The Role of ACTH in the Pathogenesis of Polycystic Ovarian Syndrome in Rats: Hormonal Profiles and Ovarian Morphology

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Summary

Numerous hypotheses have been proposed about the pathogenesis of the polycystic ovarian syndrome (PCOS). However, hormonal control of persistent follicles has not been established. The objective of the present study was to compare the follicular structure and hormonal profiles of rats treated with the adrenocorticotrophic hormone (ACTH) with two experimental models of PCOS. ACTH-treated animals were compared with those exposed to continuous light, those treated with estradiol valerate, and with control (in proestrous and diestrous). Serum hormone levels, histomorphometrical changes, and immunoexpression of vimentin, cytokeratins, cadherins, and proliferating cell nuclear antigen (PCNA) were examined. Treatment with ACTH resulted in an elevation of corticosterone secretion with LH reduction but without changes in ovarian morphology. Although stress (or ACTH) stimulation may be only one of pathophysiological mechanisms involved in follicular cyst pathogenesis in other species, we do not have important evidence to suppose that this would happen in rats.

Key words
ACTH • Ovary • Polycystic Ovarian Syndrome • Rat • Stress

Introduction

Polycystic ovarian syndrome (PCOS) is the most common and least understood endocrine disorder affecting approximately 5 % of women of reproductive age. It is considered a syndrome not a disease, although the term "polycystic ovarian disease" is often used synonymously, but incorrectly, to describe this syndrome, which is manifested by heterogeneous clinical symptoms (Xita *et al.* 2002).

Numerous experimental models to assess PCOS in rats have been developed. The following hormonal substances, among others, have been employed to induce this condition: estradiol valerate, dehydroepi-androsterone, neonatal androgenization, and continuous light exposure (Mahesh *et al.* 1987, Mahajan 1988,

Salvetti *et al.* 2003, 2004a,b).

On the other hand, stress has been implicated in the etiology of spontaneously occurring persistent follicles in cattle and sheep. In cattle, the preovulatory luteinizing hormone (LH) surge is sensitive to the inhibitory effects of exogenous adrenocorticotrophic hormone (ACTH), and such inhibition results in the development of persistent follicles (Phogat *et al.* 1997, Dobson *et al.* 2000). However, the reproductive hormonal control of the consequent development of either the persistent follicle or subsequent follicles was not examined in these studies.

The objective of the present study was to compare the follicular structure and hormonal profiles of rats treated with ACTH with two experimental models of PCOS.

Methods

Animals and treatment

Female Wistar rats (175–200 g) were provided by the Center for Experimental Biology and Laboratory Animal Sciences, Faculty of Veterinary Sciences, UNL. Before the experiment, the animals were kept under controlled cycle of light and darkness (lights on from 06:00 to 20:00 h), and a temperature range of 20-24 ℃ with free access to water and commercial food (Cargill, Argentina). Lighting was provided by banks of General Electric 4 coolwhite 40-W fluorescent tubes to obtain a light intensity of 350 lux at 1 m above the 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).

Forty animals displaying at least two normal 4-5 days of estrous cycles, just before treatment, were allocated to four groups: The **control group** received an equivalent volume of vehicle and the animals were sacrificed after 10 weeks, in proestrous **(C-P)** (*n* = 5) to obtain serum and preovulatory tertiary follicles, and in diestrous **(C-D)** (*n* = 5) to obtain serum and growing follicles. The animals in the **continuous light group (L)** were placed in the conditions described, except that the light was extended to 24 h and the rats were sacrificed after 105 days (Salvetti *et al.* 2004a). The **estradiol valerate (EV)-treated group** was injected with a single 2-mg IM dose of EV and the rats were sacrificed at 60 days (Progynon Depot, Schering, Buenos Aires, Argentina) (Schulster *et al.* 1984, Farookhi *et al.* 1985). The animals belonging to the **ACTH-treated group**

received an equivalent to 20 UI/kg (200 µg/kg) of ACTH (ACTHElea, Elea, Buenos Aires, Argentina) subcutaneously every 24 h for 18 days and were sacrificed 24 h after the last injection (Fraser *et al.* 2001, Kitamura *et al.* 2002).

