Correlation between Vaginal Cytology and Serum Progesterone in Sumatran Rhinoceros (*Dicerorhinus sumatrensis*)

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Introduction

The Sumatran rhinoceros (*Dicerorhinus sumatrensis*), with a population of less than 400 in the world, is the most critically endangered of all the five existing rhino species. Its distribution that used to range from northern India to Borneo is now fragmentarily found in Sumatra, Peninsula Malaysia, and Sabah. In other parts of South-East-Asia, such as Sarawak, Thailand, Myanmar, and Laos, their existence has yet to be confirmed and the viability of any population is unlikely. Poaching for horns, habitat degradation for timber and land conversion into agriculture, industries and other human uses are major threats to these rhinos (WWF, 1996). Worldwide, captive breeding for over a hundred years has yet to produce a progeny. Aggressive courtship behaviour may be the major factor for difficult breeding in Sumatran rhinos. Such occasion usually occurs due to untimely introduction of the male to a female that is not in oestrus (Zainal-Zahari et al., 1995). In order to determine oestrus, various methods including plasma hormonal assay (Zainal-Zahari et al., 1995), excretory metabolites assay (Heistermann *et al.*, 1998; Wasser *et al.*, 1996; Schwarzenberger *et al.*, 1993; Hindle *et al.*, 1992) and ultrasonography (Schaffer, *et al.*, 1994) have been deployed. These methods may although proved to be effective, are usually costly, tedious and require professional handling. Thus, vaginal cytology, a fast, inexpensive and an easy method that had been proven effective in dogs (Wright et al., 1990; England, 1992), sheep (Zourgui *et al.*, 1976), Yucatan pigs (Rodgers *et al.*, 1993) silver foxes, *Vulpes vulpes* (Boue *et al.*, 2000) was employed on Sumatran rhinoceros to test for its applicability in detecting oestrus in this species.

Materials and Methods

The research was carried out for six months at the Sumatran Rhinoceros Conservation Centre, Sungai Dusun, Malaysia. Four adult female rhinoceros (R_1, R_2, R_3, R_4) were the subjects of our study. Collection of exfoliative vaginal cells was carried out twice a week in accordance with blood sampling for hormonal assay for a period of six months. The animal was coaxed into a stanchion and then restrained. Once the tail is directed to one side, the vulva is thoroughly cleansed with mild chlorhexidine and water, and subsequently dried using a sponge. A size 18-rubber Foley catheter, measuring 35 cm with a metal stilet inside, was inserted gently into the vagina as far as the fornix. The stilet was immediately removed and a syringe filled with physiological saline was attached to the catheter. The anterior end of the vagina was flushed with 20-40 ml of the physiological saline and re-infused 2-3 times before being aspirated and transferred into a calibrated capped tube. Then, the aspirated fluid was centrifuged for 10 minutes at 1,600 r.p.m. to sediment the epithelial cells.

The supernatant was discarded, leaving only 0.1-0.2 ml of the mixture. A drop of the mixture was placed on a slide to make a smear. Two smears were made from each sample and air-dried. Prior to staining, the air-dried smear was rehydrated with distilled water for 5 minutes and then fixed with 70% ethanol for 3 minutes. The first 200 cells were counted, identified and classified into parabasal, small intermediate, large intermediate, superficial and cornified based on Schutte's classification (Christie *et al.*, 1972). Eosinophilic Index (EI) of each smear was obtained using the formula by (Mestre *et al.*, (1990). Blood samples were collected from these females at an interval of three to four days throughout the period of this study. The site of blood collection was the medial coccygeal blood vessels. Blood samples were centrifuged within one hour after collection for 15 minutes at 2,500 r.p.m. Plasma was aspirated and stored in a labeled cryovial at –20°C pending analysis. Plasma progesterone concentration was measured using a commercial kit, Coat-A-Count® Progesterone (Diagnostic Products Corporation, Los Angeles, California 90045-5597, U.S.A.) by radio immunoassay.

Results and Discussion

A total of 330 smears from the four subjects were examined and five types of epithelial cells were described (parabasal, small intermediate, large intermediate, superficial, and cornified), based on Schutte's cell classification (Christie *et al.*, 1972). Parabasal, small intermediate and large intermediate cells were classified as non-keratinised cells. The superficial and cornified cells were grouped as keratinised cells as they contain keratin precursors (characteristic of oestrus), and thus stained red with Modified Shorr's trichrome.

Results of vaginal cytology indicated that the oestrous cycle of Sumatran rhinoceros was 22±6.36 days, compared to 28±7.64 days as indicated by serum progesterone radio immunoassay. From eosinophilic curves and serum progesterone profiles shown in Figures 1, 2, 3 and 4, oestrous cycle of each subject was irregular, with the exception of R_2. Statistical analysis using SPSS programme showed that correlation between vaginal cytology and serum progesterone was weak (0.235). However, when statistical analysis was done on individual animal, correlation coefficient (r) between the two reached as high as 0.745 in R_1, 0.408 in R_2, 0.183 and 0.172 in R_3 and R_4 respectively. The eosinophilic

index (EI) averaged at 57.42±12.96. Of the twelve peaks recorded in the four animals, two animals showed three first EI peaks followed by second peaks, 13.33±2.08 days after the first highest peak. For these subjects, the percentage of non-keratinised cells ranged between 49.1 and 65.0 per cent.

Although the period of study was for six months, due to breeding programme and stress factors, sampling from subjects ranged from 121 to 204 days. Cell types, shape, size and staining properties in vaginal cytology of the Sumatran rhinoceros is similar to that reported in bitches (Concannon et al., 1986) and cows (Miroud et al. and Noakes, 1990).

Occurrence of EI peaks were noted when progesterone profile was at its lowest range, which could possibly be the oestrus stage. However, this profile was inconsistent, EI peaks had occurred when progesterone profile was at a relatively high level, 0.79 ng/ml and 0.97 ng/ml respectively. This may account for the weak correlation coefficient (0.235) between vaginal cytology and progesterone profile. Also, irregularity of oestrous cycles, variations of EI and progesterone concentrations between individuals and within different oestrous cycles of each animal led to the weak correlation.

A higher frequency of sampling may give us a better picture of the reproductive status of these animals. However, due to fear of imposing great stress on the animals, samplings were limited to only twice per week. Furthermore, presence of large cysts and tumours in the uteri of most of the females in this study (Schaffer *et al.*, 1994) may also influence reproductive abilities of these animals.

Conclusions

Results showed that vaginal cytology was not a reliable method to detect oestrus in Sumatran rhinoceros. Accuracy of oestrus detection may increase with frequency of sampling though stress may also hamper their reproductive abilities. Furthermore, the reproductive status of these animals was not established. A number of matings had not resulted in pregnancy, not to mention production of viable offspring. Thus, further study on the reproductive status of these animals should be pursued.

Benefits from the study

The benefits obtained form this study is the knowledge that exfoliative vaginal cytology is not a fast and reliable technique to determine the oestrous cycle of this Malaysian endangered species. Since the Sumatran rhinoceros is considered near extinct, it is imperative that we pursue a research on superovulation and embryo transfer of embryos, and on the collection and cryopreservation of Sumatran rhinoceros semen.

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Project Publications in Refereed Journals

None.

Project Publications in Conference Proceedings

None.

Graduate Research

Choong Siew Shean. 2002. Wild life reproduction. [M.V.Sc.]. Universiti Putra Malaysia.

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