Sex hormone concentrations in the blood of sei whales (*Balaenoptera borealis*) off Iceland

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ABSTRACT

Blood samples were collected postmortem at sea, from 195 sei whales (127 females and 68 males) caught southwest of Iceland between 1983 and 1988. The reproductive status of the whales was determined by anatomical/histological methods. The blood samples were measured by radioimmunoassays for progesterone (P), testosterone (T) and oestradiol concentrations, which were then related to the reproductive status, the length of the whales and the days of the hunting season. Serum P concentrations in females were found to be clustered mainly into two groups, one with values at or below the detection limit (0.1nmol/L) of the assay (Group I) and the other with values about two orders of magnitude higher (Group III) with intermediate values (Group II) in between. Anatomical results showed that Group I ($n = 73$) was largely a mixture of immature and anoestrous mature females. Group III ($n = 39$), with a significantly ($p < 0.01$) greater mean body length than Group I, had a distinct frequency distribution of serum P values with a mean (SD) concentration of 10.3nmol/L (4.1) and consisted predominantly of pregnant females. Many foetuses were lost at sea due to a slit in the abdomen for cooling purposes, but all 13 foetuses (1.5-3.7m in length) recovered belonged to females of Group III. Group II ($n = 15$) consisted mainly of anoestrous mature animals. When pregnancy was estimated by serum P values and sexual maturity by the anatomical findings, the apparent pregnancy rate of mature females was 0.37, agreeing reasonably with earlier reports. Male sei whales were classified into immature, pubertal and mature groups by anatomical/histological methods and had mean T concentrations (nmol/L, ranges) of 0.85, 0.1-4.5; 3.3, 0.1-14.7 and 4.8, 0.1-14.8, respectively. Serum T concentrations did not correlate significantly with body length in the groups but pubertal and mature males had significantly higher geometric mean T values than immature males. Mean serum T concentrations in males, classified as sexually mature by anatomical/histological methods, rose approximately 3.2-fold every 30 days during July-September indicating a seasonal breeding cycle. It is concluded that measurements of sex hormone concentrations in sei whales make a powerful addition to the earlier anatomical/histological methods for determination of reproductive status, not only corroborating them but apparently surpassing them in sensitivity of detecting pregnancy and cyclical changes in serum T values during the male reproductive cycle.

KEYWORDS: SEI WHALE; SEX HORMONES; REPRODUCTION; BREEDING SEASON; OVULATION; PREGNANCY; HORMONES; SEASONALITY

INTRODUCTION

Sei whales (*Balaenoptera borealis*) migrate seasonally every year, feed on their main prey (euphausiids and copepods) at higher latitudes, and breed about every second year at lower latitudes (Boyd *et al.,* 1999). Their presence in Icelandic waters in summer and autumn months is irregular and their abundance varies (Martin, 1983; Cattanach *et al*., 1993), but in the North Atlantic this species has been found to be genetically uniform (Árnason, 1995).

The gestation period of female sei whales is not known exactly but is reported to be more than 10.75 months, with lactation lasting about 6 months (Lockyer, 1984). The reproductive state of sei whales has been assessed by postmortem studies of various anatomical parameters of their sex organs, both macro- and microscopically (Gambell, 1968; Mitchell and Kozicki, 1974; Masaki, 1976; Lockyer, 1984). Pregnancy of females has been assessed by the presence of a foetus in the uterus, a large corpus luteum in the ovaries, the width of the uterine cornua and by histological study of the uterine mucosa (Lockyer and Smellie, 1985). The presence of a corpus luteum or corpus albicans in either ovary has been used as an indication of sexual maturity in females. Using these methods, an apparent pregnancy rate (lactating females with calves not included) of 0.40-0.44 and an ovulation rate of 0.59 has been calculated for Icelandic mature female sei whales (Lockyer and Martin, 1983).

The testicular weight, the diameter of the seminiferous tubules and the presence of spermatozoa in the tubules have been used to assess the sexual condition of males (Gambell, 1968; Mitchell and Kozicki, 1974; Masaki, 1976; Lockyer, 1984). While Gambell (1968) found no evidence of a sexual cycle for the Southern Hemisphere male sei whale, Mitchell and Kozicki (1974) reported an increase in testes weight from May/June until September/October for the North Atlantic male sei whale and increasing sperm counts during late summer.

Life span related cyclic reproductive events are programmed to begin after sexual maturity in most mammals. Considerable effort has, therefore, been put into age estimation of the whales. The age estimates, however, have been variably successful in different species as briefly reviewed by Lockyer (1984) and Horwood (1987). Sex hormones are obviously involved in the process of sexual maturation and their concentrations might relate more strongly to growth than age. Age versus length curves have been reported for the sei whale (Mitchell and Kozicki, 1974; Lockyer and Martin, 1983).

