Plasma Progesterone Levels during Pregnancy in the Little Brown Bat Myotis Lucifugus (Vespertilionidae)\textsuperscript{1}

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ABSTRACT

Plasma progesterone was measured by radioimmunoassay in individual female Myotis lucifugus throughout pregnancy and lactation. Progesterone levels, which averaged 6.7 ± 0.7 ng/ml in late hibernation, rose to a mean of 18.9 ± 6.7 ng/ml in unimplanted bats collected in the first two weeks after arrival at a maternity roost. Analysis of progesterone levels in bats in which the developmental stage of the embryo was known revealed two sharp, transient increases in plasma progesterone during the preimplantation period. The first, with values of 30–45 ng/ml, occurred at ovulation. The second, with values of 20–30 ng/ml, coincided with blastocyst formation. Progesterone levels increased exponentially from a mean of 7.4 ± 1.0 ng/ml during early implantation to peak values of 100–200 ng/ml (X = 136.2 ± 15.6) in the last two weeks of pregnancy, and showed no evidence of either midpregnancy or prepartum decline. Despite involution of the corpus luteum at the end of pregnancy, progesterone levels averaged 9.0 ± 1.0 ng/ml during lactation and did not decline until the end of lactation. In bats undergoing abortion, mean levels of plasma progesterone were already less than 6 ng/ml, equivalent to levels in nonbreeding females.

The results indicate that the progesterone profile of pregnant M. lucifugus, though generally resembling those of other bats, exhibits several distinctive features. The sharp rise in plasma progesterone coinciding with blastocyst formation has not been reported in other mammals and suggests a possible role of progesterone in the cavitation process. In addition, peak values of plasma progesterone in late pregnancy were conspicuously higher than levels reported in other vespertilionid bats. The levels did not appear to fall before parturition, although such falls have been reported in other bats.

INTRODUCTION

Circulating levels of sex steroids reflect gonadal activity; their changes during the reproductive cycle have been studied extensively in several mammalian species (Yoshinaga, 1973; Gustafson and Shemesh, 1976). In contrast, there are only a few reports concerning plasma steroids in bats; until the advent of sensitive radioimmunoassay techniques (Chard, 1983), accurate measurement of steroids in the small blood samples obtainable from bats (Kallen, 1960) was not feasible. In recent years, plasma testosterone levels have been reported for male Nyctalus noctula (Racey, 1974), Pipistrellus pipistrellus (Racey and Tam, 1974) and Myotis lucifugus (Gustafson and Shemesh, 1976). Both estrogen and progesterone levels during pregnancy have been measured in Macrotus californicus (Phyllostomatidae) (Burns and Wallace, 1975; Burns and Easley, 1977) and Antrozous pallidus (Vespertilionidae) (Oxberry, 1979). Plasma progesterone values have been reported for Tadarida brasiliensis (Molossidae) (Jerrett, 1979) and P. pipistrellus (Vespertilionidae) (Racey and Swift, 1981). As part of a study of basic reproductive physiology in another vespertilionid, Myotis lucifugus lucifugus, we have measured circulating progesterone levels in females from the end of hibernation through lactation.

MATERIALS AND METHODS

Most of the bats used were captured in mist nets set outside a large maternity roost at Red Bay, Bruce

\begin{footnotesize}
\begin{enumerate}
\item This work was supported by grants from the Medical Research Council of Canada. Portions of these data were presented at the 18th Annual Meeting of the Society for the Study of Reproduction, July 22–25, 1985, Montreal, Canada, and appeared in Biol. Reprod. 32 (Suppl. 1):120.
\item Reprint requests: Dr. G. Dale Buchanan, Faculty of Health Sciences, McMaster University, 1200 Main St. W., Room 2J32, Hamilton, Ontario, Canada L8N 3Z5.
\end{enumerate}
\end{footnotesize}
RESULTS

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Adams and separated Plasma is estrone, testosterone, androstenedione, estradiol, and 20a-dihydroprogesterone, which have crossreactivity with 20a-dihydroprogesterone, testosterone, dihydrotestosterone, androstenedione, estrone, and estradiol. Corticosterone crossreactivity is 1.2%. The labeled ligand used was [1,2H]proges-
terone (57 Ci/mmole, New England Nuclear, Boston, MA). The sensitivity limit of the assay is 10 pg/tube. Plasma samples (10–41 μl) were precipitated with 1 ml absolute ethanol, and suitable aliquots of the ethanol extract were taken for assay. Free steroid was separated from bound with dextran-coated charcoal, and the bound fraction was counted in a Beckman LS233 liquid scintillation counter. In a number of instances, sufficient plasma was available to assay the same sample on three separate occasions. Coefficients of variation for low (2.9 ± 0.3 ng/ml) and medium (11.8 ± 1.1 ng/ml) sample concentrations were 16.5% and 15.6%, respectively. For 18 samples (2.8–30.0 ng/ml) run on different occasions, the percentage difference from the mean was 26.2 ± 3.2%. Comparison made on another series of samples showed that the difference between precipitation with alcohol and extraction with ether before assay was 31.0 ± 4.4% (n=8). Although it was possible to measure progesterone in all individual samples, estradiol levels were below the sensitivity (10 pg) of our assay system.

