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The Quality of Fresh Semen of Bulls at 5^0C and 24^0C With or Without Diluent

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ABSTRACT

The aim of this study was to determine the quality of fresh sperm cattle at 5°C and room temperature and used of diluent. The study was conducted at the Laboratory Animal Husbandry Faculty of Kanjuruhan University. Research material used was fresh sperm cattle that obtained from the Institute for Artificial Insemination (BIB) Singosari Malang. Decrease in motility and viability without extender were higher (P <0.01) compared with extender. Motility of spermatozoa decreased to 10% at 12 hours without diluent. While the decrease in motility with diluent was 10% at 24 hours. It is also demonstrated motility of 0% at 96 hours without diluent while the semen with extender had 12.3 ± 2.16%. Also on the viability, the reduction reached 20% in diluent usage at 9 hours and without diluent at 3 hours. Decrease in motility and viability without diluent were higher (P <0.01) compared with diluent. Motility of spermatozoa decreased to 10% at 6 hours without diluent. While motility with diluent decreased 6% at 6 hours. It is also demonstrated motility 1% at 42 hours without the diluent while the semen with diluent had 14.5 ± 0.53%. Also on the viability, on the reduction reached 20% in the use of diluent at 15 and 57 hours without diluent, 0% viability while the diluent at 57 hours was 31.171±0.37%. Abnormality in six hours without a diluent has shown a decrease of 20% whereas the diluent is still down 15%. The conclusion of this study is the quality of spermatozoa in the storage temperature 5°C higher than storage at room temperature. The quality of spermatozoa at 5°C temperature and room temperature with diluent is higher than without diluent.

Key Words: Sperm Quality, Time Storage, Temperature, Fresh Sperm, Diluent

INTRODUCTION

The success of a Artificial Insemination program (AI) in cattle does not only depend on the quality and quantity of sperm ejaculated, but also depends on the ability to maintain the quality and increase the volume of semen for a while longer after ejaculation so that more cows will be inseminated. The effort to maintain the quality of the semen and multiply the results of an ejaculation of male superiority by using some diluents. Terms of each diluents is to provide nutrients for the needs of spermatozoa during storage, should allow the sperm to move progressively, non toxic to sperm, becomes a buffer for the sperm, can protect sperm from cold shock for both frozen semen and semen which is not frozen (liquid semen).

Some problems in dilution and especially semen storage can be solved by taking the path of freezing sperm. But for AI activities that utilize liquid semen due to the absence or scarcity of frozen sperm in an area that has had the same type of superior male used for frozen semen, the dilution and storage will be a problem. Solihati and Peter (2011) reported that diluents Egg Yolk Citrate was more able to maintain the vitality of spermatozoa Simmental cattle up to 4.67 days of storage; Yellow Skim milk-egg for 3.86 days and fresh milk for 4.00 days and the lowest was obtained from coconut water diluents yolk ie 3.33 days after dilution at three storage temperatures - 5^0C. Sperm motility liquid sperm Simmental cattle in this study were still above the motility of at least worthy of

AI, which is 40% only limited storage time the fourth day of the fourth diluents, although a diluents Citrate-Egg Yolk and Milk Fresh-Egg Yolk could still be reached until the fifth day.

Arifiantini and Purwantara (2010) reported that the percentage of motility and viability of semen every 24 hours during 144 hours of storage at a temperature of 5 ° C showed that the decline in motility as much as 4.3% to 8.6% for every 24 hours of observation and a decrease in viability between 5.1% to 5.8%. Yudi et al. (2008) reported that the shelf life of fresh semen based motility and viability, after 3 and 9 hours of storage at room temperature row - succession was 48.33 ± 10, 52 ± 7% and 20.00 , 98%. While on the 5 °C temperature was 41.67 ± 8.88% and 12.92 ± 7.22%. Meanwhile, the viability of the same retention time was 71.49 ± 6.32% and 50.40 ± 7.3% at room temperature and 65.82 ± 6.68% and 41.07 ± 8.34% at a temperature 5 °C. Differences in motility and viability, were significant (P <0.05) between the storage at room temperature and a temperature of 5 °C can be seen after 3 hours. Semen stored at a temperature of 5 °C showed a decrease in motility and viability faster than the sperm stored at room temperature. So that at any observation sperm stored at room temperature showed motility and viability was significantly higher. Based on this background it is necessary to know the quality of fresh semen bulls at 5 °C and room temperature storage with a long shelf by using different diluents.