After the radioimmunoassay (RIA) revolution in the early 1970s, blood sex hormone measurements have been used in domestic animals to confirm pregnancy by monitoring interrupted progesterone cycling and male fertility by measuring serum Testosterone (T) values, replacing less sensitive urine measurements before that (Edquist and Stabenfeldt, 1989). Studies on progesterone (P)

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concentrations in blood from small toothed whales in captivity, where serial measurements can be made on the same animal, have shown that serum P is a good indicator of ovulation and pregnancy (Sawyer-Steffan *et al.*, 1983; Ozharovskaya, 1990). Serum P together with its metabolites and bioactive follicle-stimulating hormone in urine has been measured in captive killer whales (*Orcinus orca*) to study their ovarian cycles and gestation period, which is 17 months (Walker *et al.*, 1988). Serum T concentrations in the captive male bottlenose dolphin (*Tursiops truncatus)*, a seasonal breeder, were reported to reflect sexual maturation and sexual activity (Schroeder and Keller, 1989). There are few reports on serum sex hormone concentrations in both sexes of the large baleen whales and only on two species, i.e. the North Atlantic fin whale, *Balaenoptera physalus* (Kjeld and Árnason, 1990; Kjeld *et al.*, 1992) and the Antarctic minke whale, *Balaenoptera acutorostrata* (Yoshioka *et al.*, 1990; Yoshioka and Fujise, 1992; Iga *et al.*, 1996).

This paper presents the first data on reproductive hormone concentrations in sei whales. Both sexes of sei whale were classified by anatomical methods into reproductive groups, in which the hormonal levels were then studied and compared. The study also involved the distributional pattern of the hormones, their relationship to the body length of the whales and date of capture during the hunting season.

MATERIALS AND METHODS

Blood samples were obtained from a total of 195 sei whales (127 females and 68 males) which were caught southwest of Iceland from late June to late September-October during the summers of 1983-1988 (Fig. 1). The collection and use of postmortem blood samples collected in the same way and the excellent short-term stability of steroid hormones in serum has been described (Kjeld *et al.*, 1981; Kjeld, 2001). Briefly, within about 15 minutes following the death of the animal, the skin of the fluke was dried with a cloth followed by cutting its lateral third off and blood from the wound was collected into plastic test tubes. The samples were centrifuged at 2,000 rpm at sea and the supernatant serum kept frozen at −20°C; 3-6 months later, the samples were stored again at −80°C for half a year up to eight years until analysed. Serum concentrations of T and P measured in 18 serum samples after three months storage at −20°C and then again after about eight years storage at −80°C, showed no significant difference for either sex hormone. This stability of the steroids in frozen serum samples is borne out by their stability in the human serum controls used in the assays, where they showed no significant change in concentration over 18 months. Foetuses were collected from the uteri of the whales by biologists at the whaling station who recorded their sex and length. However, until 1986, a number of foetuses were lost at sea, when the belly of the adult was cut open to cool the meat. Sometimes one or both ovaries were lost as well, making the diagnosis of pregnancy by anatomical indicators uncertain.

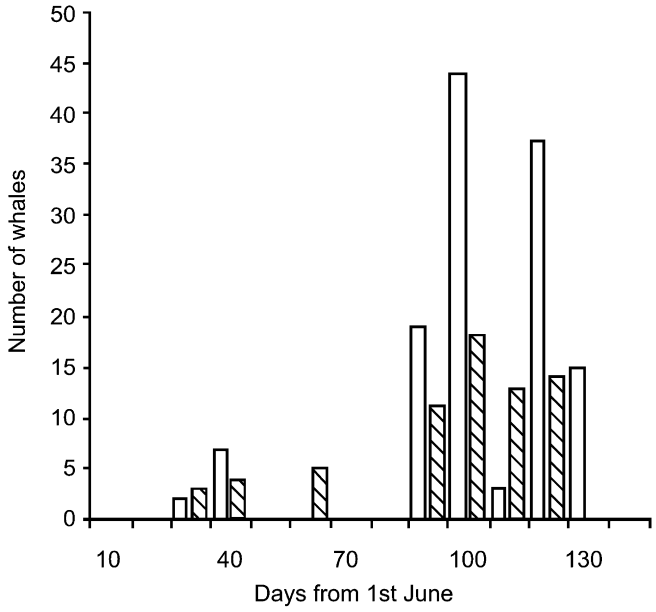

Fig. 1. Frequency distribution of sei whales caught every 10 days during the summer hunting season. Open columns: females; hatched columns: males.

Radioimmunoassays (RIAs)

RIAs with extraction and internal standards were chosen to measure the hormones. Precision of RIAs is generally less than that of enzyme immunoassays and Elisa assays, but RIAs are robust and the extraction step avoids possible matrix effects from the little known serum of whales. The assays for the total (protein bound and free) sex hormone concentrations in serum have been described in some detail by Kjeld *et al.* (1992) with regard to their sensitivity and specificity.

