

Semen Characteristics of Banteng (*Bos javanicus*) Collected by Electroejaculation Method

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Keywords: banteng, semen, electroejaculation, artificial insemination.

INTRODUCTION

Banteng (*Bos javanicus*) is listed as endangered species in IUCN Redlist. Only few thousand wild banteng survive and their numbers are decreasing as a result of hunting and the loss of habitat due to deforestation and conversion to agricultural land. One of the conservation measures is to preserve this species in ex-situ breeding center. The breeding centre with the largest number of individuals is Ragunan Zoo in Jakarta, with 21 animals. Despite those numbers of individuals available, the sex ratio was far from ideal at 16 males and five females. While in Taman Safari Prigen, there were with 16 individuals and a sex ratio of five males and 11 females [1] Since number of males are usually less than females, and also to avoid inbreeding in order to keep high heterozygosity population, therefore, assisted reproductive technology such as artificial insemination is considered to be necessary.

For artificial insemination, semen collection sufficient quantity and quality of semen from males are needed. Thus, best semen collection method should be considered. Though transrectal massage procedure does not require the male to be anesthetized prior to semen collection, semen collected by this technique contains urine contamination. Besides, volume of semen sample obtained by transrectal massage is lesser compared to electroejaculation. Electroejaculation is also suitable for wild animal because in this particular semen collection procedure, males are not required to be trained.

The information about semen characteristics of banteng bulls collected by electroejaculation is less known. To date, study done by Johnston *et al.* (2002) is the only available data. That study only provided data from one semen sample from one 4-year-old banteng bull located at Western Plains Zoo [2]. Moreover such volume parameter and consistency was not reported from that study. Thus, this study is aimed

to provide more reliable data from larger number of semen samples collected by electroejaculation and to add information to previous data that are available.

MATERIALS AND METHODS

• Animas and Husbandry

The study was undertaken at Taman Safari Indonesia Cisarua, Bogor, Indonesia and Ragunan Zoo, Jakarta, Indonesia. The study used two banteng bulls (B#1 and B#2) at Taman Safari Indonesia, Cisarua, Bogor and one bull (B#3) at Ragunan Zoo. Four ejaculates samples (S-1B#1, S-2B#2, S-3B#3, S-4B#3) were collected from 3 banteng (*Bos javanicus*) with age of 4-15 years old and body weights of 400-600 kg.

• Semen Collection

Prior to semen collection, the bulls were anesthetized by the authorized zoo veterinarian using combination of ketamine (1 mg/kg) and xylazine (0,5 mg/kg). As bulls were anesthetized, they were restrained in lateral recumbency. Immediately, after the bulls were anesthetized preputial hairs were clipped. The preputial area was then washed with soap, rinsed with clean water and rubbed with towel. Inner portion of the preputium was flushed with sterile physiological saline, then rubbed gently with paper towel. Semen collection was performed by the authorized veterinarian using electroejaculator immediately after anesthesia. Electroejaculator rectal probe was lubricated then inserted into the anus until 20 cm in depth and directed against the pelvic floor. Each stimulus lasted for 5 s and was followed by a pause of 5 s. The stimulus was repeated until penile erection appeared followed by ejaculation. Electroejaculation was attempted once per banteng bull, except one banteng bull at Ragunan Zoo, the electroejaculation was attempted twice with one month of interval.

• Semen Evaluation

Ejaculates were evaluated immediately after collection. Four ejaculates collected from 3 banteng bulls were evaluated either macroscopically and microscopically.

Macroscopically, semen samples were evaluated for volume, consistency, color and pH. The semen volume was measured by reading the scale on collection tube, the colour assessed by eye. Semen pH value was examined using pH indicator paper (Merck, Germany). Microscopic parameter of semen characteristics were assessed subjectively. Semen samples were evaluated for progressive motility, velocity, viability, sperm concentration and percentage of morphologically normal spermatozoa. Progressive motility was subjectively assessed by placing semen sample with physiological saline on glass slide. The percentage of live sperm and the percentage of morphologically normal spermatozoa were determined using eosin-negrosin staining. Sperm concentration was determined by calculating spermatozoa per 1 milliliter semen, counted using Neubauer chamber.

