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LIVABILITY AND RECOVERY RATE OF BALI CATTLE SPERMATOZOA DURING PRESERVATION IN TRIS-BASED EGG YOLK DILUENT WITH DIFFERENT SUCROSE LEVELS

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ABSTRACT

This study aimed to determine the effect of sucrose addition in tris-based egg yolk diluent in maintaining and protecting spermatozoa during preservation. The design of this study was a completely randomized design (CRD) which consisted of five treatments with ten replications. The treatments were tris-based egg yolk diluent without sucrose (P0, control), tris-based egg yolk diluent with 0.2% sucrose (P1), tris-based egg yolk diluent with 0.3% sucrose (P2), tris-based egg yolk diluent with 0.4% sucrose (P3) and tris-based egg yolk diluent with 0.5% sucrose (P4). The parameters measured were motility, livability, abnormality, intact plasma membrane, and recovery rate. Semen was evaluated 2 times, freshly after being collected and post thawed. The results showed that the increase level of sucrose addition from 0.2% to 0.5% increased the motility value from 43.59% to 48.15%, the livability value from 51.24% to 55.45%, the intact plasma membrane value from 44.66% to 48.21%, the recovery rate value from 54.6% to 60.2 %, and reduce the abnormality value from 13.49%-10.24%. It can be concluded that the addition of 0.2-0.5% sucrose in tris-based egg yolk diluent could increase motility, livability, intact plasma membrane, recovery rate, and could reduce the abnormalities of Bali cattle spermatozoa during preservation.

Key words: abnormality, intact plasma membrane, livability, motility

ABSTRAK

Penelitian ini bertujuan mengetahui penambahan persentase sukrosa pada pengencer tris kuning telur dalam mempertahankan dan melindungi spermatozoa selama preservasi. Rancangan yang digunakan dalam penelitian ini adalah Rancangan Acak Lengkap (RAL) yang terdiri atas lima perlakuan dengan sepuluh ulangan. Perlakuan yang diberikan adalah pengencer tris kuning telur tanpa tambahan sukrosa (P0, kontrol), pengencer tris kuning telur dengan 0.2% sukrosa (P1), pengencer tris kuning telur dengan 0.3% sukrosa (P2), pengencer tris kuning telur dengan 0.4% sukrosa (P3) dan pengencer tris kuning telur dengan 0.5% sukrosa (P4). Parameter yang diukur adalah, motilitas, livabilitas, abnormalitas, membran plasma utuh dan recovery rate. Semen dievaluasi setelah ditampung dan setelah post thawing kembali. Hasil penelitian menunjukkan bahwa penambahan sukrosa dari 0.2% - 0.5% meningkatkan nilai motilitas dari 43.59% ke 48.15%, nilai livabilitas dari 51.24% ke 55.45%, nilai membran plasma utuh dari 44.66% ke 48.21% dan nilai recovery rate dari 54.6% ke 60.2% serta menurunkan angka abnormalitas 13.49%-10.24%. Disimpulkan bahwa penambahan sukrosa hingga 0.5% dalam pengencer tris kuning telur dapat meningkatkan motilitas, livabilitas, membran plasma utuh dan recovery rate serta dapat menurunkan abnormalitas spermatozoa sapi Bali selama preservasi.

Kata kunci: abnormalitas, membran plasma utuh, livabilitas, motilitas

INTRODUCTION

Freezing process (cryopreservation) of semen at a very low temperatures (-196° C) will cause damage to cells due to ice crystals formed and changes in electrolyte concentration (Holt, 2000). This condition will affect the movement/motility of sperm. In order to maintain the nature of spermatozoa movement during freezing process, protecting the integrity of the spermatozoa membrane is very important in the preservation process.

