RESEARCH ARTICLE

Monitoring the Gestation Period of Rescued Formosan Pangolin (*Manis pentadactyla pentadactyla*) With Progesterone Radioimmunoassay

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Eight species of pangolin have been identified in the world. However, understanding of pangolin reproductive biology has been limited to fragmentary records. In this study, the concentration of serum progesterone in three pregnant and two nonpregnant rescued female Formosan pangolins (*Manis pentadactyla pentadactyla*) was monitored using a commercial progesterone radioimmunoassay kit. During gestation, the serum progesterone of pregnant pangolins A, B, and C remained at 28.5–55 ng/ml ($n = 31$ samples), 10.9–50.1 ng/ml ($n = 34$), and 12.4 and 33.5 ng/ml with a peak at 47.6 ng/ml ($n = 19$), respectively, whereas the serum progesterone of nonpregnant pangolins D and E remained at 1.99 ± 1.62 ng/ml ($n = 80$) and 2.27 ± 1.64 ng/ml ($n = 27$), respectively. From this study, it was found that female pangolin weighing as low as 2.14 kg was already capable of reproduction. For pregnant pangolins to give birth to viable offspring, their body weight must increase significantly, 63.89 and 134.0% in the study, from the time of inception or early pregnancy until parturition. In addition, study has found that both viable offspring were born fully developed and exceeded 80 g in weight. The period of gestation was found to be as short as 318 or longer than 372 days. Therefore, the Formosan pangolin should only be able to reproduce once a year. This study is the first insight into hormone assay for determining the

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gestation period of pangolin. Further investigations on the same subject are necessary to establish criteria for the recognition of reproductive status in pangolins. Zoo Biol 30:1–11, 2011.

Keywords: Formosan Pangolin; *Manis pentadactyla pentadactyla*; progesterone; radioimmunoassay; gestation period

INTRODUCTION

The Formosan Pangolin (*Manis pentadactyla pentadactyla*) is isolated in Taiwan, and is one of three subspecies of Chinese pangolins in Asia [Schlitter, 1993; Wu et al., 2002]. Pangolins are nocturnal animals, spending daylight hours in self-dug burrows over 1.5 m below the ground, which adds to the great difficulty of tracking pangolins for research in the field [Chao et al., 1993; Wu et al., 2004]. Understanding of pangolin reproductive biology has been limited to fragmentary reproductive records, primarily from interviews with hunters, birthing records of rescued individuals in shelters, and a handful of dead fetus anatomy records [Chao et al., 1993; Chen et al., 2000; Chevenix-Trench, 1922; Masui, 1967; Sandrine and Chin, 2009; Van Ee, 1966; Wu et al., 2002; Wu, 1998]. The gestation period of Chinese pangolin was estimated to be 3–4 months to 8 months, with the possibility of reproducing once or twice a year [Chao et al., 1993; Chen et al., 2000; Liu and Xu, 1981; Wu, 1998]. There are few birth records for Cape [Van Ee, 1966], Indian [Chevenix-Trench, 1922], and Malayan Pangolins [Sandrine and Chin, 2009; Lekagu and McNeely, 1988]. Raising pangolins in captivity had been largely unsuccessful and little information could be accumulated from captive animals [Yang et al., 1999, 2007].

There was no direct hormone evidence to confirm the pangolin's gestation period. Reproductive hormone assay technology is applied to many other endangered species [Bowers et al., 2005; Hesterman et al., 2008; Katsumata et al., 2006; Putranto et al., 2007; Schwarzenberger et al., 2000]. In mammals, pregnancy is associated with Progesterone(P4) concentrations greater than those observed during nonpregnant diestrus [Capezzuto et al., 2008; Dionysius, 1991; Engeland et al., 1997; Karen et al., 2003]. In goats, a progesterone assay provides an accurate pregnancy diagnosis [González et al., 2004]. Radioimmunoassay (RIA) reagent has been very effective (100%) in detecting pregnant animals [Dionysius, 1991; Engeland et al., 1997; González et al., 2004]. The aim of this study is to evaluate the gestation period of limited number of rescued Formosan pangolin using P4 RIA detection in serum samples. Identifying the exact gestation period is beneficial in the captive management and wild population investigations within the conservation program of Formosan pangolin.

