Effect of Vitamin C Administration in Diluent Media to Quality of Dairy Cattle Thawed Spermataozoa

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ABSTRAK

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Proses pembekuan dan *thawing* semen, dapat menyebabkan kematian spermatozoa dan spermatozoa yang bertahan hidup mempunyai fertilitas yang rendah. Tujuan dari penelitian ini untuk mendapatkan konsentrasi vitamin C yang optimum dalam media pengencer untuk meningkatkan kualitas spermatozoa sapi perah (*Bos taurus*) tanpa *sexing* dan hasil *sexing* setelah *thawing*. Metode yang digunakan adalah metode eksperimental dengan rancangan acak lengkap pola faktorial 3x4. Rancangan ini terdiri dari dua faktor yaitu pemisahan spermatozoa dan konsentrasi vitamin C. Faktor pemisahan spermatozoa yaitu spermatozoa tanpa *sexing* dan hasil *sexing* (X dan Y). Faktor konsentrasi vitamin C yaitu 0% (K), 0,25% (P_1), 0,50% (P_2) dan 0,75% (P_3). Data dianalisis menggunakan analisis variansi (ANAVA) dan uji Jarak Berganda Duncan 95%. Konsentrasi vitamin C yang optimum dalam media pengencer untuk meningkatkan motilitas spermatozoa sapi perah (*Bos taurus*) tanpa sexing setelah thawing adalah 0,25% dan 0,5%. Sedangkan untuk meningkatkan motilitas spermatozoa sapi perah (*Bos taurus*) hasil sexing, baik spermatozoa X maupun spermatozoa Y setelah thawing adalah 0,50%. Viabilitas spermatozoa tanpa sexing dengan penambahan vitamin C 0,75% pada media pengencer lebih rendah dibanding dengan semen dalam pengencer tanpa penambahan viamin C. Penambahan vitamin C pada pengencer tidak mempengaruhi tingkat abnormalitas spermatozoa tanpa sexing dan spermatozoa X, tapi penambahan vitamin C pada pengencer 0,25% tidak berbeda dengan penambahan vitamin C 0,5%. Penambahan vitamin C dalam media pengencer dapat mempengaruhi tingkat motilitas, viabilitas, abnormalitas dan integritas membran plasmaa spermatozoa tanpa *sexing* dan hasil *sexing* pada sapi perah (*Bos taurus*) setelah *thawing*.

Kata Kunci: Spermatozoa, Vitamin C, *Sexing*, Pengencer, *Thawing*

ABSTRACT

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The process of freezing and thawing of semen could lead spermatozoa death and low fertility for alive spermatozoa. This research was subjected to determine the optimum concentration of vitamin C in diluent media to improve the quality of non-sexed and sexed of thawed dairy cattle (*Bos taurus*) spermatozoa. The method used was completely randomized design with 3x4 factorial consisting of spermatozoa isolation and vitamin C concentration. Spermatozoa isolation factors were non-sexed and sexed (X and Y) spermatozoa. Vitamin C concentrations factors were 0% (K); 0.25% (P_1); 0.50% (P_2) and 0.75% (P_3). Data were analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test 95%. The optimum concentration of vitamin C in diluent media to improve the quality of non-sexed thawed dairy cattle (*Bos taurus*) spermatozoa was 0.25%. While the optimum concentration of vitamin C in diluent medium to improve quality of thawed dairy cattle (*Bos taurus*) spermatozoa both X and Y was 0.50%. Administration of vitamin C in diluent media could improve the quality of thawed non-sexed and sexed (X and Y spermatozoa) spermatozoa in dairy cattle (*Bos taurus*).

Kata Kunci: Spermatozoa, Vitamin C, Sexing, Diluent, Thawing

INTRODUCTION

Dairy milk yield has not fulfilled national milk requirement due to the small population of dairy cattle. National dairy cattle population is about 525,171 heads (CSA 2015). It only increases by 4.31% (502,516 heads) from 2014. The National requirement of fresh milk is 3.3 million ton/year, otherwise, its availability only supplies about 690 hundred ton/year (21%) and the rest are fulfilled by imported 2.61 million ton/year (79%) (Ministry of Industry 2013). Dairy cattle population could be increased by Artificial Insemination (AI) and use of sexing process to increase desired sex of cattle.

Currently, the most AI used frozen semen. Frozen semen may be used for long term. However, freezing process leads to spermatozoa death up to 50% and low fertility for the alive one (Lessard et al. 2000). Thawing is a re-melting process using certain media and duration before the spermatozoa used. This thawing process generated heat shock effect or contamination of oxygen to spermatozoa influencing plasma membrane stability and then it influenced the quality of spermatozoa (Salim et al. 2012).

