Steroid hormones and prolactin in white whales (*Delphinapterus leucas*) from West Greenland

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Testosterone, progesterone, oestrogen and prolactin concentrations were determined in serum and urine samples collected from white whales (*Delphinapterus leucas*) taken by Greenlanders in the autumn of 1989–1991 and during May 1992. Hormone levels following long vs short intervals between hunting and isolation of serum samples were compared, and a significant difference was seen in the oestrogen concentrations. In male white whales the testosterone levels were significantly higher in sexually mature animals as compared to immature animals, 4.14 vs 0.96 nmol/l in serum samples and 0.62 vs 0.11 creatinine index in urine samples, respectively. Hormone levels of immature, pregnant, lactating and resting female white whales were compared, and the pregnant animals had higher levels of progesterone than the nonpregnant animals, 29.1 vs 1.76 nmol/l in blood and 22.1 vs 0.54 creatinine index in urine, respectively. The oestrogen level was higher in urine from pregnant females, 2.43 vs 0.37 creatinine index, but concentrations in serum were not significantly elevated. Lactating females had higher serum prolactin concentrations than nonlactating females, 4.35 vs 1.97 µg/l, respectively. In sexually mature males oestrogen concentrations were higher and progesterone concentrations were lower in May than in autumn. The reproductive states were indicated by both serum and urine concentrations of steroid hormones, but the two sample types did not show proportionality in hormone concentrations when taken from the same animal. Based on between-group variations in hormone content, models for diagnosis of sexual maturity in males and pregnancy in females are suggested.

Key words:
White whale, beluga, *Delphinapterus leucas*, testosterone, progesterone, oestrogen, prolactin.

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Introduction

The reproductive biology of the white whale (*Delphinapterus leucas*) has been incompletely characterized. Most literature describes general characteristics such as occurrence, growth, maturation, diets and pregnancy rates (see Sergeant & Brodie 1975, Stewart & Stewart 1989 for reviews). Few reports present details of the characteristics of the reproductive organs (Sergeant 1973, Heide-Jørgensen & Teilmann 1994). Recently Stewart (1994) reported the progesterone levels in female white whales from the eastern Canadian Arctic, but no reports are available on white whale sex hormones from elsewhere. Endocrine parameters have been described for some other cetaceans such as the fin whales (*Balaenoptera physalus*, Kjeld *et al.* 1992), the minke whale (*Balaenoptera acutorostrata*, Yoshioka *et al.* 1990, Yoshioka & Fujise 1992), various small delphinids (*Tursiops truncatus*, *Stenella longirostris* and *Delphinus delphis*, Sawyer-Steffan *et al.* 1983, Kirby 1984, Kirby & Ridgway 1984, Wells 1984, Ozharovskaya 1990, Schroeder 1990), the killer whale (*Orcinus orca*, Walker *et al.* 1988) and Dall's porpoise (*Phocoenoides dalli*, Temte 1991). The main purpose of most endocrinological studies of cetaceans has been to use hormonal parameters as diagnostic markers of pregnancy and male maturation, thus providing methods for predicting the dynamics of populations.

The literature on reproductive endocrinology of the odontocetes confirms the synthesis of progesterone from corpus luteum. The role of this hormone in luteal maintenance during pregnancy has been established for several

terrestial species and one might extrapolate the mechanisms to marine animals. Thus the findings by Stewart (1994) confirm that pregnant white whales have high concentrations of progesterone in the blood. In killer whales oestrogens, *i.e.* oestradiol-17β and oestrone, peak in the pre-ovulatory phase of the oestrous cycle (Walker *et al.* 1988). However the elevated concentrations seen in most species during pregnancy have not been fully documented, although increased levels were reported for pregnant dolphins by Kirby & Ridgway (1984). Testosterone, the dominant androgen steroid, has been measured in elevated concentrations in sexually mature male Dall's porpoise (Subramanian *et al.* 1987, Temte 1991) and in the dolphins *Stenella longirostris* (Wells 1984) and *Tursiops truncatus* (Schroeder & Keller 1989). The presence and distribution of gonadotropins within reproductive groups have not yet been described in the literature, although early studies have indicated the existence of follicle stimulating, luteinizing and growth hormones, together with prolactin, as judged from isolation of the hormones from (unspecified) whale pituitaries followed by characterization in biological assays (Wallace & Ferguson 1964). Furthermore, prolactin as well as growth hormone producing cells have been identified immunocytochemically in pituitaries from several odontocetes (*Stenella plagiodon (=frontalis)*, *Feresa attenuata* and *Orcinus orca,* Schneyer & Odell 1984).

The present investigation was based on serum and urine samples collected from the Inuit catch of white whales in West Greenland during 1989–1992. Among the purposes were to analyse the variation in concentrations of steroid hormones in peripheral blood and in excreted urine between groups of female white whales representing different reproductive states, and between groups of male white whales to judge whether maturation and/or influence from the mating season could be detected. Also, the content of the peptide hormone prolactin in the blood was used to identify hormonal correlates to lactation in female white whales. Finally, the measured hormone levels were used in diagnostic models to predict pregnancy and lactation in females and sexual maturation in males.

It was also possible to evaluate the influence of procedural changes on the subsequent hormone analyses, since our procedures for collecting and processing samples improved during the sampling period. Sampling methods were compared with a view to defining convenient methods for collection of serum from cetaceans and other marine vertebrates under extreme field conditions.