The data were examined by a one-way analysis of variance, and the difference in group means was tested for statistical significance by Duncan’s Multiple Range Test (Duncan, 1955). Additionally, values for each stage of pregnancy and lactation were compared with pregnant (late hibernation) levels by computing the t statistic for the distribution of the difference of means of unpaired samples (Snedecor and Cochran, 1967).

RESULTS

The data have been grouped by developmental stage rather than collection date, since pregnancy in M. lucifugus is not highly synchronized within the colony (Tuttle and Stevenson, 1982). Pregnancy was asynchronous by at least three weeks in the Red Bay colony, since newly parturient bats were collected first on 29 June. The percentages of pregnant bats in the later collections were 4 July, 90%; 12 July, 50%; 19 July, 10%. Most of the asynchrony is due to individual variation in time of arousal from hibernation. However, the time of ovulation with respect to arousal varies by at least 48 h (Buchanan, 1984), which is significant during early pregnancy.

Pregnancy, 50–60 days in M. lucifugus (Wimsatt, 1945), was divided into five stages as shown in Figure 1. Stage I, the preimplantation period, lasts about 10 days (Buchanan, 1984). Both parous and nulliparous uteri were variably hyperemic and edematous, but not different in size or shape from before ovulation (Figs. 1A,B). In Stage II, the early implantation period, the entire uterus becomes turgid, the right uterine horn becomes markedly convex along the antimesometrial (cranial) border (Fig. 1C), and the implantation site is often visible as an erythematous spot. During both Stages I and II, exact developmental status
can be determined only by actually examining the conceptus. In subsequent stages, uterine width, the distance between the uterotubal junctions, increases progressively and can be used as a convenient index of relative development within stages. During Stage III, the period of embryogenesis, the uterus nearly doubles in width. The right uterine horn assumes an ovoid shape, but the left uterine horn is still recognizable (Fig. 1D). In the second half of pregnancy, the fetal growth period, uterine width increases from 7.5 to 22+ mm. At the beginning of this period, the left uterine horn becomes incorporated into the wall of the expanding uterus and is no longer distinguishable (Fig. 1E). Near the end of pregnancy, the thinly stretched wall conforms to the fetal contours (Fig. 1F). In the absence of any significant developmental landmarks, the fetal period was divided arbitrarily into equal time periods, Stages IV and V.

Plasma progesterone values from 35 bats examined during Stages I and II are shown in Figure 2 arranged according to the developmental status of the conceptus. All but four of these bats were from the Craigmont colony and were autopsied at precise intervals after arousal from hibernation. The variation in time of ovulation with respect to arousal is reflected in the occasional time overlaps between successive developmental stages (e.g., uniovulated bats vs. bats with fertilized ova) and in the recovery of the same developmental stage at different times after arousal (e.g., fertilized ova, 4-cell embryos). Morulae were arrayed by increasing cell number and blastocysts by maximum diameter. Figure 2 reveals two sharp, transient increases in plasma progesterone during the preimplantation period: the first at the time of ovulation, and the second about three days later at the beginning of blastocyst formation. Figure 2 also shows that
progesterone levels did not change noticeably at implantation.

Table 1 shows mean plasma progesterone levels at different stages of pregnancy in bats from the Red Bay colony plus values from eight hibernating bats from Craigmont for comparison. Since experimental protocols precluded examination of embryos in bats collected during early pregnancy, Stage I bats collected in the first two weeks of the maternity season were analyzed separately from those collected later. Due to variation in arousal times and hence ovulation, the former should contain bats representing all phases of preimplantation development, whereas the latter should consist primarily of bats bearing blastocysts. As shown, several of the bats in the 3–11 May collections had high progesterone levels, similar to those seen in Figure 2; the mean value, 18.9 ng/ml, agreed substantially with the mean of 14.4 ± 2.0 ng/ml for the 30 unimplanted bats in Figure 2. In contrast, progesterone levels in bats in the Stage I collections of 14–17 May and levels in Stage II collections did not differ from preovulatory levels. Plasma progesterone increased modestly during embryogenesis (Stage III), then rose rapidly during the fetal period to reach peak values in the last two weeks of pregnancy (Stage V). Despite involution of the corpus luteum, progesterone values in lactating bats remained above preovulatory and early implantation levels and showed no evidence of a decline during lactation.

