Immunohistochemical and Morphometric Study of Pituitary Pars Distalis Thyrotrophs of Male Viscacha (*Lagostomus maximus maximus*): Seasonal Variations and Effect of Melatonin and Castration

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ABSTRACT

The physiology and behavior of viscacha vary along the year according to the modifications of environmental signals such as the photoperiod length, temperature, rainfall pattern, food composition, and social interactions. The pituitary pars distalis thyrotrophs (TSH cells) of male viscacha were immunohistochemically identified, and the morphometric parameters: percentage of immunopositive area (% IA), cell percentage in pars distalis (% PDC), number of cells per reference area (no. cells/RA), and major cellular and nuclear diameters were analyzed. Three different groups of adult male viscachas were used: (1) captured in their natural habitat during the year, (2) melatonin-administered, and (3) castrated. The thyrotrophs were localized in the ventromedial sector, mainly in the pars distalis cephalic extreme. They were oval or pyramidal in shape, and their immunostaining intensity was heterogeneous. The % IA, % PDC, and no. cells/RA exhibited a significant decrease in June–July (winter, gonadal regression period) in relation to February–March (summer–early autumn, reproductive period), and they were recovered in August–September (later winter–early spring, gonadal recovery period). No morphometric variations of TSH cells were observed in melatonin-treated animals, whereas a decrease of the % IA, % PDC, and no. cells/RA was observed in castrated animals in relation to the intact animals. Our results show TSH cell morphometric variations during the year in agreement with the animal's different physiological conditions during the reproductive cycle, and probably in response to the environmental signals changes. Melatonin does not have a direct effect on the TSH cells. However, castration modifies some thyrotroph morphometric parameters, reinforcing the hypothesis that androgens affect the cells activity. Anat Rec, 291:400–409, 2008.

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The pituitary gland is located in the central part of the hypothalamus–pituitary–thyroid (HPT) axis. The thyrotrophs (TSH cells), thyrotropin (TSH) producers, are stimulated or inhibited by the hypothalamus neuropeptides (De Groef et al., 2005), the thyroid hormones (Rosa and Bryant, 2003; Malaguti et al., 2004), and the gonadal steroids (Borges et al., 1998; Banu and Aruldhas, 2002).

Several studies on different species such as bullfrog (Tanaka et al., 1990), rat (Kum et al., 2006), dog (el-Etreby and el-Bab, 1978), bat (Mikami et al., 1988), and monkey (Girod and Trouillas, 1980) have described the morphological characteristics and localization of the TSH cells in the pituitary pars distalis (PD). Some investigations on woodchuck (Frink et al., 1980), bat (Anthony and Gustafson, 1984), and rat (Yoshimura et al., 1982; Kum et al., 2006) have reported variations in the cells activity, number, and ultrastructure in relation to the reproductive season. Other authors have investigated the relation between the pineal gland and the HPT axis. In Djungarian hamsters, the number and distribution of PD TSH cells (Wittkowski et al., 1988), and the immunoreactivity to TSH (Bergmann et al., 1989) did not differ between animals maintained under long and under short photoperiods. Brammer et al. (1979), in an in vitro study with rat pituitary cells, observed that melatonin did not affect TSH secretion, whereas other in vivo experiments in rat and hamster demonstrated that the short photoperiod, darkness, and melatonin administration caused a decrease in the serum TSH levels (Vriend, 1981; Baltaci et al., 2004).

Ahlquist et al. (1990) observed in rat that androgens administration decreased the TSH α and β mRNA levels in the cytoplasm of rat pituitary cells, but no changes were observed in the serum TSH concentration nor in the TSH pituitary content. The authors suggested that androgens exert a differential effect upon the synthesis and secretion of this pituitary hormone. In addition, Banu and Aruldhas (2002) reported that serum TSH and TSH-R patterns change when testicular steroidogenesis is reactivated.

Our experimental model, the *Lagostomus maximus maximus* (viscacha), is a rodent of nocturnal habits. Its physiology and behavior vary during the year according to the modifications of environmental signals such as the photoperiod length, temperature, rainfall pattern, food composition, and social interactions. The adult male viscacha in its natural habitat exhibits an annual reproductive cycle synchronized by the environmental photoperiod through the pineal gland and its main hormone, melatonin (Dominguez et al., 1987; Fuentes et al., 1991, 1993; Pelzer et al., 1999). The viscacha annual reproductive cycle presents three well-defined periods: reproductive period (summer–early autumn, February–March, long photoperiod), gonadal regression period (winter, June–July, short photoperiod), and gonadal recovery period (later winter–early spring, August–September; Fuentes et al., 1993, 2003; Muñoz et al., 1997, 2001; Aguilera-Merlo et al., 2005). Previous studies on the pituitary PD cells of adult male viscacha have shown variations in the morphometric parameters of the luteinizing hormone (LH), follicle-stimulating hormone (FSH), adrenocorticotropic hormone (ACTH), and growth hormone (GH) cells during the year, depending on the reproductive cycle and the environmental conditions. In addition, different effects of melatonin administration upon every cell population have been reported (Filippa et al., 2005; Filippa and Mohamed, 2006a,b).

