bovine semen cryopreserved in different concentrations (0, 1, 5, 10, and 20mM) of jambolan extract (*Syzygium cumini*)

Kinetic parameters	Treatments					p-value
	Control	1	5	10	20	
TM	38,13±31,94 ^{AB}	15,91±06,99 ^B	54,65±17,11 ^A	36,27±09,78 ^{AB}	30,43±11,13 ^{AB}	0,0189
PM	11,57±11,93 ^B	06,52±03,11 ^B	24,67±03,09 ^A	15,68±04,30 ^{AB}	12,48±06,02 ^B	0,0012
VCL	46,13±08,55 ^{AB}	29,73±18,98 ^B	54,98±10,76 ^A	54,90±02,53 ^A	55,23±03,35 ^A	0,0011
VSL	18,45±1,72	25,26±1,27	24,77±5,48	24,60±1,99	23,78±2,80	0,0886
VAP	27,58±8,40	34,92±2,04	33,60±6,14	33,48±1,99	33,13±1,93	0,1204
LIN	38,98±11,06	38,56±1,27	45,45±7,26	44,77±2,91	43,25±6,19	0,2615
SRT	65,18±9,37	72,49±0,56	73,33±6,90	73,45±2,01	71,65±5,60	0,1074
WOB	58,87±8,8	53,24±2,10	61,42±4,73	60,97±2,62	60,12±4,21	0,0644
ALH	3,40±0,20 ^{AB}	4,49±0,56 ^A	3,22±0,70 ^B	3,42±0,1 ^{AB}	3,53±0,44 ^{AB}	0,0176
BCF	6,72±2,70 ^B	9,64±0,20 ^A	7,92±1,24 ^{AB}	8,05±0,42 ^{AB}	8,32±0,45 ^{AB}	0,0203
HIPER	2,35±3,11 ^B	2,16±0,93 ^B	6,62±2,69 ^A	4,12±0,88 ^{AB}	3,63±2,00 ^{AB}	0,0092

Values are expressed as mean ± standard deviation (SD). TM - Total motility; PM - Progressive motility; VCL - Curvilinear velocity; VSL - Straight-line velocity; VAP - Average path velocity; LIN - Linearity; STR - Straightness; WOB - Wobble; ALH - Amplitude of lateral head displacement; BCF - Beat cross frequency and HYPER - Hyperactivation.

Means with different uppercase letters in the same row indicate a significant difference ($P < 0.05$) by the Tukey test.

Spermatozoa cryopreserved under extender containing different concentrations of jambolan extract (*S. cumini*) showed significant values ($P < 0.05$) for TM, PM, VCL, ALH, BCF, and hyperactivation among the groups. TM was higher in the extender with 5mM (54.65±17.11), differing ($p < 0.05$) from the extender with 1mM. For PM, the extender with 5mM was superior to the other extenders (24.67±03.09), without, however, differing ($p > 0.05$) from the 10mM extender. For the VCL parameter, it was observed that the extender with 5mM, 10mM, and 20mM maintained high values, differing ($p < 0.05$) from the extender with 1mM. The extender with 1mM was superior (4.49±0.56), differing statistically ($p < 0.05$) from the extender with 5mM for ALH, and from the control for BCF. The extender with 5mM showed the highest hyperactivation index (6.62±2.69), differing ($p < 0.05$) from the extenders with 1mM of jambolan extract (*S. cumini*) and the control.

ATP availability in the seminal extender is fundamental for sperm motility and velocity. The VCL and VSL parameters correlate differently with the percentage of motile sperm, where the former shows high correlation and the latter low

correlation, with the VCL value being a good indicator of sperm vigor (Perreault, 2002). In this study, for VCL and post-thaw sperm vigor, a relationship between parameters was observed during the physiological thermoresistance test, where at 180 minutes (data not published), it was found that the extenders with 5, 10, and 20mM of jambolan extract (*S. Cumini*), compared to the other experimental extenders, showed significantly higher values.

Parameters of hyperactivation, motility, progressive motility, and sperm velocity are compromised during cryopreservation (Sohail et al., 2013). In this study, BCF values were higher in the extenders supplemented with jambolan extract (*S. cumini*). This parameter indicates the frequency of sperm movement, and when increased, it causes modifications in the movement pattern, altering the linear path due to sperm hypermotility (Ratnawati et al., 2018). Since cryopreservation pre-capacitates the spermatozoon and causes changes in the sperm membrane, accompanied by Ca^{2+} influx, it leads to sperm hyperactivation (Schmidt and Kamp, 2004).

In the present study, the ALH value was significantly higher ($p < 0.05$) for the extender with 1mM compared to the extender with 5mM of jambolan extract (*S. cumini*). This parameter

shows the average width of sperm head oscillation during its movement (Kathiravan *et al.*, 2011), and its measurement is related to the ability to penetrate the oocyte's zona pellucida (Matos *et al.*, 2008), which tends to increase after cooling (Tardif *et al.*, 1996).

Although there are no specific studies on the effect of jambolan extract (*S. cumini*) on these parameters in bulls, Sousa Filho (2023), when studying seminal extenders for sheep, observed a lower ALH value in spermatozoa cryopreserved with jambolan extract (*S. cumini*), differently from what was observed in this study.

Table 3. Plasma membrane integrity, acrosomal membrane integrity, and mitochondrial membrane potential post-thaw of bovine semen cryopreserved in different concentrations (0, 1, 5, 10, and 20mM) of jambolan extract (*Syzygium cumini*)

Parameters	treatments mMol/mL					SEM ¹	Significance	
	Control	1	5	10	20		Lin ²	Quad ³
PMI	59,33	71,66	81,00	74,66	64,50	11,358	0,713	0,165
AMI	81,33	84,50	77,00	82,33	77,16	3,172	0,305	0,749
MMP	62,33 ^A	40,16 ^B	47,00 ^{AB}	51,16 ^{AB}	37,83 ^B	4,038	0,006	0,330

¹SEM- Standard Error of the Mean (%), ²Lin- Significance for linear effect, ³Quad- Significance for quadratic effect.

