Biosaintifika 11 (1) (2019) 117-124

Biosaintifika
Journal of Biology & Biology Education

http://journal.unnes.ac.id/nju/index.php/biosaintifika

Cryopreservation of Aceh Cattle Semen with Date (*Phoenix dactylifera*) Extract Supplementation

Yonadiah Dwitya[1], ✉Kartini Eriani[1], Hendra Saputra[2], Al Azhar[3], Muhammad Rizal[4]

DOI: http://dx.doi.org/10.15294/biosaintifika.v11i1.18033

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Indonesia
[2]Animal Husbandry Office of Aceh Province, Indonesia
[3]Faculty of Veterinary Medicine, Universitas Syiah Kuala, Indonesia
[4]Department of Animal Science, Faculty of Agriculture, Universitas Lambung Mangkurat, Indonesia

History Article

Received 8 February 2019
Approved 1 April 2019
Published 30 April 2019

Keywords

Phoenix dactylifera; Cryopreservation; Spermatozoa Quality; Aceh Cattle

Abstract

Cryopreservation process could affect spermatozoa quality during from reactive oxygen species (ROS) produced in cellular metabolism and the environment. Spermatozoa damage caused by ROS during cryopreservation can be reduced with the addition of natural antioxidant which commonly found in fruits like date palm. This research was done to investigate the influence of date extract on semen quality after cryopreservation. This experimental study used a completely randomized design with 4 treatments and 6 replications. Semen collected from two aceh cattle bulls was diluted in tris egg yolk extender contained different concentrations (v/v) of date extract: 0% (P0, control), 0.75% (P1), 1% (P2), and 1.25% (P3) before cryopreserved at -196 °C for 7 days. Semen quality prior to and after cryopreservation as well as sperm DNA integrity were determined by standard microscopic and laddering methods, respectively. The results showed that the addition of 1% date extract could maintain viability (68.67%), plasma membrane integrity (62.33%), and abnormality (18.58%) of aceh cattle spermatozoa, but unable to maintain its motility above 40%. There was no DNA fragmentation observed in both treated and fresh semen. This is the first study investigates the influence of supplementation of date palm extract on preserved aceh cattle spermatozoa diluted in egg yolk tris based extender.

How to Cite

Dwitya, Y., Eriani, K., Saputra, H., Azhar, A., & Rizal, M. (2019). Cryopreservation of Aceh Cattle Semen with Date (*Phoenix dactylifera*) Extract Supplementation. *Biosaintifika: Journal of Biology & Biology Education*, 11(1), 117-124.

✉ Correspondence Author:
Jl. Syeh Abdur Rauf No. 3, Banda Aceh 23111, Aceh, Indonesia
E-mail: kartini_eriani@unsyiah.ac.id

p-ISSN 2085-191X
e-ISSN 2338-7610

INTRODUCTION

Aceh cattle has been stated as one of Indonesian beef cattle germplasm by the Ministry of Agriculture of The Republic of Indonesia (Kementan, 2011). This ruminant plays an important role in the economic, social, and culture of Aceh community life due to its big contribution for the meat fulfillment in the region (Mirza & Rahayu, 2017). Efforts to increase productivity and genetic quality of aceh cattle are done by implementation of artificial insemination (AI) program nationally, from which semen cryopreservation becomes one of important parts (Layek et al., 2016). Based on the report of Aceh Husbandry Office (2017), the successful rate of AI program in Special Effort of Mandatory Breeding Cattle (Upsus Siwab) 2017 in the entire Aceh regions using frozen semen from the Artifical Insemination Agency of Lembang and Singosari is 21.47% (12,952 out of 60,341 heads of breeding target). Around 62.55% (37,742 head) become pregnant from natural mating and 15.98% (9,647 heads) are not pregnant. Although semen quality of post thawing has increased for decades, low fertilization occures due to 50% immotil or damage spermatozoa from cryopreservation (Layek et al., 2016).

Cryopreservation-related low quality of spermatozoa such as decreased viability and fertility (Bansal et al., 2011), and DNA damage (Liu et al., 2016) was assumed caused by reactive oxygen species (ROS). These oxygen derivatives, highly reactive compounds are produced in the cellular metabolism and environment (Bansal et al., 2011). Spermatozoa damage caused by ROS in the cryopreservation process can be reduced with the addition of natural antioxidants, compounds found in high concentration in the date extract (Al Farsi et al., 2005; Al Juhaimi & Ghafoor, 2014).

