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Reproductive Hormone Levels within Captive Female Northern Fur Seals (Callorhinus ursinus) with and without Chemical Contraceptives

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Abstract

Northern fur seals (*Callorhinus ursinus*) are held in only a few institutions within the United States and the world. The Seattle Aquarium was the first institution to successfully breed and raise captive Northern fur seals from conception to adulthood. The captive group that was the focus of this study consisted of one adult male and five adult females. One female was related to the adult male and was placed on chemical contraceptives to prevent inbreeding. Another female was exposed to a chemical spill in the wild while *in utero*, had some health problems throughout the study period, and never became pregnant. The remaining three females were thought to be reproductively normal throughout most of the study period, although only one became pregnant and gave birth. Serum estrogen, progesterone, and testosterone levels were measured using standard competitive binding antibody radio and enzymeimmunoassay techniques. Individual animal longitudinal data are reported for samples collected over the 7-year study period. The hormone data revealed changes associated with chemical contraceptives, pregnancy, and animals becoming sexually mature and senescent.

Key Words: captive reproduction, reproductive hormones, estrogen, progesterone, testosterone, contraceptives, immunoassay, pinnipeds, Northern fur seal, *Callorhinus ursinus*

Introduction

Northern fur seals (*Callorhinus ursinus*) are found in the North Pacific in the Bering and Okhotsk Seas. Adult males measure 2.1 m in length and weigh 175 to 272 kg, while adult females are much smaller at 1.4 m, weighing only 30 to 50 kg (Riedman, 1990; Reynolds & Rommel, 1999). Females reach sexual maturity at 4 to 5 y while males are able to breed from 6 to 7 y, but usually cannot hold territories in the wild until they are 8 to 9 y old (Riedman, 1990; Reynolds & Rommel, 1999). Longevity in the wild is thought to be in the late teens (Riedman, 1990).

The reproductive biology of fur seals is typical for that of a pinniped. They observe a synchronous seasonal breeding season in which the females give birth to a single pup and come into a postpartum estrus cycle to conceive the next offspring while caring for the recently born pup (Gentry & Holt, 1986). The Northern fur seal's annual breeding season occurs in July. Annual pregnancy rates are thought to be relatively high, with approximately 90% pregnancy rate for females over 8 y old (York, 1987). All females exhibit an obligatory delayed implantation phase of the newly fertilized egg that lasts 3 to 4 mo while they are nursing their new pups and foraging at sea. The end of the lactation stage (around October) marks the time when the fertilized egg implants into the uterus and begins to grow (York & Scheffer, 1997). This growth phase begins in October-November and lasts through the following July, thus making the gestation period for Northern fur seals 9 to 10 mo (York & Scheffer, 1997; Boyd et al., 1999).

Reproductive hormones measured in wild Northern fur seals during delayed implantation show that following estrus there is an elevation of progesterone, even during delayed implantation, simulating a pseudopregnancy (Boyd et al., 1999). Physiologically, there is little difference between pseudopregnancy and delayed implantation in Northern fur seals, and there is little difference in serum progesterone (P) levels between the delayed and implanted phase of pregnancy or active gestation and subsequent growth of the fertilized egg or embryo (Boyd et al., 1999). Other studies of both captive and wild female Northern fur seals report lower levels of serum estrogen (E) and P throughout delayed implantation, with an increase in both sex steroids around embryo implantation (Daniel, 1974, 1975; Kiyota et al., 1999). This pattern of sex steroids during delayed implantation and pregnancy is similar to that found within other marine mammals such as sea otters (*Enhydra*

lutris), harbor seals (*Phoca vitulina*), California sea lions (*Zalophus californianus*), and Steller sea lions (*Eumetopias jubatus*) (Odell, 1975; Boulva & McLaren, 1979; Hoover, 1998; Larson et al., 2003).

