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Immunohistochemical Study of Somatotrophs in Pituitary Pars Distalis of Male Viscacha *(Lagostomus maximus maximus)* in Relation to the Gonadal Activity

V. Filippa F. Mohamed

Cátedra de Histología y Embriología, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, San Luis, Argentina

Key Words
Lagostomus · Pituitary · Somatotrophs · Gonadal activity

Abstract
Somatotrophs were identified and quantified in pituitary pars distalis of male viscachas *(Lagostomus maximus maximus)* during the annual reproductive cycle, after the administration of melatonin, after castration and in different growth stages by immunohistochemistry and morphometric analysis. In adult male viscachas, the somatotrophs were distributed throughout the pars distalis during the reproductive cycle. They were oval, pyramidal or round in shape with a large round nucleus. The percentage immunopositive area, the major cellular diameter and the number of cells decreased during the gonadal regression period in relation to the values found in the reproductive period. The administration of melatonin did not provoke any variations of the morphometric parameters studied. On the contrary, a significant decrease in the percentage immunopositive area, in the major cellular diameter and in the number of somatotrophs in castrated viscachas was observed. The study of different growth stages showed that these morphometric parameters increased from immature to adult animals in the reproductive period. The results obtained suggested that the variations of the morphometric parameters of somatotrophs are more related to the gonadal development and activity than to a direct effect of melatonin.

Introduction

In species that undergo seasonal reproduction, there exists a strong correlation between the reproductive status and the secretion of the growth hormone (GH). The physiological importance of GH in the reproductive function and in the sexual maturation has been demonstrated [Hull and Harvey, 2002]. In animals that present a reproductive cycle synchronized by environmental photoperiod and regulated by melatonin, this hormone acts on

Abbreviations used in this paper

ACTH	adrenocorticotropic hormone (corticotropin)
D	darkness
DAB	3,3′-diaminobenzidine tetrahydrochloride
GH	growth hormone
L	light
LH	luteinizing hormone
PBS	phosphate-buffered saline

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Dr. Verónica Filippa
Cátedra de Histología y Embriología, Facultad de Química, Bioquímica y Farmacia
Universidad Nacional de San Luis, Av. Ejército de los Andes, 950-2° Piso
5700 San Luis (Argentina)
Tel./Fax +54 2652 422 644, Fax +54 2652 430 224, E-Mail vpfilipp@unsl.edu.ar

the hypothalamus-pituitary gonadal axis modulating the hormonal secretion. Several investigations have shown direct or indirect effects of melatonin on the synthesis and secretion of GH in fish [Falcon et al., 2003], poultry [Zeman et al., 1999], rat [Griffiths et al., 1987; Diaz-Rodriguez et al., 2001] and human beings [Forsling et al., 1999]. However, data on the relationship between melatonin and the morphologic characteristics of the somatotrophs are scarce. Falcon et al. [2003] reported in fish that changes in the photoperiod modulate growth and development, and the pinealectomy and/or melatonin administration affect the size or number of the GH cells. Hira et al. [2001] demonstrated that in hamsters the GH cells (somatotrophs) are not affected by age, photoperiod or melatonin administration.

Investigations in mouse [Sinha et al., 1979], rat [Hertz et al., 1989; González-Parra et al., 1996] and bovine [Kineman et al., 1990] have demonstrated that the variations in the serum levels of gonadal steroids modulate the synthesis, content and secretion of GH from the pituitary gland. It has been reported that in male rats the number of GH cells varies according to the presence of gonadal steroids during the neonatal period [González-Parra et al., 1998, 2000]. Moreover, the relationship between the somatotrophs and animals growth stages or age was studied in *Bufo arenarum* [Miranda et al., 1996] and pig [Lee et al., 2004]. Jurado et al. [1998] demonstrated that the GH cell area and perimeter decreased in old rats as compared to young animals, suggesting that aging affects the immunohistochemical and ultrastructural features of these cells. Kuwahara et al. [2004] reported in rat a decrease in the proportion, number and size of pituitary GH cells through age.

The viscacha *(Lagostomus maximus maximus)* is a rodent of seasonal reproduction with nocturnal habits. Adult male viscachas in their natural habitat exhibit a reproductive cycle synchronized by environmental photoperiod through the pineal gland and its main hormone, the melatonin [Dominguez et al., 1987; Fuentes et al., 1991, 1993; Pelzer et al., 1999]. The reproductive cycle is characterized by three periods: (1) gonadal regression period, (2) gonadal recovery period, and (3) reproductive period. In the gonadal regression period during the short days of winter, a small number of follicular structures and luteinizing hormone (LH) cells were observed in the pituitary gland [Mohamed et al., 2000; Filippa et al., 2005]. In the testis, a minor number of spermatids and mature sperms, hypotrophic Leydig cells with scanty organelles and a minor number of Sertoli cells with scanty organelles were reported [Muñoz et al., 1997, 2001]. In the epididymis, a decrease in its diameter and in the number of cells was determined [Aguilera Merlo et al., 2005]. Moreover, a minimum concentration of serum testosterone and a maximum concentration of melatonin were reported [Fuentes et al., 1993, 2003]. During the spring, the modifications at pituitary and gonadal levels start, causing the recovery of the gonadal activity, which is maximal during summer and early autumn during the reproductive period [Fuentes et al., 1991, 1993; Muñoz et al., 1997, 2001; Filippa et al., 2005].

