The Quality of Buffalo Sperm in Tris Egg Yolk Diluent with Addition of Different Levels of Mangosteen Peel Extract

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Abstract

This study aimed to determine the quality of buffalo sperm in tris egg yolk diluent added with mangosteen peel extract at different levels. This study was conducted using an experimental method using a completely randomized design consisting of four treatment concentrations of mangosteen peel extract (T1: 0%, T2: 5%, T3: 10%, and T4: 15%) and four replications, respectively. Parameters observed were motility, abnormalities, intact plasma membrane, and acrosome intact. In the results, the addition of mangosteen peel extract had a significant effect ($p > 0.05$) on the motility of buffalo sperm that had been diluted in egg yolk tris diluent. This study concluded that the addition of 5–10% mangosteen peel extract in egg yolk tris diluent has a significant effect on sperm motility, could minimize the abnormality, and minimize the decrease in the intact plasma membrane of the buffalo sperm.

Keywords: abnormalities, buffalo, intact plasma membrane, motility

Received: 10 June 2023 **Revised:** 27 October 2023 **Accepted:** 27 November 2023

INTRODUCTION

The buffalo population in Riau has decreased from 2017, 34,542 heads to 23,677 heads in 2019. One of the factors that cause the low population of buffalo is the lack of males in the field (Yendraliza *et al*., 2010). Artificial insemination (AI) is a solution to overcome the shortage of males in the field. The application of AI in buffalo in Indonesia has been started since 1978 but the development is little compared to cattle so study on buffalo is far behind compared to cattle.

The characteristics of buffalo semen besides having a low volume also have low motility compared to cattle (Mughal *et al*., 2018). This is because the high content of phosphatidylcholine in the sperm membrane is higher in buffalo than in cattle (Andrabi, 2009). Dilution is one solution to increase the volume of buffalo semen. The characteristics of a good diluent can provide energy, buffer, and become an antibiotic for sperm (Rehman *et al*., 2013).

Tris is a type of diluent that has buffering ability, osmotic activity, and low toxicity. Egg yolk has a low-density protein that can protect the plasma membrane surface during freezing (Akhter *et al*., 2017). Jung *et al*., (2006) stated that the mangosteen fruit (*Garcinia mangostana L*.) is also thought to have potential as a natural antioxidant. The addition of mangosteen fruit extract in Tris Egg Yolk diluent is expected to strengthen the plasma membrane of buffalo during freezing. The purpose of this study was to evaluate the quality of frozen buffalo semen with the addition of mangosteen peel extract in egg yolk tris diluent.

MATERIALS AND METHODS

Ethical Approval

This study has been approved by the ethics committee of the Faculty of Agriculture and Animal Husbandry of UIN Suska Riau number: KE/KEP-FPP/03/04/2021.

Study Period and Location

This study was performed at the Reproduction Laboratory, Animal Research

Institute (Balitnak) Ciawi, Bogor, West Java, from April–June, 2021.

Sample Collection

The semen collected from male buffalo that were reared intensively at the Balitnak Ciawi Bogor weighed of ±700 kg, aged ±14 years. Buffaloes were reared for according to Balitnak maintenance standards with forage elephant grass and concentrates. Semen collections were carried out once a week for 6 weeks at 8 am. Semen storage was done with an artificial vaginal at a temperature of 40–45°C. The Semen used in this study has a motility of 70%.

Mangosteen Peel Extract

Mangosteen peel extract was prepared according to Chaovanalikit *et al*. (2012) and Caridi *et al*. (2007) with modification. The mangosteen peel used was derived from Garcia products. First, mangosteen peel and aquabidest were mixed in a ratio of 1:40 (mangosteen peel: aquabidest). Then, the resulting supernatant was filtered using a filter cloth followed by centrifugation for 15 minutes at 3000 rpm, and separated from the residue. The solution and the residue were separated using a filter cloth. Inactivation was carried out in an oven at 56°C for 30 minutes and stored at a cold temperature in the refrigerator for further evaluation.

Diluent Manufacturing

The composition of the diluent consists of Tris Aminomethane, citric acid, fructose, penicillin-streptomycin, and egg yolk (Table 1). The procedure for preparing the diluent was started by dissolving Tris Aminomethane (Sig), citric acid, and fructose (Merck) in 80 mL of aquabidest and homogenized. Then, penicillin and streptomycin were added and homogenized again. Finally, added the egg yolks that have been separated from the albumin and mixed properly. This procedure was adopted by Balitnak Ciawi.

