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Seasonal Variations in the Testis and Epididymis of Vizcacha (*Lagostomus maximus maximus*)[1]

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ABSTRACT

Seasonal changes in reproductive activity in the adult male vizcacha (*Lagostomus maximus maximus*), a South American rodent, were investigated. Monthly, for 2 yr, the animals were killed and decapitated during the night near their burrows in the vicinity of San Luis, Argentina. The testes, epididymides, and pineal glands were removed and used for biochemical and structural studies. Significant changes associated with seasonal cycles were found. 1) In July–August (winter in South America), a short hibernal period of sexual quiescence, decline in testicular and epididymal weights, arrest of spermatogenesis, and decrease of serum testosterone were observed. The gonads regressed during this period, with regression most pronounced in August. 2) During September–November (spring), a recovery period—without arrest of spermatogenesis—was observed, with significant expression of gonadal activity during April–May (autumn). In this season, gonadal weight was increased and spermatogenesis was complete. These results indicate an increase in sexual activity as well as in the ability to secrete testosterone. A gradual reduction of testicular activity appeared in June–July (early winter). Conversely, in this period, the pineal hydroxyindole-*O*-methyl transferase activity decreased in contrast to the highest values observed in winter.

Our findings indicate that the male adult vizcacha under natural conditions exhibits an annual reproductive cycle. A possible relationship between increased pineal activity and gonadal regression is also suggested.

INTRODUCTION

Many mammals that are seasonal breeders are able to respond to annual changes by adaptive alterations in physiological as well as in behavioral status in anticipation of the coming season. The switching on and off of reproductive function during the annual breeding cycle is the most striking example of such a photoperiodically induced process [1, 2]. The relation between photoperiodic changes and the sexual cycle of mammals has long been recognized [3]. The pineal gland has also been implicated in the regulation of seasonal reproductive cycles in several photoperiodic mammalian species. One of the most studied of these species is the Syrian hamster (*Mesocricetus auratus*), in which gonadal regression is induced by exposure to short photoperiod [4]. Removal of the pineal prevents this response to short days [5].

The present study was designed to examine the histological, morphometric, and biochemical changes in the testis and epididymis of the adult male vizcacha (*Lagostomus maximus maximus*) in different seasonal photoperiods and the relationship of photoperiod to pineal activity.

The vizcacha lives in large colonies in extensive burrow systems. The animals emerge at dawn and at dusk to feed [6]. The gestation period is 5.5 mo, and March–April is the main breeding season—although matings occur at other times of the year [7]. Llanos and Crespo [6] reported that vizcachas in the wild have only one breeding season per year, which begins in March or April, and that 36% of female vizcachas have a second pregnancy later in the year.

Reproduction in the male vizcacha is not well understood. According to Llanos and Crespo [6], vizcachas are fertile throughout the year in their natural habitat. Because of their particular habitat and short exposure to light in some periods of the year, vizcachas would appear to provide an interesting study of environmental regulation of reproduction.

MATERIALS AND METHODS

Ninety-six male vizcachas (4 animals/mo weighing 4–7 kg) were used in the study. The animals were killed by shooting, at night, in their natural habitat near their burrows in the vicinity of San Luis, Argentina (33° 20′ south latitude, 760 m altitude) and immediately decapitated (March 1986 to March 1988).

In San Luis, summer days have 14 h of light and an average temperature of 25°C. In winter, the light phase is 10 h, and the average temperature is 10°C. The average rainfall is 107 mm during summer and 10 mm in winter.

Morphometric Studies

Body weight was measured to the nearest 0.1 g, and the testes and epididymides were weighed (sensitivity, 0.01 mg). The right testis, right epididymis, and pineal gland were quickly removed and placed in vials on dry ice. The vials were then sealed, kept frozen, and shipped to the labora-

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tory for biochemical assays. Mixed arterial-venous samples of blood from the trunk were collected for serum testosterone determinations. The left testis and left epididymis were fixed in Bouin's fluid and processed for routine histology.

Structural stereological studies were performed using the testes and epididymides of vizcachas killed during April, August, and October (4 animals/mo). The samples were selected according to the seasonal variations of the gonadal weights and their histological characteristics (maximal, minimal, and intermediate activity). Cross sections were examined by light microscopy in an Orthoplan Leitz microscope. Photomicrographs were taken at random at 25× and enlarged up to 100× during printing. A total of five photographs were used for each animal. About 10–15 cross sections of seminiferous tubules and 6 cross sections of epididymal duct were examined per photograph. To estimate the tubular surface of testis epididymis, the prints were examined in a Bitpad 1 (Summagraphics, Co., Hartford, CT) digitizing tablet and processed according to Peachey [8], with a morphography program in a Hewlett-Packard (Palo Alto, CA) HP 150 computer. Data were subjected to ANOVA.