Components that can be added to the diluent to maintain the integrity of the sperm membrane are cryoprotectants (Toelihere, 1993). There are two types of cryoprotectants, namely intracellular cryoprotectants and extracellular cryoprotectants. Glycerol is one example of intracellular cryoprotectants, whereas egg yolk and sugar/sucrose are the extracellular cryoprotectants (Holt, 2000). The combination of the two types of cryoprotectants is expected to provide an optimal protection against spermatozoa during the cryopreservation process

Tris (hydroxymethyl) aminomethan is a buffer that is often used for buffering due to its low toxicity. Egg yolks have lipoprotein and lecithin which maintain and protect the integrity of spermatozoa lipoprotein envelope. Sucrose acts as an extracellular cryoprotectant to protect membranes from damage during low temperatures storage (Aisen *et al*., 2002). The components in the diluent such as buffer are expected to be able to maintain the pH of the solution so that it remains neutral for the life of spermatozoa and protect it from the effects of cold shock (Arifiantini and Purwantara (2010).

Previous research has observed that the addition of sucrose in the diluent succeeded in improving the semen quality of Garut sheep (Yulnawati and Herdis, 2009), enhancing the characteristics of semen of Boer goat after freezing (Nainga *et al*., 2010), improving the quality of cow semen after thawing (Jian *et al*., 2010), and increasing the quality of frozen semen from cauda epididymis in sheep (Herdis *et al*., 2016). Sucrose is expected to be an additional source of substrate for cells during storage that will protect spermatozoa cell membranes from the effects of cold shocks due to low temperatures storage. So far, the concentrations of sucrose addition in egg yolk diluents in Balinese cattle semen have not been reported yet.

Therefore, this research aimed to find out the best concentration percentage of sucrose addition in the tris-based egg yolk diluent that can maintain and protect spermatozoa during preservation.

MATERIALS AND METHODS

This study used semen from a 450 kg weighed, 7-year-old Balinese bull that was kept at the Tenayan Raya Artificial Insemination Center (*Balai Inseminasi Buatan*/BIB). The bull was fed with Napier grass *(Pennisetum purpureum)* and concentrate every day. Drinking water was provided on ad libitum basis. The bull exercised every day and the semen collection was performed once a week.

Diluents preparation

The diluents consisted of 3.028 g of Tris (hydroximethyl) aminomethane crystals, 1.25 g of fructose crystal, and 1.7 g of citric acid monohydrate that were dissolved with aquabidest to reach a volume of 100 mL (solution 1). Solution 1 was homogenized for 15 minutes and 80 mL of solution 1 was then mixed with 20 mL of egg yolk, stirred slowly for up to 60 minutes (solution 2). The 0.5 mL penicillin and 0.4 mL streptomycin was added to solution 2 and homogenized for 15 minutes. Solution 3 was prepared by adding 6% glycerol to the solution 2. Solution 3 was then divided into 5 treatments with 10 replications. The diluent solution for each treatment and replication were placed in a water jacket with a temperature of 37° C. Sucrose was added to solution 3 in treatment group of P1, P2, P3, and P4. The solution was also homogenized for 15 minutes. Composition of the diluent in each treatment was presented in Table 1.

Percentage of sucrose added to the tris-based egg yolk diluent was converted in grams as followed: Sucrose density = 1.59 g /cm^3 (1 mL).

$$density = \frac{mass}{volume}$$

Sucrose concentration of 0.2% was equivalent to 1.59 g/cm^3 = $\frac{m}{0,2}$ g/cm^3

m = 1.59g/cm^3 x 0.2 g/cm^3, = 0.318 g/cm^3 or 0.318 g

Sucrose concentration of 0.3%, 0.4%, and 0.5% were equivalent to 0.477 g, 0.636 g, and 0.795 g, respectively

Semen dilution

The volume of diluent used was determined using the procedures by Shukla (2011).

Diluted spermatozoa were packaged into mini straws (0.25 mL) and were equilibrated in a cool-top at 5° C for 4 hours. After equilibration, spermatozoa were frozen by placing the straws at 10 cm from the surface of liquid nitrogen (temperature around -130° C) for 15 minutes, then the straws were kept in the liquid nitrogen container (temperature around -196° C).

