Changes in progesterone concentrations in the Japanese long-fingered bat, *Miniopterus schreibersii fuliginosus**

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Summary. In the Japanese long-fingered bat, when compared with the baseline values during non-pregnancy in the autumn, plasma progesterone concentrations were not significantly elevated during the delayed implantation stage that begins before the bats enter hibernation. However, progesterone concentrations were significantly lower during the delayed development stage that occurs during hibernation and rose significantly during the rapid embryogenesis that occurs after arousal from hibernation in the spring. Changes in the corpus luteum volume corresponded closely with those of plasma progesterone values. Maintenance of gravid females at 25°C for 2 weeks in winter resulted in significant increases in the plasma progesterone concentration and the corpus luteum volume.

Introduction

Miniopteryine bats (*Miniopterus* species), widely inhabiting the Old World, have a unique reproductive pattern (Uchida, 1957; Wimsatt, 1969; Richardson, 1977; van der Merwe, 1980). In the Japanese long-fingered bat, *Miniopterus schreibersii fuliginosus*, copulation in mid-October is followed closely by ovulation and fertilization (Mōri & Uchida, 1980, 1981a, b, 1982). In November, the females enter into hibernation in a pregnant condition and after having experienced a delayed implantation of about 60 days (mid-October to mid-December), the blastocyst begins to implant from mid-December to early January and then undergoes a subsequent period of delayed development (early January to March) (Kimura & Uchida, 1983). Embryonic development, however, proceeds rapidly after arousal from hibernation in early April (Kimura & Uchida, 1984), and the young are born in late June or early July (Uchida, 1957).

Uchida *et al.* (1984) showed that elevated temperatures (23–25°C) and abundant food supply during winter accelerated the rate of embryonic development and that the corpus luteum cells in bats kept at an elevated temperature (25°C) became active while those in hibernating bats were less active. However, information which might explain an hormonal regulation of such a reproductive mechanism is scarce. The present study was therefore carried out to measure the plasma progesterone concentrations during pregnancy in this bat.

Materials and Methods

Animals. Forty-nine gravid bats (*M. s. fuliginosus*) were captured from abandoned mines (33·6°N) in the Wakayama Prefecture, and 2 precopulatory bats which had a maturing follicle with a primary oocyte in only the left ovary at Ohstedō Cave (32·5°N) in the Kumamoto Prefecture (see Table 1). Blood was taken in heparinized tubes from bats under ether anaesthesia by a cardiac puncture 38–40 h after capture in the pregnant bats captured before (5

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November 1983) or after (1 April 1984 and 17 May 1984) hibernation and 4–5 h after capture in the unmated bats captured on 13 September 1985. Plasma was separated by centrifugation and stored at -20°C until assay. Specimens collected on 5 January 1984 were divided into two groups, i.e. the hibernating and aroused, as follows: the hibernating bats were maintained under artificial hibernation in a cold room ($5-7^{\circ}\text{C}$, humidity 60%) for 14 days to eliminate possible arousal caused by capture; the aroused bats were kept without hibernation in a constant temperature and humidity room ($25 \pm 1^{\circ}\text{C}$, 90%) and fed on sufficient meal worms and water for the same period. Afterwards, the bats of both groups were anaesthetized with ether and exsanguinated from the heart, and the separated plasma was stored at -20°C until assay.

Radioimmunoassay. Plasma progesterone concentrations were measured by radioimmunoassay using progesterone antiserum, standard progesterone and [$1,2\text{-}^3\text{H}$]progesterone purchased from Sorin Biomedica (Saluggia, Italy). According to the information provided with the antiserum, it had been prepared in a rabbit against 11α -hydroxyprogesterone hemisuccinate-BSA and the following materials were checked for cross-reactivity: progesterone, 100%; cortexone, 0.65%; testosterone, 0.5%; 17α -hydroxyprogesterone, 0.37%; 20α -dihydroprogesterone, 0.2%; pregnenolone, 0.05%; androstenedione, 0.04%; oestradiol, 0.01%; $3\beta,17\alpha$ -dihydroxyprogesterone, 0.01% and androsterone, cortisol and epitestosterone, all $<0.01\%$. The antiserum and labelled progesterone were used according to the supplier's specifications. The minute volume of plasma obtained from each bat did not allow division into duplicates, and therefore a $100\text{ }\mu\text{l}$ sample for each bat was twice extracted with 2 ml diethyl ether by vortexing for 2 min. Procedural losses were determined by monitoring the recovery of labelled progesterone added to plasma samples from 18 specimens of 49 bats before extraction, and the average recovery ratio was 95.7%. Bound and free hormones were separated by dextran-coated charcoal. The inter- and intra-assay coefficients of variation were 13.1% and 12.4%, respectively, and the assay sensitivity was 10 pg/tube . Ewe plasma collected in the anoestrous season was used to verify the measurement of progesterone in the assay; values obtained ($0.85 \pm 0.32\text{ ng/ml}$ (s.e.m.), $n = 3$) were comparable to those reported by Saba *et al.* (1975).

