Addition of Trehalose of Duck Egg Yolk-Tris as an Extender Medium on Buffalo Frozen Semen

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ABSTRACT
This study aimed to determine the effect of adding trehalose of duck egg yolk-tris extender medium on the quality of frozen semen at buffalo. The design used was experimental a Randomized Block Design (RBD) method with 4 treatments and 4 replications as a group. The addition of trehalose (T) with several concentrations in the extender of tris duck-egg yolk as the first treatment, namely the control consisted of egg yolk tris 80% + 20% + 0% trehalose (T0), the second treatment 0.5% (T1), the third treatment % (T2) and the fourth treatment 1.5% (T3). The observed variables included: motility, viability, abnormality, and membrane integrity. The results of this study showed that the addition of trehalose of duck egg yolk-tris had a non-significantly different effect (P>0.05) on the percentage of spermatozoa motility with the average successively at T0, T1, T2 and T3, namely 25.00±5.80. %, 27.50±5.00%, 30.00±8.17%, 32.50±5.00%, the effect was very significant (P<0.01) on the percentage of spermatozoa viability and membrane integrity, with the mean successively at treatment T0 that is 42.25±2.60%, 43.25±2.90%, T1 44.00±2.68%, 46.00±2.40, T2 46 ,63±1.11%, 48.25±1.26%, T3 48.25±1.50%, 49.50±2.39%, significantly different effects (P<0.05) on spermatozoa abnormality with the mean successively at T0, T1, T2 and T3 that is 12.38±1.66%, 18.00±3.24%, 19.63±9.07%, 25.50±9.06% . The results of the study concluded that the addition of trehalose to the tris of duck egg yolks could maintain the motility, viability, membrane integrity of spermatozoa, but the addition of trehalose was unable to maintain the abnormality of Buffalo spermatozoa.

Keywords: *Buffalo, egg yolk-tris, frozen semen, trehalose.*

1. INTRODUCTION

A livestock sector has an important role in the life and development of Indonesian human resources. The increase in people's welfare will be followed by an increase in consumption of livestock products which will also move the economy in the livestock sub-sector. However, the reality shows that the consumption of animal products in Indonesia is relatively low. Buffalo is one of the animals that has great benefits for human life. Buffaloes are livestock that produce red meat and milk [1].

The implementation of reproductive technology, especially artificial insemination (AI) on buffalo in Indonesia still has many obstacles, which are indicated by the high rate of service per conception (S/C) and repeated breeding. This will have an impact on the development of the buffalo population and the income of farmers. Low semen quality was one of the causes of AI failure. Related to this problem, various strategies to improve the quality of semen have been carried out, especially through improving the freezing process or cryopreservation, so that frozen semen can be used for a long time [2, 3].

Besides, the quality of the semen produced can affect the success of AI, right extender methods and storage of semen are also known to be able to influence the success of AI. The addition of an extender medium has an important role to support the improvement of semen quality. Currently, there are many kinds of extension media, both simple and commercial, such as skim egg yolk, citrate or tris egg yolk and andromed. Each of these extender media has a different performance. The limitation of using the extender medium often leads to low cement motility due to the effect of cold shock on frozen semen and intracellular changes due to water loss associated with the formation of ice crystals, so that it cannot maintain the quality of frozen semen for a longer period of time [4].

Trehalose is one of the ingredients that can act as an antioxidant. The addition of trehalose into the diluent medium will increase cell resistance to free radicals (H2O2) which is a by-product of mitochondria in producing ATP. The addition of Trehalose with a concentration of 1.5% in the diluent was able to improve the quality of the semen of Pampita sheep [5, 6], it was also reported that the addition of trehalose in the diluent with a concentration of 1% could improve the frozen semen of cattle with concentrations of 1.5%, 2.5%, and 3.5% and obtained optimal results at concentrations of 1.5% [7], and concentrations of 0.2% to 0.4% is able to improve the quality of frozen semen of arrowroot sheep. This study aimed to determine the effect of adding trehalose in tris duck-egg yolk extender on the quality of frozen semen at buffalo.

2. MATERIALS AND METHODS

2.1 Semen Collection.

This study was conducted from 3 ejaculations of 2 males. Semen collection used an artificial vagina.

