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Pituitary Pars Intermedia of Male Viscacha *(Lagostomus maximus maximus)*: A Morphometric Study of Seasonal and Age-Related Changes in Immunohistochemistry

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Key Words

Lagostomus · Pituitary pars intermedia · Season · Age · Immunohistochemistry

Abstract

The aim of this work was to study the pituitary pars intermedia in seasonally captured adult male and immature viscachas by immunohistochemistry and image analysis. The pituitary pars intermedia exhibited a well-developed parenchyma with scarce connective tissue and vascularization. It was formed by a close association of melanotrophs and folliculostellate cells. The folliculostellate cells were stellate in shape with cytoplasmic processes, and they originated follicles with PAS-positive colloid inside. The morphometric parameters of melanotrophs, follicular colloid and folliculostellate cells (S-100-ir and GFAP-ir) varied seasonally and in relation to age. These parameters showed minimal values in the adult males captured in winter and in immature animals, and they were maximal in summer. The percentage of vimentin-positive area of the folliculostellate cells was maximal in immature animals, decreased in relation to age and did not vary seasonally in the adult animals. The greatest development of pars intermedia in the adult animals in relation to the immature ones is probably related to the adults' adaptation to the semiarid environment. The expression of the tested proteins suggests a probable neuroectodermic origin for the folliculostellate cells of the viscacha pituitary pars intermedia. In addition, the cytoplasmic processes of folliculostellate cells might originate an intercellular communication network inside the pars intermedia. The decrease in the morphometric parameters melanotrophs, follicular colloid and folliculostellate cells in winter suggests a low endocrine activity of this zone. This fact might be due to the effect of the short photoperiod and high melatonin serum levels.

Introduction

The pituitary pars intermedia (PI) exhibits morphological variations among mammalian species [Ben-Jonathan et al., 1991]. Cytoarchitectural changes related to the reproductive cycle, age and sex of several vertebrate species have been reported in this zone [Anthony and Gus-

Abbreviations used in this paper

α-MSH	α-melanocyte stimulating hormone
FSC	folliculostellate cells
GFAP	glial fibrillary acidic protein
PBS	phosphate buffered saline
PD	pars distalis
PI	pars intermedia
PN	pars nervosa

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tafson; 1984; Ben-Jonathan et al., 1991; Anthony et al., 1998]. Another unusual feature of the PI is the relative lack of vascular supply to the endocrine tissue [Saland, 2001]. The main endocrine cellular type is the melanotroph, which secretes the α-melanocyte stimulating hormone (α-MSH) [Takeuchi, 2001]. It is well known that this hormone is an important regulator of mammalian skin and coat colour [Slominski et al., 2004]. Lincoln and Baker [1995] have reported a decrease in α-MSH concentrations in Soay sheep during winter. Lebaili et al. [1999] have described a marked hypersecretion of melanotrophs in hydrated gerbils. Corticotroph-like cells and folliculostellate cells (FSC) have also been observed in PI [Gary and Chronwall, 1995; Lebaili et al., 1999; Takeuchi, 2001].

The pituitary FSC, also called follicular, stellate, agranulated or glial-like cells, have been reported in mammalian [Soji et al., 1994; Gary and Chronwall, 1995; Lebaili et al., 1999; Cardin et al., 2000; Inoue et al., 2002; Mabuchi et al., 2004; Marettová and Maretta, 2004; Shirasawa et al., 2004] and non-mammalian species [De Rijk et al., 1991; Chowdhury and Yoshimura, 2002]. At present, there is no agreement in the literature in relation to the exact function and embryological origin of these cells. In pituitary PI, they were chromophobic, stellate in shape with cytoplasmic processes [Takeuchi, 2001]. The FSC of the pituitary gland have been described in humans [Morris and Hitchcock, 1985; Coates and Doniach, 1988; Redecker and Fechner, 1989], rats [Nakajima et al., 1980; Shirasawa et al., 1983; Tsuchida et al., 1991; Gary and Chronwall, 1995], pigs [Marettová and Maretta, 2004], mink [Cardin et al., 2000], dogs and horses [Méndez et al., 1998] using the anti-S-100 protein antiserum. A subpopulation of these cells expressed the glial fibrillary acidic protein (GFAP) whose presence varied under numerous physiological conditions, probably indicating a regulatory function [Gary and Chronwall, 1995; Sands and Chronwall, 1996]. In addition, some authors have reported that the FSC were immunostained with the anti-GFAP and anti-vimentin antisera in rats [Sands and Chronwall, 1996] and in gerbils [Lebaili et al., 1999]. Soji et al. [1994] have shown an increase in the number of these cells in relation to age, suggesting that the expression of the S-100 protein indicates the cells` morphologic maturation stage in rats. The FSC in most pituitary glands of the studied species originated follicular structures with colloid in their lumen [Anthony and Gustafson, 1984; Ben-Jonathan et al., 1991; Kameda, 1991; Ogawa et al., 1996; Mohamed et al., 2000; Claudius et al., 2005]. In pigs and guinea pigs, a relation between the follicular colloid, the FSC and the pituitary hormone-secretory cells has been suggested [Kameda, 1991; Ogawa et al., 1997]. Some studies have reported variations in the pituitary follicular colloid related to the reproductive cycle, age and sex in pigs [Ogawa et al., 1996], guinea pigs [Kameda, 1990, 1991] and bats [Anthony and Gustafson, 1984].

