

Delayed development in Fischer's pygmy fruit bat, *Haplonycteris fischeri*, in the Philippines

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Summary. A long delay in post-implantation embryonic development was detected in Fischer's pygmy fruit bats (palaeotropical fruit bats of the suborder Megachiroptera), the first time such a delay has been demonstrated outside the bat suborder Microchiroptera. Samples of bats were obtained from the Philippines over 5 years, and reproductive tracts were preserved and examined using standard histological techniques. Most parous female pygmy bats were impregnated in June, within a few weeks of parturition, and the embryos underwent superficial implantation at the anterior end of the uterus contralateral to the previously gravid uterus. Shortly thereafter, the rate of embryonic growth slowed tremendously for up to 8 months. During the period of delay, the mean length of the embryoblast increased only from 280 μm to 520 μm . In March of the following year, the developmental rate increased, and the embryos completed development in the next 3 months. The 8-month delay gives these bats a gestation period of 11.5 months, the longest known in bats. Most nulliparous females become pregnant at an age of 3–5 months, and their embryos entered a similar delay that terminated in March or April, after 2–6 months of delay. Males showed signs of fertility throughout the entire year, but testis volume was highest during May, June and July, at about the time when most females become receptive.

Keywords: fruit bat; delayed development; *Haplonycteris*; reproduction

Introduction

Delays between copulation and parturition have been intensively studied as model systems for the timing and control of reproduction. Delayed implantation is the most common delay process and has been found in species in 7 distantly-related orders of mammals (Renfree & Calaby, 1981). Delayed fertilization (by spermatozoa stored in the uterus) is the norm in temperate-zone bats (Gustafson, 1979; Oxberry, 1979; Racey, 1982), and has also been recorded in several species of subtropical and tropical bats (Myers, 1977; Krutzsch, 1979). Post-implantation delayed development is the least common form of delay in mammals, and is known from only a few species of bats in the suborder Microchiroptera (Bradshaw, 1962; Fleming, 1971; Bernard & Meester, 1982; see also Racey & Swift, 1981). While much is known about delayed implantation (e.g. Flint *et al.*, 1981), the causes and controls of delayed development have been less tractable (Burns *et al.*, 1972; Burns & Wallace, 1975; Burns & Easley, 1977; Burns, 1981), and descriptions of delay periods are available for only two species (Bradshaw, 1962; Fleming, 1971).

This study describes a delay in post-implantation development that leads to a pronounced seasonal reproductive pattern in Fischer's pygmy fruit bat, *Haplonycteris fischeri* (Suborder Megachiroptera; Family Pteropodidae), a species restricted to the humid tropics. The objectives of

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this paper are: (1) to demonstrate the occurrence of a post-implantation delay in development in Fischer's pygmy fruit bat (referred to hereafter as the pygmy bat) in the Philippines; (2) to describe differences in the timing of delay between parous adult females and primiparous females; (3) to provide evidence that the timing of reproductive events is similar in each year; and (4) to propose some possible mechanisms for control of the delay.

Materials and Methods

The study was conducted during the period 1979–1987 on Negros Island in the central Philippines. The specimens were obtained in a forested valley, the Lake Balinsasayao watershed, at elevations of 800–1200 m (9°11'N, 121°23'E). A detailed description of the site and its climate and flora is presented elsewhere (Heideman, 1987). Rainfall at the site is mildly seasonal in annual distribution, and the usually short and mild dry season is generally centred around April. Flower and fruit production were mildly seasonal, with slight peaks in the dry season and first half of the wet season (Heideman, 1987).

These pygmy bats are small frugivores weighing 16–20 g. The genus *Haplonycteris* is monotypic and endemic to the Philippine Islands, where it is restricted to primary forest habitats. Pygmy bats feed mostly on fruits, including species of *Ficus* (figs) and probably fruits of plants in the genus *Piper*, but may also depend upon flowers during parts of the year (Utzurum, 1984; P. D. Heideman & R. C. B. Utzurum, unpublished data).

Specimens were obtained in June 1979, June 1981, and monthly from June 1982 to June 1983, supplemented with live captures in February 1984, and monthly from April 1984 to July 1984. Monthly sample sizes for 1979–1983 are presented in Table 1.

Of 578 individuals captured during 1979–1983, 259 were marked and released after external examinations of reproductive condition, including palpation of females to check for embryos or fetuses. Embryos were detectable by palpation when the diameter of the portion of the uterus containing the conceptus was 3 mm or greater (see Heideman, 1988). Reproductive autopsies were performed on the remainder. I recorded position and greatest length of uterine swellings/embryos (if present), size of the nipples, degree of development of mammary tissue, lactational

Table 1. Sample sizes* of pygmy fruit bats (*Haplonycteris fischeri*) from Negros Island from 1979 to 1983

Period	Males		Females		Total
	Subadults†	Adults‡	Subadults†	Adults‡	
12–13 June 1979	0	0	0	2 (2) [2]	2
3–15 June 1981	0	13 (13) [0]	0	15 (15) [12]	28
1982–83					
28 June/8 July	4 (4) [0]	13 (13) [2]	2 (1) [0]	22 (20) [19]	41
29 July/3 August	15 (5) [0]	15 (11) [1]	11 (6) [1]	41 (15) [14]	82
27 August/1 September	16 (5) [0]	7 (7) [2]	34 (12) [4]	28 (21) [11]	85
4–12 October	11 (6) [2]	4 (4) [2]	5 (1) [1]	5 (5) [3]	25
4–13 November	17 (6) [0]	9 (6) [2]	10 (5) [4]	24 (7) [3]	60
6–11 December	14 (4) [0]	11 (6) [3]	5 (4) [2]	16 (8) [3]	46
8–13 January	8 (4) [0]	6 (5) [3]	3 (3) [3]	18 (9) [6]	35
4–12 February	7 (6) [0]	2 (2) [1]	1 (1) [1]	2 (2) [2]	12
4–9 March	12 (5) [1]	18 (8) [3]	5 (3) [3]	31 (8) [7]	66
7&9, 14, 26–28 April	6 (3) [0]	11 (6) [2]	8 (3) [3]	27 (9) [4]	52
2–8, 19–21, 25 May	9 (3) [0]	12 (5) [2]	4 (1) [0]	23 (3) [1]	48
5–12 June	9 (8) [0]	7 (6) [2]	3 (3) [2]	25 (14) [7]	44
Total	128 (59) [3]	128 (79) [25]	91 (43) [24]	279 (138) [94]	626

*The numbers do not sum to the total number of animals captured (578), as 48 of the captures were recaptures from previous months. The numbers in parentheses are totals autopsied, and the numbers in brackets are totals sectioned (7–9 April and 2–8 May).

†Up to 12 months of age.

‡> 12 months of age.

status, and differences in ovary size and colour. For males, I recorded the extent and colour of glandular throat patches, testis length, testis width, testis colour, seminal vesicle length, and a qualitative assessment of seminal vesicle volume. Testis volume was approximated using the formula for a prolate spheroid ($V = 0.523 \times \text{length} \times \text{width}^2$) (Lidicker, 1973). Reproductive tracts were removed, fixed in Bouin's solution, dehydrated in a graded ethanol series, embedded in Paraplast, serially sectioned at 5–10 μm , and stained with haematoxylin and eosin.

All animals were classed as young or adult on the basis of epiphyseal fusion within the phalanges. Animals aged 8–10 months were more difficult to recognize by degree of epiphyseal fusion, and many males and females aged 10–12 months were indistinguishable from older animals by this technique alone. For males, the degree of development of throat glands was also used to assess age in 8–14-month-old individuals. Female pygmy bats from 8–12 months of age could be recognized by their relatively small nipples, which did not attain the size of those of older adults until they were first suckled, usually at an age of about 12 months.

In order to describe the stages of embryonic development during the delay, I defined substages of gastrulation and embryonic disc formation. Stages were defined on the basis of observable changes in pygmy bat embryos, and are described in the results.

Most statistical tests were carried out on the MIDAS computer program on the MTS computer system of The University of Michigan. The contrasts used in the analyses of variance were constructed from a-priori predictions. Binomial tests (Sokal & Rohlf, 1981) were carried out on a hand calculator.