Beside antioxidant, date palm contains other nutrients like energy, sucrose, glucose, fructose, Ca, Mg, K, P, Fe, Zn, Cu, B, vitamin C, protein (Al Juhaimi & Ghafoor, 2014) and antimicrobial (Taleb et al., 2016). All of which could be a requisite of good extender for maintaining spermatozoa quality after cryopreservation. Since ancient time, date palm has been used as herbal remedy (Mehraban et al., 2014). Bahmanpour et al. (2006) also reported that date extract can increase concentration, motility, morphology, and quality of DNA spermatozoa of adult rats. Hassan et al. (2012) stated that date pollen is used to increase women and men fertility. El-Neweshy et al. (2012) reported that date seed is also used as a traditional treatment for improving semen quality and overcoming infertility.

Effect of the addition of palm date extract on the quality of fresh, diluted and equilibrated semen of Limousin cattle has been done (Perbawati, 2018), but not on those of fresh or frozen semen of Aceh cattle. This study was done to investigate the quality and DNA spermatozoa integrity of aceh cattle semen diluted in egg yolk tris extender containing different concentrations of palm date extracts. The results obtained can be used as a basis of using palm date extract for improving or maintaining the quality of frozen aceh cattle semen. This might help effort to support genetic improvement and breeding selection of aceh cattle through artificial insemination-based breeding program nationally.

METHODS

Study Location

The research was carried out in the Laboratory of Balai Inseminasi Buatan (BIB) Saree of Aceh Husbandry Office and the Laboratory of Balai Perikanan Budidaya Air Payau (BPBAP) of Aceh Fisheries Office.

Palm Date Extraction

Date extract (tamar phase) was created by maceration process in 96% methanol guided Nafiah and Lusiana (2017) and Setianingsih et al. (2017) with some modification. In brief, date dried by exposure to sunlight, were mashed and maceration using 96% methanol over 82 hours. Then filtered and evaporated using rotary evaporator at temperature of 55°C as far as to obtain thick extracts of dates.

Semen collection and Cryopreservation

Completely Randomized Design (CRD) was applied to this study with 4 replications. Fresh semen was collected from two 5 years old of aceh cattle bulls (*Bos taurus indicus*) and subjected for macroscopic evaluation for its color, volume, pH and consistency as well as microscopic analysis for mass movement, concentration, motility, viability, and secondary abnormality. The semen fulfilled cryopreservation criteria was diluted in tris egg yolk extender containing cryoprotectant 7% glycerol and different concentrations (v/v) of date extract namely 0% (P0, control), 0.75% (P1), 1% (P2), and 1.25% (P3). Diluted semen were packed in straws, equilibrated in 5°C refrigerator for 3 hours and then frozen in deep freezer (-20 °C) for 15 minutes. After 14 minutes storage above liquid nitrogen of -120 °C in styrofoam box (Mukhlis et al., 2017) straws were stored in liquid

nitrogen container (-196°C) for 7 days.

Semen Quality Analysis

Frozen semen was thawed at 37 °C water bath for 60 seconds and subjected for standard semen quality analysis (Mukhlis et al., 2017; Rizal & Riyadhi, 2016). Sperm motility, viability, plasma membrane integrity, and secondary abnormality were determined by using an Olympus stereo microscope with 100 times magnification (Rahmiati et al., 2015) and presented in percentage (0 to 100%) a with scale of 5% (Eriani et al., 2017). Motility was observed by putting a drop of semen above object glass and marked by forward progressive movement. For viability and secondary abnormality analysis, a thin semen smear was prepared on an object glass, stained it in eosin staining solution for 10 minutes and microscopically observed. The numbers of cells observed were 200 cells. Living spermatozoa were marked by white head and those dead one were marked with a red head (Eriani et al., 2017). Abnormal Spermatozoa were characterized by their abnormal morphology such as having tail or head only, broken tail or coiled tail (Rahmiati et al. 2015). Plasma membrane integrity was determined by hypoosmotic swelling (HOS-test). Here semen was diluted in hypoosmotic solution (1:1), incubate it at 37°C for 30 minutes and microscopically evaluated. Analysis was done by observing 200 cells. Spermatozoa have intact plasma membrane were characterized by their coiled or bulged tails whereas those having broken plasma membrane characteristically has straight tail (Eriani et al. 2018).

