

Plasma progesterone concentrations in the female Natal clinging bat (*Miniopterus schreibersii natalensis*)

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Summary. Plasma progesterone concentrations measured by radioimmunoassay in the Natal clinging bat remained below 2.01 ng/ml during lactational anoestrus but increased significantly during the period of delayed implantation. Values peaked at implantation but were followed by a significant decrease thereafter. Concentrations remained low (<6.0 ng/ml) during the initial period of fetal development (153–180 days *post coitum*) and attained peak values (85.6–181.3 ng/ml) 216–222 days after fertilization. The marked post-implantation increase in progesterone concentrations coincided with a significant increase in placental weight.

Keywords: bats; progesterone; implantation; pregnancy; seasonal

Introduction

The reproductive cycles of heterothermic bats are affected by hibernation which apparently arrests the progress of several reproductive events (see Oxberry, 1979; Buchanan & YoungLai, 1988, for references). In some vespertilionid and rhinolophid bats spermatozoa are stored in the female reproductive tract until after permanent arousal during spring when ovulation, fertilization and implantation occur. In others copulation during autumn is followed by ovulation, fertilization and initial embryogenesis. In the latter group, females start hibernating while pregnant but implantation is delayed during hibernation and only occurs after permanent arousal in spring (Oxberry, 1979).

The timing of reproductive events in the Natal clinging bat is similar to the latter pattern. Lactational anoestrus lasts almost 9 weeks, and ovulation and fertilization after migration from the Transvaal bushveld to the Transvaal Highveld (van der Merwe, 1973, 1975) is followed by a preimplantation period which lasts about 120 days (van der Merwe, 1979, 1980). Females enter hibernation, characterized by periods of deep, compulsory torpor and bouts of intensive activity, in the pregnant condition (Norton & van der Merwe, 1978). During this period the conceptus only develops as far as the bilaminar blastocyst, which lies freely in the uterine lumen and is not surrounded by a zona pellucida (van der Merwe, 1979, 1980, 1982). Implantation is followed by an 8-week period of relatively slow embryonic and fetal development. This is followed by more rapid fetal development to reach parturition size approximately 16 weeks after implantation.

No published information is as yet available on steroid concentrations in these bats and the present study was undertaken to evaluate seasonal and gestational changes in Natal clinging bats with reference to plasma progesterone concentrations in free-ranging adult females studied in their natural environment.

Materials and Methods

Collection and handling. Twelve to 16 adult female Natal clinging bats were collected monthly during 1988 from breeding rookeries in Long One Cave (25°54'S, 27°46'E) or Sandspruit Cave No. 1 (24°37'S, 27°40'E) situated on the

Transvaal Highveld and Transvaal bushveld respectively. In the laboratory heparinized blood samples (0.2–0.4 ml) were collected by cardiac puncture from anaesthetized bats (fluothane: halothane, ICI, Macclesfield, UK) between 15:00 and 17:00 h on the day of capture. Plasma fractions were stored at -20°C after separation by centrifugation at $\sim 1500\text{ g}$ and 4°C for 15 min. The reproductive tracts of bats, killed by cervical dislocation, were examined *in situ*, excised and processed for histological examination as described by van der Merwe (1982). Non-pregnant females collected between 11 March and 17 October were excluded from the present analysis. To correct for intra-sample variability, fetal age and/or gestation stage were determined as described by van der Merwe (1979). When present the bidiscoidal placentae were removed from the uterus and weighed.

Progesterone assay. The radioimmunoassay procedure used to determine plasma progesterone was with minor modifications similar to that described by van Aarde (1985). Antiserum raised in a rabbit against progesterone-21-hemisuccinate-bovine serum albumin was used at a dilution of 1:10 000. Cross-reactivities, as determined by the supplier (R. P. Millar, Department of Chemical Pathology, University of Cape Town, South Africa), were: 11β -hydroxyprogesterone, 47.1%; 11-hydroxyprogesterone, 25.8%; 5-pregnane-3,20-dione, 24.8%; pregnenolone, 31%; 17β -hydroxyprogesterone, 1.9%; 11-deoxycorticosterone, 2.2%; 11-deoxycortisol, 1.5%; 3-hydroxy-5-pregnane-20-one, 0.4%. Cross-reactivity with testosterone, 4-androstenedione, 17β -oestradiol and oestrone was $<0.001\%$.

The recovery of $[1,2,6,7\text{-}^3\text{H}]$ progesterone added to plasma pools was $80.2 \pm 5.48\%$ (mean \pm s.d., $n = 6$) and the sensitivity of the assay was 0.089 ± 0.061 ng/ml ($n = 6$). The intra-assay coefficient of variation was 4.66% and the inter-assay coefficients of variation were 15.88% (0.156 ng/ml), 16.43% (5.0 ng/ml) and 7.10% (10 ng/ml). Serially diluted samples of pooled plasma from pregnant and non-pregnant females yielded progesterone values parallel to the standard curve. Water and ether blanks included in each assay were consistently below the sensitivity of the assay.