Materials and methods

Collection of samples

Over a four-year period (1989–92) blood and urine samples were taken from white whales in various age, sex and reproductive states off West Greenland. The white whales had been killed in the Inuit hunt as described elsewhere (Heide-Jørgensen & Teilmann 1994). Blood samples were obtained from 75 females and 52 males, urine samples from 32 females and 20 males, all from a total of 131 animals (four animals were represented solely by urine samples). All samples were taken from whales caught during the autumn (September–October), with the exceptions that three serum samples and one urine sample came from sexually mature males and one serum sample from a lactating female killed in May. The reproductive states – immature, resting mature, pregnant and lactating in females; immature and sexually mature in males – were judged by examining the reproductive organs (see Heide-Jørgensen & Teilmann 1994). Fifty-three blood samples came from immature females, four from pregnant animals, 15 from lactating females and one from a resting female; in addition, two females were simultaneously lactating and pregnant. Forty-six males were immature and six were mature. For the urine samples the distribution was 24, 2, and 6 for immature, pregnant and lactating females, and 18 and 2 for immature and mature males, respectively. The following sampling procedures were carried out:

The samples in 1989–90 were taken by puncturing a large vein usually several hours or even days *post mortem,* and isolating the serum by passive coagulation for 24 hours (treatment 1). This collection comprised 28 blood samples, all of which appeared brownish-black due to extreme hemolysis and partial degradation. In 1991–92 the animals were brought to the research vessel *Adolf Jensen* within a few hours after being killed. The blood samples (N = 99) were isolated by cardiac puncture, and serum was harvested by centrifugation immediately after collection (treatment 2). When the latter sampling method had been used, serum samples were only weakly hemolysed. Serum samples were aliquotted and stored at −28°C. Urine samples were taken from the bladder into 50 ml plastic tubes and stored at −28°C.

Hormone analyses

All common reagents for construction and performance of hormone analyses were of at least analytical grade. Progesterone and oestrone for preparation of calibrator series were dilutions of reference standards from Sigma® (Sigma Chemical Compagny, St. Louis, Mo., USA), which also delivered the horseradish peroxidase, type VI for synthesis of enzyme conjugates. Steroid derivatives for coupling to horseradish peroxidase came from Steraloids® (Steraloids Inc., London, UK).

Serum and urine samples were subjected to steroid hormone analyses after appropriate processing. The measured concentrations of steroid hormones are reported in SI units, *i.e.* nmol/l.

Initially hormone levels in urine were indexed against the protein content. However due to occasional contam-

ination of the urine with other body fluids unrealistic protein concentrations were indicated, and creatinine was used instead for indexing. Creatinine was measured by a kinetic picrate method without deproteinization according to Jaffé (Creatinine UNI-KIT II, Roche®, Switzerland), and the index was calculated as the concentration of the hormone in pmol/l divided by the creatinine content in μmol/l (Creatinine index = 10^6* molecular ratio). Creatinine content was not measured for six female and four male urine samples that had been collected more than six months before the time of analysis because this molecule is too unstable. To estimate steroid hormone levels from these samples a standard value for creatinine content was derived from the remaining samples (4343 and 4799 μmol/l for males and females, respectively). The group means were used in order to avoid introduction of bias in the statistical treatment of missing observations (Weisberg 1985).

Acid hydrolysis of urine samples

Urine samples were subjected to hydrolysis of conjugated steroids prior to steroid extraction: Urine was diluted with hydrochloric acid to a final concentration on 2 mol/l, and samples were then placed in a boiling water bath for 15 min. After cooling, hydrolysates were neutralized with NaOH until a pH of 6–8 was achieved.

Steroid extraction procedures

For the purpose of isolation of the steroid hormones, serum samples and urinary hydrolysates were subjected to organic solvent extraction as previously described (Rice *et al.* 1993). Briefly, sodium chloride was added to all samples prior to extraction to a final concentration of 0.5 mol/l. The extraction was executed twice, each time with four volumes of freshly opened diethyl ether. Ether phases were evaporated, and the extracts were resuspended in assay buffer in a volume so that the response was within 20–80% of displacement in the assays. The recovery of radiolabelled controls averaged >90% for progesterone and >95% for oestrone, oestradiol-17β and testosterone.

Progesterone analysis

Progesterone was quantified using an enzyme-linked immunosorbent assay (ELISA) as described by Rice *et al.* (1993). The antiserum was raised against 11α-hydroxyprogesterone-11-hemisuccinate: BSA, and the conjugate was progesterone3-O-(carboxymethyl)oxime: horseradish peroxidase. The antibody showed the following cross-reactivities at 50% of displacement (CR): progesterone 100%; 11α-hydroxyprogesterone 5%; pregnandione 0.4%; other steroids tested < 0.05%. Each sample was assayed in duplicate. The limit of detection (LDC) defined as 2* standard deviation of zero binding value was < 0.2 nmol/l. The inter- and intraassay coefficients of variation (CV) were < 10 and 8%, respectively.

Oestrogen analysis

The ELISA method described by Rice *et al.* (1993) was used to determine the oestrogen content in the sample extracts. The conjugate was 17β-oestradiol-3-hemisuccinate coupled to horseradish peroxidase, and the antibody (Dr. B. Svenstrup, States Serum Institute of Denmark) was raised in a rabbit against oestradiol-17β-6-O-(carboxymethyl)oxime: BSA. The antibody was previously characterized in radioimmunoassay (Callesen *et al.* 1986). In ELISA the major CRs were: oestradiol-17β 100%; oestrone 17%; oestriol 0.3%. The ELISA was characterized with respect to assay variation, inter- and intraassay CV 12 and 10%, respectively, and sensitivity as LDC was 4–10 pmol/l. There was parallelism between dilutions of controls and the dose response curve. However, when assaying white whale samples the requirement for parallelism between dilutions of sample extracts and the measured concentrations could not be met. The displacement curves for dilutions of extracts had slopes which were more similar to the displacement curve for oestrone than to the one for oestradiol-17β. This indicates that oestrone is present in high concentrations relative to oestradiol-17β in the white whale extracts, as has been reported for killer whales (Walker *et al.* 1988). Consequently oestrone was used for calibration of the oestrogen analysis, although a possible presence of oestradiol-17β might lead to overestimation of oestrogen concentrations.