Straws from each treatment group were thawed using water at 37° C (in a water bath) for 5 minutes to evaluate the quality of the spermatozoa. Semen evaluation was carried out twice. The first evaluation was carried out freshly after collection with parameters as follows: pH, odor, volume, color and consistency, concentration, motility, individual motion, and mass motion. The second evaluation was done after post-thawed with parameters of motility, abnormality, livability, intact plasma membrane, and recovery rate.

The parameters measured in this study were;

a. Percentage of motility.

Sperm motility could be seen from the percentage of sperm moving forward, which was observed using light microscope with a 400x magnification. The range was from 0-100% with a scale of 5% (Toelihere, 1993)

$$\text{Motility (\%)} = \frac{number\ of\ motile\ spermatozoa}{number\ of\ spermatozoa\ counted} \times 100\%$$

b. Spermatozoa Abnormalities

Spermatozoa abnormalities were abnormal sperm shape due to dilution, freezing and thawing, such as head without tails and bent tails because they were associated with fertility (Yendraliza *et al.*, 2015). The level of sperm abnormality in the semen samples was determined through the formula:

$$\text{Abnormalities (\%)} = \frac{number\ of\ abnormal\ spermatozoa}{number\ of\ spermatozoa\ counted} \times 100\%$$

c. Intact plasma membrane (IPM) of Spermatozoa

Evaluation of the spermatozoa plasma membrane integrity (percentage of IPM) was carried out using the hypo-osmotic swelling (HOS) method test. Observation of the intact plasma membrane of spermatozoa was conducted by dripping a drop of sperm mixture (a mixture of sperm and osmolality solution that had been incubated) on the slide and covered with cover glass, then observed under a microscope with 400x magnification. Spermatozoa with intact membranes would hold hypoosmotic fluid inside the cell, so that the tail looked curved or bent, while spermatozoa with straight tails indicated that the plasma membrane had been damaged, since it was unable to hold water from entering.

d. Spermatozoa Livability

Livability of spermatozoa was determined by calculating the percentage of live spermatozoa after stained with 2% eosin (Bucak *et al.*, 2007). A drop of semen sample with two drops of eosin was placed on a warm glass object then mixed and immediately evaluated with a 400x magnification light microscope. Livespermatozoa was marked by a white head, while the dead spermatozoa wasmarked by a red head. A minimum of 200 spermatozoa were counted, with the following formula:

$$\text{Live Spermatozoa (\%)} = \frac{Number\ of\ living\ spermatozoa}{Number\ of\ spermatozoa\ counted} \times 100\%$$

e. Recovery Rate

Recovery rate percentage (RR) was calculated according to Garner and Hafez (2000) calculation as followed:

Percentage of motile sperm post thawing divided by the motility of fresh sperm multiplied by 100.

Data Analysis

The data were analizsed using one way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Balinese Cattle Fresh Semen Quality

Fresh semen Characteristics of Balicattle in this study was shown in Table 2. The characteristics of fresh semen in this study met the eligibility standard of SNI (National Standard of Indonesia) (2017) for frozen semen. Garner and Hafez (2016) stated that semen that could be diluted was the one which had 40% motility.

Bali Cattle Semen Quality Post-Thawed Motility

The addition of sucrose to tris-based egg yolk diluent significantly affected the spermatozoa motility of Bali cattle after thawing process (P>0.05) (Table 3). The motility rate of Balie cattle was increased from 43.59% to 48.15%. These results were still in accordance with the provisions of SNI 01.4869.1-2005 which stated that the quality of cow semen after undergoing the freezing process must contain at leats 40 % live and motile spermatozoa (Ditjennak, 2000). Increase in the sperm motility of Bali cattle in this study was suspected due to lactose that was able to be a source of energy to maintain the motility and survival of spermatozoa during the freezing and post-thawing process. Sugar such as sucrose will produce ATP which was very important for the contraction of fibrils in the sperm tail which cause movement (motility) in spermatozoa (Hammerstedt, 1993), and it also considered as an energy source and an extracellular cryoprotectant at the same time (Rizal, 2017).

