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Collection and characterization of semen in Mithun (*Bos frontalis*) bulls

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Abstract

The objective of this study was to collect semen from semiwild Mithun (*Bos frontalis*) bulls using an artificial vagina (AV) and to determine semen characteristics. Collection of semen with an AV was attempted in five Mithun bulls using both anestrous and estrous Mithun females. No Mithun bull mounted an anestrous female Mithun during 60 trials, but satisfactory mounting, including extension of the penis, occurred in 25 trials with estrous Mithun females. In 15 of these trials, semen was successfully collected in an AV with an internal temperature of 42 to 46 °C. However, in 10 trials with an AV with an internal temperature of 36 to 40 °C, semen was not collected. Mean (± SEM) intervals to first mount and to ejaculation in the AV were 27.9 ± 3.6 sec and 113.8 ± 6.6 sec, respectively. Semen volume and pH were 3.1 ± 0.35 mL and 6.59 ± 0.04, and mean mass activity (scale, 0 to 4), initial sperm motility, live sperm count, sperm concentration, total number of sperm in the ejaculate, and overall sperm length were 2.2 ± 0.3, 78.6 ± 2.6%, 80.7 ± 2.2%, 710.8 ± 66.8 × 10^6/mL, 2114 ± 364.4 sperm, and 67.9 ± 0.6 μm, respectively. The proportion of morphologically normal sperm was 80.6 ± 0.2%, whereas the proportion with a morphologically abnormal head, midpiece, tail, and acrosome were 4.2 ± 0.4%, 1.6 ± 0.5%, 6.1 ± 1.1%, and 7.1 ± 0.9%, respectively. The mean incidence of tail-less heads and proximal and distal protoplasmic droplets were 0.5 ± 0.1%, 0.3 ± 0.2%, and 2.4 ± 0.3%, respectively. In conclusion, we successfully collected semen from semiwild Mithun bulls with an AV maintained at 42 to 46 °C, and overall, the semen was within the normal range of that collected from fertile domestic bulls.

Keywords: Artificial vagina; Breeding soundness evaluation; Mithun bulls; Semen collection and evaluation; Sperm characteristics

1. Introduction

The Mithun (*Bos frontalis*), a semiwild species found in the hilly tracts of the northeastern states of India, namely Arunachal Pradesh, Nagaland, Manipur, and Mizoram, belongs to the family Bovidae, order Artiodactyla, and class Mammalia [1]. It plays an important role in the socioeconomic life of the people of this region living at an altitude of 2700 to 3000 m above sea level [2]. Only recently has the attention of animal scientists been drawn toward economic exploitation of this unique animal.

Evaluation of semen is an important tool in determining the breeding potential of a bull. Semen collection with an artificial vagina (AV) apparently

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yields the most representative sample of a bull's semen. Although semen has been collected from Mithun bulls by transrectal massage [3], there is apparently no published report regarding semen collection with an AV in this species. Therefore, the objective of the current study was to determine the feasibility of semen collection from Mithun bulls using an AV and to assess semen characteristics.

2. Materials and methods

2.1. Animals and location

Five apparently healthy Mithun bulls, approximately 4 to 6 yr of age, were selected from the herd derived from various hilly tracts of the northeastern region of India. The average body weight of the bulls was 501 kg (493 to 507 kg) at 4 yr, which increased to 530 kg (523 to 538 kg) at 6 yr of age. The bulls were housed at the National Research Centre on Mithun, Jharnapani, Nagaland, India.

2.2. Climate

The National Research Centre on Mithun has a cold climate with abundant rainfall. According to the Meteorological Observatory of the Indian Council of Agricultural Research (Jharnapani, Nagaland), minimum and maximum average temperatures are 25 and 31 °C during summer and 9 and 25 °C during winter. Rainfall and relative humidity varied from 9.4 to 289.5 mm and from 72.5% to 83.8% when the study was conducted (April to October).

2.3. Bull feeding and management

The animals were maintained under a semi-intensive system of management. They were provided with concentrate (4 kg/animal per day), evenly distributed in the morning and evening, and were allowed to graze on pasture in the intervening period. The ingredients of the concentrate included crushed maize, wheat bran, rice bran, and mustard cake, with 9%, 15%, 12.4%, and 35% crude protein, respectively. However, crude protein of the concentrate mixture was 19%. Each bull was also fed 500 g of chick peas (*Cicer arietinum*, containing 20% crude protein) daily in the morning. The bulls had access to drinking water ad libitum.

2.4. Training

To enable semen collection, all five bulls were trained to mount a Mithun. The AV used was identical to that used for domestic bulls, based on a previous report that the length of the penis of a Mithun bull was similar to that of buffalo bulls [4]. Initially, the bulls were introduced to the site of semen collection. An anestrous female Mithun (of an appropriate size) was placed inside a service crate at the collection site, and each bull was brought there daily and made to approach the female. The anestrous female teaser in the crate was subsequently replaced with another in an attempt to increase the bull's interest. When possible, an estrous female Mithun was used in the service crate in lieu of the anestrous female, and the bulls were allowed to approach and mount it, but coitus was prevented. The estrous female in the crate was again replaced by an anestrous female Mithun. Thus, the Mithun bulls repeatedly had exposure to either an estrous or anestrous female at the same site of collection.

2.5. Semen collection and evaluation

A total of 60 trials, 12 from each Mithun bull, were done for collection of semen with an AV, using an anestrous female as a mount animal. Further, a total of 25 trials were made using an AV to collect semen from the bulls utilizing an estrous female. In 10 trials, the internal temperature of the AV was maintained at 36 to 40 °C (average, 38 °C), whereas in the remaining 15, it was between 42 and 46 °C (average, 44 °C). Temperature of the AV was measured within the lumen with a breeder's thermometer, 5 min after the AV was assembled.

The interval to the first mount and to ejaculation were recorded [5]. Semen color was deemed milky or creamy, and pH and ejaculate volume were recorded [6]. Mass activity of semen and initial sperm motility were noted [7], the proportion of live sperm was assessed [8], and sperm concentration was determined with a hemocytometer using bright-field microscopy [6]; mass activity was assessed under × 100 magnification, whereas all other end points were assessed under × 400 magnification. Sperm biometry was conducted [9], and morphologic abnormalities were enumerated under oil immersion (×1000) with bright-field microscopy [10]. Acrosomal morphology was studied [11] with phase-contrast microscopy using Giemsa stain under oil immersion (×1000). However, for the purpose of sperm counting with respect to live sperm, sperm biometry, head abnormalities, midpiece and tail abnormalities, acrosomal morphology and other abnormality, a total of 200 sperm were counted for each character independently, and the value was expressed as a percentage.