Results

Reproduction in males

Spermatozoa were present in the cauda epididymidis of adult males in all months of the year. However, there were small but significant changes in testis volume of adult males during the year (one-way analysis of variance; $F = 2.02$; $df = 10$; $P < 0.05$; Fox & Guire, 1976), and a pre-planned comparison indicated that testis volume was significantly higher in the months of May and June (one-way analysis of variance; $F = 17.19$; $P < 0.001$; Fox & Guire, 1976), at about the time when most parous females became receptive (Fig. 1a). For males born in the previous May or June, testis volume remained very low throughout October, and then began a gradual increase in volume that continued until mean testis volume of this group at 12 months of age (in June) was equivalent to that of adults at the same time of year (Fig. 1b). A pre-planned comparison indicated that testis volume for these young males was significantly higher during May and June, at about the time when most parous females became receptive, than in other months (one-way analysis of variance; $F = 43.59$; $P < 0.0001$; Fox & Guire, 1976). In these young males, changes in seminal vesicle length and relative volume paralleled those in testis volume.

Reproduction in females

Female pygmy bats have a long duplex uterus (as defined by Mossman, 1987). The female reproductive tract is very similar to that of *Cynopterus sphinx* (Gopalakrishna & Karim, 1980; Sandhu, 1984), except that the two uteri have separate cervical canals, which fuse subterminally and centrally in the relatively shorter cervix. Quiescent uteri are generally about 1 mm in diameter in multiparous females, and about half that diameter in prepubertal females. The ovary is almost entirely enclosed in a bursa, with a small slit-shaped opening to the peritoneal cavity. The oviduct arises medially and posteriorly on the ovarian bursa, adjacent to the opening, and loops cranially and laterally around the ovary before meeting and opening into the uterus just posterior to its cranial tip.

Follicular development

Throughout pregnancy, primordial, primary, secondary, very early antral, and atretic follicles were found in both ovaries. Larger antral follicles and Graafian follicles (Fig. 2) were found only in May, June or July in females that were near term (3 of 5 cases) or post-parturient (5 of 8 cases).

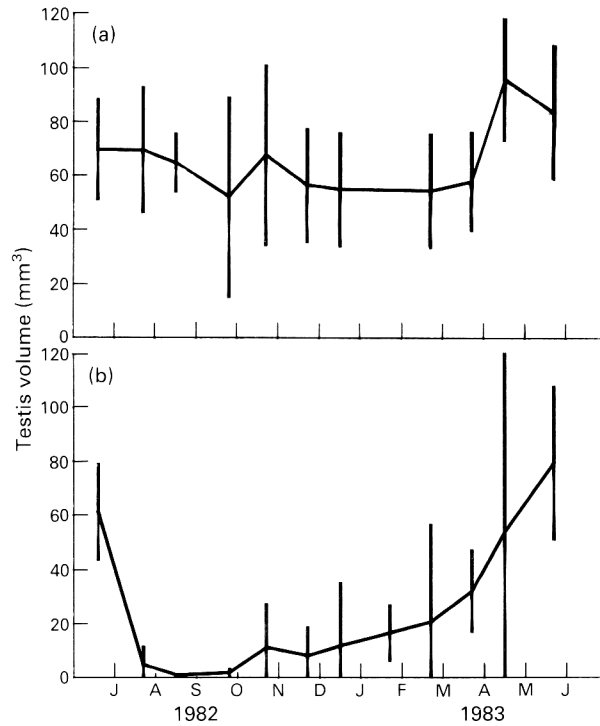


Fig. 1. Mean monthly testis volume of pygmy bats captured from June 1982 to June 1983. The bars indicate Scheffé 95% confidence intervals for each mean. (a) Adults (12 or more months in age), (b) young (1-12 months in age).

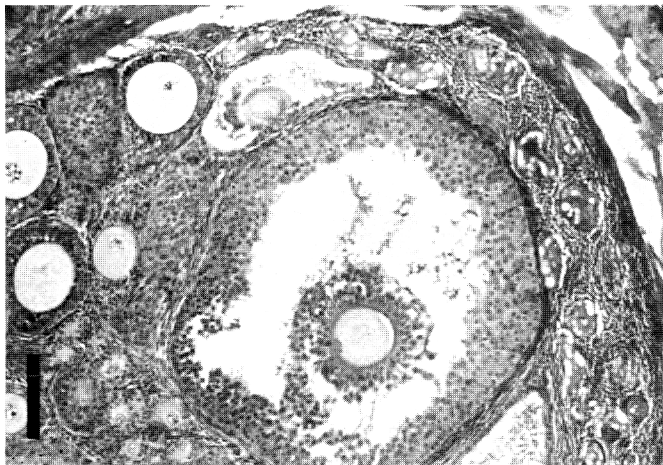


Fig. 2. Ovary with a large Graafian follicle near ovulation on 5 July 1982. Primary and secondary follicles are visible at the left of the figure, and old atretic follicles are visible right of the large follicle. Scale bar = 100 μ m.

Progestational reaction

Post-parturient uteri were identifiable for at least 1 month after parturition by their large size (diameter up to 5 mm), near absence or lack of uterine glands, extensive damage to the endometrium, and large quantities of blood and loose tissue within the lumen. In contrast, quiescent uteri from multiparous adults had a small lumen and moderately developed uterine glands. Sectioned quiescent uteri of parous adults averaged 1.00 mm in diameter (Table 2). In preovulatory females, the diameter of the cranial end of quiescent uteri had increased by 13%. Uteri with an ovum or blastocyst within the oviduct or uterine lumen had increased in diameter by an additional 12%. Figure 3 contrasts uteri of a female carrying a preimplantation embryo. The non-pregnant uterus in this female (Fig. 3b) showed no apparent progestational reaction, and is similar in appearance to sections through quiescent uteri. Quiescent uteri in reproductively mature nulliparous and primiparous females were generally smaller than in parous females, but in primiparous females the diameter of the cranial end of the uterus ipsilateral to a preimplantation embryo had increased nearly to adult size. The hypertrophy of the cranial end of the uterus at the beginning of pregnancy (Table 2) is similar to that described as a 'progestational reaction' for other pteropodid bats (Marshall, 1953; Gopalakrishna & Karim, 1971), except that it is much milder in pygmy bats.

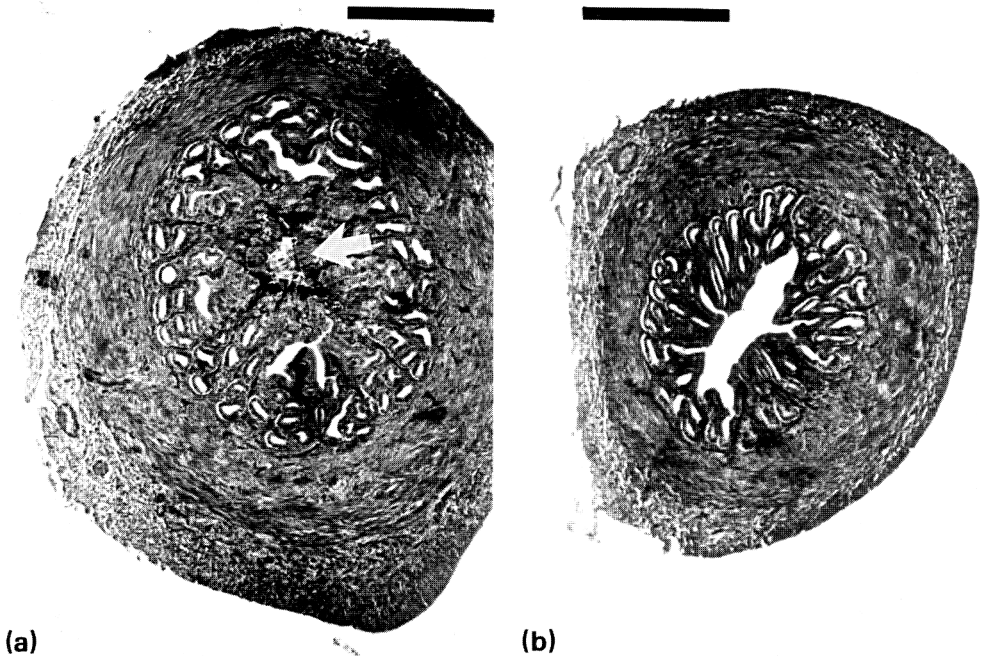


Fig. 3. Cross sections through the cranial ends of the pregnant, progestational uterus (a) and the non-pregnant uterus (b) from a primigravid female captured on 8 January 1983. The larger progestational uterus (a) holds an unimplanted blastocyst (white arrow) composed of an inner cell mass and trophoblast. The more darkly staining tissue in the region of the blastocyst is uterine tissue that is being eroded. Scale bars = 250 μ m.

