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ESTROUS CYCLE IN LABORATORY AND FIELD CONDITIONS IN TWO SPECIES OF SOUTH AMERICAN MURIDAE (*Akodon azarae* and *Calomys laucha*)

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RESUMEN: Ciclo estral en condiciones de laboratorio y de campo en dos especies de múridos sudamericanos *(Akodon azarae* y *Calomys laucha).* Muchos múridos sudamericanos son reservorios de agentes etiológicos de enfermedades endémicas que son transmitidas al hombre. El estudio de su fisiología reproductiva es esencial para el conocimiento de estas enfermedades. Estudiamos los cambios en la morfología celular vaginal para determinar las características del ciclo estral en *Akodon azarae* y *Calomys laucha* en condiciones de laboratorio. Usamos esta información para establecer en estas especies la distribución del ciclo estral en condiciones de campo a través de las estaciones de un año. El largo del ciclo observado más frecuente fue de 6 y 7 días para *A. azarae* y *C. laucha* respectivamente. En ambas especies los extendidos vaginales fueron estudiados durante el ciclo estral. Los cambios en los extendidos vaginales fueron también estudiados durante la gestación en *C. laucha.* Los datos de campo mostraron que no hay una completa interrupción de la ciclicidad estral en estos roedores en invierno, sin embargo no se capturaron hembras preñadas (determinadas por palpación abdominal) en esta estación.

ABSTRACT: Many South American murids are reservoirs of etiologic agents of endemic diseases that are transmitted to man. The study of their reproductive physiology is essential for the knowledge of these diseases. We studied the changes in the vaginal cell morphology to determine the characteristics of the estrous cycle in *Akodon azarae* and *Calomys laucha* in laboratory conditions. We used this information to establish in these species the distribution of the estrous cycle throughout the seasons during a year in field conditions. The length of the cycle most frequently observed was 6 and 7 days for *A. azarae* and *C. laucha* respectively. In both species vaginal smears were studied during the estrous cycle. The changes in the vaginal smears were also studied during gestation in *C. laucha.* Field data showed that there is not a complete interruption of estrous cyclicity in these rodents in winter, however pregnant females (as determined by abdominal palpation) were not captured in this season.

Key words: estrous cycle, *Akodon azarae, Calomys laucha,* reproduction, field, laboratory, rodents.

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INTRODUCTION

Species of the genera *Akodon* and *Calomys* have been recognized as natural reservoirs of the etiologic agents of hemorrhagic fevers (Junín, Machupo and Hantaan viruses) (Johnson et al., 1966; Sabattini et al., 1977; Weissembacher et al., 1985, 1990), lymphocytic choriomeningitis (Webb et al., 1958) and Chagas disease *(Trypanosoma cruzi)* (Basso et al., 1977; Moretti et al., 1980). Due to the important role of these murids in the trasmission to the human of the mentioned diseases some reproductive studies have been carried out (Pinter, 1970; Merani and Lizarralde, 1980, Merani et al., 1983; Pérez Zapata et al., 1987; Hodara et al., 1989). However, their reproductive physiology has not been studied in depth. Some authors reported a seasonal breeding in the field for *Akodon azarae* (Lizarralde, 1981; Zuleta et al., 1988), *Calomys laucha* (Kravetz and De Villafañe, 1981) and *Calomys musculinus* (Mills et al., 1992a). The causes of this seasonal breeding pattern (with no reproduction in winter) are not clear, male or female factors could be envolved. It is known that hormonal changes during the estrous cycle are correlated with changes in the vaginal cell morphology in many mammals (Mc Donald, 1975). In this paper we studied in *Akodon azarae* and *Calomys laucha* the changes in the vaginal cell morphology during the estrous cycle under laboratory conditions to establish the presence of periodical pattern changes; and then we used this information to observe the distribution of these vaginal cell patterns throughout the different seasons during a year under field conditions. The aim of the work was to determine if the females of *A. azarae* and *C. laucha* were responsible for the lack of reproduction in winter stopping their estrous cycle (anestrus).

MATERIALS AND METHODS

Laboratory studies

The *A. azarae* colony derived from twelve pairs which were established with animals captured in Canal 9, Balcarce (37° 45' S, 58° 15' W, province of Buenos Aires, Argentina). Three pairs of *C. laucha* from Diego Gaynor (34° 18' S, 59° 14' W, province of Buenos Aires, Argentina) constitued the first animals of the other colony. Colonies were established in 1977 and 1982 for *A. azarae* and *C. laucha* respectively.

