Original Paper

Cells Tissues Organs

Cells Tissues Organs 2008;188:310–319
DOI: 10.1159/000123202

Accepted after revision: November 8, 2007
Published online: March 28, 2008

Characterization of Pituitary Cell Populations in Rats with Induced Polycystic Ovaries

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Key Words
Pituitary · Polycystic ovaries · Rat

Abstract
Several hypotheses have been proposed about the pathogenesis of polycystic ovarian syndrome (PCOS), however, the fundamental physiological interactions that initiate the development of follicular cysts have not yet been elucidated. Hence, in this study the proliferation, density and population of gonadotrophs, mammotrophs, somatotrophs and corticotrophs of the pituitary glands of rats with induced follicular cysts have been investigated by 2 experimental models (continuous light exposition and estradiol valerate-treated rats). Specific immunoreactivity associated with follicle-stimulating hormone, luteinizing hormone, prolactin, growth hormone and adrenocorticotropic hormone was evaluated by immunohistochemistry with specific hormone antibodies and proliferation of secretory cells by their colocalization (double-labeling) with proliferating cell nuclear antigen. The results indicate a reduction in the density and proliferation of gonadotrophs in both experimental groups. A reduction in the average density, proliferation and population of lactotrophs and corticotrophs was also observed in estradiol valerate-treated animals. However, no significant differences were found in somatotrophs. The present study contributes to the information about alterations of some cell populations that occur in the pituitary gland of rats with polycystic ovaries, and will enhance our understanding of the pathogenesis of this disease.

Abbreviations used in this paper

ACTH	adrenocorticotrophic hormone
AEC	amino-ethyl-carbazol
ANOVA	analysis of variance
DAB	diaminobenzidine
EV	estradiol valerate
FSH	follicle stimulating hormone
GH	growth hormone
GnRH	gonadotrophic releasing hormone
LH	luteinizing hormone
PCNA	proliferating cell nuclear antigen
PCO	polycystic ovaries
PCOS	polycystic ovarian syndrome
PRL	prolactin
PVC	persistent vaginal cornification
RIA	radioimmunoassay
SEM	standard error of mean

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Introduction

Ovarian cysts are persistent anovulatory follicular structures in the absence of corpus luteum that spontaneously occur in several animal species (cattle, sheep, goats, pigs, dogs and rodents) and human beings [Savio et al., 1990; Campion et al., 1996; López-Dıaz and Bosu, 1997; Abbott et al., 2002; Xita et al., 2002; Vanholder et al., 2006].

In women, polycystic ovarian syndrome (PCOS) is characterized by reproductive manifestations that include anovulation, hyperandrogenism, reduced fertility, increased fetal wastage and associated metabolic features comprising insulin resistance, dyslipidemia and premature atherosclerosis [Barontini et al., 2001]. Many studies have demonstrated the heterogeneity of this disorder and unveiled multiple additional abnormalities within and outside the hypothalamic-pituitary-ovarian axis. However, the primary pathophysiological mechanisms fundamental in this disorder remain unknown.

Although the pathogenesis of PCOS is still controversial, an array of plausible pathophysiologies has emerged over the last several decades of study. Inappropriate gonadotrophin secretion with elevated luteinizing hormone (LH) and relatively low follicle-stimulating hormone (FSH) secretion is typical. This pattern of gonadotrophin secretion is related to accelerated gonadotrophin-releasing hormone (GnRH) pulse generator activity and could promote hyperandrogenism and impaired follicle maturation.

Several studies have confirmed an increase in LH levels with normal to suppressed levels of FSH in PCOS compared with expected gonadotrophin output in healthy women during the follicular phase of the menstrual cycle [Barontini et al., 2001]. These findings have led to the notion that an elevated LH/FSH ratio is a neuroendocrine hallmark of PCOS.

Ovarian cysts are not regarded as a primary disease, but as a symptom of an impaired endocrine function. The association between the occurrence of cysts and endocrine alterations in humans and farm animals suggests that the origin of the disorder is at the hypothalamic and/or pituitary level. Studies about spontaneous cases of PCOS have several limitations. It is impossible to determine for how long the cystic structures might have existed before the diagnosis. In addition, the circumstances affecting ovarian function during the diagnosis may be different from the conditions prevailing during the study. Therefore, most of the earlier studies on PCOS are based only on endocrine data, without information about the cellular dynamics of the pituitary gland [Barontini et al., 2001; Doi et al., 2005]. As a result, it is helpful to induce ovarian cysts experimentally in order to monitor their development closely [Baravalle et al., 2007; Ortega et al., 2007].