A one-way analysis of variance indicated significant interactions (F(4,9) = 50.89, d.f. = 7, 59, p<0.001) between plasma progesterone levels at different stages of pregnancy. Duncan’s Multiple Range test showed that mean values for Stage V differed statistically from all other means, and at Stage IV, means differed from all other means except the Stage I mean for 3–11 May. No statistically significant difference appeared among other values. However, comparison of the various stages of pregnancy against late hibernating (prepregnant) levels, by computing the difference in means for unpaired samples, showed statistically significant differences between all except the material from late Stage I and Stage II.

In this study, we collected several nulliparous adult females that were anovular. Their ovaries possessed neither mature follicles nor corpora lutea or corpora albicantia. The small, essentially symmetrical uteri of these bats showed no histological evidence of hor-
TABLE 1. Plasma progesterone levels in *Myotis lucifugus* during pregnancy and lactation.

<table>
<thead>
<tr>
<th>Reproductive status</th>
<th>Days postovul.</th>
<th>Uterine width (mm.)</th>
<th>No. bats</th>
<th>Plasma progesterone range (ng/ml)</th>
<th>X ± SEM</th>
<th>Diff. from hib.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hibernating</td>
<td>---</td>
<td>4</td>
<td>(8)</td>
<td>5.2 - 9.7</td>
<td>6.7 ± 0.7d</td>
<td>---</td>
</tr>
<tr>
<td>Stage I</td>
<td>1 - 10</td>
<td>4</td>
<td>(7)</td>
<td>2.6 - 45.0</td>
<td>18.9 ± 6.7d,e</td>
<td>*</td>
</tr>
<tr>
<td>(3 - 11 May)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>1 - 10</td>
<td>4</td>
<td>(7)</td>
<td>3.8 - 14.5</td>
<td>6.9 ± 1.3d</td>
<td>ns</td>
</tr>
<tr>
<td>(14 - 17 May)</td>
<td>4 - 7</td>
<td>4</td>
<td>(7)</td>
<td>5.2 - 13.7</td>
<td>9.3 ± 0.9d</td>
<td>**</td>
</tr>
<tr>
<td>Stage II</td>
<td>10 - 14</td>
<td>4</td>
<td>(9)</td>
<td>20.3 - 56.0</td>
<td>33.4 ± 4.4c</td>
<td>***</td>
</tr>
<tr>
<td>Stage III</td>
<td>14 - 28</td>
<td>7.5 - 12</td>
<td>(7)</td>
<td>80.9 - 206.0</td>
<td>136.2 ± 15.6c</td>
<td>***</td>
</tr>
<tr>
<td>Stage IV</td>
<td>28 - 42</td>
<td>13 - 22+</td>
<td>(9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage V</td>
<td>42-term</td>
<td>13 - 22+</td>
<td>(7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactating</td>
<td>---</td>
<td>---</td>
<td>(10)</td>
<td>6.1 - 16.0</td>
<td>9.0 ± 1.0d</td>
<td>*</td>
</tr>
<tr>
<td>Anovular⁸</td>
<td>---</td>
<td>---</td>
<td>(10)</td>
<td>1.7 - 10.6</td>
<td>5.7 ± 0.9d</td>
<td></td>
</tr>
<tr>
<td>Abortingʰ</td>
<td>---</td>
<td>---</td>
<td>(7)</td>
<td>2.4 - 19.1</td>
<td>5.9 ± 2.3d</td>
<td></td>
</tr>
<tr>
<td>Aborted</td>
<td>---</td>
<td>---</td>
<td>(7)</td>
<td>2.6 - 15.3</td>
<td>6.3 ± 1.7²</td>
<td></td>
</tr>
</tbody>
</table>

*Hibernating bats were collected in late March; stages of pregnancy are described in text.

¹Intervals for Stages I–III determined from timed pregnancies (Buchanan, 1984), other intervals estimated from field collections.

²Statistical difference between means of unpaired samples for hibernation vs. respective stages: n = not significant, * = p<0.05, ** = p<0.025, *** = p<0.005.

³Figures without common superscripts differ (p<0.05) by Duncan’s Multiple Range Test.

⁴Nulliparous adults with neither mature follicles nor corpora lutea.

⁵Exhibiting some intrauterine bleeding.

⁶Figures not included in statistical analysis.

monal stimulation. On the basis of other data (Buchanan, unpublished), we believe these bats were yearlings that did not complete folliculogenesis before entering hibernation, and that the low plasma progesterone levels shown in Table 1 represent basal levels for sexually inactive *M. lucifugus*. A number of bats collected in June aborted shortly after capture. Most of the bats were in Stage IV or V, although a few may have been in late Stage III. As Table I shows, even in bats just beginning to abort, plasma progesterone had already fallen to levels approximating those found in anovular bats.

