Effect of hyperandrogenism on ovarian function

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Abstract

The objective of this work was to study the ovarian function when follicular development is induced during a hyperandrogenic condition. Female rats were injected with either equine chorionic gonadotropin (eCG group) to induce folliculogenesis or eCG together with DHEA to induce folliculogenesis in a hyperandrogenic condition (eCG + HA group). The control group was injected with vehicle. Ovarian mRNA levels of the peroxisome proliferator-activated receptor gamma (PPARγ) co-activator PGC1α, the PPARγ co-repressor NCoR, the main enzymes involved in the ovarian steroidogenesis (CYP17, 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-HSD, and CYP19A), and cyclooxygenase 2 (COX2) were evaluated only by real-time PCR. COX2 was evaluated by both real-time PCR and western blot. Serum steroid hormones and both the oxidative and inflammatory statuses were also quantified. We found that eCG-induced folliculogenesis induced increased mRNA levels of PGC1α and decreased those of NCoR when compared with controls. In addition, we found an increase in serum estradiol (E2) levels and enhanced mRNA expression of CYP19A. A pro-inflammatory status and a pro-oxidant status were also established. When folliculogenesis was induced in a hyperandrogenic condition, the mRNA levels of the PPARγ co-repressor NCoR remained higher than in controls and the pro-inflammatory and pro-oxidant statuses were enhanced. In addition, the enzymes involved in ovarian steroidogenesis were altered leading to the accumulation of testosterone and an unfavorable E2/testosterone ratio. These alterations led to abnormal follicular development.


Introduction

Fuel sensors, such as metabolites (e.g., glucose, fatty acids, and amino acids) and hormones (e.g., insulin, leptin, and ghrelin), are involved in the regulation of fertility at each level of the hypothalamic–pituitary–gonadal axis (Froment et al. 2006). The discovery of the peroxisome proliferator-activated receptor (PPAR) family of transcription factors revealed the link between lipid/glucose availability and long-term metabolic adaptation (Issmann & Green 1990). PPARs have three subtypes, α, β/δ, and γ (Issmann & Green 1990, Komar 2005), which are detected in the ovary of several species, including the rat (Komar 2005). PPARγ is expressed primarily in the granulosa cells of developing follicles (Komar & Curry 2002), where it regulates the synthesis of steroid hormones (Huang 2008). At the end of follicular development, the luteinizing hormone surge down-regulates the expression of ovarian PPARγ (Komar et al. 2001, Froment et al. 2003). In the rat, PPARγ expression is low in newly forming luteal tissue and higher in luteal tissue present from previous ovulations (Komar & Curry 2002).

PPARγ is activated by binding either to endogenous factors (such as prostaglandins (PGs) or fatty acids) or to exogenous factors (including non-steroidal anti-inflammatory drugs, fibrates, polycyclic aromatic hydrocarbons, traditional medicines, and thiazolidinediones (such as pioglitazone and rosiglitazone)) (Yu et al. 1995, Jaradat et al. 2001, Lim & Dey 2002, McIntyre et al. 2003, Seli & Duleba 2004, Huang et al. 2005, Kanayama et al. 2005, Kim et al. 2005, Banerjee & Komar 2006). In addition, PPARγ inactivation can be prevented by thiazolidinediones (Choi et al. 2010, Kono et al. 2012).

The binding of PPARγ to ligands modulates its transcriptional activity by increasing the recruitment of co-activators, such as PGC1α (Puigserver et al. 1998), but, in the absence of ligands, the recruitment of co-repressors, as NCoR, down-regulates the transcriptional activity of PPARγ (Yu et al. 2005). After its activation, PPARγ modulates lipid metabolism (Curti et al. 2011) and the inflammatory response (Celinski et al. 2012, Rezvanfar et al. 2012), whereas, during folliculogenesis, PPARγ modulates steroidogenesis and cellular proliferation (Froment et al. 2006). Given the
important role of the PPARγ system in modulating endocrine and metabolic pathways, the management of the PPARγ system represents a fundamental tool in treating metabolic disorders.