MATERIALS AND METHODS

Subjects

Wild-born Formosan pangolins, rescued between 2005 and 2007, were used in the study. After arrival at Taipei Zoo, quarantine was conducted for over a month. During quarantine, fecal analysis was performed every other week until two successive parasite-free results were obtained. Health examination was followed by

X-rays and a routine physical examination. Female Formosan pangolins with normal feeding behavior and feces were chosen for sampling and monitoring. A total of five females were monitored, in which three were pregnant and two were not. The complete pregnancy of one pangolin (A), the partial pregnancies of two others and the nonpregnancies of two more (B, C) were opportunistically monitored and the nonpregnancies of two more (D, E) were monitored, too.

Pangolin A was rescued and then sheltered in the Zoo. She was out of quarantine and was housed with a male in the same pen for mating from December 23–27, 2005, then was separated from the male and housed individually. Two female pangolins (B and C) were isolated individually at the time of their rescue and throughout the quarantine period. Pangolin D and E were isolated and housed in individual pens.

- Pangolin A was hormonally monitored for $15\frac{1}{2}$ months (including a gap of $3\frac{1}{2}$ months surrounding parturition), beginning 3 months after arrival; this female mated $5\frac{1}{2}$ months after arrival, and gave birth $10\frac{1}{2}$ months later, 16 months after arrival (Table 1).
- Pangolin B was monitored for $8\frac{1}{2}$ months, beginning 2 months after arrival; she gave birth $10\frac{1}{2}$ months after arrival (Table 1).
- Pangolin C was monitored for 12 months, beginning 3 months after arrival; she gave birth 12 months after arrival (Table 1).
- Pangolin D was monitored for 21 months, beginning 7 months after arrival (Table 1).
- Pangolin E was monitored for 12 months, beginning $7\frac{1}{2}$ months after arrival (Table 1).

Housing

Indoor pens measured $120 \times 120 \times 80\,\text{cm}^3$ and were filled to a depth of 10 cm with a mixture of dirt and wood shavings as substrate. Each pen with a wooden nest box ($40 \times 60 \times 50\,\text{cm}^3$), divided into two compartments, a mixture of dirt and wood shavings, was prepared as bedding in pen and box (Fig. 1). Room temperature was controlled so as not to exceed 28°C (82°F) in the summer and ranged between 24 and 26°C (75–79°F) in the winter. Humidity was maintained above 60% year round. Photoperiod was maintained at 10 hr of artificial light per day year round.

Diet

The pangolins were fed a cooked diet (Table 2) based on published research [Yang et al., 1999, 2007], provided at 4 PM every afternoon.

Blood Drawing

Blood samples were collected weekly from 2005 to 2006 (A, B, and D) and once every 2 weeks in 2007 (C and E). Each animal was physically restrained while a 23-G 1¼-inch needle was inserted into the tail vein (not visible) to a depth of more than 2 cm from the ventral side of tail (Fig. 2). Then, 2–3 ml of blood was drawn from their tail vein using a 5-cc syringe. Blood samples were poured into serum tube. Whole blood samples were allowed to clot, they were then centrifuged and the serum was collected for assays. Following serum separation, all samples were stored in a freezer at −20°C before P4 assay.

TABLE 1. Basic Data of Pregnant and Nonpregnant Pangolins

No.	Microchip no.	Female arrived time (BW, g)	Mated time (BW, g)	Begin of monitoring (BW, g)	End of monitoring (BW, g)	Birth time (BW, g)	Cub no.	Sex of cub	Cub BW(g)	Note
A	0064E68E9	July 9, 2005 (2,280)	December 23–27, 2005 (3,600)	October 3, 2005 (2,300)	February 12, 2007 (5,104)	November 9, 2006 (6,050)	1	F	110	Cub survived for 9 months Gestation period—318 days
B	0064D3289	November 7, 2005 (2,800)	Before arrived (?)	January 16, 2006 (2,600)	October 2, 2006 (2,420)	September 20, 2006 (1,850)	1	F	52	Cub died after umbilical cord was cut Mother died 40 days later Gestation period—over 318 days
C	0066AB73B	October 2, 2006 (2,140)	Before arrived (?)	January 2, 2007 (3,500)	December 31, 2007 (4,360)	October 9, 2007 (4,780)	1	M	80	Cub survived for 3 days Gestation period—over 372 days
D	0064E44C5	June 1, 2004 (1,800)	None	January 3, 2005 (3,100)	October 2, 2006 (3,630)	None	–	–	–	
E	0066C126F	May 22, 2006 (2,340)	None	January 2, 2007 (4,100)	December 31, 2007 (4,690)	None	–	–	–	

Fig. 1. Separated chamber of the nest box where each pangolin was housed, with a mixture of dirt and wood shavings as bedding.

