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The Effect of *Jamblang* Leaves Extract (*Syzygium cumini*) Inclusion Skim Milk-Egg Yolk Extender on Motility, Abnormality and Viability of Aceh Cattle Spermatozoa Stored at 4°C

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ABSTRACT

This research aimed to know the effect of the addition of *jamblang* leaves extract (*Syzygium cumini*) in skim milk-egg yolk extender on motility, abnormality, and viability of Aceh cattle spermatozoa stored at 4°C. The research design used was Complete Randomized Design consisting of 5 treatments and 5 replications. The treatments consisted of J0 (egg yolk-skim milk), J1 (egg yolk-skim milk + *jamblang* leaf extract 0.2 g/100 ml), J2 (egg yolk-skim milk + *jamblang* leaf extract 0.4 g/100 ml), J3 (egg yolk-skim milk + *jamblang* leaf extract 0.6 g/100 ml) and J4 (egg yolk-skim milk + *jamblang* leaf extract 0.8 g/100 ml). The data related to fresh semen characteristics were analyzed descriptively, while spermatozoa quality following dilution and equilibration were analyzed using analysis of variance (ANOVA). The difference between treatment were compare using the Duncan's multiple range test. The results indicated that fresh semen quality was still met the requirement of semen processing for freezing. Moreover, the addition of *jamblang* leaves extract in skim milk-egg yolk extender significant effect ($P < 0.05$) on spermatozoa motility after equilibration and spermatozoa viability after dilution, while for spermatozoa motility after dilution, spermatozoa viability after equilibration, abnormal spermatozoa after dilution and equilibration did not have a significant effect. Based on result, it can be concluded that the addition of *jamblang* leaves extract in skim milk-egg yolk extender could maintain the quality of Aceh cattle spermatozoa, while the use of incorrect dose becomes toxic and disrupt the spermatozoa activities and leading to death.

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Introduction

Aceh cattle has been officially recorded as one of germplasms according to the Decree of Ministry of Agriculture of the Republic of Indonesia Nomor: 2907/KPTS/OT.140/6/2011. Aceh cattle population decreases annually. In 2002, there were 711,143 Aceh cattle, in 2010 it decreased to 671,086 and in 2015 it decreased to 580,287 cattle (Diskeswannak Aceh, 2016). Therefore, efforts need to be done to increase Aceh cattle population in order to reach a better quality and quantity.

Arifiantini (2016) stated that artificial insemination (AI) is one of the technologies which is able to increase the population and livestock genetic quality. The extender availability was an urgent aspect in preparing spermatozoa for AI application. The extender could increase the volume of semen, provide energy source for the spermatozoa, provide buffer to maintain pH, the osmotic pressure, and electrolyte balance, protect

the spermatozoa from cold shock condition as well as to prevent and to decrease free radical (Susilawati, 2013).

Buffer and cryoprotectant extender can protect and maintain the quality of spermatozoa (Arifiantini and Yusuf, 2006). One of the extender that as buffer system is egg yolk-skim milk extender. Skim has the function to protect spermatozoa from pH changes. While the egg yolks contain lipoproteins and lecithins that protect spermatozoa from cold shock condition and provide nutrition for spermatozoa. Marliani *et al.* (2014) stated that the addition of antioxidants in spermatozoa dilution can reduce damage to the spermatozoa membrane due to the lipid peroxide reaction. Antioxidants are nucleophilic compounds are those that have the ability to reduce, extinguish or suppress free radical reactions. One of the natural antioxidants sources is *jamblang* leaf. The qualitative test for the antioxidant of *jamblang* leaves produced a better antioxidant activity than the fruit, so that it has the potential to

be a natural antioxidant. The compound content of *jamblang* leaves is alkaloids, flavonoids, saponins, quinones, tannins, steroids and polyphenols (Marliani *et al.* (2014). Research conducted by Sari (2017) reported that the activity test results of the IC₅₀ value of *jamblang* leaves were 8.85 bpj, and in the research of Marliani *et al.* (2014) showed that the activity of the IC₅₀ value on *jamblang* leaves was 12.84 bpj. *Jamblang* leaves also contain several compounds such as flavonol glycosides, myrisetin 3-O-4 acetyl L-rhamnopyranoside, tannins, triterpenoids, quercetin (Ayyanar dan Subash-Babu, 2012; Ramya *et al.*, 2012). Besides that, use of *jamblang* leaves in dilution is expected to reduce the cost of frozen semen production, because *jamblang* leaves are easy to obtain. Based on this background, it is necessary to addition of *jamblang* leaves extract in egg yolk-skim milk media as an alternative extender to maintain the quality of Aceh cattle spermatozoa during the storage at temperature of 4°C.

