# Monthly changes in the reproductive organs of female Miniopterus schreibersi natalensis (A. Smith, 1834)

By R.T.F. BERNARD

Department of Zoology, University of Natal, Pietermaritzburg, Republic of South Africa

Receipt of Ms. 1. 9. 1979

# Abstract

Investigated monthly changes in the ovaries, uterine horns and vagina of *Miniopterus schreibersi*. Correlation between these changes is used to suggest that the reproductive cycle of M. *schreibersi* is under typical mammalian hormonal control. The estrous cycle of M. *schreibersi* is described and is characterised by an extended pregnancy/luteal stage, and a short anestrus. The estrous cycle of M. *schreibersi* is compared with those of other vespertilionid bats.

# Introduction

There is considerable literature on the reproduction of vespertilionid bats. Four reproductive strategies have been reported as occurring in the Vespertilionidae. In all members of this family occurring in temperate latitudes, excluding the Miniopterinae, copulation occurs in autumn, spermatozoa are stored over winter, and ovulation and fertilization occur in spring (REEDER 1939, for *Myotis lucifugus*; KITCHENER 1975, for *Chalinolobus gouldii*; KITCHENER and HALSE 1978, for *Eptesicus regulus*). In members of the Miniopterinae from temperate latitudes, copulation and fertilization occur in autumn and embryonic implantation is delayed for a period approximately corresponding to the period of hibernation, (DWYER 1963; VAN DER MERWE 1977; WALLACE 1978, for *Miniopterus schreibersi*; DWYER 1963, 1968; RICHARDSON 1977, for *M. australis*).

Outside temperate latitudes two further reproductive strategies have been described. Both *Myotis nigricans* (WILSON and FINDLEY 1970) and *Myotis adversus* (DWYER 1970) are seasonally polyestrus, both producing approximately three litters each year.

In members of the Miniopterinae from tropical latitudes, copulation and fertilization occur approximately synchronously and pregnancy ensues without an intervening period of delayed implantation.

The estrous cycle and associated changes in the reproductive tract have been described for *Myotis lucifugus* (REEDER 1939), *Scotophilus wroughtoni* (GOPALAKRISHNA 1949), *Chalinolobus gouldii* (KITCHENER 1975), *Pipistrellus hesperus* (KRUTZSCH 1975) and *Eptesicus regulus* (KITCHENER and HALSE 1978).

In this paper the monthly changes in the ovaries, uterine horns and vagina are described and these changes are used to ellucidate the estrous cycle of *Miniopterus schreibersi*.

# Materials and methods

49 adult female *M. schreibersi* were collected from caves in the Natal Midlands (C. 29°S–30°15'S) during 1977. Specimens were collected every two weeks, the average sample size for any two-week period was two.

U.S. Copyright Clearance Center Code Statement: 0044-3468/80/4504-0217 \$ 2.50/0 Z. Säugetierkunde 45 (1980) 217-224 © 1980 Verlag Paul Parey, Hamburg und Berlin ISSN 0044-3468/ASTM-Coden ZSAEA 7

Specimens were killed by asphysiation with carbon dioxide and the female reproductive tract was removed under a dissecting microscope. Tissues were fixed in Bouin's fixative for approximately one week and thereafter stored in 70 per cent alcohol.

Tissues were dehydrated through a graded sequence of alcohols, cleared in toluene and embedded in Paraplast (Sherwood Medical Industries, Inc., St. Louis, Missouri, 63103), and all sections were cut at 5  $\mu$ m. All sections were stained with Ehrlich's haematoxylin and counter stained with eosin.

All measurements of reproductive tissues were made with an optical micrometer. Individual follicular diameters were calculated from two measurements made at right angles to each other, so that one measurement always included the largest follicular diameter. Measurements of follicular diameter included the theca folliculi. For the purpose of this study a secondary follicle was defined as a follicle in which the stratum granulosum was comprised of two or more cell layers, but where these cell layers were not interrupted by an antrum. A Graafian follicle was one in which the cell layers of the stratum granulosum were interrupted by an antrum.

Thickness of the vaginal epithelium was measured in approximately 20 places in each bat, and these measurements were used to obtain a mean monthly epithelial thickness. Thickness of the vaginal epithelium was measured from the basement membrane, to the outer cell layer. When a superficial cornfied layer was present it was measured separately.

