HAYATI Journal of Biosciences March 2010
Vol. 17 No. 1, p 27-30
EISSN: 2086-4094

Available online at:
http://journal.ipb.ac.id/index.php/hayati
DOI: 10.4308/hjb.17.1.27

Quality of Epididymal and Ejaculated Sperms of Spotted Buffalo in Dextrose Supplemented Extender

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Received January 27, 2009/Accepted February 19, 2010

Quality of epididymal and ejaculated sperms of spotted buffalo in three different extenders (i.e. Andromed, Andromed supplemented with 0.2% dextrose, and Andromed supplemented with 0.4% dextrose) was studied. The results showed that there was no significantly different (P > 0.05) quality of ejaculated and epididymal sperms. The motility percentage of post thawing epididymal and ejaculated sperms was 41-46% and 41-45%, respectively. And percentage of cytoplasmic membrane integrity of the post thawing epididymal and ejaculated sperms was 66-67% and 47-54% respectively. There was also no significant different (P > 0.05) in the percentage of cytoplasmic membrane integrity between the extenders of both ejaculated and epididymal sperms. This result suggested that epididymal sperms were reliable for artificial insemination as good as ejaculated sperms.

Key words: ejaculated sperm, epididymal sperm, dextrose, spotted buffalo

INTRODUCTION

Indonesia has a unique species of buffalo which is their existence are endanger due to decreasing of the population every year. One of the endemic species is spotted buffalo (*Bubalus bubalis*), and local people usually call it as *Tedong Bonga*. Their natural habitat is in Tana Toraja, South Sulawesi. The body of spotted buffalo is relatively bigger than that of local buffalo, with straight and massive back. The average weight of adult buffalo is approximately 700-800 kg. Population of this species is decrease every year due to local tradition and culture to protect male buffalo from sexual/reproductive activities and to make use of them for funeral traditionally ceremonies.

Application of AI technology could be a solution to increase population of the endemic buffalo species in Tana Toraja, Indonesia. However, traditionally socio-cultural role of the Torajan to protect male buffalo from sexual activity cannot be disturbed. Utilization of epididymal sperms instead of ejaculated sperms is a great alternative source of sperms for AI application of spotted buffalo in Tana Toraja. Epididymis tissue is a place for sperms storage before they are ejaculated. Some researchers reported that sperms collected from the cauda epididymis of the buffalo had the same quality as ejaculated sperms and they were reliable to be used for AI application (Lambrechts *et al.* 1999; Herold *et al.* 2004; Herrick *et al.* 2004; Harshan *et al.* 2005; Herold *et al.* 2006).

Determination of appropriate extenders used for ejaculated and epididymal sperms of spotted buffalo is need to be conducted in order to maintain the quality of the sperms after storage in the form of both liquid and frozen sperms. In this study, quality of ejaculated and epididymal sperms is compared. Furthermore, observation of post equilibration and post thawing sperm quality of both ejaculated and epididymal sperms in dextrose supplemented extenders is presented.

MATERIALS AND METHODS

Ejaculated Sperm Collection. Ejaculated sperms was collected using artificial vagina. Evaluation of the fresh semen quality was conducted by measuring concentration, percentage of motility, abnormality and percentage of cytoplasmic membrane integrity. The collected semen should be matched with standard quality of fresh sperms for AI (i.e. motility percentage was more than 70%, concentration of sperms more than 1 x 10^9 cell ml^{-1} and percentage of abnormality was less than 15%). Then the samples were undergone for further processing to be frozen sperms (Bearden & Fuquay 1997).

Epididymal Sperm Collection. Cauda epididymis tissues were collected during the slaughter process of spotted buffalo at a traditional ceremony held in Pangli village, Rante Pao district, Tana Toraja, Sulawesi, Indonesia. The tissues were washed and separated from

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the testis and other tissues. And they were kept in a solution of NaCl 0.9%. Collection of the sperms was conducted according to method of Rizal *et al.* (2004). The suspension was then centrifugated at 500 g for 20 min at room temperature (26-28 °C). The pellet of epididymal sperms was examined for the quality using similar parameters to that of ejaculated sperms.

Extender Treatments and Sperm Quality Analysis. Ejaculated and epididymal sperms were diluted in three different extender treatments, i.e. Andromed, Andromed supplemented with 0.2% dextrose, and Andromed supplemented with 0.4% dextrose. The diluted sperms were packed into 0.25 ml of straw and equilibrated at 5 °C for 3 h. For freezing process, the straws were initially placed on 10 cm above surface of liquid nitrogen for 15 min, then they were dipped into and stored in the liquid nitrogen (-196 °C).

