Quality of boer goat liquid semen in coconut water diluent with egg yolk addition during cold storage

Domingos Cruz Pinto[1*], *Nurul* Isnaini[2], and *Trinil* Susilawati[1]

[1] Department of Animal Production, Institute Polytechnic of Betano. Same-Manufahi, Timor Leste
[2]Faculty of Animal Science, Brawijaya University, Malang Indonesia

Abstract. The research aimed to analyze the effect of young green coconut water diluent with egg yolk in Boer goat liquid semen quality at cold storage. This study used an experimental laboratory with 4 treatments and 10 replications. The treatments tested were: P0 = CEP-3 + 10% egg yolk + 0.4% egg white, P1 = 90% coconut water + 10% egg yolk, P2 = 85% coconut water + 15% egg yolk, and P3 = 80% coconut water + 20% egg yolk. The variables analysis was individual motility, viability, abnormality, intact plasma membrane, and intact acrosome hood. The data were analyzed using Analysis of Variance (ANOVA), and the difference between treatments continued with Duncan Multiple Range Test. The result showed significant differences in individual motility, viability, intact plasma membrane, and intact acrosome hood ($P<0.01$), and abnormality showed not significant difference ($P>0.05$). In conclusion, the combination of coconut water + 20% egg yolk could be recommended for the diluent of Boer goat liquid semen in cold storage for up to 3 days.

1 Introduction

Boer goat is a type of goat originally from South Africa, which has been domesticated by the community for a long time to produce meat and milk, as well as other by-products for the community's needs. The effort to improve local goats population and genetic quality is by using artificial insemination technology using liquid semen of Boer goat [11]. Artificial insemination can be done using liquid or frozen semen, which can be applied depending on its availability. Artificial insemination using frozen semen has long been widely recognized by farmers but is less efficient and has many obstacles in its implementation. [15] stated that the process of making frozen semen is very complicated, due to high price of liquid nitrogen in global market. In addition, the quality of frozen semen decreases during freezing and thawing, resulting in a decrease in fertility [19]. It's also explained by [22] that the process of freezing semen can cause damage to membrane and deoxyribonucleic acid (DNA) which results in decreased fertilization ability. The alternative to overcome the obstacles experienced by technical personnel and farmers in the field can be liquid semen. The use of liquid semen for artificial insemination has been

* Corresponding author: dcruzpinto122@gmail.com

proven that it's more effective in overcoming the limitations of both liquid nitrogen and frozen semen [10]. [21] explained that artificial insemination using liquid semen is of better quality than frozen semen. Using liquid semen for artificial insemination certainly needs a diluent in order to maintain the quality of semen during cold storage period [2]. The quality of liquid semen is largely determined by the diluent used, because the diluent can function as a source of nutrients, anti-cold shock, buffer, isotonic, prevent bacterial growth and provide extracellular cryoprotectants that can protect spermatozoa during the cooling process [18].

The diluent used for making liquid semen must be relatively cheap, simple, practical, and easy to obtain. The alternative diluent that is found in many residential areas is coconut water. Using young coconut water as a main semen diluent aim to achieve the best production of liquid semen due to its several advantages as follows, easily obtained, relatively cheap, easy and practically to apply. [21] stated that green coconut water used as a semen diluent is expected to minimize damage to spermatozoa and can be stored at cold temperatures that do not require liquid nitrogen. Green coconut water contains simple carbohydrates such as glucose, fructose, and sucrose and has antioxidant compounds from the flavonoid group that differ according to the level of maturity [22]. In addition, green young coconut water also contains lipids and proteins, B vitamins and vitamin C, and is rich in minerals (N, P, K, Ca, and Mg) [5]. With the presence of simple carbohydrates and macro and micro molecules contained in coconut water, it's hoped that it can function as a source of nutrition and cryoprotectant for spermatozoa during the storage process in cold storage. However, using green coconut water alone can reduce the protection of spermatozoa at low temperatures. Hence, it must be combined with egg yolk because it contains lipoproteins and lecithin, which act as extracellular cryoprotectants to protect spermatozoa [4]. In connection with this description, a study was conducted using young green coconut water diluent combined with egg yolk with different percentages to analyze the quality of Boer goats is liquid semen during cold storage.