The aim of this work was to localize and study different morphometric parameters (percentage of immunopositive area, percentage of immunoreactive cells in PD, number of cells per reference area, and major cellular and nuclear diameters) of the pituitary PD thyrotrophs of adult male viscacha during the year, after melatonin administration and castration. These parameters can be considered cellular activity indicators (Takahashi, 1991; Torres et al., 1995; Vidal et al., 1995; Filippa et al., 2005; Filippa and Mohamed, 2006a,b).

MATERIALS AND METHODS

Experimental Animals

The viscachas weighing 5–7 kg were captured in their habitat near San Luis, Argentina (33° 20' south latitude, 760 m altitude) during 2004–2005, using traps placed in their burrows. The reproductive condition of viscachas was carefully assessed on the basis of observations by light microscopy of testes. All male viscachas of June–July bimester were in the gonadal regression period. Values of solar irradiation expressed as heliophany and monthly mean values of precipitations and temperature were provided by the Servicio Meteorológico Nacional San Luis (www.smn.gov.ar). In June–July, the lowest values of heliophany, precipitation, and temperature were observed (Table 1).

Twenty animals were used for the seasonal study, four per bimester: summer–early autumn (February–March), autumn (April–May), winter (June–July), later winter–early spring (August–September), and spring (October–November). After being captured, animals were immediately taken to the laboratory, anesthetized with Nembutal (pentobarbital) and killed by decapitation. The brain was rapidly exposed, and the pituitary gland was excised, fixed in Bouin's fluid, processed for light microscopy and embedded in paraffin. The pituitary gland was sagittally sectioned and each hemi-pituitary was completely cut following the same design of the previously reported work (Filippa and Mohamed, 2006b). Immunostaining was analyzed at low magnification (×20 objective), which showed that the sections obtained in the middle sector 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. The experimental design was approved by the local Ethics Committee and was in agreement with the guidelines of the National Institute of Health (NIH, Bethesda, MD) for the use of experimental animals.

TABLE 1. Environmental conditions during the year

	Feb–Mar	Apr–May	June–July	Aug–Sept	Oct–Nov
Heliophany	9.38	7.09	6.11	7.21	9.09
Precipitation (mm)	90	27	11	15	58.5
Temperature (°C)	19.5	13	10.25	14.5	21.5

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 9:00 AM and 5:00 PM, for 9 weeks. The control group received only the diluent. In both groups, the histological study of the testes was carried out to confirm the effect of melatonin on the reproductive status. In the melatonin-administered viscachas an inhibitory effect of this hormone on the spermatogenic activity was observed. These results were similar to those previously found in our laboratory (Muñoz, 1998).

Castration

Eight adult male viscachas captured during the month of May (autumn) were used. The castrated and intact animals 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.

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 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 mouse monoclonal antibody against human pituitary TSH (BioGenex, San Ramon, CA). 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) at 20°C. The Biotin–Streptavidin Amplified system (B-SA) was used as follows: sections were incubated for 30 min with diluted biotinylated anti-mouse 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 of 3,3′-diaminobenzidine tetrahydrochloride 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 all cases, two controls for specificity of the primary antibody were made: (1) omission of primary antibody, and (2) absorption of primary antibody with homologous antigen. No positive structures or cells were found in these sections.

Morphometric Analysis

Computer-assisted image analysis system was used to measure the percentage of immunopositive area, percentage of immunoreactive cells in PD, the number of thyrotrophs per reference area, and the major cellular and nuclear diameters. The system consisted of an Olympus BX-40 binocular microscope (magnification ×200), 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 parameters were measured with the image analysis system. Before counting, a standard area of 76,241 μm^2 (reference area, RA) 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: four tissue sections from a pituitary gland were used, and all the microscopic fields were analyzed in every section (50–70 microscopic fields according to the section). Therefore, between 200 and 280 microscopic fields were analyzed in each gland, and four pituitary glands were analyzed in each group of animals. Finally, 800–1,120 microscopic fields or measures were carried out per group. The following morphometric parameters were determined:

Percentage of immunopositive area (% IA) of thyrotrophs was calculated using the formula % IA = ΣAc/ΣRA × 100, where ΣAc was the sum of the area of immunolabeled cells and ΣRA was the sum of the PD area of every microscopic field. The % IA represents the volume density and it was calculated according to the concept usually accepted and used by several authors (Miranda et al., 1996; Cónsole et al., 2001; Filippa and Mohamed, 2006b).

The percentage of immunoreactive cells in PD (% PDC) in each image was obtained (A/A+B × 100). Each image contained approximately 700–900 cells. The number of immunoreactive cells (A) and the number of nuclei in unstained cells (B) were counted (Dada et al., 1984).