Mating

There was evidence of recent mating, assuming that pygmy bats do not store spermatozoa, in the form of spermatozoa in uteri, or of females bearing preimplantation embryos, in all months

Table 2. Mean of greatest length of inner cell mass (ICM) and maximum diameter of gravid uterus during developmental stages occurring within the period of delayed development and as development began accelerating at the end of delay

Developmental stage	No.*	Maximum diam. of gravid uterus (mm)	Maximum length of ICM (μ m)
Early gastrulation	15	2.4	220
Mid-gastrulation	49	2.8	350
Late gastrulation	8	3.1	450
Early plate	10	3.6	545
Mid-plate	5	4.5	1300
Late plate	1	3.5	1400

*Includes some females captured in 1984 on Negros and two other islands.

except February, April and December, suggesting that at least some males were reproductively capable in any given month. Spermatozoa were not found in the reproductive tracts of any near-term females (0 of 5 near-term females), but spermatozoa were found in post-parturient, preovulatory females (5 of 7 cases, including 2 of 3 females lacking tertiary follicles). Some spermatozoa were found in the uteri of 8 of 11 females with embryos between the 16-cell stage and implantation and in 6 of 15 females with implanted embryos 1–2 months in age, but were not found in 6 females with 4–5-month embryos. A female captured on 4 March 1983 contained a 16-cell morula and a corpus luteum with a large, blood-filled central cavity, indicative of ovulation and mating as late as February or early March.

Ovulation, fertilization and ovarian dominance

Ovulation in most parous females occurred in late May, June or early July (Fig. 4). Ovulation followed parturition by a few weeks (Fig. 4), occurring before full reduction in size of the post-parturient uterus (11 of 11 cases). One parous female with a tubal morula and a blood-filled cavity within the corpus luteum was found in early March, indicating ovulation as late as February or early March (Fig. 4). In that female, the contralateral ovary held a regressed corpus luteum. No evidence of polyovulation was seen (nor were normally developing twins ever observed; Heideman, 1988).

There was no significant difference between the number of ovulations from the right and left ovaries (78 right, 62 left; $P > 0.10$; binomial test; because only one corpus luteum was found in all females, corpora lutea in an ovary were taken as evidence of ovulation from that ovary). In all cases, large tertiary follicles, a tubal ovum, or a tubal embryo were contralateral to the post-parturient uterus (12 of 12 cases; $P < 0.05$, binomial test), indicating that ovulation alternated between ovaries. Ovarian dominance would be expected to be more apparent in primigravid females than in parous females if it is characteristic of a species in which ovulation typically alternates between ovaries. This was not the case for pygmy bats, as the number of cases of ovulation and/or implantation on each side was not statistically different for primigravid females (13 right, 11 left; $P > 0.75$; binomial test) or multiparous females (65 right, 51 left; $P > 0.10$; binomial test). All embryos were found in the uterus ipsilateral to the corpus luteum. Unfertilized ova and early embryos were found only near the ovarian end of the oviduct. All tubal embryos were still enclosed by zonae pellucidae, with the exception of one degenerating tubal morula.

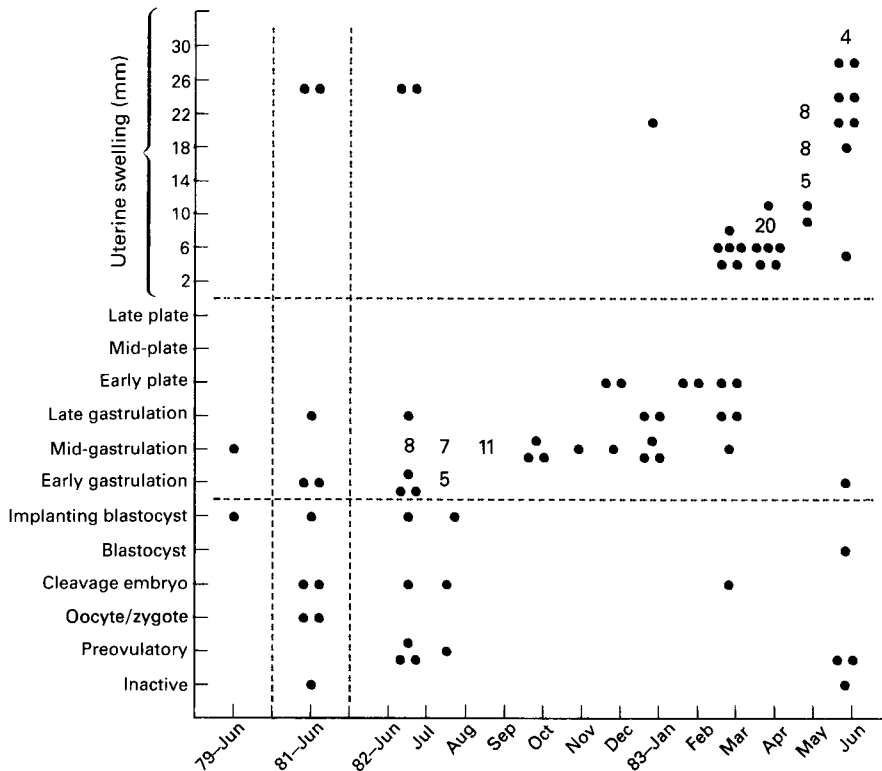


Fig. 4. Non-pregnant parous female pygmy bats and the developmental stages of embryos from pregnant multiparous females captured from June 1979 to June 1983. Solid circles and numbers represent individual embryos and a number of embryos at the same stage, respectively. Only sectioned reproductive tracts from non-pregnant females and those with embryos at stages from ovulation through to the completion of plate formation have been included on the figure, except for females with a uterine swelling of 5 mm or greater. Marks on the x axis indicate midpoints of months; y axis labels are defined in the text.

Most young females ovulated in August, September or October, although one young female with a tubal blastocyst was captured in early January (Fig. 5).

Implantation

In most parous females, implantation occurred in June, with a few in July or August, and one occurring as late as March (Fig. 4). In most primiparous females, implantation occurred in October or November, with evidence of implantation in one female as late as January (Fig. 5).

The embryo apparently usually reached the uterus as a blastocyst still enclosed by the zona pellucida; in 2 of 4 cases the zona pellucida either still surrounded part of the blastocyst or was present near the blastocyst in the uterus. Unimplanted and implanting blastocysts were located centrally within the preferentially stimulated portion of the uterus ipsilateral to the corpus luteum. Implanting blastocysts conformed to the shape of the uterine lumen until the yolk sac cavity expanded with fluid. Where the developing trophoblast was in contact with the endometrium, the uterine epithelium was either missing or disintegrating.