Different experimental models have been used to study the animals' seasonal adaptations to their environments. The viscacha *(Lagostomus maximus maximus)* is a rodent of nocturnal habits and seasonal reproduction, inhabiting the semiarid region in the centre of Argentina. The adult male viscacha in its natural habitat exhibits an annual reproductive cycle synchronized by the environmental photoperiod and modulated by the pineal gland and its main hormone, melatonin [Dominguez et al., 1987; Fuentes et al., 2003]. This cycle presents 3 well-defined periods: reproductive (summer, early autumn), gonadal regression (winter) and gonadal recovery (spring) [Fuentes et al., 1991, 1993; Muñoz et al., 2001; Aguilera Merlo et al., 2005; Filippa et al., 2005]. In winter, the pinealocites cellular activity and serum values of melatonin were maximal [Dominguez et al., 1987; Cernuda-Cernuda et al., 2003; Fuentes et al., 2003]. In pituitary pars distalis (PD), the lowest number of PAS-positive follicular colloids has been reported in the regression period of the adult male and immature viscachas [Mohamed et al., 2000]. Scardapane et al. [1983] have described the effect of chronic melatonin administration in pituitary PI and have demonstrated a decrease in number, diameter and area of follicles in the treated animals.

The aim of this work was to study the pituitary PI in seasonally captured adult males and in immature viscachas. The melanotrophs, follicular colloid and FSC were studied by immunohistochemistry and image analysis.

Materials and Methods

Study Types

Seasonal Study. Sixteen adult male viscachas (>5 kg body weight) were captured in their natural habitat near San Luis, Argentina (33° 20' south latitude, 760 m altitude), in the following periods: February to March (summer), April to May (autumn), July to August (winter) and November to December (spring).

Age-Related Study. Four male animals (<5 kg body weight) were captured in spring and carefully classified as immature (sexually immature) according to body weight (1–2 kg) and light microscopy observations of testes [Llanos and Crespo, 1954; Mohamed et al., 2000; Filippa and Mohamed, 2006b].

Values of solar irradiation expressed as heliophany and seasonal mean values of precipitation and temperature (table 1) were provided by the Servicio Meteorológico Nacional San Luis

(www.smn.gov.ar). The lowest values of heliophany, precipitation and temperature were observed in winter.

After being captured, animals were immediately taken to the laboratory, anaesthetized with Nembutal (pentobarbital) and killed by decapitation. The brain was rapidly exposed and the pituitary gland was excised, sagittally sectioned, fixed in Buin's fluid, processed for light microscopy, embedded in paraffin and serially sectioned in the horizontal plane. The experimental design was approved by the local ethics committee and was in agreement with the guidelines of the National Institutes of Health (Bethesda, Md., USA) for the use of experimental animals.

Table 1. Solar irradiation and seasonal precipitation and temperature data

	Summer	Autumn	Winter	Spring
Heliophany, h	9.38	7.09	6.82	9.09
Precipitation, mm	90	27	11	58.5
Temperature, °C	22	13	12	19.66

PAS Technique

Serial sagittal sections (5 μm thick) were cut and carried through xylene and graded alcohols to water. Slides were incubated for 5 min in periodic acid. Then they were rinsed with distilled water, incubated for 15 min in a solution of Schiff reactive and rinsed with water. The slides were counterstained with hematoxylin for 1 min, dehydrated and mounted.

Immunohistochemistry

The immunohistochemistry technique was carried out as follows: serial sagittal sections (5 μm thick) were cut and carried through xylene and graded alcohols to water. Slides 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). Non-specific 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 moist chamber at 4°C with the following primary antisera: polyclonal anti-α-melanocyte stimulating hormone, polyclonal anti-S-100 protein, polyclonal anti-GFAP, and monoclonal anti-vimentin V-9 (BioGenex, San Ramon, Calif., USA). After the slides 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 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 a 3.3′diaminobenzidine-tetrahydrochloride chromogen solution in 2.5 ml PBS and 50 μl of an 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. No positive structures or cells were found in these sections.

Morphometric Analysis

Computer-assisted image analysis system was used to measure the different morphometric parameters. The system consisted of an Olympus BX-40 binocular microscope, 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: images acquisition, automatic analogous adjust, thresholding, background subtraction, distance calibration, area and diameter measuring, and diskette data logging. The images were displayed on a colour monitor, and the parameters were measured with the image analysis system. Before counting, 2 reference areas of 3,000 μm^2 (×100 objective) and 18,141.82 μm^2 (×40 objective) were defined on the monitor, and distance calibrations were performed using a slide with a micrometric scale for microscopy (Reichert, Austria).

Melanotrophs

The morphometric study of melanotrophs was carried out as follows: 6 tissue sections from a pituitary gland were used, and all the microscopic fields captured with ×40 objective were analyzed in every section (24 microscopic fields according to the section). Therefore, 144 microscopic fields were analyzed in each gland, and 4 pituitary glands were studied in each group of animals. Finally, 576 microscopic fields or measures were carried out per group. The following morphometric parameters were determined.

Percentage of α-MSH-positive area (%IA-MSH) was calculated using the formula $\%IA\text{-}MSH = \Sigma Ac/\Sigma RA \times 100$, where ΣAc was the sum of the area of immunolabelled cells for anti-α-MSH and ΣRA was the sum of the PI area of every microscopic field. The percentage of immunopositive area 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, 2008].

The number of melanotrophs (MSH cells, n/RA) with a visible nucleus was counted in 24 microscopic fields per section. The result was expressed as number of melanotrophs per reference area.

