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The pituitary of non-pregnant and pregnant viscachas (*Lagostomus maximus maximus*): a comparative study by immunohistochemistry and morphometric analysis

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ABSTRACT

The neuroendocrine hypothalamic–pituitary axis undergoes morphological and biochemical changes throughout gestation. In viscacha, pregnancy lasts approximately 154 days, and three stages can be described: early, mid- and late pregnancy. The aim of this work was to study the pituitary LH-gonadotrophs, FSH-gonadotrophs, somatotrophs, corticotrophs and thyrotrophs of non-pregnant and pregnant adult viscachas by immunohistochemistry and morphometric analysis. Immunopositive percentage area (%IA), cell percentage in the pars distalis (%PDC), number of cells per reference area (n°cell/RA), and major cellular (MCD) and nuclear (ND) diameters were analyzed. The different cell populations showed a well-defined morphology, immunolabeling patterns and regionalization in the pars distalis (PD). In the early pregnancy of animals the morphometric analysis demonstrated that %IA, %PDC and n°cell/RA increased in the FSH-gonadotrophs and decreased in the somatotrophs in relation to non-pregnant animals. In mid-pregnancy, there was an increase in %IA, %PDC, and n°cell/RA of LH-gonadotrophs, FSH-gonadotrophs, somatotrophs, and thyrotrophs. The MCD of LH-gonadotrophs and FSH-gonadotrophs increased. In late pregnancy, the %IA, %PDC and n°cell/RA of LH-gonadotrophs, FSH-gonadotrophs, somatotrophs and corticotrophs decreased whereas the values of the thyrotrophs remained constant. The MCD of LH-gonadotrophs, FSH-gonadotrophs and corticotrophs decreased. No significant changes were observed in the ND of the studied cell types. In conclusion, this work provides evidence for histological and morphometric changes in the different cell types of the pituitary PD in viscachas during pregnancy, probably according to the requirements of this physiological stage.

1. Introduction

Pituitary pars distalis (PD) hormones are synthesized, stored and secreted by different cell types which undergo variations from the state of non-pregnancy to the end of pregnancy. Pregnancy is a special physiological condition in which hypothalamus, pituitary, ovary, adrenal and thyroid glands exhibit significant histological changes, and the hormonal variations are marked (Gibori et al., 1984).

Studies carried out in different species with seasonal reproduction have shown morphological or morphometric variations of some pituitary cell types during pregnancy. Wise et al. (1986) have observed in pregnant sheep a decrease in the number, size, and hormonal content of LH-gonadotrophs. In this species, it has also been indicated that the pituitary and serum concentration of luteinizing hormone (LH) remain low during the last third of pregnancy (O'Reilly and Dziuk, 1973) whereas increasing concentrations of steroid hormones may be responsible for the downregulation of gonadotrophin-releasing hormone (GnRH) secretion and the synthesis and release of gonadotrophins (Thomas et al., 1994). Porter et al. (1990, 1991) have reported in rat that serum concentration of growth hormone (GH) decreases from non-pregnancy to the end of pregnancy and throughout lactation, and the proportion of pituitary somatotrophs, which express and synthesize prolactin (PRL) during pregnancy, also decreases. Morphological changes of lactotrophs and somatotrophs related to different physiological stages like puberty, pregnancy and lactation have been demonstrated in goats (Vásquez et al., 2002). Moreover, corticotrophs increase in number and size during pregnancy in goats (Navarro et al., 1991). Likewise, Anthony and Gustafson (1984a) have reported that in pregnant bats there is a hypertrophy of periodic acid-Schiff positive cells (possible lactotrophs) at the end of gestation. This selective increase of one cell type may result in a reduction of the volume fractions of other cell types, probably thyrotrophs. In addition, it

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Table 1
Environmental conditions during the year. The seasonal average values of solar irradiation (heliophany), temperature and precipitation (mean ± SD) were obtained from the monthly average values provided by the Servicio Meteorológico Nacional San Luis. Temperature data correspond to the average daily temperatures.

	Summer	Autumn	Winter	Spring
Heliophany (h/d)	9.38 ± 1.06	7.09 ± 0.97	6.82 ± 0.31	9.09 ± 1.23
Precipitation (mm/month)	90.0 ± 2.82	27.0 ± 3.05	11.0 ± 1.41	58.5 ± 5.65
Temperature (°C)	19.5 ± 2.12	13.0 ± 2.82	12.0 ± 1.41	21.5 ± 2.12

has been demonstrated that sex steroids render these cells more sensitive to negative feedback regulation with thyroid hormones (Ahlquist et al., 1987), and studies carried out by Šošić-Jurjević et al. (2006) in rats have confirmed morphometric variations of thyrotrophs according to estrogen levels.

The viscacha (*Lagostomus maximus maximus*) is an autochthonous South American rodent inhabiting an area extending from the south of Paraguay and Bolivia to the center of Argentina (Llanos and Crespo, 1954). In its natural habitat, this rodent is a seasonal breeder. Several studies have demonstrated variations of the pineal–pituitary–gonadal axis in adult male viscachas throughout the year (Dominguez et al., 1987; Fuentes et al., 1991, 1993, 2003; Muñoz et al., 1997, 2001; Mohamed et al., 2000; Filippa et al., 2005; Aguilera-Merlo et al., 2005, 2009). Seasonal variations of the PD cell types have been reported in relation to modifications of environmental signals, principally photoperiod length, in adult male viscachas (Filippa et al., 2005; Filippa and Mohamed, 2006a,b, 2008, 2010).

Some reproductive characteristics of these monoestral animals, such as polyovulation, implantation, and birth of their offspring, have been described in previous studies (Weir, 1971; Roberts and Weir, 1973). Viscachas usually have an estrous period in autumn, and they are pregnant in winter (Jackson, 1989). Gestation lasts approximately 154 days, an unusually long period for a rodent, and it finishes in spring when the probabilities of survival are ideal for the mother and offspring (Weir, 1971; Keefe and Turek, 1985). The pregnancy stages of viscachas were estimated on the basis of capture time, number and size of the embryos and fetuses. Three stages were classified: early pregnancy (autumn), mid-pregnancy (winter), and late pregnancy (late winter and early spring) (Gil et al., 2007; Jensen et al., 2008; Filippa and Mohamed, 2010). Some morphological aspects of pituitary PD corticotrophs have been reported in adult female viscachas (Filippa and Mohamed, 2006a). Recently, lactotrophs have been described in a morphological and morphometric study in adult male and female viscachas (Filippa and Mohamed, 2010). Variations of the morphometric parameters of this cell type were found between non-pregnant and pregnant animals.

The purpose of this work was to study the morphological and morphometric characteristics of LH-gonadotrophs, FSH-gonadotrophs, somatotrophs, corticotrophs and thyrotrophs of pituitary PD in non-pregnant and pregnant viscachas. The analyzed morphometric parameters, immunopositive percentage area, PD cells percentage, number of cells per reference area, and major cellular and nuclear diameters were considered indicators of the histological changes (Takahashi, 1991; Vidal et al., 1995; Filippa et al., 2005; Filippa and Mohamed, 2006a,b, 2008, 2010).

2. Materials and methods

In this work, 6 non-pregnant and 18 pregnant viscachas weighing 2–4 kg were used for the morphological study. The animals were captured in their habitat near San Luis, Argentina (33°20′ south latitude, 760 m altitude) during 2007–2008. The seasonal mean values of solar irradiation (expressed as heliophany), precipitation and temperature were provided by the Servicio Meteorológico Nacional San Luis (Table 1).

The reproductive condition of the viscachas was carefully assessed on the basis of the following criteria: (i) the examination of the uterine horns, and (ii) the analysis of the ovaries by light microscopy. The viscachas captured in summer (February) were non-pregnant. All females captured from autumn to early spring (April–September) were pregnant. Pregnancy stages were classified on the basis of the number and size of embryos or foetuses into: (i) early pregnancy (6 animals), captured in autumn (April), with two or more embryos from 1 to 3 cm; (ii) mid-pregnancy (6 animals), captured in winter (July), with two foetuses from 9 to 11 cm; (iii) late pregnancy (6 animals), captured in late winter and early spring (September), with two foetuses measuring more than 19 cm. This classification was done according to previous reports by Gil et al. (2007), Jensen et al. (2008) and Filippa and Mohamed (2010).

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 was excised, fixed in Bouin's fluid, processed for light microscopy and embedded in paraffin. The pituitary was sagittally sectioned (5 μm thick), and each hemi-pituitary was completely cut following the same design as used in a previously reported study (Filippa and Mohamed, 2006b; Acosta et al., 2010). 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.

2.1. Immunohistochemistry

For immunohistochemical analysis, the streptavidin–biotin immunoperoxidase method was used, and the details, suppliers, dilution, time and temperature of the incubation of the antibodies used are reported in Table 2. The tissue sections were first deparaffinized with xylene and hydrated with decreasing concentrations of ethanol. Microwave pre-treatment (antigen retrieval) was performed by incubating the sections in 0.01 M citrate buffer (pH 6.0). After incubation for 20 min in a solution of 3% H_2O_2 in water in order to inhibit endogenous peroxidase activity, they were washed (3× 10 min) in phosphate-buffered saline (PBS, 0.01 M, pH 7.4). Non-specific binding sites for immunoglobulins were blocked by 15 min incubation with 0.25% casein in PBS, washing in PBS and incubation with the primary antibodies. The slides were subsequently washed (3× 10 min) in PBS. Immunohistochemical visualization was carried out using the Super Sensitive Ready-to-Use Immunostaining Kit (QD000-5L; BioGenex, San Ramon, CA, USA) at 20 °C. The sections were incubated for 30 min with biotinylated anti-IgG, and after being washed (3× 5 min) in PBS, they were incubated for 30 min with horseradish peroxidase-conjugated streptavidin, and finally washed in PBS. The reaction site was revealed by a 100 μl 3.3′-diaminobenzidine tetrahydrochloride (DAB) chromogen solution in 2.5 ml PBS and a 50 μl H_2O_2 substrate solution, resulting in a brown precipitate. The sections were counterstained with Harris' hematoxylin for 30 s, washed for 10 min in running water, dehydrated in increasing graded ethanol, cleared in xylene and mounted with Entellan (Merck, Darmstadt, Germany). Labeling was assessed using an Olympus BX-40 light microscope (Olympus, Hamburg, Germany).