Initial adhesion of the blastocyst in the pygmy bat is superficial, although trophoblast cells did grow preferentially into the lumina of the uterine glands near the blastocyst. Three preimplantation or very early implanting blastocysts were available for study, and these consisted only of an inner

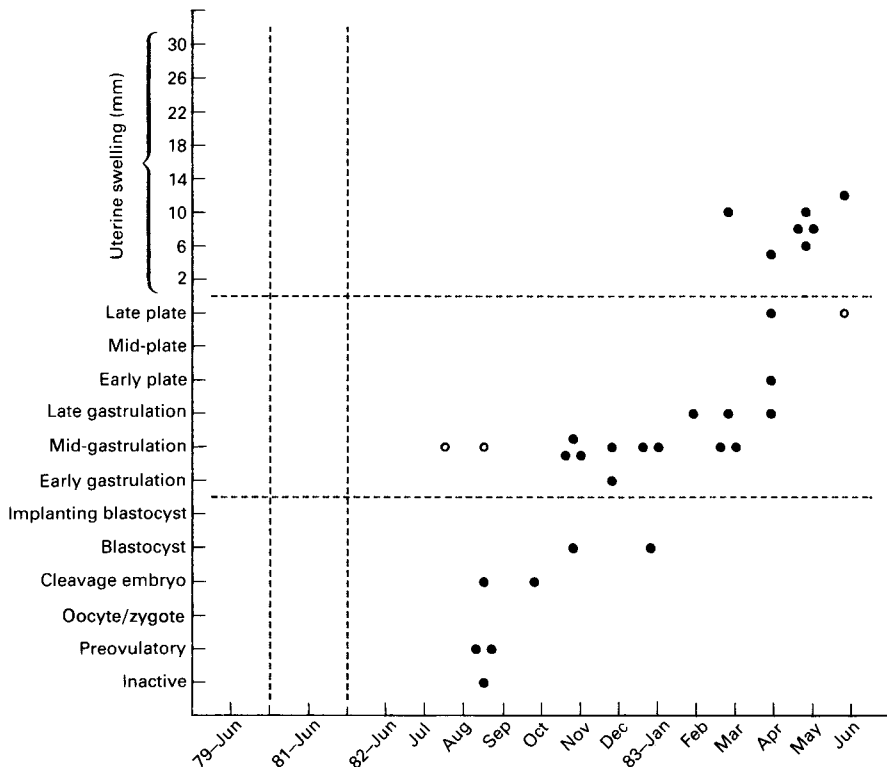


Fig. 5. Non-pregnant subadult female pygmy bats and the developmental stages of embryos from pregnant primigravid and non-parous females captured from June 1979 to June 1983. Solid circles represent individual embryos from females < 1 year old; open circles represent embryos from non-parous females judged to be > 1 year old. Only sectioned reproductive tracts from non-pregnant females and those with embryos at stages from ovulation through the completion of plate formation have been included on the figure, except for embryos for which the uterine swelling was 5 mm or greater. Marks on the x axis indicate midpoints of months; y axis labels are defined in the text.

cell mass and a single layer of trophoblast (Fig. 3). In 2 of these blastocysts, uterine tissue adjacent to the trophoblast showed signs of erosion, suggesting that implantation may have begun. In 2 blastocysts at a later stage, the inner cell mass was attached to one embryonic pole of the trophoblast within a blastocoele. At the end of implantation (4 animals examined) syncytiotrophoblast had begun to form outside the cytotrophoblast (Fig. 6), and the inner cell mass consisted of a sphere of cells surrounded by a layer of endoderm. The endoderm lined the yolk sac cavity and was separated from the cytotrophoblast by Reichert's membrane (Fig. 7). In one of these specimens, the endodermal layer and Reichert's membrane were still incomplete at the abembryonic pole. In another one of these, the central sphere of the embryoblast had differentiated into an outer layer of columnar ectodermal cells and an inner sphere of cells (Fig. 8).

There were thick accumulations of cells of the extraembryonic endoderm of the yolk sac in the region of the embryoblast of many embryos in early delay (Figs 8 & 10). These areas occasionally included extracellular material similar in staining properties to Reichert's membrane. J. J. Rasweiler (personal communication) noted that the situation in pygmy bats (in some of this material he examined) was reminiscent of that in the phyllostomid bat *Glossophaga soricina* (Rasweiler, 1974).

A layer of large, multinucleate cells morphologically similar to those described as 'giant cells' in other mammals (Mossman, 1939; Wimsatt, 1951) appeared in the uterus at this stage (Fig. 6). By this point the syncytiotrophoblast and cytotrophoblast of the embryo had engulfed maternal blood spaces in the adjacent endometrium (Fig. 9); I considered this stage to mark the completion of implantation. Determination of the precise relationship between trophoblast and maternal tissue at this stage requires further study at the ultrastructural level.

Since relatively few specimens were found with embryos in stages of implantation, the period of development up to this latter stage may be rapid, possibly less than 1 or 2 weeks. All of the evidence suggests that when the embryos reached this point (usually by June or July in most parous females; Fig. 10), their developmental rate suddenly slowed tremendously.

Stages of delay

In the unbroken monthly series of samples from July 1982 to February 1983, the diameter of the uterus at the site of implantation increased beyond 4 mm in only 1 of 94 females examined. Uterine swellings remained at about 3–4 mm for about 8 months, but in March uterine swellings up to 10 mm in diameter were found. Regular increases in average size were found in all succeeding months until parturition (Heideman, 1988). With two exceptions, all parous females captured from August through February possessed embryos undergoing amniogenesis and embryonic disc formation (Figs 11 & 12).

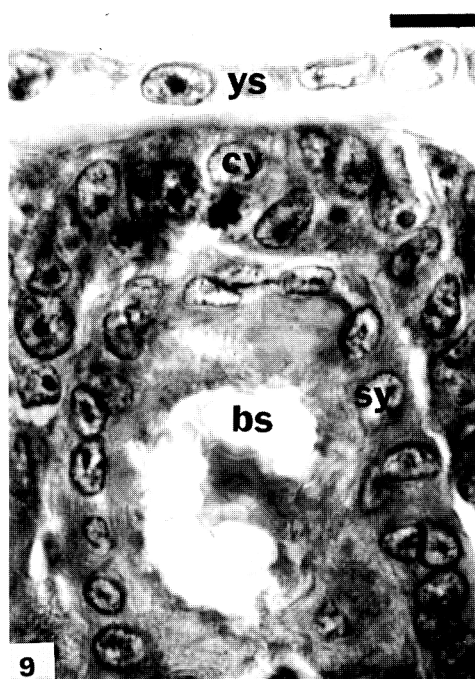
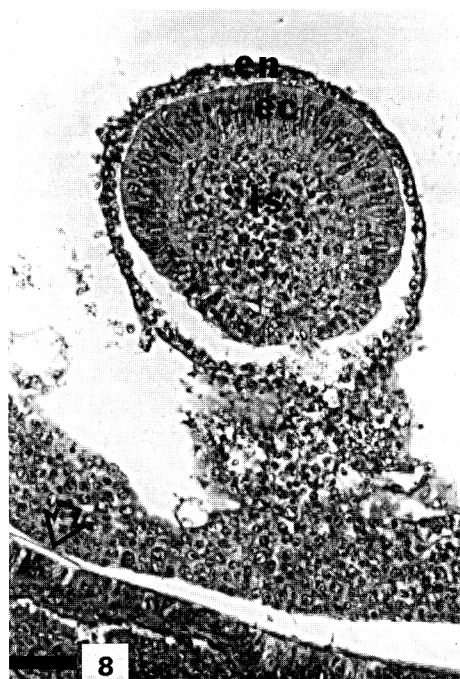
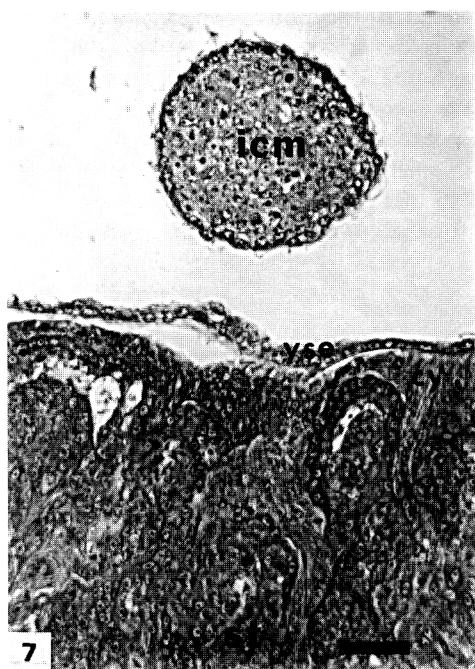
During the period of delayed embryonic development no changes were observed in the trophoblast or endoderm. However, slight modifications of the inner cell mass continued. Cells within the inner cell mass (Fig. 11) began to dissociate or disintegrate, forming a schizamniotic cavity ('early gastrulation' on Figs 4, 5 & 8). At about the same time or slightly later, cells in the outer, columnar ectodermal layer began to flatten and separate from the inner, solid sphere of cells proximally to their attachment to the trophoblast, forming a second cavity ('mid-gastrulation' on Figs 4 & 5). As fluid accumulated in these cavities, they continued to enlarge, eventually coalescing to form a single amniotic cavity ('late gastrulation' on Figs 4, 5 & 11). After about 5 months of delay, some embryos had a primitive streak and had begun to flatten into an embryonic disc ('early plate' on Figs 4, 5 & 12). The embryos of most, but not all, parous females had attained stages of embryonic plate formation (Figs 12 & 13) in February and March. In March, the rate of development of the embryos of parous females increased tremendously. Development continued at a rate typical for small pteropodid fruit bats for the next 3 months until parturition in late May or early June.