2 Materials and Methods

This research was conducted at Animal Reproduction Laboratory of Sumber Sekar of the Faculty of Animal Science, Brawijaya Universitas, from August 1st to September 24th 2023. The materials used was fresh semen of 2 heads of Boer goat aged 3–4 years, and semen was collected using an artificial vagina. The semen diluent used was green coconut water with a maturity level of 6-8 months. The egg yolk used was native chicken yolk, antibiotics (sulfate streptomycin and sulfate penicillin), NaHCO3, CEP-3, NaCl 0,9%, aquabides and eosin-nigrosin, hypoosmotic swelling test (HOST), and formalin 1%. The experiment was conducted with a randomized design group comprising 4 treatments and 10 replications. Semen was diluted with coconut water and egg yolk with the dilution composition in each treatment as follows: P0 (control) = CEP-3 + 10% egg yolk + 0.4% egg white albumen, P1 = 90% coconut water + 10% egg yolk, P2 = 85% coconut water + 15% egg yolk, and P3 = 80% coconut water + 20% egg yolk. The variables observed were individual motility of spermatozoa, viability of spermatozoa, and abnormality of spermatozoa, intact plasma membrane, and intact acrosome hood. The data were analyzed using Analysis of Variance (ANOVA), and differences between treatments were continued with the Duncan Multiple Range Test [16].

3 Result

3.1 Fresh semen quality of Boer goat

The evaluation of fresh semen quality was tested, after collected by using an artificial vagina. The fresh semen quality evaluated through both macroscopic and microscopic observations as presented in Table 1.

Table 1. The fresh semen of Boer goats observations

Semen quality test	Observation result
Macroscopic	
Volume (ml)	0.99 ± 0.10
Color	milky white and thick
Odor	Typical of goat semen
Consistency	Spoon-thick
pH	6.59 ± 0.18
Microscopic	
Mass motility	2+
Individual motility (%)	73 ± 3,50
Viability (%)	77.19 ± 4.54
Abnormality (%)	8.96 ±1.05
Concentration of spermatozoa (x 10^7 million/ml)	4.120 ± 441.46

3.2 Individual motility of spermatozoa during cold storage

The individual motility of spermatozoa was evaluated at a temperature drop of 5^0C, starting from the first hour on the first day after dilution and storage, and repeated every 24 hours. The observation procedure for individual motility was carried out by taking semen using an ose, then dropping a drop of semen on glass object covered with a cover glass, and observing using binocular microscope light with a magnification of 400 times [18]. The individual motility was evaluated based on the progressive movement of spermatozoa, which is forward motion, compared to spermatozoa in which does not move (rotating, backward, and swinging) [17]. The observation was conducted in five different points of view, with the results of the assessment percentage (0-100%), and good spermatozoa having motility >70% [18]. The results individual motility of sparmatozoa as shown in Table 2.

Table 2. The average motility of spermatozoa after cold storage

Treatment	Length of observation day (%)		
	D1	D2	D3
P0	68,40±3,04^{b}	60,50±6,44^{c}	54,00±5,66^{b}
P1	58,60±9,64^{a}	48,70±9,81^{a}	34,08±7,99^{a}
P2	60,90±8,02^{a}	51,63±7,32^{ab}	36,83±2,84^{a}
P3	63,20±6,63^{ab}	52,75±6,90^{b}	41,40±5,81^{a}

Note: Different notations in the same column indicate significantly different (P<0.01).

3.3 Viability of spermatozoa during cold storage

The viability test of spermatozoa after storage at cold temperatures was used to determine the live and dead spermatozoa. The viability of spermatozoa observation done by dripping a drop of semen at the top of the object glass using an ose. The eosin nigrosin solution was dripped closely as much as one drop using an ose. Semen and eosin solution on the object

glass were stirred slowly until evenly mixed using a glass object, and the solution at the end of the glass object placed on another glasses object with a tilt angle of 35^0, then pulled towards the other end so that a thin smear was formed. The smear results were observed utilizing a light microscope with magnification 400 times [17]. The results of spermatozoa viability observation can be seen in the Table 3.

Table 3. The average viability of spermatozoa after cold storage

Treatment	Length of observation day (%)		
	D1	D2	D3
P0	74,69±7,59^{c}	71,01±9,11^{b}	62,04±6,45^{b}
P1	67,51±11,18^{a}	61,32±11,25^{a}	46,14±9,70^{a}
P2	68,43±8,81^{ab}	60,54±9,57^{a}	47,05±5,86^{a}
P3	70,80±7,66^{b}	64,16±9,14^{a}	51,75±6,75^{a}

Note: Different notations in the same column indicate significantly different (P<0.01).

