Short Communication

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2023.52.008232

Antioxidant Effect of *Abarema Cochliacarpos* on Freezing Ram Semen

Dyoggo Mendonça de Souza Abelenda, Giovanna Beatriz Vilela Sampaio, Maria Eduarda Miranda Cavalcanti Santiago, Maria Fernanda Alves Do Nascimento and Kauany Amaral Lima José Adelson Alves Do Nascimento Junior*

Afya Faculty of Medical Sciences Garanhuns, Brazil

***Corresponding author:** Jose Adelson Alves Do Nascimento, Afya Faculdade De Ciências Médicas Garanhuns, Brazil

ARTICLE INFO

Received: August 10, 2023

Published: August 18, 2023

Citation: Dyoggo Mendonça de Souza Abelenda, Giovanna Beatriz Vilela Sampaio, Maria Eduarda Miranda Cavalcanti Santiago, Maria Fernanda Alves Do Nascimento and Kauany Amaral Lima José Adelson Alves Do Nascimento Junior. Antioxidant Effect of *Abarema Cochliacarpos* on Freezing Ram Semen. Biomed J Sci & Tech Res 52(2)-2023. BJSTR. MS.ID.008232.

ABSTRACT

Sheep farming in the Northeast region of Brazil is an important component of the local economy. The objective of this work was to test the effect on seminal quality of ovine plant-derived antioxidants extracted from *Abarema cochliacarpos*, aiming to decrease reactive oxygen species (ROS) in cryopreserved ovine semen samples. Sheep semen was collected in triplicate, frozen with cryopreservation extender extract with addition of *Abarema cochliacarpos* oil, at the following concentrations: 0 (control), 2.5 mg; 1.25 mg; 0.67mg; 0.31 mg and 0.2 mg. The data obtained by the sinusoidal arc were submitted to analysis of variance. The results indicate that there were no significant differences between treatments for membrane integrity or mitochondrial potential, and in evaluating computer-assisted sperm analysis (CASA) between concentrations on the parameters used in the study. There was, however, a difference in the parameters of progressive motility, linearity, straightness, and frequency of cross-flagellar beating, with emphasis on the concentration of 1.25 mg of *A. cochliacarpos*, not indicating an adverse effect. The 1.25 mg concentration was the most effective in this experiment.

Keywords: Sheep Farming; Seminal Quality; Oil Extract; Cryopreservation

Abbreviations: ROS: Reactive Oxygen Species; AI: Artificial Insemination; CASA: Computer Assisted Sperm Analysis; ROS: Reactive Oxygen Species; CEUA: Committee for Ethics in the Use of Animals; PMI: Plasma Membrane Integrity; *A. Cochliacarpos*: *Abarema Cochliacarpos*; LABRAPE: Animal Reproduction Laboratory of Pernambuco; UFRPE: Federal Rural University of Pernambuco; UAG: Garanhuns Academic Unit; CBRA: Brazilian College of Animal Reproduction; PBS: Phosphate Buffered Saline Solution; CFDA: 6 Carboxyfluorescein; MMP: Mitochondrial Membrane Potential; DIC: Completely Randomized Design; ANOVA: Analysis of Variance; MP: Progressive Motility; PIM: Progressive Individual Motility; LIN: Linearity; STR: Straightness; BCF: Crossed Flagellar Beat Frequency; MT: Total motility; VCL: Track Speed; VSL: Progressive Speed; VAP: Route Speed; ALH: Amplitude of Lateral Displacement of the Head; WOB: Oscillation Index

Introduction

One of the most used techniques to improve the productive potential of animals is artificial insemination (AI). It is among the oldest biotechnological techniques applied for animal genetic improvement, being the one that produced the greatest genetic gain due to the ease of its practical domain, making it the most widespread [1]. To perform AI, semen from tested animals may be used, handled fresh or stored under refrigeration and freezing. Semen, when frozen, suffers several structural, chemical, and physical damages to the spermatozoa, leading to a decrease in motility, viability, and fertility parameters [2]. These damages are linked to the process of cooling, freezing, and thawing [3]. The occurrence of this damage differs between species, due to variation in sperm size and shape, as well as in lipid composition. The alterations caused by this process are due to changes in temperature, oxidative injuries via reactive oxygen species, formation of ice crystals, DNA damage, alterations in the sperm membrane, osmotic stress, in addition to the toxicity of cryoprotectants [4]. To

minimize oxidative damage, several studies such as Banday, et al. [5] used media with antioxidant substances, thus improving post-thawing sperm quality.

