Progesterone patterns in Siamensis Eld's deers (*Rucervus eldii siamensis)* and Thamin *(Rucervus eldii thamin*) assessed by fecal hormone assay

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1.Introduction

The Eld's deer has experienced the most severe decline in numbers during the last 60 years. The greatest threat to the extinction in the wild of Siamensis and Thamin Eld's deers especially in Thailand continues to be poaching and habitat losses. In several Zoos and Wildlife sanctuary has a generally valuable female in a management breeding program but not given birth. Unlike in some population group, the reproductive rate of captive wildlife is highly variable until over population and inbreeding, because not enough of data to supporting or determine reproductive status which important for breeding program. A high priority is understanding the reproductive status of the captive population. Hormones drive the reproductive process, and endocrine patterns can indicate reproductive health (Brown *et al*., 2001). Remarkable progress has been made in assessing ovarian function in female, primarily through the analysis of excreted progestogen matabolites (Schwarzenberger *et al*., 1998). By Using non-invasive hormone monitoring by collecting fecal sampling. It is will provide valuable information on each female (Plame, 2005) because not all species or individuals permit the required level of handling. Fecal samples should be collected to the planned administration of the contraceptive implant to provide valuable reproductive status (ovarian activity, ovulation, pregnancy diagnosis, contraceptive assessment), assay validation and customized advice for breeding and research programs.(Morrow, 2007)

2. Objectives

2.1 Evaluating progesterone patterns in Females Ru*cervus eldi siamensis* and *Rucervus eldi thamin* by Fecal steroids extract

2.2 To determine individual reproductive physiological status of each animal, in order to use as a tool to evaluate assisted reproductive technologies to be appropriate.

2.3 To rule out on hormonal database for cage management and success breeding in population group of Eld's deer.

3. Methods

3.1 Animals and Fecal Sample Collection

Animals in this case study included 2 female Siamensis Eld's deer (*Rucervus eldii siamensis)* and 8 female Thamin Eld's deers at 3 institutions (2 zoos and 1 university). All animals were of adult age. Both species were put for data base

requirement to breeding management plan by assisted reproductive technologies such as artificial insemination (AI) and embryo transfer (ET)

Individual fecal samples 10-50 g were collected one to three times weekly for 6- to 10- month periods. Criterions for fecal collection are fresh, no material contamination, and homogeneous feces so the metabolite is eventually distributed in fecal sample. After collections, samples were frozen and stored at -20 °C until analysis.

3.2 Fecal Steroid Extraction

Frozen feces were dried in hot air oven at 60 °C until no change in weight of the sample is not detected, pulverized, and the fecal powder was stored at -20°C until steroid extraction. A 0.1-g aliquot of well-mixed powder was boiled twice in 5 mL of 90 % ethanol for 20 minutes. After centrifuging at 2,500 g for 20 minutes, supernatants were collected, sediment were dissolved in 5 mL of 90 % ethanol. Extractants were vortexed (30 seconds) after that bring up centrifuged at 2,500 g for 15 minutes, supernatants were collected and combined in same tube before that, supernatants were recovered, dried, and redissolved in 1 mL dilution buffer. Extractans were vertexed (1 minute) and then stored at -20°C until steroid assays.

3.3 Assay Protocols

Hormonal assay is performed by Competitive ELISA (Enzyme Linked Immuno Sorbent Assay) according to Brown *et al.*(2004). The progesterone EIA relied on a Monoclonal Antibody: Pregnane CL-425 (Provided by Quidel Corporation), progesterone standards (known concentrations), unknown (samples) concentrations of hormone (unlabeled antigen) and the hormone-specific enzyme conjugate (HRP) (the labeled antigen) are added to the well. The labeled and unlabeled antigens compete for binding sites on the antibody during the incubation phase. The substrate is added and reacts with the bound enzyme conjugate and changes color. The product of the substrate catalysis by enzyme is measured by transmitting light of a specific wavelength (405 nm.) through the product and measuring the amount of light adsorbtion by a plate reader (spectrophotometer).

3.4 Data analysis

Mean data are presented as ±SEM. Definition of the estrous cycle was based on fecal progestogen profiles. For each female, a nonpregnant baseline progestogen value was calculated using an iterative process in which values that exceeded the mean plus 1.5 standard deviations (SD) were excluded. The average was then recalculated and the elimination process was repeated until no values exceeded the mean plus 1.5 SD [Brown *et al*., 1994, 2001]

Onset of the luteal phase was defined as the first point after values increased above the baseline by 50 % and remained elevated for at least 2 consecutive weeks. The end of the luteal phase was defined as the first of two consecutive values that returned to baseline concentrations. Estrous cycle length was calculated as the beginning of the one luteal phase to the beginning of the next (Brown *et al*., 1994).