The major cellular and nuclear diameters were measured using the length tool of the Image Pro Plus 5.0 software on each melanotroph with a visible nucleus. These parameters were measured for 60 immunoreactive α-MSH cells per group.

The analyzed morphometric parameters can be considered as a measure of the cellular activity [Takahashi, 1991; Torres et al., 1995; Filippa et al., 2005; Filippa and Mohamed, 2006a, b, 2008].

Follicular Colloid

For the morphometric study of the follicular colloid, 576 microscopic fields (×40 objective, see details in *Melanotrophs*) were carried out per group. The following morphometric parameters were determined.

Percentage of PAS-positive colloidal area (%PAS) was calculated using the formula $\%PAS = \Sigma Ap/\Sigma RA \times 100$, where ΣAp was the sum of the area of PAS-positive follicular colloid and ΣRA was the sum of the PI area of every microscopic field.

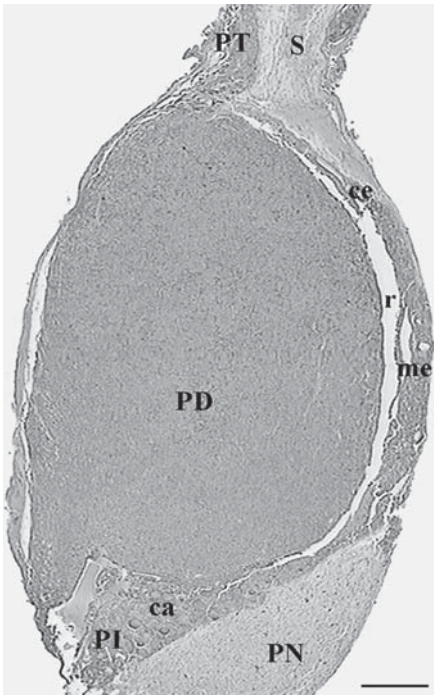

Fig. 1. Pituitary gland of adult male viscacha captured in February (summer). PT = Pars tuberalis; S = pituitary stalk; PD = pars distalis; PI = pars intermedia; PN = pars nervosa; r = Rathke's pouch; ce = cephalic zone; me = medial zone; ca = caudal zone. Hematoxylin-PAS. Scale bar = 1,000 μm.

The number of PAS-positive colloids (Col, n/RA) was counted in 24 microscopic fields per section. The result was expressed as number of PAS-positive colloid per reference area.

The major colloidal diameter was measured using the length tool of the Image Pro Plus 5.0 software on each PAS-positive follicular colloid. This parameter was measured for 220 colloids per group.

Folliculostellate Cells

The morphometric study for the FSC was carried out as follows: 6 tissue sections from a pituitary gland were used, and all the microscopic fields captured with ×100 objective were analyzed in every section (110 microscopic fields according to the section). Therefore, 660 microscopic fields were analyzed in each gland, and 4 pituitary glands were studied in each group of animals. Finally, 2,640 microscopic fields or measures were carried out per group. The following morphometric parameters were determined.

Percentage of immunopositive area for each antiserum (%IA-S-100, %IA-GFAP, %IA-vim) was calculated using the formula $\%IA = \Sigma Ai/\Sigma RA \times 100$, where ΣAi was the sum of the area of immunopositive cells for each antiserum and ΣRA was the sum of the PI area of every microscopic field.

The number of anti-S-100 immunostained cells (FSC, n/RA) with a visible nucleus was counted in 110 microscopic fields per section. The result was expressed as number of FSC per reference area.

Statistical Analysis

The results were expressed as mean ± standard error of the mean for all data sets. The different groups were evaluated using 1-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. $p < 0.05$ was assumed to be significant.

Results

Seasonal Study

The pituitary gland of the adult male viscacha exhibited a well-developed PI, separated from the PD by the Rathke's pouch and from the pars nervosa (PN) by long blood vessels. Three zones were distinguished: cephalic, medial and caudal, the latter being well developed in the inferior extreme (fig. 1). The PI presented little development of the connective tissue and vascularization. It was formed by a close association of melanotrophs and FSC, the last ones originated follicles with PAS-positive colloid in their lumen.

The melanotrophs were found throughout the PI parenchyma, mainly located near the limit of PI with PN, and disposed in groups or isolated. These cells were observed in a basal position of the follicular structures without contacting the lumen. They were round, oval or elongated in shape, presented abundant cytoplasm and a round or oval nucleus with an evident nucleolus. The percentage of immunopositive area, the number and the major cellular diameter of these cells decreased significantly ($p < 0.01$, $p < 0.001$ and $p < 0.05$, respectively) in winter in relation to summer (table 2, fig. 2). No seasonal variation was observed in the nuclear diameter.

The PAS-positive follicular colloid showed seasonal variations, presenting minimal values of percentage of area, number and major colloidal diameter in winter. These parameters were maximal in summer. Some follicular structures presented immunostained colloid with anti-S-100 (table 3, fig. 3 and 4).

The FSC were distributed in all the PI parenchyma among the melanotrophs. These cells exhibited irregular nuclei, were stellate in shape with long cytoplasmic processes and originated follicles. The cytoplasmic processes were in contact with the melanotrophs and communicated follicles with each other or with the Rathke's pouch (fig. 4). The FSC presented cytoplasmic immunostaining for S-100, GFAP and vimentin proteins. However, the maximal values of percentage of immunopositive area were observed with anti-S-100. Immunostaining for S-100 protein was observed in the cytoplasm, in the cytoplasmic processes and occasionally in the nucleus. The

percentage of immunopositive area for S-100 and GFAP, and number of cells presented seasonal variations, being minimal in winter (table 4, fig. 4 and 5). No seasonal variation was observed in the percentage of immunopositive area with anti-vimentin (table 4, fig. 5).

