Tissue and Cell 44 (2012) 351–357

Contents lists available at SciVerse ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

Cellular associations of pituitary gonadotrophs in a rodent (*Lagostomus maximus maximus*) with photoperiod-dependent reproduction

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ARTICLE INFO

Article history:
Received 18 November 2011
Received in revised form 17 May 2012
Accepted 25 May 2012
Available online 28 June 2012

Keywords:
Lagostomus
Gonadotrophs
Cellular associations
Photoperiod
Immunohistochemistry

ABSTRACT

The morphological characteristics and percentage of the cellular associations between gonadotrophs (LH- and FSH-secreting cells) and other cellular types were studied in pituitary pars distalis of adult male viscachas (*Lagostomus maximus maximus*) by double immunohistochemistry using specific antibodies to LH, FSH, PRL, GH, ACTH, TSH and S-100 protein (by folliculostellate cells; FSC), during long and short photoperiods. Bihormonal gonadotrophs were observed in ventro-medial and dorsal regions, interspersed between monohormonal gonadotrophs, and their number increased in short photoperiod. LH- and FSH-gonadotrophs were found around lactotrophs, enclosed by somatotrophs in the dorsal region, and associated with irregular corticotrophs. Gonadotrophs and thyrotrophs were associated along blood vessels and follicular structures. The cytoplasmic prolongations of FSC were in contact with both gonadotrophs. The percentage of LH–FSH, LH–ACTH, LH–FSC, FSH–LH, FSH–PRL, FSH–GH, FSH–ACTH, FSH–TSH and FSH–FSC associations decreased, whereas LH–PRL increased in short as compared to long photoperiod. The most abundant associations were LH–GH and LH–TSH during long photoperiod, but LH–GH and LH–PRL during short photoperiod. FSH–GH and FSH–PRL were the most numerous associations, and LH–FSC and FSH–FSC were the less abundant ones in both photoperiods. These results provide the morphological evidence for specific cellular associations between gonadotrophs and other cellular types of viscacha pituitary.

1. Introduction

Changes in the histophysiology of the pituitary gland are induced by annual changes in daylength (photoperiod) in many seasonally breeding species (Goldman, 2001). Pituitary pars distalis (PD) cells are not randomly distributed in the parenchyma, but have special arrangements related to intercellular communication, which is important for the regulation of hormonal secretion (Noda et al., 2001). The gonadotrophs (LH- and FSH-secreting cells) are an important link in the pituitary–gonadal axis and thus in the regulation of reproduction. However, these cells do not work alone. The pituitary homeostasis and adaptation to reproductive needs requires a coordinated action among cells that are part of different hypothalamic–pituitary–target organs axes (Denef, 2008). Our experimental model, the viscacha (*Lagostomus maximus maximus*), is a rodent of seasonal reproduction which exhibits a reproductive cycle synchronized by the environmental photoperiod through the pineal gland and its main hormone, melatonin (Dominguez et al., 1987; Fuentes et al., 2003). Its reproductive activity occurs during the long days of summer and early autumn, and experiences an important testicular regression on short winter days (Aguilera-Merlo et al., 2005; Muñoz et al., 2001). LH-gonadotrophs, lactotrophs, somatotrophs, corticotrophs, thyrotrophs and FSC of pituitary PD presented the highest values of their morphometrical parameters (immunopositive area, number of immunostained cells, major cellular and nuclear diameters) in the reproductive period (long photoperiod). All these cellular types have presented the lowest values of their morphological parameters in the gonadal regression period (short photoperiod), with the exception of the FSH-gonadotrophs, whose morphometrical parameters have shown the lowest values in the reproductive period (Acosta et al., 2010; Filippa et al., 2005; Filippa and Mohamed, 2006a,b, 2008, 2010a). Thus, the distribution and number of cells may affect their topographical relationships, and to analyze cell-to-cell contact is of interest. Moreover, variations of cellular associations resulting from the changes of photoperiod have been poorly investigated. A clarification of the morphological relationships among the different pituitary cellular types constitutes an important step towards the understanding of the nature of cellular association within the pituitary of *Lagostomus maximus maximus*. For this reason, the objectives of this work were to study the morphological characteristics, and to quantify the cellular associations

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0040-8166/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tice.2012.05.003

between gonadotrophs (LH- and FSH-cells) and other cellular types, hormone-secreting cells and folliculostellate cells (FSC) in pituitary PD of adult male viscacha during the long (reproductive period) and short (gonadal regression period) photoperiods.