Histology. Reproductive organs from all bats were fixed in Bouin's fluid, serially sectioned at $7\text{ }\mu\text{m}$, stained with haematoxylin and eosin, and the developmental stage of the embryo was determined. From the ovarian sections the volumes of the corpora lutea were estimated as described by Rowlands & Heap (1966).

Results

Progesterone concentration

The September samples represent non-pregnant animals and the mean plasma progesterone value was 7.0 ng/ml . At the delayed implantation stage in November (just before hibernation), plasma progesterone values were greater than in September but were not significantly elevated. However, in January, during delayed development, progesterone concentrations were significantly lower ($P < 0.05$) in the hibernating bats with an implanting blastocyst than in non-pregnant bats in September (Table 1). After arousal from hibernation, the progesterone concentrations increased in

Table 1. Plasma progesterone concentrations and corpus luteum volumes in Japanese long-fingered bats

Date captured	Date of plasma sampling	Stage or size of embryo	Progesterone conc. (ng/ml)	Corpus luteum volume (mm^3)
Non-pregnancy				
13 September 1985	13 Sept.	No embryo	7.0 ± 0.1^a (2)	No corpus luteum
Pregnancy				
5 November 1983	7 Nov.	Free blastocyst	11.1 ± 1.8^{ac} (5)	0.139 ± 0.011^{ac} (5)
5 January 1984				
Hibernating*	21 Jan.	Implanting blastocyst	4.7 ± 0.6^b (6)	0.123 ± 0.015^a (4)
Aroused†	21 Jan.	Implanting blastocyst	10.5 ± 1.1^{cd} (6)	0.190 ± 0.008^b (4)
1 April 1984	3 Apr.	Early placentation	8.0 ± 0.9^{ad} (5)	0.175 ± 0.011^{bc} (5)
17 May 1984	19 May	12 mm conceptus	11.8 ± 1.5^e (10)	0.180 ± 0.009^b (9)

Values are mean $\pm$ s.e.m. for the no. of bats indicated in parentheses. Means with at least one superscript in common are not significantly different ($P > 0.05$, Student's *t* test or Cochran-Cox test).

*Maintained under artificial hibernation in a cold room ($5-7^{\circ}\text{C}$) from 7 January.

†Kept at an elevated temperature ($\sim 25^{\circ}\text{C}$) from 7 January.

bats at the early placentation stage (April) to an extent comparable to that in the September samples, and were significantly ($P < 0.05$) greater in bats with a 12-mm embryo (May). The progesterone values in the aroused bats of the January samples with an implanting blastocyst were significantly ($P < 0.01$) higher than those in the hibernating bats of the January samples.

Corpus luteum volume

The changes in the corpus luteum volume corresponded closely with those in plasma progesterone concentration during pregnancy: the volume increased after awakening from hibernation (Table 1). In the aroused bats prevented from hibernating in January, the volume was significantly ($P < 0.01$) larger than that in the hibernating bats in the same month.

Discussion

In non-hibernating bat such as the Mexican free-tailed bat, *Tadarida brasiliensis mexicana* (Jerrett, 1979), which exhibited no delayed implantation and development, plasma progesterone concentrations increase steadily until the mid-point of pregnancy and then decline after parturition. On the other hand, in hibernating bats such as the pallid bat, *Antrozous pallidus* (Oxberry, 1979), and pipistrelle bat, *Pipistrellus pipistrellus* (Racey & Swift, 1981), the progesterone concentrations fluctuate until the time of a peak of progesterone just before parturition; nevertheless the progesterone concentrations during pregnancy in the 2 species do not fall to the values at the beginning of gestation and/or the baseline value. In the Japanese long-fingered bat, progesterone values were not significantly elevated during delayed implantation but were significantly depressed during retarded embryonic development after implantation (delayed development), and rose significantly after arousal from hibernation.