The testosterone antiserum was raised in rabbits against testosterone–3-carboxymethyl-oxime-bovine serum albumin. It had a 66% cross-reaction with 5a–dihydrotestosterone, but 3% and 2% with 5α-androstane–3β, 17β–diol and 5α-androstane–3α, 17β–diol, and less than 0.7% for a number of other structurally related steroids. The cross-reaction of 5α–dihydrotestosterone was not considered a problem as it is an androgen of which testosterone is the main precursor and, in human serum, known to be of ten times lower concentration. After addition of tritium labelled internal T standard, serum samples (0.5ml) were extracted with six volumes of diethyl ether, and then evaporation at 40°C under a gentle airstream followed by dissolution in 0.5ml of assay buffer. The assay had a mean inter-assay imprecision of 14% for a sample with T concentration of 3.6nmol/L and intra-assay imprecision of 8% for a sample with T concentration of 4.9nmol/L. The lower detection limit for this assay was 0.1nmol/L. Mean recovery of T from the internal standard was 82%.

Progesterone was measured by a modified radioimmunoassay method, using a more sensitive and specific antiserum. The antiserum was a rabbit anti-progesterone-11; (Fitzgerald, USA; cat no.: 20-PR20) with a crossreactivity of < 1% for 17-hydroxyprogesterone, pregnenolone, cortisol and 11-deoxycorticosterone and none for androstenedione. Serum samples (0.5ml), after addition of internal tritiated P standard, were extracted in eight volumes of petroleum ether (boiling range 40-80°C; Merck, Darmstadt, Germany). Mean inter- and intra-assay imprecision was 12% and 7% respectively for a serum sample with a P concentration of 4.8nmol/L. The detection limit was 0.1nmol/L. An internal tritiated standard used to assess procedural losses had a mean recovery of 76%.

A highly specific and sensitive antiserum raised against oestradiol-6-carboxymethyloxime-bovine serum albumin was used for the oestradiol assay. Structurally related steroids such as oestriol, oestrone and ethynyl-oestradiol had a cross-reaction of 0.4, 0.2 and 0.16%. Serum samples of 0.4ml were extracted in 4.0ml of diethyl ether, which was evaporated at 40°C under a gentle airstream. The assay had

a mean inter- and intra-assay imprecision of 16 and 9% respectively for a serum pool of 141pmol/L. The detection limit for this assay was 15pmol/L.

Conversion factors for the hormones are: testosterone, nmol/L × 0.288 = ng/ml; progesterone, nmol/L × 0.315 = ng/ml; estradiol, pmol/L × 0.272 = pg/ml.

Anatomical/histological measurements

The anatomical measurements used to decide the reproductive status of the female sei whales were conducted according to earlier reports on the species (Gambell, 1968; Mitchell and Kozicki, 1974; Masaki, 1976). All ovaries were inspected and the presence of corpora lutea (CL) and albicantia recorded. To confirm pregnancy in females with CL, but a slit uterus without a foetus, the width of the uterine cornua were measured in order to decide further if the whale had been pregnant (Lockyer and Smellie, 1985).

For males, the methods of the above cited references dealing with sei whales were also used. The testes were weighed and the width of the seminiferous tubules and their preponderance in the testes studied. Spermatogenesis and the presence of spermatozoa in the tubuli were recorded. From these studies the males were divided into three groups: immature, pubertal (intermediate) and mature (Masaki, 1976). These histological techniques are further described in more recent publications on studies of odontoceti (Collet and Saint Girons, 1984; Sørensen and Kinze, 1994).

Total body length was measured from snout tip to tail fluke notch in a straight line on the whaling platform. Measurements were originally made in feet (ft), but they have been converted into meters (m). The mean (SD, ranges) length (m) of females and males averaged 13.7m (0.98, 10.4-15.8) and 12.9m (0.7, 10.97-14.63), respectively. The mean lengths at sexual maturity for males and females have been reported to be 12.9m (42.5ft) and 13.3m (43.5ft), respectively (Mitchell and Kozicki, 1974; Lockyer and Martin, 1983; Boyd *et al.*, 1999).

Statistical analysis

The student's t-test was used to compare groups by their means. A linear regression model was adapted to the $\log_{10}$ of the T values related to the days (daycount) of the hunting season counted from the 1st of June. Association between variables was assessed by the Pearson correlation coefficient. The significance level was set at 0.05.

