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EXPRESSION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS IS REGULATED BY GONADOTROPINS AND STEROID HORMONES IN *IN VITRO* PORCINE OVARIAN FOLLICLES

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The peroxisome proliferator-activated receptors (PPARs) are a family of transcription factors which are involved in the regulation of different processes such as lipid metabolism, inflammation, angiogenesis, tissue remodeling and steroidogenesis. Our previous data described the ovarian expression of PPAR isoforms (-α, -γ and -β), and the increase of PPAR-α and PPAR-γ in porcine ovarian follicles during the estrous cycle. The current studies were undertaken to test the hypothesis that gonadotropin and steroid hormones can regulate the expression of PPAR isoforms in the ovary. Medium follicles (4 – 6 mm) at 10 – 12 days of the estrous cycle were obtained from mature crossbred gilts. Ovarian follicles were exposed to gonadotropins FSH and LH at 50, 100 and 150 ng/ml, and to steroid hormones such as progesterone (P4), testosterone (T) and estradiol (E2) at 10^{-8}, 10^{-7}, 10^{-6} M for 24 hours. Then mRNA and protein expression of PPAR-α/γ/β *via* real time PCR and Western blot, respectively, were measured. We observed that FSH increased both mRNA and protein expression of all PPAR isoforms, while LH only increased PPAR-α/γ. We have also noted that P4 and E2 significantly increased expression of PPAR-α/γ without having an effect on β isoform, while T had no effect on all PPARs expression. Our study clearly showed that local regulators of ovarian activity, both gonadotropin and steroid hormones are regulators of PPAR isoforms expression in porcine ovarian follicles.

Key words: *ovarian follicle, estrous cycle, peroxisome proliferator-activated receptors-α, -γ, -β, steroid hormones, luteinizing hormone, follicle stimulating hormone, gonadotropin*

INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs) are a subfamily of three ligand-inducible transcription factors, which belong to the superfamily of nuclear hormone receptors. In mammals, the PPARs subfamily consists of three members: α, β and γ, which control the expression of a large number of genes involved in metabolic homeostasis, lipid, glucose, energy metabolism, adipogenesis, inflammation in addition to reproduction (1). PPARs receptor subtypes (α, β and γ) have different physiological activities and tissue localization. For example, PPAR-α and -β/-δ participate in energy combustion, while PPAR-γ enhances adipogenesis by taking part in energy storage (2). PPARs are expressed in almost all tissues involved in lipid metabolism such as the liver, kidney, intestines and skeletal muscles (1, 2). However, all the PPAR isoforms have been detected in the ovary of many species like rats (3, 4), mice (5), sheep (6), cows (7, 8), buffalos (9), pigs (10, 11) and humans (12). Our recent data described that the expression of PPAR isoforms α/γ increased in porcine ovarian follicles during estrous cycle (10). Several studies have indicated the important role of PPARs in ovarian functions such as the cell cycle, apoptosis and steroidogenesis (13, 14). Data of Froment *et al*. (13) documented that PPAR-γ regulated follicular development, oocyte maturation, ovulation and regression of corpus luteum (CL) in sheep. Furthermore, PPARs control ovarian steroidogenesis; stimulatory effect of PPAR-γ activators on progesterone (P4) secretion was observed in rats, bovines (3, 13), pigs (15-17) and humans (18). Agonists of PPAR-γ have been reported to modify steroid production by both follicular (3, 7, 16, 19, 20) and luteal cells (8, 21).

A recent study indicated that PPAR expression can be regulated by some hormones and by different factors like endocrine chemicals disruptor. For example, in pancreatic beta cell line MIN6, adiponectin, an adipocyte-derived factor, induced PPAR-γ expression at the mRNA and protein levels (22). Our previous report demonstrated that in ovarian follicles, resistin increased PPAR-γ expression in a dose-dependent manner (10). In rat granulosa cells, lower mRNA and protein PPAR-γ expression was observed after luteinizing hormone (LH) peak (3, 23). However, Long *et al*. (4) described that decrease level of follicle stimulating hormone (FSH) during the neonatal period had no effect on expression of mRNA for PPAR-γ in mice ovary. Particularly, the data obtained in rhesus monkey showed that LH/hCG rapidly reduced PPAR-γ expression and its target gene NR1H3 enzyme in preovulatory follicles (24). Moreover, polyphenolic compounds significantly increased PPAR-α mRNA in liver (25, 26), while tetrabromobisphenol A increased the expression of the PPAR-γ protein in placenta human JEG-3 cells (27).