Several experimental models for polycystic ovaries (PCO) have been developed in rats. Estradiol valerate (EV) has been employed to induce this syndrome and cause a sudden appearance of PCO due to disturbances in metabolic and physiologic processes [Schulster et al., 1984; Farookhi et al., 1985]. A simple method to induce the disease is to expose mature rats to an environment with constant light. This induces cysts gradually, similar to PCOS, and is also the least invasive of all methods developed till now [Salvetti et al., 2003, 2004a, b; Baravalle et al., 2007].

In this study, utilizing different experimental models, we tested the hypothesis that the pituitary gland plays a role in the pathogenesis of PCO. Although serum hormone profiles and ovarian function after the establishment of the PCO state have been characterized extensively in both animals and women, the fundamental physiological interactions that initiate the development of ovarian follicular cysts have yet to be elucidated. Any experimental model could be ideal for studying this complex disease, since the hypothalamic-pituitary system is altered in all models. Hence, in this study we have investigated the proliferation, density and population of gonadotrophs, mammotrophs, somatotrophs and corticotrophs from the pituitary glands of rats with PCO induced in 2 experimental models.

Materials and Methods

Animals and Treatment

Female Wistar rats (175–200 g) were provided by the Center for Experimental Biology and Laboratory Animals Sciences, Faculty of Veterinary Sciences, National University of Litoral. Before the experiment, the animals were kept under controlled cycles of light and darkness (lights on from 6:00 to 20:00 h), at 18–22°C with free access to water and commercial food (Cargill, Buenos Aires, Argentina). Lighting was provided by banks of General Electric 4 cool white 40W fluorescent tubes at a light intensity of 350 lx 1 m above floor [Salvetti et al., 2004a]. All procedures were carried out according to the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996).

Thirty animals displaying at least 2 normal 5-day estrus cycles just before treatments were allocated in 3 groups (n = 10). Rats in the control group (C) received an equivalent volume of vehicle and were sacrificed after 10 weeks, in proestrus. Animals in the continuous light group (L) were placed in the conditions described, except that light exposure was extended to 24 h and the

Table 1. Used antibodies, suppliers and dilutions

Antibodies	Clone	Supplier	Dilution
Primary antibodies			
PCNA	PC-10	Novocastra, Newcastle, UK	1:200
LH	polyclonal	NIDDK-NIH, Bethesda, USA	1:5,000
FSH	polyclonal	NIDDK-NIH	1:2,000
ACTH	polyclonal	NIDDK-NIH	1:3,000
GH	polyclonal	BioGenex, San Ramon, Calif., USA	prediluted
PRL	polyclonal	BioGenex	prediluted
Secondary antibodies			
Anti-rabbit IgG	polyclonal	Zymed, San Francisco, Calif., USA	1:300
Anti-mouse IgG	polyclonal	Chemicon, San Francisco, Calif., USA	1:120

rats were sacrificed after 105 days [Salvetti et al., 2004a]. In the EV-treated group, the animals were injected intramuscularly with a single dose of EV 2 mg (Progynon Depot; Schering, Buenos Aires, Argentina) and sacrificed after 60 days following protocols described previously [Farookhi et al., 1985; Baravalle et al., 2007]. Animals of the C and EV groups were kept with lights on from 6:00 to 20:00 h.

Vaginal Smears

Vaginal smears of all animals were examined daily under a phase contrast microscope for the relative abundance of nucleated epithelial cells, cornified cells and leukocytes. Five-day cycles were considered regular. The observation of cornified cells in the smears for a minimum of 10 consecutive days was defined as persistent vaginal cornification (PVC) and considered an indication of follicular cystic development [Salvetti et al., 2004a].

Tissue Sampling

All animals were killed by decapitation at around 9:00 h, trunk blood was collected and the serum stored at –20°C until use for hormone assays. Pituitary glands were carefully dissected, weighed and fixed in 10% buffered formalin for 6 h at room temperature, then washed in phosphate-buffered saline. For light microscopy, fixed tissues were dehydrated in an ascending series of ethanol, cleared in xylene, and embedded in paraffin. A series of 5-μm-thick axial sections were cut from each pituitary for immunohistochemistry and histological analysis. The sections were mounted on slides previously treated with 3-aminopropyltriethoxysilane (Sigma-Aldrich, St. Louis, Mo., USA) and stained with hematoxylin and eosin for preliminary observation. The ovaries were processed with the same protocol and stained with hematoxylin and eosin.