DNA Fragmentation Analysis

Quality of spermatozoa DNA was determined analyzing its integrity laddering method (Zilli et al., 2003). Here DNA was extracted from both fresh and frozen semen by using Genomic DNA Purification Kit (Promega, 2017). DNA extract was then subjected for electrophoresis in 1.5% agarose at 135 V for 30 minutes and probed visualized using Uvidoc. The DNA damage was characterized by its fragmentation and laddering formation after the electrophoresis (Yusoff et al., 2018).

Data Analysis

Data were analyzed with ANOVA using SPSS 17 application. Differences between treatments were analyzed by Tukey test. DNA fragmentation data analyzed descriptively and presented in figure.

RESULTS AND DISCUSSION

Fresh Semen Quality

The results of macroscopic and microscopic evaluations of fresh semen of aceh cattle are presented in Table 1. The fresh semen has met the standards to use in cryopreservation as the following: motility >70% (SNI, 2017), mass movement (+++), concentration >800x10^6/ml viability >75%, and abnormality <20% (Zenichiro et al., 2002).

Table 1. Average quality of fresh semen of aceh cattle

Parameter	Result
Macroscopic Volume (ml)	4.92±0.60
Color	Milk white
Stink	Odor
Consistency	Medium-strong
pH	6.62±0.10
Microscopic Motility (%)	84.17±2.04
Mass movement	+++
Concentration (10^6/ml)	1,256.67±28.61
Viability (%)	93.33±1.99
Abnormality (%)	7.17±1.25

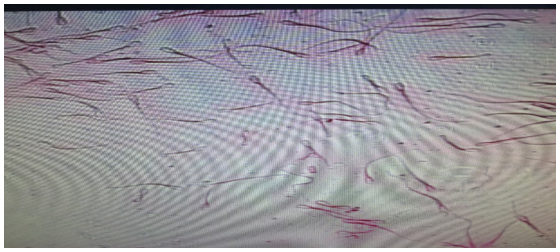

Figure 1. Spermatozoa viability of fresh semen of aceh cattle

Semen Quality After Cryopreservation

Sperm motility is the most important parameter for success fertilization. Motility data of aceh cattle spermatozoa is showed in Table 2.

Motility after cryopreservation of spermatozoa in the P0 (control) was 33.33%. This below standard required for artificial insemination i.e. 40%. Low (33.3% and 25.8%) post cryopreservation motility of spermatozoa diluted in tris egg yolk extender was also previously reported for aceh cattle (Rizki et al., 2018) and bali cattle (Salmah, 2014). Similarly, spermatozoa of aceh cattle semen diluted in Andromed™ had motility rate around 36.4% (Aslam et al., 2014) and 30.69% (Mukhlis et al., 2017).

Spermatozoa motility of aceh cattle semen

Table 2. Motility percentage of spermatozoa of aceh cattle

Phase	N	Treatment			
		P0	P1	P2	P3
Dilution	6	82.50±2.74[a]	70.83±3.76[b]	80.83±3.76[a]	73.33±2.58[b]
Equilibration	6	75.00±3.16[a]	65.00±5.48[b]	74.17±3.76[a]	69.17±4.92[ab]
Post thawing	6	33.33±2.58[a]	12.5±2.74[d]	29.17±2.04[b]	21.67±2.58[c]

Note: Numerals in the same row followed by the same superscript show no significant difference (P>0.05). P0, P1, P2, P3 = 0%, 0.75%, 1%, 1.25% date extract.

Table 3. Viability (%) of aceh cattle spermatozoa

Phase	N	Treatment			
		P0	P1	P2	P3
Dilution	6	90.92±1.69[a]	91.17±1.91[a]	93.50±2.17[a]	80.67±1.89[b]
Equilibration	6	85.42±5.48[ab]	86.50±5.41[a]	88.92±4.74[a]	78.50±0.55[b]
Post thawing	6	65.50±1.41[a]	66.17±3.53[a]	68.67±5.58[a]	59.92±0.58[b]

Note: Numbers in the same row followed by the different superscript show significant difference (p<0.05). P0, P1, P2, P3 = 0%, 0.75%, 1%, 1.25% date palm extract.

