Seasonal Changes in Reproductive Hormones of Female Formosan Black Bears (*Ursus thibetanus formosanus*) in Captivity

圈養雌性台灣黑熊繁殖內分泌季節性變化之初探

Keng-Ruel Chang[1], Frank Chiahung Mao[1], Chieh-Chung Yang[2] and Feng-Tse Chan[2]

張耿瑞[1] 毛嘉洪[1] 楊吉宗[2] 詹芳澤[2]

[1]Department of Veterinary Medicine, National Chung-Hsing University, Taichung, Taiwan

[2]Endemic Species Research Institute, Chichi, Nantou, Taiwan

[1]國立中興大學獸醫學系 台中市國光路250號

[2]行政院農業委員會特有生物研究保育中心 南投縣集集鎮民生東路1號

Abstract

Seasonal changes in serum reproductive hormones of three female Formosan black bears (*Ursus thibetanus formosanus*) in captivity were studied monthly from April 2001 to May 2003 at the Low Altitude Experimental Station of Endemic Species Research Institute. The LH concentration was significantly higher (ANOVA, $p < 0.05$) in spring than those in other three seasons, but no significant seasonal differences ($p > 0.05$) were found for the concentrations of FSH, prolactin, estradiol and progesterone. Also, there were significantly positive correlations in concentrations between FSH and progesterone, between LH and estradiol, between LH and progesterone, and between estradiol and progesterone. The female Formosan black bears seem to be capable for breeding all the year round, but spring is the major mating season. The results of this study provide important reproductive information useful for the preservation of the Formosan black bear.

摘 要

本實驗爲從2001年4月起至2003年5月，針對季節性影響圈養在特有生物研究保育中心低海拔試驗站之三隻台灣黑熊繁殖內分泌所做的研究。結果發現四季血清中激濾泡素 (FSH)、泌乳素 (prolactin)、雌二醇 (estradiol)及助孕素 (progesterone) 濃度無季節性的差異 ($p > 0.05$)，但血清中排

卵素 (LH) 濃度在春季是最高且具有顯著差異 ($p < 0.05$)。此外，在雌性台灣黑熊的血清激濾泡素和助孕素、排卵素和雌二醇、排卵素和助孕素及雌二醇和助孕素間存有正相關關係。由結果可發現台灣黑熊爲全年均可繁殖，而春季爲主要的發情交配季節。本研究成果提供關於台灣黑熊重要的繁殖資訊，希望能作爲台灣黑熊保育的參考。

Key words : Formosan black bear, reproductive hormone, seasonality

關鍵詞：台灣黑熊、繁殖內分泌、季節性

Received: November 14, 2003 Accepted: January 28, 2004

收件日期：92年11月14日 接受日期：93年1月28日

Introduction

The family Ursidae consists of two subfamilies: Ailurinae and Ursinae. The subfamily Ursinae has 4 genera and 7 species, of which brown bear (*Ursus arctos*), polar bear (*Ursus maritimus*), American black bear (*Ursus americanus*), and Asiatic black bear (*Ursus thibetanus*) belong to the genus *Ursus*. The Formosan black bear (*Ursus thibetanus formosanus*) is a subspecies of the Asiatic black bear (Wilson and Reeder 1993), and is the largest carnivore endemic to Taiwan. It is an omnivorous animal that lives widely in low to high elevations of 2,000m to 2,500m, and active in both day and night (Hwang *et al*. 2000). In recent years it is at the verge of nearly extinction, because of habitat destruction and human interference. According to the wildlife conservation law of Taiwan, in 1989 the Council of Agriculture of the Executive Yuan listed the Formosan black bear as an endangered species, that is prohibited for hunting, possessing, or killing. Because its population was extremely low and rarely seen, very few studies had been conducted, and seasonal changes in its reproductive hormones was lacking.