Northern fur seals are held in only a few institutions within the United States and the world. Longevity in captivity has been observed to be 25 y for females and 18 y for males (Seattle Aquarium, unpub. data). Northern fur seals were first successfully bred from captive-reared individuals by the Seattle Aquarium in 1983. Since that time, Mystic Aquarium in Connecticut, the New York Aquarium, and facilities in Japan have successfully bred captive fur seals. For captive management purposes, once the offspring reach adulthood, they must be reproductively isolated from their parents either physically or chemically to prevent inbreeding. Moving large marine mammals is expensive and often not desirable for the facility exhibiting the animals or for the animals themselves. Chemical contraception or permanent sterilization then becomes crucial if all the animals must share the same captive environment or exhibit. The Seattle Aquarium has housed related individuals together since its opening in 1977 and has used chemical contraception to prevent parent/offspring mating since the early 1990s.

While reproductive strategies vary among marine mammals and terrestrial vertebrates, chemical signals via sex steroids that control reproduction remain fairly constant across taxa. Thus, measuring circulating sex steroids (E, P, and testosterone [T]) should elucidate understanding of the Northern fur seal's reproductive cycle and the effectiveness of chemical contraception. Commonly, E and P have been measured to determine reproductive activity within female mammals, while T has been measured to determine sexual activity within males (Norris, 1996). Recently, Browne et al. (2006) reported that P and E were not found to be elevated in serum samples taken from wild Northern fur seal females with recently implanted embryos (determined by time of year sampled). Rather, they found that T was significant at the time of embryo implantation (October-November), suggesting that this androgen may be superior to E and P when measuring reproductive activity within adult female Northern fur seals. It is thought that circulating androgens (T) have active roles within many female mammals by acting as a substrate for estrogen synthesis within the ovary (Norris, 1996).

Herein we report longitudinal hormone data, specifically, E, P, and T, collected over seven years within five captive female Northern fur seals housed together with an adult male at the Seattle Aquarium. The goal of this study was to compare hormone levels within an adult female on chemical contraceptives with four others that were not.

Materials and Methods

Five captive female Northern fur seals sampled during this study were housed at the Seattle Aquarium (Female #1, Female #2, Diana, Baabs, and Woodstock) (see Table 1). All individuals were maintained on public display in the same exhibit with a sexually mature male, Buster. The display was outdoors and thus exposed to natural light cycles. The Northern fur seals were exhibited in a 3.96-m deep pool that contained 238,480 l

Table 1. Birth or arrival date and reproductive history of captive Northern fur seals housed at the Seattle Aquarium during this study

Animal	Arrived	Captive/wild caught	Birthdate	Reproductive history/birth control
Female #1	13/10/77	Wild caught from the Pribilof Islands, AK	Approx. 1976	Several pups born before the study began, including Buster, the dominant male housed with the females; birth control: melengestrol implant (MGA) 4.65 gr 11/8/93 and porcine zona pellucida (PZP): 11/4/96, 5/5/96, 26/5/96, 1/4/97, and 24/6/98
Female #2	13/10/77	Wild caught from the Pribilof Islands, AK	Approx. 1976	Several pups born before the study began, including Baabs and Woodstock
Diana	1981	Captive born at Pacific Biological Research Station in Nanaimo, BC, from a wild born mother; transferred to SA on 04/84	7/8/80	No reproductive history
Baabs		Captive born	2/8/88	Pup 1: Stillborn pup on 7/7/98
Woodstock		Captive born	16/8/89	No reproductive history

of filtered, natural salt water. In addition to pool space, the animals were given unlimited access to 31 m^2 of dry resting space. Feedings of herring, capelin, and squid were offered three times per day. The amount of food consumed varied depending on age, season, and reproductive status. Blood samples were taken approximately every other month from the females for a period of seven years (1991 to 1998).

Since one of the females, Female #1, was the male's biological mother, she was given chemical contraceptives (melengestrol implant [MGA], 4.65 gr on 8/11/93, and porcine zona pellucida [PZP] injections [65 µg intramuscular on 4/11/96, 5/5/96, 26/5/96, 1/4/97, and 24/6/98]) to prevent conception. The actions of the two contraceptives differ physiologically. The MGA implant is synthetic P that suppresses ovarian development and estrus by simulating physiological pregnancy or luteal phase (Wood et al., 2001), while the PZP vaccine stimulates the production of antibodies that prevent the attachment of sperm to an ovulated egg, thus blocking fertilization (Frank & Kirkpatrick, 2002). The other adult female fur seals (Female #2, Baabs, Diana, and Woodstock) did not receive contraceptives during the study period.