The aim of this work was to study the somatotrophs in the pituitary pars distalis of this rodent during the annual reproductive cycle, after the administration of melatonin, after castration and in different growth stages. The cell distribution and their morphometric parameters (immunopositive area, major cellular and nuclear diameters and number of cells) were studied by immunohistochemistry and image analysis system. The morphometric parameters analyzed can be considered as a measure of the cellular activity [Takahashi, 1991; Torres et al., 1995; Vidal et al., 1995; Filippa et al., 2005; Filippa and Mohamed, 2006].

Materials and Methods

Experimental Animals

The viscachas were captured in their habitat near San Luis, Argentina (33° 20' south latitude, 760 m altitude) during 2004, using traps placed in their burrows. In San Luis, in summer, there is up to 14 h light daily (14L:10D) with an average temperature of 25°C. In winter, the light phase decreases to 10 h (10L:14D) and the average temperature is 10°C. In spring, the light phase increases to 12 h (12L:12D) and the average temperature is 15°C. After being captured, animals were immediately taken to the laboratory, anesthetized with Nembutal (pentobarbital) and killed by decapitation. The experimental design was approved by the local Ethics Committee and was in agreement with the guidelines of the National Institutes of Health (NIH, USA) for the use of experimental animals.

Twelve adult male viscachas weighing 5–7 kg were captured during the most representative months of their reproductive cycle, for a period of over 1 year: 4 animals during the reproductive period in summer to early autumn (February to April), 4 animals in the gonadal regression period in winter (July to August), and 4 animals in the gonadal recovery period in spring (September to October). The animals with a body weight lower than 5 kg were carefully classified into immature (1–2 kg) and prepubertal (3–4 kg) according to body weight and light microscopy observations of testis [Llanos and Crespo, 1954; Branch et al., 1993; Mohamed et al., 2000; Filippa and Mohamed, 2006].

Administration of Melatonin. Eight adult male viscachas captured during the month of February (summer) were used. The rodents were kept in isolated boxes with free access to water and

food at 20 ± 2°C. They were maintained under a 14L:10D photoperiod. The experimental group received two daily subcutaneous injections of melatonin (Sigma, 100 μg/kg body weight in oil solution) at 09:00 and 17:00 h, for 9 weeks. The control group received only the diluent.

Castration. Eight adult male viscachas captured during the month of May (autumn) were used. The castrated and control groups were kept in isolated boxes for 6 weeks. They were maintained under a 14L:10D photoperiod with free access to water and food at 20 ± 2°C.

Tissue Preparation

The pituitary gland was excised from the brain, sagittally sectioned (fig. 1, line A), fixed in Bouin's fluid, processed for light microscopy and embedded in paraffin. Each hemipituitary was completely cut from A to C, and the number of sections (5 μm thick) ranged from 120 to 250. Immunostaining was analyzed at low magnification (×20 objective), which showed that the sections obtained in the A-B sector (fig. 1) exhibited the greatest immunostained areas in all the groups of the studied animals. Therefore, four regularly spaced serial sections in the mentioned sector were chosen in every group for morphometric analysis.

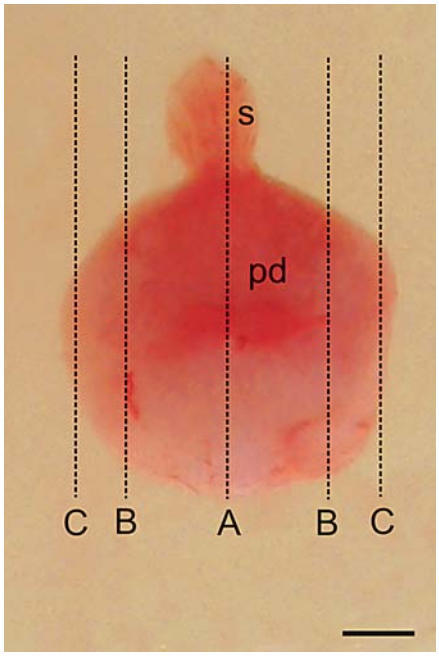

Fig. 1. Recently removed pituitary gland of adult male viscacha. Line A represents vertical cut to obtain two hemipituitaries. The A-B sector was selected for the morphometric study. pd = Pars distalis; s = pituitary stalk. Scale bar = 0.1 mm.

