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Progesterone concentrations and contents in the plasma, ovary, adrenal gland and placenta of the pregnant Natal clinging bat *Miniopterus schreibersii natalensis*

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Reproduction in the Natal clinging bat in South Africa at 25° S is characterized by fertilization in April, implantation during July and parturition in November. In the present study plasma progesterone concentrations peaked two months before implantation, this coinciding with significant peaks in ovarian progesterone content and concentration. Plasma progesterone concentrations remained relatively low during and after implantation, but increased significantly during the limb bud stage to peak levels one month before parturition. Adrenal progesterone content and concentration did not follow a specific trend; post-implantation ovarian progesterone content and concentration remained at baseline values. Placental progesterone content increased significantly with foetal weight (age). The present study showed that pregnancy in these bats is characterized by two major sources of progesterone, with the ovary as the primary source before implantation and the placenta the primary source during embryonic and foetal development.

Contents

Introduction

Reproduction in Schreibers' long-fingered bats (*Miniopterus schreibersii*) is characterized by delayed implantation, with the delay in Japan at 33° N and Australia at 37° S lasting two months (Kimura & Uchida, 1983; Crichton, Seamark & Krutzsch, 1989) and in Africa at 25–33° S four to five months (van der Merwe, 1979; Bernard, Bojarski & Millar, 1991). In the former populations implantation is followed by a period of delayed embryonic development, but this does not occur in the African populations at 33° S (Bernard *et al.*, 1991).

Published information on plasma progesterone concentrations during pregnancy in these bats is contradictory. In Japanese and Australian long-fingered bats, plasma concentrations remain low throughout developmental delay (Kimura, Takeda & Uchida, 1987; Crichton *et al.*, 1989). In

South Africa at 33° S, progesterone concentrations increase slightly after fertilization but remain low throughout delayed implantation (Bernard *et al.*, 1991). However, in South Africa at 25° S, concentrations follow a bimodal pattern, with the first peak occurring at implantation and the second 216–222 days after fertilization (van der Merwe & van Aarde, 1989). Because of this, and the eight-week time lapse from implantation to limb-bud formation, van der Merwe & van Aarde (1989) suggested that the reproductive cycle of these bats also is characterized by a period of slow embryonic and foetal development. Since this contradicts the pattern described for the same species at 33° S (Bernard *et al.*, 1991), the present paper re-investigated the plasma progesterone profile of these bats at 25° S in South Africa. Furthermore, since the biphasic pattern of plasma progesterone concentrations recorded by van der Merwe & van Aarde (1989) suggests different phases of progesterone secretion, possibly attributable to different structures, an attempt was made to ascertain the source(s) of progesterone secreted during pregnancy by relating ovarian, adrenal and placental concentrations of progesterone to gestational stage.

Materials and methods

Collection and handling

Animals and plasma were collected from March to November 1989 from roosting sites in Long One Cave (25° 54′ S, 27° 46′ E) or Sandspruit Cave No. 1 (24° 37′ S, 27° 40′ E) as described by van der Merwe & van Aarde (1989). Plasma, ovaries and adrenals were kept at −20 °C until assayed and placentae were stored in a 10 ml solution of alcohol, formalin and acetic acid (AFA). All organs were weighed before being processed for progesterone assays.

Extraction of progesterone from organs and tissues

Monthly collections of ovaries and adrenals were pooled owing to the small mass of these organs (≈0·2 mg). Representative samples of placentae were individually extracted. The pooled organs and tissue samples were weighed and then homogenized in a small ground glass homogenizer containing 0·2 ml gelatin-free phosphate buffer. The homogenate was pipetted into a centrifuge tube and the homogenizer rinsed with 0·8 ml of phosphate buffer. After the homogenate had been centrifuged at 1500 * g for 10 min, the supernatant was pipetted off for direct assay.

