The oestrous cycle of the spiny mouse (*Acomys cahirinus*)

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Summary. The oestrous cycle of the spiny mouse (*Acomys cahirinus*), as determined by vaginal smears, is 11.1 ± 1.9 (s.d.) days ($n = 110$). The vaginal smears show cell patterns similar to those seen in the rat, but secretion of mucus is greater than in the rat. The average age at vaginal opening is 45 ± 2.77 (s.e.m.) days, but the first litter (sired by litter mates) did not occur until 103 ± 4.04 (s.e.m.) days. The decidual response to uterine trauma indicates that there is an active luteal phase. The ovaries are otherwise histologically similar to those of other murids.

Introduction

The spiny mouse (*Acomys cahirinus*) is a desert-dwelling murid rodent found in Egypt, Israel and other areas of the Middle East. They are nocturnal animals with peaks of activity at dawn and dusk (Bodenheimer, 1949). The species has frequently been used for studies of renal physiology because its kidneys are able to concentrate urine to 4.8 M and because it conserves plasma volume during dehydration (Shkolnik & Borut, 1969; Horowitz & Borut, 1970; Borut, Horowitz & Castel, 1972). They have also been used for studies of diabetes and obesity (Strasser, 1968; Hefti & Flückiger, 1967; Pictet, Orci, Gonet, Rouiller & Renold, 1967; Gonet, Stauffacher, Pictet & Renold, 1965; Cameron, Stauffacher, Orci, Amherdt & Renold, 1972). Most of these studies on diabetes were carried out on colonies derived from one established in Basel (Young, 1976) but diabetes may not be widespread. Urine collection and analysis did not give evidence of diabetes in my colony (B. Peitz, unpublished). This species has also been used for a number of behavioural studies on pheromones (Porter, Doane & Cavallaro, 1978; Porter & Doane, 1979).

Aside from comments on their relatively long gestation period, 38–39 days, and small litter size, 1–4 but generally 2 (Dieterlen, 1963; Strasser, 1968; Young, 1976), very little is known about the basic reproductive physiology of the spiny mouse. Dieterlen (1961) reported that behavioural oestrus followed parturition by about 15 h. If mating was prevented or was infertile, oestrus recurred in about 11 days (range 9–15 days). Ruch (1967) demonstrated that there is no lactational delay in implantation in this species. Kang & Anderson (1975) examined the ultrastructure of primary oocytes in preantral follicles but did not discuss cyclic changes in the ovary or the structure of antral or Graafian follicles.

This paper presents a detailed study of the oestrous cycle, including a description of vaginal smears, the effects of light on the cycle and evidence for the existence of an active luteal phase in this murid species. It also includes a histological description of the corpora lutea and an estimate of the age at vaginal opening.

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Materials and Methods

Animals

The animals used in this study were part of a laboratory colony begun in 1968 from stock collected in Israel by A. Shkolnik. The animals were housed in wooden cages of various sizes. The number of animals in each cage varied with the size of the cage and with the size of the family group. Animals were kept in family groups to decrease fighting and achieve the best breeding performance. Adults raised in different cages do not readily accept each other when moved into new caging so new breeding groups were established by using weanlings. The spiny mice were fed a mixed diet of sunflower seeds, shelled corn, rolled toasted barley and Purina cat chow. Temperature was maintained at 22-5°C and lighting was on a 12 h light/12 h dark cycle.

Oestrous cycle in normal adults

Adult females used to study the normal oestrous cycle were separated from males and housed in all-female groups. Daily vaginal smears were taken in the mid-afternoon with small cotton-tipped swabs. Smears were taken from a total of 108 females for various periods. Some of the animals were killed at different stages of the oestrous cycle for histological studies. The ovaries were fixed in Bouin's solution, sectioned at 10 µm and stained with haematoxylin and eosin. Five of the females were pregnant at the time they were included in the study. They were separated from their mates 1-3 weeks before birth of the litters and vaginal smears were started on the day following normal parturition so that the length of time between parturition and the next oestrus could be determined.

Demonstration of a uterine decidual reaction

Many of the adult females used to examine the stages of the oestrous cycle were subsequently used to demonstrate the uterine response to trauma at different times during the cycle. Only animals which had had at least one complete normal cycle, as determined by the vaginal smear pattern, were used. The animals were anaesthetized by intraperitoneal injection of 2-25 mg pentobarbitone sodium (Nembutal: Abbott) in 0-15 ml saline (9 g NaCl/l). One uterine horn was traumatized by inserting a needle into the uterus near the oviducts and scratching the inner surface. The scratches were done on alternate sides in each experimental group and sham operations without the trauma were also performed. Animals were killed 1-7 days after operation for histological studies. Tissues were weighed on a microbalance to the nearest 0-1 mg and were fixed in Bouin's solution, sectioned at 10 µm and stained with haematoxylin and eosin. Vaginal smears were taken daily from 5 animals after uterine traumatization on the second day of dioestrus to determine whether the uterine decidual reaction had any effect on the length of the oestrous cycle.