RESULTS

Females

The serum P values in female sei whales clustered mainly into two groups, one with values at or below the detection limit (0.1nmol/L) of the assay (Group I) and another one with values about two orders of magnitude higher (Group III). In between these two groups was a smaller number with intermediate values (Group II). There is no distribution for Group I but the frequency distribution of the serum P concentrations ($\log_{10}$) for Group II and Group III is presented in Fig. 2. Group III ($n = 39$) displays a distinct frequency distribution. The hatched upper parts of the columns show the females in which foetuses were present. The lower limit of the serum progesterone for Group III is apparently between $\log_{10}$P = 0.4-0.6 or 2.5-4.0nmol/L. The concentration of 3.5 nmol/L ($\log_{10}3.5 = 0.54$) was chosen as a working limit and using that limit the mean (SD) for Group III was 10.3 (4.1) nmol/L. Group II is more scattered but with values sufficiently above 0.1nmol/L ($\log_{10}0.1 = -1$) to make it a separate group. The classification by serum P values is further analysed in Table 1. The number of individuals, mean P concentration with range, mean length with range and the number of foetuses found are recorded for each group in Table 1. The mean body length of Group III (which has been divided into those with and without foetuses) was significantly longer than that of Group II ($p = 0.039$) and Group I ($p < 0.0001$), but there was no significant difference between the latter groups. No foetuses were found in females from Group I or Group II. By contrast, 13 foetuses were found in females of Group III. Table 1 also shows that of the 73 females in Group I, 33 were classified anatomically as immature and of the 39 in Group III, 27 were classified as pregnant.

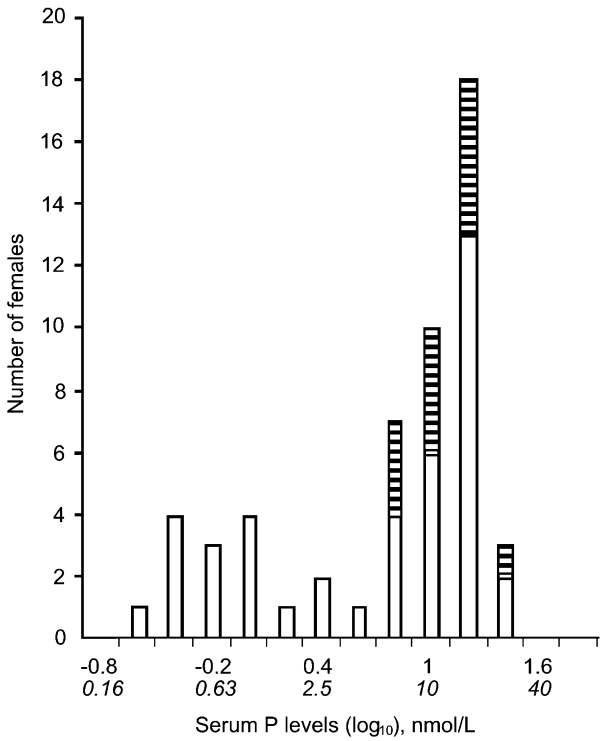

Fig. 2. Frequency distribution of serum $\log_{10}$P values above 0.1nmol/L (detection limit of assay). Striped parts of columns indicate females with foetus present. A near normal distribution curve for serum values above $\log_{10}$2.5 to 4.0nmol/L (0.4 to 0.6) is observed. On the x-axis, below the $\log_{10}$P values, the actual concentrations in nmol/L have been inserted.

In Table 2 the anatomical classification of reproductive status of the females is further compared with the serum progesterone levels. Besides the classes of immature, anoestrous, pregnant and lactating females, there are six females that could not be definitely classified and five that were not classified at all, probably because the ovaries were missing as well as the uterus. Table 2 shows that 95% of Group I was made up of immature and anoestrous females. Of the 39 females in Group III (P ≥ 3.5nmol/L), 27 were classified as pregnant and six and three were indecisive and not classified, respectively. The shortest whale of the anatomically defined pregnant group was 12.8m. The apparent pregnancy rate of that group (ratio of mature females pregnant, females with calves excluded) is 0.38 by the anatomical data, but 0.37 if pregnancy is judged by serum P values and sexual maturity, which P values do not indicate, by anatomical data. If, however, the six indecisive cases (Table 2), which all have serum P values above 3.5nmol/L, are added to both groups (mature and mature pregnant females) the rate becomes 0.41.

Table 1

Female sei whales classified by serum progesterone (P) values (nmol/L). Group III has been divided into those with and without foetuses detected in the uterus. Mean values of serum P (range) and length are given together with foetuses detected and the number of immature and pregnant whales, decided by anatomical methods, counted in each group.

Groups (G I, G II, G III) with P limits, nmol/L	*n*	Mean P values (range)	Mean body length (range), m	Foetuses detected	Anat. immat.	Anat. pregn.
G I, P ≤ 0.1	73	≤ 0.1	13.4 (10.4-15.9)	0	33	1
G II, 0.1 < P < 3.5	15	0.81 (0.2-2.5)	13.7 (11.6-15.2)	0	3	2
G III, P ≥ 3.5 (no foetus)	26	10.4 (3.9-19.5)	[a]14.2 (13.1-15.2)	0	1	14
G III, P ≥ 3.5 (foetus)	13	10.0 (4.9-19.7)	[a]14.2 (12.8-14.9)	13	0	13

[a]Significantly different (p<0.01) from Group I.

Table 2

Female sei whales classified into reproductive groups by anatomical findings. Mean values of serum P (range) and length are given together with foetuses detected and the number of low (≤ 0.1 nmol/L) and high (≥ 3.5 nmol/L) serum P values in each group.