Semen Processing

Fresh semen was divided into four treatment concentrations of mangosteen peel extract (T1: 0%, T2: 5%, T3: 10%, and T4: 15%) and four replications, respectively. Then the diluent was added gradually until the dilution volume was reached. Homogenized for 30 minutes and evaluated. Equilibration was carried out for 4 hours in a refrigerator at 40°C and evaluated. Next, the filling process was carried out on the 0.25 mL straw, followed by sealing, and finally, the straw was printed. Before freezing the straws were placed on a counting rack and placed 4 cm above the surface of the liquid N2 in the freezing box (43 cm long, 27 cm wide). This pre-freezing process was carried out for 5 minutes. After that, the straw was dipped into Liquid N2. Semen was thawed after 24 hours in liquid N2 using ordinary tap water. After thawing evaluation was carried out.

Motility Calculation

The individual movements of the spermatozoa were indicated by placing semen on an object glass and covered with a cover glass and observed under a microscope at 400x magnification. Total spermatozoa sperm count (minimum of 200 sperm). Motility (%) = [(Number of spermatozoa with forward movement x Number of spermatozoa^{-1}) x 100].

Abnormality Calculation

A smear preparation from a drop of semen mixed with 2 drops of eosin on a warm glass object and viewed under a light microscope with 400x magnification. The percentage of abnormal spermatozoa count was calculated from the total number of spermatozoa counted. Abnormality (%) = [(Number of abnormal spermatozoa x Number of spermatozoa^{-1}) x 100].

Intact Plasma Membrane Calculation

The intact plasma membrane was calculated using the Host Test solution (Yendraliza *et al*., 2019) (Table 2). Observation of the intact plasma membrane of spermatozoa was carried out by mixing a drop of sperm mixture (a mixture of sperm and the incubated host test solution) on a glass slide and a cover slip, then observed under a microscope with a magnification of 400x. Intact plasma membrane (%) = [(Number of

spermatozoa with an intact plasma membrane x Number of spermatozoa^{-1}) x 100].

Data Analysis

The data obtained in this study were statistically analyzed using ANOVA followed by Duncan's test. All process was performed using SPSS v20 software.

RESULTS AND DISCUSSION

Quality Fresh Buffalo Semen

The average volume of fresh buffalo semen in this study was 2.27 mL (Table 1). Yendraliza *et al*. (2019) stated that the average volume of fresh buffalo semen was in the range of 1.44–1.76 mL. The volume of fresh Aceh buffalo semen was 1.2 mL. The color and consistency of buffalo semen in this study were creams and slightly thick. In contrast to Sianturi *et al*. (2012) who found the consistency of buffalo semen was slightly runny and Eriani *et al*. (2018) who stated that Jaffrabadi buffalo semen was white cream. The mass movement of buffalo semen in this study was ++ – +++. This finding followed the national standard for buffalo Semen (Badan Standardisasi Nasional, 2017). The semen motility of buffalo in this study was different from that of Nilli Ravi and Jaffrabadi buffalo (70% VS 75%; 75–81%) (Ghodasara *et al*., 2016; Ansari *et al*., 2017). The percentage of abnormalities, viability, and the intact plasma membrane of buffalo sperm in this study (10% and 85.75%) were different from the results of previous studies (Ghodasara *et al*., 2016; Ansari *et al*., 2017; Eriani *et al*., 2018). The difference in the quality of fresh buffalo semen between several studies was caused by different types of livestock, environment, and different buffalo breeding systems (Das *et al*., 2017; Hanifah *et al*., 2020).

Quality Semen Buffalo After Diluent, Equilibration, and Frozen

The mean motility, abnormalities, acrosomal cap and intact plasma membrane of buffalo sperm treated with mangosteen peel extract in egg yolk tris diluent after thawing were significantly different ($p > 0.05$). The administration of 5–10% mangosteen peel extract in Tris Egg Yolk diluent of buffalo semen resulted in high motility values of 41.25–52.50%, low sperm abnormalities, intact acrosomal cap, and high intact plasma membrane compared to 15% mangosteen peel extract administration (Table 3).