Immunohistochemistry

The tissue sections were first deparaffinized with xylene and hydrated through decreasing concentrations of ethanol. They were incubated for 20 min in a solution of 3% H_2O_2 in water in order to inhibit endogenous peroxidase activity. Then they were rinsed with distilled water and phosphate-buffered saline (PBS, 0.01 M, pH 7.4). Nonspecific binding sites for immunoglobulins were blocked by incubation for 15 min with 0.25% casein in PBS and rinsed with distilled water and PBS. Sections were then incubated overnight in a humidified chamber at 4°C with the primary antiserum raised in rabbits against human pituitary GH (polyclonal; DakoCytomation, Carpinteria, Calif., USA). After they were rinsed with PBS for 10 min, the immunohistochemical visualization was carried out using the Super Sensitive Ready-to-Use Immunostaining Kit (BioGenex, San Ramon, Calif., USA) at 20°C. The Biotin-Streptavidin Amplified system (B-SA) was used as follows: sections were incubated for 30 min with diluted biotinylated anti-rabbit IgG, and after being washed in PBS, they were incubated for 30 min with horseradish peroxidase-conjugated streptavidin, and finally washed in PBS. The reaction site was revealed by 100 μl 3,3′-diaminobenzidine tetrahydrochloride (DAB) chromogen solution in 2.5 ml PBS and 50 μl H_2O_2 substrate solution. The sections were counterstained with hematoxylin for 1 min, dehydrated and mounted.

In order to confirm the specificity of the immunoreactive procedures, adjacent sections were stained according to the above described protocol, but incubation in the primary antiserum was omitted. In addition, normal rabbit serum was used instead of primary antiserum. No positive structures or cells were found in these sections.

Morphometric Analysis

Computer-assisted image analysis system was used to measure the major cellular and nuclear diameters, the immunopositive area and the number of somatotrophs in pituitary pars distalis. The system consisted of an Olympus BX-40 binocular microscope (magnification 400×), interfaced with a host computer, image processing and recording system. The images were captured by a Sony SSC-DC5OA camera and processed with Image Pro Plus 5.0 software under control of a Pentium IV computer. The software allowed the following processes: image acquisition, automatic analogous adjust, thresholding, background subtraction, distance calibration, area and diameter measuring, and diskette data logging. The image was displayed on a color monitor, and the immunopositive areas were measured with the image analysis system. Before counting, a standard area of 18,141.82 μm^2 was defined on the monitor, and distance calibration was performed using a slide with a micrometric scale for microscopy (Reichert, Austria). The morphometric study was carried out as follows: 4 tissue sections from a pituitary gland were used (fig. 1), and all the microscopic fields were analyzed in every section (80–100 microscopic fields according to the section). Therefore, between 320 and 400 microscopic fields were analyzed in each gland and 4 pituitary glands were analyzed in each group of animals. Finally, 1,280–1,600 microscopic fields or measures were carried out per group. The percentage immunopositive area (% IA) of somatotrophs was calculated using the formula % IA = $\Sigma Ac/\Sigma At \times 100$, where ΣAc was the sum of the area of immunolabeled cells and ΣAt was the sum of the pars distalis area of every microscopic field. The major cellular and nuclear diameters were measured using the length tool of the Image Pro Plus 5.0 software on each somatotroph with a visible nucleus. These diameters were measured for 50 immunoreactive GH cells per group. The number of immunostained cells with a visible nucle-

