Blood plasma concentrations of progesterone, sperm storage and sperm viability and fertility in Gould’s wattled bat (Chalinolobus gouldii)

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The fertility and viability of spermatozoa stored by male and female Gould’s wattled bats, Chalinolobus gouldii, was investigated in a captive colony of ten bats (three males and seven females). Bats were housed in outdoor flight cages. Plasma progesterone concentrations, measured using double antibody radioimmunoassay, isolation experiments plus sperm motility and sperm membrane stability tests were used to evaluate the viability and fertility of stored spermatozoa. Mean plasma progesterone concentrations were lowest during midwinter (< 0.5 ng ml⁻¹) with a 20-fold increase recorded in late winter to early spring. During pregnancy, plasma progesterone concentrations increased to about 13 ng ml⁻¹ and returned to basal values soon after parturition. The results of the plasma progesterone assays and the isolation experiments indicate that female C. gouldii can store fertile spermatozoa for at least 33 days. The investigation of spermatozoa stored by male C. gouldii revealed that 6–7 months after peak spermatogenesis about 60% of the stored spermatozoa were motile and more than 60% had stable membranes, indicating that the spermatozoa stored by males were viable and likely to be fertile. The results of this study clearly indicate that both male and female C. gouldii are capable of storing fertile spermatozoa for prolonged periods.

Introduction

Hibernating, insectivorous bats from temperate latitudes have a number of reproductive peculiarities (Wimsatt, 1980). In these bats, hibernation impinges upon the reproductive period and as a result, the events begun in late summer are not completed until the following spring (Oxberry, 1979). This hiatus involves either a period of prolonged sperm storage, in conjunction with delayed ovulation and fertilization, delayed implantation of the early fetus (Oxberry, 1979) or embryonic diapause (Richardson, 1977). Each of these modes of reproduction allows mating and parturition to be optimally timed, independently (Sandell, 1990), but sperm storage may have the added advantage of allowing selection to occur after mating via sperm competition (Birkhead and Moller, 1993). Seasonally delayed fertilization is found in most vespertilionid and rhinolophid bats that hibernate (Oxberry, 1979). Matings typically occur before hibernation, although mating during and after hibernation can occur and spermatozoa are stored by both male and female bats (Racey 1979).

While prolonged sperm storage has been documented in many bats (more than 30 species are currently known to store spermatozoa), there have been few investigations of the fertility of the stored spermatozoa. In a review by Racey (1979), only eight species were listed as being confirmed stokers of fertile spermatozoa, with the maximum recorded storage of fertile spermatozoa being 198 days (Racey 1973). Clearly, without investigating the fertility of stored spermatozoa the significance of storage is difficult to establish, since storage is only adaptive if the stored spermatozoa are fertile (for review see Fenton, 1984).

A number of Australian bats are known to store spermatozoa. These include Chalinolobus gouldii (Kitchener, 1975), C. morio (Kitchener and Costa, 1981), Mormopterus planiceps (Krutzsch and Crichton, 1987), Nyctophilus gouldii (Phillips and Inwards, 1985), Vespertulus regulus (Kitchener and Halse, 1978; Tidemann, 1993) and V. vulturnus and V. darlingtoni (Tidemann, 1993). However, in spite of the abundance of these and other examinations that have documented prolonged sperm storage, there have been no studies on the viability and fertility of the stored spermatozoa in Australian Microchiroptera.

This study aims to investigate the viability and fertility of spermatozoa stored by captive Gould’s wattled bat, C. gouldii (Chiroptera: Vespertilionidae). This tree-roosting vespertilionid bat (body mass 10–20 g) is one of the most common and widespread Australian bat and is found throughout most of the continent (Hall and Richards, 1979; Reardon and Flavel, 1987). Studies by Kitchener (1975) and I. Schlawe (unpublished data) indicate that both female and male C. gouldii store spermatozoa over the winter. Females in the south-west of Western Australia are found to be inseminated as early as May (autumn), while ovulation and pregnancy did not ensue until
late August/early September (late winter/early spring), with two young born 3 months later (Kitchener, 1975). C. gouldii is reported to enter torpor in the cooler parts of its range (Dixon, 1983) and recent work has confirmed that this species is extremely thermally labile and able to enter torpor at ambient temperatures as high as 25°C (Hosken and Withers, in press). We report here on the results of experiments designed to investigate the viability and fertility of spermatozoa stored by male and female C. gouldii.

