

ANNUAL MALE REPRODUCTIVE CYCLE OF A HANTAVIRUS RESERVOIR, THE LONG-TAILED MOUSE *Oligoryzomys flavescens* (RODENTIA; CRICETIDAE, SIGMODONTINAE) FROM URUGUAY

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ABSTRACT: We studied the reproductive cycle in males of the cricetid rodent species *Oligoryzomys flavescens* (Waterhouse, 1837), collected in northern Uruguay. From February 2001 to February 2002, specimens were captured in grasslands and exotic forests. Determination of the reproductive condition was based on external characteristics of gonads as well as histological and histometric analysis. The testicular volume was measured and testes position (whether scrotal or intra-abdominal) was verified by dissection. Correlations between these anatomical features and some environmental factors that included temperature, precipitation and photoperiod, were analyzed using linear regression analysis. The annual reproductive cycle of *O. flavescens* was characterized by a period of quiescence during winter months (June to August) and a main period of reproductive activity from spring to early fall (October to April). Spermatogenic activity ceased completely in winter and was gradually restored in early spring (September). Reproductively active animals had larger testes and thicker seminiferous tubules than non-reproductive individuals. In addition the epididymides of the former were filled with sperm. Ambient temperature and photoperiod appear to be the main environmental factors that are directly correlated with the reproductive cycle. Finally, in spite of its wide use, it is shown that testes position is an inaccurate predictor of reproductive activity since many individuals showing full spermatogenic activity had intra-abdominal testes. Testicular volume and histological analysis are more appropriate in determining the male reproductive condition of the species.

RESUMEN: Ciclo reproductivo anual masculino de un reservorio de Hantavirus, el ratón colilargo *Oligoryzomys flavescens* (Rodentia, Cricetidae, Sigmodontinae) de Uruguay. El ciclo reproductivo masculino de *Oligoryzomys flavescens* (Waterhouse, 1837) fue establecido en una población del norte de Uruguay. Los animales fueron colectados entre Febrero 2001 y Febrero 2002 en praderas y áreas forestales. La determinación del estado reproductivo se basó en la posición testicular, así como en el análisis histológico e histométrico de las gónadas. La posición testicular (escrotal o intra-abdominal) se verificó mediante disección. Las correlaciones entre estas características anatómicas y algunos parámetros ambientales —temperatura, precipitación y fotoperíodo— fueron analizadas empleando regresiones lineales. El ciclo reproductivo anual de *O. flavescens* se caracterizó por un período de quiescencia durante los meses de invierno (junio-agosto) y un período de máxima actividad desde primavera hasta principios de otoño (octubre-abril). Durante el invierno cesó completamente la actividad espermatogénica, la cual se restableció gradualmente en primavera (setiembre). Los animales reproductivamente activos presentaron testículos más grandes y túbulos seminíferos de mayor diámetro en relación con los no activos, además de presentar abundante esperma en el epidídimo. La temperatura

ambiental y el fotoperíodo parecen estar directamente correlacionados con el ciclo reproductivo. No obstante su amplio empleo, la posición de los testículos no resultó ser un buen indicador de la actividad reproductiva, observándose varios animales con testículos intra-abdominales y actividad espermatogénica. El volumen testicular y el análisis histológico son más apropiados para determinar el estado reproductivo en esta especie.

Key words. Hantavirus reservoir. Male reproductive cycle. Oryzomyini. Spermatogenic activity.

Palabras clave. Ciclo reproductivo masculino. Espermatogénesis. Oryzomyini. Reservorio de Hantavirus.

INTRODUCTION

Seasonal breeding in mammals synchronize the energetically expensive reproductive activities to coincide with the most favorable annual environmental conditions (Bronson, 1989; Bronson and Heideman, 1994). Usually for non-tropical rodents, breeding occurs during spring and summer when environmental conditions favor reproductive success. These species frequently are able to detect environmental cues that reliably predict oncoming spring conditions to coordinate breeding. Day length has been recognized as the most commonly used environmental cue (Edmonds and Stetson, 1993, 1995), but reproduction may also be triggered by rainfall, temperature, and/or secondary plant compounds (Berger et al., 1981; Nelson et al., 1995). In many seasonally breeding rodents there is a considerable testicular regression and reduction of both steroidogenesis and gametogenesis at the end of the breeding season. After a period of quiescence there is a spontaneous gonadal recrudescence (i.e. testis enlargement and restoration of gametogenesis) at the beginning of a new breeding season (Fuentes et al., 1991; Parreira and Cardoso, 1993; Dacar et al., 1998; Lee et al., 2001; Couto and Talamoni, 2005).