In the majority of species which show seasonal or obligatory delay of implantation, e.g. armadillo (Labhsetwar & Enders, 1968; Peppler & Stone, 1976), stoat (Gulamhusein & Thawley, 1974), European badger (Bonnin *et al.*, 1978), spotted skunk (Mead, 1981), northern fur seal (Daniel, 1981) and black bear (Foresman & Daniel, 1983), the corpora lutea are less active during embryonic diapause, and the plasma progesterone concentrations stay slightly above the values in non-pregnant animals until shortly before implantation, when the concentrations begin to increase with activation of the corpora lutea. However, the roe deer, *Capreolus capreolus*, is unique in having an active corpus luteum throughout the diapause period, although no significant change is detected in the progesterone concentration until after implantation (Hoffman *et al.*, 1978; Aitken, 1981). A high progesterone concentration and the presence of active corpora lutea are important for maintenance of lactational delayed implantation in the mouse and rat (Yoshinaga, 1974; Gidley-Baird, 1981). The high progesterone value during delayed implantation in Japanese long-fingered bats is therefore of interest. According to histological studies (Peyre & Herlant, 1963a, b, 1967) on the long-fingered bat inhabiting the south of France, it is said that the corpus luteum is poorly developed throughout delayed implantation for 5 months of the entire hibernation period and that an arrest in LH secretion after ovulation, together with insufficient prolactin, is responsible for delayed implantation.

In the Japanese long-fingered bat, the plasma progesterone concentration was low and the corpus luteum volume was small at the delayed development stage. These results support our belief that the corpus luteum is less active during delayed development in this bat (Kimura & Uchida, 1983; Uchida *et al.*, 1984). The so-called delayed development was first described in the California leaf-nosed bat, *Macrotus californicus* (Bradshaw, 1962), and occurs also in the Mexican fruit bat, *Artibeus jamaicensis* (Fleming, 1971). In the California leaf-nosed bat, the progesterone values are low throughout delayed development and a biphasic pattern of plasma progesterone is observed during pregnancy (Burns & Easley, 1977). Elevated ambient temperatures and ample food supplies

failed to shorten the period of delayed development in California leaf-nosed bats (Burns, 1981); however, such a condition not only hastened embryonic development (Uchida *et al.*, 1984) but also resulted in significant increase in both the plasma progesterone concentration and the corpus luteum volume (this study) in Japanese long-fingered bats. Therefore, the retarded embryonic development of the long-fingered bat is a direct and passive response to the cold and depressed metabolism as indicated by Kimura & Uchida (1983), and seems to differ essentially from that of the above 2 non-hibernating species. On the other hand, in the pipistrelle bat, delayed development can take place experimentally under cold conditions after fetuses have reached a palpable size (Racey, 1973); under natural conditions also, the gestation period is extended in cold weather, and the duration of the low progesterone period that occurs between the apparent initial rise and fall in early pregnancy and the later high levels just before parturition is lengthened (Racey & Swift, 1981), and this prolonged period of low progesterone seems to coincide with the period of cold weather. This phenomenon in the pipistrelle bat may be analogous to the low progesterone period that appears to occur in response to the cold during delayed development in the Japanese long-fingered bat.

The baseline level (7 ng/ml) of plasma progesterone in the Japanese long-fingered bat during a period when no luteal tissue was present was similar to that (~ 5 ng/ml) reported for the pallid bat (Oxberry, 1979) and the Mexican free-tailed bat (Jerrett, 1979), but the baseline values in the California leaf-nosed bat appear as 'zero' (Burns & Easley, 1977). Relatively high baseline values of plasma progesterone observed during non-pregnancy in the former 3 bat species suggest that the measured progesterone is secreted by the ovarian interstitial gland tissue as suggested for the Mexican free-tailed bat (Jerrett, 1979) and/or by the adrenal cortex as in the ewe (McCracken & Baird, 1969) and women (Baird, 1976). On the other hand, ACTH stimulation can increase plasma progesterone values 350–500% over baseline levels in post-menopausal women (Vermeulen, 1976). It is still possible, therefore, that stress of bats by artificial incidents such as capture and transport may influence the plasma progesterone concentrations. Although the adrenal glands of many hibernators undergo seasonal changes in size and activity (Wang, 1982), such changes have not been studied in Japanese long-fingered bats. The endocrine control mechanisms of delayed implantation and development in Japanese long-fingered bats require further study.

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