Diana was thought to be a special case because she had been exposed to an unknown chemical spill while *in utero* that caused her mother to fall into a coma. Diana's mother was held in captivity briefly in Nanaimo, British Columbia, during the summer of 1980 when she gave birth to Diana. The adult female was later released, but Diana was held and raised in captivity. She was later transported to her permanent home at the Seattle Aquarium in 1981 as an apparently healthy juvenile. When she was 6 y old, however, she became completely blind for no apparent reason. It was thought that her blindness was a result of her early chemical exposure as the other fur seals in the exhibit at that time did not show any health problems. Thus, Diana may not be a physiologically normal fur seal and may show different hormone profiles when compared to the healthy females.

Serum and plasma samples were run on competitive protein binding radioimmunoassays (RIA) for E, P, and a competitive binding protein enzymeimmunoassay (EIA) for T. Assays were performed at the Seattle Aquarium and the Center for Conservation Biology, University of Washington, Seattle, using previously described methods (Wasser et al., 1994, 1996; Larson et al., 2003).

Estrogen was measured according to manufacturer's specifications by the total Estrogens I125 RIA kit (ICN Diagnostics Division, Costa Mesa, California). Assay sensitivity was 2.5 pg/ml at 90% of maximal binding.

Progesterone levels were measured by a monoclonal P antiserum made against 4-P-11-ol-3, 20-dione hemisuccinate: BSA (Grieger et al., 1990) as described in Wasser et al. (1994). This antiserum cross-reacts 100% with P, and with a variety of epimers of 5-pregnane-3-diol and 5-pregnane-3-olone (Wasser et al., 1994). The assay was incubated at 4° C for 2.5 h in a total volume of 500 µl: 100 µl of standards and samples in duplicate incubated with 200 µl of assay buffer, 100 µl of H^3 tracer (mean of 10,000 cpm), and 100 µl of antibody (1:25,000). After the first incubation, 500 µl of charcoal were added and the assay was incubated again (20 min), centrifuged (20 min), the supernatant was decanted into 5 ml of scintillation fluid (Ultimagold), and the percent bound-unbound hormone/antibody complexes were counted on a Beckman LS5801. Assay sensitivity was 0.04 ng/ml at 90% maximal binding.

Testosterone was measured according to manufacturer's specifications by the Assay Designs Testosterone kit (Assay Designs, Ann Arbor, Michigan). Assay sensitivity was 5.67 pg/ml at 90% of maximal binding. Samples were diluted 1:5 in assay buffer before analysis.

Estrogen, T, and P hormone assays were validated using the parallelism test. Parallelism was demonstrated between serial dilutions of fur seal serum and the standard curve. The standard curve and the dilution curve slopes should be close to equal for the curves to be parallel. Significant differences in slope were determined by *t* tests assuming unequal variances (*Excel*, Version 8 Office 1997).

Results

Assay Validation and Accuracy

Serially diluted E, T, and P samples were parallel to their respective standard curves with non-significant slope differences validating the effectiveness of each assay (paired *t* tests of slopes [standard curve vs dilution curve respectively]: E, slopes: -0.14 vs -0.18, $p = 0.14$; T, slopes: -0.08 vs -0.07, $p = 0.14$; and P, slopes: -0.08 vs 0.08, $p = .29$). Intra-assay coefficients of variation of all assays were found to be less then 5% while inter-assay coefficients of variation were less than 10% for E, T, and P.

Longitudinal Data

Baseline values were calculated for P and E by taking an average of these levels from the three fur seals (Baabs, Diana, and Woodstock) that were not pregnant and were not on chemical contraceptives outside of the summer breeding season (July and August). Progesterone baseline was found to be 50.73 ng/ml while the E baseline was 206.85

pg/ml. Longitudinal data for each Northern fur seal is shown in Figure 1.

