

Progesterone levels and reproductive status of white whales (*Delphinapterus leucas*) from the Canadian Arctic

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Serum progesterone levels were compared to the presence and size of ovaries, corpora lutea and fetuses in white whales sampled at three sites in the eastern Canadian Arctic. Generally, a progesterone level greater than 3.00 ng/ml indicated a pregnancy although there was some overlap in the range of values with mature, nonpregnant whales. This overlap may be an artifact of sample management or may indicate failed or failing pregnancies. Data are not available to test these hypothesis. Progesterone concentration was not significantly correlated with ovarian mass, corpus luteum mass or fetus mass. Ovaries of immature whales were significantly smaller than those of mature-but-not-pregnant and pregnant whales.

Key words:

White whale, beluga, *Delphinapterus leucas*, Monodontidae, Odontoceti, progesterone, reproduction.

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Introduction

Understanding the nature of reproductive cycles is basic to understanding the ecology of organisms and populations (Smith 1976). Although assessing the reproductive status of wild white whales (*Delphinapterus leucas*) has relied on examination of ovarian structures from harvested whales (e.g. Kleinenberg *et al.* 1969, Sergeant 1973, Burns & Seaman 1984), live whales handled for tagging, captures for aquaria and other purposes could provide information on reproduction. For some small stocks it may be useful to capture white whales alive specifically to determine reproductive status if a non-lethal method for doing so is available and the need warrants. Here I report on an initial effort to relate circulating levels of progesterone to the ovarian structures, hence reproductive status, of white whales.

Materials and methods

Reproductive tracts, blood samples and lower jaws were obtained from white whales landed by Inuit hunters during their subsistence hunts near Arviat (formerly Eskimo

Point, 61°05'N, 94°06'W), Pangnirtung (66°07'N, 65°43'W) and Grise Fiord (76°25'N, 82°52'W) N.W.T., Canada, from July through September 1984–1987. Data collected included the date and approximate time of death, the presence and nature of mammary secretions and the presence of term fetuses. At Arviat, where some whales were taken in nets, associations of newborn white whales with adults in the net were recorded to help assess female status. This method was quickly discredited when newborn calves were also found with only males.

Blood was drawn 0.5 to 48 h after death by chest puncture using a 50 cc syringe and delivered into untreated blood tubes. Most blood samples were centrifuged within 2 h after collection and the serum was drawn off and frozen separately. Some blood samples were frozen whole until they were thawed and separated just prior to being analysed. Information was not available to assess the effect of this difference in treatment but the treatment was independent of reproductive class. Reproductive tracts were frozen or stored in 10% neutral buffered formalin until examined later. There was possibly some change in mass associated with the different preservation methods for ovaries (Heide-Jørgensen & Teilmann 1994) but tracts were frozen or fixed irrespective of reproductive class.

In the laboratory the ovaries were removed from the

reproductive tract and weighed individually to the nearest 0.01 g. If a large corpus luteum was visible externally, it was removed intact from the ovary, weighed to the nearest 0.01 g and measured to the nearest 0.1 mm across three orthogonal diameters. Here the overall diameter of the corpus luteum for each white whale is defined as the mean and the maximum diameter is defined as the largest of these three measurements. Ovaries were then sectioned serially with a scalpel at 5 mm intervals and the presence of other corpora lutea, corpora albicantia and follicles recorded. The uterus was subjectively graded by size (small, medium, large) (Burns & Seaman 1984). The horns and body of the uterus were opened and examined for signs of pregnancy, such as increased vascularization, smoothing of the internal walls and the presence of a fetus. Fetuses were removed and weighed to the nearest 0.1 g.

Females were categorized as immature, mature but not pregnant and pregnant. Immature whales had no corpora lutea or corpora albicantia, were not lactating, were not pregnant, and had small uteri. Mature-but-not-pregnant white whales displayed some of the following features: presence of corpora albicantia or regressing corpora lutea (Kirby 1990), evidence of lactation, and a medium-sized, empty uterus with sharp ridges on the internal walls, with little evidence of vascularization (Burns & Seaman 1984). Initially, pregnant white whales were defined by the presence of a large corpus luteum lacking obvious connective tissue or by the presence of a fetus. However no large corpora were found without a fetus and the presence of a fetus became the sole criterion. Fetus size was used as an index of the time the female had been pregnant.