4. Result and Conclusions

Fecal progesterone assay has been used as noninvasive mean for assessing ovarian cyclicity in several species. However, progesterone pattern in Siamensis Eld's deer (*Rucervus eldii siamensis)* has never been reported. The objective of the study was to assess progesterone pattern of Siamensis and Thamin Eld's deers *(Rucervus eldii thamin*). Progesterone profile was detected in Siamensis (n=2) and Thamin Eld's deers (n=8) by Enzyme immunoassay (EIA). The average estrous cycle length of Siamensis (n=11 cycles) and Thamin Eld's deers (n=20 cycles) were 21.64±1.83 and 20.75±1.13 days, in range 12-32 and 14-30 days, respectively. In follicular phase, overall baseline of progesterone concentrations in Simensis and Thamin Eld's deers were 298.91±11.99 and 172.0±28.09 ng/g, respectively. In luteal phase, progesterone concentrations in Simensis and Thamin Eld's deers were ranging from 448 to 3,112 and 258.96 to 1,840 ng/g. Progesterone concentration of three pregnant Thamin Eld's deers; one to term gestation (34 weeks after artificial insemination) and two premature births (32 weeks after embryo transfer) was investigated. During the first several weeks, progesterone concentrations remained at 400-1,000 ng/g and increased markedly during mid pregnancy (especially at 23rd week) with a peak concentrations reaching to 14,720 ng/g. Progesterone concentration in these Eld's deers abrupt decline coincided with parturition (Fig 1). A similar gestation in Thamin Eld's deers has been reported by Monfort *et al*.(1990), Hosack *et al*.(1997) and Morrow (2007). In conclusion, the study demonstrated the first report of Siamensis Eld's deer progesterone profile (Fig.2a, b) compared to that of Thamin Eld's deer. Fecal progesterone analysis would be useful for determining reproductive status (ovarian activity, estrous cycle, baseline concentrations and pregnancy diagnosis). Progesterone data base of individual Eld's deer is important for breeding management by assisted reproductive technologies to be appropriate.

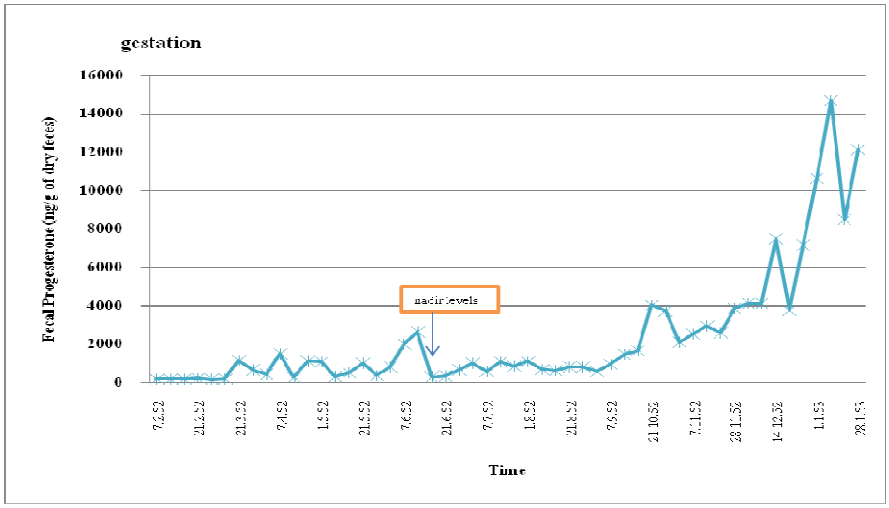

Fig 1. Fecal progesterone concentrations in an individual Eld's Deer female (*Rucervus eldi thamin*) throughout gestation.

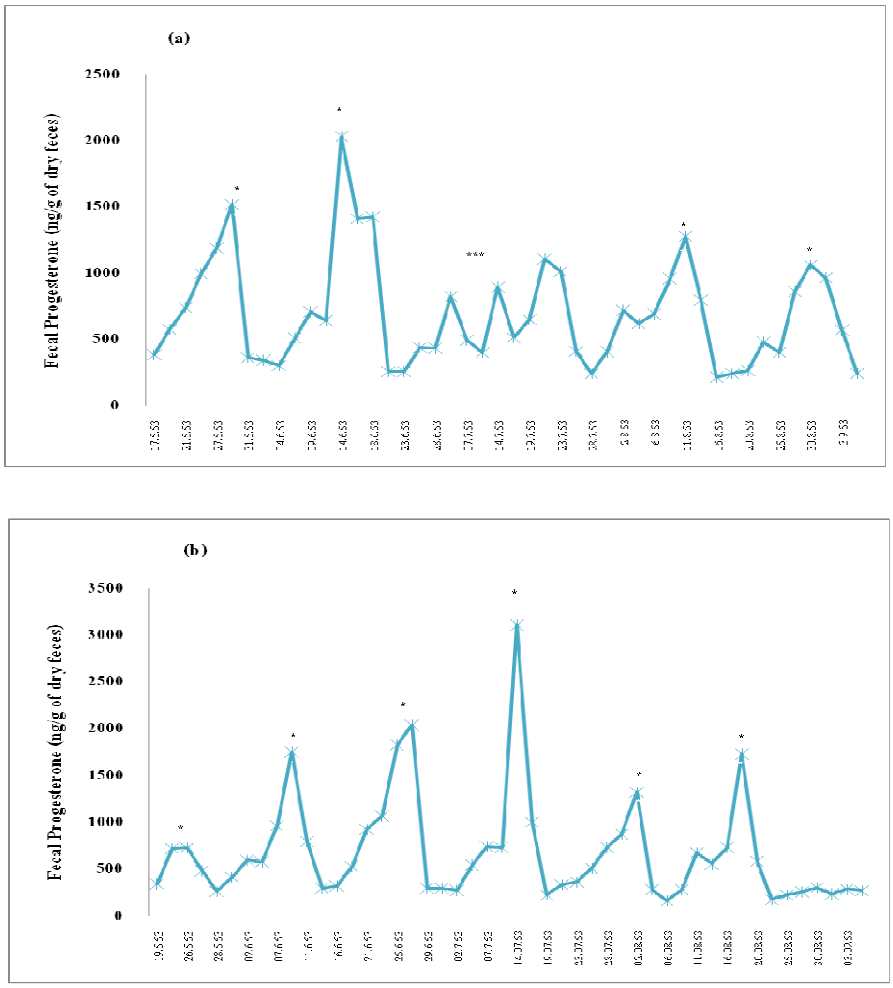

Fig. 2a, b. Individual profiles of fecal progesterone concentrations in two representative Eld's Deer females (*Rucervus eldi siamensis*). Reproductive cycles are designated by an asterisk.

5. References

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