Reproductive groups by anatomical methods	*n*	Mean P values (range), nmol/L	Mean body length (range), m	Foetuses detected	P ≤ 0.1, number in group	P ≥ 3.5, number in group
Immature	37	0.40 (0.1-10.2)	12.6 (10.4-14.3)	0	33	1
Anoestrous	45	0.49 (0.1-7.3)	*14.2 (12.8-15.9)	0	36	2
Pregnant	30	*9.43 (0.1-19.7)	14.0 (12.8-14.9)	13	1	27
Lactating	4	*0.40 (0.1-0.8)	13.9 (13.1-14.9)	0	2	0
Indecisive	6	*9.73 (5.9-12.1)	14.3 (13.7-14.6)	0	0	6
Not classified	5	8.08 (0.1-19.5)	14.5 (14.0-15.2)	0	1	3

*Significantly different (p < 0.01) from the value above.

Serum P concentrations did not change significantly with daycount in any of the reproductive groups during the summer. Serum P concentrations (10 ± 4.48, range 4.9–19.7nmol/L) of the 13 females with foetuses in their uteri were not significantly different from the pregnant ones without foetuses, and did not have any relationship with the size of the foetuses, the length of which however, increased significantly ($p < 0.01$) during the summer (Fig. 3). The equation for the regression line in Fig. 3 agrees well with the equation given by Lockyer and Martin (1983) for the sei whales off Iceland and their 'best fit' birth date of 29 November with a foetus length of 4.5m.

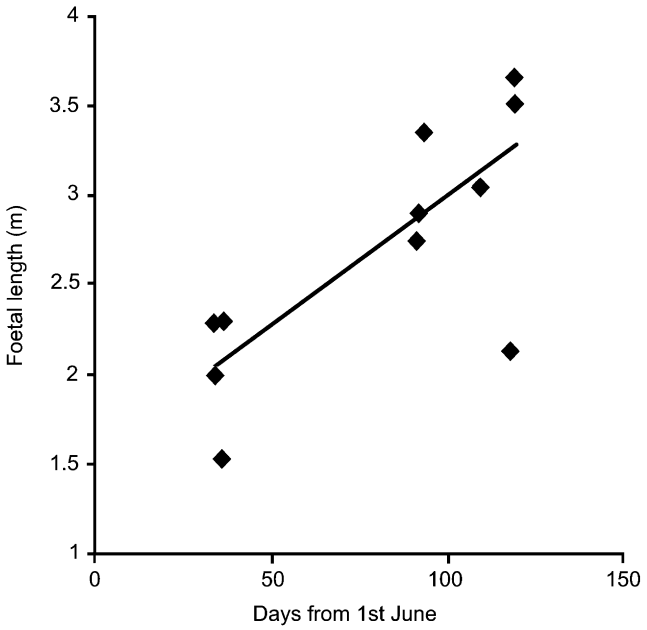

Fig. 3. The length of the 13 recorded foetuses plotted against day of catch. The equation for the regression line was $y = 0.0144x + 1.5544$ ($R^2 = 0.592$, $p = 0.002$).

Serum 17ß-oestradiol (E2), T and P concentrations were measured randomly in a different group of 26 females, 6 immature, 13 non-pregnant mature and 7 pregnant individuals. E2 concentrations varied widely and did not correlate with changes in serum concentrations of P or T. However, in this limited number of individuals the mean E2 concentrations were found to be significantly ($p < 0.01$) higher in pregnant females (171 ± 50pmol/L) than in the non-pregnant mature females (49 ±10pmol/L), which in turn were not significantly different from the immature females (41 ± 6pmol/L). Mean serum T concentrations in the above female groups, with an overall mean of 1.5 ±0.3nmol/L, did not differ significantly between each other.

Males

Using the anatomical/histological methods the 68 male sei whales were classified into three reproductive groups in Table 3: immature, pubertal and mature. Three individuals were unclassified. Mean values with ranges are given for serum T and body length for each class of whales. The number of whales with T values equal or below 0.1nmol/L and equal or above 1.0nmol/L for each group is also given. The number between the two limits is obtained by subtraction (14 immature whales), with 19 (86%) below 1.0nmol/L. In contrast, about 80% of the pubertal and mature males have serum values above 1.0nmol/L. Serum T values do therefore agree reasonably with the anatomical and histological classification of reproductive status in male sei whales.

While pubertal males had significantly higher mean serum T values than the immature males, there was no difference between the pubertal and mature whales (Table 3). The mean

Table 3

Male sei whales by anatomical classification in three reproductive classes: immature, pubertal and mature. Mean levels (SD) and ranges (nmol/L) for serum testosterone (T) and length are given as well as the number of T ≤ 0.1 and T ≥ 1.0 in each group.

