

Seasonal differences in spermatogenesis, testicular mass and serum testosterone concentrations in the grizzly bear

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Abstract: The objectives of this study were to determine whether there are seasonal changes in spermatogenesis for pre-pubertal and post-pubertal grizzly bears (*Ursus arctos horribilis*), and investigate the seasonal association between testis mass and serum testosterone (T) concentrations for post-pubertal grizzly bears from the continental US from May through October. Testes from 25 grizzly bears were collected from bears killed by federal and state wildlife personnel in Montana and Wyoming from 1978 to 1995. Fifty blood samples were obtained from wild, post-pubertal (≥ 5.5 years) male grizzly bears from May through October in Montana and Wyoming from 1993 through 1995. In pre-pubertal bears, the seminiferous tubules were small and surrounded by abundant interstitial tissue in May. Tubules were enlarged and closely packed July through September. Tubules began to degenerate in November. Although spermatogonia and spermatocytes were present from May through September, spermatids never occurred within seminiferous tubules. The epididymal tubules in pre-pubertal bears were well organized from May through September, although they never contained spermatozoa. In post-pubertal bears, spermatogenesis changed seasonally: the entire spermatogenic population from spermatogonia through spermatids was present May through August, and spermatogonia and spermatocytes were present in October and November. The seminiferous epithelium began to deteriorate in July. The epididymal tubules contained spermatozoa May through August only. Both testis mass and T concentrations peaked in June. Mean T concentrations during May and June were greater ($P = 0.02$) than those during July through October. These results suggest that in grizzly bears in the continental US, seasonal changes in spermatogenesis are accompanied by changes in testis mass and T concentrations and both are associated with photoperiod.

Key words: epididymal tubules, grizzly bear, Montana, seminiferous tubules, testis, testosterone, *Ursus arctos horribilis*, Wyoming

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Research on reproductive biology of male bears has been limited to American black bears (*Ursus americanus*), Asiatic black bears (*U. thibetanus japonicus*), brown bears (*U. arctos*), and polar bears (*U. maritimus*) and has concentrated primarily on characterizing seasonal testosterone (T) concentrations (McMillin et al. 1976, Palmer et al. 1988, Tsubota and Kanagawa 1989, Horan et al. 1993, Garshelis and Hellgren 1994, Tsubota et al. 1999) and gonadal activity (Erickson and Nellor

1964, Erickson et al. 1968, Tsubota and Kanagawa 1989, Garshelis and Hellgren 1994, Komatsu et al. 1996, Tsubota et al. 1997). In temperate climates, male bears are annual breeders with regression of testicular structure and function between breeding periods. Although regional variation occurs (Garshelis and Hellgren 1994), generally, T concentrations, testis mass or size, and spermatogenesis increase from January through June, implicating increasing daylength (or decreasing night-length) as an important regulator of the seasonal control mechanism of the hypothalamic–pituitary–testis axis. Spermatogenesis, with spermatozoa in the epididymides, typically occurs from April through August, although it

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can occur as early as March (Tsubota and Kanagawa 1989) and as late as November (Erickson et al. 1968).

Most of what is known about brown bear gonadal activity, however, is limited to the non-breeding or early breeding season (Sep–May) in Alaska (Erickson et al. 1968), the Yukon (Pearson 1975), and Hokkaido, Japan (Tsubota and Kanagawa 1989, Tsubota et al. 1991). The seasonal relationship between T concentrations and testicular activity has not been reported for grizzly bears. Therefore, the objectives of this study were to: (1) identify seasonal differences in spermatogenesis for pre-pubertal and post-pubertal grizzly bears, and (2) investigate the seasonal association between testis mass, T concentrations, and photoperiod for post-pubertal grizzly bears from the continental US from May through October.

Methods and materials

Tissue sources and processing

Testes and epididymides from 14 pre-pubertal and 11 post-pubertal grizzly bears were collected from bears killed by federal and state wildlife personnel in Montana and Wyoming from 1978 to 1995. Therefore, we did not measure seasonal differences in the testis and epididymis by repeated measures of the same animals. Usually within a few hours after death, the testes and epididymides of each bear were removed from the scrotum and dissected free of the tunica vaginalis. The epididymides were removed from the testes and each was weighed separately. Each testis and epididymis was fixed in neutral-buffered formalin for several days then stored in 10% formalin or 70% ethanol.