TABLE 2. Daily Formula for Formosan Pangolin

Feed	Unit	Weight
Bee larvae	g	100
Apple	g	65
Mealworms	g	22.5
Mazuri Insectivore Diet[a]	g	7.5
Egg yolk	g	10
Mix powder[b]	g	5–10
Vitamin and mineral supplement[c]	g	1.5
Chitin	g	10
Water	ml	60
Vitamin K	g	1/15
Soil	g	5

[a]Mazuri 5MK8 Catalog ♯0050819.
[b]Mix powder = coconut powder 300 g + yeast powder 600 g + $CaCo_3$ 100 g.
[c]Vitamin and Mineral Supplement for Pig, China Chemical & Pharmaceutical Co., LD, Taiwan.

Fig. 2. A 23-G $1\frac{1}{4}$-inch needle with 5-cc syringe was inserted into the tail vein (not visible) to a depth of more than 2 cm from the ventral side of tail. Two to three milliliters of blood was drawn from the tail vein.

P4 RIA

The P4 RIAs of serum samples were performed at the RIA lab (Department of Nuclear medicine, Chiu Clinic 3rd Floor No. 2 Nan-King West Road, Taipei, 104, Taiwan, People's Republic of China) The P4 concentration in serum samples was determined in duplicate using commercial PROGESTERONE DSL-3400 (Kit DSL-3400; Diagnostic System Laboratories, Webster, TX), as previously described for bovine [Nilsson and Skinner, 2009]. The smallest detectable dose was 0.1 ng/ml and the intra-assay coefficient of variation was 5.4%. Steroid recovery was $>95\%$ and linearity recovery was $>91\%$ within acceptable criteria for the RIA lab. Inter-assay coefficient of variation was 3.8%. Cross-reactivity data are available from the manufacturer.

Body Weight

The pangolins were weighed at regular intervals to help ascertain their relative health in captivity. Each animal was kept into a plastic box and weighed by electric scale every week.

RESULTS

P4 Assay

The serum P4 concentration results of Pangolin A, obtained during the 2 months before mating, were 2.97–18.5 ng/ml ($n = 13$). The serum P4 concentration increased to 41.2 ng/ml quickly 3 weeks after mating. It was then maintained between 28.5 and 55 ng/ml ($n = 31$) until 4 weeks before parturition (Fig. 3). She gave birth successfully and cub weighed 110 g (Fig. 4).

The serum P4 level of Pangolin B after quarantine was 28.5 ng/ml. The P4 concentration remained between 10.9 and 50.1 ng/ml ($n = 34$; Fig. 3). P4 concentrations fell to 11.7 ng/ml at 4 days before parturition. She gave birth to a female cub weighing 52 g. Unfortunately, the cub died 3 min after the umbilical cord was cut. After parturition, the P4 concentration dropped to 1.26 ng/ml 12 days later.

After quarantine was completed, Pangolin C showed a P4 concentration of 18.2 ng/ml. P4 concentration was maintained between 12.4 and 33.5 ng/ml ($N = 19$) with a peak at 47.6 ng/ml. The concentrations fell to 4.9 ng/ml at 1 day before parturition. She gave birth to a male cub weighing 80 g. The young pangolin died 3 days after birth due to the mother's lactation deficiency. The concentration after parturition decreased to 0.24–6.7 ng/ml (Fig. 3).

The concentration of serum P4 of Pangolin D was between 0.1 and 8.88 ng/ml and the mean was 1.99 ± 1.62 ng/ml ($n = 80$; Fig. 3).

The concentration of serum P4 of Pangolin E was between 0.8 and 8.03 ng/ml and the mean was 2.27 ± 1.64 ng/ml ($n = 27$; Fig. 3).

The variation patterns of P4 concentrations of the pregnant pangolins were significantly different from that of the nonpregnant pangolins. Serum concentrations were maintained at higher concentration in pregnant pangolins (over 10 ng/ml) during gestation, whereas they remained lower in nonpregnant pangolins (between 0.1 and 8.88 ng/ml). It might be close to the time of parturition, when the P4 concentration decreased under 10 ng/ml. Consequently, based on the data of P4 concentration and parturition records, the gestation period of pangolin A was at least 318 days, whereas the gestation periods of pangolin B and C were estimated to be over 318 and 372 days, respectively (Fig. 3).

