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Effect of Melatonin and Vitamin E on SOD-Cu / Zn Levels and Membrane Permeability During Weed Semen Conservation

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Abstract

In species such as the hair, oxidative stress is related to sperm damage during sperm conservation, which affects its fecundity. This research aimed to determine the effect of the addition of melatonin and vitamin E on the levels of Dismutasa-Cu / Zn Superoxide (SOD-Cu / Zn) during the conservation of diluted bristle semen. Seminal maestras of 7 to 18 months old verracos were collected. The optimal parameters are diluted and divided into aliquots: control (with diluent), control with vehicle (diluent and ethanol) and semen with melatonin (1.25 mM) and vitamin E (1 mM. at 16 °C, if enzyme activity (SOD-Cu / Zn), with commercial kit (Cayman®-Chemical Company Ann Arbor, MI, USA) and Hypo-osmotic Test (HOST +), to evaluate the integrity of Plasma membrane Results were analyzed by ANOVA, with SPSS 15.0 statistical package for Windows with p≤0.05 If SOD-Cu / Zn was observed in melatonin-based women, but significantly (p <0.05) higher rates and increased percentage of HOST + sperm, the conservation day with respect to the different groups Melatonin has an antioxidant effect on the conservation of seminal hair at 16 °C with an increase in enzymatic activity of CuZnSOD, which improve the seminal quality to increase the of the plasma membrane (HOST +) in sperm cells.

Keywords: Semen; Conservation; Cerdo; Radicales libre

Introduction

The cryopreservation of semen is one of the most important procedures in the development of biotechnology for assisted reproduction [1], which allows for maximum distribution of germinal material of interest specimens, facilitating the development of genetic improvement programs [2]. However, during the 24-hour cooling of semen, there is an increase in concentrations of reactive oxygen species (ROS) affecting seminal quality (decreased sperm motility and loss of membrane permeability) events. which have been associated with the lipoperoxidation process [3] and antioxidant enzymes such as the Dismutasa Superoxide (SOD) in its three isoforms (SOD-Cu / Zn- the cytosolic SOD, the SOD-Mn, the mitochondrial SOD and the SOD-Cu / Extracellular or secreted zn) [4], have the protective effect during frozen or refrigerated semen storage [5-8], improving sperm motility and membrane integrity [9].

In this sense, in recent years there have been studies on the use of different antioxidants (melatonin, vitamin C and vitamin E) in seminal diluents to prevent deleterious effect of RL during sperm conservation [10,11]. On the basis of this background, the present research has aimed to determine the effect of adding melatonin and vitamin E on the activity of SOD-Cu / Zn during the conservation of diluted bristle semen.

Materials and Methods

Población y muestra: The study was carried out in UCLA (Barquisimeto, Venezuela). The 20 weeks of semen for the studio were taken from 7-18-month-old Landrace, Large White and Duroc verrace warts from the Porcine Inversion Breeders' farm, located in the Yaritagua-Barquisimeto range, state Yaracuy-Venezuela. Breeding males selected as donors of semen and healthy animals, maintained in optimum health conditions, in a clean and balanced nutrition habitat, established in the Code of Ethics for the Life of the Bolivarian Republic of Venezuela (2010) in their second part chapter 3 [12]. Makes a big deal. The collection of the seminal seminal is realized by means of penean massage with the manguado in a suitable foal for such purpose. The ability to obtain experimental trials has been analyzed in the farm laboratory and those that meet the seminal selection criteria for artificial insemination programs have been diluted for conservation at 16 °C with commercial dilution for bristle semen (MR -A®), taking into account the volume and the optical density of the eyelid.

Handling of the Master

From each selected sample randomly, the first day (day 0) prepared 3 5ml aliquots, which were labeled control (diluent soil MR-A®), vehicle control (ethanol), and aliquot with melatonin (1.25 mM (Sigma®)) and 1 mM vitamin E (Sigma-Aldrich), added at a ratio of 20µl per ml of diluted semen.

