Morphological and Morphometric Changes of Pituitary Lactotrophs of Viscacha (*Lagostomus maximus maximus*) in Relation to Reproductive Cycle, Age, and Sex

VERÓNICA FILIPPA[1] **AND FABIAN MOHAMED**[2*]

[1]Fellow of the Research Career, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

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

ABSTRACT

Lactotrophs in pituitary pars distalis (PD) of viscacha were studied by immunohistochemistry and morphometric analysis in the following groups: 1) adult males throughout the reproductive cycle (reproductive, gonadal regression, and recovery periods), 2) melatonin-treated adults, 3) castrated adults, 4) prepubertal, 5) non-pregnant females, and 6) pregnant females (early, mid, and late pregnancy). Immunopositive percentage area (%IA), cell percentage in PD (% PDC), number of cells per reference area (no.cell/RA), major cellular and nuclear diameters were analyzed. Lactotrophs were mainly localized in the ventro–medial region and the caudal extreme of PD. In the male viscachas, they were isolated in small and big groups, close to blood vessels and near follicles. These cells were pleomorphic and with a heterogeneous cytoplasmic immunolabeling pattern. In the adult males of the gonadal regression period the morphometric parameters were the lowest. Most parameters of lactotrophs in the prepubertal were significantly lower than in the adult males in the reproductive period. In the melatonin-treated animals and in castrated animals there was a decrease in %IA, %PDC, and no.cell/RA. In the females, the morphometric parameters increased at the end of pregnancy. Non-pregnant females exhibited a higher immunopositive area and number, but a smaller size of cells than males. Our results showed that in the adult male viscacha, lactotrophs vary seasonally, probably due to the photoperiod effect through melatonin. Besides the changes observed after castration, in prepubertal animals, in adults of different sex, and during pregnancy suggest that the gonadal steroid hormones might modify the lactotrophs activity. Anat Rec, 293:150–161, 2010.

Key words: *Lagostomus*; pituitary gland; lactotrophs; reproductive cycle; melatonin; age; sex

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*Correspondence to: Fabian Mohamed, Cátedra de Histología y Embriología- Área de Morfología, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Av. Ejército de los Andes 950-2° Piso (5700) San Luis, Argentina. Fax: 54-2652-422644/430224. E-mail: fhmo@unsl.edu.ar

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The pituitary pars distalis (PD) prolactin-producing cells (PRL cells, lactotrophs or mammatrophs) have been widely studied. The morphology, distribution, and ultrastructure of these cells have been described in the pituitary of several mammals, such as rat (Nakane, 1970; Tougard and Tixier-Vidal, 1994), mouse (Baker and Gross, 1978), bat (Mikami et al., 1988), sheep (Mikami and Daimon, 1968), deer (Schulte et al., 1980), equine (Rahmanian et al., 1997), and humans (Halmi et al., 1975; Baker and Yu, 1977). According to the electronic characteristics of the cytoplasm secretory granules, different types of lactotrophs were identified in rat (Nogami, 1984; De Paul et al., 1997), mouse (Iwama and Sasaki, 1989), and equine (Rahmanian et al., 1997).

In some rodents of seasonal reproduction, a decrease in the immunopositive area, number, and size of the lactotrophs has been observed in the non-reproductive period or in short artificial photoperiods (Kuwahara et al., 2000; Cónsole et al., 2002). In addition, it has been reported in different species that the secretion of prolactin (PRL) is correlated with the length of day, independently of the seasonal reproduction pattern. The PRL serum levels were high in the reproductive season of equine (Thompson et al., 1986), deer (Delgadillo et al., 1993; Bubenik et al., 1996), hamster (Lerchl et al., 1993; Cónsole et al., 2002), and other photoperiodic rodents (Blank and Desjardins, 1986; Smale et al., 1988). Several mammals adapt their physiology to environmental seasonal changes through the pineal melatonin secretion. Fernández-Alvarez et al. (2000) reported that the melatonin inhibitory effect upon the pituitary hormones is mainly exerted at the level of the secretory processes.

There is no agreement in the literature in relation to the effect of castration upon this pituitary population. Some researchers have not found variations in the pituitary PRL after castration in mouse (Sinha et al., 1979), whereas others have observed cellular degranulation in rat (Purves and Griesbach, 1952) or decrease in the number of lactotrophs in horse (Tortonese et al., 2001).

In golden hamster (Taniguchi et al., 1989) and Japanese black steers (Sato et al., 1999) a decrease in the proportion of PD lactotrophs in relation to age has been reported.

In rat, an increase in the liberation of PRL and in the number of lactotrophs has been observed during pregnancy, with the proportion of these cells being higher in females during lactation (Porter et al., 1990). Several studies have reported differences in the proportion of lactotrophs in relation to sex (Sasaki and Iwama, 1988; Gonzalez-Parra et al., 1996). In most of the studied species, such as humans (Baker and Yu, 1977) and rats (Nogami, 1984), the amount of lactotrophs was higher in females than in males.

