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Research Article

Effect of Vitamin C and E Supplementation and Combination of Both in Egg Yolk Tris Diluter on the Quality of Sapera Goat Spermatozoa in the 5 °C Cooling Process

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ABSTRACT

This study was aimed to determine the effect of vitamin C, E supplementation and both combination in the egg yolk tris diluent on the quality of Sapera goat spermatozoa in 5˚C cooling process. The samples were fresh semen collected from Sapera goat. It was divided into four different treatment groups. The control treatment or P0 contained semen sample + egg yolk tris diluent, P1 contained semen sample + egg yolk tris diluent + vitamin C 0,9 mg/ml, P2 contained semen sample + egg yolk tris diluent + vitamin E 1 mg/ml, P3 contained semen sample + egg yolk tris diluent + vitamin C 0,9 mg/ml + vitamin E 1 mg/ml. The least decrease of spermatozoa motility, viability and membrane integrity was found in P3, which was significantly different to P0 ($p<0,05$). However, P1 and P2 did not show significant difference in the results of motility and viability evaluation ($p>0,05$). It can be concluded that the combination of vitamin C and E supplementation can be an optimal antioxidants supplement in egg yolk tris diluent on the quality of Sapera goat spermatozoa in 5˚C cooling process.

Key words: Sapera Goat, Vitamin C, Vitamin E, Quality of Spermatozoa, 5˚C Cooling Process.

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INTRODUCTION

The increase in population in Indonesia is directly proportional to the need for food and the need for animal proteins such as eggs, meat and milk.[1] Food products that have high nutritional value and have a great opportunity to be developed, one of which is milk. Consumption / needs of fresh milk and its derivative products are estimated to continue to increase in line with population growth, economic growth, improvement in education levels, nutritional awareness and lifestyle changes.[2] Ditjennak,[2] said that national milk production in 2016 was recorded at 852,951 thousand tons, an increase of 2.13% compared to production one year earlier. Domestic fresh milk production from goats to date is around 24.8% of national needs.[3] Some types of dairy goats that can produce milk in Indonesia include Peranakan Etawa, Saanen and Sapera (saanen cross with Peranakan Etawa).[4] Sapera goats produce milk 2-4 liters / day higher than PE goats which vary between 2-3 liters / day.[5] Milk production will increase along with the increase in the population of Sapera goats. One of the efforts to increase the sapera goat population is with artificial insemination (IB) reproductive technology. Artificial Insemination (IB) is a technique of introducing semen using a special device called an insemination gun into the female reproductive tract.[6]

The IB program in its implementation requires a good supply of cement in quality as well as the quantity of livestock. The way that can be done to minimize the decline in cement quality during storage is by diluting cement using an additional qualified diluter. Semen diluters generally use egg yolk Tris because they have met the dilution requirements, namely containing nutrients for spermatozoa, protecting the spermatozoa membrane from cold shock, and buffering.[7]

Artificial Insemination (IB) in goats the success rate is still very low in Indonesia.[8] This can be caused by the low quality of frozen semen which is characterized by a decrease in the motility of spermatozoa after thawing.[9] The decrease occurs due to an increase in free radicals that trigger lipid peroxidation.[10] The addition of antioxidants to the diluent material can minimize damage to the spermatozoa membrane due to lipid peroxidation during the cooling process.[11]

Some studies mention that vitamin C and vitamin E in the diluter can maintain the quality of spermatozoa. According to Lubis *et al*,[12] vitamin C is able to ward off free radicals and prevent the occurrence of a free radical reaction chain, so as to avoid damage to lipid peroxidation which affects the viability, motility and fertility of spermatozoa). According to Sitohang *et al*,[13] in their research mentioned vitamin E is the most effective antioxidant, because vitamin E is fat-soluble and very important because most of the damage by free radicals occurs in cell membranes and low-density lipoproteins and all of these things are made of fat molecules. Research conducted by Pavlovic *et al*,[14] vitamin C together with vitamin E can inhibit oxidation reactions by binding to vitamin E radicals formed in the process of breaking free radical reactions by vitamin E into free vitamin E which functions again as antioxidants. With different mechanisms of action between vitamins C and E, if these two vitamins are used it can inhibit the activity of free radicals.

