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Supplementation of Cysteine on Plasma Membrane Integrity of Buck Spermatozoa

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ABSTRACT: The purpose of the study was to determine Cysteine supplementation in the extender to plasma membrane integrity. Semen was collected using artificial vagina from buck aged 2 to 2.5 years in normal reproduction. The design used was randomized block design with ten replication. Semen collection was done once a week. Only fresh semen with a minimum of 70% motile sperm and 80% morphologically normal were used in this research. Andromed as a based extender was diluted using aquabidest with a ratio of 1:4. The treatment was different concentration of Cysteine as follows : 0.0 mM (P0); 0.5 mM (P1), 1.0 mM (P2) and 1.5 mM (P3). Data analysis using analysis of variance (ANOVA). If there were any differences, Duncan test will used for further analysis The results showed that the percentage plasma membrane integrity on before freezing at 1 mM (75.64%) was higher (P <0.05) compared with a dose of 0.0 g (73.78%), and dose of 0.5mM (72.93%), but did not differ (P> 0.05) with a dose of 0.5 mM g (75.46%). Plasma membrane integrity on post thawing for P2 (73.16%)was higher (P <0.05) than P0 (69.8%), P1 (71.53%) and P3 (70.7 %). It was concluded that supplementation of Cysteine 1.0 mM is optimum concentration to maintain plasma membrane integrity of buck frozen semen.

Keywords: Andromed, Cysteine, Plasma membrane integrity, Semen,Buck

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 (Bergeron *et al.*, (2006). Previous results showed that although buck spermatozoa have motility after freezing to thawing about 40-60%, but only about 10-30% who do not have biological damage (Gadea, 2005). Moreover, freezing and thawing of sperm will increased 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). Sperm were 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. 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 was use antioxidants that have the ability to reduce, extinguish or suppress free radical reactions (Agarwal *et al.*, 2005). Supplementation of Cysteine in Andromed was expected to prevent free radicals during processing and storage of frozen semen so that it will maintain quality of frozen semen. The purpose of this study was to determine effect of Cysteine supplementation on plasma membrane integrity.

MATERIALS AND METHODS

Semen collection and Semen dilution

Semen was collected from buck aged from 2 to 2.5 years using an artificial vagina. Semen

collection was done once a week. Only fresh semen with a minimum of 70% motile sperm and 80% morphologically normal were used in this research. Andromed as a based extender was diluted using aquabidest with a ratio of 1: 4. Cysteine in different concentration was supplemented in based extender as follows : 0.0 mM (P0); 0.5 mM (P1), 1.0 mM (P2) and 1.5 mM (P3)

Freezing and thawing procedure

Semen put in the straw with concentration 75 million/ml, cooled for 2 h at 5°C. Freezing is done by putting straw in the steam of nitrogen (N2) liquid for 10 min. and then stored for 24 h. Thawing was done by dipping the straw into water for 30 sec.

Evaluation of plasma membrane integrity

Evaluation of plasma membrane integrity using a solution of Hypo-Osmotic Swelling (HOS) test as follows : 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 magnification.

Research Design and Data Analysis

The design used was randomized block design. Each treatment was repeated ten times. Data analysis using analysis of variance (ANOVA). If there were any differences, Duncan test will used for further analysis

RESULTS AND DISCUSSION

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 because of loss of cellular components and inactivation of proteins essential enzyme in the acrosome. (Dorado *et al.,* 2010). Percentage of plasma membrane integrity of spermatozoa at freezing stage and dose Cysteine can be seen in Table 1.

Table 1. Percentage of Plasma Membranes Integrity and Freezing Stages

Freezing stages	Concentration of Cysteine **(mM)**			
	0.0	**0.5**	**1.0**	**1.5**
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 that the percentage plasma membrane integrity at Cysteine concentration of 1.0 mM (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 0.5 mM (75.46%). The equilibrated phase was carried out for 2 h at a temperature of 3-50C, predicted Cysteine has a role in protection on plasm membrane (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. Optimal concentration of Cysteine as antioxidant effect was to 1.0 mM, whereas at concentration of 1.5 mM have the possibility of negative influence of Cysteine that cause deterioration in the plasma membrane integriy. Membrane damage also occurs due to cold stress as presented by Watson (2000), that the 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 and the structure of the membrane causes an increase in opportunities

for membrane damage as a result of many hydrogen bonds are weaken 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 (Kumar *et al.*, 2003). 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 (Chatterjee and Gagnon.,. 2001;Fonseca *et al.,* 2005).

Normal metabolic process will generate many free radicals, especially onion superoxide (Zhao *et al*., 2009). Initiation phase of free radical formation has been ongoing since the semen was collected and when the dilution occurred due to contact with oxygen. Cysteine 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 Cysteine as antioxidants in maintaining membrane integrity against lipid peroxidation reaction.

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 which lead to restructuring of the membrane and affect membrane fluidity due to increased side chain fatty acids (Cerolini *et al.*,2000; Munsi *et al*., 2007).

The negative effect of Cysteine 1.5 mM on the plasma membrane integrity may be due to too high Cysteine concentrations that cause ineffective antioxidant action even become a prooxidant (free radicals) that precisely reproduce the formation of radicals. Changes in antioxidant function becomes prooxidant or free radicals cause more unsaturated fatty acids that are subjected to free radicals (Nur *et al.*, 2005). 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.

CONCLUSIONS

Cysteine concentration in Andromed extender will affect the integrity of the plasma membrane. Cysteine supplementation with 1.0 mM concentration is most well maintain the integrity of the plasma membrane.

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