FULL PAPER *Theriogenology*

Seasonal Changes in Spermatogenesis and Testicular Steroidogenesis in Wild Male Raccoon Dogs (*Nyctereutes procynoides*)

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ABSTRACT. Testes of 15 wild adult male raccoon dogs (*Nyctereutes procynoides*) obtained from September 2000 to April 2001 were studied to clarify seasonal changes in spermatogenesis and testicular steroidogenesis. There were marked seasonal variations in the testis weight and size with values relatively low in September and highest in March. Spermatogonia and primary spermatocytes were observed in September, while spermatogonia, spermatocytes and round spermatids were present in January, and all types of spermatogenic cells including mature spermatozoa were found in the mating season (February and March). The number of spermatogenic cells reached th eir peak values in February and March. In addition, steroidogenic enzymes were immunolocalized using polyclonal antisera raised against bovine adrenal cholesterol side-chain cleavage cytochrome P450 (P450scc), human placental 3β-hydroxysteroid dehydrogenase (3βHSD), porcine testicular 17α-hydroxylase cytochrome P450 (P450c17), and human placental aromatase cytochrome P450 (P450arom). P450scc and P450c17 were identified in Leydig cells and spermatids in February, whereas these enzymes were present only in Leydig cells in September. 3βHSD was found in Leydig cells in September and February with more intense staining in February. The localization of P450arom changed seasonally: no immunostaining in September; more extensive immunostaining in Leydig cells, Sertoli cells, and elongating spermatids in February. These results suggest that seasonal changes in the testis weight and size of wild male raccoon dogs are correlated with changes in spermatogenesis. Seasonal changes in testicular steroidogenesis suggest that the synthesis of androgen and estrogen reaches its peak in the mating season.

KEY WORDS: immunocytochemistry, *Nyctereutes procynoides*, raccoon dog, spermatogenesis, steroidogenesis.

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Mammals in temperate regions exhibit a seasonal cycle in reproduction with spermatogenesis and testicular steroidogenesis limited to a specific time of the year. Seasonal changes in spermatogenesis, morphology of the testis, and peripheral testosterone concentrations have been reported in numerous species, including brown bear (*Ursus arctos yesoensis*) [33], blue fox (*Alopex lagopus*) [29], ferrets (*Mustela putorius furo*) [22], fallow deer (*Dama dama*) [2], Cape Mountain zebras (*Equus zebra zebra*) [26] and Hawaiian monk seals (*Monachus sschauinslandi*) [3]. Further information regarding seasonal changes in testicular function is available in an extensive review by Lincoln [20]. These seasonal changes in testicular size and peripheral testosterone concentrations suggest significant changes in spermatogenesis and testicular steroidogenesis.

The raccoon dog (*Nyctereutes procyonoides*) is a typical seasonal breeder with a short sexually active period lasting about 6 weeks in February and March, followed by a long period of sexual dormancy from April to January [38]. During the mating period, male raccoon dogs are willing to mate and produce mature spermatozoa. The oestrus of female raccoon dogs lasts for about only 4 days, followed by a gestation of about 61 days when fertilization is successful [39]. Male raccoon dogs exhibit a marked seasonal variation in both testicular size and serum testosterone concentrations, which begin to increase during autumn, reach peak values in the mating season, and return to basal values during May to August when daylight hours are long [36].

Although much information on reproduction in the raccoon dog has been reported recently [10, 16, 36–39], many gaps in our understanding of the mechanisms of their reproduction remain. The aim of the present study was to investigate the mechanisms that regulate spermatogenesis and testicular steroidogenesis by immunocytochemical and histological observations in wild male raccoon dogs.

MATERIALS AND METHODS

Animals: Fifteen wild male raccoon dogs which were suspected to be adult based on their body weights (2.32–5.24 kg) and had died due to traffic accidents were collected within 72 hr of death from 18 September 2000 to 24 April 2001 in Gifu and Kanagawa Prefectures in Japan. Testicular weight was measured using scales, and testicular size was expressed in mm after measuring the (length $\times$ width $\times$ height)$^{1/3}$ following autopsy. Testicular tissues obtained were immediately fixed for 13 hr in Bouin's solution for histological and immunocytochemical observation.