The inner cell mass doubled in greatest length during the delay. The greatest length of the inner cell mass at the beginning of the delay in late June averaged 280 μm ($N = 12$), and reached 520 μm ($N = 7$) in February and March, near the end of delay. Much of this increase was through the formation and expansion of the schizamniotic cavity. Both the diameter of the uterus and the mean greatest length of the inner cell mass increased slightly with developmental stage (Table 2). The fact that few embryos were found in mid- or late plate formation suggests that these 2 stages were often reached after the termination of delay.

Three females captured in July 1982, August 1982 and June 1983, respectively, showed no sign of having undergone parturition (no identifiable post-parturient uterus, no remnant of corpus luteum, nipples small), and had embryos in the initial stages of amniogenesis (Fig. 5). These females could have failed to reproduce in their first year, or their embryos may have failed to terminate the delay synchronously with others.

Corpus luteum

The corpus luteum was always ipsilateral to the reproductive duct carrying the newly-ovulated ovum or conceptus. Although a very few cases of partly everted corpora lutea were observed, in most instances the corpus luteum formed a spherical mass that filled 25–90% of the ovary (Fig. 14).



By the time the blastocyst had reached the uterus, the corpus luteum was vascularized. The corpus luteum of early pregnancy and early delay typically had large cells with large nuclei and prominent nucleoli (Fig. 14). Corpora lutea of females with embryos in delay or soon after the end of delay also contained large lutein cells (Figs 15 & 16). In most females with embryos of about 5–10 mm, large luteal cells were still abundant, but their nuclei appeared to be very slightly smaller (Fig. 17). During late pregnancy (embryos ≥ 20 mm), most lutein cells were much smaller and the corpora lutea had therefore decreased considerably in size (Fig. 18). By the last month of gestation, the involuting corpus luteum had diminished to about 1% of the volume of a new corpus luteum. Involuting corpora lutea were still detectable in post-parturient females, but by the time of implantation of the next embryo they were no longer apparent.

First pregnancy

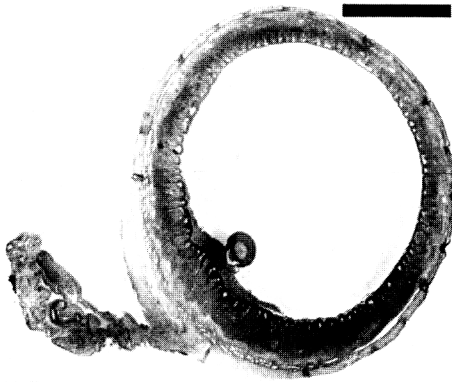
Young females became pregnant early in their first year. Spermatozoa were found in the reproductive tracts of 2 of 3 young females captured in late August, when they were only about 3 months old. The ovaries of these 3 females had tertiary follicles. Proliferation of the endometrium and uterine glands was apparent in both uteri of all 3 females and the uteri had increased from the typical subadult diameter of about 0.5 mm to a typical adult diameter of about 1 mm. After ovulation, a progestational reaction was apparent in both uteri (4 of 5 cases), although in 2 bats the reaction was greater in one uterus (see Fig. 3). The first primigravid female was captured in early September (Fig. 5) and most fertilizations occurred in late September or October when these females were 4 or 5 months old (1 of 10 pregnant in September; 6 of 6 pregnant in October and early November). One young female held an early implanting embryo in January, indicating a late impregnation in late December or January (Fig. 5). Delay began at the same developmental stage in embryos of these primigravid females as in those of adults and followed a similar

Fig. 6. Cross-section of the uterus and embryonic membranes from a female with an embryo near the beginning of the delay on 1 July 1982. The yolk sac endoderm has thickened, and the trophoblast has differentiated into a cytotrophoblast (cy) and syncytiotrophoblast (open arrow) (also see Fig. 9). Maternal blood spaces are apparent (solid black arrow) and may be in direct contact with syncytiotrophoblast. Multinucleate giant cells (g) are clearly visible within the uterine stroma. Below the giant cells can be seen an intact uterine gland adjacent to the myometrium (m). The embryo of this female is shown in Fig. 8, and the upper right of this figure overlaps with the lower left of Fig. 8. cy = Cytotrophoblast; g = giant cell; m = myometrium; st = uterine stroma; yc = yolk sac cavity; yse = yolk sac endoderm. Scale bar = 100 μ m.

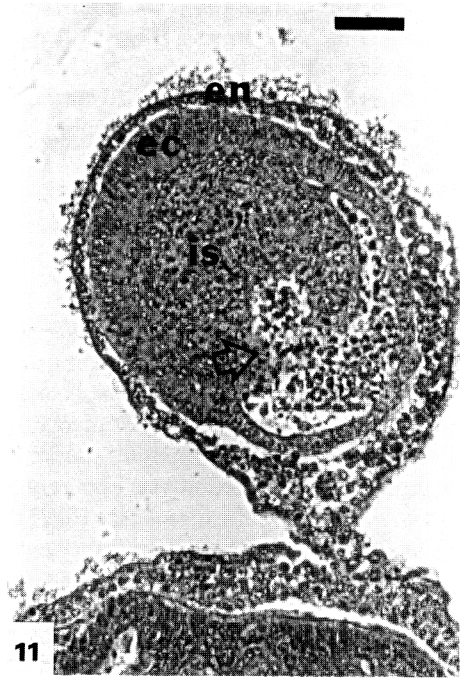
Fig. 7. Embryoblast (inner cell mass) of an implanted embryo just before delay or early in delay on 30 July 1982. The inner cell mass consists of a layer of endoderm surrounding a layer of ectoderm. The cylinder of cells that joins the embryoblast to the yolk sac is perpendicular to the plane of sectioning. cy = Cytotrophoblast; icm = inner cell mass; st = uterine stroma; yse = yolk sac endoderm. Scale bar = 50 μ m.

Fig. 8. Embryo in 'early gastrulation' just before the start of amniogenesis early in delay on 30 July 1982. The embryo is composed of a well-defined layer of endoderm surrounding a columnar layer of ectoderm that in turn surrounds an inner sphere of cells. The space between the endoderm and ectoderm is probably an artefact of processing. The open arrow indicates Reichert's membrane where the tissues have pulled apart during processing. The uterus from this section is shown in Fig. 6, which overlaps with part of this figure. ec = Ectoderm; en = endoderm; is = inner sphere of cells. Scale bar = 50 μ m.

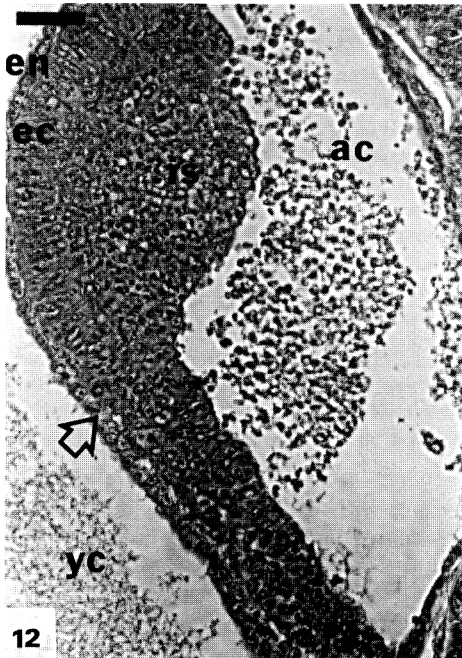
Fig. 9. Fetal and placental membranes from a female captured on 29 August 1982 with an embryo early in delay. The embryo of this female is shown in Fig. 10. ys = Yolk sac endoderm; cy = cytotrophoblast; sy = syncytiotrophoblast; bs = maternal blood space. Scale bar = 10 μ m.