The sexing process also induced damage to the plasma membrane, mitochondria veil, releasing of various enzymes, the decrease of lipoprotein and amino acid level, agglutination of spermatozoa head leading to decrease of motility and fertility of spermatozoa, even the spermatozoa death.

Winarto (2010) said that spermatozoa damage by oxidative stresses developed by free radicals against normal cells, protein, and fat. Yuliani & Lukman (2013) also said that the alive spermatozoa were very sensitive to the external environment. Temperature change and extreme osmolarity during AI process might lead to Reactive Oxygen Species (ROS) production (Nebel 2007; Moore et al. 2005; Sukmawati et al. 2014). The quality of sperm might be improved after freezing process by administration of Vitamin C in diluent media. Vitamin C was an antioxidant leading to decrease the sensitivity of plasma membrane of spermatozoa against lipid peroxidation due to ROS process (Sugiarti et al. 2001).

The quality of thawed sperm administered by 0.02% vitamin C into diluent media did not different in motility, abnormality and viability with the control, but showed significant different ($P<0.05$) in the integrity of plasma membrane (Afiati et al. 2014). Franco et al. (2013) reported that supplementation of ascorbate acid in semen diluent did not show a positive effect in 0.45 g/L but positive in 0.9 and 1.8 g/L concentration against integrity and stability of plasma membrane.

Study of antioxidant administration has been widely provided, however, the study of the administration of vitamin C into diluent media to determine the quality of spermatozoa of dairy cattle (*Bos taurus*) without and with good sexing after thawing has not been widely done. Therefore, the study of the effect of vitamin C administration into diluent media against the quality of dairy cattle of non-sexed and sexed spermatozoa after thawing is needed to be done. This study aim was to obtain optimum vitamin C concentration in diluent media to improve the quality of dairy cattle (*Bos taurus*) of thawed non-sexed and sexed spermatozoa.

MATERIALS AND METHODS

This research was conducted in Laboratory of Breeding Reproduction and Animal Cells Culture of Indonesian Research Institute for Biotechnology and Bioindustry, Bogor from April – July 2015. Semen samples were from one 4-years old Friesian Holstein. Semen was collected using artificial vagina equipped by scale container glass tube. Collected semen was immediately observed macroscopically (for color, odor, volume, pH, and its consistency) and microscopically (for its concentration, mass movement, motility, viability, abnormality, the integrity of whole plasma membrane and nucleus integrity). Good semen categories were having minimal mass movement (thin, infrequent, and slow movement), motility ≥70%, abnormality <20%, and concentration >1000 x 106 ml (Arifiantini 2012).

Spermatozoa sexing process

Good semen and spermatozoa were sexed using multilevel albumin method using Bovine Serum Albumin (BSA 5% for the upper column and 10% for under column). Then 1 ml semen was slowly added and aged for 60 minutes on that upper multilevel albumin column. Upper albumin column (BSA 5%) was predicted as spermatozoa X and the under albumin column (BSA 10%) as spermatozoa Y (Hendri 1992; Kaiin et al. 2003).

Dilution, packaging, equilibration, freezing and thawing

Non-sexed and sexed spermatozoa were then diluted using Tris-Stirat buffer (Table 1). Interval time between semen collections to the dilution was no more than 15 minutes. Administration of vitamin C in diluent media was adapted with treatments (0, 0.25, 0.5, and 0.75% concentration).

Table 1. Composition of tris-citric buffer diluent

Material	Amount
Tris (Hydroxymethyl aminomethane) (g)	3.028
Citric acid (g)	1.675
Fructose (g)	1.25
Penicillin (g)	0.0525
Streptomycin (g)	0.075
Aquabides (ml)	100
Yolk (ml)	20.0
Glycerol (ml)	6.00

(Anggraeny et al. 2004)

