The Quality of Chilled Fat Tail Sheep Ram's Semen with Antioxidant Addition, Vitamin C and Vitamin E In Citrate Egg Yolk Extender

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ABSTRACT

The study was conducted to compare the effect of antioxidant supplementation in citrate egg yolk (CEY) extender on quality of chilled fat tail sheep (FTS) ram semen under field condition. This study used Citrat egg yolk (CEY) extender with the addition of antioxidant Vitamin C (0.1 gr/100 ml) and Vitamin E (0.4 gr/100 ml). Semen was collected from FTS ram using artificial vagina. Only semen has motility 20% and abnormality under 20% were used for frozen semen process. Collected semen was divided into three tubes and extended with CEY (PO), CEY+Vitamin C (P1), CEY+Vitamin E (P2). The extended semen samples were chilled to 4°C, and observed four hours after stored in refrigerator. The result indicated PO had a greater quality than other treatment ($P<0.01$) on the motility, abnormality and viability of chilled ram semen, but no significant different in membrane integrity. In conclusion, Citrat egg yolk added with antioxidant Vitamin C and Vitamin E has not provided a protective effect in early hours of storage. Moreover, the addition of antioxidants could a slightly decreased the semen quality after dilution process.

Keywords: Citrate egg yolk, Antioxidant, DEG, Chilled semen

INTRODUCTION

Fat tail sheep rams is one of an important Indonesian breed for meat production, characterized by enlarges in edge of tail containing lipid and narrowed in the end of the tail. One of reproduction problem that FTS ram has a poor fertility, it caused by big tail hinder mating process. Application of artificial insemination (AI) can be a solution to overcome that problem. AI which is performed by using fresh or cooled Storage in 1 C can be alternative cryopreservation when AI performed within a short time. An acceptable fertility of cryopreserved semen still has been a challenge. Forming reactive oxygen species (ROS) leads a detrimental effect to spermatozoa during the chilling process. The main target of ROS is cell membrane and induced lipid peroxidation which can damage sperm membrane structure and changes the concentration of lipid structure. Lipid peroxidation can be inhibited by the supplementation antioxidants that can bind to the peroxidation (Hammersted, 1993). The antioxidants commonly used are vitamins C and vitamin E (Herdis, 2002).

Vitamin C represents the major water5soluble antioxidant in plasma. Ascorbic acid is required in vivo as a cofactor for at least eight enzymes and can also act as an antioxidant by reacting with free radicals (Michael et al., 2002). The concentration of vitamin C in seminal plasma is 10 times greater than in blood plasma (364 vs. 40 µmol L51) (Gangwar et al, 2011). Vitamin E, a lipophilic molecule present in the cell membrane, is considered as both a membrane5stabilizer and a potent antioxidant molecule protecting cell membrane against lipid peroxidation and ROS attacks (Urano et al., 192x, 1922; Niki et al., 2004).

Recent studies reported that addition antioxidant vitamin C 0.151 g/100 ml extender (Hasan et.al, 2014) and vitamin E 0.4 g/100 ml extender (Alawiyah and Hartono, 2006) can prevent the quality of frozen spermatozoa. Therefore, the study need to know the effects of various antioxidants toward quality on chilled FTS ram semen.

MATERIALS AND METHODS

Semen collection

FTS rams (aged 2 years) were used in this study, nine ejaculates were collected twice a week. Semen was collected in the morning used artificial vagina and immediately transferred to the laboratory for macroscopic and microscopic observation. Volume ejaculate was determined by the scale in the collecting tube. Thereafter, semen observed by a microscope to determine the motility and concentration of sperm cell.

Semen processing and evaluation

Only semen with minimum concentration 3710^9 sperm cell/ml and 20% motility were a process to make a chilled semen. The ejaculates were treated by three treatments: CEY (P0); CEY+vitamin C (P1); CEY+vitamin E (P2), respectively. The composition of CEY extender based on Paulenz et al (2002) consist 2.9 g natrium sitrat, 0.1 g fructose, 20% egg yolk and dissolved in 100 ml distillated water. Vitamin C were added to 0.2 g/100 ml and 0.4 g/100 ml for vitamin E extender. Semen was diluted with an extender to a final concentration 100710^6 sperm/ml. Semen was loaded into a tube and equilibrated at refrigerator (1°C) for four hours. After equilibration, semen were analyzed motility, viability, abnormality, and membrane integrity. Sperm motility was measured subjectively by putting drop semen under coverslip, then counting live and death sperm from minimum five microscopic fields. The mean of five microscopic fields recorded as the motility. The percentage of viability measured using eosin nigrosin staining. Sperm suspension was mixed with stain and spreading on the slide, the viability was assessed by counting alive (colorless) and death cell (absorb stain) about 200 cells. The morphologically normal spermatozoa were measured using eosin nigrosin stain (Evan and Maxwell, 1927). Membrane integrity was measured by using HOS test (100 mOsm) consisting of 9 gr fructose, 4.9 gr sodium citrate and diluted in 100 ml distillated water. 10 µl semen diluted with 100 µl HOS solution in eppendorf tube then incubated at 37°C for 60 min. 200 cell counting to determine percentage of cell membrane integrity, sperm with swollen tail were classified to undamaged membrane.