Vaginal smears

Smears obtained by vaginal washing were examined under a microscope for the relative abundance of nucleated epithelial cells, cornified cells, and leukocytes. Cycles with duration of 4–5 days were considered regular. The observation of cornified cells in the smears during a minimum of 10 serial days was defined as persistent vaginal cornification (PVC) and considered to be an indication of follicular cystic development (Salvetti *et al.* 2004a).

Tissue sampling

All the animals were killed by decapitation, at around 9:00 h, trunk blood was collected, and the serum stored at –20 ℃ until used for hormone assays. The ovaries were dissected and fixed in 10 % buffered formalin for 6 h at room temperature and washed in a phosphate buffer saline (PBS) solution. For light microscopy, fixed tissues were dehydrated in an ascending series of ethanol, cleared in xylene, and embedded in paraffin. Five-micrometer thick sections were mounted in slides previously treated with 3-aminopropyltriethoxysilane (Sigma-Aldrich, St. Louis, MO) and stained with hematoxylin-eosin for preliminary observation.

Immunohistochemistry

Details and concentration of the antibodies used are summarized in Table 1. Each antibody was assayed in at least five sections of each ovary from each individual. The streptavidin-biotin immunoperoxidase method was done as previously described (Ortega *et al.* 2004, Salvetti *et al.* 2004b). In brief, sections were deparaffinized and hydrated, and given microwave pretreatment (antigen retrieval). The endogen peroxidase activity was inhibited with 1 % H_2O_2 and nonspecific binding was blocked with 10 % normal goat serum. All sections were incubated with the primary antibodies for 18 h at 4 ℃ and then for 30 min at room temperature with rat-preabsorbed biotinylated secondary antibodies selected specifically to each of the two types of primary antibodies used (monoclonal or polyclonal). The visualization of antigens was achieved by the streptavidin–peroxidase method

Table 1. Used antibodies, suppliers and dilutions.

Antibodies	Clone	Supplier	Dilution
Primary antibodies			
Vimentin (VIM)	V9	Zymed	Prediluted
Cytokeratins (1/5/6/8/10/14/18) (CKs)	LP 34, 34βE12 & 35βH11	Dako	Prediluted
pan-Cadherin (GFAP)	Polyclonal	Zymed	1:50
Proliferation cellular nuclear antigen (PCNA)	PC10	Novocastra	1:200
Secondary antibodies			
Anti-rabbit IgG	Polyclonal	Zymed	1:300
Anti-mouse IgG	Polyclonal	Chemicon	1:120

(BioGenex, San Ramon, CA) and 3.3-diaminobenzidine (Liquid DAB-Plus Substrate Kit – Zymed, San Francisco, CA) was used as chromogen. Finally, the slides were washed in distilled water and counterstained with Mayer's hematoxylin, dehydrated, and mounted. To verify specificity, adjacent control sections were subjected to the same immunohistochemical method, replacing primary antibodies by rabbit and mouse nonimmune serum.

Image Analysis

Image analysis was performed using Image Pro-Plus 3.0.1® system (Media Cybernetics, Silver Spring, MA, USA). Images were digitized by a CCD color video camera (Sony, Montvale, NJ, USA) mounted on top of a conventional light microscope (Olympus BH-2, Olympus Co., Japan) using ×4, ×10, and ×40 objective lenses. Microscopic fields covering the entire follicular wall area were digitized and stored in a 24-bit true color TIFF format.

Follicular mean diameter and the granulosa and theca thickness were analyzed using specific tools of the software. The immunohistochemical stained area (IHCSA) was calculated as a percentage of total area, assessed by color segmentation analysis, which produces quantification by locating all objects of a specific color (brown stain). The images were then transformed to a bilevel scale TIFF format (Salvetti *et al.* 2004b). The IHCSA (black area) was calculated from at least 25 images of each area (granulosa, theca externa, and theca interna) in each slide.

Hormone assays

Follicle-stimulating hormone (FSH) and LH serum levels were determined by radioimmunoassay (RIA) using the kit provided by NIDDK (USA) as previously described (Ortega *et al.* 2004). Intra- and inter-assay 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^{-1} 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 (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 inter-assay coefficients of variation were 7.5 % and 11.9 %, respectively (Chamson-Reig *et al.* 1999).