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MATERIAL AND METHODS

This study used Sapera goat semen as research material that was accommodated from 1 male Sapera goat aged ± 2.5 years which was taken 2 times a week using an artificial vagina. This research was carried out in June 2020, sampling was carried out at the Bumi Kesilir Farm farm, Banyuwangi and the research was carried out at the Instrument Laboratory of Universitas Airlangga PSDKU Banyuwangi. The materials used in the examination in this study were both macroscopic and microscopic examinations, namely Physiological NaCl, Eosin-Nigrosin, glycerol, egg yolk tris, citric acid, fructose, penicillin, streptomycin, vaseline, aquades and warm water. The tools used in this study include artificial vaginas, scaled tubes, refrigerators, test tubes, micropipettes, binocular microscopes, tricocular microscos, glass objects, glass covers. Semen shelter is carried out using an artificial vagina. Macroscopical inspection of fresh cement includes the volume, consistency, color, smell and degree of acidity of the cement. Microscopic examination of fresh semen includes motility, viability and abnormalities of spermatozoa.

This study is an experimental study using the Complete Randomized Design (RAL) method. This study used 4 treatments with 5 times ualnagan. P0 as a control group with cement and diluter tris yolk without the addition of vitamin C and vitamin E. P1 as a treatment group with cement and diluter tris yolk and the addition of vitamin C 0.9 mg / ml, P2 as a treatment group with cement and diluter tris yolk and the addition of vitamin E 1 mg / ml and P3 as a treatment group with semen and diluter tris yolk with the addition of vitamin C 0.9 mg / ml and vitamins E 1 mg/ml. The cement sample is then stored at 5°C. The examination includes motility, viability and the intact plasma membrane of spermatozoa. The data obtained were analyzed using the Multivariate Analysis of Variants (Manova), if there is a significant difference ($p<0.05$) then continued with the Duncan Test.

RESULTS

The sapera goat fresh cement examination which includes macroscopic and microscopic examinations is carried out to determine the feasibility of the sample before being treated. The results of macroscopic and microscopic examination of Sapera goat cement can be seen in table 1 and the results of the influence of vitamin C and E supplementation and the combination of the two in the time period can be seen in table 2.

A picture of the viability of the spermatozoa of Sapera goats can be seen in Figure 1 which has been colored using *Eosin-negrosin*. A picture of the intact plasma membrane of the Spermatozoa of sapera goats can be seen in Figure 2

DISCUSSION

The average volume of Sapera goat semen produced in this study was higher than the previous study of 0.94 ml.[15] According to Syawal,[16] in his research stated that the volume of goat semen ranges from 0.5 - 2 ml with an average of 1 ml. Tambing *et al*,[17] stated that the varying volume of cement was caused by several factors, namely the way the cement was taken, the frequency of storage, the age and the nation of livestock. Hidayati,[9] stated that the color of semen is often associated with consistency (viscosity) and concentration of spermatozoa, if the semen is thicker, the higher the concentration. The consistency of fresh semen of Sapera goats in this study is thick, cream-colored and has a distinctive goat smell, namely prengus. This agrees with Hastono *et al*;[18] Hidayati,[9] who said that the consistency of sapera goat cement is thick, cream-colored and smells of prengus. The acidity (pH) of fresh

Tabel 1: The quality of fresh semen of Sapera goats.

Quality	Average Defiation Standards
Macroscopic	
Volume (ml)	1.05 ± 0.05
Smell	Typical Goats
Color	Beige
Consistency	Viscous
Ph	6.6 ± 0.49
Microscopic	
Motility (%)	84.00 ± 1.10
Concentration (million/ml)	3.888 ± 116.34
Viability (%)	88.8 ± 0.98
Mass Movement	+++
Secondary Abnormalities (%)	3.40 ± 0.8

Table 2: Results of the effect of Vitamin C and E supplementation and the combination of the two in a period of time.

Examination results	Treatment	Average Defiation Standards (%)			
		24 H	48 H	72 H	96 H
Motility	**P0**	70.40^{a} ± 0.9	59.60^{a} ± 0.9	49.00^{a} ± 2.0	38.60^{a} ± 1.7
	P1	73.80^{b} ± 1.9	63.00^{b} ± 2.2	53.80^{b} ± 1.6	41.60^{b} ± 1.1
	P2	74.60^{c} ± 1.5	63.40^{b} ± 3.3	53.00^{b} ± 3.0	41.60^{c} ± 1.1
	P3	77.60^{d} ± 2.0	67.80^{c} ± 0.8	57.20^{c} ± 1.6	47.20^{d} ± 1.5
Viability	**P0**	74.60^{a} ± 1.7	63.80^{a} ± 1.9	54.00^{a} ± 1.6	43.40^{a} ± 2.1
	P1	79.80^{b} ± 1.3	70.20^{b} ± 3.1	58.60^{b} ± 2.1	47.60^{b} ± 1.7
	P2	80.60^{c} ± 1.7	71.20^{c} ± 2.9	59.40^{c} ± 2.6	48.20^{c} ± 2.1
	P3	83.20^{d} ± 1.5	74.60^{d} ± 1.8	63.00^{d} ± 2.0	52.00^{d} ± 1.9
Intact plasma membrane	**P0**	72.20^{a} ± 0.8	61.60^{a} ± 0.5	52.60^{a} ± 1.5	41.60^{a} ± 1.8
	P1	76.00^{b} ± 1.6	66.60^{b} ± 2.4	56.00^{b} ± 1.6	44.20^{b} ± 1.6
	P2	77.60^{c} ± 1.5	67.20^{c} ± 3.3	56.80^{b} ± 2.6	46.20^{c} ± 1.6
	P3	**80.80^{d} ± 1.3**	**70.40^{d} ± 0.5**	**60.20^{c} ± 1.3**	**49.80^{d} ± 0.4**