Fifteen sigmodontine rodent species have been reported to occur in Uruguay. However, there is hardly any information about their ecology and reproductive biology. Most available information represents scattered data that were opportunistically obtained by collectors. Nevertheless, Barlow (1969) compiled some biological data of most Uruguayan rodent spe-

cies, including their habitat preferences, diet, breeding activities, molts, parasites, and predators.

Oligoryzomys flavescens (Waterhouse, 1837) together with *O. nigripes* (Olfers, 1818) are the two species of the genus currently recognized for Uruguay (see Frances and D'Elia, 2006). *O. flavescens*, vernacularly known as colilargo (long-tailed), has been recognized as a reservoir of Hantavirus in Argentina and Uruguay (Levis et al., 1998; Delfraro et al., 2003). *O. flavescens* has a wide distribution that extends from northern and central Argentina (Buenos Aires, Catamarca, Córdoba, Corrientes, Entre Ríos, Jujuy, La Pampa, Mendoza, Misiones, Salta, San Luis, and Tucumán Provinces; Cirignoli et al., 2006), south-eastern Brazil (from Bahía to Rio Grande do Sul State; Wesksler and Bonvicino, 2005) to Uruguay (Langguth, 1963). Adult *O. flavescens*, on average between 20-30 g, are mainly found in stands of tall grass in marshes and along rivers and streams (Barlow, 1969). While in Uruguay there were trapped breeding adult females (pregnant or lactating) only between January and May and males with scrotal testes between March and May (Barlow, 1969), in the Argentinean Buenos Aires Province, pregnant females and males with scrotal testes were found all year round except in winter months (Mills et al., 1992a).

The aim of this study is to present a first approximation to the reproductive cycle of males of *O. flavescens* from Uruguay based on external characteristics of gonads as well as histological and histometric analysis. In addition, correlations between environmental

factors that include temperature, precipitation and photoperiod, and the annual reproductive cycle are discussed.

MATERIALS AND METHODS

Adult males of *Oligoryzomys flavescens* ($n = 147$; see **Appendix**) housed at the Colección de la Facultad de Ciencias, Universidad de la República, Uruguay, were examined. These specimens were captured using pitfall traps during a faunal survey by Cravino et al. (2002) between February 2001 and February 2002 at Compañía Forestal Uruguaya Sociedad Anónima, Department of Rivera, Uruguay ($30^{\circ} 59' - 31^{\circ} 10' S$; $55^{\circ} 22' - 55^{\circ} 38' W$; **Fig. 1**). The vegetation of this area mainly comprises *Eucalyptus grandis* and *Pinus elliottii* plantations, as well as grasslands and native forest. Data on environmental parameters of the study area that included temperature, precipitation and photoperiod (**Fig. 2**) were obtained from the Dirección Nacional de Meteorología, Montevideo, Uruguay.

All specimens were fixed in 10% formalin and stored in 70% alcohol. Specimen measurements were taken from the collection catalog (ZVC-M); unfortunately specimen weight was not available, therefore body length was used as a proxy for body mass. Animals were classified as adults based on dental eruption and wear following Pearson (1992). Testes position as either scrotal or intra-abdominal was verified by dissection. Testicular volume (Tv) was estimated from the right testis length (L) and width (W), measured by one observer (LB) with a pair of calipers to the nearest 0.1 mm (SOMET, Check Republic), by the equation: $Tv = L \times W^2 \times 0.523$ following Heideman et al. (2000). Testes and epididymides from 69 specimens (selected from the total of 147 on the basis of preservation and to have an equal representation of all months) were post-fixed in buffered 10% formalin, dehydrated in an increasing concentration alcohol series, cleared in chloroform and embedded in paraffin. Paraffin sections (7 μm thick) were stained with haematoxylin and eosin, mounted in Entellan (Merck, Darmstadt, Germany) (Ganter and Jolles, 1970) and examined and photographed under an Olympus-Vanox light microscope (Olympus, Japan). The diameter of seminiferous tubules was measured using an ocular micrometer (1/100 mm; E. Leitz, Wetzlar, Germany).

