Plasma progesterone concentrations in pregnant and non-pregnant black bears (*Ursus americanus*)

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Summary. Plasma levels of progesterone were determined from serial samples taken from 2 black bears over 3 consecutive fall periods. Each animal was pregnant during the 1st and 3rd years. Variations in progesterone levels were seen between animals and within each animal between pregnancies. Average baseline levels during the mid-to-late preimplantation period were 5.0–12.5 ng/ml and increased 2–3-fold at the approximate time of implantation. Values during non-pregnancy were detectable but much lower (0.6–2.5 ng/ml). The observed pattern of progesterone secretion in this species appears consistent with that reported for other species exhibiting obligate delayed implantation.

Introduction

The American black bear (*Ursus americanus*) is one of several mammalian species which exhibits the reproductive phenomenon termed obligate delay of implantation. After conception in mid-June and embryonic development to the blastocyst stage shortly thereafter, a period of embryonic quiescence occurs (Wimsatt, 1963). The unattached blastocyst lies free in the uterine lumen; the dormant condition is characterized by a very low mitotic index and reduced metabolic activity (Gulyas & Daniel, 1967; Daniel, 1974) although some growth is apparent (Wimsatt, 1963). Upon reactivation and implantation in late November, development proceeds through to parturition in late January. The gestation period is approximately 225 days in length of which about 145–165 days are spent in arrested development. Commonly the young remain with the female through the first summer. The prolonged lactational period during this time is thought to prevent the next oestrous cycle and so breeding only occurs every other year (Rauch, 1961; Erickson, Nellor & Petrides, 1964).

Numerous investigations have focussed on the hormonal changes associated with the delay state in such species as the mink (Cochrane & Shackelford, 1962; Møller, 1973), western spotted skunk (Mead & Eik-Nes, 1969a, b; Foresman & Mead, 1974; Foresman, Reeves & Mead, 1974), European badger (Canivenc, Bonnin-Laffargue & Lajus, 1967; Bonnin, Canivenc & Ribes, 1978), northern fur seal (Daniel, 1975), and roe deer (Short & Hay, 1966; Aitken, 1974). Similar studies in the black bear have not, for obvious reasons, been so readily performed. The availability of several bears maintained in a zoological park in Knoxville, Tennessee and the interest in breeding programmes at this facility made the present investigation possible.

Materials and Methods

Two female bears were anaesthetized (2 mg ketamine hydrochloride/2 mg xylazine, i.m.) twice weekly during 2 consecutive fall periods and monthly during the third fall season. Both females were pregnant during the first and last periods (late September–early December 1977 and 1979).

Cubs from the first pregnancies were removed during the fall of 1978 (13 November—Bear 1; 14 September—Bear 2) so both females were in anoestrus (or lactational anoestrus, Bear 1) during the middle year (early October—early December 1978). Because both animals were involved in an ongoing breeding programme and successful pregnancies were desired, sampling was discontinued in December when the females withdrew into denning facilities and became lethargic.

Blood samples withdrawn from the jugular vein into 20 ml syringes and transferred into heparinized vials were centrifuged at 600 *g* and 4°C for 10 min. The plasma was stored at -20°C until assay. Plasma progesterone concentrations were determined by radioimmunoassay using progesterone antisera and [³H]progesterone obtained from New England Nuclear (Boston, Massachusetts, U.S.A.). The antiserum had been prepared in rabbits against progesterone-11 β -succinyl-bovine serum albumin. Antiserum and labelled progesterone were used according to the supplier's specifications. All other procedures followed those previously published (Foresman & Mead, 1978) with one exception. The initial extraction steps were performed using 2 ml petroleum ether rather than hexane.