3.4 Abnormality of spermatozoa during cold storage

The morphology observations were done by examining the visible abnormalities and defects in certain parts of the spermatozoa or entirely after storage. The evaluation of spermatozoa abnormality was done in the same way as viability by making a review preparation by dripping one drop of semen on the tip of the glass object using an ose and one drop of eosin nigrosin solution dripped nearby using an ose. Semen and eosin nigrosin solution was stirred slowly until evenly mixed using a glass object, and the solution at the end of the glass object was placed on another glass object with a tilt angle of 35^0, then pulled to the end of thin smear. Furthermore, to identify the abnormality actually using the light of microscope with a magnification of 400 times. [17]. The results of abnormality is shown in Table 4.

Table 4. The average of spermatozoa abnormality after cold storage

Treatment	Length of observation day (%)		
	D1	D2	D3
P0	8,14±1,80	9,41±0,99	9,28±1,50
P1	8,20±1,47	9,62±1,27	10,24±1,46
P2	9,03±2,07	9,33±1,99	9,95±0,90
P3	8,82±2,19	9,12±1,41	9,31±1,64

3.5 Intact plasma membrane during cold storage

The intact plasma membrane observed using the hypoosmotic swelling test solution [17]. The observation was done by putting a solution of Host 0.50 µl and 0.30µl semen into a microtube and homogenized by vortexing 20 times, then incubated in a water bath with a temperature of 37ºC for 30 minutes. The incubation results were taken with an ose, and one drop was dropped on a glass object. Make a review using the tip of another glass object, then pull to the other tip without being covered with cover glass, then make observations using a binocular light microscope with a magnification of 400 times and count as many as 200 spermatozoa [18]. The results of intact plasma membrane as presented in Table 5.

Table 5. Intact plasma membrane average after cold storage

Treatment	Length of observation day (%)		
	D1	D2	D3
P0	71,14±5,98^{c}	65,17±6,87^{b}	58,48±6,50^{b}
P1	63,05±11,17^{a}	56,02±10,92^{a}	40,81±9,55^{a}
P2	64,35±9,77^{ab}	57,98±9,83^{a}	41,54±5,70^{a}
P3	67,34±8,13^{bc}	59,05±10,13^{a}	46,70±5,16^{a}

Note: Different notations in the same column indicate significantly different (P<0.01).

3.6 Intact acrosome hood during cold storage

Observation of intact acrosome hood was performed at a temperature drop of 3-5^{0}C and repeated every 24 hours. The an intact acrosome hood was observed using 1% formalin mixed with 0.9% physiological NaCl. The solution uses 99 ml of physiologic NaCl and 1 ml of formalin, which was added and homogenized using a magnetic stirrer for 5-10 minutes. The process of observing an intact acrosome hood was taken by inserting 10µl of semen and 30µl of formalin droped into microtube, and homogenizing by vortexing 20 times. The solution incubated in a water bath at 37^{0}C during half hours. The results of the semen dropped on a glass object using an ose as much as 1 drop and without being covered with a covered glass and observed using microscope light binocular with magnification 400 times, and minimum number of 200 spermatozoa counted [18]. The results of the observation of an intact acrosome hood are shown in Table 6.

Table 6. The average of intact acrosome hood after cold storage

Treatment	Length of observation day (%)		
	D1	D2	D3
P0	69,29±6,52^{b}	64,69±7,39^{b}	58,83±6,26^{b}
P1	61,61±10,83^{a}	54,89±10,64^{a}	40,76±11,42^{a}
P2	63,48±10,22^{a}	56,36±9,13^{a}	41,89±5,67^{a}
P3	65,30±6,55^{ab}	57,57±9,18^{a}	45,62±5,29^{a}

Note: Different notations in the same column indicate significantly different (P<0.01).