Testosterone analysis

Testosterone concentrations were measured using a commercially available radioimmunoassay kit from DPC® (Diagnostic Products Corporation, Los Angeles, California), developed for use on extracts of blood and urine. LDC was 0.2–0.5 nmol/l, and inter- and intraassay CV was < 10%. The antiserum displayed the following CR's, taking testosterone as 100%: Dihydrotestosterone 34%; 5βandrostan-3α,17β-diol 3.8%; 11-hydroxytestosterone 3.3%; 5α-androstan-3α,17βdiol 2.9%; 5α-androstan-3β,17β-diol 2.7%; androsterone 2.1%; and other steroids tested (*e.g.* 11-deoxycortisol, 11-keto-testosterone and androstenediol) below 1%; all according to the manufacturer.

Prolactin analysis

A commercial kit, Prolactin Serozyme® (Serono Diagnostics, Switzerland), designed for the determination of prolactin in human blood samples, was used to quantify prolactin in white whale serum samples. The antibody of the kit had previously shown a recognition of essential structures of the hormone by reaction with prolactin from the antarctic fur seal (*Arctocephalus gazella*) by Boyd (1991). Data on cross-reactivity were only available for human gonadotropins: Luteinizing, follicle stimulating and thyroid stimulating hormones and placental lactogen did not show any interference in the analysis; the human growth hormone showed a slight cross-reactivity in the assay corresponding to 0.35 µg/l for each IU/l of 1st IRP 66/217.

In order to increase the sensitivity and ease the processing, we modified our method slightly with respect to volumes of samples and reagents, and with respect to temperature and time of incubations: Fifty µl of sample or standard in duplicates was mixed with 100 µl of anti-prolactin reagent and incubated for 60 min at room temperature. Following this, 100 µl of the separation reagent was added, and the incubation continued for 20 min. The next steps were magnetic separation and a single washing with 500 µl of wash solution, ending with enzyme reaction for 40 min at room temperature. The colour development of the enzyme assay was monitored at two wavelengths, 492 and 550 nm, so that a wide-range calibration curve was obtained, as suggested by the manufacturer. The assay characteristics stated by the manufacturer were not altered, as judged from control determinations: Interassay CV 5–6%, intraassay CV 2–4% and LDC 0.2 µg/l. The reference preparation 2nd IRP 83/562 was used for calibration in µg/l.

The specific recognition of white whale prolactin in the kit was investigated by analysis of partially purified prolactin. The prolactin was isolated from the pituitary of a lactating female which had given birth shortly before her death; this pituitary was expected to contain high amounts of prolactin. The preparation followed the diethyl aminoethyl ion exchange chromatography described by Wallace & Ferguson (1964). Growth hormone and luteinizing hormone did not bind to the ion exchanger, while prolactin did bind and was eluted from the column by a change in pH of the elution buffer. Subsequently, pH of the effluent was adjusted to 5.1, and a precipitation with 40% (V/V) of ethanol was performed (Hartree 1966). In this step of purification the follicle stimulating and luteinizing hormones remained unprecipitated whereas prolactin could be isolated by centrifugation. The resulting precipitate was dried under vacuo, resuspended in the kit's serum diluent and used for characterization of the assay.

Statistical methods

Calculations of calibration curves for the hormone analyses were assisted by regression analysis of the log/logit transformation of the response versus the calibrator dose.

Routine descriptive statistical methods as described by Box *et al.* (1978) were used. Nonbalanced analysis of variance and comparison between group means was performed in SAS® (SAS Institute Inc. 1987) using the general linear models (GLM) procedure. Group mean comparisons were made with the Hochberg's GT2 method, which was reported to be suitable for unequal group sizes (SAS Institute Inc. 1987). Linear regression analyses were performed via the GLM procedure in SAS® (SAS Institute Inc. 1987). Regression analysis of logarithmically transformed observations was introduced in order to achieve homogeneity of the variance (Weisberg 1985). The logarithmic regression analysis has an additional advantage in an expression of proportionality, since $\ln Y = \ln(a*X) = \ln a + \ln X$, giving the slope equal to 1, when proportionality exists.

Models for predicting state of reproduction according to hormone levels were computed with the assistance of the categorical data modeling procedure in SAS® (SAS Institute Inc. 1987). Briefly, an iterative procedure of parameter estimation was performed in conjunction with an estimation of the likelihood ratio, and the probability of model fitting and the significance of the individual parameters were used to judge statistical significance (see Shott 1991 and Jensen & Høier 1993 for reviews).