Table 2
Features of the primary antibodies used in immunohistochemistry.

Antibody	Immunostained cell type	Specifications	Time/temperature of incubation
Anti-human (h) LHβ (luteinizing hormone)	LH-gonadotroph	Clone 3LH 5B6 YH4 Cat. No. AM030-5M Dilution 1:200 BioGenex	12 h, 4 °C in a moist chamber
Anti-hFSHβ (follicle-stimulating hormone)	FSH-gonadotroph	Clone 83/12/2A8 2C7 Cat. No. MU026-UC Dilution 1:100 BioGenex	12 h, 4 °C in a moist chamber
Anti-hGH (growth hormone)	Somatotroph	Polyclonal (developed in rabbit) Cat. No. N1561 Ready to use DakoCytomation	6 h, 4 °C in a moist chamber
Anti-hACTH (adrenocorticotropin hormone) (synthetic human ACTH 1-24)	Corticotroph	Polyclonal (developed in rabbit) Cat. No. AR035-5R Ready to use BioGenex	60 min, 4 °C in a moist chamber
Anti-hTSH (thyroid-stimulating hormone)	Thyrotroph	Clone 5404 Cat. No. MU 033-UC Dilution 1:50 BioGenex	6 h, 4 °C in a moist chamber

In all cases, two control experiments for the specificity of the primary antibody were made: (i) omission of primary antibody, and (ii) adsorption of primary antibody with a homologous antigen. No positive structures or cells were found in these sections. Pituitaries of rats were used as positive controls.

2.2. Morphometric analysis

A computer-assisted image analysis system was used to measure the percentage of immunopositive area, the percentage of immunoreactive cells in PD, the number of cells 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 (Sony Corp., Tokyo, Japan) and processed with Image-Pro Plus 5.0 software (Media Cybernetics Inc., Bethesda, MD, USA) under control of a Pentium IV computer. The software allowed the following processes: image acquisition, automatic analogous adjustment, thresholding, background subtraction, distance calibration, area and diameter measuring, and disk 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). Immunostaining for all the antibodies used was analyzed at low magnification (20× objective). The sections obtained in the middle sector of the pituitary exhibited the greatest immunostained areas in all the studied animals. Six pituitaries were analyzed in each group of animals, 4 regularly spaced serial sections (100 μm each) from the pituitary sector mentioned above were used, and all the microscopic fields were analyzed in each section (50–70 microscopic fields according to the section). Thus, between 200 and 280 microscopic fields were analyzed in each gland. In total, 1200–1680 microscopic fields or measures were analyzed per group. The following morphometric parameters were determined:

- Percentage of immunopositive area (%IA) of each cell type was calculated using the formula $\%IA = \sum Ac / \sum RA \times 100$, where $\sum Ac$ is the sum of the area of immunolabeled cells and $\sum RA$ is 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).
- The percentage of immunoreactive cells in PD (%PDC) in each image was obtained according to the formula $A/(A+B) \times 100$. Each image contained approximately 700–900 cells. The number of immunoreactive cells (A) and the number of nuclei in unstained cells (B) were counted.
- The number of immunostained cells (n°cell/RA) with a visible nucleus was counted in 10 microscopic fields per section. The result was expressed as number of cells per RA.
- Major cellular (MCD) and nuclear (ND) diameters were measured using the length tool of the Image-Pro Plus 5.0 software on each cell with a visible nucleus. These parameters were measured for 40 immunoreactive cells per pituitary gland.

2.3. Statistical analysis

The results were expressed as means ± standard error of the mean (SEM) for all data sets. The software GraphPad Prism (v. 3.02; GraphPad Software Inc., La Jolla, CA, USA) was used for the statistical analysis of morphometric measurements. The data were tested for normality and evaluated using one-way analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparison test. A probability of less than 0.05 was assumed to be significant.

3. Results

As in other species (Desantis et al., 1998; Ferrandino et al., 2001), the pituitary PD of adult female viscachas exhibited distinctive regions or zones in relation to their position, which were called ventral (anterior), medial and dorsal (posterior, close to Rathke's pouch). In addition, two ends were distinguished: a rostral one (superior, connected with the pars tuberalis) and a caudal one (inferior) (Fig. 1A).

In non-pregnant viscachas, LH-gonadotrophs were localized in the PD ventral and medial regions, and at the rostral end. These cells were occasionally also observed on the posterior edge in the dorsal region of the PD (Fig. 1A). They were found isolated or in clusters along the blood vessels and next to the follicular structures. The LH-gonadotrophs were oval or spherical in shape with a nucleus in eccentrical position, and some cells had a cytoplasmic prolongation surrounding non-stained cells or reaching to blood vessels. Most cells presented a homogeneous immunolabeling pattern, whereas