The viscacha (*Lagostomus maximus maximus*) is a seasonal reproduction rodent of nocturnal habits. The environmental photoperiod synchronizes the annual reproductive cycle of male adults through the pineal gland and its main hormone, melatonin. This cycle presents three well-defined periods: reproductive (summer-early autumn, long photoperiod), gonadal regression (winter-short photoperiod), and gonadal recovery (spring). Minimal activity of the pineal gland and low melatonin serum levels have been observed during the reproductive period (Dominguez et al., 1987; Pelzer et al., 1999; Fuentes et al., 2003). In pituitary PD, a higher number of PAS-positive follicular colloids and an increase in the morphometric parameters of gonadotrophs and somatotrophs have been reported (Mohamed et al., 2000; Filippa et al., 2005; Filippa and Mohamed, 2006b). In testis, maximum spermatogenic activity, abundant Sertoli cells, hypertrophic Leydig cells, and a high concentration of testicular receptors for LH (luteinizing hormone), FSH (follicle stimulating hormone), and PRL have been observed (Fuentes et al., 1991, 1993, 2003; Muñoz et al., 1997, 2001). The epididymis also exhibited morphological characteristics of higher activity in this period (Fuentes et al., 1991; Aguilera-Merlo et al., 2005). In the gonadal regression period, lower numbers of follicular structures, LH cells, and somatotrophs were observed in the pituitary gland. The testis exhibited hypotrophic Leydig cells and a lower number of spermatides, mature spermatozoids, and Sertoli cells. In epididymis, a decrease in its diameter and in the number of cells were observed. Moreover, minimal serum levels of testosterone and maximal serum levels of melatonin were determined. In the gonadal recovery period, gradual modifications were described due to the reinitiating testicular activity. In prepubertal viscachas, variations of the morphometric parameters of pituitary somatotrophs and corticotrophs have been related to the gonadal development and activity (Filippa and Mohamed, 2006a,b). In female viscacha, some characteristics of reproductive organs, gestation, and pregnancy have been studied (Weir and Rowlands, 1974; Gil et al., 2005). The viscacha presents a long pregnancy (145–166 days; Mossman and Duke, 1973), during which three stages have been described: early, mid, and late pregnancy (Gil, 2005; Jensen et al., 2008).

The purpose of this work was to perform an immunohistochemical and morphometric study of PD lactotrophs in adult male viscachas throughout their reproductive period, after melatonin administration and castration. In addition, lactotrophs were studied in relation to age, sex, and pregnancy. The analyzed morphometric parameters, immunopositive percentage area, PD cells percentage, number of cells per reference area (no.cells/RA), and major cellular and nuclear diameters were considered as indicators of cellular activity (Takahashi, 1991; Torres et al., 1995; Vidal et al., 1995; Filippa et al., 2005; Filippa and Mohamed, 2006a,b, 2008).

MATERIALS AND METHODS

Experimental Animals

The viscachas were captured in their habitat near San Luis, Argentina (33° 20′ south latitude, 760 m altitude) during 2004–2005, using traps placed in their burrows. In San Luis, in summer, the light phase is upto 14 hr light daily (14L:10D) with an average temperature of 25°C. In winter, the light phase decreases to 10 hr (10L:14D), and the average temperature is 10°C. In spring, the light phase increases to 12 hr (12L:12D), and the average temperature is 15°C.

Twelve adult male viscachas weighing 5–7 Kg were captured during the most representative months of the reproductive cycle: four animals during the reproductive period in summer–early autumn (February to April); four animals in the gonadal regression period in winter (July), and four animals in the gonadal recovery period in spring (September to October). Four male animals

with body weight lower than 5 Kg were captured during spring and carefully classified as prepubertal (sexually immature) according to body weight (3–4 Kg) and light microscopy observations of testis (Llanos and Crespo, 1954; Branch et al., 1993; Mohamed et al., 2000; Filippa and Mohamed, 2006b). Four non-pregnant female animals with adult ovary histology captured in summer were used. The pregnant animals were classified on the basis of the number and size of embryos or foetuses into: 1-early pregnancy (four animals), captured in summer and early autumn, with two or more embryos from 1 to 3 cm; 2-mid pregnancy (four animals), captured in winter, with two foetuses from 9 to 11 cm; 3-late pregnancy (four animals), captured in late winter and early spring, with two foetuses measuring more than 19 cm (Gil, 2005; Jensen et al., 2008).

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 hemipituitary was completely cut, following the same design used in a previously reported work (Filippa and Mohamed, 2006b). Immunostaining was analyzed at low magnification (X 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, USA) for the use of experimental animals.

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 hr, for 9 weeks. The control group received only the diluent. 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). The experimental design was similar to that reported in previous works (Filippa et al., 2005; Filippa and Mohamed, 2006a,b, 2008).

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. The experimental design was similar to that reported in previous works (Filippa and Mohamed, 2006b, 2008).

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 for 60 min in a humidified chamber at 4°C with the mouse monoclonal antibody against pituitary PRL (DakoCytomation, Carpinteria, CA, USA). After rinsing with PBS for 10 min, 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-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 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. The immunohistochemical procedure was similar to that reported in previous works (Filippa et al., 2005; Filippa and Mohamed, 2006a,b, 2008).

Morphometric Analysis

A computer-assisted image analysis system was used to measure the percentage of immunopositive area, percentage of immunoreactive cells in PD, the number of lactotrophs per RA, and the major cellular (MCD) and nuclear diameters (ND). The system consisted of an Olympus BX-40 binocular microscope (magnification 200X), 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 (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). Thus, 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:

1. Percentage of immunopositive area (% IA) of lactotrophs was calculated using the formula % IA = $\sum$Ac/$\sum$RA × 100, where $\sum$Ac was the sum of the area of immunolabeled cells, and $\sum$RA 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, 2008).
2. 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).
3. The number of immunostained cells (no. cell/RA) with a visible nucleus was counted in 10 microscopic fields per section. The result was expressed as no. cells/RA (Miranda et al., 1996).
4. MCD and ND were measured using the length tool of the Image Pro Plus 5.0 software on each lactotroph with a visible nucleus. These parameters were measured for 40 immunoreactive lactotrophs per pituitary gland.

Statistical Analysis

The results were expressed as means ± standard error of the mean (SEM) for all data sets. The data 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

Adult Male Viscachas: Reproductive Cycle

In the adult male viscachas, the lactotrophs were mainly localized in the ventro–medial region and the caudal extreme of PD during the reproductive annual cycle (Fig. 1A,E,H). In the cephalic extreme, long blood vessels were observed delimiting a region with intensely marked cells in the ventral region.

During the three periods of the reproductive cycle, these cells were found to be covering the blood vessel wall and extending the cellular surface in contact with them. In addition, they were next to the colloidal lumen of follicular structures, which occasionally exhibited immunolabeling (Fig. 1C). The lactotrophs were distributed in big groups (from 10 to 15 cells) or small groups (five or six cells) and some of them were isolated. Some groups included regularly shaped, mainly oval lactotrophs and other groups consisted of cells with cytoplasmic prolongations in contact (Fig. 1B,I). The latter groups were more frequent in the reproductive and gonadal recovery periods, whereas in the gonadal regression period cells were frequently found isolated or forming small groups (Fig. 1F,G).