Results

Evaluation of the prolactin analysis

A white whale pituitary preparation, enriched with respect to prolactin, was tested for response in the analysis at various dilutions. The recovery of white whale prolactin (in arbitrary units: mU/l) measured in the kit as human prolactin is shown in Fig. 1. The regression equation was, (X, Y) = (added mU/l, recovered µg hPRL/l), $Y = -1.64 + 2.34*X$, where the intercept did not differ from 0 ($P = 0.63$). The logarithmic regression analysis gave $\ln Y = 0.83 + 0.97*\ln X$, and the slope was not different from unity ($P = 0.34$). Fig. 1 also depicts the determination of a white whale serum sample in different dilutions with the kit's serum diluent. The resulting regression analysis gave a straight line correlation, (X, Y) = (dilution, measured prolactin): $Y = -0.10 + 0.16*X$, $R = 0.98$, intercept not different from 0 ($P = 0.93$), and the logarithmic regression equation was: $\ln Y = -2.59 + 1.18*\ln X$, the slope was not different from unity ($P = 0.32$). Thus, proportionality apparently exists between the kit's human

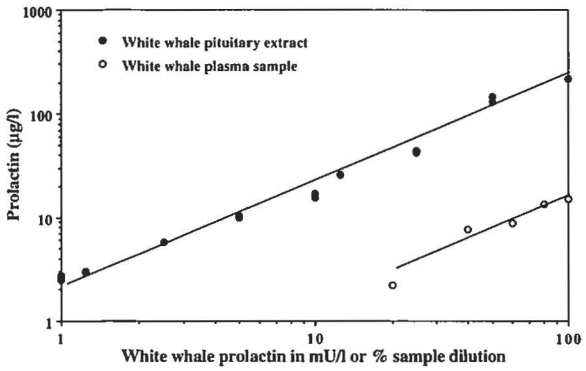

Fig. 1. Determination of prolactin content (µg/l) in dilutions of white whale pituitary extract and of a white whale plasma sample. White whale pituitary extract as mU/l, sample dilutions as percent dilution.

calibrators and the response from white whale prolactin assayed as a prolactin-enriched pituitary preparation as well as from white whale serum samples.

Effect of changes in sampling technique

In order to see whether the difference in sampling technique, treatment 1 and 2, influenced the analyses of hormone contents in the serum samples, the concentrations measured with the two types of treatment were compared within the various groups of reproductive activity by unbalanced analysis of variance. A general tendency towards elevated steroid concentrations was seen for treatment 1 as compared with treatment 2, but only the oestrogen determinations showed a significant difference (Table 1). No between-treatment difference could be discerned for the prolactin measurements.

Correlation between plasma and urine samples

Urine samples less than 6 months of age at the time of analysis were checked for creatinine content. The analysis resulted in: mean – standard error of the mean ± SE (range), 4343 ± 314 (2980 – 7719) µmol/l for males and 4799 ± 291 (1843 – 8089) µmol/l for females; means of the two groups were not different (P = 0.14).

Material from animals subjected to both blood and urine sampling was used to investigate the relationship between the levels of unconjugated steroid hormones in the blood and the amount of a given hormone secreted into the urine, the latter being a measure of the total metabolism of that hormone when indexed against creatinine. Linear regression analyses showed a significant influence of the measured concentrations in serum samples on the corresponding steroid concentrations in urine, whereas only the analysis of progesterone observations gave a significant correlation between serum and urine concentrations in the logarithmic regressions (Table 2). Moreover all slopes in logarithmic regressions were sig-

Table 1. Mean concentrations of progesterone, oestrogen, testosterone and prolactin in serum from various reproductive classes of white whale using different isolation procedures. preg&lact: lactating pregnant females. nd: not determined. P(treat) = Probability for identical results from the two treatments in unbalanced analyses of variance.

	Male white whales		Female white whales				
	immature	mature	immature	pregnant	lactating	preg&lact	P(treat)
Number of observations:							
Treatment-1	15	1	16	3	3	1	
Treatment-2	31	5	37	1	12	1	
Progesterone (nmol/l):							
Treatment-1	2.06	1.91	2.39	29.1	2.00	24.1	
Treatment-2	1.08	0.60	1.28	24.0	2.40	38.9	
Difference	−0.99	−1.33	−1.11	−5.08	0.40	14.7	0.12
Oestrogen (nmol/l):							
Treatment-1	6.27	3.36	7.22	9.48	7.58	4.15	
Treatment-2	2.40	8.22	2.42	5.91	2.82	0	
Difference	−3.87	4.87	−4.81	−3.57	−4.76	−4.15	<0.001
Testosterone (nmol/l):							
Treatment-1	1.35	5.95	nd	nd	nd	nd	
Treatment-2	0.77	3.78	nd	nd	nd	nd	
Difference	−0.58	−2.17	nd	nd	nd	nd	0.08
Prolactin (µg/l):							
Treatment-1	3.38	2.07	3.19	1.49	1.15	2.40	
Treatment-2	2.73	1.64	1.73	2.83	4.56	13.3	
Difference	−0.68	−0.57	−1.46	1.34	3.41	10.9	0.95

Table 2. Statistical parameters for comparison between serum and urine levels of steroid hormones in white whales. R = coefficient of correlation for the regression model. P(model) = probability for the regression model in analysis of variance. $P(\beta_0 = 0)$ = probability for Y-axis scission = 0. $P(\beta_1 = 1)$ = probability for slope = 1. Serum-P4, -OEST, -TES = Serum concentrations of progesterone, oestrogen and testosterone in nmol/l. Urine-P4, -OEST, -TES = urine concentrations of the steroids as creatinine index.

Linear regression, $Y = \beta_0 + \beta_1 * X$:					
Y	X	N	R	$P(\beta_0 = 0)$	P(model)
Serum-P4	Urine-P4	48	0.844	0.0040	0.0001
Serum-OEST	Urine-OEST	47	0.296	0.0029	0.0435
Serum-TES	Urine-TES	15	0.812	0.0098	0.0002
Logarithmic regression, $\ln Y = \beta_0 + \beta_1 * X$:					
Y	X	N	R	$P(\beta_1 = 1)$	P(model)
Serum-P4	Urine-P4	48	0.618	0.0059	0.0001
Serum-OEST	Urine-OEST	47	0.088	0.0001	0.56
Serum-TES	Urine-TES	15	0.397	0.0001	0.1434

nificantly different from 1; therefore a direct proportionality between serum and urine steroid levels could not be confirmed.

