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QUALITY OF BOER GOAT LIQUID SEMEN ON DIFFERENT COCONUT WATER DILUENT *(COCOS NUCIFERA)* DURING COLD STORAGE

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Abstract– Optimization of liquid semen quality of Boer goat with non-synthetic diluent raw materials using coconut water, because it has a biochemical component that functions like a synthetic diluent. The objectives of this study were to observe the quality of Boer goat liquid semen using different coconut water diluted varieties and the age of the fruit was added 10% yolk. Semen was collected from 3 Boer goat using an artificial vagina once a week. (T1) Green Young Coconut Water (*viridis*), (T2) Green Old Coconut Water, (T3) Red Old Coconut Water (*rubescens*) and (T4) Red Young Coconut Water and (P0) Tris Aminomethane as a Control. Variables analysis was individual motility, viability, abnormality and membrane integrity. Randomized completed block design (RCBD) was used with 4 treatments and 1 control and 7 replications. Data were analyzed by Analysis of Variance (ANOVA), the difference between treatments was performed with Duncan's Multiple Range Test using Genstat 18.2 program. The results showed that treatments have a significantly effect (P <0.05) on individual motility at D0 and D2; while on the other day individual motility significant decreased (P <0.01). Further test showed that the best treatment was T0. The viability of spermatozoa showed that treatment had no effect (P> 0.05) on D0; D1 and D2 but significantly (P <0.05) in D3 and had a very significant effect (P <0.01) on D4 - D8. Further test results showed that P0 has the best viability value during cooling. The spermatozoa abnormality showed had no effect of treatments (P> 0.05) of storage D1 to D7 but significantly (P <0.05) in D0 storage. Further test results showed that T0 and T1 had the lowest values of 1.5 ± 0.6% and 1.1 ± 0.9%. The integrity membrane showed that the treatment had no effect (P> 0.05) on storage D1 to D3 but had a very significant effect (P <0.01) on storage D4 to D8. Further test results showed that T0 has the highest value on storage D4 to D8. In conclusion, the best quality is T0 up to 7 days storage with the motility = 40.0 ± 5.0%, viability = 40.7 ± 9.5%, abnormality = 1.6 ± 0.6%, membrane integrity = 42.5 ± 8.55% and total motile spermatozoa = 80.0 ± 10.0 Million / mL. For the coconut water showed that (viridis) Young green coconut water stored at 2 days with the motility = 60.0 ± 7.0b%, viability = 69.3 ± 8.1%, abnormality = 69.3 ± 8.1% membrane integrity = 59.8 ± 9.23% and total motile spermatozoa = 120.0 ± 15.3 million / mL.

INTRODUCTION

Artificial Insemination (AI) is a reproductive technology that can improve the genetic quality of livestock. During this time AI uses frozen semen that is stored on liquid nitrogen. The main constraint in some area in Indonesia is the absence of liquid nitrogen, therefore it needs AI technology using liquid semen (Susilawati *et al*., 2017) which has some storage techniques for AI applications (Hafez, 2008).

Several semen diluents that have been used by Susilawati (2017) is Extender of CEP-2 + 10% egg yolk which has the best extender to maintain quality of stored semen at refrigerator temperature (4-5^{o}C) that is suitable for artificial insemination with the longest storage of CEP-2 + 10% coconut milk or CEP-2 + 5% skim milk Extender. Susilawati *et al* (2018) has reported that CEP2 egg yolk can maintain the sperm motility better than other diluent for Bali cattle sperm and supported by Ratnawati *et al* (2017) that the usage of CEP-2 diluent in the processing of liquid semen can support sperm motility on PO, Bali and Madura bulls until the 5th days of cold

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storage. Costa *et al.,* (2016[b]) reveales that dilution based on tris aminomethane + egg yolk can maintain the semen quality including spermmotility, viability and cattle sperm membrane integrity during cooling process. AI using semen in tris aminomethane + 20% egg yolk dilution agent and cold stored for the first and fifth day of storage resulted CR 86.67% and 83.33% while S / C were 1.31 and 1.44 respectively (Costa *et al.,* 2016a).

