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The Sperm Motility of Ongole Crossbreed Cattle in Coconut Water Based Diluents During Storage at 3-5°C

Dedi Muhammad[1*], Nurul Isnaini[1], Aulia Puspita Anugra Yekti[1], Kuswati[1], Muchamad Luthfi[2], Lukman Affandhy Sunarto[2], Trinil Susilawati[1*]
[1]Faculty of Animal Husbandry, University of Brawijaya, Malang, East Java, Indonesia-65145
[2]Beef Cattle Research Center Grati, Pasuruan, East Java, Indonesia-67184
*Corresponding authors: dedi.Pendidik@gmail.com, trinil_susilawati@yahoo.com

Abstract*—The implementation of the artificial insemination program using liquid semen can reduce the level of damage to the spermatozoa. Coconut water diluent is the alternative semen diluent. This diluent is made from local and cheap materials. The aims of this research were to examine the the sperm motility of Ongole Crossbreed cattle in coconut water based diluents during storage at 3-5°C. This research was carried out at Beef Cattle Research Center Pasuruan Laboratory on April, 2018. The research used laboratory experimental method. The experimental design was Randomized Completely Block Design. The data were analyzed by Analyze of Variance. There are three treatments in this research (T1 = Coconut water + yolk egg 20% , T2 = Coconut water + yolk egg 20% + white egg 0,4% + fructose 1 mg/ 0,1 ml, and T3 = Coconut water + egg yolk 20% + white egg 0,4% + fructose 2 mg/ 0,1 ml). There are ten replications of each treatment. The result of this research show were not significant differences in sperm motility and viability between coconut water diluents with variuos level of fructose during chilled storage.*

***Keywords*—** *Liquid semen, Motility, Total motil sperm*.

I. INTRODUCTION

The implementation of the artificial insemination program using liquid semen can reduce the level of damage to the spermatozoa. [1] explained that spermatozoa stored in cold temperature will experience a decrease in quality of about 30 to 60%. Liquid semen is found to be quite effective as the solution to overcome the limited liquid nitrogen supplies and the high cost of infrastructure for frozen semen storage.

Liquid semen is semen that has been added with diluents then stored at 3-4°C [2]. The addition of diluents aims to fulfill nutritional needs and provide a suitable environment for spermatozoa. In addition, diluents should be able to protect spermatozoa from any damage due to temperature and should be made from materials that are not expensive and are easy to get.

Coconut water can be used as an alternative diluent because of its wide availability in the surrounding environment and affordable price. [3] have reported that coconut water contains carbon elements in the form of simple carbohydrates, such as: glucose, sucrose, and fructose. Moreover, [4] also stated that coconut water contains carbohydrates, fats and proteins that is necessary for spermatozoa.

The semen dilution process can decrease the concentration of substances contained in seminal plasma such as the level of amino acid and ions that can change the osmotic pressure stability in diluents which later can affect semen motility and viability [5]. The addition of energy sources (fructose) and macromolecules (in the form of exogenous proteins) to coconut water based diluent is expected to substitute some of the lost substances from seminal plasma due to the dilution so that it can support the sperm viability.

Coconut water based diluents with the supplementation of egg yolk as cryoprotectant, fructose as an additional energy source, and egg white as macromolecules are expected to maintain the sperm quality optimally. [6] has conducted a research regarding the use of green coconut water based diluents and she found that those diluents can maintain the percentage of sperm motility of Boer goats above 40% until day 2 during cold storage at s of 4-5°C.

Based on the explanation above, it is necessary to conduct a study on the the sperm motility of Ongole Crossbreed cattle in coconut water based diluents during storage at 3-5°C.

II. MATERIALS AND METHODS

A. Coconut Water Based Diluents

The diluents were made from coconut water obtained from 5 to 8 months old coconuts. The coconut water were heated at 56°C for 20 minutes in order to deactivate the enzymes in it. Furthermore, egg yolk 20%, 1 mg/ml NaHCO3, 1 mg/ml penicillin and 1 mg streptomycin sulfate were added into the coconut water. Then egg white 0.4%, 10 and 20 mg/ml of fructose were also added as a treatment.

B. Ongole Crossbreed Semen

Title Semen samples used in this study were obtained from Ongole Crossbreed cattle which were maintained in Beef Cattle Research Station, Grati Pasuruan. The criteria set for the samples include mass motility of ≥ 2+ and individual motility of ≥ 70%. The semen collection was done twice a week for each bull by using the artificial vaginal method.