Hormone levels in male white whales

Testosterone, progesterone and oestrogen were measured in serum and urine samples from immature and mature male white whales, and prolactin was measured in the serum samples (Table 3). Group mean comparisons in analysis of variance indicated that the testosterone levels were unevenly distributed: 0.96 and 4.14 nmol/l in serum (P = 0.001) and 0.11 and 0.61 creatinine index in urine (P = 0.01) from immature and mature animals, respectively. Oestrogen levels changed insignificantly in serum from 3.66 nmol/l in immature to 7.41 nmol/l in mature animals, while the urine creatinine index increased from 0.47 in immature to 1.33 in mature animals (P = 0.01). Progesterone did not vary significantly between the groups, although a trend towards elevated levels was observed in serum from immature males (P = 0.07). Prolactin concentrations had a very high individual variation, as can be seen from the measured ranges, and no between-group difference was observed.

The samples from male white whales offered the possibility of investigating the influence of hunting season on hormone concentrations. A thorough statistical analysis of the combined effects of seasonality and maturity could not be performed because we had insufficient material, but the data from sexually mature males could be compared between spring (N = 3) and autumn (N = 3) seasons of collection. Testosterone and prolactin did not differ

Table 3. Concentrations of testosterone (TES, nmol/l), progesterone (P4, nmol/l), oestrogen (OEST, nmol/l) and prolactin (PRL, µg/l) in male white whale serum and urine (as creatinine index) according to sexual-maturity status. N = number of observations, mean = mean concentration, SE = standard error of the mean, range = minimum and maximum concentration measured, P(diff) = probability for difference between groups in analysis of variance.

Serum hormones:															
	TES				P4				OEST				PRL		
N	mean	SE	range	N	mean	SE	range	N	mean	SE	range	N	mean	SE	range
Immature males															
46	0.96	0.13	0–4.72	46	1.40	0.11	0.57–3.88	46	3.66	0.79	0–32.7	36	2.82	0.95	0.22–34.7
Mature males															
6	4.14	1.33	0.69–8.53	6	0.80	0.28	0.22–1.91	6	7.41	2.26	3.36–17.8	6	1.72	0.20	1.04–2.22
P(diff)		0.0001				0.07				0.11				0.64	

Urine hormones:											
	TES				P4				OEST		
N	mean	SE	range	N	mean	SE	range	N	mean	SE	range
Immature males											
14	0.11	0.03	0.004–0.39	18	0.64	0.04	0.40–1.00	18	0.47	0.06	0–1.13
Mature males											
2	0.61	0.52	0.09–1.13	2	0.66	0.26	0.40–0.92	2	1.33	0.99	0.34–2.32
P(diff)		0.01				0.92				0.01	

Table 4. Concentrations of progesterone (P4, nmol/l), oestrogen (OEST, nmol/l), and prolactin (PRL, μg/l) in serum from female belugas at different reproductive states. N, mean, SE, and range as in Table 3, all pregnant = pool of pregnant plus lactating, pregnant animals; all lactating = lactating plus pregnant lactating animals. Columns having different superscripts of the mean concentrations are significantly different ($\alpha = 0.05$).

Reproductive states	P4				OEST				PRL			
	N	mean	SE	range	N	mean	SE	range	N	mean	SE	range
Immature	53	^{b}1.61	0.19	0.54–9.86	53	3.87	0.69	0.15–25.2	44	2.00	0.26	0–12.8
Pregnant	4	^{a}27.9	4.52	22.4–41.4	4	8.39	2.81	2.99–16.1	4	1.82	0.35	1.23–2.83
Lactating	15	^{b}2.32	1.02	0.73–16.5	15	3.77	1.28	0–18.5	15	3.88	1.34	0.15–20.9
Resting	1	^{b}1.08	–	–	1	1.33	–	–	1	1.54	–	–
Pregn. lactating	2	^{a}31.5	7.39	24.1–38.9	2	2.09	2.06	0–4.15	2	7.87	5.47	2.40–13.3
all pregnant	6	^{a}29.1	3.52	22.4–41.4	6	6.42	2.31	0–16.1	6	3.84	1.91	1.28–13.3
all nonpregnant	69	^{b}1.76	0.26	0.54–16.5	69	3.81	0.60	0–25.2	60	2.46	0.40	0–20.9
all lactating	17	5.76	2.60	0.73–38.9	17	3.57	1.15	0–18.5	17	^{a}4.35	1.31	0.2–20.9
all nonlactating	58	3.41	0.94	0.54–41.4	58	4.15	0.67	0–25.5	49	^{b}1.97	0.26	0–12.8

between seasons, whereas progesterone concentrations decreased significantly ($P < 0.05$) from autumn to spring: 1.34 ± SE 0.31 nmol/l for autumn samples and 0.26 ± SE 0.02 nmol/l for spring samples (Fig. 2). Oestrogen also appeared to differ between the seasons: 4.22 ± SE 0.76 nmol/l in autumn and 10.6 ± SE 3.82 nmol/l in spring ($P = 0.05$).