Figure 1a represents all sample data for Female #1. Her P levels were below baseline for the majority of the study except for sustained high P for approximately 1.5 y after the MGA implant from 1993 to 1995. Following the MGA implant, between 1996 and 1998, Female #1 was given the vaccine PZP to prevent conception. During that time, only her E levels fluctuated with some significant spikes above baseline during the summer months, indicating ovarian activity associated

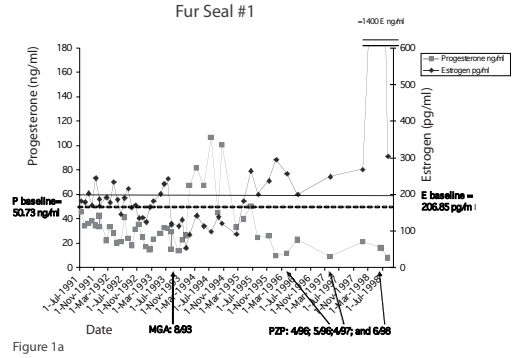

Figure 1a

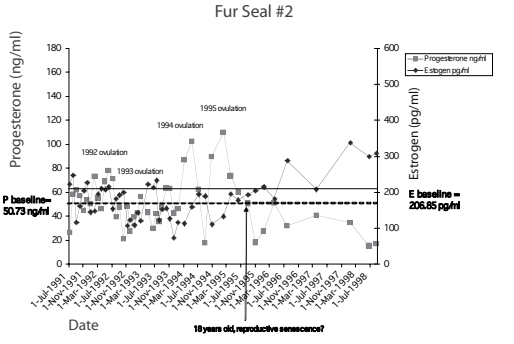

Figure 1b

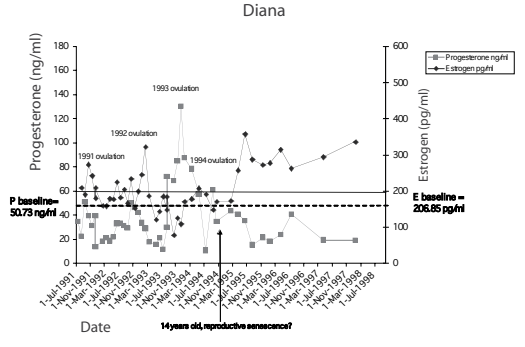

Figure 1c

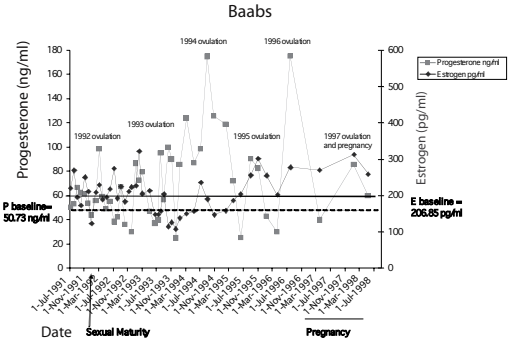

Figure 1d

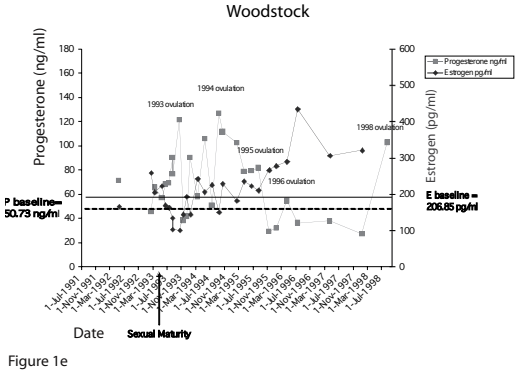

Figure 1e

Figure 1. Progesterone and estrogen longitudinal data for each captive female Northern fur seal during the study period from 1991 to 1998; Figure 1a is data from Female #1 who was sexually mature throughout the duration of the study and was given the following contraceptives: melengestrol implant (MGA) on 8/11/1993 and porcine zona pellucida (PZP) on 11/4/96, 5/5/96, 26/5/96, 1/4/97, and 24/6/98. Figure 1b is data from Female #2 who was also sexually mature throughout the study but was not given chemical contraceptives. Figure 1c is data from Diana who was sexually mature during the study but thought to have physiological abnormalities relating to exposure to an unknown chemical *in utero*, causing her to go blind at the age of 6 years. Figure 1d is from Baabs who became sexually mature in 1992 and gave birth to a stillborn pup in the summer of 1998. Figure 1e is from Woodstock who also became sexually mature during the study in 1993.

with egg development but no rise in P associated with an ovulation (Norris, 1996).