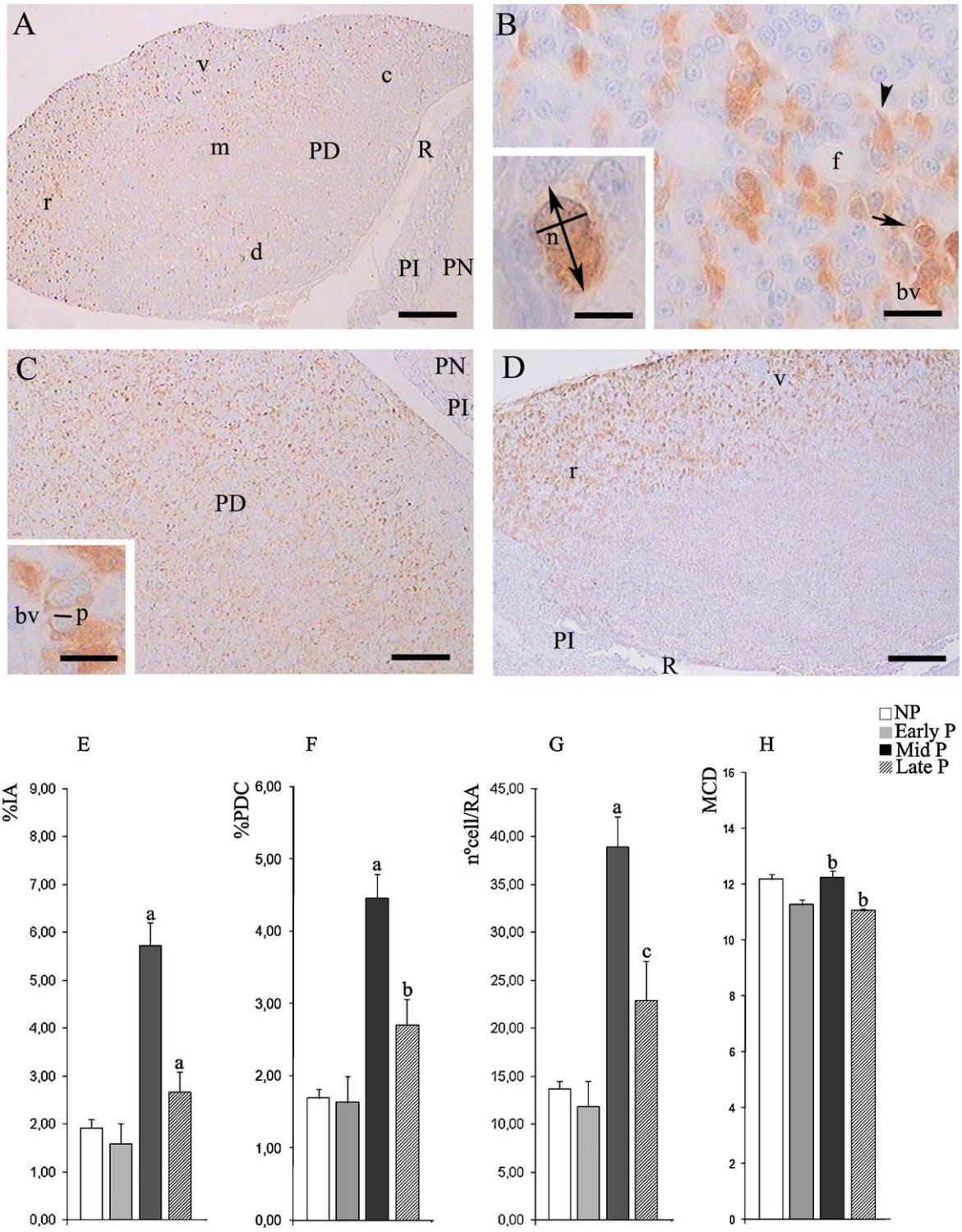

Fig. 1. LH-gonadotrophs of the pituitary pars distalis (PD) of non-pregnant (A and B) and pregnant (C and D) viscachas. (A) These cells are mainly localized in the PD ventral (v) and medial (m) regions and at the rostral end (r). c, caudal end; d, dorsal region; PN, pars nervosa; PI, pars intermedia; R, Rathke's pouch. Scale bar: 500 μm. (B) LH-gonadotrophs are isolated (arrowhead) or forming groups (arrow) along blood vessels (bv) and next to follicular structures (f). Scale bar: 25 μm. Inset: Higher magnification of an oval LH-gonadotroph with a nucleus in eccentrical position (n). The double-headed arrow indicates the major cellular diameter and the bar indicates the nuclear diameter. Scale bar: 10 μm. (C) Numerous LH-gonadotrophs are distributed throughout the PD parenchyma in mid-pregnancy. PN, pars nervosa; PI, pars intermedia. Scale bar: 250 μm. Inset: There are cells with cytoplasmic prolongations (—p) surrounding non-stained cells or reaching blood vessels (bv). Scale bar: 25 μm. (D) In late pregnancy the LH-gonadotrophs are less numerous and they are mainly in the ventral region (v) and at the rostral end (r). PI, pars intermedia; R, Rathke's pouch. Scale bar: 250 μm. (E) Immunopositive percentage area (%IA); a: $p < 0.001$ mid-pregnancy (5.73 ± 0.46) vs. early pregnancy (1.59 ± 0.42), late pregnancy (2.67 ± 0.41) vs. mid-pregnancy. (F) Cell percentage in PD (%PDC); a: $p < 0.001$ mid-pregnancy (4.46 ± 0.34) vs. early pregnancy (1.64 ± 0.36); b: $p < 0.01$ late pregnancy (2.71 ± 0.35) vs. mid-pregnancy. (G) Number of cells per reference area (n°cells/RA); a: $p < 0.001$ mid-pregnancy (38.89 ± 3.15) vs. early pregnancy (11.84 ± 2.64); c: $p < 0.05$ late pregnancy (22.86 ± 4.09) vs. mid-pregnancy. (H) Major cellular diameter (MCD), b: $p < 0.01$ mid-pregnancy (12.25 ± 0.23) vs. early pregnancy (11.26 ± 0.19), late pregnancy (11.07 ± 0.06) vs. mid-pregnancy. Bars represent mean ± SEM and the values of LH-gonadotrophs were compared with the values of the previous stage. Significant differences were determined by analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparison test. NP, non-pregnant; Early P, early pregnancy; Mid P, mid-pregnancy; Late P, late pregnancy.

Table 3
Major cellular and nuclear diameters (μm) of non-pregnant and pregnant adult female viscachas.

	NP	Early P	Mid P	Late P
LH-gonadotrophs	5.53 ± 0.14	5.47 ± 0.16	5.92 ± 0.18	5.55 ± 0.15
FSH-gonadotrophs	5.11 ± 0.14	5.23 ± 0.14	5.24 ± 0.10	5.16 ± 0.05
Somatotrophs	5.15 ± 0.10	4.39 ± 0.13	4.76 ± 0.15	4.51 ± 0.17
Corticotrophs	5.96 ± 0.10	5.17 ± 0.34	5.81 ± 0.18	5.40 ± 0.17
Thyrotrophs	5.25 ± 0.25	5.57 ± 0.10	5.43 ± 0.11	5.44 ± 0.05

The values are expressed as mean ± SEM (n = 6). NP, non-pregnant; Early P, early pregnancy; Mid P, mid-pregnancy; Late P, late pregnancy. No significant differences were determined by analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparison test; $p > 0.05$.

other cells exhibited intense immunostaining in the cytoplasm periphery (Fig. 1B). The LH-gonadotrophs did not present significant changes in shape and immunolabeling pattern during the entire pregnancy. However, in mid-pregnancy, these cells were found widely distributed in the PD (Fig. 1C). In late pregnancy, LH-gonadotrophs were mainly localized in the ventral region and at the rostral end (Fig. 1D). The %IA, %PDC, n°cell/RA, and MCD of these cells increased significantly in mid-pregnancy in relation to early pregnancy, and decreased significantly in late pregnancy (Fig. 1E–H). No significant changes were observed in the ND of the LH-gonadotrophs throughout pregnancy (Table 3).

The FSH-gonadotrophs of non-pregnant viscachas were mostly distributed on the anterior edge in the ventral region and at the rostral end (Fig. 2A). They were found in the proximity of follicular structures and in contact with blood vessels, forming cords or clusters, and some of them were isolated. Most cells were oval or spherical in shape, occasionally pyramidal, and with an eccentric nucleus. Cytoplasmic immunostaining was homogeneous although some cells were more intensely stained than others (Fig. 2B). The FSH-gonadotrophs did not present significant changes in their shape and immunolabeling pattern during gestation. In mid-pregnancy, these cells were found widely distributed in the PD, mainly in medial and dorsal regions (Fig. 2C). In addition, intensely labeled FSH-gonadotroph clusters were observed in this stage (Fig. 2D). FSH-gonadotrophs were also observed in the medial region in late pregnancy (Fig. 2E). In early pregnancy, the %IA, %PDC and n°cell/RA of the FSH-gonadotrophs increased significantly in relation to values in non-pregnant animals. The %IA, %PDC, n°cell/RA, and MCD of these cells increased significantly in mid-pregnancy in relation to early pregnancy. In contrast, in late pregnancy a significant decrease of these parameters was observed (Fig. 2F–I). No significant changes were observed in the ND of the FSH-gonadotrophs throughout pregnancy (Table 3).

Somatotrophs were distributed throughout the PD parenchyma while at the rostral end, the cellular regionalization was limited by blood vessels (Fig. 3A). The somatotrophs were less numerous and less intensely labeled at this end (towards the ventral region), whereas towards the dorsal region they were numerous and lightly labeled. Moreover, immunolabeling in the intercellular space was also observed. These cells were found isolated or forming small groups, near follicular structures and in contact with blood vessels. They were oval or spherical in shape, with scanty homogeneously labeled cytoplasm and a nucleus in central position (Fig. 3B). Somatotrophs did not present significant changes in their shape and immunolabeling pattern throughout pregnancy. The cellular regionalization at the rostral end was less evident in late pregnancy than in mid-pregnancy (Fig. 3C–D). The %IA, %PDC and n°cell/RA of somatotrophs decreased significantly in early pregnancy in relation to non-pregnant animals. These parameters increased in mid-pregnancy, and decreased significantly in the last period of pregnancy (Fig. 3E–G). No significant changes were observed in the MCD and ND of the somatotrophs throughout pregnancy (Fig. 3H, Table 3).

Corticotrophs were localized throughout the PD, mainly at the caudal end and in the dorsal region of the parenchyma in non-pregnant viscachas (Fig. 4A). A great quantity of these cells was found to be closely associated with follicular structures. The corticotrophs were polygonal, oval or spherical in shape, some were stellate with cytoplasmic prolongations. The nucleus was spherical or oval and eccentrically or centrally placed. The cytoplasmic immunolabeling was homogeneous; however, some cells in the periphery of the cellular cytoplasm presented an intense immunolabeling (Fig. 4B). The corticotrophs did not show significant changes in their shape and immunolabeling pattern throughout gestation; they were more isolated and scarce in the dorsal region and at the caudal end in late pregnancy than in mid-pregnancy (Fig. 4C–D). In late pregnancy, a significant decrease in the %IA, %PDC, n°cell/RA, and MCD of corticotrophs was observed in relation to other pregnant stages and non-pregnant viscachas (Fig. 4E–H). No significant changes were observed in the ND of these cells throughout pregnancy (Table 3).

The non-pregnant viscacha thyrotrophs were mainly localized in the ventral region and at the rostral end (Fig. 5A). Occasionally, they were also observed in the dorsal region and on the posterior edge of PD next to Rathke's pouch. The cellular regionalization at the rostral end was limited by blood vessels (Fig. 5A). These cells were found isolated or in clusters and forming fences along the blood vessels' surfaces. The thyrotrophs were generally oval in shape and some of them presented a short cytoplasmic prolongation, reaching blood vessels. They exhibited a spherical nucleus with an eccentric or central position. The cytoplasmic immunostaining intensity was different among thyrotrophs; it was generally more intense in isolated than in clustered cells. In some thyrotrophs more intensity of the immunolabeling was observed at the cellular ends that were in contact with blood vessels (Fig. 5B). The shape and immunolabeling pattern of these cells did not present significant changes throughout pregnancy. Numerous thyrotrophs in the ventral and medial regions were distributed forming big clusters in mid- and late pregnancy (Fig. 5C–E). The %IA, %PDC and n°cell/RA of thyrotrophs increased significantly in mid-pregnancy in relation to early pregnancy, and they remained constant in late pregnancy (Fig. 5F–H). No significant changes were observed in the MCD and ND of these cells throughout pregnancy (Fig. 5I, Table 3).