The number of immunostained cells (no. cells/RA) with a visible nucleus was counted in 10 microscopic fields per section. The result was expressed as number of cells per RA (Miranda et al., 1996).

The major cellular and nuclear diameters were measured using the length tool of the Image Pro Plus 5.0 software on each thyrotroph with a visible nucleus. These parameters were measured for 40 immunoreactive TSH cells per group.

Statistical Analysis

The results were expressed as means ± standard error of the mean (SEM) for all data sets. The different groups were studied every bimester and the data were evaluated using one-way analysis of variance 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 *Lagostomus maximus maximus* pituitary PD exhibited different regions or zones called ventral (anterior) and dorsal (posterior, close to Rathke's pouch). In addition, two extremes were distinguished: a cephalic one (superior, connected with pars tuberalis) and a caudal one (inferior).

The thyrotrophs were localized in the PD ventromedial region, mainly in the cephalic extreme in all studied groups (Fig. 1A–E). The cells regionalization inside the PD parenchyma was limited by blood vessels (Figs. 1B, 2A,D). They were occasionally observed in the dorsal region next to Rathke's pouch. The thyrotrophs were isolated or in clusters next to the follicular structures lumen, forming fences along the blood vessels surface (Fig. 2B,E), but they were mainly found isolated during the June–July bimester (Figs. 1C, 2F). Most cells were oval or pyramidal in shape, and some of them had a short cytoplasmic prolongation, reaching blood vessels or surrounding nonstained cells. They exhibited a spherical nucleus of an eccentric or central position. A fine cytoplasmic immunostaining, generally more intense, was observed in the cellular extreme that was in contact with a blood vessel wall (Fig. 2B,C,E,F). The PD cells immunostaining did not exhibit the same intensity because the cells cytoplasms were either intensely stained or lightly stained (Fig. 2E).

Most of the analyzed morphometric parameters values showed variations during the year as follows: % IA, % CPD, and no. cells/RA reached their maximum in the February–March bimester (reproductive period). They showed a significant decrease in June–July (gonadal regression period; $P < 0.001$; $P < 0.01$; and $P < 0.05$; respectively) and a significant increase in August–September (gonadal recovery period; $P < 0.001$; $P < 0.01$; $P < 0.05$; respectively). No variations in the values of the major cellular and nuclear diameters were observed (Table 2).

The distribution, localization, morphology, immunostaining pattern, and the morphometric parameters analyzed in melatonin-administered viscachas did not show differences in relation to the control ($P > 0.05$; Table 3).

In castrated animals, the thyrotrophs were mainly found isolated in the cephalic extreme of PD. In these animals, a significant decrease ($P < 0.05$) in % IA, % CPD and no. cells/RA was observed in relation to the intact animals. The major cellular and nuclear diameters did not show significant variations ($P > 0.05$; Table 4; Fig. 3).

DISCUSSION

Environmental conditions such as photoperiod length, temperature, and water and food availability, and social interactions modify the physiological state of wild animals (Ruby and Zucker, 1992; Dark et al., 1994). These environmental signals determine the beginning or ending of specific seasonal adaptations, carrying out the necessary endocrine adjustments to ensure survival and reproductive success. Previous studies have confirmed that the *Lagostomus maximus maximus* is a long-day breeder, that is, its reproductive period occurs during summer and early autumn. It exhibits a short period of gonadal regression during short winter days and a gonadal recovery period at early spring (Dominguez et al., 1987; Fuentes et al., 1991, 1993, 2003; Pelzer et al., 1999; Muñoz et al., 1997, 2001; Mohamed et al., 2000; Aguilera-Merlo et al., 2005; Filippa et al., 2005).

In this work on TSH cells, we demonstrate that (1) there are variations during the year of immunopositive percentage area, PD cells percentage and cells number/RA in the pituitary PD of adult male viscachas, with the minimum values in June–July (winter, gonadal regression period); (2) melatonin administration did not cause variations in the studied morphometric parameters; (3) castration provoked a significant decrease of the immunopositive percentage area, PD cell percentage, and cell number/RA.

The localization and morphology of PD TSH cells were studied in species such as monkey (Girord and Trouillas, 1980), bat (Anthony and Gustafson, 1984; Mikami et al., 1988), rat (Kum et al., 2006), toad (Miranda et al., 1996), and bullfrog (Tanaka et al., 1990), and the results show a similar distribution pattern in different species. It is interesting to point out that the TSH cells are generally restricted to the PD anterior and medial zones, and they are rarely observed in the dorsal region next to pars intermedia (PI). Our results in viscacha are similar to those previously reported for other species. TSH cells were mainly located in the ventromedial zone, especially in the PD cephalic extreme. They were principally oval or pyramidal in shape, and they were frequently distributed near the blood vessels.

