Semen collection in the Sumatran rhinoceros (*dicerorhinus sumatrensis*, Fischer 1814) for breeding attempt to sustain biodiversity

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Abstract

Sumatran rhino is the most endangered rhino species. Population is less than 300 individuals estimated to remain in the wild with highly declining rate to 50% in the last 15 years. Assisted reproduction technology such as semen collection and cryopreservation or artificial insemination are new methods in conservation of rhinoceros. The objectives of this study was a) to determine the male reproductive status, b) to establish a reliable semen collection methods and c) to assess semen parameters of the fresh collected sample. Three methods for semen collection were compared to determine one male rhinoceros fertilizing potential: a) stimulated combination of artificial vagina (AV), penile massage (PM) and accessory gland massage (AGM), b) AV and PM, c) only with PM. AV combined with PM and AGM gave the best semen collection result with an ejaculation success of 85.71% (6/7, n=7). The combination of AV with PM and PM only obtained an ejaculation success rate of 40% (2/5, n=5) and 33.33% (1/3, n=3), respectively. Full penis erection can be induced in 80% (8/10, n=10) of the collection when stimulated in the morning. Stimulation in the afternoon resulted in only 60% (3/5, n=5) of collection in a full erection. However regardless of the daytime, the ejaculation success rate was equivalent, 60% (6/10, n=10) in the morning and 60% (3/5, n=5) in the afternoon. The collected ejaculates has a volume of 1-12.4 ml, colored purify turbid to cream turbid and pH 6.90 – 6.99. Semi quantity concentration was 0.05 – 0.1 x 10^6 sperm/ml. Motility was very weak with forward slow motion scored from 0 to 1. 80% of spermatozoa were immature (prox. cytoplasmic droplet) with head (macro-, microcephalic) and tail (broken tail) abnormalities. Semen quality increased after semen collection has been conducted for several times, sperm concentration increased to approximately 0.2 – 0.25 x 10^6 sperm/ml and the amount of immature sperm decreased to 5%. In conclusion, the combination of AV, PM, and AGM showed better results for semen collection compared to other collection methods. Repeated semen collection increased semen quality, although the male has low fertilizing capacity due to low sperm concentration (oligozoospermia) and small volume of ejaculate (oligospermia).

Key words: Sumatran rhino, semen collection, semen quality, oligozoospermia, oligospermia

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Introduction

The Sumatran rhinoceros is well known as the most critically endangered rhino species among the rhino species in the world (Anonymous, 2004a). The population in natural habitat is only 300 individuals surviving with very high declining rate about 50% of the population lost in the last 15 years. The animal is also known as absolutely solitary and also very slow breeders (Anonymous, 2004b). Most of the breeding programs in captivity were not successful since 100 years ago, until two offspring were recently born in Cincinnati Zoo in 2001 and 2004 (Foose, 2004). Moreover, than 80% of captive population that kept at the Zoo and Sanctuary in England, USA, Indonesia and Malaysia died since 1989. Beside very limited number of captive population, there is also only a few healthy pair of rhinos kept in the captivity (Anonymous, 2003).

General problems that could be appeared in all Sumatran rhinos due to limited information and knowledge on the reproductive biology of the species (Foose, 1995), such as violent behaviour displayed when male and female rhinos were put together not in the proper time for mating (Anonymous, 2004c), they will fight seriously and it can result in an injury or death animals, and some pathologies were found in the reproductive organs of some animals (Schaffer, *et al.*, 1994 and 2001). In the other hand, there is almost no information available on the reproductive potency of the male rhinos. Therefore, captive breeding programs of the Sumatran rhinos is facing many problems that affected its success to produce offspring including the rhinos in SRS Way Kambas.

Since there is no pregnancy recorded from the female in Way Kambas, despite many mating have been occurred until now. Therefore, there is an urgent need to assess the reproductive potency of the male (or Torgamba) since the female is performed to have clearly a regular pattern of reproductive cycle which is already approved by using hormone analysis and ultrasound scanning. In order to possible analyzing semen quality of Torgamba, it is fortunately required the development of a reliable semen collection methods, since there is no methods performed for the Sumatran rhinoceros. Therefore, the research was conducted in order to support success breeding programs of the species, with the objectives were (1) to establish a reliable semen collection methods, (2) to assess semen parameters of the fresh ejaculates, and (3) to determine Torgamba's reproductive potency.