4. Discussion

In this study, LH-gonadotrophs, FSH-gonadotrophs, somatotrophs, corticotrophs and thyrotrophs of the pituitary PD of adult female viscachas were identified by immunohistochemistry. The morphology of these cells and their morphometric variations from non-pregnancy to late pregnancy stages were described. The different cell populations presented a clear regionalization. This suggests that the localization in the pituitary parenchyma is not at random; it is probably related to the distribution of blood vessels and the regulatory factors that reach the endocrine cells through blood circulation. The morphological characteristics of the endocrine cells of the pituitary PD of female viscachas were similar to those described in females of various species, such as bat (Mikami et al., 1988), mink (Vidal et al., 1995), mice (Kuwahara et al., 2004), rat (Kum et al.,

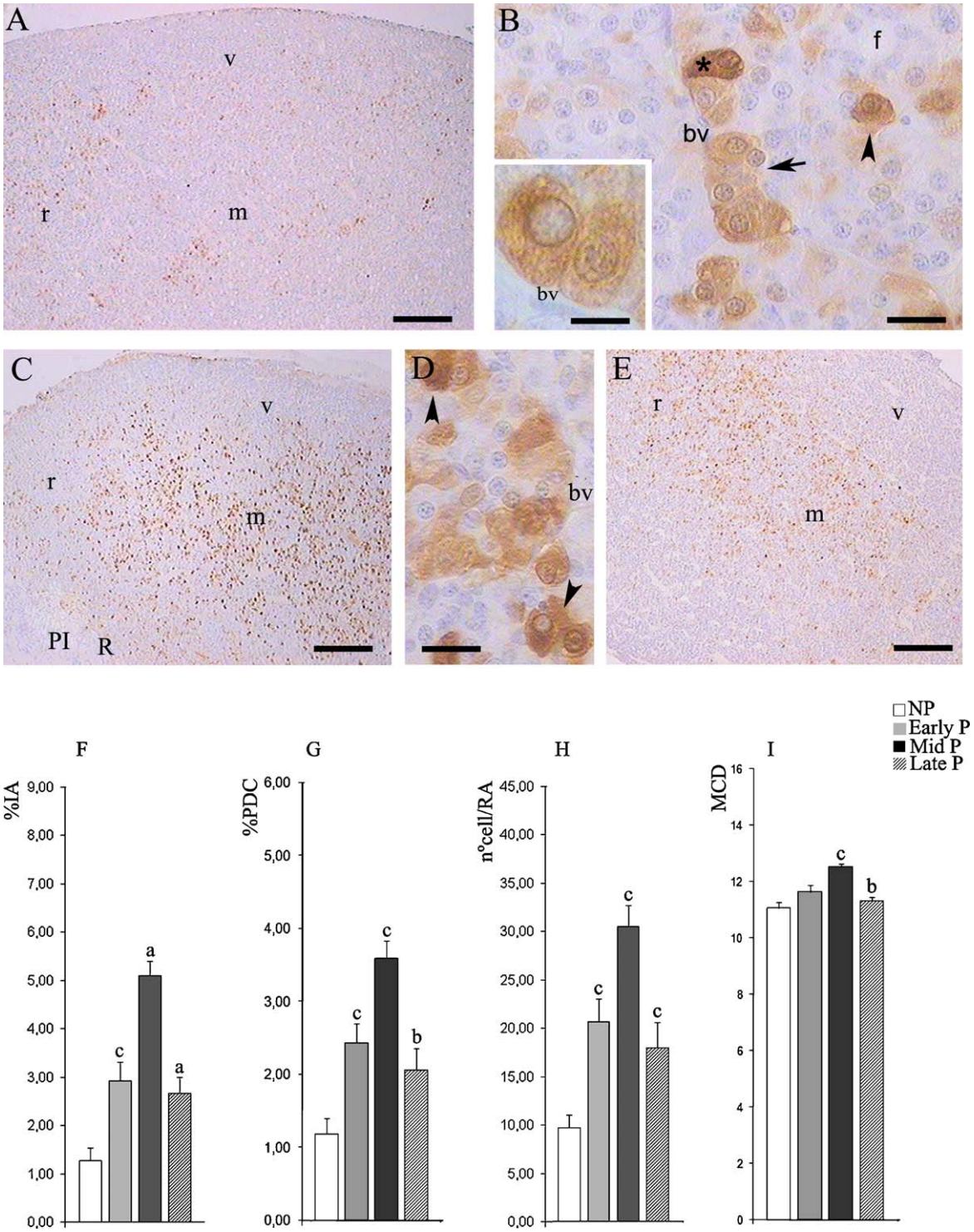

Fig. 2. FSH-gonadotrophs of the pituitary pars distalis (PD) of non-pregnant (A and B) and pregnant (C–E) viscachas. (A) FSH-gonadotrophs localized in the PD ventral (v) and medial (m) regions, and at the rostral end (r). Scale bar: 250 μm. (B) These cells are forming cords (arrow) or clusters along the blood vessels (bv), and some of them are isolated (arrowhead) in the vicinity of a follicular structure (f). Some FSH-gonadotrophs exhibit more intense immunostaining (*). Scale bar: 25 μm. Inset: Higher magnification of oval FSH-gonadotrophs in contact with blood vessels (bv). Scale bar: 10 μm. (C) Several FSH-gonadotrophs are widely distributed in the PD parenchyma in mid-pregnancy. m, medial region; r, rostral end; v, ventral region; PI, pars intermedia; R, Rathke's pouch. Scale bar: 250 μm. (D) In this stage, there are clusters of FSH-gonadotrophs intensely stained (arrowheads). bv, blood vessel. Scale bar: 25 μm. (E) The number of these cells is lower in late pregnancy; they are mainly located in the medial region (m). r, rostral end; v, ventral region. Scale bar: 250 μm. (F) Immunopositive percentage area (%IA); c: $p < 0.05$ early pregnancy (2.92 ± 0.38) vs. non-pregnancy (1.27 ± 0.26); a: $p < 0.001$ mid-pregnancy (5.10 ± 0.29) vs. early pregnancy, late pregnancy (2.66 ± 0.33) vs. mid-pregnancy. (G) Cell percentage in PD (%PDC); c: $p < 0.05$ early pregnancy (2.44 ± 0.26) vs. non-pregnancy (1.19 ± 0.21), mid-pregnancy (3.60 ± 0.24) vs. early pregnancy; b: $p < 0.01$ late pregnancy (2.07 ± 0.29) vs. mid-pregnancy. (H) Number of cells per reference area (n°cells/RA); c: $p < 0.05$ early pregnancy (20.60 ± 2.38) vs. non-pregnancy (9.67 ± 1.30), mid-pregnancy (30.48 ± 2.17) vs. early pregnancy, late pregnancy (17.97 ± 2.58) vs. mid-pregnancy. (I) Major cellular diameter (MCD); c: $p < 0.05$ mid-pregnancy (12.51 ± 0.14) vs. early pregnancy (11.63 ± 0.25); b: $p < 0.01$ late pregnancy (11.32 ± 0.13) vs. mid-pregnancy. Bars represent mean ± SEM and the values of FSH-gonadotrophs were compared with the values of the previous stage. Significant differences were determined by analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparison test. NP, non-pregnant; Early P, early pregnancy; Mid P, mid-pregnancy; Late P, late pregnancy.