Although there is evidence that different factors modulate PPARs expression, however, local regulators of ovarian activity

such as a gonadotropins and steroid hormones have not been reported to regulate PPARs in the ovary. Thus, in this study, for the first time, we used *in vitro* model of whole porcine ovarian follicles (including theca and granulosa cells) to investigate dose- response effects of both gonadotropins LH, FSH and steroid hormones like P4, testosterone (T) and estradiol (E2) on mRNA and protein expression of all isoforms of PPARs. As an experimental model, we used porcine ovary because pigs are becoming a valid alternative to traditional non-rodent species in pharmacological, physiological and toxicological studies, in addition to many of their physiological characteristics that resemble those of humans (28).

MATERIALS AND METHODS

Reagents and antibodies

M199 medium and phosphate-buffered saline (PBS) were purchased from CytoGen, Poland. Antibiotic-antimycotic solution (100×), TRIS, Na-deoxycholate, Nonidet NP-40, sodium dodecyl sulfate (SDS), protease inhibitor (EDTA-free), dithiothreitol (DTT), Tween 20, bromophenol blue, dimethyl sulfoxide (DMSO), anti-β-actin antibody, FSH from porcine pituitary (cat. # F2293), luteinizing hormone (LH) from sheep pituitary (cat. # L5269), synthetic steroids: P4, T, E2 (cat. # P0130, cat. # 86500, cat. # E2257, respectively) and Western blotting luminol reagent (cat. # sc-2048) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Mouse monoclonal PPAR-γ (sc-271392), goat polyclonal PPAR-α (sc-1985) and rabbit polyclonal PPAR-β (sc-7197) antibodies were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). A Bradford protein assay kit was obtained from Bio-Rad Laboratories (Hercules, CA, USA).

Sample collection and ovarian follicle incubation

Porcine ovaries were collected from mature (7 – 8 months of age) crossbred gilts (Large White and Polish Landrace) at a local abattoir. Average weight of these animals is 130 ± 10 kg. A veterinarian determined the age, weight and gender of all animals before slaughter. Ovaries were collected in a bottle filled with sterilized ice-cold saline containing an antibiotic-antimycotic solution before being transported to the laboratory. Approximately 1 hour elapsed between slaughter and ovary collection. Individually, each ovary derived from mature, estrous cycling pigs was checked for their size and morphology of the CL. Medium size follicles (4 – 6 mm) were obtained from ovaries of pigs on days 10 – 12 of the estrous cycle as described previously (29). Estrus was designated as day zero. After isolation, ovarian follicles were cut using small scissors to facilitate the penetration of the compounds into the tissue. Ovarian follicles were exposed to LH and FSH at 50, 100, and 150 ng/ml doses, and the steroids P4, T and E2 at 10^{-8}, 10^{-7}, 10^{-6} M doses for 24 hours. The doses of hormones were chosen based on previous observations (29). After incubation, the medium was removed and follicles were stored at –20°C for PPARs protein expression. Status of health of follicles at the end of culture was measurement using cell viability test (Alamar blue). To analyze PPARs mRNA expression, a part of ovarian follicles was immediately frozen in liquid nitrogen and stored at –70°C. The total number of ovarian follicles for each experiment was 64 (*Fig. 1*). Each treatment was conducted in four wells and each experiment was repeated three times (n = 3). So, the total number of ovarian follicles both for Western blot and PCR analysis was 384.

Real-time PCR

Isolation of total RNA, including a 15 min DNAse I treatment, was carried out using the High Pure RNA Tissue Kit (12033674001, Roche Applied Science, Mannheim, Germany) according to the manufacturer's protocol. The RNA concentration was quantitated by spectrophotometry using optical density measurements at 260 and 280 nm (BioPhotometer Plus, Eppendorf, Germany). One microgram of RNA was used for reverse transcription.

cDNA synthesis was performed using the Transcriptor First Strand cDNA Synthesis Kit (04379012001, Roche Applied Science, Mannheim, Germany) with a mixture of 50 pmol/μL anchored oligo(dT)18 primer and 600 pmol/μL random hexamer primers according to the manufacturer's protocol. The reverse transcription reaction was incubated for 10 min at 25°C, followed by 60 min at 50°C and 5 min at 85°C, with subsequent cooling to 4°C. The samples were stored at –20°C or future analysis.

