

## Evidence of sperm storage in *Pipistrellus kuhlii* (Chiroptera: Vespertilionidae) in western Iran

Mozafar SHARIFI<sup>1</sup>, Rostam GHORBANI<sup>2</sup>, Alireza FAZELI<sup>3</sup> and William HOLT<sup>4</sup>

<sup>1</sup> Department of Biology, Faculty of Science, Razi University, Baghabrisham 67149, Kermanshah, Iran; e-mail: sharifimozafar@hotmail.com

<sup>2</sup> School of Medicine, Kermanshah Medical Science University; e-mail: rostamgh@yahoo.com

<sup>3</sup> Department of Obstetrics and Gynaecology, Sheffield University, Sheffield S10 4RY, England, U.K.; e-mail: a.fazeli@sheffield.ac.uk

<sup>4</sup> Institute of Zoology, Nuffield building, Zoological Society of London, NW1 4RY, U.K.; e-mail: Bill.Holt@ioz.ac.uk

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**A b s t r a c t.** Several species of the genus *Pipistrellus* (Vespertilionidae) store spermatozoa for prolonged periods prior to ovulation, but the reproductive strategy used by *Pipistrellus kuhlii* remains unknown. In order to investigate the prevalence of sperm storage in the bats of this genus we documented endometrial sperm storage in the uterus of two female *Pipistrellus kuhlii* collected during mid-December in western Iran. The uteri were distended with densely packed spermatozoa, those at the periphery exhibiting perpendicular orientation toward the endometrium. This orientation, together with the fact that copulation is unlikely to occur at this time is used to infer that *P. kuhlii* stores sperm over the winter.

### Introduction

Retention of viable spermatozoa within the reproductive tract of either male or female for an extended period of time is defined as sperm storage (Neweiler 2000). Sperm storage typically occurs in females whose gametogenic cycle is not synchronized with that of the male or in males that can retain spermatozoa beyond the cessation of spermatogenesis. The retention of spermatozoa in the reproductive tract of bats residing in temperate regions is associated with delayed ovulation and fertilization and has been reported for over 40 species of bats (Crichton & Krutzsch 2000). The vast majority of species that store spermatozoa belong to either the rhinolophid or vespertilionid families (Racey 1979). Most of these bats reside in temperate regions of the world where a period of hibernation interrupts the reproductive cycle. Several species of the genus *Pipistrellus* (Vespertilionidae) have been reported to store spermatozoa. These include *Pipistrellus subflavus*, *P. minimus*, *P. endoi*, *P. coreensis*, *P. savii*, *P. rusticus* (Crichton & Krutzsch 2000), the Japanese house bat, *Pipistrellus abramus* (Hiraiwa & Uchida 1956), the Indian pipistrelle, *Pipistrellus ceylonicus* (Gopalakrishna & Madhavan 1971), and *Pipistrellus pipistrellus* (Courrier 1921, Racey & Potts 1970).

Three species of the genus *Pipistrellus* including *P. pipistrellus*, *P. savii* and *P. kuhlii* occur in Iran (DeBlase 1980). *P. kuhlii* has been reported from various places in Africa, Middle East, SW Europe and Kashmir (Corbet & Hill 1991). In Iran this bat has been reported in numerous localities east, south and west of the central deserts although it is

conspicuously absent from the northern part of the country (E t e m a d 1983). This vast distribution covers several climatic zones (S h a r i f i et al. 2000) for which no information is available on the reproductive biology of *P. kuhlii*. Many reproductive patterns that have been described for bats of the genus *Pipistrellus* focused on bats from temperate regions of Europe, North America and Japan with one exception concerning a semi-tropical Indian pipistrelle. The present study adds to our knowledge of reproductive patterns of *P. kuhlii* in a temperate mountainous region in mid-Zagros in western Iran.