Blocks of tissue were cut from each testis and mid-corpus epididymis and dehydrated in a graded series of ethanol concentrations and embedded in paraffin. White et al. (1998) demonstrated that local sampling of testicular parenchyma is valid in grizzly bears as their testes exhibit uniformity between regions. Tissue sections were cut 5- μ m thick, deparaffinized, and stained with hematoxylin and eosin. Tissue images were captured using a digital camera attached to a light microscope (400 \times).

Age of each bear was determined by counting the cementum annuli of premolars (Willey 1974). Age at puberty, the age at which spermatozoa first accumulate within the seminiferous and epididymal tubules in quantity, is attained in grizzly bears in Montana and Wyoming in 5.5 years (White et al. 1998). Testes were considered pre-pubertal when the seminiferous tubules were completely aspermatic and the epididymis was

devoid of spermatozoa. As testis collection was opportunistic, it was not possible to obtain specimens for all seasons and ages.

Spermatogenesis

To investigate seasonal differences in spermatogenesis for grizzly bears from the continental US, we visually examined testicular and mid-corpus epididymal tissue sections from 14 pre-pubertal (killed in May [1], Jul [3], Aug [4], Sep [5], and Nov [1]) and 11 post-pubertal (killed in May [1], Jun [1], Jul [3], Aug [3], Sep [2], and Oct [1]) bears. Several morphological characteristics were observed. For testicular tissue, we recorded relative shape and size of seminiferous tubules, general condition of seminiferous epithelium (organized versus deteriorating), germ cell types present, relative amount of interstitial tissue, presence of vacuoles in the interstitial tissue, and presence of spermatozoa. For epididymal tissue, we recorded general condition of the tubules, presence of microvilli and spermatozoa, and relative thickness of smooth muscle fibers surrounding the tubules. If we documented distinct differences in histological characteristics by month, we concluded that seasonal differences occurred in the testis and epididymis.

For months with >1 bear, we observed little histological variation within months (in Jul–Sep for pre- and post-pubertal bears). Therefore, light micrographs of seminiferous and epididymal tubules from pre- and post-pubertal bears in Fig. 1 and 2, respectively, illustrate general differences in tubular architecture between months. The tissue sections are not necessarily the same cell association (Hess 1999), nor are they intended to illustrate all cell associations within the seminiferous epithelia of grizzly bears.

Blood sampling and testosterone radioimmunoassay

Blood samples were collected opportunistically in 1993 through 1995 as bears were captured as part of other research projects in Montana and Wyoming. All bears were captured in culvert traps or snares and immobilized with 4 mg/kg ketamine. Blood samples were taken from 50 different male grizzly bears with vacutainers or syringes from a femoral vein shortly after immobilization. Whole blood was stored frozen at -20°C for <2 years, centrifuged at 1,850 \times gravity for 20 min at 4°C , then assayed for testosterone (T). Testosterone was assayed without extraction by radioimmunoassay (Coat-a-count, Total testosterone, Diagnostic Products Co., Los Angeles, California, USA). Assay sensitivity was 0.05

