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Serum testosterone, progesterone, and estradiol concentrations and sexual maturation in spotted seals (*Phoca largha*)

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ABSTRACT

Spotted seals (*Phoca largha*) are ice-breeding phocid found in eight different breeding colonies all over the world. They exhibit a seasonal breeding pattern, with annual and synchronous cycles; however, little is known about their reproductive endocrinology. In this study, we measured serum testosterone, progesterone, and 17β-estradiol concentrations in captive spotted seals (simple number: female n = 68; male n = 89) throughout a full reproductive cycle. Males that were older than 4 years had significant testosterone fluctuations and were, therefore, classified as sexually mature. These animals show significant seasonal changes in testosterone levels, with average peak concentrations of 10.81 ± 9.57 nmol/L (±SD) from November to February, compared with mean concentrations of 1.42 ± 3.09 nmol/L throughout the remainder of the year. Females that reported a significant variation in progesterone concentrations and were older than 4 years were considered to be sexually mature. In these females, progesterone levels increased in February, remained elevated for 7 months with a mean value of 37.39 ± 17.03 nmol/L, and then dropped to 0.74 ± 0.54 nmol/L. Serum 17β-estradiol levels were also found to be significantly increased in January, remained so for 8 months (15.80 ± 14.15 ng/L), and then declined after August (7.77 ± 6.78 ng/L). In seals, mating typically occurs in February and March, 1 month after the observed peaks in testosterone and estradiol concentrations and corresponding to the increase in progesterone. A moderate positive correlation between testosterone and progesterone concentrations in sexually mature males was also observed (Spearman rho, r = 0.63, P < 0.01). In sexually immature females, progesterone and estradiol concentrations were found to be significantly lower than those in mature females. Finally, the observed patterns of estradiol and progesterone in sexually mature females suggest that embryonic diapause or successful implantation occurs in August.

1. Introduction

The spotted seal (*Phoca largha*) is a small bodied, phocid species (82–123 kg) that gives birth on sea ice [1]. They are distributed over a large geographic area ranging from 120° E to 160° W and from 38° N to 65° N, gathered into eight separate breeding colonies [2]. Spotted seals exhibit a seasonal reproductive pattern with synchronized annual breeding cycles. Their breeding season depends on both reproductive biology and habitat climate factors: after on-ice births, pup weaning and ice melting occur simultaneously, which ensures that the ice does not block the pups'

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access to prey [1,3,4]. The timing of the ice melt varies across colonies because of differences in latitude and environmental factors, resulting in breeding seasons that differ between colonies. Naito and Konno [5] reported that the breeding season of spotted seals in the southern sea of Okhotsk was between June and July, whereas Beier and Wartzok [6] reported that, in the northern Bering Sea, the breeding season was between April and May. In Liaodong Bay (38°43′–40°58′ N, 119°50′–122°18′ E), China, Wang and Wang [3] reported that the breeding occurred between March and April. On the basis of data provided by the Dalian Sun Asia Aquarium (DSAA, 38°52′ N, 121°34′ E), spotted seals under human care typically give birth from late December to early March, with most births occurring in January and February.

The reproductive biology of pinnipeds has been described predominantly in conjunction with behavioral observations and complemented by morphologic data of fetal and maternal reproductive organs [6–15]. Recently, there has been increased concern about the potential effects of organic marine pollutants on reproductive health in seals [16–20], which has led to further studies aimed at profiling normal reproductive hormone concentrations [21]. Additional investigations on the reproduction of pinnipeds have also been published, estimating the reproductive hormone concentrations over a limited period [21–28], with only a few reporting hormonal changes during a complete reproductive cycle [29,30]. In recent years, effects of food limitation on hormones in seals were also concerned by scientists [31]. To date, there is still little information available about reproductive organ histology or hormone levels in spotted seals. In this study, we measured serum testosterone (T_2), progesterone (P_4), and 17β-estradiol (E_2) concentrations throughout the annual reproductive cycle of spotted seals from Liaodong Bay, China. We also examined the relationships between these hormonal concentrations and the onset of sexual maturation.