PGs modulate different ovarian functions, such as the rupture of ovarian follicles associated with ovulation (Husein & Kridli 2003, Medan et al. 2003) and luteolysis (Motta et al. 1999, 2001). We have previously reported that hyperandrogenism induces a pro-inflammatory status mediated by the PG system in both mice (Luchetti et al. 2004, Elia et al. 2006) and rats (Amalfi et al. 2012). It has been recently reported that, in breast cancer, a PPARγ agonist inhibits P450 aromatase expression by means of the inhibition of the PGE pathway (Margalit et al. 2012).

Hyperandrogenism also induces an increase in the production of reactive oxygen species by the ovary, thus altering oxidant–antioxidant balance (Luchetti et al. 2004, Diamanti-Kandarakis & Economou 2006, Elia et al. 2006).

In this work, we studied the effect of hyperandrogenism on the regulation of ovarian function. Specifically, we designed a murine model that allowed us to investigate events linked to early follicular development. We studied: i) the histology of ovarian tissue, ii) the transcript levels of the PPARγ co-activator PGC1α and the PPARγ co-repressor NCoR, iii) the main enzymes involved in ovarian steroidogenesis: CYP17, 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-HSD, and CYP19A, iv) the serum levels of progesterone, testosterone, and estradiol (E2), v) the ovarian inflammatory status characterized by both the gene and protein expression of cyclooxygenase 2 (COX2), the limiting enzyme of PG synthesis, and the ovarian PGE content, and vi) the systemic oxidant–antioxidant balance, evaluated by the lipid peroxidation index and by the production of the antioxidant metabolite glutathione (GSH) in serum samples.

Materials and methods

Animal model

The animal model consisted of immature (22–25 days old) female Sprague–Dawley rats intraperitoneally injected with 25 IU of chorionic gonadotropin (eCG; Sigma–Aldrich) in 0.1 ml saline solution (eCG group) (Faut et al. 2011). The hyperandrogenized (HA) group consisted of rats intraperitoneally injected with 25 IU/rat eCG together with a s.c. injection of 0.1 ml sesame oil (eCG + HA group). The control group consisted of rats injected with vehicle (sesame oil). Rats were housed under controlled temperature (22 °C) and illumination (14 h light:10 h darkness cycle; lights on at 0500 h) and allowed to access Purina rat chow and water freely. All procedures involving animals were conducted in accordance with the Animal Care and Use Committee of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina).

This study was approved by the Ethics Committee of the School of Medicine of University of Buenos Aires (Argentina). In this model of follicular development, the peak of ovarian steroidogenesis is obtained 8 h after eCG treatment (Faut et al. 2011). Thus, rats were anesthetized with carbon dioxide and killed by decapitation after 8 h of treatments. Trunk blood was collected and serum was separated by centrifugation at 1000 g for 15 min and stored at −80 °C until progesterone, E2, testosterone, lipid peroxidation index, and GSH levels were determined. Ovarian tissue from a total of 20 rats from each group was used as follows: ten freshly dissected ovaries from different rats were immediately fixed in 4% (w/v) paraformaldehyde for morphological studies, whereas other 30 were frozen at −80 °C. Of the latter, ten were used for mRNA analysis by real-time PCR, ten for western blotting, and ten for PGE quantification by RIA.

Histological studies of ovarian tissue

To study the effect of hyperandrogenization on early folliculogenesis, serial sections of ten ovaries from each of the three groups and from different animals, fixed as described earlier in this study, were consecutively cut (5 μm/section), placed on gelatin-coated slides (Biobond, British Biocell International, Cardiff, UK), air dried for 2 h, and fixed for 5 min in acetone at 4 °C. Then, consecutive sections from each ovary were washed in PBS (137 mmol/l NaCl, 2.7 mmol/l KCl, 4.3 mmol/l Na2HPO4, 7H2O, 1.4 mmol/l KH2PO4, and pH 7.3) and stained with hematoxylin and eosin (DAKO Corporation, Carpinteria, CA, USA) for histological analysis. This resulted in 200 sections for each ovary. Histological serial sections were analyzed independently by three of the authors, and ovarian follicles were classified and quantified. Follicular atresia was also quantified.

