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Semen characteristics and freezing capability of Madura's cattle

Wilmintje Marlene Nalley^{a)}, Iis Arifiantini^{b)}, Eros Sukmawati^{c)}

^{a)} Faculty of Animal Science, University of Nusa Cendana Kupang 85148, Indonesia

^{b)} Department of Veterinary Clinic, Reproduction and Pathology, Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor 16680, Indonesia.

^{c)} Artificial Insemination Centre, Lembang Bandung 40391, Indonesia.

Abstracts

Madura cattle are a stable, inbred hybrid of Zebu and banteng (*Bos Javanicus*). They originated on the island of Madura near northeastern Java where the original cattle population was banteng, very similar to Bali cattle. Nowadays, Madura cattle frozen semen in Indonesia were produced by two National Artificial Insemination (AI) centre. The information of semen characteristic and the freezing capability of this breed is limited. This research aimed to study the characteristics of Madura's bull fresh semen and freezing capability from one of National AI Centre. Five Madura bull belong to National Lembang AI centre used for this research, in total 185 ejaculate during 2015. The semen was collected using artificial vagina and subsequently evaluate macro- and microscopically according to AI Centre procedure. Semen were diluted with skim milk egg yolk extender, packed into ministraw (0.25 ml) and equilibrate at 5°C for 4 hours and freeze using automatic freezing machine. Result demonstrated the color and aspect of the ejaculates ranged from milky white to creamy, the mean \pm SD semen volume ranged from 5.16 ± 1.04 to 5.98 ± 1.39 ml, with progressive motility and sperm concentration were 70.00 to 71.9 ± 2.94 % and 746.6 to $1.305.52 \times 10^6$ sperm respectively. Post thawing motility were varies among bull. All bulls showed moderate post thawing motility which was only $40 \pm 1.76\%$ to $42.88 \pm 3.93\%$. This result was lower than other native breed. In conclusion, the presented results indicate that there is variability of sperm concentration among individual and Madura sperm had a moderate freezing capability.

Key word : Madura cattle, Semen characteristics, Freezing capabilty

Introduction

Madura cattle is a Bali-Ongole-Java native composite breed, develop from Bali (*Bibos banteng*), Java-native (*Bos javanicus*) and Ongole (*Bos-indicus*). This breed are conserved pure in Sapudi island, east Java. Madura cattle are raised for beef/ draught, racing ("Karapan sapi") and beauty contest calls "sonok" (Risqina *et al.*, 2014). "Karapan sapi" is a traditional bull racing festival on Madura island. Every year from about July through October, local bulls are yoked to wooden skids and raced for 130 meters. Their bodies are neat, compact, and deep, with well-developed forequarters. The cows attain an average weight of about 210 kg and bulls range from 350 to 375

kg at maturity (Lamoureux, 2003). The reddish brick-brown Madura cattle has a non specific white pattern on the back-bottom. The uniformity of the breed was developed through a tuft selection by the people in Madura. Cryopreservation of germplasm is widely used in agriculture. To improve quality of Madura cattle, Lembang artificial insemination (AI) center produce frozen semen of this breed. This research aimed to study the characteristics of Madura's bull fresh semen and freezing capability to predict number of straw produce of each ejaculate.

Material and Methods

Five Madura bulls age 5-7 years old, body weigh 500-600 kg, belong to Lembang AI Center was used as a sperm source. In total 185 ejaculate collected during 2015. The bull were kept under natural light and maintained under a uniform and constant nutrition regime. Each bull being fed on a daily diet of 6 kg concentrate and 30 kg of king grass, and water provide ad libitum.

a. Extender Preparation

Milk extender was prepared by using 10 g of skim milk (Tropicana slim) powder and 0.9 g of glucose in 100 mL of distilled water, heated to 95 °C for 10 min, and then cooled to room temperature before the addition of 10% egg yolk and 8 % glycerol. Finally the extender were added with 0.5 mg Streptomycin and 1000 IU Penicillin ml⁻¹ extender (Kulaksiz *et al.*, 2012).