After ethyl-ether extraction, the serum testosterone was determined by RIA using a specific antiserum provided by Dr. G.D. Niswender (NIDDK). Labeled hormone was purchased from New England Nuclear (Boston, MA, USA). Assay sensitivity: the lowest point in the testosterone standard curve was 12.5 pg and the lowest detectable concentration when extracting 150 µl serum was 208.3 pg/ml. Intra- and inter-assay coefficients of variation were 7.8 % and 12.3 %, respectively (Bianchi *et al.* 2004).

Corticosterone concentration was determined by extracting 25 µl of serum sample with 1 ml of dichlormethane (Merck, Westpoint, PA, USA). The solvent was decanted and dried at 50 ºC. Fifty microliters of 1-alpha-2-alpha-N(^{3}H) corticosterone (Amersham, UK; 10000 cpm approximately) were diluted with 200 µl of standard solutions or unknown samples and 50 µl of anticorticosterone rabbit serum. The tubes were incubated overnight at 4 ºC and the separation of bound and free hormone fractions was achieved by the addition of 100 µl of charcoal (1 g %)-dextran (0.1 g %) suspension. Bound

Table 2. Histomorphometric analysis of follicular wall of ovaries in control and treated rats[*].

	C-P	L	EV	ACTH
Follicle mean diameter	526.45 ± 32.53 [a]	765.37 ± 29.56 [b]	684.79 ± 48.98 [b]	561.31 ± 25.33 [a]
Granulosa thickness	55.42 ± 5.24 [a]	62.46 ± 10.09 [a]	33.04 ± 4.17 [b]	63.00 ± 2.50 [a]
Theca interna thickness	14.17 ± 1.24 [a]	16.04 ± 1.66 [a]	9.94 ± 0.74 [b]	16.08 ± 1.70 [a]
Theca extrena thickness	11.80 ± 1.01	10.65 ± 1.38	11.57 ± 1.34	8.79 ± 0.86

[*] Values represent mean ± SEM (μm). Different letters within a row indicate significant differences ($p < 0.05$)

Table 3. Distribution of immunoreactivity in the follicular wall of ovaries in controls and treated rats.

	C-P	L	EV	ACTH
Vimentin				
Granulosa	7.76 ± 0.46 [a]	30.62 ± 2.48 [b]	23.70 ± 3.23 [c]	8.90 ± 2.01 [a]
Theca interna	5.98 ± 0.64	5.62 ± 0.76	7.32 ± 2.47	4.40 ± 1.13
Theca externa	2.29 ± 0.28 [a]	2.31 ± 0.45 [a]	6.32 ± 1.66 [b]	2.94 ± 0.89 [a]
Cytokeratins				
Granulosa	0.94 ± 0.20 [a]	19.96 ± 1.48 [b]	15.28 ± 2.00 [c]	1.19 ± 0.44 [a]
Theca interna	-	-	-	-
Theca externa	-	-	-	-
Pan-Cadherin				
Granulosa	11.02 ± 1.44 [a]	26.74 ± 2.04 [b]	31.14 ± 4.13 [b]	9.76 ± 3.06 [a]
Theca interna	5.88 ± 0.47 [a]	16.96 ± 2.27 [b]	14.38 ± 1.44 [b]	6.61 ± 2.37 [a]
Theca externa	0.43 ± 0.28	0.65 ± 0.17	0.60 ± 0.19	0.53 ± 0.17
PCNA				
Granulosa	8.29 ± 0.76 [a]	2.34 ± 0.54 [b]	1.72 ± 0.26 [b]	6.89 ± 0.96 [a]
Theca interna	9.49 ± 0.76 [a]	2.08 ± 0.62 [b]	0.76 ± 0.17 [b]	8.15 ± 0.99 [a]
Theca externa	3.53 ± 0.64 [a]	1.01 ± 0.31 [b]	0.38 ± 0.13 [b]	3.30 ± 0.54 [a]

[*] Values represent mean immunohistochemical stained area (IHCSA) ± SEM. Different letters within a row indicate significant differences ($p < 0.05$)

radioactivity was determined in an LKB β-counter. The intra- and inter-assay coefficients of variation ranged between 4-7 %, and 8-10 %, respectively (Spinedi *et al.* 1991).

Statistics

A statistical software package (SPSS 11.0 for Windows, SPSS Inc., Chicago, IL, USA) was used for performing the statistical tests. The statistical significance of differences was assessed by one-way ANOVA, followed by Duncan's multiple range test as a multiple comparison test. *P*<0.05 values were considered significant. Results were expressed as mean ± SEM.