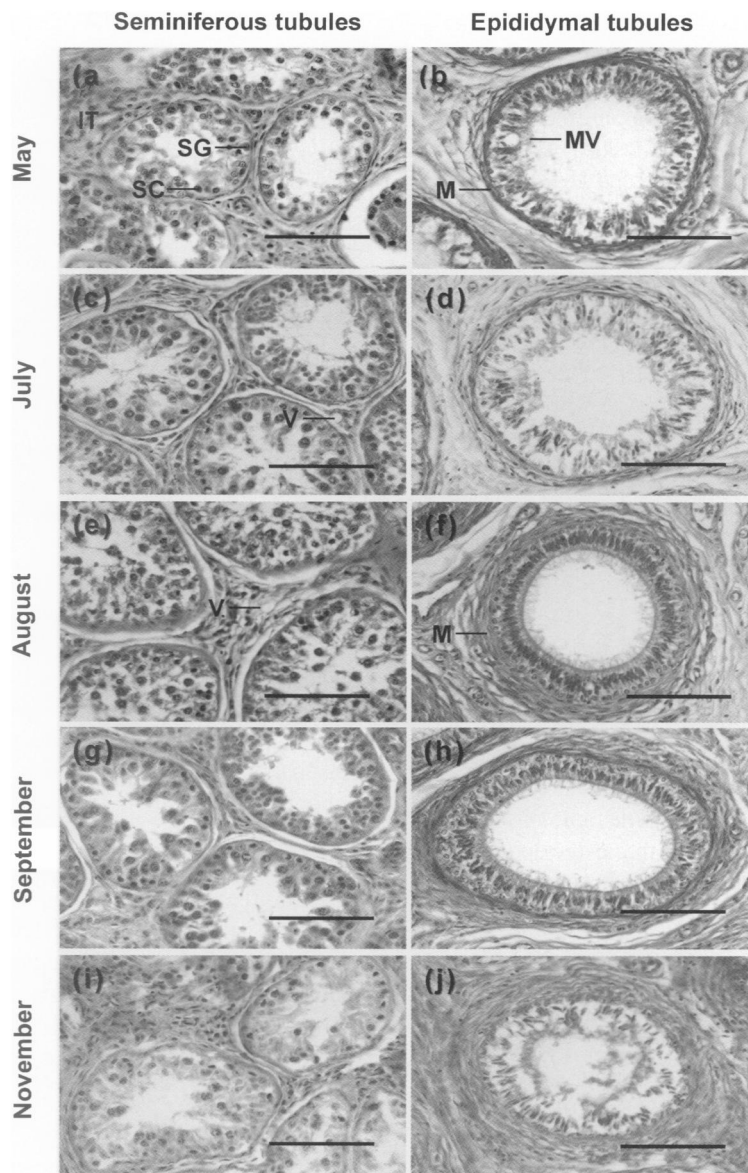


Fig. 1. Seasonal changes in seminiferous epithelium and mid-corpus epididymal epithelium in 14 pre-pubertal grizzly bears killed in Montana and Wyoming in May (1), Jul (3), Aug (4), Sep (5), and Nov (1). Testicular and epididymal sections within each month were from the same bear. All scale bars = 150 μm . (a) spermatogonia (SG) and spermatocytes (SC) present; interstitial tissue (IT) abundant. (b) microvilli (MV) lining the lumen of tubules dispersed but common; tubules surrounded by a narrow band of smooth muscle fibers (M). (c) tubules closely packed (little interstitial tissue); vacuoles (V) in interstitium common. (d) microvilli abundant and common; tubules surrounded by narrow band of smooth muscle fibers. (e) tubules closely packed together; vacuoles (V) in interstitium common. (f) microvilli abundant; tubules surrounded by thick band of smooth muscle fibers (M). (g) tubules closely packed together; vacuoles in interstitium not as common as in Aug. (h) microvilli dispersed; tubules surrounded by a thick band of smooth muscle fibers. (i) tubules not as rounded and inflated as in Jul, Aug, and Sep; interstitial tissue abundant (tubules not tightly packed together); few vacuoles in interstitium. (j) epithelium deteriorated; microvilli absent; tubules surrounded by thick band of smooth muscle fibers.

ng/ml. Both inter- and intra-assay coefficients of variation were <10% for concentrations of T \geq 0.5 ng/ml.

Concentrations of T from May through October were analyzed by one-way ANOVA. The Holm-Sidak test was used to test for differences between means. Statistical significance was assumed when the type I error was <0.05. Blood samples used in this study did not originate from the same bears in which testes and epididymides were collected. Testis mass and T concentrations from post-pubertal bears (\geq 5.5 years old) only were reported; we did not have these data for pre-pubertal bears.

Results

Spermatogenesis—pre-pubertal bears

May. The seminiferous tubules in May were the smallest of all months examined (Fig. 1a). The seminiferous epithelia contained spermatogonia and spermatocytes; spermatids were absent from the epithelium. Lumina were absent or indistinct in most tubules. Interstitial tissue was abundant, comprising a large proportion of the volume of the testis. The corpora epididymides were well organized, lumina were quite distinct, and microvilli were dispersed but common (Fig. 1b). A thin band of smooth muscle fibers surrounded each tubule (Fig. 1b). No spermatozoa were observed in this portion of the epididymus.

July. Evidence of testicular maturation appeared in July (Fig. 1c). The seminiferous tubules of these bears were larger in diameter and were closely packed and the area occupied by interstitial tissue appeared smaller. Large vacuoles appeared in the interstitium. The height of the seminiferous epithelium was uneven, giving the lumen a star-shaped appearance. Spermatogonia and spermatocytes were present but no spermatids were observed. The corpora epididymides were well organized, lumina were quite distinct, and microvilli were abundant (Fig. 1d). A thin band of smooth muscle fibers surrounded the tubules (Fig. 1d). No spermatozoa were observed in this portion of the epididymus.