Female #2's longitudinal data is represented in Figure 1b. Her P levels were below baseline except during late spring and the summer months (May through August), from 1991 until the summer of 1995, when she was 18 y old. Her E levels were also similarly cyclic, with higher than baseline levels occurring primarily during the summer months (Figure 1b). Her E levels continued to cycle with peaks at or above baseline in 1996, 1997, and 1998 but with no associated rise in P, suggesting failure to ovulate and form a *corpus luteum*. She was 18 y old on 1995 and could have reached the end of her reproductive life or senescence.

Diana's data showed seasonal fluctuations in both E and P levels (Figure 1c). She had rises in both E and P at or above baseline in the summers of 1991, 1992, 1993, and 1994. She had a sustained P rise in the late summer/fall of 1993 lasting until the spring of 1994 that could have been associated with a sustained *corpus luteum* or a pseudopregnancy. After 1995, her E levels continued to be above baseline, indicating ovarian activity with no ovulation as her P levels do not cross baseline. At that time, she was 15 y old and was potentially reaching the end of her reproductive activity.

Baabs's longitudinal data (Figure 1d) was significantly different than the older females, with most of her P and E levels above baseline after 1992 when she was 4 y old and sexually mature (Riedman, 1990). Each year, she cycled and ovulated with resulting high P levels. She had an elevated and sustained P level in 1994 that could have been a sustained *corpus luteum* that then turned into a pseudopregnancy. Her P and E levels were also higher near the end of the study in 1998 because she was pregnant, resulting in a full-term stillborn pup in July 1998.

Woodstock's data (Figure 1e) was most similar to Baabs's profile. Woodstock is a year younger than Baabs, however, and did not start cycling until she reached sexual maturity in 1993 when she was also 4 y old. Her profile is typical in that she exhibits an annual seasonal cycling, with E and P peaks associated with egg development and ovulation in each year except for 1997.

Longitudinal T data for all female fur seals is shown in Figure 2. All females in the study had elevated T levels during the summer breeding months and lower levels in the fall/winter. There were no obvious significant patterns between individuals.

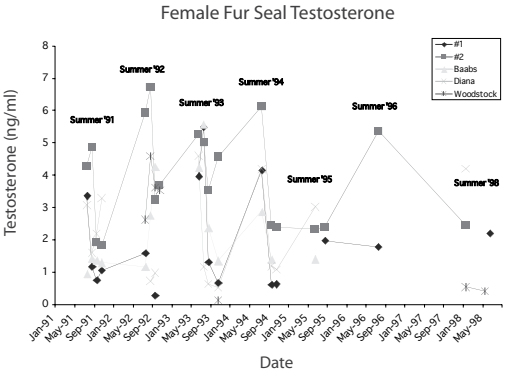

Figure 2. Longitudinal fur seal testosterone data; note that most females had high T levels during the summer breeding season rather than in the fall/winter. The high T levels in the summer are likely associated with ovarian activity and estrus. Browne et al. (2006) suggested high-circulating T levels during implantation within pregnant fur seals. In this study, there was only one known pregnant female, Baabs, 1997-1998, and there may be inadequate samples during that year to show the high T levels associated with the embryo implantation suggested by Browne et al.

Discussion

Results of this study demonstrated the ability to determine differences among captive female fur seals on chemical contraceptives within P only. Diana, Female #1, and Female #2 had the lowest P levels throughout the duration of the study, most likely due to their ages and reproductive senescence, while Baabs had the highest P levels associated with her regular seasonal cycling and pregnancy near the end of the study. The E data was relatively similar between individuals, indicating most females exhibited regular seasonal ovarian follicular activity of the theca and granulosal cells that produced E. In addition, the T levels within all females were similar, suggesting routine follicular activity surrounding the production of androgens (T) and its eventual conversion to E in the granulosal cells during the breeding season (Norris, 1996). Browne et al. (2006) found that T levels were significant around the time of embryo implantation within wild fur seals, while E and P were not significant. They sampled females only once during the fall around the time of embryo implantation, however, and may have missed the time during the reproductive cycle during which the females may have elevated P. In this study, the females only had high T levels during the breeding season. The difference between the T results in this study and those presented in Browne et al. (2006) may be due to the fact that there was only one female in this study that was pregnant and the samples taken during that time may have been insufficient to show elevated T during embryo implantation.