Materials and Methods

Animals

Seven female Chalinolobus gouldii were collected under licence in April 1994 from the state forest 70 km south of Perth, Western Australia (32°S). These bats were held in captivity over winter before isolation trials were undertaken. This ensured that the females were more than one year old. This precaution was necessary since many bats are not sexually mature in their first year (Tuttle and Stevenson, 1982) and it is difficult to distinguish between mature bats and young of the year once the latter animals have obtained adult size. The three male bats that were used in the experiments were captured in March 1995. The male bats were chosen on the basis of their enlarged testes which indicated that spermatogenesis was underway (I. Schlawe, unpublished data).

All bats were housed at the Department of Zoology, University of Western Australia in two outdoor flight cages (5 m x 2 m x 2.2 m). Bats were maintained on a diet that primarily consisted of mealworms (larval Tenebrio molitor), with the occasional addition of other insects, including bushcrickets and moths. Mealworms were dusted with powdered milk and once a week a vitamin supplement (Pentavite®) was added. Water was supplied ad libitum.

Methods

Three procedures were used in the investigation: isolation experiments were used to investigate sperm fertility, and sperm motility examinations and sperm membrane stability tests were used to investigate sperm viability.

Isolation experiments involved separating female and male bats during winter before ovulation but after copulation. Females were separated from males on 14 July, some 2.5 months after spermatozoa are usually found in wild animals (Kitchener, 1975). Animals had cohabited for 4.5 months at the time of separation. Plasma progesterone concentrations were monitored to ensure that females were not pregnant before separation and thus the possibility of falsely asserting that stored spermatozoa caused pregnancy could be circumvented. Blood samples had been taken from female bats throughout the year to establish basal plasma progesterone concentrations and it was assumed that animals with basal concentrations at the time of separation were not pregnant.

Blood was taken by puncturing a vein in the uropatagial membrane with a 26 gauge needle and collecting the outflow in a heparinized micro-haematocrit tube. The volume of blood collected in this way never exceeded 150 μL. Blood was immediately centrifuged at 13 000 g for 4 min, after which blood haematocrit values were recorded. Capillary tubes were then fractured at the cell–plasma interface, the blood solids were discarded and the plasma portion was transferred to Eppendorf tubes, frozen and stored until assays were performed. In addition, when two females were killed (see below), more than 300 μL of blood was collected via cardiac puncture with a 26 gauge needle. Blood was transferred to heparinized micro-haematocrit tubes and treated as above. The relatively large volume of blood obtained via heart punctures was used to check for parallelism with the standard curve and validate assays performed on smaller volumes of plasma. The concentration of progesterone in the plasma samples was measured in hexane extracts of small aliquots of plasma using double antibody radioimmunoassay. Two millilitres of hexane was added to each sample which was then vortexed for 2 min. The solvent was removed, and the sample was dried with N₂ gas, reconstituted with buffer and assayed. The assay method followed that of Martin et al. (1983) with some slight modifications. These were: (1) the volumes of plasma used were smaller. Volumes varied but were always greater than 25 μL and a minimum of two replicates per sample was used in each assay. (2) The antiserum used in this study was raised in a rabbit against progesterone-IIα-carboxymethylexolime (CMO)–HSA. It had the following crossreactivity: 100% progesterone deoxy corticosterone (2%), 20α-OH progesterone, 17β-OH progesterone, 17α-OH progesterone and alloprogrenolone (all 1%). The limit of the assay was 0.02 ng per tube. The intra-assay variation (mean ± coefficient of variation) was 0.15 ± 0.24, 5.21 ± 0.19, and 8.14 ± 0.34 for quality controls containing 4.25, 2.13 and 0.92 ng mL⁻¹, respectively. The interassay coefficients of variation were 3.76, 5.16 and 6.52, respectively.