The concentration of progesterone in serum was determined by radioimmunoassay (RIA) following Yuthas-trakosol *et al.* (1974). Briefly, extraction was accomplished by combining 250 μ l of serum with 750 μ l of phosphate-buffered saline (PBS, pH 7.2) and 5.5 ml of ethyl ether. The mixture was vortexed for 4 min, equilibrated for 15 min and lyophilized on dry ice/ethanol (-43°C). The decanted supernatant was dried under a stream of nitrogen gas in an oscillating water bath (37°C), then reconstituted with 1.250 ml PBS. For the RIA the P4 antisera developed in rabbits (A18, N. Rawlings, University of Saskatchewan, Saskatoon, SK) was used at 1:2500 initial dilution in PBS. Labeled P4[1,2-³H(N)] (New England Nuclear, Boston, MA) was diluted in PBS to ~10 000 cpm/100 μ l. The scintillation cocktail was Ecolume (ICN Biomedicals Inc., Irvine, CA). Typical ranges for inter- and intra-assay coefficients of variation in this lab are 6–12% and 4–9%, respectively. The mean sensitivity of the assay at 95% binding is 12 pg per tube.

Jaws were kept frozen until the teeth were extracted. Longitudinal thin sections (approximately 0.3 mm) of teeth were prepared (Wainwright & Walker 1988) and used to estimate ages (see Stewart 1994 for details). The sections were viewed using reflected light with a variable-power dissecting microscope. Ages, in years, were

based on the formation of two growth layer groups per year (Goren *et al.* 1987, Brodie *et al.* 1990).

All data were tested for normality (Kolmogorov-Smirnov with Lilliefors' correction) and equal variances (Levene medial test) (Kuo *et al.* 1992) at $P > 0.05$. All data sets failed at least one of these tests and were subsequently analysed using Kruskal-Wallis ANOVA on ranks and Dunn's multiple comparisons (Kuo *et al.* 1992). Within a category, correlations were examined between progesterone level and maximum ovarian mass, and, among pregnant whales, corpus luteum and fetal mass using Spearman rank order correlations (Kuo *et al.* 1992). For statistical analysis the ovaries of each female were identified as large-ovary and small-ovary rather than as left or right to group those with a corpus luteum together. Average age of maturity was calculated following DeMaster (1978). SigmaStat™ software was used for all other analyses (Kuo *et al.* 1992). Means are presented ± 1 SD.

Results

Reproductive status was determined for 63 female white whales (52 from Arviat, 5 from Pangnirtung, 6 from Grise Fiord) which provided data on two or more of progesterone level, age, ovary mass and ovarian structures. Differences in sample sizes for some analyses resulted from missing data. The data from the three locations have been combined.

The mean ages of the females in the three reproductive classes were significantly different (Table 1, Kruskal-Wallis ANOVA on ranks, $P < 0.001$). Immature females were significantly younger than either mature-but-not-pregnant or pregnant females (Dunn's multiple comparison, $P < 0.05$) but ages of mature, nonpregnant females and mature, pregnant females were not significantly different ($P > 0.05$). The oldest immature whale was 6.5 years old. The youngest mature, nonpregnant whale was 5 years old and had a single corpus albicans. The youngest pregnant whale was 4 years old and had one corpus luteum and a small fetus. The average age of maturation was 6.43 ± 0.11 ($N = 59$).

The estimated time from the whale's death to sample collection was recorded infrequently. The average time was 8.9 ± 12.9 hours (range 0.5 to 48 h, $N = 19$). The correlation between this time and progesterone level was not significant ($P = 0.64$) for immature ($R = -0.17$, $N = 9$) or for mature-but-not-pregnant whales ($R = -0.50$, $P = 0.23$, $N = 7$). Time-to-sampling was available for only three pregnant females. They were 1.5, 1.5 and 6.5 h associated with progesterone levels of 11.42, 14.10 and 6.50 ng/ml, respectively.

Mean progesterone levels varied significantly among the three classes of females (Table 1, $P < 0.001$). Progesterone levels in immature and mature-but-not-pregnant females were significantly lower than in pregnant ones (P

Table 1. Mean progesterone concentrations in the serum, ages, mass of the large-ovary, mass of the small-ovary, and mass of the fetus in white whales (*Delphinapterus leucas*) according to reproductive status. Subscripts indicate means in a column which were not significantly different (Kruskal-Wallis ANOVA at $P < 0.001$, and Dunn's multiple comparison at $P < 0.05$).