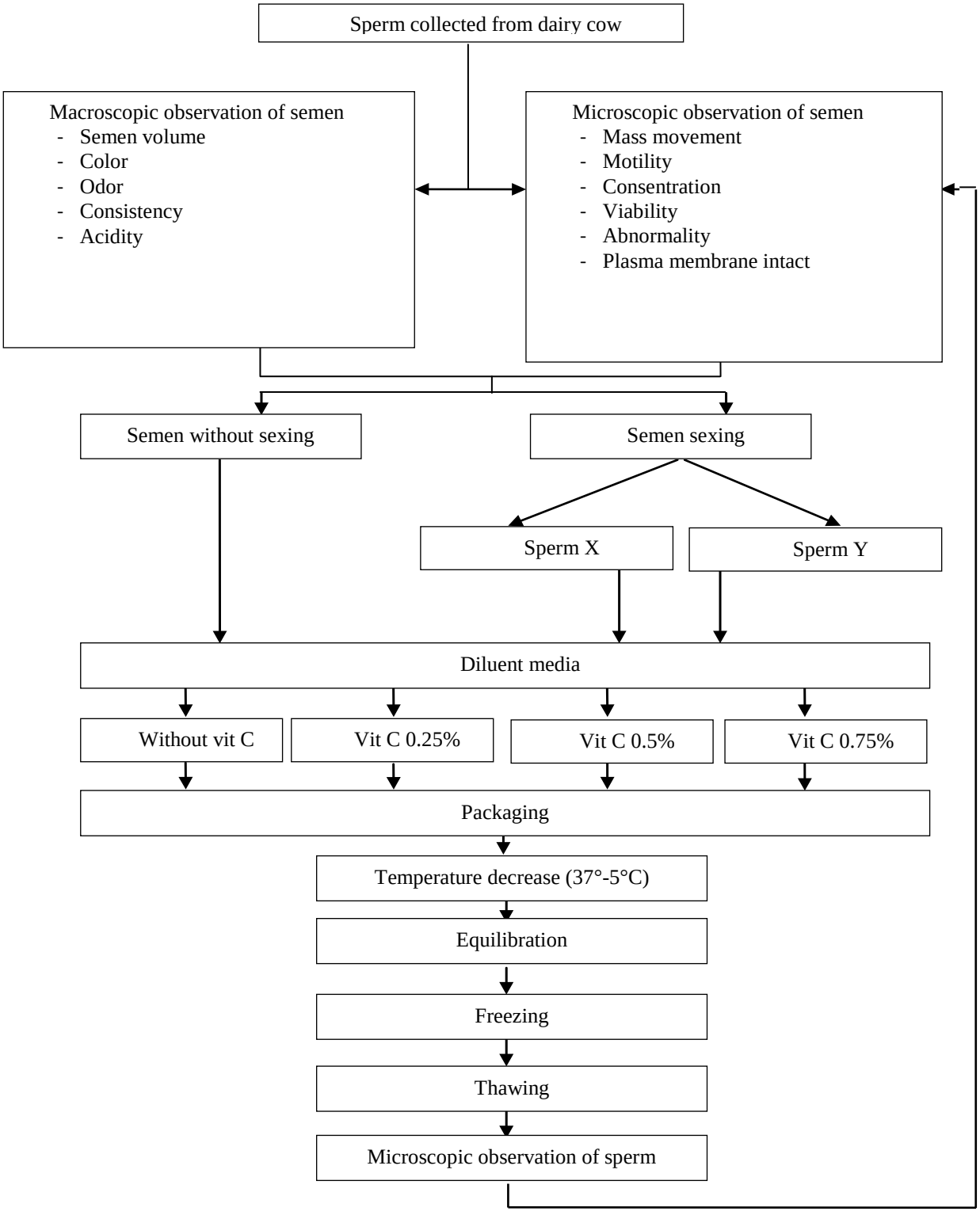

Figure 1. Flow diagram of assessment process (modified Kaiin et al. 2003).

In packaging process, 0.5 ml straw was labeled using printing tool. Semen was put in that straw using filling tool. Then the straw was sealed using sealing tool. Semen was equilibrated by placing it in 5°C temperature for 4 hours before freezing process. Sealed straws were organized in the cooling rack and then were equilibrated. Freezing process was conducted in nitrogen steam by putting sealed straw rack 10 cm

above liquid nitrogen for 15 minutes (about -130°C) and then put in -196°C liquid nitrogen for 10 minutes (Sugiarti et al. 2001). Frozen semen was thawed by putting those frozen straws into 37°C water for 30 seconds (Pratiwi et al. 2011). Observation of spermatozoa quality was conducted microscopically.

Assessment and observation of microscopic parameters

Microscopic parameters observed after thawing were the mass movement, motility, viability, abnormality, the integrity of plasma membrane and spermatozoa nucleus. This study process is showed in Figure 1.

Data analysis

This study was designed by two factors that were spermatozoa isolation and vitamin C concentration. Spermatozoa isolation factors were non-sexed and sexed (X and Y). Vitamin C factors were 0% (K), 0.25% (P1), 0.50% (P2) and 0.75% (P3). The method used in this study was an experimental method with completely randomized design 3x4 factorial pattern and 3 repetitions. Data obtained were then analyzed by 95% ANOVA (Steel & Torrie 1995).