Age-Related Study

The organization and morphology of pituitary PI of immature male viscachas were similar to those of adult males. The FSC of immature males did not present the typical stellate-like shape of adult animals, and only 1 or 2 cytoplasmic processes were observed. These cells exhibited immunostaining for the 3 tested antisera. The morphometric parameters of melanotrophs, follicular colloid and FSC (S-100-ir and GFAP-ir) were lower than in adult male viscachas (tables 2–4). On the contrary, the percentage of immunopositive area with anti-vimentin was maximal in immature males and decreased in relation to age (table 4, fig. 6).

Discussion

In several mammalian species, the photoperiod synchronizes the reproductive cycle, body weight, energetic metabolism and pelage moult [Lincoln, 2006]. The viscacha is a rodent of nocturnal habits whose physiology and behaviour vary during the year according to modifications in environmental signals such as photoperiod length, temperature, rainfall pattern, food composition and social interactions. The environmental signals determine the beginning or ending of the specific seasonal adapta-

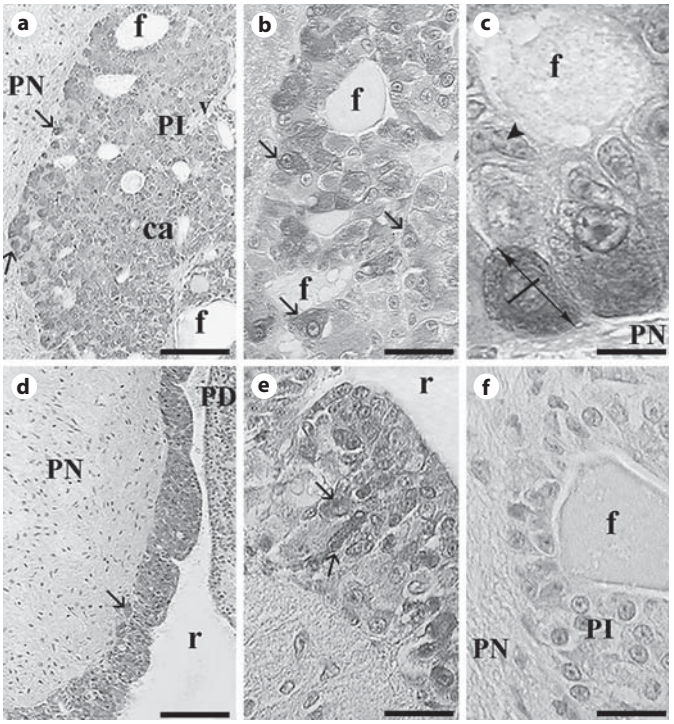

Fig. 2. Images of pituitary pars intermedia (PI) of adult male viscachas immunostained for α-MSH. **a–c** Animals captured in February (summer). The melanotrophs (arrows) are distributed throughout the PI parenchyma, especially near the limit with pars nervosa (PN). They are pleomorphic, round or oval in shape and are located in a basal position near the follicles (f) without contacting the lumen. Double-headed arrow = Major cellular diameter; bar = nuclear diameter; arrowhead = folliculostellate cell; v = blood vessel; ca = caudal zone. **d**, **e** A smaller number of melanotrophs (arrows) is observed in viscachas PI captured in July (winter). PD = Pars distalis; r = Rathke's pouch. **f** Negative control of immunoperoxidase staining. Scale bars = 100 μm (**a**, **d**); 25 μm (**b**, **e**, **f**); 10 μm (**c**).

Table 2. Seasonal and age-related morphometric study of pituitary pars intermedia melanotrophs

	Adult				Immature
	summer	autumn	winter	spring	
%IA-MSH	10.25 ± 0.90	7.13 ± 0.41[c]	5.17 ± 0.16[b]	7.35 ± 0.31[c]	1.08 ± 0.03[a, b]
MSH cells, n/RA	7.76 ± 0.21[a]	5.02 ± 0.20	3.82 ± 0.16[c]	5.35 ± 0.29[b]	0.92 ± 0.17[a]
MCD, μm	15.07 ± 0.49[c]	13.47 ± 0.02	12.59 ± 0.14	13.38 ± 0.27	11.69 ± 0.37[a, c]
ND, μm	7.12 ± 0.56	6.55 ± 0.34	6.83 ± 0.13	6.51 ± 0.04	6.50 ± 0.29

The values are expressed as mean ± SEM (n = 4).

%IA-MSH = Percentage of α-MSH-positive area; MSH cells, n/RA = number of melanotrophs per reference area; MCD = major cellular diameter; ND = nuclear diameter.

Significant differences were determined by analysis of variance followed by the Tukey-Kramer multiple comparison test.

%IA-MSH: [a] $p < 0.001$, immature vs. summer, autumn and spring; [b] $p < 0.01$, winter vs. summer and immature vs. winter; [c] $p < 0.05$, autumn vs. summer and spring vs. summer.

MSH cells, n/RA: [a] $p < 0.001$, summer vs. autumn and winter, and immature vs. summer, autumn, winter and spring; [b] $p < 0.01$, spring vs. summer; [c] $p < 0.05$, winter vs. autumn and spring.

