CHANGES IN SERUM PROGESTERONE LEVELS AND GROWTH OF FETUSES IN HOKKAIDO BROWN BEARS

TOSHIO TSUBOTA, Department of Theriogenology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan
YOSHIYUKI TAKAHASHI, Department of Theriogenology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan
HIROSHI KANAGAWA, Department of Theriogenology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan

Abstract: Serum progesterone levels in 5 female Hokkaido brown bears (*Ursus arctos yesoensis*) were determined from April 1983 to April 1985. Four females were mated with males, while the 5th female was segregated to serve as a control. The growth of fetuses in 2 females was monitored by ultrasonography at 10-day intervals during December and January. Serum progesterone levels increased from basal levels of 0.4–1.1 ng/ml on day −300 (300 days before parturition) to higher levels at approximately day −210. The levels, which ranged from 1.6 to 5.4 ng/ml, were maintained until approximately day −60, when they sharply increased to 8.3–9.7 ng/ml. The levels then dropped on day 0 and remained at 0.4–0.5 ng/ml between day 30 and day 90. Change in serum progesterone level in the unmated female was similar to that of the mated animals. The images of fetuses in 2 bears were first observed on day −37 and day −33 when their crown-rump length was 1.5–2.0 cm. Thereafter fetus growth was sigmoidal. The females carried 2 and 3 fetuses and had 1 and 3 cubs 1 month postpartum, respectively.

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Delayed implantation occurs in several mammalian species. It is known among most genera in the Macropodidae, Tarsipedidae, Mustelidae, Ursidae, Otariidae, and Phocidae; several genera in the Burramyidae, Cricetidae, Muridae, and Chinchillidae; and a few genera in the Soricidae, Talpidae, Pteropodidae, Vespertilionidae, Dasypodidae, and Cervidae (Renfree and Calaby 1981). Delayed implantation in the Ursidae has been suggested by Hamlett (1935), Wimsatt (1963), Kordek and Lindzey (1980), and Foresman and Daniel (1983). We use the term "delayed implantation," although this phenomenon has also been called embryo diapause because embryo development is arrested (Renfree and Calaby 1981).

Plasma progesterone levels in captive black bears (*U. americanus*) were different during pregnancy and nonpregnancy (Foresman and Daniel 1983), and the free blastocysts in the uteri of black bears were observed June–August (Wimsatt 1963). We expected the Hokkaido brown bears would also exhibit delayed implantation.

Currently, ultrasonography is 1 of the reliable methods for pregnancy diagnosis. Using ultrasonography, the embryo was first observed at gestational day 10 in dogs (*Canis domesticus,* Robert and Terri 1984) and the embryonic vesicle was first visible at gestational day 12–14 in the heifer (*Bos* spp., Pierson and Ginther 1984). We used ultrasonography to determine fetal growth during the postimplantation period.

The purpose of this study was to observe the characteristic reproductive physiological changes accompanying delayed implantation in Ursidae.

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METHODS

Five captive Hokkaido brown bears housed at the Noboribetsu Bear Park, Hokkaido, Japan, were used in this study. Bears 1–4 were allowed to mate; bear 5 was segregated from the males and was not allowed to mate. The sexual behavior of bears 1 and 2 was observed from 30 April to 3 July 1983.

Serum Progesterone

We measured the serum progesterone levels of 5 adult female brown bears monthly from April 1983 to April 1985. The bears were anesthetized with 4 mg/kg ketamine hydrochloride and 0.9 mg/kg xylazine or 0.5 mg/kg suxamethonium and 5 mg/kg ketamine hydrochloride. Blood samples were drawn from the jugular vein and were centrifuged at 3,000 rpm for 15 min. The serum was stored at −20 C until assay. Serum progesterone concentrations were determined by radioimmunoassay.

Ultrasonography Examination

We used ultrasonography on 2 females 7 times between September 1984 and January 1985 to monitor fetal growth (Table 1). It was used monthly from September to December and every 10 days from 16 December to 15 January. We used an electronic linear

Table 1. Period of serum progresterone measurement and ultrasonography (USG) examination of 5 Hokkaido brown bears.

Bear	Age (years)	Period of serum progesterone measurement	Period of USG examination
1	13	Apr 1983–Mar 1984	
2	11	Apr 1983–Feb 1984	
3	14	May 1984–Apr 1985	Sep 1984–Jan 1985
4	11	May 1984–Apr 1985	Sep 1984–Jan 1985
5[a]	11	May 1984–Mar 1985	

[a] Segregated from male bears from 21 Dec 1983 to 30 Sep 1984.

scanner with a 3.5 MHz transducer (Tomosonic EUB-200, Hitachi Medical Corp., Tokyo). The technique was based on a positive identification of fetal echoes on a standard B-mode scan. When fetal heart movements were located, a record was made using a M-mode scan, in which moving objects were recorded as regular wave forms and stationary objects were represented as straight lines.