The lactotrophs constituted a highly pleomorphic population. They were oval, round, pyramidal, or cup-like in shape, with an oval or spherical nucleus in eccentrical position. The cytoplasmic immunolabeling pattern was heterogeneous. Most cells presented fully labeled cytoplasms, whereas in other cells immunolabeling was limited to a region, an extreme or the cytoplasm periphery (Fig. 1B,D). Some exhibited cytoplasmic prolongations reaching blood vessels or surrounding non-labeled cells (Fig. 1I,J).

In the gonadal regression period, the % IA, % PDC and no.cell/RA decreased significantly ($p < 0.001$; $p < 0.01$; $p < 0.001$, respectively) in relation to the reproductive period values. These parameters increased significantly ($p < 0.001$; $p < 0.01$; $p < 0.001$, respectively) in the recovery period. No significant changes ($p > 0.05$) were observed in the cellular diameters (MCD and ND) throughout the reproductive cycle (Table 1).

Prepubertal Male Viscachas

The localization, distribution, shape, and immunostaining pattern of lactotrophs in prepubertal male animals were similar to those observed in adult males. However, in the prepubertal animals, isolated cells or cells forming small groups predominated (Fig. 2). The parameters % IA, no. cell/RA, MCD, and ND were significantly lower than in adults in the reproductive period and in the gonadal recovery period ($p < 0.05$ for all parameters). In relation to adult males in the gonadal regression period, no significant differences were observed ($p > 0.05$) for % IA, but the % PDC and the no. cell/RA were significantly higher ($p < 0.01$ and $p < 0.05$, respectively), and the MCD and ND were significantly lower ($p < 0.05$, Table 1).

Melatonin Administration

In the melatonin-treated animals isolated cells predominated and cells forming big groups were occasionally present. These cells were oval, spherical, and cup-like in shape, but cells with cytoplasmic prolongations were scarce. Cellular immunostaining was less intense and homogeneous, and immunostaining of the colloidal material of the follicular structures was rarely observed (Fig. 3). The % IA, % PDC, and no. cell/RA parameters decreased significantly ($p < 0.01$; $p < 0.05$; $p < 0.05$, respectively) in relation to the control group. No significant changes were observed in the MCD and ND, $p > 0.05$ (Table 2).

Castration

In castrated animals, lactotrophs were localized in a lower proportion in the ventro–medial region and in the caudal extreme. They were isolated or forming small groups. They were pleomorphic, and very few cells presented cytoplasmic prolongations. The cytoplasmic immunostaining pattern was more homogeneous than in intact animals (Fig. 4). The % AI, % CPD and no. cell/AR were significantly lower ($p < 0.001$; $p < 0.01$; $p < 0.001$, respectively) than in intact animals. No significant differences ($p > 0.05$) were observed in the values of DCM and DN between the groups (Table 3).