2. Material and methods

2.1. Experimental animals

The viscachas were captured in their habitat near San Luis, Argentina (33°20′ south latitude, 760 m altitude) during 2007–2008, using traps placed in their burrows. The light phase in San Luis lasts up to 14 h (14L:10D) during summer, with an average temperature of 25 °C. In winter, the light phase decreases to 10 h (10L:14D), and the average temperature is 10 °C. Eight adult male viscachas weighing 5–7 kg were captured during summer (February to March; long photoperiod; four animals) and during winter (July; short photoperiod; four animals). After being captured, the animals were immediately taken to the laboratory and they were intraperitoneally anesthetized with a ketamine (Ketamine Clorhidrate, Holliday Scott S.A.): xilacine (Xilacine Clorhidrate, Richmond Laboratories, Veterinary Division) solution (10:1, w/v, 0.3 ml/kg of body weight), and sacrificed by an intracardiac injection of Euthanyle (0.25 ml/kg body weight, Sodic Penthobarbithal, Sodic Diphenilhidanthoine, Brouwer S.A.). The pituitaries were processed for light microscopy, sagittally sectioned (5 μm thick), and each hemi-pituitary was completely cut following the same design used in previously reported studies (Acosta et al., 2010; Filippa and Mohamed, 2006b). The reproductive condition of viscachas was carefully assessed on the basis of observations by light microscopy of testes. Appropriate procedures were performed to minimize the number of animals used. The experimental design was approved by the local Ethics Committee, and was in agreement with the National Institute of Health (NIH, USA) guidelines for the use of experimental animals. Moreover, the Biodiversity Control Area of the San Luis Ministry of the Environment (Argentina) approved a study protocol to conduct scientific research within the territory of this province (Resolution No. 03 PRN-2011).

2.2. Double immunohistochemistry

This technique was performed with the objective of examining the associations between gonadotrophs (LH and FSH) and other pituitary cells (hormone-secreting cells and FSC). Thus, the studied associations were LH-gonadotrophs–FSH-gonadotrophs (LH–FSH), LH-gonadotrophs–lactotrophs (LH–PRL), LH-gonadotrophs–somatotrophs (LH–GH), LH-gonadotrophs–corticotrophs (LH–ACTH), LH-gonadotrophs–thyrotrophs (LH–TSH), LH-gonadotrophs–FSC (LH–FSC), FSH-gonadotrophs–LH-gonadotrophs (FSH–LH), FSH-gonadotrophs–lactotrophs (FSH–PRL), FSH-gonadotrophs–somatotrophs (FSH–GH), FSH-gonadotrophs–corticotrophs (FSH–ACTH), FSH-gonadotrophs–thyrotrophs (FSH–TSH), and FSH-gonadotrophs–FSC (FSH–FSC).

Details, suppliers, dilution, time and temperature of the incubation of the antibodies used in the immunohistochemical techniques are reported in Table 1. The double immunohistochemical technique was similar to that reported in a previous work (Acosta et al., 2010). Mouse monoclonal antibodies against pituitary LH, or FSH were used for the first labeling (BioGenex, San Ramon, CA, USA). The reaction sites of the first primary antibody (against LH or FSH) were revealed by 3,3′-diaminobenzidine tetrahydrochloride (DAB) chromogen solution in PBS and H_2O_2 substrate solution (Catalog n° QD000-5L; BioGenex, San Ramon, CA, USA), resulting in a brown precipitate. For the second labeling, the slides were incubated with the second primary antibody (against LH, FSH, PRL, GH, ACTH, TSH or S-100 protein). The reaction sites were revealed by New Fuchsin Chromogen Kit (Catalog n° HK 183-5K; BioGenex, San Ramon, CA, USA), resulting in a fuchsia precipitate. DAB and New Fuchsin were selected as chromogens to visualize the antigens, since this combination is known to provide good contrast. The sections were counterstained with Harris' hematoxylin for 30 s, washed for 10 min in running water, and mounted with a permanent aqueous mounting medium (SuperMount, BioGenex). Labeling was assessed using an Olympus BX-40 light microscope.

Table 1
Features of the primary antibodies used in the immunohistochemistry.