Materials and Methods

The research sample used was semen from a 10 year old Aceh cattle which is in the Balai Inseminasi Buatan Daerah (BIBD) Dinas Kesehatan Hewan dan Peternakan Aceh located in Saree Aceh Besar District. Completely randomized design (CRD) with 5 treatments and 5 replications was used in this experiment. The five treatments were J0= egg yolk-skim milk; J1= egg yolk-skim milk + *jamblang* leaves extract of 0.2 g/100 ml; J2= egg yolk-skim milk + *jamblang* leaves extract of 0.4 g/100 ml; J3= egg yolk-skim milk + *jamblang* leaves extract of 0.6 g/100 ml; J4= egg yolk-skim milk + *jamblang* leaves extract of 0.8 g/100 ml.

The making of *jamblang* leaves extract was started by aerating *jamblang* leaves until it dried and grinding it to form a simplicia powder. The simplicia powder was macerated with a ratio of every 1000 grams of simplicia powder macerated used 4 liters of 96% ethanol. After 4 (four) days of the maceration process, it was filtered and concentrated using a vacuum rotary evaporator at a temperature of 40°C at a speed of 60 rpm until the extract became paste.

The dilution of egg yolk-skim milk is a diluting solution using skim milk, egg yolk, aquadest, fructose, penicillin and streptomycin. Aquadest was heated until the temperature reached 37°C using an erlenmeyer, then added by skim milk and homogenized. Fructose was then added as well and homogenized. Furthermore, egg yolk was added and homogenized. The solution was then steamed for 15-20 minutes until dew appeared on the inside of the erlenmeyer. The solution was removed and left cool to room temperature of 27°C. After it cools down, penicillin and streptomycin were added into the solution. As much as 1000 IU/ml of penicillin and 1000 ug/ml of streptomycin was added. The solution was then stored in refrigerator and deposited for 3

(three) days, so that there is separation between the sediment and the supernatant, the supernatant is used, while the sediment is removed (Susilawati, 2011).

The semen was collected in the morning at 08.00 WIB using an artificial vagina. Subsequently, the quality of fresh semen was examined macroscopically (volume, color, consistency, pH and odor) and microscopically (mass movement, concentration, motility, viability and abnormalities). The sample used was those which had minimal mass movement ++, live motility of more than 70% and abnormalities of less than 20%. After evaluating the quality of fresh semen, semen was divided into 5 (five) groups of egg yolk-skim milk extender which had been added by *jamblang* leaves extract and homogenized. The concentration desired was 100 million spermatozoa/ml of extender. The time interval between the semen storage until the dilution was not more than 15 minutes in accordance with the reference issued by BIB Lembang (2019). The semen that has been diluted by diluent solution will be cooled into a test tube and stored in a refrigerator at 4°C for 4 hours. Equilibration time is the time required for the spermatozoa before freezing to adjust to the diluent. The semen should remain in the extender with or without glycerol for approximately 4 hours (Toelihere, 1985).

Evaluation of semen is carried out in 3 (three) stages, the first stage is carried out after dilution, the second stage after equilibration. At both stages, a microscopic evaluation was carried out which included motility, viability and abnormalities. The third stage is evaluating the ability of spermatozoa to survive at 4°C which is observed every 24 hours as long as the motility of the spermatozoa has motility above 40%.

The data related to fresh semen characteristics were analyzed descriptively, while spermatozoa quality following dilution and equilibration were analyzed using analysis of variance (ANOVA). The difference between treatment were compare using the Duncan's multiple range test.

Results and Discussion

The quality of Aceh cattle fresh semen

The quality evaluation of the fresh semen is the first step to determine the feasibility of semen for further processing into frozen semen. The results of the research on the quality of fresh semen from Aceh cattle in two reservoirs was presented in Table 1 as follows.