RESULTS AND DISCUSSION

Assessment of fresh semen (Table 2) showed good result in accordance with the good quality requirement of spermatozoa, so then was continued by isolation process. The quality of sexed spermatozoa showed good score and was feasible to be frozen (Table 3). Spermatozoa quality of fresh FH semen (Table 2) used was categorized as normal spermatozoa according to Hafez & Hafez (2000): had normal ejaculation volume by 5-8 ml. Normal semen odor was similar with chlorine or acacia flower odor (Subrata 1999). Garner & Hafez (2000) said that normal ejaculation color of cattle was beige to white milk, while spermatozoa with low concentration would appear clearly and transparent. That color change was matched with pH meter. Hafez & Hafez (2000) reported that pH of fresh cattle semen was around 6.4-7.8. Those normal-categorized spermatozoa were qualified for further process.

Table 2. Quality of fresh dairy cattle semen

Parameter	Result (Average) (±SD)
Macroscopic observation	
Color	Beige – White-milk
Odor	Specific
Volume (ml)	10.5±2.18
pH	7
Consistency	aqueous – dense
Microscopic Observation	
Consentration (10^6/ml)	1440.67±699.84
Mass movement	Good
Motility (%)	79.63±7.47
Viability (%)	87.19±6.63
Abnormality (%)	6.01±1.46
Integrity of plasma membrane (%)	84.92±5.25
Integrity of nucleus (%)	96.88±5.40

Spermatozoa X and Y had qualified score for artificial insemination (Table 3). Obtained result was in accordance with research result of Afiati (2004) and Pratiwi et al. (2006) showing that percentage of motility, viability and integrity of plasma membrane, and abnormality of sexing result spermatozoa showed a qualified score for freezing.

Table 3. Quality of sexing result spermatozoa

Parameter	Spermatozoa X	Spermatozoa Y
Concentration (10^6/ml)	1022±625.63	1280±890.31
Motility (%)	82.13±6.52	85.72±4.74
Viability (%)	70.57±7.36	77.35±8.96
Abnormality (%)	5.07±1.06	4.69±1.82
Integrity of plasma membrane (%)	82.29±7.86	83.49±8.98
Integrity of nucleus (%)	99.69±0.54	99.89±0.18

Table 4. Motility of thawed dairy cattle spermatozoa

Concentration of vitamin C	Spermatozoa type (± SD) (%)		
	Non-sexed	Spermatozoa X	Spermatozoa Y
0% (control)	43.44±3.03$^{(A)a}$	48.88±6.23$^{(A)a}$	43.29±4.99$^{(A)a}$
0.25% (P1)	52.01±3.82$^{(B)a}$	41.81±3.65$^{(A)b}$	43.37±1.76$^{(A)b}$
0.50% (P2)	46.21±5.41$^{(AB)a}$	58.74±2.19$^{(B)b}$	59.99±1.47$^{(B)b}$
0.75% (P3)	42.82±2.05$^{(A)a}$	46.35±5.98$^{(A)a}$	38.24±4.28$^{(A)a}$

Different capital words in one column show significant difference (P<0.05). Different lowercase in one row shows significant different (P<0.05)

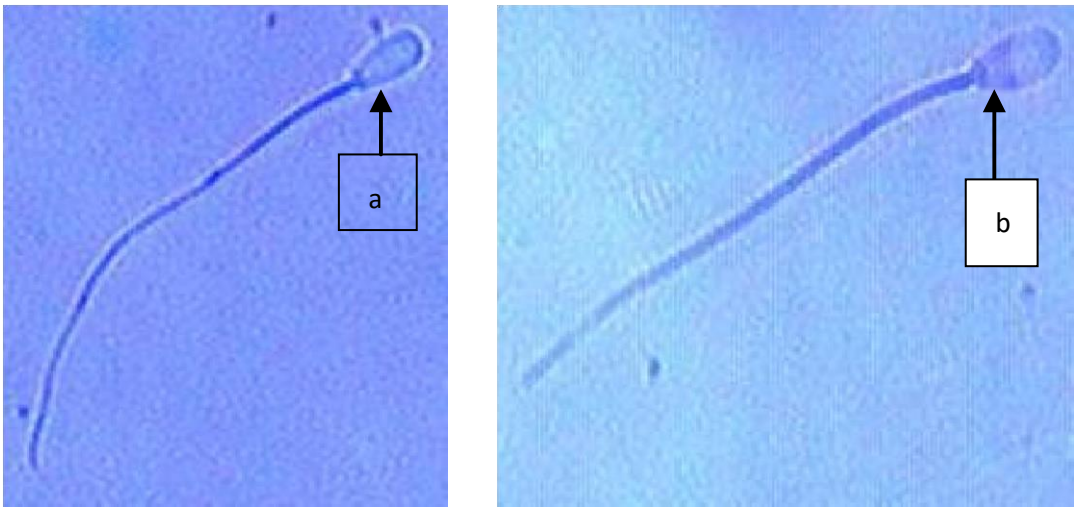

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Figure 2. Viability of dairy cattle spermatozoa, (a) alive spermatozoa, (b) dead spermatozoa.

