Evidence of sperm storage in *Myotis capaccinii* (Chiroptera: Vespertilionidae) in western Iran

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Abstract: **Several species of the family Vespertilionidae store spermatozoa for prolonged periods prior to ovulation, but the reproductive strategy used by *Myotis capaccinii* remains unknown. Reproductive cycle of *Myotis capaccinii* has been determined using macroscopic and microscopic examinations on two captured bats in spring and one male and one female reared in a flight cage during winter. Microscopic slides prepared from one male collected in late July showed spermatids indicating that spermatogenesis develops in summer. Estimation of the volume of testes and epididymis based on photographs taken on weekly intervals during late summer until late winter in male, *M. capaccinii* shows that the rate of epididymis to testes volume increases by more than one order of magnitude from late summer until late winter. Microscopic slides prepared from this bat indicate that decapacitated spermatozoa are packed in the tubules in the epididymis in late winter.**

Key words: sperm storage, reproductive strategy, *Myotis capaccinii*.

Introduction

Because of the short summer in temperate regions, the breeding cycle in insectivore bats should typically be synchronized with abundance of insects and birth should occur over a very short period of time (Racey, 1982). In order to adjust to the short supply of food different reproductive strategies have been evolved in bats (Neuweiler, 2000). Through these strategies both offsprings and females obtain greater chance of survival by accurate timing of the birth. Delayed ovulation and fertilization (e. g. in *Pipistrellus ceylonicus*, Racey, 1979), delayed implantation (e. g. in *Rhinolophus rouxi*, Rasweiler 1993) and embryonic diapause (e. g. in *Artibeus jamaicensis*, Altringham, 1996) are amongst the reproductive strategies that are used by bats in order to cope with changing environments. Retention of viable spermatozoa within reproductive tract of either female or male for an extended period of time is defined as sperm storage (Crithonn, 2000).

The majority of species that perform special reproductive strategy 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 intervenes the reproductive cycle.

Several species of the genus *Myotis* have been reported to store spermatozoa. These include *Myotis tricolor* (Bernad, 1982), *M. formosus*, *M. ikonnikovi*, *M. leucogaster* (Uchida and Mori, 1987), *M. lucifugus* and *M. grisescens* (Miller, 1942). Six species of the genus *Myotis* including *M. mystacinus*, *M. emarginatus, M. nattereri, M. bechsteini, M. blythii* and *M. capaccinii* occur in Iran (Sharifi et al.,

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2000), none of which have been documented to store spermatozoa. The Iranian distribution of *Myotis capaccinii* is not well known and only seven localities have been reported for this bat (DeBlase, 1980; Akmali et al., 2004). However, it is possible that this bat occurs in Zagros range in western Iran and possibly in Elbourz mountains as well. The vast distribution of this species covers several climatic regimes (Sharifi et al., 2000) in which no information is available on reproduction biology of *Myotis capaccinii*. Therefore the aim of present study is to determine the reproductive cycle of both male and female of this bat.

Materials and Methods

Bats included in this study were restricted to *Myotis capaccinii* specimens captured from two new localities in Mahidasht cave (33°, 23'N and 47°, 30'E) and a fish pond in Qasre e Shirin (34°, 31'N and 45°, 35'E) in Kermanshah Province. These specimens had been fixed with 10% formalin and stored in 70% alcohol. Upon capture, bats were sexed and aged as adult and juvenile on the basis of the wing epiphyseal gap. We conducted this study in a flight cage at the Department of Biology, Razi University, in Western Iran. Two male *Myotis capaccinii* licensed by the regional Office of the Environment were collected and reared in the flight cage during the autumn and winter of 2003. Bats captured were marked individually with numbered aluminum alloy ring (Porzana Ltd, London) and then released in a flight cage (six m long two m wide two m height). The cage was covered from outside by sheets of cardboards in order to isolate the bats from excess of light and noise in the laboratory and maintained under natural light regime. The bats had unlimited access to fresh water in shallow dishes and to mealworm in plates placed at the floor of the cage and also on a desk at the height of 1m. Initially, bats were fed by hand, but from two-five days after capture they fed themselves. For males, the degree of testicular and epididymal enlargement was used as a measure of reproductive activities. Dimensions of testes and epydymes have been measured on the basis of photographs taken on weekly intervals during late summer until late winter