American black bear has hibernation and delayed implantation. It is a seasonal breeder, and its estrus is in May to July (Domico and Newman 1988; Foresman and Daniel 1983; Renfree and Calaby 1981; Tsubota *et al*. 1987; Tsubota *et al*. 1997). For Asiatic black bear it is suspected to be in heat and to mate in March to December (Domico and Newman 1988). In Taiwan the studies on the Formosan black bear were limited to a few observations on its distribution and activity in natural environments. In terms of breeding, no hibernation has been observed so far for this subspecies (Kenneth and Wendell 1989; Hwang *et al*. 2000), and it processes a peculiar reproductive status characterized by induced ovulation, delayed implantation for 5-6 months, and pseudo-pregnancy (Chang *et al*. 1994; Yang *et al*. 2003).

Concentrations of serum reproductive hormones, such as the follicle stimulating hormone (FSH), luteinizing hormone (LH), and prolactin, have been reported in female bears. FSH and LH surge coincidently with an acute

increase at the peaks of urinary oestrone conjugate, behavioral oestrus, and urinary estradiol in female giant panda (*Ailuropoda melanoleuca*) (Monfort *et al*. 1989; Shi *et al*. 1991). The LH concentration is not significantly different between March and December in captive female American black bears housed at Bear Country of USA, but higher in November to December in free-ranging female bears (Tsubota *et al*. 1998). The LH concentration is low in January to April and higher in December in Japanese black bears (*Ursus thibetanus japonicus*) kept at the Akita, Japan (Sato *et al*. 2000). For female American black bears the estradiol concentration is highest in March prior to mating and in June during the mating season, and then, followed by a significant decrease in November and December (Tsubota *et al*. 1998). In captive female Japanese black bears, serum estradiol concentration is low in November and December, high in January, and varies in April to October (Sato *et al*. 2000). The prolactin concentration pattern is similar to those of estradiol, with elevated levels during the mating season in June, decreasing slightly in July, and low level in November to December in female American black bears (Tsubota *et al*. 1998). In free-ranging female American black bears, progesterone concentration increases gradually after mating to the peak in November to December (Tsubota *et al*. 1998). In captive female Japanese black bears, which include animals of both known and unknown reproductive status, serum progesterone concentration is low from April to July, higher after August and much higher in November and December, and then returns to low in March (Sato *et al*. 2000). Progesterone concentration is higher in March to June than non-mating season for non-pregnant giant pandas, and its estradiol concentration is higher during the spring mating season (Lindburg *et al*. 2001; Etsuo *et al*. 2003).

This study was intended to obtain basic information on reproductive hormones, FSH, LH, and prolactin, in captive female Formosan black bears, pertaining to their seasonal changes, relationships, and reproductive traits.

Materials and Methods

Animals studied

Three female Formosan black bears were used in this study. Each of them was paired with a male bear in the breeding season each year. Bear No.1 was from private donation and 9 years old, Bear No.2 was confiscated from a hunter and 8 years old, and Bear No.3 was obtained from a private amusement park and its age was unknown. No.1 was kept with her male partner in the enclosure from April to September 2001, but without expressing mating behavior. No.2 was mated on March 22-23, 2001, May 5-9, 2002, and April 15-18, 2003. It produced a cub in each of early November of 2001 and 2002. However, the cub produced in 2002 died two days after the birth. No.3 had an infection with serious inflammation on the back in 2001, and had no mating.

The bears studied were housed in covered outdoor enclosures with ambient light and temperature at the Low Altitude Experimental Station of Endemic Species Research Institute (120°56'52.47"E and 24°16'24.39"N) near Taichung. The breeding climate and latitude were fairly similar to those in their nature habitat. The proper diet was provided according to Yang *et al*. (2001). The times of estrus and mating were observed and recorded.