Immunohistochemistry

Details and concentrations of the antibodies used are summarized in table 1. The streptavidin-biotin immunoperoxidase method was used as described earlier [Ortega et al., 2004; Salvetti et al., 2004b; Sanchez-Criado et al., 2005]. Diaminobenzidine (DAB, Liquid DAB-Plus Substrate Kit; Zymed, San Francisco, Calif., USA) and aminoethylcarbazol (AEC; BioGenex, San Ramon, Calif., USA) were selected as chromogens to visualize the antigens because this combination is known to give good contrast in double-labeled immunohistochemistry.

Sections were deparaffinized, hydrated and given microwave pretreatment (antigen retrieval). Endogenous peroxidase activity was inhibited with 1% H_2O_2 and nonspecific binding was blocked with 10% normal goat serum. All sections were incubated with proliferating cell nuclear antigen (PCNA) antibody for 1 h and with rat-preabsorbed biotinylated goat anti-mouse antibody for 30 min at room temperature. Antigens were visualized by the streptavidin peroxidase method (BioGenex) and DAB was used as the first chromogen. For the second labeling, sections were microwaved at 800 W for 5 min in citrate buffer (0.01 M, pH 6) to eliminate massive nonspecific binding and then incubated with 10% normal goat serum for 30 min at room temperature. The sections were incubated with each pituitary hormone antibody for 18 h at 4°C and then processed as mentioned above, with goat anti-rabbit streptavidin peroxidase followed by AEC substrate instead of DAB. Finally, the slides were washed in distilled water and counterstained with Mayer's hematoxylin, washed in distilled water, and mounted with nonaqueous permanent mounting medium (Ultramount; Dako, Carpinteria, Calif., USA). To verify specificity, adjacent control sections were subjected to the same immunohistochemical method, replacing primary antibodies with rabbit and mouse nonimmune sera.

Each pituitary hormone antibody was probed (absorption test) with the specific hormone (100 mg/ml; supplied by Dr. Parlow, NIH-NHIDDK and Sigma-Aldrich). To exclude the possibility of nonsuppressed endogenous peroxidase activity, some sections were incubated with DAB reagent alone.

Image Analysis

Image analysis was performed using Image Pro Plus 3.0.1 (Media Cybernetics, Silver Spring, Mass., USA). Images were digitized by a CCD color video camera (Motic 2000; Motic China Group, Xiamen, China) mounted on top of a conventional light microscope (Olympus BH-2; Olympus Co., Tokyo, Japan) using an objective magnification of ×40. Resolution of the images was set to 1,200 × 1,600 pixels. Each pixel of the image corresponded to 0.13 μm at the used magnification and each field represented a tissue area of 0.031 mm^2. The system captured each image and automatically corrected for background. This prevented differen-

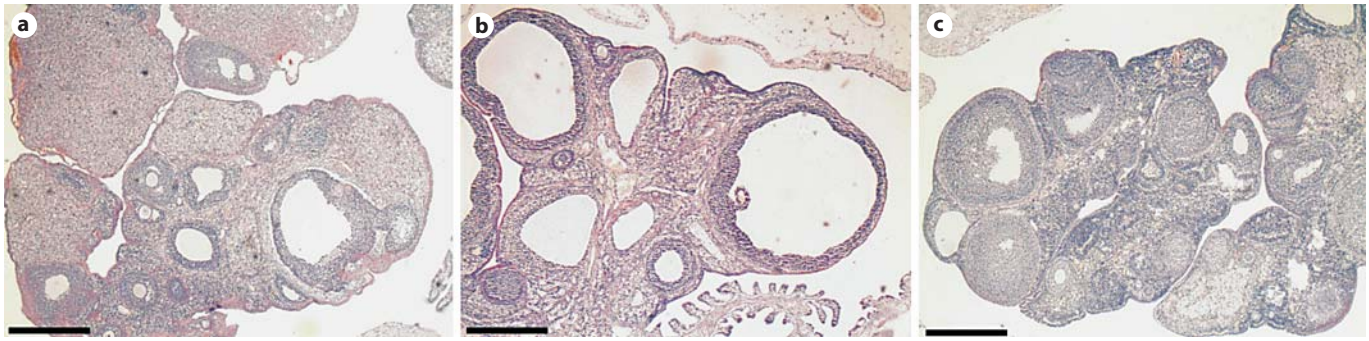

Fig. 1. Sections of ovaries from control (proestrus; **a**), constant light-exposed (**b**) and EV-treated (**c**) rats with hematoxylin and eosin staining. Scale bars = 500 μm.

tial readings due to different lighting conditions. No further image processing was done. Since the pituitary gland does not have an even distribution of secretory cells due to a ventromedial sex zone, the image analysis was performed in microscopic fields covering the entire axial section.