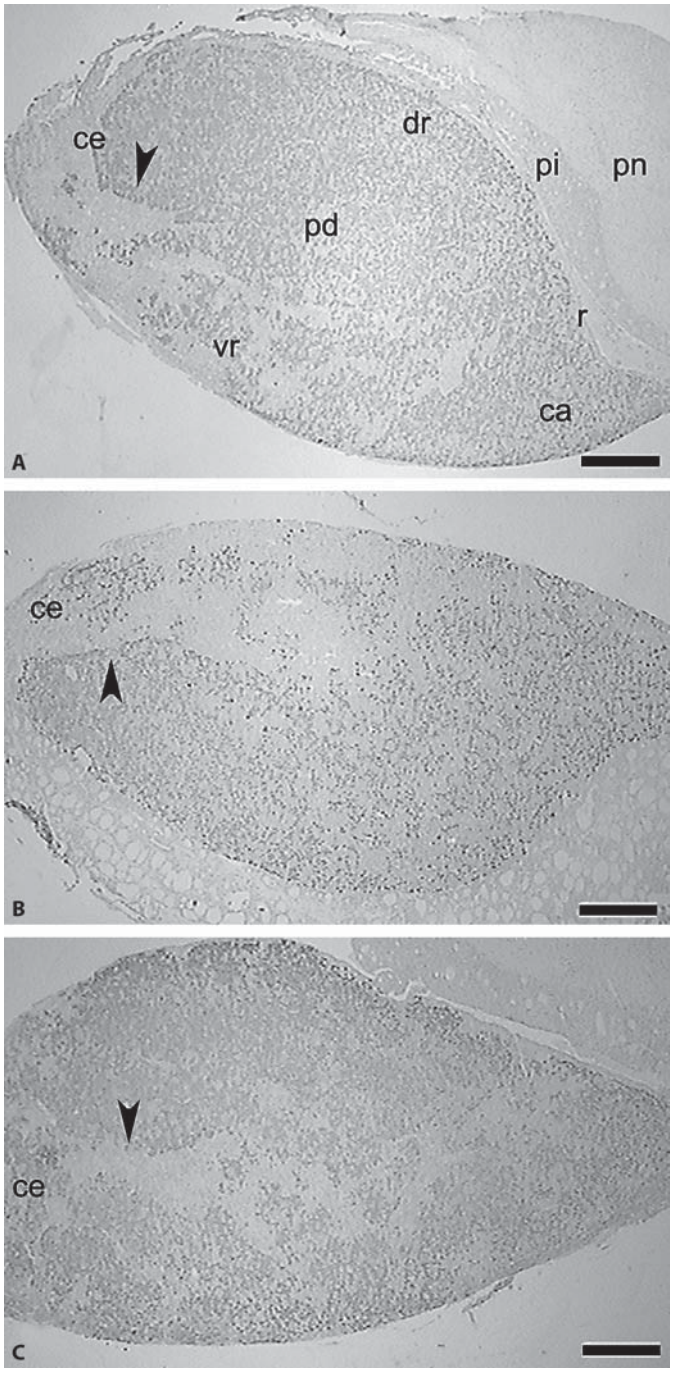

Fig. 2. Pituitary gland of adult male viscachas captured during the reproductive (**A**), gonadal regression (**B**), and gonadal recovery periods (**C**). Somatotrophs are distributed throughout the pars distalis parenchyma during the annual reproductive cycle. In the cephalic extreme long blood vessels are delimiting a region where there are no immunopositive cells (arrowheads). pd = Pars distalis; pi = pars intermedia; pn = pars nervosa; r = Rathke's pouch; ce = cephalic extreme; ca = caudal extreme; dr = dorsal region; vr = ventral region. Scale bar = 500 μm.

us was counted in 10 microscopic fields per section. The result was expressed as number of cells per microscopic field area (18,141.82 μm^2).

Statistical Analysis

The results were expressed as means ± standard error of the mean (SEM) for all data sets. All data of the different groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. Differences between experimental and control groups were evaluated using Student's t test. A probability of less than 0.05 was assumed to be significant.

Results

The *L. maximus maximus* pituitary pars distalis exhibited different regions or zones called ventral (anterior) and dorsal (posterior, close to Rathke's pouch). Besides, two extremes were distinguished: a cephalic one (superior, connected with pars tuberalis) and a caudal one (inferior).

Somatotrophs of adult male viscachas were distributed throughout the parenchyma of pars distalis during the reproduction cycle (fig. 2A–C). In the cephalic extreme, a small sector delimited by long blood vessels where there were no immunopositive cells was observed (fig. 3A). The somatotrophs were usually found as part of the follicle in basal position, without contacting the colloidal lumen (fig. 3B), and along the sinusoidal surface, scattered or grouped in small clusters (fig. 3C). These cells were mainly found isolated during the gonadal regression period (fig. 3D). They were oval, pyramidal or round with a voluminous nucleus that occupied most of the cytoplasm (fig. 3E, F). The cytoplasm fine immunolabeling was more intense in cells localized in the cephalic region. In some samples of the reproductive and gonadal recovery periods, immunolabeling was observed in the intercellular space in the sectors where there were many immunolabeled cells. The percentage immunopositive area, the major cellular diameter and the number of somatotrophs decreased significantly ($p < 0.01$, $p < 0.05$ and $p < 0.01$, respectively) during the gonadal regression period in relation to the reproductive period. These parameters showed a significant increase ($p < 0.01$, $p < 0.05$ and $p < 0.01$, respectively) during the gonadal recovery period (fig. 4, table 1). No variations were observed in the nuclear diameters.

The study in relation to different growth stages showed that the somatotrophs of prepubertal (fig. 5) and immature (fig. 6) viscachas were morphologically similar to those of adults. However, in the cephalic extreme, the

