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The influence of trehalose, taurine, cysteamine and hyaluronan on ram semen Microscopic and oxidative stress parameters after freeze–thawing process☆

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Abstract

There is a lack of information regarding lipid peroxidation and antioxidant capacity in cryopreserved ram semen, and cryopreservation is associated with the production of reactive oxygen species (ROS) which lead to lipid peroxidation (LPO) of sperm membranes, resulting in a loss of motility, viability and fertility of sperm.

The aim of this study was to determine the influence of certain additives and their different doses on standard semen parameters, lipid peroxidation and antioxidant activities after the cryopreservation/thawing of ram semen. Ejaculates collected from four Akkaraman rams, a native breed of sheep, were evaluated and pooled at 33 °C. Semen samples which were diluted with a Tris-based extender containing additives including trehalose (50, 100 mM), taurine (25, 50 mM), cysteamine (5, 10 mM), and hyaluronan (0.5, 1 mg/ml), and an extender containing no additives (control) were cooled to 5 °C and frozen in 0.25 ml French straws, being stored in liquid nitrogen. Frozen straws were thawed individually at 37 °C for 20 s in a water bath for evaluation.

The use of a Tris-based extender supplemented with 50 mM trehalose, 25 mM taurine, and 5 and 10 mM cysteamine led to higher percentages of post-thaw motility, in comparison to the control group ($P < 0.01$). No significant differences were observed in the percentages of acrosome and total abnormalities, and the hypoosmotic swelling test upon the supplementation of the freezing extender with antioxidants after the thawing of semen. In biochemical assays, the addition of antioxidants did not cause significant differences in levels of malondialdehyde (MDA), glutathione (GSH), and glutathione peroxidase (GSH-Px), after thawing, when compared to groups with no additives. In this study, catalase (CAT) activities were higher in the group that was applied 25 mM taurine as an antioxidant, than in all of the other groups ($P < 0.001$). Compared to the controls, antioxidant treatment with 100 mM trehalose, 50 mM taurine, 5 mM cysteamine and 0.5 mg/ml hyaluronan, significantly elevated vitamin E (vit E) levels in samples ($P < 0.001$).
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Keywords: Ram semen; Freezing; Additives; Semen parameters; Antioxidant activities

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1. Introduction

The cryopreservation of spermatozoa has allowed specific opportunities for the conservation of genetic resources through sperm banks, the guarantee of a constant commercial supply of semen, and collaboration in breed improvement programs by means of the artificial insemination (AI) technique [1,2]. However cervical AI, in which frozen semen is used, is limited by low fertility rates in sheep. For that reason, it requires the application of the intrauterine technique to achieve an acceptable result in fertility. However, this technique is not easy to perform under field conditions [3]. Alternative methods for the freezing of ram spermatozoa require further improvement, as well as the development of a greater understanding of the cell changes that occur during the freeze–thawing process.

The membrane of ram spermatozoa has a particular composition that renders successful freezing difficult [4,5]. Additionally, the lipid composition of the sperm membrane is a major determinant of the motility, viability, lipid peroxidation and cold shock of spermatozoa [6,7]. Differences in fatty acid composition and lipid class ratios in spermatozoa among species are important factors in the freezability of the male gametes [8]. The presence of high concentrations of long chain polyunsaturated fatty acids (PUFA) within the lipid structure of sperm cells requires efficient antioxidant systems to defend against peroxidative damage and associated sperm dysfunction [9–11]. The protective antioxidant systems in spermatozoa are primarily of cytoplasmic origin. Spermatozoa discard most of their cytoplasm during the terminal stages of differentiation, and lack the significant cytoplasmic component containing antioxidants that counteract the damaging effects of reactive oxygen species (ROS) and lipid peroxidation. Due to this, spermatozoa are susceptible to lipid peroxidation during cryopreservation and thawing [12,13]. Cryopreservation also produces physical and chemical stress on the sperm membrane, associated with oxidative stress and ROS generated by dead spermatozoa and atmospheric or molecular oxygen of the environment, inducing decreases in the motility, membrane integrity and fertilizing potential of spermatozoa for AI [14–16]. The controlled and spontaneous release of molecular oxygen, forming low concentrations of ROS, is required for the maintenance of the fertilizing ability and capacitation/acrosome reaction of spermatozoa. Excessive ROS impairs motility and capacity of fertilization [17–19]. Additionally, cold shock arising from other stress plays an important role in the molding of membranes by determining their sol–gel balance and dynamic status that affects the fusion of the plasma membranes of the male and female gametes [20,21].

The antioxidant system comprising reduced glutathione (GSH), glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD), have been described as functioning as a defense against lipid peroxidation in semen, and are important in maintaining sperm motility and viability [22–24]. In recent years, studies have also been conducted on ram semen diluents, including additives such as, taurine, trehalose, selenium, and surfactant compounds, so as to improve post-thawed motility, viability and membrane integrity of spermatozoa [25–28].

Trehalose, a non-permeant disaccharide, has a protective action related both to osmotic effect and specific interactions with membrane phospholipids, rendering hypertonic media, causing cellular osmotic dehydration before freezing, and then decreasing the amount of cell injury by ice crystallization [29–32]. Amongst compounds of the epididymal and oviduct fluids, sulfonic amino acids (vg. taurine) are important protectors of cells against the accumulation of ROS, in case of exposure to aerobic conditions and the freezing/thawing process [33–35]. Thiols such as cysteamine and GSH are a large class of antioxidants. Cysteamine stimulates GSH synthesis during the in vitro maturation of oocytes, and promotes embryo development and quality [36]. The GSH is a ubiquitous molecule found at mM range in a number of cells, and is able to react with many ROS directly. The GSH is also a cofactor for GSH-Px that catalyzes the reduction of toxic H_2O_2 and hydroperoxides, protecting mammalian cells from oxidative stress, and the intracellular level of thiols in sperm is reduced by cryopreservation [23,37]. Hyaluronan, an essential component of the extracellular matrix and non-sulfated glycosaminoglycan, is involved in important physiological functions such as motility, capacitation of the spermatozoa [38,39], and preserves post-thaw spermatozoa viability and in vitro membrane stability [40].

To our knowledge, the use of cysteamine and hyaluronan in the cryopreservation of ram semen has not been reported. Therefore, this study was conducted in order to determine the influence of the addition of certain additives, including trehalose, taurine, cysteamine and hyaluronan, at different doses during cryopreservation, on standard semen parameters, lipid peroxidation, and antioxidant activities (GSH, GSH-Px, CAT, vit E) after thawing.