Antibody	Immunostained cellular type	Specifications	Time/temperature incubation
anti-human (h)LHβ (luteinizing hormone)	LH-gonadotroph	Clone 3LH 5B6 YH4 Cat. N° AM030-5 M Dilution1:200 BioGenex	12 h, 4 °C in a moist chamber
anti-hFSHβ (follicle stimulating hormone)	FSH-gonadotroph	Clone 83/12/ 2A8 2C7 Cat. N° MU026-UC Dilution 1:100 BioGenex	12 h, 4 °C in a moist chamber
anti-hPRL (prolactin)	Lactotroph	Polyclonal (developed in rabbit) Cat. N° N1549 Ready to Use DakoCytomation	60 min, 4 °C in a moist chamber
anti-hGH (growth hormone)	Somatotroph	Polyclonal (developed in rabbit) Cat. N° 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. N° AR035-5R. Ready to Use BioGenex	60 min, 4 °C in a moist chamber
anti-hTSH (thyroid stimulating hormone)	Thyrotroph	Clone 5404 Cat. N° MU 033-UC Dilution 1:50 BioGenex	12 h, 4 °C in a moist chamber
Anti-S-100 protein	Folliculostellate cell (FSC)	Polyclonal (isolated from bovine brain) Cat. N° AR 058-5R. Ready-to-Use BioGenex	12 h, 4 °C in a moist chamber

In all cases, two control experiments for the specificity of the primary antibody were performed: (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. Rat pituitaries were used as positive controls.

2.3. Morphometric analysis

A computer-assisted image analysis system was similar to that reported in previous works (Acosta et al., 2010; Filippa et al., 2005; Filippa and Mohamed, 2006a,b, 2008, 2010a,b). The sections obtained in the middle sector of the pituitary exhibited the greatest immunostained areas in all the studied animals. Four pituitaries were analyzed in each group of animals, 3 regularly spaced serial sections (100 μm each) were used, and microscopic fields were examined under a 40× objective. One hundred cells (LH-gonadotrophs or FSH-gonadotrophs) were counted in each section with a standard area of 18141.82 μm^2, and the number of contacts with one or more than one cells of the second cellular population (gonadotrophs, lactotrophs, somatotrophs, corticotrophs, thyrotrophs and FSC) were expressed as percentage of cellular association. The number of double immunostained gonadotrophs with a visible nucleus was counted in 10 microscopic fields per section. The result was expressed as the number of cells per microscopic field area.

2.4. Statistical analysis

The results were expressed as means ± standard error of the mean (SEM) for all data sets. Differences between two groups were evaluated using Student's *t*-test. Comparisons of cellular associations of each of the gonadotrophs during long and short photoperiods were evaluated using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test. A probability of less than 0.05 was assumed to be significant.

3. Results

The pituitary PD of adult male 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 (or cephalic, superior, connected with the pars tuberalis) and a caudal one (inferior) (Fig. 1a).

Immunostaining for gonadotrophs revealed the presence of bihormonal cells containing both gonadotrophins, LH and FSH. In the ventro-medial and dorsal regions, numerous cells were doubly labeled. These cells were arranged in groups or isolated, and others were interspersed between monohormonal gonadotrophs, covering the blood vessels and near follicular structures. The number of bihormonal gonadotrophs per field increased significantly during short photoperiod (winter, 4.68 ± 0.78), in relation to the long photoperiod (summer, 2.98 ± 0.62) (Fig. 1b, c).

FSH-gonadotrophs were observed between LH-gonadotrophs grouped or arranged in cellular cords. FSH-gonadotrophs isolated or forming small groups were frequently surrounded by LH-gonadotrophs (Figs. 1b and 2a). The percentage of LH–FSH and FSH–LH associations decreased significantly in short photoperiod with respect to long photoperiod (Fig. 3).

Groups of LH-gonadotrophs and lactotrophs close together were observed in the ventro-medial region. Some cells in these groups had cell-to-cell associations. In addition, LH- and FSH-gonadotrophs were found around lactotrophs, isolated or arranged in small groups of two and three cells. The edge of the lactotrophs in contact with the gonadotrophs was generally observed to be slightly concave in cell-to-cell associations. Occasionally, a short cytoplasmic prolongation of lactotroph extended towards gonadotrophs. In addition, follicular colloids immunostained with anti-prolactin were observed to be surrounded by FSH-gonadotrophs (Figs. 1d and 2b). The percentage of the LH–PRL association increased, while FSH–PRL decreased significantly during winter in relation to summer (Fig. 3).

Isolated gonadotrophs were seen to be enclosed by somatotrophs throughout the PD parenchyma, especially towards the dorsal region. A LH-gonadotroph cytoplasmic prolongation was often found to contact somatotrophs; FSH-gonadotrophs–somatotrophs associations were found to be in contact with blood vessel walls, and in basal position in relation to follicular structures. In the cephalic end, a small sector delimited by long blood vessels where there were numerous gonadotrophs, some of these cells were in contact with only one somatotroph (Figs. 1e and 2c). LH–GH association was not modified by changes in the photoperiod, whereas the FSH–GH association decreased significantly in short photoperiod as compared to long photoperiod (Fig. 3).