⁴PMI – plasma membrane integrity. ⁵AMI - acrosomal membrane integrity. ⁶MMP – mitochondrial membrane potential. *Data were evaluated by regression analysis at 5% probability.

Means with different uppercase letters in the same row indicate a significant difference (P <0.05) by the Tukey test.

Although there was no statistical difference (P>0.05) for plasma and acrosomal membrane integrity among the extenders, it was observed that in all extenders, the integrities were preserved and evaluated with a high percentage of plasma and acrosomal membrane integrity.

In the present study, it was verified that the mitochondrial membrane potential was mostly below 50%, with a statistical difference between the extenders (P<0.05). And even though there was no statistical difference for plasma membrane integrity, it is possible to infer that there is a relationship between damage to the plasma membrane and impaired mitochondrial membrane potential, as presented by Kanno *et al.* (2016).

With the supplementation of jambolan extract (*S. cumini*) in the experimental extenders, it was expected that the antioxidants present in jambolan would preserve the fluidity, functionality of the plasma membrane, acrosomal and mitochondrial functionality, given that mitochondrial activity is ATP-dependent (Silva

In Table 3, no variation (P>0.05) was observed, after thawing, in the integrity of the plasma and acrosomal membranes. For the mitochondrial membrane potential, a significant difference (P<0.05) was observed, with higher potential in the extender without the addition of jambolan extract (*S. cumini*), compared to the extenders with 1 and 20mM/mL of jambolan extract (*S. cumini*), suggesting membrane destabilization. The bioactive compounds of jambolan in the extenders supplemented with 5 and 10mM of jambolan extract (*S. cumini*) did not differ (P>0.05) from the control extender.

and Gadella, 2006), and that low ATP production indicates reduced mitochondrial membrane potential, with a response in lower metabolic efficiency, increased production of free radicals, and cellular metabolic imbalance (Amaral *et al.*, 2013).

The absorbance and malondialdehyde (MDA) quantification results in the different treatments are presented in Table 4.

The measured absorbance varied among the experimental extenders, with the control showing the highest absorbance (0.198±0.070), while the extenders supplemented with jambolan extract (*S. cumini*) showed significantly lower values.

MDA quantification was also analyzed, with the control showing the highest average MDA concentration (0.006±0.003µMol/mL). The extenders supplemented with jambolan extract showed lower MDA quantification (P<0.05), ranging from 0.001±0.001 to 0.003±0.001µMol/mL.

Table 4. Mean and standard deviation of absorbance and malondialdehyde quantification in bovine semen cryopreserved in different concentrations (1; 5; 10; 20 mM) of jambolan extract (*Syzygium cumini*), and control group without experimental treatment

Treatments mMol/mL	Absorbance	MDA (μ Mol/mL)
Control	0.198 ± 0.070^A	0.006 ± 0.003^A
1	0.116 ± 0.038^B	0.002 ± 0.001^B
5	0.131 ± 0.020^B	0.003 ± 0.001^B
10	0.090 ± 0.005^B	0.001 ± 0.001^B
20	0.117 ± 0.007^B	0.002 ± 0.001^B

Means with different letters in the same row differ significantly (ANOVA and TUKEY test, $p < 0.05$).

The results indicate a significant reduction in absorbance and MDA concentration in all experimental extenders compared to the control, suggesting a potentially beneficial effect of the treatments on the extent of cellular lipid peroxidation.

The study demonstrated that the extenders with jambolan extract (*S. cumini*) significantly reduced ($p < 0.05$) the production of malondialdehyde, a marker of oxidative damage, and the absorbance in cryopreserved bovine semen.

The chemical composition of *S. cumini* includes several phytochemical compounds, among which polyphenols stand out, exhibiting antioxidant potential and beneficial health properties (Ayyanar and Bandu-Subash, 2012). Furthermore, they are water-soluble, produced by the plant's secondary metabolism, and their chemical organization is established by having hydroxyls linked to their aromatic rings, which confer antioxidant action (Sureda et al., 2014).

The number of hydroxyls influences the antioxidant potential, and the greater the presence of hydroxyls, the greater the antioxidative capacity of phenolic compounds, since their action in reducing free radicals occurs by donating an electron, present in the hydroxyl, to the free radical (Markovic et al., 2012).

In this research, the measurement of the antioxidant capacity of the *S. cumini* extract occurred through the quantification of malondialdehyde in the sperm cryopreservation extender medium. In the TBARS reaction, the MDA present in the sample reacted with thiobarbituric acid (TBA) in an acidic medium

and under heating, forming a pink-colored complex. This complex is a chromophore, a substance that absorbs visible light, which under certain conditions, establishes a directly proportional relationship with absorbance. In the treatments with *S. cumini*, the donation of an electron, present in the hydroxyl, to the free radical, resulted in less oxidative damage and lower MDA production (Ruan et al., 2008; Santos et al., 2020).

CONCLUSION

Jambolan extract preserved sperm viability post-cryopreservation, with reduced oxidative stress and maintenance of cellular functions.

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CONTRIBUTORS

N Almeida, N Almeida, A Maciel, T Santos, V Ribeiro, AS Sousa, J Teixeira, I Vieira: Investigação.

R Marchão, I Nascimento: Supervisão, Análise formal.

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DATA AVAILABILITY STATEMENT

The research data are available upon request.

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