Seasonal changes in testicular size and serum LH, prolactin and testosterone concentrations in male polar bears (*Ursus maritimus*)

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Little is known about the reproductive endocrinology of the male polar bear, *Ursus maritimus*, except that serum testosterone concentrations are high in April and May during the mating season and are low from August to November during the non-mating season. The objective of this study was to describe the relationship between seasonal changes in testicular size and serum concentrations of testosterone, LH and prolactin. Blood samples and testicular measurements were obtained from free-ranging male polar bears in Canada in April ($n = 5$) and May ($n = 15$) near Resolute Bay, Northwest Territories and near Churchill, Manitoba in July ($n = 15$) and October ($n = 22$). Testis size was greater in May ($39.4 \pm 3.5 \text{ cm}^2$) than in October ($27.3 \pm 2.0 \text{ cm}^2$) ($P = 0.002$). Serum testosterone concentrations were approximately three-fold higher in April ($5.8 \pm 0.8 \text{ ng ml}^{-1}$) than in May

($1.7 \pm 0.5 \text{ ng ml}^{-1}$), July ($0.6 \pm 0.2 \text{ ng ml}^{-1}$) and October ($1.1 \pm 0.2 \text{ ng ml}^{-1}$). Similarly, serum LH concentrations were high in April ($0.14 \pm 0.04 \text{ ng ml}^{-1}$) and low in May ($0.09 \pm 0.01 \text{ ng ml}^{-1}$), July ($0.10 \pm 0.02 \text{ ng ml}^{-1}$) and October ($0.08 \pm 0.00 \text{ ng ml}^{-1}$). Serum prolactin concentrations were high in April ($1.9 \pm 0.3 \text{ ng ml}^{-1}$), highest in May ($2.5 \pm 0.2 \text{ ng ml}^{-1}$), lower in July ($1.3 \pm 0.1 \text{ ng ml}^{-1}$) and lowest in October ($0.8 \pm 0.07 \text{ ng ml}^{-1}$). The present study demonstrates a positive relationship between testicular size and serum concentrations of LH, prolactin and testosterone in the male polar bear and confirms the previously reported seasonal changes in serum testosterone concentrations. Data from the present study provide important baseline and comparative endocrine information that can be used to aid captive breeding programmes in zoos and to further ecological-behavioural studies of polar bears.

Introduction

The polar bear (*Ursus maritimus*) is one of eight species of bear alive today. Polar bears live in coastal habitats of the circumpolar Arctic, including Canada, USA, Norway, Greenland and Russia. The polar bear was once considered to be a threatened species; however, the current world population is believed to be stable and estimated to be between 22 000 and 27 000 bears (Derocher *et al.*, 1998). Although much is known about the ecology of polar bears, there is little information regarding their reproductive biology. As in other species of temperate region bears (black, *Ursus americanus*; brown, *Ursus arctos*), polar bears mate during periods of increasing daylength in the spring, but implantation of the blastocyst is delayed until autumn, when pregnant females enter their dens (Wimsatt, 1963; Lønø, 1970, 1972; Mead, 1989). In support of these

findings, studies have shown increased serum progesterone concentrations in female polar bears near the time of implantation (Ramsay and Stirling, 1988; Derocher *et al.*, 1992).

Little is known about the reproductive biology of male polar bears. Males reach sexual maturity at approximately 6 years of age but do not begin mating until 8–10 years of age (Stirling, 1988). Palmer *et al.* (1988) reported that the profile of serum testosterone concentrations in male polar bears is similar temporally to that of male black bears. Serum testosterone concentrations were low in autumn (August–November) during the non-breeding season and then increased markedly in spring (April–May) during the breeding season. Although not determined in this study, Palmer *et al.* (1988) assumed that the seasonal changes in serum testosterone concentrations were correlated with changes in testis size, as observed in the black bear (McMillen *et al.*, 1976). Other parameters of reproductive endocrinology, such as seasonal changes in serum concentrations of LH and prolactin have not been examined in male polar bears.

The purpose of the present study was to expand the current knowledge of reproductive endocrinology of male

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polar bears. The objectives of the study were (i) to determine whether there was an association between testicular size and serum testosterone concentrations and (ii) to determine whether serum concentrations of LH and prolactin change seasonally.