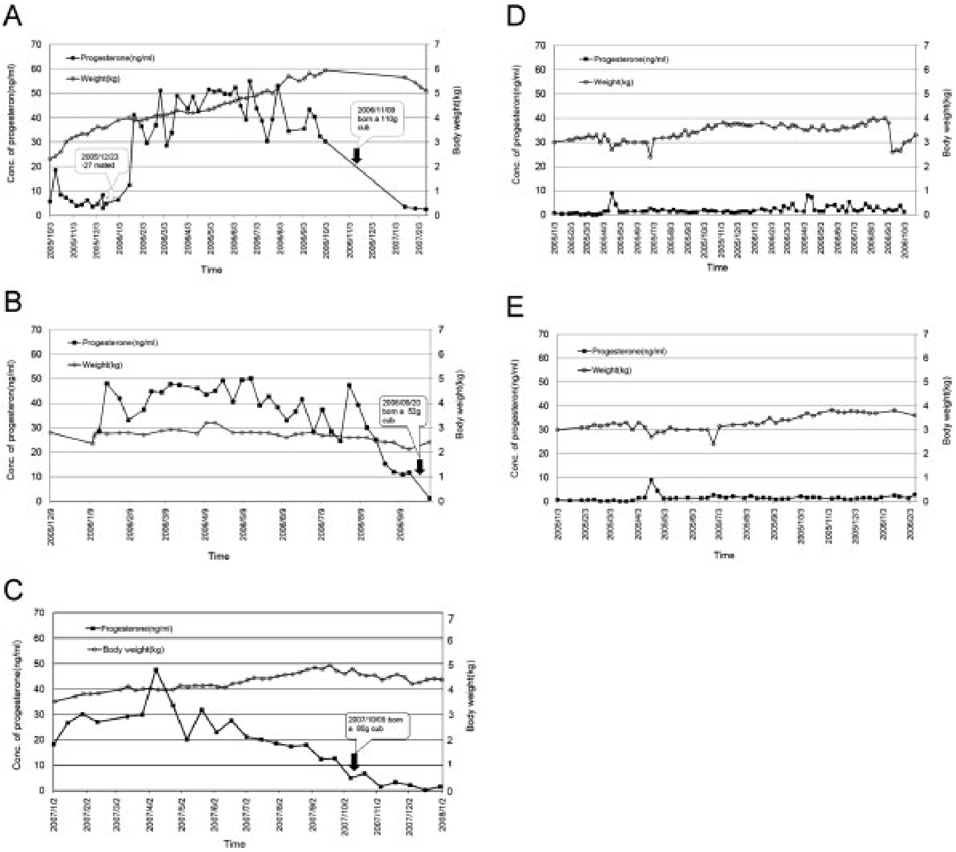

Fig. 3. Serum progesterone concentration (SPC) (—■—) and body weight (BW) (—○—) of the pregnant pangolins **A, B, C** and nonpregnant pangolins **D, E**. Pangolin A mated in captivity (white arrow) and SPC elevated dramatically 3 weeks later and declined gradually at 4 weeks before parturition (black arrow). The SPC of Pangolin B and C were high indicating mating occurred before rescue and declined since 2 months and 5 months before parturitions. SPC of the nonpregnant pangolins D, E was generally lower than that of the pregnant group. The BW of pregnant pangolins A and C increased faster than nonpregnant pangolins D and E. The pangolin B could not increase BW during gestation and lost the weight gradually before parturition.

Fig. 4. Pangolin A gave birth successfully in captivity. The female cub weighed 110 g.