Histology: Testicular samples were dehydrated in an ethanol series and embedded in paraffin wax. Serial sections (4 μm) were mounted on slides coated with poly-L-lysine (Sigma, St. Louis, MO, U.S.A.). Some sections were stained with hematoxylin-eosin (HE) for observations of general histology. Ten seminiferous tubules per raccoon dog were evaluated histologically using an Olympus photo-

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microscope with a × 40 objective lens. A movable cursor dot (approximately 0.1 μm in diameter) was used for measuring the diameter of the testis. Jandel Scientific Sigma Scan^R Image Analysis software (Jandel Scientifics Corporation, Montgomeryville, PA) was used for processing measurements. The repeatability of measurements, expressed as a coefficient of variation for ten measurements, was 0.74% at 10 μm and 5.8% at 0.7 μm.

Evaluation of spermatogenesis: The score count for determining the stage of spermatogenesis [14] was modified and used to evaluate spermatogenesis in the aspirates of testes. The modified criteria of the score count, principally based on the most advanced spermatogenetic cells, were as follows: Score 1, spermatogonia A only; Score 2, no cells further than spermatogonia B; Score 3, no cells further than L (leptotene) primary spermatogocytes; Score 4, no cells further than Z (zygotene) primary spermatogocytes; Score 5, no cells further than P(pachytene) primary spermatogocytes; Score 6, no cells further than round spermatids; Score 7, no cells further than the small head of the spermatozoon; Score 8, a few mature-phase spermatozoa (≤5 in each field under × 400); and Score 9, many mature-phase spermatozoa (>5 in each field under × 400). The counting was usually performed while the whole sample was examined under a light microscope, using a low (× 100) and then a high (× 400) magnification.

Immunocytochemistry: Adjacent sections were incubated with 10% normal goat serum to reduce background staining caused by the second antibody. The sections were then incubated with primary antibody (1:500 or 1:1000) raised against bovine adrenal cholesterol side-chain cleavage cytochrome P450 (P450scc) [1], human placental 3β-hydroxysteroid dehydrogenase (3βHSD) [6], porcine testicular 17α-hydroxylase cytochrome P450 (P450c17) [9], and human placental aromatase cytochrome P450 (P450arom) [17] for 16 hr at room temperature. The antibodies of 3βHSD and P450arom were kindly supplied by Dr. Mason and Dr. Harada, respectively, and the antibodies of P450scc and P450c17 were kindly supplied by Dr. Kominami. The sections were then incubated with a second antibody, goat anti-rabbit lgG conjugated with biotin and peroxidase with avidin, using a rabbit ExtrAvidin^{TM} staining kit (Sigma), followed by colouring with 30 mg 3,3-diaminobenzidine (Wako) solution in 150 m*l* of 0.05 mol Tris-HCl l^{-1} buffer, pH 7.6, plus 30 μl H_2O_2. Finally, the reacted sections were counterstained with haematoxylin solution (Merck). The control sections were treated with normal rabbit serum (Sigma) instead of the primary antisera.

RESULTS

Testicular size and weight: Seasonal changes in testis size and weight in wild male raccoon dogs are shown in Figs. 1 and 2. The largest values were found during the mating season in March (mean testicular size, 20.56 mm; mean testicular weight, 5.89 g) and the lowest values were found during the non-mating season in September (mean testicular size, 11.564 mm; mean testicular weight, 1.82 g).

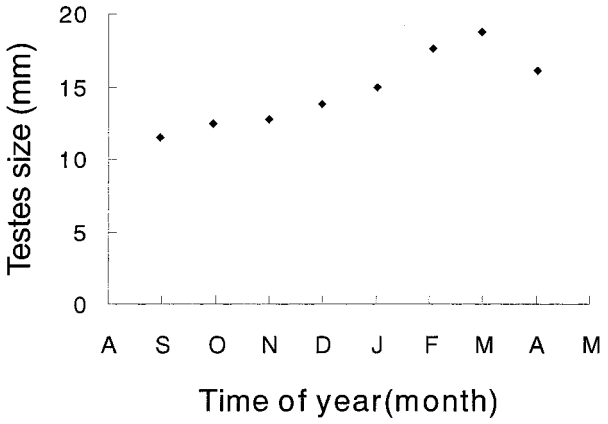

Fig. 1. Seasonal changes in testis size of wild male raccoon dogs.