The average density for each antibody reaction was calculated in all images as a percentage of total area evaluated through color segmentation analysis, which extracts objects by locating all objects of a specific color (red stain). Red stain (AEC) was selected and a mask was applied to make a permanent separation of colors. The images were then transformed to a bilevel scale TIFF format [Salvetti et al., 2004a; Ortega et al., 2007]. Changes in percentages and proliferation of each secretory cell type were evaluated by counting 2,000 labeled and unlabeled cells per pituitary gland.

Hormone Assays

Serum levels of FSH and LH were determined by radioimmunoassay (RIA) using the kit provided by NIDDK (Bethesday, Md., USA) as described earlier [Ortega et al., 2004; Baravalle et al., 2007]. Intra- and interassay coefficients of variation for LH and FSH were less than 8 and 12%, respectively. Minimum detectable concentrations were 0.16 and 1.18 ng/ml of serum for LH and FSH, respectively.

Serum estradiol and progesterone were estimated by RIA using highly specific antiserum provided by Dr. G.D. Niswender (NIDDK) [Korenman et al., 1974]. Labeled hormones were purchased from Amersham (Little Chalfont, UK). Assay sensitivity for estradiol was 1.7 pg and intra- and interassay coefficients of variation were 9.3 and 11.4%, respectively. Assay sensitivity for progesterone was 50 pg and intra- and interassay coefficients of variation were 7.5 and 11.9%, respectively [Chamson-Reig et al., 1999; Baravalle et al., 2007].

Serum testosterone was determined by RIA using a specific antiserum provided by Dr. G.D. Niswender (NIDDK) after ethyl ether extraction. Labeled hormone was purchased from New England Nuclear (Boston, Mass., USA). Assay sensitivity was as follows: the lowest point in the testosterone standard curve was 12.5 pg and the lowest detectable concentration while extracting 150 μl serum was 208.3 pg/ml. Intra- and interassay coefficients of variation were 7.8 and 12.3%, respectively [Bianchi et al., 2004; Baravalle et al., 2007].

Statistics

The number of 10 individuals per group resulted from a sample size calculation that evaluated the number of individuals necessary to produce an estimation of immunoreactive cell density which would fall within 0.4 units of the real value. The formula used was: $n = Z^2 \times SD^2/d^2$, where n is the sample size, Z the level of confidence (1.96 for 95%), SD is the standard deviation (0.3) and d = 0.4. Because we were able to reject the null hypothesis in most cases, type 2 errors were not considered a problem.

Distribution of data was tested for normality using the Kolmogorov-Smirnov test and data were analyzed by one-way ANOVA. Means were compared using Duncan's multiple range test. Pearson's coefficient was used to analyze a possible correlation between the different variables studied. All statistics were calculated using the SPSS software (version 11.0 for Windows; SPSS Inc., Chicago, Ill., USA). For all tests, statistical significance was accepted at $p < 0.05$. Results were expressed as means ± SEM.

Results

Estrus Cycles

The animals of the C group showed normal estrus cycles during the whole experiment. In the L group, all animals showed irregular cycles within 3 weeks and PVC within 13 weeks of permanent light exposure. In the EV group, all animals displayed irregular cycles within 1 week of EV treatment and most (90%) showed PVC by 3 weeks after injection.

Ovarian Morphology

Ovaries from the C group exhibited follicles in various stages of development. Growing primary, secondary and tertiary follicles were observed in proestrus. In the L group, follicles in development were observed in addition to follicles with evidence of atresia. Many large cysts with thickened granulosa cell layer or large cystic follicles with scant granulosa cells were also seen. Corpus luteum was

Table 2. Weight, proliferation and cellular density of pituitary glands of controls (C), continuous light-exposed (L) and EV-treated rats (EV)

	C	L	EV
Weight of pituitary glands, mg	13.64 ± 1.06[a]	17.8 ± 1.09[a]	29.71 ± 3.15[b]
Total cellular proliferation, %	1.85 ± 0.28[a]	0.49 ± 0.26[b]	1.19 ± 0.25[a]
Cellular density, cells/mm^2	8,842.69 ± 190.07[a]	9,711.89 ± 615.46[a]	8,719.69 ± 674.05[a]

Data are presented as means ± SEM. Different superscript letters indicate statistical differences ($p < 0.05$).

