Plasma concentrations of oestrone, progesterone and corticosteroids during late pregnancy and after parturition in the harbour seal, *Phoca vitulina*

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Summary. Blood samples were taken at weekly intervals during the last 2 months of pregnancy from a mature (7-year-old) harbour seal which had conceived and was kept in captivity. The concentrations of oestrone, oestrone sulphate, progesterone and glucocorticoids were determined by radioassays. The plasma levels of unconjugated oestrone were slightly greater than those of oestrone sulphate. Total oestrone declined steadily over the last month from a peak of 2.3 ng/ml at 30 days before parturition. Plasma progesterone concentrations rose to 61 ng/ml at 2 weeks before parturition and fell to about half that value by 2 days before birth of a normal male pup. Plasma glucocorticosteroids reached a peak of 164 ng/ml, also at 2 weeks, but showed only a slight decline thereafter. A higher value (392 ng/ml) was recorded 5 days after parturition.

Introduction

Although some specific information exists on reproductive events in aquatic mammals, there is very little on the underlying hormonal controls (Ronald, Hanly, Healey & Selley, 1976). Such knowledge is needed both as basic information on comparative reproduction and as a requirement for studies on the breeding of endangered species, such as the Mediterranean monk seal, *Monachus monachus*. The present report arises from the opportunity to obtain samples from a pregnant harbour seal, *Phoca vitulina*, during late pregnancy and near the time of parturition. The harbour seal is known to exhibit delayed implantation (Fisher, 1954).

Materials and Methods

Animal

The harbour seal was born at Boothbay Harbor, Maine, at the beginning of June 1972, and brought into captivity at Guelph, Ontario, on 23 June 1972. It was held in confinement with a male captured at the same time and place. The two seals were moved from Guelph to outdoor holding tanks at St Andrews, New Brunswick, during the periods of June to August in 1974 and 1978. The female was retained at Guelph in 1979.

The seal's diet consisted of *Clupea harengus* (normally 2 kg/day, but during pregnancy 3 kg/day), supplemented with vitamins A, B-complex, C, D, E, extra thiamine hydrochloride, and sodium chloride. Although treated for an infected lower jaw in 1977, the female exhibited no other health problems.

By February the seal was evidently pregnant and was observed by biweekly weighings to have gained 25 kg over its normal weight in November. X-rays confirmed the presence of a developing fetus. Just before the birth the seal weighed 94 kg, a marked increase from its normal weight of 60 kg (± 1 kg). The parturition occurred on 7 June 1979 on a dry ledge and the 9 kg pup was delivered immediately and deliberately into water. The pup was subsequently reared normally by the mother.

Blood samples

Quarterly blood samples were taken from the mother for analysis. The samples were taken from veins in the left or right hind flippers into vacutainers, with the animal restrained on a V-board as described previously (Ronald, Foster & Johnson, 1969).

Hormone estimations

Oestrone and oestrone sulphate determinations were performed in duplicate on 0.5 ml plasma diluted to 1.0 ml with normal saline (9 g NaCl/l), and extracted twice with 5 ml methylene chloride. Measurements were made by radioimmunoassay (RIA) on aliquots of the aqueous phase for oestrone sulphate, without hydrolysis, and of the extracted steroids for oestrone, using a specific antiserum for oestrone purchased from Dr P. N. Rao (Southwest Foundation for Research and Education, San Antonio, Texas). This antiserum was raised in rabbits to the 3-O-carboxymethyl ether of oestrone conjugated with bovine serum albumin (BSA). It had little or no cross-reaction with other steroids (Rao & Moore, 1977), but showed a cross-reaction of 65% with oestrone sulphate (P. N. Rao, personal communication, and confirmed in our laboratory). This represented almost 100% cross-reaction in terms of the steroid moiety. Assays were carried out as previously described (Raeside & Rosskopf, 1980). Recoveries were about 90% for both oestrone and oestrone sulphate, using ^3H -labelled standards. The lowest detectable amount of oestrone was 20 pg/ml plasma (charcoal-treated plasma from an ovariectomized sow) and blank values for the assay were less than the lowest point on the standard curve (10 pg). The coefficients of variation between and within assays were, respectively, 6.3 and 5.5% for oestrone, and 11.1 and 7.8% for oestrone sulphate ($n = 12$). No corrections were made for losses and the results were expressed as oestrone equivalents for the combined estimations of oestrone and oestrone sulphate.

Progesterone concentrations were determined by RIA (Lindner, Perel, Friedlander & Zeitlin, 1972) using anti-progesterone-11 α -hemi-succinyl-BSA serum (Miles-Yeda Ltd, Miles Research Division, Elkhart, Indiana, U.S.A.). Plasma samples (0.25–0.5 ml) were extracted twice with 10–20 volumes of petroleum ether (b.p. 40–60°C, analytical grade, Fisher Sci. Co., Toronto, Ontario) and aliquots of the dried extracts were taken for assay. Recovery of an internal standard of [^3H]progesterone was >90%. All samples were assayed in a single run. The apparent sensitivity of the method was 0.25 ng/ml. Specificity was examined by partition chromatography of [^3H]progesterone and steroids extracted from two plasma samples, using small Celite columns (0.9 $\times$ 8.0 cm) and a solvent system of *n*-hexane/80% aqueous methanol.