**DISCUSSION**

Transient peaks in plasma progesterone, such as we found in *M. lucifugus* at ovulation, and blastocyst formation have not been reported in *Antrozous pallidus* (Oxberry, 1979), *Tadarida brasiliensis mexicana* (Jettret, 1979) or *Macrotus californicus* (Burns and Easley, 1977); in all of these bats, progesterone levels are reported to rise at ovulation and to continue to rise subsequently. The plasma progesterone pattern in *M. lucifugus* may, of course, be species-specific. However, in the studies mentioned, data were grouped by time of examination and collections were less frequent. As a result, transient changes in plasma progesterone could have been obscured or gone undetected. In any event, it seems certain that the periovulatory rise in progesterone is due to the ovulatory surge of the pituitary luteinizing hormone (LH), since similar LH-induced progesterone peaks occur in rats (Morishige et al., 1973), mice (McCormack and Greenwald, 1974; Michael et al., 1975), hamsters (Leavitt and Blaha, 1970), and guinea pigs (Challis et al., 1971). Humans, on the other hand, exhibit ovulatory peaks of both 17-hydroxyprogesterone and progesterone (Strott et al., 1969), and rabbits show an ovulatory peak of 20α-hydroxyprogesterone (Challis et al., 1973).

The presence of a discrete peak in plasma progesterone correlated with the beginning of blastocyst formation is intriguing; so far as we are aware, similar progesterone peaks have not been noted in other mammals. In fact, progesterone peaks do occur when blastocysts are forming in rats, mice, and guinea pigs (loc. cit.); however, in these species, blastocysts form 24 h or less before implantation, and the rise in progesterone appears intuitively to be related to nidation. On the other hand, transient peaks in plasma progesterone are not seen in carnivores (Heap and Hammond, 1974) or in ungulates (Yoshinaga, 1973), although both have long preimplantation periods, and blastocysts formation and implanta-
tion are clearly separated. In both groups, the corpus luteum is the paramount source of progesterone, and plasma levels, which are quite low at ovulation, rise steeply and continuously during early pregnancy. In contrast, steroidogenetic activity is prominent in the interstitial tissue of several bats (Myotis grisescens, Guraya and Greenwald, 1964; A. pallidus, Oxberry, 1979) (T. brasiliensis mexicana, Jerrett, 1979) throughout the year including pregnancy, and unpublished data from this laboratory show the same to be true in M. lucifugus. Further, the corpus luteum of M. lucifugus does not appear to be a significant source of steroidogenesis until after implantation; a recent study showed that ovarian progesterone content did not differ between ovaries with or without corpora lutea during Stages I and II of pregnancy (Buchanan and Younglai, 1985). In any event, the present data suggest that progesterone may play a role in formation of blastocysts. This concept is also supported by the report of Wang et al. (1984), who showed that progesterone antibodies administered to mice after coition not only prevented implantation, but blocked embryonic development at the morula stage.

Plasma progesterone levels rose exponentially after implantation and reached peak values in the final two weeks of pregnancy (Stage V). The peak values we obtained were considerably higher than those reported in two other vesperilionid bats, A. pallidus, 50 ng/ml (Oxberry, 1979) and Pipistrellus pipistrellus, 10–12 ng/ml (40 nmol/l) (Racey and Swift, 1981). However, Jerrett (1979) reported a progesterone peak of 106 ng/ml in the molossid bat, T. brasiliensis mexicana; and although Burns and Easley (1977) found high values of just over 30 ng/ml in the phyllostomatid bat, M. californicus, Jerrett (1979) cited unpublished data of P. H. Krutzsch showing individual values as high as 119 ng/ml in this species.

Oxberry (1979) reported a significant decrease in plasma progesterone in A. pallidus during the middle third of pregnancy, and Racey and Swift (1981) show a similar but not statistically significant decline just after midpregnancy in P. pipistrellus. We saw no evidence of a midpregnancy fall in plasma progesterone levels in M. lucifugus even when the data in Table 1 were arranged by examination time or increasing fetal size within stage. Likewise, we saw no evidence of a prepartum decline in plasma progesterone, although such a decline has been reported in all other bats studied. The present data do not exclude the possibility of a prepartum fall in progesterone values; however, the two Stage V bats listed in Table 1 with highest plasma values (191 and 206 ng/ml) carried fetuses which were indistinguishable from neonesates, suggesting that any prepartum decline must occur quite close to the end of gestation.

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REFERENCES


Gustafsson AW, Shemesh M, 1976. Changes in plasma testosterone levels during the annual reproductive cycle of the hibernating bat, Myotis lucifugus lucifugus, with a survey of plasma testosterone levels in adult male vertebrates. Biol Reprod 15:9–24


