Pseudopregnancy in harp seals (*Phoca groenlandica*)

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Concentrations of circulating progesterone were monitored in a captive breeding group of harp seals. Five females were monitored for 2 years, over which time they either became pregnant (four instances) or displayed an overt pseudopregnancy (five instances). Only one female exhibited consecutive pseudopregnancies. In all pseudopregnancies, serum progesterone values were high and remained high until July, which is close to the time when implantation normally occurs. After this time progesterone concentrations remained high, gradually declining to baseline values over the following four months. In the three females that were pregnant one year and pseudopregnant in the other year, the average progesterone concentration during pseudopregnancy was lower than that exhibited during pregnancy ($P < 0.05$). All females (except one) increased their mass, girth and condition index over the normal gestation period whether they were pregnant or pseudopregnant. The potential consequences of such a high rate of pseudopregnancy on the use of ovarian scars for estimating reproductive rates and population size are discussed.

Introduction

Pseudopregnancy is operationally defined as a prolonged increase of circulating progesterone concentrations in the absence of a fetus, often accompanied by various clinical signs typical of pregnancy, such as mass gain, mammary gland enlargement and lactogenesis. In some species the signs are covert, but in others, for example dogs, the female can display a full array of symptoms, including a mock birth, followed by attachment to inanimate objects, and heavy lactation (Arthur *et al.*, 1989). Ovulation and subsequent pseudopregnancy typically occur as a result of infertile mating, but can be induced by clitoral stimulation (Freeman and Neill, 1972; Pepe and Rothchild, 1974). During pseudopregnancy, progesterone is produced by the corpus luteum, the steroidogenic function of which, at least in laboratory animals (for example, rats and rabbits) is mediated by oestradiol, LH and prolactin surges (Freeman and Neill, 1972; Ross, 1978; Bill and Keyes, 1983; Arthur *et al.*, 1989).

Pseudopregnancy in wild animals has not often been documented since it would be evident only when an apparently pregnant female did not produce offspring. Full confirmation necessitates the repeated acquisition of progesterone concentrations, as well as proof of the absence of a fetus. Gathering such information on marine mammals is practicable only with captive animals. There have been two reports of pseudopregnancy in seals. Noonan (1989) described cycles of increased progesterone (up to about 30 ng ml^{-1}) with no

evidence of a fetus upon X-ray in captive hooded seals (*Cystophora cristata*). Reijnders (1990) suggested that the increased progesterone concentrations (about 9.5 ng ml^{-1}) in nonpregnant harbour seals (*Phoca vitulina*) which lasted throughout diapause could be interpreted as pseudopregnancy. The progesterone changes throughout pregnancy have been measured in other phocid species and show increases over the course of gestation, with a large decline a few days prepartum and then a fall to baseline values soon after the birth of the pup (Raeside and Ronald, 1981; Boyd, 1984a; Kirby, 1990; Reijnders, 1990).

Pseudopregnancy and pregnancy have been compared only once in the same species (harbour seals: Reijnders, 1990) since captive pinnipeds breed so rarely. In the present study, serum progesterone concentrations were monitored over 2 years in a group of captive harp seals (*Phoca groenlandica*). Some of the females in the colony became pregnant, while others displayed overt pseudopregnancy. This study compares the progesterone profiles and some associated morphological changes of pregnant, pseudopregnant and nonpregnant females.

Materials and Methods

Five female harp seals were captured during the pupping season in the Gulf of St Lawrence. Two adult females were acquired in March 1989, and three in March 1990. These females were flown with their approximately one-week old pups to the seal holding facility of the Ocean Sciences Centre of Memorial University in Logy Bay, Newfoundland. Two adult males were also acquired, one from the Quebec Aquarium in May of 1989, and the other in April 1991.

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Male and female seals and pups were housed communally in wooden outdoor tanks, ranging in size from 7.5 m diameter $\times$ 1.5 m deep to 12.3 m diameter $\times$ 2.5 m deep, through which sea water was continuously pumped at ambient temperature from the adjacent bay. The tanks were surrounded by wooden decking to which the seals had free access. When the water in these tanks froze over during the winter, the seals hauled out onto the ice pans as well as on to the decking.

The seals were fed herring (*Clupea harengus*) once a day on an *ad libitum* basis, and were given as much as they would eat during 2 h at midday, supplemented with Seatabs multivitamins (Pacific Research Labs, El Cajon, CA). The animals were weighed on a Howe platform scale (± 250 g; Model 11717) once every two weeks from May 1990 to November 1990 and once a week thereafter. Axillary girths and straight lengths were also measured at this time (Scheffer, 1967). A condition index was calculated using the equation (axillary girth $\times 100$)/length (McLaren, 1958; Scheffer, 1967; Sergeant, 1973).

