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Hasanuddin J. Anim. Sci.
Vol. 3, No. 1:8 -14 May 2021
pISSN 2621-9182 eISSN 2621-9190
DOI:10.20956/hajas.v3i1.14171

Motility, Viability, and Abnormality of the Frozen Bali Bull Semen with Andromed and Egg Yolk-Tris Extender

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Abstract

This study aimed to compare the quality of frozen semen (motility, viability and abnormality of the sperms) of Bali bull using Andromed and tris egg yolk (TEY) extenders. The semen of one Bali bull was collected using an artificial vagina. The semen was collected five times, in which, each semen collection was divided into two different extender treatments. Treatment 1 (T1) was Andromed+aquadest and T2 was TEY+glycerol. The parameters measured in the study were motility, viability, and abnormalities of the Bali bull post-thawing sperms. Each parameter in each treatment was compared using the T-test (t-test independent sample). This studyindicated that the quality of post-thawing semen in T1 was much better incomparison toT2;the motility and viability ofpost-thawing semen using Andromed were still above 40% (50.80±6.14% and 54.20±5.03%). On the other hand, the motility and viability of post-thawing semenin TEYwas25.80±4.43% and 33.30±2.64%, respectively. In conclusion, the used of Andromed as an extender could maintain the motility and viability of the Bali bull sperms after thawing in comparison to TEY. However there was no different in sperms abnormality within the two extenders (13.20±2.73% vs 14.30±3.51).

Keywords : Abnormality, andromed, motility, viability percentage, yolk tris

INTRODUCTION

Semen cryopreservation is a technique for storing genetic material in a frozen state that aims to make optimal use of males, especially in Bali cattle's development. The productionof frozen semen must be based on quality requirements by the National Standardization Agency (BSN) by the Indonesian National Standard (SNI) number 4869: 2008; the assessment of morality and individual movement (Sukmawati *et. al*., 2014). The spermatozoa fertility rate is determined through acrosome status testing, where initial identification is obtained by observing

the type of abnormality obtained. The success rate of artificial insemination (AI) in Bali Bull was related to the quality of sperms. The quality of sperms is influenced by external factors (feeding, environment, and processing of semen) and internal factors (age, housing, and genetic). In general, the semen that are usually used for insemination can be in the form of fresh or frozen. On an intensive farm, frozen semen was more widely used because it is more long-lasting period. A series of freezing processes to thawing can cause several things such as cold shock, osmotic stress, and the formation of ice crystals (Holt, 2000).As an extender, several criterion must be meet for the requirement that are needed to maintain the quality of semen during processing and storage. Several semen extenders are available for commercial used, however the problem faced to get those extenders sometimes is difficult and expensive as well as should be long time waiting for transportation. Therefore, it is a good idea to develop an alternative extender to avoid a dependence from difficult and extensive commercial extender. In order to know the efficacy of an alternative extender, it must be compared to the commercial extender one. Therefore, this study aimed to compare two different types of extenders; Andromed+aquabidest and Tris Egg Yolk+glycerol in maintaining the quality of post-thawing Bali bull sperms.

MATERIALS AND METHODS

Materials

The materials used in this study were fresh Bali bull semen that obtained from Artificial Insemination (AI) Station of Livestock and Animal Health Services, South Sulawesi Province, Indonesia. The other supporting materials included eosin 20%, alcohol 70%, egg yolk, aquabidest, tissue, 0.9% NaCl solution, straw, Andromed, glycerol, tris (hydroxymethyl) amino methane, glucose crystals and citric acid monohydrate.

Methods

This study was used diluted Bali bull ejaculated semen by comparing the effectiveness of two types of treatment extenders, namely Andromed+Aquabidest (T1) and Tris egg yolk (TEY)+Glycerol (T2). The ejaculated semen was collected five times in the morning with an interval of three days. The semen was taken immediately to the laboratory for macroscopically and microscopically evaluation, where the results were obtained as information for further treatment.

Semen Dilution Process

The ejaculated semen was separated into two different tubes containing different extenders as treatments. The tube in T1was added with Andromed+Aquabidest, while in tube T2was added with tris egg yolk+glycerol+Aqubidest. The amount of diluted semen volume was calculated to achieve the amount of dilution, including concentration and progressive motility of the sperms. After knowing the number of doses, then multiplied by the volume of insemination and reduced by the volume of fresh cow semen, to obtain the amount of extender to be added (Nilna, 2001).