Effects of constant light or constant darkness on the oestrous cycle

Females were selected at random from the breeding cages and most of them had had at least one litter before being placed in constant light (N = 6) or constant dark (N = 6). Daily vaginal smears were taken in the mid-afternoon with small cotton-tipped swabs.

Age at sexual maturity

Several breeding cages were checked daily and births were recorded. Twenty-six (26) of the young females were examined daily and their age and weight were recorded on the day of vaginal opening. These young females were either left with their original family groups or housed with

male litter mates throughout the observation period. Those housed with male litter mates were observed until the birth of their first litter. Two of the young females were killed on the day of vaginal opening and their ovaries were prepared for histological examination as described above.

Results

Oestrous cycles

Of the 108 adult females that were included in the study 88 (81.5%) showed definite smear stages at regular intervals; the other 20 (18.5%) animals exhibited irregular patterns. Of those which had regular cycles 68 were followed for only one complete cycle, 20 for two cycles and 2 for three complete cycles, for a total of 110 normal cycles. Of the 88 which showed at least one regular cycle 11 (10.2% of all animals) also showed one or more irregular cycles.

The average length of the normal cycles was 11.1 ± 1.9 (s.d.) days. The distribution of the normal cycle lengths was slightly skewed with a mode of 11 days and a range of 6–18 days. The average number of days spent in dioestrus was 7.7 ± 1.7 (s.d.) days. The distribution of the length of dioestrus was also slightly skewed with a mode of 7 and a range of 3–15. Animals with the longer cycles spent more time in dioestrus: the correlation coefficient (r) between the length of the cycle and the length of dioestrus was 0.82.

Of the 22 animals followed for more than one cycle 9 had cycles of identical length. The average difference between the lengths of 22 consecutive cycles was 1.36 ± 0.36 (s.d.) days. The average time between normal parturition and the onset of the next oestrus was 21 ± 7.6 (s.d.) days ($n = 5$).

The vaginal smears showed the same types of cells as those seen in vaginal smears from rats. However, it was not clear which stages correspond to the rat stages pro-oestrus, oestrus and metoestrus since many of the smears contained mixtures of the cell types seen in each of the rat stages. For this reason each smear was designated by a number or combination of numbers to indicate the types of cells present: nucleated epithelial cells were indicated by 1, cornified cells by 2, cornified clumps by 3, 4 indicates a mixed smear and 5 indicates leucocytes and mucus (dioestrus). Many of the smears were best described by combinations of the numbers. A complete description of the most common patterns is presented in Table 1. The percentage of normal cycles in which each type of smear was seen is also given in Table 1.

Table 1. Description of the common types of vaginal smears during the oestrous cycle in the spiny mouse

Type	Appearance	% of normal cycles in which each type occurred
1	Nucleated epithelial cells only; same as pro-oestrus in the rat	7.3
1-2	Nucleated epithelial cells and individual cornified cells	82.2
2	All cornified cells, but not masses	23
2-3	Single cornified cells and cornified masses	35.2
3	Extensive cornified masses, often the whole cornified layer of the vagina will slough	36.1
3-4	Many cornified masses as in 3 with leucocytes and mucus	40.7
4	All possible cell types with mucus	23.6
5	Leucocytes and mucus with some nucleated epithelial cells; more cells and much more mucus than in the rat dioestrous smear	100
5-1	Larger numbers of nucleated epithelial cells mixed with leucocytes and less mucus than in type 5; this type of smear always precedes type 1 or 1-2 in those animals in which it occurs	37.8

The irregular smear patterns could be classed into two types: persistent dioestrus (possibly anoestrus) and atypical patterns of oestrous smears. Six of the animals (30% of those with irregularities) were in oestrus at the time smearing started but did not show oestrus again while

smearing continued. The average number of days these animals spent in dioestrus was 33.8 ± 12.6 (s.d.). Two animals showed only a type 5 smear. It is possible that the process of taking vaginal smears was stressful to these animals and interrupted their normal cycles. Another 9 animals (45% of those with irregularities) showed atypical smears and unusual sequences of smears. The most common irregularities were the appearance of type 4 smears before smear types 1, or 1–2, prolonged duration of type 4 smears (up to 5 consecutive days) and alternation of type 4 smears with other types including type 5. Two of the animals showed very short periods of non-dioestrous smears (1 day) with prolonged periods of dioestrus. The longest period of persistent oestrus seen in any of the cycles was 8 days. The irregular cycles of those 11 animals that had both regular and irregular cycles, all showed irregularities in the sequence of smears.