Table 3. Quantitative analysis of cellular population of pituitary cells in controls (C), continuous light-exposed (L) and EV-treated rats (EV)

	C	L	EV
LH immunoreactive cells			
Average density	1.69 ± 0.28[a]	0.87 ± 0.09[b]	0.61 ± 0.16[b]
Proliferation	4.43 ± 0.25[a]	0.14 ± 0.03[b]	0.21 ± 0.04[b]
Population	1.16 ± 0.34[a]	0.08 ± 0.03[b]	0.29 ± 0.18[b]
FSH immunoreactive cells			
Average density	1.87 ± 0.26[a]	0.69 ± 0.06[b]	1.04 ± 0.14[b]
Proliferation	2.11 ± 0.68[a]	0.23 ± 0.18[b]	0.21 ± 0.19[b]
Population	1.95 ± 0.28[a]	2.04 ± 0.51[a]	1.52 ± 0.43[a]
ACTH immunoreactive cells			
Average density	1.03 ± 0.41[a]	0.93 ± 0.23[a]	0.44 ± 0.12[b]
Proliferation	3.49 ± 1.12[a]	0.42 ± 0.27[b]	0.28 ± 0.17[b]
Population	2.46 ± 0.76[a]	1.83 ± 0.31[a]	0.77 ± 0.14[b]
GH immunoreactive cells			
Average density	0.29 ± 0.05[a]	0.31 ± 0.09[a]	0.55 ± 0.13[a]
Proliferation	1.14 ± 0.31[a]	1.65 ± 0.47[a]	0.95 ± 0.59[a]
Population	2.57 ± 0.93[a]	3.06 ± 1.57[a]	2.24 ± 0.58[a]
PRL immunoreactive cells			
Average density	0.49 ± 0.04[a]	0.37 ± 0.16[a]	0.15 ± 0.02[b]
Proliferation	1.73 ± 0.28[a]	1.56 ± 0.64[a]	0.03 ± 0.01[b]
Population	1.22 ± 0.08[a]	1.27 ± 0.22[a]	0.48 ± 0.09[b]

Data are presented as means ± SEM. Different superscript letters indicate statistical differences ($p < 0.05$).

absent and hyperplasia of interstitial glands was observed. The ovaries from the EV group showed follicular cysts, although primary and early secondary follicles were present. There was a conspicuous absence of both large secondary and tertiary follicles as well as corpus luteum (fig. 1).

Pituitary Gland Weight and Proliferation

Pituitary gland weights were similar in C and L rats (13.64 ± 1.65 mg and 17.8 ± 1.09 mg, respectively). However, pituitary glands from EV rats were significantly heavier (29.71 ± 3.15 mg, $p < 0.05$) than those from C and L rats.

Total cellular proliferation was significantly reduced in the pituitary gland of L rats (0.49 ± 0.26%) compared to C and EV rats (1.85 ± 0.28% and 1.19 ± 0.25%, respectively, $p < 0.05$). No differences were found among the groups in cellular density ($p > 0.4$; table 2).

Quantitative Analysis of Cellular Populations

Results of the quantitative analysis are summarized in table 3. Specific immunoreactivity associated with each antibody was seen clearly after immunodetection of FSH, LH, prolactin (PRL), growth hormone (GH) and adrenocorticotropic hormone (ACTH) and was evaluated as average density (percentage of the area occupied by the reaction). Colocalization studies allowed the observation of proliferating secretory cells when double-labeled with PCNA and specific hormone antibodies were evidenced (fig. 2).

Gonadotrophs. The average density, proliferation and population of LH cells were smaller in treated animals (L and EV) than in controls ($p < 0.05$). Similar observations were made for FSH cells, except for the population of positive cells that did not differ significantly ($p > 0.7$).

Lactotrophs and Corticotrophs. No significant differences were found between C and L animals. In the EV group, a reduction in the average density, proliferation and population of PRL and ACTH immunoreactive cells was observed ($p < 0.05$).