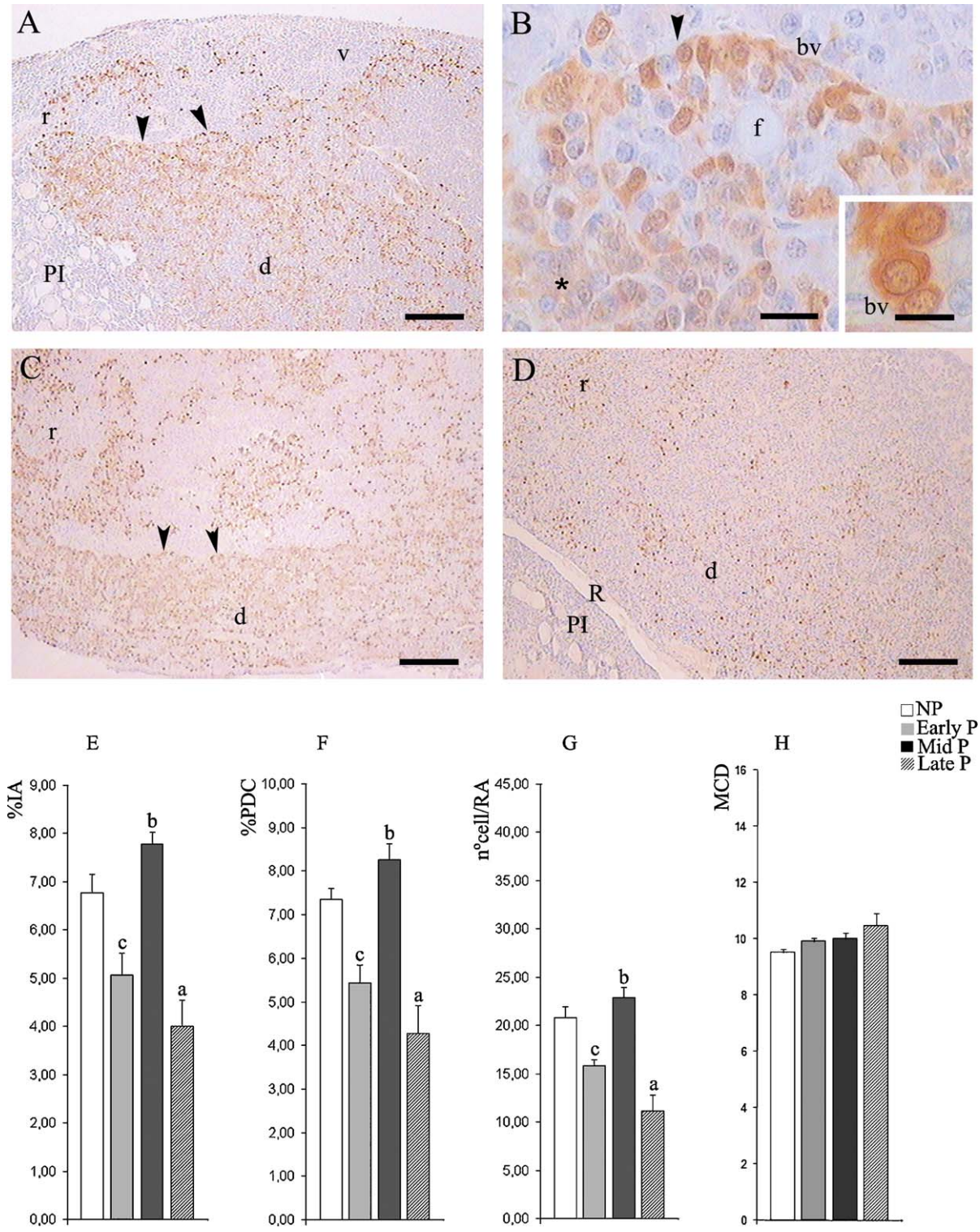

Fig. 3. Somatotrophs of the pituitary pars distalis of non-pregnant (A and B) and pregnant (C and D) viscachas. (A) At the rostral end (r) the regionalization of the somototrophs is limited by blood vessels (arrowheads). The somatotrophs from the ventral region (v) are less numerous, but they are abundant in the dorsal region (d). PI, pars intermedia. Scale bar: 250 μm. (B) The cellular regionalization (arrowhead) is limited by blood vessels (bv) at the rostral end. Some somatotrophs are in the proximity of follicular structures (f), and there is immunolabeling in the intercellular space (*). Scale bar: 25 μm. Inset: Higher magnification of oval and spherical somatotrophs with scanty homogeneously labeled cytoplasm and central nuclei. bv, blood vessel. Scale bar: 10 μm. (C) The arrowheads indicate the regionalization of somototrophs at the rostral end (r) in mid-pregnancy. d, dorsal region. Scale bar: 250 μm. (D) A smaller number of these cells are present in the PD and the cellular regionalization at the rostral end (r) is less evident in late pregnancy. d, dorsal region; PI, pars intermedia; R, Rathke's pouch. Scale bar: 250 μm. (E) Immunopositive percentage area (%IA); c: $p < 0.05$ early pregnancy (5.06 ± 0.45) vs. non-pregnancy (6.77 ± 0.39); b: $p < 0.01$ mid-pregnancy (7.78 ± 0.25) vs. early pregnancy; a: $p < 0.001$ late pregnancy (4.00 ± 0.55) vs. mid-pregnancy. (F) Cell percentage in PD (%PDC); c: $p < 0.05$ early pregnancy (5.44 ± 0.41) vs. non-pregnancy (7.35 ± 0.25); b: $p < 0.01$ mid-pregnancy (8.26 ± 0.37) vs. early pregnancy; a: $p < 0.001$ late pregnancy (4.28 ± 0.64) vs. mid-pregnancy. (G) Number of cells per reference area (n°cells/RA); c: $p < 0.05$ early pregnancy (15.80 ± 0.67) vs. non-pregnancy (20.76 ± 1.18); b: $p < 0.01$ mid-pregnancy (22.88 ± 1.08) vs. early pregnancy; a: $p < 0.001$ late pregnancy (11.18 ± 1.61) vs. mid-pregnancy. (H) Major cellular diameter (MCD); no significant changes were observed in this parameter. Bars represent mean ± SEM and the values of somatotrophs were compared with the values of the previous stage. Significant differences were determined by analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparison test. NP, non-pregnant; Early P, early pregnancy; Mid P, mid-pregnancy; Late P, late pregnancy.

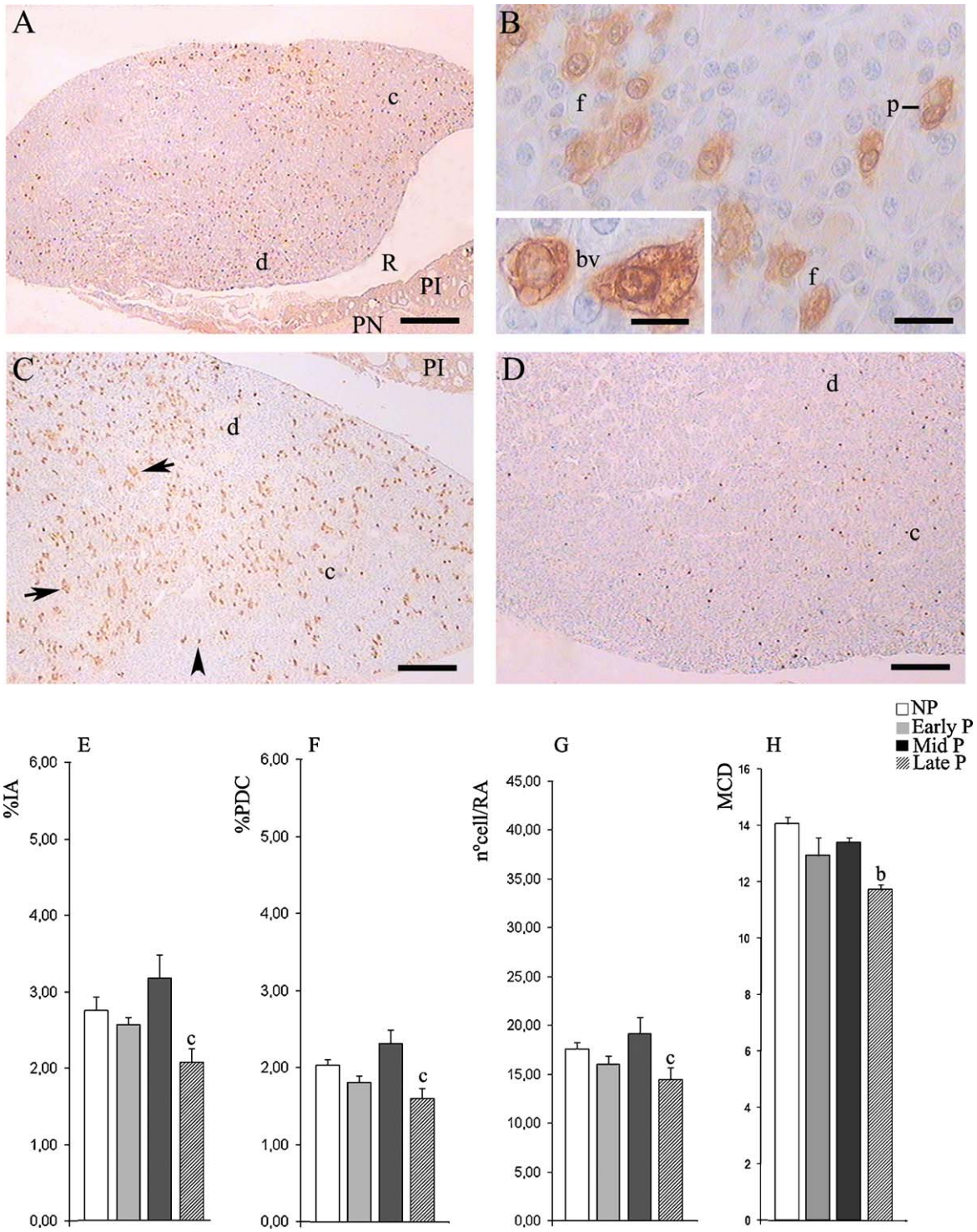

Fig. 4. Corticotrophs of the pituitary pars distalis (PD) of non-pregnant (A and B) and pregnant (C and D) viscachas. (A) Corticotrophs are mainly localized in the dorsal region (d) and at the caudal end (c). PN, pars nervosa; PI, pars intermedia; R, Rathke's pouch. Scale bar: 500 μm. (B) There are corticotrophs in a basal position of follicular structures (f). These cells are polygonal, oval, spherical and stellate in shape. Some cells have short cytoplasmic prolongations (—p). Scale bar: 25 μm. Inset: Higher magnification of corticotrophs with heterogeneous immunolabeling, mainly in the cellular periphery. bv, blood vessel. Scale bar: 10 μm. (C) In mid-pregnancy, several corticotrophs are observed at the caudal end (c) and in the dorsal region (d). There are cells forming groups (arrows), others are isolated (arrowhead). PI, pars intermedia. Scale bar: 250 μm. (D) In late pregnancy, the corticotrophs are less numerous and they are mainly isolated. c, caudal end; d, dorsal region. Scale bar: 250 μm. (E) Immunopositive percentage area (%IA); c: $p < 0.05$ late pregnancy (2.07 ± 0.18) vs. mid-pregnancy (3.17 ± 0.30). (F) Cell percentage in PD (%PDC); c: $p < 0.05$ late pregnancy (1.60 ± 0.13) vs. mid-pregnancy (2.31 ± 0.18). (G) Number of cells per reference area (n°cells/RA); c: $p < 0.05$ late pregnancy (13.64 ± 1.17) vs. mid-pregnancy (18.11 ± 1.60). (H) Major cellular diameter (MCD); b: $p < 0.01$ late pregnancy (11.74 ± 0.16) vs. mid-pregnancy (13.39 ± 0.18). Bars represent mean ± SEM and the values of corticotrophs were compared with the values of the previous stage. Significant differences were determined by analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparison test. NP, non-pregnant; Early P, early pregnancy; Mid P, mid-pregnancy; Late P, late pregnancy.