The gonadotrophs and corticotrophs were well regionalized in the pituitary PD of viscacha, and both cellular types were located in some areas. Most of the associated gonadotrophs and corticotrophs were isolated cells in the parenchyma. However, corticotrophs were also surrounded by gonadotrophs. The corticotrophs associated with gonadotrophs were often irregular in shape and with cytoplasmic prolongations (Figs. 1f and 2d). LH–ACTH and FSH–ACTH associations decreased significantly during winter in relation to summer (Fig. 3).

The localization in the PD parenchyma described for gonadotrophs and thyrotrophs was very similar. Thus, there were many associated cells along the surface of blood vessels and around follicular structures. Thyrotrophs forming groups were usually observed to be enclosed by gonadotrophs. One or two FSH-gonadotrophs were sometimes found to be encircled by thyrotrophs (Figs. 1g and 2e). In addition, some LH-gonadotrophs had a cytoplasmic prolongation which extended up to thyrotrophs. The LH–TSH association was unchanged with the photoperiod, while FSH–TSH decreased significantly during winter (Fig. 3).

LH- and FSH-gonadotrophs were observed to be associated with FSC that were forming follicular structures, and with those arranged between the endocrine cells. The FSC cytoplasmic prolongations were in contact with or involving gonadotrophs. Gonadotrophs were generally associated with follicular structures in winter (Figs. 1h, i and 2f). The percentage of LH–FSC and FSH–FSC associations decreased significantly in the short photoperiod in relation to the long photoperiod (Fig. 3).

Furthermore, a comparison was made between the associations of each gonadotroph during long and short photoperiods (by ANOVA with data of Fig. 3). In the long photoperiod, the percentages of LH–GH and FSH–GH associations were significantly higher in relation to the other associations. During the short photoperiod, an increase in the percentage of LH–PRL association was observed with respect to LH–FSC, LH–FSH and LH–ACTH associations. The percentages of LH–FSC and FSH–FSC associations were significantly lower than other associations in both photoperiods.

4. Discussion

Pituitary PD cellular types were previously studied in adult male viscachas throughout their annual reproductive cycle. Seasonal variations of the morphological characteristics and morphometric parameters, considered as indicators of cellular activity, were observed in different cellular populations. LH-gonadotrophs, lactotrophs, somatotrophs, corticotrophs, thyrotrophs and FSC presented a higher activity in the reproductive period (summer and