Gene expression analysis

To determine whether hyperandrogenism altered the gene expression of NCoR, PGC1α, CYP17, 3β-HSD, 17β-HSD, CYP19A, and COX2, their mRNA levels were measured by real-time PCR analysis. Total mRNA from ovarian tissue from the control, eCG, and eCG + HA groups was extracted using RNAzol RT (MRC gene, Molecular Research Center, Cincinnati, OH, USA) following the manufacturer’s instructions. cDNA was synthesized from 400 ng mRNA using random primers. real-time PCR analysis was performed from this cDNA by means of the real mix B124-100 (Biodynamics SRL, Buenos Aires, Argentina) and those primers according to the analysis. The amplified products were quantified by fluorescence using the Rotor Gene 6000 Corbett. Results are expressed in arbitrary units. The primers are shown in Table 1.

Western blotting

Ovarian tissue was lysed for 20 min at 4 °C in lysis buffer (20 mM Tris–HCl, pH 8.0, 137 mM NaCl, 1% Nonidet P-40, and 10% glycerol) supplemented with protease inhibitors (0.5 mM phenylmethylsulphonyl fluoride, 0.025 mM N-p-tosyl-L-phenylalanine chloromethyl ketone, 0.025 mM N-p-tosyl-lysine chloromethyl ketone, and 0.025 mM 1-1-tosylamide-2-phenyl-
Table 1 List of primers used in real-time PCR.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Forward primer (5′–3′)</th>
<th>Reverse primer (5′–3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ncor1</td>
<td>TAT CGG AGC CAT CTT CCC AC</td>
<td>ACT TCG GTA TCC TGG GGT TG</td>
</tr>
<tr>
<td>Ppsar1a</td>
<td>AAT GCA GCG GTC TTA GCA CT</td>
<td>GTG TGA GGA GGG TCA TGG TT</td>
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<tr>
<td>Pgs2</td>
<td>ATG AGT ACC GCA AAC GCT TC</td>
<td>CCC CAA AGA TAG CAT CTG GA</td>
</tr>
<tr>
<td>Rpl32</td>
<td>TGG TCC ACA ATG TCA AGG</td>
<td>CAA AAC AGG CAC ACA AGC</td>
</tr>
<tr>
<td>Cyp17a1</td>
<td>TCT CAT TAC ACC CAC GCA GA</td>
<td>CGG GGC AGT TGT TTA TCA TC</td>
</tr>
<tr>
<td>Hsd3b</td>
<td>GAC ACC CCT CAC CAA AGC TA</td>
<td>TTG TAA AAT GCG CAG AGC AG</td>
</tr>
<tr>
<td>Hsd17b1</td>
<td>TCT CAT TAC ACC CAC GCA GA</td>
<td>CGG GGC AGT TGT TTA TCA TC</td>
</tr>
<tr>
<td>Cyp19a1</td>
<td>CCT GGC AAG ACC TCC TTA TC</td>
<td>CCA CGT CTC TCA GCG AAA AT</td>
</tr>
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PG RIA

Ovarian PGE content was determined by RIA as reported previously (Motta et al. 1999). Results are expressed in pg/μg protein. Protein concentration in ovarian tissue was determined by the Bradford method (Bradford 1976).

Oxidative stress-related parameters

Lipid peroxidation

The amount of malondialdehyde (MDA) formed from the breakdown of polyunsaturated fatty acids was measured as an index of peroxidation (Motta et al. 2001). Results are expressed as content of MDA (nmol MDA formed/ml serum).

GS content

The antioxidant metabolite GSH was quantified as described previously (Elia et al. 2006). The reduced form of GSH comprises the bulk of cellular protein sulphydryl groups. Results are expressed in μmol GSH/ml serum.

Statistical analysis

Statistical analyses were performed using the Instant program (GraphPad Software, San Diego, CA, USA). ANOVA followed by Tukey’s test was used to compare all pairs of columns. The Bonferroni’s correction for multiple testing was used to adjust the threshold for statistical significance to \( P<0.05 \).

Results

Ovarian histology

Ovaries from the control group showed a total of 5440 ± 120 follicles while ovaries from the eCG group had 11 115 ± 150 follicles and those from the eCG + HA group had 8350 ± 140 follicles. The ovarian follicles were classified and quantified as primordial (PrF), primary (PF), secondary (SF), and antral follicles (AF) (Fig. 1A). The percentage of PrF was higher in the eCG group than in controls and that corresponding to eCG + HA group was higher than in the eCG and control groups (Fig. 1A). PF was lower in the eCG + HA group than in the eCG and control groups (Fig. 1A). The percentage of atretic follicles decreased in the eCG when compared with control (Fig. 1B), while that in the eCG + HA group did not differ from that in the eCG or control groups (Fig. 1B).
The histological examination of ovaries from the eCG group showed the following: PrF were located in the ovarian cortex, formed by an oocyte surrounded by a flattened layer of pre-granulosa cells (Fig. 1C); PF presented at least one cuboidal layer of granulosa cells (Fig. 1C); SF showed more than one layer of cuboidal granulosa cells and an incipient theca layer (Fig. 1D); and AFs presented a cavity called antrum, a fully grown oocyte with the surrounded zona pellucida, and a basal lamina between granulosa and theca cells (Fig. 1E); AF showed good differentiation of the basal lamina (Fig. 1F).