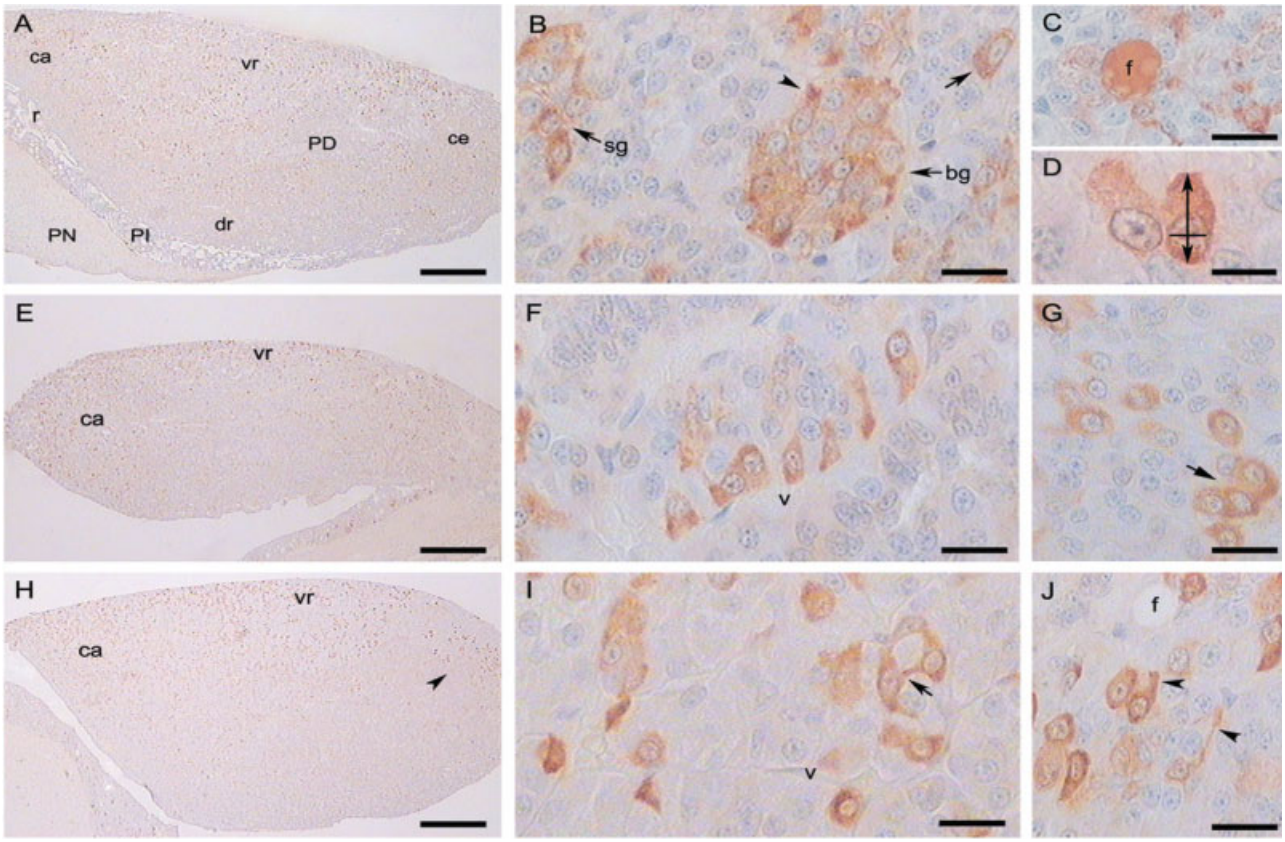

Fig. 1. **A–D:** Pituitary gland of adult male viscacha captured in February (reproductive period, summer). A: Lactotrophs localized in the caudal extreme (ca) and the ventro–medial region of pars distalis (PD). B: Isolated cells (arrow), forming big groups (arrow-bg) or small groups (arrow-sg) and cells with heterogeneous immunostained cytoplasms (arrowhead). C: An immunolabeled colloidal lumen of follicular structure (f). D: Oval lactotrophs with nuclei in eccentrical position, (↔): Major cellular diameter; (–): Nuclear diameter. **E–G:** Pituitary gland of adult male viscacha captured in July (gonadal regression period, winter). A smaller number of lactotrophs are present in PD. The pleomorphic lactotrophs are in contact with blood vessels (v) and forming a small group (arrow). **H–J:** Pituitary gland of adult male viscacha captured in September (gonadal recovery period, spring). H: Intensely marked cells (arrowhead) located in the cephalic extreme. I: A group of cells with cytoplasmic prolongations in contact (arrow). J: Lactotroph in contact with a colloidal lumen of follicular structure (f) and other pleomorphic cells with cytoplasmic prolongations (arrowheads). PN, pars nervosa; PI, pars intermedia; r, Rathke's pouch; ce, cephalic extreme; dr, dorsal region; vr, ventral region. A, E, and H, scale bar = 500 μm; B, C, F, G, I, and J, scale bar = 25 μm; D, scale bar = 10 μm.

TABLE 1. Morphometric parameters of lactotrophs in adult male throughout the reproductive cycle and in prepubertal male viscachas

	Adults			
	Rep. P.	Reg. P.	Rec. P.	Prepubertal
% IA	2.62 ± 0.16	1.23 ± 0.13[a]	2.81 ± 0.23	1.73 ± 0.19[d]
% PDC	2.82 ± 0.25	1.59 ± 0.09[b]	2.86 ± 0.25	2.27 ± 0.21[b]
no. cell/RA	16.18 ± 1.09	6.69 ± 0.73[a]	17.66 ± 1.36	11.78 ± 1.15[c]
MCD (μm)	12.15 ± 0.28	11.80 ± 0.16	11.90 ± 0.24	10.73 ± 0.30[c]
ND (μm)	6.06 ± 0.06	5.95 ± 0.07	5.88 ± 0.08	5.55 ± 0.06[c]

The values are expressed as mean ± SEM ($n = 4$).
% IA, immunopositive percentage area; % PDC, percentage of immunoreactive cells in pars distalis; no. cell/RA, number of cells per reference area; MCD, major cellular diameter; ND nuclear diameter; Rep. P., reproductive period; Reg. P., gonadal regression period; Rec. P., gonadal recovery period.
Significant differences were determined by analysis of variance followed by the Tukey-Kramer multiple comparison test.
[a] $p < 0.001$: Reg. P. versus Rep. P., Reg. P. versus Rec. P.
[b] $p < 0.01$: Reg. P. versus Rep. P., Reg. P. versus Rec. P., Prepubertal versus Reg. P.
[c] $p < 0.05$: Prepubertal versus Rep. P., Prepubertal versus Reg. P., Prepubertal versus Rec. P.
[d] $p < 0.05$: Prepubertal versus Rep. P., Prepubertal versus Rec. P.

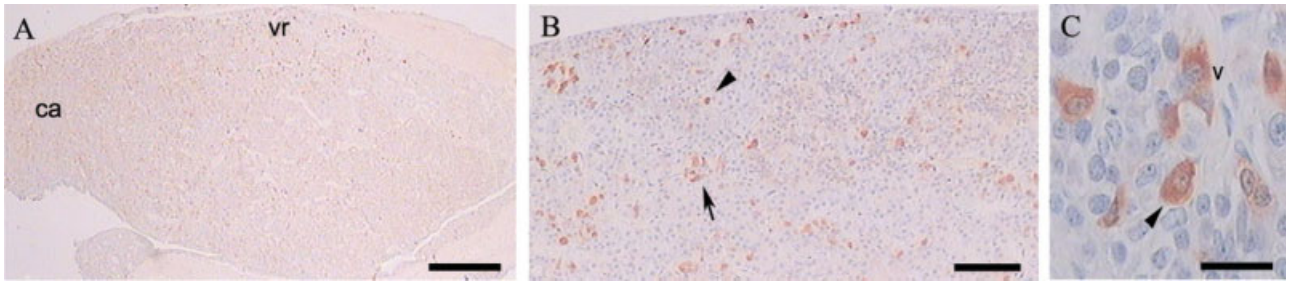

Fig. 2. **A–C:** Pituitary gland of prepubertal male viscacha captured in October (spring). Lactotrophs are localized in the caudal extreme (ca) and the ventro–medial region of PD. There are cells forming small groups (arrow) and some isolated (arrowheads) in contact with a blood vessel (v). vr, ventral region. A, scale bar = 500 μm; B, scale bar = 100 μm; C, scale bar = 25 μm.

