



The Role of Diluents in Maintaining Quality of Bull Sperm During Freezing

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Abstract

This study aimed to determine the effect of diluent in maintaining the quality of bull sperm during freezing and physiological processes of bull sperm that occur during freezing. This research was conducted by observing samples of frozen bull sperm that uses diluents, as well as through the assessment of data research with assessment parameters such as macroscopic and microscopic assessment of cattle semen. Data were analyzed using descriptive statistical analysis. The results of this study indicate that the ratings fresh semen is done in two ways, namely macroscopically and microscopically. Grossly obtained volume of average ejaculate of 6 ml with a beige color and acidity levels on average 5.8. The concentration used is 'medium' which is a category of consistency and decent for further processing until the stage of freezing to rethawing. Microscopic assessment that includes an average frequency of motility in the fourth shelter is 70% with 3+ mass motility, percentage of live of fresh semen was obtained in this study was 80%. This value is the percentage of normal life. Based on these results it was concluded that the addition of diluents can reduce the rate of loss of quality (motility and percentage of survival) of bull sperm during the stages of preservation. Andromed diluent best in reducing the rate of decline in the quality of bull sperm during the stages of preservation.

Keywords: Diluent of sperm, freezing of bull sperm, processing of sperm

A. Introduction

In Indonesia, the beef cattle business requires special attention in connection with efforts to maintain and support the increase in population, where appropriate technology, especially in the field of reproduction can be implemented easily and efficiently. Optimizing the use of artificial insemination (IB) of whom are to pursue any bulls to be able to produce a calf every year with gender as desired, i.e. male or female. Male animals of choice given the ability of farmers to maintain the growth and development of body cells faster than the female so it is very appropriate if male animals farmed for the purpose of fattening (fattening); while female animals reared for its ability to produce children, and milk (Pratiwi, et al., 2006).

In the frozen storage, despite the fertility of bull semen frozen until thawed normally be used to IB, but the technique of frozen storage at that time resulted in loss of life percentage is still 40-50% for sperm during the freezing process until melting back (Prathalingam et al., 2006). During the production of bull sperm frozen, sperm cells can undergo a number of risks that could potentially degrade the quality of sperm. Sperm freezing techniques, for example the use

of diluents, incubation, observation for color DNA and freezing can affect the durability of the viability and ability of sperm fertility. Nonetheless, Johnson, et al., (2000) reported that the danger of freezing sperm induced by freezing to thawing back can be minimized by optimizing the cooling rates and freezing material.

One of the ingredients have been used are chicken egg yolk which has become a common component of a diluent for freezing sperm from different types of livestock over 60 years ago. It has been shown that egg yolks can help in the resistance to the cold stress and improving the ability of sperm fertility (Lamia, et al., 2004). In addition, protective action yolk is widely identified with the content of low-density lipoprotein (LDL) (Hu et al., 2009; Moussa et al., 2002).

The diluent containing LDL (tris yolk) may improve / enhance sperm motility, acrosome reaction and plasma membrane integrity, DNA integrity Boer after freezing to thawing back and can be used as a freeze protection medium (Hu, et al., 2009). Bergeron et al. (2004) suggest that the possible components LDF (low-densityfraction) -lipoprotein of diluent yolk protein contaminated with seminal plasma (BSP proteins) and this may represent a common mechanism of protection of sperm with egg yolk. Given this research are expected to evaluate the effect of diluent on motility, percentage of sperm bulls during freezing until thawed. Addition of diluent in sperm processed until a freeze is expected to provide better results. With the addition of the diluent in the process of pickling liquid semen can maintain the quality of sperm during freezing to thawing back. As a source of scientific information that the effort to address the decline in the quality of sperm bulls during the freezing process or preservation of sperm.

B. Methodology

Research materials

The material in this study is a microscope, glass object and glass deck, semen / sperm Cattle were accommodated by using an artificial vagina (VB), frozen sperm, egg yolk diluent, and diluent andromed.