RESULT AND DISCUSSION

Electroejaculation was successfully done in 2 banteng bulls at Taman Safari Prigen, Indonesia and one banteng bull at Ragunan Zoo, Indonesia (Figure 1). The result of macroscopic and microscopic evaluation of semen obtained are shown in Table 1. Semen samples collected by electroejaculation showed various results which were dirty white, milky white, and cream in colour. This finding agreed with Johnston *et al* (2002) that reported banteng bull semen had watery milk in colour [2]. Semen consistency obtained from three bulls in this study was vary from watery to moderately thick.

study were 6,4-6,8 in range, while the mean±SE value for this parameter was 6,625±0,10. This result was comparable with previous study that reported pH value of 6,6 [2].

Velocity scores obtained in this study were close in agreement with earlier report although one sample showed lower score [2]. The mean±SE value of percentage of viability of banteng bull sperm were lower compared to that of Bali bulls [4]. Some samples were lower in percentage of viability compared to tprevious report [2].



Figure 1 Semen collection of banteng bull using electroejaculator. (a) Rectal probe was inserted through the anus of anasthetized male banteng, (b) Semen sample was recovered inside collection tube.

Tabel 1 Macroscopic and microscopic characteristics of semen in banteng (*Bos javanicus*) subjected to electroejaculation

| Sample No. | Macroscopic evaluation | | Microscopic evaluation | | | | |
|----------------|------------------------|-------------------|--------------------------|------------------|-------------------|---|----------------------------------|
| | Volume (ml) | pH | Progressive motility (%) | Velocity score | Viability (%) | Sperm concentration (10 ⁶ /ml) | Morphologically normal sperm (%) |
| S-1B#1 | 4 | 6,8 | 25 | 3 | 63,5 | 230 | 67,22 |
| S-2B#2 | 7 | 6,5 | 25 | 2 | 70,09 | 510 | 63,2 |
| S-3B#3 | 6,8 | 6,4 | 75 | 4 | 78 | 1240 | 81 |
| S-4B#3 | 7,4 | 6,8 | 70 | 4 | 76,5 | 1100 | 86,75 |
| Mean±SE | 6,3±0,78 | 6,625±0,10 | 48,75±13,75 | 3,25±0,48 | 72,02±3,32 | 770±239,62 | 74,54±5,58 |

Volume of semen samples that were successfully collected in this study ranged from 4-7,4 ml with mean±SE value of 6,3±0,78 ml. This result was lower compared to the volume of semen collected by electroejaculation from closely related species, Bali bull, which had mean±SE value of 6,98±0,29 ml (4). The pH value of the semen in this

The lowest sperm concentration in this study comparable with previous finding [2]. However, the mean±SE value of sperm concentration in this study which was still above the sperm concentration of semen from Bali bulls [4]: Iswadi *et al* (2007) had reported sperm concentration of 400 million to 10200 million sperm ml from gaur semen collected by

electroejaculation. That range was considered as very good sample [3].

Percentage of progressive motility and viability were vary among samples and relatively lower than semen volume reported by previous study which were 70% and 83%, respectively [2]. Percentage of progressive motility was also vary among samples. This finding also reported by Iswadi *et al.* (2007) that found the percentage of progressive motility that vary from 16% to 70% in range from gaur semen collected by electroejaculation [3].

Morphologically normal sperm is necessary for fertilisation. In this study, percentage of morphologically normal sperm ranged from 67,22 to 86,75, with mean \pm SE value of 74,54 \pm 5,58. The highest value of sperm concentration of this study was higher than previous study which was only 77% [2], The mean value \pm SE was also higher compared to those in Bali bulls [4].

CONCLUSION

The present study has successfully provided data of semen characteristics of banteng collected by electroejaculation method with larger number of samples. The result will be useful for assisted reproductive technology in this species and for further research on male reproductive biology.

ACKNOWLEDGMENTS

The authors would like to thank Taman Safari Bogor, Indonesia and Ragunan Zoo, Jakarta, Indonesia for the permission to publish the data. This study is supported by USAID through Sustainable Higher Education Research Alliances (SHERA) Program-Center for Collaborative Research Animal Biotechnology and Coral Reef Fisheries (CCR ANBIOCORE) and Directorate General of Higher Education (DGHE) Indonesia through the PMDSU Study Grant Program.

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