Sex Steroid and Prolactin Profiles in Male American Black Bears (*Ursus americanus*) during Denning

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ABSTRACT. Serum sex steroid and prolactin profiles were examined in the male American black bear, *Ursus americanus* during denning. Sera collected in December and the following March from 8 denning male black bears in Minnesota, U.S.A. were assayed for testosterone, estradiol-17β and prolactin. Eight bears were confirmed to be the denning mode based on a serum urea to creatinine ratio less than 10. Serum testosterone concentrations tended to increase from December to the subsequent March whereas serum estradiol-17β concentrations tended to decrease during this period. There were few changes in serum prolactin concentrations between December and March. These findings suggest that spermatogenesis and testicular steroidogenesis initiated during denning may be influenced by changes in serum sex steroid concentrations in the American black bear.—KEY WORDS: bear, denning, sex steroid.

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The American black bear, *Ursus americanus* is a seasonal breeder with a mating season from May to August [6]. Male bears can breed during a more extended period than the breeding period of female bears. It has been reported that active spermatogenesis occurs from the denning period (approximately December to subsequent April) and remains to the post-breeding season accompanied with an increase in peripheral testosterone concentrations during the pre-breeding season or breeding season in some species of the Ursidae [9, 24, 25]. The black bears are alert, can defend themselves without eating, drinking, urinating or defecating in the winter season. This phenomenon is called 'denning' which is characterized by sleep at near normal body temperature (approximately 35°C) burning up to 4,000 or more kcal/day [13, 14]. The bear's physical condition during denning is characterized by a low (<10) serum urea to creatinine (U/C) ratio in comparison to the higher one (>10) during active seasons [15]. During denning, spermatogenic and testicular steroidogenic activity resumes in male black bears [5, 11, 17]. Recently, Tsubota *et al.* [24] reported the presence of P450arom in Sertoli cells and an increase in serum estradiol-17β concentrations were coincident with the initiation of testicular recrudescence in denning black bears. Apparently males undergo testicular recrudescence before emergence from the den and the onset of mating [8]. The objectives of this study were to measure serum sex steroid and prolactin concentrations in male black bears during denning, and to determine if there was a relationship between denning and reproductive profiles in these animals. Unfortunately, serum FSH and LH profiles were not determined in this study.

Blood samples were obtained from 8 free-ranging male black bears during denning in December (5 bears) of 1992 and the subsequent March (3 bears) in Minnesota, U.S.A. Radiocollared bears were tracked to their den sites where they were immobilized with the combination of tiletamine and zolazepam (4.4 mg/kg; Telazol, Elkins-Sinn Inc, Cherry Hill, NJ, U.S.A.), removed from the den, and subjected to blood sampling and body weight measurements. Blood was taken three-to-seven times at 10 min intervals and was centrifuged at 1,800 × g for 20 min at 4°C. Sera were separated from blood cells and stored at −30°C until assayed. Serum concentrations of urea (U) and creatinine (C) were determined using the methods of Nelson *et al.* [15] and U/C ratio was calculated as a marker of denning.

Serum testosterone and estradiol-17β concentrations were measured by radioimmunoassay as described by Palmer *et al.* [17]. Sera for testosterone and estraidol-17β assay were double-extracted with anesthesia grade ether followed by equal volumes of 75% methanol and hexane. Dual extracts were reconstituted in phosphate buffer saline-0.1% gelatin and were used for radioimmunoassay using ^{3}H-testosterone and ^{3}H-estradiol-17β, respectively, as tracers. The cross-reactivities of the antisera were reported previously [1]. Testosterone and estradiol-17β assays for black bear serum were previously validated by column chromatography, parallelism and recovery of unlabeled ligand [17]. The sensitivity of the testosterone and estradiol-17β assay was 8 *p*g/tube and 4 *p*g/tube, respectively. Intra- and inter-assay coefficients of variation were 7.7% and 7.0% for the testosterone assay and 5.7% and 6.0% for the estradiol-17β assay, respectively.

Serum prolactin concentrations were measured by radioimmunoassay as described by Tsubota *et al.* [26]. Briefly, a heterologous radioimmunoassay using ^{125}I-labeled porcine prolactin and goat anti-porcine prolactin serum as a primary antibody was used. Purified porcine prolactin (USDA-pPRL-B-1) for iodination and a standard and the primary antiserum were obtained from Dr. D. J. Bolt (USDA Animal Hormone Program). The prolactin assay for black bear serum was previously validated by parallelism and measurement of prolactin in response to TRH injection into bears [26]. Intra- and inter-assay coefficients of variation were 5.5% and 5.7%, respectively. The hormone

concentrations are expressed as the mean of samples collected several times from each bear. Student's *t* test was used for the statistical analysis in this study.