The preservation condition of specimens from the collection did not allow us to complete the assessment of the testicular cell types. Thus, to accomplish this task, we collected 15 adult *O.*

flavescens in Parque Lecoq, Department of Montevideo ($34^{\circ} 48' S - 56^{\circ} 20' W$; **Fig. 1**) and Roosevelt, Department of Canelones ($34^{\circ} 52' S - 56^{\circ} 00' W$; **Fig. 1**), in southern Uruguay, with Sherman traps between October 2004 and June 2005 (**Appendix**). These animals were sacrificed with an overdose of pentothal anaesthesia. Both testes and epididymides were immediately removed and fixed in Bouin's solution, dehydrated and embedded as above. Sections (4 μm thick) were stained, examined and photographed as sections from collection specimens (Ganter and Jolles, 1970). Testicular volume was estimated and the diameter of the seminiferous tubules was recorded as previously described.

Data were analyzed using the statistical software InfoStat/Profesional 1.1 (Universidad Nacional de Córdoba, Argentina). Non-parametric univariate analyses Kruskal-Wallis ANOVA (Sokal and Rohlf, 1981) were used to test for statistically significant differences between testicular volume, body length and the diameter of the seminiferous tubules. Where statistically significant differences were detected, maximally non-significant subsets ($P > 0.05$) were derived by the a posteriori Dunn's post hoc test procedure (Sokal and Rohlf, 1981) using ranked means. Lineal regression analysis (Sokal and Rohlf, 1981) was used to assess the relationships between environmental parameters and testicular volume.

RESULTS

Testes size and spermatogenic activity in *Oligoryzomys flavescens* varied seasonally (**Table 1**). Most specimens captured between February and April 2001 and between October 2001 and February 2002 showed large testes and spermatogenic cells up to the spermatid stage (**Fig. 3A**), and the epididymides filled with sperm (**Fig. 3B**) (**Table 1**). During May, there was a clear reduction in mean testicular volume because some animals had large testes, whereas others had regressed testes. Statistically significant smaller ($H = 47.17$; $d.f. = 3$; $P < 0.001$) testes than those from the spring and summer and complete absence of spermatogenic activity was observed during winter months (June to August) (**Figs. 3C, D**) (**Table 1**). During September, an increase in testes size compared to winter values was observed, however it did not attained statistical significance ($H = 0.41$; $d.f. = 3$; $P = 0.52$)



Fig. 1. Map of Uruguay showing the localities of *Oligoryzomys flavescens* examined in the present study. A) Compañía Forestal Uruguaya Sociedad Anónima; B) Parque Lecoq; C) Roosevelt. See locality details in the **Appendix**. Insert: map of South America showing the relative position of Uruguay.

(**Table 1**). During September, males had seminiferous tubules lined with few elongated spermatids (**Fig. 3E**) and epididymides still without spermatozoa (**Fig. 3F**), indicating that they were in a state of testicular recrudescence.

Active animals in summer showed relatively abundant tissue, including large Leydig cells, in the interstitial space. However, few small cells were observed in the intertubular space in animals collected during winter (**Figs. 3A, C**). Mean seminiferous tubule diameters also changed in animals collected in different time periods (**Fig. 4**). Animals captured between February and April 2001 and between October 2001 and February 2002 showed thicker seminiferous tubules than animals collected between June and August [$(H = 15.27; d.f. = 4; P < 0.001)$; $(H = 5.90; d.f. = 4; P = 0.02)$; **Fig. 4**]. Although animals collected during September had thicker seminiferous tubule diameters than animals collected in winter, this difference was not statistically significant ($H = 0.84; d.f. = 4; P = 0.36$; **Fig. 4**). During May, seminiferous tubule diameters were highly variable because some specimens showed spermatogenic activity while others had regressed testes.