b. Semen collection, evaluation and Prosessing

The semen was collected using artificial vagina, twice a week. Immediately after collection the semen were evaluate macro and microscopically. Semen color was assessed visually in the collecting tube. The volume of semen was measured directly from the graduated collecting tube. All these samples were evaluated for sperm motility and sperm concentration. To evaluate sperm motility, a small drop (10 µl) of semen was placed on a pre-warmed (37°C) slide, mix well with 40 µl of saline solution, covered by a coverslip and examined under phase contrast microscope (400X). Two investigators scored the sperm motility and the average scores were recorded. Only semen having > 1.000x10⁶ sperm concentration and > 70% of progresif sperm motility were selected for cryopreservation. The semen diluted with diluent to a final concentration of 100x10⁶ sperm/ml. Diluted semen were loaded into 0.25 ml straws (Minitube Germany) using automatic filling and sealing machine (Combo System, Minitube Germany), equilibrated at 4° C for 3 hours and freeze at automatic freezing machine (Digitcool 5300 ZB 250, IMV Prancis) for 9 minute and the straws were then plunged into the liquid nitrogen, and where stored until thawing (Hafez 2000).

c. Evaluation of post thawing quality and recovery rate.

After 24 hours of storage, the semen straws were thawed in a water bath (37° C for 30 second). Sperm motility evaluation was asses immediately after thawing.using a phase contrast microscope (Olympus BX 53) X 200 magnification with a warm stage maintained at 37°C. A wet semen mount was made using 5 µL semen placed directly on a microscope slide and covered by a cover slip. Motility estimations were performed from five different microscopic fields in each sample. All data of raw semen quality and post thawing motility and recovery rate were recorded, tabulated using Microsoft Excel 2007 and expressed presented as average and standard deviation.

Results and Discussion

Result showed the semen color ranged from milky white to creamy, moderate in density. The mean ± SD semen volume ranged from 5.16±1.04 to 5.98 ±1.39 ml, with progressive motility and sperm concentration were 70.00 to 72 ± 0.03% and 746.6 to 1.305.52×10⁶ sperm ml⁻¹

respectively (Table 1). The semen volume in this study was higher than semen volume of Maduras bull reported by Romadhoni *et al.*, (2014), which was only 3 ± 0.38 ml.

Table 1 The quality of Madura bull semen*

Semen Characteristics	Bull Number				
	161001	161002	161203	161204	161205
Volume (ml)	5.16 ± 1.04	5.34 ± 1.34	4.42 ± 1.00	5.98 ± 1.39	5.72 ± 1.17
Sperm Motility (%)	70 ± 0.01	70 ± 0.01	72 ± 0.03	70 ± 0.00	70 ± 0.00
Sperm Concentration ($\times 10^6$)	$1.071.75 \pm 267.81$	$1.160.53 \pm 280.48$	$1.305.52 \pm 228.77$	919.39 ± 210.79	746.67 ± 187.74

*185 ejaculate during 2015

The production of straw per semen collection is varies for each collection and each bull. The number of straws produce from single ejaculation can yield ranged from 163.33 ± 52.44 to 237.97 ± 93.58 straws (Table 2). Single ejaculation of bull, according to Bhakat *et al.* (2015) and Roy (2006) can yield 180 to 220.70 straws and in buffalo, entire year the frozen semen production can reach 3546.46 ± 540.30 straws (Ghudasara *et al.*, 2016).

Table 2 Number of straws yield per ejaculate and Recovery rate of Madura bull cattle

Bull number	Semen attribute			
	Raw Semen Motility (%)	Number of Straw per ejaculation	Post Thawing Motility (%)	Recovery rate (%)
161001	70 ± 0.01	209.40 ± 64.89	52.41 ± 16.27	74.87
161002	70 ± 0.01	237.97 ± 93.58	59.50 ± 23.37	85.00
161203	72 ± 0.03	218.09 ± 66.87	54.43 ± 16.73	75.59
161204	70 ± 0.00	209.00 ± 61.54	52.32 ± 15.41	74.74
161205	70 ± 0.00	163.33 ± 52.44	40.78 ± 12.96	58.26

Post thawing motility of frozen thawed Madura's bull semen was moderate (40.78 ± 12.96 %) for bull number 161205, and good for bull number 161001, 161203 and 161204. Bull number 161002 demonstrated the highest motility which was $59.50 \pm 23.37\%$. The success of freezing can also be seen by assessed its recovery rate (RR), by comparing the sperm motility of raw semen with post-thawing. The RR varies from 58.26 to 85.00% (Table 2). This result was higher than FH bull RR, reported by Arifiantini *et al.*, (2005) which were only 59.40 ± 11.24 to $69.56 \pm 11.32\%$. The success of spermatozoa cryopreservation depends on many factors, including individual (Härtlová *et al.*, 2013), the interaction between cryoprotectants and the extender type (Špaleková *et al.*, 2014; Stádník *et al.*, 2015). Present study revealed that semen characters of Madura's bull, number straw produced from single ejaculation are good and had a high percentage of recovery rate.

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