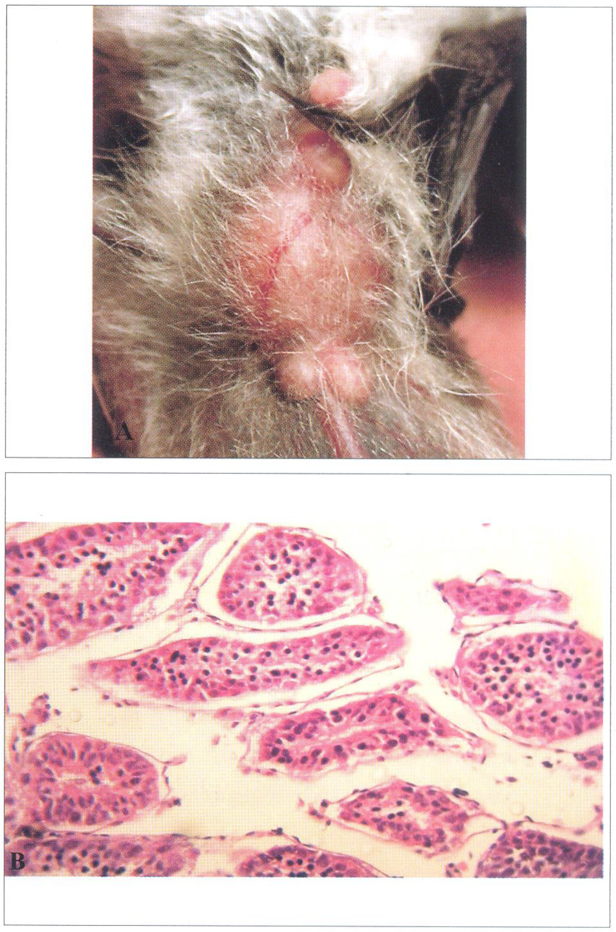

Figure 1. Enlarged testes in late spring (A) and a slide showing (B) early spermatogenesis with spermatids in seminiferous tubules.

in one male *M. capaccinii* reared in a flight cage. Testicular volume was calculated using the formula for volume of an ellipsoid (Woodall and Skinner, 1989). The ratio of the epididymis to testes was used to evaluate the speed of development in the reproductive cycle in male M. capaccinii. Bat specimens were dissected under light stereomicroscope; testis and cauda epididymidis of male were separated.

Tissues were dehydrated in a graded alcohol series, cleared in xylene and wax embedded. The separated organs were then fixed in formalin (10 %). Microscopic sections were prepared from various reproductive organs through generating serial sections with a thickness of 6 microns. Following fixation, tissues were stained using hematoxylin and eosin. The sections were then examined and photographed under a camera microscope (Leitz Dialux 22). Testes and cauda epididymis were

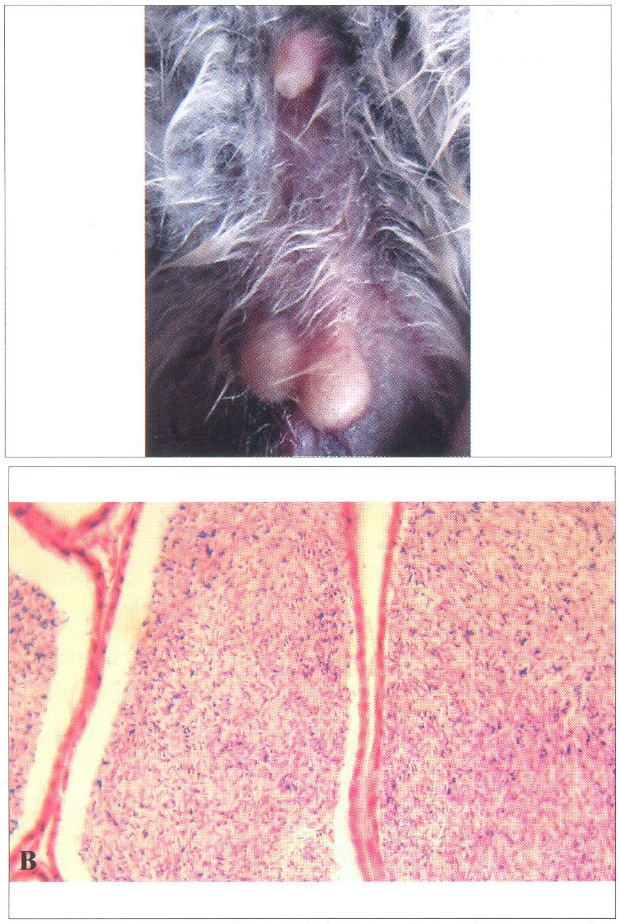

Figure 2. Enlarged epididymedis in late winter (A) and a slide showing spermatozoa (B) in epididymedis.

