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The Effect of α-tocopherol on Plasma Membrane Integrity of Goat Spermatozoa

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ABSTRACT

The purpose of the study was to determine α-tocopherol supplementation on plasma membrane integrity. Semen was collected using artificial vagina from goat aged of 2 to 2.5 years in normal reproduction. The design used was randomized block design with treatment dose of a different α-tocopherol (0 g, 0.2 g, 0.4 g, 0.6 g) in 100 ml of extender. The results showed that the percentage of plasma membrane integrity before freezing at dose of 0.4 g (75.46%) was higher (P <0.05) compared with a dose of 0.0 g (73.78%), and dose of 0.6 g (72.93%), but did not differ (P> 0.05) with a dose of 0.2 g (75.46%). Plasma membrane integrity examination results at post thawing at dose of 0.4 g (73.16%)was higher (P <0.05) than a dose of 0.0 g (69.8%), 0.2 g (71.53%), 0.6 g (70.7 %) respectively. It was concluded that supplementation of α-tocopherol 0.4 g/100 ml extender was the best dose to maintain plasma membrane integrity of frozen semen of goat.
Keywords: α-tocopherol, Plasma membrane integrity, Goat, Spermatozoa

INTRODUCTION

One of the factors that influence the success of the artificial insemination application is the quality of frozen semen. It has been demonstrated that cryopreservation is associated with oxidative stress.[1][2]. Previous results showed that although goat spermatozoa were able to maintain motility after freezing to thawing about 40-60%, but only about 10-30% who do not have biological damage [3]. Peroxidation effects on sperm motility permanently suspected cover the loss, inhibition of fructolysis and respiration, intracellular enzyme binding and damage the plasma membrane structure, especially on the acrosome [4].

Moreover, freezing and thawing of sperm increase the reactive oxygen species (ROS) , producing DNA damage cytoskeleton alterations, inhibition of the sperm–oocyte fusion and affecting the sperm axoneme that is associated with the loss of motility [5].

Goat spermatozoa are sensitive to peroxidative damage due to the high content of unsaturated fatty acids in the phospholipids of the plasma membrane and the relative low antioxidant capacity of goat seminal plasma [6]. The formation of ROS generated by destruction of the plasma membrane caused a decrease in the ability of sperm motility and increases the damage that would affect morphology of sperm capacitation and acrosome reaction.

Efforts to minimize lipid peroxidation can use antioxidants that have the ability to reduce, extinguish or suppress free radical reactions [7]. α-tocopherol is one antioxidant that can reduce damage due to peroxidation [8]. Supplementation with α-tocopherol in semen diluent medium is expected to prevent emergence of free radicals during processing and storage of frozen semen so that it will maintain quality of frozen semen.

The purpose of the study was to determine concentration of α-tocopherol supplementation on plasma membrane integrity during cryopreservation of goat semen.

MATERIALS AND METHODS

a. Semen collection
Semen collected from goats aged from 2 to 2.5 years using an artificial vagina. Collecting semen was carried out once a week. Only samples with a minimum of 70% motile sperm and 80% morphologically normal spermatozoa were frozen.

b. Freezing and thawing
Andromeda extender was diluted using aquabidest with a ratio of 1: 4. Semen put in the straw, cooled for 2 h at 5^{o}C. Freezing was done by putting straw in the steam of nitrogen (N_2) of liquid for 10 min. and then stored for 24 h. thawing was done by dipping the straw into water for 30 sec.

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c. Evaluation of Plasma Membrane Integrity
Evaluation of Plasma Membrane Integrity is using a solution of Hypo-Osmotic Swelling (HOS) test. Observations using the HOS test conducted by testing 0.1 ml of semen in 1 ml solution of fructose and sodium citrate, then incubated 30-60 min and observed swelling of the tail with 400 x magnifications [9].

d. Research Design and Data Analysis
The design used was randomized block design with treatment dose of a different α-tocopherol, namely:
P1 = 0 g of α-tocopherol/100 ml extender
P2 = 0.2 g of α-tocopherol/100 ml extender
P3 = 0.4 g of α-tocopherol/100 ml extender
P4 = 0.6 g of α-tocopherol/100 ml extender
Each treatment was repeated 10 times. Data analysis using analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Cryopreservation of spermatozoa led to a series of adverse results marked by a decline in fertility. Among these changes damage, the integrity of the plasma membrane or acrosome cap is an indication of the greatest damage from the lost function. Membrane integrity is not only important for metabolism but also certain changes in membrane components, especially during fertilization. Plasma membrane damage would cause the loss of sperm motility and ability to conceive because of loss of cellular components and inactivation of proteins essential enzyme in the acrosome [10]. Percentage of plasma membrane integrity at freezing stage and dose α-tocopherol can be seen in Table 1.