Results

Estrous cycles

The animals in the control group showed normal cycles during the whole experiment. In the constant light-exposed group, all animals showed irregular cycles within 3 weeks and PVC within 13 weeks of permanent light exposure. In the EV-treated group, all animals displayed irregular cycles within 1 week of EV treatment and most (90 %) showed PVC by 3 weeks after injection. In the ACTH group, all animals showed irregular cycles during the whole experience.
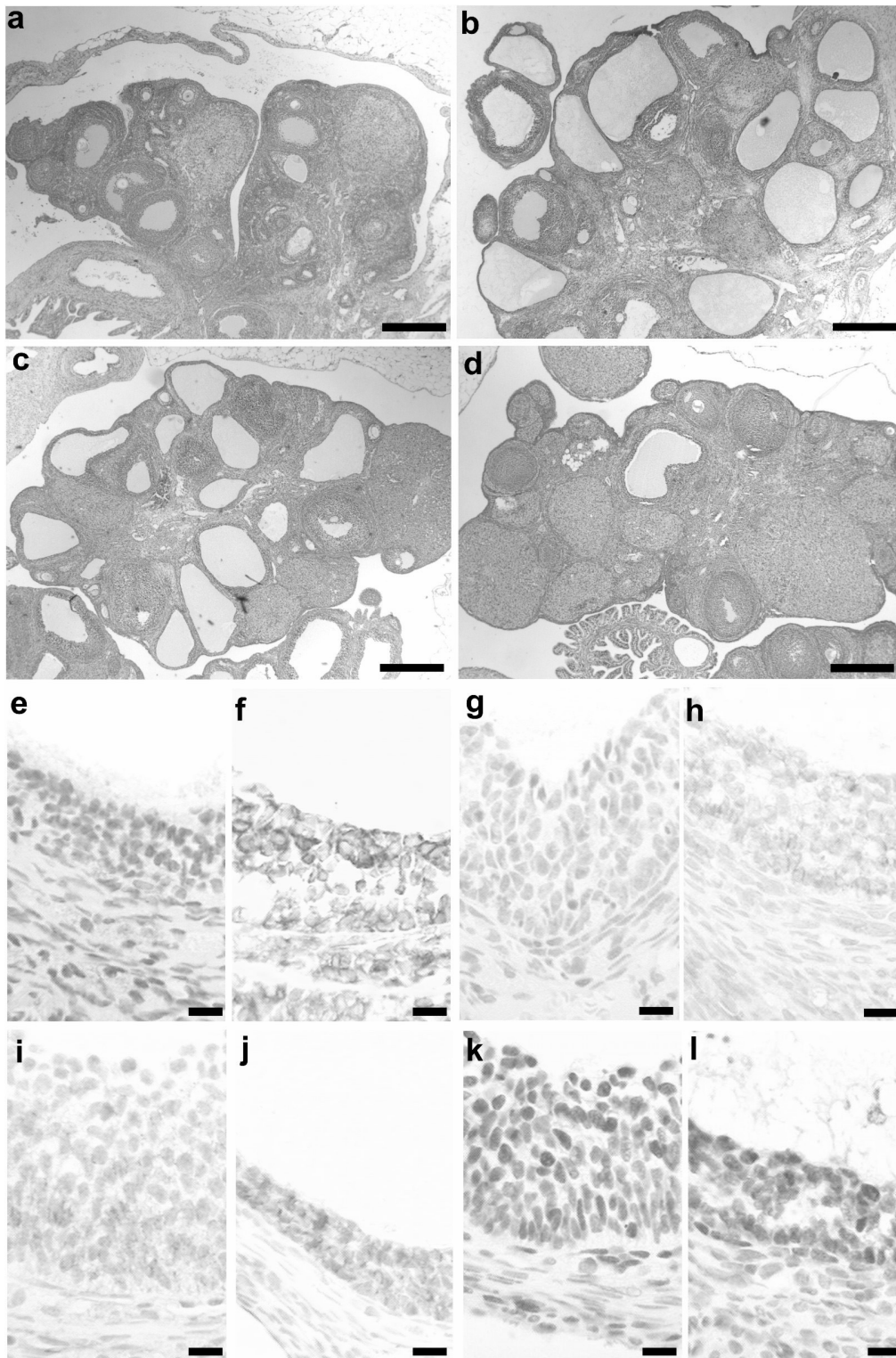
Fig. 1. Sections of ovaries from control (proestrous) (a,e,g,i,k), constant light-exposed (b,f,h), estradiol valerate-treated (c,j,l) and ACTH treated (d) rats staining with hematoxylin-eosin (a,b,c,d) and immunostaining with vimentin (e,f), cytokeratins (g,h) and PCNA (k-l) antibodies. a-d bars = 500 µm; e-l bars = 20 µm.