The other two procedures involved killing bats and examining their reproductive tracts for the presence of live spermatozoa. Four animals (two female and two males) were killed with an i.p. injection of Valarabarb® (Pitman Moore, North Ryde) administered at a dosage of 200 mg kg⁻¹. Anaesthesia is reported to have no effect on the vitality of spermatozoa (J. Cummins, personal communication). Within minutes of death, the reproductive organs were removed. The procedure for females was as follows: a ventral incision was made from the mid-chest region posterior to the pelvis, allowing the organs to be accessed. The alimentary canal was displaced and the corpus uterus, uterine horns and ovaries were removed. Only this portion of the female tract was examined, as Kitchener (1975) reported that spermatozoa are stored in uterine glands and in the uterine portion of the oviducts. The dissected piece of the reproductive tract was then placed into a 50 ml Petri dish containing a modified Tyrode’s Medium (TM) (composition in g L⁻¹ unless specified otherwise: NaCl: 5.689; KCl: 0.356; CaCl₂·2H₂O: 0.294; NaHCO₃: 2.10; MgCl₂·6H₂O: 0.096; Na₂HPO₄: 0.048; sodium pyruvate: 0.053; sodium lactate: 2.0 mL; glucose: 1.00; Hepes: 5.206; Phenol Red: 0.0055; BSA: 3.00). The left uterine horn and left ovary were then separated from the uterus with an incision approximately mid-way between the utero–tubal junction and the point of uterine bifurcation. This portion of the uterine complex was then transferred to another 50 mL Petri dish containing a small volume (one or two drops) of TM. The ovary was then removed and using a binocular dissecting microscope, a
blunted 30 gauge needle was inserted into the exposed infundibulum. A retrograde flush of the oviduct and attached uterine horn then took place, using a small volume of TM gently expelled from a syringe attached to the needle. A number of samples of the medium were then slide-mounted and examined for the presence of motile spermatozoa using interference contrast microscopy × 500 magnification. The portion of tract that had been flushed was placed into another dish containing a small volume of TM and the uterine horn was cut open and further washed with TM. This medium was also examined for the presence of spermatozoa.

The intact uterine horn and both left and right ovaries were fixed in Bouin’s fluid, dehydrated in a graded alcohol series, embedded in wax and serially sectioned at 6 μm. Sections were slide-mounted, stained with Harris’s haematoxylin and counterstained with eosin (Humason, 1979). Sections were then examined under a light microscope for the presence of spermatozoa. This examination was used as a check for the presence or absence of spermatozoa in the tract if no spermatozoa were detected after flushing of the contralateral oviduct and uterine horn.

The cauda epididymides were removed immediately from the two males after death. The epididymides were placed into a small volume of TM and forceps were used to gently squeeze spermatozoa from the vas deferens end of the epididymis. Samples of the medium were slide-mounted and examined as above.

As soon as spermatozoa were detected, the numbers of motile and non-motile spermatozoa were counted. This involved using interference contrast microscopy (×500) to count the number of motile and non-motile spermatozoa within the area demarked by a graticule. Visual assessments of this nature are quite a precise method of counting spermatozoa (Jequier and Crich, 1986). The stability of the sperm membrane of other samples was assessed by a hypo-osmotic swelling test (Weiske and Maleika, 1987) in which small volumes of spermatozoa in TM were transferred to test tubes containing a hypo-osmotic solution of sodium citrate and fructose (150 mosmol L⁻¹). Test tubes were incubated in a water bath at 35°C for 30–45 min. This solution was then slide-mounted and examined using interference contrast microscopy for spermatozoa with functional membranes, as detected by curling of fibres in the tail membrane or tail swelling (Paz et al., 1990).