Serum U/C ratio tended to decrease from December to the following March, although exhibiting no significant difference ($P>0.05$) (Table 1). Individual data of Bear No. 722 which was examined in both December and March also exhibited a decrease in serum U/C ratio from December (3.1) to March (1.7). Body weights of male bears during denning were not significantly different between December and March ($P>0.05$). However, Bear No. 722, the only bear weighed in both December and March, exhibited a expected decrease (21 kg) in body weight from December (162 kg) to March (141 kg) [2]. The disparity between total and individual data was most likely due to different body weights when the bears entered their dens.

Serum testosterone concentrations tended to increase from December (1.0 ± 1.4 *n*g/m*l*) to March (2.4 ± 2.1 *n*g/m*l*), although there were no significant differences between December and March (Table 2). Bear No. 722 also exhibited an increase in serum testosterone concentrations from December (0.3 *n*g/m*l*) to March (1.9 *n*g/m*l*). Bear No. 744 in December exhibited an extremely high level of testosterone (3.7 *n*g/m*l*) but this reason is unknown. On the other hand, serum estradiol-17β concentrations tended to decrease from December (31 ± 12 *p*g/m*l*) to March (17 ± 7 *p*g/m*l*). Bear No. 722 also exhibited a decrease in serum estradiol-17β concentrations from December (44 *p*g/m*l*) to March (15 *p*g/m*l*). Serum prolactin concentrations changed few between December (1.5 ± 0.5 *n*g/m*l*) and March (1.6 ± 0.8 *n*g/m*l*), although Bear No. 722 exhibited an increase in serum prolactin concentrations from December (1.9 *n*g/m*l*) to March (2.5 *n*g/m*l*).

This is the first report of peripheral sex steroid and prolactin profiles in the male bear during denning. A number of researchers have determined peripheral sex steroid profiles throughout an entire year including the denning period [6, 9, 11, 17, 24]. However, the changes in peripheral hormone concentrations including prolactin during denning have never been the focus of any reproductive study of bears.

The presence of denning bears can be identified by a seurm U/C ratio of <10 [15]. In this study, all of the bears except for a bear exhibited ratios below 10 in both December and March. Moreover, the ratio tended to decrease from December to March, which suggests that the degree of denning deepended with time.

It has been established that the onset of spermatogenic activity occurs during the denning period in black bears [5, 24] and brown bears [25] as well as in other deep hibernating mammals such as ground squirrels [10, 12], European hedgehogs [4, 21], and golden hamsters [22]. Serum testosterone profiles of black bears indicated that the activity of the reproductive endocrine system begins to increase during denning [11, 17, 24]. In the present study, we demonstrared an increase in serum testosterone concentrations between December and March. This finding supports the onset of spermatogenic and testicular steroidogenic activity during denning just prior to March in black bears. It is known that the increase in testosterone released from the testis terminates denning in Turkish hamsters [7] and European hedgehogs [20]. However, it has been reported that castration terminated denning in the bear [16]. More studies are necessary on the role of testosterone in the termination of denning in the bear.

Our previous study of black bears demonstrated that testicular recrudescence was associated with presence of aromatase in Sertoli cells in January [24]. We suggested the possibility that estrogen from Sertoli cells may be important for resumption of spermatogenesis in seasonal breeding as well as development of spermatogenesis in pubertal animals [3, 18, 19, 23]. In this study, we found that serum estradiol-17β concentrations tended to be higher in December than in March. This observation may indicate that estradiol-17β synthesized in Sertoli cells plays some

Table 1. Serum urea/creatinine (U/C) ratio and body weight of male denning bears

Month	Bear No.	U/C	Body Weight (kg)
December	704	3.2	145
	716	4.8	137
	722	3.1	162
	726	11.2	124
	738	3.4	176
	Mean	5.1	149
	S.D.	3.1	18
March	267	0.5	227
	722	1.7	141
	867	4.3	111
	Mean	2.2	160
	S.D.	1.9	60

Table 2. Serum hormonal profiles of denning male bears

Month	Bear No.	Testosterone (ng/m*l*)	Estradiol-17β (pg/m*l*)	Prolactin (ng/m*l*)
December	704	3.7	38	2.1
	716	0.2	38	1.4
	722	0.3	44	1.9
	726	0.5	15	1.0
	738	0.3	18	1.0
	Mean	1.0	31	1.5
	S.D.	1.4	12	0.5
March	267	1.1	25	0.8
	722	4.9	15	2.5
	867	1.4	12	1.7
	Mean	2.4	17	1.6
	S.D.	2.1	7	0.8

role in establishing an appropriate milieu for resumption of spermatogenesis in December as described previously [24].