Class	Progesterone (ng/ml)	Age (yr) (g)	Large-ovary Mass (g)	Small-ovary Mass (g)	Fetus Mass (g)
IMMATURE					
\bar{x}	1.18 _a	3.1	9.53	8.03	
SD	0.56	1.8	5.08	4.11	
Range	0.50–2.60	0–6.5	1.43–19.88	1.24–15.30	
N	24	24	22	22	
MATURE, NOT PREGNANT					
\bar{x}	1.59 _a	13.2 _b	38.78 _c	29.93 _d	
SD	1.07	6.2	18.28	14.47	
Range	0.50–5.40	5–26	12.31–85.36	10.30–68.59	
N	21	22	20	20	
MATURE, PREGNANT					
\bar{x}	9.26	10.4 _b	60.65 _c	22.64 _d	379.2
SD	5.09	4.1	21.03	6.13	239.9
Range	1.48–16.20	4–18.5	36.44–118.79	8.32–32.90	76.0–923.3
N	12	13	14	14	13

< 0.05). The level in the one newborn whale sampled was 2.00 ng/ml, slightly greater than 1 SD (0.56) above the mean for immature whales (1.18 ng/ml). The mean for immature and mature-but-not-pregnant whales was 1.37 ± 0.86 ng/ml ($N = 45$).

The mass of the large-ovary varied among classes (Table 1, $P < 0.001$). Changes in ovary mass due to formalin fixation (Heide-Jørgensen & Teilmann 1994) are insufficient to account for the differences found among reproductive classes. The large-ovary was lighter in immature whales than in mature-but-not-pregnant and pregnant whales (Dunn's multiple comparison, $P < 0.05$). Large-ovaries of mature, nonpregnant and pregnant whales were not significantly different ($P > 0.05$). The average difference in large-ovary mass between mature, nonpregnant whales and mature, pregnant whales (approximately 22 g) was similar to the mass of the corpus luteum in pregnant whales (23.64 ± 10.27 g, $N = 13$).

The average of maximum diameters of corpora lutea was 40.60 ± 5.35 mm and the average of the overall diameters was 34.40 ± 4.76 mm ($N = 14$). Corpus luteum mass was best predicted by using all three diameters (mass = $0.0005 * D_1 * D_2 * D_3 + 2.62$, $R^2 = 0.93$, $F_{1,12} = 127.9$, $P < 0.01$). Overall and maximum diameters explained less of the variation in mass ($R^2 = 0.69$ and 0.87 , respectively).

The mass of the small-ovary also varied with reproductive class of the females (Table 1, $P < 0.001$) due to lighter ovaries in the immature whales ($P < 0.05$). The small ovaries of mature-but-not-pregnant and pregnant whales were not significantly different ($P > 0.05$).

Progesterone level and large-ovary mass were not correlated in immature (Spearman rank order: $R = -0.13$, $P = 0.58$, $N = 20$) or mature-but-not-pregnant whales ($R = 0.41$, $P = 0.09$, $N = 18$). Among pregnant whales, progesterone level did not correlate with large-ovary mass ($R = -0.12$, $P = 0.72$, $N = 12$) with the mass of the corpus

luteum ($R = -0.08$, $P = 0.78$, $N = 12$), or with the mass of the fetus ($R = 0.19$, $P = 0.58$, $N = 11$). Fetus mass did not correlate with corpus luteum mass ($R = -0.36$, $P = 0.24$, $N = 12$) or large-ovary mass ($R = -0.10$, $P = 0.74$, $N = 13$).

Discussion

Assessing hormonal dynamics in wild whales has several limitations not present with captive animals (Høier & Heide-Jørgensen 1994). Post-mortem changes before and after sampling are difficult to control. The inability to centrifuge blood in some field situations introduces another variable. However in this study these variables were distributed among samples without regard to reproductive class of the females. With small sample sizes there was no relationship between the lag in sampling and progesterone levels. Also the serum levels of progesterone in this sample of white whales were within the ranges reported for other whales.

Kirby (1990) summarized data on circulating progesterone levels for many odontocetes. Typically, baseline values were less than 1 ng/ml; levels at ovulation were over 1 ng/ml, but only for short periods; and levels during pregnancy were over 3 ng/ml. All values for pregnant whales except one killer whale (*Orcinus orca*, 3.8 ng/ml) exceeded 5 ng/ml up to 13 days before giving birth (Kirby 1990) when progesterone levels may decline (Temte & Spielvogel 1985). Other studies on odontocetes also reported nonpregnant progesterone levels of 0.5 to 1.5 ng/ml (Joseph *et al.* 1987) and values for pregnant whales over 5 ng/ml (Temte & Spielvogel 1985, Joseph *et al.* 1987).

Diagnostically values above 3.00 ng/ml over an extended period were used to indicate pregnancy in the

bottlenose dolphin (*Tursiops truncatus*); baseline was less than 1.00 ng/ml (Sawyer-Steffan *et al.* 1983). Schroeder (1990) recommended the following progesterone levels for clinical assessment: anestrous or non-pregnant, < 1 ng/ml; ovulation = 1 ng/ml; pregnancy = 3 to 52 ng/ml in three separate tests at two-week intervals. He reported 100% accuracy in predicting pregnancy using this method for 28 pregnancies and four species.