Quality of spermatozoa after thawing

Motility of thawed spermatozoa (Table 4) without vitamin C (0%) administration was significantly lower than X and Y spermatozoa. However, motility of non-sexing spermatozoa without vitamin C administration (0%) was not significantly different with the 0.5% and 0.75%, but lower than 0.25% vitamin C administration.

Motility of non-sexing spermatozoa with 0.25% vitamin C administration was significantly lower than X and Y spermatozoa. Administration of 0.25% vitamin C in non-sexing spermatozoa showed the same motility with spermatozoa in 0.5%, but was significantly higher than spermatozoa in 0% and 0.75% vitamin C administration.

Motility of non-sexing spermatozoa with 0.5% vitamin C administration was significantly lower than Spermatozoa X and Y. Administration of 0.5% vitamin C in non-sexing spermatozoa had no different motility with that at 0.25%, but was significantly different with the 0% or 0.75% vitamin C administrations.

Motility of non-sexing spermatozoa with 0.75% vitamin C administration did not show significant different than Spermatozoa X or Y. Administration of 0.75% vitamin C in non-sexing spermatozoa was not different with that 0% or 0.5%, but significantly lower than the 0.5% vitamin C administration.

Motility percentage of spermatozoa (Table 4) observed was in accordance with Savitri et al. (2014) and Aslam et al. (2014). Savitri et al. (2014) reported that motility percentage of Bali cattle thawed spermatozoa was 40.00±5.00% with administration of 26.43% vitamin C in diluent media. Aslam et al. (2014) reported that administration of 0.50% vitamin C showed higher motility in Aceh cattle thawed spermatozoa than control (40.16±3.50%). Administration of vitamin C was able to improve motility of spermatozoa due to optimization of fructolysis fulfilling energy requirement in the form of ATP (Sumargono 1998). Aslam et al. (2014) said that vitamin C was able to bind radical oxygen in the cells preventing lipid peroxidation that might prevent motility. Vitamin C was a soluble vitamin which was able to protect spermatozoa from damage caused by oxidative stress by neutralizing hydroxyl, super-oxidation and peroxide hydrogen

Table 5. Viability of thawed dairy cattle spermatozoa

Concentration of vitamin C	Spermatozoa type (± SD) (%)		
	Non-Sexing	Spermatozoa X	Spermatozoa Y
0% (control)	68.31±7.28$^{(AB)}$	73.14±7.34$^{(A)}$	70.30±5.98$^{(A)}$
0.25% (P1)	75.04±7.03$^{(B)}$	73.29±9.56$^{(A)}$	74.47±7.93$^{(A)}$
0.50% (P2)	73.70±7.99$^{(B)}$	74.23±8.73$^{(A)}$	76.89±8.87$^{(A)}$
0.75% (P3)	65.49±9.74$^{(A)}$	67.72±7.48$^{(A)}$	71.37±8.09$^{(A)}$

Different capital words in one column shows significant difference (P<0.05)

Table 6. Abnormality of thawed dairy cattle spermatozoa

Concentration of Vitamin C	Spermatozoa type (± SD) (%)		
	Non-Sexing	Spermatozoa X	Spermatozoa Y
0% (control)	6.74±0.99$^{(A)}$	6.97±1.17$^{(A)}$	6.95±1.31$^{(A)}$
0.25% (P1)	5.96±1.51$^{(A)}$	6.37±1.78$^{(A)}$	6.11±1.50$^{(AB)}$
0.50% (P2)	6.60±1.64$^{(A)}$	5.92±0.93$^{(A)}$	5.36±1.61$^{(B)}$
0.75% (P3)	6.68±1.27$^{(A)}$	6.76±1.65$^{(A)}$	6.86±1.01$^{(A)}$

Different capital words in one column shows significant difference (P<0.05)

radical and prevent spermatozoa agglutination (Agarwal & Sekhon 2010; Sitohang et al. 2015).