Quantitative studies have shown that the thyrotrophs generally constitute a small proportion of the PD cells, 4–6% (Childs et al., 1983) and 2.09% (Dada et al., 1984) in rat, 1.36% in bat (Anthony and Gustafson, 1984), 0.43% in mouse (Messier, 1965), 5% in voles (Clarke and Forsyth, 1964), and 1% in black-tailed deer (West and Nordan, 1976). In viscacha, the values observed were between 2.39% and 1.16% (summer–early autumn and winter, respectively), showing a similar proportion to that reported for other species.

Studies carried out in Djungarian hamster have demonstrated that the PD TSH cells were not modified by the photoperiod variations (Wittkowski et al., 1988; Bergmann et al., 1989). In this work, seasonal variations of the morphometric parameters of PD thyrotrophs of adult male viscacha were observed, demonstrating that these cells respond to environmental conditions changes, probably through different factors regulating this cellular population activity.

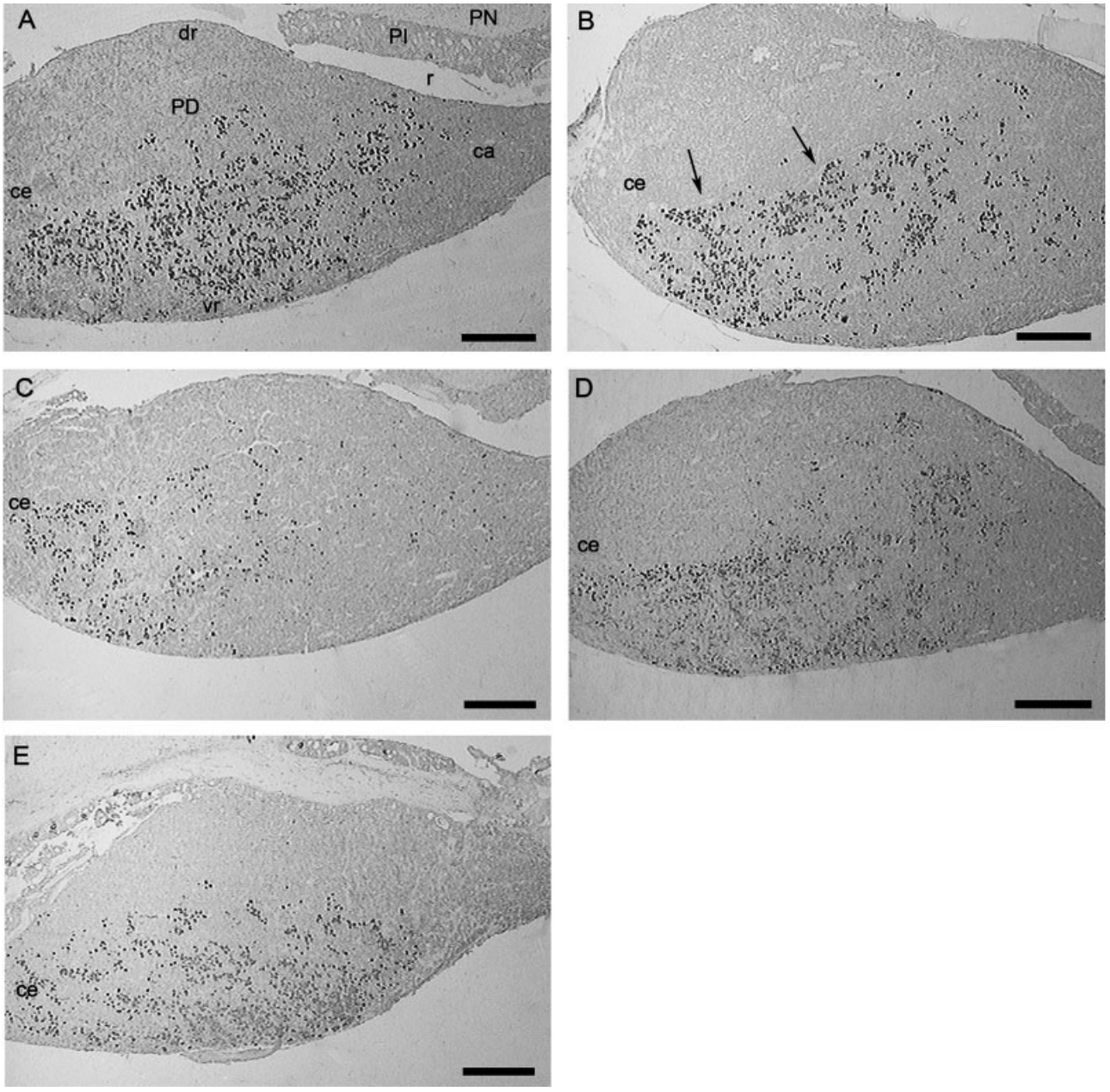

Fig. 1. **A–E:** Pituitary glands of adult male viscachas captured during February (summer, A), May (autumn, B), July (winter, C), September (early spring, D), and November (spring; E). Thyrotrophs are localized in the pars distalis (PD) ventromedial region, mainly in the cephalic extreme (ce). The cells regionalization is limited by blood vessels (arrows). C: A small number of thyrotrophs (TSH cells) is present in PD during July. PI, pars intermedia; PN, pars nervosa; r, Rathke' pouch; ca, caudal extreme; dr, dorsal region; vr, ventral region. Scale bar = 500 μm.