The anesthetized bears were examined in the supine position. The skin surface of the ventral area to be examined was clipped and shaved. The area of the body surface in which the uterus was located was explored with a transducer. The ultrasonogram was viewed on the scanner monitor with the real-time function. After an image was frozen on the screen, photographs were taken. The crown-rump length (CRL) of the fetuses was measured from the photographs.

RESULTS

Litter sizes and copulation and parturition dates for the 4 bears are presented in Table 2.

Serum Progesterone

Figure 1 shows the changes in the serum progesterone levels of 4 pregnant bears. Serum progesterone was at basal levels of 0.4–1.1 ng/ml between day −300 and day −210. The levels gradually increased from day −210 to day −60 (range 1.6–5.4 ng/ml) and were elevated to 8.4–9.7 ng/ml on approximately day −60. The levels began to fall on day −30 and decreased to 1.8–2.5 ng/ml shortly before parturition. The levels after parturition were 0.3–0.9 ng/ml.

The change in serum progesterone level of the segregated bear was similar to those of the pregnant bears (Fig. 2).

Table 2. Dates of observed copulation and parturition and litter size in 4 Hokkaido brown bears.

Bear	Dates of observed copulation[a]	Parturition date	Litter size
1	30 Apr & 15 Jun 1983	31 Jan 1984	3
2	30 Apr & 18 Jun 1983	27 Jan 1984	2
3	Not observed	22 Jan 1985	3
4	Not observed	18 Jan 1985	1

[a] Represents 1st and last observed copulation.

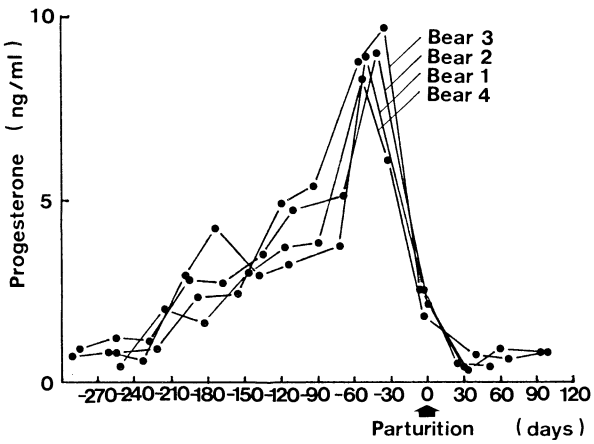

Fig. 1. Serum progesterone levels during pregnancy in 4 Hokkaido brown bears (bears 1–4).

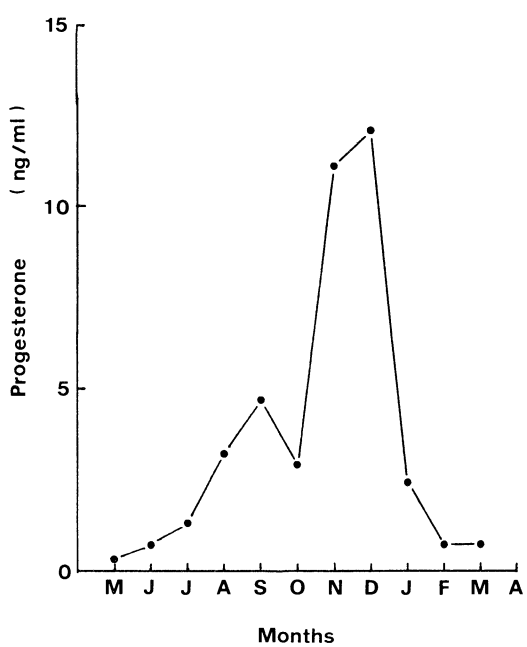

Fig. 2. Serum progesterone levels in a nonpregnant Hokkaido brown bear (bear 5).

Fetal Growth

Fetuses and fetal sacs within the intrauterine lumen were first observed by ultrasonography on day −37 and day −33 in bears 3 and 4, respectively. The fetal sac imaged as a nonechogenic area (black) and the fetus was observed as a echogenic area (white), but no fetal details could be discerned. The fetal CRL of bears 3 and 4 at that time were 1.5 cm and 2.0 cm, respectively.

Fetal heart beats were first observed on day −27 in bear 3 and on day −23 in Bear 4. The fetal CRL of bears 3 and 4 had increased to 4.5 and 6.0 cm.

Two fetuses were found on the ventral right side and 1 on the ventral left side of bear 3 on day −17. In bear 4, 2 fetuses, whose ribs, head, and heart were distiguishable, were found on the ventral left side on day −13. Though the CRL measurements were not made on day −7 and day −3 because the fetuses were too large to fit on the monitor screen, the CRL of bears 3 and 4 were estimated to be 14 cm and 17 cm, respectively. Figure 3 shows the changes in fetal heart rate. Fetal heart rate decreased with fetal growth.