The result of the study (Table 1) show that the semen volume of 10 years old aceh cattle obtained in this study was 3.75±0.35 ml/ejaculate, lower than the research conducted by Wahyuni (2018) which report the volume of semen of aceh cattle aged 3 years, namely 4.36±0.77 ml/ejaculate, however, it is relatively the same as research conducted by

Table 1. The quality of Aceh cattle fresh semen

Parameter	Observation Result
Macroscopic	
Volume (ml)	3.75 ± 0.35
Color	Milky white
Consistence	Medium
Odor	Fishy (typical)
pH	7.00 ± 0.00
Microscopic	
Mass movement	++
Motility (%)	81.5 ± 7.78
Concentration (10 ⁹ /ml)	1.68 ± 0.60
Percentage of living spermatozoa (%)	92.68 ± 1.76
Abnormality (%)	5.86 ± 0.02

Zulyazaini *et al.* (2016) who obtained the semen volume of aceh cattle aged 3.0±3.5 years, namely 3.82±0.47 ml/ejaculate. The difference in the volume of semen for cattle is likely influenced by the age of the cattle, which is different according to what Melita *et al.* (2014) stated that the quality of semen is influenced by age and the holding interval. The color and consistency of semen have a positive correlation. The results of this obtained a milky white semen color with moderate consistency. The pH of aceh cattle semen obtained in this study was 7.00±0.00. The same results were also obtained for 3 years old aceh cattle semen in Wahyuni (2018) research, namely 7.00±0.00, and PO cattle semen, namely 7.00±0.00 (Sholikah *et al.*, 2016). The smell of aceh cattle semen in this study is unique, according to the opinion of Risal and Herdis (2010) that the smell of cattle semen is a distinctive smell. The mass movement of fresh semen from aceh cattle in this study is (++) . According to Toelihere (1985), cattle semen that is suitable for processing must have spermatozoa mass movements ranging from (++) to (+++). The percentage of spermatozoa motility of fresh semen in this study was 81.5±7.78%, this result was higher than the percentage of spermatozoa motility of fresh semen from PO cattle, namely 70.00±0.00% (Sholikah *et al.*, 2016), but it is equivalent to the percentage of spermatozoa motility of rambon banyuwangi cattle, namely 81.11±4.16% (Safitri, 2018). The concentration of spermatozoa is an important factor in describing the quality of the semen to be used (Bearden and Fuquay, 1984). The results of the mean concentration of spermatozoa in the semen of aceh cattle in this study were 1.68±0.60 x 10⁹ spermatozoa/ml. Susilawati (2013) reports that the concentration of cattle semen varies from 800-2000 million spermatozoa per military, this means that the results of this study have relatively good spermatozoa concentration.

The percentage of Aceh cattle spermatozoa motility after dilution and after equilibration using egg yolk-skim milk extender with the addition of *Jamblang* leaves extract (*Syzygium cumini*)

The progressive spermatozoa in egg yolk-skim milk extender added by *jamblang* leaves extract, then equilibrated for 3-4 hours and followed by an assessment of motility after equilibration. The average percentage of spermatozoa motility after dilution and after equilibration using egg yolk-skim milk extender with the addition of *jamblang* leaves extract can be seen in Table 2.

Based on the results of the analysis of variance, there was no significant effect on spermatozoa motility after dilution, but after equilibration showed a significant effect ($P < 0.05$) on the motility of spermatozoa. Based on further tests, it was found that the treatment of egg yolk-skim milk extender + 0.4 g/100 ml of *jamblang* leaves extract (J2) had the highest average percentage of spermatozoa motility compared to other treatments (J0, J1, J3 and J4). This situation is assumed since the treatment has optimized the rate of fructolysis which causes sufficient energy requirements to live and move spermatozoa. *Jamblang* leaves extract which contains antioxidants with a dose of 0.4 g/100 ml (J2) can bind free radicals so as to prevent lipid peroxidation which can inhibit glycolysis and motility (Aurich *et al.*, 1997).

The addition of *jamblang* leaf extract with a high dose of 0.8 g/100 ml (J4) had the lowest percentage of motility compared to other treatments. Rahardianto *et al.* (2012) stated that the addition of a diluent media solution that is not suitable as a living medium for spermatozoa can be toxic to spermatozoa. This condition is thought to be due to the high concentration of *jamblang* leaf extract which causes high concentrations of vitamin C, tannins and alkaloids in the semen

Table 2. The average percentage of spermatozoa motility of Aceh cattle after dilution and after equilibration using egg yolk-skim milk extender with the addition of *Jamblang* leaves extract