MCD: [a] $p < 0.001$, immature vs. summer; [c] $p < 0.05$, summer vs. winter and spring, and immature vs. spring.

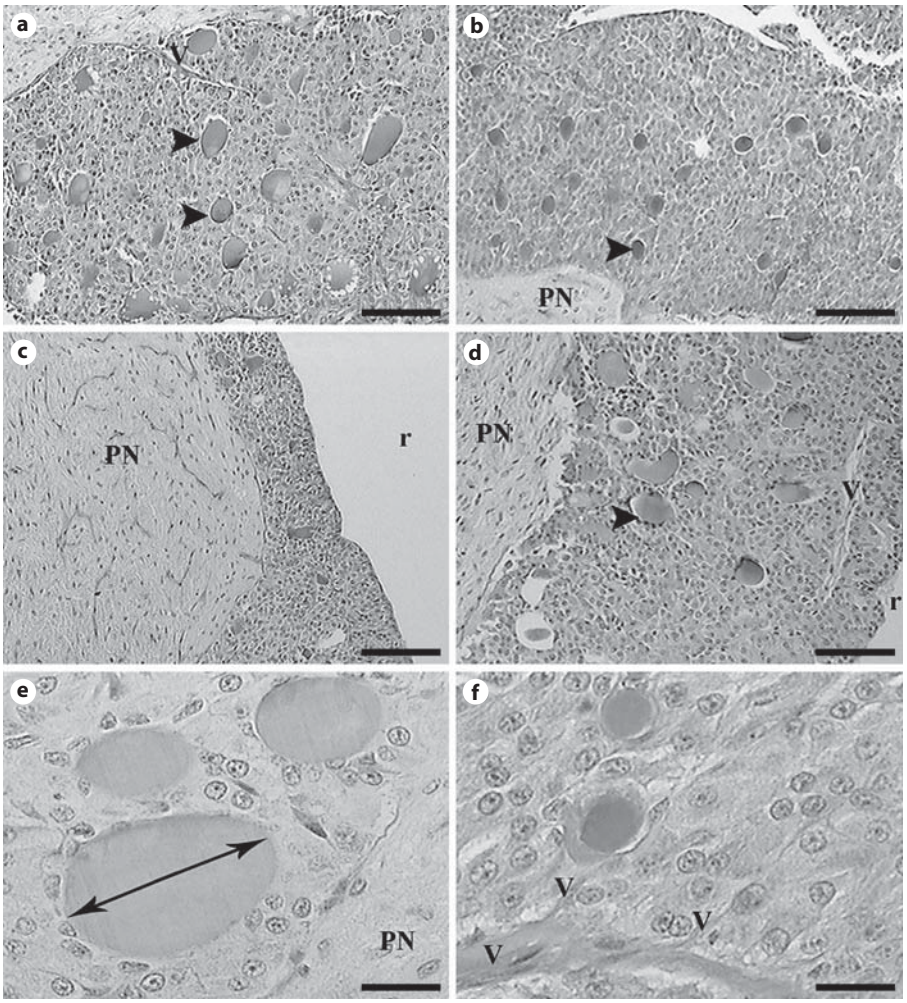

Fig. 3. PAS-positive follicular colloids (arrowheads) in pituitary pars intermedia (PI) of adult male viscachas captured in February (summer; **a**, **e**), April (autumn; **b**), July (winter; **c**, **f**) and October (spring; **d**). Long blood vessels (v) constitute the limit with pars nervosa (PN) and branch out in PI. Double-headed arrow = Major colloidal diameter; r = Rathke's pouch. Scale bars = 100 μm (**a–d**); 25 μm (**e**, **f**).

Table 3. Seasonal and age-related morphometric study of pituitary pars intermedia follicular colloid

	Adult				Immature
	summer	autumn	winter	spring	
%PAS	21.03 ± 0.37	13.02 ± 0.21[c]	9.54 ± 0.45[b, c]	16.64 ± 2.25	2.05 ± 0.60[a–c]
Col, n/RA	4.30 ± 0.26[a–c]	2.68 ± 0.14	1.98 ± 0.15[c]	3.14 ± 0.08	1.86 ± 0.05[c]
Col-D, μm	39.87 ± 0.49	36.30 ± 0.88	30.41 ± 1.10[b]	37.20 ± 0.18	21.91 ± 0.18[a, b]

The values are expressed as mean ± SEM (n = 4).

%PAS = Percentage of PAS-positive colloidal area; Col, n/RA = number of follicles per reference area; Col-D = major colloidal diameter.

Significant differences were determined by analysis of variance followed by the Tukey-Kramer multiple comparison test.

%PAS: [a] $p < 0.001$, immature vs. summer; [b] $p < 0.01$, winter vs. summer, immature vs. autumn and spring; [c] $p < 0.05$, autumn vs. summer, winter vs. spring and immature vs. winter.

Col, n/RA: [a] $p < 0.001$, summer vs. winter and immature; [b] $p < 0.01$, summer vs. autumn; [c] $p < 0.05$, summer vs. spring, winter vs. spring and immature vs. spring.

Col-D: [a] $p < 0.001$, immature vs. summer, autumn and spring; [b] $p < 0.01$, winter vs. summer, autumn and spring, and immature vs. winter.