August–September. Seminiferous tubules in August and September were rounded, enlarged, and closely packed. (Fig. 1e,g). Lumina were relatively indistinct and star-shaped. Spermatogonia and spermatocytes were present. No spermatids were observed. Small vacuoles were present in the interstitium. Interstitial tissue was more abundant in September than July and August. The corpora epididymides were well organized and their lumina distinct (Fig. 1f,h). Tall microvilli were common in August but dispersed in September. A thick band of

smooth muscle fiber surrounded the tubules (Fig. 1f,h). No spermatozoa were observed in this portion of the epididymus.

November. Evidence of testicular regression appeared in November (Fig. 1i). Interstitial tissue was abundant (tubules were not closely packed) and the number of spermatocytes were fewer than in August–September. The epithelia of the corpora epididymides were in decline (Fig. 1j). Microvilli were absent. A thick layer of smooth muscle fibers surrounded the tubules (Fig. 1j). No spermatozoa were observed in this portion of the epididymus.

Spermatogenesis—post-pubertal bears

May. The seminiferous epithelia in May produced and released spermatozoa (Fig. 2a). All generations of spermatogenic cells, from spermatogonia to fully formed spermatozoa, were present. Large vacuoles were present in the interstitium. The corpora epididymides were well organized and spermatozoa almost completely filled the lumina. Tall microvilli lining the lumen of the tubules were common (Fig. 2b).

June. Seminiferous tubule regression was evident in June (Fig. 2c). Although the seminiferous tubules were still well organized, round spermatids were fewer in number and smaller, and fewer vacuoles were present in the interstitium. The corpora epididymides were well organized and spermatozoa completely filled the lumina (Fig. 2d). Tall microvilli were common (Fig. 2d).

July–August. Seminiferous tubules in July and August (Fig. 2e,g) were similar in appearance to those in June (Fig. 2c). Few round spermatids were present and few vacuoles were observed in the interstitium. The corpora epididymides in July (Fig. 2f) were well organized, although the quantity of spermatozoa within the tubules appeared to be lower than in bears collected from May and June. The earliest signs of epididymal regression occurred in August (Fig. 2h); although spermatozoa were still present, epithelial deterioration was evident (Fig. 2h).

September–October. Seminiferous tubules were in decline by September and October (Fig. 2i,k). Only spermatogonia and spermatocytes were present; vacuoles in the interstitium were small or absent. Lumina of corpora epididymides (Fig. 2j) contained what appeared to be apoptotic cellular debris, and spermatozoa were absent. Microvilli were dispersed or absent in most individuals. The corpora epididymides for bears killed in October (Fig. 2l) were reduced in diameter and

