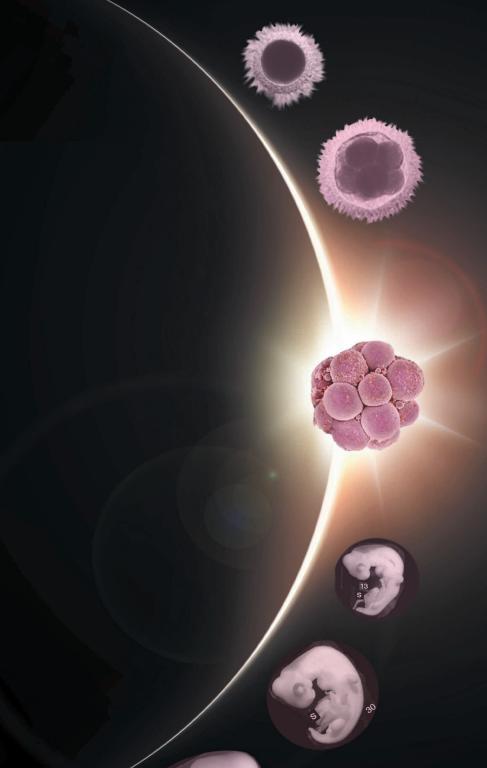


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Assessment of Male Fertility in Endangered Banteng, Gaurs and White Rhinoceros: A Case Report

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¹ Bureau of Conservation, Research & Education, Zoological Park Organization under the Royal Patronage of H.M. the King ² Khao Kheow Open Zoo, Zoological Park Organization under the Royal Patronage of H.M. the King *Corresponding author: ampialaska@hotmail.com **Keywords:** banteng, gaur, semen quality, sperm morphology, white rhinoceros

Introduction

Wild population endangered megaherbivores such as gaur (Bos gaurus), banteng (Bos javanicus) and white rhinoceros (Ceratotherium simum simum) has been declining due to natural habitat loss and overhunting. There are good members of captive population in zoos which can be a valuable resource for research and conservation programs such as gamete banking and reproductive biology research. However, some of these endangered animals fail to propagate offsprings due to unknown causes even complete copulation was observed. Clinical examination of reproductive organs and assessment of semen quality and sperm morphology would certainly be necessary for diagnose male fertility and further sperm preservation. Moreover, recent advances in the development of assisted reproductive technologies show feasibility to overcome reproductive problems and genetic diversity (2). The objective of the study was to assess semen quality and sperm morphology in captive banteng, gaurs and white rhinoceros.

Materials and Methods

Mature banteng (n=1, estimated body weight 500 kg), gaurs (n=2, estimated body weight 400-500 kg) and white rhinoceros (n=1, estimated body weight 2000 kg) in Khao Kheow Open Zoo (KKOZ) were anesthetized for physical examination and semen collection (Fig 1).

Banteng and gaurs (gaur 1 and 2) were anesthetized using two different formulas

of anesthetic drugs. A combination of thaifentanil (A3080), medetomidine HCl, and ketamine HCl was used in banteng and gaur 2. Gaur 1 was anesthetized by a combination of carfentanil, xylazine HCl and ketamine HCl. Rectal probe (7 cm in diameter) three longitudinal with electrodes was inserted and placed on the base of accessory sex glands. Three series of stimulation was conducted by using an electro-stimulator (P.T. Electronics, Boring, OR) starting at 2 V (1-2 stimulations) and gradually increased 0.5 V of each stimuli up to 10 V (18-36 stimulations per series).

A male white rhinoceros was anesthetized by using a combination of medetomidine hydrochloride, butophanol tartrate and ketamine hydrochloride. Ketamine HCl and/or Guaifenesine was given IV to maintained throughout the procedure. A modified rectal probe (11.5 cm in diameter) was used. Stimulation was performed for three series. Each series was started at 2 V (2-5 stimulations) and gradually increased to 8 V (2-5 stimulations).

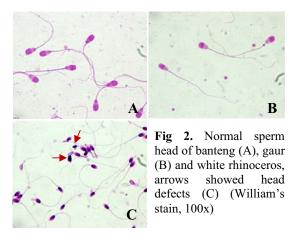
Ejaculated semen was divided for sperm evaluation and cryopreservation. Sperm viability was assessed by eosin-nigrosin staining. William's staining was used for assessing head morphological abnormality. Fresh semen was fixed in formal saline for assessing tail morphological abnormality.

Results and Discussion

Semen characteristic and sperm morphology of banteng, gaurs and white rhinoceros were shown in table 1. Semen quality and sperm morphology of all animals except gaur 2 were normal. Semen of gaur 2 was diagnosed low sperm motility and low sperm concentration. Sperm morphology of banteng and gaur was similar, but different in white rhinoceros, which had smaller head (Fig 2). Major abnormalities of sperm morphology were coiled and bent tails. All semen samples except that from gaur 2 could be cryopreserved.



Fig 1. Electroejaculation in banteng (A), gaur (B) and white rhinoceros (C). Penis characteristic of white rhinoceros (D).



The present study shows the first time of anesthesia and semen collection in white rhinoceros in Thailand. Ejaculated semen of banteng, gaurs and white rhinoceros was successfully collected after general anesthesia. Semen quality of all animals except gaur 2 was in range of normal data in previous reports (4, 5). This information is valuable for fertility assessment in

captive male banteng, gaurs and white rhinoceros, which can benefit breeding management. Furthermore, ejaculated semen of the animals in the study was successfully preserved successfully and kept in the wildlife semen bank. Frozen semen would be useful for aiding low genetic diversity which found bottle necked situation of banteng and gaur populations in some areas (1, 3). Suitable semen extender, freezing protocol and postthawed success i.e. motility, viability and physiological function of the sperm will be reported in the future study.

In conclusion, anesthesia and semen collection protocols in the study could be used safely for physical examination and male fertility assessment in banteng, gaurs and white rhinoceros. Semen of these endangered species could be cryopreserved for future research and applied in reproductive technologies such as artificial insemination and *in vitro* fertilization.

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Table 1. Characteristics of ejaculated semen of captive banteng, gaurs and white rhinoceros

Species Parameters	Banteng	Gaur 1	Gaur 2	Rhino- ceros
Semen volume (ml)	24.6	5.7	7.1	19.3
Semen pH	7.5	7	8.5	8.5
Sperm concentration (x106spermatozoa/ml)	365	2,020	35	765
Sperm motility (%)	80	90	50	80
Alive sperm (%)	78.5	85.5	83	86.5
Normal head morphology (%)	99	98	95.5	94.5
Normal tail morphology (%)	73.5	84.5	65.5	78.0