ASSESSMENT OF FRESH SEMEN QUALITY OF DOMESTICATED BANTENG (*BOS JAVANICUS* D'ALTON, 1823) IN SABAH, MALAYSIA

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ABSTRACT. *Banteng cattle have been domesticated in several places in the Southeast Asia. A domesticated form of Banteng, called 'Bali cattle' considered to be the same species and occurs widely in Indonesia and has been introduced to other areas of the world. Banteng cattle were classified as endangered species by IUCN Red List. In the past few years an effort has been made by Department of Veterinary Services and Animal Industry (DVSAI) Sabah, Malaysia in Banteng research for conservation and breeding program purposes. Electroejaculation technique was applied on fifteen Banteng cattle for semen collection. Collected fresh semen then analyzed for semen volume, pH, sperm concentration and motility. The objective of this study was to determine the fresh semen quality of domesticated Banteng cattle reared in Sabah, Malaysia. Results in this study show the semen volume was between 2.2 to 11.5 ml (mean; 6.28±2.3 ml), pH 6.63 to 7.48 (mean;7.01±0.24), sperm concentration between 30 to 470 x 10^6 sperm/ml (mean; 266 x 10^6 sperm/ml) and sperm motility between 10 to 70% (mean; 56.08±16.72 %). We conclude that data generated in this study were useful and important hedge against the complete loss of threatened populations of Banteng cattle, in making the decision for conservation and breeding program where the sources were very limited.*

KEYWORDS. Banteng, Bali cattle, semen quality

INTRODUCTION

The Banteng cattle (also known as Tembadau) are a species of wild cattle found in Southeast Asia. Wild Banteng is classified as endangered species by IUCN Red List because the decline part of species range more than 80% with overall decline of at least 20% over the last three generations (International Union for Conservation of Nature, 2012). The earliest documented report on the Banteng cattle was made by Schlegel and Muller in 1836 (Martojo, 2002). In their report, the Banteng cattle was found wild in small herds with a single bull and several cows and calves in the forests of Java and Kalimantan (Borneo). Banteng cattle were

observed in some parts of dense forest and bamboo jungles in Indochina, Borneo (Sabah, Sarawak, Kalimantan) and Java. There were estimated to be less than 5000 purebred of Banteng cattle recorded in the wild in Southeast Asia (Hedges, 1996). Because purebred Banteng cattle can interbreed with domestic cattle, there are questions about the purity of the genetic status of the wild and captive remaining population. Furthermore, previous study show Banteng cattle have been domesticated in several places in Southeast Asia and later known as 'Bali cattle' (Payne, 1970). The name 'Bali cattle' derives from the fact that the first domestication of this animal was carried out on the island of Bali (Darmadja, 1980). Martojo (2002) was reported the distinguishing difference between the Banteng and 'Bali cattle' is the size and some behavioural traits. Domesticated Banteng cattle can be found in countries such as Bangladesh, Brunei, Cambodia, India, Indonesia (Bali, Java, Kalimantan), Laos, Malaysia, Myanmar, Thailand and Vietnam. Banteng cattle were domesticated and breed as an alternative for meat production and working animals (Ismail *et al.*, 2010).

Briefly, domesticated Banteng cattle were similar in size to domestic cattle (eg; Kedah-Kelantan cattle), being 1.55 to 1.65 meter tall at the shoulder, and weighing about 600 to 800 kg. Previous records show, the weight gain of Banteng cattle for the first 6 months were 0.33 kg per day in male and 0.30 kg per day in female (Copland, 1974). Characteristic of Banteng cattle show both males and females have white stockings on their lower legs, a white rump, a white muzzle and white spots above the eyes. The build is similar to that of domestic cattle, but with a rather slender neck and small head and a ridge on the back above the shoulders. Report by Copland (1974) shows the length of gestation of Banteng cattle was at an average of 302.7 days. Interestingly, Andrews (1972) reported the Banteng cattle were able to maintain good body condition despite digest poor quality food or even under nutritional stress as compared to the same condition to domestic cattle. Further report by Toelihere (2002) stated that, even under poor environmental conditions, Banteng cattle were able to maintain their productivity by producing calves every year and quickly recover of the body weight after exposure to poor nutritional conditions or heavy work.