Table 1 Percentage of Plasma Membranes Integrity and Freezing Stages According to dose α-Tocopherol

Freezing stages	**dose α-Tocopherol (g/100 ml)**			
	0.0	0.2	0.4	0.6
Before freezing (%)	73.78±2.52 [a]	75.64±1.37 [b]	75.46±3.17 [b]	72.93±3.55 [a]
Post thawing (%)	**69.80±3.20 [a]**	**71.53 ± 2.18 [b]**	**73.16 ± 1.96 [c]**	**70.7 ± 4.19 [a]**

Different superscript in the same row indicate significantly different ($p < 0.05$)

The results showed the percentage of plasma membrane integrity before freezing at a dose of 0.4 g (75.46%) was higher ($P < 0.05$) than 0.0 g (73.78%), and 0.6 g (72.93%), but did not differ ($P > 0.05$) with a dose of 0.2 g (75.64%). The equilibrated phase is carried out for 2 h at a temperature of 3-5^{0}C, predicted α-tocopherol has a role in warding off free radicals that are formed from the shelter and during dilution and equilibrated while in the diluent without α-tocopherol, there are no antioxidants that counteract free radicals resulting in increased damage due to peroxidation of sperm plasma membrane [11][12].

Results of radical chain reaction of lipid peroxidation peroxide only be stopped by an antioxidant that has the ability to break the chain reaction. At this stage, α-tocopherol slows the course of peroxidation reactions because of its ability to capture free radicals, break the peroxidation reaction by releasing hydrogen ions with electrons. Stability and radical formation tocopherol which was slower than the propagation or propagation of lipid peroxide radicals can suppress and slow the course of peroxidation chain reaction.

Doses of 0.2 g and 0.6 gr α-tocopherol have no optimal antioxidant effect when compared to 0.4 g , whereas at doses 0.6 g there was the possibility of negative effect of α-tocopherol that cause deterioration in the plasma membrane integrity was higher to 0.4 g dose ($P < 0.05$). Membrane damage also occurs due to cold stress. Primary membrane damage occurs during the freezing process at a temperature of 15 ° C to -60 ° C. The decreased quality of spermatozoa to cold stress due to temperature changes associated with the high ratio of saturated fatty acids and unsaturated phospholipids and low in cholesterol in membrane composition [13] and the structure of the membrane causes an increase in opportunities for membrane damage as a result of many hydrogen bonds are weakened and easily bound by free radicals. Once free radicals are formed, will lead to the formation of new free radicals through a chain reaction between the lipid proxies radical occurs [14]. The ongoing chain reaction of lipid peroxidation can affect membrane integrity because free radicals can react with membrane components, especially structural components, such as membrane proteins, so the damage take place not only at the plasma membrane but also on the internal cell [15]

Plasma membrane integrity examination at post thawing stage showed that the highest plasma membrane integrity at a dose of 0.4 g α-tocopherol (73.16%). Normal metabolic process will generate many free radicals, especially anion superoxide [16]. Initiation phase of free radical formation has been ongoing since the semen was collected and when the dilution occurred due to contact with oxygen. The formation of free radicals occurs very quickly without requiring any energy, so the percentage difference in value between retailers plasma membrane integrity supplemented α-tocopherol has been shown in this phase. Supplementation of α-tocopherol showed suppression effect against lipid peroxidation chain reaction. There was significant

effect on post thawing stage, probably because of a role in an optimal α-tocopherol as antioxidants in maintaining membrane integrity against lipid peroxidation reaction.

That phase propagation or propagation of free radical formation has taken place is indicated by the high decrease in the plasma membrane integrity to the dilution of fresh semen. Damage to the plasma membrane other than that due to peroxidation can be caused also by osmotic stress when exposed to a hypertonic medium. Cryoprotectant glycerol also has a direct protective effect on the plasma membrane. Glycerol is directly bonded with polar heads of membrane phospholipids and interacts with membrane proteins and induces the formation of membrane structures such as cleft border. This can lead to restructuring of the membrane and affect membrane fluidity due to increased side chain fatty acids [8]. Provision of α-tocopherol may play a role in supporting the role of glycerol as a cry protectant and a source of energy.

Decrease of percentage of plasma membrane integrity at before freezing stage until post thawing due to membrane damage, whether caused by cold stress, osmotic stress and damage caused by lipid peroxidation [17]. There was a rearrangement of the lipid membrane during cooling and thawing relations back so there was disorder of lipid-lipid and lipid-protein that disrupt membrane function. In this case, the role of glycerol as cryo protectant and maintains very large membrane flexibility to deal with the changes due to the cryopreservation process[18,19].

The negative effect of tocopherol 0.6 g on the plasma membrane integrity may be due to too high tocopherol concentrations that cause ineffective antioxidant action even becomes a prooxidant (free radicals) that precisely reproduce the formation of radicals. The type of phenolic antioxidants (such as tocopherol) in excessive concentration will lose its effectiveness as an antioxidant and even to form a prooxidant. Changes in antioxidant function become prooxidant or free radicals cause more unsaturated fatty acids that are subjected to free radicals . This situation further accelerates and expands the incidence of lipid peroxidation of sperm plasma membrane damage due to loss of some essential unsaturated fatty acids making up the membrane [20] This tendency was reinforced by the fact that the content of MDA as a lipid peroxidation product that is toxic to spermatozoa was found higher at a dose of 0.6 g tocopherol compared to the three other doses [21]

CONCLUSION

Supplementation of α-tocopherol in semen extender had effect on the plasma membrane integrity of sperm. Dose of α-tocopherol 0.4 g/100 ml extender was the best dose to maintain plasma membrane integrity of frozen goat semen.

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