Morphology

Ovaries from the control group exhibited follicles in various stages of development. Preovulatory tertiary follicles and growing secondary follicles were observed in proestrous, whereas only secondary follicles and fresh corpora lutea were seen in diestrous. Primordial, primary, and atretic follicles, as well as interstitial glands, were found in both control groups. In

constant light-exposed animals, small follicles in early development were observed, in addition to follicles showing evidence of atresia, and many large cysts with thickened granulosa cell layer or large cystic follicles with scant granulosa cells. Corpus luteum was absent and hyperplasia of interstitial glands was observed. The ovaries of EV-treated animals 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. In ACTH-treated animals, the ovarian morphology was similar to the control animals.

Histomorphometry

Histomorphometric results are listed in Table 2. The average diameter of cystic follicles was significantly higher in constant light-exposed and EV-treated rats. The thickness of granulosa, theca interna, and theca externa did not show significant differences among C-P, C-D, L, or ACTH groups. In the EV-treated rats, a significant reduction in the thickness of the granulosa and theca interna was evident.

Immunohistochemistry

A summary of the immunohistochemical (IHCSA) expression for the different antibodies is given in Table 3 and Figure 1. The granulosa cell layer of cystic follicles in the L and EV groups had a significantly greater IHCSA for vimentin than the C-P and ACTH antral follicles. The theca interna cells in C-P ovaries, similarly as in the treated ovaries, showed a moderate positive immunostaining without differences in the IHCSA. Some theca externa cells showed weak staining in all follicles studied, and a higher IHCSA was observed in EV-treated rats.

Cytokeratins (CKs) showed a low expression in the granulosa cells of antral follicles in the C-P and ACTH-treated animals, whereas in granulosa cells of cystic follicles, L and EV groups showed significantly higher IHCSA. No immunostaining was detected in theca cells.

Immunostaining for p-cadherins was observed in the granulosa and theca interna of cystic follicles (L and EV groups) and weak and irregular immunostaining was seen in the theca externa.

Cellular proliferation, evaluated through the expression of proliferating cell nuclear antigen (PCNA), showed a high index in the whole follicular wall in antral follicles of C-P and ACTH. This was significantly decreased in L and EV groups.

Hormone levels

Hormone serum levels are shown in Figure 2. The three treatments induced a significant reduction in LH and FSH levels as compared to C-P animals. Estradiol serum levels in EV-treated and L animals showed significant increments, while in ACTH-treated animals, the levels were similar to those of proestrous animals. The levels of progesterone in the treated animals were similar to those of proestrous animals. Testosterone serum concentrations varied with a significant reduction in L and EV animals in relation to C-D. Corticosterone showed an increase in the ACTH group and a significant reduction in the EV group.

Discussion

Why and how ovaries become polycystic have been the subject of considerable speculation as well as the object of meticulous studies with different approaches (Barberi 1991, Agarwal and Shankar 1998, Hoo and Shaw, 2005, Raja *et al.* 2005). Nevertheless, little is known about the mechanisms that cause their development. The etiology and pathogenesis of follicular cysts have been the subject of numerous hypotheses since the middle of the 19th century. However, to this day, they are still not completely clarified either in humans or in animals (Barberi 1991, Hamilton *et al.* 1995, Garverick 1997, Silvia *et al.* 2002, Peter 2004).

In this work, both L and EV groups develop polycystic ovaries. The hormonal levels; vimentin, CKs, and cadherins immunoexpression; and follicular wall morphometry differed markedly from those of the control group, indicating that polycystic ovaries do not define a single disorder, but rather a general class of conditions encompassing a variety of individual expressions.