T2 Extender Media

Tris aminomethane crystals was weighed for 3.63g; 0.5g of glucose crystals, with 1.99 g of citric acid monohydrate. All the components were put into a 100 mL volumetric flask, then add aquabidest until the solution reaches 100 mL. The solution was homogenize for 15 minutes, then kept in a water bath at 37 °C.

In a different volumetric flask, a 20 mL of egg yolk were prepared then added with 80 mL of solution made previously and homogeneous them with yolk for 30 minutes. Furthermore, 100.000 IU of penicillin and 100 mg of streptomycin and 6% glycerol were added when the solution was ready for use.

Processing of Semen

The diluted semen with an certain extender was packed into a 0.25 mL mini straw and equilibrated at 5°C for four hours in the refrigerator. After that, the straw containing the semen was removed from the refrigerator into the box filled with liquid nitrogen for a period of 10-15 minutes. Furthermore, the thawing process was carried out by taking frozen straws and then immersing them in warm water at 37 °C for 30 seconds. The frozen semen (straw) was confirmed to be dry using a microscopic quality evaluation as a form of post-thawing spermatozoa quality status. This process was applied to the treatments T1 and T2.

Parameters and data analysis

The parameters measured in this study were the macroscopic and microscopic determination of the quality of fresh semen. Quality of post-thawing spermatozoa examined microscopically for what was included in the macroscopic test, namely, seeing the volume, viscosity, pH, microscopic observations included motility with a standard value of 90% (moving very active or fast); 75-80% (move active or rather fast); 40-65% (active movement, seen thin and rare waves and slow mass movement). Secondly, the percentage of life (viability) is calculated based on the ratio between the numbers of living cells divided by the total cells counted. The last abnormality is a deviation in spermatozoa's morphology, which can reduce the fertility of spermatozoa.The data obtained were analyzed using a comparative T-test (t-test independent sample) (Sudjana, 1996).

RESULTS AND DISCUSSION

Macroscopic Quality of Bali Cattle Fresh Semen T1 and T2

The quality of Bali bull semen that are indicated by motility, viability, and abnormality of the sperms at two different extender treatments used in the present study are shown in Table 1.

Table 1. Motility, viability, and abnormality of Bali bull sperms at two different extender treatments

Parameter (%)	Treatment (T)	
	T1	**T2**
Motility	50.80±6.14[a]	25.80±4.43[b]
Viability	54.20±5.03[a]	33.30±2.64[b]
Abnormality	13.20±2.73	14.30±3.51

Note: Different superscript in the same column indicates significant different (P<0.01) T1=Andromed+ aquadest; T2=TEY+glycerol

Motility of Bali Bull Sperms at Different Extenders

In general, evaluation of post-thawing Bali bull semen that were diluted using two different extenders (T1 and T2), the quality of sperms differed significantly (P<0.01). Table 1 shows that the motility, viability and abnormality of post-thawing semenT1 was 50.80±6.14%, significantly (P<0.01) lower than in T2 (25.80±4.43%). This results are lower than the post-thawing motility of the previous study using tris egg yolk of 58.38 ± 4.63 (Viquez *et. al*., 2020).

The main target expected from egg yolk is the lecithin contain. Egg yolk lecithin protects spermatozoa cells' plasma membrane from damage during the processing, cooling, freezing, and thawing of frozen sperms. Andromed does not use egg yolk because it is feared that egg yolk will become a medium for spreading various kinds of infectious bacteria (Minitub, 2001). As a substitute for the yolk, soybean extract is used as a mixture of andromed diluents.

In the frozen semen process, cryopreservation is a method that causes the physiological conditions of spermatozoa cells to adapt to changes in temperature and osmotic pressure that occur during the dilution to thawing process (Andrabi, 2009). Usually, this process attacks cell organelles, especially in the nucleus, and impacts the viability and different metabolic factors such as ATP concentration in spermatozoa cells (Blesbois, 2007).

The high viscosity nature of egg yolk causes the cell diffusion process's limitations (Morris *et al*., 1988). Tris-based extenders released the least amount of lactate dehydrogenase and sorbitol dehydrogenase in spermatozoa during cryopreservation. Furthermore, the use of tris in this study is useful for maintaining the acrosome hood's condition so that there is no early reaction (Singh *et. al*., 1991). The drastic decrease in motility in egg yolk Tris diluent is suspected of physical changes during adding diluent because spermatozoa's friction with fat globally causes spermatozoa death.

