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Analysis of Sperm Freezing Capability of Various Bulls at the Singosari AI Center

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INTRODUCTION

Artificial insemination (AI) has been widely applied to improve genetic quality in cattle worldwide. One of the most important factors in AI program is the quality of semen. Many procedures in semen processing have been developed to preserve the sperm quality. The Singosari AI center produces the frozen semen through cryopreservation method. During this process, the number of semen collected will be rejected if the quality is low. Fresh semen with less than 70% of sperm motility, before freezing semen with less than 55%of sperm motility and less than 40% of sperm post-thawing motility evaluation will be rejected, so that it was only the viable sperm will be processed to be frozen semen commercial. Therefore, comparative study was carried out to analyze the freezing capability of sperm.

MATERIALS AND METHODS

This study was done using secondary data from the Singosari AI center in 2016. The data presented were the percentage of fresh, before freezing and post-thawing semen rejected during equilibration and cryopreservation. The method that used was a comparative study using some references.

RESULT AND DISCUSSION

Semen processing in Singosari AI center was started from the evaluation of the male feasibility until the processing of their semen becomes frozen semen. It was evaluated by libido assessment, serving capacity erection, copulatory thrust, lifting at the ground thrust and semen volume. Average of male feasibility percentage was 90.1% consisted of 11 breeds of bulls (Friesian Holstein, Bali, Madura, Ongole, Brahman, Angus, Simental, Limousin, *Japanese Black*, Galekan, and *Banteng Cross*), 5 breeds of bucks (Peranakan Etawa, Boer, Boerawa, Senduro and Saanen), and 2 breeds of rams (Sapudi and Ekor Gemuk). The semen collected, then was evaluated by the macro and microscopic evaluation. Further, microscopy evaluation of semen collected was observed in 3 steps. They were named fresh semen, before freezing and post-thawing motility evaluation. Poor quality semen will be rejected. The result showed the highest percentage of rejected semen was in the fresh semen evaluation, and it has decreased after equilibration and cryopreservation (fig. 1).

Fresh semen has many requires to diluted, they are pH: 6.2-6.8 for bulls and 6.2-7.0 for bucks and rams; minimum motility: 70%; and maximum abnormality: 10%. The biggest percentage of semen rejected is fresh semen. It means that they are sperm motility with less than 70% motility. The maximum target percentage of fresh semen rejected in the Singosari AI center is 10%. Therefore, the percentage of fresh semen rejected in every month in 2016 was exceeding the maximum targeted, especially in the early year. It might be affected by the quality and quantity of nutrition. According to the data on feeding management, the number of feedings was reduced from May (40.7 kg of forage, 5 kg of silage, 4.85 kg of the concentrate, and 0.04 kg of minerals) and increasingly reduced in October (38.52 kg of forage, 4.05 kg of silage, 4.14 kg of the concentrate, and 0.06 kg of minerals) below the average. It was carried out to lose weight bulls. The influence of nutrition on the quality of the sperm does not occur directly at the same time. However, it takes about 7 weeks to recover the spermatogenesis process in ram among semen volume, sperm concentration and the total number of spermatozoa after treating with the ideal diet in the treatment group compared with control group (1). Therefore, the result of spermatogenesis recovery will have occurred starting from July until the end of the year. But the increasing semen rejected was occurred in December, out of the expectation. It might be caused by many cases of the disease significantly occur in December (102 cases of the disease occurred in bulls). Fresh semen quality was

not only determined nutrition factor, but also determined by health condition, animal species, breed, age, hormonal regulation, the level of exploitation, environmental temperature and relative humidity during semen collection (2).

Fresh semen will be diluted using the extender and equilibrated. The role of semen extender is to supply the important materials protecting sperm cells from various shocks during processing, storage, and transportation. There are many components added to fresh semen as the extender composition, such as egg yolk, ions, cryoprotectants, fructose, and antibiotics. The maximum target percentage of before freezing semen rejected was also determined in 10%. It was only reached in September, October, and December. The equilibration is the temperature transition to avoid sperm cold shock at cryopreservation. The minimum standard for chilled semen is 55% for progressive motility accepted. Percentage of rejected semen before freezing decreased significantly. It might be affected by the components of the extender, and the equilibration time and condition.

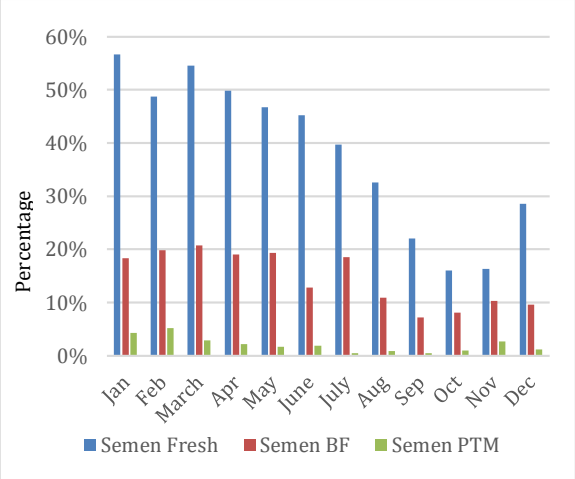

Fig. 1: Average of semen rejected each step in Singosari AI center in 2016 (3).

Post-thawing motility (PTM) assessment was to select the viable frozen semen to commercialized. There are many parameters allowed for frozen semen commercial based on SNI for frozen semen, number 4869.1:2008 for bulls and 4869.3:2014 for goats. They are 40% for minimum motility, 2 for minimum individual motility, 25 million sperm cells for minimum dose in 1 bull straw and 50 million sperm cells in 1 goat straw. PTM assessment showed the lowest percentage of semen rejected commercialized was 49.90%. It was in the same range with acceptable post-thaw motility of Madura bulls, that was 40.0±1.76 – 42.9±3.93% (4).

Overall, the data showed that there was the decline of semen rejected toward to the end of 2016. All of the semen processing steps were carried out according to SNI.ISO 9001:2008, and SNI.ISO 17025 for fresh, before freezing and frozen semen evaluation. The percentage of viable frozen semen to This result indicates the freezing capability of sperm is getting better over time. Freezing capability of sperm plays an important role to determine fertilization occurred.

The minimum standard to determine whether the semen accepted at the Singosari AI center was motility parameter for every evaluation during cryopreservation, and added with pH and percentage of sperm abnormality in fresh semen evaluation, meanwhile individual motility and sperm concentration per dose were added in the post-thawing evaluation. To ensure the fertility of sperm, the sperm quality assessment might be added by more parameter, such as the level of the intact acrosome, membrane intact cells, and mitochondrial activity. It can be carried out with a multiparametric approach using CASA/flow cytometry, and it showed 77.4% in agreement with the standard QC (5). This method might be used to improve evaluation the quality of sperm using genomic approaches.

CONCLUSION

In conclusion, the quality of semen was influenced by nutrition, health condition, hormonal regulation, animal species, breed, age, environmental temperature, relative humidity during semen collection, extender components and equilibration time. The total percentage of rejected sperm was quite high, but reduced increasingly when the nutrition managed well. To more improve the method of semen evaluation, we also suggest using CASA/flow cytometry.

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