Table 4. Plasma membrane integrity (%) of aceh cattle spermatozoa

Phase	N	Treatment			
		P0	P1	P2	P3
Dilution	6	84.50±3.22[ab]	85.17±2.66[a]	88.25±2.93[a]	80.92±0.92[b]
Equilibration	6	80.25±2.96[a]	82.75±2.40[a]	83.67±2.75[a]	74.75±0.76[b]
Post thawing	6	60.33±1.08[a]	62.25±1.47[a]	62.33±1.94[a]	55.83±0.52[b]

Note: Numbers in the same row followed by the different superscript show significant difference (p<0.05). P0, P1, P2, P3 = 0%, 0.75%, 1%, 1.25% date palm extract.

treated with date extract ranged from 0.75% to 1.25% (P1, P2, and P3) was significantly lower than that of control (P0). This condition was probably caused by several factors such as cryopreservation, and higher viscosity and thickness of extender due to date extract supplementation. Eriani et al. (2018) reported cryopreservation significantly decreases spermatozoa motility. Mitochondria, energy-producing organelle for spermatozoa movement is the most sensitive to cellular stress induced by semen processing (Leon et al. 2014). According to Ferrusola et al. (2008), destruction of mitochondrial structure during cryopreservation is correlated with decreased motility of spermatozoa post thawing.

Higher viscosity and thickness of extender due to supplementation of date extract are related to the ingredient of the extract. *Khalas* date contains high concentration of sugar, minerals, ions, and antioxidant bioactive compounds in the extract (Al Juhaimi & Ghafoor, 2014). Antioxidants compounds found in *khalas* date are anthocyanin, carotenoids, phenol (Al Farsi et al., 2005) and vitamin C (Al Juhaimi & Ghafoor, 2014). Telis et al. (2007) argued that the occurrence sugars (sucrose, glucose, and fructose) could increase solution viscosity proportional to concentration and temperature decrease.

The addition of egg yolk to extender might thickening extender that in turn reduced sperm motility (Rizal & Riyadhi, 2016; Bathgate, 2006). This study also found that increased thickness of egg yolk tris extender due to date extract supplementation was not only resulted in lower spermatozoa motility but also unusual spermatozoa moving pattern. Microscopic observation done in this study showed that spermatozoa in semen treated with date extract moved toward left and right sides of date extract occurred in the extender.

In addition to lower motility, higher viscosity tends to lower viability of spermatozoa (Hafez, 2000). This study also evaluated viability and membrane plasma integrity, two important parameters in accessing semen quality and spermatozoa fertility, of aceh cattle spermatozoa treated in different concentration of date extract as

Table 5. Secondary abnormality of frozen aceh cattle spermatozoa

Phase	Treatment				
	N	P0	P1	P2	P3
Dilution	6	13.00±0.32[b]	13.50±0.55[ab]	13.58±0.49[ab]	13.83±0.52[a]
Equilibration	6	15.58±0.49[b]	16.00±0.71[ab]	16.25±0.42[ab]	16.58±0.58[a]
Post thawing	6	18.25±0.61[a]	18.42±0.49[a]	18.58±0.49[a]	18.67±0.41[a]

Note: Numbers in the same row followed by the different superscript show significant difference ($p<0.05$). P0, P1, P2, P3 = 0%, 0.75%, 1%, 1.25% date palm extract.

presented in Table 3 and 4.

Danang et al. (2012) described plasma membrane serves as protector of organelle cells from mechanical damage and from undesired intermembrane trafficking of molecules and ions. Plasma membrane damage, therefore, might result in viability loss due to disruption of cellular metabolic and physiological processes. Data in Table 3 and 4 showed that P2 had the highest post thawing viability (68.67%) and plasma membrane integrity (62.33%), but no differences were observed among P0, P1 and P2. Post thawing viability of P3, on the other hand, was significantly lower ($p<0.05$) than that of other treatment groups. These findings were predicted caused by optimum osmolarity created by the addition of 1% date extract on extender. Date contains a variety of nutrients such as carbohydrate, protein, antioxidant, and vitamin C that might act as spermatozoa protectants. Sugar contents like sucrose (Rizal et al., 2007), glucose, fructose, and others (Castelo et al., 2010) in extender serve as extracellular cryoprotectants that might protect plasma membrane from damage during cryopreservation (Mukminat et al., 2014; Rizal et al., 2007). Karp (2010) stated that carbohydrate in a cellular environment can be associated with lipid or protein of plasma membrane of cells. Aisen et al. (2002) reported that cryoprotective effect of sugars resulted from hydrogen bond formation between their hydroxyl group and polar head of plasma membrane phospholipids.