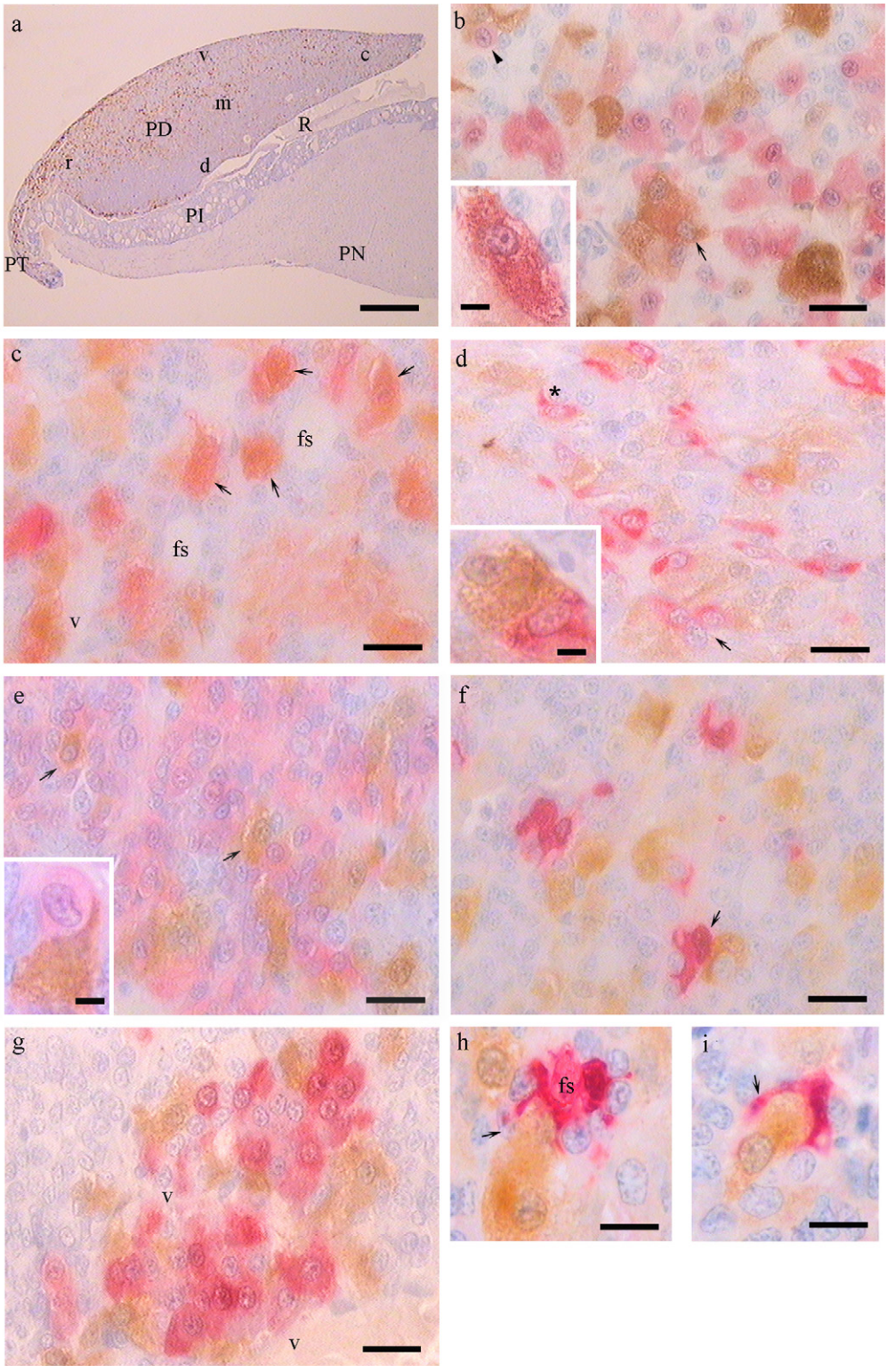

Fig. 1. (a) Sagittal section of an adult male viscacha pituitary showing its different regions: PD, pars distalis; PI, pars intermedia; PT, pars tuberalis; PN, pars nervosa; R, Rathke's pouch; c, caudal end; r, rostral end; v, ventral region; m, medial region; d, dorsal region. Immunohistochemical technique with anti-LH. Scale bar: 500 μm. (b–i) Double immunohistochemistry images of LH-gonadotrophs (brown) and FSH-gonadotrophs, lactotrophs, somatotrophs, corticotrophs, thyrotrophs and FSC-S-100 positive (fuchsia). (b and c) LH–FSH association during long and short photoperiods, respectively. Bihormonal cells (arrows) are arranged in groups or isolated, some of them are near follicular structures (fs). FSH-gonadotroph (arrowhead) is surrounded by LH-gonadotrophs. v, blood vessel. Scale bar: 25 μm. Inset: Higher magnification of a bihormonal gonadotroph. Scale bar: 10 μm. (d) LH–PRL association, there is a small group of lactotrophs in contact with LH-gonadotrophs (arrow). A short cytoplasmic prolongation of lactotroph is extended towards a LH-gonadotroph (*). Scale bar: 25 μm. Inset: Higher magnification showing that the edge of a lactotroph in contact with a gonadotroph is slightly concave. Scale bar: 10 μm. (e) LH–GH association in dorsal region of PD. LH-gonadotrophs are enclosed by somatotrophs (arrows). Scale bar: 25 μm. Inset: Higher magnification of a LH-gonadotroph cytoplasmic prolongation involving a somatotroph. Scale bar: 10 μm. (g) LH–ACTH association, corticotrophs associated with LH-gonadotrophs are irregular in shape and exhibit cytoplasmic prolongations (arrow). Scale bar: 25 μm. (f) LH–TSH associations are along the surfaces of blood vessels (v). Groups of thyrotrophs are enclosed by LH-gonadotrophs. Scale bar: 25 μm. (h and i) LH–FSC association, LH-gonadotrophs are in contact with cytoplasmic prolongation (arrows) of FSC that are forming follicular structures (fs) and with isolated FSC, respectively. Scale bar: 10 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