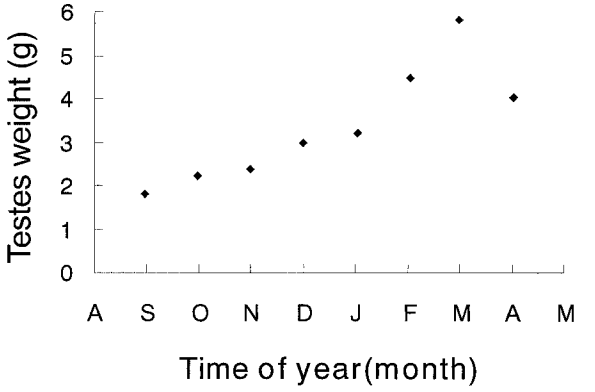

Fig. 2. Seasonal changes in testis weight of wild male raccoon dogs.

Seminiferous tubule diameter: There were seasonal changes in seminiferous tubule diameters in wild male raccoon dogs (Fig. 3). The values of the seminiferous tubule diameters were low in September (78.45 ± 9.7 μm, mean ± SD), began to increase in October, and thereafter increased steadily until the mating season of the following year (February and March) when they reached their highest values (160.52 ± 18.3 μm, mean ± SD). The seminiferous tubule diameter then started to decline in April.

Evaluation of spermatogenesis: Marked seasonal changes were observed in the histological appearance of seminiferous epithelium from September to April of the following year. The mean score count of spermatogenesis also changed seasonally during this period (Fig. 4). Spermatogonia and primary spermatocytes were present in September. Spermatogonia, spermatocytes and round spermatids were present in January. All types of spermatogenic cells were found, including mature-phase spermatozoa in February. Spermatogonia and degenerating spermatocytes were present in April.

Immunocytochemistry: P450scc, 3βHSD and P450c17

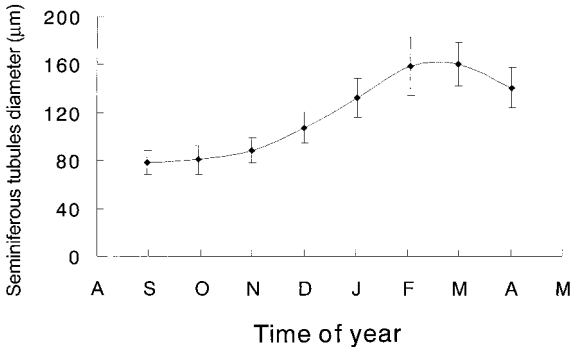

Fig. 3. Seasonal changes in seminiferous tubule diameter of wild male raccoon dogs.

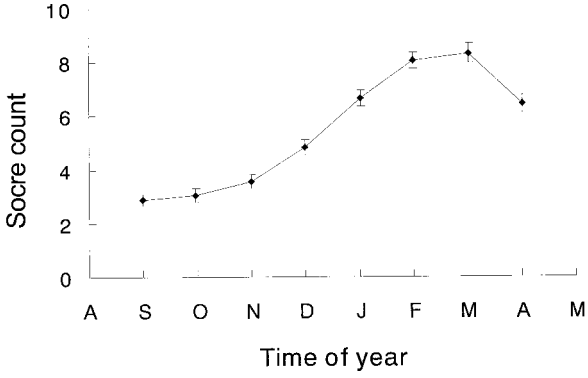

Fig. 4. Seasonal changes in the mean score count of spermatogenesis of wild male raccoon dogs.