The addition of antioxidants based on substrates of plant origin is a viable alternative [6]. *Abarema cochliacarpos*, a plant species native to Brazil, has numerous medicinal properties. For example, Silva, et al. [7] reported the presence of catechins, which show evidence of antioxidant activity and ability to fight free radicals [8]. Therefore, the aim of this investigation was to test the effect on seminal quality of ovine plant-derived antioxidants extracted from *A. cochliacarpos* to decrease ROS in cryopreserved ovine semen samples. The results of the study will be valuable to improve biotechnologies aimed at sheep farming and its reproduction, in addition to increasing savings and reducing damage to sperm membranes.

Materials and Methods

Extraction and purification of *Abarema cochliacarpos*

Animals, Place and Period of Experiment

The research was carried out in January 2018 at the Animal Reproduction Laboratory of Pernambuco (LABRAPE), located on the premises of the Federal Rural University of Pernambuco (UFRPE), Academic Unit Garanhuns (UAG). Sheep from rural properties in the municipality of Garanhuns, in the Mesoregion of Agreste Pernambucano, were used. The experiment was approved by the Committee for Ethics in the Use of Animals (CEUA), under license number 127/2017.

Semen Collection

Two adult rams were selected after andrological examination. After being conditioned to collection with an artificial vagina, semen collections were performed on alternate days. During semen collection, ewes were used as dummies, not necessarily in estrus, which was manually contained. The semen was received in a silicone collection cone and graduated Falcon® tubes.

Fresh Semen Evaluation

As the semen was collected, it was sent to the Animal Reproduction Laboratory of Pernambuco (LABRAPE) to carry out the spermogram, quantifying the following parameters: volume (mL), sperm concentration (x109 spermatozoa/mL), progressive individual motility (PIM , 0 -100%) and vigor (0-5), according to the criteria recommended by the Brazilian College of Animal Reproduction (CBRA, 2013). An aliquot of 2.5µL of semen diluted in 997.5 µL of phosphate buffered saline solution (PBS) was pipetted, thus obtaining a saline solution at a concentration of 1:400, based on the sperm count, the concentration was defined camera-assisted sperm.

Adding Compound to Semen

The semen was diluted by the addition of the diluent medium, with the addition of *A. cochliacarpos* oil. The semen received pre-established concentrations of the extract. The oil obtained by aqueous extraction was subjected to several tests of antioxidant activity [7,8] and later, the analysis was performed together with ram semen where the concentrations were used: C1:0 (control); C2: 2.5; C3: 1.25; C4: 0.67; C5: 0.31; C6: 0.2 milligrams of extract.

Semen Freezing

After dilution the semen was packaged in 0.25 ml straws and subjected to freezing in the TK3000® machine (TK Freezing Technology LTDA, Uberaba, Brazil), using a quick-freezing curve (P3:S1) with a stabilization time of 1 hour according to previous studies in our laboratory [9]. Then, the freezing curve started (-20°C /min) until reaching -120°C. Then, the straws were immersed and stored in liquid nitrogen (-196°C) until later use.

Thawing evaluation

After freezing the desired samples, sperm parameters were evaluated after thawing (37 °C for 30 seconds).

Computer-Assisted Sperm Analysis

Analysis of sperm kinetics was performed using Computer-Assisted Sperm Analysis (CASA). An aliquot (3 µL) of the diluted sample was placed on a slide and covered with a coverslip (18 x 18 mm), both preheated (37°C), and evaluated under a phase contrast microscope (Eclipse 50i, Nikon, Japan, 100x). Images were captured by a video camera (Basler Vision Technologies TM A312FC, Germany). For each sample, five random fields were selected, with records of at least 500 spermatozoa. The analyzed variables were: Total motility (MT, %), Progressive motility (MP, %), Linearity (LIN, %), Straightness (STR, %); Track Speed (VCL, µm/s), Progressive Speed (VSL, µm/s); Velocity of travel (VAP, µm/s), Amplitude of lateral displacement of the head (ALH, µm/s); cross-flagellar beat frequency (BCF,%). CASA system values were measured with the following settings: temperature 37°C; magnification, 100x; number of images, 25; images per second, 25; head area, 20 to 70 µm2; PAV: slow 10 µ/s < medium 45 µ/s < fast 75 µ/s; progressiveness, 80% STR; circular, 50% LIN. Plasma membrane integrity (PMI) [10].