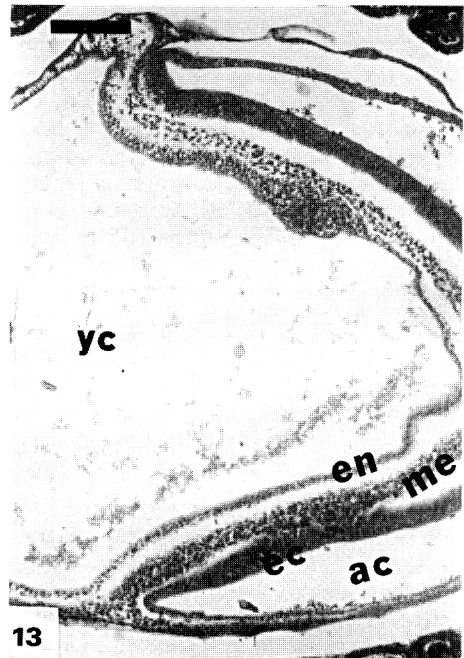
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pattern. Primigravid females may have terminated the delay at a slightly earlier stage of development than parous females, as none of their embryos had mesoderm in February and March (Fig. 5).

By the last 2 months of gestation it was difficult to age females accurately, as the epiphyses of the phalanges had fused in some yearling females, but a few females could still be classed as primigravid on the basis of small teat size. In this latter group, parturition apparently occurred about 2 weeks to 1 month later than in parous females (Heideman, 1988). This suggests that the majority of females undergoing parturition in late May or early June were parous, while primigravid females tended to have young later in June.

Annual timing of parturition

Multiparous female pygmy bats produced a single young in May or June during each of 6 years of sampling, including 1984 and 1987 as well as the 4 years shown in Fig. 4 (Heideman, 1988). The typical delay varied in length from 8 months for multiparous females to 4 or 5 months for primiparous females. Delay terminated in March (most multiparous females) or March and April (primigravid females) and was followed by about 3 months of development at a more rapid rate. In the 3 years in which larger samples were obtained, parous females were again pregnant shortly after parturition, and thus had a gestation length of 11.5 months, the longest reported for any bat.

Two parous females and one primiparous female were exceptions to this pattern. One parous adult was near term in January, and another had a 16-cell tubal embryo in March. One primiparous female had an implanting blastocyst in January.

Embryos from females taken in February and April 1984 were at developmental stages paralleling those of the samples in the same months in the previous year. As in 1983, no lactating females, non-pregnant females or flying juveniles were found in these months. This indicates that the reproductive pattern and the length of the delay was similar in both years. In June of 1979, 1981, 1982, 1983, 1984 and 1987 almost all females captured were either near term or showed evidence of recent parturition (Heideman, 1988), indicating that reproductive timing was also similar in each of those years ($\pm \sim 2$ weeks).

Fig. 10. Cross-section of a uterus with an embryo near the beginning of delay on 1 July 1982. The two cavities visible within the inner cell mass have not yet joined to form a single amniotic cavity. The fetal and placental membranes of this embryo are shown in Fig. 9. Scale bar = 1000 μ m.

Fig. 11. Embryo in delay in 'late gastrulation' on 8 July 1982. A single amniotic cavity has formed from the joining of two cavities (open arrow), one formed by degeneration or separation of cells within the centre of the inner sphere of cells, the second formed by the separation of the inner sphere of cells from the overlying ectodermal tissue. ec = Ectoderm; en = endoderm; is = inner sphere of cells. Scale bar = 50 μ m.

Fig. 12. Embryo in early plate formation late in the delay period on 8 February 1983. The amniotic cavity has enlarged, and contains abundant cellular debris. In this embryo, the primitive streak was becoming apparent. ac = Amniotic cavity; ec = ectoderm; en = endoderm; is = inner sphere of cells; yc = yolk sac cavity. Scale bar = 50 μ m.

Fig. 13. Embryo in late plate formation (primitive streak stage) just starting normal development after the delay (8 April 1983). The embryo has become a curved plate composed of endoderm, mesoderm, and ectoderm. ac = Amniotic cavity; ec = ectoderm; en = endoderm; me = mesoderm; yc = yolk sac cavity. Scale bar = 150 μ m.

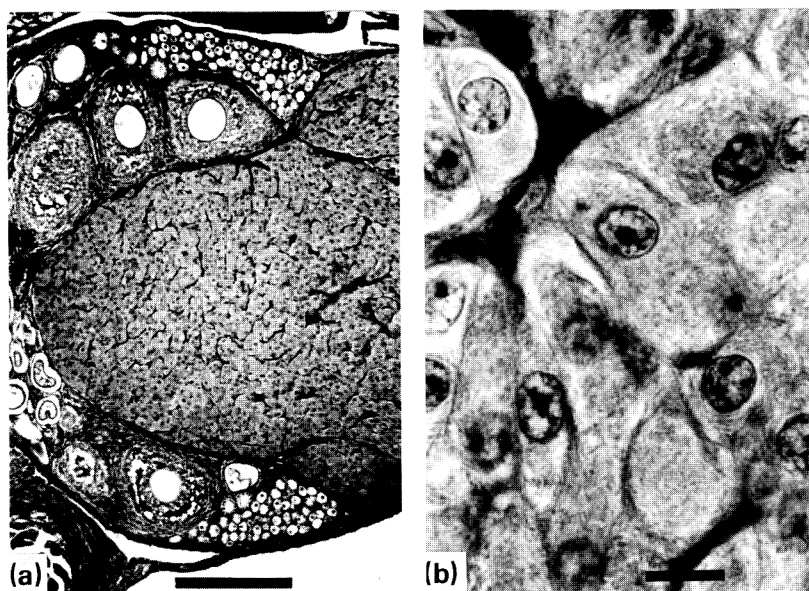


Fig. 14. Ovary with corpus luteum from a parous female with an embryo in the early delay on 28 June 1982 (a) and higher magnification of the luteal cells (b) showing that, at this time, the luteal cells are uniformly large and have large nuclei. Scale bar = 250 μ m (a), 10 μ m (b).

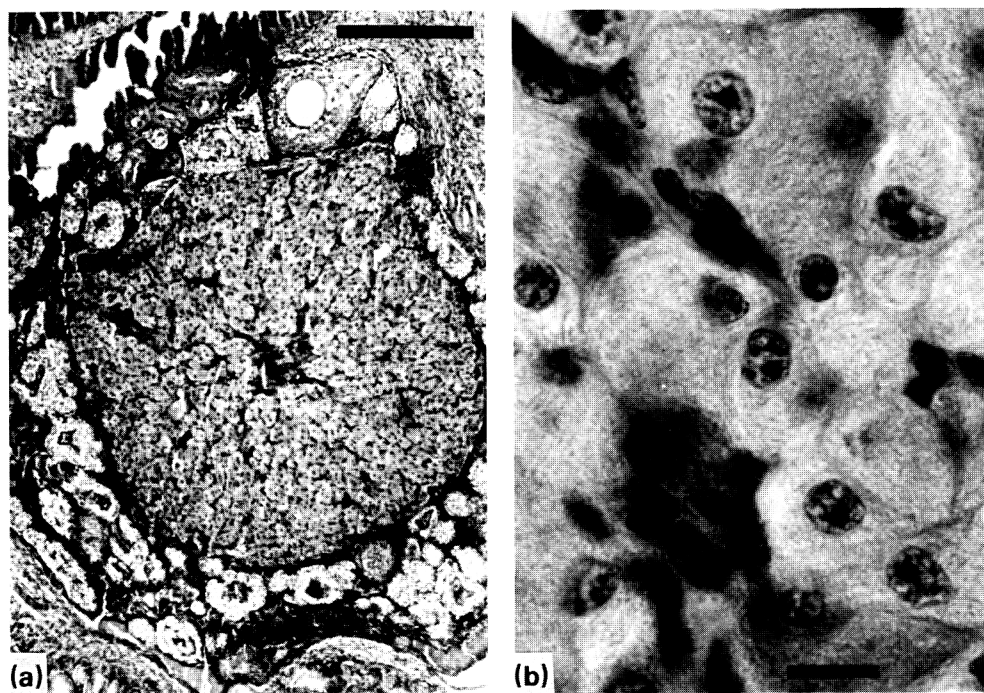


Fig. 15. Ovary with corpus luteum from a parous female with an embryo in delay on 8 January 1983 (a) and higher magnification of the luteal cells (b). Scale bar = 250 μ m (a); 10 μ m (b).