Real-time PCR analyses were performed using the StepOne Real-Time PCR system (Applied Biosystems). The mRNA expression of all investigated genes was quantified in each sample using *Taq*Man Gene Expression Assays (Applied Biosystems) (*Table 1*). GAPDH expression was used as an endogenous control. Quantitative PCR was performed with 100 ng of cDNA, 1 μL of the gene expression assay, and 10 μL of *Taq*Man PCR master mix (4369016, Applied Biosystems) in a final volume of 20 μL. After 2 min of incubation at 50°C, the thermal cycling program was as follows: 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. The cycle threshold number (Ct) was determined for quantitative

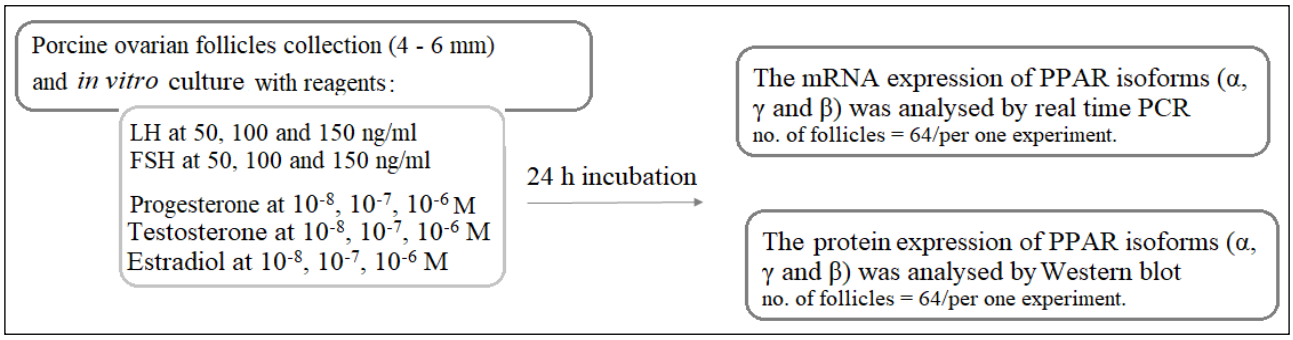

Fig. 1. Diagram of the experimental plan. After ovarian follicles isolation, follicles were exposed to reagents with reagents for 24 hours. The total no. of ovarian follicles in one experiment was 64. Each treatment was conducted in four wells and each experiment was repeated three times (n = 3). So, the total number of ovarian follicles both for Western blot and real time PCR analysis was 384.

Table 1. Genes investigated in the present study. *Taq*Man Gene Expression Assays were used to quantify mRNA expression. [A]Catalog numbers refer to Life Technologies (Grand Island, NY, USA).

Gene Symbol	Gene Name	Catalog[A] #s	Reference Sequence
PPARA	peroxisome proliferator-activated receptor-α	Ss03380164_u1	NM_001044526.1
PPARG	peroxisome proliferator-activated receptor-γ	Ss03394828_m1	NM_214379.1
PPARBETA	peroxisome proliferator-activated receptor-β	Ss03394198_g1	NM_001130241.2
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	Ss03375629_u1	NM_001206359.1

measurement, and the data analysis was performed using the comparative threshold cycle (Ct) method. Relative quantification (RQ) was performed using the $2^{-\Delta\Delta Ct}$ method by adjusting the target gene expression to GAPDH and comparing the adjusted expression with that of the control group (RQ = 1).

Western blot analysis

Tissue preparation, lysis, Western blotting and quantification were performed as previously described (10). Briefly, whole ovarian follicles were homogenized in ice-cold lysis buffer (50 mM Tris-HCl pH 7.5, 100 mM NaCl, 0.5% Na-deoxycholate, 0.5% NP-40, 0.5% SDS and EDTA-free protease inhibitors) and the protein content was determined using the Bradford reagent with bovine serum albumin (BSA) as per the standard procedure. After homogenization, the supernatants were collected and stored at –20°C until further analysis. 30 µg of proteins were reconstituted directly in the appropriate amount of sample buffer, which consisted of 125 nM Tris (pH 6.8), 4% SDS, 25% glycerol, 4 mM EDTA, 20 mM DTT, and 0.01% bromophenol blue and then the samples were separated by 12% SDS-PAGE (BioRad Mini-Protean II Electrophoresis Cell) and transferred to nitrocellulose membranes (BioRad Mini Trans-Blot apparatus). Following the transfer, the membranes were washed and blocked with 5% BSA/0.2% Tween-20/0.02 M TBS for 1 hour. The membranes were then incubated overnight with antibodies diluted 1:200 in TBS/Tween at 4°C. After incubation with the primary antibody, the membranes were washed with TBS and 0.02% Tween-20 and incubated for 1 hour with a horseradish peroxidase-conjugated antibody diluted 1:500 in TBS/Tween. An anti-β-actin antibody diluted 1:3000 was used as a loading control. Signals were detected by chemiluminescence using Western Blotting Luminol Reagent and visualized using the ChemidocTM XRS + System (BioRad Laboratories). All bands visualized by chemiluminescence were quantified using a densitometer and ImageLabTM 2.0 Software (BioRad Laboratories).