Ovarian histology

The histological appearance of the ovaries was similar to that of the rat and house mouse. Ovulation occurred between smear stages 2 and 3. Ovulation occurred from only one ovary in 5 animals but from both ovaries in 4 others. Corpora lutea were formed after ovulation from granulosa cells streaming into the antrum (Pl. 1, Fig. 1; Pl. 2, Fig. 4). The average number of corpora lutea formed during each cycle was 2.5 ± 1.1 (s.d., $n = 16$).

Mature luteal cells were arranged in whorls and had strongly basophilic cytoplasm (Pl. 1, Fig. 2; Pl. 2, Fig. 5). The corpora lutea were usually oval in shape and the average values for the length and width of the largest cross-section at different times during the cycle are presented in Table 2. Luteal regression usually started by the 8th day of dioestrus. Regression was associated with a decrease in cytoplasmic basophilia, particularly in the centre, a decrease in luteal cell size and the appearance of pycnotic nuclei (Pl. 1, Fig. 3; Pl. 2, Fig. 6).

Table 2. Average maximal diameters of corpora lutea at various stages of the oestrous cycle

Stage	No. of animals	Corpora lutea		
		No.	Length (μ m)	Width (μ m)
Smear type 3	3	4 (newly forming)	673 ± 68	569 ± 73
Day 5 of dioestrus	3	9	745 ± 66	659 ± 64
Day 8 of dioestrus	3	8	670 ± 157	537 ± 84
Smear type 1-2	4	11	700 ± 76	603 ± 69
Smear type 2-3	3	8	634 ± 98	578 ± 77
Smear type 3	3	8	644 ± 51	541 ± 88

Values are mean $\pm$ s.d.

PLATE 1

Fig. 1. Corpus luteum on the first day of dioestrus showing the luteal cells invading the antrum. $\times 75$.

Fig. 2. Fully formed and active corpus luteum on the fifth day of dioestrus. $\times 75$.

Fig. 3. Corpus luteum at pro-oestrus (smear type 1-2) of the next oestrous cycle showing the early phases of regression which are most apparent in the centre. $\times 75$.

PLATE 2

Fig. 4. Higher power view of the corpus luteum in Pl. 1, Fig. 1. $\times 300$.

Fig. 5. Higher power view of the corpus luteum in Pl. 1, Fig. 2, showing the arrangement of cells and cytoplasmic basophilia. $\times 300$.

Fig. 6. Higher power view of the regressing corpus luteum in Pl. 1, Fig. 3, showing a region of decreased cell density and cytoplasmic basophilia and an increase in pycnotic nuclei. $\times 300$.

PLATE I

(Facing p. 456)

PLATE 2

Demonstration of a uterine decidual reaction

Traumatization of one horn of the bicornuate uterus during early dioestrus increased the weight of that horn compared to the other (Table 3). The sensitivity was less when the trauma was applied immediately after ovulation (smear types 3 and 4) than on the 1st or 2nd day of dioestrus. Histological examination showed typical decidual reactions in the traumatized uterine horns, detectable after the first 24 h following traumatization. Decidual tissue induced on the 2nd day of dioestrus was maintained until the 6th day. Decidual tissue examined on the 7th day of dioestrus after traumatization on Day 1 showed some pycnotic cells and there was bleeding into the lumen of the uterus. Traumatization did not delay the appearance of the next oestrus: the difference in length between a normal cycle and a cycle in 5 animals subjected to uterine trauma was 0.8 ± 1.53 days (s.e.m.). Red blood cells were seen in vaginal smears at the end of dioestrus in 3 of the 5 animals with uterine trauma.

Table 3. The effects of endometrial scratching on uterine horn weight in spiny mice

Treatment	No. of animals	Wt of uterine horn (mg)		Mean $\pm$ s.e.m. difference (mg)	t	P
		Control	Traumatized			
None	4	42.5	48.4	5.8 ± 2.0	2.9	0.1
Sham operation	5	42.5	42.8	0.4 ± 3.88	0.001	0.5
Trauma during late oestrus, autopsy 4 days later	4	28.3	34.3	$5.5 \pm 0.88^\dagger$	6.25	0.01
Trauma on the 1st day of dioestrus, autopsy 24 h later	3	37.0	46.8	9.8 ± 4.56	2.16	0.2
Trauma on 1st day of dioestrus, autopsy 4 days later	6	31.3	135.1	$103.8 \pm 37.5^*$	2.77	0.05
Trauma on 2nd day of dioestrus, autopsy 4 days later	5	35.7	66.7	$31.0 \pm 9.19^*$	3.37	0.05
Trauma on 1st day of dioestrus, autopsy 7 days later	5	32.5	60.4	27.9 ± 11.96	2.33	0.1

Significantly different: * $P < 0.05$, $^\dagger P < 0.01$ (Paired t test).