Observation of spermatozoa viability (Table 5) was conducted to eosin-nigrosin colored spermatozoa. Spermatozoa which absorbed color was dead spermatozoa, meanwhile, spermatozoa which did not absorb color (green) was alive spermatozoa (Figure 2). Putra et al. (2012) said that permeability of dead spermatozoa membrane was increase, so that eosin-nigrosin dye easily passed the membrane and entered spermatozoa. Meanwhile, the permeability of alive spermatozoa membrane remained normal, so that eosin-nigrosin dye could not pass the membrane. Thawed spermatozoa were more sensitive and easily dead. Park & Graham (1992) said that spermatozoa lost their viability during freezing process due to change of structure and membrane function.

Viability (Table 5) in non-sexing spermatozoa without vitamin C (0%) administration was not different with the 0.25% and 0.5%, but was significantly higher than the 0.75% vitamin C administration.

Administration of 0.25% vitamin C to non-sexed spermatozoa showed the same viability with 0% and 0.5%, but was significantly higher than the 0.75% vitamin C administration.

The viability of non-sexed spermatozoa administered by 0.5% vitamin C was not different with 0% and 0.25%, but was significantly higher than 0.75% vitamin C administration.

The viability of non-sexed spermatozoa administered by 0.75% vitamin C was not different with the 0%, but was significantly higher than 0.25% and 0.5% vitamin C administration.

The viability of X and Y spermatozoa did not significantly different either in the 0.25% or 0.75% vitamin C administration.

Research results showed that administration of vitamin C in diluent media might be a reference for spermatozoa viability. Viability percentage obtained in this study was in accordance with Savitri et al. (2014) reporting that viability percentage of thawed Bali cattle spermatozoa with 44.03% vitamin C administration in diluent media was 54.33±3.51%.

Administration of vitamin C in diluent media is one of the factors increasing spermatozoa viability. Vitamin C, in addition to counteracting free radicals, was also to optimize fructolysis decelerating damage of spermatozoa membrane permeability and consequently extend the life of spermatozoa (Hidayaturrahmah 2007).

Abnormality (Table 6) of non-sexing and spermatozoa X did not different in each vitamin C administration level. Abnormality in spermatozoa Y with 0% vitamin C administration did not different with the 0.25% and 0.75%, but was significantly higher than 0.5% vitamin C administration.

Abnormality observed in this study was morphological abnormalities. Abnormality of spermatozoa was caused by various factors such as: diseases, temperature, season, treatments during cryopreservation and technique of semen collection and

Table 7. Integrity of plasma membrane of thawed dairy cattle spermatozoa

Concentration of Vitamin C	Spermatozoa type (± SD) (%)		
	Non-Sexing	Spermatozoa X	Spermatozoa Y
0% (control)	37.72±4.08$^{(A)a}$	32.11±4.15$^{(A)b}$	36.03±3.35$^{(A)a}$
0.25% (P1)	38.38±5.98$^{(A)a}$	36.54±4.73$^{(AB)a}$	36.49±4.31$^{(A)a}$
0.50% (P2)	37.70±4.78$^{(A)a}$	37.44±4.18$^{(B)a}$	37.20±6.53$^{(A)a}$
0.75% (P3)	27.77±5.77$^{(B)a}$	34.69±5.57$^{(AB)b}$	34.25±3.20$^{(A)b}$

Different capital words in one column shows significant difference (P<0.05). Different lowercase in one row shows significant different (P<0.05)

Table 8. Integrity of nucleus of thawed dairy cattle spermatozoa

Concentration of vitamin C	Spermatozoa type (± SD) (%)		
	Non-*Sexing*	Spermatozoa X	Spermatozoa Y
0% (control)	99.78±0.44	99.18±0.86	99.73±0.54
0.25% (P1)	99.89±0.33	99.63±0.58	99.73±0.57
0.50% (P2)	99.79±0.41	99.78±0.44	99.90±0.29
0.75% (P3)	99.82±0.36	99.68±0.48	99.58±0.84