In Malaysia, an initiative by FELDA Farm Product Sdn. Bhd., a subsidiary of Federal Land Development Authority (FELDA) in breeding program was carried out by using the domesticated crossbred Banteng cattle or 'Bali cattle' in their ranching program in oil palm plantation (Johari and Jasmi, 2009; Ismail *et al.*, 2010). The cattle ranching in the oil palm plantation was found beneficially support both livestock and plantation sector due to high demand for meat. However, there are few publications on their fertility status, production and reproductive performances. It is the aim of this paper to present analysis on their semen which related to the fertility status of this breed.

MATERIAL AND METHODS

Semen collection

Fresh semen samples were collected from fifteen Banteng cattle bulls reared in Department of Veterinary Services and Animal Industry (DVSAI), Sabah. Electroejaculation technique was used as previously mentioned by Iswadi *et al*. (2012). Briefly, the electroejaculation technique was accomplished using an automated semen collection unit with automatic and manual settings (ElectroJac5, Ideal Instruments, Neogen Corporation, USA) and a 66 mm rectal probe with three ventrally oriented electrodes. The semen was collected as it was emitted from the preputial orifice into a graduated test tube.

Fresh semen analysis

Immediately after collection, the progressive motility was evaluated under a light microscope at 100x magnification. The volume of each ejaculated sample was measured in a graduated test tube. A Makler counting chamber (Sefi-Medical Instruments Ltd) was used to determine the sperm concentration. pH meter (Mettler Toledo, Switzerland) was used to measure collected fresh semen pH.

Statistical analysis

Statistical analysis was performed using SPSS 12.0 software (SPSS Inc., Chicago, IL). Data are expressed as the range (mean±s.d.). Correlation tests were used to determine the relationship among various groups. Difference between means was considered statistically significant at $P<0.05$.

RESULTS

Semen collection

Following electroejaculation technique by electrical stimulation, the semen was collected from all fifteen Banteng bulls. As a result, all Banteng bulls in this study achieved an erection with a voltage to range between 1 and 3 volt (mean; 1.61±0.58 volt), electroejaculation time to range between 20 and 164 seconds (mean; 66.74±36.42 seconds) and electroejaculator pulse to range between 7 and 30 seconds (mean; 20.65±7.05 seconds) (Table 1). During electrical stimulation, musculoskeletal movement consisted of hindlimb extension and pelvic thrusting which is typical when using electroejaculation. There was no evidence of rectal trauma by electrical stimulation in all those Banteng bulls. Based on the result of this study, voltage was found correlate significantly with electroejaculation time $r(21)=0.708$, $P\leq0.01$ and electroejaculator pulse $r(21)=0.496$, $P\leq0.05$.

Table 1. Results of observation and semen collection of Banteng bulls by electroejaculation technique.

Parameters	Range value (mean±s.d.)
No. of Banteng bulls (n)	15
Age (month)	17 – 50 (31.20±8.02)
Total no. of ejaculates	23
Body weight (kg)	204 – 309 (263.07±39.40)
Electroejaculator voltage (V)	1 – 3 (1.61±0.58)*, **
Electroejaculation time (sec)	20 – 164 (66.74±36.42)**
Electroejaculator pulse (sec)	7 – 30 (20.65±7.05)*

* Correlation is significant at $P<0.05$.

** Correlation is significant at $P<0.01$.

Banteng cattle observation

In this study, Banteng bulls were observed for their body weight and age. During the time of this study, the weight of Banteng bulls recorded to range between 204 and 309 kg (mean; 263.07±39.40 kg) and the age to range between 17 to 50 months old (mean; 31.20±8.02 month). Body weight was found correlate significantly with age $r(13)=0.577$, $P\leq0.05$.