Thickness of the uterine wall and endometrium was measured in approximately 20 places in each specimen, and these measurements were used to obtain a mean monthly thickness. Thickness of the endometrium was measured from the edge of the lumen to the beginning of the myometrium. Total thickness of the uterine wall was measured from the edge of the lumen to the epithelium lining the outside of the uterine horn.

#### Results

# Monthly cycles in the ovary

The ovarian cycle is characterised by two processes; the growth and development of follicles and the atresia of excess follicles. These two processes have been described by VAN DER MERWE (1977) for *M. schreibersi* and by BERNARD (in prep.) for *M. fraterculus*.

An indication of ovarian activity was obtained by measuring the diameters of all secondary and Graafian follicles and calculating mean monthly diameters for these follicles. There were two periods of Graafian follicle production (Fig. 1). The first during March and April resulted in an ovulation, while the second period, from August to December, did not.



Fig. 1 Monthly changes in ovarian activity. Open squares indicate mean ( $\bar{x}$ ) monthly Graafian follicle diameters and closed squares indicate mean monthly secondary follicle diameter. Vertical lines indicate  $\bar{x} \pm 2SD$ 

#### Changes in the reproductive organs of female Miniopterus schreibersi

The process of ovulation is followed by the formation of a corpus luteum. An early corpus luteum was seen in the left ovary of a bat collected on 17th April and the last specimen with a corpus luteum was collected on 2nd December, approximately seven and a half months later.

The diameter of the early corpus luteum, from the specimen collected on 17th April was  $550\,\mu$ m,  $70\,\mu$ m smaller than the largest Graafian follicle. The small size of the early corpus luteum was a result of shrinkage and folding of the stratum granulosum. The centre of all early corpora lutea were filled with erythrocytes, leucocytes and fibrous material. The corpus luteum reached its maximum size of  $750\,\mu$ m at the beginning of July and this size was maintained until mid-August (Fig. 2). Between mid-August and December the corpus luteum diameter decreased and from September increasing amounts of fibrous material were present in the centre of the corpus luteum. During the period of corpus luteum growth, prior to implantation, the cells of the interstitial tissue of the corpus luteum were larger than those of the interstitial tissue.



*Fig.* 2. Monthly changes in corpus luteum diameter. Solid squares indicate mean  $(\bar{x})$  monthly diameter and vertical lines  $\bar{x} \pm 2$ SD. Approximate date of implantation has been indicated



Fig. 3. Monthly changes in the thickness of vaginal epithelium. Closed circles indicate mean  $(\bar{x})$  monthly thickness of vaginal epithelium, open cirles, mean monthly thickness of superficial cornified layer. Vertical lines indicate  $\bar{x} \pm 2SD$ 

FONDA and PEYRE (1965) and PEYRE and MALARSINE (1969) demonstrated the localisation of  $5-3\beta$  hydroxysteroid dehydrogenase in the placental discs of *Miniopterus schreibersi*, suggesting their function in the production of the steroid hormones estrogen and progesterone. ELS (1978) has shown that during early pregnancy in *M. schreibersi* the interstitial gland tissue in the ovary is the most active steroidogenic centre in the ovary. The importance of the corpus luteum in *M. schreibersi* for the maintenance of pregnancy must therefore be questioned.

The cells of the corpus luteum of *M. schreibersi* appeared to remain active up to one month prior to parturition, indicating that the placental discs do not completely take over the production of estrogen and progesterone after implantation. From November the nuclei of the luteal cells appeared to shrink, their borders becoming corrugated, the luteal blood supply decreased and the border of the corpus luteum became indistinct.

#### Monthly cycles in the vagina

There were two periods of growth of the epithelium in *M. schreibersi*, the first from March to April and the second from August to October (Fig. 3). These periods coincided with the development of Graafian follicles in the ovaries. Between early November and February the vaginal epithelium appeared to be in a resting stage. No mitotic divisions were seen in the stratum germinativum between November and January, but a few were apparent in late February. During this period the epithelium maintained a more or less constant thickness and was comprised of nucleated cells only.

From the beginning of March to mid-April, the stratum germinativum was actively dividing and there was a resultant increase in the mean monthly thickness of the epithelium from  $28\mu$  m in March to  $52\mu$  m in April. During this period the epithelium was comprised of nucleated cells and a few scattered leucocytes. During late March the first superficial layers of cornified cells were present, and by mid-April this layer had reached a maximum thickness of  $15\mu$ m. Delamination of the vaginal epithelium occurred between mid-April and early May.