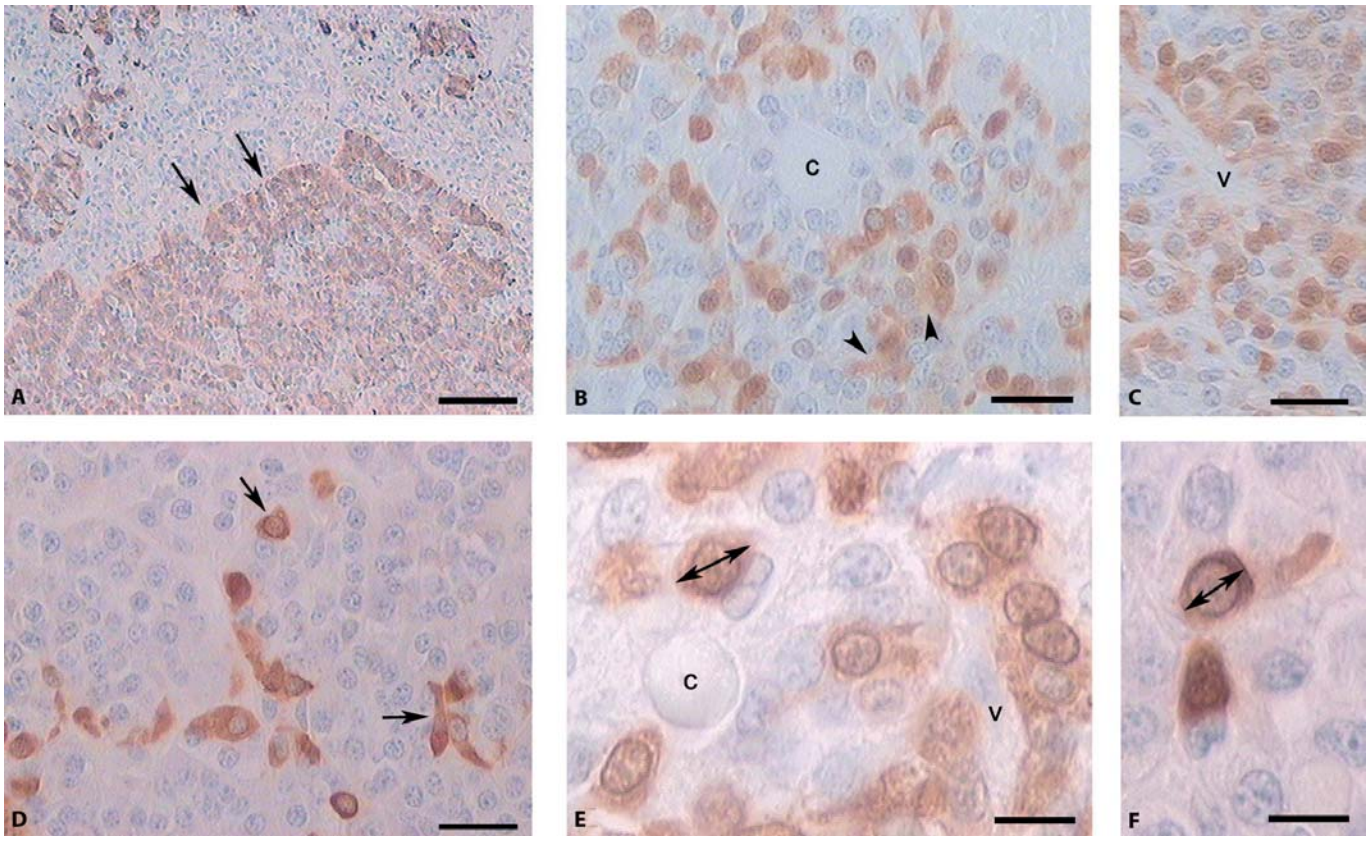

Fig. 3. A Detail of cephalic extreme of figure 2A, where there is a long blood vessel (arrows) delimiting a region where there are no somatotrophs. Scale bar = 100 μm. **B** Somatotrophs in basal position of the follicular structure, without contacting the colloidal lumen and there is immunolabeling in the intercellular space (arrowheads) during the reproductive period. Scale bar = 25 μm. **C** Somatotrophs are in contact with a blood vessel. The immunolabeling in the intercellular space is observed during the gonadal recovery period, too. Scale bar = 25 μm. **D** A small number of somatotrophs (arrows) are present in pars distalis during the gonadal regression period. Scale bar = 25 μm. **E**, **F** Oval and round somatotrophs are shown during the reproductive and gonadal regression periods, respectively. c = Colloidal lumen; v = blood vessel; ↔ = major cellular diameter. Scale bar = 10 μm.

small sector delimited by long blood vessels was not observed in the immature animals (fig. 6A). In prepubertal viscachas only the major cellular diameter was significantly smaller ($p < 0.05$) than in adult viscachas in the reproductive and gonadal recovery periods. Besides, the number of cells was significantly higher ($p < 0.001$) than in the viscachas in the gonadal regression period. In immature viscachas, the percentage immunopositive area and major cellular diameter were significantly smaller ($p < 0.01$ and $p < 0.05$, respectively) than in adult viscachas of the reproductive and gonadal recovery periods. The number of somatotrophs in immature viscachas was significantly smaller than in prepubertal ($p < 0.01$) and adult viscachas ($p < 0.05$) in the reproductive period (fig. 4, table 1). No variations were observed in the nuclear diameters.

In adult males administered melatonin, no significant variations ($p > 0.05$) were observed in the values of the percentage immunopositive area, the number of somatotrophs and the major cellular and nuclear diameters of the experimental group in relation to the control group (table 2).

After castration, a significant decrease in the percentage immunopositive area ($p < 0.001$), of the number of somatotrophs ($p < 0.001$) and of the major cellular diameter ($p < 0.05$) was observed. No variations were observed in the nuclear diameters (table 3).