In this sense, the PCOS has been considered a progressive multiglandular endocrinopathy where the delicate balance of the hypothalamic–pituitary–adrenal–ovarian axis is disturbed, resulting in a failure of the cyclic reproductive mechanism (Mahesh *et al.* 1987, Hamilton *et al.* 1995). A total loss of the cyclic reproductive changes appears to follow a phase of irregular rhythmicity in rats experimental PCOS (Salvetti *et al.* 2004a).
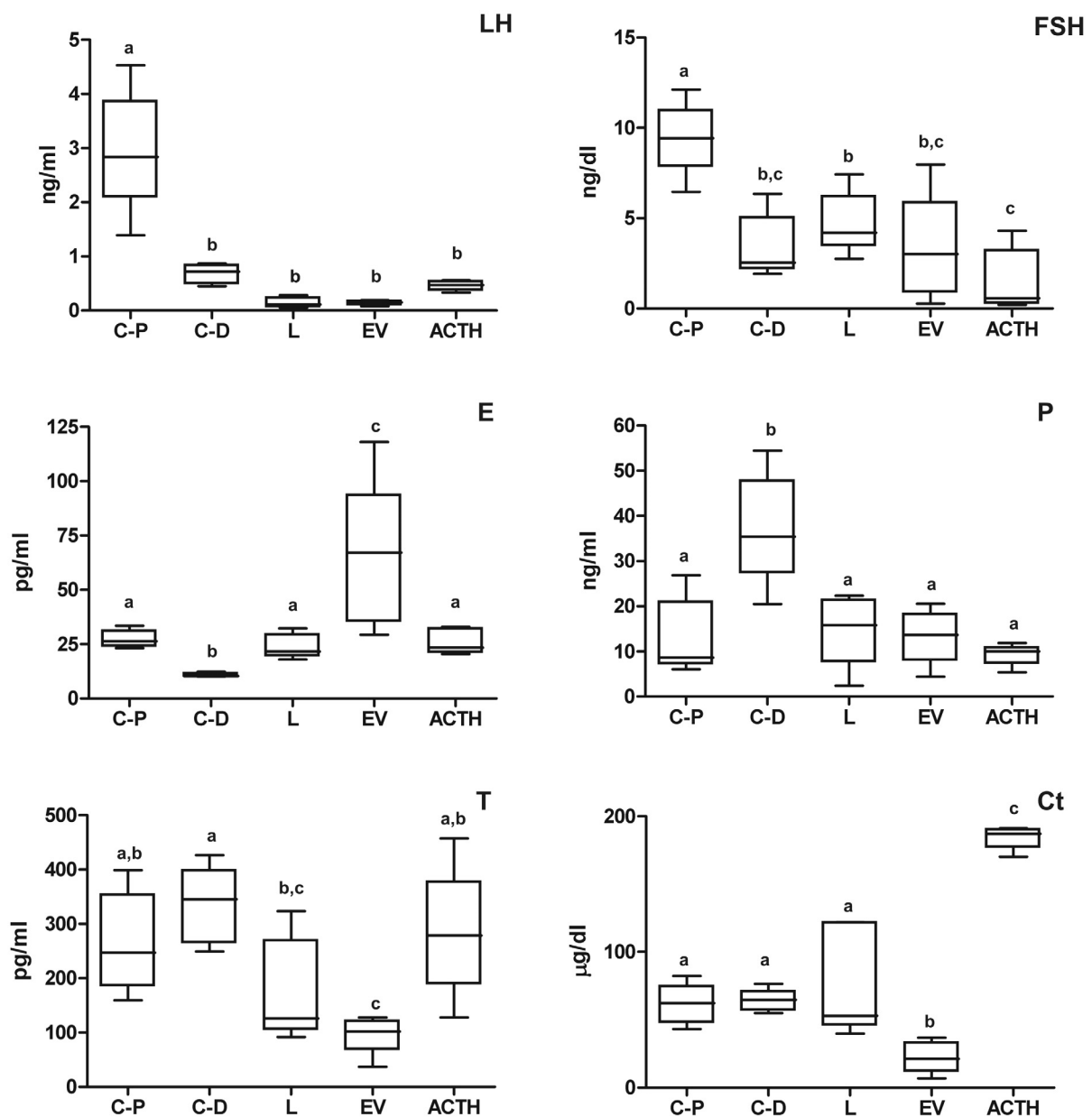
Fig. 2. Medial values, interquartile intervals (box) and range (whiskers) for LH, FSH, estradiol (E), progesterone (P), testosterone (T) and corticosterone (Ct) in control-proestrous (C-P), control-diestrous (C-D), constant light-exposed (L), estradiol valerate-treated (EV) and ACTH treated (ACTH) rats. Different letters indicate statistical differences ($p < 0.05$).