Dismutasa Superoxide Enzyme (SOD)

For the determination of the enzyme Dismutas-Cu/Zn Superoxide enzyme (SOD-Cu / Zn) using a commercial kit (Cayman®-Chemical Company Ann Arbor, MI, USA) For the preparation of the enzyme If 1.5 ml of diluted semen were taken into an Eppendorf® tube and 2 ml of lysis buffer (20mM tris base, 1 mM EDTA, 210mM mannitol and 70 mM sucrose at pH 7) were added. 2) Chilled by a large grain and mixed in a Thermoline® Model 376000 vortex for minutes to centrifuge at 1500 X g for 10 minutes at 4 °C in the microcentrifuge. The supernatant obtained was used to quantify SOD-CuZn by means of Sunrise model ELISA, Tecan® model 0,3360093, Austria) at 450nm.

Hypo-osmotic test (HOST +)

To carry out the preparation, a hypoosmotic solution (100mosmol/L) with 490 mg sodium citrate and 900 mg fructose per 100mL distilled water is prepared. From this solution 100µL were taken for each 25µL of diluted semen seed and incubated at 37 °C for 30 minutes. If those sperm are considered positive in those which are observed any degree of helical torsion of the glue ("swelling"), counting 100 sperm for each month.

Results

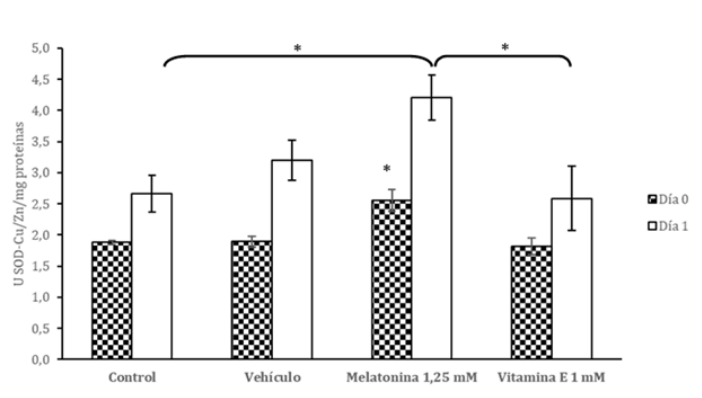

Figure 1: Significant differences in semen with melatonin with respect to other controls, vehicle and vitamin E day 0, and significant differences with other controls and with vitamin E day 1 of conservation. ANOVA ($p < 0.05$).

In order to evaluate the SOD-Cu/Zn in melatonin monuments it is observed significantly ($p < 0.05$) the highest conservation day with respect to the different groups. This difference was maintained only on day 1 ($p < 0.05$) with the control group and vitamin E, the latter being the one presenting the poorest enzymatic activity (Figure 1 & Graphic 1). SOD-Cu/Zn levels in diluted semen of 7-19-month-old bristles, day 0 and 1 of preservation at 16 °C with melatonin (1.25 mM) and vitamin E (1mM). *Significant differences in semen with melatonin with respect to other controls, vehicle and vitamin E day 0, and significant differences with other controls and with vitamin E day 1 of conservation. ANOVA ($p < 0.05$).

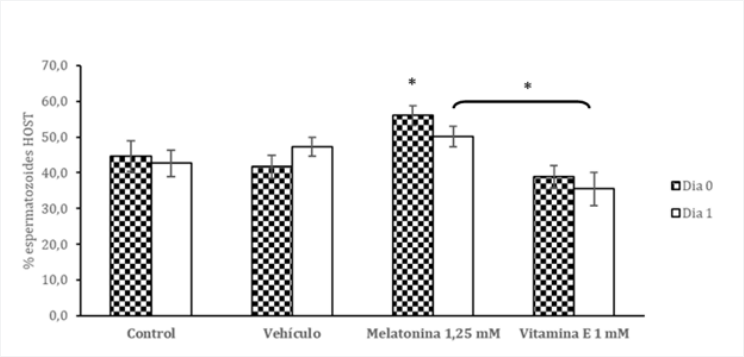

Figure 2: Membrane integrity (positive HOST) in diluted semen of 7-19-month-old hairs, day 0 and 1 of preservation at 16 °C with melatonin (1.25 mM) and vitamin E (1mM). *Significant differences in semen with melatonin and conservation day 0 with respect to control, vehicle and vitamin E, and conservation day 1 with protein by vitamin E. ANOVA ($p < 0.05$).