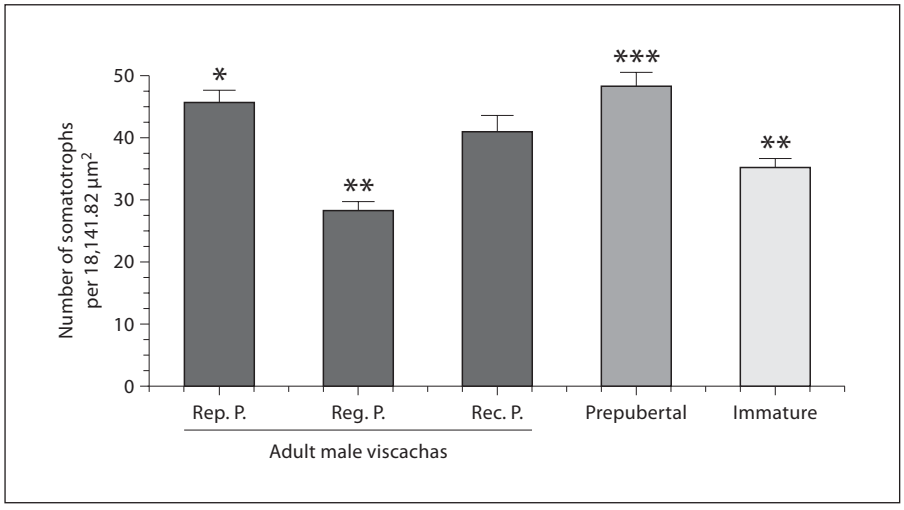

Fig. 4. Number of somatotrophs of adult male viscachas in the three periods of the reproductive cycle: reproductive (Rep. P., 45.84 ± 1.62), gonadal regression (Reg. P., 28.19 ± 1.61) and gonadal recovery (Rec. P., 41.08 ± 2.53), and in prepubertal (48.06 ± 2.33) and immature animals (35.20 ± 1.56). Bars represent mean ± SEM. * $p < 0.05$ Rep. P. vs. Immature; ** $p < 0.01$ Reg. P. vs. Rep. P., Reg. P. vs. Rec. P. and Immature vs. Prepubertal; *** $p < 0.001$ Prepubertal vs. Reg. P.

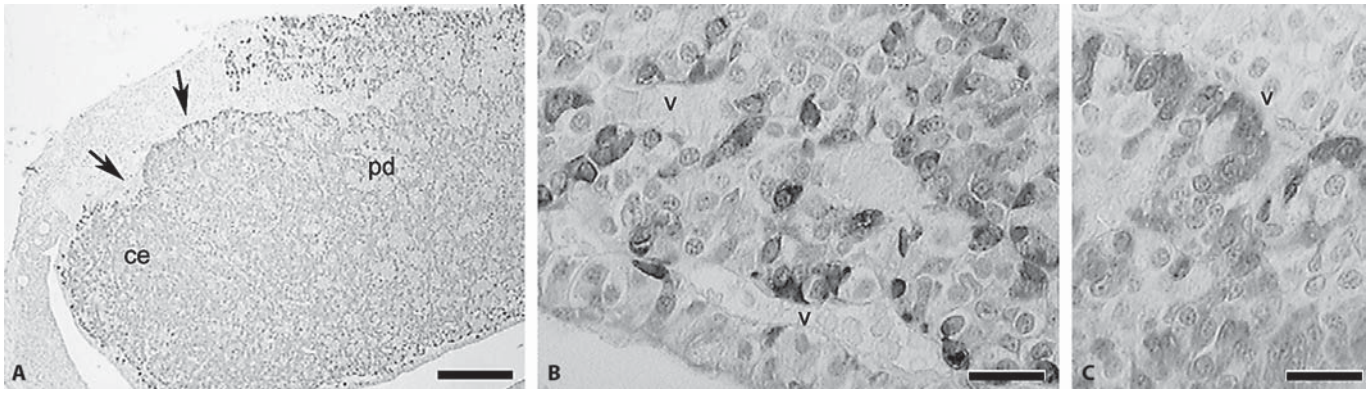

Fig. 5. Pituitary gland of prepubertal male viscacha. **A** Cephalic extreme (ce) of pars distalis (pd) where there is a long blood vessel (arrows) delimiting a region where there are no somatotrophs. Scale bar, 250 μm. **B**, **C** There are numerous somatotrophs isolated or grouped in small clusters along the surface of blood vessels (v). Scale bar = 25 μm.