The remaining pellet was reconstituted in 1·0 ml of 0·6 M NaOH and progesterone was extracted from the NaOH solution by adding 2 ml petroleum ether. This mixture was vortexed for 5 min and then frozen at −20 °C for 1 h. The petroleum fraction was decanted into a glass extraction tube and dried under a stream of nitrogen in a waterbath at 37 °C. This extraction was repeated twice. Petroleum ether (1·0 ml) and 70% methanol (1·0 ml) were added to the dried extract and vortexed for 2 min. The petroleum ether and methanol fractions were then pipetted into separate glass extraction tubes and evaporated under a stream of nitrogen at 37 °C. Extracts were reconstituted in 1·0 ml phosphate buffer for progesterone determination.

The storage solution (AFA for the placentae) contained alcohol which absorbed some progesterone. The AFA volume of each storage vial was determined and made up to 10 ml to correct for evaporative loss. The possible degradation of gestagens in the storage solution was not investigated. A 1·0 ml aliquot of each of these samples was removed and dried under a stream of nitrogen at 37 °C. This was reconstituted in phosphate buffer for progesterone determination.

Progesterone determination

Progesterone concentrations of homogenate supernatants, pellet extracts and plasma were determined by using the method described by van Aarde (1985) and an antiserum supplied by R. F. Millar (Department of

TABLE I

Plasma progesterone concentrations and ovarian and adrenal progesterone content and concentration in adult Natal clinging bats. All means are followed by one standard deviation of the mean and values in parentheses represent sample size

Collection date	Reproductive development stage	Plasma progesterone (ng/ml)	Ovarian progesterone		Adrenal progesterone		Placental progesterone	
			Content (ng/ovary)	Concentration (ng/mg)	Content (ng/adrenal)	Concentration (ng/mg)	Content (ng/placenta)	Concentration (ng/mg)
17 Mar. 1989	Non-pregnant	3·38 ± 0·41 (6)	0·834 (10^P)	3·09	9·77 (10^P)	25·05	—	—
12 Apr. 1989	Tubal (zygote/morula)	2·49 ± 0·37 (8)	2·542 (8^P)	6·20	8·36 (10^P)	17·07	—	—
17 May 1989	Free-lying bilaminar blastocyst	6·19 ± 0·44* (8)	20·614 (9^P)	64·42	6·96 (^{P})	21·75	—	—
14 Jun. 1989	Endometrium well developed	3·24 ± 0·28* (14)	3·136 (11^P)	19·60	6·44 (12^P)	14·30	—	—
19 Jul. 1989	As above	4·92 ± 0·68* (12)	8·307 (11^P)	22·45	7·72 (13^P)	14·04	—	—
28 Aug. 1989	Foetus	9·19 ± 0·80* (14)	2·703 (11^P)	9·01	8·40 (13^P)	24·01	—	—
20 Sep. 1989	Foetus	12·30 ± 1·71 (8)	2·659 (9^P)	8·31	7·61 (10^P)	23·07	—	—
24 Oct. 1989	Foetus	35·60 ± 1·42* (7)	1·198 (9^P)	3·63	8·88 (10^P)	11·39	1163 ± 272	8·87 ± 2·86 (8)
16 Nov. 1989			0·959 (11^P)	3·69	12·61 (17^P)	20·02	1756 ± 271	10·25 ± 2·30 (10)
	Pre-partum	31·29 ± 2·42 (4)						
	Post-partum	5·70 ± 0·62 (5)						

^{P}—Values for samples pooled from number of females (n) given in parentheses

*—Mean significantly (F test; $P \leqslant 0{\cdot}05$) different from preceding mean

Chemical Pathology, University of Cape Town, South Africa). Cross-reactivity of other steroids with the antibody was provided by van der Merwe & van Aarde (1989). The recovery of [1, 2, 6, 7,-^{3}H] progesterone added to residue pools was 86·9% ± 6·97 (n = 6) and to plasma 86·1% ± 7·28 (n = 6). The sensitivity of the assay was 0·06 ± 0·037 ng/ml (n = 15). The intra-assay coefficient of variation was 2·9% and the inter-assay coefficients of variation were 12·4% (0·312 ng/ml), 7·2% (5·0 ng/ml) and 3·3% (10·0 ng/ml). Serially diluted adrenal, ovarian and placental homogenates and extracts and plasma yielded progesterone values parallel to the standard curve. Water and petroleum ether blanks included in assays were consistently below the sensitivity of the assay.