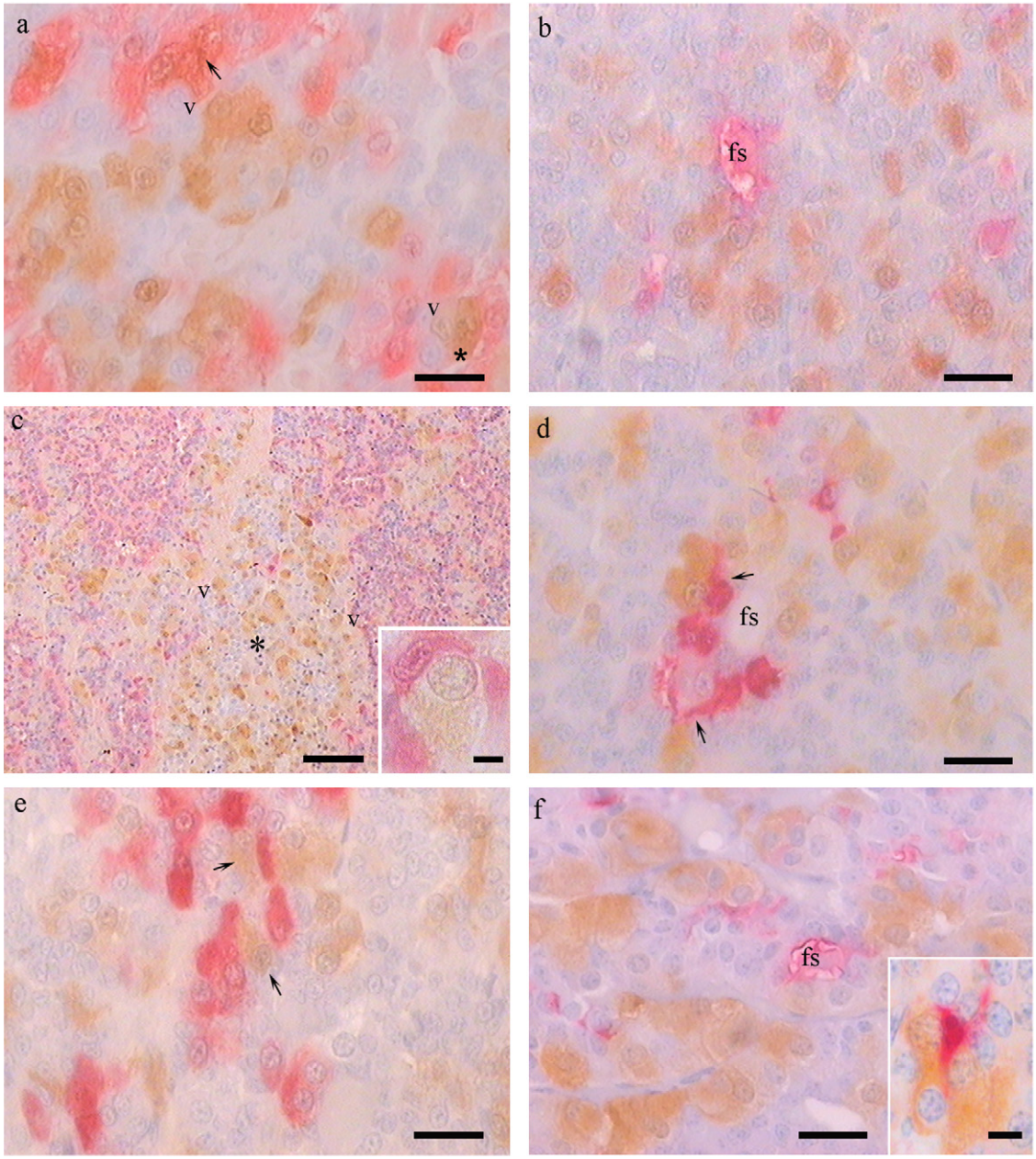

Fig. 2. (a–f) Double immunohistochemistry images of FSH-gonadotrophs (brown) and LH-gonadotrophs, lactotrophs, somatotrophs, corticotrophs, thyrotrophs and FSC-S-100 positive (fuchsia). (a) FSH–LH association, there are FSH-gonadotrophs encircled by LH-gonadotrophs (*). Bihormonal cells (arrow) are interspaced with monohormonal cells along the blood vessels (v). Scale bar: 25 μm. (b) FSH–PRL association, a follicular colloid is immunostained with anti-PRL (fs) and FSH-gonadotrophs are near the follicle. Scale bar: 25 μm. (c) FSH–GH association, in the cephalic end are blood vessels (v) limiting an area with numerous FSH-gonadotrophs (*). Scale bar: 100 μm. Inset: FSH-gonadotroph surrounded by numerous somatotrophs in the dorsal region. Scale bar: 10 μm. (d) FSH–ACTH association, these cells are near a follicular structure (fs) and some cytoplasmic prolongation of irregular corticotrophs are in contact with FSH-gonadotrophs (arrows). Scale bar: 25 μm. (e) FSH–TSH association, there are some FSH-gonadotrophs (arrows) surrounded by thyrotrophs. Scale bar: 25 μm. (f and inset) FSH–FSC association, FSH-gonadotrophs are associated with FSC of the follicle structures (fs) or with isolated FSC. Scale bar: 25 μm and 10 μm, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