2.2 Evaluation of fresh semen.

Fresh semen was evaluated macroscopically and microscopically to determine its quality for further processing. Macroscopic evaluation included; volume, color, odor, pH, color and consistency, while microscopic included concentration, motility, survival, abnormalities and integrity of spermatozoa membranes [8, 9].

2.3 Glycerolization.

As much as 6% was spent on extender, then 6% glycerol blank was added to the extender and mixed with semen.

2.4 Filling and sealing.

It is the process of filling semen that has been diluted and clamped using an automatic filling and sealing machine. Before being put into liquid N_2, semen was placed on the surface of liquid N_2 at a temperature of ±-110 °C for 9 minutes [10, 11] after semen can be frozen by placing cement in liquid N_2 and stored in a container [12, 13].

2.5 Evaluation of semen post thawing.

Motility, viablity, abnormality and sperma tozoa membrane integrity were assessed daily post thawing [14, 15].

3. RESULTS AND DISCUSSION

3.1 Evaluation of Buffalo Fresh Semen

The results of this research on the quality of fresh semen in buffalo cattle is shown in Table 1.

The results of the evaluation of the volume of fresh semen above show that the first ejaculation is 5.5 ml, the second ejaculation is 3.5 ml, the third ejaculation is 1.5 ml, and the fifth ejaculation is 3 ml. The average volume of fresh buffalo semen was 3.38±1.65 ml with a range of 1.5 ml to 5.5 ml. The smell of buffalo semen in the results of this study was a normal fishy odor, this condition indicates that the semen is in normal condition [16, 17]. The color of fresh buffalo semen obtained in this study is cream. The results show that the semen is in normal condition and meets the eligibility requirements for further processing into frozen semen. The consistency of fresh buffalo semen in the first ejaculation is thick, while the second, third and fifth ejaculations are moderate [5].

Meanwhile, the pH (Degree of Acidity) of semen is measured with litmus paper. Evaluation of fresh buffalo semen shows that the pH in the first ejaculation shows a pH of 6, while the 2^{nd}, 3^{rd}, and 4^{th} ejaculation is pH 7. Spermatozoa motility obtains a result of 70%, while the 2^{nd} ejaculation, 3^{rd}, and 4^{th} obtain 60%. The concentration of fresh buffalo semen in this study shows that the result in the 1^{st} ejaculation is 1400 × 106, the 2^{nd} ejaculation is 1000 × 106 and the 3^{rd} ejaculation and 4^{th} ejaculation is 1200 × 106. Mass Movement of Spermatozoa in the 1^{st} ejaculation gets (+++), while 2nd, 3rd and 4th ejaculation get (++) [18, 14].

3.2 Evaluation of Frozen Buffalo Semen Post

Thawing the use of an extender medium with the addition of Trehalose as an antioxidant is able to

Table 1. Characteristics of fresh Buffalo semen

Characteristic	Treatment				Average ± SD
	1	2	3	4	
Volume (ml)	5.5	3.5	1.5	2	3.13±1.80
Odor	Specific	Specific	Specific	Specific	Specific
Color	Cream	Cream	Cream	Cream	Cream
Consistency	Thick	Mild	Mild	Mild	Mild to thick
pH	6	7	7	7	6.8±0.5
Motility (%)	70	60	60	60	63±5
Mass movement	+++	++	++	++	++ - +++
Concentration (million/ml)	1,400	1,000	1,200	1,200	1,200±163.30

Note: The quality of buffalo semen is evaluated macroscopically and microscopically

Table 2. Average Motility, Viability, Abnormality and Membrane Integrity of Buffalo spermatozoa post thawing

Variable	Trehalose Treatment			
	T0	T1	T2	T3
Spermatozoa Mortality (%)	25.00±5.80^{ns}	27.50±5.00^{ns}	30.00±8.17^{ns}	32.50±5.00^{ns}
Spermatozoa Viability (%)	42.25±2.60^{Daa}	44.00±2.68^{CABa}	46.63±1.11^{BBb}	48.25±1.50^{Aba}
Spermatozoa Abnormality (%)	12.38±1.66^{Daa}	18.00±3.24^{CABa}	19.63±9.07^{BBb}	25.50±9.06^{Abb}
Spermatozoa Membrane Integrity (%)	43.25±2.90^{DA}	46.00±2.40^{CA}	48.25±1.26^{BB}	49.50±2.39^{Ac}