Material and Methods

For the research, we used only one male Sumatran rhino available is named Torgamba, he is about 26 years old and the rhino is kept in pair in the Sumatran Rhino Sanctuary, Way Kambas National Park, Indonesia. The study was conducted in two parts. The first part is to compare an application of different semen collection techniques i.e., massage technique, artificial vagina technique, and electroejaculation technique. Massage technique, where focused on accessory gland massage (AGM) in order to stimulate accessory gland secretion and ejaculation and penile massage (PM) in order to stimulate penile erection and ejaculation (Schaffer, *et al.*, 1990). While artificial vagina (AV) is conducted in order to stimulate an ejaculation (Pickett, *et al.*, 1987). However, electroejaculation technique is used to stimulate penile erection and ejaculation through electrical stimulation (Howard, *et al.*, 1983). The study was conducted to compare several combinations of the techniques that might be reliable for the Sumatran rhinoceros. First combination is to combine all three techniques, second combination is just penile massage and AV, and third was with penile massage only, while electroejaculation technique was conducted separately without combination with any other techniques. The combination of all three techniques was conducted as follow: first step, someone was to stimulate accessory gland through rectal palpation, and the second person massaged the penis until it was erected. As soon as the penis erected then artificial vagina was inserted to the penis. While the penile massage was conducted continuously through putting regular pressure to the artificial vagina until an ejaculate was obtained (for max. 45 minutes) and it will be stopped when the ejaculate was not obtained after 45 minutes. Moreover, the second combination was conducted with the same procedures as described above but only for penile massage and AV, while the third technique was only done to massage the penis. Electroejaculation technique was using electroejaculator machine with electrical voltage and current used within 3-10 volt and 50-350 mA, respectively. The second part of the study was semen analysis, the analysis was conducted that was only suitable to be done directly in the field, such as (a) macroscopic analysis for assessing volume and pH of ejaculates, and (b) microscopic analysis for sperm concentration, morphology and abnormality assessment.

Result and Discussion

Although only limited numbers of ejaculates could be obtained, the study provided the first data on characterization of fresh ejaculate obtained from semen collection methods in the

Sumatran rhinoceros. Combination of AV, PM, and AGM method is the best semen collection method with 85.71% (6/7, n=7) of the collection can obtain an ejaculate. The other collection methods have less successful, combination of AV and PM can only provide 40% (2/5, n=5) ejaculate, and the lowest result 33,3% (1/3, n=3) of ejaculate was obtained by single stimulation PM. The collected ejaculates had a volume of 1- 12.4 ml (*Oligospermia*) (Schaffer, *et al.*, 1990), colored purify turbid to cream turbid and pH 6.90-6.99. Semi quantity concentration was 0.05 0.1 x 10^6 sperms/ml. Motility was very weak with forward slow motion scored from 0 to 1. 80% of the spermatozoa were immature *(prox. cytoplasmic droplet)* with head (*macro-, microcephalic*) and tail (broken tail) abnormalities. Semen quality increased after semen collection has been conducted for several times, sperm concentration increased to approximately 0.2-0.25 x 10^6 sperms/ml and the amount of immature sperm decreased to 5%.

Semen collection was same whether it was collected in the morning or in the afternoon, ejaculation has been obtained about 60% (6/10, n=10) and 60% (3/5, n=5) respectively. Although, full penile erection was obtained 80% in the morning and only 60% obtained in the afternoon. Continue semen collection has relatively improved semen quality.

Semen collection using an open system of artificial vagina (*Hannover type*) could not be carried out because it is difficult to purchase the inner-liner of artificial vagina. As substitute to the technique, semen drippings from vagina and penis after copulation has been collected. Ejaculate from natural copulation and electroejaculator was also *azoospermia*, although the ejaculation could produce about 12-20 ml semen. Using electroejaculation technique could obtain 34 ml of ejaculate. Electroejaculator technique could stimulate an optimal erection and ejaculation process. Electroejaculator technique could stimulate to produce higher volume ejaculate compare to other techniques, it due to high stimulation on semen plasma secretion (Hafez, 2000). The rhino has improved spermatogenesis process during the rainy season, it showed when an ejaculate collected post coitus contained life and motile sperm although it was *oligozoospermia.*

Conclusion

The conclusion revealed from the study that were (1) the study has provided the first data on the characterization of the fresh ejaculates obtaining from semen collection methods. (2)

although only small numbers of ejaculates have been obtained, but the results showed that a combination of AGM, PM and AV yielded a higher success rate in stimulating ejaculate compared to the other collection methods, (3) however in comparison to other rhino species by using the same methods, the volume of ejaculate and sperm numbers were low, and finally (4) the result of study has indicated that Torgamba appears to have low fertilization capacity as result of low sperm concentration, so called *oligozoospermia* and small volume of the ejaculate, so called *oligospermia*.

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