Materials and Methods

General

Fifty-seven free-ranging, adult male polar bears were captured between May 1995 and November 1996. All bears were located from a helicopter and then anaesthetized with Telazol® (Fort Dodge Laboratories Inc., Fort Dodge, IA) using remote injection equipment (Stirling *et al.*, 1989). Twenty male bears were captured during the breeding season (April, $n = 5$; May, $n = 15$) on sea ice in the Canadian central Arctic, near Resolute Bay, Northwest Territories. Thirty-seven bears were captured during the non-breeding season (July, $n = 15$; October–November, $n = 22$) on land near Churchill, Manitoba. Straight-line body length was measured to the nearest cm between the distal tips of the rostrum and tail with the bear positioned in sternal recumbency and its head and vertebral column aligned linearly. The age of the bears was determined by extracting a vestigial premolar tooth and then sectioning the tooth and counting the cementum annuli (Calvert and Ramsay, 1998). Testicular dimensions were determined with calipers and recorded as the length from the cranial to the caudal pole, and the width at the greatest sagittal circumference. The dimensions for each bear were recorded as the mean values of both testes. Blood samples were collected via an i.v. catheter that was aseptically secured in the jugular vein of anesthetized bears and 20 ml of blood was removed via an infusion cap. Blood collection was performed within 25 min of the initial disturbance of the bears. Blood samples were rapidly cooled and then centrifuged at 2134 g for 10 min to collect serum. The frozen serum was subsequently shipped on dry ice to the University of Illinois at Urbana-Champaign and stored at -20°C until analysed by radioimmunoassay.

Radioimmunoassays

The heterologous radioimmunoassay used to determine LH concentrations in bear serum was established previously and validated in our laboratory by parallelism and recovery of unlabelled LH (Horan *et al.*, 1993). A monoclonal anti-bovine LH antibody (lot no. 2, 518B7), which detects LH from many different mammalian species (Matteri *et al.*, 1987) was provided by J. Roser at the University of California-Davis. Purified bovine LH (USDA-bLH-B-6) was provided by J. Proudman (USDA Animal Hormone Program). Intra- and interassay coefficients of variation were 3.9 ($n = 6$) and 6.3% ($n = 7$), respectively. Sensitivity of the assay was 80 pg. Bear serum prolactin concentrations were determined by a heterologous radioimmunoassay established

previously and validated in our laboratory (Tsubota *et al.*, 1995). Purified pig prolactin (USDA-pPRL-B-1) used for iodination and standards and primary antiserum (goat anti-pig prolactin) were provided by D. J. Bolt (USDA Animal Hormone Program). Intra- and interassay coefficients of variation were 7.1 ($n = 6$) and 9.2% ($n = 7$), respectively. Sensitivity of the assay was 30 pg. Serum testosterone concentrations were determined in double extracted bear serum with a radioimmunoassay previously validated in our laboratory by column chromatography, parallelism and recovery of unlabelled ligand (Palmer *et al.*, 1988). The crossreactivity of the antiserum was reported by Bahr *et al.* (1983). Sensitivity of the testosterone assay is 8 pg. Recovery of labelled testosterone (1000 c.p.m.) added to serum before extraction was $> 70\%$. Intra- and interassay coefficients of variation were 3.5 ($n = 4$) and 10.2% ($n = 16$), respectively. Hormone concentrations were determined using the RIAEIA Parallelism Program with Hot Recovery written by M-C. J. Wu (Taiwan Livestock Research Institute, Hsinhua).

Statistical analysis

Mean testicular size and hormone concentrations were compared by season using a one-way ANOVA with PROC GLM (SAS, 1985). Means were log transformed before statistical analysis to reduce the heterogeneity of variance. When a significant difference was found, multiple comparisons of means were made using the least significant differences test. Two values for testosterone concentrations in April and one value for testosterone concentrations in May were removed from the final analysis of data because these values were more than two standard deviations different from the means. Data are presented as means $\pm$ SEM. Differences were considered significant at $P < 0.05$.