Viability of Bali bull sperms at different extenders

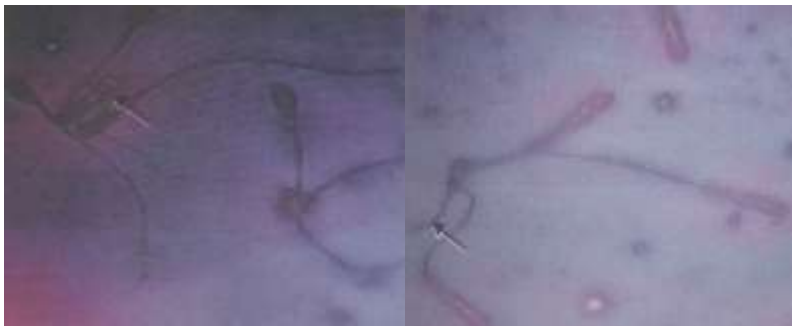
Tabel 1 shows that the percentage of live post-thawing Bali bull sperms in T1 is 54.20±5.03%. It is significantly (P<0.01) higher than inT2 (33.30±2.64%). As with other cell plasma membranes, the spermatozoa plasma membrane is composed of proteins and phospholipids. In general, cells will use certain mechanisms to prevent uncontrolled membrane damage (Markie *et. al*., 2001). The cell membrane plays an important role in maintaining cell organelles physically and regulating the traffic in and out of cells, all substrates and electrolytes needed in cell biochemical processes. In this study, dead and living spermatozoa could be distinguished using eosin dye. Stained cells were defined as dead cells, contrary were accounted as living cells.

Abnormality of Bali bull sperms at different extenders

Number and types of abnormalities of Bali bull sperms at different extenders are shown in Table 2.

(a) (b)

Figure 1. Abnormalities of spermatozoa in the head: a) microcephalus; b) the head is cut off



(a) (b)

Figure 2. Abnormalities of caudal spermatozoa: a) Tail arch; b) short tail.

Table 2. Abnormalitiy of post-thawing spermatozoa

Type of sperm abnormality	Treatment	
	T1	T2
Primary (%)	10.60±1.61	11.00±2.20
Secondary (%)	2.60±0.96	3.30±1.82
Total (%)	13.20±2.73	14.30±3.51

The results of the study found that after treating using two types extenders, the abnormalities of the Bali bull sperms can be divided into two categories; primary and secondary abnormalities, with the respective values between T1 and T2 being 13.2±2.73% and 14.3±3.51% ($P>0.05$) (Table 2). In general, the type of primary abnormality is categorized as occurring in the head of the spermatozoa (Menon *et. al*., 2011), while the secondary abnormality is the condition of the severed membrane in the head and tail relationship. The two diluents used did not give significantly different results but can maintain conditions with an abnormality level below 20%. Therefore it is still classified as suitable for use (Susilawati, 2011). Abnormalities of the tail's spermatozoa are related to immotility, where this condition shows that they cannot move progressively so that the initiation of natural fertilization cannot occur (Nikolettos *et. al.*, 1999;

Kawakami *et. al*., 2005). This study shows tail abnormalities such as a curved tail (Figure 2a) and a short tail (Figure 2b).

Referring (Kavak *et. al*., 2004; Patricia, 2013), the types of head abnormalities were found, namely microcephalus (Figure 1a) and severed head (Figure 1b). Abnormalities in the head are related to spermatozoa's inability to carry out acrosome reactions and penetrate the zona pellucida (Nikolettos *et. al.*, 1999). The microcephalus case showed the size of the spermatozoa head smaller than the normal size (variable size). Variable size abnormality is indicated to have abnormalities in the acrosomal hood and core chromatin, causing spermatozoa to lose their ability to fertilize egg cells (Sailer *et. al*., 1996).

CONCLUSION

As conclusion, there was a significant different ($P<0.01$) the quality of Bali bull post-thawing semen at different extender used. The used of Andromed as an extender could maintain the motility and viability of the Bali bull sperms after thawing (50.80±6.14% vs 25.80±4.43% and 54.20±5.03% vs 33.30±2.64%) in comparison to TEY. However there was no different in sperms abnormality within the two extenders (13.20±2.73% vs 14.30±3.51).

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