Estrous Cycle Based on Blood Progesterone Profiles and Changes in Vulvar Appearance of Malayan Tapirs (*Tapirus indicus*)

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Abstract. The progesterone (P4) profiles and macroscopic vulvar changes of female Malayan tapirs were investigated in order to understand their fundamental reproductive physiology and to search for visual indicators of estrus. Blood was collected once or twice a week from seven female Malayan tapirs kept at four zoos. Serum or plasma P4 concentrations were determined by radioimmunoassay. The P4 concentrations changed cyclically throughout the years, and a total of 56 cycles was confirmed in the seven females. The length of the estrous cycle based on the P4 profiles was 43.6 ± 2.0 days; however, this mean includes great variation in length, from 21 to 84 days. Mucous discharge from the vulva and vulvar swelling were seen when the P4 concentrations were low before the beginning of a rise in most cases. In conclusion, captive female Malayan tapirs have variations of approximately 1 to 3 months in estrous cycle length, and visual changes in the vulva are helpful in estimating estrus in female Malayan tapirs.

Key words: Estrous cycle, Malayan tapir (*Tapirus indicus*), Mucus, Progesterone, Vulva

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ing in captivity, the current status of Malayan tapirs in captivity is not good. As of April 2006, a total of 154 Malayan tapirs are registered at International Species Information System (ISIS) registration facilities [7]. However, there has been a trend towards a decrease in the number of captive populations in recent years. According to the Japanese Studbook of Tapirs [8], 29 Malayan tapirs are kept at 9 zoological institutes in Japan (December 31, 2004), the number of births from 1999 to 2004 was approximately one per year and the number of deaths was one or more per year. To maintain the Japanese captive population is proving difficult at present, because the numbers of parous females and pairs showing copulation behavior are limited. Recently, with the goal of improving captive breeding in Japanese zoos, females were housed together with a male only during estrus in consideration of their lifestyle, which in the wild is solitary except during estrus. Therefore, it is important to determine when females are in estrus. It has been reported that the estrous cycles of all 4 species in captivity are approximately one month in length [1, 5]. These cycle lengths have been presumed based on the interval of courtship and/or copulatory behaviors. If neither courtship nor copulation is observed, it is difficult to detect estrus in the female [9].

There are limited reports concerning the endocrinology of the estrous cycle in tapirs. In Baird’s tapirs, longitudinal profiles of serum progesterone (P4) and estradiol have been investigated during the estrous cycles and pregnancy [10], and the estrous cycle in Brazilian tapirs has been determined based on plasma P4 profiles [12]. Only recently, a short communication was published concerning the estrous cycle of one Malayan tapir based on the profiles of plasma P4 and estradiol [13]. Only three reports seem to be available concerning the endocrinological estrous cycle of tapirs.

In the present study, the longitudinal profiles of the blood serum/plasma P4 concentrations were monitored to understand the fundamental reproductive physiology, in particular the estrous cycle, of the Malayan tapir. In addition, the relationship between P4 profiles and macroscopic vulvar changes was investigated to determine the utility of vulvar observation as a visual indicator of estrus.

### Materials and Methods

**Animals and management**

A total of seven female Malayan tapirs kept at the Tama Zoological Park (TZP), Yokohama Zoological Gardens (YZG), Preservation and Research Center, City of Yokohama (PRC), and Nagoya Higashiyama Zoo (NHZ), Japan, were studied for a period of 3 months to 4.5 years (Table 1). The animals were fed a daily diet of timothy or alfalfa hay, fresh grass, branches and leaves, pellets, sweet potato, carrots, apples and other components (bananas, cabbages and/or sliced bread). Drinking water was available ad libitum. Female #44 was housed with a male from September 13, 2002 to December 7, 2003 and on a few other days; female #61 was housed with a male from February 1, 2002 to February 23, 2004 and on a few other days. Female #62 were weighed weekly or monthly since she was new to the PRC.