### Statistical analyses

All statistics were performed using the Statview SE + Graphics (Abacus Concepts) statistical package. Regression analysis, Student’s t tests and ANOVA were used, with all tests using a maximum \( P = 0.05 \) to determine significance. Where appropriate, means are presented with ±1 standard error and the sample size (n).

### Results

**Females: haematocrit, plasma progesterone and isolation experiments**

Blood haematocrit values varied from 38.0 to 66.0. Haematocrit was generally lowest during late winter and early spring and highest in late summer, early autumn. Lowest mean monthly values were recorded in October, mean = 43.3 ± 0.99 (n = seven measurements on five bats), and highest mean monthly levels were obtained in April, mean = 63.6 ± 0.86 (n = seven measurements on seven bats). There was no obvious variation in blood haematocrit values due to reproductive condition as values for pregnant and non-pregnant females were identical, nor was there any consistent pattern of variation due to repeated blood sampling.

Plasma progesterone concentrations varied considerably throughout the year (Table 1), with a steady decrease from February until July (late summer until mid-winter). Regression analysis revealed that the negative relationship between blood plasma progesterone concentrations and the date of sampling was statistically significant from 28 February to 26 July 1995, although the sampling date explained only 40% of the variation observed in plasma concentrations (\( P < 0.0001, r^2 = 0.4, n = 48 \) measurements on seven bats). Lowest plasma progesterone concentrations (\(< 0.5 \text{ ng ml}^{-1}\)) were recorded in June and July (early to mid-winter), but because of the large variation in progesterone concentrations of individuals from the end of March until the end of July, mean concentrations were not statistically different (ANOVA Fisher’s PLSD; all \( P \) values > 0.2). By mid-August (late winter), mean blood plasma progesterone concentrations had increased to more than 20 times (\(> 6 \text{ ng ml}^{-1}\)) higher than the minimum previously

<table>
<thead>
<tr>
<th>Date</th>
<th>Stage of cycle¹</th>
<th>Plasma progesterone (ng ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 February</td>
<td>Pro-oestrus</td>
<td>2.39 (± 0.09; n = 3)</td>
</tr>
<tr>
<td>17 March</td>
<td></td>
<td>2.63 (± 0.73; n = 5)</td>
</tr>
<tr>
<td>28 March</td>
<td></td>
<td>2.34 (± 0.77; n = 7)</td>
</tr>
<tr>
<td>17 April</td>
<td>Oestrus</td>
<td>1.16 (± 0.29; n = 7)</td>
</tr>
<tr>
<td>17 May</td>
<td></td>
<td>0.98 (± 0.28; n = 6)</td>
</tr>
<tr>
<td>6 June</td>
<td></td>
<td>0.47 (± 0.16; n = 7)</td>
</tr>
<tr>
<td>3 July</td>
<td></td>
<td>0.30 (± 0.05; n = 7)</td>
</tr>
<tr>
<td>26 July</td>
<td></td>
<td>0.40 (± 0.16; n = 7)</td>
</tr>
<tr>
<td>18 August</td>
<td></td>
<td>0.56 (± 2.95; n = 6)</td>
</tr>
<tr>
<td>12 September</td>
<td>Pregnant</td>
<td>12.50 (± 0.32; n = 2)</td>
</tr>
<tr>
<td>22–25 September</td>
<td>Pregnant</td>
<td>13.07 (± 0.81; n = 2)</td>
</tr>
<tr>
<td>9 October</td>
<td>Pregnant</td>
<td>3.32 (± 0.55; n = 4)</td>
</tr>
<tr>
<td>19 October</td>
<td>Pregnant</td>
<td>4.34 (± 3.47; n = 3)</td>
</tr>
<tr>
<td>3–8 November</td>
<td>Pregnant</td>
<td>1.70 (± 0.71; n = 4)</td>
</tr>
<tr>
<td>17 November</td>
<td>Lactating</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>4 December</td>
<td>Lactating</td>
<td>0.86</td>
</tr>
<tr>
<td>20 December</td>
<td>Lactating</td>
<td>0.45 (± 0.21; n = 4)</td>
</tr>
<tr>
<td>15 January</td>
<td>Lactating</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>Not lactating</td>
<td>0.16 (± 0.08; n = 3)</td>
</tr>
</tbody>
</table>