Classification	*n*	T	Range	Length	Range	T ≤ 0.1	T ≥ 1.0
Immature	22	0.85 (1.32)	0.1-4.5	12.12 (0.50)	11.0-13.1	5	3
Pubertal	11	*3.27 (3.94)	0.1-14.7	*12.89 (0.36)	12.2-13.4	1	9
Mature	32	4.82 (4.12)	0.1-14.8	*13.43 (0.48)	12.8-14.6	2	25
Not classified	3	0.57	0.1-1.5	13.31	13.1-13.7	2	1

*Significantly different ($p<0.01$) from the value above.

length, on the other hand, increased significantly for each group with increased maturity.

Serum T concentrations in sexually mature male sei whales did not have a significant correlation with the body length of the whales. There was no significant change in the mean length of males caught during the summer hunting season. The mean (geometric) measurable serum T concentrations of the 30 sexually mature (anatomical/histological) males, on the other hand, increased significantly ($p < 0.001$) with daycount during the hunting season. This is shown in Fig. 4 where the $\log_{10}$ values of the T concentrations of the sexually mature males are plotted against the days (daycount) of the summer season. The equation for the regression line was: $\log_{10}T = -1.10 + 0.017$daycount; $R^2 = 40$, $n = 30$. This signifies an approximate 3.2-fold increase in the geometric mean of the T concentration for each interval of 30 days during the observation period. The two mature males with T values of 0.1nmol/L or less (detection limit of assay) are shown as open diamond marks in Fig. 4. The pubertal and immature males (Table 3) also showed a tendency of increase in serum T values with daycount, but not significantly so.

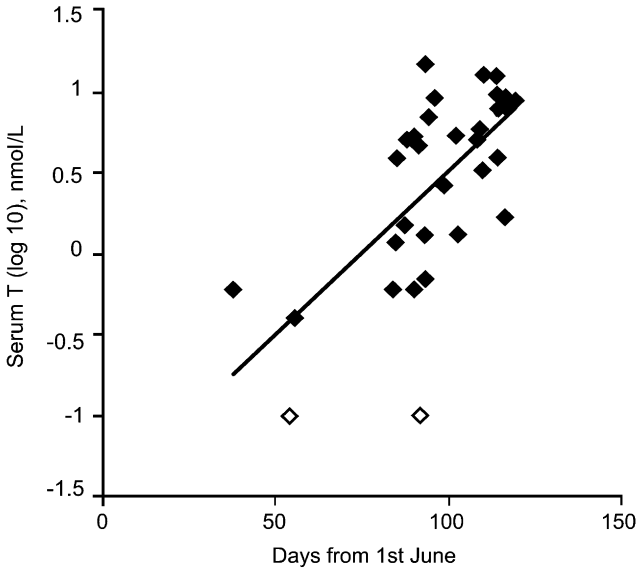

Fig. 4. Scatter plot of $\log_{10}$ serum T concentrations in mature male sei whales (decided by anatomical/histological methods) versus days (daycount) of the hunting season. A regression line has been adapted to the data, $\log_{10} T = -1.10 + 0.017$daycount ($R^2$, adjusted = 40%; $n = 30$; $p < 0.001$). Serum T values of 0.1nmol/L (assay detection limit) from two mature whales are shown by open diamonds but have not been included in the regression analysis.

Mean (SD) serum oestradiol of 39.9 (27.7) pmol/L and P of 2.3 (3.2) nmol/L concentrations in 11 randomly selected mature males were not significantly different from the respective mean concentrations in 13 non-pregnant mature females.

DISCUSSION

Sample limitations

The material used is a selected sample since the whalers work under strict regulations with regard to the smallest size and the maximum number they may catch. Moreover, a punishment is incurred if they accidentally catch a nursing cow with a calf. The sample material is therefore selected with regard to size and sexual status in general. Furthermore, the short hunting season from June to September and fluctuations in the abundance of sei whales off western Iceland (Martin, 1983) allow us to study only a limited part of the yearly cycle of hormonal changes in the whales. This paper represents the first data on sex hormones in the sei whale.

A number of mammalian hormones show diurnal variation in their serum concentrations. This applies to several pituitary hormones and hormones of their target organs such as ACTH and cortisol, which show one of the largest variations, besides responding to stress with elevation. Growth hormone and prolactin, both with diurnal variation, also respond to stress. Sex hormone levels with episodical changes have smaller diurnal variation, but are also known to be influenced by stress (Schroeder and Keller, 1989). In this study the sex hormone concentrations were compared with the time of day at which the whales were caught. No pattern was detected. In a recent study on fin whales, cortisol levels were studied in relation to time of day and the length of time they were chased by the whaling boats (mostly 0 to 90mins). Serum cortisol did not correlate with either time of day or chase time (Kjeld, 2001). Hence, sex hormone concentrations in this study are not likely to be influenced by either diurnal variation or stress due to the capture process.