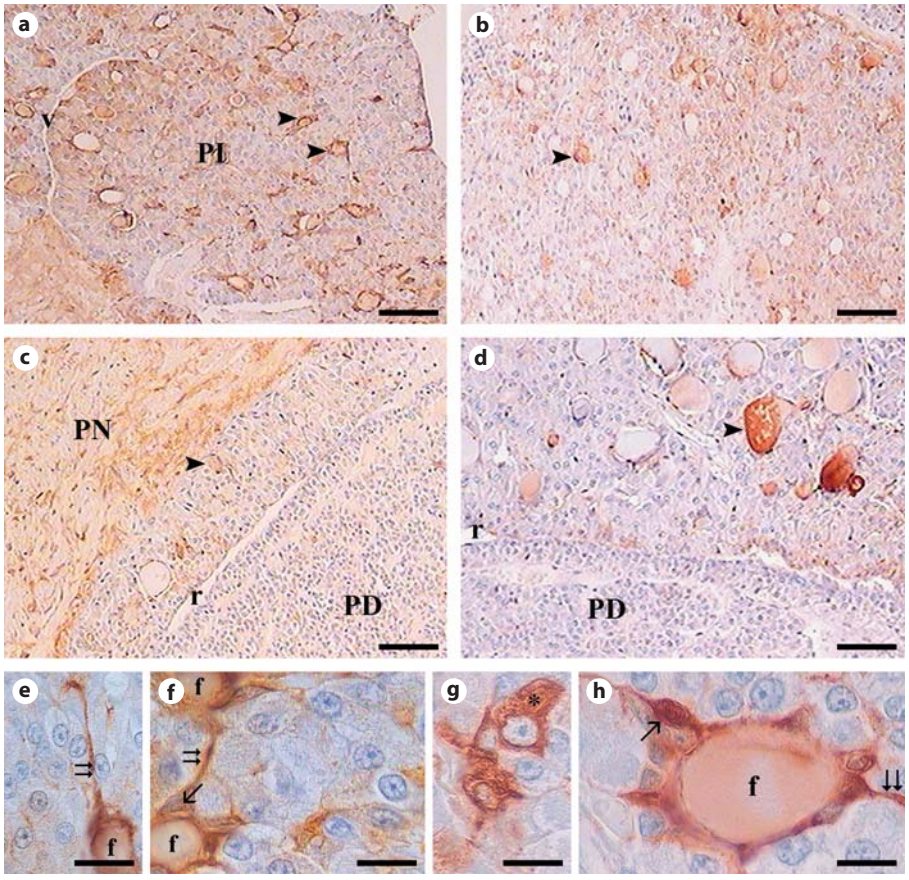

Fig. 4. Folliculostellate cells of adult male viscachas captured in February (summer; **a**, **f–h**), April (autumn; **b**, **e**), July (winter; **c**) and October (spring; **d**) immunostained with anti-S-100. These cells are distributed in all the pars intermedia (PI) parenchyma, and originate follicles (f). Some cells exhibit nuclear and cytoplasmic immunostaining (arrows) and others only cytoplasmic immunostaining (asterisk) for S-100. The folliculostellate cells are stellate in shape and show irregular nuclei. They exhibit long cytoplasmic processes (double arrows), which are in contact with the melanotrophs and communicate follicles among themselves (**e**, **f**). Various follicles present immunostained colloid with anti-S-100 (arrowheads). PN = Pars nervosa; r = Rathke's pouch; PD = pars distalis; v = blood vessels. Scale bars = 100 μm (**a–d**); 25 μm (**e**); 10 μm (**f–h**).

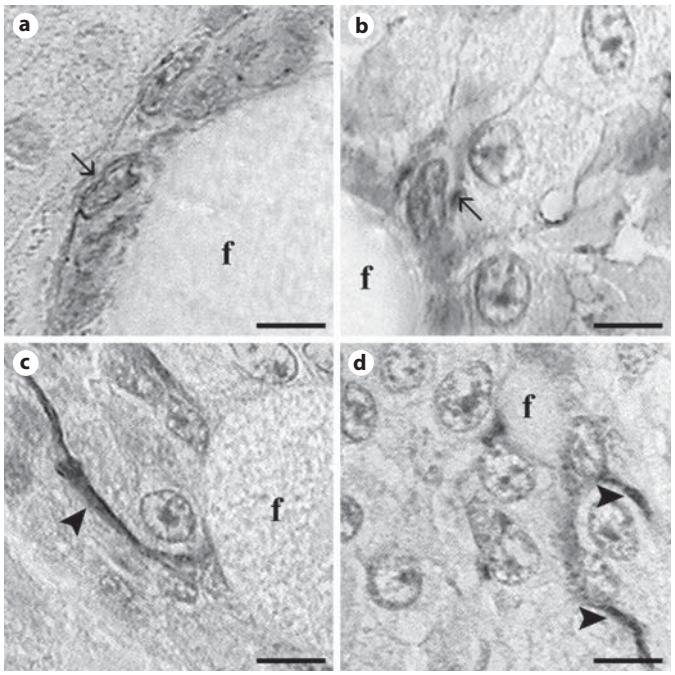

Fig. 5. Images of folliculostellate cells originating follicles (f) of adult male viscachas captured in February (summer; a, c) and July (winter; b, d) immunostained with anti-GFAP (**a**, **b**) and anti-vimentin (**c**, **d**). The immunostaining for GFAP is observed in the cytoplasm (arrows) around the nucleus of the FSC. The immunostaining for vimentin is mainly observed in the cytoplasmic processes (arrowheads) of the FSC. Scale bars = 10 μm.