Body Weight

- Pangolin A weighed 3.6 kg when she was introduced to a male for mating. Before giving birth to a female cub weighing 100 g on November 9, 2006, her weight was 6.05 kg. Her total body weight increased 63.89% (2.35 kg) during the gestation period (Fig. 3).
- Pangolin B was rescued on December 7, 2005, weighing 2.8 kg and dropped to 2.35 kg by January 11, 2006 when quarantine was finished. The gradual weight loss continued down to 1.8 kg on September 20, 2006, when she gave birth to a female weighing 52 g. Her weight before parturition decreased 35.7% (1 kg; Fig. 3).
- Pangolin C was rescued on October 2, 2006, weighing 2.14 kg. Before giving birth to a male cub weighing 80 g on October 9, 2007, her weight was 4.78 kg, with a total increase in bodyweight of 134% (2.64 kg; Fig. 3)
- Pangolin D arrived at Taipei Zoo on June 1, 2004, weighing 1.8 kg. On January 2, 2005, her weight was 3.1 kg. She weighed 3.63 kg on October 31, 2006, resulting in a total increase in body weight of 17% (0.53 kg; Fig. 3).
- Pangolin E arrived at Taipei Zoo on May 22, 2006, weighing 2.34 kg. On January 2, 2007, her weight was 4.1 kg. She weighed 4.69 kg on December 31, 2007, which calculates to a total increase in body weight of 14.9% (0.59 kg; Fig. 3).

Results of this study suggest that a female Formosan pangolin weighing 2.14 kg (pangolin C) could be ready for pregnancy. Pregnant pangolins might increase in body weight to enable birth of viable fetus generally. Even when born alive, pangolin cubs weighing under 80 g, seem to could not survive well after their birth. Also, pregnant pangolins seem to gain weight faster than nonpregnant pangolins.

DISCUSSION

From the study, the serum P4 concentration of pregnant and nonpregnant female Formosan pangolins could be detected consistently and successfully by commercial P4 RIA reagent. There are obviously variations in hormone patterns between pregnant and nonpregnant pangolins (Fig. 3). It is an effective and convenient method to determine reproductive status of rescued female Formosan pangolins.

The gestation period of the Formosan pangolin from mating to parturition can be as short as 318 or longer than 372 days. Consequently, the Formosan pangolin can only reproduce once a year. The results of this study refute the gestation periods, estimated from 3 to 8 months and breeding twice a year from the interview of hunters or birth records, published previously without hormone evidence [Chao et al., 1993; Chen et al., 2000; Liu and Xu, 1981; Masui, 1967; Wu, 1998]. The gestation period of the great anteater (*Myrmecophaga tridactyla*) is considered to be 190 days [Patzl et al., 1998], whereas that of the silky anteater (*Cyclopes didactylus*) is 120–150 days [Chiarello et al., 2008]. Although they are similar in terms of feeding habits and body temperature, based on this study, the gestation period of Formosan pangolins is longer than anteaters. The variation of gestation period from 318 to 372 days of Formosan pangolins might be associated with the embryo delay implantation such as bear, armadillo, or marsupial [Bagatto et al., 2000; Hesterman et al., 2008; Sato et al., 2001], but the sharp P4 peak of pregnant bear before implantation could not be identified in the limited data of pregnant pangolins [Dehnhard et al., 2006]. More advance investigation should be necessary.

Judging by their weight upon entering the Zoo, which ranged between 1.8 and 2.8 kg, the five pangolins in this study were estimated to be 1–1.5 years old [Liu and Xu, 1981], which female Formosan pangolins might be ready to breed at 1–1.5 years old, or early than that. At the same time, pregnant young pangolins were still growing to adult. However, that energy or food supply from ants or termites with a seasonal variation cannot support a high level of metabolic energy expenditure by Chinese pangolin [Heath and Hammel, 1986; Ke et al., 1999; Wu et al., 1999]. Pregnant pangolins have more stable body temperature gradient than nonpregnant pangolins. The need for heat production would be greater because of heat loss would be higher [Heath, 1987; Heath and Hammel, 1986]. The role of special metabolic mechanism for maintaining in fetus and pregnant young pangolin development should not ignore the importance of energy requirement of the primitive mammal.

