Study of Soya Addition in Tris Base Extender on the Quality of Senduro Goat Spermatozoa and Membrane Integrity on Storage Temperature 4-5°C

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Abstract. Soya is an ingredient that is added to semen extender as a component of macromolecules. This study aims to examine the effect of adding soy in the Tris base diluent on the quality and integrity of the Senduro goat spermatozoa membrane. Spermatozoa membrane integrity was observed using the HOST method. The results showed the greatest motility, viability, and integrity of the spermatozoa membrane at the beginning of storage were found in the tris + soya diluent of 2%. But at the end of storage, ie on the second day after storage, the quality of spermatozoa in the control group and the treatment group (2% and 3%) did not differ only. Based on the results of the study it can be concluded that the use of soy in the tris base diluent has not been able to provide optimal protection in Spermatozoa Senduro Goat during storage at a temperature of 4-5^{0}C.

Keywords: spermatozoa od Goat Senduro, soya, tris base extender, temperature of 4-5^{0}C

1 Introduction

Senduro Goat is a type of Etawa goat which is an Indonesian goat race. The Senduro Goat is the result of cross-breeding between the Jamnapari Etawa Goat and the Lumajang local goat in Indonesia, the Penggolo Goat [1]. The etawa goat has two uses, which are being able to produce meat and milk. The special characteristic of etawa crossbreed goats is that they have long ears that hang and droop, have high productive and reproductive potency [2].

The increased population of Senduro Goats is needed to strengthen the economy of Indonesian farmers. An effective method for accelerating and increasing the livestock population is by applying reproductive biotechnology, like Artificial Insemination (AI). The application of AI requires the supply of semen from superior males, to obtain superior offspring. Before AI is done, fresh semen that has been obtained then diluted using diluent media, and then carried out storage at low temperatures. Therefore dilution media are needed to maintain the quality of spermatozoa during the storing process [3,4,5].

The development of simple diluent media for goat semen needs to be done. It is because most Indonesians still use commercial diluents at high prices. Simple diluents that can be used for the process of diluting and storing goats were basic tris [6,7]. Basic tris diluent already contain buffers, anti-microbial agents, and energy sources. But it lacks some of the macromolecules. So, it needs the addition of macromolecules [8,9] to protect sperm extracellularly during the dilution process. Macromolecules that are often added in semen diluent include egg yolks, skim milk, soya, albumin, and coconut milk [10,3]. The purpose of

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this study was to examine the effect of adding soya as macromolecules in the basic tris diluent on the quality and integrity of the Senduro Goat sperm membrane during 4-5 ° C storage temperature.

2 Material and Method

2.1 Tris Base Diluent with Soya Supplementation

Chemical ingredients for manufacturing basic tris diluent include: 2.96 gr basic tris (Bioworld, USA); 1.65 gr of citric acid (Bioworld, USA); 2.00 gr Fructose (Bioworld, USA); 100,000 IU penicillin (Meiji, Japan), 0.1 gr streptomycin (Meiji, Japan), and 100 ml of sterile distilled water (Milli-Q-water). All ingredients are dissolved in aliquots. At the end of the process, then soybean was added with concentrations: 0%, 1%, 2%, and 3%.

2.2 Preparation and Dilution Process of Senduro Goat Semen

The Senduro Goat Cement was obtained from the Technical Implementation Unit, Animal Husbandry Department, East Java Province, Indonesia. An intake of fresh semen is done using an artificial vagina. Fresh semen is then subjected to quality tests, which are macroscopic (volume, pH, viscosity, color, odor) and microscopic (motility and viability). Good quality semen (pH 6.8 - 7.4; volume 1-3 ml; beige color, motility ≥ 70%, viability ≥ 80%) will then proceed to the dilution process. The semen dilution process using a basic tris diluent with various soya treatments. The dilution process is carried out at a water bath at 37-40^{0}C.

2.3 Sperm Quality Test

The sperm quality observed both before and during the storage processes are motility and viability.

Sperm Motility. Sperm motility was observed by two people using a microscope. Fresh semen, as well as the results of dilution and storage, were taken using an ose glass, then dripped on an object glass and observed using a 200 X microscope magnification at 37 ° C temperature.

Sperm Viability. Sperm viability was observed under a microscope using the eosin-nigrosin staining method. Fresh semen, the results of dilution and storage are taken using an ose, then dripped on an object glass and mixed with eosin-nigrosin dye, then made smear. Sperm were counted which were dead and which were living. Living sperm were colorless on the head, while dead sperm will be purple. 200 sperm were counted, then the percentage was calculated between live and dead sperm.

2.4 Sperm Membrane Integrity

Sperm membrane integrity was observed using the HOST method [11]. Diluted and storage semen were taken as much as 0.1 ml, then added 1 ml of 150 mOsm hypoosmotic solution consisting of sodium citrate (25 mmol / l) and fructose (75 mmol / l) and incubated at 37 ° C for 30 minutes. The incubated semen was placed on the slide, covered with a cover glass and

observed under a 200 X microscope magnification. Sperm with intact membrane integrity are marked by a curled tail. Sperm with intact plasma membrane were marked with a swelling or bulging tail, while sperm with a damaged membrane have their tails remaining straight or not bulging.

2.5 Data Analysis

Data in the form of a percentage were transformed into arcsin, then normality was tested. The results of the normality test were continued with the ANOVA test, and to analyze the differences between treatments, Duncan's test was performed.

3 Result and Discussion

3.1 Sperm Quality

The observation of sperm quality included motility and viability during the storage process. The observations of sperm motility and viability can be seen in Table 1.

Table 1. Observation Results of Senduro Goat Sperm Motility and Viability during 4-5 ° C Storage Temperature.