C. Researcg Design

Randomized Block Design was used as the research method along with 3 treatments and 10 replications. The

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grouping was done based on different collection times. The three treatments performed include: first (T1) = coconut water based diluent + egg yolk 20%; second (T2) = coconut water based diluent + egg yolk 20% + egg white 0.4% + 1 mg/ 0,1 ml fructose; and third (T3) = coconut water based diluent + egg yolk 20% + egg white 0.4% + x 2 mg/ 0,1 ml fructose.

D. Observation of Sperm Motility

The sperm motility was observed under a light microscope at magnification of 400 times. One drop of liquid semen was taken using inoculating loop then placed on top of an object glass and covered with cover glass. This sperm motility was assessed by observing the individual movement of spermatozoa that moves progressively (Hafez, 2008; Garner and Hafez, 2008; Susilawati, 2011).

III. Result and Discussion

A. The Quality of Ongole Crossbreed Semen

Number The examination of Ongole Crossbreed cattle semen before being diluted includes macroscopic examination which consist of volume, pH, and color. Then followed by microscopic examination which are mass motility, individual motility, viability, abnormality, concentration, and total motile sperm. The average and standard deviation of the semen quality of Ongole Crossbreed cattle can be seen in Table I.

TABLE I. Semen Quality of Ongole Crossbreed Cattle.

Variable	Mean ± standard deviation
Macroscopic	
Semen volume (ml)	4,00 ± 1,41
Semen Color	Cream
pH	6,50 ± 0,12
Mikroskopis	
Massa motility	2+
Individual motility (%)	71,25 ± 2,50
Sperm Viability (%)	92,31 ± 2,67
Sperm Abnormality (%)	2,60 ± 0,78
Concentration (million/ml)	1245,00 ± 184,30
Total motile sperm	886,75 ± 129,85

The observation results of average Ongole Crossbreed cattle semen volume used are 4.00 ± 1.41 ml. These results are low compared with the statement of [7] that the volume of cattle semen collected ranges from 5-8 ml. The semen color of Ongole Crossbreed cattle obtained in the study is cream. [8] explained that the color bull semen is generally yellowish white or almost as white as milk due to the presence of riboflavin in it. As for the average pH of semen is 6.50 ± 0.12 which indicates that the semen pH used for the study is normal. [9] explained that the pH of bull semen ranges from 6.5 to 7.9.

The microscopic examination done includes mass motility, individual motility, viability, abnormality, and concentration. The average percentage of semen mass motility found is 2+. This number has met the requirement for mass motility in order for semen to be processed [10]. Meanwhile, the average motility of individual (Ongole Crossbreed cattle) fresh semen found is 71.25 ± 2.50%. [11] stated that the minimum requirement for semen motility to be diluted is 70%. Further, the semen concentration of Ongole Crossbreed cattle used is 1245.00 ± 184.30 million/ml. [7] explained that the semen concentration varies from 1,000-2,000 million of spermatozoa per milliliter or 800-2,000 million of spermatozoa per milliliter.

The average sperm viability of Ongole Crossbreed cattle used for the present study is 92.31 ± 2.67%. This means that Ongole Crossbreed semen used are eligible to be used. [10] stated that fresh semen which will be diluted should at least has 70% of live sperm. The average percentage of Ongole Crossbreed semen abnormality used for the study is 2.60 ± 0.78%. This number shows that spermatozoa that has normal morphology are 97.40%. It means the percentage of normality of Ongole Crossbreed cattle semen is in a good category. [12] explained that semen is categorized as poor and has low fertilization power if its percentage of abnormality is more than 20%.

B. The Motility of Ongole Crossbreed Sperm after Treatments

Number Motility is an important parameter in processing semen for artificial insemination program. The average and standard deviations of Ongole Crossbreed cattle liquid semen motility with various treatments of coconut water based diluents, are shown in Table II.

TABLE II. The Motility of Ongole Crossbreed after Treatments.