Coconut water has the potential to be used as a diluent, since its content includes nutrients such as glucose, fructose and sucrose for energy sources and balance (Yong *et al.,* 2009) and antioxidants as well as amino acids that ensure the durability of spermatozoa during cryopreservation . The use of coconut water as small ruminant semen diluent yields varied results. Daramola *et al.,* (2016) study on African goats using Coconut Water added different yolk concentration gave an excellent results on motility, viability and integrity of the membrane. Dwaadmadji *et al.,* (2007) reported Coconut and egg yolk dilution with a ratio of 75%: 25% and 50%: 50% can maintain the quality of Nubian goat semen for 2 days storage, while Kewilaa (2013) reported Coconut water and egg yolk 20% has a motility 40 - 50% for 4 days storage at 4-5^{o}C in thin tail sheep.

Coconut water has different biochemical components based on coconut varieties and fruit maturity levels, thus affecting the nutritional, antioxidant and isotonic properties. The Old Green Coconut (*viridis*) has higher sugar content than the Younger Coconut (*viridis*) and the Red Coconut (Rubescens) as well as the Yellow Coconut (Yong *et al.,* 2009). Differences in varieties and fruit maturity, affect the level of biochemical content and its preservation power to spermatozoa during cold storage at 4-5^{o}C because of differences in chemical composition and molecular weight (Naing, 2010).

MATERIALS AND METHODS

This research was conducted at Animal Reproduction Laboratory of Sumber Sekar Unit of Animal Husbandry Faculty, Brawijaya University Malang. The material used was fresh semen of 3 heads Boer goat aged 3-5 years. Coconut water used were green (*viridis*) and red coocnut (*rubescens*) with fruit maturity including young coconut (5-7 months) and old coconut more than 12 months old fruit, egg yolk, tris aminomethan, aquabidest, alcohol 70%, eosin -negrosin, antibiotics, 3% NaCl, $NaHCO_3$. The method used was experimental with 4 treatments and 1 control, namely T0 (control): Tris aminomethane; (T1): Green Young Coconut Water; (T2): Green Old Coconut Water; (T3) Red Old Coconut Water (T4): Red Young Coconut Water, which each treatment was added with 10% Egg yolks and repeated for 7 times.

Control Diluents Preparation (Trisaminomethan) + 10% Egg yolk

Preparation of trisaminomethan + 10% egg yolk was for 100 mL as Susilawati (2013) with the composition were tris amino methan 1.363 g, citric acid 0.762 g, lactose 1.5 g, fructose 0.5 g, egg yolk 10 g, raffinosa 2.7g, streptomycin, 0.1, aquabidest 80 mL, penicillin 0.1. all the materials were homogenizedand mixed with antibiotics. After that egg yolk was added as much as 10% then homogenized. The diluents were taken as much as 5 mL, then centrifuged for 2 times in 30 minutes with 1500 rpm. The last, the supernantant was taken as much as 3 mL.

Coconut water diluents preparation + 10% Egg yolk

Young and Old Green Coconut Water (viridis) as well as Young and Old Red Coconut Water (rubescens) were each taken for 100 mL, filtered with Whattman filter paper, placed on 4 beaker glass of 100 mL size which has been labeled according to the treatment. All were inactivated by heating temperature at 56^{o}C for 20 minutes. Taken pH was measured for each 50 mL diluents as a sampling sampling, when acid was indicated then buffers was added. Furthermore, mixed with egg yolk as much as 10%.

Semen collection

Ten ejaculates were collected from a healthy and proven fertile Boer cross buck (36 months old and weighing 75 kg) by artificial vagina at 7 day intervals. Ejaculates were immediately sent to Laboratory for initial evaluation (volume, motility, concentration). The quality of each ejaculate needed were : more than 0.8 mL in volume, more than 70% in motility and 2.5 – 3 x 10^6 spermatozoa/mL in concentration, to be suitable for a further process (Susilawati, 2013).

Semen and Diluents Preparation

Diluents and semen were added for each 0.07 mL at water jacket temperature 33^{o}C then homogenized.

Furthermore, it was placed in the refrigerator in a cool bottom position until the temperature reach to 25°C, then diluents were added as a much as 0.93 mL at 25°C, 2 mL at 20°C and 2 mL at 10°C respectively. The total dilution volume was 5 mL after that, placed it on a cool top position until the temperature reached steadily at 4-5°C. the quality of spermatozoa was evaluated 1 hour after the temperature reached at 4-5°C as D0.