The histological examination of ovaries from the eCG+HA group showed increased follicular atresia, detected mainly in AFs. In addition, degenerative changes were observed in granulosa cells, which shrank and became evident as pyknotic bodies (Fig. 1G). In a later stage of atresia, the oocyte showed signs of reinitiated meiosis and fragmentation with decreased or absent granulosa layer (Fig. 1H). In addition, we found invasion of the theca cells over granulosa cells (Fig. 1I).

**Effect of hyperandrogenism on the PPAR system**

The induction of ovarian folliculogenesis decreased the mRNA levels of the PPARγ co-repressor NCoR (Fig. 2A) and increased the mRNA levels of the PPARγ co-activator, PGC1α (Fig. 2B), when compared with controls. In the hyperandrogenic condition (eCG+HA group), the mRNA levels of NCoR increased and remained high relative to those of the control and eCG groups (Fig. 2A), whereas the mRNA levels of PGC1α decreased to control values (Fig. 2B).

**Effect of hyperandrogenism on enzymes involved in the ovarian steroidogenesis**

Folliculogenesis induced by eCG did not modify the mRNA levels of CYP17 (Fig. 3A), 3β-HSD (Fig. 3B), or 17β-HSD (Fig. 3C), but increased the mRNA levels of CYP19A, an enzyme that synthesizes E2 from testosterone (Fig. 3D). In the hyperandrogenic condition, the mRNA levels of CYP17, 3β-HSD, and 17β-HSD (Fig. 3A, B and C respectively) significantly increased when compared with both the control and eCG groups whereas those of CYP19A decreased to control values (Fig. 3D).

**Effect of hyperandrogenism on progesterone, testosterone, and E2 levels**

Folliculogenesis induced by eCG increased serum progesterone levels (Fig. 4A), did not modify serum testosterone levels (Fig. 4B), and increased serum E2.
levels when compared with controls (Fig. 4C). In the hyperandrogenic condition, progesterone levels decreased to control values (Fig. 4A), while testosterone levels increased when compared with both the control and eCG groups (Fig. 4B) and serum E2 levels increased when compared with both the control and eCG groups (Fig. 4D). It is important to note that the E2/testosterone ratio, a marker of follicular development, increased with the induction of folliculogenesis (Fig. 4D) but decreased in the HA condition when compared with both the control and eCG groups (Fig. 4D).

**Effect of hyperandrogenism on the ovarian pro-inflammatory status**

The ovarian pro-inflammatory status was evaluated by the mRNA and protein levels of COX2 and by the ovarian content of PGE. Folliculogenesis induced by eCG increased both mRNA (Fig. 5A) and protein (Fig. 5B, C and D) levels of COX2 and PGE content (Fig. 5E) when compared with the eCG group.

**Effect of hyperandrogenism on circulating oxidant–antioxidant balance**

Regarding the ovarian oxidant–antioxidant balance, the lipid peroxidation index was evaluated by the content of ovarian MDA and the antioxidant response by the ovarian content of GSH. Neither MDA content nor GSH content was modified by the induction of folliculogenesis (Fig. 6A). However, when folliculogenesis was induced in a hyperandrogenic condition, MDA content was higher and GSH content was lower than in the eCG and control groups (Fig. 6A and B respectively).

**Discussion**

The battery of animal models used for the study of polycystic ovaries has allowed the different aspects of the pathology to be focused. In that context, the letrozole model in rats is suitable to mimic the ovarian features of human polycystic ovary syndrome (PCOS), while the dihydrotestosterone model is suitable for studies of both ovarian and metabolic features of the syndrome (Mannerás et al. 2007). In agreement with these findings, our previous findings (Luchetti et al. 2004, Elia et al. 2006, Faut et al. 2011) and data presented herein reveal that the DHEA model in rats induces a polycystic phenotype that allows to focus on both ovarian and metabolic aspects.