The testes were assessed histophysiologically by rating slides of 4 animals by month according to the method described by Grocock and Clarke [9]. A spermatogenic index was calculated on the basis of inspection of ten slides per testis. Numerical values were assigned according to the criteria established by Johnston and Zucker [10].

Biochemical Studies

Serum testosterone concentrations were determined by use of specific antibody against testosterone-3-O-carboxymethyloxime-BSA with the following cross-reactivities: 0.2% with cholesterol, cortisol, corticosterone, progesterone, estrone, pregnenolone, dehydroepiandrosterone, androsterone, and estrone; 71% with dihydrotestosterone; 0.7% with androstenedione; 0.6% with 3β,17β-dio-5α-androstane; 2.4% with 3β,17β-diol-5α-androstane; 4.8% with 3β,17β-diol-5α-androstane; and 0.4% with 3β,17β-diol-5α-androstane, as previously described [11].

The pineal glands were individually weighed and assayed for hydroxy-O-methyl transferase (HIOMT) activity [12]. [^{14}C]Methyl-S-adenosylmethionine, 54 mCi/mmol, was purchased from New England Nuclear (Boston, MA). A liquid scintillation spectrometer (Beckman LS-233, Beckman Instr., Palo Alto, CA) was used to measure radioactivity. Serum testosterone and HIOMT activity were determined only in May and August 1987.

RESULTS

Testicular and Epididymal Weights

The testicular and epididymal weights of vizcachas relative to body weight are shown in Figure 1. These data were registered during the period 1986–1987 and indicate important changes associated with the annual seasonal cycle. The more elevated values were observed during April–May (autumn). These maximal values were followed by a rapid and significant (ANOVA; $p < 0.05$) decrease in July–August. Redevelopment began in September (late winter and early spring), and rose progressively in summer to reach the peak already described.