Cortisol is the major glucocorticoid in the plasma of the seal (Liggins, France, Knox & Zapol, 1979). Total corticosteroids were determined by a competitive protein-binding radioassay using [1,2- ^3H]corticosterone (Radiochemical Centre, Amersham) and diluted plasma (2% in distilled water) from a male dog as described previously (Liptrap & Raeside, 1975). Separation of free and bound steroids was made with Florisil (Fisher Sci. Co., Toronto). Samples were included in a single run of the assay which had a coefficient of variation of 5.2%. Recovery for the method was >90% and the limit of detection was 0.2 ng/ml plasma.

Results

The only significant decreases seen among the biochemical tests during pregnancy were in blood urea nitrogen (45.3–16.0 mg/100 ml) and glucose (144.6–78.0 mg/100 ml), which occurred before the birth. Total bilirubin increased 3 weeks before the birth and remained high for 1 week *post partum* (1.5 against a normal 0.7 mg/100 ml). Haematological changes were limited to decreased red blood cell count (5.61 to $4.75 \times 10^6/\text{mm}^3$), haemoglobin (21.3 to 19.1 g/100 ml), and haematocrit (57.4 to 48%) which became significant 3 weeks before the birth, but regained normal levels 5 days *post partum*.

Concentrations of total oestrone, progesterone and corticosteroids in the plasma of a pregnant seal are shown in Text-fig. 1. the day of parturition was designated as Day 0, with Day 1 commencing 24 h after delivery. In all but one sample (Day -44), there were slightly greater amounts of unconjugated oestrone than oestrone sulphate with a ratio of 1.5 ± 0.4 (mean $\pm$ s.d.) as oestrone equivalents. Total oestrone reached a peak of 2.3 ng oestrone equivalent/ml plasma at Day -30, and declined steadily during the last month of the pregnancy. A further decrease in concentration was noted in the sample obtained 5 days after parturition.

Text-fig. 1. Plasma concentrations of total oestrone (○, oestrone + oestrone sulphate); progesterone (●) and corticosteroids (▲) in late pregnancy and after parturition (Day 0).

Progesterone values varied between 40 and 60 ng/ml over the last 6–7 weeks of pregnancy, but <30 ng/ml was found at 48 h before parturition. At 5 days after the birth the level had fallen to 2.5 ng progesterone/ml. With two plasma samples (Days –51 and –9) partition chromatography on small Celite columns revealed only one peak, which was coincident with that of an internal standard of [³H]progesterone.

High levels of corticosteroids with a peak of >160 ng/ml plasma were found at 1–2 weeks before term. A slightly lower value was seen at 2 days before parturition and a very high concentration (392 ng/ml) at 5 days after the birth.

Discussion

Endocrine patterns of the seal at term have not been described previously, so that a comparison of the present findings can be made only with distantly related species. The gradual decrease in the plasma concentrations of oestrogens over the last month of pregnancy differs from the pattern shown by most species, but is similar to the protracted decline in the pregnant mare (Lovell, Stabenfeldt, Hughes & Evans, 1975; Nett, Holtan & Estergreen, 1975). A relationship between the fetal gonads and the placental formation of oestrogens has been demonstrated in the mare (Raeside, Liptrap, McDonell & Milne, 1979). The remarkable enlargement of the fetal gonads of the grey seal, which was noted at term by Amoroso, Harrison, Matthews & Rowlands (1951), suggests a similar involvement of the fetus in the hormonal changes in late pregnancy.

The plasma concentrations of progesterone were notably higher than found in the domestic animals, including the bitch (Concannon, Powers, Holder & Hansel, 1977), but only slightly above that reported for the 9th month of gestation in a blue-nosed dolphin (Richkind & Ridgway, 1975). More frequent sampling is needed to decide whether an apparent decline in plasma levels of progesterone over the last 2 weeks of gestation is typical for the seal, as it is for the cow, ewe and sow (see Thorburn, Challis & Currie, 1977). Early microscopic signs of regression of the corpus luteum in late pregnancy have been noted in the harbour seal (Fisher, 1954). The possible contribution of placental production of progesterone remains unknown.

Liggins *et al.* (1979) have reported high corticosteroid levels in plasma of adult and fetal Weddell seals with no difference between pregnant and non-pregnant or stressed and unstressed animals. Our findings in a single harbour seal maintained in captivity showed lower concentrations than in Weddell seals in their natural habitat, but suggest an increase in plasma corticosteroids in late pregnancy. The highest value (392 ng/ml) was seen, however, in the sample taken from the lactating female 5 days after parturition. For comparison, the corticosteroid levels in an immature female (2-year-old) and in a mature male (7-year-old) harbour seal were 144 ng and 544 ng/ml plasma, respectively. These estimations were confirmed later by radioimmunoassay. While the data are mainly from a limited number of observations from a single animal they add support to the suggestion of Liggins *et al.* (1979) that high levels of corticosteroids may be a characteristic of diving mammals. This preliminary study raises the possibility of a relationship between glucocorticoid concentrations and the reproductive status in the female seal.

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