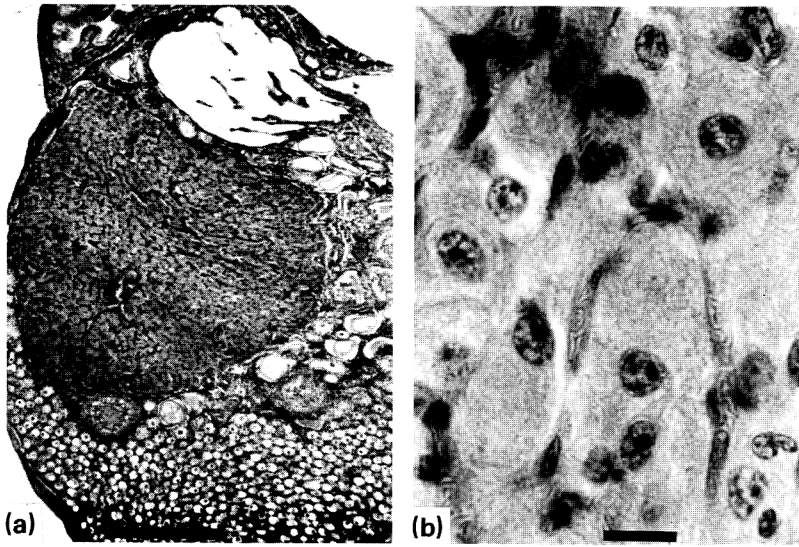


Fig. 16. Ovary with corpus luteum from a primiparous female with an embryo at the end of delay on 8 April 1983 (a) and higher magnification of the luteal cells (b). Scale bar = 250 μ m (a); 10 μ m (b).

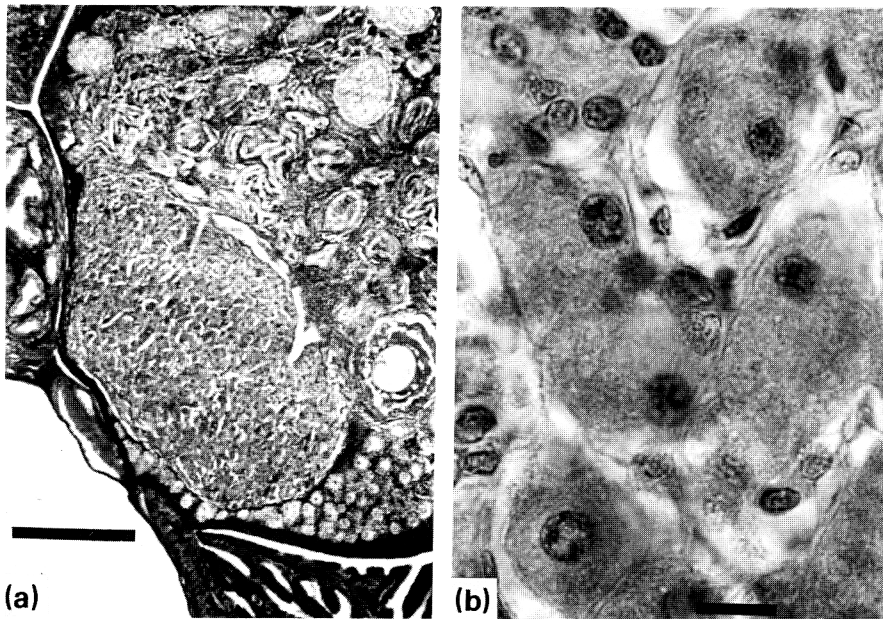


Fig. 17. Ovary with corpus luteum from a parous female with an 8-mm embryo on 7 April 1983 (a) and higher magnification of the luteal cells (b). Scale bar = 250 μ m (a); 10 μ m (b).

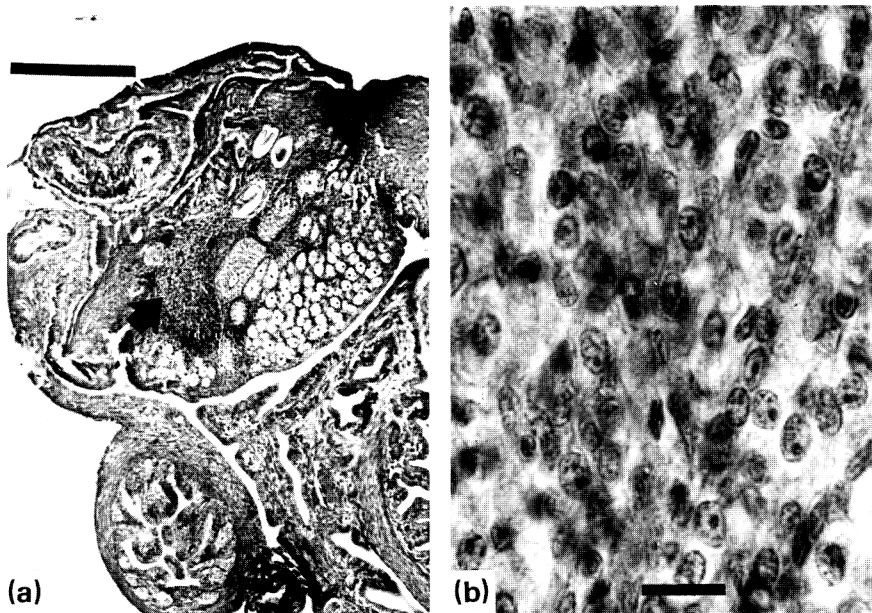


Fig. 18. Ovary with corpus luteum (arrowed) from a parous female with a 21-mm embryo on 5 May 1983 (a) and higher magnification of the luteal cells (b). Scale bar = 250 μ m (a); 10 μ m (b).

Discussion

Typical gestation periods among the smaller members of the family Pteropidae are 3–4 months (Moghe, 1956; Kingdon, 1974; Sreenivasan *et al.*, 1974; Start, 1974; Bhat *et al.*, 1980; Krishna & Dominic, 1983) but longer lengths have been reported in large species (e.g. 5–6 months: Baker & Baker, 1936; Marshall, 1953; Kingdon, 1974; Thomas & Marshall, 1984). Development in pygmy bats seems to require about 2–4 weeks before the delay and \sim 3 months after the delay, or a total of about 3.5–4 months of active development. Except for the period of delay, the pattern of early development in pygmy bats is similar to that described for a number of megachiropterans (Keibel, 1922; Moghe, 1956; Rasweiler, 1979).

The alternation of ovulation between ovaries in successive pregnancies in pygmy bats is similar to that found in other megachiropterans (Marshall, 1947, 1949, 1953; Ramakrishna, 1950; Gopalakrishna & Murthy, 1960; Gopalakrishna, 1964, 1969; Gopalakrishna & Choudhari, 1977), and is the only pattern reported for the group (Wimsatt, 1979).

The progestational reaction in pygmy bats is slight, but otherwise similar to that described for other pteropodid bats (Marshall, 1953; Gopalakrishna & Karim, 1971). In multiparous female pygmy bats, it occurs only in the part of the uterus adjacent to the ovary containing the corpus luteum. The swelling from the local proliferation of the uterine endometrium and uterine glands became macroscopically detectable before the arrival of the blastocyst. Rasweiler (1979, 1982) suggested that the progestational reaction in *Pteropteryx* may preclude movement of the blastocyst past the zone of expansion; this is possible for pygmy bats as well.