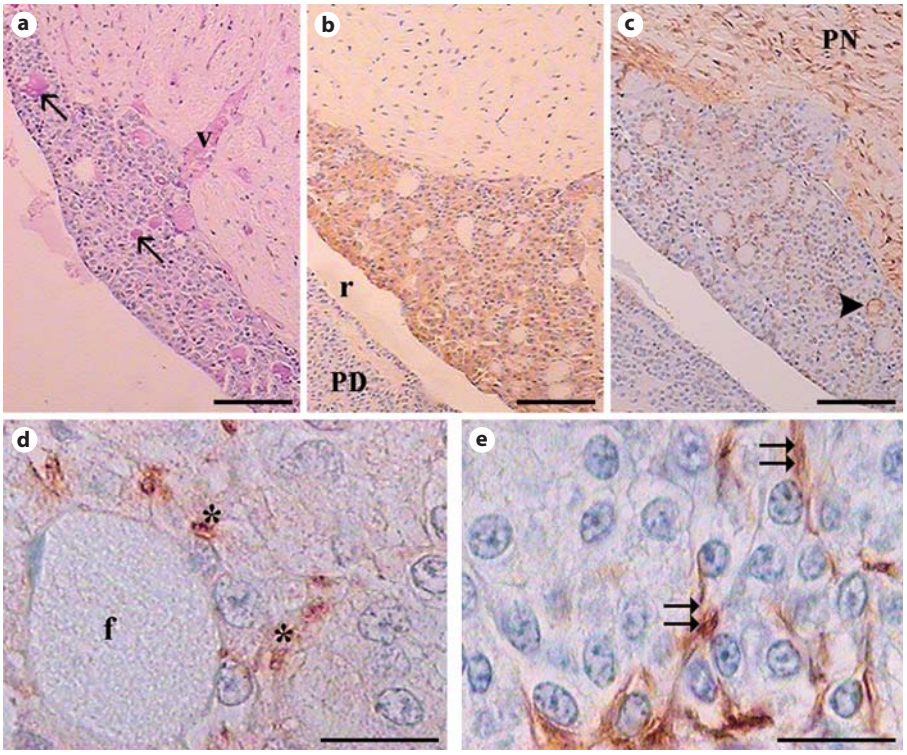

Fig. 6. Pituitary pars intermedia (PI) of immature male viscachas with PAS technique (**a**) and immunostained with anti-α-MSH (**b**), anti-S-100 (**c**), anti-GFAP (**d**) and anti-vimentin (**e**). Arrows = PAS-positive follicular colloid; arrowhead = follicle immunostained with anti-S-100; asterisks = the immunostaining for GFAP is observed in the cytoplasm of the FSC; double arrows = cytoplasmic processes of FSC immunolabelled with anti-vimentin; PD = pars distalis; PN = pars nervosa; v = blood vessels; r = Rathke's pouch; f = follicle. Scale bars = 100 μm (**a–c**); 10 μm (**d**, **e**).

Table 4. Seasonal and age-related morphometric study of folliculostellate cells of pituitary pars intermedia

Antisera	Parameters	Adult				Immature
		summer	autumn	winter	spring	
S-100	%IA-S-100	6.22 ± 0.15^{a}	4.22 ± 0.05	2.95 ± 0.09^{a}	3.20 ± 0.02^{b}	1.39 ± 0.13^{a}
	FSC, n/RA	4.11 ± 0.30	3.84 ± 0.29	2.48 ± 0.40^{c}	3.07 ± 0.26	$2.10 \pm 0.13^{b, c}$
GFAP	%IA-GFAP	1.66 ± 0.08^{a}	0.24 ± 0.03	0.18 ± 0.03^{c}	0.40 ± 0.02	0.12 ± 0.02^{b}
Vimentin	%IA-vim	0.25 ± 0.02	0.31 ± 0.02	0.29 ± 0.08	0.39 ± 0.05	$0.82 \pm 0.06^{a, b}$

The values are expressed as mean ± SEM (n = 4).

%IA-S-100 = Percentage of S-100-positive area; FSC, n/RA = number of folliculostellate cells per reference area; %IA-GFAP = percentage of GFAP-positive area; %IA-vim = percentage of vimentin-positive area.

Significant differences were determined by analysis of variance followed by the Tukey-Kramer multiple comparison test.

%IA-S-100: [a] $p < 0.001$ = summer vs. autumn, winter, spring and immature, winter vs. autumn, and immature vs. autumn, winter and spring; [b] $p < 0.01$, spring vs. autumn.

FSC, n/RA: [b] $p < 0.01$, immature vs. summer; [c] $p < 0.05$, winter vs. summer and immature vs. autumn.

%IA-GFAP: [a] $p < 0.001$, summer vs. autumn, winter, spring and immature; [b] $p < 0.01$, immature vs. spring; [c] $p < 0.05$, winter vs. spring.

%IA-vim: [a] $p < 0.001$, immature vs. summer and winter; [b] $p < 0.01$, immature vs. autumn and spring.

tions to maintain a positive energetic balance. In this way, the viscacha can adapt physiologically to the climate seasonal variations, performing the necessary endocrine adjustments to survive and to increase reproductive success [Filippa and Mohamed, 2006a]. In viscacha, the pineal gland modulates the behavioural responses through melatonin according to the photoperiod seasonal variations. The pinealocites cellular activity and serum values of melatonin were maximal in winter [Dominguez et al., 1987; Cernuda-Cernuda et al., 2003; Fuentes et al., 2003].