Moreover, proteins in extender had a role to maintain stability and permeability by wrapping the plasma membrane, so that just a little bit of plasma membrane phospholipids were oxidized (Putranti et al., 2010). High antioxidant and vitamin C contents of date palm (Al Farsi et al. 2005; Al Juhaimi and Ghafoor 2014) also help in protecting plasma membrane phospholipids from damage (Hu et al., 2014). The protection was achieved by capturing free radicals to prevent peroxidative chain reactions (Bansal et al., 2011) that increased proportional to cellular respiration rate in the mitochondria during cryopreservation (Fiqri et al., 2014). Increased integrity of plasma membrane post thawing due to administration of vitamin C was reported in aceh cattle spermatozoa (Aslam et al., 2014).

Another aspect of semen quality measured in this study was spermatozoa abnormality, a physical disorder might occur during cryopreservation. Spermatozoa abnormality accessed in this study was secondary abnormality that covers head or tail only, broken tail or coiled tail, all of which could happen on spermatozoa after leaving the seminiferous tubules. Secondary abnormality of aceh cattle spermatozoa recorded in this study is presented in Table 5.

Data in Table 5 indicates that P0 had the lowest (18.25%) secondary abnormality, but no significant difference observed among treatment groups. Spermatozoa abnormality increased in line with cryopreservation stages, but still below 20%. This is good because accuracy of abnormality evaluation is an important parameter in differentiating good or bad semen for fertilization (Januškauskas & Žilinskas, 2002).

Herdiawan (2004) reported the spermatozoa abnormality might occur due to physical changes of medium with a decreasing temperature. The change occurred can be osmotic pressure change or the formation of intracellular ice crystals. Rizal & Riyadhi (2016) also explained that extender containing synthetic compounds could maintain its chemical and physical properties better that supplemented by natural compounds during freezing. This is because the later can be damaged at the time of freezing process, resulting in depreciation of protective materials to spermatozoa.

Spermatozoa DNA Integrity

DNA integrity analysis was done to see the damage of spermatozoa during cryopreservation. Result of DNA laddering analysis on frozen semen diluted in extender added with different concentrations of date extract is presented in Figure 2.

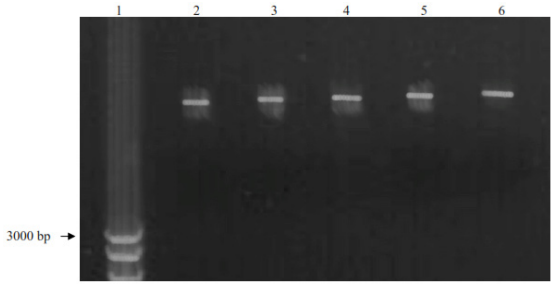

Figure 2. Electrogram of spermatozoa DNA of aceh cattle. Line 1 = DNA marker 100 kb, line 2, 3 , 4, and 5 are DNA extracted from semen treated with date extract of 0% (P0), 0.75% (P1), 1% and 1.25% (P3). Line 6 = DNA extracted from fresh semen.

Based on this result, date extract contains various nutrients as reported by Al Juhaimi & Ghafoor (2014), Taleb et al. (2016), and Al Farsi et al. (2005); which protect spermatozoa from damage (Mukminat et al., 2014; Putranti et al., 2010). Although motility in this research unable to be used for AI, however, it can be applied to intracytoplasmic sperm injection (ICSI) technology in an attempt to improve the genetic quality of Aceh cattle germplasm preservation in the future. Based on Eriani et al. (2013), ICSI does not need motile spermatozoa but need an intact DNA chain.

Novelty of the study

This study is the first in investigating post cryopreservation quality and DNA of aceh cattle spermatozoa frozen using egg yolk tris extender supplemented with different concentration of date extract.

Benefit and Contribution of Study for Science and Society

The results obtained can be used as a basis of using palm date extract for improving or maintaining the quality of frozen aceh cattle semen. This might help effort to support genetic improvement and breeding selection of aceh cattle through artificial insemination-based breeding program nationally.

CONCLUSION

Addition 1% date palm extract into tris egg yolk extender could maintain viability, plasma membrane integrity, and secondary abnormality as well as DNA integrity aceh cattle spermatozoa, but could not maintain motility above 40%. DNA laddering analysis showed there was no DNA fragmentation in all treatments.

ACKNOWLEDGEMENT

The author would like to thank Aceh Husbandary Office who gave valuable contribution on this research.

REFERENCES

Aisen, E. G., Medina, V. H. & Venturino, A. (2002). Cryopreservation and Post-Thawed Fertility of Ram Semen Frozen in Different Trehalose Concentrations. *Theriogenology,* 57, 1801-1808.

