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Supplementation Of α Tocopherol On Plasma Membrane Integrity Of Goat Sperm After Freezing.

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ABSTRACT

Problems that often arise in the process of freezing semen is destruction of the plasma membrane because the sperm membrane contains unsaturated fatty acids are highly susceptible to peroxidation damage. α-tocopherol is an antioxidant that reduce damage due to peroxidation. The purpose of the study was to determine α-tocopherol antioxidant supplementation in diluter on plasma membrane integrity after freezing process. Semen was collected using artificial vagina from goat aged 2 to 2.5 years in normal reproduction. The design used was randomized block design with treatment dose of a different α-tocopherol (0 grams, 0.2 grams, 0.4 grams, 0.6 grams) in 100 ml of extender. The results showed that the percentage to plasma membrane integrity equilibrated at a dose of 0.4 g (75.46%) was higher (P <0.05) compared with a dose of 0.0 g (73.78%), and the 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 antioxidant α-tocopherol 0.4 ml is the best dose to maintain membrane integrity of semen frozen of goat..

KEYWORDS

Antioxidant, Plasma membrane integrity, Goat Semen

INTRODUCTION

One of the factors that influence the success of the artificial insemination (AI) application is the quality of frozen semen. It has been demonstrated that cryopreservation is associated with oxidative stress.(Chatterjee, 2001). Previous results showed that although goat spermatozoa to maintain motility after freezing to thawing about 40-60%, but only about 10-30% who do not have biological damage (Gadea, 2005). 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 (Kumar et al., 2003).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 (Breininger et al.,2005). 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 (Watson et al., 2000). The formation of ROS generated by destruction of the plasma membrane caused a decrease in the ability of sperm motility and increase 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 (Agarwal et al., 2005). α-tocopherol is one antioxidant that can reduce damage due to peroxidation (Munsi et al., 2007). 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 done once a week. Only samples with a minimum of 70% motile and 80% morphologically normal spermatozoa were frozen.

b. Freezing and thawing
Materials used semen extender Andromed diluted using aquabidest with a ratio of 1: 4. After semen put in the straw, cooled for 2 hours at 5oC. Freezing is done by putting straw in the steam of nitrogen (N2) of liquid for 10 minutes. (Temperature-140oC) and then lowered until it touches the surface of liquid nitrogen, immersed and stored for 24 hours. Thawing was done by dipping the straw into room temperature water for 30 seconds.

c. Evaluation of Plasma Membrane Integrity
Evaluation of Plasma Membrane Integrity using a solution of Hypo-Osmotic Swelling (HOS) test. Observations using the HOS test conducted by testing 0.1 ml of cement in 1 ml solution of fructose and sodium citrate, then incubated 30-60 min and observed swelling of the tail with 400 x magnification (Jayendra et al., 1984; Lechniak et al., 2002)

d. Research Design and Data Analysis
The design used was randomized block design with treatment dose of a different α-tocopherol, namely:
P1 = 0 grams α-tocopherol/100 ml extender
P2 = 0.2 grams of α-tocopherol/100 ml extender
P3 = 0.4 grams of α-tocopherol/100 ml extender
P4 = 0.6 grams of α-tocopherol/100 ml extender
Each treatment was repeated 6 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 acrosomal 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.. (Dorado et al., 2010). Mean percentage of Plasma membrane integrity of spermatozoa at freezing stage and dose α-tocopherol can be seen in Table 1.

Table 1. Mean Percentage of Plasma Membranes Integrity and Freezing Stages According 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)

Examination results showed the percentage plasma membrane integrity equilibrated at a dose of 0.4 g (75.46%) was higher (P <0.05) compared with a dose of 0.0 g (73.78%), and a dose of 0.4 g (72.93%), but did not differ (P> 0.05) with a dose of 0.2 g (75.46%). The equilibrated phase is carried out for 2 hours at a temperature of 3-50C, 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 that was not α-tocopherol (dose 0.0 g), no there are antioxidants that counteract free radicals resulting in increased damage due to peroxidation of plasma membrane of spermatozoa (Meseguer et al., 2004).