were immunolocalized in Leydig cells in wild male raccoon dogs in September and February (Fig. 5b, c, d, f, g, h). No immunostaining was detected in control sections when normal rabbit serum was substituted for the primary antibody (Fig. 5a). Seasonal changes in the immunolocalization of P450scc and P450c17 were very similar. These enzymes were present in both Leydig cells and spermatids in February during the mating season, but only in Leydig cells in September during the non-mating season. 3βHSD was immunolocalized weakly in Leydig cells in September and more intensely in February (Fig. 5d, h). Marked seasonal changes in immunolocalization of P450arom were observed. P450arom was not observed in Leydig cells in September. The most extensive immunostaining was present in Leydig cells, Sertoli cells and round and elongating spermatids in February during the mating season (Fig. 5e, i).

DISCUSSION

The results of this study demonstrate interacting seasonal alterations among testicular weight, size and spermatogenesis in wild male raccoon dogs, which are closely associated with variations in the immunolocalization of testicular steroidogenic enzymes.

The cyclical alternation in the growth and involution of testes is a well-known phenomenon [5], and the seasonality of testicular mass and spermatogenesis shown in this study is in general agreement with some reports on this species [36–38]. We observed testicular recrudescence and regression in the adult male raccoon dog as judged by testis weight, size and score count of spermatogenesis with highest values of weight and size in the mating season. In addition, the results of seminiferous tubule diameter also showed that the highest values were in the mating season. These results suggest that seasonal changes in testicular weight and size are correlated with changes in the numbers of germ cells in the wild male raccoon dog. This finding is similar to that observed in other mammals such as the black bear [35], roe deer [5] and horses [12, 13].

The survival of spermatogenic cells is dependent on gonadotrophins as well as intratesticular androgens induced by LH [4, 21, 32]. Testosterone is essential for male sexual differentiation during fetal development, as well as for the initiation and maintenance of spermatogenesis and the expression of male secondary sex characteristics during postnatal development. Testosterone regulates spermatogenesis at specific germ-cell transformation steps [5]. An important function of androgen is the conversion of round spermatids to elongated ones in the rat [24]. Testosterone also plays an important role in preventing apoptotic cell death in androgen-dependent tissues [30, 31]. Unfortunately, serum testosterone concentrations could not be determined in wild male raccoon dogs in this study, but there has been a report on farmed raccoon dogs by Xiao [36] of marked seasonal changes in serum testosterone concentrations with a significant rise in October, a peak in the early breeding season, and a rapid drop at the end of the breeding season.

The biosynthesis of sex steroid hormones such as progestin, androgen and estrogen requires the activity of each specific steroidogenic enzyme. Immunolocalization of steroidogenic enzymes was first observed in the raccoon dog in this study, showing that P450scc, 3βHSD and P450c17 were localized in Leydig cells in the non-mating season, and that P450scc, P450c17 and P450arom were localized in Leydig cells and spermatids in the mating season. These findings are similar to those observed in other species such as the Hokkaido brown bear [34] and the Japanese black bear [18]. In this study, the results strongly suggest that the synthesis of progestin, androgen and estrogen occurs in Leydig cells; and pregnenolone, androgen and estrogen synthesis occur in spermatids in male raccoon dog testis.

It was of even more interest that there was a seasonal change in immunolocalization and staining intensity of the steroidogenic enzymes P450scc, 3βHSD, P450c17 and P450arom in male raccoon dogs in this study. P450scc, 3βHSD and P450c17 were localized in Leydig cells in both the non-mating (September, December and April) and mating seasons (February); However, the staining of P450scc, 3βHSD and P450c17 in Leydig cells was more intense dur-

