The 6th International Seminar on Tropical Animal Production
Integrated Approach in Developing Sustainable Tropical Animal Production
October 20-22, 2015, Yogyakarta, Indonesia

Preservation of Bull Cement Technology Applications without Freezing Proceed and Utilization of Epididymis as a Slaughterhouse as a Waste Product to Optimized on Bali Cattle Artificial Insemination in Remote Areas

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ABSTRACT: Currently the application of Artificial Insemination (AI) Reproductive Technology has been urged to be developed in the remote area. The applications of these technologies were to accelerate the population of Bali Cattle in remote areas. The increase in income of farmers is done by increasing the number of births, the number of pregnancy and the number of breeding. Increasing the genetic quality becomes an important factor for remote areas for accelerate the increase in the income of farmers. The limitation of Artificial Insemination Equipment such as Liquid Nitrogen (N_2) for maintaining the frozen cement Bali cattle is the big problem recently. Without liquid N_2 would impossible to maintain the spermatozoa still a live to apply the AI in the difficult area. To solve the problem we need to preservation the cement without frozen method, which will not need N_2 liquid. The availability of good cement becomes a critical success factor. Waste from slaughterhouses (RPH) in the form of a bull testicle and good genetic quality can be utilized in the form of spermatozoa using semen collection method from epididymis are expected to be used as a liquid cement to AI. Liquid nitrogen must be produced by special chemical plant and are usually located in urban areas or areas that are easily affordable transportation. This research has used the tannins as a substance that can prolong life of bull sperm without freezing methods. These researches showed that two methods production of cement could improve the AI program and increased the pregnancy rate of Bali Cattle at remote area. By this cement/sperm we can serve the sperm for AI in the remote area. The program will continue until the result of NTT as a source of national cattle and calf birth Bali cattle. This succeed will be a pilot project to applied this technology in the different areas where lack of equipment and facilities to increase the cattle population in Indonesia.

Keywords: sperm, artificial insemination, Bali Cattle, pregnancy, epididymis

INTRODUCTION

Artificial insemination is one important method and has been applied to improve the quality and quantity of genetic using insemination using a sperm in the cervix to place the estrus females (Hafez, 2000, Bearden 1980 and 1997, Evans 1987). Spermatozoa were first discovered by Leuwenhoek on cement and testes (Austin and Short, 1982). According to Garner and Hafez, (1987) spermatozoa is a long cell consisting of a flat head and a tail containing nucleus containing the device for cell movement. Sperm cells enveloped by plasma membrane called plasmalemma. The big problem of application of AI in Indonesia is availability of Liquid Nitrogen (N_2) to keep

the sperm of frozen condition. The problem of transportation and distance will make very difficult to serve the appropriate management. Reproductive failure is a major source of economic loss in the beef industry. The majority of this loss occurs because cows do not become pregnant during a defined breeding season. Therefore, the goal of any breeding program (AI or natural service; synchronized or not) is to maximize the number of females that become pregnant (Perry *et al,* 2011).

Post thawing motility 40 % is National standard in Indonesia; need the N_2 liquid, so by this research we have tried to maintain the live sperm without cryopreservation procees. Tannins were collected from Lamtoro leaves (*Leucaena leucocephala)* as divulging chemicals capable of binding substances that cause the cessation of the process of energy metabolism without the freezing process. Tannin is a derivative of polyphenols in the form of condensed tannins (Arranz *et al.,* 2009), also known as polymer proanthocyanids 2-50 until flavonoids. Tannin as already known contain the flavonoids that can be a decapacitation factor of sperm on female reproductive track. Decapacitation factors are expected to restrain the use of nutrients for the metabolism so that the power of his life can be extended and will return to work when the decapacitation factors released by chemical or manually by a centrifuge at a certain rotation speed. These factors increase the stability of the membrane decapacitation with, among others maintaining the physiological ratio cholesterol / phospholipid. Pressing the influx of Ca AMP, press the increase in Ca intracellular, and is able to bind the protein complex and are bound to the ion Ca, Mg, Na and K. In the absence of the metabolism of nutrients in spermatozoa can survive a long time and does not happen any changes in the spermatozoa to put the female goat reproductive tract and release reversible bonding process occurs tannin (Ponci *et al.,* 2000). Tannins have a strong ability to bind to proteins with high affinity, large molecular, and open. Tannins are also capable of binding protein-protein or protein complex that is bound with ions of Ca, Mg, Na and K; carbohydrates, and fats. Tannin supplementations (such as tannins Leucocephala) in the preparation of liquid sperm are expected to maintain the viability of spermatozoa during storage without freezing. It is suspected, the function of tannins can bind and retain the decapacitation factors on spermatozoa and seminal plasma membrane, thereby preventing premature capacitation and the early death of spermatozoa during storage (Calvi L. *et al.,* 1995 and Mirajuddin, 2012). This researched have done to define two steps of semen preparation which fix with the local situation of area, which has limitation for AI equipment to increasing the population of Bali cattle.