Animals captured between June and August had intra-abdominal testes. During the rest of the year, animals having both scrotal and in-

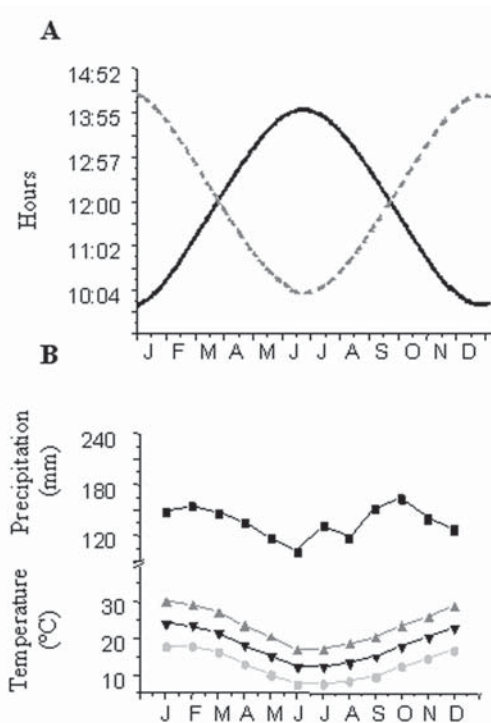


Fig. 2. Day length (- -; A) and night length (-; B), averaged between 1961 and 1990, at Compañía Forestal Uruguaya Sociedad Anónima where male *Oligoryzomys flavescens* were collected, ▼ = monthly temperature (°C); ● = minimum temperature (°C); ▲ = maximum temperature (°C); ■ = monthly precipitation (mm).

tra-abdominal testes were observed. Nevertheless, only 30 of 147 specimens (20.4 %) analyzed by dissection had complete scrotal testes. In addition, most animals examined which were spermatogenetically active had intra-abdominal testes (**Table 1**). Despite animal body lengths being generally similar throughout the year, body lengths of animals collected between June and September were statistically significant smaller than animals collected between February and April 2001 ($H = 15.48; P < 0.01$) and between October 2001 and February 2002 ($H = 23.10; P < 0.01$) (**Table 1**).

Among the environmental factors examined, photoperiod (P) and ambient temperature (T) were both positively and significantly correlated with testicular volume (Tv) ([Photoperiod: $Tv = 0.8615P + 0.0329; R^2 = 0.72; F = 20.18; d.f. = 1, 9; P = 0.002$]; [Temperature:

Table 1

Samples sizes (n), mean (\pm SE) body length, and mean (\pm SE) testicular volume used to assess male reproductive cycle in male *Oligoryzomys flavescens* from northern Uruguay. The number of specimens with scrotal testes and with active spermatogenesis are also indicated.

Year/Months	n (Total)	n (histology)	Body length (mm)	Testes volume (mm ³)	Scrotal testes	Active sperm.
2001						
February-April	33	15	71.8 \pm 4.4	52.6 \pm 26.2	10	14
May	11	6	71.9 \pm 5.8	30.9 \pm 25.1	1	5
June	7	4	68.3 \pm 3.5	5.8 \pm 2.7	0	0
July	9	6	67.0 \pm 2.4	5.3 \pm 3.4	0	0
August	7	5	68.3 \pm 2.0	6.4 \pm 1.8	0	0
September	5	5	67.2 \pm 1.9	11.1 \pm 4.3	1	1
October	9	6	70.8 \pm 3.3	51.3 \pm 25.2	2	6
November	7	6	74.9 \pm 7.1	59.1 \pm 24.4	4	6
December	23	6	72.2 \pm 4.4	43.9 \pm 17.4	4	6
2002						
January	21	5	73.7 \pm 4.0	47.6 \pm 17.2	6	5
February	15	5	70.0 \pm 4.8	44.6 \pm 16.9	2	5