early autumn). All these cellular types presented a lower activity in the gonadal regression period (winter) (Filippa et al., 2005; Filippa and Mohamed, 2006a,b, 2008, 2010a; Acosta and Mohamed, 2011). By contrast, FSH-gonadotrophs activity was minimal in the reproductive period, it increased during the gonadal regression period, and it reached its maximum level during the gonadal recovery period. This shows that FSH-gonadotrophs play an important role during spring, when the recovery of the testicular activity of adult male viscachas starts. The variations found among viscachas gonadotroph cells during the annual reproductive cycle suggest FSH and LH have different roles in testicular steroidogenesis and spermatogenesis (Filippa et al., 2005).

The present study shows a complete analysis of gonadotrophs cellular associations with other cellular types of pituitary PD in a rodent that has a particular reproductive cycle, mainly synchronized by the environmental photoperiod. The variations of these associations during the long and short photoperiods in adult male viscacha are also demonstrated. In addition, the presence of bihormonal gonadotrophs has been proved for the first time in this rodent of seasonal reproduction.

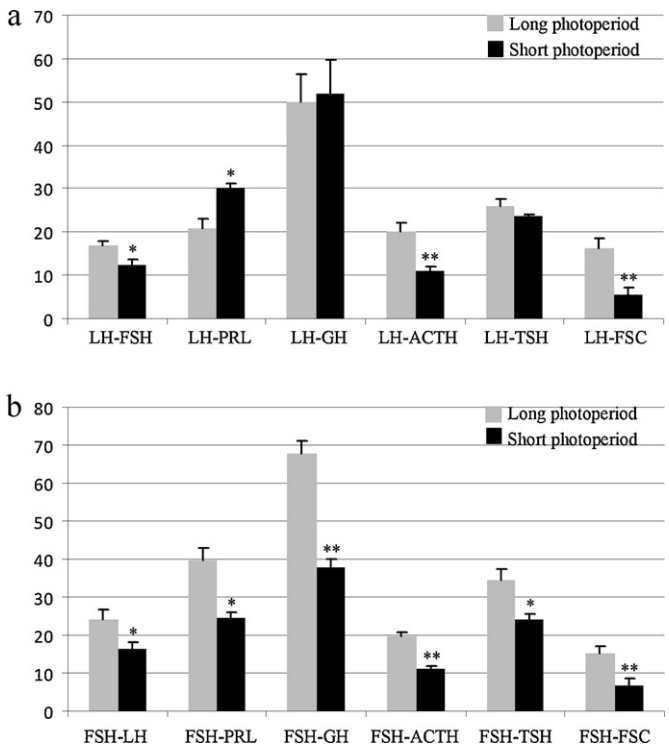

Fig. 3. (a, b) LH- and FSH-gonadotroph associations with other cellular types, respectively. The values represent cellular association percentages (%) and they are expressed as mean ± SEM ($n = 4$). For each cellular association, significant differences were determined by the Student's t-test. $^{*}p < 0.05$: Short photoperiod vs. long photoperiod. $^{**}p < 0.01$: Short photoperiod vs. long photoperiod.

A functional diversity in the hormonal content of gonadotrophs has been reported in other species, some of which have seasonal reproduction (Eagle and Tortonese, 2000; Meeran et al., 2003; Taragnat et al., 1998; Townsend et al., 2004). Filippa et al. (2005) have reported a decrease in the number of LH-gonadotrophs in the short photoperiod as compared to the long photoperiod, and an increase in the number of FSH-gonadotrophs in *Lagostomus*. The present study shows an increase of bihormonal gonadotrophs in the short photoperiod, thus suggesting that some LH-gonadotrophs are turned into bihormonal cells during winter. The heterogeneity in the hormonal storage pattern might represent different functional states of a single cellular type, probably according to the period of the annual reproductive cycle. These results agree with those reported in previous studies (Filippa et al., 2005), suggesting that an increased FSH is needed for promoting spermatogenesis in the gonadal recovery period (spring). Morphological associations between gonadotrophs and lactotrophs have been widely studied in different species (Allaerts et al., 1991; Tortonese et al., 2001; Townsend et al., 2004). The concavity of cup-shaped lactotrophs (CSL) has been reported to be the site of contact with gonadotrophs, and they have also been found to be surrounded by lactotrophs clusters (Cónsole et al., 1999; Denef et al., 1989; Nogami and Yoshimura, 1982; Sato, 1980). Townsend et al. (2004) have shown that these intercellular associations are affected by both gonadal status and season in the equine pituitary. Other authors have reported that gonadotrophs are surrounded by somatotrophs (Wong et al., 1998) and that LH cells cytoplasmic processes surround GH cells (Watanabe, 1985), which are both morphologic facts that suggest that some peptides in gonadotrophs perform a paracrine regulation on GH synthesis and secretion stimulation (Denef, 2008).