Currently there is no standard on the long shelf use fresh semen at a temperature of 5 °C and room temperature that are still able to guarantee the quality of fresh sperm so that it can be processed further. Aim of this research is how the quality of fresh sperm bulls at the storage temperature of 5°C and with a long shelf space and the use of different diluents. So it is necessary to study the quality of fresh semen of bulls at the storage temperature of 5 ° C and with a long shelf space and the use of different diluents. Based on the research results Yudi, et al (2008) showed that the semen stored at a temperature of 5 °C showed a decrease in motility and viability faster than the sperm stored at room temperature. In this study, treatment was added by the use of diluent. So sperm stored at a temperature of 5 °C showed a decrease in motility and viability faster than the semen stored at room temperature by using diluent than those without diluent.

MATERIALS AND METHODS

Research material used is fresh bulls semen were obtained from the Institute for Artificial Insemination (BIB) Singosari Malang.

The research method used is laboratory research using completely randomized design (CRD) factorial. This study was carried out to determine the quality of spermatozoa fresh semen in storage at room temperature and 5 ° C, with the use of diluents and without diluents on a long shelf 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48, 51, 54, 57, 60, 63, 66, 69, 72, 75, 78, 81, 84, 87, 90, 93, 96 hours, each repeated 10x. The variables measured were: motility, viability and sperm abnormalities. Data were analyzed using factorial completely randomized design. If the treatments show significant effect then continued with Least Significant Difference test.

RESULTS AND DISCUSSION

Fresh Semen Quality

Examination of fresh semen in the study includes volume, color, concentration, mass motility, individual motility, percentage of survival, abnormal spermatozoa which can be seen in Table 1.

The quality of the fresh semen on the research showed that the semen could be used for further processing. The percentage of fresh semen motility Limousin beef obtained from microscopic examination was 70% at a concentration of 1333 million spermatozoa / ml. The percentage of fresh semen motility and concentration that meets the requirements for further processing, as a minimum percentage of motility and concentration produced must be 70% and not less than 500 million spermatozoa / ml (Zenichiro, et al, 2002). Further Hafez and Hafez (2008) states that fresh

spermatozoa used must have motility percentage of more than 50% with a concentration of more than 500 million spermatozoa / ml. The percentage of fresh semen motility in this study was higher. According to Susilawati (2005) semen had a motility percentage over 70% more than when survival is lower than 70%. Examination of the concentration needs to be done. The concentration of spermatozoa could be used to predict the fertility of bulls. The percentage of fresh semen abnormality of 3% indicates that fresh semen could be used to process more feasible because, according to Hafez and Hafez (2008) spermatozoa abnormalities should not exceed 20%. The quality of the fresh semen used in this study is the semen that has good quality.

Table 1. Quality of fresh semen

Examination	Mean
Volume (ml)	12.8 ml
Color	white
pH	6.4
Mass motility	++
Individual motility (%)	70%
Concentration (million / ml)	1333
Viability (%)	93%
Abnormalities (%)	3%

The results showed that the longer the storage of spermatozoa at a temperature of 5 °C, the motility and viability will fall, where the motility and viability of spermatozoa without dilution showed a higher decrease (P <0.01) than the motility and viability with the diluent. It was followed by the increase in sperm abnormalities in semen without diluent wherein abnormality was higher (P <0.01) than the abnormality with the diluent. Quality of spermatozoa at different time intervals and use of diluent also showed a very significant difference (P <0.01).Decrease in motility and viability without diluent was higher (P <0.01) compared to semen with diluent . Sperm motility decreased to 10% at the 12 hours period without diluent. While by diluting a 10% decrease was observed on a long shelf 24 hours. It was also observed motility 0% 96 hours without diluent while with diluent it was 12.3 ± 2.16%. Likewise in viability, the reduction reached 20% with the use of diluents with a long shelf of 9 hours and without diluting in 3 hours.

