

The use of transrectal massage combined with artificial vagina as semen collection in javan banteng (*Bos javanicus*) bull

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Abstract. Semen collection in wild animals may be challenging for some methods such as electro ejaculator may cause pain and distress to the animals. Transrectal massage has been widely used in domestic and wild animals semen collection. Even though an artificial vagina may provide a condition that mimics the female reproductive tract and known to provide better results in some species, the application may face obstacles in the field. This study was conducted to evaluate the possibility of modified semen collection in banteng bull by combining the transrectal massage method followed by the use of an artificial vagina. The bull was habituated and trained for semen collection in chute under veterinarian supervision in Taman Safari Indonesia, Cisarua, Bogor, West Java. Semen samples from one Javan banteng bull were collected once a week. Banteng bull showed stud and penile protrusion during semen collection. Watery semen with volume ranged from 1-2 ml were successfully collected, with sperm progressive motility ranged from 25%-40%. Our study also showed sperm abnormalities comprising 18.11% abnormal sperm heads, 28.82% abnormal sperm tails and 0.94% teratoid form. The results demonstrated the possibility of this modified method as an alternative procedure for semen collection in banteng bull which may also be useful for other wild animals in field conditions

Keywords: artificial vagina, banteng, semen, transrectal massage.

1 Introduction

Banteng is one of the endangered animals as listed in IUCN Redlist. Conservation strategy for Javan banteng, to be specific, is regulated in PerMenHut No. P.58/MENHUT-II/2011 in Indonesia. One of the strategies is to meet the international population targets as many as 300 individuals descended from 30 individuals without interfering with the population in nature. On the other hand, inbreeding should also be avoided to maintain biodiversity. Semen collection has been done in several wild animals as one of the methods to preserve biodiversity, especially in endangered animals.

Previously, some papers reported banteng semen collection using electro ejaculator [1,2]. Electroejaculator had also been applied to other species such as mouse deer, black bear, red deer [3,4,5]. Because pain and distress caused by electroejaculation in conscious animals are welfare concerns, this protocol should always be done under general anesthesia. Nevertheless, the study had reported that yak semen obtained with electroejaculation was not better than that obtained with artificial vagina [6]. Artificial vagina offers promising semen quality and

quantity in bull breeding soundness for domesticated bulls, but it is not popular for wild animals that are not docile. Besides, this original protocol needs dummy cow or female to perform. On the other hand, transrectal massage had been used as semen collection in several animals closely related to banteng, which were Mithun and gaur [7,8]. This method is considered to be easier and simpler to perform since it needs no expensive tool like electro ejaculator, dummy cow or female presence and needs a shorter time to train. Some disadvantages to being underlined in this method are the bull did not always show penile protrusion during the semen collection and the semen was lacking in sperm [9,10]. Thus, in this study, we tried to combine transrectal massage with an artificial vagina. We wanted to see the possibility of this modified method to collect semen from banteng in the field.

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2 Materials and Methods

2.1 Habituation process

One banteng bull aged around 2.4 years old, weighed about 530 kg was used in this study and habituated by the zookeeper under the supervision of a veterinarian at Taman Safari Indonesia, Cisarua, Bogor, West Java. Banteng was trained to enter modified chute by putting certain foods as baits at the end of the chute. Once the banteng bull entered the chute, the veterinarian would gently put his hand into the banteng's rectum to make the bull get used to per rectal palpation. This procedure was done repeatedly once a week until the bull feels comfortable and stay still for semen collection.

2.2 Semen collection

First, all materials needed for semen collection were prepared. Once banteng entered the chute, its preputium was washed using a warm saline solution and wiped using a paper towel. Semen collection was done by massaging the ampulla of different ducts until the bull showed signs of response such as penile protrusion. Artificial vagina featured with a collection tube was then directed near the penis to let the penis penetrate it. After semen was ejaculated, the artificial vagina was pulled back slowly then the semen was evaluated macroscopically and microscopically. Semen collection was conducted once a week.

2.3 Semen evaluation

Semen samples were evaluated macroscopically for its color, volume, and consistency. Microscopically, semen samples were evaluated for its mass motility and progressive motility. All these evaluations were done in the field soon after collection. For morphological evaluation, one drop of semen diluted with four drops saline solution was placed on the glass object, smeared and air-dried. Two smeared samples per each period of collection time were then transported to Reproduction Rehabilitation Unit (URR) at IPB University for staining and evaluation. A minimum of 500 cells was evaluated for sperm head, tail, and teratoid form abnormality.

2.4 Statistical analysis

Data from macroscopic evaluation, mass, and progressive motility were shown descriptively. Data from morphological evaluation from two smear slides per period of the collection were shown as the mean value.

3 Results and Discussion

According to this study, the banteng bull was adapted enough for semen collection after approximately five months of the habituation process. Banteng bull showed docility to enter the chute and stay still during semen collection. The length of the period needed for an animal to be adapted to repeated treatment or certain repeated

stimuli may vary among species. This also can be influenced by the frequency of the animal exposed to the habituation process. A study had reported semen collection in rhino that took only two months of habituation until the semen could be collected [11]. Semen collection approach also can be considered as another factor, where semen collection involving general anesthesia administration may not need the habituation process.