Seasonal variations studies of the thyrotroph cells number and cellular activity carried out in woodchuck (*Marmota monax*) have shown that these parameters vary according to the thyroid gland activity and the animal physiological activities (Frink et al., 1980). A higher proportion of these cells in June after hibernation and a lower proportion in December during mating were observed in bat (Anthony and Gustafson, 1984). This finding suggests that the increase of thyrotrophs frequency in spring coincides with a metabolic activity increase and also with early stages of the spermatogenesis. In addition, TSH cells percentages in rat were observed to vary under different physiological and experimental conditions (Childs, 1983; Sekulic et al., 1998). These variations can be directly correlated to the levels of stimulation and/or inhibition of the specific secretory activity. The stimulation of the hormone secretion decreases the TSH content stored in the pituitary cells secretory granules, whereas its inhibition increases the TSH content stored in the pituitary cells secretory

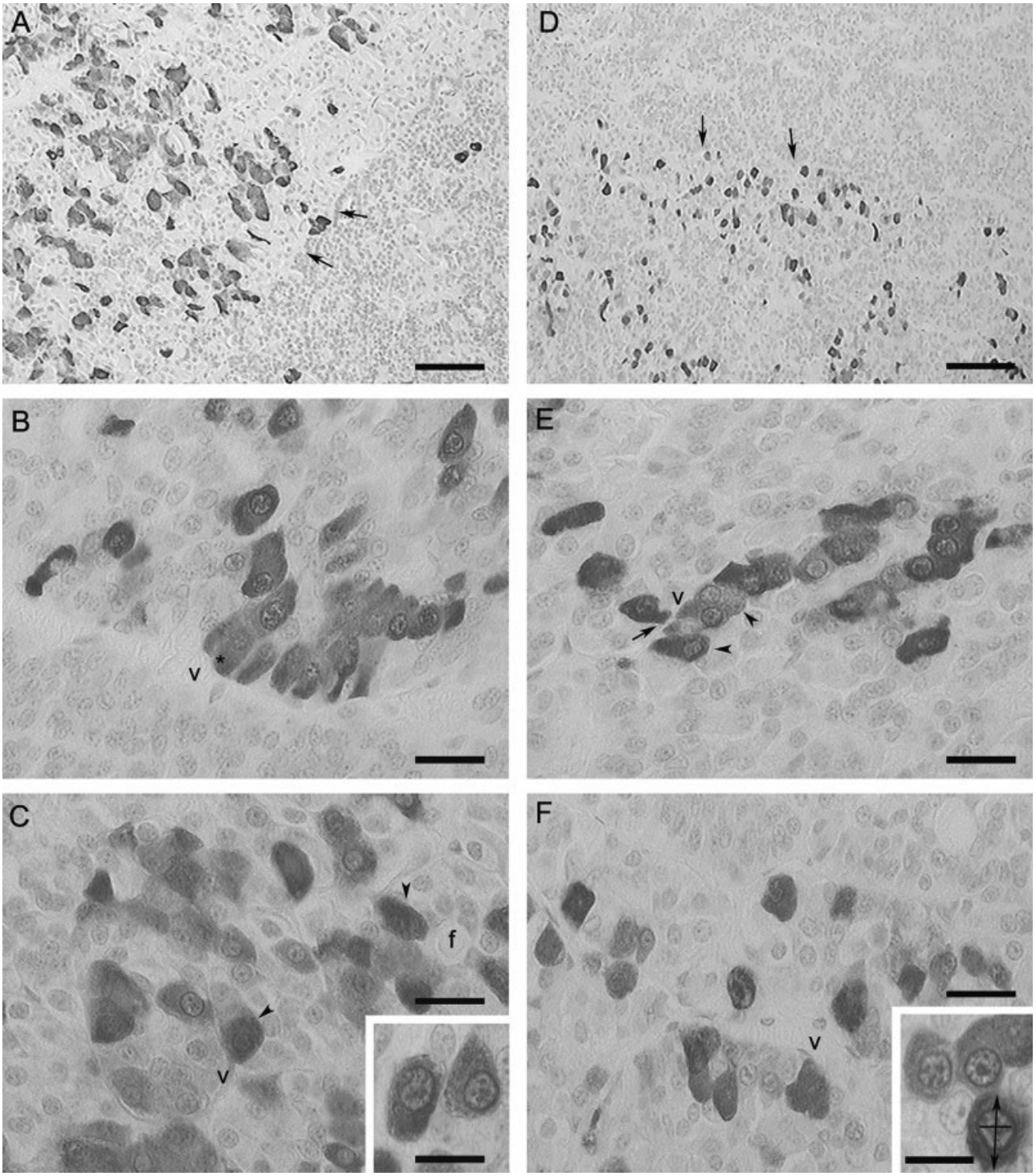

Fig. 2. **A–F:** Pituitary glands of adult male viscachas captured during February (A–C) and July (D–F). **A:** Detail of the cephalic extreme of Figure 1A, where there are blood vessels delimiting the cells regionalization (arrows). Scale bar = 100 μm. **B:** Thyrotrophs (TSH cells) forming fences along the blood vessels surface (v). The fine cytoplasmic immunostaining is more intense in the cellular extreme (*) that is in contact with a blood vessel. Scale bar = 25 μm. **C:** Numerous thyrotrophs (arrowheads) are in contact with a blood vessel wall (v) and some of them next to the follicular structures (f). Scale bar = 25 μm. Right inset: higher magnification of oval and pyramidal thyrotrophs. Scale bar = 10 μm. **D:** Detail of cephalic extreme of Figure 1C. The arrows show the regionalization of the thyrotrophs. These cells are mainly isolated during July (winter). Scale bar = 100 μm. **E:** Thyrotrophs forming fences along the blood vessels surface (v). There is a TSH cell with a cytoplasmic prolongation reaching the surface of blood vessel (arrow). The TSH cells immunostaining did not exhibit the same intensity (arrowheads). Scale bar = 25 μm. **F:** There are few thyrotrophs near to blood vessel (v). Right inset: The double-headed arrow indicates the major cellular diameter; the horizontal bar indicates the nuclear diameter. Scale bar = 10 μm.

TABLE 2. Morphometric parameters of TSH cells during the year[a]

	Feb–Mar	Apr–May	June–July	Aug–Sept	Oct–Nov
% IA	3.35 ± 0.09	2.76 ± 0.30	1.43 ± 0.15 a, b	2.98 ± 0.21	2.63 ± 0.13
% PDC	2.39 ± 0.10	1.95 ± 0.23	1.16 ± 0.10 c, d	2.25 ± 0.17	2.05 ± 0.07
No. cells/RA	18.81 ± 2.58	18.10 ± 2.42	9.98 ± 0.96 e	18.04 ± 1.38	16.98 ± 0.79