Type and Data Resource

To obtain the required data in this study required primary data and secondary data, i.e.:

1. Primary data is data obtained directly from observations until freezing fresh sperm were used yolk and Andromed diluents.
2. Secondary data is data obtained by reviewing the literature data (books and journals)

Research design

a. Making solution of Diluent

Diluent solution used in this study consisted of 2.42 g Tris, 1.48 gr of citric acid, 1.00 grams of fructose, 6.6 ml glycerol, 25 mg of gentamicin, 50,000 IU penicillin to 100 ml of water non-pyrogenicsteril. One by one the ingredients put into distilled water while stirring with a magnetic stirrer for 20 minutes. After the diluent provided, then stored at a temperature of 5 ° C

b. Semen reservoirs

Semen from ejaculation results obtained from cattle using artificial vagina. The semen used in this study for preservation process assessment volume, sperm concentration and percentage of motile sperm and have requirements with motility > 70% and normal sperm morphology > 85%. Then semen ejaculation results are used equally divided between different diluent solutions.

c. Semen processing

(1) Dilution of semen

After the evaluation of the quality of fresh semen completed, the semen is divided into two same fractions, a fraction diluted with diluent solution to the yolk, and the other with the diluent solution treated with Tris and andromed, which is diluted in the ratio 1 semen: 4 diluent and then homogenized. Providing two pieces of test tubes, wherein each tube contains each medium Tris and Andromed. Semen diluted, sucked up as much as 1 ml is then inserted into the tube containing the diluent medium and left for 20 minutes at a temperature of 28°C.

(2) Processing to Packaging in straw

After the sperm of dilution available, further sperm cooled from 37°C into 4°C for 1.5 hours. Then fill Straw Polyvinyl chloride (PVC) (0.25 ml) (Biovet, France) using a filling sealing machine and the temperature is maintained at 4°C for 2.5 hours.

(3) The freezing of sperm

Straw containing sperm has been stored at a temperature of 4°C subsequently placed 3 cm above the surface of liquid nitrogen, which is approximately -120°C temperature for 15 minutes. After 15 minutes, the straw directly immersed in liquid nitrogen (-196°C) for storage in containers.

(4) Reimbursement of sperm

Bull thawed sperm by placing a straw in a water bath at 37 ° C for 45 seconds for further quality assessment.

(5) Ratings for Motility

Semen dropped on object glass and covered with a glass deck is then observed under a microscope with a magnification of 40 x 10. Penilaian mass movement set with a score of 0; 1; 1+; 2; 2+; 3; 3+ (Wahjuningsih, et al., 1998). This assessment is carried out as many as four stages, namely the fresh semen, sperm after the addition of diluents, sperm after the addition of diluents pre filling sealing and sperm after melting back.

(6) Calculation of Percentage of Life

Calculation of percentage of survival using a pillowcase preparations were observed under a microscope with a magnification of 40 x 10. Preparations pillowcase made by dripping a drop of semen on a glass object plus a drop of dye eosin and homogenized. After it reviews the use of glass deck is then dried and evaluated. Red sperm counted dead and sperm which do not absorb colors or slight color absorbing countless lives. The assessment was performed as many as four stages, namely the fresh semen, sperm after the addition of a diluent, sperm after the addition of diluents pre filling sealing and sperm after rethaw.

Assessment parameters

Parameter assessment in this study is:

1. Ratings for macroscopic. This assessment includes semen volume, pH, color and consistency. This assessment is performed on fresh sperm and did not get any treatment.
2. Microscopic Ratings for. This assessment includes assessing motility and the percentage of life lived four times. The first assessment on fresh semen covering mass motility, individual motility, concentration and percentage of life. The second assessment in sperm processed. The third Ratings for in sperm pre filling sealing. The fourth assessment in sperm after melting back

Technique of Data Analysis

Data were analyzed by analysis of variance with treatment that shows the real effect, then tested using Significant Difference test (LSD) and qualitative analysis of data obtained.

C. Result and Discussion

The Characteristics of Fresh Semen

Based on research conducted on fresh semen cattle, obtained results are shown in Table 1.