Hormone levels in female white whales

Serum progesterone levels were high in pregnant females (mean = 27.9 nmol/l) and in females that were simultaneously lactating and pregnant (mean = 31.5 nmol/l) (Table 4). All nonpregnant females had low serum progesterone concentrations (mean progesterone = 1.76 nmol/l), except one animal killed in May that had a high concentration (16.5 nmol/l). She was lactating and had probably calved less than 14 days prior to catch. Concentrations of oestrogen and prolactin did not differ significantly between the reproductive states, although pregnant females had a higher mean value for oestrogen. Progesterone and oestrogen were significantly elevated ($P < 0.05$) in the urine from pregnant animals compared with that of lactating or immature animals (Table 5). When serum levels of all pregnant animals were compared with those of all nonpregnant animals, progesterone concentrations were significantly higher ($P < 0.05$) in the former than the latter (Table 4). Furthermore, a comparison of all lactating with all nonlactating females showed that prolactin levels in serum were significantly higher ($P < 0.05$) in the former than the latter (Table 4).

Diagnosis of reproductive state following hormone determinations

The between-group comparisons with respect to hormone content of serum and urine indicated differences between whales in the various reproductive classes. In order to clarify whether these differences could be used to diagnose the reproductive states of individuals, hormone concentrations were correlated to group specificity by using a categorical model (Table 6). The male serum samples collected in May were excluded from this investigation due to an effect from the time of sampling (Fig. 2), and no analysis was made on male urine samples owing to the low number of observations (Table 3). As an illustration of the model fitting, Table 6 shows the numbers of correct

Table 5. Concentrations of progesterone (P4) and oestrogen (OEST) in urine from female belugas in different reproductive states. Concentrations are given as the creatinine index. N, mean, SE, and range as in Table 3. Columns having different superscripts of the mean concentrations are significantly different ($\alpha = 0.05$) according to group mean comparison following unbalanced analysis of variance.

Reproductive states	P4				OEST			
	N	mean	SE	range	N	mean	SE	range
Immature	24	^{b}0.55	0.03	0.28–0.88	23	^{b}0.34	0.05	0.11–1.04
Pregnant	2	^{a}22.1	11.8	10.2–33.8	2	^{a}2.43	1.92	0.68–4.17
Lactating	6	^{b}0.53	0.05	0.38–0.67	6	^{b}0.40	0.08	0.26–0.76

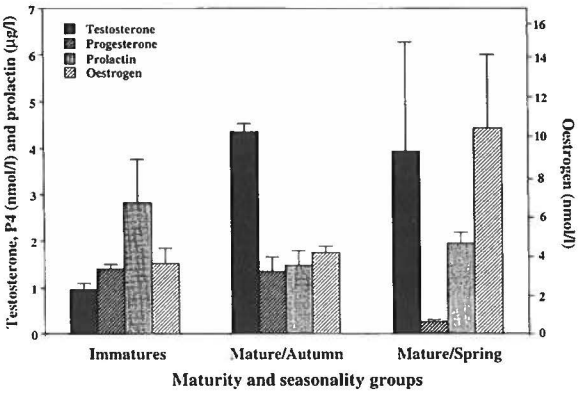

Fig. 2. Mean concentrations of testosterone, progesterone, oestrogen (nmol/l) and prolactin (µg/l) in immature and mature male white whales sampled in autumn and spring.

and incorrect predictions when using the models on the set of observations and taking the cut-off value to be a probability of 0.50. Serum (threshold = 19.5 nmol/l) and urine (6.19 creatinine index) progesterone levels are found to be powerful indicators of pregnancy, and serum testosterone level (3.30 nmol/l) can be used to diagnose male sexual maturity.

Discussion

Evaluation of prolactin analysis

White whale serum samples responded in the analysis, which had been originally designed for the determination of human prolactin levels, and sample dilutions reacted proportionally to the calibration curve. Serum components other than prolactin might have reacted with the antibody of the kit. There is, for example, a close structural relationship between prolactin and growth hormone in several species (Posner 1977). Also, Nicoll *et al.* (1977) have described the binding of serum components from hypophysectomized pigeons to an antibody raised against mammalian prolactin. It is therefore essential to investigate whether the kit actually measured prolactin and not another substance in the serum samples, and for this purpose a white whale pituitary extract was prepared. It was enriched with respect to the content of prolactin using techniques which deselected other gonadotropins. The result confirmed that dilutions of the preparation reacted proportionally to the calibration curve, and this allowed us to conclude that the molecule recognized by the kit's antibody was of pituitary origin. Moreover, since growth, luteinizing and follicle stimulating hormones were preferentially removed by the methods used to prepare the extract, according to data on whale gonadotropin migration in the chromatographic method used (Wallace & Ferguson 1964), it is likely that the kit measures white whale prolactin specifically. It can therefore be concluded that the method does measure white whale prolactin in a way which depicts proportionality between white whale prolactin and the calibration curve, although the absolute concentrations cannot be determined.

Effect of changes in sampling technique

A comparison between the two sampling techniques (treatments 1 and 2) within the reproductive groups of both sexes indicated a trend towards overestimating concentrations of oestrogen and testosterone in treatment 1

Table 6. Logistic regression models for the diagnosis of male maturity and of pregnancy and lactation in females based on the concentrations of progesterone (P4), oestrogen (OEST), testosterone (TES) and prolactin (PRL) in serum samples, and indexed concentrations of P4, OEST and TES in urine samples. Serum concentrations in nmol/l (PRL in µg/l), urine concentrations as creatinine index. The regression equations represent the significant model (P = 0.05). Response in test: correct(+) or incorrect(-) result in analysis using odds ratio equal to 0.50 as the threshold value. Threshold values are the concentrations corresponding to P(odds ratio) = 0.5.