4. Discussion

4.1 Fresh semen quality of Boar goat

Table 1 shows that the average value of fresh semen volume is 0,99 ± 0.10 ml. This result shows little difference in the semen volume of Boer goats research by [15], which is 0,93 ± 0,16 ml. The color of fresh semen of Boer goat is milky white with a medium and thick consistency. The consistency of Boer goat semen indicated that the consistency is still in the normal stage, namely medium and thick. [18] stated that the consistency of spermatozoa has a positive correlation with the concentration of spermatozoa produced by a stud with a relative assessment of dilute, medium, and thick. The smell of Boer goat semen is distinctive, such as fresh male goat semen, and does not cause a foul smell, so it was categorized as normal. The average pH value of Boar goat semen obtained in this research was 6.59 ± 0.18, according to [17], it was reported that goat semen has a pH ranging from 6,2 to 6,8. The result of the microscopic examination in fresh semen revealed that the mass motility was 2+. [17] states that good semen to be diluted into liquid semen has a minimum mass motility of 2+ and a maximum of 3+. The average percentage of individual motility was 73±3.50%, the average viability percentage of spermatozoa in this study was 77.19±4.54%, and the average percentage of abnormality was 8.96±1.05%. The average value of semen concentration is 4.120 ± 441.46 million/ml. This result was lower than the

research of [2], which is 5.093 ± 188.92 million / ml. Based on the macroscopic and microscopic results of tested semen quality, it shows that Boer goats semen has met the criteria for further processing into liquid semen for artificial insemination in local goats. According to [18], good quality fresh semen for dilution is semen with a percentage of spermatozoa motility >70% and abnormality <20%.

4.2 Individual motility of spermatozoa

The results as shown in Table 2 shows that use of green coconut water as a spermatozoa diluent significantly affect (P<0.01) the motility on the first, second, and third-day observations. The P3 treatment come up with the best results compared with P1 and P2 treatments, except for the control treatment (P0). The addition of 20% egg yolk percentage in P3 treatment proved to be better and able to maintain the motility of liquid semen at 3-5^{0}C temperature storage until the third day. This might be caused by the nutritional content in green coconut water, especially the simple sugar component and lecithin in egg yolk, which is still available to protect and maintain the ability of spermatozoa in cold storage. [4] stated that egg yolk is an extracellular cryoprotectant material containing lipoproteins and lecithin, which functions as a food provider, energy source, and spermatozoa extracellular protector from cold shock.

The percentage of spermatozoa motility in liquid semen diluted using egg yolk with coconut water decreased from the first day to the third day, along with the storage time. This result is thought to be caused by the availability of simple carbohydrate sources contained in coconut water and lipoproteins and lecithin in egg yolk has been reduced, so that spermatozoa experience a lack of energy needed. [2] stated that the reduced energy is due to the metabolism of spermatozoa during storage. In addition, green coconut water diluent stored at cold temperatures for a long time will experience color changes; originally bright yellow turned yellowish white and increased viscosity to thicken. This condition is likely that during storage at cold temperatures, there is a process of fermentation and accumulation of lactic acid. [14] stated that spermatozoa stored at a cold temperature of 3-5^{0}C will form lactic acid in the diluent, which causes the semen plasma that has been mixed with the diluent to be a toxin, and metabolic activity still continues anaerobically and aerobic, which can drain spermatozoa energy, resulting in a decrease in spermatozoa motility.

4.3 Viability of spermatozoa

The results in Table 3 show that the use of green coconut water as a semen diluent had a significant effect (P<0.01) on viability on the first, second and third days of cold storage These results showed that the viability of liquid semen in P3 until the third day was best compared to the treatment of P1 and P2, except in the control (P0). This might be caused by the simple carbohydrates that serve as a source of energy, and the flavonoid content in green coconut water and lecithin in egg yolk are still sufficiently available to prevent damage to the spermatozoa membrane during storage at cold temperatures. (5) explains that coconut water contains carbohydrates, lipids, proteins, and minerals that are needed by spermatozoa. The use of coconut water as a liquid semen diluent is because coconut water diluent contains simple carbohydrates and flavonoids, according to [14], which are antioxidants from the enzyme class that play a role in protecting membranes from oxidative damage. (3) states that lecithin contained in egg yolk is important in protecting the spermatozoa membrane by maintaining the phospholipid bilayer arrangement of spermatozoa at cold temperatures. The decrease in the percentage of viability of liquid semen spermatozoa began to decrease drastically, along with the length of storage time on

the second and third days. This was thought to be because the energy source in the form of simple carbohydrates in coconut water diluent and lecithin in egg yolk to maintain the spermatozoa membrane during cold storage has been reduced, resulting in spermatozoa death. [13] reported that long storage of semen at cold temperatures can affect the quality of semen, which is due to the availability of energy substances that are decreasing and the stability of the buffer solution has decreased, so that the metabolic process of spermatozoa can be disrupted, which has an impact on reducing viability and increasing spermatozoa death. (12) added that storage of semen at low temperatures will cause damage to the plasma membrane, due to the effect of cold shock. Live and dead spermatozoa are indicated by a bright color and dead spermatozoa are reddish purple as seen in (Figure 1)