Results

Male polar bears become adults at 5 years of age. The polar bears sampled ranged in age from 5 to 20 years. Body length did not differ with season, location of sampling or age. Body length ranged from 196 to 247 cm. Testicular size changed seasonally (Fig. 1); testicular size was greater in May ($39.4 \pm 3.5 \text{ cm}^2$) than in October ($27.3 \pm 2.0 \text{ cm}^2$) ($P = 0.002$). Serum testosterone concentrations (Fig. 2) were approximately three-fold higher in April ($5.8 \pm 0.8 \text{ ng ml}^{-1}$) than in May ($1.7 \pm 0.5 \text{ ng ml}^{-1}$), July ($0.6 \pm 0.2 \text{ ng ml}^{-1}$) and October ($1.1 \pm 0.2 \text{ ng ml}^{-1}$) ($P < 0.05$). Serum testosterone concentrations were lowest in July with similar concentrations in May and October. Serum LH concentrations (Fig. 3) were highest in April ($0.14 \pm 0.04 \text{ ng ml}^{-1}$) during the breeding season and low in May ($0.09 \pm 0.01 \text{ ng ml}^{-1}$), July ($0.10 \pm 0.02 \text{ ng ml}^{-1}$) and October ($0.08 \pm 0.0 \text{ ng ml}^{-1}$). Serum prolactin concentrations (Fig. 4) were high in April ($1.9 \pm 0.3 \text{ ng ml}^{-1}$), highest in May ($2.5 \pm 0.2 \text{ ng ml}^{-1}$), lower in July ($1.3 \pm 0.1 \text{ ng ml}^{-1}$) and lowest in October ($0.8 \pm 0.07 \text{ ng ml}^{-1}$).

Fig. 1. Seasonal changes in testicular size in male polar bears (*Ursus maritimus*). Testicular dimensions (length and width) were determined for the right and left testes and the average measurement was taken. Bars represent testis size in cm² (length × width) (mean ± SEM). **P* = 0.002.

Fig. 2. Seasonal changes in serum testosterone concentrations in male polar bears (*Ursus maritimus*). Each bar represents serum testosterone concentrations in ng ml⁻¹ (mean ± SEM). ^{abc}Different letters indicate significant differences among months (*P* < 0.05).

Fig. 3. Seasonal changes in serum LH concentrations in male polar bears (*Ursus maritimus*). Each bar represents serum LH concentrations in ng ml⁻¹ (mean ± SEM). ^{ab}Different letters indicate differences among months.

Fig. 4. Seasonal changes in serum prolactin concentrations in male polar bears (*Ursus maritimus*). Each bar represents serum prolactin concentrations in ng ml⁻¹ (mean ± SEM). ^{abc}Different letters indicate significant differences among months (*P* < 0.05).

Discussion

The present report is the first to describe the relationship between serum testosterone, LH and prolactin concentrations and testicular size in male polar bears. The present study determined that (i) testis size was greater in April and May during the breeding season than in July or October during the non-breeding season; (ii) serum testosterone concentrations were highest in April, decreased in May and were very low in July and October; (iii) serum LH concentrations were highest in April, coincident with peak serum testosterone concentrations; and (iv) serum prolactin concentrations were higher in April and May coincident with increasing daylength and lower in July and October coincident with decreasing daylength. These findings contribute significantly to the understanding of the reproductive endocrinology of male polar bears.

This is the first report of seasonal changes in testis size in polar bears. In the black bear, testicular regression occurs from July to December, whereas testicular recrudescence occurs from mid-January to May (Erickson and Nellor, 1964; McMillen *et al.*, 1976; Horan *et al.*, 1993; Tsubota *et al.*, 1997). The seasonal pattern in testicular size in the polar bear was similar to that of the black bear; however, the overall change in size was smaller. In October, the regressed testis of the polar bear was 69% of the seasonal maximum measured in May in contrast to the black bear in which the regressed testis in January was 45% of the seasonal maximum measured in May (Howell-Skalla *et al.*, 2000). Typically, the most marked examples of testicular regression occur in species with the shortest breeding season (Lincoln, 1989). Therefore, a greater difference in testis size was expected on the basis of the short mating season of the polar bear.

In general, testicular size was positively correlated with serum testosterone concentrations both of which were higher during the mating season in April and May than during the period of reproductive inactivity in July and October. This profile in serum testosterone concentrations is similar to that observed by Palmer *et al.* (1988); however,

a much greater difference was observed in the present study between serum testosterone concentrations collected in April and May. This difference could be attributed to the small sample size used in April. Palmer *et al.* (1988) sampled 43 bears in April and 20 in May, whereas in the present study, only five bears were sampled in April and 15 bears in May. The sample size used in April was reduced to $n = 3$ and to $n = 14$ in May because of extremely high values (18, 32 and 40 ng ml⁻¹) that were considered statistical outliers. However, it should be noted that Palmer *et al.* (1988) demonstrated a linear relationship between age and serum testosterone concentrations in male polar bears during the breeding season. Indeed, the values excluded from analysis in the present study were from older bears (11–21 years of age) and these values did fall within the range observed in older bears by Palmer *et al.* (1988).