Female #1's P levels were most likely low due to the chemical contraceptives that she was given

during the study that suppressed ovarian activity. Even though the MGA implant she was given in August 1993 was a synthetic P and increased her circulating P levels for approximately 1.5 y (until mid 1995) (see Figure 1a), the long-term effect of this implant is thought to be complete ovarian suppression with no *corpus luteum* formation and thus no production of P (Norris, 1996). Unfortunately, an ultrasound was not performed to verify the status of the ovary and the follicles. The only measurement of the *corpus luteum* formation reported in this study was the rise in P. Later, in 1996, 1997, and 1998, she was given a different chemical contraceptive, PZP vaccine, which acts to block the development of a fetus within an ovulated egg by breaking down the zona pellucida, the outer covering of the egg (Frank & Kirkpatrick, 2002). During the time that Female #1 was on PZP, her E levels began to increase, indicating estrus and ovarian activity, but her P levels remained depressed (Figure 1a). This has been found in several other species given the PZP vaccine following the MGA implant (Frank & Kirkpatrick, 2002; AZA Contraceptive Center, pers. comm., 2007).

Diana's relatively low P levels may be due to her medical history and her age. She became permanently blind in both eyes by the age of 6. The aquarium is unaware of any other physiological effects relating to her chemical exposure; however, although she did ovulate regularly (P levels above baseline) until 1995 when she was 15 y old (Figure 1c), she never became pregnant. The other adult-aged female that was not on chemical contraceptives was Female #2. She cycled normally and ovulated in 1992, 1993, 1994, and 1995 (Figure 1b). It wasn't until Female #2 was 18 y old did she apparently stop ovulating and reach reproductive senescence. Perhaps Diana's other medical problems caused her to become reproductively senescent earlier than healthy females.

The fur seal with the highest overall P levels was Baabs (Figure 1d). She started producing high P levels in 1992 and showed P peaks every year since then, suggesting regular, seasonal ovarian cycling with ovulation production of a *corpus luteum*. She was also pregnant at least once during the study with a full-term stillborn pup born in July of 1998. She may have also been pregnant or pseudopregnant in 1997, judged by the high and sustained high P levels, but the fetus may not have been carried to term and a pup was never found. In addition, no ultrasound or x-rays were performed to determine the presence of a fetus. After the study ended in 1999, Baabs successfully gave birth to a live male pup, Isaac, who currently resides at the Seattle Aquarium.

During the study, two of the Northern fur seal females—Baabs and Woodstock—became sexually mature. This increase in reproductive activity after maturity was evident on the longitudinal graphs (Figure 1d & 1e). Neither Baabs nor Woodstock had P levels above baseline until they were close to 4 y old (Baabs, P = 98.39 ng/ml on 3/5/92, 3.75 y old, Figure 1d, and Woodstock, P = 90.61 ng/ml on 8/5/93, 4 y old, Figure 1e). This data corroborates the average age of 4 y for the onset of sexual maturity in wild Northern fur seals found in the literature (Riedman, 1990; Reynolds & Rommel, 1999).

In addition to two of the females becoming sexually mature, two and possibly three of the females entered reproductive senescence or ovulatory failure during the study: Female #1, Female #2, and Diana. Both Females #1 and #2 were 18 y old in 1995, and their profiles show a reduction in P past the baseline after that time, suggesting no further development or ovulation of eggs. It is unclear whether or not the decrease in P in Female #1 is associated with her age or the contraceptives.

The ability to monitor reproductive activity, including effective chemical contraception, sexual maturity, reproductive senescence, and pregnancy is essential for proper captive management of Northern fur seals. This study revealed that longitudinal P levels were the best indicator of reproductive activity (i.e., ovulation with *corpus luteum* formation and pregnancy) and ovarian suppression via the contraceptives employed (MGA and PZP). We will continue to monitor reproductive hormones within Northern fur seals with and without chemical contraception, focusing on P to determine relative reproductive activity within captive Northern fur seals housed at the Seattle Aquarium and elsewhere.

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