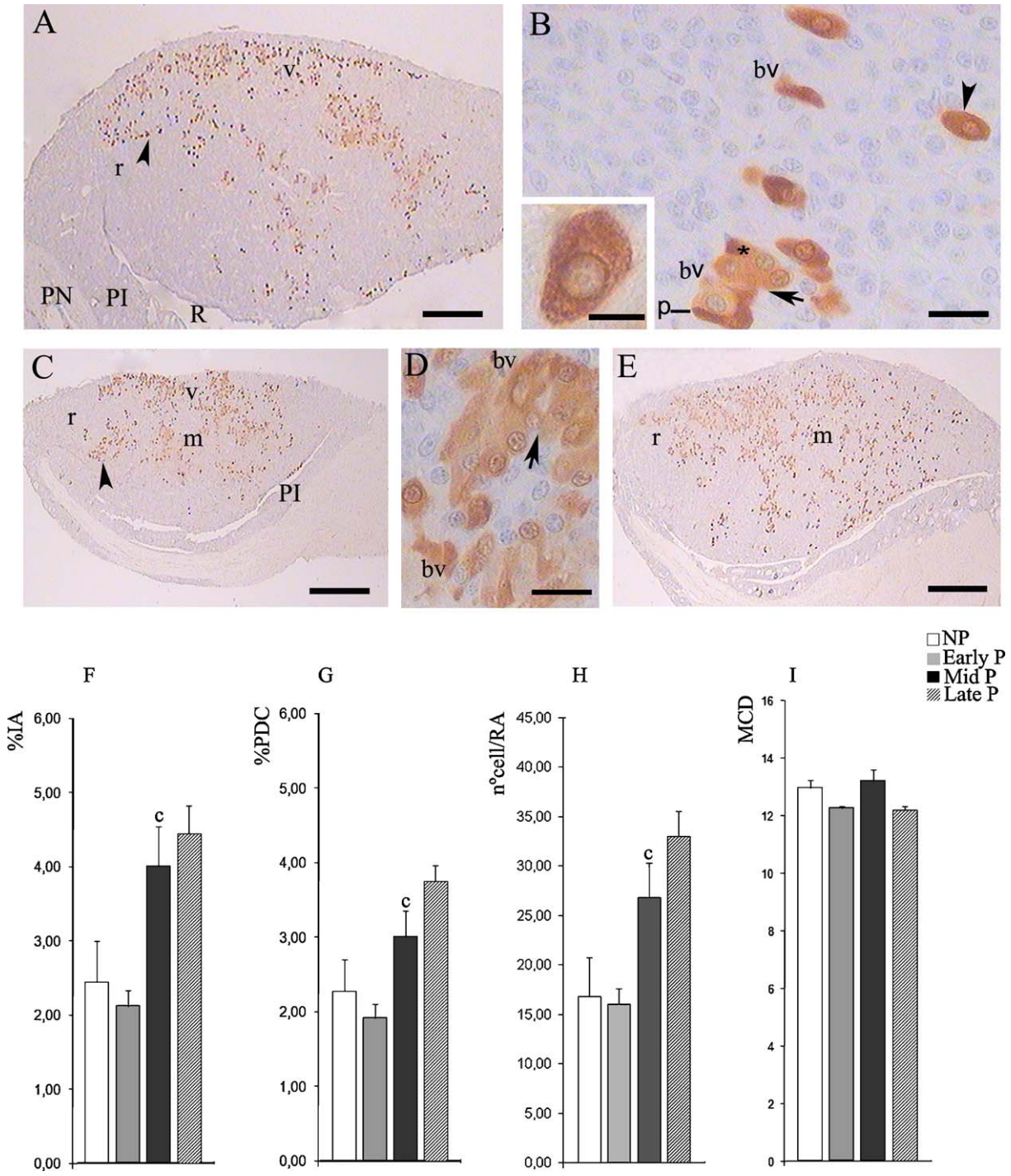

Fig. 5. Thyrotrophs of the pituitary pars distalis (PD) of non-pregnant (A and B) and pregnant (C–E) viscachas. (A) The thyrotrophs are localized in the ventral region (v) and at the rostral end (r). At this end, the cellular regionalization is limited by blood vessels (arrowhead). PN, pars nervosa; PI, pars intermedia; R, Rathke's pouch. Scale bar: 500 μm. (B) These cells are isolated (arrowhead) and forming fences (arrow) along the blood vessel surface (bv). The image shows a thyrotroph with a cytoplasmic prolongation (—p) reaching the blood vessel (*: intense immunostaining at the cellular end that is in contact with the blood vessel). Scale bar: 25 μm. Inset: An oval thyrotroph with an eccentric nucleus. Scale bar: 10 μm. (C) Numerous thyrotrophs in the ventral (v) and medial (m) regions of the pars distalis parenchyma are present in mid-pregnancy. The arrowhead indicates the cellular regionalization at the rostral end (r). PI, pars intermedia. Scale bar: 500 μm. (D) In this stage, there are thyrotrophs forming big groups (arrow) and disposed along the blood vessels (bv). (E) There are numerous thyrotrophs in late pregnancy. r, rostral end; m, medial region. Scale bar: 500 μm. (F) Immunopositive percentage area (%IA); c: $p < 0.05$ mid-pregnancy (4.00 ± 0.54) vs. early pregnancy (2.12 ± 0.21). (G) Cell percentage in PD (%PDC); c: $p < 0.05$ mid-pregnancy (3.01 ± 0.35) vs. early pregnancy (1.92 ± 0.19). (H) Number of cells per reference area (n°cells/RA); c: $p < 0.05$ mid-pregnancy (26.82 ± 3.47) vs. early pregnancy (15.99 ± 1.58). (I) Major cellular diameter (MCD); no significant changes were observed in this parameter. Bars represent mean ± SEM and the values of thyrotrophs were compared with the values of the previous stage. Significant differences were determined by analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparison test. NP, non-pregnant; Early P, early pregnancy; Mid P, mid-pregnancy; Late P, late pregnancy.

2006; Šošić-Jurjević et al., 2006) and mare (Eagle and Tortonese, 2000).

It has been reported that the proportion of LH-gonadotrophs diminishes after ovulation and remains low during pregnancy and lactation in bats (Anthony and Gustafson, 1984b). Thompson et al. (1991) have demonstrated that in pony mares estradiol inhibits the synthesis of LH subunits, whereas progesterone reduces LH secretion and stimulates follicle stimulating hormone (FSH) storage. Several studies have discussed the significance of GH to the reproductive system. Childs et al. (2006) have indicated that, due to the lipolytic effects of GH, a reduction of this hormone is needed during the postovulatory period to promote sufficient fuel to support pregnancy. In addition, Chandrashekar and Bartke (1998) have observed in rat non-parallel responses of LH and FSH secretion, indicating that GH modifies gonadotroph activities. Childs (2000) has reported that GH might regulate LH secretion or alter the sensitivity of the gonadotrophs to GnRH and steroids. In the present study, the occupied area, percentage and number of cells of LH-gonadotrophs were not modified significantly during the early pregnancy of viscachas in relation to non-pregnant animals. On the contrary, these parameters showed a significant increase in FSH-gonadotrophs and the somatotrophs exhibited a significant decrease. These results suggest an inverse relationship between the secretory activities of these cell types in early pregnancy. However, as has been reported in other species (Girord et al., 1981; Childs et al., 1994; Desantis et al., 2000; Eagle and Tortonese, 2000), the presence of gonadotrophs containing LH and FSH should not be discarded as an explanation for the variations observed in viscacha.

LH-gonadotrophs, FSH-gonadotrophs, somatotrophs, and thyrotrophs have shown a wide distribution in the PD parenchyma in mid-pregnancy of viscacha. The increase in the number and size of these cells suggests a higher secretory cell activity during this pregnancy stage to support the metabolic requirements. The differences observed in the intensity of cytoplasmic immunolabeling of thyrotrophs might indicate variations in the cellular activity or the existence of cell subpopulations that respond to different stimuli. Gil et al. (2007) have demonstrated morphological and morphometric characteristics of a higher steroidogenic activity in the ovarian interstitial tissue of viscacha in mid-pregnancy. Thus, the ovarian steroids might stimulate the activity of those pituitary cell types. On the other hand, there is evidence that gonadotrophins, principally LH, modulate the development and steroidogenic activity of the ovarian interstitial tissue in mouse (Clarke and Brook, 2001). Other authors have described that GH promotes the metabolic activity and the optimal body composition to support pregnancy (Giustina and Veldhuis, 1998; Hull and Harvey, 2002). In addition, Escalada et al. (1997) have demonstrated in rat that an increase of the pituitary immunoreactive GH content in mid-pregnancy is necessary to achieve an increase in serum GH levels during late gestation. Several authors have studied the effects of the gonadal steroidal hormones on thyrotrophs (Kimura et al., 1994; Banu and Aruldhas, 2002; Šošić-Jurjević et al., 2006). Ahlquist et al. (1987) have shown in rat that sex steroids render thyrotrophs more sensitive to negative feedback regulation by thyroid hormones. Villalobos et al. (2004) have demonstrated in female rats that most of the thyrotrophs were polyhormonal, also containing GH and adrenocorticotrophic hormone (ACTH). These authors regarded these cells as constituting a mosaic of different cellular phenotypes rather than a single cell type whose relative abundance varies in different physiological and pathophysiological conditions.

A decrease of the morphometric parameters of LH-gonadotrophs, FSH-gonadotrophs and somatotrophs was observed in late pregnancy of viscachas. The lower activity of these cells might be due to the negative feedback effect of gonadal steroids, mainly progesterone and androstenodione whose serum levels increased during late pregnancy (Gil et al., 2007). In relation to these results, Jensen et al. (2008) have demonstrated that the values of serum progesterone increase from early to late pregnancy. Fowler et al. (2003) observed during late pregnancy of sheep that the amount of pituitary LH diminished, and LH-gonadotrophs were hypotrophic. It has been recently reported in viscacha that lactotrophs increase in number and size in late pregnancy (Filippa and Mohamed, 2010). In the present work, a decrease in the number of somatotrophs was observed in this stage. Some somatotrophs might probably begin to synthesize and secrete prolactin (PRL) in late pregnancy in preparation for the lactation period, as demonstrated in other species (Porter et al., 1990). A decrease of somatotrophs and an increase of lactotrophs have been demonstrated in rat during pregnancy, probably due to an interconversion from somatotrophs to lactotrophs through an intermediate cell type, called mammosomatotrophs (Porter et al., 1991).