2. Materials and methods

2.1. Animals and blood sampling

The animals used in this study were from DSAA. Seals were either rescued under yearling from Liaodong Bay, China, between 2000 and 2006, or were born under human care at the DSAA from 2005 to 2011. The total population number was 62. Seal age was determined through studbook or historical data. Over the course of 1 year, we sampled an average of 13 randomly selected individuals on the same day each month. Pregnant females were not used in this study. Approximately 3 mL of blood was collected for each sample (female samples, n = 68; male, n = 89. It should be noted that these numbers represent the sample number rather than the animal number; individuals could be sampled more than once a year.) from veins in the hind flippers from 7:00 to 9:00 AM when the animals were restrained on a V-shaped bench. Blood samples were stored in medical biochemical tubes with coagulation gel for serum extraction. The serum was separated by centrifugation (1500× *g*, for 15 minutes) after collection and stored at 4 °C. Assays were conducted within 6 hours.

2.2. Assays

Serum concentrations (20–35 μL per serum sample) of T_2, P_4, and E_2 were measured for both sexes *via* electrochemiluminescence immunoassay, using a Cobas e 411 (Roche Diagnostics, Mannheim, Germany) and its corresponding human hormone assay kits (Cobas, e pack series) as per the manufacturer's instructions. A Tris(2,2′-bipyridyl)-ruthenium(II) complex was used as a label in these assays. The T_2 kit (sheep-antibody, intra-assay: coefficient variation [CV] 5.6%; interassay: 9.0%, n = 18) had an analytical sensitivity range of 0.087 to 52.0 nmol/L and a functional sensitivity (hormone concentration level below which interassay CV >20%) of 0.416 nmol/L. The total cross-reaction of the T_2 antiserum with E_2 was ≤0.16%, whereas that with P_4 could not be detected. The P_4 kit (mouse antibody, intra-assay: CV 8.6%, interassay: 9.5%, n = 18) had an analytical sensitivity range from 0.095 to 191 nmol/L and a functional sensitivity of 0.48 nmol/L. The total cross-reactivity values for the P_4 antiserum with E_2 and T_2 were 0.009% and 0.02%, respectively. The E_2 kit (rabbit-antibody, intra-assay: 6.6%, interassay: 2.4%, n = 18) had an analytical sensitivity range from 5.00 to 4300 ng/L and a functional sensitivity of 12 ng/L. The total cross-reaction of the E_2 antiserum with both P_4 and T_2 was 0.001%. The kits used in this study were validated by running serial dilutions of lyophilized and reconstituted pools of serum from spotted seals and comparing the resulting slope with that of the standard curve (data are provided in the Supplemental Fig. 1).

For internal quality control, Elecsys PreciControl Universal 1 and 2 from the Roche Diagnostics GmbH (Mannheim, Germany) were used after each calibration and during each run for the routine testing. External quality assessment of the assays was continuously performed and certified by the accredited Reference Institute for Bioanalytics (Bonn, Germany; www.dgkl-rfb.de).

2.3. Data analysis

Data analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). The relationships between age and maturity were determined by analyzing scatter plots of T_2 in males and P_4 and E_2 in females. Bivariate correlation analysis was used to examine the relationship between two hormones. Comparisons between two groups for different seasons were analyzed by paired samples *t* test; a mean value was used if an individual was sampled more than once in each group. Comparisons between two groups of different ages were analyzed by two-factor ANOVA with reproductive maturity and month as factors. Hormone values under determination were recorded as the lower boundary of analytical sensitivity for each kit when conducting data analysis. A P value of 0.01 was considered to indicate statistical significance in this study.

3. Results

3.1. Sexual maturation

A total of 89 samples from 27 males and 67 samples from 19 females were collected and assayed.