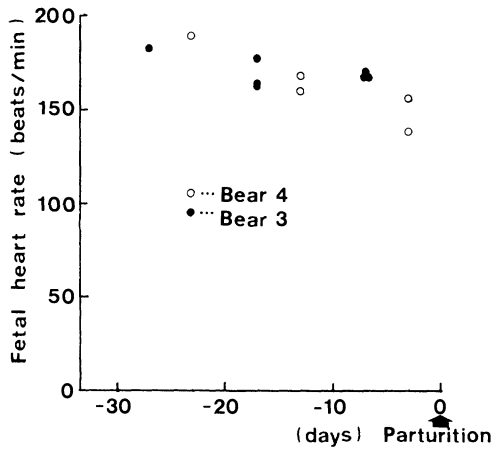

Fig. 3. Changes in fetal heart rate in Hokkaido brown bears (bears 3 and 4).

DISCUSSION

Serum Progesterone

Foresman and Daniel (1983) suspected that implantation in the black bear occurred in late November, when the plasma progesterone levels increased sharply in 2 pregnant bears. The progesterone levels increased when implantation occurred in the European badger (*Meles meles* [Bonnin et al. 1978]), spotted skunk (*Spilogale putorius* [Mead and Eik-Nes 1969]), and marsupials (Tyndale-Briscoe and Hinds 1981). In this study, serum progesterone levels were greatly elevated at approximately day −60 (late Nov-early Dec, Fig. 1). Therefore, we suspected that implantation in the Hokkaido brown bear also occurs approximately 60 days before parturition.

This small elevation in serum progesterone levels on day −210 during the breeding season was considered to be a hormonal change accompanying ovulation. Corpora lutea were probably formed in the ovary, and there was slight secretion of progesterone after ovulation and fertilization. Wimsatt (1963) described corpora lutea in the delay period as nonfunctional compared to those after implantation. He observed that there was a 3.2-fold increase in the luteal volume after implantation. If fertilization occurred on day −210 and implantation on day −60, the difference is the delay period. Thus, serum progesterone levels during the delay period were higher than those before fertilization but lower than after implantation. This suggests that the existing corpora lutea in the ovary were nonfunctional during the delay period. Foresman and Daniel (1983) believed progesterone levels during the delay period would probably be much lower. However in this study, we observed that progesterone levels during the delay period were higher than the basal levels before fertilization.

Serum progesterone levels began to decline on day −30; therefore, we suspected that low secretion of progesterone could maintain the pregnancy during the 2nd half of pregnancy.

Because the change in serum progesterone level in the nonpregnant bear was similar to those of the pregnant bears, we suggest that corpora lutea formed independently of fertilization, a slight progesterone secretion occurred, and the corpora lutea became functional in the appropriate season. We expect that this is an intrinsic mechanism, which occurs spontaneously and independently of fertilization, implantation, and parturition. In most marsupials in which delayed implantation occurred, ovulation was followed by the growth of a functional corpus luteum regardless of whether fertilization took place (Renfree 1981). It is probable that ovulation was followed by the formation of nonfunctional corpora lutea and the activation of the corpora lutea in bears. Foresman and Daniel (1983) reported that plasma progesterone levels during nonpregnancy were much lower in black bears with cubs. It was assumed that no corpora lutea

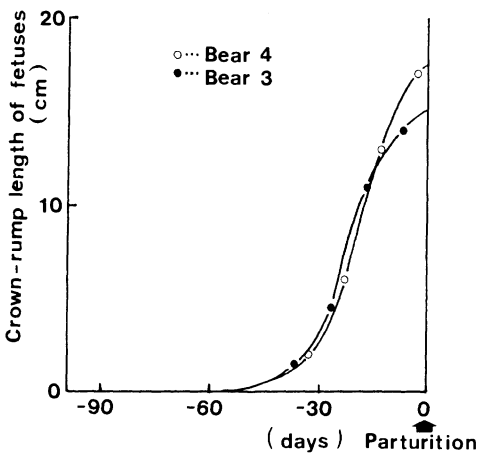

Fig. 4. Fetal growth curves for Hokkaido brown bears (bears 3 and 4).

existed in the ovary and progesterone was not secreted because of the influence of hormones during lactation.

Fetal Growth

The growth curves of fetuses were drawn according to the changes of CRL (Fig. 4). These were drawn as sigmoid curves, with the assumption that the growth of fetuses began on day −60, when serum progesterone levels were greatly elevated. The decrease in fetal heart rate in these bears agrees with the report that the fetal heart rate in domestic animals decreased with fetal growth during the 2nd half of pregnancy (Too et al. 1974, Mitchell 1973).

In the present study, we confirmed that delayed implantation occurred in the Hokkaido brown bear and that the fetuses grew during a 60-day period; this can be observed from the change in serum progesterone levels and the growth of fetuses imaged by ultrasonography. If fertilization occurred on the last day of observed copulation and implantation occurred 60 days before parturition, when serum progesterone levels were greatly elevated, the calculated gestation period was 222–229 days in bears 1 and 2. The period of fetal growth is 60 days and is equal to one-fourth of the gestation period; the delay period is approximately 170 days.

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