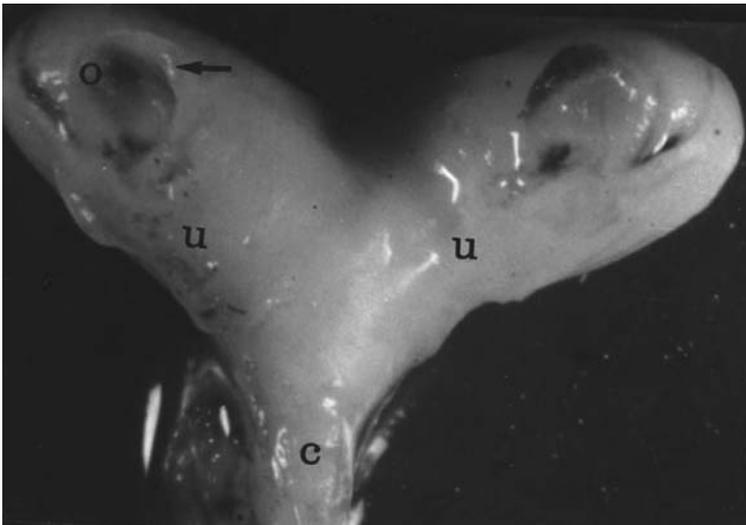
## Material and Methods

Two female *Pipistrellus kuhlii* were collected in Ghassre Shirin in western Iran (34°30' N and 45°25' E) from an old building which was under reconstruction. These two bats were selected from a larger group (15 individuals) which was brought to the Razi University by a curious person on 15th December 2000. All bats had apparently been in deep torpor. The bats weighed 4.9 and 5 grams and had body lengths of 78.7 and 77.5 mm respectively. The bats were kept alive in a wooden cage and were sacrificed using chloroform in a stoppered glass bottle in the laboratory.

The bat specimens were dissected under a light stereo-microscope and the uterus, oviduct, ovaries and urinary system were separated. The separated organs were then fixed in buffered formalin (10 %). Histological sections were prepared from ovary and other tissues. Tissue sections were stained using haematoxylin and eosin. The sections were then examined by light microscopy.

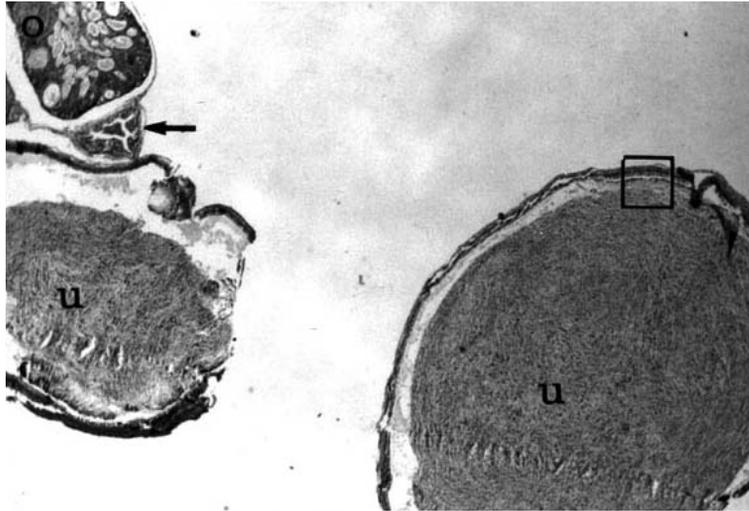
## Results

The reproductive tracts of *Pipistrellus kuhlii* captured in mid-December 2000 were massively distended with spermatozoa (Fig. 1). Examination of sections through the uterus revealed that



**Fig. 1.** Parts of reproductive tract of *Pipistrellus kuhlii* showing (O) ovary (U) uterus and (C) cervix. Arrow showing oviduct. Bar=3mm.

it was densely packed with spermatozoa (Fig. 2). Closer examination of the interaction between spermatozoa and the epithelial cells showed that the spermatozoa at the periphery exhibited perpendicular orientation toward the endometrium (Fig. 3). It is evident that the line up of spermatozoa is most evident in the apical parts of the uterus. However, a relatively small population of spermatozoa was also located in the middle and lower parts of the uterus. Close contact between spermatozoa and the plasma membrane of adjacent epithelial cells is not apparent. This is likely to be due to the shrinkage of the uterus contents as a result of the preparation procedures. In the present study no spermatozoa were found in the oviduct, the uterotubal junction and cervix. Several large follicles were observed in sections of the ovaries from this animal (Fig. 2).



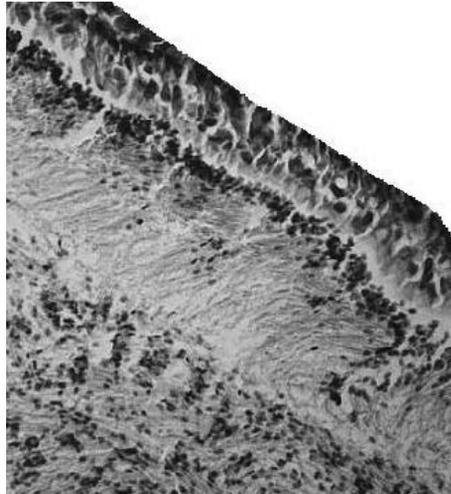
**Fig. 2.** Section showing the junction of the horns of the uterus. Stain: hematoxylin-eosin. 300X.