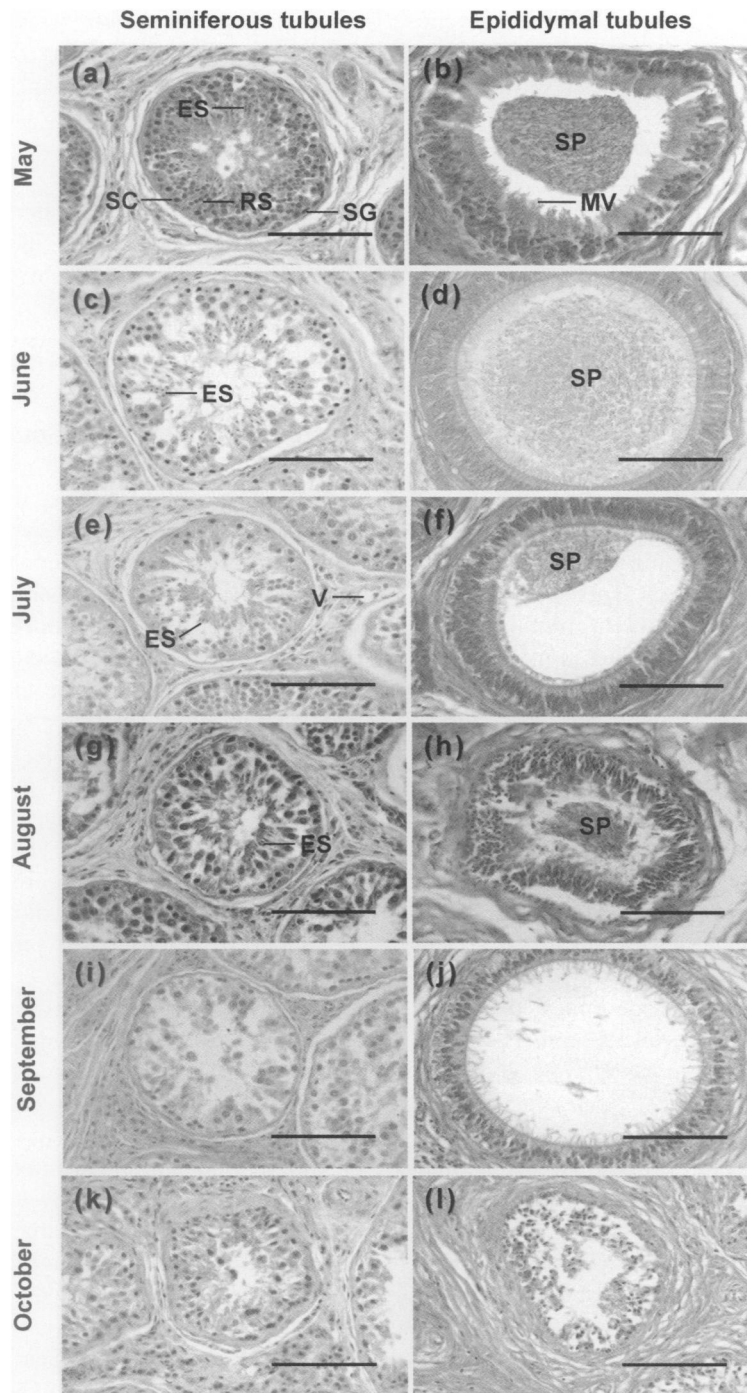


Fig. 2. Seasonal changes in seminiferous epithelium and mid-corpus epididymal epithelium in 11 post-pubertal grizzly bears killed in Montana and Wyoming in May (1), Jun (1), Jul (3), Aug (3), Sep (2), and Oct (1). Testicular and epididymal sections within each month were from the same bear. All scale bars = 150 μ m. (a) entire spermatogenic population including spermatogonia (SG), spermatocytes (SC), round spermatids (RS), and elongated spermatids (ES) present. (b) spermatozoa (SP) almost completely filled lumen; microvilli (MV) common. (c) spermatogonia, spermatocytes, round spermatids, and elongated spermatids (ES) present. (d) spermatozoa (SP) completely filled lumen; microvilli common. (e) elongated spermatids (ES) present; large vacuoles (V) in

the epithelium was atrophic. Spermatozoa within the lumina were absent.

Seasonal effects on testis mass and testosterone concentrations

Both testis mass and T concentrations were greatest in June, concurrent with maximum daylight (Fig. 3). Mean T concentrations during May and June were higher ($P = 0.02$) than those during July through October (Fig. 3).

Discussion

Seasonal differences in gonadal activity and T concentrations in grizzly bears from the continental US conforms generally with the standard ursid pattern described for American black bears (Erickson and Nellor 1964, Erickson et al. 1968, McMillin et al. 1976, Palmer et al. 1988, Tsubota et al. 1999), Asiatic black bears in Japan (Komatsu et al. 1996, 1998; Okano et al. 2003), Hokkaido brown bears (Tsubota and Kanagawa 1989; Tsubota et al. 1991, 1993), brown bears in Alaska (Erickson et al. 1968), and grizzly bears in the Yukon (Pearson 1975). Bears are long-day breeders with a breeding season extending from May to July (Erickson and Nellor 1964, Erickson et al. 1968, Craighead et al. 1969, Ballard et al. 1982, Garshelis and Hellgren 1994). Spermatogenesis and steroidogenesis begin before the breeding season. Round and elongated spermatids can be found within the seminiferous epithelia and spermatozoa in epididymides as early as March and April (Erickson et al. 1968, Tsubota and Kanagawa 1989). Testosterone concentrations peak in April or May (McMillin et al. 1976, Palmer et al. 1988, Tsubota and Kanagawa 1989, Garshelis and Hellgren 1994, Tsubota et al. 1997). Spermatogenesis and steroidogenesis typically decline in August, although spermatids and spermatozoa are present as late as October and November (Erickson et al. 1968).