Sample collections and analyses

Blood samples were collected from the 3 female bears monthly from April 2001 to May 2003, except at the time of pregnancy, bearing, nursing, and healthy problems. All bears were anaesthetized with Zoletil 50 (10 mg/kg; Virbac Co. Ltd., Taipei, Taiwan) and then, blood samples were collected via jugular vein with the intravenous method. Blood samples were kept at room temperatures for 2 to 3 min for cooling, and then centrifuged at 3,000 rpm for 10 min to collect serum. The serum samples were frozen in ice cube and sent to Apex' Medical Laboratory in Taichung for analysis. FSH, LH, prolactin, estradiol, and progesterone concentrations were measured by an automatic immunoassay instruments (Axsym™ system; Abbott Laboratories, Taipei, Taiwan) with the enzyme-linked immunosorbent assay (ELISA). Sample, anti-hormone coated microparticles, hormone assay buffer, and line diluent (0.1M phosphate buffer) were combined in a well of the reaction vessel (RV). Alkaline phosphatase conjugate was added to the second well of the RV. After being incubated, the reaction mixture was washed to remove unbound materials. The substrate (4-methylumbelliferyl phosphate) was added, and the fluorescent product formed was measured by the ELISA optical assemble. The assays specifically quantified hormones with minimal cross-reactivity (0.24%) with other endogenous steroids. The assay sensitivity was 0.37 mIU/ml, 0.5 mIU/ml, 28 pg/ml, 0.6 ng/ml, and 30 pg/ml for the FSH, LH, estradiol, prolactin, and progesterone, respectively.

Statistical analyses

Data collected were grouped into 4 seasons: spring (March-May), summer (June-August), autumn (September-November) and winter (December-February). Individual reproductive hormone concentrations for each season were calculated as mean±S.E., and compared among the four seasons by ANOVA and Duncan's New Multiple Rang tests. Relationships among the hormones in the serum were also analyzed by the correlation analysis.

Results

FSH concentrations were higher in winter (1.72±0.37 mIU/ml), and then, followed by autumn (1.62±0.3 mIU/ml), spring (1.45 mIU/ml), and summer (1.2±0.13 mIU/ml) (Table 1), while the LH concentrations were significantly higher ($p < 0.05$) in spring (4.3± 3.3 mIU/ml) than those in summer (1.0 mIU/ml), autumn (1.01±0.01 mIU/ml) and winter (1.02 ±0.01 mIU/ml). The estradiol concentrations were higher in spring (1.76±0.76 ng/ml) than those in autumn (1.4±0.4 ng/ml), summer (1.36 ±0.23 ng/ml), and winter (1.15±0.11 ng/ml), but the differences were not statistically significant ($p>0.05$). The prolactin concentrations were higher in summer (2.85± 0.86 ng/ml) and then, followed by autumn (2.60 ± 0.85 ng/ml), winter (1.85±0.94 ng/ml), and spring (1.49 ng/ml). The progesterone concentrations were higher in spring (2.24± 0.83 ng/ml) and winter (2.24±1.32 ng/ml), highest in autumn (3.83±1.02 ng/ml), and lowest in summer (0.56±0.14 ng/ml). Significant positive correlations were found for the concentrations between FSH and progesterone ($r = 0.76$, $p<0.05$) and between LH and estradiol ($r = 0.89$, $p<0.05$), but not between LH and progesterone ($r = 0.01$, $p>0.05$) and between estradiol and progesterone ($r = 0.06$, $p>0.05$).

Discussion and Conclusions

This report first describes the seasonal changes in concentrations of serum FSH, LH, prolactin, progesterone and estradiol and their relationships for the Formosan black bear. FSH concentrations were highest in winter, lowest in summer, while estradiol concentrations were higher in spring (Table 1), suggesting that FSH raises follicular action and started the estrus cycle in spring. These phenomena are fairly similar to those of other species of Ursidae; the animals begin their reproductive cycle after hibernation (Howell-Skalla *et al*. 2000; Lundberg *et al*. 1976; Mead 1989; Tsubota *et al*. 1998).