Although there were more ovulations from the right than left ovaries, there was no statistical difference in the proportion of ovulations from each ovary, suggesting that neither ovary is dominant. However, it is possible that the right ovary exhibits partial dominance.

There was evidence of mating in at least 6 (and probably 9) months of the year, assuming that delay begins only after implantation. This is consistent with the observation that epididymal

spermatozoa were present in all months of the year, although testis volume was greatest during the period when most parous females were impregnated. The presence of spermatozoa in the reproductive tracts of some pregnant females indicates that either small numbers of spermatozoa persist in the uterus for up to 2 months following fertilization or that these females had copulated up to 2 months following fertilization.

The delay in pygmy bats is not of fixed length. Almost all parous females became pregnant in June and July, but one female with a 16-cell tubal morula was captured in March (Fig. 4), and impregnations of young females occurred from September to December (Fig. 5). Despite the occurrence of fertilizations over this 8-month period, the delay ended in March or April in almost all cases. Only one embryo emerged from the delay early (Fig. 4). This great variation in length of the delay makes it likely that some environmental cue plays a role in controlling the duration of the delay. Variation in the duration of this kind of post-implantation developmental delay has not been reported in other species (but see Racey & Swift, 1981).

Although I have distinguished particular stages of embryonic development during delay, there is no clear evidence that the stage of embryonic growth has an effect on the duration of the delay. The embryos may need to attain a certain developmental stage before delay can end, but it is also possible that they reach the stages observed merely as a consequence of the very slow continuing development during delay.

During the delay period in pygmy bats, the mean length of the embryos approximately doubled, due partly to growth and partly to the formation of the amniotic cavity. This continuation of growth and development, albeit at a very low rate, is consistent with the post-implantation delays found in the insectivorous bats *Hipposideros caffer* (Bernard & Meester, 1982) and *Macrotus californicus* (Bradshaw, 1962; Bleier, 1975), as well as the frugivore, *Artibeus jamaicensis* (Fleming, 1971).

A post-implantation period of retarded embryonic growth is relatively rare in mammals. It has been demonstrated with histological material for only 3 species of microchiropteran bats, 2 in the family Phyllostomidae (Bradshaw, 1962; Fleming, 1971; Bleier, 1975) and one in the family Hipposideridae (Bernard & Meester, 1982). Delays have been implicated or demonstrated in several other microchiropteran species, including 3 additional families (Rhinolophidae: Ramakrishna & Rao, 1977; Emballonuridae: Krishna & Dominic, 1982; Natalidae: Wimsatt, 1975), and have been invoked to explain a disparity between gestation lengths of *Miniopterus australis* at two different tropical localities (Medway, 1971). A related form of delay, in which development seems to be more moderately slowed for all or much of gestation, has been described in the bat family Vespertilionidae (Racey & Swift, 1981).

In the megachiropteran bats, a possible post-implantation delay has been reported in one of the two annual pregnancies in *Cynopterus sphinx* (Krishna & Dominic, 1983). However, the nature of this delay is not yet clear, as it was demonstrated using weight changes of whole uteri, and the results are also compatible with delayed implantation accompanied by a uterine weight increase due to a progestational reaction at the beginning of the delay. Delayed implantation following such a pattern has been described for the megachiropteran bat *Eidolon helvum* (Mutere, 1965).

Developmental delay was first reported by Bradshaw (1962) for *Macrotus californicus* (Phyllostomidae; Microchiroptera) from Arizona, with the suggestion that the delay might be caused by low winter food supplies. However, Burns *et al.* (1972) found that neither food provided in excess (with consequent weight gain) nor elevated temperatures affected the embryonic growth rate. They did find lowered plasma thyroxine concentrations during part of the delay, and suggested that "... temporary hypothyroidism is probably the functional end point through which a complicated and highly integrated endocrine control system is manifested." In their animals, however, once-daily injections of thyroxine given for up to 40 days did not increase the rate of embryonic development (as cited in Burns & Wallace, 1975). Burns & Wallace (1975) described biphasic peaks in plasma oestrone and oestradiol during development, the first peak corresponding to the period that encompasses copulation and the early part of the delay, and the second to the

period that covers development after delay. Burns & Easley (1977) found a similar biphasic pattern of plasma progesterone. They speculated that the elevated hormone concentrations might be required for implantation, and suggested that the subsequently lowered hormone concentrations might be significant for the delay. In contrast, A. C. Enders (p. 65, in Flint *et al.*, 1981), noted that he had examined Bleier's material (Bleier, 1975), and suggested that the delay might be due to vascular lack in the endometrium. Richardson (1979) described increases in the size and number of prolactin-secreting cells in the pituitary at the end of delay, and suggested that prolactin might play a role in the control of delay in *Macrotus californicus*. He also noted decreases in the size and number of gonadotrophic cells (secreting LH and/or FSH) in late pregnancy and lactation. Despite this work, the nature of the environmental and neuroendocrine factors controlling the onset and end of the delays is still unknown for all species.

In *Artibeus jamaicensis* the delay occurs when the uterine glands are hypertrophied, and at a later stage in implantation (Fleming, 1971) than in pygmy bats. While the corpus luteum does not change in size between the 2-5-month delay and the non-delay periods in *Artibeus jamaicensis*, luteal cells were smaller during the delay, and Fleming (1971) suggested that lowered luteal hormone production might cause the delay. In pygmy bats, there were no clear changes in the luteal cells over the delay period, but this does not preclude the possibility that there are changes in steroid production during the delay. My data provide little evidence for or against this hypothesis for pygmy bats.

In pygmy bats, the pattern of variation in the start and end of the delay permits an evaluation of possible initiation and termination cues. Environmental or endogenous cues should control the three points that time each female's annual reproductive cycle: ovulation and mating, the beginning of delay, and the end of delay. At least one environmental cue is necessary at some point in the cycle to maintain the quite precise annual timing and synchrony of parturition in pygmy bats. For two of these points, the initiation of delay and ovulation and mating, the data presented here are equally consistent with endogenous or environmental control (or both). However, because termination of delay occurred relatively synchronously in March despite the 5-7-month range of conception dates, and hence of delay initiation dates, it seems likely that an environmental cue is involved in the termination of delay. Pygmy bats may also use environmental cues to control other parts of the cycle. It is also possible that only a single environmental cue (to terminate delay) could control reproductive timing in females if endogenous cues controlled ovulation and mating (e.g. a post-partum oestrus) and the entry into delay. The use of an endogenous signal to begin delay might explain the fact that embryos conceived over a 5-7-month period all entered delay.

In the relatively mildly seasonal tropical climate of Negros Island, few seasonal changes are predictable enough to be likely reproductive cues for pygmy bat females. Environmental events that varied in timing between years (e.g. periods of food abundance or wet and dry seasons) can be rejected as possible termination cues because the timing of parturition was similar in each of 5 years (Heideman, 1988). For the same reason, a simple change in nutritional status is unlikely to trigger termination of the delay, as this would be expected to vary considerably among females from year to year. Daily photoperiod changes quite predictably on Negros, but the amount of change is very low. The circadian rhythms of pteropodid bats can be entrained by very low light intensity differences (Erkert, 1982), but their threshold level for the lengths of light and dark periods have not yet been determined. Pygmy bats on Negros Island are close to the equator (9°N), where maximum daily photoperiodic change is only 40 sec (a maximum of 17 min per month), and such a low rate of change has not yet been shown to trigger reproductive changes in mammals. Variation in photoperiod therefore offers a precise signal to pygmy bats, but is only usable if the animals are physiologically capable of detecting the changes. Populations of pygmy bats on some other islands in the Philippines give birth during months different from those of the Negros Island population described here (Heideman, 1988). This suggests that, if a photoperiodic change functions as a termination cue, then the bats have been able to evolve a response to a variety of photoperiods.

Pygmy bats are extremely unusual in being small tropical mammals living in a rather equable

climate that none the less have an unusually long period of delay (8 months) between copulation and parturition, and thereby the longest gestation period reported for a bat (11.5 months). This first clear demonstration of a post-implantation delay in development outside the suborder of microchiropteran bats allows the comparison of delayed development between the two divergent suborders of bats. The system is worthy of more detailed study, both to determine its mechanism and control, and as a possible model for the study of patterns of early development.

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