Blood samples were taken from the hind flipper, usually twice each month, though occasionally more frequently, from April 1990 until April 1992. The only hiatus in this sampling regimen was in late winter, when ice prevented access to the seals. The blood was drawn into 10 ml clot (silicone-treated) vacutainers, from which the serum was collected after centrifugation at 3080 g for 20 min and stored frozen in 1 ml aliquots at -70°C for subsequent analysis.

Progesterone was determined with a no-extraction, solid phase ^{125}I -radioimmunoassay (sensitivity 0.05 ng ml^{-1}) designed for the quantitative measurement of progesterone in serum or plasma (Coat-A-Count Progesterone; Diagnostic Products Corporation, Los Angeles, CA). With this assay, the ^{125}I -labelled progesterone competes for a fixed time with progesterone in the subject sample for antibody sites in antibody-coated polypropylene tubes. The supernatant was decanted to terminate the competition and isolate the antibody-bound fraction of the radiolabelled progesterone; the radioactivity of the supernatant was measured in a gamma-counter and converted to progesterone concentration via calibration.

The antiserum is highly specific for progesterone, with very low crossreactivity (maximum expected for 11-deoxycortisol 2.4%) to other steroids that might be present in the samples. Lipaemia, bilirubin or haemolysis do not have any clinically significant effect on the assay.

Statistical analysis

The coefficients of variance (CV) were low and uniform, with no 'end of run' effect observed in assays involving up to 200 tubes. The interassay CVs (Lot 011 QC) were low: 12.9%; medium: 14.2%; high: 8.2%. The intra-assay CVs were low range: 0.5%; entire range $< 5.0\%$.

All statistical analyses were performed on individual seals. One-way analysis of variance was used to compare mean progesterone concentrations across years. Pearson's product-moment correlation coefficients were calculated to compare the relationship between progesterone concentrations and morphometric variables in true pregnancy and pseudopregnancy.

Results

There were four pregnancies over the course of this study. Mating was never observed. One female (Emma) carried a pup to full-term, giving birth on 28 March 1992. The rest of the pregnancies ended prematurely with live pups being aborted (Florence: November 1991; Madeleine: February 1991; Sybil: December 1990). Madeleine died from an apparently congenital heart defect shortly after her pup was aborted.

All pregnancies were accompanied by an increase in serum progesterone concentrations (Fig. 1). In the three animals that aborted their fetus, there was an abrupt increase in the already raised values, most often observed in October. Just before this peak, progesterone concentrations had declined in the three animals. Progesterone concentrations fell before all births and abortions, and returned to baseline values postpartum.

There was steroidal and morphological evidence of all the pseudopregnancies (Fig. 1). Babette showed an increase in mass during both seasons; however, she did not deliver a pup, and an X-ray in May 1992 confirmed the absence of a fetus. In both years monitored, her progesterone concentrations increased until July, falling thereafter until November. Similarly, in 1990 Emma and Florence showed pseudopregnancies with the chronology of progesterone changes similar to that shown by Babette. Emma also exhibited an increase in mass concurrent with changing hormone concentrations; Florence did not. Sybil showed no mass or progesterone change when she was not pregnant; her progesterone concentrations remained at baseline values after her pup was aborted in 1990 until the end of this study. The average progesterone concentrations in all pseudopregnancies were significantly lower than those reached by pregnant animals (Table 1).

There was no consistent relationship between progesterone concentrations and mass, axillary girth or condition index (Table 2). Only three significant correlations were evident. Mass was positively correlated with progesterone concentration for Sybil's pregnancy, but negatively related for Babette's first pseudopregnancy. The positive correlation between axillary girth and progesterone during Madeleine's pregnancy is based on only six observations. In most instances, there was a strong positive correlation between mass and axillary girth. Mass was positively associated with condition index except during Florence's pseudopregnancy and Madeleine's pregnancy for which there were only four condition indices.

Discussion

Phocid seals pup at the same time each year, and after a short lactation, mate and undergo a period of delayed implantation (Boyd, 1991). Harp seals are no exception to this pattern, giving birth to a single pup in March, nursing it for 10–14 days, followed by mating, and a 4–4.5-month diapause before implantation of the blastocyst in July or August (Lavigne and Kovacs, 1988; Stewart *et al.*, 1989; Sergeant, 1991). Some workers contend that in marine mammals the corpus luteum is active during the entire gestation period and that the placenta, therefore, does not take over steroid production (Noonan, 1989; Kirby, 1990). Hobson and Boyd (1984)

Fig. 1. (□) Serum progesterone concentrations (ng ml⁻¹), (■) mass (kg) and (—) condition indices for female harp seals (a) Babette; (b) Florence; (c) Sybil; (d) Emma and (e) Madeleine. When progesterone is shown as 0 ng ml⁻¹, no sample was collected on that date. The x axis represents two years beginning with the breeding season in April 1990. Births and abortions are indicated on each graph by the annotated arrow (B: birth; A: Abortion).