Variables of Semen Quality

Individual Motility of Spermatozoa

Individual motility evaluation was performed to observe the progressive movement of spermatozoa in one point of view by using a 400x light magnification microscope at constantly temperature covered with a cover glass. Progressive movement is a toward movement that is the best movement for spermatozoa (Susilawati, 2013).

Viability of Spermatozoa

The procedure of viability is by making smear of 1 drop of eosin negrosin with 1 drop of semen, then observed in a 400x magnification microscope without glass cover with a total count of 200 spermatozoa. The live spermatozoa would not absorb the eosin negrosin, however the dead spermatozoa will absorb it. The viability value is the percentage of total live spermatozoa divided by total spermatozoa was observed, multiplied by 100%.

Abnormalities of Spermatozoa

Spermatozoa abnormalities was observed by looking at morphological abnormalities both due to physiological and technological causes. The morphological observation procedure is similar to the viability evaluation. Abnormal sperms were indicated by tailless, abnormal heads and abnormal tail formation with a proximal cytoplasmic droplet and abnormal tail formations with a distal droplet (Ax *et al*., 2008).

Membrane Integrity of Spermatozoa

Evaluation of spermatozoa membrane integrity was used hypo-osmotic swelling test solution. 1 mL hypoosmotic solution 150m osmol (made from 7.35 g of sodium citrate, $2H_2O$, 13.52 g fructose dissolved in 1000 mL of aquabides). Subsequently dripped 0.1 mL of sample semen in 1 mL of HOST solution and incubated at 37°C for 30 minutes. Next was calculated spermatozoa that are circular and straight tail using 400x magnification microscope totaling about 200. The percentage of membrane integrity is calculated from the total circular tail spermatozoa divided by the total observed x 100%. Swollen or coiled tails were considered as intact and active sperm membranes, while unswollen head and uncoiled tails were considered as disrupted and inactive sperm membranes (Susilawati, 2013).

Statistical analysis

The statistical significance of the result was evaluated by a two way completely randomized blocks design analysis of variance using Genstat 18.2 for statistical software. Data presented as means $\pm$ SD with probability $P<0.05$ considered as significantly different.

RESULTS AND DISCUSSION

The characteristics of fresh semen used in this study were Volume = 0.8 ± 0.3 mL, color = Milk cream, Consistency = thick, pH = 7, Concentration = 501 ± 46.6 x 107 / mL, Mass Motility = 3+, Individual Motility = 78.3 ± 2.6%, viability = 81.4 ± 3.5%, abnormality = 4.2 ± 1.1% and has a good membrane integrity = 83.1 ± 2.4%. The criteria of semenis good based on the opinion of Susilawati (2011); Ax *et al* (2008).

Individual Motility of Spermatozoa

Based on the result of variance analysis showed that the treatment gave a real effect ($P <0.05$) on the motility of Boer goat spermatozoa on the first and second day; while on the other day showed a significant decreasing ($P <0.01$) on the motility of Boer goat spermatozoa. The T0 diluent maintains spermatozoa motility up to 40% on day 7, whereas in T1, T3, T4 diluents until the second day, furthemore for T2 only day 1.

The best result was on T0 as a control using tris aminomethane and egg yolk. The presence of nutritional components available in tris amino methane combined with extracellular cryoprotectant from egg yolk ensures the sperm motility abilities during cold storage persist on 40% to 7 days. According to Amirat *et al.,* (2004) and Aboagla and Terada (2003) tris aminomethane and egg yolks diluent contain Low Density Lipoprotein (LDL) especially phospholipid which protects spermatozoa from cold shock effect because it is macromolecule substance, maintaining

phosphobillayer membrane configuration of spermatozoa through membrane coating mechanism to become barrier of cold shock.

The ability of coconut water to maintain the motility is caused by the available nutrients, antioxidants and buffer so can ensure the optimal physiological conditions for sperm motility according to Kewilaa (2013). In addition there is an egg yolk which functions as extra cellular cryoprotectant because it contains phospolipid.