Table 1. Characteristics of fresh bull semen

Parameter	Average
A. macroscopic	
▪ Volume (cc)	6
▪ Color	Beige
▪ Consistency	moderate
▪ pH	5.8
B. microscopic	
▪ motility	70
▪ Movement mass	3 +
▪ Viability Percentage (%)	80

Source: Results of Measurement, 2016

Table 1 shows that the assessment of fresh semen is done in two ways, namely macroscopically and microscopically. This is in accordance with the opinion raised by Toelihere (1985) that the semen quality assessment carried out macroscopically (pH, color, consistency) and microscopic (motility, percentage of survival, concentration and morphology of sperm).

The volume of the ejaculate obtained an average of 6 ml. This indicates the excellent quality of the assessment and normal volume, as the opinion of Pratiwi et al. (2006) suggested that the volume of the ejaculate cattle 6-10 ml.

Assessment of color on fresh semen veal was obtained by beige. Beige on cattle semen is quite normal and is of good quality. As Pratiwi et al. (2006) that the color of the fresh semen good beef is yellowish white to cream color.

The level of consistency of fresh semen cattle used in this study was moderate. This assessment category was also a category of good consistency and feasible for further processing until the stage of freezing to thawing back. This is in line with the opinion of Pratiwi et al. (2006) which states that the consistency of semen bull is medium to thick.

The degree of acidity (pH) of cattle semen was observed in this study was an average of 5.8. Although the pH value is not in accordance with that raised by the Pratiwi et al. (2006) which states that cattle semen pH is 7, but the value of 5.8 is still considered good and still worth further processed to the point of freezing to rethawing.

For microscopic assessment that includes an average frequency of motility in the fourth shelter is 70% by mass motility 3+. This is in accordance with the opinion of Pratiwi et al. (2006) that the standard of fresh semen bull that must be met for the processing of > 70% (range 70-90%) with a mass movement 2+ to 3+. Similarly, the opinion raised by Arifiantini & Yusuf (2006), which classifies the mass motility in 3 criteria and criterion 3+ is the most excellent mass motility. Further suggests that the criteria +++ is a mass movement of the most well characterized by big waves, fast moving dark, and on the move. In general, the concentration of fresh semen of cattle presented by the Pratiwi et al. (2006) that the fresh bull semen concentration ranges 800-1160 million / ml.

The percentage of live fresh semen obtained in this study was 80%. This value is the percentage of life that is in line with the normal. This is stated by Hafez (1993) that the normal sperm 80-95%. The percentage of sperm was obtained by observation of sperm after staining the preparations pillowcase. Mixture pillowcase obtained by adding dye eosin staining, so as to facilitate the calculation of the live of sperm,

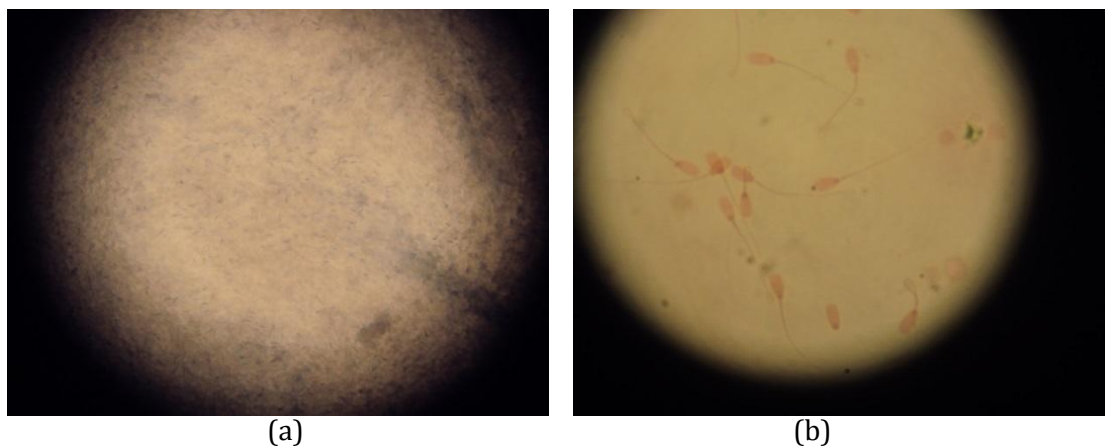


Figure 1. (a) The appearance of mass Motility of Fresh Semen; (B) sperm in eosin staining (viability Percentage of sperm)

Motility of Sperm

Based on observations of sperm motility using Tris and Andromed during processing sperm can be seen in Figure 2.