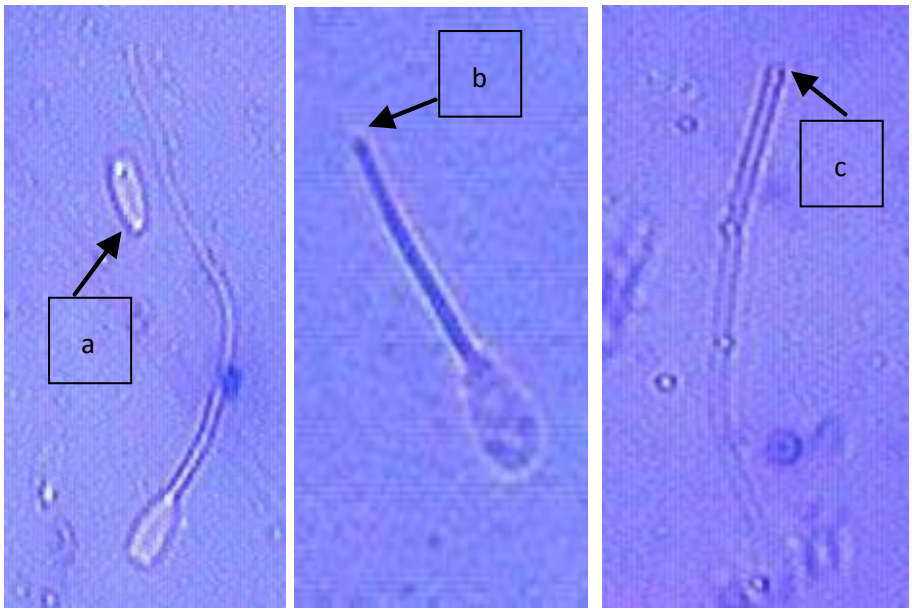

Figure 3. Abnormality of dairy cattle spermatozoa. (a) head without tail, (b) break tail, (c) tail without head.

staining (Sukmawati et al. 2014). Results of abnormality observation (Table 6) showed that abnormality commonly found was secondary abnormality such as separated head-tail and broken tail (Figure 3).

Abnormality on spermatozoa tail is allegedly caused by lipid peroxidation. Silva (2006) said that mid-piece and posterior part of spermatozoa tail were very vulnerable to lipid peroxidation. Percentage of the lowest abnormality in this study was in the vitamin C administration. This showed that vitamin C played a role as a free-radical scavenger. Vitamin C protected lipid from oxidation reaction by extracellular (Fraga et al. 1991; Sitohang et al. 2015). It is possible due to the vitamin C administration may protect spermatozoa tail by extracellular that may prevent lipid peroxidation.

The integrity of plasma membrane (Table 7) of non-sexing spermatozoa without vitamin C (0%) was not

different with spermatozoa Y, but was significantly lower than spermatozoa X.

Administration of 0.25% or 0.5% vitamin C administration in non-sexing spermatozoa was not different with both spermatozoa X or Y, meanwhile, administration of 0.75% vitamin C in non-sexing spermatozoa was significantly lower than spermatozoa X and Y.

Non-sexed spermatozoa showed the same plasma membrane integrity in 0%, 0.25%, and 0.5% but significantly higher than 0.75% vitamin C administration.

Vitamin C administrations in spermatozoa X showed the same plasma membrane integrity in 0%, 0.25%, and 0.75%, but significantly lower than 0.5% vitamin C administration. Spermatozoa Y plasma membrane showed the same integrity in each vitamin C concentration.

Observation of plasma membrane integrity using hypoosmotic swelling (HOS) test marked by coiling tail for good plasma membrane and straight tail for the damage one (Figure 4). Plasma membrane served as physical protector of cell organelles and regulating exit and entry of nutrients and ions and maintaining electrolyte balance of extra and intra-cellulars (Sukmawati et al. 2014).

Percentage of plasma membrane integrity in this study (Table 7) increased by vitamin C administration. This proved that vitamin C was one of the vitamins serving as the soluble antioxidant which might prevent reactive oxygen activities with polyunsaturated fatty acids in the plasma membrane of spermatozoa (Wijaya 1995).

Research result in this study was in accordance with Sumargono (1998) who reported that administration of 1.5 mM vitamin C in tris-yolk might maintain integrity percentage of thawed spermatozoa plasma membrane of mud buffalo from damage due to lipid peroxidation. Besides, administration of 0.50 g/100 mL in Andromed diluent after thawing resulted percentage of plasma membrane integrity of Aceh cattle by 42.65±5.34% (Aslam et al. 2014). Difference of concentration of vitamin C used to integrity percentage of spermatozoa plasma membrane was allegedly due to difference in