Statistical analysis

Each treatment was conducted in quadruplicate and each experiment was repeated three times (n = 3). Distribution of normality was checked by Shapiro-Wilk test. A one-way analysis of variance (ANOVA) was used for multiple comparisons involving more than two treatment groups. The Tukey honest significant difference (HSD) multiple range test was performed *post hoc* (GraphPad PRISM v. 4.0; GraphPad Software, Inc., San Diego, CA). All data are expressed as the mean ± SEM. Statistical significance is indicated by different letters ($P < 0.05$) or by *($P < 0.05$), **($P < 0.01$), and ***($P < 0.001$).

RESULTS

Effect of gonadotropins on mRNA expression of PPARs isoforms

Fig. 2 showed that both gonadotropins LH and FSH at all doses significantly increased mRNA expression of PPAR-α: 1.8-, 1.7-, and 1.8-fold after 50, 100 and 150 ng/ml of LH, and 1.9-, 1.8-, and 1.8-fold after 50, 100 and 150 ng/ml of FSH compared with control (*P*<0.05, $P < 0.01$) (*Fig. 2A*). Similar effect was observed in PPAR-γ mRNA expression; it was 1.6-, 1.9-, and 1.5-fold after 50, 100 and 150 ng/ml doses of LH and 1.7-, 1.8-, and 1.5-fold after 50, 100 and 150 ng/ml doses of FSH compared with control ($P < 0.05$, $P < 0.01$) (*Fig. 2C*). PPAR-β mRNA expression of 1.8- and 1.5-fold compared with control was higher in ovarian follicles treatment only by FSH at doses 100 and 150 ng/ml ($P < 0.05$, $P < 0.01$), but not LH (*Fig. 2B*).

Effect of gonadotropin on protein expression of PPARs isoforms

We observed that both gonadotropins stimulated PPARs protein expression: LH at 100 and 150 ng/ml only PPAR-γ (54/57 kDa) ($P < 0.05$), while FSH at all doses for both PPAR-α (55 kDa) and PPAR-γ ($P < 0.05$, $P < 0.01$) and only at doses 150 ng/ml PPAR-β (52 kDa) expression ($P < 0.05$) (*Fig. 3A*). Densitometry analysis confirmed presented results (*Fig. 3B*).

Effect of steroids on mRNA expression of PPARs isoforms

Statistically significant increases in PPAR-α mRNA expression of 1.7-, 1.5-, and 1.9-fold compared with control were observed with P4 treatments of 10^{-6}, 10^{-7} and 10^{-8} M, respectively and 1.5-, 1.7-, and 2.4-fold compared with control with E2 doses of 10^{-6}, 10^{-7} and 10^{-8} M, respectively ($P < 0.05$, $P < 0.01$, $P < 0.001$) (*Fig. 4A*). *Fig. 4B* showed that all steroid hormones had no effect on PPAR-β mRNA expression. PPAR-γ mRNA expression was significantly higher by 1.6-, 1.6-, and 1.5-fold compared with control by P4 at 10^{-6}, 10^{-7}, 10^{-8} M, respectively and 1.7-, 1.5-, and 1.8-fold by E2 at 10^{-6}, 10^{-7}, 10^{-8} M, respectively ($P < 0.01$) (*Fig. 4C*). Additionally, testosterone had no effects on mRNA levels of PPAR-α, -β and -γ.

Effect of steroids on protein expression of PPARs isoforms

PPAR-α protein expression was significantly higher after P4 and E2 treatment at doses 10^{-7} and 10^{-8} M ($P < 0.01$, $P < 0.001$). We observed that protein expression of PPAR-β was significantly increased only after P4 at 10^{-7} M ($P < 0.05$), while PPAR-γ after P4 at 10^{-7} M and E2 at 10^{-7} and 10^{-8} M ($P < 0.05$, $P < 0.01$, $P < 0.001$) (*Fig. 5*). We observed that T had no effect on protein expression of all PPAR isoforms.