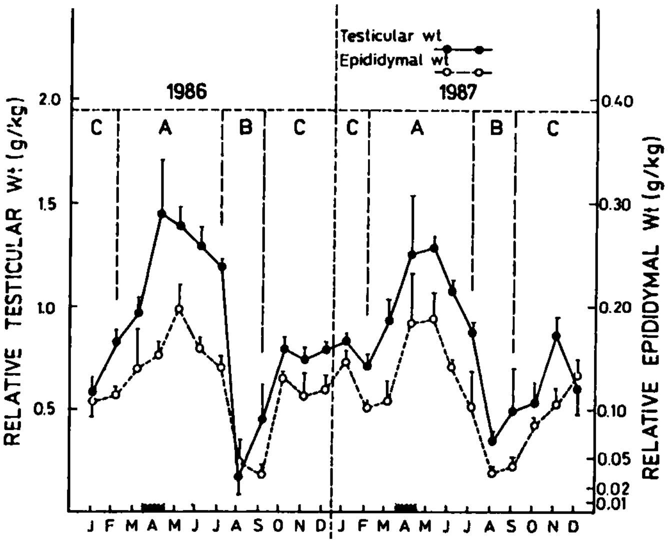


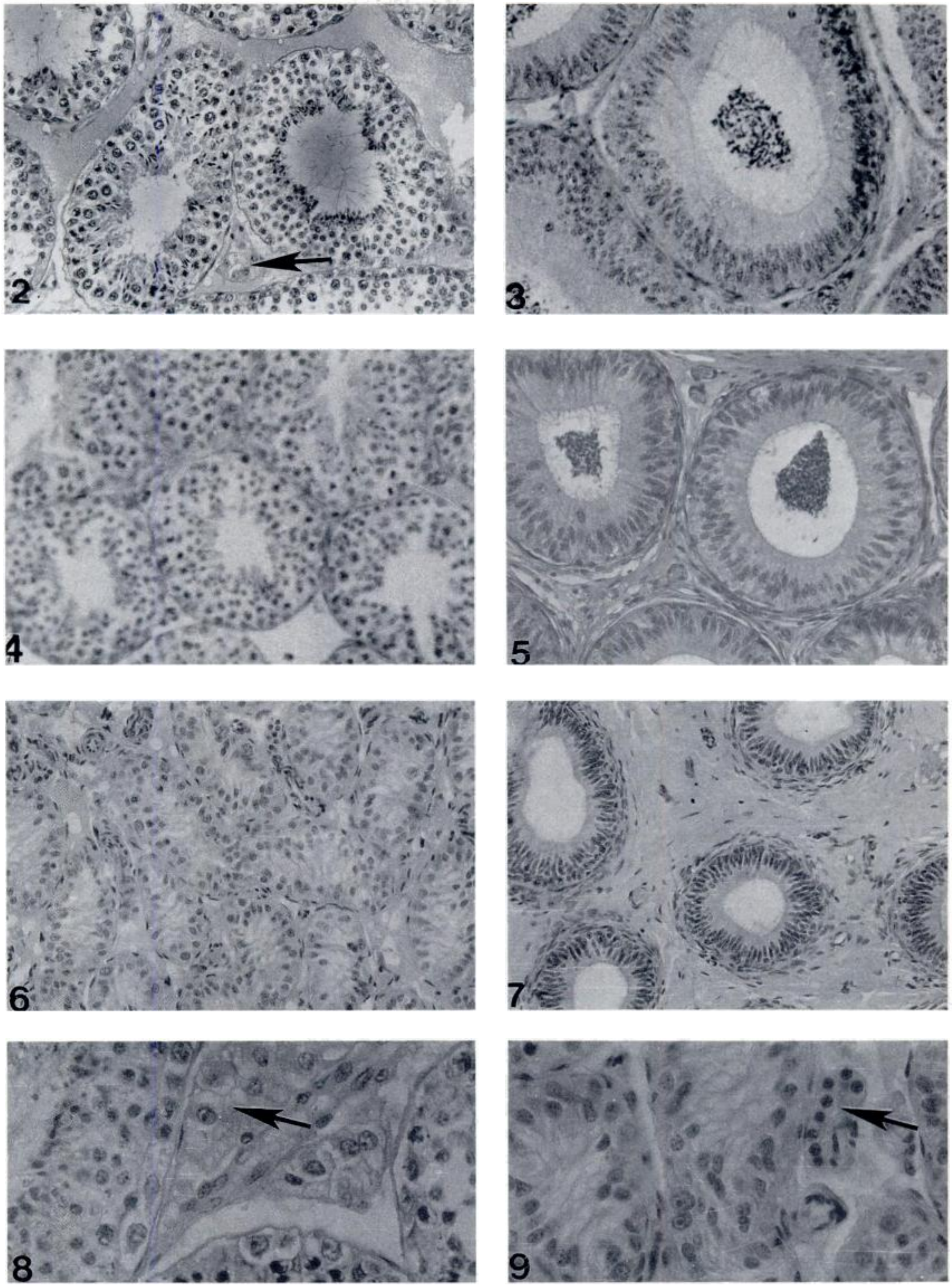
FIG. 1. Annual variations in the weights of the right testis and epididymis for the adult male vizcacha. The breeding period is indicated by the cross-hatched bar. Values are means ± SEM from 4 animals in each month. Statistical analysis was performed by ANOVA: A ≠ B ($p < 0.05$), B ≠ C ($p < 0.05$), A ≠ C ($p < 0.05$).

Histological Changes

Significant structural changes associated with the seasonal cycles were found throughout the year (see Figs. 2–9). During summer and autumn (Fig. 2), the seminiferous tubules were large in diameter and spermatozoa were present in the lumen. The interstitial cells showed a voluminous cytoplasm and round nucleolus (Figs. 2 and 8). In the beginning of spring (Fig. 4), there were histological signs of gonadal recovery.

An inactive gonadal state was observed during July and August (winter). It was characterized by a gradual reduction of seminiferous tubules and their transformation into cords (Fig. 6). Spermatozoa were absent. In August, only Sertoli cells and spermatogonia were detectable. The Leydig cells were small in size, showing a small, dense nucleus and reduced cytoplasm (Fig. 9).

A comparison of Figures 3 and 5 with Figure 7 clearly shows seasonal changes in the epididymis. In April and October, sperm were abundant, and were not present in August; in April and October, tubule diameter and epithelial height were greater than in August, and there was less intertubular connective tissue.



FIGS. 2 to 9. Paraffin sections (6 μm) of seminiferous tubules and epididymal ducts of vizcachas. Stained with hematoxylin and eosin.

FIGS. 2 and 3. Testis and epididymis from a vizcacha killed in April (autumn). Arrow: cluster of Leydig cells. Note height of the epididymal epithelium. Spermatozoa are present in the lumen. ×250.

FIGS. 4 and 5. Testis and epididymidis of a vizcacha killed in October (spring) showing gradual recovery of gonadal histology. ×250.