Table 1. Individual information for the female Malayan tapirs investigated in this study

<table>
<thead>
<tr>
<th>Individual No.</th>
<th>Name</th>
<th>Age</th>
<th>Birth history</th>
<th>Facility</th>
<th>Period of blood sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>#14 2)</td>
<td>Yumeko</td>
<td>23</td>
<td>Uniparous</td>
<td>TZP</td>
<td>Sep 3–Dec 20, 2003</td>
</tr>
<tr>
<td>#44</td>
<td>Koro</td>
<td>12</td>
<td>Nulliparous</td>
<td>TZP</td>
<td>Sep 24, 2001–Dec 13, 2003</td>
</tr>
<tr>
<td>#56</td>
<td>Ume</td>
<td>9</td>
<td>Nulliparous</td>
<td>PRC</td>
<td>Mar 7–May 25, 2001</td>
</tr>
<tr>
<td>#60</td>
<td>Mimi</td>
<td>8</td>
<td>Nulliparous</td>
<td>YZG</td>
<td>Jul 23–Oct 29, 2001</td>
</tr>
<tr>
<td>#61</td>
<td>Maya</td>
<td>5</td>
<td>Nulliparous</td>
<td>YZG</td>
<td>Sep 23, 2001–Apr 23, 2004</td>
</tr>
<tr>
<td>#86</td>
<td>Sachiko</td>
<td>2</td>
<td>Nulliparous</td>
<td>NHZ</td>
<td>Jan 14, 2005–Feb 19, 2006</td>
</tr>
</tbody>
</table>

1) Japanese Studbook of Tapir. 2) Died as a result of decrepitude at 23 years of age (December 21, 2003). 3) At the beginning of study. 4) Estimated. 5) Successful breeding after this study period. 6) TZP: Tama Zoological Park; PRC: Preservation and Research Center, City of Yokohama; YZG: Yokohama Zoological Gardens ZOORASIA, Japan.
血小板收集

操作性条件技术 [14] 用于从七只马来貘收集血小板。所有马来貘对训练作出了反应，并学会了因身体刷洗和按摩而卧倒或侧卧。血小板每星期或每两周收集一次。当每只马来貘被鼓励采取侧卧姿势时，从前肢的cephalic静脉或后肢的medial saphenous静脉抽取血样，无需物理或化学约束。从雌性#14，#44，#60，#61和#86（TZP，YZG，NHZ）的血清和雌性#56和#62（PRC）的血浆分离血清并储存在-30°C备用。

放射免疫分析

血小板P4被乙醚提取，血小板的水平通过放射免疫分析确定，如先前所述 [16, 17]。大于97%的P4在提取后被恢复。本实验所用的抗血清的交叉反应性α-P4为100%，5α-pregnanedione为62.2%，pregnenolone为3.88%，11-deoxycorticosterone为2.25%，17α-hydroxyprogesterone为1.23%，11α-hydroxyprogesterone为1.23%。基于下限的实验范围为<30 pg/ml。每个实验的内和交叉反应系数的变异分别为7.9和16.2%。

观察外阴外观

雌性#44和#61的阴道和阴唇肿胀被观察和记录，作为0分（正常阴道）和1分（阴唇和阴道分泌物）或2分（大分泌物或肿胀）的分数。一个动物饲养员几乎每天同时观察雌性以保持侧卧姿势。雌性的评分1或2不能标准地在两个雌性之间比较，因为这是根据每个检查点所见的每个饲养员可视化确定的。

数据分析

数据以平均值±标准误表示。当雌性P4浓度第一次上升到下一个周期的水平时，可以计算出发情周期的持续时间。

结果

血小板P4浓度

图2展示了雌性#44，#44，#60，#61和#86（TZP，YZG，NHZ）的血清和雌性#56和#62（PRC）的血浆浓度变化。P4浓度的变化显示了周期变化；例如，雌性#44（图2a）和#86（图2f）保持了全年的周期。雌性#44，在8个周期（图2a）中观察到配种前P4的升高。雌性#61的血清P4在2002年10月开始时保持在基线水平。从2002年12月24日到2003年4月30日，雌性#61因皮肤病、营养不良和意外贫血及肝硬化而接受治疗。在没有医疗干预的期间，#61的血清P4浓度呈周期性变化，但未与雄性配种。雌性#62的血清P4水平仅在大约4.5年的时间内观察到七次（图2g）。然而，雌性#62在大约1年的时间内没有进行血液收集，所以可能未包含周期。雌性#62在图2g中确认了第一个周期，该雌性在2000年6月-7月（大约2年）时体重为62.0 kg，在大约3-4年的年龄时体重达到约300 kg。

图1。雌性马来貘#44的外阴外观。 (a) 分数0：正常外阴（2002年4月6日）。 (b) 分数1：阴道分泌物和阴道肿胀（2002年3月2日）。
A total of 56 cycles were determined for the seven females by serum/plasma P4 profiles, and the mean cycle length was 43.6 ± 2.0 days (n=56; range, 21–84 days). The individual distributions of the cycle lengths of the seven females are shown in Fig. 3.