Results of radical chain reaction of lipid peroxidation peroxide can only be stopped by an antioxidant that has the ability to break the chain reaction. At this stage, α-tocopherol slow 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.

At doses of 0.2 g, α-tocopherol appears not to have optimal antioxidant effect when compared to 0.4 g dose, whereas at a dose of 0.6 g the possibility of negative influence of α-tocopherol that cause deterioration in the plasma membrane integrity a higher percentage, compared to 0.4 g dose (P <0.05). At this stage equilibrated membrane damage also occurs due to cold stress as presented by White (1993), that the primary membrane damage occurs during the freezing process at a temperature of 15 ° C to -60 ° C. There is a substance that is missing from the total plasma membrane phospholipids of spermatozoa during sudden cooling to a temperature -79 ° C and melting time again. 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 (White, 1993) 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 peroxy radical occurs. 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 can take place not only at the plasma membrane but also on the internal cell .

Plasma membrane integrity examination results at this stage of post thawing stage , revealing the highest plasma membrane integrity values remain at a dose of 0.4 g (73.16%), higher (P <0.05) compared with a dose of 0.0 g (69.8%), 0.2 g (71.53%), 0.6 g (70.7 %).

Normal metabolic process will generate many free radicals, especially onion superoxide (O2). 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. Diluent in α-tocopherol supplementation showed suppression effect against lipid peroxidation chain reaction. There is significant effect on the dilution stage, probably because this stage play a role in an optimal 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 interact with membrane proteins and induce 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 (Munsi et al., 2007). Provision of α-tocopherol may play a role in supporting the role of glycerol as a cryoprotectant and a source of energy.

Higher percentage decline against the plasma membrane integrity from dilution until post thawing because of membrane damage, whether caused by cold stress, osmotic stress and damage caused by lipid peroxidation. According to Parks and Graham (1992), there was a rearrangement of the lipid membrane during cooling and thawing relations back so there is disorder of lipid-lipid and lipid-protein that disrupt membrane function. In this case, the role of glycerol as cryoprotectant and maintain very large membrane flexibility to deal with the changes due to the cryopreservation process. Changes in the shape of the solid phase into liquid phase, a change from the freezing temperature to a temperature below 0 ºC and metabolic activity that took place since contact with oxygen during post thawing are all factors that could cause damage to the plasma membrane and death of spermatozoa.

The negative influence on tocopherol 0.6 g for the plasma membrane integrity may be due to too high tocopherol concentrations that cause ineffective antioxidant action even become a prooxidant (free radicals) that precisely reproduce the formation of radicals. This condition is in accordance with Fouad (2009) who states that the type of phenolic antioxidants (such as tocopherol) in excessive concentration will lose its effectiveness as an antioxidant and even to form a prooksidan. Changes in antioxidant function becomes prooxidant or free radicals cause more unsaturated fatty acids that are subjected to free radicals. This situation further accelerate and expand the incidence of lipid peroxidation of sperm plasma membrane damage due to loss of some essential unsaturated fatty acids making up the membrane. This tendency is reinforced by the fact that the content of MDA as a lipid peroxidation product that is toxic to spermatozoa ever found at a dose of 0.6 g tocopherol compared to others.

CONCLUSIONS and SUGGESTION

Supplementation antioxidant α-tocopherol 0.4 ml of Andromed diluent able to maintain optimal plasma membrane intact frozen goat semen

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REFERENCES

[1] Agarwal, S.A. Prabakaran, T.M. Said. 2005. Prevention of oxidative stress injury to sperm. J. Andrology 26 : 654-660

[2] Bilodeau. J.F., S. Chatterjee, M.A. Sorard and C. Gagnon. 2000. Levels of antioxidants defences are decreased in bovine spermatozoa after a cycle of freezing and thawing. Mol.Reprod Dev.55:282-288.