Females

The females with the lowest P concentrations in Group I (Table 1) were the most numerous. Since these concentrations were at the detection limit of the assay, their distribution remains unknown but the values of the group were well separated from the rest. Table 2 shows that about one half of this group consisted of resting (non-oestrous, non-lactating) sexually mature females. A more sensitive assay might show whether these groups had a different distribution of serum P values. The number of resting females may have been relatively high during the observation period as the mating season was approaching. With a gestation period of about 11 months and a suckling period of 6-8 months there should be at least four to five months rest in the reproductive cycle for mature sei females.

The serum P concentrations of the 39 females in Group III clearly show a distinct group with respect to their distribution, and this is born out by data from the anatomical

studies in Table 2. Of the 30 females judged pregnant by the anatomical study, 27 belonged to Group III; nine other females from Group III could not be definitely judged by the anatomical method because of an abdominal slit at sea, injuring or removing the internal sex organs. Thirteen foetuses were, however, recovered from uteri, mainly after 1985 when the whalers dropped the practice of slitting the belly at sea for cooling purposes. The serum P concentrations of these mothers of the 13 foetuses recovered were not significantly different from the other females in Group III (Table 1). This is further illustrated in Fig. 2, where the values from these females have been hatched and added to the other Group III females.

The lowest P concentration in a female with a foetus present was 4.9nmol/L. However, the mean (SD) P concentration of Group III was 10.3 (4.1) nmol/L, and 2.1nmol/L would therefore be the cut off point for the 2.5% fractile of pregnancy or the lower tail of the 95% confidence intervals. If so, two additional females with serum P values of 2.5 (judged pregnant by anatomical methods) and 2.1nmol/L (mature anoestrous) would have been added to Group III.

Using anatomical methods, three females of Group III were judged not to be pregnant, one immature and two anoestrous (Table 2). These (13.4m or longer) had serum P values of 10.2, 5.1 and 7.3nmol/L, respectively, all well above the lower limits of Group III discussed above. While the immature case might best be explained by some mishandling of a specimen, the other two raise the question of non-pregnant ovulators in Group III. Serum P elevations in these ovulations last for about 12 to 28 days in odontocetes (Schroeder, 1990; Robeck *et al.*, 1993). In man and some other mammal species (Niswender and Nett, 1994), including captive killer whales (Robeck *et al.*, 1993) and bottlenose dolphins (Kirby, 1990), P concentrations are considerably higher in pregnancy than during ovulation. Lockyer and Martin (1983) stated that summer ovulations in sei whales were undoubtedly rare and, for the closely related fin whale, Lockyer and Sigurjonsson (1991) reported that for the months of June and July almost all the corpora lutea present indicated pregnancy. However, further studies are needed to see if some of the mature non-pregnant sei females might be ovulating in July-September.

Individuals from Group II consisted mainly of anoestrous mature females as shown in Table 1. Of 15 in the group, 3 were considered immature (serum P values, 0.3, 0.4, 0.7; length 12.5m or less) and 2 pregnant (serum P values, 0.3, 2.5; length 13.7m, 12.8m) but the 10 remaining were judged to be anoestrous, mature females. One can only speculate as to why these animals have higher levels than the majority (Table 2), which has values of 0.1 or less. Some might have aborted recently with decreasing P concentrations, although most abortions happen in the very early months of pregnancy (Niswender and Nett, 1994). Little is known about abortion rates in baleen whales, particularly in early pregnancy (Lockyer, 1984). Foetus death *in utero* in Antarctic fin whales was estimated to be a minimum of 0.14% (Ichihara, 1962). The serum P values might also have been rising slightly in preparation for the upcoming mating period. Such preliminary ovarian follicular development in ruminants occurs in wave-like patterns where P levels have a definite modulating role (Adams, 1999). Increasing serum P values have been observed in minke whale females during a similar period of the year (Kjeld *et al.* 2004).

Serum P concentrations after ovulation without pregnancy are not known in rorquals, nor is the relative role of the placenta in P production. Since the corpus luteum remains functional until the end of pregnancy although not increasing in size during its last seven months (Gambell, 1968), it seems likely that the placenta produces a cetacean chorionic gonadotropin, the measurement of which in blood or urine, like in other mammals, should be the most reliable pregnancy index.

The apparent pregnancy rate of mature females was 0.37 to 0.38, but 0.41 if indecisive results from the anatomical assessments are included. The shortest female decisively pregnant by both anatomical and hormonal methods was 12.8m (42ft). The pregnancy rate and the length at sexual maturity estimated by the P concentrations agreed closely with earlier reports based on biological and anatomical data for the Icelandic sei whale mentioned in Materials and Methods (Lockyer and Martin, 1983).

In a small number of sei whales from a separate group, serum oestradiol values did not change with length, daycount, sex or serum P or T values. Oestradiol values were, however, significantly higher in pregnant than in non-pregnant females, which does not agree with fin whale results (Kjeld *et al.*, 1992). Serum oestradiol values in sei whales need further study.