Sperm quality was analyzed based on parameters of progressive motility, life span and cytoplasmic membrane integrity on the stage of freezing process, after equilibration and thawing. Percentage of progressive motility of the sperms was calculated on eight different visual fields using a light microscope (400 X) (Rasul *et al.* 2001). Percentage of cytoplasmic membrane integrity was observed by hypo osmotic swelling test (HOST), using a solution of 0.13 g ml^{-1} of fructose (Sigma, USA), and 0.07 g ml^{-1} of Na Citrate (Sigma, USA) (Rodriquez-gil *et al.* 1994). As much as 10 µl of sperm suspension was mixed with 990 µl of HOST solution and incubated at 37 °C for 30 min. The sperms with intact membrane condition were indicated by performing swollen tail, whereas the sperms with damaged membrane condition were indicated by a straight tail (not swollen tail).

The treatments were conducted in three replications and the data were analyzed using variance analysis (ANOVA). The differences between treatments was tested using smallest significant different test (Steel & Torrie 1993).

RESULTS

Quality of Fresh Sperms. The result showed that concentration of the sperms collected from the cauda epididymis tissue was 10.71 x 10^9 cell/ml (Table 1). This amount was higher than that of ejaculated sperms (2.69 x 10^9 cell/ml). Motility percentage of ejaculated sperms was also higher than that of epididymal sperms. Motility percentage of epididymal sperms was 65.0%. This value was still reliable to be used for further study.

The percentage of abnormality cells of sperms from epididymis was higher than that of ejaculated sperms (Table 1). This result confirms that maturation process of sperm cells is still going on in the epididymal tissues. The most abnormality found in epididymal sperms was the existence of cytoplasmic droplet at distal area. Whereas, the abnormality found in ejaculated sperms was secondary abnormality, such as detached tail from the head of the sperms. Percentage of cytoplasmic membrane integrity of epididymal sperms, however, was relatively higher than that of ejaculated sperms.

Quality of the Sperms After Freezing. In general, percentage of the progressive motility of ejaculated sperms equilibrated in the three kinds of extenders was higher than that of epididymal sperms (Table 2). Nevertheless, the motility percentage of post thawing sperms showed that epididymal sperms had higher value than that of ejaculated sperms. The motility percentage of post thawing sperms of both ejaculated and epididymal sperms was still matched with the standard quality requirements of storage sperms for AI application, which it should be higher than 40%. Post thawing sperms equilibrated with 0.2 and 0.4% dextrose supplemented extenders had higher value of motility percentages than the control group ($P > 0.05$). Percentage of cytoplasmic membrane integrity of the sperms (Table 3) showed that percentage of cytoplasmic membrane integrity of epididymal sperms was higher than that of ejaculated sperms in either post equilibration and post thawing ($P < 0.05$).

Table 1. Average quality of ejaculated and epididymal sperms of spotted buffalo

Parameter	Ejaculated sperms	Epididymis sperms
Concentration (cell/ml)	$2695.10^6 \pm 1045$	$10710.10^6 \pm 49$
Motility (%)	70.0 ± 0.0	65.0 ± 0.0
Abnormality (%)	6.5 ± 1.5	15.0 ± 3.0
Cytoplasmic membrane interity (%)	77.5 ± 1.5	79.0 ± 0.0

Table 2. Average of motility percentage in three different compositions of extenders

Sperm sources	Timing	Andromed	Andromed + dextrose 0.2%	Andromed + dextrose 0.4%
Ejaculated	Post equilibration	60.00 ± 0.00a	63.33 ± 2.36a	63.33 ± 2.36a
	Post thawing	41.67 ± 2.36a	43.33 ± 2.36a	45.00 ± 0.00a
Epididymal	Post equilibration	50.00 ± 0.00b	56.67 ± 4.71a	56.67 ± 4.71a
	Post thawing	41.67 ± 2.36a	45.00 ± 0.00a	46.67 ± 2.36a

Different superscript in different row and colomn showed significantly different ($P < 0.05$).

Table 3. Average of percentage of cytoplasmic membrane integrity in three different compositions of extenders

Sperm sources	Timing	Andromed	Andromed + dextrose 0.2%	Andromed + dextrose 0.4%
Ejaculated	Post equilibration	67.67 ± 0.94a	64.33 ± 0.47a	67.33 ± 0.47a
	Post thawing	47.33 ± 0.94a	53.00 ± 2.16a	54.67 ± 0.94a
Epididymal	Post equilibration	72.00 ± 0.82b	71.33 ± 0.94b	73.00 ± 0.82b
	Post thawing	67.33 ± 0.94b	66.67 ± 1.25b	66.67 ± 1.70b

Different superscript in different row and colomn showed significantly different ($P < 0.05$).