Animals were housed in cages (38 x 28 x 15 cm) provided with softwood shavings and cotton. In all experiments, bedding was changed weekly and commercial balanced

Table 1: Annual field distribution of *Akodon azarae* pregnant females (as determined by abdominal palpation) and vaginal smears of non-pregnant females. (*)Values are the average registered during the 9 days before each capture.

trapping month	weather conditions*			captured females	pregnant females	non pregnant females	
	light period h:min	max. temp. °C	min. temp. °C			smears with epithelial or cornified cells	smears with leucocytes
February	14:40	30.7	18.0	9	1	4	4
May	11:14	16.7	7.8	0	0		
June	10:43	18.2	5.9	30	0	15	15
August	11:38	18.4	8.3	38	0	12	26
November	14:43	25.0	12.3	7	2	5	
December	15:30	28.8	18.8	8	6	1	1
Total				92	9	37	46

mouse food for reproduction and water were provided ad libitum. Room temperature was maintained at 20-25 °C with a constant light-dark cycle of 12 h light : 12 h dark photoperiod initiated at 08:00 a.m. Newborn animals were counted and sexed at birth, weaned at 21 days (King, 1963) and identified with ear marks.

Females of both species were housed in groups of 4-5 animals of the same age. Estrous cycle length was studied in 9 virgin females of *A. azarae* and 8 virgin females of *C. laucha*. They were 70 and 120 days old respectively at the begining of the study. Vaginal smears were made daily at the same hour, by flushing the vagina with tap water. Wet smears were examined under a phase contrast microscope at 10x magnification. The proportion of leucocytes, nucleated epithelial cells and cornified cells was determined. For the analysis, cycles were considered to begin when cornified cells dominated the smears (day 0) and to continue until the day preceding next appearance of the same morphology. Each female was studied during a period of time that allowed the follow-up of three consecutive estrous cycles. Thirteen virgin females of *C. laucha* were used to study the day of parturition and the changes in the vaginal cell morphology during this period. Each female was mated when 120 days old with a male of proven fertility and then she was examined daily for sperms in the vaginal smears. Presence of sperms was considered as day 1 of gestation. Smears continued to be taken during gestation.

Field studies

Rodents were obtained by live trapping during 1989 in Diego Gaynor (34° 18' S, 59° 14' W, province of Buenos Aires, Argentina). Sherman live traps (8 x 8 x 23 cm) were baited with peanut butter and were placed by pairs in lines of ca. 40 traps at 5 m intervals. Habitats sampled included three adjacent weedy fencerows of cultivated fields of 700 m long. The trapping months were February, May, June, August, November and December. For each month, samplings were carried out during 4 days, being the first day of prebaiting. Captured animals were collected each morning and were carried to a field laboratory and the species, sex, weight and occurrence of pregnancy (as determined by abdominal palpation) was registered for each individual.

The number of females captured of *A. azarae* and *C. laucha* in each month are described in **Tables 1** and **2** respectively. In these trapping months the number of pregnant females was recorded and the others females were sampled for their vaginal citology. Vaginal smears of both species were taken, air-dried and then evaluated as we described previously. We

Table 2: Annual field distribution of *Calomys laucha* pregnant females (as determined by abdominal palpation) and vaginal smears of non-pregnant females. (*)Values are the average registered during the 9 days before each capture.

trapping month	weather conditions*			captured females	pregnant females	non pregnant females	
	light period h:min	max. temp. °C	min. temp. °C			smears with epithelial or cornified cells	smears with leucocytes
February	14:40	30.7	18.0	15	5	4	6
May	11:14	16.7	7.8	15	1	5	9
June	10:43	18.2	5.9	4	0	1	3
August	11:38	18.4	8.3	12	0	1	11
November	14:43	25.0	12.3	4	2	2	
December	15:30	28.8	18.8	5	3	1	1
Total				55	11	14	30

obtained the average of the light period of the capture days and the means of the minimum and maximum temperatures respectively registered during the 9 days before each capture to provide an overview of the weather conditions (**Tables 1** and **2**).

RESULTS

Estrous cycle in *A. azarae* and *C. laucha*

As not all females displayed regular cycles, only individuals which showed at least three consecutive cycles were classified as cycling. Eight out of 9 *A. azarae* females were to fulfil this conditions. The average estrous cycle length, as determined from vaginal smears was 6.26 ± 0.88 days, with a range of 5-8, being 6 days the most frequent value. Cycle length was relatively constant for each female.

Samples which presented a predominance of cornified cells were considered as estrous smears for both species, corresponding with day 0 of the estrous cycle. In particular, *A. azarae* estrous smears had the exclusive presence of cornified cells. All smears from the day before were dominated by nucleated epithelial cells, containing also some cornified cells with or without a few leucocytes. On the day after, smears showed a consistent return to nucleated epithelial cells, 80 % of these smears contained mainly nucleated epithelial cells with only a few cornified cells and leucocytes, 10 % with nucleated epithelial cells and leucocytes and 10 % with cornified cells and leucocytes. The remaining days vaginal smears were dominated by leucocytes, but there were a few cornified and nucleated epithelial cells too (**Fig. 1**).