Administration of mangosteen peel extract up to 15%, was able to protect the intact plasma membrane of the mud buffalo sperm after thawing (55–63.50%), but the sperm motility of the mud buffalo after thawing was only able to be maintained according to Indonesian National Standard (SNI) 2017 on administration 10% mangosteen peel extract in Tris Egg Yolk diluent

Table 1. Composition of treatment diluent

Ingredient	Addition of mangosteen peel extract			
	T1	T2	T3	T4
Tris *aminomethane* (g)	6.098	6.098	6.098	6.098
Fructose (g)	2.5	2.5	2.5	2.5
Citric acid (g)	3.4	3.4	3.4	3.4
Egg yolk (%)	20	20	20	20
Penicillin (g)	0.2	0.2	0.2	0.2
Streptomycin (g)	0.2	0.2	0.2	0.2
Mangosteen peel extract (µm)	-	75	150	225

Table 2. Composition of test hosts

Composition	Amount
Aquadest (mL)	100
Fructose (g)	1.35
Sodium citrate (g)	0.75
Total	100 mL (in liquid form)

Table 3. Mean of motility, abnormalities, intact acrosome, and intact plasma membrane of buffalo sperm after dilution, equilibration, and thawing

Treatment	After dilution	After equilibration	After thawing
		Motility	
T1	68.75 ± 2.50	58.75 ± 9.57	52.50^{ab} ± 10.80
T2	68.75 ± 2.50	58.75 ± 10.31	51.25^{ab} ± 11.09
T3	68.75 ± 2.50	58.75 ± 10.31	41.25^{b} ± 11.09
T4	68.75 ± 2.50	58.75 ± 9.13	35.00^{b} ± 8.54
		Abnormality	
T1	17.25^{a} ± 1.26	17.25^{ab} ± 1.41	20.50^{a} ± 1.29
T2	15.00^{ab} ± 0.82	15.00^{ab} ± 1.15	20.75^{b} ± 0.50
T3	19.25^{b} ± 1.71	20.25^{b} ± 1.29	23.75^{c} ± 1.26
T4	21.50^{b} ± 2.08	22.75^{b} ± 2.06	25.75^{d} ± 1.50
		Intact acrosome	
T1	67.25^{a}	66.00^{a} ± 2.16	60.50^{b} ± 1.91
T2	65.00^{ab}	64.25^{a} ± 1.50	63.50^{a} ± 1.29
T3	69.25^{b}	62.50^{b} ± 1.73	57.75^{a} ± 1.26
T4	61.50^{b}	60.50^{ab} ± 2.08	55.00^{ab} ± 2.16
		Intact plasma membrane	
T1	70.00^{a} ± 0.82	66.00^{ab} ± 2.45	60.25^{b} ± 3.10
T2	72.50^{a} ± 1.25	68.75^{b} ± 2.99	63.50^{b} ± 3.42
T3	69.00^{ab} ± 0.82	64.00^{b} ± 2.58	57.75^{b} ± 2.63
T4	66.75^{a} ± 1.20	61.75^{a} ± 2.50	55.00^{ab} ± 2.94

(41.25–51.25%). This shows that the antioxidants present in mangosteen peel extract can prevent free radicals to prevent peroxidation reactions during freezing (Purnama, 2019). Phospholipid structure of the plasma membrane is very susceptible to temperature changes (Wajdi *et al*., 2021; Fiqih *et al*., 2021). During freezing sperm metabolism continues which produces energy and free radicals that will cause damage to the plasma membrane (Iskandari *et al*., 2020). However, the presence of up to 10% mangosteen peel extract in egg yolk tris diluent was able to prevent damage to the plasma membrane of the mud buffalo sperm (Prayogo *et al*., 2022; Wijayanti *et al*., 2023). This is supported by data from the intact acrosomal cap of the mud buffalo sperm after thawing which is still above the 2017 SNI standard. Several studies on the addition of mangosteen peel extract in semen dilution are different from this study. Nanda *et al*. (2019) and Effendi *et al*. (2015) found that giving mangosteen peel extract up to 4% could maintain the motility of goat sperm and cattle sperm. This difference is due to respective livestock sperm having a different response to the type of diluent used during freezing (Saputro *et al*., 2022).

CONCLUSION

The administration of 5–10% mangosteen peel extract in egg yolk tris diluent in buffalo semen can maintain motility, intact acrosome, intact plasma membrane, and abnormalities of buffalo sperm.

ACKNOWLEDGEMENTS

The authors express their gratitude to the Reproduction Laboratory, Animal Research Institute (Balitnak) Ciawi, Bogor for providing facilities for this study.

AUTHORS' CONTRIBUTIONS

Y: Conceptualization and drafted the manuscript. AP, JH, and RSGS: Treated the animal laboratory. DAK and Y: Validation, supervision, and formal analysis. DAK: Performed the statistical analysis and the preparation of tables. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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