The following procedures were performed to analyze plasma membrane integrity (PMI). Aliquots (40 µL) of semen from each experimental group were distributed in microtubes (1.5 mL) for each evaluation, to which 5 µL of propidium iodide, 5 µL of CFDA (6 carboxyfluorescein) and 5 µL of of paraformaldehyde. Then, 5 µL of each sample were placed on a slide and covered with a coverslip (18 x 18 mm) and submitted to evaluation under a fluorescence microscope. For each sample, five random fields were selected, with a record of 200 spermatozoa, those that presented green color being classified as intact.

Mitochondrial Membrane Potential

For mitochondrial membrane potential (MMP) analysis, 5 µL of JC-1 (Stock solution 5 mg/mL in DMSO; Working solution 153 µM in DMSO) was added to semen samples containing 40 µL, distributed in microtubes of 1 .5ml. Subsequently, the samples were incubated for 5 min at room temperature and evaluated. Cells with intermediate partsstained orange were classified as having high mitochondrial membrane potential. For each sample, five random fields were selected, with a record of 200 spermatozoa.

Statistical Analysis

Completely randomized design (DIC), with six treatments and six replications (3 per animal). The treatments were composed by the concentrations: C1:0 (control); C2: 2.5; C3: 1.25; C4: 0.67; C5: 0.31; C6: 0.2 mg of the extract. The data obtained were submitted post-transformation by sinusoidal arc (arcsen P/100) to analysis of variance (ANOVA) by the "F" test, with the averages of the treatments compared with the Tukey test at 5% significance.

Results

(Table 1) The results of five extracts (Table 2) show that there were no significant differences between concentrations after semen thawing in the following parameters: TM, VCL, VSL, VAP, WOB and ALH.

Table 1: Shows that the semen collected is healthy and complies with the standard contained in the Manual for Andrological Examination and Evaluation of Animal Semen. This material will be used as a basis for analysis and comparison with later results.

	Animal 1	Animal 2	Overall Average
Volume (mL)	0.93 ± 0.20	0.92 ± 0.22	0.93 ± 0.19
Concentration	107.25 ± 11.17	115.75 ± 11.78	111.5 ± 11.56
motility (%)	80.00 ± 8.16	77.5 ± 6.45	78.75 ± 6.94
Vigor	3.50 ± 0.57	3.75 ± 0.50	3.62 ± 0.51

Note: mL: Milliliter.

Table 2: Mean values after thawing of ram semen with addition of vegetable extract, with evaluation in the CASA computerized system.

Evaluated parameters	Control	C.2.5	C.1.25	C.0.67	C.0.31	C.0.2
MT (%)	49.40a	51.01a	50.37a	45.37a	51.72a	47.36a
MP (%)	27.34a,b	30.80a,b	33.91b	25.76a	29.87a,b	28.07a,b
VCL (µm/s)	64.68a	69.13a	73.00a	62.25a	74.23a	72.50a
VSL (µm/s)	41.06a	49.81a	54.58a	40.95a	47.96a	44.90a
VAP (µm/s)	50.25a	62.18a	60.80a	49.63a	58.48a	55.62a
LIN (%)	52.82a,b	53.56a,b	59.01b	54.15a,b	53.13a,b	51.92a
STR (%)	64.75a	64.12a	70.69b	65.25a,b	64.61a	64.05a
WOB (%)	61.80a	63.30a	65.17a	63.30a	62.39a	61.11a
ALH (µm/s)	2.36a	2.28a	2.26a	2.03a	2.35a	2.54a

Note: Progressive motility (PM); Linearity (LIN); Straightness (STR); Trail speed (VCL); Progressive Speed (VSL); Speed of travel (VAP); Amplitude of lateral head displacement (ALH); Total Motility (MT); wobble index (WOB); Micromeres per second (µm/s).

However, there was a significant difference between treatments when the parameters of progressive motility (PM), linearity (LIN), straightness (STR) and cross-flagellar beat frequency (BCF) were evaluated. However, it was demonstrated that concentrations of less than 1.25 mg of the extract showed lower percentage values of the parameters evaluated in the CASA. This may lead to the conclusion that concentrations below 1.25 mg may not have the expected antioxidant action. However, a hypothesis that could explain the fact that values higher than this could compromise sperm quality after thawing is also not validated using higher concentrations of this extract in this study. This hypothesis corroborates Marsico [11] that different extracts at different concentrations can present different biochemical behaviors.