Ovarian changes

Ovarian morphology provides both a convenient and appropriate measurement to describe cystic status as differences were found in the morphometric characteristics, and presence or absence of follicular cyst and corpus luteum. On the other hand, we have recently described changes that occur in the expression of vimentin and CKs in the ovary of rats with ovarian cysts, which are probably due to structural and functional modifications that occur during cystogenesis (Salvetti *et al.* 2004b). The present study demonstrates a similar pattern of expression of these proteins in cystic follicles and confirms the absence of cellular changes in the follicular wall of ACTH-treated animals.

Numerous lines of evidence suggest that important growth-related cellular functions such as cell proliferation, expression of differentiated phenotype, response to tissue-specific hormones, and tissue

morphogenesis are modulated through changes in cellular shape (Ben-Ze'Ev and Amsterdam 1987, Marettova and Maretta 2002). Since cytoskeletal elements are responsible for determining cell morphology and shape, this study about cytoskeletal-related protein expression was directed towards investigating the possible existence of a relation between their immunolocalization and the organization of the follicular wall in follicular cysts. The occurrence of vimentin-containing granulosa cells has been related to mitotic activity, and with follicular atresia and dedifferentiation associated with loss of cell-to-cell contact (Raymond and Leong 1989, Van den Hurk *et al.* 1995). However, if cell proliferation activity in the granulosa and theca interna layers is considered to decrease in association with the induction of follicular cysts, the changes in vimentin expression observed would be due to cellular dedifferentiation. The idea that an increase in intermediate filaments is a degenerative change is also supported by their increase reported in many papers (Ghadially *et al.* 1978, Pieraggi *et al.* 1984).

Cell adhesion molecules (cadherins) have been identified in the ovaries and linked with cyclic remodeling. Although cadherin proteins have been identified in cells of developing follicles and corpora lutea, their role in ovarian function is unclear (Machell and Farookhi 2003). There is evidence that cadherins contribute to the structural integrity of follicles and corpora lutea. For example, in atretic pig follicles, the loss of granulosa cell adhesion and follicle wall integrity is associated with a significant decrease in the expression of E-cadherin by granulosa cells (Ryan *et al.* 1996). Likewise, a similar decrease in E-cadherin protein has been reported in baboon regressing corpora lutea during the late luteal phase of the reproductive cycle (Khan-Dawood *et al.* 1996). Our results agree with data that suggest that disturbances in ovarian cell adhesion *in vivo* are linked with increased atresia, cyst formation, and insufficient luteal function (Khan-Dawood *et al.* 1996, Makrigiannakis *et al.* 1999). *In vitro* studies indicate that cell-cell communication and aggregation are important for the differentiation of rat granulosa cells by enhancing FSH induction of both aromatase and LH receptors (Farookhi and Desjardins 1986).

Cystic follicles showed extremely low PCNA reaction in the granulosa and theca cell layers, compared with healthy follicles, confirming the findings in cows with ovarian cystic disease (Isobe and Yoshimura 2000). These suggest that the disorder of cell proliferation is also associated with the occurrence of follicular cysts in rats.

Endocrinological aspects

Although the exact mechanism involving the hypothalamic-pituitary-axis in the PCOS still has to be elucidated, it should be noted that closely related neuroendocrine mechanisms are involved in the cyclic release of ACTH and gonadotropins. In addition, there is evidence from both *in vitro* perfusions and *in vivo* experiments to show that exogenously increased ACTH concentrations or stress reduce the amount of LH released. In domestic ruminants, treatment with ACTH resulted in a reduction of pulsatile LH secretion and in the abolition of LH surge without changes in the secretion of FSH. In fact, FSH ability to stimulate follicular emergence remains despite the development of PCOS (Ribadu *et al.* 2000). Cows with ovarian follicular cysts frequently have an abnormal, intermediate concentration of progesterone (Hatler *et al.* 2003). Even at these low concentrations of progesterone, the preovulatory surge of LH and subsequent ovulation can be blocked resulting in the persistence of ovulatory size follicles (Duchens *et al.* 1994). One possible source of this progesterone is the adrenal gland, due to treatment of cows with ACTH-induced rapid secretion of progesterone (Verkerk *et al.* 1994, Silvia *et al.* 2005). However, in adrenalectomized rats subjected to stress, there was a decrease in LH level and an increase in progesterone (Galvez *et al.* 1999). Thus, these hormonal changes could represent a reflex action of an extra-adrenal mechanism.