DISCUSSION

To the best of our knowledge, the present study is the first to demonstrate that both gonadotropins and steroid hormones regulate PPARs expression in porcine ovarian follicles; we observed that FSH increased both mRNA and protein expression of all PPARs isoforms, while LH only increased PPAR-α and -γ. We have also noted that progesterone and estradiol significantly increased expression of PPAR-α and -γ without affecting the -β isoform, while testosterone had no effect on all PPARs expression. In details, we obtained a difference in results between mRNA and protein expression of PPARs; for example LH stimulated PPARs isoform α and γ at mRNA levels, while finally we observed higher protein expression for only for PPAR-γ. Several possible mechanisms have been reported which may help explain this discrepancy, for example, high protein expression may suppress mRNA expression, and high gene expression may diminish post-transcriptional processes (30).

The findings of the present study provide interesting and novel insights into PPARs expression in the ovary. A previous study published by Komar *et al*. (3) and Banerjee and Komar (23) demonstrated that in rat granulosa cells, mRNA and protein expression of PPAR-γ were markedly decreased in response to the LH surge. Moreover, cells that responded to LH lost PPAR-γ mRNA expression, whereas those that did not respond to the surge maintained their level of PPAR-γ expression (31). A study by Long *et al*. (4) documented that FSH is not a primary factor initiating PPAR-γ expression in rat ovaries, and suggested the involvement of other hormones. Here, we observed that gonadotropin stimulated PPAR-α and -γ expression, while FSH increased PPAR-β. These differences are likely to be attributed to the culture model used. In our experiments we used *in vitro*

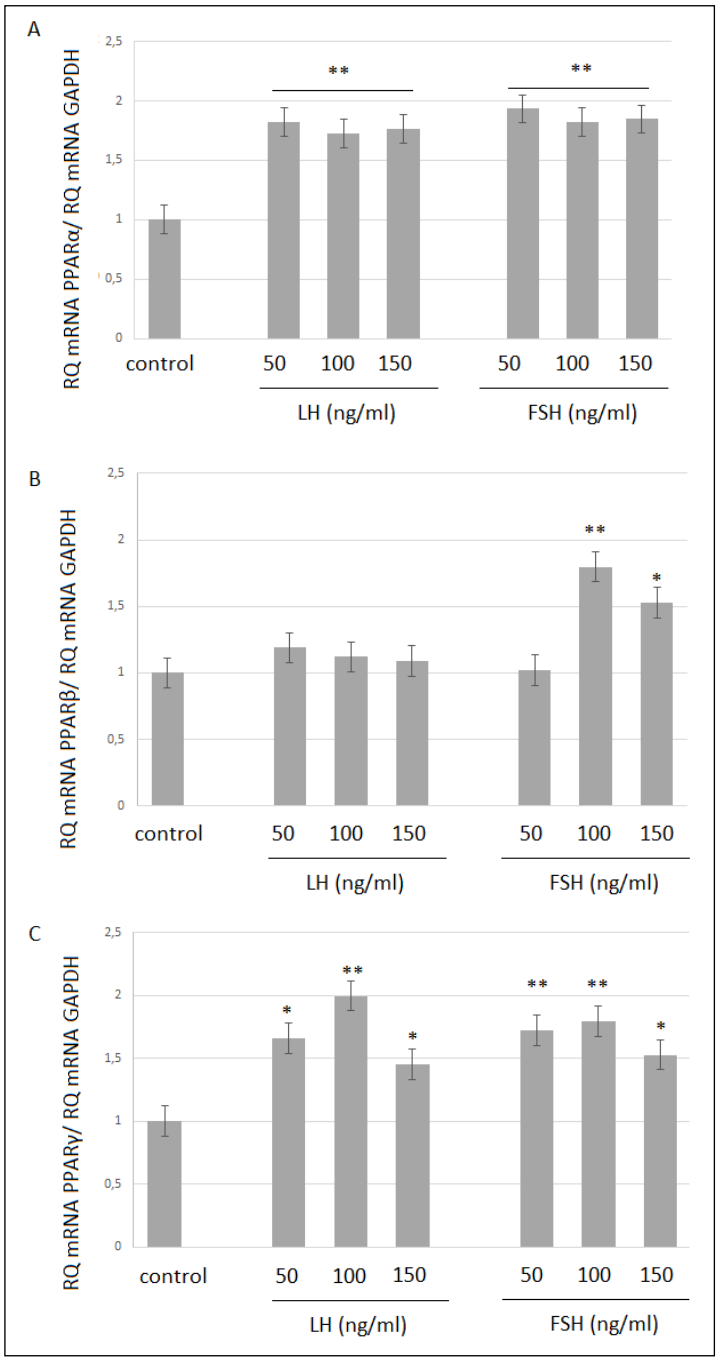

Fig. 2. Effect of LH and FSH at doses of 50, 100 and 150 ng/ml on mRNA expression of PPARs: A) α, B) β and C) γ. The mRNA expression level was determined by real-time PCR using *Taq*Man Gene Expression Assays. The expression of PPARs was normalized to the expression of GAPDH. Real-time PCR were independently performed and repeated three times. The data are plotted as the mean ± SEM. Significance between control and gonadotropin treatments is indicated by *P < 0.05 and **P < 0.01.