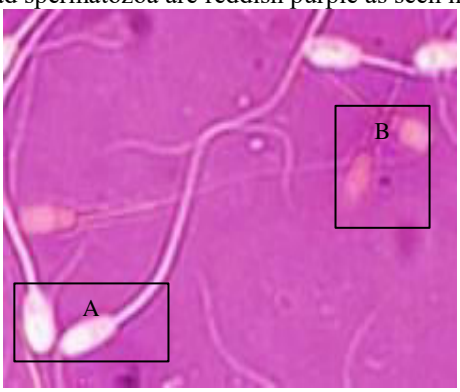

Fig. 1. Viability of spermatozoa: (A) live spermatozoa and (B) dead spermatozoa

The examining viability of spermatozoa using eosin-nigrosin was used to distinguish living and dead spermatozoa because eosin-nigrosin can react with the spermatozoa, membrane. Living spermatozoa were transparent and did not absorb color, especially on the head, because the membrane remains intact [18]. Dead spermatozoa can absorb eosin dyes and are colored purple-red, this is because the membrane has been damaged and unable to withstand exposure to eosin-nigrosin solution, so the eosin-nigrosin solution penetrates into the spermatozoa membrane, which is characterized by a purple-red color.

4.4 Abnormality of spermatozoa

Table 4 shows that the use of coconut water showed abnormalities in treatments P1, P2, and P3 were not significantly different (P>0.05) on the first, second and third days. The results showed that the abnormality of the four treatments namely P0, P1, P2 and P3 during cold storage, the abnormality did not increase significantly. The average value of abnormality on the first day observation until the third day, the highest percentage was in the P1 treatment which was 10.24 ± 1.46% and the lowest in P0 which was 9.28 ± 1.50%. This was proven from the four treatments; the percentage value of abnormality is still less than <15%, so it was feasible for artificial insemination applications. This is supported by the opinion of [18] that good spermatozoa abnormality for the application of artificial insemination must have a spermatozoa abnormality value of less than <20%. The low abnormality in treatments P1, P2 and P3, except for the control (P0), is still in the normal stage. This is thought to be because simple carbohydrates and flavonoids in coconut water and lecithin in egg yolk are still available, and the physical and physiological conditions of the diluent material have not been damaged. [14] stated that the availability of nutritional elements that are still complete and not many spermatozoa have died is a determining factor because the diluent is still in a good physiological condition for spermatozoa. Damage abnormalities

found in the four treatments during observation were secondary abnormalities, which occurred during the process of making the preparation of the ulcer, causing spermatozoa whose tails are cut off and heads without tails, as shown in (Figure 2).

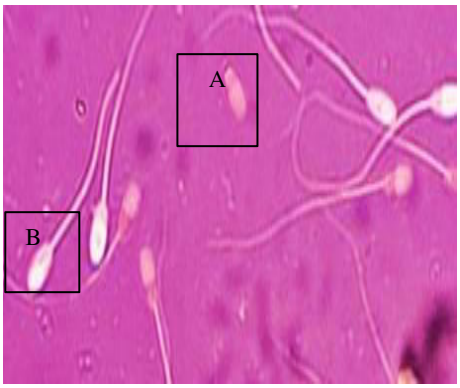

Fig. 2. The abnormality of spermatozoa: (A) head without tail and (B) tail broken off

The secondary abnormality damage to the spermatozoa was thought to be caused during the process of making the screw preparation, causing the spermatozoa to have their tails cut off and heads without tails. In addition, there were spermatozoa whose tail shape was circular in the center around the head of the spermatozoa; this is thought to be due to cold shock during temperature reduction and long storage at cold temperatures. [17] stated that secondary abnormality damage occurs after the process of spermatozoa formation, until ejaculation, and also during the process of spermatozoa processing, which includes folding tails, heads without tails, or tails without heads.