Males

Mean serum T concentrations in all male sei whales (3.10nmol/L) were a little higher than in male fin whales (2.0nmol/L; Kjeld *et al.*, 1992) but five times higher than in male minke whales (0.6nmol/l) off Norway during a similar time of year (Kjeld *et al.*, 2004).

When the serum T levels within reproductive groups classified by anatomical/histological methods were compared (Table 3), reasonable agreement was evident. Most of the immature males shorter than 12.9m had serum values below 1.0nmol/L and the pubertal and mature males had values generally above 1.0nmol/L.

Serum T concentrations in the mature males increased exponentially and significantly with daycount during the summer, with a 3.2-fold rise for every 30 days (Fig. 4). Since the mean length of caught whales remained more or less unchanged, the T increase with daycount must have been caused by other factors. The rise of serum luteinizing hormone (LH) and follicular stimulating hormone (FSH), with increased mass of the testicular parenchyma, could bring about such an effect. Mitchell and Kozicki (1974) found only a modest monthly increase in testicular weight during four months (see below), so the serum T value is obviously a more sensitive index of the male reproductive cycle than testis weight. The additional effect of the increasing number of grown bulls reacting to reproductive cues may probably also influence this exponential serum T elevation. As can be seen in Fig. 4, two of the mature males (length 12.8m and 13.1m) had serum T values of 0.1nmol/L or less. If these two were included in the regression calculation the increase would be faster still, or fourfold every 30 days.

It is not known when this rise in serum T concentrations stops. At the finish of the hunting season towards the end of September, serum T values are about 8.0nmol/L and at the end of October they will be 25nmol/L according to the equation, if they do not level off before that. At the end of September when the catch stops, the results did not indicate a halt in the serum T increase. Shortly after the serum T levels stop increasing, the mating season usually begins (Bronson and Heideman, 1994). For the captive bottlenose dolphin (Schroeder and Keller, 1989) and terrestrial herbivores with seasonal breeding (Lincoln and Short, 1980;

Bronson and Heideman, 1994), the serum T concentrations generally reach a peak or an elevated plateau before the rutting period starts and then begin to fall during the rut.

While Gambell (1968) did not find any evidence of a male sexual cycle in Southern Hemisphere sei whales based on testis examination, Mitchell and Kozicki (1974) reported strong evidence for such a cycle in sei whales of the northwest Atlantic Ocean. The main evidence was supported by two observations: (1) a 60% increase in the weight of the testes of mature males from June and July until September and October; and (2) the increasing presence of sperm in the seminiferous tubuli from June to October. In this study, the serum T rise with daycount during the summer supports the results of Mitchell and Kozicki and indicates a yearly seasonal breeding cycle in the North Atlantic male sei whale as reported for the North Atlantic fin whale (Kjeld *et al.*, 1992). Recent articles (Yoshioka and Fujise, 1992; Mogoe *et al.*, 2000) on Antarctic male minke whales report no increase in serum T concentrations or testicular weight from December to March. This does not agree with results of studies on the Northern Hemisphere minke whale (Kjeld *et al.*, 2004). Gambell's (1968) data on southern sei whales together with the above reports on minke whales in the Southern Hemisphere suggest lower serum T levels or some physiological differences between the whale counterparts of the Southern and Northern Hemispheres, or that they might somehow be out of phase.

The serum T rise with daycount observed in this study in the mature male sei bulls is estimated to be faster than that for fin and minke whales during the same period of the year (Kjeld *et al.*, 1992; Kjeld *et al.*, 2004). The most likely explanation is an earlier mating period for sei whales, but could also indicate a shorter endocrine preparation time corresponding to a shorter breeding period for sei whales than for the other two species. Lockyer (1999) reviewed the peak time of conception for baleen whales, as has Horwood (1987) for the sei whale. The North Atlantic sei whale was estimated to have a peak conception rate from November to February, whereas fin and minke whales had their peaks in December and February, respectively. The results from this study would seem to support the months of November/December as the peak conception months, or even December/January as suggested by Lockyer and Martin (1983) for sei whales caught off Iceland.

It is concluded that measurement of serum concentrations of progesterone and testosterone are a potent addition to biological anatomical methods for investigating reproduction in sei whales, apparently surpassing them in sensitivity of detecting pregnancy by serum P values and cyclical changes by serum testosterone values during the male seasonal reproductive cycle. The present results do corroborate earlier results by the anatomical/histological methods and suggest new possibilities in the study of baleen whale reproduction, besides constituting a reference for much needed hormonal measurements on the species in different locations at different times of the year.

ACKNOWLEDGEMENTS

We wish to thank K. Loftsson for his constant encouragement and assistance. The help of the staff of the Endocrine Unit Laboratory, University Hospital and the Marine Research Institute, especially that of Arndís Theódórs, is much appreciated. A grant from The Icelandic Research Council is acknowledged.

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Date received: 28 May 2002.
Date accepted: 1 April 2003.