Evaluation of fresh semen quality

Briefly, semen quality measured were based on semen volume collected range between 2.2 ml to 11.5 ml (mean 6.28±2.30 ml), pH level in collected semen in range from 6.63 to 7.48

(mean 7.01±0.24), sperm concentration in range from 30 to 710 x 10^6 sperm/ml (mean 254 x 10^6 sperm/ml) and progressive motility between 10 to 70% (mean 56.09±16.72 %) (Table 2).

Table 2. Fresh semen parameters of domesticated Banteng bull collected by electroejaculation technique.

No. of bull	Observation		Macroscopic				Microscopic	
	Weight (kg)	Age (month)**	No. of ejaculate	Color	Volume (ml)**	pH	Progressive motility (%)*	Sperm concentration (x10^6 sperm/ml)*
1	232	24	1(i)	Dark	6.5	7.19	50	140
			1(ii)	Creamy white	2.2	7.40	60	237
2	250	50	2	Dark	6.8	6.66	60	30
3	204	24	3	Creamy white	2.8	7.07	70	410
4	285	38	4	Creamy white	8.8	6.85	70	670
5	309	36	5(i)	Creamy white	8.8	6.90	70	290
			5(ii)	Creamy white	11.5	6.75	60	580
6	211	24	6	Creamy white	5.5	6.68	70	150
7	209	17	7(i)	Dark	3.5	7.13	10	30
			7(ii)	Dark	7.0	7.06	10	50
8	222	27	8	Creamy white	7.5	6.80	60	173
9	278	25	9(i)	Creamy white	7.0	6.63	70	710
			9(ii)	Creamy white	5.5	6.89	50	470
10	309	33	10	Creamy white	6.5	7.48	60	290
11	308	33	11(i)	Creamy white	3.0	7.13	70	76
			11(ii)	Creamy white	10.0	7.37	70	460
12	265	32	12(i)	Creamy white	5.0	6.93	50	372
			12(ii)	Creamy white	5.0	7.29	60	74
13	305	36	13(i)	Creamy white	5.5	7.25	60	170
			13(ii)	Creamy white	8.0	7.03	60	35
14	263	32	14	Creamy white	6.8	6.81	60	270
15	296	37	15(i)	Creamy white	4.3	6.94	40	120
			15(ii)	Creamy white	7.0	7.13	50	50
Mean	263.07	31.20	-	Creamy white	6.28	7.01	56.09	254.65
s.d.	39.40	8.02	-	-	2.30	0.24	16.72	209.84

* Correlation is significant at $P<0.05$.

** Correlation is significant at $P<0.01$.

Statistically, semen volume correlate significantly with age r(13)=-0.642, $P \leq 0.01$ and progressive motility correlate significantly with sperm concentration r(21)=0.439, $P \leq 0.05$. No significant correlation was found between; 1) age and pH, progressive motility, sperm concentration, 2) pH and progressive motility, sperm concentration. Semen in four out of fifteen Banteng bulls experience with high debris. One bull shows very low percentage (10%) of progressive motile sperm. Seven bulls show very high percentage (70%) of progressive motile sperm. No contamination of urine or blood was recorded in all semen ejaculated in this study.

DISCUSSION

The definition of the age puberty in bulls can be defined as the age at which spermatozoa are present in the ejaculate (Bearden and Fuquay, 1997). Banteng bull was reported reach puberty at the age of 12 to 18 months, at a body weight of 170.4 to 225.2 kg (Talib *et al*., 2002). Toelihere (2002) reported Banteng cattle reach puberty at around 600 days (20 months) or even longer at body weight of 140 to 165 kg. On the other hand, report by Purwantara *et al*. (2012) shows that Banteng cattle, both male and female reach puberty between 12 to 24 months of age at a body weight of 100 to 150 kg. Difficulty in measure the exact time of puberty in male occurs because the first differentiation of spermatogenic cells precedes the release of the first spermatozoa from the seminiferous tubules by a month or more (Hafez, 1974). Uniquely, Banteng bull that reach puberty can be recognized with the red hair on their body begins to darken. Despite calculating the age of the bulls, size and weight measurement were found more important in determining the onset of puberty and their relation to semen quality. Factors affecting the age and weight at puberty such as genotype, environmental conditions, temperature, nutrition and humidity were a major importance in the evaluation of semen quality in which puberty to be the major factor that dictates reproductive competence in bulls (Brito *et al*., 2002). Mathevon *et al*. (1998) reported the semen volume per ejaculates, sperm motility and concentration were improve with an advance in age of the bull. Study by Van Denmark & Free (1970) reported the semen volume was found increase with age and body weight. Furthermore, high correlation (0.90) was recorded between the body size and semen production (Mamabolo, 1999).