4.5 Intact plasma membrane of spermatozoa

Table 5 shows that the use of coconut water as a Boer goat liquid semen diluent has a very significant effect ($P<0.01$) on intact plasma membranes on the first, second and third days. These results indicate that the intact plasma membrane in the control (P0) treatment showed the best results compared to the P1, P2, and P3 treatments. However, the P3 treatment can provide better results compared to the P1 and P2 treatments. This was thought to be because green coconut water diluent contains simple carbohydrates such as glucose, fructose, sucrose and flavonoid that can act as a protector of spermatozoa plasma membrane at cold temperature. [5] stated that coconut water contains carbohydrates, lipids, proteins, and antioxidants from the flavonoid. In addition, it was suspected that lecithin in egg yolk could protect the plasma membrane from the effects of cold temperatures. [4] mentioned that egg yolk as an extracellular cryoprotectant containing lipoproteins and lecithin that can function to protect the spermatozoa membrane and prevent cold shock during cooling. The decrease in the percentage of intact plasma membrane began to decrease with the length of storage time on the second and third days. This condition is thought to be due to the reduced content of simple carbohydrates in coconut water diluent and lecithin in egg yolk, as well as the occurrence of spermatozoa metabolic processes during storage, resulting in an increase in lactic acid in the diluent which causes damage to the plasma membrane. [20] mentioned that the metabolic process of spermatozoa can affect spermatozoa survival because spermatozoa with high metabolic activity will produce high lactic acid, which can cause spermatozoa death. The results of observations of intact plasma membranes of spermatozoa whose tails are circular, as shown in (Figure 3), indicate that

the plasma membrane was still intact because it could withstand hypoosmotic solutions, and spermatozoa whose membranes are damaged are characterized by straight tails.

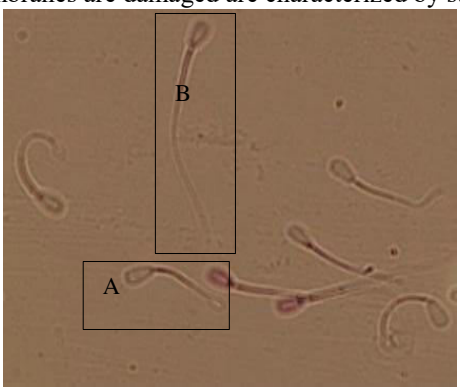

Fig. 3. Intact plasma membrane: (A) intact and (B) not intact

Spermatozoa whose membranes are intact, if exposed to a host solution and incubated in an incubator with a temperature of 37^0C, the spermatozoa try to increase the volume of water in the spermatozoa body so that the liquid inside and outside the spermatozoa body remains in balance. Spermatozoa with a good plasma membrane will try to withstand the host solution that causes constriction of the spermatozoa membrane that can cover the tail, thus forcing the spermatozoa tail to curl at the end. Spermatozoa whose membrane has been damaged could not withstand the host solution, causing the tail to be straight, because the methylation process in the spermatozoa cell has lost it's function [17].

4.6 Intact acrosome hood of spermatozoa

Table 6 shows that the use of green coconut water as a Boer goat liquid semen diluent gives a significant effect ($P < 0.01$) on intact acrosome hood on the first, second and third days. The P3 treatment with the addition of 20% egg yolk to coconut water can provide better results than the P1 and P2 treatments in maintaining the quality of intact acrosome hoods in cold storage until the third day, except for the control (P0). This result was expected due to the presence of simple carbohydrate components, such as glucose, fructose and sucrose in coconut water [5]. In addition, lecithin in egg yolk plays an active role as an extracellular cryoprotectant that serves as a source of nutrients for spermatozoa and protects the acrosome hood from membrane damage during cold storage. [1] stated that the presence of sucrose contained in coconut water, as well as lipoproteins and lecithin in egg yolk, is able to protect the spermatozoa membrane against cold shock and the denaturing effect of glycoproteins and glycolipids, membrane constituents with the length of storage. [14] reported that antioxidant compounds contained in coconut water, such as vitamin C and flavonoids, will act as protectors of spermatozoa from lipid peroxidation during cold storage by inhibiting and preventing lipid peroxidation of spermatozoa membranes by binding reactive oxygen species (ROS). [8] added that vitamin C could capture free radicals and prevent chain reactions so as to avoid peroxidative damage that can affect the viability of spermatozoa during cooling.