Types of Diluent Treatment	Motility Percentage on the Day – after storage		Viability Percentage on the Day – after storage	
	1	**2**	1	2
Basic Tris	21,67 ± 1,05^{b}	9,17 ± 1,01^{b}	22,62 ± 1,05^{c}	19,68 ± 1,13^{b}
Basic Tris + Soya 1%	39,67 ± 1,03^{ab}	13,67 ± 1,04^{b}	57,78 ± 1,02^{a}	26,19 ± 0,93^{a}
Basic Tris + Soya 2%	59,33 ± 0,93^{a}	18,33 ± 1,07^{a}	65,82 ± 1,03^{a}	27,90 ± 1,04^{a}
Basic Tris + Soya 3%	37,50 ± 1, 04^{ab}	0,3 ± 0,01^{c}	35,70 ± 1,05^{b}	17,78 ± 1,02^{b}

Note: different notation (a, b, c,....) indicated a significant different

Based on the results of the study in Table 1 showed that the motility and viability of spermatozoa membrane from Senduro Goat has decreased dramatically during storage at a temperature of 4-5^{0}C. Spermatozoa motility of Senduro Goat on the first day after storage there was a significant difference in all treatments. On the first day after storage, the greatest motility was in the tris base diluent with 2% soya addition, the lowest motility was in the tris base diluent without adding soya. On the second day after storage, the greatest motility was in the tris base diluent with 2% soya addition, while the smallest motility was in the tris base diluent with 3% soya addition.

Observation of spermatozoa viability showed significant differences in all treatments, both on the first and second days after storage. On the first day after storage, the greatest viability was in the tris base diluent with 2% soya addition, and the smallest viability was in the tris base diluent without soya addition. On the second day after storage, the greatest viability was in the tris base diluent with 1% and 2% soya addition, while the smallest viability was in the tris base diluent with 3% soya addition.

3.2 Membrane Integrity

Observation of membrane integrity was carried out to assess the integrity of the spermatozoa membrane which was stored in the tris base diluent with the addition of soya and without soya. The observation of spermatozoa membrane integrity can be seen in Table 2.

Table 2. The Membrane Integrity of Goat Sperm During Storage at Temperature of 4-5ºC.

Types of Diluent Treatment	Membrane Integrity on The Day ---After Storage	
	1	2
Basic Tris	36,78 ± 1, 05[b]	19,84 ± 1,01[b]
Basic Tris + Soya 1%	57,77 ± 1,03[a]	17,05 ± 1,05[c]
Basic Tris + Soya 2%	59,51 ± 1,04[a]	26,10 ± 1,06[a]
Basic Tris + Soya 3%	23,16 ± 1,68[c]	20,58 ± 1,35[b]

Note: different notation (a, b, c,....) indicated a significant different

Based on observations in Table 2 showed that on the first day after storage, the integrity of the spermatozoa membrane was greatest in the tris base diluent with 1% and 2% soybean addition. On the second day after storage, the greatest membrane integrity was in the tris base diluent with a 2% soya addition.

Based on the results of the research showed that the addition of soy in the tris base extender was able to protect against the motility and viability of the Spermatozoa Senduro Goat. Soya is one of the macromolecules that can be added to an extender. Macromolecules provide extracellular protection against sudden changes in temperature during the dilution process [4, 6]. Fresh semen before to be implemented in AI, a dilution process was first carried out. The next step was the storage process at low temperatures. Therefore it needs diluents that can increase the volume of semen and maintain the quality of spermatozoa so as not to decrease too drastically which can cause spermatozoa death.

In semen diluent, there are always buffers, energy sources, antibacterial agents, and extracellular protective macromolecules. The results of this study indicated that the addition of 2% soybean was able to provide the best protection Spermatozoa Senduro Goat during storage at a temperature of 4-5^{0}C. The results of this study were similar to those of Narwade et al. [10] and Akourky et al. [12] which showed that the using of soy in extender goat semen can protect against the quality of spermatozoa during the storage process at low temperatures. Soya contained lecithin which can bind to the spermatozoa membrane and provide protection to the outside of the membrane.

The observations in Table 2 showed that soy can protect against the integrity of the spermatozoa membrane, especially at a concentration of 2% both on the first day and the second

day after storage. But the results of this study were different from the study of Chelucci et al., [13] where the best concentration was 1% and different from the results of Vidal et al. [14].

The lecithin component in soy can bind to the spermatozoa membrane to provide extracellular protection. The mechanism of protection of spermatozoa membranes was assumed in two ways. The first mechanism, exogenous phospholipids can replace membrane phospholipids so that they can maintain the membrane structure [15]. Another possibility was that lecithin phospholipids do not enter the cell membrane, but form protection outside during the dilution or storage process at low temperatures, covering the cell membrane [16].

In this study, the greater concentration caused the motility, viability, and integrity of the membrane to decrease. This was due to the higher concentration of soy that will be toxic to spermatozoa. Based on the results of the study also showed that soy was only able to protect for two days of storage, on the second day it appeared the quality and integrity of spermatozoa dropped dramatically. This showed that soya in the tris base diluent has not been optimal in providing spermatozoa protection during the storage.

4 Conclusion

Based on the results of the research it can be concluded that giving soya with different concentrations, giving a different effect on the protection of Spermatozoa Senduro Goat. The best concentration of soy in providing protection against spermatozoa The senduro goat was 2%. However, soya in the Tris base diluent has not been able to provide optimal protection against Spermatozoa Senduro Goats during storage at 4-5 ° C.

Based on the results of the study, further research was needed to optimize the tris base diluent with the addition of soy as an extracellular protective material Spermatozoa Senduro Goat.

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