Statistics

Data on plasma concentrations for each month were exposed to 1-way analysis of variance followed by Tukey's standardized multiple range test (SAS GLM). Regression analysis was used to seek relationships between foetus size, placental progesterone concentrations and placental progesterone content. Significance was accepted at the 5% level.

Results

Plasma progesterone concentration showed two statistically significant peaks during pregnancy. The first (6·19 ± 0·44 ng/ml) occurred before implantation during May and the second (35·60 ± 1·42 ng/ml) commenced in August and culminated at parturition in November (Table I).

Both monthly ovarian progesterone concentration and content showed one very distinct peak (64·42 and 20·61 ng/ml, respectively) in May. Concentrations decreased gradually to reach basal

TABLE II

Individual foetal weights (g) and placental progesterone contents (ng/placenta) and concentrations (ng/mg) in Natal clinging bats

	Progesterone	
Foetal weight (g)	Concentration (ng/mg)	Content (ng/placenta)
1·167	9·26	1027·86
1·252	10·55	1636·31
1·266	13·51	1337·49
1·411	15·35	1535·00
1·496	9·17	1097·65
1·520	4·57	825·34
1·534	7·86	1219·87
2·002	4·88	776·41
2·008	8·66	1280·81
2·012	11·23	1381·29
2·289	11·97	1618·34
2·320	9·54	1460·57
2·348	11·07	1971·57
2·474	7·51	1139·27
2·494	12·20	2020·32
2·568	7·56	1404·65
2·600	9·20	1520·76
2·809	9·42	1811·47

levels (3·63 ng/ml) in October (Table I). There was no noticeable trend in the monthly adrenal progesterone concentration and content (Table I). Progesterone content varied from 6·44 ng/adrenal to 12·61 ng/adrenal and concentrations ranged from 11·39 ng/mg to 24·01 ng/mg (Table I).

Placental progesterone concentration ranged from 4·57 to 15·35 ng/mg and placental content from 776·41 to 2020·32 ng (Table II). Total placental progesterone content increased significantly with an increase in foetal weight ($r = 0{\cdot}59$; $P < 0{\cdot}01$) but no correlation was found between foetal weight and placental progesterone concentration ($r = -0{\cdot}17$; $P > 0{\cdot}20$). Nor was there any significant correlation between plasma progesterone levels and either total placental progesterone ($r = 0{\cdot}16$; $P > 0{\cdot}20$) or placental progesterone concentration ($r = 0{\cdot}13$; $P > 0{\cdot}20$) (Table II).

Discussion

Our results confirm the seasonal bimodal plasma progesterone concentration pattern of *Miniopterus schreibersii natalensis* reported previously (van der Merwe & van Aarde, 1989). However, in the present study the first peak occurred before implantation and coincided with a peak in ovarian progesterone content and concentration. A similar bimodal seasonal pattern in plasma concentration has been reported in *Myotis lucifugus* (Buchanan & Younglai, 1986) and in *Macrotus californicus* (Burns, 1981). Pre-implantation increases in plasma progesterone concentrations have also been recorded in other species with delayed implantation, i.e. Antarctic fur seals, *Arctocephalus gazella* (Boyd, 1991), black bears, *Ursus americanus* (Foresman & Daniel, 1983) and mustelids (Mead, 1981). In the Japanese long-fingered bat *M. s. fuliginosus*, progesterone values were not significantly elevated during delayed implantation (Kimura *et al.*, 1987), suggesting that the corpus luteum is less active during delayed development in these bats (Kimura & Uchida, 1983). This, however, is not supported by our observation on ovarian contents and concentration which are then 10 times higher than at fertilization (see Table I). In *M. s. natalensis* at 33° S the peak in May, just after fertilization, was not statistically significant (Bernard *et al.*, 1991) and in the European badger the first progesterone peak also was not attributed to implantation (Canivenc & Bonnin, 1981).