In previous birth records for the Formosan pangolin, the mother weighed 4 kg at the time of parturition, and the stillborn offspring weighed 70 g [Chao et al., 1993]. Two live cubs, 92 g and 93 g, were born from two 3 kg Chinese pangolins, but died at 24 hr and 5 days later [Heath and Vanderlip, 1988]. In Hua-nan pangolin reproduction records, the mother weighed 3.86 kg and gave birth to a 75-g stillborn neonate [Wu, 1998]. For young pregnant pangolins to give birth to viable offspring, their body weight must increase significantly, 63.89 and 134.0% in the study, from the time of inception or early pregnancy until parturition. Meanwhile, the hormone levels gradually declined through most of the monitored pregnancies for pangolin B and C whose infants promptly died, whereas a decline in hormone level toward the very end of the pregnancy of the pangolin A whose infant survived for several months (Fig. 3). The higher body weight of prepregnant and preparturient female represent the advantage in environment adaption and energy obtaining in most animals [Bagatto et al., 2000; Dehnhard et al., 2006; Heath, 1987; Heath and Hammel, 1986; Ke et al., 1999], which might be good for survival rate of the newborn infants. The body weight of prepregnant and preparturient females and newborn cubs should be impact criteria of successful breeding of pangolins. However, due to the low number of subjects, in order to confirm this conclusion, wider investigations on the subject are necessary to establish criteria for the successful parturition in these species.

CONCLUSION

1. P4 RIA is a reliable and effective method to evaluate the reproductive status of rescued Formosan pangolins.
2. The gestation period of the Formosan pangolin was determined to be from 318 to 372 days.
3. Formosan pangolins should be ready to breed at 1–1.5 years old. The body weight of prepregnant and preparturient females and newborn cubs should be impact criteria of successful breeding.

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REFERENCES

Bagatto B, CrossleyII DA, Burggren WW. 2000. Physiological variability in neonatal armadillo quadruplets: within-and between-litter differences. J Exp Biol 203:1733–1740.

Bowers S, Gandy S, Paul K, Woods L, D'Angelo D, Horton C, Tabaka C, Willard S. 2005. The relationship between vaginal electrical impedence and hormone profiles during pregnancy and parturition of a white rhinoceros (*Ceratotherium simum simum*). J Zoo Wildl Med 36:451–456.

Capezzuto A, Chelini MOM, Felippe ECG, Oliveira CA. 2008. Correlation between serum and fecal concentrations of reproductive steroids throughout gestation in goats. Anim Reprod Sci 103:78–86.

Chao JT, Chen YM, Yeh WC, Fang KY. 1993. Notes on newborn Formosan pangolin, *Manis pentadactyla pentadactyla*. J Taiwan Mus 46:43–46.

Chen DY, Lee CS, Yu CB. 2000. Evaluation of pangolin parturient time. Res Dev Mark 16:287.

Chevenix-Trench CG. 1922. Notes on a young Indian pangolin or scaly anteater *Manis crassicaudata*. J Bombay Nat Hist Soc 24:590.

Chiarello A, Miranda F, Samudio R, Members of the IUCN SSC Edentate Specialist Group. 2008. *Cyclopes didactylus*. IUCN 2008. IUCN Red List of Threatened Species.

Dehnhard M, Hildebrandt TB, Knauf T, Jewgenowa K, Kolter L, Göritz F. 2006. Comparative endocrine investigations in three bear species based on urinary steroid metabolites and volatiles. Theriogenology 66:1755–1761.

Dionysius DA. 1991. Pregnancy diagnosis in dairy goats and cows using progesterone assay kits. Aust Vet J 68:14–16.

Engeland IV, Ropstad E, Andresen Ø, Eik LO. 1997. Pregnancy diagnosis in dairy goats using progesterone assay kits and oestrous observation. Anim Reprod Sci 47:237–243.

González F, Cabrera F, Batista M, Rodríguez N, Alamo D, Sulon J, Beckers JF, Gracia A. 2004. A comparison of diagnosis of pregnancy in the goat via transrectal ultrasound scanning, progesterone, and pregnancy-associated glycoprotein assays. Theriogenology 62:1108–1115.

Heath ME. 1987. Twenty four hour variations in activity, core temperature, metabolic rate, and respiratory quotient in captive Chinese pangolins. Zoo Biol 6:1–10.

Heath ME, Hammel HT. 1986. Body temperature and rate of O2 consumption in Chinese pangolins. Am J Physiol 250:377–382.

Heath ME, Vanderlip SL. 1988. Biology, husbandry, and veterinary care of captive Chinese pangolins. Zoo Biol 7:293–312.

Hesterman H, Jones SM, Schwarzenberger F. 2008. Reproductive endocrinology of the largest dasyurids: characterization of ovarian cycles by plasma and fecal steroid monitoring. Part I. The Tasmanian devil (*Sarcophilus harrisii*). Gen Comp Endocrinol 155:234–244.