Al Farsi, M., Alasalvar, C., Morris, A., Baron, M. & Shahidi, F. (2005). Comparison of Antioxidant Activity, Anthocyanins, Carotenoids, and Phenolics of Three Native Fresh and Sun-Dried Date (*Phoenix dactylifera* L.) Varieties Grown in Oman. *Journal of Agricultural and Food Chemistry*, 53 (19), 7592–7599.

Al Juhaimi, F. & Ghafoor, K. (2014). Physicochemical Properties and Mineral Contents of Seven Different Date Fruit (*Phoenix dactylifera* L.) Varieties Growing from Saudi Arabia. *Environ Monit Assess*, 186, 2165–2170.

Aslam, H., Dasrul, & Rosmaidar. (2014). Pengaruh Penambahan Vitamin C dalam Pengencer Andromed Terhadap Persentase Motilitas dan Membran Plasma Utuh Spermatozoa Sapi Aceh Setelah Pembekuan. *Jurnal Medika Veterinaria*, 8 (1), 20–26.

Bahmanpour, S., Talaei, T., Vojdani, Z., Panjehshahin, M. R., Poostpasand, A., Zareei, S. & Ghaeminia, M. (2006). Effect of Phoenix Dactylifera Pollen on Sperm Parameters and Reproductive System of Adult Male Rats. *Iranian Journal of Medical Sciences*, 31 (4), 208–212.

Bansal, A.K. & Bilaspuri, G.S. (2011). Review Article: Impacts of Oxidative Stress and Antioxidants on Semen Functions. *Veterinary Medicine International*, 1-7.

Bathgate, R., Maxwell, W. M. C. & Evans, G. (2006). Studies on The Effect of Supplementing Boar Semen Cryopreservation Media with Different Avian Egg Yolk Types on In Vitro Post-Thaw Sperm Quality. *Reproduction in Domestic Animals*, 41 (1), 68–73.

Castelo, T. S., Bezerra, F. S. B., Lima, G. L., Alves, H. M., Oliveira, I. R. S., Santos, E. A. A., Peixoto, G. C. X. & Silva, A. R. (2010). Effect of centrifugation and sugar supplementation on the semen cryopreservation of captive collared peccaries (T*ayassu tajacu*). *Cryobiology*, 61, 275–279.

Danang, D. R., Isnaini, N. & Trisunuwati, P. (2012). Pengaruh Lama Simpan Semen Terhadap Kualitas Spermatozoa Ayam Kampung dalam Pengencer Ringer's pada Suhu 4°C. *Journal of Tropical Animal Production*, 13 (1), 47-57.

Dinas Peternakan Aceh. (2017). *Upaya Khusus Sapi/ Kerbau Indukan Wajib Bunting (Upsus Siwab)*. Dinas Peternakan Aceh, Banda Aceh.

El-Neweshy, M. S., El-Maddawy, Z. K., & El-Sayed, Y. S. (2012). Therapeutic Effects of Date Palm (*Phoenix dactylifera* L.) Pollen Extract on Cadmium-Induced Testicular Toxicity. *Andrologia*, 20, 1–10.

Eriani, K., Azhar, A., Ihdina, M., Rosadi, B., Rizal, M. & Boediono, A. (2018). Quality Enhancement of Aceh Swamp Buffalo (*Bubalus bubalis*) Frozen Semen by Supplementing β-Carotene. *Tropical Animal Science Journal*, 41 (1), 1–7.

Eriani, K., Sari, N., Rosnizar, R., Dasrul, D., Suhartono, S. & Rizal, M. (2017). Cryopreservation of Aceh Swamp Buffalo (*Bubalus bubalis*) Semen with Combination of Glycerol and Lactose. *Biosaintifika: Journal of Biology & Biology Education*, 9 (3), 409-416.

Eriani, K., Sukra, Y., Boediono, A., Djuwita, I. & Sumarsono, S. H. (2013). Produksi Embrio Kucing Secara in Vitro dari Spermatozoa Hasil Preservasi Melalui Fertilisasi Mikro. *Jurnal Kedokteran Hewan*, 7 (1), 37-42.

Ferrusola, C. O., Galán, Y. S., Fernández, E. V., Bolaños, J. M. G., Muriel, A., Fernández, L. G., Tapia, J. A. & Peña, F. J. (2008). Detection of "Apoptosis-Like" Changes During the Cryopreservation Process in Equine Sperm. *Journal of Andrology*, 29 (2), 213-221.