[3] Breininger E, N.B.Beorlegui, M.T.Beconi, 2005. Alpha-tocopherol improves biochemical and dynamic parameters in cryopreserved boar semen. Theriogenology 63 : 2126–2135

[4] Chatterjee S., Gagnon C. 2001. Production of reactive oxygen species by spermatozoa undergoing cooling, freezing and thawing. Mol Reprod Dev 59:451–458.

[5] Cerolini S, Maldjian A, Surai P, Noble R. 2000. Viability, susceptibility to peroxidation and fatty acid composition

of boar semen during liquid storage. Anim Reprod Sci 58: 99–111.

[6] Dorado, J., A.M. Serrano and M. Hidalgo. 2010. The effect of cryopreservation on goat semen characteristics related to sperm freezability. J. Reprod. Sci 121 : 115-123

[7] Fouad, T. 2009. Antioxidants, nature and chemistry: Non-enzymatic antioxidants alpha tocopherol (Vitamin E). www.doctorslounge.com / primary / articles / antioxidants / antioxidants5.html

[8] Gadea. J., FG. Vasquez, C. Matas, J.C. Gardon, S. Canovas and D. Gumbao. 2005. Cooling and freezing boar spermatozoa: suplementation of the freezing media with reduce gluthatione preserves sperm function. J. Andrology.6:3.

[9] Janice,L., Bailey, N. Cormier. 2000. Semen cryopreservation in domestic animals : A damaging and capacitating phenomenon. J. Andrology vol. 21. no.1.

[10] Chatterjee S., Gagnon C. 2001. Production of reactive oxygen species by spermatozoa undergoing cooling, freezing and thawing. Mol Reprod Dev 59:451–458.

[11] Jayendra, R.S and L.J.D. Zaneceld. 1984. Instructions for hypoosmotic swelling test (HOS) semen analysis. Reproductive Resources Centre. Lab Grant Hospital of Chicago

[12] Lechniak, D., A. Kedzierski and D. Stanislawski. 2002. The Use of HOS test to evaluate membrane functionality of boar sperm capacitated In vitro. Reproduction in Domestic Animals., Vol.37, No.6. (http://www. blackwellpublishing.com.)

[13] Kumar,S., J.D.Millar and P.F.Watson. 2003. The effect of cooling rate on survival of cryopreserved bull,ram and boar spermatozoa : A comparison of two controlled-rate cooling machines. Cryobiology 46 : 246-253.

[14] Meseguer, M. N. Garrido , C. Simon, A. Pellicer and J. Remohi. 2004. Concentration of glutathione peroxidases 1 and 4 in fresh sperm provide a forecast of the outcome of Cryopreservation of Human Spermatozoa. Journal of Andrology vol 25 : 773-780.

[15] Munsi, M.N., MMU. Buiyan, M.G.S. Alam. 2007. Effects of Exogenous Glutathione on the Quality of Chilled Bull Semen. Reproduction in Domestic Animal Vol 42:358-362.

[16] Nur. Z.., I. Dogan, U. Gunay, and M. K. Soylu. 2005. Relationships between Sperm Membrane Integrity and other Semen Quality Characteristics of the Semen of Saanen Goat Bucks. Bull Vet Inst Pulawy. 49: 183-187.

[17] Parks JE, Graham J.K. 1992. Effects of cryopreservation procedures on sperm membranes. Theriogenology;38:209–22.

[18] Sum. A.K., R. Faller and J.J. de Pablo. 2003. Molecular Simulation Study of Phospholipid Bilayer and Insights of the Intertactions with Disaccharides. J. Biophys.85:2830-2844.

[19] Watson,P.F. 2000. The causes of reduced fertility with cryopreserved semen. Anim Reprod Sci ;60–61:481–92.