The semen motility rate of Bali cattle in this study was different from Simmental cattle (65.12±5.53%), Limousin cattle (63.44±3.22%), and FH cattle (63.12±3.53%) (Komariah *et al*., 2013), and from Bali cattle (79.3%) (Susilawati *et al*., 2018). This difference was caused by different breed, types of diluent, and maintenance systems (Garner and Hafez, 2016). Other studies also reported that the addition of sugar in the form of sucrose and trehalose in diluent significantly increased the percentage of motile spermatozoa from cattle frozen semen (Woelders *et al*., 1997). The positive effect of raffinose and trehalose have been observed in frozen semen of mice (Storey *et al*., 1998), dogs (Yildiz *et al*., 2000), and goat (Eiman and Takato, 2004).

Livability

Addition of sucrose to tris-based egg yolk diluent increased the spermatozoa livability value of Bali cattle after thawing process (Table 3). Livability value obtained in this study was different from the El-Sheshtawy and El-Nattat (2018) who observed the livability of cattle semen with tris fructose diluent (74%) addition. Sucrose was the raw material for producing energy through the glycolysis pathway by spermatozoa. The process of glycolysis would produce adenosine triphosphate (ATP), therefore sperm motility and survival could be maintained (Barbonetti *et al*., 2010). The energy was more used to maintain the motility and vitality of spermatozoa during storage (Murray *et al*., 1999). As an energy source substrate, glucose, fructose, and sucrose entered the cell by two mechanisms, namely active transport and diffusion (Mansjur, 2001). These carbohydrate molecules would be metabolized through the glycolysis pathway or the Krebs cycle. The produced energy in the form of ATP would be utilized by spermatozoa in motion (Manjunath, 2012).

Intact Plasma Membrane (IPM)

Addition of sucrose in this study increase the IPM value of Bali cattle spermatozoa after thawing process (Table 3). This was presumably because the addition of 0.5% sucrose to the diluent could protect cell organelles from mechanical and biochemical damage. Plasma membrane was needed by spermatozoa because it served to protect cell organelles from mechanical and biochemical damage. Sugar played an important role in reducing the salt content of dilution solutions, therefore reducing the solution effect (Meryman, 2007). This cryoprotective effect of sugar resulted from the formation of hydrogen bonds between the sugar hydroxyl group and the polar phospholipid head of the cell plasma membrane, so that sugar replaced the position of water molecules in the dehydration process during freezing (Aisen *et al*., 2002). The integrity of the plasma membrane would affect the motility and vitality of sperm. Plasma membrane had many macromolecules such as proteins, lipoproteins, glycoproteins, and others that could function as enzymes, receptors, channels, or carrier (Lehninger, 1994). These macromolecules function to facilitate traffic in and out of cells throughout the substrate and electrolytes.

Uchida *et al*. (2007) stated that sugars would bind with water molecules to protect the spermatozoa membrane from nucleation and the formation of ice crystals. Bakas and Disalvo (1991) stated that sugar could stabilize cell plasma membranes during cryopreservation. The integrity of the plasma membrane would keep the enzyme aspartate aminotransferase (AspAT) from leaving the cell because this AspAT enzyme was the main mitochondrial enzyme in producing ATP (Colenbrander *et al*., 1992). The same source stated that ion leakage could be minimized by interaction between the enzyme ATPase and sodium-potassium pump so that motility and membrane integrity could be maintained after preservation. As a stable compound, it was not easy to change sucrose structure into ionic form which can change the osmotic pressure of the semen diluent solution. Changes in the osmotic pressure of the diluent solution could cause spermatozoa death.