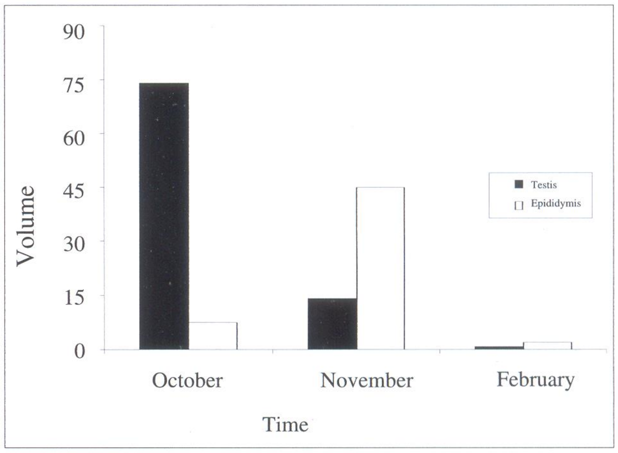

Figure 3. Changes in length of testis and epididymis of one male M capaccinii kept in captivity.

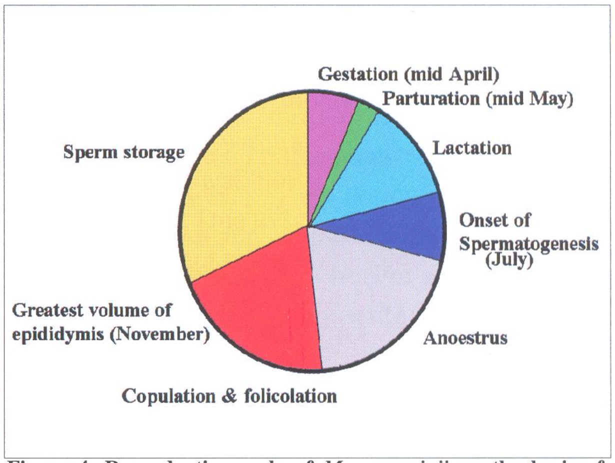

Figure 4. Reproductive cycle of *M. capaccinii* on the basis of information obtained from male bats reared in a flight cage during autumn and winter, parturition time of females gave birth in the flight cage and a gestation period of five weeks.

separated and weighed.

Results

Size of the Testes in *Myotis capaccinii* began to increase in early July and before the end of summer testicular size reached to its maximum. This increase in testes mass is mainly due to the development of spermatogenic cells up to the spermatid stage. During this period no spermatozoa were released into epididymis. In late summer the spermatozoa began to move into epididymis. This was clearly illustrated by the decrease in testicular mass and concomitant increase in epididymal mass by the end of summer. Comparison between enlarged testes in August and the recessed testes in winter time is shown in Figure 1A and Figure 2A. In contrast with testis mass, epididymal volume peaked in November (Figure 3). Histological examinations of male testes and epididymes were in accordance with the reproductive pattern apparent from macroscopic data (Figure 1B and Figure 2B). Few spermatozoa were recorded in testes during November, while at the same time considerable volume of sperm existed in the cauda epididymides (Figure 2B). In autumn the proportion of bats with enlarged testis declined when no males showed signs of testis enlargement. In contrast, male bats began to have enlarged epididymis. Table 1 shows length, width and volume of testis and epididymidis in bat captured and kept in cage on during autumn and winter.

Discussion

Normally, sperm storage has been documented by several methods including isolation experiment (e. g. *Pipistrellus ceylonicus*, Gopalakrishna and

Table 1.Length, width and volume of testis and epididymidis in bat captured and kept in the flight cage during autumn and winter.

TIME	L.T	L.E	W.T	W.E	V.T	V.E
2003/10/28	7.2	2.2	8.4	3.5	84.46	4.48
2003/11/04	6.8	2.5	8	4.5	72.35	8.42
2003/11/11	6.5	3.2	7.3	4.6	57.59	11.26
2003/11/18	6	3.7	6.5	5	42.14	15.38
2003/11/25	5.4	4.8	6	5.2	32.32	21.58
2003/12/02	5	6.1	5.4	5.8	24.24	34.12
2003/12/16	4.5	6.4	4.3	6.4	13.83	43.58
2003/12/30	4	6.5	3.6	6.1	8.62	40.21
2004/01/20	3.6	4.5	3	5.8	5.39	30.20
2004/02/09	2.8	4.2	2.7	4.3	3.39	12.91
2004/02/17	2	2.8	2	3.4	1.33	5.38
2004/03/01	1.1	1.4	1.6	2.7	0.47	1.70

Madhavan 1971), demonstrating a time lag between mating and ovulation (e. g. *Lasiurus ega*, Myers 1977) and by demonstrating packing and orientation of spermatozoa toward the secretory epithelium in the uterus (e. g. *Macroglossus minimus*, Hood and Smith 1989).

In all these methods female bats are subjected to the experiments, however, changes in male reproductive organs can equally be indicative of storage (Crichton and Krutzsch, 2000).