Motility of sperm ranged between 40-75% (Garner and Hafez, 2008). Indonesian National Standard (SNI) requires that a qualified semen used in the IB program must have a minimum percentage of motile spermatozoa 40%. The existence of a highly significant difference (P <0.01) in the old store spermatozoa using diluents on percentage motility associated with the supplies the nutrients needed by the spermatozoa to acquire the energy used to support the movement. Supplies nutrients derived spermatozoa of diluent used in this study. the longer the storage time, means that the energy required decreased because due to the nutrients that are available already on the wane. Temperature adjustment to 5^0C temperature can also affect the movement since the sperm must be able to adjust the physical condition of the environment.

Percentage viability of fresh semen of normal cow by 60 – 80% (Hafez, 2008; Kusumawati, et al., 2007). The optimum temperature for the survival of spermatozoa is 37-38^{o}C (Zenichiro, et al, 2002). Therefore, when the ambient temperature below the optimum temperature for life then the spermatozoa will be under pressure (cold shock). The longer the storage time the pressure faced by spermatozoa will also be greater.

To survive spermatozoa requires a constant supply of nutrients as an energy source. Nutrition of spermatozoa derived from a diluent which has the substance or substances required by spermatozoa which is a food source for them, among others, such as fructose, lactose, raffinose, amino acids and vitamins in the yolk so that spermatozoa can obtain energy resources in sufficient quantities for the motility. Reported by Tambing et al (2003) the longer the old store at a

temperature of 5^{0}C, the intake of nutrients derived from diluents diminishing this reduction will affect energy to power live spermatozoa.

The temperature can kill sperm because the longer the storage time when the supply of nutrients and automatically the less energy that is used by fewer and fewer spermatozoa and if the time is longer sperm will die. Supplies nutrients derived spermatozoa of diluents used in this study. The longer the storage time, means decreasing the energy required because due to nutrients available already on the wane.

The longer sperm storage at room temperature, the motility and viability will fall where the motility and viability of spermatozoa without dilution showed a higher decrease (P <0.01) than the motility and viability with the diluent. It was followed by the increase in sperm abnormalities in spermatozoa without diluent wherein abnormality was higher (P <0.01) than the abnormality with the diluent. Quality of spermatozoa at different time savings and use of diluent also showed a very highly significant (P <0.01). A decrease in motility and viability without diluent higher (P <0.01) compared with diluent motility. Sperm motility decreased to 10% at 6 hours long shelf without diluent. While by diluting a decrease of 6% on a long shelf 6 hours. It is also indicated motility 1% with long shelf 42 hours without diluent while with the diluent it was 14.5 ± 0.53%. Likewise on the viability, the reduction reached 20% with use of diluent and without diluent with a long shelf 15 hour with diluent by long shelf 57 hours without diluent, viability showed 0% while the old store diluent with 57 hours still 31.171 ± 0.37%. Abnormalities in the old store 6 hours without diluent has shown a decrease of 20%, while the diluents is still down 15%.

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Processing techniques, including diluent and dilution rate, and the type of carbohydrate as carbohydrates as an energy source in the media as well as protective spermatozoa (anti-cold shock) becomes important, because it will affect the quality (Hafez and Hafez, 2008). According to the research Yudi, et al. (2008) that the characteristics of the fresh semen is still quite good with notilitas at 3 and 12 hours after storage at room temperature was 48.33% and 10.42%

CONCLUSION

Quality of spermatozoa at 5 °C temperature storage was higher than storage at room temperature. Quality of spermatozoa at a temperature of 5 °C and room temperature by diluting was higher than without diluent. Longer storage, quality of sperm was declining either with or without diluent at a temperature of 5 °C and room temperature.

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