Treatment	Observation Period	
	After Dilution	After Equilibration
J0 (0 g/100 ml)	70.24±1.77	67.73±14.06 ^{ab}
J1 (0.2 g/100 ml)	70.14±2.89	64.79±7.65 ^{ab}
J2 (0.4 g/100 ml)	71.89±1.99	69.27±15.97 ^b
J3 (0.6 g/100 ml)	68.09±9.66	52.50±10.06 ^{ab}
J4 (0.8 g/100 ml)	66.66±2.81	49.88±10.49 ^a

Note: different superscripts indicate significant differences ($P < 0.05$).

thinner medium. The higher the concentration of vitamin C in the diluent medium will cause an accelerated rate of fructolysis resulting in an accelerated accumulation of lactic acid and a decrease in pH. According to Solihati *et al.* (2008) stated that a decrease in semen pH causes a decrease in the activity of metabolic enzymes so that the energy requirements for spermatozoa motility are not met. The higher the tannins contained in the jamblang leaf extract, the higher the nutrient transport in the membrane. According to Wurlina *et al.* (2020) if the permeability of the membrane as the entry point for nutrients in producing energy is disrupted, it can cause nutrient transport to be disrupted, so that the spermatozoa experiences a lack of energy and reduces sperm motility.

The data in Tabel 2 indicated that the average percentage of spermatozoa motility of Aceh cattle has decreased in each treatment. It was estimated that this decrease occurred due to the decreased availability of nutrients in the extender, the increased acidity (pH) of the semen and the occurrence of cold shock in the spermatozoa. Blanco *et al.* (2011) explained that since cold stress can destabilize the membrane, it increased membrane permeability to calcium ions in the mitochondria and cytosol. The energy reserves used by spermatozoa to move progressively will decrease as the increase in calcium ions in the mitochondria that decrease the synthesis of ATP. The percentage of decreased motility after equilibration in the addition of 0.4 g/100 ml (J2) jamblang leaf extract treatment was 3.64%, while in the addition of 0.8 g/100 ml (J4) jamblang leaf extract treatment, the decrease in spermatozoa motility was 25%.

The percentage of Aceh cattle spermatozoa abnormalities after dilution and after equilibration using egg yolk-skim milk diluent with the addition of *Jamblang* leaves extract (*Syzygium cumini*)

Spermatozoa abnormalities were assessed after the semen was diluted with various treatments, then equilibrated for 3-4 hours and evaluated. The average percentage of Aceh cattle spermatozoa abnormalities after equilibration using egg yolk-skim milk extender with the addition of *jamblang* leaves extract can be seen in Table 4 as follows.

The results of spermatozoa abnormalities from various treatments were still relatively good. This is because the results obtained were not more than 20%. The spermatozoa abnormality did

not exceed 20% because it affects fertility (Garner and Hafez, 2008). Parera *et al.* (2009) stated that an abnormality with a result of 8-10% has no significant effect on motility but an abnormality of more than 25% can affect fertility.

The results of this research showed that there were various primary and secondary abnormalities. Primary abnormalities include short tail, double tail, coiled tail, bent neck, macrocephalic and taped head. Secondary abnormalities include a headless tail and a head without a tail. Primary abnormalities occur due to failure of spermatozoa in the spermatogenesis process. The most common abnormality is a severed head with a tail. This may be caused by an improper review process. Carelessness in the review can cause the head and tail of the spermatozoa to be damaged due to pressure on the review (Yatusholikhah *et al.*, 2015). Garner and Hafez (2000) stated that secondary abnormalities occurred in the storage and treatment processes during the staining.

Based on Table 4 above, the average percentage of spermatozoa abnormalities has increased in each observation period phase. The spermatozoa abnormalities increased during the freezing process due to imbalance in osmotic pressure of the spermatozoa metabolism during storage temperature of 3-5°C and cold shock (Solihati *et al.*, 2008). Abnormality can also be caused by inaccurate process of making the pulp preparations, causing the spermatozoa to have their heads and tails off. The abnormal spermatozoa in this study are shown in the images below.

The percentage of Aceh cattle spermatozoa viability after dilution and after equilibration using egg yolk-skim milk extender with the addition of *Jamblang* leaves extract (*Syzygium cumini*)

The viability of spermatozoa was assessed in two steps, after dilution and after equilibration. The average percentage of viability of Aceh cattle spermatozoa following dilution and equilibration was presented in Table 4.