In the present study both macroscopic and histological evidences have been obtained from males to infer this bat store spermatozoa. Macroscopic data available from this study implies that spermatogenesis only occurred until late summer. This is based on changes in testicular development and testis mass in captured and fixed specimens and also on the assumption that maximum testes size is associated with maximal spermatogenic activity (Gustafson 1979; Hosken et al., 1998).

These data resembled those from many other temperate zone vespertilionid and rhinolophid bats such as *Myotis lucifugus* and *M. gricescens* (Miller 1942), *Rhinolophus hipposideros* (Gaisler 1966), *Nyctalus noctula* (Racey, 1974), and *Pipistrellus pipistrellus* (Racey and Taam 1974) and *Pipistrellus kuhlii* (Sharifi et al., 2004). All these species undergo seasonal spermatogenic activity during summer and autumn. The spermatogenic activity ceases before winter hibernation. In addition to testicular changes, the activity of accessory sex glands also typifies vespertilionid bats inhibiting temperate regions in both northern and southern hemisphere. Generally, in these bats prostate gland and epididymal activity increased to its highest level after spermatogenesis was terminated in late summer (Racey and Taam 1974). In order to estimate the total sperm storing period one need to have data on mating time in this bat. In Iran there is only one report on captive parturition of three *M. capaccinii* which is in mid May (Akmali et al., 2004).

Sperm storage like other reproductive delays occurs mostly in hibernating species, although several tropical species have also shown the ability to store spermatozoa. Racey and Entwistle (2000) have identified several potential selective advantages that might favor the development of reproductive delays including the functional relationship between sperm storage and heterothermy, seasonal constraints on reproduction, timing of reproductive event between males and females, mate selection and provision for sperm competition. Sperm storage would enable the bats to synchronize the beginning of their gestation with the period in which food supply has improved. The ability to store spermatozoa could also extend the mating season and ensure insemination in separated populations. In bats with an extended period of mating, there is a risk of desynchronized parturition pattern. Therefore, the ability to store sperm might have evolved to promote reproductive synchrony when male and females mated over an extended period.

Information obtained from current study can not provide adequate data necessary to estimate the duration of fertilizing life of the stored spermatozoa because there is no information regarding the onset of mating in *M. capaccinii*. However, if we assume that at the time when epididymis reaches to its greatest volume the reproducing female receive adequate spermatozoa to store during the entire hibernation period in winter and fertilize the oocyte in spring, it would be possible to provide a rough estimate of duration of various reproductive events for this species. Considering that this bat give birth to neonates in mid May and assuming that the gestation period for this bat is equal to other similar size bat, the reproductive cycle of *M. capaccinii* can be shown in Figure 4.

In two reviews on sperm storing bat by Crichton and Krutzsch (2000) and Racey (1979) 41 species of bats have been shown to store spermatozoa. In another study (Sharifi et al., 2004) one more species (*Pipistrellus kuhlii*) has been shown to store spermatozoa for 5.5 months. In the available list of sperm storing bats there is not mention of *M. capaccinii* as a species that store spermatozoa although several other species of the genus *Myotis* are known to have this capability. Therefore, we regard this incident as the first report of sperm storage in *M. capaccinii*.

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ذخیره اسپرم در خفاش «انگشت دراز» (Chiroptera: Vespertilionidae) *Myotis capaccinii* در غرب کشور

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چکیده

تعدادی از گونه‌های خفاش از خانواده "خفاش‌های شب" (**Vespertilionidae**) تا قبل از اوولاسیون اقدام به ذخیره طولانی مدت اسپرم می‌نمایند ولی هیچگونه اطلاعاتی در مورد استراتژی تولید مثل خفاش انگشت دراز وجود نداشته است. با استفاده از مشاهدات میکروسکپی و ماکروسکپی از دو خفاش نمونه برداری شده در فصل تابستان و همچنین دو خفاش نر و ماده‌ای که در طول زمستان در قفس پرواز نگهداری شدند سیکل تولید مثل این خفاش معین گردید. وجود اسپرماتید در اسلایدهای تهیه شده از خفاش نر در اوائل تابستان نشان داد که برخلاف بسیاری از پستانداران دیگر اسپرماتوژنز در تابستان کامل می‌گردد. برآورد حجم بیضه و اپیدیدیم در طول پائیز و زمستان بر اساس عکس‌های گرفته شده نشان داد که از اواخر تابستان تا اواخر زمستان حجم اپیدیدیم در این خفاش بیش از ده برابر می‌گردد. اسلایدهای تهیه شده از خفاش‌های نر در طول زمستان نشان می‌داد که توده‌های فراوانی از اسپرم در لوله‌های اپیدیدیم وجود دارند.

واژه‌های کلیدی: ذخیره اسپرم، استراتژی تولید مثلی، *Myotis capaccinii*.

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