			Response in test			
Male sexual maturity:			immature		mature	
	Estimated regression equation (E)	threshold	+	−	+	−
Serum	−4.0821+1.2385*(TES)	3.30	46	0	5	1
Female pregnancy:			nonpregnant		pregnant	
			+	−	+	−
Serum	−59.58+3.064*(P4)	19.5	60	0	6	0
Urine	−12.94+2.091*(P4)	6.19	29	0	2	0
Urine	−5.684+4.7094*(OEST)	1.21	29	0	1	1

P(odds ratio) = exp(E)/(1 + exp(E)), where E is the expression derived from logistic regression.

relative to treatment 2. This trend was observed despite the use of methods designed for extracted material which should have eliminated any effects from steroid-binding globulins and other nonspecific effects from the blood matrix. The reasons for the discrepancy are most likely related to metabolic changes in the steroids, *e.g.* from hydratization of conjugated steroids or from oxidation of the steroids. Whether such changes occur during hunting, sample collection or the subsequent storage period (up to 2 years in a freezer) cannot be determined. However hemolysis reportedly affects the results of progesterone analysis, possibly by releasing enzymes that modulate the molecule so that it cannot be recognized by the antibody used in the analysis (*e.g.* Reimers *et al.* 1983, Vahdat *et al.* 1984, Inns & Cecchini 1989, Yoshioka & Fujise 1992). Also, Van der Molen & Groen (1968) have described the 20α-hydroxysteroid-dehydrogenase-dependent interconversion between progesterone and 20α-dihydroprogesterone and between androstenedione and testosterone in hemolyzed blood.

Based on the probable presence of hydrolytic enzymes and oxidoreductases in hemolysed blood, we conclude that treatment 1 possibly results in elevated steroid concentrations. Thus the more laborious treatment 2 must be recommended for the collection of blood samples. Nevertheless the difference between the two treatment groups is not unambiguous, and some of the difference might be explained by factors other than the treatment procedures. For instance there might be some individual variation within the groups owing to changes in hormone concentrations, *e.g.* in the period immediately prior to the onset of maturity in the male and female immature groups, when steroid release could be expected. Also, the group of pregnant females might be nonhomogeneous, since the secretion of oestrogen, for example, probably varies during gestation. It is important to bear in mind that the number of observations per group is small, especially when the groups are further divided into two treatment categories.

Prolactin determinations apparently were not influenced by the difference in sampling technique. The antigenic structures of prolactin seem to have been maintained even under extreme hemolysis. However another possible explanation is that the prolactin measurements reflect a large individual variation within the reproductive groups. For instance the largest measured concentration of prolactin was found in an immature male. The finding of high concentrations of the hormone in nonlactating females and in males emphasizes the complexity of prolactin action, since its secretion is regulated by many factors. In addition to the direct influence of hormone-regulating mechanisms, factors such as time of day, photoperiod and temperature affect prolactin secretion (see McNeilly 1980 for a review). Also, physiological stress might be an essential factor for the secretion of prolactin from the pituitary, and the animals sampled in this study may have been hunted for several hours before being killed.

Correlation between serum and urine samples

A comparison between serum steroid concentrations and the amounts of the same steroid excreted into the urine confirmed a covariation, although proportionality between the two types of steroid determination could not be confirmed by logarithmic regression analysis.

The unconjugated steroids in the blood reflect the amounts active in regulation of reproductive events at the time of sampling. The concentrations are not constant, since they change following episodic secretions leading to diurnal and long-term variations. The urinary steroids, in contrast, characterize the hormonal turnover integrated over a longer period and thus reflect prolonged processes, *e.g.* luteal maintenance or placental activity. Thus the two types of sample depict different expressions of hormonal activity, and the lack of proportionality might be expected. We suggest that the urinary steroids are better indicators of hormonal activity owing to their low sensitivity to short-term variations. On the other hand, when hormones were used to describe different reproductive classes both sample types proved equally useful, except that serum oestrogen did not vary significantly between pregnant and nonpregnant females.

Hormones in male white whales

In male white whales the group comparisons were confounded by the presence of three samples from mature animals collected in May; all other samples were obtained in autumn.

The most obvious features that distinguished sexually mature males were the elevated testosterone concentrations in serum and urine samples, regardless of the time of catch. Our results showed a four-fold increment in testosterone concentration between sexually immature and mature males. Male white whales have not been previously characterized with respect to testosterone content in immature and mature animals, so the only available data for comparison are from the investigations of other cetaceans: Testosterone concentrations of sexually mature Dall's porpoises averaged 10.46 ± SE 1.20 ng/ml (36.2 ± 4.2 nmol/l), while those of immature animals averaged 1.00 ± SE 0.52 ng/ml (3.5 ± 1.8 nmol/l) (Temte 1991). Comparable results were obtained when following a male Dall's porpoise throughout one year (Subramanian *et al.* 1987). Testosterone was measured in a few male delphinids (Wells 1984, Schroeder & Keller 1989), indicating summer levels of 50–200 nmol/l, in contrast to winter levels of 0–20 nmol/l. Similar seasonal variation in testosterone secretion has also been documented in mysticete cetaceans (fin whales and minke whales, Kjeld *et al.* 1992, Yoshioka & Fujise 1992) with high concentrations measured during summer vs low winter levels.

Thus care must be taken in interpreting hormone levels in species that have seasonal mating.

The progesterone content of serum and urine from male white whales varied according to the time of hunting and sampling rather than the state of maturity. The measured levels were comparable to the progesterone content in serum and urine from nonpregnant females, and this possibly reflects the release of progesterone from cortex of the adrenal glands. Further investigation is needed, but the seasonal variation might be explained by the fact that secretion of progesterone and other corticosteroids increases in autumn, and possibly winter, owing to an overall change in metabolic activity. Another possible explanation is that the growth of testes which occurs in May (Heide-Jørgensen & Teilmann 1994) affects the levels of progesterone and other steroid hormones.