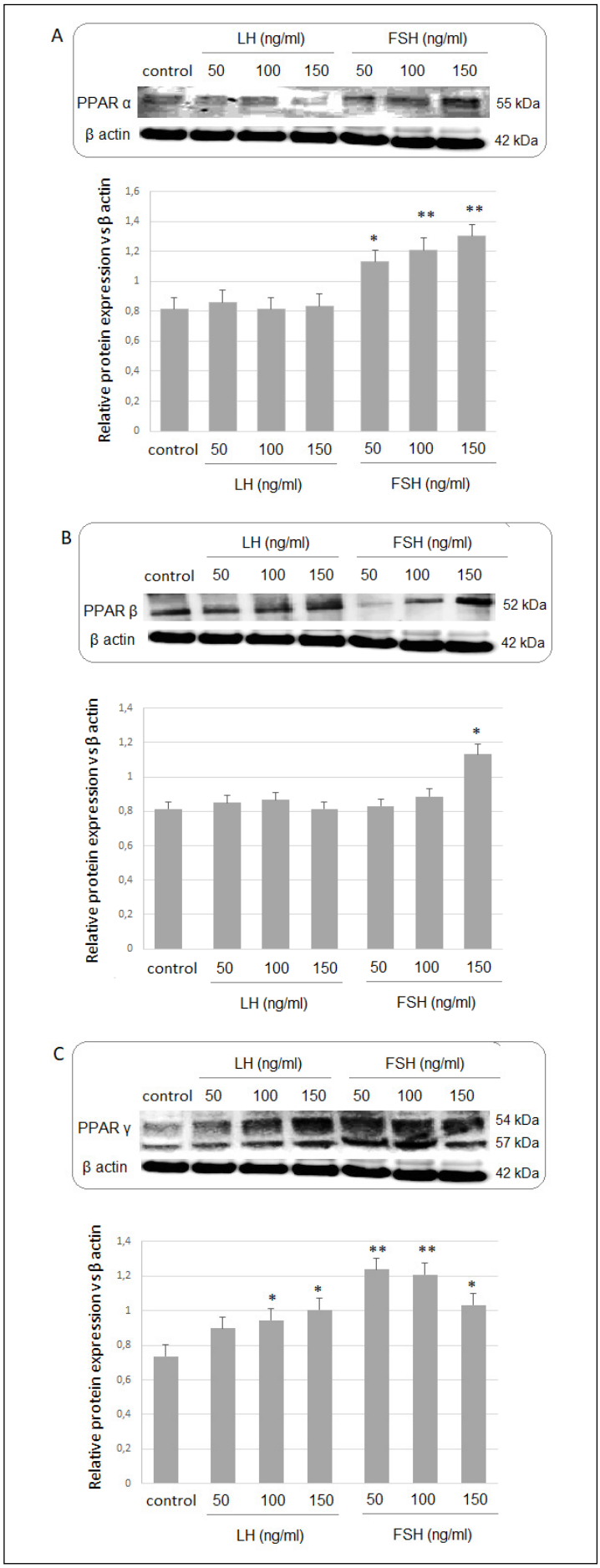

Fig. 3. Effect of LH and FSH at doses of 50, 100 and 150 ng/ml on protein expression of PPARs: A) α, B) β and C) γ. The amount of protein (30 μg) in each sample was confirmed by immunoblotting using an anti-β-actin antibody. The proteins levels of PPARs α (55 kDa), β (52 kDa) and γ (54/57 kDa) were densitometrically scanned. Western blotting experiments were independently performed and repeated three times. The data are plotted as the mean ± SEM. Significance between control and gonadotropin treatments is indicated by *P < 0.05 and **P < 0.01.

culture of whole ovarian follicles including both compartments granulosa and theca cells. This is a more physiological model of ovarian physiology than monoculture of granulosa cells because interaction between both compartment of follicles was maintained in *in vitro* condition. Moreover, mRNA of the FSH receptor is present on granulosa cells surface, while LHR on theca cells and both cells respond to gonadotropin activity in the ovary. Additionally, interactions of the hypothalamic-pituitary-ovarian axis are accountable for normal ovarian activity. FSH plays a role in weaning and regulating the number of ovarian follicles that mature, in addition to granulosa cell proliferation and apoptosis (32), whereas LH is the principal luteotrophic signal in pigs, cows and sheep, and it is necessary for normal functioning of theca cells and development of the CL and maintenance of its action (33, 34). Last data of Nagyova *et al*., (35) showed the interesting findings on the expression of an oocyte-derived growth factor in regulation of the activity of gonadotropin in the ovary using primary porcine culture.