*Fig.* 4. Monthly changes in thickness of uterine wall and endometrium. Triangular symbols indicate mean  $(\bar{x})$  monthly thickness of uterine wall, circular symbols, mean monthly thickness of endometrium. Closed symbols are measurements from the right uterine horne, open symbols, of the left uterine horn. Thickness of the myomtrium is approximately represented by the distance between the two sets of lines

#### Changes in the reproductive organs of female Miniopterus schreibersi

Two specimens collected in the first week of May had shed the cornified layer and some of the underlying nucleated cells, so that the epithelium was comprised of approximately three layers of nucleated cells.

Between late May and late August the vaginal epithelium maintained a more of less constant thickness of  $22\mu$ m. During this period there were no cell divisions in the stratum germinativum and the epithelium was comprised of nucleated cells only.

From late August to mid-October mitotic divisions were again apparent in the stratum germinativum and there was a resultant increase in epithelial thickness from a monthly mean of  $25\mu$  m in August to  $53\mu$  m in October. During this period there was no production of cornified layer and the epithelium was comprised of nucleated cells only.

During October and November delamination of the vaginal epithelium occurred again and leucocytes were abundant in the epithelium.

Contents of the vaginal lumen changed with changes in the vaginal epithelium. Nucleated cells were present throughout the year, but were abundant during the two periods of delami-



Fig. 5. Estrous cycle of six vespertilionid bats. Note the prolonged estrus in Myotis lucifugus, Chalinglobus gouldii and Eptesicus regulus and the period of anestrus in Scatophilus wroughtoni, Miniopterus fraterculus and Miniopterus schreibersi



Prostrus Estrus Metestrus Pregnancy/luteal stage Lactation/anestrus Anestrus

nation. Cornified epithelial cells were present during the second half of April, during the first period of delamination. Leucocytes were present prior to and during the two periods of delamination. Spermatozoa were present in the vagina of three bats collected on 14th April, prior to ovulation.

## Monthly changes in the uterine horns

From late December to mid-Marc<sup>1</sup> the endometrium appeared to be in a resting stage and there was little change in the uterine dimensions (Fig. 4). The uterine glands were short, straight and few in number.

Between mid-March and mid-May the thickness of the endometrium increased from a mean of  $100\mu$  m in March to a mean of  $520\mu$  m in May. This increase was partly the result of hyperplasia and hypertrophy of endometrial cells, and partly of an increase in thickness of the myometrium. Figure 4 shows that during this period the proportion of uterine wall thickness, as represented by endometrial thickness, decreased, indicating an increase in myometrial thickness. During this period the uterine glands became elongated and coiled and the sub-mucosa became increasingly vascularised.

Between April and mid-August there was a decrease in the mean monthly endometrial and uterine wall thickness, although during this period the uterine glands became increasingly coiled and numerous, and the sub-mucosa increasingly vascularised.

The epithelium lining the uterine horn lumen was columnar in shape, with clear apical cytoplasm and basal nuclei. After parturition which occurred in early December the lumen of the uterine horns was filled with cellular debris, the result of breakdown of the endometrium.

Spermatozoa were first seen in the uterine horns in April, at the time of ovulation, and last seen in August, four months after fertilization. During this period the spermatozoa were stored in the uterine glands.

#### Estrous cycle of M. schreibersi

The estrous cycle, including pregnancy, in eutherian mammals has been divided into five stages: proestrus; estrus; metestrus; pregnancy/luteal stage; and lactation/anestrus. From the study of monthly changes in the reproductive organs of M. schreibersi it is possible to recognise these five stages, and in figure 5 the estrous of M. schreibersi has been compared with other members of the Vespertilionidae.

The proestrus condition occurred between mid-March and early April, when developing Graafian follicles were present in the ovaries and the vaginal epithelium was undergoing a period of growth.

Estrus occurred in mid-April, when there was a single preovulatory Graafian follicle in the left ovary, a superficial layer of cornified cells on the vaginal epithelium and spermatozoa were present in the vagina.

Estrus was followed by a short of metestrus, during which delamination of the vaginal epithelium occurred and the ovum was present in the oviduct.

The pregnancy of M. schreibersi was divided into two stages, approximately four months of delayed implantation and four months of active fetal growth. The period of delayed implantation was characteristic of the luteal stage of most mammlas, the vaginal epithelium was thin, the ovaries contained a single corpus luteum and no Graafian follicles and the endometrium was highly developed. During the period of active fetal growth Graafian follicles were present in the ovaries and the vaginal epithelium underwent a period of growth.