Basal LH concentration in the American black bear does not have seasonal difference (Horan *et al*. 1993; Howell-Skalla *et al*. 2000), but the concentration was highest statistically in spring for the female Formosan black bear (Table 1). This indicates that ovulation of the Formosan black bear occurs in spring. This result was supported by Tsubota *et al*. (1998) who reported that the rise in serum LH concentration during the peri-implantation period suggests that LH may be luteotropic and concomitant with the higher serum progesterone concentration. The above demonstrates that there is a positive relationship between LH and progesterone concentrations.

Estradiol concentration of the Formosan black bears was high in all the year round without seasonal changes (Table 1), suggesting that it has a yearlong polyestrous cycle with a long reproductive capacity. Its reproductive season in Taiwan is similar to that of male American black bears in Virginia, while those in North Carolina in the lower latitude zone were earlier and longer (Garshelis and Hellgren 1994). The above may indicate that the Formosan black bears mate in spring, as that of the giant panda (Lindburg *et al*. 2001). The mating season of captive polar bears in Canadian Central Arctic and Manitoba is in spring and of the North American bears is in summer (Howell-Skalla *et al*. 2002; Renfree and Calaby 1981). Therefore, the breeding season is species specific in the family Ursidae.

For American black bears housed in the United States Department of Agriculture, their serum prolactin concentration is lowest in

Table 1. Seasonal changes in concentrations (mean±S.E., n=20) of reproductive hormones for the female Formosan black bears in captivity

Items	Spring	Summer	Autumn	Winter
FSH, mIU/ml	1.45±0.001	1.20 ± 0.13	1.62 ± 0.30	1.72 ± 0.37
LH, mIU/ml	4.30 ± 3.3[a]	1.00 ± 0.001[b]	1.01 ± 0.01[b]	1.02 ± 0.01[b]
Estradiol, ng/ml	1.76 ± 0.76	1.36 ± 0.23	1.40 ± 0.40	1.15 ± 0.11
Prolactin, ng/ml	1.49±0.001	2.85 ± 0.86	2.60 ± 0.85	1.85 ± 0.94
Progesterone, ng/ml	2.24 ± 0.83	0.56 ± 0.14	3.83 ± 1.02	2.24 ± 1.32

[a, b] The concentrations were significantly different (ANOVA, $p<0.05$) between the numbers with superscript a and superscript b.

autumn and winter when daylight is short, and then increases steadily during spring (Tsubota *et al*. 1995). This supports this study that the serum prolactin concentration in the Formosan black bears rose with increasing day length (spring to summer) and decreased with decreasing day length (autumn to winter)(Table 1). Furthermore, prolactin concentration plays an important role in seasonal testicular function and cyclical progesterone changes (Curlewis 1992). In contrast, increasing serum prolactin inhibits luteinic function and decreases serum prolactin after activating corpus luteum (Tsubota *et al*. 1998). The results of this study showed that the highest prolactin concentration was in summer at the time when the progesterone concentration was the lowest. The prolactin concentrations decreased in autumn and winter at the time of short daytime, allowing a subsequent blastocyst implantation (Tsubota *et al*. 1998).

Progesterone concentration was higher in spring (Table 1) for functional corpus luteum formation, and increased in autumn to winter, as the case found for the Hokkaido brown bear, Japanese black bear, American black bear in Rhode Island (Seager and Demorest 1986; Tsubota *et al*. 1987; Foresman and Daniel 1983), and giant panda without mating (Monfort *et al*. 1989; Shi *et al*. 1991). For Japanese black bears, progesterone concentration increases in November to February without mating (Sato *et al*. 2001), similar to that of Formosan black bears in this study. Progesterone concentration was low in summer after oestrus, similar to that reported for captive female American black bears (Tsubota *et al*. 1998). The surge of progesterone concentration in autumn is possible with embryo implantation (Foresman and Daniel 1983; Tsubota *et al*. 1987; Palmer *et al*. 1988; Hellgren *et al*. 1990). Therefore, the progesterone concentration in Formosan black bears changed as those of other animals with delayed implantation, increased slightly during the delayed implantation, and were higher during the embryo implantation, such as the cases found for American black bears and polar bears captured in Manitoba and Beaufort Sea, and Hokkaido brown bears (Foresman and Daniel 1983; Palmer *et al*. 1988; Tsubota *et al*. 1987).