T	Mean ± standard deviation								
	D1	D2	D3	D4	D5	D6	D7	D8	D9
T1	70,1 ± 0,2	69,1 ± 2,2	67,1 ± 3,6	66,1 ± 3,8	57,2 ± 9,7	37,3 ± 5,4	27,1 ± 9,6	6,2 ± 6,7	4,6 ± 6,3
T2	70,6 ± 1,6	69,6 ± 1,7	67,1 ± 3,6	66,1 ± 3,8	58,7 ± 7,7	39,3 ± 3,8	27,6 ± 7,3	6,6 ± 3,5	3,6 ± 3,5
T3	71,1 ± 2,3	68,6 ± 2,5	67,1 ± 4,3	65,6 ± 4,5	57,2 ± 7,4	37,6 ± 5,5	27,1 ± 8,3	6,2 ± 2,3	3,6 ± 2,5

Note : T = Treatments, D = Day storage. No significant difference (P > 0.05)

The variance analysis results for the sperm motility of Ongole Crossbreed cattle during storage at 2-5°C showed no significant difference (P > 0.05) at Day 1 until Day 9 of storage between T1, T2, and T3 treatments.

The results showed no difference between treatments of coconut water based diluents with the addition of fructose in different amounts which means that fructose supplementation to the coconut water based diluents does not have any significant effects to the sperm motility of Ongole Crossbreed cattle. To keep living, spermatozoa requires energy in the form of ATP obtained from the anabolism process of energy sources. Naturally, there is an energy source in the form of fructose both in seminal plasma and coconut water. So if until Day 9 of storage the energy sources needed by spermatozoa have been fulfilled from seminal plasma and coconut water, the addition of fructose will not have any significant effects on the sperm motility. [8] stated that fructose is the simple sugar found in the seminal. Fructose is a good energy source for spermatozoa because of its shorter metabolic pathway through the process of fructolysis compared to glycolysis process that

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is taking longer

Treatments of T1, T2, T3 are coconut water based diluents. The observation results in Table I show that the motility lasted above 40% until Day 5 of storage. Although in term of maintaining the sperm motility it needs shorter time compared to CEP or Tris Aminomethan diluents, coconut water has the potential to be developed into semen diluent that has a good quality. [4] stated that coconut water can be the alternative diluent that is easy to get because it is widely available in the surrounding environment and the price is affordable. Coconut water content varies depending on its variety, age and also climate factors. [13] confirmed that coconut water is one of the ingredients that can be used for semen diluents that meet the criteria because coconuts in tropical countries such as Indonesia are very easy to get at low prices compared to the synthetic chemicals. Moreover, coconut water contains carbohydrates which can be energy source for the life of spermatozoa.

C. Tolat Motile Sperm of Ongole Crossbreed Cattle after Treatments

Number Total motile sperm is the product of the sperm motility with the concentration of sperm in a liquid semen. This definition is supported by [14] who stated that the motile sperm count can be done by multiplying the progressive motile of sperm percentage with the sperm concentration. If the total motile sperm is known, it can also be seen whether the liquid semen used meets the requirements for artificial insemination.

TABLE III. Tolat Motile Sperm of Ongole Crossbreed after Treatments.

Treatments	Mean ± standard deviation
T1	42,8 ± 8,6
T2	44,5 ± 4,9
T3	43,0 ± 9,1
Expectation value	40,0

Note : No significant difference (P > 0.05)

Table III shows that the average of Ongole Crossbreed cattle total motile sperm at Day 6 of storage, from the largest to the smallest, respectively are 44.5 million /ml in T2, 43.0 million/ml in T3, and 42.8 million /ml in T1. These results indicate that T2 treatment is able to maintain the best total motile sperm while the lowest total motile sperm in Ongole Crossbreed cattle is T1.

All treatments (T1, T2 and T3) at Day 6 of storage had higher values than the expected value (40 million/ml). This means that liquid semen of Ongole Crossbreed cattle with coconut water based diluent, based on the total motile sperm value at Day 6 of storage still met the SNI standards as semen for IB. The expectation value of 40 million/ml is determined based on SNI standard on bull frozen semen. The SNI standard for sperm concentration in straw doses is 25 million/mini straw dose (100 million/ml) with individual motility of 40%. [15] stated that total motile sperm greatly influences the chance of fertilization. [11] also added that liquid semen with a total motile sperm value of above 40 million/ml is suitable to be used for IB.

IV. CONCLUSIONS

1) The addition of fructose and egg white to coconut water-based diluents (T1, T2, T3) do not show any significant differences.

2) Coconut water based diluents (T1, T2, T3) can maintain motility, viability, abnormality of spermatozoa according to the standard to be used in the implementation of artificial insemination until the Day 5.

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