Parturition occurred in early December and was followed by a period of lactation/anestrus. Although lactation lasted until only mid-January, the anestrus condition continued until mid-March. The anestrus condition was characterised by the absence of Graafian follicles from the ovaries, and an undeveloped vaginal epithelium.

# Discussion

The monthly changes in the reproductive organs of M. schreibersi are very similar to those of M. fraterculus (BERNARD, in prep.), and are essentially similar to those reported for other vespertilionid bats.

A comparison of figures 1, 3 and 4 shows that in the present study the peaks of Graafian follicle development, vaginal epithelium thickness and endometrial development coincide. This correlation would be expected if one could assume that the reproductive cycle of M. schreibersi was under typical mammalian hormonal control. WIMSATT (1960) stated that "it appears unlikely that the immediate factors controlling reproductive phenomena of bats differs in fundamental quality from those operative in other mammals, for the reproductive organs of bats respond in the same way to gonadotrophic and sex hormones."

The correlation in the changes reported for the ovary, uterine horn and vagina of *M. schreibersi* make it probable that the hormonal control of reproduction in this species is essentially similar to that ellucidated for other mammals.

MYERS (1977) reported that in four species of vespertilionid bats from Paraguay (*Lasiurus ega, Eptesicus furinalis, Myotis albescens* and *Myotis nigricans*) the corpus luteum reaches its maximum size at the time of implantation and gradually reduces in size towards the time of parturition. While all the above mentioned species do not have an extended pregnancy, in *Miniopterus schreibersi* where the gestation period is extended by a four month period of delayed implantation. In *M. fraterculus*, where the period of delayed implantation is approximately two-and-a-half months, the corpus luteum reaches its maximum size at about the time of implantation (BERNARD, in prep.). The significance of this delayed growth of the corpus luteum in *M. schreibersi*, and also whether delayed implantation causes delayed corpus luteum growth are not known.

It can be seen from figure 5 that there are prominent differences in the estrous cycles of some vespertilionid bats. *Myotis lucifugus*, *Chalinolobus gouldii* and *Eptesicus regulus* are all from temperate latitudes, they copulate in autumn, store sperm over winter and ovulate in spring. The period from copulation to ovulation has been described as estrus or sub-maximal estrus. In these three species, there is no period of anestrus (Fig. 5), presumably because the extended estrus leaves no time for a reproductively quiescent period. In *Scotophilus wrough-toni* from Bangalore (12°58'N), there is no period of sperm storage, copulation and fertilization occurring in spring. In this species there is a four month anestrus. In *Miniopterus schreibersi*, although pregnancy is extended by four months, there is a two month period of anestrus is also two months. In *M. fraterculus* proestrus is approximately one-and-a-half months longer than in *M. schreibersi*.

#### Acknowledgements

I would like to thank Professor J. MEESTER for critically reading this manuscript and Mrs. D. HAVERLY for typing the manuscript. Financial assistance was received from the C.S.I.R. and the University of Natal Research Committee.

#### Zusammenfassung

#### Monatliche Veränderungen an den Geschlechtssorganen weiblicher Miniopterus schreibersi natalensis (A. Smith, 1834)

Im Laufe des Jahres erfahren Vagina und Ovarien von *Miniopterus schreibersi* zwei Aktivitätsperioden. Die erste Periode von März bis April fällt mit der Zeit der Kopulation und der Entwicklung des Endometriums zusammen. Das Corpus luteum ist zur Zeit der Implantation maximal vergrößert, trotz einer Implantationsverzögerung von 4 Monaten. Die Art der Veränderungen in Vagina, Ovarien und Uterushörnern und ihre zeitlichen Beziehungen lassen vermuten, daß die Fortpflanzung bei *Miniopterus schreibersi* in ähnlicher Weise wie bei anderen Säugetieren hormonal gesteuert wird. Der Oestrus-

zyklus von *M. schreibersi* ähnelt sehr dem von *M. fraterculus*. Er ist durch ein langes Trächtigkeitsstadium und das Auftreten eines Anoestrus charakterisiert. Die Periode der Implantationsverzögerung bei *M. schreibersi* ähnelt dem Lutealstadium anderer Säugetiere darin, daß Graafsche Follikel in den Ovarien fehlen und das Epithel der Vagina dann dünn ist. Dagegen entwickeln sich während des Fötalwachstums Graafsche Follikel, und die Dicke des Vaginalepithels nimmt dann zu.