In conclusions, there are seasonal changes in reproductive hormones in female Formosan black bears, and the changes in the LH concentrations are statistically significant. These findings provided the important information for the reproductive physiology and the management of the Formosan black bear.

Acknowledgements

The authors thank all members of Low Altitude Experimental Station for assisting in collecting samples, especially for the Station Chief Mr. Tung-Chi Ho and Mr. Wen-Hui Chan. We are grateful to Mr. Gary Wang for performing the serum laboratory assays.

Literature Cited

Chang, S. S., S. L. Lee, Y. Y. Chen, M. C. Chao, C. J. Yang, P. C. Chen, J. H. Lin and L. S. Wu. 1994. A preliminary study on fecal progesterone level in Formosan black bear (*Selenarctos thibetanus formosanus*). Taipei Zoo Bulletin 6: 67-71.

Curlewis, J. 1992. Seasonal prolactin secretion and its role in seasonal reproduction: A review. Reproduction, Fertility and

Development 4: 1-23.

Domico, T. and M. Newman. 1988. Bears of the world. Facts on File. pp. 189. New York.

Etsuo, N., H. Takako, H. Tatsuoko, N. Nagahiro and K. Teruyuki. 2003. Changes in urinary concentrations of total estrogen and pregnanediol in a female giant panda (*Ailuropoda melanoleuca*) from 1991 to 2000. Zoo Biology 22: 383-387.

Foresman, K. R. and J. C. Daniel. 1983. Plasma progesterone concentrations in pregnant and non-pregnant black bears (*Ursus americanus*). Journal of Reproduction and Fertility 68: 235-239.

Garshelis, D. L. and E. C. Hellgren. 1994. Variation in reproductive biology of male black bears. Journal of Mammalogy 75: 175-188.

Hellgren, E. C., M. R. Vaughan, F. C. Gwazdauskas, B. Williams, P. F. Scanlon and R. L. Kirkpatrick. 1990. Endrocine and electrophoretic profiles during pregnancy and nonpregnancy in captive female black bears. Canadian Journal of Zoology 69: 892-898.

Horan, K., R. Nelson, S. Palmer and J. M. Bahr. 1993. Seasonal response of the pituitary and testes to gonadotropin-releasing hormone in the black bear (*Ursus americanus*). Comparative Biochemistry and Physiology 106: 175-182.

Howell-Skalla, L. A., D. Bunick, R. A. Nelson and J. M. Bahr. 2000. Testicular recrudescence in the male black bear (*Ursus americanus*): Changes in testicular luteinizing hormone, follicle-stimulating hormone, and prolactin-receptor ribonucleic acid abundance and dependency on prolactin. Biology of Reproduction 63: 440-447.

Howell-Skalla, L. A., M. R. L. Cattet, M. A. Ramsay and J. M. Bahr. 2002. Seasonal changes in the testicular size and serum LH, prolactin and testosterone concentrations in male polar bears (*Ursus americanus*). Reproduction 123: 729-733.

Hwang, M., Y. Wang and D. L. Garshelis. 2000. Preliminary study on activity patterns of Formosan black bears (*Ursus thibetanus formosanus*) in Yushan National Park, Taiwan. Journal of National Park 10: 26-40. (in Chinese)

Kenneth, D. E. and E. D. Wendell. 1989. Factors affecting black bear reproductive success and cub survival. Journal of Wildlife Management 53: 962-968.

Lindburg, D. G., N. M. Czekala and R. R. Swaisgood. 2001. Hormonal and behavioral relationships during estrus in the giant panda. Zoo Biology 20: 537-543.

Lundberg, D. A., R. A. Nelson, H. W. Wahner and J. D. Jones. 1976. Protein metabolism in the black bear before and during hibernation. Mayo Clinic Proceedings 51: 716-722.

