

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

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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 1×10^9 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 centrifuged 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 10.71×10^9 cell/ml (Table 1). This

amount was higher than that of ejaculated sperms (2.69×10^9 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 integrity (%)	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	$60.00 \pm 0.00a$	$63.33 \pm 2.36a$	$63.33 \pm 2.36a$
	Post thawing	$41.67 \pm 2.36a$	$43.33 \pm 2.36a$	$45.00 \pm 0.00a$
Epididymal	Post equilibration	$50.00 \pm 0.00b$	$56.67 \pm 4.71a$	$56.67 \pm 4.71a$
	Post thawing	$41.67 \pm 2.36a$	$45.00 \pm 0.00a$	$46.67 \pm 2.36a$

Different superscript in different row and column 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	$67.67 \pm 0.94a$	$64.33 \pm 0.47a$	$67.33 \pm 0.47a$
	Post thawing	$47.33 \pm 0.94a$	$53.00 \pm 2.16a$	$54.67 \pm 0.94a$
Epididymal	Post equilibration	$72.00 \pm 0.82b$	$71.33 \pm 0.94b$	$73.00 \pm 0.82b$
	Post thawing	$67.33 \pm 0.94b$	$66.67 \pm 1.25b$	$66.67 \pm 1.70b$

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

DISCUSSION

Motility percentage is one of main criteria for determining quality and reliability of the sperms for AI application. Higher percentage value of ejaculated sperm motility (70.0%) than that of epididymal sperm motility (65.0%) was due to a high correlation with the cell density of the epididymal sperms, so that movement of the sperms was very limited. In addition, seminalis cytoplasmic solution in ejaculated sperms could decrease cell density and the solution had also an important role in increasing sperm motility.

Movement of sperms occurs due to the existence of a particular protein called dynein at external microfibrils of sperms's tail. This protein has function as ATP-ase enzyme to convert intermediate adenosin tri phosphate (ATP) to adenosin monophosphate (AMP) and two anorganic phosphate ions (Pi). This process generates high energy that can induce contraction of the microfibrils and creates twisted movement of the sperms (Rizal *et al.* 2003). The ATP is obtained by breakdown the sugar via Embden-Mayerhof pathway (Garner & Hafez 2000).

The component of seminalis cytoplasmic solution was fructose, sorbitol, citric acid, inositol, glyceryl phosphoryl choline (GPC), ergothionine, sodium, potassium, calcium, magnesium, and chloride (Hafez & Hafez 2000). These components are important substances for membrane and also as energy resources for ATP generating. Then it can generate movement of ejaculated sperms more active than the movement of epididymal sperms.

Abnormality of epididymal sperms was higher than that of ejaculated sperms. The abnormality in epididymal sperms mostly found was distal cytoplasmic droplet. This could be occurred due to on going maturation process at cauda epididymis (Bearden & Fuquay 1997). However, epididymal sperms could be used for fertilizing the ovum, because they had the same capability to fertilize ovum as ejaculated sperms (Hafez & Hafez 2000).

Previous studies showed that epididymal sperms of several species had good quality and still meet the requirement to fertilize ovum *in vitro* or *in vivo* (Axner *et al.* 1998; Sankai *et al.* 2001; Tsutsui *et al.* 2003; Herrick *et al.* 2004; Hori *et al.* 2004; Rizal *et al.* 2004; Setiadi *et al.* 2007). Fertility capability of the sperms is determined by the motility and abnormality of the cell (Hafez & Hafez 2000).

Plasma membrane integrity of the ejaculate sperms is relatively lower than that of the epididymal sperms. The percentage of MPU of the epididymal sperms was relatively higher (79%) than that of ejaculated sperms (77.5%). This could be due to the presence of seminalis plasma in ejaculated sperms. Seminalis plasma that consists of several kinds of protein has great impact on composition of the lipoprotein and of cytoplasmic membrane (Hafez & Hafez 2000). Ejaculated sperms that had seminalis plasma were already conditioned for ovum fertilization. The presence of seminalis plasma, composition of cell membrane was changed to be ready for capacitation process and acrosomal reaction. This condition caused the percentage of cell plasma membrane integrity of

ejaculated sperms was lower than that of epididymal sperms.

In general, the addition of glycerol as a cryoprotectant to the extender was able to maintain quality of post thawing sperms better than that of without glycerol (Fabbrocini *et al.* 2000). Andromed® is a commercial extender that consists of Tris hydroxy aminomethane, fructose, citric acid, glycerol, and several kinds of antibiotics (Minitub 2001). Andromed as a commercial extender already supplemented with glycerol as a cryoprotectant that is an appropriate extender to obtain quality of buffalo's frozen sperms for AI application.

Cytoplasmic membrane integrity results indicated there was a change in osmotic pressure during freezing process. Osmotic pressure difference between intra dan extracellular caused damages of cytoplasmic membrane, so that percentage of cytoplasmic membrane integrity was found lower after freezing and thawing process. The percentage of cytoplasmic membrane integrity of epididymal sperms was higher than that of ejaculated sperms on post equilibration and post thawing (Table 3). According to Harshan *et al.* (2005) epididymal sperms tended to be more resistant to cold shock than ejaculated sperms.

Composition of cell membrane lipoprotein of epididymal sperms was different from ejaculated sperms, which they have an additional cytoplasmic semen. However, the percentage of cytoplasmic membrane integrity of post thawing sperms showed different trend of results with the percentage of motility. This results might be due to instability of cytoplasmic membrane and lipoprotein composition in cell membrane of epididymal sperms.

Intact cytoplasmic membrane had positive correlation with motility, viability, and membrane integrity of acrosome head of the sperms. Intact cytoplasmic membrane could assure normal metabolism so that processes of substrate and electrolyte transports in and out of the membrane could occur properly (Rizal *et al.* 2003). The role of cytoplasmic membrane integrity was not only for metabolism processes to support motility of the sperms, but also it had function to attain fertilization process such as capacitation, acrosomal reaction and adsorption of the sperms to the surface of the ovum (Jeyendran *et al.* 1994).

Sperms could utilize sugar as the main source of energy during the freezing and storage process. Sugar used in Andromed extender was fructose. In extender treatments, dextrose was added as an additional substrate for energy. Dextrose was consists of 90% glucose that can be breakdown through glycolysis pathway to generate energy for maintaining motility and life span of the sperms (Murray *et al.* 1999). According to Rizal *et al.* (2003), motility of the sperms was depend on energy in the form of ATP. Aisen *et al.* (2002) suggested that metabolism process of the sperms could occur properly in extender supplemented with sugar that was easy to be metabolized.

Quality of ejaculated and epididymal sperms in extenders supplemented with 0.2 and 0.4% (w/v) was better than that of control extender ($P > 0.05$). This result indicated that dextrose could have optimal functions as

external cryoprotectant and additional energy source apart from fructose as the main energy source in Andromed extender.

This study concluded that the quality of ejaculated and epididymal sperms of the spotted buffalo was the same reliable to be used for AI application. Nevertheless, with the supplementation of dextrose sugar at the concentration of 0.2 and 0.4% (w/v) into the Andromed extender did not show any significant effect in increasing the quality of ejaculated and epididymal sperms during either freezing process, post equilibration and post thawing.

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