In the present study the pre-implantation progesterone peak was approximately one month earlier than that found in the previous year at implantation (van der Merwe & van Aarde, 1989). The time of parturition, however, is the same in both studies. Buchanan & Younglai (1988) showed that hibernating little brown bats, *Myotis lucifugus* frequently display elevated progesterone concentrations, probably due to rhythmic activity of the hypothalamus and pituitary. This conceivably may affect peak concentrations and the shift in the peaks recorded by us may be ascribed to variation in metabolic state which may affect ovarian activity. On the other hand, the peak recorded during May in the present study coincided with an obvious increase in uterine endometrial activity. Seasonal bimodal plasma progesterone concentrations have not been reported in other bat species having delayed implantation such as *Antrozous pallidus* (Oxberry, 1979), *Macrotus californicus* (Burns & Easley, 1977), *Tadarida brasiliensis mexicana* (Jerrett, 1979) or in the mink (Martinet, Allais & Allain, 1981).

Plasma progesterone may be increased by stress-induced changes in adrenal function (Vermeulen, 1976) but this should not affect the bimodal pattern recorded in the present study, as our capture methods were constant for the duration of the study. Furthermore, it is unlikely that the plasma progesterone pattern was affected by adrenal progesterone since no seasonal adrenal pattern was evident (see Table I). Although the present plasma progesterone pattern was comparable to a study in the previous year (van der Merwe & van Aarde, 1989), the very high peak

in that study (127·74 ng/ml) was not observed in the present study, suggesting that there is a critical time period for the peak; further research should elucidate this.

The pre-implantation peak in plasma as well as ovarian progesterone levels recorded during the present study coincides with the formation of the blastocyst, as has been reported for the little brown bat by Buchanan & Younglai (1986), suggesting that ovarian progesterone may play a role in the formation of the blastocyst. Bernard (1980) showed a significant increase in the size of the corpus luteum in *M. schreibersii natalensis* up to implantation and a gradual decrease thereafter, suggesting that the increase in plasma and ovarian progesterone before implantation during the present study results from increased luteal activity before implantation. However, in *Lasiurus ega*, *Eptesicus furinalis*, *Myotis albescene* and *Myotis nigricans* the luteal volume reaches its maximum size only at implantation (Myers, 1977). Discrepant data exist for *Miniopterus schreibersii*. Peyre & Herlant (1967) reported that the corpus luteum of *M. schreibersii* from Southern France became increasingly vascular and active at implantation, whereas Wallace (1978) observed the corpus luteum of Australian members of this species to exhibit no histological evidence for an increase in activity at the end of delayed implantation. Kimura *et al.* (1987) thought that the corpus luteum plays an important role in maintaining pregnancy in *M. fuliginosus*.

The luteal cells of *M. s. natalensis* apparently decreased in size up to implantation, increasing rapidly thereafter (Bernard *et al.*, 1991). These observations suggest a reduction in secretory activity during delayed implantation. However, since the interstitial cells of the ovary were reported to be 'large with clear nuclei, resembling secretory tissue prior to implantation' (Bernard, 1980) and since Els (1978) showed that the interstitial cells in these bats are steroidogenically active during early pregnancy, we suggest that the peak in plasma and ovarian progesterone content can be ascribed to the steroidogenic activity of the interstitial tissue.