#### References

- DWYER, P. D. (1963): The breeding biology of *Miniopterus schreibersi blepotis* (Temminck) (Chiroptera) in north-eastern New South Wales. Aust. J. Zool. 11, 219-240.
- DWYER, P. D. (1968): The biology, origin, and adaptation of *Miniopterus australis* (Chiroptera) in New South Wales. Aust. J. Zool. 16, 49–68.
- (1970): Latitude and breeding season in a polyestrous species of *Myotis*. J. Mammalogy 51, 405-410.
- ELS, D. A. (1978): Histochemistry of the reproductive tract of *Miniopterus schreibersi*: the ovary. Unpubl. report, Mammal Res. Inst., Pretoria Univ., South Africa.

FONDA, E.; PEYRE, A. (1965): Localisation de la 5–3 hydroxystéroidodés-hydrogénase dans le placenta de Minioptère au cours de la gestation. C. r. hebd. Séanc. Acad. Aci., Paris 261, 2963–2969.

GOPALAKRISHNA, A. (1949): Studies on the embryology of Microchiroptera. Part III. The histological changes in the genital organs and accessory reproductive structures during the sex-cycle of the vespertilionid bat – *Scotophilus wroughtoni* (Thomas). Proc. Ind. Acad. Sci. **30**, 17–46.

KITCHENER, D. J. (1975): Reproduction in female Gould's Wattled bat, *Chalinolobus gouldii* (Gray) (Vespertilionidae), in Western Australia. Aust. J. Zool. 23, 29–42.

- KITCHENER, D. J.; HALSE, S. A.; (1978): Reproduction in female *Eptesicus regulus* (Thomas) (Vespertilionidae) in South-western Australia. Aust. J. Zool. 26, 257-267.
- KRUTZSCH, P. H. (1975): Reproduction of the Canyon bat, *Pipirtrellus hesperus*, in southwestern United States, Am. J. Anat. 143, 163–200.
- MERWE, M. VAN DER (1977): Reproduction of the Natal Clinging bat, *Miniopterus schreibersi natalensis* (A. Smith, 1834). Unpubl. Ph. D. thesis, Pretoria Univ., South Africa.

MYERS, P. (1977): Patterns of reproduction of four species of vespertilionid bats in Paraguay. Univ. Calif. Publ. Zool. 107, 41 pp.

PEYRE, A.; MALARSINE, A. (1969): L'équipement stéroidodeshydrogénasique et la fonction endocrine du placenta de Minioptère (Chiroptère). C. r. Séanc. Soc. Biol. 163, 914–917.

REEDER, E. M. (1939): Cytology of the reproductive tract of the female bat Myotis lucifugus lucifugus. J. Morph. 64, 431–453.

RICHARDSON, E.G. (1977): The biology and evolution of the reproductive cycle of *Miniopterus* schreibersi and *M. australis* (Chiroptera: Vespertilionidae). J. Zool. (Lond.) 183, 353-375.

WALLACE, G. I. (1978): A histological study of the early stages of pregnancy in the bent-winged bat (*Miniopterus schreibersi*) in north-eastern New South Wales, Australia, (30° 27'S). J. Zool. (Lond.) 185, 519–538.

WILSON, D. E.; FINDLEY, J.S. (1970): Reproductive cycle of a neotropical insectivorous bat, Myotis nigricans. Nature 225, 1155.

WIMSATT, W. A. (1960): Some problems of reproduction in relation to hibernation in bats. Bull. Mus. Comp. Zool. 124, 249–270.

Author's address: RICHARD T. F. BERNARD, Department of Zoology, University of Natal, P.O. Box 375, Pietermaritzburg, Natal, Republic of South Africa

224

# **ZOBODAT - www.zobodat.at**

Zoologisch-Botanische Datenbank/Zoological-Botanical Database

Digitale Literatur/Digital Literature

Zeitschrift/Journal: <u>Mammalian Biology (früher Zeitschrift für</u> <u>Säugetierkunde</u>)

Jahr/Year: 1979

Band/Volume: 45

Autor(en)/Author(s): Bernard Richard T.F.

Artikel/Article: <u>Monthly changes in the reproductive organs of female</u> <u>Miniopterus schreibersi natalensis (A. Smith, 1834) 217-224</u>