The oestrogen content of male serum and urine samples did not differ between immature and mature animals. Comparisons between May and autumn samples from mature animals indicated that oestrogen is possibly found in higher concentrations in May, coinciding with the growth of testes in males (Heide-Jørgensen & Teilmann 1994) and the onset of oestrous in females (Stewart & Stewart 1989).

The prolactin content of serum from males apparently was unaffected by either sexual maturity or the time of capture. The dominant feature of the prolactin analysis was the very high variation between individuals. When all white whale samples were compared the highest prolactin concentration was measured in serum from an immature male. This animal's sexual-maturity state should not have affected the result because the most common function of the hormone is to induce milk-producing tissue (McNeilly 1980). The more generalized effects of prolactin, such as those related to responsiveness to changes in photoperiod, might be invoked to explain the finding of a maximum concentration in an immature male killed in autumn.

Hormones in female white whales

The main variation in hormone levels of female white whales was seen for progesterone in pregnant vs nonpregnant animals. Progesterone concentrations varied significantly and specifically between the total group of pregnant females and the group of nonpregnant females, and this was seen whether the pregnant animal was lactating or nonlactating. The mean concentrations were 29 nmol/l for pregnant and 1.8 nmol/l for nonpregnant animals, the latter being near to the concentrations seen in males, thereby indicating a lack of luteal activity. The only exception was a lactating female that had a serum progesterone concentration between the two group means (16.5 nmol/l). Stewart (1994) reported the mean progesterone level in pregnant white whales caught in the eastern Canadian Arctic to be 9.26 ng/ml (29.4 nmol/l), with a range between 1.48 and 16.20 ng/ml (4.7–51.5 nmol/l). Immature and nonpregnant females in his study had mean concentrations of 1.37 ng/ml (4.4 nmol/l), ranging from 0.50 to 5.40 ng/ml (1.6–17.2 nmol/l). Thus there is a good agreement between our results and those of Stewart (1994) with respect to progesterone concentrations in pregnant white whales. However nonpregnant females in our study had lower progesterone concentrations than those described for nonpregnant females by Stewart (1994). This difference may be explained by the use of different analytical procedures. Also, one cannot exclude the possibility of a population difference between the white whales in West Greenland and the Canadian Arctic.

Stewart (1994) reported one lactating female in his sample of nonpregnant white whales to have had a high serum progesterone concentration (5.40 ng/ml = 17.2 nmol/l). It was suggested that she had recently calved, and this would agree with our observation of high progesterone levels shortly after parturition. The reason for this phenomenon is not clear, but an explanation could be that the progesterone which maintains pregnancy in white whales is of placental origin.

The high individual variation in serum prolactin levels which characterized males was also observed in females. When comparing all female reproductive groups no particular one could be identified by the prolactin concentration alone, although both nonpregnant and pregnant lactating animals had higher mean prolactin levels. When the data were pooled, lactating animals showed a significantly higher prolactin level than nonlactating animals. This would appear to indicate that prolactin concentrations are higher in lactating than in nonlactating females. However, due to the high individual variation within the different reproductive groups it is difficult to make a clear distinction between prolactin concentrations in lactating and nonlactating females.

The oestrogen content in samples from females was not affected by either pregnancy or lactational amenorrhea as one would expect it to be, although the measurements did show tendencies towards elevated and lowered concentrations, respectively. Our inability to separate mean concentrations of oestrogen with respect to reproductive group might be explained by the small number of observations in each group, combined with a high variability within the groups. The latter effect could have been at least in part due to the between-treatment difference, which was significant for oestrogen.

Diagnosis of reproductive state following hormone determinations

Concentrations of progesterone in female serum or urine and of urine oestrogen can be used to diagnose pregnancy. Serum testosterone can be used to diagnose sexual maturity in males. The models can be used for calculating threshold values between pregnancy and nonpregnancy

in females, and sexual maturity and immaturity in males (Table 6). The overall oestrogen content in serum was not found to be useful for specifying female reproductive classes. Prolactin concentration exhibited too much individual variation to be a reliable predictor of lactation in female white whales.

We compared our model for diagnosing pregnancy by reference to a threshold value of 19.5 nmol/l progesterone in white whale serum with the corresponding threshold value of 3 ng/ml equal to 9.5 nmol/l proposed by Stewart (1994). Our logistic regression model clearly suggests a higher cut-off value. We did not observe any false predictions of pregnancy in our investigation, whereas Stewart (1994) found that in two cases out of 12, pregnant white whales were judged to be nonpregnant. The discrepancy could be due to the low number of observations in our study (4 pregnant white whales), or to the variability in methods employed for sample treatment and progesterone analysis in both studies.

Conclusions

This study reports the concentrations of progesterone, testosterone, oestrogen and prolactin found in serum and urine of white whales hunted off West Greenland. Our results reflect the hormonal parameters of animals which have been subjected to stress during hunting for up to several hours. The time span between killing and sample collection may have influenced the hormone levels in the blood and urine samples. We also cannot exclude the possibility of an effect from the sample treatment performed under extreme field conditions. This study will therefore be most instructive for samples collected under the same conditions. Care should be taken if our results are used, for example, in a comparison with white whales living in captivity. The quality of blood samples from relatively unstressed, living animals is much different from the quality of the blood samples used in this study.

Progesterone appears to be suitable for predicting pregnancy in female white whales when measured in serum or urine, and testosterone in both serum and urine could be used for determining the maturity status of male white whales. Urine oestrogen might be used for detecting pregnancy in females. The concentrations of steroid hormones in urine are probably more reliable for characterizing reproductive state than are the corresponding serum concentrations.

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