Fiqri, M. M., Ducha, N. & Raharjo. (2014). Motilitas Spermatozoa Sapi Brahman dengan Berbagai Konsentrasi dalam Pengencer CEP-D yang Disimpan dalam Refrigerator. *Lentera Bio*, 3 (3), 181–185.

Hafez, E. S. E. (2000). *Reproduction in Farm Animals, 7th edition*. Baltimore: Lippicott Williams & Wilkins.

Hassan, W. A., El-kashlan, A. M. & Ehssan, N. A. (2012). Egyptian Date Palm Pollen Ameliorates Testicular Dysfunction Induced by Cadmium Chloride in Adult Male Rats. *Journal of American Sciences*, 8 (4), 659–669.

Herdiawan, I. (2004). Pengaruh Laju Penurunan Suhu dan Jenis Pengencer terhadap Kualitas Semen Beku Domba Priangan. *Jurnal Ilmu Ternak Dan Veteriner*, 9 (2), 98–107.

Hu, J. H., Zan, L. S., Zhao, X. L., Li, Q. W., Jiang, Z. L., Li, Y. K. & Li, X. (2014). Effects of Trehalose Supplementation on Semen Quality and Oxidative Stress Variables in Frozen-Thawed Bovine Semen. *Journal of Animal Science*, 88: 1657–1662.

Januškauskas, A. & Žilinskas, H. (2002). Bull Semen Evaluation Post-Thaw and Relation of Semen Characteristics to Bull's Fertility. *Veterinarija Ir Zootechnika*, 17 (39): 1-8

Karp, G. (2010). *Cell Biology Sixth Edition*. United States of America: Wiley.

Kementerian Pertanian. (2011). *Keputusan Menteri Pertanian Republik Indonesia Nomor 2907 Tahun 2011 tentang Penetapan Rumpun Sapi Aceh*. Jakarta: Kementerian Pertanian.

Layek, S. S., Mohanty, T. K., Kumaresan, A. & Parks, J. E. (2016). Cryopreservation of Bull Semen: Evolution from Egg Yolk Based To Soybean Based Extenders. *Animal Reproduction Science*, 172, 1–9.

Leon, J., Castroviejo, D. A., Sainz, R. M., Mayo, J. C., Tan, D. X. & Reiter, R. J. (2004). Minireview: Melatonin and mitochondrial function. *Life Sciences,* 75, 765-790.

Liu, T., Gao, J., Zhou, N., Mo, M., Wang, X., Zhang, X., Yang, H., Chen, Q., Ao, L., Liu, J., Cui, Z. & Cao, J. (2016). The Effect of Two Cryopreservation Methods on Human Sperm DNA Damage. *Cryobiology*, 72, 210–215.

Mehraban, F., Joodi, B., Jafari, M., Toori, M. A., Sadeghi, H., Mostafazade, M. & Sadeghi, H. (2014). Effects of Date Palm Pollen (*Phoenix dactylifera* L.) and Astragalus ovinus on Sperm Parameters and Sex Hormones in Adult Male Rats. *Iranian Journal of Reproductive Medicine*, 12 (10): 705-712.

Mirza, I. & Rahayu, W. (2017). Model Pengembangan Kawasan Peternakan Sapi Aceh di Kabupaten Aceh Jaya Propinsi Aceh. *Jurnal Peternakan Indonesia*, 19 (3), 152–160.

Mukhlis, Dasrul, & Sugito. (2017). Analisis Motilitas Spermatozoa Sapi Aceh Setelah Pembekuan dalam Berbagai Konsentrasi Andromed®. *Agripet*, 17 (2), 112–120.

Mukminat, A., Suharyati, S. & Siswanto. (2014). Pengaruh Penambahan Berbagai Sumber Karbohidrat pada Pengencer Skim Kuning Telur Terhadap Kualitas Semen Beku Sapi Bali. *Jurnal Ilmiah Peternakan Terpadu*, 2 (2), 87–92.

Nafiah, F. & Lusiana, N. (2017). Pengaruh Ekstrak Daging Buah Kurma Ajwa (*Phoenix dactylifera*) Terhadap Hitung Jenis Monosit Mencit (Mus musculus) Bunting. In *Prosiding Seminar Nasional III Tahun 2017* (pp. 17–21). Malang.