There were no notable differences in the minimum and maximum temperatures to which these bats were exposed in each year (data not shown). Similarly the summer rainfall (September to April) was not different (1988: 82 mm; 1989: 78 mm). However, the rainfall from December to March differed appreciably (1988: 107 mm; 1989: 142 mm). This period coincides with the period of lactational anoestrus in *M. s. natalensis*—an energetically demanding time. Rainfall during the 1989 lactational anoestrus was also notably higher than the normal monthly total of 113 mm. Unfortunately no data on insect abundance are available for these periods, but if related to rainfall it may help explain the difference in time of plasma progesterone peaks observed in the present study. The length of gestation in *P. pipistrellus* varied by 10 days in two consecutive years and corresponded to changes in weather and insect availability (Racey & Swift, 1981). Sandell (1990) discussed the evolutionary advantage of shifting the time of mating and implantation and suggested that the condition of the males was the deciding factor. In this regard it is of interest to note that implantation in *M. s. natalensis* at 33° S takes place at least one month later than at 25° S which may explain subtle differences in monthly progesterone profiles. The possible effects of environmental variables on progesterone secretion stress the importance of not pooling samples from different years to compile a profile.

It also should be noted that pregnancy in *M. s. natalensis* is not highly synchronized and that implantation within a specific colony can take place over a period of six weeks (see van der Merwe, 1979; van der Merwe & van Aarde, 1989), which will affect progesterone profiles based on pooled samples. The time-specific frequency distribution of reproductive events also differs from year to year (pers. obs.) which conceivably will induce annual variations in steroid profiles.

The decline in ovarian progesterone levels after the formation of the blastocyst suggests that the contribution of the ovaries to the plasma pool, although still of relevance (see Bernard *et al.*, 1991),

diminishes and that placental progesterone becomes an important source during foetal development. No notable increase in ovarian progesterone occurred in the second half of pregnancy when plasma levels increased tenfold (Table I). The gradual increase in plasma progesterone until the second peak corresponds to the significant positive correlation between total placental progesterone and foetus weight and hence age. This and the recorded decline in ovarian progesterone content and concentration (see Table I) suggest that the placenta is an important source of progesterone in *M. s. natalensis* during the second half of pregnancy. Contrary to this, Racey & Swift (1981) believed that in *P. pipistrellus* the corpus luteum was the major source of progesterone until parturition. The ovary is essential for gestation throughout pregnancy in *M. californicus* even though the placenta is enzymatically active (Burns, 1981). However, steroidogenesis was not investigated during the present study and the ovary may also be a source of increased plasma progesterone during the second half of pregnancy: gestagens are not stored in the luteal tissue and increased production may be associated with increased release, resulting in low ovarian levels.

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REFERENCES

Bernard, R. T. F. (1980). Monthly changes in the reproductive organs of female *Miniopterus schreibersi natalensis* (A. Smith, 1834). *Z. Säugetierk.* **45:** 217–224.

Bernard, R. T. F., Bojarski, C. & Millar, R. P. (1991). Plasma progesterone and luteinizing hormone concentrations and the role of the corpus luteum and LH gonadotrophs in the control of delayed implantation in Schreibers' long-fingered bat (*Miniopterus schreibersii*). *J. Reprod. Fert.* **93:** 31–42.

Boyd, I. L. (1991). Changes in plasma progesterone and prolactin concentrations during the annual cycle and the role of prolactin in the maintenance of lactation and luteal development in the Antarctic fur seal (*Arctocephalus gazella*). *J. Reprod. Fert.* **91:** 637–647.

Buchanan, G. D. & Younglai, E. V. (1986). Plasma progesterone levels during pregnancy in the little brown bat *Myotis lucifugus* (Vespertilionidae). *Biol. Reprod.* **34:** 878–884.

Buchanan, G. D. & Younglai, E. V. (1988). Plasma progesterone concentrations in female little brown bats (*Myotis lucifugus*) during hibernation. *J. Reprod. Fert.* **83:** 59–65.

Burns, J. M. (1981). Aspects of endocrine control of delay phenomena in bats with special emphasis on delayed development. *J. Reprod. Fert.* (Suppl.) **29:** 61–66.

Burns, J. M. & Easley, R. G. (1977). Hormonal control of delayed development in the California leaf-nosed bat, *Macrotus californicus*. III. Changes in plasma progesterone during pregnancy. *Gen. comp. Endocr.* **32:** 163–166.