Data are expressed as means \pm one standard error of the mean and Student's *t* test values were calculated to determine statistical significance between means.

Results

Plasma progesterone concentrations in females included in the present analysis remained below 2.0 ng/ml during lactational anoestrus (January/February collections), but increased significantly ($P < 0.05$) after fertilization and attained a mean value of 4.8 ± 1.96 ng/ml during the tubular stage of embryonic development (March collection). During the period of preimplantation development (March–July) mean progesterone concentrations increased steadily and attained a peak value of 21.07 ± 3.76 ng/ml during July when bilaminar blastocysts were enclosed by uterine endometrium or implanted (Table 1).

Table 1. Plasma progesterone concentrations (ng/ml) in adult Natal clinging bats collected during 1988

Collection date	Reproductive developmental stage	Progesterone conc. (ng/ml)
19 January	Lactating	1.78 ± 0.48 (4)†
8 February	Lactating	1.35 ± 0.27 (4)†
11 March	Tubal (zygote/morula)	$4.80 \pm 1.96^{**}$ (6)†
21 April	Morula, unilaminar blastocyst	$12.89 \pm 3.45^{**}$ (5)†
27 May	Free lying bilaminar blastocyst	18.53 ± 3.17 (4)†
4 July	Implantation, embryonic disc	21.07 ± 3.76 (5)†
10 August	Embryonic disc, primitive streak and mesoderm formation	$6.85 \pm 0.78^*$ (12)
16 September	Fetus (limb bud)	5.41 ± 0.74 (16)
17 October	Fetus	$127.74 \pm 9.44^*$ (15)
17 November	Fetus	$49.02 \pm 5.62^*$ (8)
	<i>Post partum</i>	0.76 ± 0.17 (5)

Values are mean \pm s.e.m. for the number of samples given in parentheses.

†Each sample comprises plasma pooled from 2 or 3 females.

*Mean values significantly (*t* test; $P < 0.001$) different from that of the preceding month.

**Mean value significantly (*t* test; $P < 0.05$) different from that of the preceding month.

The peak in plasma progesterone concentrations at implantation was followed by a significant ($P < 0.001$) decline during August when implanted blastocysts were expanding, and values remained below 7.0 ng/ml until September (Table 1), when a gestational age of 153–180 days was reached (Table 2). A second peak in progesterone values occurred during October (127.57 ± 9.44 ng/ml) when advanced fetuses were found (Table 1).

Table 2. Changes in mean plasma progesterone concentrations (ng/ml) and placental weights (mg) in pregnant Natal clinging bats with gestation age

Fetal age† (days)	Progesterone conc. (ng/ml)		Placental weight (mg) (mean \pm s.e.m.)
	Mean \pm s.e.m.	Range	
153–166	5.01 \pm 0.74 (8)	2.94– 9.57	—
167–180	5.80 \pm 1.34 (8)	1.76– 14.20	54 — (1)
202–208	—	—	84.23 \pm 6.59 (3)
209–215	124.53 \pm 14.94 (5)	78.25–143.32	139.20* \pm 13.42 (5)
216–222	144.54 \pm 12.33 (7)	85.64–181.29	133.46 \pm 8.12 (7)
223–229	93.86 \pm 19.91 (3)	67.67–132.94	126.38 \pm 6.47 (3)
237–240	49.02* \pm 5.62 (8)	28.31– 78.05	140.55 \pm 5.76 (10)
<i>Post partum</i>	0.76* \pm 0.17 (5)	0.17– 1.2	—

*Mean value significantly different (t test; $P < 0.05$) from that of the preceding fetal age.

Values in parentheses indicate no. of bats.

†Determined as described by van der Merwe (1979).

In an attempt to correct for variability in gestational age during a specific day of sampling, the information in Table 2 reflects changes in progesterone values during pregnancy. Plasma progesterone concentrations remained relatively low (< 6.0 ng/ml) from Day 153 to Day 180 *post coitum*, increasing sharply thereafter to peak at 144.54 ± 12.33 ng/ml ($N = 7$) 216–222 days after fertilization. This increase coincided with a significant ($P < 0.05$) increase in placental weight (Table 2). Progesterone concentrations decreased gradually during the last 20 days of pregnancy and immediate pre-partum values ranged from 28.3 to 78.1 ng/ml (49.02 ± 5.62 ng/ml; $N = 8$). Values in samples taken within 2 days after parturition varied from 0.17 to 1.2 ng/ml (0.76 ± 0.17 ng/ml; $N = 5$).

Fetuses collected on 16 September differed in age by as much as 27 days while those collected on 17 October differed by 20 days. Although similar age differences can be expected during earlier embryonic development, progesterone concentrations stayed low, even in females with small fetuses (i.e. before the bidiscoidal placentae were macroscopically discernible).