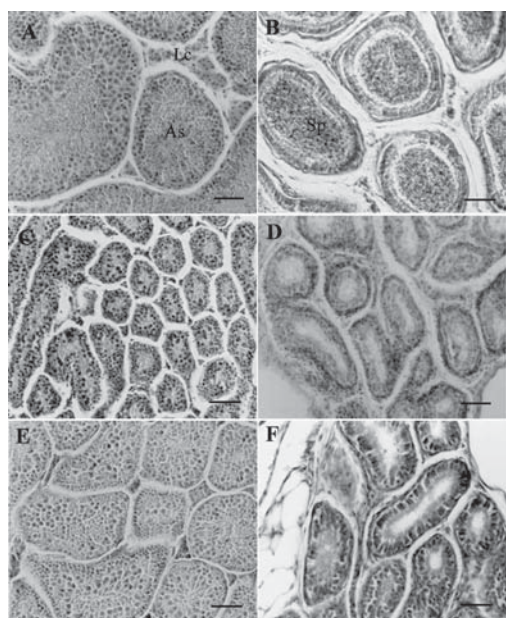


Fig. 3. Light microscope micrographs of male gonads of *Oligoryzomys flavescens* from northern Uruguay (locality A in **Fig. 1**) examined in the present study. A and B denote active testes and epididymides, respectively, of a specimen collected in October 2001. C and D represent non-active testes and epididymides in a specimen collected in June 2001. E and F denote recrudescing testes and epididymides in a specimen collected in September 2001. As = advanced spermatids; Lc = Leydig cells; Sp = sperm; the bar = scale of 50 μ m.

$Tv = 0.7518T - 2 \times 10^{-5}$, $R^2 = 0.56$; $F = 10.35$; $d.f. = 1,9$; $P = 0.012$], while monthly precipitation showed no significant correlation with Tv .

Studies of fresh specimens collected between October 2004 and May 2005 in Roosevelt and Lecoq, showed that seven were reproductively active (body mass = 21 \pm 4 g, body length = 89 \pm 8 mm). Their testes had an average testicular volume of 41.2 \pm 14.1 mm³ and an average seminiferous diameter of 157 \pm 18 μ m (120 tubules). The seminiferous epithelium comprised the whole male germ line—spermatogonias, spermatocytes and spermatids—as well as Sertoli cells (**Fig. 5A**). The interstitial tissue had numerous Leydig cells with rounded nuclei, lymphatic sinusoids and blood vessels (**Fig. 5B**). In addition between April and June 2005, eight reproductively quiescent male *O. flavescens* (body mass = 15 \pm 1 g, body length = 85 \pm 4 mm) were collected in Roosevelt. The testicular volume of quiescent animals was 9.2 \pm 5.6 mm³ and the average seminiferous diameter was 58 \pm 11 μ m (118 tubules). The seminiferous epithelium was composed mainly by Sertoli cells, and some primary spermatocytes and spermatogonias (**Fig. 5C**). The interstitial tissue had Leydig cells with an irregular shaped nuclei and minimal cytoplasm (**Fig. 5D**).

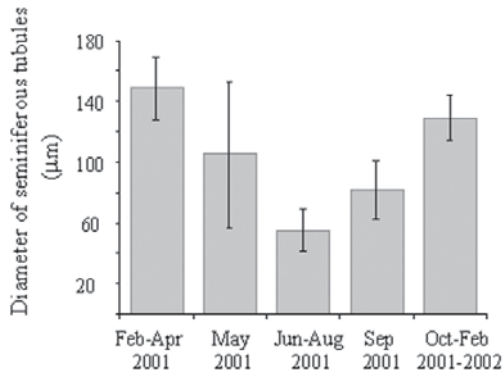


Fig. 4. Mean (\pm SE) diameter of seminiferous tubules of male *Oligoryzomys flavescens* from northern Uruguay (locality A in **Fig. 1**) examined in the present study that were collected in February-April 2001 ($n = 6$; 99 tubules), May 2001 ($n = 4$; 80 tubules), June- August 2001 ($n = 5$; 82 tubules), September 2001 ($n = 3$; 60 tubules) and October 2001-February 2002 ($n = 6$; 114 tubules).

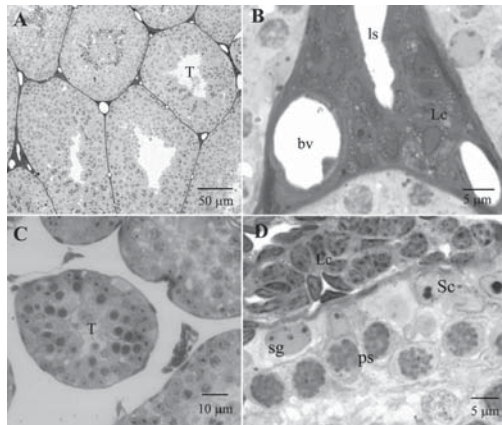


Fig. 5. Light microscope micrographs of male gonads of *Oligoryzomys flavescens* from Roosevelt (locality C in **Fig. 1**). Testis sections from a reproductively active male showing seminiferous tubules (A) and the interstitial space (B). Testis sections from a quiescent male showing one seminiferous tubule (C) and the interstitial tissue (D). bv = blood vessel; Lc = Leydig cells; ls = lymphatic sinusoid; ps = primary spermatocyte; sg = spermatogonia; T = seminiferous tubule.