Several studies have reported that sex hormones such as E2, T, and dehydroepiandrosterone (DHT) exert an effect on PPAR-γ expression in insulin-target tissues such as the liver, muscles and fat (36, 37). In this work we demonstrated that P4 and E2 increased expression of PPAR-α and -γ in the ovarian follicles. Our results are in good agreement with the previous data published by Sato *et al*. (38), who demonstrated that a long period of exposure to E2 significantly increased PPAR-γ protein expression in mature adipocytes. Interestingly, our observation can by also supported by previous data. In the porcine, during the development of ovarian follicles, PPAR-γ and -α protein expression was increased, which was correlated with P4 and E2 levels in the follicular fluid (10). Moreover, in rat granulosa cells, high levels of mRNA for PPAR-γ was observed during follicular development when gonadotropin concentration was also increased (3). Our data also showed that testosterone had no effect on all PPARs ovarian expression, such as DHT on mRNA expression of PPAR-γ in the adipose tissue (38). Our results is

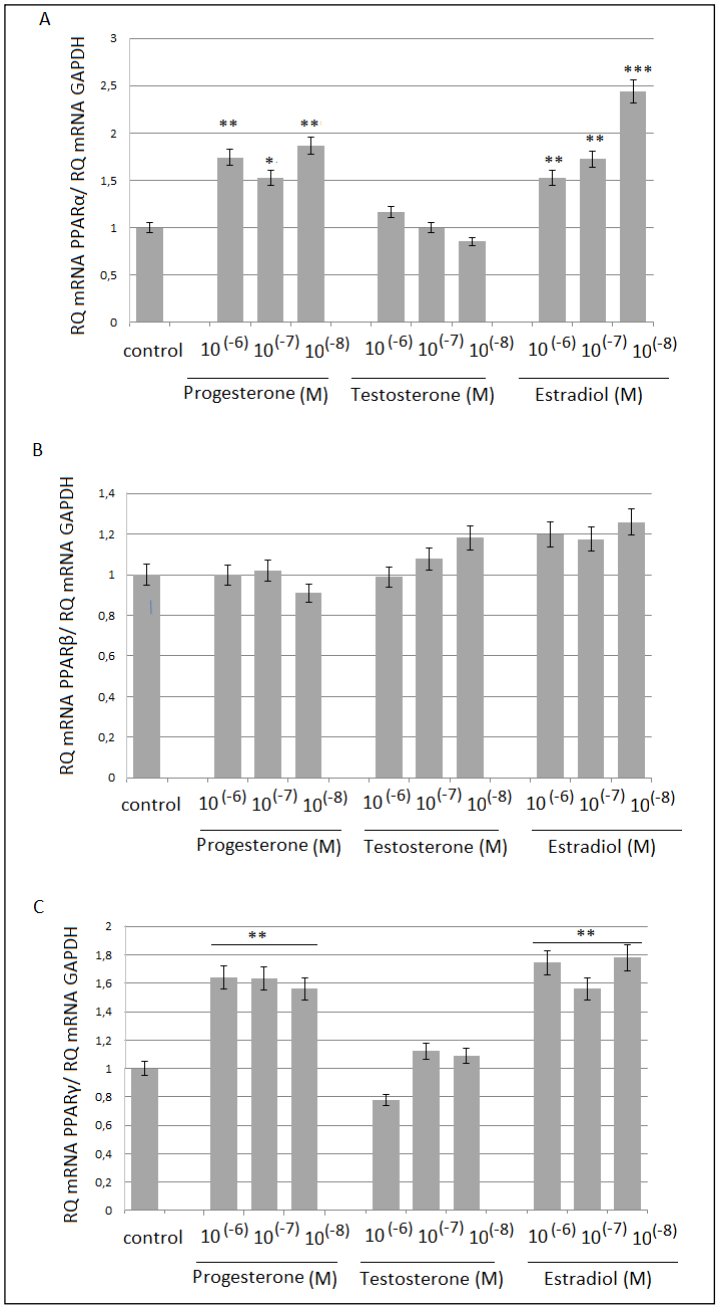

Fig. 4. Effect of progesterone, testosterone and estradiol at doses 10^{-6}, 10^{-7} and 10^{-8} M on mRNA expression of PPARs: A) α, B) β and C) γ. The mRNA expression level was determined by real-time PCR using *Taq*Man Gene Expression Assays. The expression of PPARs was normalized to the expression of GAPDH. Real-time PCR were independently performed and repeated three times. The data are plotted as the mean ± SEM. Significance between control and steroids hormone treatments is indicated by *P < 0.05, **P < 0.01, and ***P < 0.001.