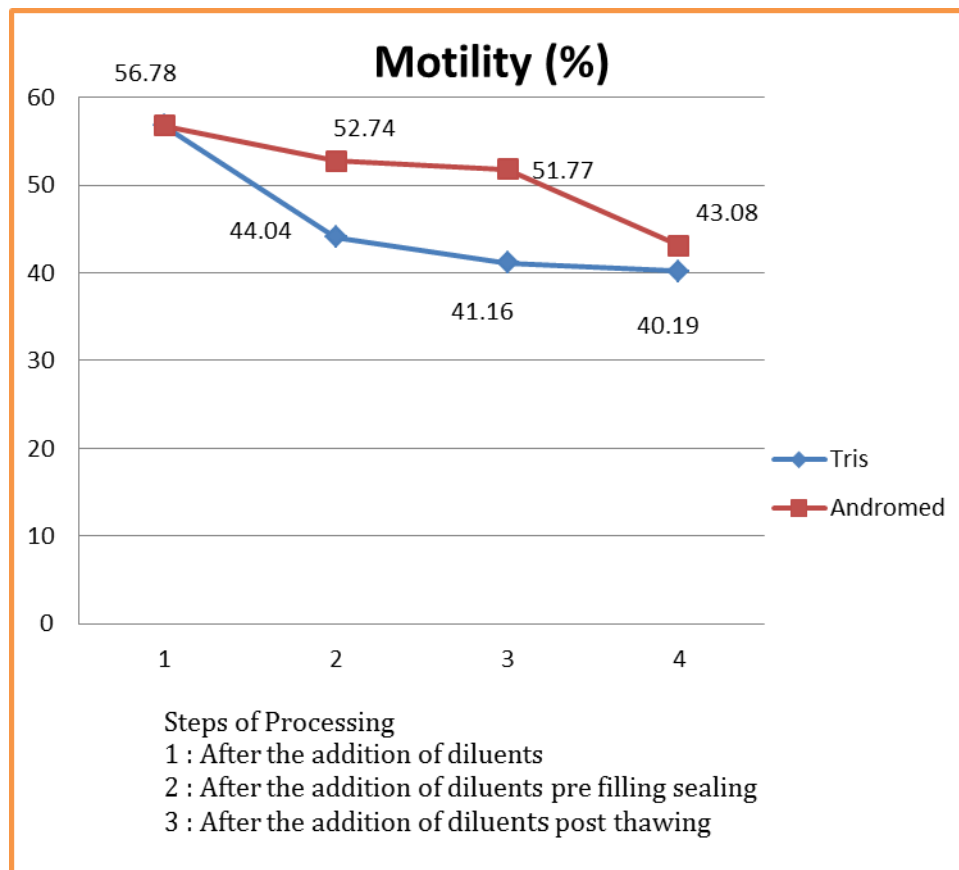


Figure 2. Sperm Motility by using Tris and Andromed

Figure 2 shows that the second diluent (Tris and Andromed) both decreased motility of sperm during freezing. Diluent andromed be able to maintain the quality of sperm during freezing. This is most likely caused by the content of diluent Andromed are better able to minimize the adverse impacts of stress (cold shock) during the stages of freezing sperm until thawing stages (reliquefaction for frozen sperm).

The occurrence of a significant reduction in treatment by using Tris yolk compared diluent andromed allow sperm decreased motility caused by the content of the diluent tris that trigger the use of energy is maximized, so that sperm undergo more moves that ultimately lead to the motility of dwindling over storage process stages of sperm.