Major diam. cellular (μm)	13.95 ± 0.31	13.83 ± 0.33	13.04 ± 0.30	13.82 ± 0.22	12.97 ± 0.41
Diam. nuclear (μm)	5.64 ± 0.16	5.60 ± 0.11	5.63 ± 0.07	5.70 ± 0.14	5.45 ± 0.19

[a]The values are expressed as mean ± SEM (n = 4). % IA, percentage of immunopositive area; % PDC, cells percentage in pars distalis; No. cells/RA, number of cells per reference area. Significant differences were determined by analysis of variance followed by the Tukey Kramer multiple comparison test: a, $P < 0.001$ June–July vs. Feb–Mar, and June–July vs. Aug–Sept; b, $P < 0.01$ June–July vs. Apr–May, and June–July vs. Oct–Nov. c, $P < 0.01$ June–July vs. Feb–Mar, and June–July vs. Aug–Sept; d, $P < 0.05$ June–July vs. Apr–May, and June–July vs. Oct–Nov. e, $P < 0.05$ June–July vs. Feb–Mar, June–July vs. Apr–May, and June–July vs. Aug–Sept.

TABLE 3. Morphometric parameters of TSH cells after melatonin administration[a]

	Control	Experimental
% IA	2.90 ± 0.57	3.06 ± 0.58
% PDC	1.99 ± 0.10	2.19 ± 0.41
No. cells/RA	17.09 ± 0.40	18.76 ± 1.40
Major diam. cellular (μm)	12.80 ± 0.54	13.86 ± 0.32
Diam. nuclear (μm)	5.44 ± 0.07	5.52 ± 0.06

[a]The values are expressed as mean ± SEM, (n = 4). % IA, percentage of immunopositive area; % PDC, percentage of immunoreactive cells in PD; No. cells/RA, number of cells per reference area. No significant differences were determined by Student's *t*-test.

TABLE 4. Morphometric parameters of TSH cells after castration[a]

	Intact	Castrated
% IA	1.28 ± 0.05	0.70 ± 0.09*
% PDC	1.11 ± 0.06	0.59 ± 0.07*
No. cells/RA	9.14 ± 0.18	4.94 ± 0.18*
Major diam. cellular (μm)	12.63 ± 0.60	13.19 ± 0.41
Diam. nuclear (μm)	5.35 ± 0.09	5.21 ± 0.01

[a]The values are expressed as mean ± SEM, (n = 4). % IA, percentage of immunopositive area; % PDC, percentage of immunoreactive cells in PD; No. cell/RA, number of cells per reference area.
*$P < 0.05$ Castrated vs. Intact group by Student's *t*-test.

granules (Torres et al., 1995). Moreover, in some species of birds (Nicholls et al., 1988), rodents (Péczely et al., 1980; Gerlach and Aurich, 2000), and mammals (Nicholls et al., 1988; Gerlach and Aurich, 2000; Billings et al., 2002) with seasonal reproduction, the thyroid hormones play an important role in the endocrine conditions maintenance during the reproductive season. In ewes, the transition toward the reproductive season is not affected by the thyroid hormones, but their presence is necessary because, during a restricted period of time, they permit neuroendocrine changes leading to the reproductive activity ending (Karsh et al., 1995). The thyroid hormones might provoke morphological changes in the GnRH neurons regulating TSH liberation (Yamamura et al., 2004). When there are no thyroid hormones, there is no seasonal reproductive rhythm (Rosa and Bryant, 2003).