FIGS. 6 and 7. Testis and epididymidis of a vizcacha killed in August (winter). Seminiferous cords are composd predominantly of Sertoli cells (Fig. 6), and reduced epididymal ducts together with insterstitial tissue (Fig. 7) are the main histological characteristics. ×250.

FIG. 8. Higher magnification of the testis of a vizcacha killed in December (summer). Note complete spermatogenesis in the seminiferous tubules and a cluster of well-maintained Leydig cells (arrow). ×600.

FIG. 9. Testis of vizcacha killed in August (winter). Cluster of reduced Leydig cells showing small, dense nuclei (arrow) and seminiferous cords. ×600.

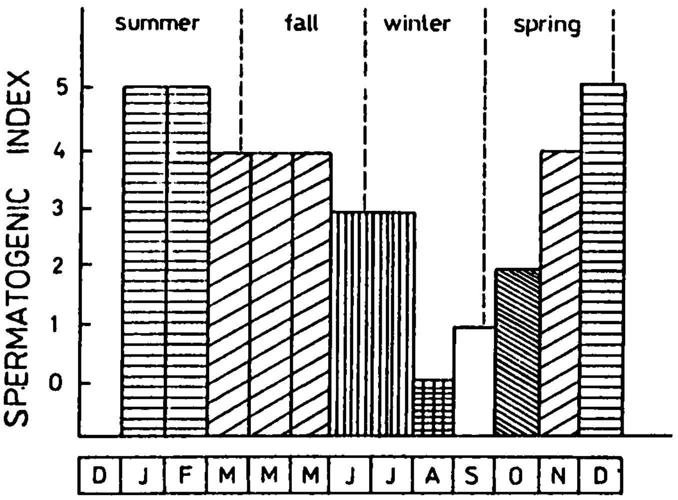


FIG. 10. Spermatogenic index showing seasonal variations. The index was calculated from the inspection of ten slides per vizcacha. Each column represents the mean of 4 animals. Numerical values were assigned according to the following criteria for stages: 5, the seminiferous tubules were large and spermatogenesis was complete; 4, spermatogenesis was complete but spermatozoa and elongated spermatids were decreased in number; 3, the number of spermatozoa was further reduced, and elongated spermatids were present; 2, elongate spermatids were absent; 1, only Sertoli cells, spermatogonia, and primary spermatocytes were present; 0, only Sertoli cells and spermatogonia were detectable (according to Grocock and Clarke [9]).

Figure 10 shows the spermatogenic index registered during the four seasons of the year. Complete spermatogenesis (stages 4 and 5) was detected during summer and autumn months. In contrast, spermatogenesis was incomplete during July–October (winter and beginning of spring). A dramatic arrest of spermatogenic activity was observed in August.

Morphometric Analysis (Table 1)

The structural stereological analyses of seminiferous tubules and epididymal ductal surfaces were performed using vizcachas killed in April, August, and October. A correlation between both weights and stereological quantification was observed. On the basis of these data, the reduction of surface in August, compared to April values, was 25% for seminiferous tubules and 83% for epididymal ducts. Middle values were obtained in October.

TABLE 1. Seasonal variations in testis and epididymis: comparison of weights and surface areas.*

	Testis		Epididymis	
Months	Tw/Bw†	Tubular surface (μm^2)	Ew/Bw‡	Ductal surface (μm^2)
April	1.47 ± 0.30	8.800 ± 0.8(a)	0.18 ± 0.07	16.588 ± 1.3(d)
August	0.25 ± 0.07	2.188 ± 0.3(b)	0.04 ± 0.01	2.770 ± 0.80(e)
October	0.66 ± 0.24	4.635 ± 0.9(c)	0.66 ± 0.24	18.051 ± 0.76(f)

*Values are expressed as means ± SE. Four animals were used by month. Statistical analysis was performed by ANOVA:
(a) ≠ (b) ≠ (c) ($p < 0.05$)
(d) ≠ (e) ≠ (f) ($p < 0.05$).
†Tw/Bw: testicular weight/body weight.
‡Ew/Bw: epididymal weight/body weight.

Serum Testosterone and Pineal HIOMT

The general pattern of results was repeated for other parameters of reproductive function. In effect, a correlation between gonadal weights and serum testosterone concentrations was observed (Fig. 11). The results were coincident to those shown in Figure 1 and show higher values in May than in August.

The values for pineal HIOMT of the vizcachas are also shown in Figure 11. In contrast to the results for testosterone levels, the HIOMT activity was lower in May than in August.