Some studies reported that the addition of sugar in the form of sucrose or trehalose in the diluent significantly increased the IPM of frozen semen of cows (Woelders *et al*., 1997). Rizal and Riyadhi (2016) reported that IPM of buffalo semen was better in diluents that was added lactose compared to the addition of palm sugar (45-58% VS 30-48%). The best

IPM value was also produced by basic diluents that were supplemented with sucrose with a concentration of 0.5% (Yulnawati and Herdis, 2009)

Abnormality

The addition of sucrose to tris-based egg yolk diluent did not significantly affect the abnormalities of Bali cattle semen (Table 3). Different sucrose concentrations in egg yolk diluent did not show different sperm abnormality values. The value of sperm abnormality in this study still met the requirements of frozen semen used for artificial insemination which was only contained 20% or less abnormal sperm (Garner and Hafez, 2016). Bearden and Fuquay (2004) stated that abnormal morphological rates in frozen sperm ranging from 8-10% did not have significant effect on fertility. This abnormality was different from striped buffalo semen (15%) (Yulnawati *et al*., 2008). According to Maxwell and Salamon (2000), frozen sugar was shaped like dull glass and it would not damage sperm cells mechanically. This could be seen from the value of sperm abnormalities in all treatments that were not significantly different.

Recovery Rate

The addition of sucrose increased the recovery rate of Bali cattle sperm after thawing process (Table 3). Garner and Hafez (2000) stated that the higher the sperm recovery rate, the better the quality of diluent used (). The sperm recovery rate after thawing in this study was above the standards stipulated in SNI 4869-1 in 2017 (50%). Bali cattle sperm recovery rate in this study (54.3-60.2%) was higher than the recovery rate of FH cattle (42.40-51%) (Zelpina *et al*., 2012) andlocal sheep (46.53%) (Solihati *et al*., 2018), but was lower than buffalo recovery rates of 43-63% (Rosadi *et al*., 2015). This difference was caused by the motility of fresh semen, use of diluent, and freezing process (Garner and Hafez, 2016).

The presence of egg yolk and glycerol in the diluent of this study strengthen the quality of the diluent. Low-density lipoprotein contained in egg yolk bound the plasma membrane of spermatozoa cells and formed a membrane between fatty acids and water (Bergeron *et al*., 2007), thus the plasma membrane stillfunctioned properly during preservation (Akhter *et al*., 2016). In addition, the presence of LDL also played a role in binding to PDC-109 protein in the plasma and prevented the occurrence of phospholipid efflux (Bergeron *et al*., 2007). Glycerol would prevent cell dehydration by replacing free water that came out of the cell and was also used for oxidative metabolic processes so that it could reduce intracellular electrolyte concentrations that caused damage to spermatozoa

Table 1. Composition of diluent in each treatment

Composition	Addition of sucrose in tris-based egg yolk diluent				
	P0	P1	P2	P3	P4
	0%	0.2%	0.3%	0.4%	0.5%
Tris (hydroximethyl) aminomethan (g)	3,028	3,028	3,028	3,028	3,028
Fructose (g)	1.25	1.25	1.25	1.25	1.25
Citric acid monohydrate (g)	1.7	1.7	1.7	1.7	1.7
Egg yolk (mL)	20	20	20	20	20
Penicillin (mL)	0.5	0.5	0.5	0.5	0.5
Streptomycin (mL)	0.4	0.4	0.4	0.4	0.4
Glycerol (%)	6	6	6	6	6
Sucrose (g)	-	0.318	0.477	0.636	0.795

P0= The treatments were tris-based egg yolk diluent without sucrose (control), P1= Tris-based egg yolk diluent with 0.2% sucrose, P1= Tris-based egg yolk diluent with 0.3% sucrose, P2= Tris-based egg yolk diluent with 0.4% sucrose, P3= Tris-based egg yolk diluent with 0.5% sucrose

Table 2. Fresh semen characteristic of Bali cattle

Fresh semen characteristics	Mean values
1. Volume (mL)	5
2. pH	6.5
3. Color	Cream
4. Consistency	Condensed
5. Mass Motion	3
6. Individuals Motion	3+
7. Motility (%)	80
8. Intact plasma membrane (%)	85
9. Concentration	1.883×10^9