As shown in Figure.1, penile protrusion was observed during semen collection with an artificial vagina combined with transrectal massage. This event demonstrated, that although there was no dummy or trained female available, semen collection with an artificial vagina can still be performed. Transrectal massage directed to the ampulla of deferent duct stimulate semen to be released to the ampullae. Usually, semen would only dribble down the preputial cavity and hair, which may affect semen quality [9]. In this study, an artificial vagina acted as a sexual stimulation by providing temperature and consistency that mimic the female reproductive tract. Once penile protruded from the preputium, it entered the artificial vagina, then became fully extended as in natural mating. To our knowledge, there were no reports available for semen collection protocol that combines transrectal massage with an artificial vagina. But, some reports demonstrated the use of transrectal massage before electroejaculation as an additional stimulant [12, 13].



Figure 1. Penile protrusion observed during semen collection

Physiologically, ejaculation is the result of sensory stimulation in the glans penis. This stimulation causes muscles to contract coordinately [14]. The time required for penile protrusion in bull stimulated with transrectal massage was 80.08 seconds [12] which was longer compared to the recent study. Palmer *et al.* (2005) reported that mean semen emission time needed for semen in bull collected with transrectal massage was 103 seconds (75-182 seconds). In that study, the threshold time determined for transrectal massage was 4 minutes.

The longer mean duration of semen collection with transrectal massage had been reported [15], which took about 7.4 minutes. In a recent study, first pressure from the bull towards the artificial vagina was at 39.08 seconds after transrectal massage stimulation and then followed by a quite strong stud at 53.67 seconds inside the artificial vagina with a total time of procedure was 3 mins 49 seconds. This may explain that adding artificial vagina to the standard transrectal massage protocol affected the duration of semen collection. One observation of this subsequent physiological event of ejaculation in detail is shown in Table 1.

Table 1. Physiological events of ejaculation observed in banteng

| Physiological Events | Duration |
|--|-----------------|
| 1st penile protrusion from the preputium | 03.08 secs |
| 2nd penile protrusion from the preputium | 19.92 secs |
| Penis started to extend | 39.08 secs |
| 1st muscle contraction (1st stud)* | 53.67 secs |
| 2nd muscle contraction (2nd stud) | 01 min 27 secs |
| 3rd muscle contraction (3rd stud) | 01 min 36 secs |
| 4th muscle contraction (4th stud) | 02 mins 55 secs |
| 5th muscle contraction (5th stud) | 02 mins 59 secs |
| 6th muscle contraction (6th stud) | 03 mins 8 secs |
| Massage stopped | 03 mins 26 secs |
| Penis exited from the artificial vagina | 03 mins 46 secs |
| Penis retracted back to preputium | 03 mins 49 secs |

From all the attempts, four semen samples had successfully been collected within 4 weeks of the collection period. Fresh semen samples characteristics per week are shown in Table 2. Semen samples volume obtained in this study were comparable to semen ejaculated in natural mating which ranged from 0.5-12 ml, with a mean of 3-5 ml [14]. A previous study [15] reported mean semen volume collected with a transrectal massage in beef bulls was 2-6 ml (1-9 ml). Lesser volume had been reported in Mithun as reported [7] which only 0.75 ml. Colors of the semen samples in this study were not different compared to the previous study in banteng [1, 2]. Mass motility was not observed in all of the period of the collection in this study. Progressive motility of sperm in this study was lesser compared to previous studies [1, 2, 16, 17]. The difference may be explained by the difference in the method used. According to previous studies, motility was not affected by the method of collection [12]. There was no significant difference between sperm progressive motility collected with an artificial vagina with transrectal massage [15]. But, [9] showed that percent of motile sperm collected with transrectal massage was lower compared to electroejaculation.

For morphological abnormality, we found several types of sperm head abnormalities which were abnormal contour, detached head, knobbed acrosome,

macrocephalus, microcephalus, narrow at the base, pyriformis, round head and tapered. Sperm tail abnormalities found in this study were as follows: abaxial tail, abnormal midpiece, accessory tail, bent principal piece, coiled tail, cytoplasmic droplet, dag-like defect, double tail, and tail stump. The mean value of abnormal sperm heads, abnormal sperm tails and teratoid forms in this study were 18.11%, 28.82%, and 0.94%, respectively. Several abnormalities were categorized as major abnormalities in which the present may interfere with bull fertility, while some of the others were related to genetic [18, 19]. Compared with previous studies, this study provides a more specific count on abnormalities. Banteng sperm with normal head morphology and normal tail morphology were 99% and 73.5%, respectively [17], which were higher compared to normal morphology in a recent study. Abnormal sperm heads and abnormal sperm tails were not significantly different among transrectal massage and artificial vagina [9]. Thus, the difference between the result in this study compared to the previous study in the same species may reveal individual variation that may be affected by the age of the banteng used.

Table 2. Macroscopic and microscopic evaluation of banteng semen collected with combination transrectal massage and artificial vagina

| Parameter | Period of collection | | | | |
|-------------|----------------------|-------------|-------------|-------------|--------|
| | Week I | Week II | Week III | Week IV | |
| Macroscopic | Volume | 1 ml | 1 ml | 2 ml | 2 ml |
| | Color | Transparent | Milky White | Transparent | Cream |
| | Consistency | Watery | Watery | Watery | Watery |
| | Mass motility | - | - | - | - |
| Microscopic | Progressive motility | 30-40% | 40% | 30-35% | 25-30% |
| | Abnormal sperm heads | 18.37% | 18.16% | 18.70% | 17.23% |
| | Abnormal sperm tails | 43.22% | 19.86% | 33.59% | 18.60% |
| | Teratoid forms | 1.37% | 1.40% | 0.58% | 0.39% |

4 Conclusion

In this study, we demonstrated the possibility of this modified method as an alternative procedure for semen collection in banteng bull. This method will be useful in the field where females or dummy does not always present. Besides, this method does not require general anesthesia and still provide the possibility to see physiological events of ejaculation.

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