The electroejaculation technique is routinely used in many countries since it is considered to be a quick, safe and reliable procedure. Electroejaculators come in a variety of shapes and sizes, and are used for untrained animals. Electroejaculation technique was an alternative for semen collection despite welfare considerations is needed due to stress or pain of electroejaculation in many countries (Falk *et al*., 2001). Report by McGowan (2004) show the electroejaculation technique when performed by a skilled veterinarian is most likely to result in more than 95% of semen collection. In this study, we show that electroejaculation technique can be used for semen collection from Banteng bulls without cause trauma or side effects to the animals and resulted in 95.6% successful of semen collection.

Bearden & Fuquay (1997) reported that the semen volume per ejaculates, sperm motility and concentration were common criteria for semen quality evaluation. Immediately after the Banteng semen has been collected, the progressive sperm motility and sperm concentration are assessed. In this study, the results show the percentage of progressive motile sperm is about 10% to 70% in range. Our result is comparable with the report by Barth (2000) where an acceptable semen sample collected in the field by electroejaculation show to have between 40% and 59% of motile spermatozoa (60% to 69% for a good sample and 80% to 100% for a very good sample).

Semen volume is an important criterion in semen evaluation. The quality of the semen was reported may decrease as the total volume of the ejaculate increases (Huat, 1973). But, generally larger volumes mean more sperm. Matured bulls generally have a larger volume of ejaculate than the younger bulls. Therefore, age should be considered with respect to this parameter of semen evaluation. Previously, Dahmani (2012) mentioned the volume of the semen ejaculate will generally be between 1 and 8 ml, with most of the bulls usually provides 6 ml of ejaculate. Fiaz *et al*. (2010) estimated the average volume from a series of collections were 2.92±0.03 ml in Jersey bulls and 4.05±0.03 ml in Holstein Friesian bulls. Other study by Wells *et al*. (1976) reported the average semen volume collected to range between 0.5 to 9.5 ml (mean; 4.9 ml) in Hereford and 2.0 to 9.4 ml (mean; 3.3 ml) in Angus bulls. Study by Arifiantini *et al*., (2006) recorded the average ejaculated semen from ten domesticated Banteng bulls in the range between 2.24 and 9.63 ml (mean; 6.30±1.8 ml) which were comparable to the result of this study. The results of semen volume measurement were varies because it depends on various multifactor such as the function of the semen vesicle and sex glands, age, breed, collection frequency and feeding regime (Al-Hakim *et al*., 1984).

The color of bull semen was reported in ranges from an opalescent/watery to creamy white (Sarder, 2007). These ranges of color have been reported by a number of authors (Shaha *et al*., 2008; Sharma *et al*., 2012; Patel and Siddiquee, 2013) and apparently do not have any bearing on the semen quality. When the semen is of good quality, it has a milky or creamy white colour and is of poor quality when its colour is similar to watered milk (Dahmani, 2012). If the ejaculate is yellow, it may be contaminated with urine and if pink/red, there may be the presence of blood which may be due to adhesions or trauma that has occurred prior to collection. A black or dark sample may indicate debris such as dirt or manure contaminating the sample due to an uncleaned prepuce or the presence of pus.