Notes: Different superscript letters (a,b,c,d) in the same column show a significant difference ($p<0.05$).

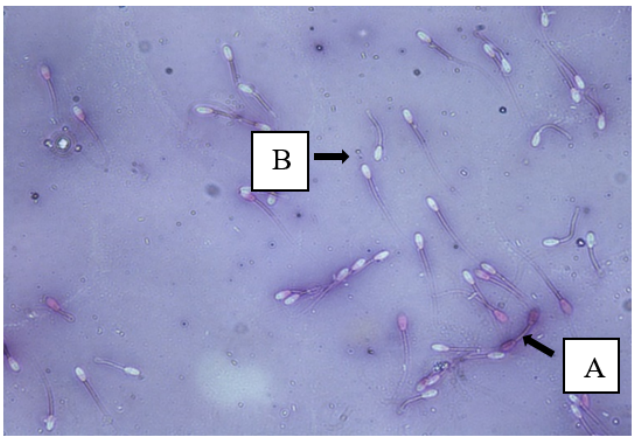

Figure 1: Viability of Sapera goat spermatozoa (*Eosin-negrosin* Staining); Magnification 400X; Nikon Mincroscope, Eclipse E200). Notes: A. Dead Spermatozoa; B. Live Spermatozoa.

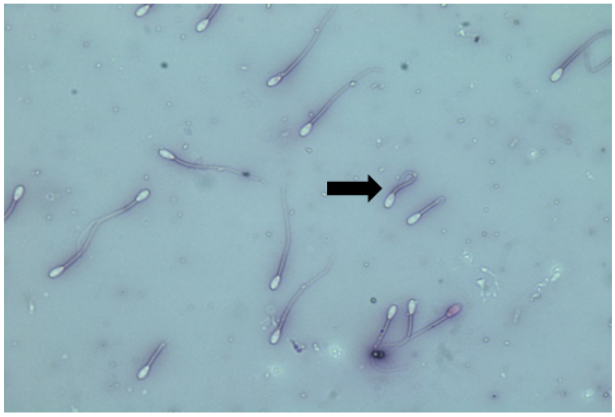

Figure 2: Intact plasma membrane of Sapera goat spermatozoa.

cement of Sapera goats in this study with an average of 6.6. This is in accordance with Bearden *et al*,[19] that cement with good quality has a pH ranging from 5.9-7.3. The same thing was shown by Susilowati *et al*,[20] that the pH of goats ranges from 6.4-6.8. Microscopic examination includes examination of concentration, motility, mass movement, viability and abnormality of cement. The concentration of fresh semen of Sapera goats in this study with an average of 3,888 (million/ml). According to Ax *et al*,[21] the normal spermatozoa concentration of goats ranges from 2500-5000 million/ml. The mass movement obtained shows results (+++) meaning that the spermatozoa waves are large and fast. This is in accordance with Susilawati *et al*,[22] that the mass movement of spermatozoa is categorized baik if it is seen to form large waves, numerous and dark and active like black clouds The percentage of motility of sapera goat spermatozoa in fresh semen examination is around 84%. The same thing is shown by Tambing,[17] that cement with a motility of 50-80% is classified as normal and fertile. The percentage of spermatozoa viability of Sapera goats in this study was 88%. This result is higher than the research of Masyitoh *et al*,[15] which was 82%. The percentage of abnormalities in the spermatozoa of Sapera goats in this study was 3.4%. According to Susilowati *et al*,[20] spermatozoa abnormalities are still feasible for treatment if the spermatozoa abnormality is less than 20%.