Table 1. Changes in mean plasma progesterone concentration in adult female Gould’s wattled bats during 1995

¹As outlined in Kitchener, 1975.

Values are means (± se where \( n > 1 \)).

\( n = \) seven measurements on five bats, and highest mean monthly levels were obtained in April, mean = 63.6 ± 0.86 (\( n = \) seven measurements on seven bats). There was no obvious variation in blood haematocrit values due to reproductive condition as values for pregnant and non-pregnant females were identical, nor was there any consistent pattern of variation due to repeated blood sampling.

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recorded. Student's t test comparison of the mean plasma progesterone values recorded in July and August revealed that this difference was statistically significant (t value = 3.66, d.f. = 16, P = 0.0021). By early September (spring), the recorded plasma progesterone concentrations reveal a clear dichotomy. Two bats, which subsequently proved to be pregnant, had high progesterone concentrations (mean = 12.6 ± 0.32 ng ml⁻¹); conversely, the mean plasma progesterone concentration of the other five bats that did not produce young remained relatively constant when compared with mid-August concentrations (5.6 ± 1.4 ng ml⁻¹ on 12 September compared with 6.56 ± 2.95 ng ml⁻¹ on 18 August). Comparison of the mean plasma progesterone concentrations of animals that were subsequently found to be pregnant and those that produced no young revealed that the plasma progesterone concentrations of pregnant animals were significantly higher than those of non-pregnant bats in all samples where comparisons were possible (Student's t tests: 12 September, t value = −2.98, d.f. = 5, P = 0.031; 22–25 September, t value = −10.13, d.f. = 4, P = 0.0005). This dichotomy was apparently maintained for the next 50 days, with the pregnant bat maintaining increased plasma progesterone concentrations, while the mean concentration for non-pregnant bats fell steadily to reach basal values by early December (mean = 0.45 ± 1.4 ng ml⁻¹, n = 4). (One of the pregnant bats was killed in September.) It should be noted that the plasma progesterone concentration of the surviving, pregnant bat fell to 4.64 ng ml⁻¹ at about mid-pregnancy (19 October), before increasing again before parturition.

Progesterone assays indicated that the pregnant bat (no. 58), that subsequently produced twins had basal plasma progesterone concentrations for at least 5 weeks after separation from male bats (Fig. 1). On 26 July 1995, 12 days after separation, her plasma progesterone concentration was lower than at any other time (0.12 ng ml⁻¹) and on 18 August concentrations had not changed appreciably, and were in fact similar to those recorded in May (0.16 ng ml⁻¹). The other bat that became pregnant had a slightly increased plasma progesterone concentration on 26 July (1.28 ng ml⁻¹), 2 weeks after separation; however, insufficient blood was obtained for assaying again before 12 September 1995, by which time plasma progesterone concentrations were very high (12.24 ng ml⁻¹).

The plasma progesterone concentrations of the female that gave birth on 11 November 1995 had fallen to undetectable values by 17 November, one week postpartum, and stayed very low for the duration of lactation (mean = 0.52 ± 0.3 ng ml⁻¹, n = 4 measurements on one bat) (Fig. 1).