MATERIAL AND METHODS

The main material was the extraction of Lamtoro leaves. Tannin is obtained by extracting the Lamtoro leaf at Integrated Research and Testing Institute of the University of Gadjah Mada (UGM LPPT). Tannin obtained was used as a supplement in cement dilution of the spemr after collection from the bull. Spermatozoa collected from Bull by the method of artificial vagina, after finished, the fresh semen were evaluated followed by dilution process. This dilution supplemented by tannin. Supplemented media with subsequent spermatozoa were stored in a refrigerator at a temperature of 4 degrees Celsius for 7 weeks. After 7 weeks, spermatozoa then ready for use artificial insemination in Bali cattle after the resipien was ready on estrus period. The waste product of Slaughter House was Semen collected the sperm from epididymis of Bull testes. The media for sperm were PBS, physiological saline and eosin-Negrosin. Furthermore, the testicles are washed using a physiological Na Cl solution and then collected evaluation of spermatozoa the epididymis is separated from the testicles. After spermatozoa regardless of caudal epididymis, sperm motility examination and counting of live-dead percentage of spermatozoa by eosin and negrosin staining.

In this study, the evaluation of the quality of spermatozoa has been carried out by examined the sperm motility under the microscope before use and the percentage of dead spermatozoa a live examined by negrosin eosin staining method. Pregnancy of Bali cattle have done checking at 2 months of age pregnancy after completed Artificial insemination by rectal palpation methods. All the data were analyzed by ANOVA and had significance difference by probability $P< 0.05$.

RESULTS AND DISCUSSION

Table 1. Mean of pregnancy rate, motility of spermatozoa and percentage of dead live sperm on three types of spermatozoa performed artificial insemination in Bali Cattle

No	Sperm	Pregnancy rate (%)	Motility (%)	Live and dead presentation
1	With tannin	21 ± 1.5	30 ± 1.8	35 ± 3.1
2	Epidemical sperm	18 ± 2.3	31 ± 2.3	36 ± 2.5
3	Control (frozen)	30 ± 3.1	40 ± 2.7	37 ± 2.8

As shown in Table 1 that no significant differences between the three types of spermatozoa that have been used, such as, first spermatozoa were not frozen but preserved with tannin on media diluent, spermatozoa results of the collection of epididymis cattle in slaughterhouse and as a control spermatozoa frozen in liquid nitrogen. Pregnancy rate, motility rate and live and dead sperm showed no significantly difference it means the quality of three types sperm as equal, so base on these resulted we can use all types of spemr for AI on cattle. Motility and viability of sperm when preserved lower quality compared to fresh semen, it is supported by a statement Lamirande *et al.* (1997) that the metabolism of spermatozoa as living cells can produce a product called Reactive Oxygen Species (ROS). ROS production is a factor responsible for the decrease in motility, vitality, and the fertilization capability of spermatozoa during the process of preservation. Another study conducted by Ansari *et al.* (2010) stated that the freezing process for the purpose of preservation of spermatozoa can accelerate the production of ROS. Research conducted Awda *et al.* (2009) and Hall, C.A dan Cuppet, S.L.(1997) states that the Reactive Oxygen Species has a dual function on spermatozoa. At low concentrations to induce ROS function in the process of sperm capacitation, hyper activation process, the integrity of the acrosome, and play a role also in the process of fusion of sperm and oocyte. Whereas at high concentrations and excess, ROS can cause DNA damage, inhibits the fusion process between spermatozoa and oocytes, as well as decrease the motility of spermatozoa. These data has been able to prove that the spermatozoa result of preservation without freezing and without liquid N_2, at day 7 was still able to produce a pregnancy in Bali cattle, suggesting that preservation without liquid N2 can be as an alternative to that areas where lack of liquid N_2 for maintaining the frozen spermatozoa. Further use epididymis spermatozoa can also be used as an alternative to the male which slaughters as a source of spermatozoa and can be used as a rescue method for superior genetically males to be slaughtered. Sperm epididymis has opportunity to collected and still alive and has the capability for fertilization as explained by Tajick *et al.* (2007) too investigate the proportion of normal sperm cells in bovine epididymis, bovine testicles, obtained from a local slaughterhouses, epididymis were incised and sperm cells were transferred into slide glasses where eosin nigrosin stain was applied either in the place or in laboratory. When sperm were stained in slaughterhouse, 88% of caput epididymis sperm were a live Moreover Briz (1995) reported that Sperm quality in the caput, corpus, and caudal regions of the epididymis of healthy and sexually mature Landrace boars. Epididymis sperm characteristics were examined by light microscopy, scanning electron microscopy, and transmission electron