Based on the analysis of variance, it showed that the addition of jamblang leaf extract to egg yolk skim extender had a significant effect ($P < 0.05$) on the viability of Aceh cattle spermatozoa after dilution, while the evaluation of viability after equilibration did not show a significant effect. J2 (egg yolk-skim milk extender + 0.4 g/100 ml of *jamblang* leaves extract) treatment had a higher spermatozoa viability than

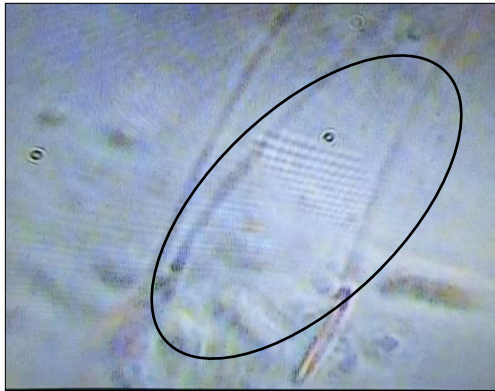
Table 3. The average percentage of spermatozoa abnormalities of Aceh cattle after dilution and after equilibration using egg yolk-skim milk extender with the addition of *Jamblang* leaves extract

Treatment	Observation Period	
	After Dilution	After Equilibration
J0 (0 g/100 ml)	6.98±0.62	9.49±2.91
J1 (0.2 g/100 ml)	5.98±0.96	10.84±3.11
J2 (0.4 g/100 ml)	6.18±0.66	8.96±1.10
J3 (0.6 g/100 ml)	6.33±2.32	10.30±2.86
J4 (0.8 g/100 ml)	6.51±1.46	9.62±1.80

Table 4. The average percentage of Aceh cattle spermatozoa viability after dilution and after equilibration using egg yolk-skim milk extender with the addition of *Jamblang* leaves extract

Treatment	Observation Period	
	After Dilution	After Equilibration
J0 (0 g/100 ml)	79.47±9.08 ^{ab}	76.72±14.30
J1 (0.2 g/100 ml)	88.21±2.03 ^{bc}	81.77±4.10
J2 (0.4 g/100 ml)	92.43±4.48 ^c	85.70±6.43
J3 (0.6 g/100 ml)	80.93±8.27 ^{ab}	79.78±8.07
J4 (0.8 g/100 ml)	75.89±9.80 ^a	68.76±7.24

Note: different superscripts indicate significant differences (P<0.05)



Picture 1. Double Tail Spermatozoa
Source: Research Dokumentation (2020)



Picture 2: Short Tail Spermatozoa
Source: Research Dokumentation (2020)



Picture 3: Coiled Tail Spermatozoa
Source: Research Dokumentation (2020)



Picture 4: Microcephalic Spermatozoa
Source: Research Dokumentation (2020)



Picture 5: Tapend Head Spermatozoa
Source: Research Dokumentation (2020)

other treatments. However, treatment J0 (without addition of *jamblang* leaves extract), J1 (egg yolk-skim milk extender + 0.2 g/100 ml of *jamblang*

leaves extract), J3 (egg yolk-skim milk extender + 0.6 g/100 ml of *jamblang* leaves extract), and J4 (egg yolk-skim milk extender + 0.8 g/100

ml of *jamblang* leaves extract) were still considered good because they were able to maintain spermatozoa viability above 70%. A decrease in cold temperatures during storage, a decrease in pH due to increased lactic acid produced by spermatozoa metabolism, reduced energy availability and damage to the plasma membrane and acrosome will reduce spermatozoa viability (Pareira *et al.*, 2010). *Jamblang* leaves extract also contains vitamin C which has the function to inhibit free radical activity so that it can avoid peroxidative damage which affects the fertility and viability of the spermatozoa (Lubis *et al.*, 2013). Previous studies have shown that adding antioxidants to semen extender can prevent free radicals from damaging the spermatozoa cell membrane which is associated with spermatozoa fertility and viability (Aslam *et al.*, 2014).

In J4 treatments (egg yolk-skim milk extender + 0.8 g/100 ml *jamblang* leaves extract) had a lower spermatozoa viability than other treatments. This is due to the more provision of *jamblang* leaves extract, the higher the number of active substances in these ingredients, such as tannins, vitamin C and saponins. The higher the active substance in *jamblang* leaves extract in the diluent medium, the spermatozoa will experience difficulty in movement, resulting in an increase in high energy consumption and the buildup of lactic acid. The decreased speed of movement due to damaged cell membranes due to the high content of lactic acid will cause spermatozoa to die (Setyaningsih, 2012).