Effects of constant light or constant darkness on the oestrous cycle

The females housed under constant light conditions were very aggressive towards each other and one was killed early in the study. The cycle length in the other 5 was 12.4 ± 0.7 days (s.e.m., 13 cycles). None of the animals showed constant oestrous smears by the end of the 50-day study. The females housed in the dark cycled very irregularly and one never had an oestrous smear. The average cycle length was 16.3 ± 2.9 days (s.e.m., 7 cycles) but this was not statistically significantly different from the normal cycle length.

Age at sexual maturity

In 26 females, vaginal opening occurred at 45 ± 2.77 days (s.e.m.) and a body weight of 36.2 ± 1.66 g (s.e.m.). In the 2 animals killed on the day of vaginal opening histological examination of the ovaries indicated that ovulation had already occurred and corpora lutea were forming. The average age of females mated with male litter mates at the birth of the first litter was 103 ± 4.04 days (s.e.m.). Since the duration of pregnancy is 38 days this indicates that fertile mating did not take place on the day of vaginal opening. However, since these females were housed with male litter mates, and the males reach sexual maturity at 55–65 days (Peitz, Foreman & Schmitt, 1979) it is possible that females could mate at their first oestrus, if housed with a mature male.

Discussion

These data on the oestrous cycle of the spiny mouse, determined by vaginal smear patterns, confirm Dieterlen's (1961) estimate based on behavioural observations. However, the time between parturition and the next oestrus reported in this study is longer than that reported by Dieterlen (1961). Since his observations were made on females in family groups while these were made on females separated from males, it is possible that the presence of the male accelerates the appearance of oestrus after parturition. The vaginal smears of the spiny mouse are more variable than those of rats, and generally contain a higher cell density. Smear type 1-2, which contains nucleated epithelial cells and individual cornified cells, occurred in 82.2% of the cycles and is probably the smear typical of pro-oestrus in this species. The type of smear seen at pro-oestrus in the rat, predominantly nucleated epithelial cells, is relatively uncommon in the spiny mouse. The appearance of oestrous smears is more variable in this species than in the rat; it may include any combination of cornified cells, cornified masses and mucus. Sometimes the whole cornified layer of the vagina came off at once during smearing.

Vaginal smears from spiny mice have more mucus than those taken from rats by the same technique. The vagina was apparently mucified as a result of progesterone secretion because the most mucus was found during the dioestrous period. Klein (1937) has demonstrated that mucification of the vagina in rodents is a result of the combined action of oestrogens and progesterone. The presence of an active luteal phase in the spiny mouse was confirmed by the induction of deciduomata in response to trauma since decidual tissue can only be formed and maintained if progesterone is present (Astwood, 1939; DeFeo, 1967). In this respect, the oestrous cycle of the spiny mouse is similar to that of the guinea-pig (Loeb, 1908) and unlike that of the rat and house mouse. The maximal sensitivity of the uterus to trauma occurred earlier in the cycle of the spiny mouse (1st day of dioestrus) than in pseudopregnancy of the rat or mouse (3rd and 4th day of pseudopregnancy respectively; Parkes, 1929; Long & Evans, 1922). Once formed the decidual tissue in spiny mice was maintained for 5–6 days, comparable to the life-span of normal decidual tissue in rats (6–8 days), house mice (5 days), and hamsters (4 days) (DeFeo, 1967).

Histologically the ovaries of spiny mice were typical of those of other murid rodents. The only unusual features were the small numbers of follicles ovulated and the formation of apparently active corpora lutea. The average number of corpora lutea formed corresponds closely to the average litter size.

The data on the age at vaginal opening and birth of the first litter indicate that these animals do not become sexually mature earlier than rats and house mice even though they are born physically precocious. The age at which the first litter is born from a mating of litter mates gives an indication of the age at which sexual maturation is complete for both sexes. However, it may be a conservative estimate since pairs that are housed together before puberty may mate later than animals of the same age that pair after puberty (Hill, 1974).

The length of the oestrous cycle and the occurrence of an active luteal phase are unusual in a murid rodent of this size (adult weight 60–80 g). The other murid rodent known to have a long oestrous cycle (26.4 ± 3.5 days) and an active luteal phase is the Australian arboreal species *Mesembriomys gouldii* but the adults of this species weigh almost 1000 g (Crichton, 1969). The spiny mouse could be a good model for studying the hormonal control of the luteal phase, and a detailed analysis of follicle dynamics and measurements of the concentrations of oestrogens, progesterone and the gonadotrophins are being undertaken.

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