Coconut water glucose plays a role in glycolysis and the krebs cycle in mithocondria spermatozoa during cooling as an energy supplier in the form of Adenosin triphosphate (ATP) to move the microtubules on the spermatozoa tail resulting in progressive movement activity (Williams and Ford, 2001). The protein content of the two varieties is not very different, but physiologically will respond slightly different to the motility of spermatozoa during storage. Naing (2010) mentioned that the differences in biochemical composition and molecular weight will respond differently to spermatozoa. The protein content in coconut water is small, but it provides potential amino acids for the activity of spermatozoa metabolism.

Coconut water also contains antioxidants that play a role in preventing oxidative damage due to lipid peroxidation. The antioxidants in this coconut water play a role in inhibiting the activity of spermtozoa membrane destruction due to lipid peroxidation through donor of hydrogen atoms, eliminating Reactive Oxygen Species (ROS) compounds that cause lipid peroxidation (Sikka 2004; Sanocka and Kurpizs 2004 and Gupta (2010). T1 has better quality than T2, T3 and T4 because the antioxidant content in Green Young Coconut Water is higher than Red Coconut, so that the quality spermatozoa was better due to smaller membrane damage so not to disturb the mitochondria to produce ATP (Juyena and Steletta, 2012). This was confirmed by the concentration of enzyme Superoxidasedysmutase (SOD) analyzed at 3 different times were higher on T1 (day 1= 36.676 ± 2.19 ng/100µL, day 3 = 36.527 ± 2.20 ng/100µL, day 8 = 24.830 ± 8.93 ng/100µL) than T2 (day 1= 30.693 ± 3.55 ng/100µL), day 3 =30.247 ± 2.82 ng/100µL, day 8= 20.455 ± 6.21 ng/100µL), T3 (day 1= 29.771 ± 3.04 ng/100µL, day 3 = 29.324 ± 2.58 ng/100µL, day 8 = 20.574 ± 5.15 ng/100µL and T4 (day 1= 32.598 ± 2.78 ng/100µL, day 3 = 30.723 ± 1.09 ng/100µL, day 8 = 20.455 ± 5.88 ng/100µL) from the other three coconut water diluents. Superoxidasedysmutase (SOD) is an enzymatic antioxidant that works in preventing free radical formation in diluents (Halliwel and Gutteridge, 2014).

Viability of spermatozoa

Based on the result of variance analysis showed that the treatment did not give an effect (P> 0,05) to viability of spermatozoa on D0; D1 and D2 but gave a significant effect (P <0.05) on D3 and had a very significant effect (P <0.01) on D4 until D8. Further

Table 1. The average of individual motility spermatozoa of Boer liquid semen after treatment

Treatment	D0 *(%)	D1** (%)	D2 * (%)	D3 ** (%)	D4 ** (%)	D5** (%)	D6** (%)	D7** (%)	D8** (%)
T0	70.7±1.9^{b}	65.0 ± 6.5^{b}	63.6 ± 4.8^{b}	52.9±8.6^{b}	50.0±7.6^{b}	47.9±8.1^{b}	42.9±8.6^{b}	40.0±5.0^{b}	36.4±6.3^{b}
T1	70.7±1.9^{b}	65.7 ± 5.3^{b}	60.0 ± 7.0^{b}	30.7± 28.5a	12.7±20.5^{a}	4.3±9.3^{a}	1.4±3.8^{a}	0.0± 0.0^{a}	0.0± 0.0^{a}
T2	52.9±24.1^{a}	46.4 ± 22.1^{a}	32.9 ± 24.8^{a}	9.9± 24.4^{a}	8.6 ± 22.7^{a}	7.9±20.8^{a}	5.7±15.1^{a}	5.7±15.1^{a}	5.0±13.2^{a}
T3	69.3±1.9^{b}	64.3 ± 5.3^{b}	58.6 ± 11.1^{b}	18.6±19.3^{a}	14.3±22.8^{a}	8.6±14.9^{a}	8.6±14.9^{a}	7.9±13.5^{a}	7.1±12.5^{a}
T4	65.7± 6.1^{a}	65.0 ± 5.0^{b}	55.8 ± 16.9^{ab}	18.6 ± 31.8^{a}	16.4±28.4^{a}	8.6±15.7^{a}	1.4±3.8^{a}	1.4±3.8^{a}	0.7±1.9^{a}

Description: * Different notations on the same column showed a significant difference (P <0.05).
** Different notations in the same column showed very significant differences (P <0.01)