Table 1. Average serum progesterone concentrations (ng ml⁻¹) in pregnant and pseudopregnant harp seals

Animal	Year 1 (1990–1991)	Year 2 (1991–1992)	ANOVA
Emma	2.59 (2.15)	13.27* (5.71)	$F = 66.82$ d.f. = 1,36 $P < 0.001$
Florence	5.99 (5.63)	12.20* (12.54)	$F = 4.53$ d.f. = 1,43 $P = 0.039$
Sybil	9.90* (9.43)	0.45 (0.12)	$F = 14.92$ d.f. = 1,36 $P < 0.001$
Madeleine	9.23* (6.25)		
Babette	4.15 (3.70)	4.03 (3.64)	$F = 0.01$ d.f. = 1,38 $P = 0.918$

*Years in which the animal was pregnant.

Values in parentheses are standard deviations.

Table 2. Pearson product-moment correlation coefficients among progesterone concentrations, body mass, axillary girth and condition index for individual harp seals

	Emma 1991–1992	Florence 1991–1992	Sybil 1990–1991	Madeleine 1990–1991
Pregnancies				
Progesterone × mass	-0.30 (<i>n</i> = 13)	+0.21 (<i>n</i> = 11)	+0.74* (<i>n</i> = 12)	+0.31 (<i>n</i> = 10)
Progesterone × axillary girth	-0.41 (<i>n</i> = 13)	+0.30 (<i>n</i> = 11)	+0.42 (<i>n</i> = 7)	+0.90* (<i>n</i> = 6)
Progesterone × condition index	-0.41 (<i>n</i> = 13)	+0.34 (<i>n</i> = 11)	-0.46 (<i>n</i> = 5)	+0.48 (<i>n</i> = 5)
Pseudopregnancies				
Progesterone × mass	Emma 1990–1991 -0.32 (<i>n</i> = 17)	Florence 1990–1991 -0.36 (<i>n</i> = 14)	Babette 1990–1991 -0.83* (<i>n</i> = 16)	Babette 1991–1992 -0.30 (<i>n</i> = 15)
Progesterone × axillary girth	+0.08 (<i>n</i> = 14)	+0.08 (<i>n</i> = 10)	-0.41 (<i>n</i> = 12)	-0.26 (<i>n</i> = 13)
Progesterone × condition index	+0.27 (<i>n</i> = 10)	+0.44 (<i>n</i> = 8)	-0.42 (<i>n</i> = 9)	-0.37 (<i>n</i> = 13)
Pregnancies				
Mass × girth	Emma 1991–1992 +0.89* (<i>n</i> = 36)	Florence 1991–1992 +0.75* (<i>n</i> = 39)	Sybil 1990–1991 +0.81* (<i>n</i> = 14)	Madeleine 1990–1991 +0.93* (<i>n</i> = 6)
Mass × condition	+0.64* (<i>n</i> = 34)	+0.60* (<i>n</i> = 39)	+0.87* (<i>n</i> = 9)	-0.48 (<i>n</i> = 4)
Pseudopregnancies				
Mass × girth	Emma 1990–1991 +0.62* (<i>n</i> = 20)	Florence 1990–1991 -0.27 (<i>n</i> = 16)	Babette 1990–1991 +0.71* (<i>n</i> = 18)	Babette 1991–1992 +0.85* (<i>n</i> = 37)
Mass × condition	+0.67* (<i>n</i> = 11)	-0.39 (<i>n</i> = 11)	+0.65* (<i>n</i> = 11)	+0.77* (<i>n</i> = 37)

* $P < 0.05$.