The lowest heliophany (short photoperiod), temperature, and rainfall pattern values were registered during June–July (winter) in San Luis (Argentina), resulting in food quality changes (Bontti et al., 1999). These environmental factors caused changes in the organism preparation at the pituitary–adrenal axis level under different environmental conditions in winter (Filippa and Mohamed, 2006a). In addition, previous studies on the thyroid gland anatomo-histology of adult male viscacha have shown that this gland presents seasonal variations with periods of maximum and minimum activity in summer and winter, respectively (Guiñazu, 1996). In addition, other studies on this rodent have shown changes in the testosterone concentration during the year, with the maximum values in summer–early autumn (February–March) and the minimum in winter (July; Fuentes et al., 1993). Our results show a higher storage of TSH in PD during summer, made evident by a bigger immunopositive area and higher cell numbers. This finding might be due to one of two reasons, either lower TSH secretion because of the negative feedback effect of the thyroid hormones or some synthesis stimulating action of the testicular androgens upon TSH synthesis. The morphometric parameters decrease in June–July (winter, gonadal regression period), suggesting that the activity of TSH cells varies according to the reproductive cycle and to the environmental signals variations. Those parameters are recovered during the gonadal recovery period in August–September (later winter–early spring), when the testicular steroidogenic and spermatogenic processes are reinitiated. In addition, the immunostaining intensity heterogeneity observed in TSH cells might represent different cellular activity states. While some thyrotrophs remain active in response to different stimuli, others remain in stand-by mode.

Different results have been reported when examining melatonin effect upon this pituitary cellular population. Brammer et al. (1979) observed in rat that melatonin does not affect in vitro TSH secretion; therefore, there would not be evidence of interaction between the pineal gland and the HPT axis. Moreover, it has been reported in rat that melatonin administration (Baltaci et al., 2004), darkness, or short photoperiod decrease TSH plasma concentration due to the antithyrotrophic effect of the pineal gland when influencing the synthesis and secretion of the hypothalamic TRH (Vriend, 1981). Mazzoccoli et al. (2004) showed in humans that the response of pituitary TSH to hypothalamic TRH and of the thyroid gland to pituitary TSH may be influenced by melatonin, thus modulating the HPT axis functioning.