Somatotrophs. No significant differences were found between groups.

Hormone Levels

Hormone serum levels are shown in table 4. The 2 treatments induced a significant reduction in LH ($p < 0.5$) without differences in FSH levels ($p > 0.8$) in relation to control animals. Estradiol serum levels were 2-fold

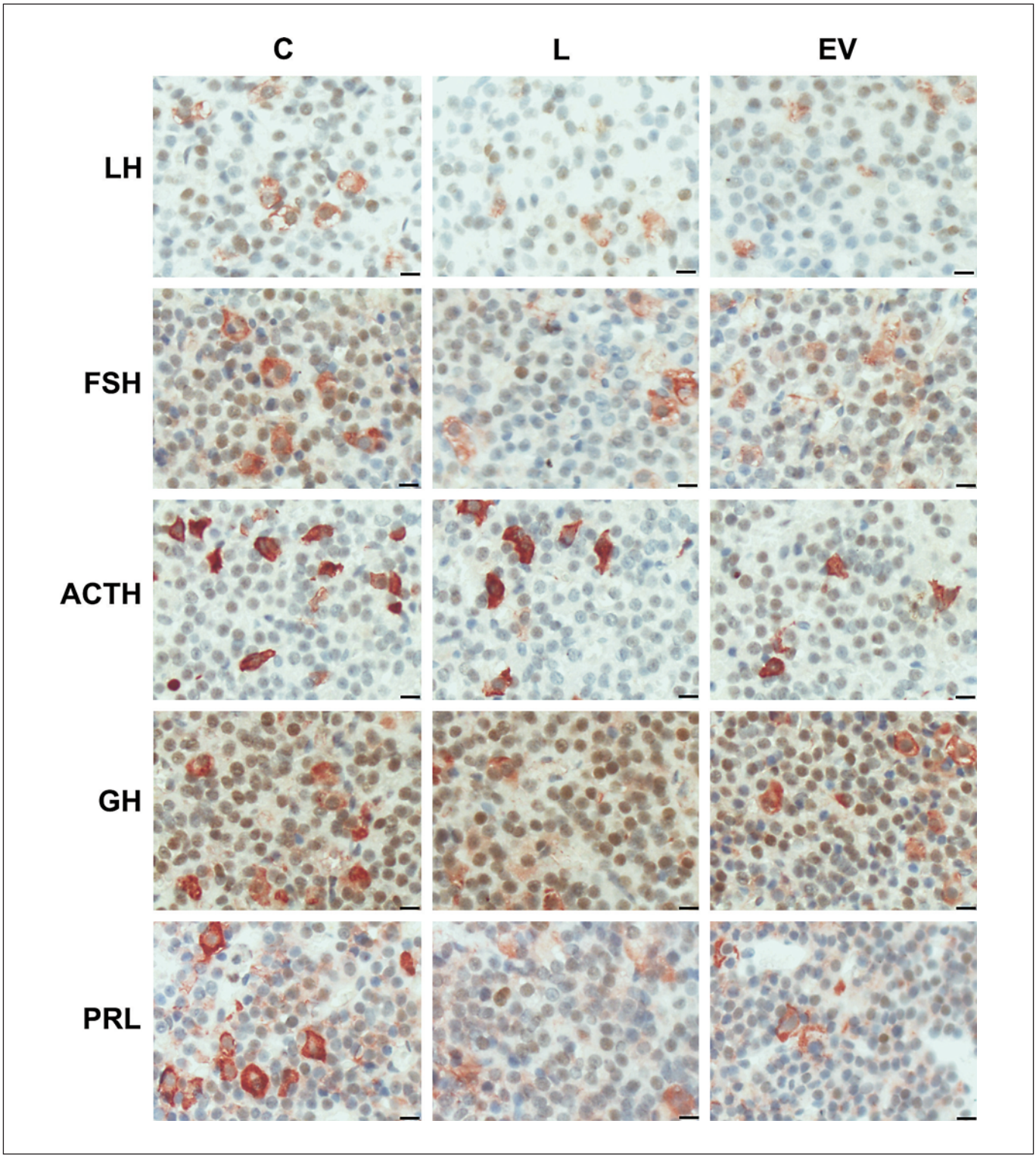

Fig. 2. Sagittal sections of the pituitary gland of controls (C), continuous light-exposed (L) and EV-treated rats (EV), showing the patterns of staining observed after immunodetection of various pituitary hormones (LH, FSH, ACTH, GH and PRL) (red) and the colocalization (double-labeling) of PCNA (brown). Scale bars = 10 μm. A reduction in the density and proliferation of gonadotrophs in both experimental groups was observed. Also, a reduction in the average density, proliferation and population of lactotrophs and corticotrophs was observed in EV-treated animals. No significant differences were found in somatotrophs.