Table 3. Mean value of motility percentage, livability, abnormality, intact plasma membrane, and recovery rate of Bali cattle semen in Tris-based Egg Yolk diluent with the addition of different sucrose concentration

Treatment	Motility (%)	Livability (%)	Abnormalities (%)	IPM (%)	RR (%)
Po Control	43.59±1.2^a	51.24±0.9^a	10.24±2.3	44.66±0.6^a	54.5^a
P1 (Sucrose 0.2%)	44.47±1.6^a	52.36± 0.9^a	11.03± 2.4	45.93± 0.5^a	55.6^a
P2 (Sucrose 0.3%)	45.62±0.7^b	53.11±0.9^b	13:49±2.7	46.98±0.4^b	57.0^b
P3 (Sucrose 0.4%)	46.94±0.5^c	54.31±0.8^c	12:19±2.8	47.22±1.4^c	58.7^c
P4 (Sucrose 0.5%)	48.15±1.3^d	55.35±1.6^d	11:49±1.6	48. 21±0.9^d	60.2^d

^{a,b,c,d} Different superscripts within the same column indicate significant difference (P<0.01)

(Hafez, 2016). The interaction of glycerol and phospholipids presented in the diluent prevented membrane dehydration by replacing bound water that was released from the cell membrane. The bond of proteins and glycoproteins membrane could also maintained membrane stability during the freezing process (Parks and Graham, 1992).

CONCLUSIONS

Addition of 0.2%-0.5% sucrose in tris-based egg yolk diluent could increase the value of Bali cattle spermatozoa quality during preservation with spermatozoa motility percentage of 43.59%-48.15%, livability of 51.24% - 55.35%, abnormality of 10.24% - 13.49%, intact plasma membrane of 44.66%-48.21%, and recovery rate of 54.5%-60.2%.

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REFERENCES

Akhter, S., B.A. Rakha, M.S. Ansari, A.U. Husna, S. Iqbal, and M. Khalid. 2016. Evaluation of quail and turkey egg yolk for cryopreservation of nili-ravi buffalo bull semen. **Theriogenology**. 87:259-265.

Aisen. E.G., H.L. Alvarez, and A. Venturino. 2002. Cryopreservation and post thawed fertility of ram semen frozen in different trehalose concentration. **Theriogenology.** 57: 1801-1808.

Arifiantini, R.I. and B. Purwantara. 2010. Motility and viability of friesian Holstein spermatozoa in three different extender stored at 5° C. **J. Indonesian Trop. Anim. Agric**. 35(4):222-226.

Bakas, L.S. and E.A. Disalvo. 1991. Effects of Ca^{2+} on the cryoprotective action of trehalose. **Cryobiology**. 28:347-353.

Barbonetti, A., M.R.C.Vassallo, D. Fortunato, S. Francavilla, M. Maccarrone, and F. Francavilla. 2010. Energetic metabolism and human sperm motility: Impact of CB1 receptor activation. **Endocrinology**. 151:5882-5892

Bergeron, A., Y. Brindle, P. Blondin, and P. Munjanath. 2007. Milk casein decrease the binding of the major bovine seminal plasma protein to sperm and prevent lipid loss from the sperm membrane during sperm storage. **Biol. Reprod**. 77:120-126.

Bearden, J.H., J.W. Fuquay, and S.T. Willard. 2004. **Applied Animal Reproduction.** 6th ed. Pearson Education Inc., New Jersey (US).

Bucak, M.N., A. Atesşahin, V. Ömer, A. Yüce, N. Tekin, and A. Akçay. 2007. The influence of trehalose, taurine, cysteamine and hyaluronan on ram semen Microscopic and oxidative stress parameters after freeze–thawing process. **Theriogenology**. 67:1060-1067.

Colenbrander, B., A.R. Fazeli, A. van Buiten, J. Parlevliet, and B.M. Gadella. 1992. Assesment of sperm cell membrane integrity in the horse. **Acta Vet. Scand**. 88:49-58.