Our results demonstrate that rats respond to ACTH administration with an elevation of corticosterone and reduction in LH and FSH (compared with proestrous levels), but without changes in the levels of other hormones or in ovarian morphology. Stener-Victorin *et al.* (2005) have suggested a metabolic shift toward corticosterone synthesis and secretion, and a hyperadrenal state in EV-induced PCOS. However, our results clearly indicate that the EV-induced PCOS is not associated with a hyperadrenal state, because we have found a decrease in corticosterone without changes in progesterone. Therefore, there was no evidence to show that basal adrenal activity differed between constantly light-induced PCOS and control rats.

In this sense, although some researchers postulated that constant light acts on the release of ACTH (Singh 1969), we have shown in present report that the constant light-induced PCOS takes place without a significant increase in the corticosterone levels (an index for adrenal activity and ACTH stimulation) (Colak *et al.* 2002, Tsilchorozidou *et al.* 2003, Silvia *et al.* 2005). This

is supported by a recent study that points to the participation of the pineal gland, but not the adrenal gland, in the pathogenesis of constant light-induced PCOS (Prata Lima *et al.* 2004). These authors showed that the ovarian morphology in PCOS induced in rats by pinealectomy is similar to that induced by constant light exposure. They also demonstrated that in both cases, the development of ovarian cysts and anovulation could be inhibited by melatonin administration. In this sense, melatonin acts on the central nervous system of the rodent, with an antigonadotropic action in the anterior hypothalamus (Reichlin 1992, Prata Lima *et al.* 2004).

However, it is interesting to mention that Paredes *et al.* (1998) showed that the exposure to stress can represent an etiological factor in the genesis of PCOS in rats. In this work, the effect on the ovarian function of exposure to 3 weeks of stress was not maintained for 11 weeks. The temporal disruption of ovulation, observed by these authors, appears to be the consequence of a poorly controlled ovarian function under increased sympathetic nerve activity. The increase in norepinephrine release after 3 weeks of stress correlates with ovarian cyst formation, and the decrease in the release found at 11 weeks of stress correlates with the recovery of ovarian function to control level (Paredes *et al.* 1998).

Among the uncertainties surrounding the etiology of the PCOS in women, the role of altered cortisol metabolism has become prominent (Tsilchorozidou *et al.* 2003). Enhanced 5α-reductase, deregulation of 11β-hydroxysteroid dehydrogenase type 1 activity, and increased total adrenal steroid production rates have been previously described in PCOS and implicated as possible pathogenetic mechanisms. However, in normal women, circulating adrenal androgen

levels and their response to ACTH are highly individualized and genetically predetermined (Rotter *et al.* 1985, Azziz *et al.* 1999). Supporting this concept, various investigators have observed a significant degree of heritability for adrenal androgen levels with an increased risk for developing PCOS (Ibanez *et al.* 1993, Thomas *et al.* 1994, Nafziger *et al.* 1998, Yildiz *et al.* 2004). Recently, Silvia *et al.* (2005) have reported that the naturally occurring cystic condition in cows is not associated with abnormalities in adrenal secretion of either cortisol or progesterone. These results suggest that adrenal dysfunction could act as an inheritable risk factor, although this is not the only one in the pathogenesis of PCOS (Xita *et al.* 2002, Silvia *et al.* 2002, Peter, 2004).

In conclusion, treatment with ACTH in rats resulted in corticosterone stimulation with LH reduction but not changes in ovarian morphology. Although stress (or ACTH) stimulation and adrenal hyperactivity may be only one pathophysiological mechanism involved in follicular cyst pathogenesis in other species, we have not found important evidence to support that this happens in rats.

Acknowledgments

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