The decrease in percentage of intact acrosome hood of Boer goat spermatozoa during cold storage from the second to the third day decreased very drastically. This is thought to be because the nutrients contained in the coconut water diluent and egg yolk have been reduced. The spermatozoa are unable to adapt to a temperature drop of 5^0C, resulting in a cold shock that results in decreased motility and viability followed by damage to the plasma membrane and damage to the intact acrosome hood on the spermatozoa head. [6] stated that

cooling spermatozoa can damage the plasma membrane and intact acrosome hood of spermatozoa due to changes in the integrity of spermatozoa chromatin. In addition, it was thought to be caused by the ability of the diluent to provide energy sources that have been depleted and the accumulation of lactic acid from the metabolic products of spermatozoa during storage at refrigerator temperatures. In addition, it was also thought to be caused by the accumulation of lactic acid from the metabolic products of spermatozoa that have died during storage. The intact acrosome hood was characterized by the anterior part of the head being darker than the posterior part or if there was a black color at the tip of the head, and the damaged intact acrosome hood did not have a black color on the head of the spermatozoa as shown in (Figure 4).

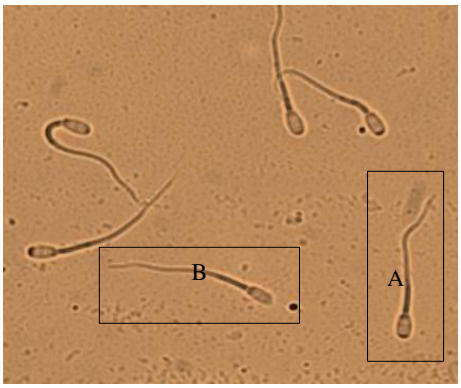

Fig. 4. Intact acrosome hood: (A) Intact acrosome hood and (B) Incomplete acrosome hood

The acrosome hood has a close relationship with the intact plasma membrane, which can protect the acrosome hood because there are several enzymes in it that can play a role in egg fertilization. [17] states that the head of the spermatozoa has an acrosome hood sheath that can function to protect the release of genetic material, and in the acrosome hood, there is a hyaluronidase enzyme that plays a role in penetrating the ovum sheath for fertilization. The spermatozoa that have an intact acrosome hood, indicated by the tip of the head there are black spots after exposure to 1% formalin solution, this was because formalin had the ability to react or fixate some hydrolytic enzymes in the acrosome hood of spermatozoa. Spermatozoa that have an incomplete or damaged acrosome cap did not have black spots at the tip of the spermatozoa head because the hydrolytic enzymes in the acrosome hood have flowed out, so there was no fixation between 1% formalin and hydrolytic enzymes in the acrosome hood on the spermatozoa head.

5. Conclusion

The use of young green coconut water and egg yolk diluents as alternative diluents for the process of making liquid semen of Boer goats is able to maintain the quality of spermatozoa in cold storage for up to 3 days, and is suitable for insemination in goats.

Acknowledge

The authors gratefully acknowledge the Government of Timor Leste, especially the National Institute of Public Administration (INAP) and Human Capital Development Fund for providing scholarship assistance (FDCH) to the author to continue the study of Master of Animal Science, Faculty of Animal Science, Universitas Brawijaya-Malang.