Discussion

Pregnancy in the Natal clinging bat is characterized by temporal changes in plasma progesterone values, with peaks in concentration occurring during implantation and 216–222 days *post coitum* (18–24 days *pre partum*). In these bats the preimplantation period is characterized by a progressive and significant increase in progesterone concentrations, followed by an 8-week post-implantation period associated with relatively low values of plasma progesterone. The preimplantation stage of development takes place while females are occupying hibernacula on the Transvaal Highveld and when they experience periods of compulsory torpor and bouts of intensive activity. The 8-week post-implantation period coincided with the last part of hibernation and migration to maternity caves. This period is characterized by a progressive decline in luteal tissue volume (see Bernard, 1979).

Retarded embryonic development as described by Kimura & Uchida (1983) in the Japanese long-fingered bat, *Miniopterus schreibersii fuliginosus*, does not occur in the Natal clinging bat in

which the embryonic period lasts about 30 days (Bernard & Meester, 1982; van der Merwe, 1979). However, the observations of Kimura & Uchida (1983) on retarded embryonic development were based on samples collected during different years. In our experience year-to-year differences in the breeding cycle can occur which may give misleading results when data from different years are grouped. Progesterone values in the Natal clinging bat stayed low throughout post-implantation embryonic development as well as part of fetal development and only increased significantly after the bidiscoidal placentae had been firmly established.

A comparison of our data with that of Kimura *et al.* (1987), however, revealed differences during the preimplantation stage of development with progesterone values not significantly elevated during delayed implantation in the Japanese long-fingered bat (Kimura *et al.*, 1987). Values recorded during delayed implantation, however, were higher than baseline values recorded in this subspecies. This is in agreement with observations on a variety of mammals experiencing delayed implantation (see Kimura *et al.*, 1987, for references) but at variance with the transient peaks in plasma progesterone found in the little brown bat, *Myotis lucifugus*, at ovulation and blastocyst formation (Buchanan & YoungLai, 1986). Since our data for the early stages of pregnancy were grouped by date of collection and since collections were made at 5–6-week intervals transient changes in progesterone concentrations may have gone undetected.

However, the progressive increase in plasma progesterone concentrations during the pre-implantation stage in the Natal clinging bat approximates the reported pattern of increase in the volume of the corpus luteum (see Bernard, 1979). This stage, furthermore, is also characterized by a progressive increase in the development of the epithelium lining and glands of the uterus (van der Merwe, 1980). Progesterone secreted during this stage may therefore be of importance in the maintenance and development of the uterine mucosa in preparation for implantation. Luteal volume also reaches a maximum volume in some vespertilionid bats, e.g. *Lasiurus ega*, *Eptesicus furinalis*, *Myotis albescens*, *M. nigricans* (Myers, 1977), at implantation and in Australian long-fingered bats the corpus luteum are well developed during winter (Wallace, 1978). Kimura & Uchida (1983) reported that the lutein cells of the Japanese long-fingered bats are active during the preimplantation period. The importance of the corpus luteum for the maintenance of early pregnancy (pre-implantation) requires further investigation since D. A. Els (personal communication) found that the interstitial gland tissue of the ovary is the most active steroidogenic centre in the ovary. This is in agreement with the observations on several other bat species, e.g. *Antrozous pallidus* (Oxberry, 1979), *Tadarida brasiliensis* (Jerrett, 1979) and *Myotis lucifugus* (Buchanan & YoungLai, 1985).

The low concentrations of plasma progesterone recorded by us during the period of relatively slower embryonic and fetal development after implantation is to some extent in agreement with the depressed values reported by Kimura *et al.* (1987) for the Japanese long-fingered bat and a variety of other mammals experiencing embryonic diapause (see Kimura *et al.*, 1987, for references). During this stage of pregnancy the corpus luteum of the Natal clinging bat decreases in volume (Bernard, 1979) but progesterone concentrations remain slightly above the values in non-pregnant females (Table 1). In the Japanese long-fingered bat the volume of the corpus luteum was small during this stage (Kimura *et al.*, 1987). Kimura & Uchida (1983) suggested that retarded development of the Japanese long-fingered bat is a direct and passive response to cold and depressed metabolism. This is in agreement with the suggestion of Racey & Swift (1981) who found that low progesterone values in the pipistrelle (*Pipistrellus pipistrellus*) coincided with the period of cold weather. Since this phase of pregnancy in the Natal clinging bat occurs only partly during the relatively cold winter months and is also characterized by periods of activity (Norton & van der Merwe, 1978) it is doubtful that the same argument is valid for these bats.

The biphasic pattern of progesterone secretion during pregnancy in the Natal clinging bat implies different phases, possibly by different structures, of progesterone production. The sharp increase with maximum values 216–222 days after conception (Table 2) coincides with the establishment of a well developed bidiscoidal placenta (Table 2). The role of the corpus luteum and placenta in progesterone secretion during the various stages of pregnancy in these bats still needs to

be quantified. Peak values during pregnancy were similar to those recorded in *M. lucifugus* (136.2 ng/ml; Buchanan & YoungLai, 1986) but higher than those recorded in *Macrotus californicus* (28 ng/ml; Burns & Easley, 1977), *A. pallidus* (55–119 ng/ml; Oxberry, 1979), and the 10–12 ng/ml reported by Racey & Swift (1981) for *P. pipistrellus*.

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