Effects of season on spermatogenesis in pre-pubertal grizzly bears

The histology of the pre-pubertal grizzly bear testis and epididymis has not been well studied; thus, comparisons with other studies are limited. In pre-pubertal

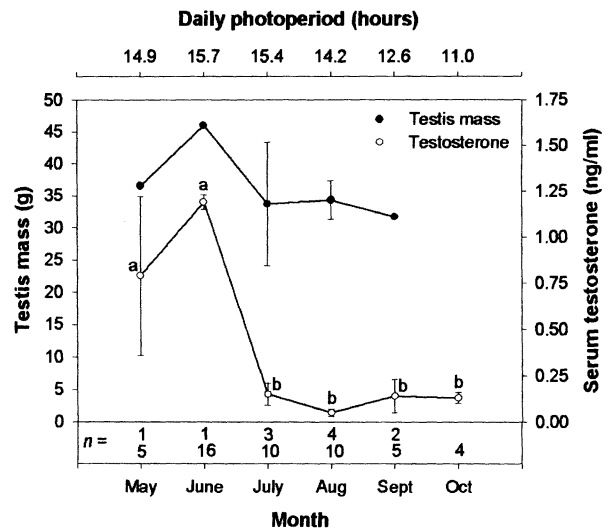


Fig. 3. Seasonal changes ($\bar{x} \pm SE$) in testis mass and serum testosterone concentration in post-pubertal male grizzly bears from Montana and Wyoming, 1993–95. Serum testosterone values with different letters are different (Holm-Sidak test, $P < 0.05$). Numbers below x-axis are sample sizes: testis mass (top row), serum testosterone (bottom row). Blood samples are not from the same bears from which testes were collected and were not obtained by repeated measures of the same bears. Hours of daylight were calculated for Bozeman, Montana (N45°41' and W111°02), the geographic center of our study area.

bears in our study, the histology of the testis differed seasonally, although not to the extent found in post-pubertal bears. The seminiferous epithelium contained only spermatogonia and spermatocytes May through November; spermatozoa never occurred (Fig. 1a,c,e,g,i). This observation indicates that the proliferation phase of spermatogenesis, but not the meiotic or differentiation phases, is activated well in advance of puberty in male grizzly bears. Although intra-testicular factors play a significant and complex role in spermatogenesis, testosterone stimulates and maintains both the meiotic and differentiation phases of spermatogenesis (Garner and Hafez 2000). Perhaps the absence of these phases in pre-pubertal grizzly bears indicates that testosterone con-

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interstitium common. (f) volume of spermatozoa (SP) reduced; microvilli common. (g) elongated spermatids (ES) present; few vacuoles present in interstitium. (h) spermatozoa (SP) present in lumen. (i) seminiferous epithelium deteriorating; spermatids absent. (j) spermatozoa absent; microvilli markedly dispersed. (k) seminiferous epithelium deteriorating; spermatozoa absent. (l) epithelium atrophied; spermatozoa absent.

centrations are not high enough to stimulate and maintain these phases.

Spermatozoa were not observed in the corpora epididymides of pre-pubertal bears. The corpora epididymides were typically well organized with numerous pseudostratified epithelial cells showing microvilli. Lumina were quite distinct from May through September (Fig. 1b,d,f,h). However, by November, the epithelia deteriorated (Fig. 1j). These histological differences suggest that there is seasonal regulation of epididymal function in pre-pubertal bears. The nature of this regulation, however, is not understood. Clearly, it is related to changes in testosterone and its cellular-active metabolite, dihydrotestosterone, the principal mediator of androgen function in the epididymus (Robaire and Hermo 1988). It may be that Leydig cells of pre-pubertal grizzly bears synthesize and secrete testosterone but in concentrations that are insufficient to stimulate and maintain spermatogenesis but are adequate to maintain the male excurrent duct system. Thus, maintenance of the epididymal structure and function deteriorates as testosterone concentrations decline due to seasonal regulation of Leydig cell function.

The seasonal patterns of seminiferous and epididymal tubule development reported by Erickson et al. (1968) for brown bears in Alaska and Erickson and Nellor (1964) for black bears in Michigan are consistent with what we found in grizzly bears from the continental US. Although the epididymal epithelium began to regress in September in Alaska (Erickson et al. 1968) and Michigan (Erickson and Nellor 1964), we did not observe regression until November. However, tissue samples from October were not available for our study.