Note: Superscripts in different capital letters showed a very significant difference (P<0.01)
Superscripts in different lowercase letters showed significantly different effects (P<0.05) Superscript on ns showed no different effect (P>0.05)

improve the quality of frozen semen in buffalo. Trehalose is a simple sugar disaccharide consisting of two glucose molecules linked to each other by a 1–1 -α glycosidic bond. This sugar is included in non-reducing sugar, has a sweetness level of about 45%. Trehalose inserts itself into the phospholipid bilayer membrane, so that the modulation of cell membrane fluidity is more stable during coagulation [10, 2]. Data on the percentage of motility, viability, abnormality and membranes integrity post-thawing of buffalo spermatozoa in tris duck egg yolk extender with the addition of trehalose used differently can be seen in Table 2.

3.3 Percentage of Motility Spermatozoa Post-Thawing

Based on the results of the average percentage of motility in buffalo in each ejaculation with different concentrations of trehalose, it can be seen that the average motility of frozen semen is the highest 20.00±0.00% at the control concentration and the lowest ie 15.00±5.77% . This is in accordance with the opinion of Arifiantini *et al*. [19] who stated that the motility of spermatozoa post thawing is at least 40%, if less than 40%, the frozen semen is not suitable for insemination [3]. The difference in the percentage of motility of buffalo spermatozoa after freezing is caused by the level of trehalose concentration used as a treatment. The higher trehalose concentration used, the better in maintaining the motility of spermatozoa. This indicates that the addition of trehalose has a good effect on the quality of post-thawing semen when compared to the control. These results are supported by research [20] using a trehalose concentration of 0.2% and 0.4% trehalose concentration which obtains a higher quality than control.

3.4 Percentage of Viability Spermatozoa Post Thawing

The results showed that the average percentage of spermatozoa in buffalo semen after freezing was highest in the trehalose treatment group with a concentration of 1.5% (T3), which was 48.25±1.50% and the lowest was in the treatment without trehalose (T0), which was 42.25±2.59%. The results of the analysis showed that the trehalose treatment of various concentrations on the percentage of live spermatozoa after freezing showed a

very significant difference (P<0.01). This study showed that the increasing number of deaths was due to trehalose not optimally functioning as an antioxidant and as a cryoprotectant in the freezing process of frozen buffalo semen.

Trehalose is known as an external membrane stabilizer which is able to maximally stabilize the lipid bilayer structure of the spermatozoa membrane during the cooling and freezing process, but the degree of protection is affected by its concentration in the extender [5].

3.5 Percentage of Abnormality Spermatozoa Post-Thawing

The results show that the highest mean abnormality is found in the T3 treatment, namely 25.50±9.06% and the lowest at T0 is 12.38±1.66%. The results of this study are still within normal limits of spermatozoa abnormalities for AI, the percentage of spermatozoa abnormalities that is good for AI is spermatozoa abnormalities of 5-20%. The plasma membrane only causes death of spermatozoa but most of the dead spermatozoa still have a normal shape [16].

3.6 Percentage of Membrane integrity Post Thawing

The results of the MPU in this study show that the average percentage of MPU of buffalo spermatozoa after freezing with tris duck egg yolk diluent with trehalose treatment after thawing is the highest at T3 namely 49.50±2.39% and the lowest is in control namely 43.25±2.90%. Statistical analysis of intact plasma membranes of post-thawing spermatozoa shows that there is no significant difference (P≥0.05). The addition of trehalose as a concentration in the diluent of tris duck egg yolk is able to maintain the quality of semen after freezing at different concentrations of trehalose [19, 11]. Meanwhile, the addition of 100 mM trehalose in the diluent results in plasma membrane integrity in cattle and buffalo.

4. CONCLUSION

Based on the results of the study, it can be concluded that the addition of a dose of trehalose in the tris diluent of duck egg yolks has a significant effect (P<0.05) on spermatozoa motility, spermatozoa survival percentage, spermatozoa abnormalities and intact plasma membranes of buffalo spermatozoa after thawing.

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