Baseline progesterone levels for the white whales examined here are represented by the progesterone concentrations in immature and nonpregnant whales. The average (1.37 ng/ml) was inflated slightly by one nonpregnant whale with a value of 5.40 ng/ml. This whale had a relatively large (9.88 mm) regressing corpus luteum and was lactating. She was sampled on 24 July 1986 at Arviat and may have calved so recently (Sergeant 1973, Brodie 1989) that progesterone levels had not yet returned to baseline levels. Repeated assays from recently parturient females (Schroeder 1990) would be required to test this interpretation. Excluding this animal, the baseline progesterone level was 1.28 ± 0.60 ng/ml ($N = 44$) with a range of 0.50 to 2.94 ng/ml. Thus a serum concentration of 3.0 ng/ml progesterone might be used to indicate pregnancy in white whales, as in bottlenose dolphins (Sawyer-Steffan *et al.* 1983, Kirby 1990, Schroeder 1990). All but two pregnant white whales in the present study had progesterone levels greater than 5 ng/ml.

Høier & Heide-Jørgensen (1994) reported the only other data on progesterone levels in wild white whales. Their values, converted to ng/ml, were 0.55 and 9.91 ng/ml for immature plus nonpregnant and pregnant classes, respectively. For pregnant white whales their values are nearly identical to those found in this study (9.26 ng/ml). Although their value for the combined immature and mature-but-not-pregnant whales appeared lower than the pooled mean in this study (1.37 ng/ml), it was within 1 SD (0.86 ng/ml).

The predictive reliability of progesterone levels to determine pregnancy is challenged by the two pregnant whales in my study which had progesterone levels lower than 3 ng/ml (EP85-12: 1.48 ng/ml with a 370.0 g fetus and PG86-13: 1.60 ng/ml with a 76.0 g fetus). Levels for the other 10 pregnant whales ranged from 5.70 to 16.20 ng/ml. Reasons for the two seemingly low values are unclear. Speculation that female (PG86-13) was in an early stage of luteal development and fetal growth (Boyd 1991) is not supported by the size of the corpus luteum, which was the heaviest examined. Moreover another female with a 78.5 g fetus had a progesterone level of 5.70 ng/ml. PG86-13 was sampled up to 10 h after death, as were other whales which had progesterone levels consistent with their reproductive class. Female EP85-12 drowned in a net during a three-day storm so the time of death is uncertain and there could have been a significant delay before she was sampled. However a mature, non-pregnant whale caught in the same net had a progesterone level of 1.48 ng/ml, almost exactly equal to the mean of her reproductive class (1.49 ng/ml). EP85-12, had the

smallest corpus luteum weighed. Sample management may have been a factor in these two low values, or both pregnancies may have been failing. In this case, progesterone level might be a more predictive measure of pregnancy than the presence of a fetus, but there are no data with which to test this hypotheses.

The lack of correlation between progesterone level and ovary mass in immature and mature-but-not-pregnant whales and the marginal correlation in pregnant ones differs from the curvilinear relationship between progesterone level and ovary mass in Dall's porpoises (*Phocoenoides dalli*) (Temte & Spielvogel 1985). Temte & Spielvogel's regression, however, included both pregnant and nonpregnant whales and was of a form requiring a zero-intercept, outside the range of their data. These authors demonstrated a significant difference in mean progesterone levels between the two groups, and pooling of such data in a regression masks the threshold concept (Sawyer-Steffan *et al.* 1983) relating progesterone to pregnancy. For white whales ovarian mass was useful in distinguishing between immature and mature-but-not-pregnant whales, although there was some overlap in ranges.

As in the present study, the relationship between progesterone level and fetus size was not significant in the Dall's porpoise (Temte & Spielvogel 1985) although a general hormone model for pinnipeds (Boyd 1991) indicates a correlation between fetus size and progesterone level. My sample of white whales represents a limited and short segment (30 days) of a long gestation (330-435 days, Brodie 1989, Heide-Jørgensen & Teilmann 1994). A greater range of gestational ages is required to test the hypothesis that hormonal patterns in white whales are fundamentally different than in pinnipeds.

In summary, serum progesterone levels ≥ 3.00 ng/ml appear to be reliable indicators of pregnancy in white whales and could be used with live whales. While the results of this study appear robust in spite of variable field conditions, it will be necessary in the future to test the effects of delays in sampling and different sample handling procedures. The effects of the latter might best be tested with captive whales. Females reached maturity at 4 to 7 years of age, with a mean of 6.43, consistent with other studies (Braham 1984, Heide-Jørgensen & Teilmann 1994).

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