DISCUSSION

The results of the present study indicate that reproductive activity in the male vizcacha is cyclic as a function of the annual seasonal periods and may be regulated by environmental conditions. This annual cycle is characterized by a short hibernal period of gonadal quiescence and arrested spermatogenesis, which is most pronounced in August, and a recovery period without arrest of spermatogenesis during spring, followed by a significant expression of gonadal activity during summer and autumn.

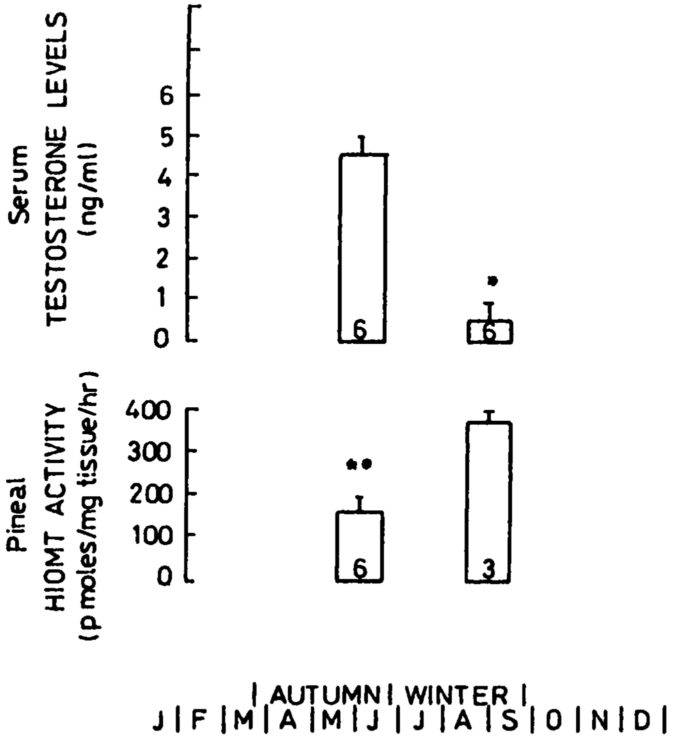


FIG. 11. Changes in the serum testosterone levels and pineal HIOMT activity of adult male vizcacha. Values are means ± SEM and the number of experiments are indicated within the bars. The asterisks indicate a statistically significant difference (*$p < 0.001$, **$p < 0.01$) between the means of May and August (Student's *t*-test).

The occurrence of spermatogenesis, the enlarged Leydig cells, and the size/weight/surface of the epididymis (target organ for male sex hormone) suggest increased male sex hormone secretion when day length is decreasing; this was borne out by the results of the assay of plasma testosterone. From these results, we conclude that spermatogenesis in vizcachas is not continuous. It has an important climax during summer and the beginning of autumn, coincident with the breeding period. A similar biological pattern has been observed in the majority of seasonally breeding mammals [13].

It is not surprising that the endocrine component of the testis, as well as gametogenic activity, exhibits rhythm fluctuations according to season. The observed changes in serum testosterone and in Leydig cells (smaller in winter and larger in spring and summer) suggest a seasonal participation of the endocrine activity.

This discontinuous gonadal activity can be attributed to seasonal changes of different environmental factors, such as ambient temperature, food availability, rainfall periods, and natural photoperiod. These parameters may be considered as important variables that control the reproductive processes of mammals [13, 14]. However, as occurs in other rodents, photoperiodic duration remains a major proximate stimulus for the regulation of seasonal reproduction of animals living in their natural habitat [15, 16]. Dependence on photoperiodic fluctuations allows animals not only to be in the suitable sexual condition at a given time, but also to actually anticipate the upcoming season and make the necessary physiological adjustment [17].

The vizcacha is rodent normally not exposed to long periods of light [6]. These animals live in burrows, where the temperature is about 18°C. The animals emerge from their burrows during the period of darkness. This natural condition may be an important factor for gonadal involution during winter. During spring and summer (14L:10D), the vizcacha is gradually exposed to twilight (crepuscular light) because the summer daylight period is more prolonged. In these seasons, vizcachas become refractory to the inhibitory effects of photoperiod, and the reproductive system is again activated in the presence of favorable conditions. It is known that the pineal gland has an essential role in mediating photoperiod information to control gonadal function in different mammals [5, 18, 19]. The pineal gland of the vizcacha may induce testicular regression during winter (short day lengths). As spring and summer approach (long day lengths), the gonads become active and are unresponsive to pineal influence. Consequently, testicular and epididymal activity increases. This hypothesis is supported by an electron microscopic study made by us [20], which demonstrated that pinealocytes during winter have cytological characteristics of a higher secretory activity than in summer and autumn. Coincidentally, the pineal HIOMT level is higher in winter than in summer.

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