

## THE INFLUENCE OF URINARY AND FECAL ODORS ON OVARIAN FUNCTION IN COEXISTING *ACOMYS* SPECIES

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### ABSTRACT

Nocturnal golden spiny mice, *Acomys russatus*, become diurnal in the presence of congeneric common spiny mice, *A. cahirinus*, or to chemical signals released by them. Here we report on the influence of signals from fresh urine and feces released by *A. cahirinus* on aspects of the estrous cycle of *A. russatus*. The results of our study show that the length of the estrous cycle of *A. russatus* is not influenced by the presence of odors released from *A. cahirinus*. However, the frequency of occurrence of post-ovulatory increases in plasma progesterone concentrations suggests that the incidence of ovulation in *A. russatus* is affected by signals produced by the urine and feces of *A. cahirinus*. The mechanisms involved in the apparent suppression of ovulation or the formation of a functional corpus luteum require further investigation.

### INTRODUCTION

Coexistence of the golden spiny mouse (*Acomys russatus*) and the common spiny mouse (*Acomys cahirinus*) in the dry, hot parts of the Rift Valley, Israel, results from temporal segregation in activity patterns. In this regard, Shkolnik (1971) demonstrated that the removal of common spiny mice from the common habitat resulted in golden spiny mice reverting their activity from a diurnal to nocturnal pattern. Haim and Rozenfeld (1993, 1995) showed that the mere exposure of golden spiny mice to the urine and feces of common spiny mice resulted in the former species changing from nocturnal to diurnal activity. Changes in the daily rhythms of body temperature and oxygen consumption of the golden spiny mice can also be induced by chemical signals derived from the urine and feces of common spiny mice (Fluxman and Haim, 1993; Haim and Fluxman, 1996).

Odors produced in the urine and feces of rodents are known to affect reproduction (see McClintock, 1983). Since artificial exposure to the urine and feces of common spiny mice does affect the activity of golden spiny mice, it may also influence their reproductive biology. Should this be the case in spiny mice, characteristics of the estrous cycle of the golden spiny mouse may be affected by the presence of fresh odors of the

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common spiny mouse. This may provide a motivation for golden spiny mice to avoid the odors of common spiny mice through a shift in activity (see Haim and Rozenfeld, 1993, 1995; Haim and Fluxman, 1996). In the present paper we address the potential influence of common spiny mice on reproduction in golden spiny mice. We report on the influence of odors in the urine and feces of common spiny mice on ovarian function in golden spiny mice, as reflected by temporal patterns in plasma progesterone concentrations. Published information on the ovarian activity of these species is limited to Pleitz (1981), who recorded estrous cycles in the common spiny mouse ranging from 13 to 22 days in length.

#### MATERIALS AND METHODS

Common and golden spiny mice were maintained in the experimental holding facilities of the University of Haifa at Oranim, as described by Haim and Fluxman (1996). All holding and handling procedures were in accordance with those sanctioned by the ethical committee of the university. Adult, nonpregnant golden spiny mouse females ( $n = 14$ ) of proven fertility were divided into two groups, with individuals of each group being kept in separate rooms and housed individually. Here they were acclimated for 3–5 weeks to a photoperiod regime of 16L:8D and an ambient temperature of  $28 \pm 1$  °C. Daily, for a period of 40 days (trial period), females in each of these groups were exposed to 1.5 ml distilled water placed on a piece of clean blotting paper in a Petri dish placed in their cages at about 24-hour intervals. For a period of 40 days thereafter (experimental period) females in the first group ( $n = 8$ ) were daily exposed to a 1.5 ml mixture of fresh feces (20 droppings per individual) and urine placed on a piece of clean blotting paper in a Petri dish placed in their cages. The Petri dish and its contents remained in the cage until replaced on the following day. Experimental animals (golden spiny mice) thus were exposed to the odors from feces and urine collected from common spiny mice throughout the trial period. The feces and urine were collected with a hypodermic needle from a tray below a wire cage holding four intact adult male common spiny mice. Female golden spiny mice in the second group ( $n = 6$ ) received a mixture of feces and urine collected in a similar way from adult female common spiny mice.

Heparinized blood samples (0.5 ml) from immobilized females were collected through cardiac puncture at three-day intervals between 09:00 and 11:00 throughout the trial periods (prior to the replacement of urine and feces as described above). Immobilization was effected by an intramuscular injection of a mixture of ketamine hydrochloride (Imalgen 1000, Rhone Merieux) and xylazine hydrochloride (Rompun, Bayer) at a dose rate, respectively, of 5.0 mg and 0.25 mg per kilogram body weight. Blood samples were centrifuged within 30 minutes after collection and an aliquot of each plasma sample was stored at  $-20$  °C until assayed.

Plasma progesterone concentrations were determined as described by Van Aarde and Van Wyk (1991) using antiserum (Code 1529) supplied by R.P. Millar (University of Cape Town, South Africa). The progesterone content of the petroleum ether extracts was determined through interpolation using the software programme SECURIA (United

Technologies Packard, Downers Grove, Illinois, USA). The sensitivity of the assays, defined as two standard deviations of the buffer blanks, was 0.078 ng/ml. Recovery of the [1,2,6,7-<sup>3</sup>H] progesterone (Code TRK 413) (Radiochemical Centre, Amersham, UK) varied from 90.3 to 92.9% ( $x = 91.3 \pm 0.86$ ;  $n = 3$ ) and intra- and inter-assay coefficients of variation were 3.7% and 8.0%, respectively. All values were corrected for procedural losses, and serially diluted plasma extracts yielded values parallel to the standard curve.