Table 1. Percentage immunopositive area, major cellular and nuclear diameters of somatotrophs of adult male viscachas during the reproductive cycle, and in prepubertal and immature animals

	Adults (5–7 kg)			Prepubertal (3–4 kg, n = 4)	Immature (1–2 kg, n = 4)
	Rep. P. (n = 4)	Reg. P. (n = 4)	Rec. P. (n = 4)		
Immunopositive area	12.66 ± 0.53	9.12 ± 0.31[a]	12.79 ± 0.38	10.48 ± 0.38	8.60 ± 0.37[a]
Major cellular diameter	10.49 ± 0.14	9.37 ± 0.10[b]	10.62 ± 0.15	9.18 ± 0.12[b]	9.32 ± 0.14[b]
Nuclear diameter	5.27 ± 0.17	5.25 ± 0.06	5.19 ± 0.14	4.95 ± 0.09	4.73 ± 0.10

The immunopositive area is expressed as mean ± SEM (%). The major cellular and nuclear diameters are expressed as mean ± SEM (μm). Rep. P. = Reproductive period; Reg. P = gonadal regression period; Rec. P. = gonadal recovery period. Significant differences were determined by analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test. [a] $p < 0.01$ Reg. P. vs. Rep. P., Reg. P. vs. Rec. P., Immature vs. Rep. P. and Immature vs. Rec. P.; [b] $p < 0.05$ Reg. P. vs. Rep. P., Reg. P. vs. Rec. P., Prepubertal vs. Rep. P. and Immature vs. Rep. P.

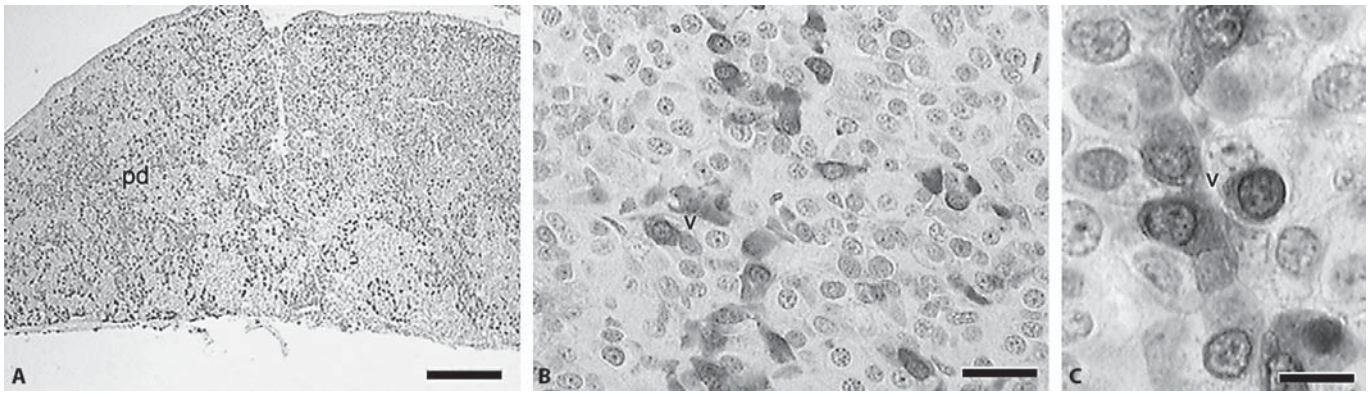

Fig. 6. Pituitary gland of immature male viscacha. **A** Somatotrophs are widely distributed throughout the pars distalis parenchyma (pd). Scale bar = 250 μm. **B** Somatotrophs are in contact with the blood vessels (v). Scale bar = 25 μm. **C** Details of oval or round somatotrophs along the surface of blood vessels (v). Scale bar = 10 μm.

Table 2. Percentage immunopositive area, number, major cellular and nuclear diameters of the somatotrophs of melatonin-administered adult male viscachas

	Control group (n = 4)	Experimental group (n = 4)
Immunopositive area	9.56 ± 0.57	10.36 ± 0.46
Number	39.15 ± 3.36	43.69 ± 2.97
Major cellular diameter	9.75 ± 0.16	9.88 ± 0.05
Nuclear diameter	5.28 ± 0.09	5.23 ± 0.06

The immunopositive area is expressed as mean ± SEM (%). The number of somatotrophs is expressed as mean ± SEM per 18,141.82 μm^2. The major cellular and nuclear diameters are expressed as mean ± SEM (μm). No significant differences were determined by Student's t test.

Table 3. Percentage immunopositive area, number, major cellular and nuclear diameters of the somatotrophs of adult male viscachas after castration

	Control group (n = 4)	Castrated group (n = 4)
Immunopositive area	10.27 ± 0.23	5.41 ± 0.17[b]
Number	53.87 ± 1.91	26.56 ± 1.12[b]
Major cellular diameter	9.43 ± 0.14	8.43 ± 0.12[a]
Nuclear diameter	5.03 ± 0.09	5.14 ± 0.10

The immunopositive area is expressed as mean ± SEM (%). Number of somatotrophs is expressed as mean ± SEM per 18,141.82 μm^2. The major cellular and nuclear diameters are expressed as mean ± SEM (μm). The significant differences were determined by Student's t test: [a] $p < 0.05$; [b] $p < 0.001$ vs. control group.