The PPARγ system is strongly expressed in granulosa cells, where it regulates early folliculogenesis (Komar 2005, Froment et al. 2006). For this reason, the PPARγ system has been the focus of several studies on
physiological and metabolic/endocrine disorders. In fact, a direct association between polymorphisms in the genes encoding PPARs and hyperandrogenism has been recently reported (San-Millan & Escobar-Morreale 2010). It has also been reported that the treatment with synthetic PPARγ ligands improves endocrine and metabolic disorders in women with PCOS (Iuorno & Nestler 1999, Girard 2001, Seli & Duleba 2004, Minge et al. 2006, Brannian et al. 2008). The PPARγ system regulates the expression of genes required for follicular development, ovulation, oocyte maturation, and corpus luteum development (Jablonka-Shariff et al. 1999, Lim et al. 1999, Feige et al. 2005, Froment et al. 2006). Long et al. (2009) found that mRNA for PPARγ was undetectable on day 1, low from days 5 to 14, and increased by day 19 post partum. Although follicle-stimulating hormone (FSH) receptor has also been detected earlier, it has been found that FSH is not a primary factor initiating the expression of PPARγ and that other agents play a role in activating its expression in the ovary (Long et al. 2009). Although the complete mechanism of ovarian PPARγ activation remains unknown as yet, it has been reported that, to act, PPARγ has to be free from its inhibitor NCoR and, to recruit, its activator PGC1α (Jablonka-Shariff et al. 1999, Lim et al. 1999, Feige et al. 2005, Froment et al. 2006).

In a previous report (Faut et al. 2011), we found that, during early folliculogenesis, gene and protein expression of PPARγ and the protein STAR is increased when compared with controls. In this study, we found that the induction of follicular development altered steroidogenic enzymes downstream of STAR: while CYP17A1, HSD3B, and HSD17B1 were not modified by eCG treatment, Cyp19a1, the gene corresponding to the enzyme that converts testosterone into E2, increased.

![Figure 4](image-url) Effect of hyperandrogenism on serum progesterone, testosterone, and estradiol (E2) levels and E2/testosterone ratio. Serum levels of (A) progesterone, (B) testosterone, (C) E2, and (D) E2/testosterone ratio from the control, eCG, and eCG+HA groups. Each column represents the mean±S.E.M. of ten measurements from different animals: a vs b: P<0.0001 and b vs c: P<0.0001 by ANOVA.

![Figure 5](image-url) Effect of hyperandrogenism on the inflammatory status. (A) Graph corresponding to the integrated optical density of mRNA of cyclooxygenase 2 (COX2) from the control, eCG, and eCG+HA groups determined by real-time PCR. Each column represents the mean±S.E.M. of ten measurements from different animals: a vs b: P<0.0001 and b vs c: P<0.0001 by ANOVA. (B) A representative western blot corresponding to protein expression of COX2 from the control, eCG, and eCG+HA groups compared with actin (C), lane 1, control; lane 2, eCG; and lane 3, eCG+HA, and (D) graph corresponding to the integrated optical density of COX2 protein from the control, eCG, and eCG+HA groups determined by western blot. Each column represents the mean±S.E.M. of ten measurements from different animals: a vs b: P<0.0001 and b vs c: P<0.0001 by ANOVA. (E) Ovarian content of prostaglandin E (PGE) determined by RIA. Each column represents the mean±S.E.M. of ten measurements from different animals: a vs b: P<0.0001 and b vs c: P<0.0001 by ANOVA.
Each column represents the mean ± S.E.M. of ten measurements from different animals: a vs b: \( P < 0.0001 \) by ANOVA.