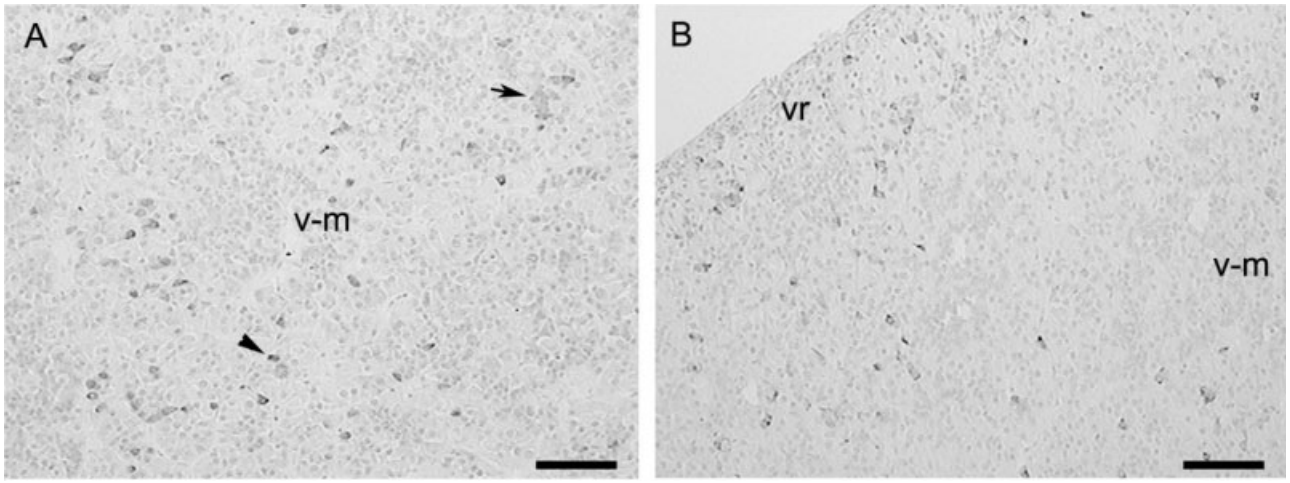

Fig. 3. **A:** Pituitary gland of adult male viscacha of control group. There are isolated (arrowhead) or grouped lactotrophs (arrow) in the ventro–medial region (v–m) of PD. **B:** Ventro–medial (v–m) and ventral regions (vr) of PD of melatonin-administered viscacha. The number of lactotrophs is smaller than in the control animal. A–B, scale bar = 100 μm.

Female Viscachas

In the non-pregnant females and in the early, mid, and late pregnant female viscachas, lactotrophs were distributed throughout the PD parenchyma. The cells localized in the cephalic extreme exhibited more intense immunostaining. These cells formed big and small groups and most of them were in contact with blood vessels and next to follicular structures, but the colloidal lumen rarely exhibited immunolabeling. This cellular population was pleomorphic, and most cells presented homogeneously immunostained cytoplasms. The heterogeneity of the immunostaining pattern was more evident in late pregnant females (Fig. 5). The % IA, % PDC and no. cell/RA parameters increased gradually and significantly ($p < 0.001$, $p < 0.01$, and $p < 0.01$, respectively) from early to late pregnancy. The MCD and ND did not show significant variations ($p > 0.05$) among the pregnant groups. Non-pregnant females presented lower % IA ($p < 0.05$) and MCD ($p < 0.05$) in relation to late pregnancy (Table 4).

TABLE 2. Morphometric parameters of lactotrophs of adult male viscachas after melatonin administration

	Control	Mel. Adm.
% IA	1.51 ± 0.05	0.95 ± 0.14[a]
% PDC	2.11 ± 0.09	1.49 ± 0.10[a]
no. cell/RA	9.67 ± 0.31	6.69 ± 0.82[b]
MCD (μm)	10.45 ± 0.65	10.46 ± 0.32
ND (μm)	5.83 ± 0.10	5.67 ± 0.15

The values are expressed as mean ± SEM ($n = 4$).
% IA, immunopositive percentage area; % PDC, percentage of immunoreactive cells in pars distalis; no. cell/RA, number of cells per reference area; MCD, major cellular diameter; ND, nuclear diameter; Mel. Adm., melatonin-administered animals.
Significant differences were determined by the Student's *t*-test.
[a] $p < 0.01$: Mel. Adm. versus Control.
[b] $p < 0.05$: Mel. Adm. versus Control.

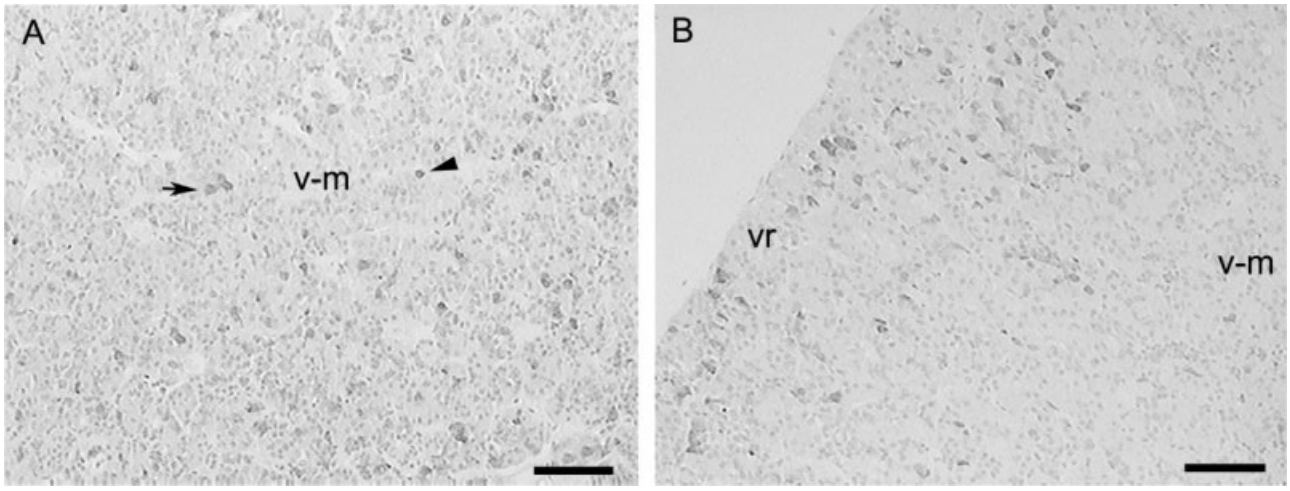

Fig. 4. **A:** Pituitary gland of adult male viscacha of intact group. There are isolated (arrowhead) or grouped lactotrophs (arrow) in the ventro–medial region (v–m) of PD. **B:** Pituitary gland of the castrated adult male viscacha. The number of lactotrophs is smaller than in the intact animal. vr, ventral region. A–B, scale bar = 100 μm.