Based on the results of the study shown in Table 4, the percentage of spermatozoa viability has decreased from the diluted semen stage with extender media until after equilibration. The decrease in the viability of spermatozoa at each observation period was caused by the increase in the number of spermatozoa that died due to the lack of energy (Nair *et al.*, 2006).

The viability of Aceh cattle spermatozoa in egg yolk-skim milk extender with the addition of *Jamblang* leaves extract (*Syzygium cumini*) after being stored at temperature of 4°C

The viability of spermatozoa observed in this study was the ability of spermatozoa to survive at 4°C which was observed every 24 hours as long as the spermatozoa had motility above 40%. The percentage of spermatozoa motility below 40% was not observed. The average survival rate of Aceh cattle spermatozoa added with *jamblang*

leaves extract in egg yolk-skim milk extender can be seen in Table 5.

Table 5 shows that the egg yolk-skim milk extender with the addition of 0.2 g/100 ml (J1) and 0.4 g/100 ml (J2) *jamblang* leaves extract resulted in a longer viability rate of Aceh cattle spermatozoa, which are 2.00±0.00 day. Then it is followed by egg yolk-skim milk extender (J0) to produce Aceh cattle spermatozoa viability of 1.00 ± 0.00 day, egg yolk-skim milk extender with the addition of 0.6 g/100 ml (J3) and 0.8 g/100 ml (J4) of *jamblang* leaves extract produces Aceh cattle spermatozoa viability of 0.00±0.00 days.

Spermatozoa which has the highest percentage of spermatozoa viability (%) was in egg yolk-skim milk extender with the addition of 0.4 g/100 ml *jamblang* leaves extract. This is due to the presence of active ingredients in *jamblang* leaves extract as a sufficient source of energy for the survival of the spermatozoa and its antioxidant activity is able to protect spermatozoa from environmental disturbances. The addition of *jamblang* leaves extract at a dose of 0.4 g/100 ml extender occurred optimally for fructolysis rates so that energy for the survival of spermatozoa is fulfilled. The antioxidant content in *jamblang* leaves extract in the form of phenols, flavonoids, vitamin C, alkaloids, saponins and tannins serves to prevent the formation of lipid peroxidation (Aurich *et al.*, 1997).

The treatment of extender material addition (J0, J3 and J4) produces low spermatozoa viability. This is because the provision of isotonic extender with inappropriate concentrations as a living medium for spermatozoa can cause maximum metabolism. The addition of *jamblang* leaves extract at high doses will cause an increase in the content of tannins, saponins, alkaloids and vitamin C. The higher the concentration of vitamin C in the diluent will cause the accumulation of lactic acid to accelerate and decrease the pH (Gangwar *et al.*, 2015). The metabolic product of spermatozoa in the form of lactic acid can also cause a decrease in pH. A pH that is too acidic can cause the viability of spermatozoa to be disturbed so that the spermatozoa will die (Emmens, 1947: Aboagla and Terada, 2004). Tannins possessed by *jamblang* leaves cause the spermatozoa to be deficient in nutrients due to disruption of nutrient transport through the membrane. According to Wurlina *et al.* (2020), high levels of tannins in diluents can interfere with the transport of nutrient into spermatozoa. Lack of nutrients can decrease the motility and viability of spermatozoa.

Table 5. The average viability of Aceh cattle spermatozoa using egg yolk-skim milk extender with the addition of *jamblang* leaves extract at storage temperature of 4°C

Treatment	Spermatozoa Viability (day)
J ₀ (0 g/100 ml)	1.00±0.00
J ₁ (0.2 g/100 ml)	2.00±0.00
J ₂ (0.4 g/100 ml)	2.00±0.00
J ₃ (0.6 g/100 ml)	0.00±0.00
J ₄ (0.8 g/100 ml)	0.00±0.00

Conclusions

Based on the analysis results of this study, it can be concluded that the addition of *jamblang* leaves extract could maintain the motility, abnormality and viability of Aceh cattle spermatozoa stored at 4°C. The addition of 0.4 g/100 ml diluent of *jamblang* leaves extract into egg yolk-skim extender resulted in the best motility, viability and abnormalities compared to other treatments. The addition of 0.2 g/100 ml and 0.4 g/100 ml *jamblang* leaves extract resulted in the best viability of Aceh cattle spermatozoa at 4°C storage compared to other treatments, because it can survive for two days

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