Ditjennak. 2000. **Statistik Peternakan Indonesia**. Direktorat Jenderal Peternakan, Jakarta.

Eiman, A. and T. Takato. 2004. Effects of the supplementation of trehalose extender containing egg yolk with sodium dodecyl sulfate on the freezability of goat sperm. **Theriogenology**. 62. 809-818.

El-Sheshtawy, R.I. and W.S. El-Nattat. 2018. Effect of tris-extender supplemented with various concentrations of strawberry *(Fragaria* spp.) on bull semen preservability. **Asian Pac. J. Reprod**. 7(2):93-96.

Garner, D.L. and E.S.E. Hafez. 2000. Spermatozoa and Seminal Plasma. In **Reproduction in Farm Animals.** Hafez, B. and E.S.E. Hafez (Eds.). 7th ed. Lippincott Williams & Wilkins, Philadelphia.

Garner, D.L. and E.S.E. Hafez. 2016. Spermatozoa and Seminal Plasma. In **Reproduction in Farm Animal.** Hafez, B. and E.S.E. Hafez (Eds.). 7th ed. Lippincott & Williams.Baltimore, Marryland, USA.

Hafez, E.S. 2016. **Reproduction In Farm Animals.** Lea and Febiger, Philadelpia.

Hammerstedt, R. H. 1993. Maintenance of bioenergetic balance in sperm and prevention of lipid peroxidation: A review of the effect on design of storage preservation systems. **Reprod. Fertil. Dev**. 5:657-690.

Herdis, I.W.A. Darmawan, and M. Rizal. 2016. Penambahan beberapa jenis gula dapat meningkatkan kualitas spermatozoa beku asal epididimis ternak Domba. **J. Ked. Hewan.** 10(2):200-204.

Holt, W.V. 2000. Basic aspects of frozen storage of semen. **Anim. Reprod. Sci**. 18(62):3-22.

Jian, H.H., L.S. Zan, X.L. Zhao, Q.W. Li, Z.L. Jiang, Y.K. Li, and X. Li. 2010. Effects of trehalose supplementation on semen quality and oxidative stress variables in frozen thawed bovine semen. **J. Anim. Sci**. 88:1657-1662.

Komariah, I. Arifiantini, and W. Nugraha. 2013. Kaji banding kualitas spermatozoa sapi Simmental, Limousin, dan Friesian Holstein terhadap proses pembekuan. **Buletin Peternakan**. 37(3):143- 147.

Lehninger, A.L. 1994. **Dasar-dasar Biokimia.** (Diterjemahkan Thenawijaya, M.). Jilid 1. Erlangga, Jakarta.

Maxwell, W.M.C. and S.Salamon. 2000. Storage of ram semen. **J. Anim. Reprod. Sci**.62:77-111.

Manjunath, P. 2012 New insight into the understanding of the mechanism of sperm protection by extender components. **Anim. Reprod**. 9:809-815.

Mansjur. 2001. **Metabolisme: Karbohidrat, Protein, Asam Nukleat**. Fakultas MIPA Institut Pertaian Bogor. Bogor.

Meryman, H.T. 2007. Cryopreservation of living cells: Principles and practice. **Transfusion**. 47:935-945.

Murray, R.K., D.K. Gardner, P.A. Mayer, and V.W. Rodwell. 1999. **Biokimia Harper**. (Diterjemahkan Hartono, A.). Ed-24. Penerbit Buku Kedokteran EGC, Jakarta.

Nainga, S.W., H. Wahida, K. M. Azamc, Y. Rosninaa, A.B. Zukib, S. Kazhala, M.M. Bukara, M. Theind, and M.M.S. Kyawe. 2010. Effect of sugars on characteristics of Boer goat semen after Cryopreservation. **Anim. Reprod. Sci**.122:23-28.

Parks, J.E. and J.K. Graham. 1992. Effects of cryopreservation procedures on sperm membranes. **Theriogenology**. 38:209-222.