Six out of 8 *C. laucha* females were to fulfil the conditions to be classified as cycling. The average estrous cycle length, as determined from vaginal smears was 7.36 ± 0.67 days, with a range of 6-8, being 7 days the most frequent value. Cycle length was relatively constant for each female.

Most of the *C. laucha* estrous smears had the exclusive presence of cornified cells and some of them also showed a few leucocytes. Smears from the day before were as follows: 17.9 % presented only leucocytes, 34.9 % nucleated epithelial cells with or without leucocytes, 23.6 % cornified and nucleates epithelial cells with or without leucocytes and 23.6 % cornified cells and leucocytes. On the day after, 17.6 % of smears presented cornified and nucleated epithelial cells, 29.4 % only nucleated epithelial cells, 17.8 % cornified cells and leucocytes, 11.8 % cornified, nucleated epithelial cells and leucocytes, 11.7 % epithelial cells and leucocytes, and 11.7 % only leucocytes. The remaining days vaginal smears were dominated by leucocytes and mucus was

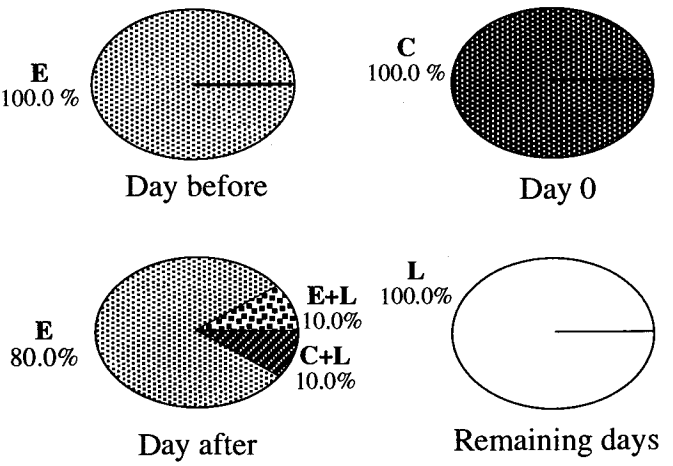

Fig. 1: Rate of the different vaginal smear types during the estrous cycle in Ako*don azarae*. E: nucleated epithelial cells; C: cornified cells; L: leucocytes. Data are referred to the dominant cell types in the vaginal smears.

found frequently (**Fig. 2**).

As smears were taken just once daily, no attempt was made to determine the exact duration of each stage of the estrous cycle. However, we were able to determine cyclic epithelial waves (i.e., the sequential appearance of nucleated and cornified cells) in both species. The duration of the epithelial waves ranged between 2 and 3 days in *A. azarae* because cornified or nucleated epithelial cells were present in all smears the days 0, before and after (**Fig. 1**). On the other hand, in *C. laucha* the epithelial waves were shorter than *A. azarae* because some smears appeared with the exclusive presence of leucocytes on the days after and before. In these days, a more varied morphology was observed in the smears of *C. laucha* because they probably represent transitional stages between the dominance of leucocytes and the predominance of cornified cells (**Fig. 2**).

Gestation in *C. laucha*

Eight of the 13 females used for the experiment became pregnant. The average day of parturition in C. *laucha* was 21.5 ± 0.75, with a range of 21-23, being day 21 the most frequent value. For the first parturition, the mean of newborn was 3.75 ± 1.38, with a range of 1-6 and 4 newborn was the most frequent value.

Vaginal smears presented mainly nucleated epithelial cells the first gestation day, 2 of them also had a few leucocytes and another 2 a few cornified cells and leucocytes as well. From day 2 to 6 of gestation all smears mainly contained leucocytes. From day 7 onwards, smears were dominated by leucocytes, although some cornified cells were also present.

Annual field distribution of *A. azarae* and *C. laucha* smears

A. azarae and *C. laucha* smears with a predominance of cornified or nucleated epithelial cells (epithelial wave), with or without leucocytes, were considered to come from females in proestrus, estrus or metestrus. On the other hand, smears with a predominance of leucocytes were considered to come from females in diestrus, anestrus or early pregnancy. In spite of finding both smear types along the year, however pregnant females (as determined by abdominal palpation) were not captured in June and August (**Tables 1** and **2**).