Statistical analyse

Results are presented as mean±SD. Motility, abnormality, viability, and membrane integrity in each treatment were obtained and were analysed by using analyse of variance (ANOVA). Significant differences ($P<0,01$) among treatment were determined using Duncan multiple range test.

RESULTS AND DISCUSSION

The effect of addition antioxidant diluted with CEY extender on sperm motility, abnormality, viability, and plasma membrane integrity in FTS ram are presented in Table 1. Addition of antioxidant influenced chilled semen and negatively affected most of in vitro parameters at early hour of cryopreservation. The higher motility was yielded from control group than other treatments. The using of vitamin C and vitamin E influenced negatively and showed significantly lower values for percentage of motility, viability, and abnormality. Results of the present experiment are presented in Table 1.

Table 1. Quality of FTS Rams Fresh Semen

Variable	Mean
Volume (ml)	1.27±0.22
Color	cream
Consistency	solid
pH	6.2
Sperm concentration (7 10^7 sel/ml)	322.2±2.13
Mass	+++
Motility (%)	20.224±1.73
Abnormality (%)	4.706±1.64
Temperature during collecting	22.22±1.06
Humidity during collecting	72.2±1.21

Table 2. Quality of FTS Ram chilled semen after 4 hour storage

variable	Fresh semen	Treatment		
		Cey control	Cey+vit.c	Cey+vit.e
Motility	20.224± 1.73	71.10 ±1.12[b]	73.63± 1.27[a]	73.90± 1.27[a]
Abnormality	4.70 ±1.64	6.17± 1.41[a]	1.21± 0.20[a]	7.22± 0.91[b]
Viability	22.196 ± 2.91	77.11± 1.29[b]	77.12± 1.32[b]	71.62± 1.62[a]
Membrane integrity	73.176 ± 3.16	62.39 ±1.11[a]	66.01 ±3.22[a]	62.91± 2.06[a]

[ab] Values in rows with different superscripts differ significantly ($P < 0.01$).

The percentage of total motility (TM), abnormality (AB), viability (VI), and membrane integrity (MI) were measured. Results showed that the TM, AB, VI, and MI were 21.03±1.31, 1.26±0.21, 27.11±3.90, 74.26±1.21 respectively in fresh semen. The results in TM, AB, VI was shown have significant difference was observed between antioxidant tested treatments. However, no significant difference in MI resulted in this study compared with control group.

Oxidative stress is a condition associated with increased production of free oxygen radicals known as reactive oxygen species (ROS). The approach of this study consisted on protection against oxidative stress using vitamin C and vitamin E as an antioxidant. Sperm membrane is susceptible to oxidative stress, especially for ram semen due to high proportion of polyunsaturated fatty acid (Muinoblanco et al, 2002). Spontaneous lipid peroxidation membrane destructs matrix lipid of membrane and leading factor of impairment sperm function (Gangwar et al, 2011).

Vitamin C is present in seminal plasma of many species and water soluble antioxidant (Azawi et al, 2013). Ascorbic acid also required as a cofactor for approximately eight enzymes and 10 times greater in seminal plasma then in blood plasma (Gangwar, 2011). Ascorbic acid gives protected of membrane by catched superoxide anion and singlet oxygen thus it can inhibit chain reaction. Higher concentrations of vitamin C (2.1mM) gave a harmful evidence to motility in frozen5thawed bull semen (Beconi et al, 1993). The similarity with Sonmez et al (2001) that supplementation vitamin C more than 2 mg/ml diluted semen has a negative effect on quality ram semen under liquid preservation. It can be explained that vitamin C is acidic (pH2) thus it can slightly reduce pH of diluter.

Vitamin E is hydrophobic molecule and present in the cell membrane, that maintain stabilize of membrane and also act as antioxidant by protecting cell from lipid peroxidation (Benhenia et al, 2016). Vitamin E is not synthesized through the seminal plasma, so it can supplemented in semen extender. Several studies reported that supplemented vitamin E give

more protection against lipid peroxidation (Niki et al, 2004). Moreover, efficacy of vitamin E depends on their accumulation in cell membrane.

In the present study, supplementation antioxidant in sperm dilutor by vitamin C and vitamin E had significantly lower value of TM, AB, VI when compared to control group. It can explain that concentrations of ROS still below concentrations that can induce damaging in early hour of liquid storage. It is also known that both enzymatic and non5 enzymatic antioxidant comprising superoxide dismutase (SOD), gluthatione reductase (GR), gluthatione peroxidase (GP7), and catalase presence in seminal plasma provided a defense against lipid peroxidation (Muioblanco et al, 2002). Otherwise, supplementation antioxidant vitamin C and vitamin E directly causing cell death, both of them have a different mechanism to damage sperm cell. Vitamin C is acidic (pH2) that can slightly reduce pH of diluter, lower pH value proved harmful for sperm cell due to sensitively toward pH changes especially acidic environment. While, vitamin E proved to cause medium more hypertonic, moreover hypertonic medium induce imbalance of cell intracellular and extracellular fluid also reduction of supplied energy due to impaired metabolism process (Hartono, 2002).

CONCLUSIONS

An addition of vitamin C and vitamin E are not useful to hold the quality after dilution. The protecting mechanism has not been seen in the early hour of storage.

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