For the purpose of this study, more than one consecutive progesterone value above a baseline value was considered indicative of the onset of a luteal phase of an estrous cycle and thus of ovulation and the post-ovulatory formation of a corpus luteum. Estrous cycle lengths were calculated as the interval in days between the onset of two luteal phases, resulting in cycle lengths only being recorded for females that experienced at least two post-ovulatory luteal phases. Difficulties experienced in collecting blood from two of the golden spiny mice females exposed to the urine of females resulted in the number of progesterone concentrations estimated for them being insufficient to estimate estrous cycle variables. As a consequence, we will only report on the results obtained from 12 females exposed to heterospecific urine.

## RESULTS

Mean plasma progesterone values for females before and during exposure to the urine collected from common spiny mice were similar (Table 1), probably as a result of cyclical changes in progesterone concentrations. Plasma progesterone values for golden spiny mice females exposed to the odors of female and male common spiny mice were also similar (Mann-Whitney  $U = 13$ ,  $p = 0.683$ ) and therefore data for them were combined. Based on temporal changes in plasma progesterone values indicative of the onset of post-ovulatory luteal phases, 13 of the 14 golden spiny mice in the control group experienced changes in luteal and ovarian activity over the 40-day period while exposed to distilled water. This ratio did not differ from that recorded for experimental females (9 of 12) exposed to conspecific urine (Fisher's exact test,  $p = 0.306$ ).

However, while 9 of the 14 females in the control trial experienced incidences of luteal activity (thus estrus) twice, this happened in significantly fewer of the females in the experimental group (only 3 of 12) exposed to heterospecific urine (Fisher's exact test,  $p = 0.192$ ). With estimates of cycle length depending on the occurrence of at least two incidences of luteal activity as indicated by surges in plasma progesterone concentrations, estimates of estrous cycle lengths ranged from 13 to 24 days. The mean value for the control group ( $18.4 \pm 3.4$  days;  $n = 9$ ) was similar to that ( $20.7 \pm 3.7$  days;  $n = 3$ ) for the experimental group (Table 2). Peak concentrations of plasma progesterone were also similar prior to and during exposure to heterospecific urine and feces (Table 2).

## DISCUSSION

The coexistence of golden and common spiny mice possibly results from the former being displaced from nocturnal activity by the latter (Shkolnik, 1971). Since odors released by the common spiny mouse alter the body temperature daily rhythm (Fluxman

and Haim, 1993) as well as oxygen consumption daily rhythm of golden spiny mice (Haim and Fluxman, 1996), the present investigation focussed on their potential influence on the ovarian activity of the golden spiny mouse. Although exposure to urine from common spiny mice had no apparent effect on the length of the estrous cycle, nor on mean peak plasma progesterone values, it did suppress the incidence of luteal phase activity in golden spiny mice. This suggests that odors in the urine and feces of common spiny mice do inhibit the frequency of occurrence of estrus in the golden spiny mice.

Table 1

The mean and difference between the maximum and minimum (range) of plasma progesterone concentrations (pg/ml) in female golden spiny mice before and during exposure to the urine of female or male common spiny mice. n = the number of plasma samples collected during the treatment period. M and F following the code number denote origin of urine to which a given female was exposed

Female Code	Before exposure				During exposure				U-value	Level of significance
	Mean	Standard deviation	n	Range	Mean	Standard deviation	n	Range		
6M	522	68	7	172	488	146	5	399	15	ns
7M	738	685	6	1797	698	329	6	786	9	ns
11M	503	212	6	629	475	153	3	286	7	ns
15M	1307	1053	7	2839	629	26	3	52	6	ns
16M	573	466	8	1121	431	308	4	697	12	ns
21M	411	79	7	210	71	94	2	132	0	<0.05
22M	728	312	8	817	581	386	6	963	13	ns
23M	582	207	8	667	429	180	6	486	15	ns
8F	168	125	6	261	255	99	6	304	11	ns
10F	360	98	6	262	837	887	4	1821	8	ns
12F	433	99	8	314	460	22	4	53	9	ns
17F	327	118	7	306	185	64	6	153	6	<0.05

Table 2

Ovarian activity parameters in female golden spiny mice prior to (n = 14) and during (n = 12) exposure to the urine of common spiny mice. Mean value  $\pm$  standard deviation, sample size in parentheses

	Mean length of estrous cycle in days	Mean peak plasma progesterone concentrations (pg/ml)	Number of females experiencing luteal ovarian activity	Number of progesterone surges indicative of cyclical changes in ovarian activity
Before treatment	18.4 $\pm$ 3.4 (9)	1317 $\pm$ 832	13 (14)	9 (14)
During treatment	20.7 $\pm$ 3.7 (3)	1180 $\pm$ 638	9 (12)	3 (14)*

\* Significantly different from before treatment ( $p < 0.05$ ).

The axis through which this apparent inhibition of ovarian activity operates is not known and requires further investigation. However, it may be ascribed to the possible stressful influence of the apparent continual presence of a competitive conspecific. Our study does imply a benefit through isolation, as far as that golden spiny mice may be able to reproduce optimally in the presence of common spiny mice by avoiding (through a shift in activity rhythms) factors such as fresh odors which may inhibit reproductive activity. As golden spiny mice females, under our experimental conditions, could not escape the influence of odors released by common spiny mice, this resulted in the suppression of the estrous incidence. This was the most significant result obtained in this study. Therefore it seems that the shifting of activity rhythms of these mice under natural conditions stands to benefit the individual's reproductive output.

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