High serum testosterone concentrations in April are coincident with the peak of the mating season, which extends from late March to May in polar bears (Lønø, 1970). High testosterone concentrations are likely to be critical for the reproductive success of a male polar bear. Polar bears do not maintain stable territories because of the unpredictability of the sea ice and consequently of the location of their main food source, the ringed seal (Stirling and McEwan, 1975; Smith and Stirling, 1978; Smith, 1980; Nemoto and Harrison, 1981; Ramsay and Stirling, 1986). The distribution of female polar bears is unpredictable and results in intense competition among males for mates. Female polar bears remain with their cubs for 2 to 3 years and, therefore, are available for breeding only every 3 years. This situation creates a skewed sex ratio of at least two to three males available for every female and greatly intensifies the competition among males (Ramsay and Stirling, 1986). Breeding opportunities are determined by intrasexual fighting and dominance displays and as a result, male polar bears are extremely aggressive during the mating season (Ramsay and Stirling, 1986). Testosterone is known to facilitate aggressive behaviour (Lincoln, 1971; Lincoln and Davidson, 1977; Lincoln, 1989) and is linked directly to increased male–male interactions during the breeding season.

The rapid decrease in serum testosterone concentrations in May appears to be associated with the end of the breeding season and may bring an end to social aggression among males. This idea is supported by Bartsh *et al.* (1992) who reported that in Antarctic Weddel seals, serum testosterone concentrations decreased suddenly either after males were rebuffed from attaining a breeding territory or if they were forced into submission. In addition, peak serum testosterone concentration in male Weddel seals was attained 2 weeks before the actual mating period and then decreased steadily throughout the remainder of the breeding season. More frequent blood sampling correlated with behavioural observations are needed to determine the exact relationship between serum testosterone concentrations and breeding behaviour in male polar bears.

Serum LH concentrations were increased in April,

coincident with peak serum testosterone concentrations. This finding supports a study by Horan *et al.* (1993) in which testosterone production in the black bear was demonstrated to be closely associated with LH release from the pituitary gland in response to GnRH. Seasonal differences in basal serum LH concentrations in black bears have not been detected (Horan *et al.*, 1993; Howell-Skalla *et al.*, 2000); however, average serum testosterone concentrations in black bears were much lower than in sampled polar bears.

Finally, the present study is the first report of seasonal changes in serum prolactin concentrations in polar bears. Circulating prolactin concentrations in male polar bears increased with increasing daylength (April to May) and decreased with decreasing daylength (July and November) as has been described in all other seasonal breeders (Curlewis, 1992). In black bears, serum prolactin concentrations are lowest during the short days of autumn and winter and then increase steadily during the spring (Tsubota *et al.*, 1995). Changes in prolactin secretion appear to play a role in seasonal testicular function although the exact role of prolactin varies with species (Bartke *et al.*, 1975; Bex *et al.*, 1978; Curlewis, 1992). Howell-Skalla *et al.* (2000) demonstrated that, in black bears, preventing the spring increase in serum prolactin concentrations with a long-acting form of bromocriptine resulted in lower serum testosterone concentrations and prevented both an upregulation of testicular LH receptors and maximal testicular growth.

The hormone data in the present study were taken from free-ranging anaesthetized polar bears. However, the validity of the values due to the effects of the anaesthetic is of concern. Telazol has been used in many studies on bears as the anaesthetic of choice. In another study, Horan *et al.* (1993) collected repeated blood samples for LH and testosterone measurements over 3 h. During the first hour of sampling, before an injection of GnRH to determine pituitary gland responsiveness, serum concentrations of LH were very constant and injection with GnRH produced a robust increase in LH and testosterone depending on the season. Furthermore, in this study, the uniform treatment of all bears with Telazol and consistent capture and handling procedures, makes any effects of capture and handling a fairly constant factor which should not confound the analysis of the effects of other variables.

In summary, the present study reports seasonal changes in testicular endocrine function in the male polar bear. These findings contribute significantly to the field of bear reproduction because so little is currently known. Baseline endocrine information in male polar bears can be applied to captive breeding programmes in zoos and to ongoing behavioural ecology studies in the field.

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