TABLE 3. Morphometric parameters of lactotrophs of adult male after castration

	Intact	Castrated
% IA	2.15 ± 0.08	0.70 ± 0.05[a]
% PDC	2.27 ± 0.11	1.45 ± 0.04[b]
no. cell/RA	14.28 ± 0.47	5.05 ± 0.25[a]
MCD (μm)	11.20 ± 0.20	10.82 ± 0.28
ND (μm)	5.74 ± 0.02	5.65 ± 0.06

The values are expressed as mean ± SEM ($n = 4$).
% IA, immunopositive percentage area; % PDC, percentage of immunoreactive cells in pars distalis; no. cell/RA, number of cells per reference area; MCD, major cellular diameter; ND, nuclear diameter.
Significant differences were determined by the Student's *t*-test.
[a]$p < 0.001$: Castrated versus Intact.
[b]$p < 0.01$: Castrated versus Intact.

The non-pregnant females (Table 4) showed values of % IA, % PDC, and no. cell/RA significantly higher ($p < 0.001$) than adult males in the reproductive period (Table 1), but the values of MCD and ND were significantly lower ($p < 0.01$).

DISCUSSION

In this study, the population of lactotrophs in pituitary PD of viscacha was pleomorphic. The cells of the cephalic extreme exhibited a different intensity of cytoplasmic immunolabeling in relation to the cells of the caudal extreme and ventro–medial region. Additionally, some cells exhibited a different pattern of cytoplasmic immunolabeling. These results suggest that there might exist subpopulations of lactotrophs that respond to different stimuli or a subdivision of the lactotrophs in secreting cells and in other cells that are in a stand-by situation, both in a different state of reactivity or in a different stage of the secretory cycle.

Reported percentages of lactotrophs vary among species: 5–16% in equine (Rahmanian et al., 1997); 2.2% (Surks and DeFesi, 1977), 8% (Takahashi and Kawashima, 1982), 27% (Hara et al., 1998), and 49.80% (Dada et al., 1984) in rat, 29% in hamster (Wang et al., 1991), 16.4% and 22.9% in mouse during non-reproductive and reproductive periods, respectively (Kuwahara et al., 2000), 1.7–4.4% in humans (Fowler and McKeel, 1979), and 8.6–31.3% in males and nulliparous females (Asa et al., 1982; Melmed, 2002). In this study, the values obtained were between 2.82 and 5.07% in male adult viscachas in the reproductive period and late pregnant females, respectively. On the other hand, the lactotroph rates differences found between species match reports by Freeman et al. (2000) regarding the lack of homogeneity of these cells in their morphology, hormonal phenotype, and function.

Lactotrophs of adult male viscacha, as gonadotrophs (Filippa et al., 2005), somatotrophs (Filippa and Mohamed, 2006b), and tysotrophs (Filippa and Mohamed, 2008), have shown seasonal variations in response to environmental signals, mainly the photoperiod. These variations correlated with the changes described for the pineal–pituitary gonadal axis in the reproductive cycle of this rodent. The cellular activity was higher during the reproductive and gonadal recovery periods, with an increase in the immunopositive area and the number of cells. PRL, together with LH, FSH, and GH (growth hormone), might stimulate and maintain testicular spermatogenesis and steroidogenesis. On the other hand, lactotrophs exhibited the lowest cellular activity in the gonadal regression period (winter). The decrease of the immunopositive area and number of cells suggests a lower hormone amount stored in the PD lactotrophs. This might be due to a decrease in the hormonal synthesis in relation to the physiological condition of the gonadal regression period when testicular steroidogenesis and spermatogenesis decrease because of the short photoperiod effect and the high melatonin serum levels. In addition, the stress caused by adverse environmental conditions during winter, such as