**Females: retrograde flush**

Two females were killed in September. At this time females had been separated from males for 49 days. Retrograde flushing of the left fallopian tube and upper reaches of the uterine horn of the first bat (no. 53) revealed no spermatozoa. Likewise, after histological preparation and slide mounting, examination of the contralateral horn revealed no spermatozoa, nor were leucocytes detected. However, the ovaries of this bat contained two or three large corpora lutea and a recently ovulated but unfertilized ovum, still surrounded by corona radiata cells, was noted near the entrance to the oviduct.

Dissection of the second female revealed two well-developed fetuses, but again no spermatozoa. The fetuses were fixed in 10% buffered formal saline and preserved in 75% alcohol for comparisons with published data. Crown–rump lengths of the fetuses were: 11.5 mm and 13.75 mm.
Storage of spermatozoa in males

The male bats were killed in spring, one on 8 September and the other on 10 October. Records of the reproductive status of captured males (Fig. 2) and histological examinations (I. Schlawe, unpublished data) indicate that this is 6–7 months after peak spermatogenesis and that testes are at, or near, their minimum size. The cauda epididymides of both males contained live spermatozoa. The proportion of motile spermatozoa varied between individuals and samples, and the male that was killed in September had a higher mean proportion of motile spermatozoa. Counts were performed on four slides that contained spermatozoa. The percentages of motile spermatozoa for the first male were 77%, 87%, 80% and 73%, mean 79%. In the second male, the percentages were 63%, 56%, 54% and 63%, mean 59%. The results of the hypo-osmotic swell test are similar, in that the male killed in September had the highest percentage of spermatozoa that displayed some swelling, with the spermatozoa often buckling midway down the tail (Fig. 3). The mean percentage of spermatozoa that displayed swelling for the male killed in September was 62.6%, while the male killed in October had slightly fewer at 60.2%.

Discussion

The results presented here indicate that storage of fertile spermatozoa is possible in female C. gouldii as female no. 58 clearly stored spermatozoa for at least 33 days; this is at the lower end of confirmed periods of storage of fertile spermatozoa by female bats, as storage periods of up to 198 days have been recorded (Racey, 1979). Unfortunately insufficient blood was obtained from the only other female to become pregnant to determine whether her pregnancy involved sperm storage or early ovulation. It is possible that the steady supply of food in captivity leads to premature ovulation (Racey, 1982) and the large embryos this bat contained when compared with Kitchener’s (1975) study suggests that this may be the case. Alternatively, the rate of development may have been increased since captivity is reported to maximize the ability of bats to thermoregulate (McNab, 1989). In any case, based on the results of the progesterone assay and the isolation experiments, female C. gouldii can store fertile spermatozoa for prolonged periods.

The uniformly basal concentrations of plasma progesterone reported here for C. gouldii during oestrus (autumn to winter), appear extremely low. However, the pattern is similar to that reported for Antrozous pallidus, where plasma progesterone remains at baseline values from the beginning of oestrus until completion of hibernation (Oxberry, 1979). The increases in plasma progesterone concentrations found in late winter are in agreement with Kitchener’s (1975) report that corpora lutea form rapidly following ovulation in late winter/early spring. Presumably the bats in the present study experienced this increase in luteal activity which led to increased concentrations of plasma progesterone. The observation that one female (no. 53) had a more than 20-fold increase in plasma progesterone from July to early September and had two or three large corpora lutea in each ovary supports this theory.

From early September, the marked difference in plasma progesterone concentrations of two of the bats compared
with the other five was related to pregnancy. The two bats with high progesterone concentrations were pregnant, as subsequently shown at post mortem in the one case and by the delivery of twins in the other and that postpartum, plasma progesterone concentrations of female no. 58 fell back to basal values. Currie et al. (1988) reported a similar postpartum response in Myotis lucifugus, although the magnitude of the decrease reported here appears considerably larger. Interestingly, the plasma progesterone concentrations of bat no. 58 were characterized by a trough in mid- to late pregnancy. Since only one female went to full term we do not know if this is typical for C. gouldii; however, Kitchener (1975) reported that corpora lutea decrease in size towards the end of pregnancy, and decreases in plasma progesterone concentrations during mid- to late pregnancy have been noted in other bats (Oxberry, 1979; Racey and Swift, 1981). As stated, female no. 58 gave birth on 11 November, which is 1–2 weeks early when compared with Kitchener’s (1975) study where the duration of gestation was between 52 and 84 days. During lactation, plasma progesterone concentration rapidly (≤ 7 days post partum) fell back to basal values. Again, since there was only one lactating female in the study we cannot be sure that this response is typical; however, it does correspond to Kitchener’s (1975) description of invasion of the corpus luteum by connective tissue during early lactation and its complete disappearance by December.