Table 4. Hormonal levels in controls (C), continuous light-exposed (L) and EV-treated rats (EV)

	C	L	EV
Progesterone, ng/ml	42.26 ± 6.17[a]	33.36 ± 9.82[a]	4.58 ± 0.71[b]
Testosterone, pg/ml	454.79 ± 52.74[a]	175.21 ± 42.73[b]	96.20 ± 15.71[c]
Estradiol, pg/ml	30.33 ± 10.15[a]	23.51 ± 2.65[a]	65.07 ± 15.31[b]
LH, ng/ml	2.53 ± 1.26[a]	0.14 ± 0.04[b]	0.13 ± 0.02[b]
FSH, ng/ml	4.10 ± 1.54[a]	4.65 ± 1.65[a]	3.31 ± 1.31[a]

Data are presented as means ± SEM. Different superscript letters indicate statistical differences ($p < 0.05$).

greater and progesterone more than 8-fold lower in EV-treated animals compared to L and C groups ($p < 0.05$).

Stronger correlations between mean LH serum levels and the average density ($R = 0.658$; $p = 0.01$) and population of LH cells ($R = 0.781$; $p = 0.001$) were observed. In contrast, no significant correlations were detected between FSH serum levels and FSH immunoreactive cells.

Discussion

The etiology and pathogenesis of PCOS has been the subject of numerous hypotheses, however, they are not yet completely clear [Barberi, 1991; Hayden and Balen, 2006; Eggers et al., 2007; Homburg, 2007; Norman et al., 2007]. Despite being one of the most common endocrinopathies in women, a comprehensive integrated theory of the pathophysiology of PCOS has not been advanced [Singh, 2005; Homburg, 2007; Norman et al., 2007].

Cellular proliferation in the pituitary has been studied by different methods and the number of dividing cells in the anterior pituitary has been found to be high during development but diminishes in adult animals [Carbajo-Pérez et al., 1989; Carbajo-Pérez and Watanabe, 1990]. In the present study, it was found that constant light exposure lowered the total proliferation index and had similar effects on gonadotroph and corticotroph proliferation. On the other hand, EV treatment decreased the proliferation of gonadotrophs, corticotrophs and lactotrophs, but induced enlargement of the anterior pituitary. This could be due to changes in the proliferation/apoptosis balance or to a hyperplasia or hypertrophy. In this sense, chronic administration of estrogen or long-acting estrogenic hormone preparations is known to produce this effect [Díaz-Torga et al., 1998; Suárez et al., 2002].

Although several studies have described gonadotrophin abnormalities in PCOS, the mechanism underlying the altered LH/FSH ratio has remained unclear. Earlier pituitary function studies (performed at 8–10 weeks after EV injection) demonstrated a markedly reduced pituitary content of LH associated with low basal plasma LH levels and reduced pituitary capacity to respond to GnRH [Hemmings et al., 1983]. In the present study, the area occupied by LH cells, LH concentration in serum, proliferation and population were found to be higher in control animals than in both treated groups. In contrast, although proliferation and density of FSH cells diminished in treated animals, the serum level was unaffected.

Since regulation of LH is causally linked to the GnRH signal, whereas FSH is relatively independent of GnRH [Culler and Negro-Vilar, 1986; Carriere et al., 1988], the authors suggest that the alterations in LH cell function may be secondary to changes in either the amount or pattern of GnRH release. Also, in the EV model, both LH and FSH initially decrease significantly after EV treatment and both gonadotrophins then begin a trend toward recovery. The fluctuations in FSH may not be biologically significant because at its nadir (11 days), plasma FSH is still quite high, as is further indicated by the large number of follicles that develop to the large secondary stage. On the other hand, LH falls to a very low level. This decrease in LH clearly precedes the decreased ovarian weight and the increased follicular atresia [Brawer et al., 1986]. Also, it has been proposed that the lack of gonadotrophin surge (LH and/or FSH) may be the primary cause of alterations in either ovulation or atresia, thus the tertiary follicles are arrested as cysts [Schulster et al., 1984].