Barth (2000) also reported in sperm concentration evaluation, the acceptable sperm concentration should exceed 250 x 10^6 sperm per ml (a good sample at least 400 x 10^6 sperm per ml and a very good sample at least 750 x 10^6 sperm per ml). Report by Arifiantini *et al*. (2006) shows the average sperm concentration from ten domesticated Banteng bulls to range between 720 and 2160 x 10^6 sperm per ml (mean; 1340±447.85 x 10^6 sperm per ml). As compared to this study, the sperm concentration is recorded between 30 to 710 x 10^6 sperm per ml which considered between low to very good sample. Even though the sperm concentrations were varies between individuals, the sperm concentration of 15 x 10^6 sperm per ml in fresh semen was reported to be necessary for a satisfactory fertilization rate when used in artificial insemination (Ballester *et al*., 2007). The concentration of the ejaculate was found related of several parameters such as the degree of sexual preparation of the bull, the age of the bull, the time of year the collection is made, the amount of sexual rest before collection, the health of the bull, the nutritional state, inherent sperm storage and the production capacity of the bull (Brito *et al*., 2002). Since it is well known that there is a direct relationship between the number of sperm inseminated and fertility, it is important that concentration estimates are determined accurately. An over estimate may result in over dilution of the sperm and consequently reduced conception rates. The accurate measurement of semen is important when semen is to be used in artificial insemination or processed for freezing. This is of course subject to animal variation and we would suggest using higher concentrations unless semen from individuals was field tested for fertility rate versus sperm concentration.

The progressive motility of sample is defined as that percentage of the sperm in a sample that swim in a more or less straight-forward direction. Circular or reverse motion of the sperm indicates problem to the reproductive performance of the bulls. The progressive motility was determined by examining a drop of diluted semen to visualize the individual cells. Average progressive motility recorded in this study was higher than the acceptable

cutline of progressive motility that was used by many breeding societies which must not be less than 50% (Parkinson, 2004). Dahmani (2012) reported that the good quality of semen usually has 40 to 50 % sperm with progressive motility with vigour of continuous progressive movement (moderate speed) to progressive (rapid movement). Good progressive motility of spermatozoa was an indicator of both unimpaired metabolism and intactness of membranes (Johnson *et al*., 2000). Furthermore, estimation of motility has fundamental importance in daily quality control of semen collection.

In this study, the pH of the semen is measured at the time of semen collection and was made by using a pH meter. Based on our results, the pH of freshly ejaculated semen of Banteng bulls was between 6.63 and 7.48 (mean; 7.01±0.24). Roberts (1986) reported that the pH of seminal plasma recorded ranges from 6.7 to 7.4 and were common in the domestic species. If the pH of the ejaculated semen was acidic, that may not indicate poor quality since highly active sperm samples produce lactic acid as a metabolic waste product (Aghangari, 1992). However, anything that alters the pH of the semen above or below the normal range will affect the sperm ability to move and finally cause sperm death.

Analysis of semen quality has a major influence to fertility status of Banteng bulls and crucial for successful of breeding programs. Previous records by Devendra *et al*. (1973) and Kirby (1972) stated the fertility rate of domesticated Banteng cattle reaches around 80% or even up to 90-100%. The most appropriate assessment of reproductive performance or fertility will vary depending on whether emphasis is placed on semen quality or differences among females. The usefulness to measure fertility of a semen sample accurately is controversial and correlations between sperm motility and fertility have revealed large ranges of variation (Januskauskas *et al*., 2003). Evaluation of a single parameter of semen quality was stated not effective predictors of fertility. Thus, Gil *et al*. (2005) reported a combination of several parameters may provide a better prediction of fertility. The testing of a large number of parameters should lead to a higher accuracy because fertilization is a multi-factorial process (Amann, 1989). However, most of semen quality analyses are expensive and time consuming and cannot be applied routinely under field and/or commercial conditions where the instant making decision for pre-selection of the bulls is needed.

CONCLUSION

Under intensive conditions in a hot, humid environment with poor quality food, Banteng cattle may be the most suitable type of cattle. It should also be clear that we still have much to learn with respect to semen quality required for optimum fertilization in breeding programs of Banteng cattle. There is no current method to accurately determine the minimum semen quality providing optimum fertility for a given male, or precisely indentify all sub-fertile males in the population. Bull fertility status is important across the whole cow herd as bulls can affect reproductive success across several females. This problem is exacerbated in single-sire herds where an infertile bull leads to reproductive failure of the cow herd which will results in economic loss to the farmers.

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