## Discussion

Sperm storage can be inferred by documenting the existence of a time interval between mating and ovulation (e.g. *Lasiurus ega*, Myers 1977), by demonstrating packing and orientation of spermatozoa toward the secretory epithelium in the uterus (e.g. *Macroglossus minimus*, Hood & Smith 1989) and by conducting isolation experiments of females after copulation (e.g. *Pipistrellus ceylonicus*, Gopalakrishna & Madhavan 1971). In the present study histological evidence obtained from two female *P. kuhlii* collected at mid-December indicate that this species store spermatozoa in western Iran. The evidence is based on the packing and orientation of spermatozoa in the uterus of this bat. Perpendicular orientation of stored spermatozoa with their heads toward the reproductive tract epithelium have been frequently used to demonstrate sperm storage in Chiroptera (Uchida & Mori 1987). This technique has also been used in other vertebrates that store sperm such as snakes (Hoffman & Wimsatt 1972), birds (Van Krey et al 1967), fish (Jalabert & Billard 1969) and mammals (Racey 1975).

In demonstrating sperm storage using light microscopy not only is the level of perpendicular orientation of spermatozoa important but so also is the location of

spermatozoa and their distance from epithelial cell plasma membrane. Reports indicate that the sites utilized by bats for sperm storage vary (Uchida & Mori 1987). However, in this study the most widely used sites include the apical parts of the uterus. The site of sperm storage in female bats varies with species (Racey 1979). Spermatozoa have been reported in different parts of the reproductive tract. In *P. abramus* (Hiraiwa & Uchida 1956) and in *P. pipistrellus* (Racey 1975) the stored spermatozoa have been reported only from the uterus. However, in the Indian pipistrelle, *P. ceylonicus* the surviving spermatozoa have been reported in the oviduct (Racey 1979).



**Fig. 3.** Sections part of uterus. Stain: hematoxylin-eosin. 500X.

Considerable numbers of follicles are present in the ovary of *Pipistrellus kuhlii* collected in mid-December (Fig. 2) in western Iran. It has been shown that in many vespertilionid bats including one species of the genus *Pipistrellus* (*Pipistrellus mimus*) the ovaries typically possess considerable number of interstitial glands and follicles (Oxberry 1979). Similarly, the reproductive cycle of *Scotophilus heathi* in India is characterized by arrested ovarian activity between December and mid-February (Abhilasha & Krishna 1996). In this bat copulation occurs between mid-January and mid-February and spermatozoa are stored by the female until ovulation takes place. These authors have argued that the effect of androgen, secreted from the interstitial tissue, might be responsible for the inhibition of development and maturation of ovarian follicles, and cause the delayed follicular development. In those species of bats which inhabit temperate areas during weeks immediately preceding hibernation significant follicle growth becomes evident (Wimsatt et al. 1966). During hibernation which may last for several months these surviving follicles persist in the ovaries. In these bats the female reproductive tract remain in an oestrous state during hibernation. Such long term oestrous is also apparent in bats that demonstrate prolonged storage of spermatozoa.

In a recent review of sperm storage in Chiroptera (Richton & Krutzsch 2000) and also in the review published by Racey (1979) there is no mention of *Pipistrellus kuhlii* as a species that stores spermatozoa although 8 other species of the genus *Pipistrellus*

are known to have this capability. Therefore we regard this incident as the first report of sperm storage in *Pipistrellus kuhlii*.

Since there is inadequate information regarding the time of mating and parturition of *P. kuhlii* in western Iran, it is not possible to give an exact estimate of the duration of fertilizing life of the stored spermatozoa. Current observations (M. S h a r i f i , unpublished) on the reproduction patterns of other species of bats (*Myotis blythii*, *Miniopterus schreibersii* and *Rhinolophus mehelyi*) living in the area may help to estimate the fertilizing period of Kuhl's pipistrelle. These observations reveal that long before mid-December, probably in late August, females desert nursery sites and seek males for copulation. At the same time fat begins to build up for hibernation. By mid-September most of these bats disappear from their maternity roosts and become torpid in their hibernacula. However, in early March the bats gradually reappear in the nursery sites and by mid-May the *M.blythii* gives birth to pups. Within two to three weeks *M.schreibersii* and *R.mehelyi* also give birth to young. If we assume that the timing of the reproductive pattern of *P.kuhlii* follows those of the local bat species in the area the total fertilizing life of spermatozoa stored in the female bat would become approximately 5 months. This is similar to the 151 days fertilization period reported for *P. pipistrellus* by R a c e y (1979). However, it is shorter than 175 days reported for *P. abramus* (H i r a i w a & U c h i d a 1956).

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