The inter-assay coefficient of variation based on two serum samples (containing 6.6 and 12.4 ng/ml) run in each assay was 9.0% (*n* = 4) and 10.8% (*n* = 3) respectively. The intra-assay coefficient of variation based upon 8 replicates of a single sample was 6.1%. The sensitivity of the

Text-fig. 1. Plasma progesterone levels during pregnancy (1977 & 1979) and non-pregnancy (1978) in (a) Bear 1 and (b) Bear 2. Arrows depict the approximate date of implantation.

assay, defined as the mass equivalent at twice the standard deviation of zero binding, varied between 6 and 28 pg. Performance characteristics provided by the supplier of the antibody and labelled antigen gave a value of 15 pg. The average recovery after extraction was $86 \pm 2.7\%$. The assay blank consisted of 200 μ l assay buffer to which 100 μ l [3 H]progesterone had been added. After charcoal precipitation, values for the blank tubes were always well below the sensitivity of the assay. Rabbit plasma collected on Day 7 of pregnancy (Day 0 = day of copulation) was used to verify the assay's quantitation of progesterone; values obtained (6.8 ± 0.3 ng/ml (s.e.m.), $n = 4$) were comparable to those previously reported in the literature (Thau & Lanman, 1975).

Results

During both lactational anoestrus (Bear 1) and non-lactational anoestrus (Bear 2) low but detectable levels of progesterone were measured (range = 0.6–2.5 ng/ml; Text-figs 1a & b). With pregnancy, baseline levels of plasma progesterone were elevated and greater variation was observed between animals and between pregnancies for each animal. A sharp rise in progesterone began in both animals during mid-to-late November (Text-figs 1a & b). From the observed copulation dates and known dates of parturition an average gestation period of approximately 225–229 days was determined (Table 1).

Table 1. Estimation of gestation length in the black bear

	Date of observed copulation	Parturition date	Gestation period (days)	Litter size
Bear 1	4 June 1977	25 January 1978	235	3
	22 May & 4 June 1979	30 January 1980	240–253	2
Bear 2	15, 16 & 18 June 1977	20 January 1978	216–219	3
	20 June 1979	16 January 1980	210	2

Discussion

The pattern of progesterone secretion in the black bear is similar to that reported for several other species which exhibit obligate delay of implantation. Although the number of animals and pregnancies in this study were small, two general observations may be drawn. Firstly, corpora lutea are active during the second half of the delay period as indicated by the elevation of plasma progesterone levels over those found during non-pregnancy, as reported for the spotted skunk (Mead & Eik-Nes, 1969a), mink (Møller, 1973), short-tailed weasel (Gulamhusein & Thawley, 1974) and European pine marten (Canivenc & Bonnin, 1975). Information on ovarian histology during pregnancy in the black bear is not as complete as that available for the above-mentioned species, but the evidence does suggest that luteal activity changes between the early and late delay periods. Comparison of luteal tissue of 29 'summer-killed' animals (June–September) with that of 2 post-implantation specimens (December) clearly indicates a marked alteration in luteal morphology, with a 2- to 4½-fold increase in luteal volume by December (Wimsatt, 1963). Blood samples were not collected during the summer months in the present study but, from this earlier histological information, progesterone levels during this portion of the delay period would probably be much lower.

Secondly, there is a marked elevation in plasma progesterone levels in late November/early December coincident with implantation. Many carnivores with and without delay exhibit a similar rise in plasma progesterone in association with implantation (spotted skunk: Mead & Eik-Nes, 1969a; mink: Møller, 1973; Murphy & Moger, 1977; ferret: Heap & Hammond, 1974; Foresman &

Mead, 1978; cat: Verhage, Beamer & Brenner, 1976). Although there appears to be a particularly large rise for Bear 1 in 1977 (Text-fig. 1a), the magnitude of change from baseline is consistent with that shown in the other pregnancies (2–3-fold increase). The reason for the higher overall progesterone values during this particular pregnancy is assumed to be simply individual variation as the other 1977 pregnancy samples were run in the same assay and consistent values were obtained for the same samples in a second assay. Though slightly higher progesterone levels were also observed in lactational anoestrus (Bear 1, range = 1.7–2.5 ng/ml) as compared to non-lactational anoestrus (Bear 2, range = 0.6–1.4 ng/ml), the difference is not felt to be significant.

The calculated gestation periods in the present study (210–240/253 days, mean = 225 days) are similar to those reported by Ammons (1974) (182–236 days, mean = 206 days, $n = 10$). The variation in calculated gestation periods which does exist in both studies may in part be due to the difficulty in identifying successful copulations. In the present study mating behaviour was observed on more than one occasion for some females.

Studies of this nature are limited in scope due to the species involved and the necessary restrictions placed upon such research by the zoological parks but a start on the acquisition of valuable information on the reproductive physiology of this species has now been made.

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