Variations in the constitution of the pituitary PI have been reported for different species [Perry et al., 1981; Ben-Jonathan et al., 1991; Takeuchi, 2001]. In the higher primates, this pituitary zone presents its maximal development in the foetal period, disappears after birth and is not identified in the adults [Osamura and Watanabe, 1985]. On the contrary, species living in arid environments exhibit a well-developed pituitary PI, which might be related to the role of the α-MSH in the osmoregulation [Shenker et al., 1985]. Lincoln and Baker [1995] have reported a seasonal decrease of α-MSH concentrations in Soay sheep in winter, and Lebaili et al. [1999] have shown a marked hypersecretion of melanotrophs in hydrated gerbils. Salt loading in mice also decreased the synthesis and secretion of α-MSH [Elkabes and Loh, 1988]. In the Siberian hamster the pituitary content of this hormone varied seasonally, being higher in the long days of spring and summer than in the short days of autumn and winter [Logan and Weatherhead, 1980].

Our results in adult male viscacha have shown a well-developed pituitary PI formed by melanotrophs and FSC and with scarce blood vessels. The FSC originated the follicular structures with PAS-positive colloid in their lumen. The melanotrophs showed cellular activity variations in relation to season and age. In addition, the main location of melanotrophs near the limit with PN suggests the hormone release towards blood vessels of this zone. The greatest development of PI in the adult animals in relation to the immature ones might be related to the adults' adaptation to the semiarid environment.

There is little information about the follicular colloid of PI in the different studied species. However, variations in the follicular colloid of pituitary PD have been reported in relation to the reproductive cycle, age and sex in pigs [Ogawa et al., 1996], guinea pigs [Kameda, 1990, 1991], bats [Anthony and Gustafson, 1984] and viscachas [Mohamed et al., 2000]. Scardapane et al. [1983] have demonstrated a decrease in number, diameter and area of follicles in viscacha PI with chronic melatonin administration.

In this work, seasonal and age-related morphometric variations of the PI follicular colloid in male viscachas were described, which exhibited minimal values in the adult animals captured in winter and in the immature ones. The follicles constituted storage structures for different products. The follicular colloid of PI showed a variation similar to that previously described in viscacha PD [Mohamed et al., 2000]. The decrease in the morphometric parameters of melanotrophs and the follicular colloid observed in winter was probably due to the effect of the high circulating levels of melatonin. In addition, our seasonal results were similar to those found in previous reports on the effect of chronic melatonin administration on the viscacha PI colloid [Scardapane et al., 1983]. These findings reinforce the hypothesis about the photoperiod and melatonin effect on this pituitary zone.

In different mammalian species, the FSC of pituitary PI were immunostained with anti-S-100, anti-GFAP and/or anti-vimentin [Stoeckel et al., 1981; Sands and Chronwall, 1996; Méndez et al., 1998; Lebaili et al., 1999; Cardin et al., 2000; Marettová and Maretta, 2004]. The typical expression of S-100 proteins [Nakajima et al., 1980] and GFAP [Velasco et al., 1982] in the FSC suggested that they have a neuroectodermic origin [Marin et al., 1989; Renner et al., 1998]. Vimentin expression is typical of the mouse embryo neural precursors [Duprey and Paulin, 1995]. GFAP expression increases after birth with the gradual disappearance of vimentin in most of the astroglial cells [Tardy et al., 1988]. Gary et al. [1995] have demonstrated morphological changes in FSC of the rat pituitary PI during early postnatal development and a subsequent shift in protein expression from vimentin to GFAP.

The FSC of viscacha pituitary PI presented morphological characteristics similar to those described for other vertebrate species. They expressed S-100, GFAP and vimentin, considered as glial, astrocyte and immature glial cell markers, respectively, suggesting that the origin of these cells is neuroectodermic. They also showed different immunostaining for S-100 in relation to season and age. It has been reported that S-100 proteins act inside the cells as calcium-sensor proteins implicated in the calcium signal transduction and, outside the cells, as ligands for specific surface receptors in various cellular types [Donato, 2001]. In our study, the intracellular (nuclear and cytoplasmic) and extracellular (follicular colloid) immunolabelling for S-100 might indicate different functions of this protein according to its localization. In addition, the seasonal variations of S-100 and GFAP in viscacha suggest a close relation between these proteins. The S-100 proteins were involved in the GFAP polymerization [Bianchi et al., 1993]. Our findings are probably related to remodelling processes of the cytoskeleton of FSC, which modify their shape according to the follicular structure size. On the other hand, we reported a decrease in the percentage of vimentin-positive area and an increase in the S-100 and GFAP expression of the FSC in relation to age. Vimentin expression in adult male viscachas suggests the presence of a small reserve population of these cells in an immature state. It has been reported that hormone synthesis and release by melanotrophs in different species are regulated by

several cytokines produced by FSC in the PI [Takeuchi, 2001]. In viscacha, the long cytoplasmic processes of FSC might originate an intercellular communication network inside the pituitary PI. Future studies will be necessary to determine a paracrine regulation of these cells on the melanotrophs in this rodent.

Previous observations in pigs and guinea pigs have suggested the existence of a relation between the follicular colloid, the FSC and the pituitary hormone-secretory cells [Kameda, 1991; Ogawa et al., 1997]. In the viscacha pituitary PI a close association of melanotrophs and FSC was observed. The decrease in the morphometric parameters of melanotrophs, follicular colloid and FSC in winter suggests a low endocrine activity in this zone. This fact might be due to the effect of the short photoperiod and the maximal serum melatonin levels. However, the influence of temperature, hydric and dietary restrictions cannot be discarded.

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