The use of diluent in the processing stages of sperm from the provision of a diluent, freezing, thawing to stage must contain critical components. As stated by Mitchell & Doak (2004) that a diluent for freezing must contain the following components: (1) egg yolk or milk, (2) glycerol on the initial concentration of approximately 7%, (3) a simple sugar, (4) Sodium citrate dehydrate or tris (hydroxymethyl) amminomethane, and (5) antibiotic.

The viability percentage of sperm

Based on observations using the percentage of sperm with Tris and Andromed during processing of sperm can be seen in Figure 3.

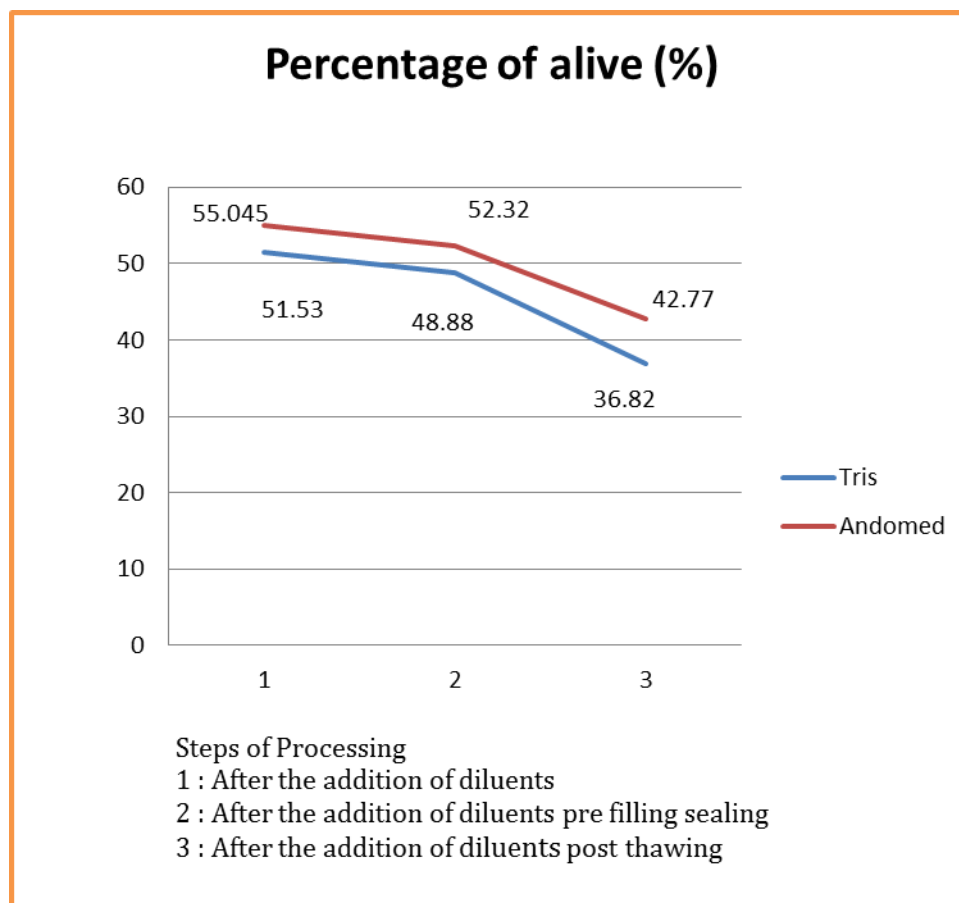


Figure 3. Percentage of sperm life by using Tris and Andromed.

Based on Figure 3, it appears that the second diluent (Tris and Andromed) both decreased quality of sperm during freezing. However, the diluent andromed better able to prevent loss of quality faster than the tris diluent during freezing sperm until thawing stages (liquefaction back for frozen sperm).

D. Conclusion

The addition of diluents can reduce the rate of loss of quality (motility and the percentage living) cattle sperm sexing results during the stages of preservation. Diluent Andromed is the best in reducing the rate of decline in the quality of sperm bulls during the stages of preservation.

To support the success of the preservation process sperm bull aiming for long term storage, then the diluent of sperm can use the egg yolk tris and the Andromed.

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