Table 2. The average of viability spermatozoa of Boer liquid semen after treatment

Treatment	D0(%)	D1(%)	D2(%)	D3*(%)	D4**(%)	D5**(%)	D6**(%)	T7**(%)	T8** (%)
T0	78.6±5.7	75.4±8,9	71.3±10.3	50.6±23.8^{b}	57.7±7.8^{b}	51.2±9.3^{b}	45.1±12.1^{b}	40.7±9.5^{b}	41.1±20.2^{b}
T1	75.0±15.2	76.3±7.5	69.3±8.1	59.1±17.9^{b}	28.3±22.3^{a}	12.3±10.1^{a}	7.8±6.2^{a}	2.6±2.4^{a}	1.9±1.5^{a}
T2	72.1±16.9	64.9±23.5	59.6±12.1	25.7±18.7^{a}	17.2±18.6^{a}	16.3±22.2^{a}	13.8±21.2^{a}	13.0±20.6^{a}	9.7±16.6^{a}
T3	81.5±9.9	76.8±7.1	62.0±12.6	48.8±16.4b	36.0±26.9^{a}	25.2±22.9^{a}	15.7±22.8^{a}	13.1±19.8^{a}	10.5±16.2^{a}
T4	79.4±3.1	75.2±5.64	66.5±10.41	56.9±10.1^{b}	28.4±24.1^{a}	23.4±3.42^{a}	16.3±21.5^{a}	12.5±17.3^{a}	11.7±17.37^{a}

Description: *) Different notations on the same column showed a significant difference (P <0.05). **) Different notations in the same column showed very significant differences (P <0.01)

test results showed the spermatozoa viability on day 3 was decreased. D0 has the best viability value in each treatment compared the other day; this condition indicates that the longer storage caused the higher percentage of spermatozoa death.

The analysis statistics results showed that between the control and the treatment of diluent were not significantly different at day 0 to day 2 of storage. This indicates that the survival of spermatozoa on liquid semen both on coconut water and tris diluent was equivalent in two days storage. The presence of protective components and nutritional providers for spermatozoa during cooling of the tris amino methane, coconut water and egg yolk ensure the supply of energy and protection from membrane damage due to cold shock and lipid peroxidation (Young *et al.*, 2009; Dwadmadji *et al.*, 2007), so that can maintain the survival of spermatozoa up to 2 days storage in coconut water with the motility around 55% and 8 days on tris aminomethane with the motility around 41%.According to analysis of variance results showed that P0 has the best motility on D2 that was 71.3 ± 10.3%. This is because the tris amino methane and egg yolks provide nutritional and protective components that ensure the life of spermatozoa during cold storage. Among all coconut water treatments, P1 had the best viability (69.3 ± 8.1%) in the day 2 of storage. Young Green Coconut Water contains glucose as much as 0.49 ± 0.02% which is one of the factors that contribute to protect the spermatozoa membrane from damage due to low temperature storage by mechanism of maintaining osmolarity through active and passive transport across the membrane (Purdy, 2006; Naing *et al.*, 2010). The Koshimoto and Mazur reports, (2002) explain that the goat spermatozoa readily utilize sugars for osmotic balance and cryoprotection. The effect of coconut water on the viability of spermatozoa is related to the presence of amino acids that protect the integrity of the membrane (Kundu *et al.*, 2001; Atessahin *et al.*, 2008; Almeida and Soares, 2002) and minerals such as potassium which synergize with potassium plasma semen for maintain osmolarity.

The protein content of all treatments did not significantly different, however physiologically will respond slightly different to the survival of spermatozoa during cooling process. The protein content in coconut water is few, less than 1%, but the protein from coconut water as the diluent which later along with the protein in seminal plasma which is the secretion of epididymis and seminal vesica will play a role in performing metabolic functions spermatozoa as well as maintaining membrane integrity. Protein from semen plasma in diluents before cooling process will prevent membrane damage by the inhibition mechanism of tyrosine phosphorylation proteins. The protein is adsorbed through thin filaments of the spermatozoa membrane thus blocking direct exposure to cold shock in spermatozoa that prevent the death of spermatozoa.