Effects of season on spermatogenesis in post-pubertal grizzly bears

The seminiferous tubules in May and June were well organized and active; the entire spermatogenic population from spermatogonia to spermatids was present (Fig. 2a,c). Additionally, large vacuoles were present in the interstitium, indicating that Leydig cells were steroidogenically active. A similar pattern of gonadal activity was found during this time of year for brown bears in Alaska (Erickson et al. 1968), grizzly bears in the Yukon (Pearson 1975), Hokkaido brown bears (Tsubota and Kanagawa 1989, Tsubota et al. 1991), and American black bears (Erickson and Nellor 1964, Tsubota et al. 1997). However, Erickson and Nellor (1964) did not find spermatozoa in epididymal tubules in black bears from Michigan in May. Unfortunately, we were unable to obtain testes from grizzly bears before May, so we do

not know precisely when spermatogenesis is initiated in grizzly bears in the continental US. Tsubota et al. (1991), however, found spermatids within seminiferous tubules of Hokkaido brown bears as early as March.

The corpora epididymides of bears in our study in May and June were lined with tall ciliated pseudostratified cells, and the lumina were packed with spermatozoa (Fig. 2b,d). This observation is consistent with those of Erickson et al. (1968) and Pearson (1975) for brown bears in Alaska and grizzly bears in the Yukon, respectively.

Although large vacuoles were present in the testicular interstitium in July, indicating Leydig cell activity, deterioration of the seminiferous epithelium in our study was not evident until July and August (Fig. 2e,g). Erickson and Nellor (1964) also found numerous, highly vacuolated Leydig cells in the interstitia of black bears in Michigan in July, and Erickson et al. (1968) found that the seminiferous epithelium of brown bears in Alaska began to degenerate in July.

The corpora epididymides of bears in July and August in our study (Fig. 2f,h) were filled with spermatozoa and lined with microvilli. Erickson and Nellor (1964) also found the epididymal tubules of black bears in Michigan filled with spermatozoa and lined with microvilli in July.

Degeneration of the seminiferous epithelium in post-pubertal bears in our study was evident in September and October (Fig. 2i,k), although the seminiferous tubules were not collapsed as Pearson (1975) found in grizzly bears in the Yukon during this time of year. A similar seasonal pattern of deterioration of the seminiferous epithelium was reported by Erickson and Nellor (1964) for black bears in Michigan, Tsubota et al. (1997) for captive black bears in Illinois, Erickson et al. (1968) for brown bears in Alaska, and Tsubota and Kanagawa (1989) for Hokkaido brown bears. Erickson and Nellor (1964) found a heavy fibrous connective tissue surrounding the seminiferous tubules in October, a phenomenon not found in any other study, including ours. The occurrence of vacuoles in the interstitium of bears in our study was noticeably less in October, as reported in other studies (Erickson and Nellor 1964, Erickson et al. 1968). We found only spermatogonia and primary spermatocytes in September and October, as did Tsubota et al. (1997) for captive black bears in Illinois. However, spermatids were found this time of year by Erickson et al. (1968) for brown bears in Alaska and Tsubota and Kanagawa (1989) and Tsubota et al. (1991) for Hokkaido brown bears.

Consistent with Erickson and Nellor's (1964) study of black bears in Michigan, the cauda epididymides of the

bears in our study were deteriorating and the lumina contained cellular debris in September and October (Fig. 2j,l); by this time spermatozoa were absent.

Effects of season on testis mass and T concentrations

Although our sample sizes were small, seasonal difference in testis mass generally paralleled seasonal differences in T concentrations (Fig. 3). The heaviest testes from a post-pubertal bear in our study were collected in June, coincident with maximum daylight and minimum darkness (Fig. 3). The effects of season on testis mass or size has been demonstrated in black bears in Michigan (Erickson and Nellor 1964), Minnesota (McMillin et al. 1976, Garshelis and Hellgren 1994), Virginia and North Carolina (Garshelis and Hellgren 1994), Idaho (Reynolds and Beecham 1980), and Illinois (in captivity, Horan et al. 1993, Tsubota et al. 1997); and in Hokkaido brown bears (in captivity, Tsubota and Kanagawa 1989). These studies show that immediately before and during the breeding season (Apr–Jun), testis mass or size is greater than at any other time of year. Testicular regression continues throughout the non-breeding season (Erickson and Nellor 1964, Howell-Skalla et al. 2000).