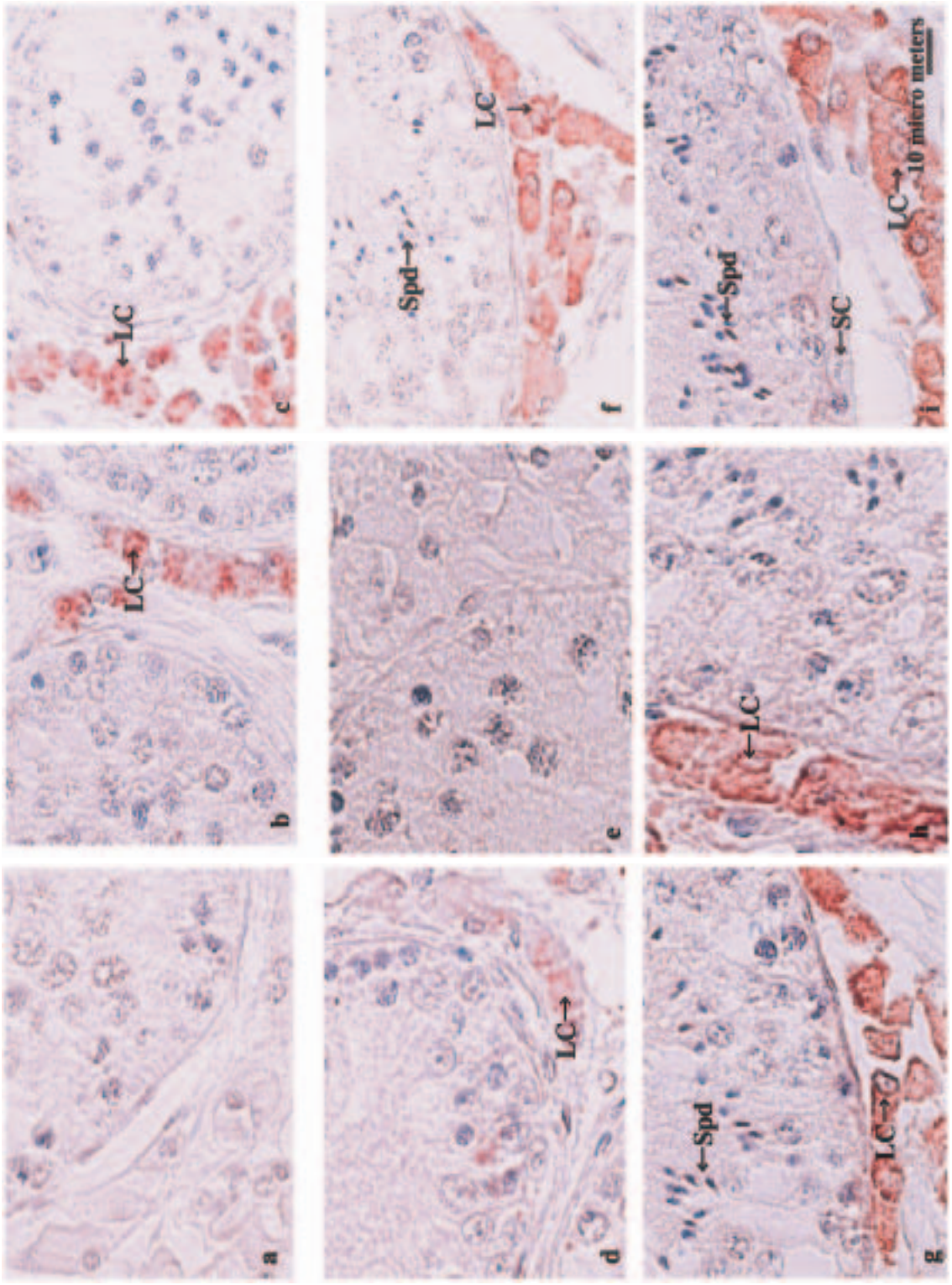

Fig. 5. Immunolocalization of steroidogenic enzymes in the testis of the non-mating (b, c, d) (September) and mating (f, g, h, i) (February) raccoon dog. Immunostaining for P450scc and P450c17, which were present in both Leydig cells and spermatids (f, g) in February but only in Leydig cells in September (b, c); 3βHSD was immunolocalized weakly in Leydig cells in September and more intensely in February (d, h); P450arom was not observed in September but was present in Leydig, Sertoli cells and round and elongating spermatids in February (e, i). Control incubated with normal rabbit serum in place of primary antiserum (a). LC: Leydig cell, SC: Sertoli cell, Spd: Spermatid.

ing the mating than the non-mating season; moreover, P450scc and P450c17 were also localized in spermatids in the mating season. These findings are similar to those observed in other species in which seasonal reproduction in staining for testicular steroidogenic enzymes was most intense when the testes were fully active [20, 35]. These findings also suggest that the synthesis of androgen and estrogen reaches their highest values in the mating season in wild male raccoon dogs.