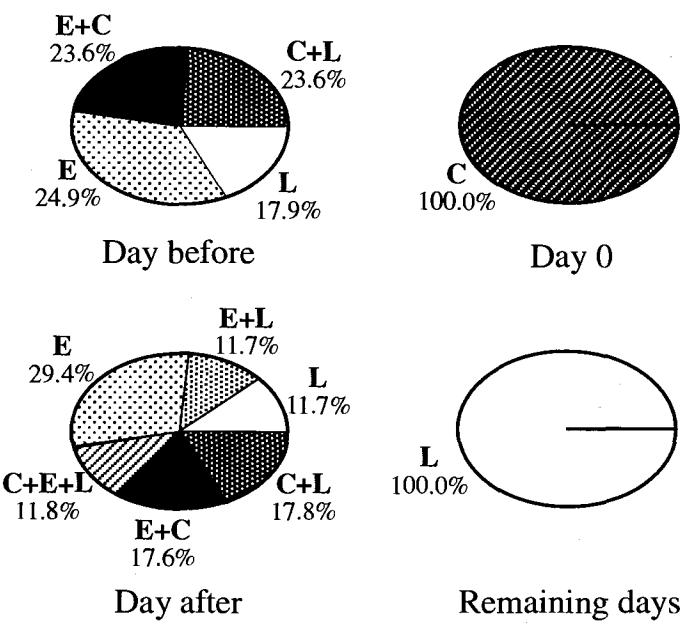

Fig. 2: Rate of the different vaginal smear types during the estrous cycle in *Calomys laucha*. E: nucleated epithelial cells; C: cornified cells; L: leucocytes. Data are referred to the dominant cell types in the vaginal smears.

DISCUSSION

The modal length of the cycle was 6 days in *A. azarae* and 7 days in *C. laucha*. Short cycles like these have been also described in many Muridae (Long and Evans, 1922; Conaway, 1954; Snell, 1956; Justines and Johnson, 1970; Dewsbury et al., 1977; Cutrera et al., 1992). Consistent short cycles appear to have been reported only in those species in which ovulation is spontaneus and pseudopregnancy is induced (Conaway, 1971; Dewsbury et al., 1977). We have also found evidence that ovulation is spontaneous in *A. azarae* and *C. laucha*, and previous results suggested that pseudopregnancy is induced (Merani, unpublished data).

A proportion of 61.54 % of C. *laucha* females became pregnant at 120 days old in agreement with the data of Hodara (1987), who reported that the 64 % of the females were pregnant between 3 and 5 months in laboratory conditions.

Also the average day of parturition (day 21.5 ± 0.75) and the size of the first litter (3.75 ± 1.38 pups) were similar to those found by this author (21 ± 1 days and 4.1 ± 1.38 newborn respectively). The first gestation day, smears were dominated by epithelial cells with or without a few leucocytes or cornified cells, like many smears of the day 1 of the estrous cycle. The remaining gestation days the smears had a predominance of leucocytes like in *Mus musculus*. This gestation morphology in *C. laucha* cannot be differentiated from that of diestrus or anestrus.

Field studies showed that females of *A. azarae* and C. *laucha* in advanced pregnancy were not captured in June and August 1989 (**Tables 1** and **2**). These results are in agreement with previous reports. In 1979 and 1980 Zuleta et al. (1988) captured only pregnant females of *A. azarae* from November to June, and newborn less than 25 days old from November to the first days of July. On the other hand, Kravetz et al. (1981) reported a long reproductive period for *C. laucha* in Córdoba (Argentina) at least from December to July. Others murids usually exhibit a seasonal breeding pattern, for example *Calomys musculinus* has a reproductive peak from October to April (Mills et al., 1992a). Both males and females were proposed to be responsible for the lack of reproduction in winter. Our field studies of *A. azarae* and *C laucha* vaginal smears revealed that the epithelial waves (proestrus, estrus or metestrus) were present throughout all the capture months along the year, thereby indicating that there was not a total inhibition of the estrous cycle in winter (anestrus). However, we could not distinguish if these cycles are regular or irregular. Mills et al. (1992b), during the winter observed mature and immature females of *Calomys musculinus* but only one of 30 was pregnant.

On the other hand, the male reproductive system could be responsible of the low reproductive performance in this season (testicular inhibition).

Probably, harsh enviromental conditions play an important role in the reproductive pattern of these murids. It is possible to propose a relationship between reproductive activity in C. *laucha* and enviromental changes (Kravetz et al., 1981). These authors found the reproductive season to begin with the increase in rains, temperature and availability of food, and to stop with the fall of these enviromental conditions. However in *C. musculinus*, which usually exhibits a seasonal breeding in the field, like *A. azarae* and *C. laucha*, under favorable climatic conditions some pregnant females were captured in autum and winter (Mills et al., 1992a). In spite of these seasonal variations in the field, pregnant females of *A. azarae* (Lizarralde, 1981) and *C. laucha* (Hodara, 1987) were found during all the year under laboratory conditions. Thus, we think that in these murids there is not an absolute reproductive season with a total and general inhibition of the estrous cycle in winter like in other mammals (Mc Donald, 1975), but the enviromental conditions may be determining in their reproductive pattern in the field.

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