For the pregnancies, only the weeks until the pup was aborted were used to calculate the correlations with progesterone.

reported luteal growth throughout pregnancy in grey seals. However, they maintained that the corpus luteum does not necessarily produce progesterone for the entire period of enlargement, and that its progestagenic function is taken over by the placenta. Hypertrophy of the corpus luteum during the last two months of pregnancy could be the result of the build-up of large lipid-filled nonsteroidogenic cells (Boyd, 1991). Survival and enlargement of the corpus luteum during the last 3–5 months of pregnancy could be the result of the secretion of chorionic gonadotrophins from the placenta. The end of pseudopregnancy may occur at the time when, in a

pregnant seal, the role of maintaining pregnancy through increased progesterone concentrations passes from the corpus luteum to the placenta. Boyd (1991) interpreted the 210 day increase in progesterone concentrations, reported by Noonan and Ronald (1989), as an indication of the functional lifespan of the corpus luteum in hooded seals. This is similar to the interpretation made by Reijnders (1990) of the 4–5 month progesterone increases in his nonpregnant harbour seals. The data reported in the present study of harp seals support these views, and suggest that the corpus luteum produces progesterone for about seven months, and that the placenta becomes

steroidogenic, thereafter. This is evident by the similarity of the progesterone changes observed until October–November, that is, the steep rise at this time in pregnant seals, but the decline in pseudopregnant animals.

Collection of blood samples began too late in 1990 to see the rise in progesterone concentrations indicative of ovulation in that season. It was apparent for Babette and Florence in subsequent seasons (4 April 1991 and 24 March 1992, respectively); however, there is no evidence of Sybil having ovulated in 1991, which may explain why she did not exhibit a pseudopregnancy in that year. Unfortunately, there were no samples for this time in 1991 for Emma, and in 1992 her progesterone concentrations had not risen by the time this study ended.

A fall in progesterone concentrations 6–7 months after mating is clear in the pregnancies that survived for this period. Although falling progesterone values during the final days of gestation seem typical, it is possible that the early decrease observed in this study was pathological, since only one pup was carried to full term. Emma's pup was delivered without incident, and nursed normally for about 24 h on what appeared to be an ample milk supply. However, it died a day later for reasons that were not determined upon necropsy.

It is difficult to assess whether the changes in mass shown during most pseudopregnancies were mediated by progesterone or were a consequence of the large seasonal changes in body condition that are not related to pregnancy. Adult males fatten over the winter to a similar extent as pregnant females, and juveniles also show large seasonal changes in body mass (Renouf *et al.*, 1993). The fact that Sybil's mass declined during year two when her progesterone concentration remained at baseline values suggests some hormonal involvement in the annual cycle of fat acquisition. Boyd (1984b) proposed that endocrine changes associated with implantation induce metabolic effects that cause fat to be stored. It seems from the present data that implantation is not necessary for such changes, although a functional corpus luteum might be. The increase in mass seen in the seals in this study began before the time when implantation should have occurred. However, the low correlation between progesterone values and changes in mass and body condition suggests, not surprisingly, that the dynamics of fat acquisition and loss involve more than just this hormone.

Boyd (1991) proposed that the reproductive system is essentially dormant during diapause and is reactivated at implantation. Insofar as progesterone increases are an indication of the activity of the reproductive system, it seems that in harp seals, at least, the reproductive system is not entirely quiescent during the interval between mating in March and implantation in late July. Boyd also found raised progesterone concentrations during diapause in grey seals (Boyd, 1983, 1984a). The increase in progesterone values observed in June 1991 in two of the pregnancies (Emma and Florence) could have been interpreted as a sign of enhanced activity upon implantation were it not that similarly timed rises are evident in all the pseudopregnancies.

It could be argued that the high rate of pseudopregnancy in this study and that of Noonan (1989) is a result of the stress of holding seals in captivity. However, this seems unlikely since we have found no evidence of pseudopregnancy in the colony

of harbour seals that are held in the same facility as the harp seals described herein. This group of harbour seals has one adult female that has produced a pup in all but one year since 1983.

It is therefore possible that the corpus luteum normally produces a pseudopregnancy in the wild when infertile matings occur, or when pregnancy is aborted. It is extremely important that the normal incidence of pseudopregnancy in wild populations is determined, since reproductive rates in pinnipeds are conventionally ascertained by examination of corpora lutea and albicantia (Sergeant, 1966, 1991; Bengston and Siniiff, 1981; Boyd, 1984a, 1985). Both of these ovarian scars would also be evident in pseudopregnancy. In any species that displays a significant rate of pseudopregnancy, errors in determining pregnancy rates, either by progesterone concentrations, or by examining ovarian scars, would increase in times of reduced food availability, given that aborted pregnancy with prolonged luteal function is more likely when condition is compromised. This is particularly relevant at the present time for the harp seal, given the current critical reductions in some Northwest Atlantic fish stocks, and the call for a renewed hunt as a means of reducing populations of seals. In any species, missed pseudopregnancy and consequent falsely raised fertility and fecundity rates would have profound consequences for the modelling of population dynamics and energy flow.

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