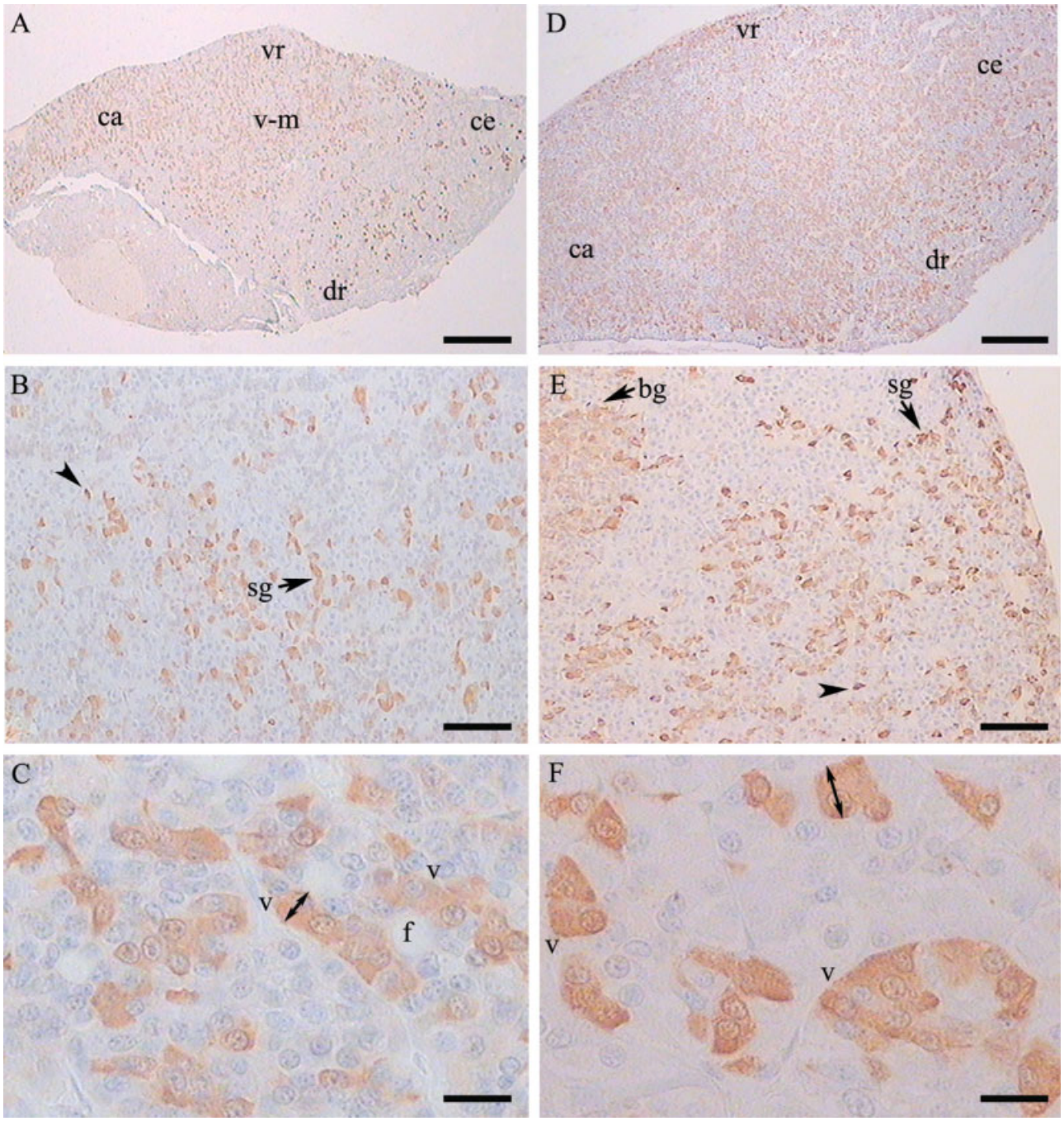

Fig. 5. **A–C:** Pituitary gland of non-pregnant female viscacha captured in February (summer). A: Lactotrophs are distributed throughout the PD parenchyma, mainly in the caudal extreme (ca) and the ventro–medial region (v–m). The cells localized in the cephalic extreme (ce) exhibit more intense immunostaining. dr, dorsal region; vr, ventral region. B,C: Isolated lactotrophs (arrowhead) and forming a small group (arrow-sg). There are cells along the blood vessels surface (v) and near follicle structures without immunolabeled colloidal lumen (f). **D–F:** Pituitary gland of late pregnant female viscacha captured in September (spring). D: Lactotrophs distributed throughout the PD parenchyma. E,F: Higher number of lactotrophs in pregnant as compared to non-pregnant female animals in the ventro–medial and ventral regions (vr) of PD. Lactotrophs are isolated (arrowhead), forming small (arrow-sg) and big groups (arrow-bg). There are a lot of cells in contact with blood vessels (v). (↔): Major cellular diameter. A, scale bar = 500 μm; D, scale bar = 250 μm; B and E, scale bar = 100 μm; C and F, scale bar = 25 μm.

TABLE 4. Morphometric parameters of lactotrophs in non-pregnant and pregnant female viscachas

	NP	Early P.	Mid P.	Late P.
% IA	4.75 ± 0.33^{c}	3.87 ± 0.24^{a}	4.86 ± 0.41^{c}	6.32 ± 0.16
% PDC	4.81 ± 0.18	4.03 ± 0.23^{b}	4.55 ± 0.20	5.07 ± 0.04
no. cell/RA	33.34 ± 2.60	27.20 ± 1.67^{b}	31.89 ± 2.89	38.63 ± 1.50
MCD (μm)	10.91 ± 0.05^{c}	11.38 ± 0.44	11.52 ± 0.12	12.32 ± 0.35
ND (μm)	5.49 ± 0.05	5.59 ± 0.07	5.67 ± 0.07	5.62 ± 0.14

The values are expressed as mean ± SEM ($n = 4$).
% IA, immunopositive percentage area; % PDC, percentage of immunoreactive cells in pars distalis; no. cell/RA, number of cells per reference area; MCD, major cellular diameter; ND, nuclear diameter; NP, non-pregnant female; Early P., early pregnant female; Mid P., mid pregnant female; Late P., late pregnant female.
Significant differences were determined by analysis of variance followed by the Tukey-Kramer multiple comparison test.
[a] $p < 0.001$: Early P. versus Late P.
[b] $p < 0.01$: Early P. versus Late P.
[c] $p < 0.05$: NP versus Late P., Mid P. versus Late P.

environmental photoperiod decrease, low temperatures, hydric and food restriction, and social interactions might affect the activity of the pituitary lactotrophs.

The results observed in the lactotrophs of the *Lagostomus* were similar to those found in other rodents, which reproduce seasonally during long days. In the Japanese wood mice (Kuwahara et al., 2000) and in hamster (Bittman et al., 1996; Cónsole et al., 2002; Johnston et al., 2003), a decrease in the size and number of lactotrophs and in the serum concentrations of this hormone were reported in the non-reproductive period in relation to the reproductive period. These results demonstrated that the lactotrophs were less active and presented lower amounts of detectable hormone due to modifications of the hormone synthesis, storage, and secretion (Stirland et al., 2001; Cónsole et al., 2002). In male animals of different species such as rat (Hondo et al., 1995), wild boar (Jedlinska et al., 1995), and ram (Regisford and Katz, 1993), PRL was involved in the regulation of the androgen-sensitive tissues activity. Lincoln (1998) has reported that PRL, together with GH and LH, controls the expression of LH testicular receptors, activates androgen synthesis, and affects spermatogenesis. This author suggests that PRL is the third gonadotrophin acting with LH and FSH to regulate the testicular activity, and with testosterone to influence other organs of the reproductive system. PRL exerts numerous actions on the organism, such as the integration of the seasonal changes of metabolism, growth, and reproduction (Freeman et al., 2000). Additionally, numerous investigations have reported that PRL secretion was considerably affected by stress. Stimulation or inhibition of the PRL secretion were different depending on the stress nature (Gala, 1990). Therefore, these results demonstrated that PRL is also necessary for maintaining the internal environment constancy (Freeman et al., 2000).