The highest coconut fat content in Old Coconut

Table 3. The average of abnormality spermatozoa of Boer liquid semen after treatment

Treatment	D0 (%)	D1 (%)	D2 (%)	D3 (%)	D4 (%)	D5 (%)	D6 (%)	D7 (%)	D8 (%)
T0	1.5 ± 0.6^{ab}	1.8 ± 1	2.1 ± 0.8	2.1 ± 1.2	2.3 ± 1.6	1.6 ± 0.9	1.8 ± 0.5	1.6 ± 0.6	2.4 ± 1.6
T1	1.1 ± 0.9^{a}	1.8 ± 1,4	1.6 ± 1	1.4 ± 0.4	3.4 ± 2	1.3 ± 0.8	2.9 ± 2.1	1.4 ± 0.5	2.3 ± 1.1
T2	1.6 ± 0.4^{ab}	2 ± 1.1	2.1 ± 1.8	1.9 ± 1.2	3 ± 2.3	1.8 ± 1.5	2.9 ± 2.9	2.6 ± 2.4	1.6 ± 1
T3	1.9 ± 0.6^{bc}	2.5 ± 1.5	1.7 ± 0.8	2.1 ± 0.8	3.2 ± 3	1.7 ± 0.8	1.7 ± 0.7	1.5 ± 1.1	2.3 ± 1.5
T4	2.4 ± 0.7^{c}	2.8 ± 1.5	1.8 ± 1.3	3 ± 1.6	2 ± 1.1	1.8 ± 0.9	1.8 ± 0.5	1.3 ± 1.1	2.3 ± 1.4

Description: *) Different notations on the same column showed a significant difference (P <0.05).

Table 4. The average of membran integrity spermatozoa of Boer liquid semen after treatment

Treatment	D0 (%)	D1 (%)	D2 (%)	D3 (%)	D4 (%)	D5 (%)	D6 (%)	D7 (%)	D8 (%)
T0	78.1 ± 8.91	75.3±9.5	76.5±9.44	69±5.89	66.1±9.64^{b}	53.3±8.77^{b}	50.1±8.62^{b}	42.5±8.55^{b}	36.3±4.59^{b}
T1	66.8 ± 15.9	63.2±14.7	59.8±9.23	57.3±12.3	38±22.4^{a}	11.9±8.98^{a}	5.75±5.57^{a}	2.32±2.07^{a}	1.71±1.34^{a}
T2	66.7 ± 24.7	66.7±26.5	56.5±25.2	50.6±23.9	28.2±21.9^{a}	14.5±18^{a}	8.4±15.7^{a}	5.32±10.6^{a}	5.32±7.15^{a}
T3	74.7 ± 6.85	67.6±5.25	65.1±5.65	64.9±6.53	38.8±22^{a}	26.4±24.6^{a}	14.2±21.9^{a}	12.7±18.3^{a}	10±16^{a}
T4	75.9 ± 7.05	72.7±4.76	66.8±4.93	59.9±3.17	31.6±21.4^{a}	21.8±17.9^{a}	12.2±14.1^{a}	7.35±11.1^{a}	6.32±9.4^{a}

Description: **) Different notations on the same column showed a very significant difference (P <0.01).

Water is 0.10 - 0.14% in 100 mL. The high fat content of coconut water has the potential to cause spermatozoa death due to the physicochemical changes in fat after the process of colling, due to increase in viscosity, as confirmed by the viability of spermatozoa on the diluent from the Old Coconut Water until the second day was not as big as the Young Coconut Water.

Coconut water also contains antioxidants of the enzyme class such as flavonoids, acting as an active substance in preventing the occurrence of free radical effects that affect the integrity of spermatozoa membrane through the mechanism of binding of peroksidan metals such as Fe and Cu, which do not cause damage to the spermatozoa membrane, causing a good viability in all treatments on day 2 of storage. This was confirmed by the high content of enzymatic antioxidant superoxidase dysmutase (SOD) in T1 when analyzed until day 8.

Abnormality of spermatozoa

The result of variance analysis showed that treatment did not give a significant effect (P> 0.05) to Boer goat spermatozoa at storage of D1 until D7 but gave a significant effect (P <0.05) on D0 storage. The further tests showed that P4 treatment had the highest abnormality value in D0 storageamong other treatments of 2.4 ± 0.7% while in treatment T0 and T1 had the lowest value of 1.5 ± 0.6% and 1 , 1 ± 0.9% respectively.