References

1. Anwar, P., Y.S. Ondho and D. Samsudewa, Quality of intact plasma membrane and intact acrosome hood of Bali cow spermatozoa preserved at 5^0c in sugarcane water extract diluent with egg yolk addition. Agromedia. **3**, 1 53-63, (2015)
2. Audia, R. P., M. A. Salim, N. Isnaini and T. Susilawati, Effect of different maturity of green coconut water as a diluent added with 10% egg yolk on the quality of Boer goat liquid semen. Journal of Tropical Livestock. **18**, 1 58-68, (2017)
3. Coester, J. S., A. Sulaiman, and M. Rizal, Viability of Limousin cattle spermatozoa preserved with tris diluent and various concentrations of soybean juice. Journal of Tropical Animal Science and Technology. **6**, 2 175-180, (2019).
4. Dwitarizki, N. D., Ismaya and W. Asmarawati, Effect of sperm dilution with coconut water and duck egg yolk and storage duration on motility and viability of Garut sheep spermatozoa at 5°C storage. Livestock Bulletin. **39**, 3 149-156, (2015).
5. Farapti and S. Sayogo, Young coconut water effect on blood pressure. Mirror of the Medical World. **41**, 12 896-900, (2014).
6. Fernandes, G. H. C., P. T. C., A.J. Serra., A. M. Crespilho., J.P.S. Peron., C. Rossato., E.C.P. Leal-Junior, and R. Albertini, The effect of low-level laser irradiation on sperm motility, and integrity of the plasma membrane and acrosome in cryopreserved bovine sperm. PLoS ONE. **10**, 3 1-11, (2015).
7. Ihsan, M. N, The use of duck eggs as goat semen diluent. Journal of Tropical Livestock. **12**, 10-14, (2011)
8. Lubis T.N., Dasrul., C. N. Thasmi and T. Akbar, Spermatozoa after Cold Storage. Journal of Agricultural Science. **3**, 1 347-361, (2013).
9. Mugiyati, M. A. Salim., N. Isnaini and T. Susilawati, Effect of young and old red coconut water as diluent on semen quality of Boer goats during cold storage. Journal of Tropical Livestock. **18**, 1 20-26, (2017).
10. Muhammad, D., N. Isnaini, Kuswati, A. P. A. Yekti, Aryogi, M. Luthfi, L. A. Sunarto, and T. Susilawati, Effect of fructose addition in green coconut water diluent on motility of PO (Peranakan Onggole) cow spermatozoa. Indonesian Journal of Animal Science. **13**, 4 318-324, (2018).
11. Rizal, M., C. Nisa, and R. Norliani, Quality of frozen semen of Boer goats cryopreserved with egg yolk tris diluent and various concentrations of moringa leaf extract. Veterinary Journal. **22**, 3 309-316, (2021).
12. Rizal, M., and M. Thahir, Viability of spermatozoa of Peranakan Etawa goats preserved with various types of diluents. JITRO. **3**, 3 1-9, (2016).
13. Rokana, E., Srigati, E.F. Lisanti, and Samudi, Effect of *Moringa oleifera* Lamm leaf powder and length of storage at 4-5°C cold temperature on the quality of liquid semen of bean goat. Journal Livestock of Indonesia. **24**, 1 43–54, (2022).
14. Salim, M. A., M. N. Ihsan., N. Isnaini, A. P. A. Yekti and T. Susilawati, Quality of Boer goat liquid semen on different coconut water diluent (*Cocos nucifera*) during cold storage. Asian Journal of Microbiology, Biotechnology and Environmental Sciences. **20,** S150-S157, (2018).
15. Sholikah, N., and S. Susilowati, Effect of egg yolk composition in green coconut water diluent on Boer goat liquid semen quality. Journal of Tropical Livestock Science and Technology. 7, 2 152-157, (2020).

16. Sudarwati H., M. H. Natsir, and V.M.A. Nurgiantiningsih, Statistics and Experiment Design Application in Animal Science. Brawijaya University Press. ISBN: 978-602-641-8, (2019).
17. Susilawati, T., Spermatology. Brawijaya University Press. ISBN: 978-602-8960-04-5, (2011).
18. Susilawati, T., Guidelines for Artificial Insemination in Livestock. Universitas Brawijaya Press. ISBN: 978-602-203-458-2, (2013).
19. Susilawati, T., F. E. Wahyudi., I. Anggraeni, N. Isnaini and M. N. Ihsan, Replacement of bovine serum albumin in CEP-2 diluent with cow blood serum and egg white on liquid semen quality. Journal of Veterinary Medicine. **10**, 2 6-10, (2016).
20. Varasofiari, L. N., E.T. Setiatin and Sutopo, Evaluation of fresh semen quality of Java Brebes cattle based on length of storage time. Journal of Livestock Agriculture. **2**, 1 201-208, (2013).
21. Yekti, A. P. A., J. Harsah., M. Luthfi., M. Dikman., A. N. Huda., Kuswati and T. Susilawati, Semen quality with various formulations of green coconut water base diluent during cold storage in Madura cattle. Journal of Tropical Animal Science and Technology. **5**, 3 49-56, (2018).
22. Yekti, A. P. A., A. R. N. Umamah., F. Safa., N. M. Andriani., N. Febrianto and T. Susilawati, Spermatozoa quality and intact acrosome hood in Friesian Holstein cattle frozen semen with different genetic quality. Agripet Journal. **24**, 1 89-95, (2024).
23. Yong, J. W. H., Ge, L., Ng, Y. F., and S.N. Tan, The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water. Molecules. **14**, 12 5144–5164, (2009).