Canivenc, R. & Bonnin, M. (1981). Environmental control of delayed implantation in the European badger (*Meles meles*). *J. Reprod. Fert.* (Suppl.) **29:** 25–33.

Crichton, E. G., Seamark, R. F. & Krutzsch, P. H. (1989). The status of the corpus luteum during pregnancy in *Miniopterus schreibersii* (Chiroptera: Vespertilionidae) with emphasis on its role in developmental delay. *Cell Tissue Res.* **258:** 183–201.

Els, D. A. (1978). *Histochemistry of the reproductive tract of* ***Miniopterus schreibersi****: the ovary*. Unpubl. report. Mammal Research Institute, University of Pretoria, South Africa.

Foresman, K. R. & Daniel, J. C., Jr (1983). Plasma progesterone concentrations in pregnant and non-pregnant black bears (*Ursus americanus*). *J. Reprod. Fert.* **68:** 235–239.

Jerrett, D. P. (1979). Female reproductive patterns in nonhibernating bats. *J. Reprod. Fert.* **56:** 369–378.

Kimura, K., Takeda, A. & Uchida, T. A. (1987). Changes in progesterone concentrations in the Japanese long-fingered bat, *Miniopterus schreibersii fuliginosus*. *J. Reprod. Fert.* **80:** 59–63.

Kimura, K. & Uchida, T. A. (1983). Ultrastructural observations of delayed implantation in the Japanese long-fingered bat, *Miniopterus schreibersii fuliginosus*. *J. Reprod. Fert.* **69:** 187–193.

Martinet, L., Allais, C. & Allain, D. (1981). The role of prolactin and LH in luteal function and blastocyst growth in mink (*Mustela vison*). *J. Reprod. Fert.* (Suppl.) **29:** 119–130.

Mead, R. A. (1981). Delayed implantation in mustelids, with special emphasis on the spotted skunk. *J. Reprod. Fert.* (Suppl.) **29:** 11–24.

Myers, P. (1977). Patterns of reproduction of four species of vespertilionid bats in Paraguay. *Univ. Calif. Publs Zool.* No. 107: 1–41.

Oxberry, B. A. (1979). Female reproductive patterns in hibernating bats. *J. Reprod. Fert.* **56:** 359–367.

Peyre, A. & Herlant, M. (1967). Ovo-implantation différée et déterminisme hormonal chez le Minioptère, *Miniopterus schreibersii* K. (Chiroptère). *C. r. Séanc. Soc. Biol.* **161:** 1779–1782.

Racey, P. A. & Swift, S. M. (1981). Variations in gestation length in a colony of pipistrelle bats (*Pipistrellus pipistrellus*) from year to year. *J. Reprod. Fert.* **61:** 123–129.

Sandell, M. (1990). The evolution of seasonal delayed implantation. *Q. Rev. Biol.* **65:** 23–42.

van Aarde, R. J. (1985). Circulating progesterone and oestradiol-17β concentration in cyclic Cape porcupines, *Hystrix africaeaustralis*. *J. Reprod. Fert.* **75:** 583–591.

van der Merwe, M. (1979). Foetal growth curves and seasonal breeding in the Natal clinging bat, *Miniopterus schreibersii natalensis*. *S. Afr. J. Zool.* **14:** 17–21.

van der Merwe, M. & van Aarde, R. J. (1989). Plasma progesterone concentrations in the female Natal clinging bat (*Miniopterus schreibersii natalensis*). *J. Reprod. Fert.* **87:** 665–669.

Vermeulen, A. (1976). Postmenopausal ovarian function. In *The endocrine function of the human ovary*: 237–244. James, V. H. T., Serio, M. & Guisti, G. (Eds). London: Academic Press.

Wallace, G. I. (1978). A histological study of the early stages of pregnancy in the bent-winged bat (*Miniopterus schreibersii*) in north-eastern New South Wales, Australia (30° 27′ S). *J. Zool., Lond.* **185:** 519–537.