microscopy. Sperm vitality decreased very slightly although progressively with the transport of sperm through the epididymis. Fukuda Y *et al.* (1990) reported that the pregnancy is the one of the parameter the successfully of spermatozoa. In this research the pregnancy had occurred, that means the sperm quality had been proved had the quality and capability to fertilized the egg bi AI methods. Local resources are also able to perform this simple technology and what is needed is assistance and guidance of the process of collection of epididymis spermatozoa from a local abattoir. The pregnancy success will make remote areas have no hope of developing reproductive technologies so that the population can be increased and the utilization of local resources will be growing

CONCLUSION

This research could be concluded that the use of spermatozoa is preserved without freezing and utilization of spermatozoa derived from the epididymis as slaughterhouse waste. These methods a alternative source of spermatozoa for artificial insemination in Bali cattle. Even, this method had pregnancy rate still did not satisfactory yet, but has been able to prove and as an alternative method to develop artificial insemination of Bali cattle in remote areas to increase the cattle population of Bali and increasing of farmer income.

REFERENCES

Austin C R and R. V. Short. 1982. Reproduction In Mammals. Cambridge University Press. London. Vol. 1.

Arranz S, Saura-Calixto F, Shaha S, Kroon PA. 2009. High contents of nonextractable polyphenols in fruits suggest that polyphenol contents of plant foods have been underestimated. J Agric Food Chem. Aug 26;57(16):7298-303. doi: 10.1021/jf9016652

Bearden, J.H. dan Fuquay, J.W. 1980. Aplied Animal Reproduction. Gadjah Mada University Press. Yogyakarta

Bearden, J.H. dan Fuquay, J.W. 1997. The male reproduction system. In : Aplie animal reproduction, 4th edition. Prentice Hall, New Jersey

Calvi L., GJ Mwalongo, BA Mwringa B ridle and JA Shields. 1995. Characteristization of Wattle-Tannin based Adhesives for Tnzania (A paper published in Holzforchung Vol 49 No 2).

Evans, G., dan Maxwell., W.N.C., 1987. Artificial Insemination of Sheep and Goats. Butherwort. Australia.

Enright BP, Lonergan P, Dinnyes A, Fair T, Ward FA, Yang X, Boland MP. 2000. Culture of in vitro produced bovine zygotes in vitro vs in vivo: implications for early embryo development and quality. Theriogenology 54, 659–673.

Fukuda Y, Ichikawa M, Naito K, Toyoda Y. 1990. Birth of normal calves resulting from bovine oocytes matured, fertilized, and cultured with cumulus cells in vitro up to the blastocyst stage. Biol Reprod 42, 114–119.

Hafez, E.S.E. 1968. Reprodeuction in Farm Animal. Second edition. Washington State University Pullman. Washington

Hafez, E.S.E daqn B. Hafez. 2000. Reproduction in farm Animal. 7th Ed. Baltimore : Lippicott Williams and Wilkins.

Hall, C.A dan Cuppet, S.L. 1997. Structure activities of natural antioxidant. Di dalam: Okezie, I.A., dan Cuppets, S.L (eds). Antioxidant Methodology in Vivo and In Vitri Concepts. AOCS Press, Champaign illnois.

Lamirande E, Lamothe G. 2010. Levels of semenogelin in human spermatozoa decrease during capacitation: involvement of reactive oxygen species and zinc. Hum Reprod. 2010 Jul;25(7):1619-30. doi: 10.1093/humrep/deq110. Epub 2010

Perry1, J.C. Dalton, and T.W. Geary South. 2011. Management factors influencing fertility in beef cattle breeding programs proceedings, applied reproductive strategies in beef cattle-northwest.

Tajik P, Arman A, Taktaz T. 2007. Bovine epididymal sperm morphology obtained from caput, corpus and cauda epididymides. Nov 1;10(21):3936-9.

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