THE QUALITY OF FREEZE-THAWED EPIDIDYMAL VERSUS EJACULATE SPERM OF SPOTTED BUFFALO

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Introduction

Spotted buffalo that lives in Tana Toraja, is endangered animal that need an effort to conserve (Anonymus, 2004). Spotted buffalo (Bubalus *bubalis*) is the most important and valuable animal in the life and culture of Toraja. South Sulawesi people. It also has the most highly prized for ritual value especially in the death ritual. The foremost part of the funeral ceremony in Tana Toraja is the sacrifice of buffalo. The predicted problem that will be faced for this species is the decline of the population in the future because of the high rate of the male to be slaughtered at the tradition ceremonies as well as male isolated and castration. To prevent the situation to be happened, efforts have lo be carried out in order to maintain and/ or to increase the population of this species, moreover, to conserve the existence of the high rank category of this species.

The conservation of the genetic material of the dead-animal can be performed by using reproductive technique that is worldwide developed. For the male, epididymal can be utilized as a genetic resource that can be stored for unlimited time (Yulnawati and Setiadi 2005; Rizal et *al.* 2004; Hori *et al.* 2004). Moreover, cauda epididymal sperm has the potential for fertilizing ovum as well as the ejaculate sperm (Hafez and Hafez 2000). The aim of this research was to compare the quality of freeze-thawed spotted buffalo epididymal and ejaculate sperm.

Material and Methods

Epididymal sperm collection and evaluation

The spermatozoa was collected from cauda epididymal by combination of slicing and flushing method using Andromed extender as treatment 1. The media and epididymal spermatozoa were then centrifuged at 3000 rpm, for 20 minutes, and the supernatant removed. Pellet that contain epididymal spermatozoa was analyzed for the quality, including concentration, motility. viability, abnormality and membrane integrity. The concentration of epididymal spermatozoa was counted using Neubauer chamber under light microscope. After observation of sperm quality, epididymal spermatozoa was diluted in Andromed.

Ejaculation sperm collection and evaluation

Semen was collected using artificial vagina three times with a week interval. The quality of ejaculate sperm was analyzed both macroscopic (volume, color, pH and density) and microscopic (concentration, mass and individual motility, viability, abnormality and membrane integrity). The quality of ejaculates which meets the requirements for freezing process (the percentage of motility ≥70%, the concentration ≥2000 million cells per ml, mass movements ++ or +++ and abnormality <15%) was diluted in the same extender as for epididymal sperm.

Cryopreservation of ejaculate and epididymal sperm

The diluted epididymal sperm was packed in ministraw (0.25 ml) which contain 50 million of motile sperm and then equilibrated in refrigerator at 5°C for 3 hours. The freezing process was started with putting down the straw after equilibration on 10 cm above the vapour of liquid nitrogen (around -130°C) for 15 minutes, and the straw was plugged into liquid nitrogen (-196°C) and store in the container.

Thawing

After storage in liquid nitrogen, the samples in the straw was thawed and analyzed for the quality, including the percentage of motility, viability and membrane integrity. Thawing was conducted by plugged the straw into the 37°C water for 30 second.

Parameters and Data Analysis

Three parameters (the percentage of progressive motility, viability and membrane integrity) was observed in fresh, diluted, equilibrated and thawed sperm. The percentage of progressive motility was assessed subjectively under light microscope by viewing more than 8 fields per slide (objective lens = 40x) (Rasul et al. 2001). A spermatozoon that move due to swimming, regardless of its speed, was considered to be motile.

A 10 microliters of sperm samples was mixed with a 10 microliters of eosin B stain on a clean microscope slide. Sperm that do not absorb the stain was considered live, in the other hand, sperm that absorb the stain was considered dead (Bissett and Bernard 2005). Evaluation of membrane integrity was carried out using hypoosmotic swelling test (HOS test) containing of 1,3 g fructose (Sigma, USA), 0,7 g Na Citrat (Sigma, USA) in 100 ml aquabidestilata (Rodriquez-gil et al. 1994). A 10 microliters of sperm suspension was mixed with 990 microliters of the HOS solution and incubated at 37°C for 30 minutes. The swollen spermatozoa characterized by coiling of the tail will be considered to have an intact plasma membrane. Data was analyzed with analysis of variance (ANOVA).

Result and Discussion

The result showed that both the percentage of progressive motility of the freeze-thawed epididymal versus ejaculate sperm were the same (41.67%). The percentage of live sperm from freeze-thawed epididymal and ejaculate were 52 and 53 %, respectively (P>0.05). Besides, the percentage of membrane integrity of freeze-thawed epididymal versus ejaculate sperm were 68 and 47.33 %, respectively (Table 1). There was a significantly different (P<0.05) between the percentage of membrane integrity of freeze-thawed epididymal and ejaculate sperm.

Table 1. The avarage quality of epididymal versus ejaculate of spotted buffalo sperm

Treatments	Post Dilution			Post Equilibration			Post Thawing		
	% M	% LS	% MI	% M	% LS	% MI	% M	% LS	%MI
Epididymal sperm	65 ± 0.0[a]	76 ± 2.8[a]	78.7 ± 0.5[b]	48.3 ± 2.4[a]	70.3 ± 0.5[a]	72 ± 0.8[b]	41.7 ± 2.4[a]	52 ± 2.9[a]	68 ± 1.4[b]
Ejaculate sperm	70 ± 0.0[b]	77.7 ± 1.3[a]	75.7 ± 0.9[a]	60 ± 0.0[b]	71.3 ± 1.3[ab]	67.7 ± 0.9[a]	41.7 ± 2.4[a]	53 ± 2.5[a]	47.3 ± 0.9[a]

Note. [a,b] Different subscribe in same colomn showed significantly different (P<0.05). M: motility, LS: Live Sperm, MI: Membane Integrity.

Data showed that sperm from ejaculate were more sensitive to damage than sperm from epididymal. In conclusion, the quality of frezee-thawed epididymal versus ejaculate of spotted buffalo sperm seemed to be met the requirements for using in artificial insemination in order to conduct the conservation program. Moreover, the quality of freeze-thwed epididymal sperm were same in general with the ejaculate sperm.

Acknowledgement

I would like to thank to Ir. Isak M. Allosomba, Flora Tonggo, MM, dr. Yulius and Mr. Slamet Sumitro for support, samples and laboratory equipment.

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