The Leydig cells of the testis are the only cells in the male with the capacity to synthesize testosterone from cholesterol [25]. Leydig cells and spermatids are the predominant sites of androgen synthesis [34]. Our results in this study suggest that the synthesis of androgen reached its peak in the mating season in male raccoon dogs. In addition, our immunocytochemical data also suggest that testicular steroidogenesis correlates with spermatogenesis in male raccoon dogs, which is similar to other species that exhibit seasonal changes in spermatogenesis and testicular steroidogenesis [18, 35].

There was a marked seasonal change in the immunolocalization of P450arom. Immunoreactivity specific to P450arom was observed in Leydig cells, Sertoli cells and spermatids in February. These findings are similar to those for American black bears [35] and Japanese black bears [18]. P450arom is a key enzyme which precedes the aromatization of testosterone to estradiol. Aromatase activity has been observed in the Leydig cells of adult rats and in the Sertoli cells of prepubertal ones [7, 8, 27, 28]. P450arom was immunolocalized in spermatids of the raccoon dog testis, a characteristic which is not unique to the raccoon dog. In mice, rats, roosters and bears [11, 18, 19, 23, 35], the P450arom was present not only in Leydig cells but also in the seminiferous tubules especially in spermatids and epididymal spermatozoa.

Our present data support the proposal that Leydig cells and germ cells are the sites of estrogen synthesis in seminiferous tubules, and that estrogen plays a role in the maturation of sperm in the epididymis [18, 35]. These findings provide new evidence for germ cells as a new source of estrogens in the male gonad and that parts of the male gonadal functions are not only androgen regulated but also estrogen controlled. Future studies will elucidate whether P450arom mRNA is expressed in the testes of raccoon dogs and what the function of the estrogen in germ cells is.

In conclusion, seasonal changes in testis weight and size in wild male raccoon dogs are correlated with changes in spermatogenesis and testicular steroidogenesis, and the synthesis of androgen and estrogen reaches their highest values in the mating season.