Perbawanti, S. A. (2018). Pengaruh Tingkat Suplementasi Sari Kurma dalam Pengencer Tris Kuning Telur terhadap Kualitas Semen Sapi Limousin yang Dipreservasi pada Suhu 3-5°C. *Skripsi*. Banjarbaru: Universitas Lambung Mangkurat.

Promega. (2017). *Wizard® Genomic DNA Purification Kit*. USA.

Putranti, O., Kustono & Ismaya. (2010). Pengaruh Penambahan Crude Tannin pada Sperma Cair Kambing Peranakan Ettawa yang Disimpan Selama 14 Hari Terhadap Viabilitas Spermatozoa. *Buletin Peternakan*, 34 (1), 1–7.

Rahmiati, Eriani, K. & Dasrul. (2015). Kualitas dan Morfologi Abnormal Spermatozoa Sapi Aceh pada Berbagai Frukuensi Ejakulasi. *Prosiding Seminar Nasional Biotik*, 2 (1), 339–344.

Rizal, M. & Riyadhi, M. (2016). Fertilitas Semen Kerbau Rawa (*Bubalus bubalis carabanensis*) yang Diencerkan dengan Pengencer Nira Aren. *Jurnal Veteriner*, 17 (15), 457–467.

Rizal, M. & Riyadhi, M. Kualitas Semen Beku Kerbau Rawa yang Dikriopreservasi dengan Pengencer Nira Aren. *Seminar Nasional Peternakan 2, Fakultas Peternakan Universitas Hasanuddin*. Makassar, 25 Agustus 2016.

Rizal, M., Herdis, Yulnawati, & Maheshwari, H. (2007). Peningkatan Kualitas Spermatozoa Epididimis Kerbau Belang yang Dikriopreservasi dengan Beberapa Konsentrasi Sukrosa. *Jurnal Veteriner*, 8 (4): 188-193.

Rizki, S., Dasrul, Hamdan, Melia, J., Riady, G. & Adam, M. (2018). Pengaruh Pemberian Gliserol dalam Medium Tris Kuning Telur Terhadap Kualitas Spermatozoa Sapi Aceh Setelah Pembekuan. *Jurnal Ilmiah Mahasiswa Veteriner*, 2 (1), 149–154.

Salmah, N. (2014). Motilitas, Persentase Hidup, dan Abnormalitas Spermatozoa Semen Beku Sapi Bali pada Pengencer Andromed dan Tris Kuning Telur. *Skripsi*. Makassar: Universitas Hasanuddin.

Setianingsih, N., Ula, A. M. & Purnamasari, R. (2017). Pengaruh Pemberian Ekstrak Metanol Daging Buah Kuema (*Phoenix dactylifiera*) Terhadap Jumlah Total Leukosit Embrio Mencit (*Mus musculus*). In *Prosiding Seminar Nasional III Tahun 2017* (pp. 111–115). Malang.

Standar Nasional Indonesia (SNI). (2017). *Semen Beku Sapi*. Jakarta: Badan Standar Indonesia.

Taleb, H., Maddocks, S. E., Morris, R. K., & Kanekanian, A. D. (2016). Chemical Characterisation and The Anti-Inflammatory, Anti-Angiogenic and Antibacterial Properties of Date Fruit (*Phoenix dactylifera* L.). *Journal of Ethnopharmacology*, 194, 457–468.

Telis, V. R.N., Telis-Romero, J., Mazzotti, H.B. & Gabas, A.L. (2007). Viscosity of Aqueous Carbohydrate Solutions at Different Temperatures and Concentrations. *International Journal of Food Properties*, 10 (1), 185–195.

Yuso , M., Hashim, F., Hassan, B. N., Ikhwanuddin, M., Sheri , S. M., Mustafa, S. & Koh, I. C. C. (2018). Cryobiology Successful Sperm Cryopreservation of the Brown-Marbled Grouper, *Epinephelus fuscoguttatus* Using Propylene Glycol as Cryoprotectant. *Cryobiology*, 30 (40), 1-6.

Zenichiro, K., Herliantien & Sarastina. 2002. *Instruksi Praktis Teknologi Prosesing Semen Beku pada Sapi*. Balai Inseminasi Buatan Singosari, Jawa Timur.

Zilli, L., Schiavone, R., Zonno, V., Storelli, C. & Vilella, S. (2003). Evaluation of DNA Damage in *Dicentrarchus labrax* Sperm Following Cryopreservation. *Cryobiology*, 47: 227-235.