We found a reduction in corticotroph proliferation in both treated groups and the area occupied by ACTH cells was lower in the EV group. Singh [1969] has postulated that constant light acts on the release of ACTH. However, their earlier work shows that constant light-induced PCO takes place without a significant increase in corticoste-

rone levels (an index for adrenal activity and ACTH stimulation) [Baravalle et al., 2007]. The study by Prata Lima et al. [2004] suggests the participation of the pineal gland, and not the adrenal gland, in the pathogenesis of constant light-induced PCO in rats. Many studies have investigated the presence of adrenal abnormalities in women with PCOS [Ayers, 1982; Ciampelli et al., 2000]. Lucky et al. [1986] found that functional hyperresponsiveness to ACTH was the most common abnormality. On the other hand, circulating levels, as well as diurnal rhythms of ACTH, were found to be similar in healthy women and women with PCOS, suggesting either altered responsiveness or adrenal stimulation by a factor other than ACTH [Horrocks et al., 1983].

In lactotrophs, it was found that the area occupied by PRL cells and their proliferation and population were significantly higher in EV-treated animals. Estrogens have been proposed to be involved in specification of cell phenotype, growth, synthesis and secretion of PRL. Estrogens have been shown to stimulate lactotroph cell growth and PRL secretion and have been linked to the development of PRL-secreting pituitary tumors [Wendell et al., 1996; Scully et al., 1997].

Women with PCOS have been reported to have lower circulating concentration of GH and reduced pituitary stores of GH than normal women [Lee et al., 1993; Micic et al., 1996]. A relationship between GH secretion and gonad function has been suggested; GH treatment has been reported to improve the reproductive function in women [Adashi et al., 1985; Homburg et al., 1990; Katz et al., 1993]. In this study, no differences in the area occupied by GH cells and their proliferation and population in rats with induced PCO were found, indicating that GH does not participate in the development of follicular cyst in these models.

In farm animals, cystic ovarian disease is a common reproductive disorder, causing infertility and economic losses [Bartlett et al., 1986; Silvia et al., 2002; Peter, 2004]. Until now, the etiology of ovarian cysts in cattle has not been clearly defined and a variety of histological patterns, endocrine abnormalities and different therapeutic responses have been observed [Vanholder et al., 2006]. Several reports indicate that in cows with ovarian cysts, there is low plasma LH concentration during the follicular phase that prevents ovulation and subsequent development of corpus luteum [Refsal et al., 1986; Robert and Reeves, 1989; Hamilton et al., 1995; Vanholder et al., 2006]. Also, LH gonadotrophs in animals with cystic ovarian disease compared with those of the diestrus and periestrus group are atrophic and contain lower hormone concentrations, suggesting reduced hormone synthesis, impaired hormone release and, perhaps, intracellular hormone degradation [Busato et al., 1995]. These changes are consistent with our findings.

In this study, both L and EV groups developed cystic ovaries. The hormonal levels and the population of some pituitary cells differed markedly from those of the C group, suggesting that cystic ovaries do not define a single disorder, but rather a general class of conditions encompassing a variety of individual expressions. Hence, PCOS has been considered a progressive multiglandular endocrinopathy, where the delicate balance of the hypothalamic-pituitary-ovarian axis is disturbed, resulting in a failure of cyclic reproductive mechanism [Mahesh et al., 1987].

In conclusion, the present study adds further information about alterations of some cell populations that occur in the pituitary gland of rats with PCO. Selective changes in pituitary gonadotroph and plasma gonadotrophin secretion may largely contribute to the complex hormonal changes responsible for the development of follicular cysts. Although much remains to be done in order to characterize these experimental models, they are very useful tools to study some aspects of cystogenesis. Further studies are needed to assess the specific role and regulation of each of these cellular populations and its participation in the pathogenesis of PCO and PCOS.

Acknowledgments

We are very grateful to Schering Laboratories (Buenos Aires, Argentina) for the provision of drugs. We also thank the National Hormone and Pituitary Program of the NIDDK (NIH) and Dr. A.F. Parlow for the RIA kits and primary antibodies and Dr. Damasia Becu-Villalobos (CONICET, Argentina) for valuable RIA contribution. H.H.O. is a Research Career Member and N.R.S., C.B. and F.R. are Fellows of the National Scientific Research Council (CONICET, Argentina).

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