The average result of the percentage of motility, viability and intact plasma membranes of Sapera goat spermatozoa from vitamin C and E supplementation and a combination of the two in the 5°C cooling process in each time period, was highest found in P3 which contained a combination of vitamin C 0.9 mg/ml and vitamin E 1 mg/ml, while the lowest percentage was found in P0. The results of the evaluation of the percentage of motility after storage at 5°C cooling hours to 24, 48, 72 and 96 hours, there was a significant difference ($p<0.05$) between P0 and P3 in each time period, while P1 and P2 did not show a significant difference ($p>0.05$) in the period of time to 48 hours and 72 hours. Putra,[23] Reactive Oxygen Species (ROS) can alter the lipid composition of spermatozoan membranes resulting in damage to spermatozoan membranes. The results of a study conducted by Tafif,[24] showed that damage to the disturbed mitochondria of spermatozoa is the main cause of decreased motility. The results of the evaluation of the percentage of viability after storage at 5°C cooling in the time period to 24, 48, 72 and 96 hours, there were significant differences ($p<0.05$) between treatment groups and there was a significant decrease pattern starting from 24, 48, 72 to 96 hours ($p<0.05$) in each treatment group. The decrease in the viability of spermatozoa can be caused by factors of decrease in temperature and duration of storage due to biochemical changes due to coldshock, osmotic disorders and membrane damage.[25] Tafif,[24] in his research stated that the effect of antioxidant administration on viability results in treatment groups with different control groups is significant, this proves that oxidative stress occurs during the cooling period of cement.

The results of the evaluation of the percentage of intact plasma membranes of spermatozoa after storage at a cooling of 5 °C hours to 24, 48, 72 and 96 hours, there was a significant difference ($p<0.05$) between P0 and P3 in each time period, while P1 and P2 did not show a significant difference ($p>0.05$) in the 72-hour time period. Rizal and Herdis,[26] in their research said that the integrity of the plasma membrane is highly correlated with the viability of spermatozoa, if the plasma membrane is damaged, the metabolism of spermatozoa will be disturbed so that spermatozoa will lose their motility and result in death. Tambing *et al*,[17] said that plasma membranes have a role in protecting intracellular organelles physically, maintaining the entry and exit of food substances and maintaining intracellular and extracellular electrolyte balance.

CONCLUSION

Supplementation of vitamins C and E, as well as the combination of the two in the yolk tris diluter can maintain the motility, viability and intact plasma membrane of sapera goat spermatozoa at a cooling process of 5 °C.

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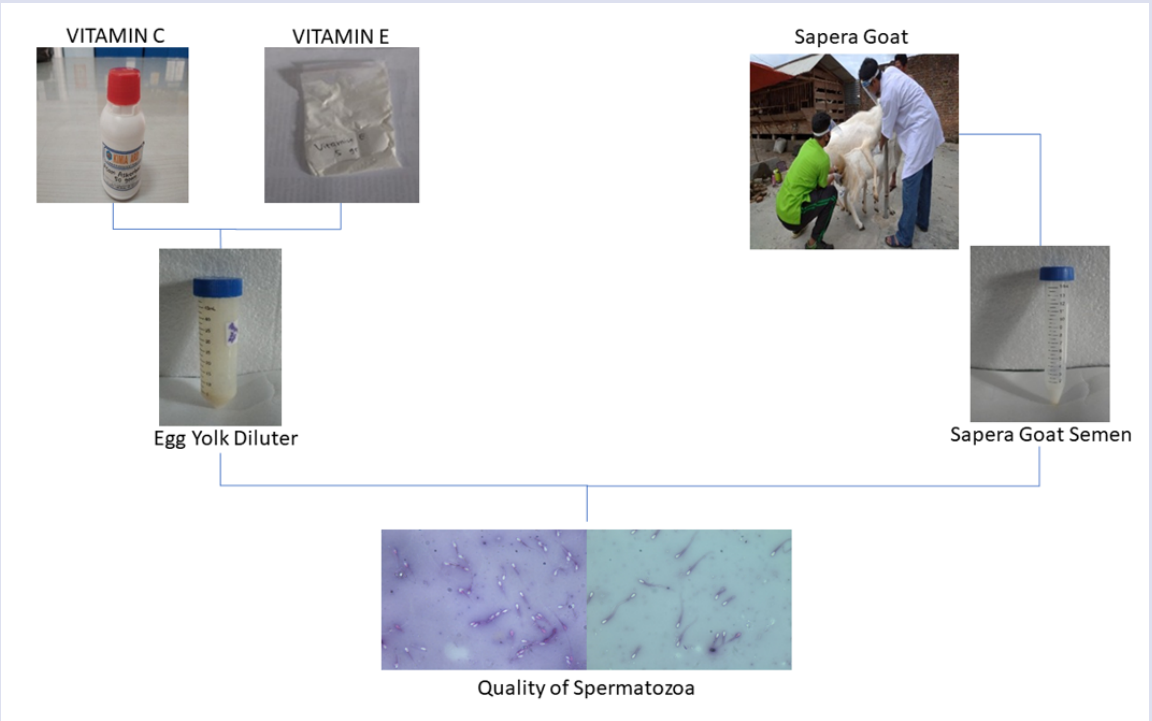
GRAPHICAL ABSTRACT



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