Thyrotrophs have shown a higher activity in the late pregnancy of viscacha, suggesting some stimulating effect of gonadal steroids on this cell population during gestation. In addition, Anthony and Gustafson (1984a) have suggested that a selective increase of one cell type may result in a reduced proportion of other cell types. Other authors have demonstrated that the pituitary cells were not irreversibly committed to the production of one single hormone and that their phenotype can change in response to functional demands (Vidal et al., 2001).

Some morphological aspects of corticotrophs in non-pregnant viscachas and during mid-pregnancy have been previously reported (Filippa and Mohamed, 2006a). In the present work, corticotrophs were studied in the three stages of pregnancy. A decrease of the morphometric parameters of these cells was observed in late pregnancy, suggesting that a reduced responsiveness of the hypothalamus–pituitary–adrenal axis during gestation contributes to some fetal protection mechanism, as has been reported in other species (Keller-Wood, 1994; Neumann et al., 1998). Similarly to chronic stress and aging, pregnancy and lactation represent challenges because of the deep physiological changes taking place in the mother. It has been demonstrated that the neuroendocrine response to different stressors was attenuated during gestation in rats (Neumann et al., 1998), sheep (Keller-Wood, 1994), and humans (Schulte et al., 1990). The reduction of the activity of the hypothalamus–pituitary–adrenal axis in pregnant rats, with a decrease of ACTH secretion, protects the pregnant animal and its offspring from the detrimental effects of higher levels of glucocorticoids (Weinstock, 1997; Neumann et al., 1998; Johnstone et al., 2001).

In adult male viscachas, the pituitary PD cell types were previously studied throughout their annual reproductive cycle, which may be divided into three periods (reproductive, gonadal regression and gonadal recovery). Seasonal variations of the morphological characteristics and morphometric parameters were observed in different cell populations in adult male viscachas. LH-gonadotrophs, lactotrophs, somatotrophs, corticotrophs and thyrotrophs presented a higher activity in the reproductive period (summer and early autumn), whereas the activity of FSH-gonadotrophs was higher in the gonadal recovery period (spring). All these cell types presented a lower activity in the gonadal regression period (winter) (Filippa et al., 2005; Filippa and Mohamed, 2006a,b, 2008, 2010). However, this was different in pregnant animals (winter, mid-pregnancy), in which the activity of most PD cells was maximal. These results in viscachas suggest that the modifications of the different cell populations during pregnancy probably occur in response to this physiological condition rather than to the environmental factors during winter such as short photoperiod, low temperature, hydric and food restrictions and the number of social interactions. In addition, the variations observed between female and male viscachas suggest that there are different effects of the gonadal steroids on each pituitary cell type.

In conclusion, this work provides evidence for histological and morphometric changes in the viscacha pituitary during pregnancy, suggesting a significant dynamism in the secretory activity of the different cell types and a great cellular plasticity according to this physiological condition.

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References

Acosta, M., Filippa, V., Mohamed, F., 2010. Folliculostellate cells in pituitary pars distalis of male viscacha: immunohistochemical, morphometric and ultrastructural study. Eur. J. Histochem. 54, 1–9.

Aguilera-Merlo, C., Muñoz, E., Dominguez, S., Scardapane, L., Piezzi, R., 2005. Epididymis of viscacha (*Lagostomus maximus maximus*): morphological changes during the annual reproductive cycle. Anat. Rec. 282, 83–92.

Aguilera-Merlo, C., Fogal, T., Sator, T., Dominguez, S., Sosa, M., Scardapane, L., Piezzi, R., 2009. Ultrastructural and biochemical seasonal changes in epididymal corpus and cauda of viscacha (*Lagostomus maximus maximus*). J. Morphol. 270, 805–814.

Ahlquist, J.A., Franklyn, J.A., Wood, D.F., Balfour, N.J., Docherty, K., Sheppard, M.C., Ramsden, D.B., 1987. Hormonal regulation of thyrotropin synthesis and secretion. Horm. Metab. Res. Suppl. 17, 86–89.

Anthony, E.L.P., Gustafson, A.W., 1984a. Seasonal variations in pituitary thyrotropes of the hibernating bat *Myotis lucifugus lucifugus*: an immunohistochemical study. Anat. Rec. 209, 363–372.

Anthony, E.L., Gustafson, A.W., 1984b. Seasonal variations in pituitary LH-gonadotropes of the hibernating bat *Myotis lucifugus lucifugus*: an immunohistochemical study. Am. J. Anat. 170, 101–115.

Banu, K.S., Aruldhas, M.M., 2002. Sex steroids regulate TSH-induced thyroid growth during sexual maturation in Wistar rats. Exp. Clin. Endocrinol. Diabetes 110, 37–42.

Chandrashekar, V., Bartke, A., 1998. The role of growth hormone in the control of gonadotropin secretion in adult male rats. Endocrinology 139, 1067–1074.

Childs, G.V., 2000. Growth hormone cells as co-gonadotropes: partners in the regulation of the reproductive system. Trends Endocrinol. Metab. 11, 168–175.

Childs, G.V., Unabia, G., Rougeau, D., 1994. Cells that express luteinizing hormone (LH) and follicle-stimulating hormone (FSH) beta-subunit messenger ribonucleic acids during the estrous cycle: the major contributors contain LH, FSH, and/or growth hormone. Endocrinology 134, 990–997.

Childs, G.V., Iruthayanathan, M., Akhter, N., Johnson, B.W., 2006. Estrogen mediated cross talk between the ovary and pituitary somatotrope. Pre-ovulatory support for reproductive activity. Mol. Cell. Endocrinol. 247, 60–63.

Clarke, J., Brook, F.A., 2001. Effect of gonadotrophins on the ovarian interstitial tissue of the wood mouse, *Apodemus sylvaticus*. Reproduction 121, 123–129.

Cónsole, G.M., Jurado, S.B., Oyhenart, E., Ferese, C., Pucciarelli, H., Gomez Dumm, C.L.A., 2001. Morphometric and ultrastructural analysis of different pituitary cell populations in undernourished monkeys. Braz. J. Med. Biol. Res. 34, 65–74.

Desantis, S., Labate, M., Corriero, A., 1998. Immunohistochemical localization of FSH and LH in the pituitary of male ruin lizards (*Podarcis sicula campestris* De Betta). Eur. J. Histochem. 42, 77–84.

Desantis, S., Labate, M., Corriero, A., Labate, G.M., De Metrio, G., 2000. Immunohistochemical evidence of seasonal changes of gonadotropes in male ruin lizard (*Podarcis sicula campestris* De Betta). Eur. J. Histochem. 44, 385–395.

Dominguez, S., Piezzi, R.S., Scardapane, L., Guzmán, J., 1987. A light and electron microscopic study of the pineal gland of the viscacha (*Lagostomus maximus maximus*). J. Pineal Res. 4, 211–219.

Eagle, R.C., Tortonese, D.J., 2000. Characterization and distribution of gonadotrophs in the pars distalis and pars tuberalis of the equine pituitary gland during the estrous cycle and seasonal anestrus. Biol. Reprod. 63, 826–832.

Escalada, J., Sánchez-Franco, F., Velasco, B., Cacicedo, L., 1997. Regulation of growth hormone (GH) gene expression and secretion during pregnancy and lactation in the rat: role of insulin-like growth factor-I, somatostatin, and GH-releasing hormone. Endocrinology 138, 3435–3443.

Ferrandino, I., Viscardi, G., Grimaldi, M.C., 2001. An immunohistochemical study of adenohypophyseal cells in the viviparous reptile *Chalcides chalcides*. Histochem. J. 33, 1–8.

Filippa, V., Mohamed, F., 2006a. ACTH cells of pituitary pars distalis of viscacha (*Lagostomus maximus maximus*): immunohistochemical study in relation to season, sex, and growth. Gen. Comp. Endocrinol. 146, 217–225.

Filippa, V., Mohamed, F., 2006b. Immunohistochemical study of somatotrophs in pituitary pars distalis of male viscacha (*Lagostomus maximus maximus*) in relation to the gonadal activity. Cells Tissues Organs 184, 188–197.

Filippa, V., Mohamed, F., 2008. Immunohistochemical and morphometric study of pituitary pars distalis thyrotrophs of male viscacha (*Lagostomus maximus maximus*): seasonal variations and effect of melatonin and castration. Anat. Rec. 291, 400–409.

Filippa, V., Mohamed, F., 2010. Morphological and morphometric changes of pituitary lactotrophs of viscacha (*Lagostomus maximus maximus*) in relation to reproductive cycle, age, and sex. Anat. Rec. 293, 150–161.

Filippa, V., Penissi, A., Mohamed, F., 2005. Seasonal variations of gonadotropins in the pars distalis male viscacha pituitary. Effect of chronic melatonin treatment. Eur. J. Histochem. 49, 291–300.

Fowler, P.A., Groome, N.P., Al-Gubory, K.H., 2003. Demonstration of a non-steroidal, non-inhibin factor in the ovine corpus luteum of pregnancy that reduces pituitary responsiveness to GnRH-induced LH secretion in vitro. Reproduction 126, 35–42.

Fuentes, L., Caravaca, N., Pelzer, L.E., Scardapane, L., Piezzi, R.S., Guzmán, J.A., 1991. Seasonal variations in the testis and epididymis of the viscacha (*Lagostomus maximus maximus*). Biol. Reprod. 45, 93–97.

Fuentes, L., Calvo, J.C., Charreau, E., Guzmán, J., 1993. Seasonal variations in testicular LH, FSH, and Prl receptors; in vitro testosterone production; and serum testosterone concentration in adult male viscacha (*Lagostomus maximus maximus*). Gen. Comp. Endocrinol. 90, 133–141.