Mead, R. A. 1989. The physiology and evolution of delayed implantation in carnivores. pp. 437-464. *In*: Gittleman, J. L. (ed.). Carnivore behavior, ecology, and evolution. Cornell University Press.

Monfort, S. L., K. D. Dahl, N. M. Czekala, L. Stevens, M. Bush and D. E. Wildt. 1989. Monitoring ovarian function and pregnancy in the giant panda (*Ailuropoda melanoleuca*) by evaluating urinary bioactive FSH and steroid metabolites. Journal of Reproduction and Fertility 85: 203-212.

Palmer, S. S., R. A. Nelson, M. A. Ramsay, I. Stirling and J. M. Bahr. 1988. Annual changes in serum sex steroids in male and female black (*Ursus americanus*) and polar (*Ursus maritimus*) bears. Biology of Reproduction 38: 1044-1050.

Renfree, M. B. and J. H. Calaby. 1981. Background to displayed implantation and embryonic diapause. Journal of Reproduction and Fertility 29: 1-9.

Sato, M., N. Nakano, T. Tsubota, T. Komatsu, T. Murase, I. Tita and T. Kudo. 2000. Changes in serum progesterone, estradiol-17β, Luteinizing hormone and prolactin in lactating and non-lactating Japanese black bears (*Ursus thibetanus japonicus*). Journal of Reproduction and Development 46: 301-308.

Sato, M., T. Tsubota, T. Komatsu, G. Watanabe, K. Taya, T. Murase, I. Kita and T. Kudo. 2001. Changes in sex steroids, gonadotropins, prolactin, and inhibin in pregnant and nonpregnant Japanese black bears (*Ursus thibetanus japonicus*). Biology of Reproduction 65: 1006-1013.

Seager, S. W. J. and C. N. Demorest. 1986. Reproduction in captive wild carnivores. pp. 852-896. *In* : Fowler, I. and E. Murray (eds.). Zoo & wild animal medicine. W. B. Saunders Campany. Philadelphia.

Shi, S., D. Lin, Y. Chen and D. Feng. 1991. Reproductive endocrinological changes during the oestrus of the female giant panda. pp. 71-77. *In*: Feng, W. and A. Zhang (eds.). A study on breeding and disease of the giant panda. Sichuan Scientific and Technical Publishers, China.

Tsubota, T., Y. Takahashi and H. Kanagawa. 1987. Changes in serum progesterone levels and growth of fetuses in Hokkaido brown bears. International Conference on Bear Research and Management 7: 355-358.

Tsubota, T., R. A. Nelson, J. D. Thulin, L. Howell, and J. M. Bahr. 1995. Annual changes in serum concentrations of prolactin in captive male black bears (*Ursus americanus*). Journal of Reproduction and Fertility 104: 187-191.

Tsubota, T., L. Howell-Skalla, H. Nitta, Y. Osawa, J. I. Mason, P. G. Meiers, R. A. Nelson and J. M. Bahr. 1997. Seasonal changes in spermatogenesis and testicular steroidogenesis in the male black bear *Ursus americanus*. Journal of Reproduction and Fertility 109: 21-27.

Tsubota, T., L. Howell-Skalla, W. R. Boone, D. L. Garshelis and J. M. Bahr. 1998. Serum progesterone, oestradiol, luteinizing hormone and prolactin profiles in the female black bear (*Ursus americanus*). Animal Reproduction Science 53: 107-118.

Wilson, D. E. and D. M. Reeder. 1993. Family Ursidae. pp. 336-340. *In*: Mammal species of the world. Smithsonian Institution, Washington.

Yang, C. C., K. J. Liaw and F. H. Hsu. 2001. Feeding habit of the Formosan black bear (*Ursus thibetanus formosanus*) in captivity. Endemic Species Research 3: 73-79. (in Chinese)

Yang, C. C., F. C. Mao, F. T. Chan and T. C. Ho. 2003. Reproduction behavior and characters of the Formosan black bear in captivity. Endemic Species Research 5(1): 1-13. (in Chinese)