Our study represents the first investigation of seasonal differences in T concentrations in grizzly bears in the continental US. Seasonal variation in T concentrations has been reported for black bears (McMillin et al. 1976; Palmer et al. 1988; Garshelis and Hellgren 1994; Tsubota et al. 1997, 1999; Howell-Skalla et al. 2000), Asiatic black bears (Okano et al. 2003), polar bears (Palmer et al. 1988), and Hokkaido brown bears (Tsubota and Kanagawa 1989). Generally, these studies show that T concentrations are elevated in the spring and decline throughout the breeding season until reaching a nadir in the fall before denning. Relative to March concentrations (and concurrent with increasing daylight and decreasing darkness), Palmer et al. (1988) demonstrated a significant increase in T concentration for polar bears near Churchill, Manitoba, in April and May. Tsubota and Kanagawa (1989) found that T concentrations in 4 captive post-pubertal Hokkaido brown bears in Japan increased from basal concentrations in February, peaked in April or May, and returned to basal concentrations by June or July. Testosterone concentrations in post-pubertal grizzly bears in our study generally conform to these seasonal patterns except that our T concentrations were greatest in June instead of April or May (Fig. 3).

The pattern of T concentration in bears in our study was of a lower amplitude in May and June than that observed in black bears (Palmer et al. 1988, Horan et al. 1993, Garshelis and Hellgren 1994, Tsubota et al. 1995, Howell-Skalla et al. 2000), polar bears (Palmer et al. 1988), and Hokkaido brown bears (Tsubota and Kanagawa 1989). Although our blood samples were taken from post-pubertal bears, their seasonal T amplitudes more closely resembled those for Asiatic black bears reported by Okano et al. (2003) and pre-pubertal (1–3 years old) American black bears reported by Garshelis and Hellgren (1994). Testosterone concentrations in our study exceeded 1 ng/ml only in May and June (Fig. 3).

Testosterone facilitates aggressive behavior and stimulates antagonistic interactions between males for breeding opportunities in a variety of vertebrates, including mammals (Wingfield et al. 1990). Garshelis and Hellgren (1994) proposed that population density and social structure, because of their influence on type and frequency of encounters among male bears, may modulate neuroendocrine–gonadal function, especially prior to and during the breeding season (Apr–Jun). A low density of post-pubertal males or a high ratio of post-pubertal females to post-pubertal males could lead to low competition (and low T concentrations) among males for estrous females (LeCount 1982, Garshelis and Hellgren 1994). Perhaps the relatively low T concentrations in the bears in our study were modulated by infrequent interactions with other post-pubertal males or high access to uncontested estrus females.

Access to estrus females by male black and brown bears is determined by a dominance hierarchy (Ramsay and Stirling 1986, Palmer et al. 1988). Dominant males are typically the largest in body mass, older, thought to be the most successful breeders, and have higher T concentrations than subordinates (Barber and Lindzey 1986, Palmer et al. 1988, Garshelis and Hellgren 1994). In a study of black bears on an island in southwestern Washington, Barber and Lindzey (1986) found that male social status was positively correlated to age and size. When multiple males contested for an estrus female, the largest (and typically older) male displaced the others without physical contact. It is conceivable that bears in our study were predominately subordinate males. Perhaps photoperiod (or night length) and population density, social structure, or both work in conjunction to modulate neuroendocrine–gonadal function in bears. Photoperiod (or night length) may regulate seasonal timing of T synthesis, and secretion and population density or social structure modulates the amplitude of T concentrations.

We acknowledge that our explanations for relatively low T concentrations in bears in our study are speculative. We did not directly measure seasonal differences in testis, epididymis, or T concentrations by repeated measures of the same animals. Instead, differences in these reproductive parameters were identified in 25 grizzly bears killed at different times of the year over 17 years (testes and epididymides) and in 50 post-pubertal grizzly bears captured over 2 years in the mid-1990s (T concentrations). We assumed the differences in the testis, epididymis, and T concentrations reported here reflect seasonal changes. Other factors, however, may also have had a significant effect, but were not considered (the effect of age on testis mass and T concentrations or the effect of capture stress on T concentrations; Sapolsky 1985).

Difficulties in obtaining reproductive material from wild bears, especially during denning and early spring (Dec–Apr), limit our understanding of male bear reproduction. Studies to greatly improve our understanding of ursid reproduction should address: (1) spermatogenesis and reproductive endocrinology during December through April, and (2) short-term gonadal responsiveness to social and nutritional factors.

Acknowledgments

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