The previously described cellular types of viscacha PD (gonadotrophs, corticotrophs, somatotrophs, and tysotrophs; Filippa et al., 2005; Filippa and Mohamed, 2006a,b, 2008) and the lactotrophs studied in this work show a particular regionalization inside the glandular parenchyma. The differential distribution might be closely associated with the blood flow pattern as well as with the nature of their responsiveness to secretagogues. Mohamed et al. (2000) have reported seasonal variations of the colloidal accumulations number in the viscacha PD. In this work, follicles with immunopositive colloid were frequently found in adult male viscachas and lactotrophs in contact with the colloidal lumen were also observed, suggesting that the follicular colloid may have a hormone storage function. Taken together, these results might indicate that lactotrophs are not homogeneous in their morphology, distribution, or function.

Some studies in several species have related cell distribution in the PD parenchyma with blood irrigation and factors or hormones that control the activity of the adenohypophyseal cells under different physiological conditions (Sasaki and Iwama, 1988; Sato et al., 1999; Lee et al., 2004). Mukherjee et al. (1991) have reported in rat that the functional heterogeneity among lactotrophs with regard to their regional distribution within the anterior lobe and their responsiveness to different secretagogues. In addition, Vitale et al. (2001) have reported that in mink the synchronization of the cellular activity inside the follicles contributed to the control of the PRL secretion during the reproductive period.

It has been reported that the inhibitory influence of melatonin on the pituitary hormones is mainly exerted at the secretory process level instead of the biosynthesis level (Fernández-Alvarez et al., 2000). In ram, deer, equine, and male hamster, PRL secretion from the PD lactotrophs was inhibited by melatonin (Gerlach and Aurich, 2000), which acts on the specific receptors localized in the pituitary PT (Hastings et al., 1989; Donham et al., 1994; Lincoln and Clarke, 1994; Morgan et al., 1996; Wittkowski et al., 1999). In addition, studies on rat (Griffiths et al., 1987) and adult golden hamster (Wang et al., 1991) have demonstrated that melatonin provoked a decrease in the number of lactotrophs and inhibited PRL production and secretion.

In the adult male viscacha, the results after melatonin administration correlated with those obtained in the regression period of the annual reproductive cycle. The treated animals showed a decrease in the morphometric parameters, suggesting a lower concentration of PRL hormone detectable in PD lactotrophs. Therefore, it is probable that the pineal hormone modulates the activity of the viscacha pituitary PD cells.

In rat (Purves and Griesbach, 1952) and in the Japanese black steer (Sato et al., 1999), it was reported that

the proportion of lactotrophs decreased with age, but their distribution pattern was not modified. Takahashi (1995) reported in rat of both sexes that the levels of PRL mRNA in lactotrophs decreased according to age, suggesting a decrease in the PRL synthesis. Vidal et al. (1994) described in mink the ultrastructural characteristics of lactotrophs in young animals during their lactation period, reporting that they were different from those observed in the adults.

The effects of castration on the lactotrophs have been reported in different species. Cellular degranulation was described in rat (Purves and Griesbach, 1952) but castration had none or very little effect on the pituitary PRL levels in mouse (Sinha et al., 1979). Bex et al. (1978) observed in the adult male golden hamster that castration during the long photoperiod did not decrease PRL serum levels. Tortonese et al. (2001) reported in horses that gonadectomy caused a significant decrease of lactotrophs.

In the prepubertal male viscacha, the number and size of lactotrophs were lower than in adults with testicular activity (reproductive and gonadal recovery periods). In the prepubertal animals, the number of cells was higher but their size was smaller than in the animals in the regression period. In castrated adult male viscachas, the decrease of the immunopositive area and the number of lactotrophs in PD demonstrated that the storage of pituitary PRL was lower than in the intact animals. These results suggest that the testicular androgens might stimulate lactotroph activity.

Porter et al. (1990) reported in rat that serum concentrations of PRL increase from non-pregnancy to lactation. In bat, a significant increase in the number of cells was observed during pregnancy and lactation (Mikami et al., 1988). Wang et al. (1991) reported in female golden hamster that lactotrophs were bigger and more numerous than in males. Other authors have reported in rat differences in lactotrophs related to sex, demonstrating that their number (Ibrahim et al., 1986) and volume (Dada et al., 1984) were higher in females than in males.

In female viscachas, the colloid rarely presented immunolabeling, and a high proportion of cells in contact with the blood vessels wall were also observed. This might be due to a higher demand of PRL serum levels in females without previous storage in the follicular structures colloid. The morphometric parameters indicated that lactotroph activity was higher at the end of pregnancy in relation to non-pregnant female animals. Also, in non-pregnant females both the immunopositive area and the number of cells were higher than in males throughout the annual reproductive cycle. In other words, in viscacha the morphometric parameters of lactotrophs are modified from non-pregnancy to pregnancy and in relation to the animal sex, suggesting that the gonadal steroid hormones might modify the synthesis activity and/or the secretion of this adenohypophyseal cellular population.

In summary, the PD lactotrophs of viscacha (*Lagostomus maximus maximus*) showed morphometric variations that suggests changes in their synthesis activity and/or secretion in relation to: 1) the annual reproductive cycle in the adult male probably due to the photoperiod effect, 2) melatonin effect, 3) gonadal steroid hormone influence according to sex and age of the animals.

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