Discussion

Adult male viscachas in their natural habitat exhibit a short gonadal regression period during winter characterized by a reduction of the testicular weight and of the diameter of the seminiferous tubules [Fuentes et al., 1991]. In addition, hypotrophic Leydig cells [Muñoz et al., 1997], seminiferous epithelium with Sertoli cells, spermatogonia and a few primary spermatocytes [Muñoz et al., 2001] and minimum concentrations of serum testosterone [Fuentes et al., 1993] were described. On the other hand, the secretory ability of the pineal gland was increased [Dominguez et al., 1987] and maximum levels of serum melatonin were determined [Fuentes et al., 2003]. The testicular activity was slowly recovered during spring and it reached its maximum during summer and early autumn in the reproductive period.

Several investigations have reported that variations of morphometric parameters of pituitary cells can be correlated to changes in their activity [Takahashi, 1991; Vidal et al., 1995; Filippa et al., 2005; Filippa and Mohamed, 2006]. It has also been suggested that variations in the secretory activity of rat somatotrophs induce variations in their volume and/or number [Torres et al., 1995], which implied variations in the overall volume of the immunopositive area of GH cells [Bonaterra et al., 2001]. In the present study of pituitary somatotrophs of male viscacha, we have demonstrated that the morphometric parame-

ters of these cells vary along the annual reproductive cycle. The lower number of cells observed in the regression period of the reproductive cycle suggests that some somatotrophs might store insufficient amounts of hormones for their detection or might be in a stand-by state of their secretory cycle. The latter has also been suggested for other pituitary cellular types in rat [Childs, 1991, 1992] and in viscacha [Filippa and Mohamed, 2006]. Besides, the immunolabeling in the intercellular space suggests that a greater secretion of GH might exist in the reproductive and gonadal recovery periods than in the gonadal regression period of the reproductive cycle.

Lee et al. [2004] reported changes in the spatial distribution through age in porcine suggesting that there may be regional specificity in the cellular differentiation and transformation during growth. In viscachas, we have also observed a small sector delimited by long blood vessels where there were no immunopositive cells, as opposed to what has been observed in immature animals in which this distribution was not so evident. These differences observed in the distribution of the somatotrophs in viscacha might depend on the variations in the endocrine regulation of these cells during growth.

In previous works on adult male viscachas, we have reported that the administration of melatonin provokes a significant decrease in the size of LH and follicle-stimulating hormone cells and in the percentage immunopositive area of LH cells in the experimental group in relation to the control group. This suggests that melatonin acts differentially on the activity of the gonadotrope cells [Filippa et al., 2005]. Moreover, melatonin provokes a decrease in the percentage of the immunopositive area of adrenocorticotropic hormone (ACTH) cells probably by affecting some secretagogue of this pituitary hormone [Filippa and Mohamed, 2006]. The results reported in some vertebrates are diverse in relation to the melatonin action on the GH cells. In rat, an in vitro study showed that melatonin inhibits the production of GH [Griffiths et al., 1987], and the melatonin effect on the pituitary gland activity is reproductive-stage-dependent, modifying the secretory capacity of GH cells [Diaz-Rodriguez et al., 2001]. In Djungarian hamster, GH cells were not affected by photoperiod or exogenous melatonin as demonstrated by Hira et al. [2001]. Meunier et al. [1988] reported that melatonin does not have any effect on the secretion of GH in cultures of mink hypophysis gland. In the present study, we did not observe any melatonin effect on the somatotrophs. For this reason we suggest that the changes of somatotrophs during the reproductive cycle of adult male viscachas might be more related to the changes of the gonadal activity than to a direct effect of melatonin.

Different researchers have demonstrated that the gonadal steroids might mediate the effects of GH on growth through the regulation of its synthesis and secretion. Somana et al. [1978] observed an increase in the pituitary GH content after treatment with androgens in rat. Sinha et al. [1979] reported a decrease in pituitary GH levels in castrated mice suggesting that the gonadal androgens are essential for maintaining normal levels in the pituitary gland. Similar results were reported by other authors who have demonstrated that testosterone has an effect on the GH cells [Wehrenberg et al., 1985; Hertz et al., 1989; Kineman et al., 1990]. Investigations carried out in mink [Vidal et al., 1994], *B. arenarum* [Miranda et al., 1996], *Anguilla anguilla* L. [Grandi et al., 2003] and pig [Lee et al., 2004] of different ages showed morphometric differences of the somatotrophs probably due to the different physiological stages. In viscacha, the castration provoked a significant decrease in the immunopositive area, in the number of cells and in the major cellular diameter, and when studying the different growth stages, a gradual increase in these parameters was observed in relation to the degree of the testicular activity.

In the present work, our results demonstrated that the morphometric parameters of somatotrophs of male *L. maximus maximus* vary according to the gonadal development and activity. However, future studies will be necessary to examine the relation between the activity of somatotrophs and the pineal-hypotalamopituitary and gonadal axis in this interesting animal of seasonal reproduction.

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