Overall, the peak plasma progesterone concentrations reported in the present study are low when compared with some studies (for example, Burns and Easley, 1977; Oxberry, 1979; Buchanan and Young-Lai, 1986). However, values are similar to those reported for Pipistrellus pipistrellus, which like C. gouldii experiences low plasma progesterone concentrations during winter and peak values of 10–12 ng ml⁻¹ (Racey and Swift, 1981).

Haematocrit values reported here are unremarkable and, as in some other studies (for example, Valdivieso and Tamsitt, 1971), no changes in haematocrit were detected that appeared to be the result of pregnancy or lactation.

The only conclusive test of sperm fertility is pregnancy (Weiske and Maleika, 1987). However, sperm viability and mobility equate with fertility in humans (Mortimer and Taylor, 1990). This is because fertility is always correlated with sperm quality (Paz et al., 1990), and motility and viability, as indicated by the hypo-osmotic swelling test, are two indicators of sperm quality (Mortimer and Taylor, 1990). In humans, when more than 60% of spermatozoa show morphological changes under hypo-osmotic conditions, the donor is considered fertile (Weiske and Maleika, 1987), and when more than 50% of spermatozoa show motility, donors are considered fertile (Mortimer and Taylor, 1990). Spermatozoa from the male bats examined in this study exceeded these values in both parameters. Racey (1973) reported that large numbers of

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Fig. 3. Comparison of spermatozoa from Gould’s wattled bats. (a) Normal spermatozoa and (b) a spermatozoon that had been subjected to a hypo-osmotic swell test. The spermatozoon in (b) shows signs of curling, which indicates that the membrane was functional. Scale bar represents 4 µm.
spermatozoa stored by male noctule bats were motile and viable and were used to impregnate female bats successfully. Unfortunately, no C. gouldii spermatozoa from pre-hibernation were available for comparison with post-hibernation spermatozoa. Still, the results presented here indicate that the spermatozoa stored by male C. gouldii were viable and motile and on that basis it is reasonable to assume that the spermatozoa retained their fertilizing capacity well into spring, some 7 months after the peak in spermatogenesis. The lack of detection of spermatozoa in female tracts using retrograde flushing was not due to the inappropriate nature of this technique when applied to bats, as histological examinations also failed to reveal any spermatozoa. As regards the female that was found to have an unfertilized ovum at post mortem, either the stored spermatozoa had been lost or else she had not been impregnated. Since no leucocytes were present in her tract, the latter explanation is favoured. With hindsight, this aspect of the study should have been carried out earlier.

The conception rate reported here (29%) is comparable to 17% reported by Racey (1973) and may reflect the difficulties in breeding captive insectivorous bats (Racey, 1973). Alternatively, it may explain why male bats store spermatozoa. It is interesting to note that in Racey’s (1973) study, 70% of female noctule bats inseminated by spermatozoa that males had stored during hibernation became pregnant, while only 60% of female noctules that stored spermatozoa during hibernation became pregnant. If the cost of sperm storage is low, selection may be expected to favour storage since the potential benefit is increased fitness. If stored spermatozoa typically retain their fertility, and evidence suggests that this is the case, then clearly storage would profoundly influence the level of sperm competition in bats.

In conclusion, this study clearly shows that fertile spermatozoa can be stored by both male and female C. gouldii for prolonged periods.

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