Karen A, Beckers J-F, Sulon J, de Sousa NM, Szabados K, Reczigel J. 2003. Early pregnancy diagnosis in sheep by progesterone and pregnancy-associated glycoprotein tests. Theriogenology 59:1941–1948.

Katsumata E, Jaroenporn S, Katsumata H, Konno S, Maeda Y, Watanabe G, Taya K. 2006. Body temperature and circulating progesterone levels before and after parturition in killer whales (*Orcinus orca*). J Reprod Dev 52:65–71.

Ke Y, Chang H, Wu SB, Liu Q, Fong G. 1999. A study on pangolin's main food nutrition. Zool Res 20:394–395.

Lekagu B, McNeely J. 1988. Mammals of Thailand 2nd ed. Bankok: The association for conservation of Thailand.

Liu ZH, Xu LH. 1981. Pangolin's habits and its resource protection. Chin J Zool 16:40–41.

Masui M. 1967. Birth of a Chinese pangolin *Manis pentadactyla* at Ueno Zoo, Tokyo. Int Zoo Yearbk 7:114–115.

Nilsson EE, Skinner MK. 2009. Progesterone regulation of primordial follicle assembly in bovine fetal ovaries. Mol Cell Endocrinol 313:9–16.

Patzl M, Schwarzenberger F, Osmann C, Bamberg E, Bartmann W. 1998. Monitoring ovarian cycle and pregnancy in the giant anteater (*Myrmecophaga tridactyla*) by faecal progestagen and oestrogen analysis. Anim Reprod Sci 53:209–219.

Putranto HD, Kusuda S, Inagaki K, Kumagai G, Ishii-Tamura R, Uziie Y, Doi O. 2007. Ovarian activity and pregnancy in the Siberian tiger, *Panthera tigris altaica*, assessed by fecal gonadal steroid hormones analyses. J Vet Med Sci 69:569–571.

Sandrine P, Chin SY. 2009. Proceedings of the Workshop on Trade and Conservation of Pangolins Native to South and Southeast Asia, June 30–2 July 2, 2008, Singapore Zoo, Singapore. TRAFFIC Southeast Asia.

Sato M, Tsubota T, Komatsu T, Watanabe G, Taya K, Murase T, Kita I, Kudo T. 2001. Changes in sex steroids, gonadotropins, prolactin, and inhibin in pregnant and nonpregnant Japanese Black Bears (*Ursus thibetanus japonicus*). Biol Reprod 65:1006–1013.

Schlitter DA. 1993. Order Pholidota. In: Wilson DE, Reeder DM, editors. Mammal species of the world: a taxonomic and geographic reference, 2nd ed. Washington, DC: Smithsonian Institution Press. p 415.

Schwarzenberger F, Rietschel W, Vahala J, Holeckova D, Thomas P, Maltzan J, Baumgartner K, Schaftenaar W. 2000. Fecal progesterone, estrogen, and androgen metabolites for noninvasive monitoring of reproductive function in the female Indian rhinoceros, *Rhinoceros unicornis*. Gen Comp Endocrinol 19:300–307.

Van Ee CA. 1966. A note on breeding the cape pangolin (*Manis temmincki*) at Bloemfontein Zoo. Int Zoo Yearbk 6:163–164.

Wu SB. 1998 Notes on a newborn Chinese pangolin (*Manis pentadactyla aurita*). J Qinghai Norm Univ (Nat Sci) 1:40–42.

Wu SB, Liu Q, Fen G, Ke Y. 1999. Preliminary study on food nutrient contents of Chinese pangolin (*Manis pentadactyla*). J Zhanjiang Norm Coll (Nat Sci) 20:74–76.

Wu SB, Ma GZ, Tang M, Chen H, Liu N F. 2002. The status and conservation strategy of pangolin resource in China. J Nat Resour 17:174–180.

Wu SB, Liu NF, Ma GZ, Tang M, Chen H, Xu ZR. 2004. A current situation of ecology study on pangolins. Chin J Zool 39:48–54.

Yang CW, Chou CS, Chao MS. 1999. The feeding of the Chinese pangolin (*Manis pentadactyla pentadactyla*) at Taipei Zoo. AZA Annual Proceedings, Minneapolis, MN. September 23–28. p 501–507.

Yang CW, Chen S, Chang CY, Lin MF, Block E, Lorentsen R, Chin JSC, Dierenfeld ES. 2007. History and dietary husbandry of pangolins in captivity. Zoo Biol 26:223–230.