**Relationships between vulvar appearance and progesterone cycle**

The relationship between vulvar appearance and serum P4 profile for females #44 and #61 is shown in Fig. 4. In most cases, mucous discharge from the vulva and/or vulvar swelling were observed at lower levels (average of 1.24 ± 0.05 ng/ml) of serum P4. However, during some cycles, changes in vulvar appearance were observed at a high P4 concentration (average of 2.70 ± 0.20 ng/ml), in the midst of the luteal phase or within periods in which serum P4 decreased (Fig. 4).
Discussion

This study is the first to demonstrate longitudinal profiles of serum/plasma P4 and individual P4 data for a group of Malayan tapirs. Monitoring of serum/plasma P4 was an effective method to determine the estrous cycle of the Malayan tapir, as confirmed by copulations at lower P4 levels. Most of the visual changes in the vulva, such as mucous discharge and vulvar swelling, were also observed in connection with lower P4 levels.

We investigated seven females between 2–23 years of age at the beginning of the study. Except for the data until 4 years of age (January, 2003) in female #62 and until 2.3 years of age (March, 2005) in female #86, all other data for the animals revealed constant cyclical changes in serum/plasma P4. It has been reported that sexual maturity is usually reached in the Malayan tapir at 2–4 years of age or 5 years of age at the latest in captiv-
The estrous cycle length of Malayan tapirs has been estimated to be 29.4 [18] or 35 days [19] based on the intervals between copulation and/or courtship behaviors. There are also reports of cycle lengths of between 28–101 days [9] and 2 months [20]. The records of estrous cycles that are 2 or 3 times longer than about one month have been considered to be due to unobserved sexual behaviors or silent estrus. However, the differences in these cycle lengths might not be due to failure to observe behaviors because the long type of estrous cycle (maximum length: 84 days) was also observed in our study. Our report is one of only a few to clarify the blood P4 profiles in order to determine the actual estrous cycle length of the tapir. Only three reports concerning endocrine profiles during the estrous cycles of tapir species were previously available (Baird’s, Brazilian and Malayan tapirs). The estrous cycle lengths of Baird’s and Brazilian tapirs based on serum/plasma P4 were 30.8 days (mean of two females) [10] and 30.2 and 29.7 days (individual means of two females) [12], respectively. In contrast, the Malayan tapirs in our study had a mean cycle length of 43.6 days (21–84 days; seven tapirs). The length of the estrous cycle based on the plasma P4 profiles of one aged female Malayan tapir has been reported to be 59.25 days (54–63 days; n=4 cycles) [13], and this is within range of the cycle length of our study. The estrous cycle lengths of Malayan tapir are longer than those of American tapir species, as described previously (approximately 1 month) [10,12], and vary from approximately 1 month to 3 months. The long estrous cycle, characterized by an extended luteal phase, has been reported in rhino species [21, 22]. Garnier et al. [21] categorized the estrous cycle of the black rhinoceros (Diceros bicornis) as 26.8 (normal type) and 46.7 days (luteal phase extended type) based on fecal pregnane profiles. Patton et al. [22] classified the estrous cycle of the white rhinoceros (Ceratotherium simum) as 35.4 (normal type) and 65.9 days (luteal phase extended type) based on fecal pregnane profiles. The cause of the extended luteal phase in these rhino species has been presumed to be a reproductive system disease, such as pyometra and endometritis [22, 23], early pregnancy loss [22, 24] or ovulation during the luteal phase, which is often observed in mares [25, 26]. However, our study could not determine whether the long cycle was caused by factors as described in rhino species, whether the normal cycle of Malayan tapir was short (approximately 1 month) or long in length (approximately 2–3 months) or whether the estrous cycle varies greatly in length.

In conclusion, the estrous cycle based on the serum/plasma P4 profiles of Malayan tapirs is 43.6 ± 2.0 days; however, this mean includes great variation in length, from 21 to 84 days. To promote captive breeding of threatened Malayan tapirs, further investigation is needed to understand the reasons for variations in the cycle length or to determine a reproductive strategy in relation to the variation in cycle length. In the present study, we also showed that visual changes in the vulva can be used as real-time indicators for estimation of a female’s estrus status. Visual observation of easy signs, such as vulvar mucus and swelling, would be helpful in captive breeding management of Malayan tapirs.

Acknowledgments

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