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REFERENCES

1. Anakwe, O. O. and Payne, A. H. 1987. Noncoordinate regulation of *de novo* synthesis of cytochrome P450 cholesterol side-chain cleavage and cytochrome P450 17α-hydroxylase/c17–20 lyase in mouse Leydig cell cultures: relation to steroid production. *Mol. Endocrinol.* **1**: 596–603.
2. Asher, G. W. and Peterson, A. J. 1991. Pattern of LH and testosterone secretion of adult male fallow deer (*Dama dama*) during the transition into the breeding season. *J. Reprod. Fertil.* **91**: 649–654.
3. Atkinson, S. and Gilmartin, W. G. 1992. Seasonal testosterone pattern in Hawaiian monk seals (*Monachus schauinslandi*). *J. Reprod. Fertil.* **96**: 35–39.
4. Billig, H., Furuta, I., Rivier, C., Tapanainen, J., Parvinen, M. and Hsueh, A. J. W. 1995. Apoptosis in testis germ cells: developmental changes in gonadotropin dependence and localization to selective tubule stages. *Endocrinology* **136**: 5–12.
5. Blottner, S., Hingst, O. and Meyer, H. H. D. 1996. Seasonal spermatogenesis and testosterone production in roe deer (*Capreolus capreolus*). *J. Reprod. Fertil.* **108**: 299–305.
6. Doody, K. M., Carr, R. R., Rainy, W. E., Byed, W., Murry, B. A., Strickler, R. C., Thomas, J. I. and Mason, J. I. 1990. 3β-hydroxysteroid dehydrogenase/isomerase in the fetal zone and neocortex of the human fetal adrenal gland. *Endocrinology* **126**: 2487–2492.
7. Dorrington, J. H. and Armstrong, D. T. 1975. Follicle-stimulating hormone stimulates estradiol-17β synthesis in cultured Sertoli cells. *Proc. Nat. Sci. U.S.A.* **72**: 2677–2681.
8. Dorrington, J. H., Fritz, I. B. and Armstrong, D. T. 1978. Control of testicular estrogen synthesis. *Biol. Reprod.* **18**: 55–64.
9. Hales. D. B., Sha, L. and Payne, A. H. 1987. Testosterone inhibits cAMP-induced de novo synthesis of Leydig cell cytochrome P45017α by an androgen receptor-mediated mechanism. *J. Biol. Chem.* **262**: 11200–11206.
10. Helle, E. and Kauhala, K. 1995. Reproduction in the raccoon dog in Finland. *J. Mammal.* **76**: 1036–1046.
11. Janulis, L., Hess, R. A., Bunick, D., Nitta, H., Janssen, S., Osawa, Y. and Bahr, J. M. 1996. Mouse epididymal sperm contain active P450 aromatase which decreases as sperm traverse the epididymis. *J. Androl.* **17**: 111–117.
12. Johnson, L. 1991. Seasonal differences in equine spermatogenesis. *Biol. Reprod.* **44**: 284–291.
13. Johnson, L. 1995. Efficiency of spermatogenesis. *Microsc. Res. Tech.* **32**: 385–422.
14. Johnsen, S. G. 1970. Testicular biopsy score count-a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. *Hormones.* **1**: 2–25.
15. Kauhala, K. 1996. Reproductive strategies of the raccoon dog and red fox in Finland. *Acta. Theriol.* **41**: 51–58.
16. Kauhala, K. and Helle, E. 1990. Age determination of the raccoon dog in Finland. *Acta. Theriol.* **35**: 321–329.
17. Kitawaki, J., Yoshida, N. and Osawa, Y. 1989. An enzyme-linked immunosorbent assay for quantitation of aromatase cytochrome P450. *Endocrinology* **124**: 1417–1423.
18. Komatsu, T., Tsubota, T., Yamamoto, Y., Atoji, Y. and Suzuki,