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Hypo-osmotic test (HOST +)

As far as the integrity of the membrane (HOST +) is concerned, the melatonin changes present a significant increase in the HOST + sperm count with respect to all groups, the conservation day; it differs from conservation day 1 in a group with vitamin E, which shows poorer HOST + percentages, even because of the lack of control (Figure 2). Figure 2 Membrane integrity (positive HOST) in diluted semen of 7-19-month-old hairs, day 0 and 1 of preservation at 16 °C with melatonin (1.25 mM) and vitamin E (1mM). *Significant differences in semen with melatonin and conservation day 0 with respect to control, vehicle and vitamin E, and conservation day 1 with protein by vitamin E. ANOVA ($p < 0.05$).

Discussion

In this investigation the semen of melatonin semen presents the greatest enzymatic activity SOD-Cu / Zn during the conservation. Similar results will be found in semen of poles and connection to add melatonin during conservation, significantly increasing the activity of endogenous enzymes (peroxidase glutathione (GSH-Px), Catalase (CAT) and SOD) in post-thawing ($P < 0.05$) [13,14] and the activity of MnSOD, CuZnSOD and catalyzing correlate significantly with various sperm parameters as progressive movement [15], which underlies their possible role in the etiology of various sperm anomalies [16].

In the case of vitamin E, it has been reported to increase the activity of SOD during conservation with liquid stem semen [17]. There are further investigations into the fact that vitamin E addition has been studied in the preservation of semen, however it has been considered that CuZnSOD activity, despite this, can be used as a predictor of lifetime. in particular, [18] that CuZnSOD is the antioxidant enzyme most affected during the process of seminal cryopreservation [19]. It is therefore important to look for mechanisms to maintain the proper levels of these antioxidant enzymes. On the basis of these results, it can be inferred that melatonin is capable of neutralizing the superoxide radical in semen, which results in the greater enzymatic activity of SOD-Cu / Zn, in addition to vitamin E in the effective display of it. that decreases the activity of the SOD-Cu / Zn, including values by the control group, could be related to the increase in mitochondrial superoxide radical production, specifically at the rate of electron carrier, such as it is the principal source of reactive oxygen species (ROS) [20] associated with alterations of the hierro-SH residues [21] based on the complete II of the electron carrier, altering the mitochondrial structure and functioning [22,23].

Evaluating plasma membrane integrity as a parameter of seminal quality, as being responsible for morphological and functional integrity of sperm [24], changes with melatonin in this investigation will reveal that my membrane integrity protection (HOST +) significant increase in HOST + sperm count, with respect to all conservation groups. This difference was maintained by day 1 of conservation with a group of women with vitamin E, a result that coincides with other investigations into the working of logs [25] and buffalo [26] and shells [13]. In the case of vitamin E the results were similar to those obtained in sheep by Gómez and col. [27] with HOST + percentage included due to other controls. This result is given by the melatonin antioxidant effect and vitamin E prooxidant effect found in the study. Alteration of membrane integrity is related to oxidative stress, disruption of SOD activity [28] causes excess of RL that interact with membrane lipids at the level of unsaturated carbonate [29] , process called lipoperoxidation, which has as a result deep changes affecting the organization and function of the sperm membrane [30,31] what it could take to the cell phone [32].

Conclusion

Melatonin has an antioxidant effect during the preservation of bristle semen at 16 °C with an increase in CuZnSOD enzymatic activity, which increases seminal quality and increases plasma membrane integrity (HOST +) in sperm.

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