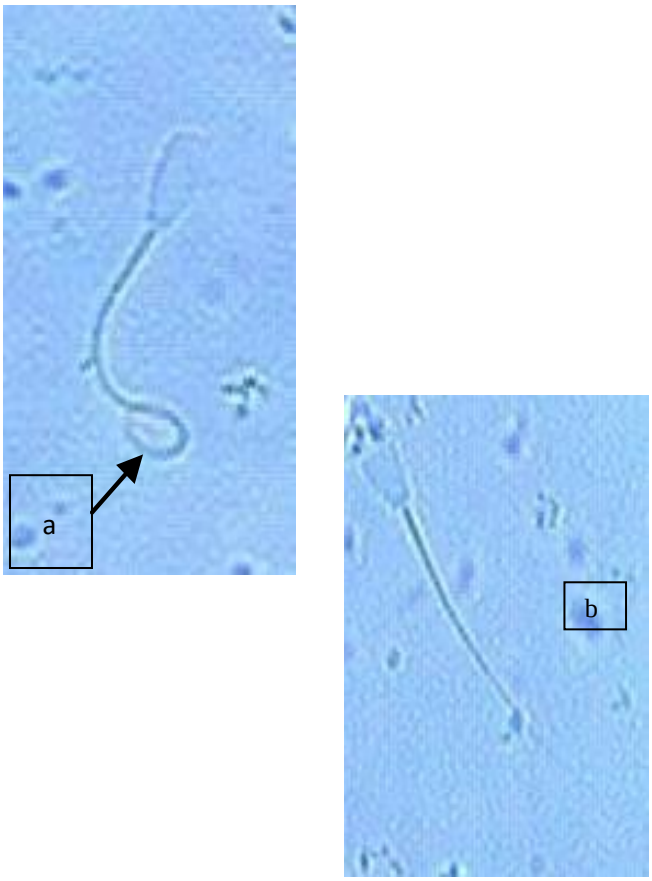

Figure 4. Integrity of plasma membrane of dairy cattle spermatozoa. (a) circle tail, good plasma membrane; (b) straight tail, damage plasma membrane.

used animal and diluent media. Toelihere (1993) explained that quality of spermatozoa was affected by diluent used.

Spermatozoa colored by acridine orange dye were used in observation of spermatozoa nucleus (Table 8). The acridine orange dye provided orange and yellow color in the dead or damage spermatozoa head and green color in alive or good spermatozoa head (Karezooni et al. 2009).

Integrity percentage of spermatozoa nucleus obtained in this study was in good condition (Figure 4). This proved that during sexing, freezing to thawing process did not cause damage in spermatozoa nucleus. Besides, high integrity percentage of spermatozoa nucleus in vitamin C treatment proved that vitamin C rolled as extracellular and intracellular free radicals scavenger (in the cytosol) (Padayatty 2003).

Observation in fresh semen and sexed spermatozoa is a basic to determine semen feasibility for further process. Komariah et al. (2013) reported quality of FH cattle spermatozoa before freezing collected from the bull in BIB Lembang (Table 9).

Table 9. Quality of FH cattle spermatozoa before freezing

Parameter	Criteria
Concentration	$1561x10^6$/ml
Mass Movement	Good
Motility	73.2%
pH	6.9

Source: Komariah et al. (2013)

Quality of thawed spermatozoa

Vitamin C was able to prevent damage to DNA caused by lipid peroxidation (Padayatty 2003). DNA damage caused by lipid peroxidation such as ring unveiling, fragmentation and DNA-protein cross-linking and DNA isolation would cause cells mutation or lethal (Awda et al. 2009). Besides, the result of lipid peroxidation such as malondialdehyde (MDA) and hydroxynonenol would trigger a modification of protein oxidation which was able to damage active sites enzyme, the conformation of protein structure and folding to form its original structure (Kumar et al. 2010; Susan & Rahayu 2013).

There was a quality decrease in non-sexing spermatozoa administered by 0.50% and 0.75% vitamin C and 0.75% vitamin C administration in spermatozoa X and Y. This is allegedly due to the decrease of pH. pH decrease in cell environment would disturb metabolic enzyme performance (Lehninger 1982). This is in accordance with the research results obtained by Sumargono (1998), Aslam et al. (2014) and Savitri et al. (2014). Sumargono (1998) said that motility of thawed mud buffalo spermatozoa decreased by administrating high vitamin C (3-5 mMolar diluent) due to the decrease of diluent pH. This is also in accordance with Aslam et al. (2014) who reported that administration of 2.00g/100ml vitamin C in Andromed diluent decreased the integrity of thawed spermatozoa plasma membrane of Aceh cattle. Administration of 4.50 mM vitamin C in diluent decreased the viability of thawed Bali cattle spermatozoa (Savitri et al. 2014).

The decrease of spermatozoa quality was also associated with the lactic acid increase in diluent media. Werdhany et al. (2000) said that longer thawed spermatozoa kept either at the time or before freezing (equilibration in 5°C) or after thawing would increase the number of dead spermatozoa due to the disability of media in maintaining pH which was more acid due to the accumulation of toxic lactic acid in spermatozoa.

CONCLUSIONS

Administration of vitamin C in diluent media improved the quality of non-sexing and X and Y spermatozoa of dairy cattle (*Bos taurus*) after thawing. The optimum concentration of vitamin C in diluent media to improve spermatozoa quality of sexed dairy cattle (*Bos taurus*) spermatozoa, both X and Y after thawing was 0.5%.

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