Y. 1997. Seasonal changes in the immunolocalization of steroidogenic enzymes in the testes of the Japanese black bear (*Ursus thibetanus japonicus*). *J. Vet. Med. Sci.* **59**: 521–529.
19. Kwon, S., Hess, R. A., Bunick, D., Nitta, H., Janulis, L., Osawa, Y. and Bahr, J. M. 1995. Rooster testicular germ cells and epididymal sperm contain P450 aromatase. *Biol. Reprod.* **53**: 1259–1264.
20. Lincoln, G. A. 1989. Seasonal aspects in testicular physiology in seasonal mammals. *In*: pp. 229–385. The Testis (Burger H and Kretser D. eds.), Raven Press, New York.
21. Marathe, G. K., Shetty, J. and Dighe, R. R. 1995. Selective immunoneutralization of luteinizing hormone results in the apoptotic cell death of pachytene spermatocytes and spermatids in the rat testis. *Endocrine* **3**: 705–709.
22. Neal, J., Murphy, B. P., Moger, W. H. and Oiphant, L. W. 1977. Reproduction in the male ferret: gonadal activity during the annual cycle; recrudescence and maturation. *Biol. Reprod.* **17**: 380–385.
23. Nitta, H., Bunick, D., Hess, R. A., Janulis, L., Newton, S. C., Millette, C. F., Osawa, Y., Shitzutu, Y., Toda, K. and Bahr, J. M. 1993. Germ cells of the mouse testis express P450 aromatase. *Endocrinology* **132**: 1396–1401.
24. O'Donnell, L., Mclachlan, R. I., Wreford, N. G. and Robertson, D. M. 1994. Testosterone promotes the conversion of round spermatids between stages VII and VIII of the rat spermatogenic cycle. *Endocrinology* **135**: 2608–2614.
25. Payne, A. H. and Youngblood, G. L. 1995. Regulation of expression of steroidogenic enzymes in Leydig cells. *Biol. Reprod.* **52**: 217–225.
26. Penzhorn, B. L. and van der Merwe, N. J. 1988. Testis size and onset of spermatogenesis in cape mountain zebras (Equus zebra zebra). *J. Reprod. Fertil.* **83**: 371–375.
27. Rommerts, F. F. G., de Jong, F. H., Brrinkmann, A. O. and van der Molen, H. J. 1982. Development and cellular localization of rat testicular aromatase activity. *J. Reprod. Fertil.* **64**: 281–288.
28. Rosselli, M. and Skinner, M. K. 1992. Developmental regulation of Sertoli cell aromatase activity and plasminogen activator production by hormones, retinoids, and the testicular paracrine factor, PmodS. *Biol. Reprod.* **46**: 586–594.
29. Smith, A. J., Mondain-Monval, M., Moller, O. M., Scholler, R. and Hansson, V. 1984. Seasonal variations of LH, prolactin, androstenedione, testosterone and testicular FSH binding in the male blue fox (*Alopex lagopus*). *J. Reprod. Fertil.* **74**: 449–458.
30. Tenniswood, M. P., Guenette, R. S., Lakins, J., Mooibroek, M., Wong, P. and Welsh, J. E. 1992. Active cell death in hormone-dependent tissue. *Cancer Metastasis Rev.* **11**: 197–220.
31. Thompson, E. B. 1994. Apoptosis and steroid hormones. *Mol. Endocrinol.* **8**: 665–673.
32. Troiano, L., Fustini, M. F., Lovato, E., Frasoldati, A., Malorni, W., Capri, M., Grassilli, E., Marrama, P. and Franceschi, C. 1994. Apoptosis and spermatogenesis: evidence from an *in vivo* model of testosterone withdrawal in the adult rat. *Biochem. Biophys. Res. Commun.* **202**: 1315–1321.
33. Tsubota, T. and Kanagawa, H. 1989. Annual changes in serum testosterone levels and spermatogenesis in the Hokkaido brown bear (*Ursus arctos yesoensis*). *J. Mamm. Soc. Jan.* **14**: 11–17.
34. Tsubota, T., Nitta, H., Osawa, Y., Mason, J., Kita, I., Tiba, T. and Bahr, J. M. 1993. Immunolocalization of steroidogenic enzymes, P450scc, 3βHSD, P450arom in the Hokkaido Brown Bear (*Ursus arctos yesoensis*) testis. *Gen. Comp. Endocrinol.* **92**: 439–444.
35. Tsubota, T., Howell-Skalla, L., Nitta, H., Osawa, Y., Mason, J. I., Meiers, P. G., Nelson, R. A. and Bahr, J. M. 1997. Seasonal changes in spermatogenesis and testicular steroidogenesis in the male black bear (*Ursus americanus*). *J. Reprod. Fertil.* **109**: 21–27.
36. Xiao, Y., Jiao, S., Tong, Y., Song, J. and Wang, G. 1994. Seasonal changes in testicular size and serum testosterone levels and the relationships between serum testosterone concentrations and reproductive performance in male raccoon dogs. *Scientifur* **18**: 39–43.
37. Xiao, Y., Forsberg, M., Laitinen, J. T. and Valtonen, M. 1995. Effects of melatonin implants in spring on testicular regression and moulting in adult male raccoon dogs (*Nyctereutes procynoides*). *J. Reprod. Fertil.* **105**: 9–15.
38. Xiao, Y., Forsberg, M., Laitinen, J. T. and Valtonen, M. 1996. Effects of melatonin implants on winter fur growth and testicular recrudescence in adult male raccoon dogs (*Nyctereutes procyonoides*). *J. Pineal. Res.* **20**: 148–156.
39. Valtonen, M. H., Rajakoski, E. J. and Makela, J. I. 1977. Reproductive features in the female raccoon dog (*Nyctereutes procyonoides*). *J. Reprod. Fertil.* **51**: 517–518.