DISCUSSION

The present study demonstrates that adult males of *Oligoryzomys flavescens* from northern Uruguay exhibit seasonal variation in their reproductive cycle. Males were reproductively quiescent in winter (June to August) as indicated by the lowest testicular volume and arrested spermatogenesis. In addition, histological and histometrical data suggest that during September, males were in recrudescence state, and during May only some individuals remained reproductively active. Between October and April (spring to middle fall), animals showed intense reproductive activity, with large testes and epididymides filled with sperm. Therefore, the main reproductive season of *O. flavescens* in northern Uruguay, based on male individuals, seem to occur between October to April. We recognize that our results are based on data from a single year and thus must be considered preliminary until further studies, probably spanning several years, are conducted. Notwithstanding, our results resemble those from many rodents from other temperate regions such as *Abrothrix longipilis* (Pearson, 1992), *Apodemus semotus* (Lee et al., 2001), *Calomys musculinus* (Mills et al., 1992b), and *Lagostomus maximus maximus* (Fuentes et al., 1991). All these species undergo seasonal spermatogenic variation, with a complete lack of spermatogenic activity during winter.

In addition, the amount of interstitial tissue also changed seasonally. Interstitial tissue was relatively abundant in spermatogenetically active testes, while in regressed testes, small Leydig cells were observed. In accordance with their endocrine role, the decrease in the number of Leydig cells during winter may be related to a reduction in androgen levels during this period. The importance of androgens in regulation of mammalian spermatogenesis is well documented (Sharpe, 1994; McLachlan et al., 1996; Holstein et al., 2003). Seasonal changes in androgen levels is correlated with seasonal variation in spermatogenic activity in other rodent species (Fuentes et al., 1991; Kenagy et al., 1999; Lee et al., 2001). A de-

tailed description of the interstitial tissue composition and its quantification in *O. flavescens*, as well as the spermatogenic wave characterization, was described elsewhere (Boiani et al., in press).

The annual reproductive cycle of *O. flavescens* from northern Uruguay appears to be associated with ambient temperature and photoperiod, although it is necessary to test these hypotheses with experimental studies. Meanwhile in sub-tropical and tropical regions (Zortéa, 2003; Couto and Talamoni, 2005), as well as in arid regions (Kenagy and Bartholomew, 1985), reproductive activity of small mammals is strongly associated with precipitation. On the other hand, in temperate humid regions, the main environmental factor affecting reproduction seems to be ambient temperature (Bronson, 1989; Heldmaier and Steinlechner, 1981). As a consequence of increased thermoregulation and food search, winter represents a period of high energy demand for small mammals. Thus, restricting sexual activity during this time provides a useful energy saving mechanism. Further, in accordance with the observed significant positive correlation between ambient temperature and photoperiod, day length might represent a proximate cue to predict fluctuations of ambient temperature in populations of *O. flavescens*.

The photo-responsiveness (i.e. the ability to react to changes in photoperiod) has been experimentally studied in many rodent species. Usually, short photoperiods (<12 h) cause gonadal regression and long photoperiods (>12 h) cause the resumption of spermatogenic activity (Edmonds and Stetson, 1993, 1995; Nelson et al., 1995; Young et al., 1999, 2000). The requirement of several weeks (8-10 weeks) of short-photoperiod exposure to attain complete gonadal regression (Young et al., 1999) may explain the presence of individuals with large testes between April and May when the length of photoperiod is below 12 hrs. On the other hand, whether other factors, such as food availability and inter-specific competition (Bronson, 1989; Bronson and Heideman, 1994) are involved in regulating reproductive function of male *O. flavescens*, is still uncertain.

Seasonal fluctuations in body size are frequently observed in many rodent populations inhabiting temperate regions (Mills et al., 1992a, b; Gockel and Ruf, 2001; Bergstrom and Rose, 2004; Rosário and Mathias, 2004). Besides seasonal variation in reproductive condition, the animals in the present study also varied seasonally in body length. We attribute differences in body size to differences in the age of individuals. Animals collected in winter may correspond to young individuals which were born late during the reproductive season, and would attain sexual maturity at the beginning of the next reproductive season.