Thus, it seems reasonable to assume that the concentration of 1.25 mg is the most appropriate, as values above and below the referent do not provide an adequate response in ram semen. Therefore, sheep farmers would need to increase the type of thawing medium for semen, thus preserving some of its primordial characteristics and, consequently, ensuring a greater probability of success in the sheep fertilization process. In this experiment (Table 3), no significant differences were observed between treatments for impairment of plasma membrane integrity and mitochondrial membrane potential. This means that *A. cochliacarpos* does not have an adverse effect on PMI and MMP at different concentrations in the present study, as also observed by Santos [12] who also used a plant extract based on green propolis as an antioxidant in the cooling of equine semen. Still, there was a decrease in sperm motility. There is no evidence, as far as we know, about the effect of *A. cochliacarpos* extract, especially on the pattern of mitochondrial activity and state of apoptosis in mammalian spermatozoa.

Table 3: Mean values of the PMI and MMP Integrity evaluation using fluorescent probes.

	Control	C2.5	C1.25	C0.67	C0.31	C0.2
PMI	28.50a	37.25a	31.50a	22.25a	21.25a	38.00a
MMP	58.25a	66.50a	69.25a	88.75a	81.25a	73.00a

Note: Plasma Membrane (PMI); Mitochondrial Membrane Potential (MMP).

Conclusion

The study analyzed five extracts (Table 2) and concluded that there were no significant differences in post-thawing semen concentrations in relation to parameters such as TM, VCL, VSL, VAP, WOB and ALH. However, there was a significant difference between treatments when evaluating PM, LIN, STR and BCF. Concentrations lower than 1.25 mg of the extract resulted in lower percentage values in the CASA, suggesting a limited antioxidant action. The hypothesis that concentrations above 1.25 mg negatively affected sperm quality was not confirmed. Thus, it seems that the concentration of 1.25 mg is the most appropriate to maintain the characteristics of ram semen and increase the chances of successful fertilization. No significant differences were found in the effects on PMI and MMP with different concentrations of *A. cochliacarpos*, which indicates its safety in these respects. However, a decrease in sperm motility was observed. So far, there is no evidence of the effects of *A. cochliacarpos* extract on mitochondrial activity and apoptosis in mammalian spermatozoa.

References

1. Baruselli OS, Catussi BLC, Abreu LA, Eliff FM, Silva LG, et al. (2019) Evolution and perspectives of timed artificial insemination in cattle. Rev Bras Playback Anim 43(2): 308-314.
2. Guerra MMP, Souza AF, Soares AT, César CNR, Silva SV, et al. (2009) Critical aspects of goat semen cryopresevation. Rev Bras Reprod Anim Supl, Belo Horizonte 6: 42-49.
3. Bittencourt RF, Ribeiro Filho AL, Alves SGG, Biscarde CE, Vasconcelos MF (2007) Cryopreservation of goat semen: The effect of freezing rate and equilibration time. science animation bras 17(2): 75-82.
4. Soares AT, Guerra MMP (2009) Effects of cryopreservation on sperm viability. Technol & Science Agropec 3(2): 53-63.
5. Banday MN, Lone AL, Rasool F, Muzamil R, Shikari A (2017) Use of antioxidants reduce lipid peroxidation and improve quality of crossbred ram sperm during its cryopreservation. Cryobiology 74: 25-30.
6. Bittencourt RF, Oba E, Ribeiro Filho AL, Chalhoub M, Azevedo HC, et al. (2013) Advances in cryopreservation of sheep semen I: Extenders and cryoprotectors. Science animation. bras, Goiânia 144(4): 522-536.
7. Silva NCB, Esquibel MA, Alves IM, Velozo ES, Almeida MZ, et al. (2009) Antinociceptive effects of *Abarema cochliacarpos*. Rev Bras of Pharmac 19(1): 46-50.
8. Bianchetti P (2014) Evaluation of the antioxidant and antibacterial activity of aqueous and ethanolic extracts of plants of the *Mytaceae* family against the microorganism Escherichia coli. Masters in biotechnology, Brazil p. 47.
9. Carneiro GF (2008) Biotechniques of Assisted Reproduction in Small Ruminants. Techn Science A group 2: 23-28.
10. Silva MSF (2015) Goat semen cryopreservation with inclusion of golden linseed oil (*Linum usitatissimum L.*) in the extender. Federal University of Bahia's Recôncavo, Center for Agrarian, Environmental and Biological Sciences, Brazil.
11. Marsico TV (2016) Oxidative stress analysis in frozen bovine sperm cells with the addition of natural antioxidants. Course completion paper (TCC III) presented to the undergraduate course in Biotechnology at the Federal University of Pampa, Brazil.
12. Santos FCC (2014) Use of green propolis extract in equine semen cooling. Federal University of Pelotas, Brazil.

ISSN: 2574-1241

DOI: 10.26717/BJSTR.2023.52.008232

Jose Adelson Alves Do Nascimento. Biomed J Sci & Tech Res