It is well known that stress-induced activation of the hypothalamic–pituitary adrenal (HPA) axis inhibits the hypothalamic–pituitary gonadal (HPG) axis (Ferin, 1999; Shalts et al., 1994). At the pituitary level, the negative interrelation between the HPA and HPG axis may be mediated by paracrine negative signals (Tilbrook and Clarke, 2006). Montuenga et al. (2000) have reported that gonadotrophs might positively and negatively interact with corticotrophs through peptides located in gonadotrophs modulating the corticotroph activity. Cónsole et al. (1999) have observed that a small number of gonadotrophs are in contact with corticotrophs in rats. Thyrotrophs have attracted relatively less attention regarding their role in paracrine interactions. However, in order to meet particular physiological needs in the body, the function of these cells also needs to be coordinated with that of other pituitary cellular types (Denef, 2008). In this sense, Watanabe (1985) has reported that thyrotrophs were closely apposed to LH-gonadotrophs in some regions of mouse PD.

In this study, variations of viscacha PD cellular associations during long (reproductive period) and short (gonadal regression period) photoperiods were observed. The number of LH–FSH, FSH–LH, FSH–PRL and FSH–GH associations decreased in the short photoperiod, whereas the amount of LH–PRL associations was seen to increase. A high degree of morphological associations of gonadotrophs with lactotrophs and somatotrophs would facilitate intercellular regulation. The morphological characteristics and variations of association percentages suggest the existence of specific paracrine mechanisms, and might represent the morphological basis for the differential regulation of the synthesis and secretion of gonadotrophins during the annual reproductive cycle. LH–ACTH and FSH–ACTH associations vary with changes in the photoperiod, being less numerous in the short than in the long photoperiod. Moreover, the most irregular corticotrophs were more frequently associated with gonadotrophs, and probably corresponded to a corticotroph subpopulation that can inhibit gonadotroph activity during winter. Gonadotrophs and thyrotrophs regionalization has been shown to be very similar in viscacha PD (Filippa et al., 2005; Filippa and Mohamed, 2008), thus a large number of associations between them are likely to be found. Unlike LH–TSH association, FSH–TSH association has been observed to decrease in winter; also, FSH-gonadotrophs have been observed to be surrounded by thyrotrophs. These data suggest that thyrotrophs might exert a differential regulation on FSH-gonadotrophs.

FSC have been determined to be the major source of follistatin, and for this reason, they are the main cells, responsible for making a difference in the regulation of gonadotrophins synthesis and secretion (Bilezikjian et al., 2003). Townsend et al. (2004) have reported a high frequency of connections between gonadotrophs and FSC, and have also suggested that FSC would act as mediators in gonadotrophs–lactotrophs interactions. The present study in viscachas PD demonstrates and quantifies the morphological associations between the gonadotrophs and FSC. These associations decreased and were mainly observed with FSC forming follicular structures in the short photoperiod. FSC between endocrine cells and those forming follicles might have different effects on the regulation of gonadotrophs. Besides, FSC may be possibly involved in the signal integration between gonadotrophs and other PD endocrine cells in this rodent.

Finally, double immunohistochemistry provided morphological evidence for the existence of specific associations between gonadotrophs and other cellular types of viscacha pituitary PD. Most gonadotroph cellular associations are probably influenced by the environmental photoperiod, suggesting the existence of specific regulatory mechanisms leading to the achievement of an integrated response of gonadotrophs to regulate seasonal reproduction of this rodent. Thus, this first study of gonadotroph associations demonstrated that there is a specific cytological configuration evidencing the presence of intrapituitary

communication between gonadotrophs and other cellular types, according to the reproductive condition requirements.

Acknowledgements

We wish to thank Mrs. A. Bernardi and Mr. J. Arroyuelo for their technical participation. This work was supported by Project 22/Q003, *Secretaria de Ciencia y Técnica*, *Universidad Nacional de San Luis*.

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