The absence of advanced germ cells (i.e. secondary spermatocytes and spermatids) in the seminiferous epithelium of quiescent animals, in the present study, could be assigned to an increased cell death rate (necrosis or apoptosis). In *Peromyscus leucopus* the re-establishment of spermatogenesis during the recrudescence period, was attributed to a decrease in testicular apoptosis (Young et al., 2001). In addition, in other small mammal populations, it has been recorded that juveniles born immediately before an unfavorable season, remain in growth diapause throughout winter. With the return of more favorable conditions in the spring, growth resumes and reproduction is triggered (Negus and Berger, 1988).

The external condition of genitalia continues to be widely used as an indicator of reproductive condition. However, it has been already shown by both histological studies and mating trials that the external condition of genitalia is an inaccurate predictor of breeding activity (Mills et al., 1992b; Parreira and Cardoso, 1993). In the present study, spermatogenetically active testes were observed in several animals with intra-abdominal testes. In addition, in laboratory-reared *Calomys musculus*, the position of testes showed daily changes and appeared to depend on ambient temperature (Mills et al., 1992b). Thus, observed testes position on wild-caught animals probably depends on environmental temperature, and may also depend on the type of trap used (i.e. live versus kill traps), han-

dling, etc. Therefore, testes position may be a misleading indicator of reproductive status. Consequently, results of studies on reproductive status based solely on testes position should be treated with caution. On the other hand, histological and histometrical analysis, together with measurements of testicular volume appear to be useful indicators of reproductive condition in rodents.

In summary, populations of *O. flavescens* from northern Uruguay show a seasonal pattern of reproduction which is correlated with annual variation in environmental temperature and photoperiod. Previous studies of rodent Hantavirus reservoirs suggest that the prevalence of infection is higher for adult males than for females and juveniles (Abbott et al., 1999; Suárez et al., 2003). In addition, horizontal virus transmission appears as the main mechanism of infection, probably as a consequence of the agonistic interactions between males during breeding season. Therefore, the present study, together with additional analyses of the reproductive cycle of females and other populations may provide an insight into the dynamics of the Hantavirus in Uruguay.

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APPENDIX

Uruguayan male specimens of *Oligoryzomys flavescens* examined in the present study. All specimens from Rivera were collected previous to the present study and are housed in the mammal collection of the Colección de la Facultad de Ciencias (ZVC-M), Universidad de la República, Uruguay. GD refers to the field catalog of Guillermo D'Elía; these specimens will be deposited at the Museo Nacional de Historia Natural y Antropología, Montevideo, Uruguay.

CANELONES, Roosevelt (34° 52' S – 56° 00' W): GD 815, 850-1, 857, 863-4, 878, 882, 902-3, 905, 912.

MONTEVIDEO, Parque Lecoq (34° 48' S – 56° 20' W): GD 742, 744-5.

RIVERA, Compañía Forestal Uruguaya Sociedad Anónima (30° 59'–31°10' S; 55° 22'–55° 38' W): ZVC-M: 2171-72, 2201, 2203-04, 2214-15, 2220-21, 2227, 2241, 2254, 2308, 2313, 2731, 2748, 2750, 2754, 2756, 2758-59, 2767, 2776-78, 2783, 2785, 2789, 2791, 2877-79, 2882, 2887, 2888, 2895, 2901-02, 2909, 2917, 2920-23, 2929, 2941, 2943, 2948, 2956, 2959-60, 2995, 3025, 3030, 3033-34, 3036, 3049, 3052, 3055, 3059, 3065, 3067, 3077, 3102-04, 3111-13, 3192-95, 3197-98, 3331-33, 3335-36, 3365, 3376, 3379-80, 3391, 3445, 3470, 3475, 3505, 3508, 3515, 3517-19, 3522-23, 3525, 3535, 3548-49, 3568-69, 3581, 3596-98, 3600, 3603, 3606, 3608, 3680, 3684, 3686, 3690-92, 3704, 3713-14, 3718-22, 3724, 3727-28, 3730, 3736, 3738, 3740, 3746, 3749, 3766-67, 3771-72, 3774, 3776-78, 3780-81, 3790.