Our previous study showed annual changes in serum prolactin concentrations in captive male black bears, which indicated that serum prolactin concentrations increase from December to the following April [26]. However, the present study indicated no change in serum prolactin concentrations between December and March, although bears in which sera were sampled in both December and March tended to have higher concentrations of prolactin in March. This disparity may come from the small sample size in this study. It is necessary to collect samples from a large population of bears to determine prolactin profiles during denning when the day-length is increasing in the Northern hemisphere.

REFERENCES

1. Bahr, J.M., Wang, S.C., Huang, M.Y. and Calvo, F.O. 1983. *Biol. Reprod.* 29: 326–334.
2. Delisi, S.M., Miers, P.G., Nelson, R.A. and Thulin, J.D. 1990. *FASEB J.* 4: 3412.
3. Dorrington, J.H., Fritz, I.B. and Armstrong, D.T. 1978. *Biol. Reprod.* 18: 55–64.
4. Dutourne, B. and Saboureau, M. 1983. *Gen. Comp. Endocrinol.* 50: 324–332.
5. Erickson, A.W. and Nellor, J.E. 1964. pp. 5–45. *In*: The Black Bear in Michigan (Erickson, A.W., Nellor, J. and Petrides, G.A. eds.), Michigan State University Agriculture Experimental Station Research Bulletin 4, East Lansing.
6. Garshelis, D.L. and Hellgren, E.C. 1994. *J. Mammal.* 75: 175–188.
7. Hall, V.D. and Goldman, B.D. 1980. *J. Comp. Physiol.* 135: 107–114.
8. Kenagy, G.J. 1980. *J. Comp. Physiol.* 135: 333–339.
9. Komatsu, T., Tsubota, T., Yamamoto, Y., Atoji, Y. and Suzuki, Y. 1997. *J. Vet. Med. Sci.* 59: 521–529.
10. McKeever, S. 1964. *Ecol. Monogr.* 34: 383–401.
11. McMillin, J.M., Seal, U.S., Rogers, L. and Erickson, A.W. 1976. *Biol. Reprod.* 15: 163–167.
12. Morton, M.L. and Gallup, J.S. 1975. *Naturalist* 35: 427–433.
13. Nelson, R.A. 1987. *Editorial, Mayo Clinic Proc.* 62: 850–853.
14. Nelson, R.A., Wahner, H.W., Jones, J.D., Ellefson, R.D. and Zollman, P.E. 1973. *Am. J. Physiol.* 224: 491–496.
15. Nelson, R.A., Beck, T.D. and Steiger, D.L. 1984. *Science* 226: 841–842.
16. Nelson, R.A. 1980. *Fed. Proc.* 39: 2955–2958.
17. Palmer, S.S., Nelson, R.A., Ramsay, M.A., Stirling, I. and Bahr, J.M. 1988. *Biol. Reprod.* 38: 1044–1050.
18. Rommerts, F.F.G., de Jong, F.H., Brinkmann, A.O. and van der Molen, H.J. 1982. *J. Reprod. Fertil.* 65: 281–288.
19. Rosselli, M. and Skinner, M.K. 1992. *Biol. Reprod.* 46: 586–594.
20. Saboureau, M. 1986. pp. 253–264. *In*: Living in the Cold: Physiological and Biochemical Adaptations (Heller, H.C., Musacchia, X.J. and Wang, L.C.H. eds.), Elsevier, New York.
21. Saboureau, M. and Dutourne, B. 1981. *Reprod. Nutr. Dev.* 21: 109–126.
22. Smit-Vis, J.H. and Akkerman-Bellaart, M.A. 1967. *Experientia* 23: 844–846.
23. Tsai-Morris, C., Aquilano, D.R. and Dufan, M.L. 1985. *Endocrinology* 116: 38–46.
24. Tsubota, T., Howell-Skala, L., Nitta, H., Osawa, Y., Mason, J.I., Miers, P.G., Nelson, R.A. and Bahr, J.M. 1997. *J. Reprod. Fertil.* 109: 21–27.
25. Tsubota, T. and Kanagawa, H. 1989. *J. Mamm. Soc. Jpn.* 14: 11–17.
26. Tsubota, T., Nelson, R.A., Thulin, J.D., Howell, L. and Bahr, J.M. 1995. *J. Reprod. Fertil.* 104: 187–191.