These data are in agreement with that reported by Gougeon (1996), according to which E2 levels are essential during follicular development. In contrast, we have previously reported that when folliculogenesis is induced in a hyperandrogenic condition, the levels of both PPARγ and StAR decrease to control values (Faut et al. 2011), and herein, we demonstrated that all enzymes downstream of pregnenolone synthesis, i.e., CYP17, 3β-HSD, and 17β-HSD, remained higher than in the control and eCG groups, except that corresponding to CYP19A, which showed the same pattern as the control group. Moreover, serum progesterone levels that increase during the induction of folliculogenesis decrease to control values during the hyperandrogenic condition. These data suggest the stimulation of ovarian steroidogenesis and the accumulation of testosterone during the induction of follicular development in a hyperandrogenic condition. This accumulation of testosterone and the adverse E2/testosterone ratio, which have been reported to impair follicular development (Gougeon 1996), result in avoiding the production of the dominant follicle able to ovulate. In addition, Dumesic et al. (2007) reported that an altered E2/testosterone ratio is responsible for poor oocyte quality in prenatally HA female rhesus monkeys and sheep. Furthermore, Amato et al. (2011) reported that a low E2/testosterone ratio is associated with oligoanovulatory cycles in women with PCOS. In agreement with these findings, herein, we found that hyperandrogenism generates altered follicular development, represented by an increased percentage of PrF and a decreased percentage of PF, suggesting accelerated follicular recruitment but in detriment of follicle development. Not only the percentage but also the total number of follicles were modified by hyperandrogenism. Ovaries from the eCG group showed an increased number of total follicles when compared with controls. This increased number of follicles decreased by hyperandrogenism when compared with those from the eCG group but remained higher than those from the control group.

The data of this study demonstrate, for the first time, that folliculogenesis induced by eCG in prepubertal rats results in increased gene expression of the PPARγ co-activator PGC1α and in decreased gene expression of the co-repressor NCoR when compared with controls and that, in a hyperandrogenic condition, NCoR remains higher than in both the control and eCG groups and PGC1α reaches control values. These results suggest that folliculogenesis induced by eCG activates the PPARγ transcriptional system. These findings and the fact that, during the hyperandrogenic condition, the mRNA levels of NCoR remain higher than those of controls, and that those of PGC1α reach control values, suggest that the decreased ovarian PPARγ system induced by hyperandrogenism might be responsible for an ovarian environment unfavorable to generate pre-ovulatory follicles.

The role of PPARγ in ovarian steroidogenesis is associated with increased COX2 activity, which, in turn, enhances PG synthesis before ovulation (Duffy & Stouffer 2001, Komar et al. 2001, Komar 2005, Banerjee & Komar 2006, Brannian et al. 2008, Kim et al. 2008). In fact, we found that folliculogenesis induction increased both gene and protein expression of ovarian COX2 and that this correlated with enhanced ovarian PGE content. We also found that hyperandrogenism exacerbated these effects, a fact in agreement with our previous findings (Amalfi et al. 2012). Taking into account the results obtained herein, both in the PPARγ system and with respect to the E2/testosterone ratio, we suggest that high COX2 expression and PG synthesis would down-regulate the PPARγ system, as in other systems (Banerjee & Komar 2006).

During early folliculogenesis, apoptosis plays a fundamental role during early folliculogenesis. We have previously demonstrated that acute hyperandrogenism induces a pro-apoptotic status (Faut et al. 2011), which alters normal folliculogenesis. Therefore, prenatally HA rats display increased ovarian oxidative stress and an anovulatory estrous cycle during their adult life.

Figure 6 Effect of hyperandrogenism on oxidative stress. (A) Lipid peroxidation was evaluated by the quantification of malondialdehyde (MDA) levels and (B) concentration of antioxidant metabolite glutathione (GSH) from the control, eCG, and eCG+HA groups. Each column represents the mean ± S.E.M. of ten measurements from different animals: a vs b: \( P < 0.0001 \) by ANOVA.

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(Amalfi et al. 2012). In this study, we found that acute hyperandrogenism induces an imbalance in the oxidant–antioxidant ratio characterized by increased systemic lipid peroxidation and decreased GSH levels. These data reveal a response of the antioxidant defenses against hyperandrogenism, which is in agreement with the so-called ‘controlled response of GSH’ reported previously (Amalfi et al. 2012).

In summary, our results demonstrate for the first time that an acute hyperandrogenic condition during follicular development alters the gene expression of the PPARγ co-repressor NCoR and co-activator PGC1α, generating an unfavorable environment that involves the dysregulation of the PPARγ system. We also found that the hyperandrogenic condition during follicular development enhances ovarian steroidogenesis, with the accumulation of testosterone and an activation of the pro-inflammatory status, and that it leads to establishment of a systemic pro-oxidant status.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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