Fuentes, L., Møller, M., Muñoz, E., Calderón, C., Pelzer, L., 2003. Seasonal variations in the expression of the mRNA encoding β1-adrenoceptor and AA-NAT enzyme, and in the AA-NAT activity in the pineal gland of viscacha (*Lagostomus maximus maximus*). Correlation with serum melatonin. Biol. Rhythm Res. 34, 193–206.

Gibori, G., Chen, Y.D., Khan, I., Azhar, S., Reaven, G.M., 1984. Regulation of luteal cell lipoprotein receptors, sterol contents, and steroidogenesis by estradiol in the pregnant rat. Endocrinology 114, 609–617.

Gil, E., Forneris, M., Domínguez, S., Penissi, A., Fogal, T., Piezzi, R.S., Scardapane, L., 2007. Morphological and endocrine study of the ovarian interstitial tissue of viscacha (*Lagostomus maximus maximus*). Anat. Rec. 290, 788–794.

Giustina, A., Veldhuis, J.D., 1998. Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. Endocr. Rev. 19, 717–797.

Girord, C., Dubois, M.P., Trouillas, J., 1981. Immunohistochemical localization of FSH and LH in the pars distalis of vervet (*Cercophitecus aethiops*) and baboon (*Papio hamadryas*) pituitaries. Cell Tissue Res. 217, 245–257.

Hull, K.L., Harvey, S., 2002. GH as a co-gonadotropin: the relevance of correlative changes in GH secretion and reproductive state. J. Endocrinol. 172, 1–19.

Jackson, J.E., 1989. Reproductive parameters of the plains vizcacha (*Lagostomus maximus*) in San Luis province, Argentina. Vida Silv. Neotrop. 2, 57–62.

Jensen, F., Willis, M.A., Leopardo, N.P., Espinosa, M.B., Vitullo, A.D., 2008. The ovary of the gestating South American plains vizcacha (*Lagostomus maximus*): suppressed apoptosis and corpora lutea persistence. Biol. Reprod. 79, 240–246.

Johnstone, H.A., Wigger, A., Douglas, A.J., Neumann, I.D., Landgraf, R., Seckl, J.R., Russell, J.A., 2001. Attenuation of hypothalamic–pituitary–adrenal axis stress responses in late pregnancy: changes in feedforward and feedback mechanisms. J. Neuroendocrinol. 12, 811–822.

Keefe, D.L., Turek, F.W., 1985. Circadian time keeping processes in mammalian reproduction. Oxf. Rev. Reprod. Biol. 7, 347–400.

Keller-Wood, M., 1994. Corticotropin responses to hypoglycemia and hypotension during ovine pregnancy. Am. J. Physiol. 266, 180–187.

Kimura, N., Arai, K., Sahara, Y., Suzuki, H., Kimura, N., 1994. Estradiol transcriptionally and posttranscriptionally up-regulates thyrotropin-releasing hormone receptor messenger ribonucleic acid in the rat pituitary cells. Endocrinology 134, 432–440.

Kum, S., Eren, U., Sandikci, M., Musal, B., 2006. A histochemical investigation of different distribution of TSH cells in adenohypophysis of female and male rats. Anat. Histol. Embryol. 35, 408–411.

Kuwahara, S., Sari, D.K., Tsukamoto, Y., Tanaka, S., Sasaki, F., 2004. Age-related changes in growth hormone (GH)-releasing hormone and somatostatin neurons in the hypothalamus and in GH cells in the anterior pituitary of female mice. Brain Res. 1025, 113–122.

Llanos, A.C., Crespo, J.A., 1954. Ecología de la vizcacha (*Lagostomus maximus maximus*) en el nordeste de la provincia de Entre Ríos. Rev. Investig. Agric. Extra Nueva Serie 10, 5–95.

Mikami, S., Chiba, S., Hojo, H., Taniguchi, K., Kubokawa, K., Ishii, S., 1988. Immunocytochemical studies on the pituitary pars distalis of the Japanese long-fingered bat, *Miniopterus schreibersii fuliginosus*. Cell Tissue Res. 251, 291–299.

Miranda, L.A., Paz, D.A., Dezi, R., Pisanó, A., 1996. Immunocytochemical and morphometric study on the changes of TSH, PRL, GH and ACTH cells during the development of *Bufo arenarum*. Cell Tissue Res. 283, 125–132.

Mohamed, F., Fogal, T., Dominguez, S., Scardapane, L., Guzmán, J., Piezzi, R.S., 2000. Colloid in the pituitary pars distalis of viscacha (*Lagostomus maximus maximus*): ultrastructure and occurrence in relation to season, sex, and growth. Anat. Rec. 258, 252–261.

Muñoz, E., Fogal, T., Dominguez, S., Scardapane, L., Guzmán, J., Piezzi, R.S., 1997. Seasonal changes of the Leydig cells of viscacha (*Lagostomus maximus maximus*). A light and electron microscopy study. Tissue Cell 29, 119–128.

Muñoz, E.M., Fogal, T., Dominguez, S., Scardapane, L., Piezzi, R.S., 2001. Ultrastructural and morphometric study of the Sertoli cell of the viscacha (*Lagostomus maximus maximus*) during the annual reproductive cycle. Anat. Rec. 262, 176–185.

Navarro, J.A., Gómez, M.A., Sánchez, J., Gómez, S., Bernabé, A., 1991. Structure and ultrastructure of adrenocorticotropic hormone cells in goat in anoestrus, gestation and milk production. Acta Anat. 140, 163–168.

Neumann, I.D., Johnstone, H.A., Hatzinger, M., Liebsch, G., Shipston, M., Russell, J.A., Landgraf, R., Douglas, A.J., 1998. Attenuated neuroendocrine responses to emotional and physical stressors in pregnant rats involve adenohypophysial changes. J. Physiol. 508, 289–300.

O'Reilly, P.J., Dziuk, P.J., 1973. Change in the level of LH in the sera and pituitaries of ovariectomized and intact ewes at parturition. Endocrinology 92, 1575–1578.

Porter, T.E., Hill, J.B., Wiles, C.D., Frawley, L.S., 1990. Is the mammosomatotrope a transitional cell for the functional interconversion of growth hormone- and prolactin-secreting cells? Suggestive evidence from virgin, gestating, and lactating rats. Endocrinology 127, 2789–2794.

Porter, T.E., Wiles, C.D., Frawley, L.S., 1991. Evidence for bi-directional interconversion of mammosomatotrophs and somatotrophs: rapid reversion of acidophilic cell types to pregestational proportions after weaning. Endocrinology 129, 1215–1220.

Roberts, C.M., Weir, B.J., 1973. Implantation in the plains viscacha, *Lagostomus maximus*. J. Reprod. Fert. 33, 299–307.

Schulte, H.M., Weisner, D., Allolio, B., 1990. The corticotrophin-releasing hormone test in late pregnancy: lack of adrenocorticotrophin and cortisol response. Clin. Endocrinol. (Oxf). 33, 99–106.

Šošić-Jurjević, B., Filipović, B., Milošević, V., Nestorović, N., Negić, N., Sekulić, M., 2006. Effects of ovariectomy and chronic estradiol administration on pituitary–thyroid axis in adult rats. Life Sci. 79, 890–897.

Takahashi, S., 1991. Immunocytochemical and immuno-electron microscopical study of growth hormone cells in male and female rats of various ages. Cell Tissue Res. 266, 275–286.

Thomas, G.B., McNeilly, A.S., Gibson, F., Brooks, A.N., 1994. Effects of pituitary–gonadal suppression with a gonadotrophin-releasing hormone agonist on fetal gonadotrophin secretion, fetal gonadal development and maternal steroid secretion in the sheep. J. Endocrinol. 141, 317–324.

Thompson Jr., D.L., Garza Jr., F., St. George, R.L., Rabb, M.H., Barry, B.E., French, D.D., 1991. Relationships among LH, FSH and prolactin secretion, storage and response to secretagogue and hypothalamic GnRH content in ovariectomized pony mares administered testosterone, dihydrotestosterone, estradiol, progesterone, dexamethasone or follicular fluid. Domest. Anim. Endocrinol. 8, 189–199.

Vásquez, F., Gómez, M.A., Serrano, J., Bernabé, A., 2002. Immunocytochemical light- and electron-microscopic studies of growth hormone, prolactin and somatomammotroph cells in female goat. Cells Tissues Organs 170, 258–265.

Vidal, S., Román, A., Moya, L., 1995. Immunohistochemical identification and morphometric study of ACTH cells of mink (*Mustela vison*) during growth and different stages of sexual activity in the adult. Gen. Comp. Endocrinol. 100, 18–26.

Vidal, S., Horvath, E., Kovacs, K., Lloyd, R.V., Smyth, H.S., 2001. Reversible transdifferentiation: interconversion of somatotrophs and lactotrophs in pituitary hyperplasia. Mod. Pathol. 14, 20–28.

Villalobos, C., Núñez, L., García-Sancho, J., 2004. Anterior pituitary thyrotropes are multifunctional cells. Am. J. Physiol. Endocrinol. Metab. 287, 1166–1170.

Weinstock, M., 1997. Does prenatal stress impair coping and regulation of hypothalamic–pituitary–adrenal axis? Neurosci Biobehav. Rev. 21, 1–10.

Weir, B.J., 1971. The reproductive organs of the female plains viscacha, *Lagostomus maximus*. J. Reprod. Fertil. 25, 365–373.

Wise, M.E., Sawyer Jr., H.R., Nett, T.M., 1986. Functional changes in luteinizing hormone-secreting cells from pre- and postpartum ewes. Am. J. Physiol. Endocrinol. Metab. 250, 282–287.