Based on Ax *et al*. (2008) reveales that ejaculate with an abnormal percentage 15% of spermatozoa can not be used for AI. Morphological spermatozoa associated with fertility, in sheep there is a positive correlation between normal morphology with motility, so that semen with abnormality 20% or more, the quality of semen is bad (Susilawati, 2011). The low abnormality in all treatments shows that the semen used is still feasible for AI because it has a good quality. The low abnormality in all of these treatments is related to the ability of each diluent in protecting the spermatozoa from mechanical damage due to the cooling process. This was showed by only a few abnormalities caused by the effects of cold shock. Abnormalities such as the tail and the central circling the head are the abnormalities caused by the effects of cold shock. The existence of simple sugar components in coconut water and lecithin compounds in egg yolk, providing protective role in the spermatozoa so that not exposed to cold shock. The protective mechanism of simple sugar compounds to the cold shock effect is through both active and passive transport mechanisms across the membrane. The mechanism of lecithin protection against the effects of cold shock on spermatozoa is as a macro molecule, which can not cross the membrane, lecithin covers the cell membrane to form a jacket that becomes the barrier for cold shock effect on spermatozoa membrane (Chenoweth and Lorton, 2014)

Membran Integrity of Spermatozoa

Based on the analysis of variance showed that the treatment did not give an effect (P> 0.05) to the membran integrity of Boer goat spermatozoa on storage of D1 to D3 but gave a very significantly effect (P <0.01) on storage of D4 until D8. Further test results showed that T0 treatment had the highest value of membrane integrity in storage of D4 to D8 among other treatments. The observed results showed the integrity of the membrane at treatment T1; T2; T3 and T4 were not different from D4 to D8 of storage. The ability of coconut water diluent in maintaining the membran integrity of spermatozoa membrane until D3 of storage, where its ability as same as the tris aminomethane diluent as a control because of the protective component of the coconut water diluent in the form of an antioxidant of the enzyme group such as flavonoids. This has been showed from the presence of high doses of superoxidase dysmutase (SOD) on the third day of storage in all treatments was still around 30,000 ng / 100µL. SOD is an antioxidant of enzyme class that plays a role in preventing oxidative damage due to peroxidation lipid in cell mebran (Halliwell and Gudtridge, 2014).

In addition, the protective components of coconut water, lippoprotein also is provided on egg yolk which has a large molecular structure as a barrier in preventing direct exposure of cold shocks to the spermatozoa membrane. Egg yolks also contain polyvinyl pyrrolidone (PVP), hydroxyethyl starch (HES) and dextran as macromolecular substances, which can not pass through the cell membrane thus inhibiting the formation of ice crystals on the spermatozoa membrane through cellular dehydration process, suppress freezing point and increase the viscosity of the media when the temperature drop (Chenoweth and Lorton, 2014). This can maintain the integrity of the spermatozoa membrane so that the integrity of the membrane is maintained. However, on the D4 until

the D8 of storage, the integrity of the membrane is no longer can be maintained except in the control, on the coconut water diluent, the integrity of the membrane has decreased dramatically. This is related to the presence of membrane damage as a result of the depletion of both protective and nutritional content as the duration of shelf life. Other components that contribute to maintain the integrity of spermatozoa membrane is the presence of amino acid content (Kundu *et al.*, 2001; Atessahin *et al.*, 2008 and Almeida and Soares, 2002)

CONCLUSION

The best quality of Boer goat liquid semen is on T0 which can be stored up to 7 days storage at cold temperature, Motility = 40.0 + 5.0%, Viability = 40.7 + 9.5%, Abnormality = 1.6 + 0.6%, good Membrane Integrity= 42.5 + 8.55% and Total motile spermatozoa = 80.0 + 10.0 Million/mL. The best quality of Boer goat liquid semen during cooling with coconut water diluent is Green Young Coconut Water (viridis) which can be stored up to 2 days storage with motility = 60.0 ± 7.0%, Viability = 69.3 + 8,1%, Abnormality = 69.3 + 8.1%, with good Membrane Integrity = 59.8 +9.23%, Total motile Spermatozoa = 120.0 + 15.3 million / mL.

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