Rizal, M., M. Riyadhi, B. Irawan, A. Wahdi, Habibah, and Herdis. 2017. Daya hidup spermatozoa epididimis sapi persilangan yang dipreservasi dengan air kelapa muda pada suhu 5° C. **J. Veteriner**. 18 (4):571-579.

Rizal, M. and M. Riyadhi. 2016. Fertilitas semen kerbau rawa (*Bubalus bubalis carabanensis*) yang diencerkan dengan pengencer nira aren. **J. Veteriner**. 17(3):457-467.

Rosadi, B., T. Sumarsono, and Darmawan. 2015. Motilitas spermatozoa kerbau lumpur pada penyimpanan suhu beku dalam es. **Jurnal-Jurnal Ilmu Peternakan XVIII**. 2:98-101.

Shukla, M.K. 2011. **Applied Veterinary Andrology and Frozen Semen Technology**. Pitam Pura. New Delhi India.

Solihati, N., S.D. Rasad, R. Setiawan, and S. Nurjanah. 2018. Pengaruh kadar gliserol terhadap kualitas semen domba lokal. **J. Biodjati.** 3(1):63-71.

Susilawati, T., D. Ratnawati, N. Isnaini, Kuswati, and A.P. Yekti, 2018. Character of liquid semen motility in various diluents on balinese cattle during cold storage. Asian J. Microbiol. **Biotech. Env. Sc**. 20(1):166-172

SNI (Standar Nasional Indonesia). (2017). **Semen Beku - Bagian 1: Sapi (SNI 4869-1:2017).** BSN (Badan Standarisasi Nasional). Jakarta.

Storey, B.T., E.E. Noiles, and K.A. Thompson. 1998. Comparison of glycerol, other polyols, trehalose and raffinose to provide a defined cryoprotectant medium for mouse sperm cryopreservation. **Cryobiology**. 37:46-58.

Toelihere, M.R. 1993. **Inseminasi Buatan Pada Ternak**. Angkasa, Bandung.

Uchida, T., M. Nagayama, T. Shibayama, and K. Gohara. 2007. Morphological investigations of disaccharide molecules for growth inhibition of ice crystals. **J. Cryst. Growth**. 299 (1):125-135.

Woelders, H., A. Matthij, and B. Engel. 1997. Effect of trehalose and sucrose, osmolality of the freezing medium, and cooling rate on viability and intactness of bull sperm after freezing and thawing. **Cryobiology**. 35:93-105.

Yendraliza, P. Anwar, and Rodiallah. 2015. **Bioteknologi Reproduksi**. Aswaja Pressindo, Yogyakarta

Yildiz, C., A. Kaya, M. Aksoy, and T. Tekeli. 2000. Influence of sugar supplementation of the extender on motility, viability and acrosomal integrity of dog spermatozoa during freezing. **Theriogenology**. 54(4):579-585.

Yulnawati and Herdis. 2009. Kualitas semen cair domba garut pada penambahan sukrosa dalam pengencer tris kuning telur. **JITV.** 14(1):45-49.

Yulnawati, Herdis, H. Maheshwari, and M. Rizal. 2008. Kualitas spermatozoa epididymis kerbau belang pada penambahan raffinosa sebagai krioprotektan ekstraseluler. **Jurnal Ilmu Ternak dan Veteriner**. 13:30-34.

Yulnawati, M. Gunawan, Herdis, H. Maheshwari, and M. Rizal. 2009. Peranan gula sebagai krioprotektan ekstraseluler dalam mempertahankan kualitas semen beku kerbau lumpur. **Prosiding Seminar Nasional Potensi dan Pengembangan Peternakan Maluku dalam Mendukung Ketahanan Pangan Nasional**. Ambon:236-250.

Zelpina, E., B. Rosadi, and T. Sumarsono. 2012. Kualitas Spermatozoa post thawing dari semen beku sapi perah. **Jurnal Ilmu-Ilmu Peternakan**. 25(2):94-102.

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