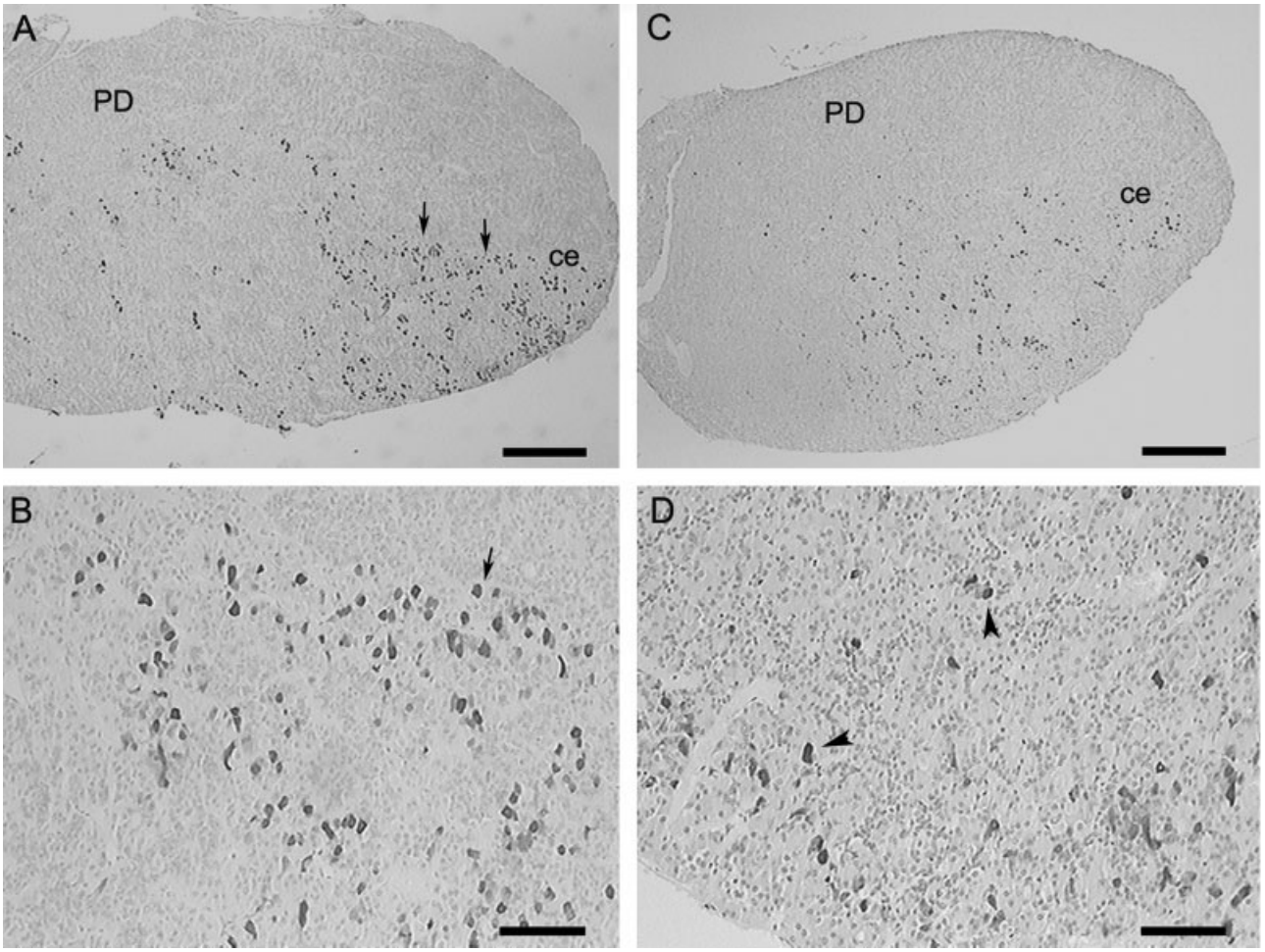

Fig. 3. **A,B:** Pituitary pars distalis (PD) of intact animals. The regionalization of the thyrotrophs (TSH cells) is limited by blood vessels (arrows). **C,D:** Pituitary PD of castrated viscachas. The number of TSH cells is lower than intact animals, and these cells are mainly isolated (arrowheads). ce, cephalic extreme. Scale bars = 500 μm in A,C, 100 μm in B,D.

The effect of melatonin administration has been reported to be different in each studied cellular population in viscacha. Previous studies have shown that the morphometric parameter variations of the LH and FSH cells (Filippa et al., 2005) and ACTH cells (Filippa and Mohamed, 2006a) may be directly or indirectly provoked by melatonin. On the other hand, the variations observed along the reproductive cycle upon GH cells are mainly dependent on the testicular androgens (Filippa and Mohamed, 2006b). In this work, no variations of the TSH cells morphometric parameters were observed in melatonin-administered animals. This finding suggests that the observed seasonal changes do not depend on a melatonin direct effect on this cellular population. These changes might be related to the histophysiological modifications from the pineal–pituitary–gonadal, pineal–pituitary–adrenal, and pineal–pituitary–thyroid axes previously reported in this rodent (Dominguez et al., 1987; Fuentes et al., 1993; Guiñazu, 1996; Muñoz et al., 1997, 2001; Aguilera-Merlo et al., 2005; Filippa et al., 2005; Filippa and Mohamed, 2006a).

The steroid hormones have been shown to modulate the thyrotroph's activity (Ahlquist et al., 1990; Moreira et al., 2000). Previous studies have shown in rat that the estrogens stimulate TSH secretion (Kimura et al., 1994). In addition, different results have been observed when studying the testosterone effect upon the cells. Ahlquist et al. (1990) have demonstrated that androgens in rat may exert a differential effect on TSH synthesis and secretion. Several investigations have analyzed the effect of castration on TSH cells. In castrated rats, the TSH cells number decrease (Kum et al., 2006). In addition, the TSH pituitary content (Borges et al., 1998), the serum TSH concentration and the serum TSH response to hypothalamic TRH decreased in castrated rats (Christianson et al., 1981). These effects were reversed with androgen administration. However, Ibrahim et al. (1986) reported in rat that the percentage of TSH-producer cells does not vary after gonadectomy.

In the present work, a decrease of the % IA, % PDC, and no. cells/RA was observed in castrated viscachas in relation to the intact animals. These results reinforce

the hypothesis that androgens affect TSH cell activity, as reported in other species. In addition, this finding might explain the observed seasonal variations of the TSH cells with minimum values during the winter when the viscacha is in the gonadal regression period.

As a conclusion, the morphometric parameters of TSH cells of adult male viscacha pituitary PD vary in response to the different environmental conditions during the year. These variations and, consequently, the cell's activity are in agreement with the animal physiological conditions during the different reproductive cycle periods. In addition, we have demonstrated that melatonin does not have a direct effect on TSH cells, whereas castration modifies some of the cell morphometric parameters and, therefore, their synthesis and/or secretion activity.

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