SHORT COMMUNICATION

SEMEN COLLECTION: A MALAYAN GAUR BULL EXPERIENCE

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Malayan gaur can be identified by their dark brown coat, lower legs being white to tan in color and weights over 1000 kg in male and 700-800 kg in female (Figure 1 and 2). In both sexes, the horns grow from the sides of their head and curve upwards. A bulging grey-tan ridge can be seen on the forehead connecting the horns and the forehead. Shoulder humps are present in adult males but none in adult females (PERHILITAN, 2009). The gestation period of the gaur in nature is about 310 to 314 days. Normally, the adult female gaur gives one calf per pregnancy. Genetically, the gaur have a chromosome complement of 2n=56 (Maslinda *et al.,* 2010).

An improvement in the management system for genetic conservation via semen collection, analysis and storage with regards to animal breeding and husbandry would result in an increase in the number of gaur in the wild (Hafiz *et al.,* 2010; Iswadi *et al.,* 2010,2012). On the other hand, increased research activities on breeding management programs such as synchronization of estrus, improvement of semen collection, semen evaluation and cryopreservation, genetic studies and increase in the use of assisted reproductive biotechnologies could be useful in increasing gaur production without interrupting their natural habitat in the wild (Fazly Ann *et al.,* 2010, 2011; Hafiz *et al.,* 2011, Iswadi *et al.,* 2011, 2012; Rosli *et al.,* 2011). Therefore by focusing on the gaur bull, the aim of this paper is to outline different techniques of semen collection

from the wild gaur to aid in their genetic conservation.

To our knowledge, limited documents have been published on the collection and analysis of the Malayan gaur semen. Therefore, this study was conducted which emphasis in using various techniques for semen collection which were transrectal massage (TM), electroejaculation (EEJ) and combination of transrectal massage-electroejaculation (TM-EEJ). Attentions were given to the number of animals used, method of semen collection and the quality of semen collected.

The semen of three matured wild Malayan gaur bulls in Seladang Conservation Center, Jenderak Selatan Wildlife Conservation Centre, Department of Wildlife and National Parks, Peninsular Malaysia were collected and analyzed. In this study, the semen was collected using three techniques which are TM, EEJ and TM-EEJ. In the TM technique, the operator carefully inserts a hand into the rectum and then alternately massages the ampullae firmly and then rhythmically strokes the urethralis muscles. The EEJ technique is performed by using the electroejaculator, 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 (Iswadi *et al*., 2012). The TM-EEJ technique on the other hand was performed with the operator inserting a hand into the rectum and then alternately massaging the ampullae and then uses the electroejaculator.

All Malayan gaur bulls in this study achieved an erection. We successfully collected the semen using all the TM, EEJ and TM-EEJ techniques. Using the TM technique, the gaurs showed very slow musculoskeletal muscle movement and penile erection. An observation during EEJ and TM-EEJ, the gaurs showed rapid musculoskeletal movement consisting of hind limb extension and pelvic thrusting which is typically seen by using EEJ. There was no evidence of rectal trauma by applying TM, EEJ and TM-EEJ technique in all three Malayan gaur bulls.

The semen was collected as it was ejaculated from the preputial orifice into a graduated test tube. Immediately after collection, the volume of each sample was measured in a graduated test tube. The progressive motility and sperm concentration was evaluated under a light microscope at 100× magnification using glass slide and Makler counting chamber (Sefi-Medical Instruments Ltd) as previously mentioned by Iswadi *et al.* (2010). The morphology of sperm was determined in at least 200 sperms using eosin-nigrosin staining protocol as previously mentioned by Hafiz *et al.* (2010).

Since only three Malayan gaur bulls were used in this study, the sampling size may not provide representative results. Due to difficulties in getting the large population of endangered gaurs in the wild,

Figure 1. Malayan gaur bull.

Figure 2. Female Malayan gaur.

Table 1. Analysis of fresh semen collected from Malayan gaur bulls using TM, EEJ and TM-EEJ technique (Mean data shown).

Observation criterion	TM technique	EEJ technique	TM-EEJ technique
Musculoskeletal movement	Slow	Rapid	Rapid
Penile erection	Yes	Yes	Yes
Time required for penile erection	Long (≥30 min)	Short (<30 min)	Short (<30 min)
Rectal trauma	No	No	No
No. of semen samples collected	12	26	7
Semen volume (ml)	0.3-11.0 (2.7)	0.5-6.0 (2.2)	0.2-8.5 (2.2)
Semen pH	8.22-8.42 (8.32)	6.58-7.95 (7.56)	6.58-7.80 (7.34)
Progressive motile sperm (%)	<10*	16-70	30-70
Semen concentration ($x10^6$ sperm/ml)	700-2350	105-3400	450-2350
Sperm viability (%)	30	86	80
Normal morphology (%)	70	87.5	85

* Semen was contaminated with urine and debris.

we managed to get only three matured wild Malayan gaur in this study.

Based on this study the semen volume recorded ranged 0.3-11.0 ml for TM, 0.5-6.0 ml for EEJ and 0.2-8.5 ml for TM-EEJ. Progressive motile sperm ranged from 16-70% for EEJ and 30-70% for TM-EEJ. Less than 10% of progressive motile sperm recorded in TM was due to contamination by urine and debris. Viable sperm recorded was about 30% in TM, 86% in EEJ and 80% in TM-EEJ. Normal morphology ranged from 70% in TM, 87.5% in EEJ and 85% in TM-EEJ (Table 1).

Many factors influence the quality of individual ejaculates of semen including interval time since the last ejaculation, sexual preparation and kind of semen collection technique and equipment used (Foote, 1978). The kind of semen collection technique and equipment affects the reservoir of sperm stored in the excurrent ducts and influence the semen characteristics. It is well established that characteristics of bull semen vary widely, not only between bulls but also between ejaculates within bulls from time to time (Nadaraja, 1967) and also the type of technique used for semen collection (Foote, 1978). Semen volume, concentration and motility of sperm are recognised as important indices of semen characteristics (Saacke, 1984). Semen producing ability is essential to ensure the supply of quality germplasm for increasing the production performance of future gaur progeny in the country.

Data on semen collection is important as a baseline for field and laboratory purpose. Thus, data generated from this study will be useful in breeding programs that would contribute to wild gaur genetic conservation. Furthermore, breeding biotechnology such as artificial insemination and semen cryopreservation is highly dependent on the successful collection of semen. The conservation and evaluation of gaur semen will provide valuable data for wild gaur conservation management practices in the future.

As a conclusion, based on the results of this study, we suggest to use the EEJ and TM-EEJ techniques for semen collection in Malayan gaur bull.

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