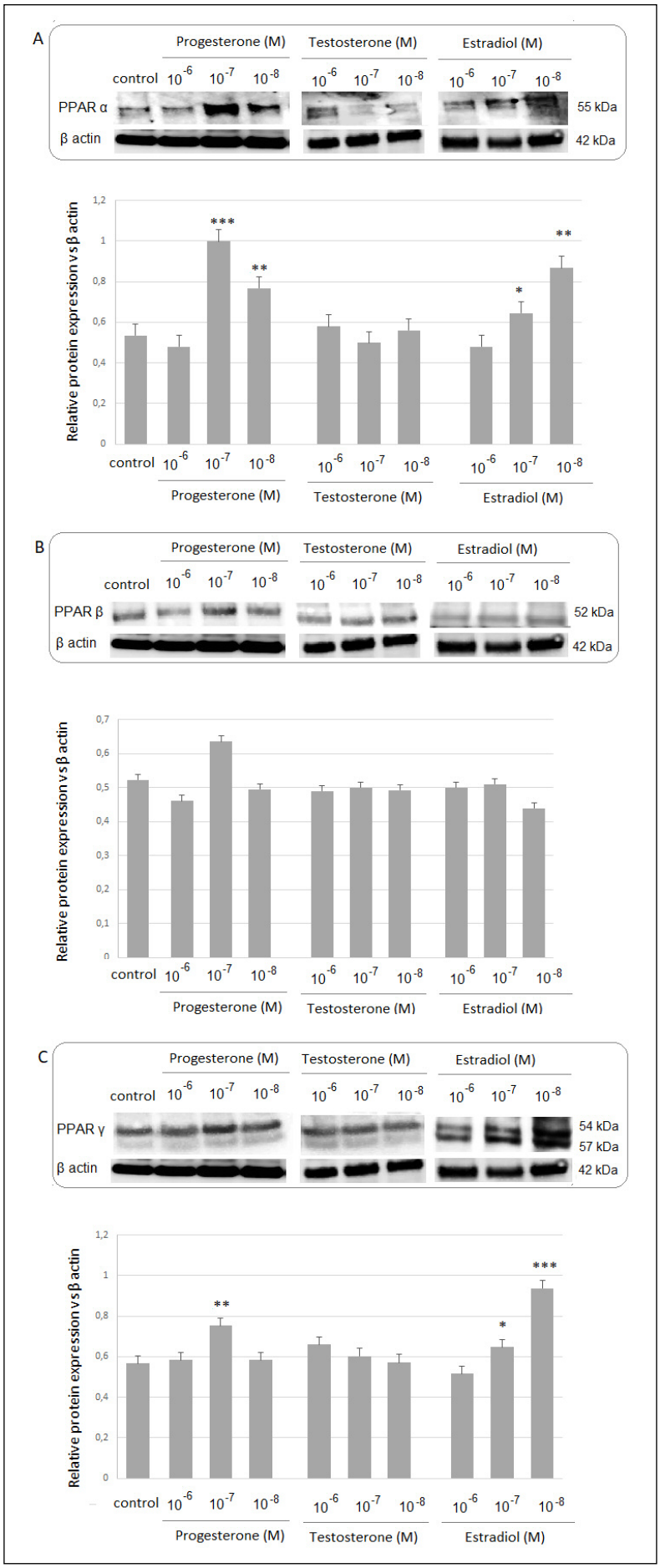

Fig. 5. Effect of progesterone, testosterone and estradiol at doses 10^{-6}, 10^{-7} and 10^{-8} M on protein expression of PPARs: A) α, B) β and C) γ. The amount of protein (30 µg) in each sample was confirmed by immunoblotting using an anti-β-actin antibody. The protein levels of PPARs α (55 kDa), β (52 kDa) and γ (54/57 kDa) were densitometrically scanned. Western blotting experiments were independently performed and repeated three times. The data are plotted as the mean ± SEM. Significance between control and steroids hormone treatments is indicated by *P < 0.05, *P < 0.01, and ***P < 0.001.

good agreement with data of Sato *et al*. (38), who shown the main difference between the actions of E2 and androgen (DHT or T) involves their effect on PPAR-γ expression at the protein level. Moreover, data of Dieudonne *et al*. (39) explain that T acted as a negative effector of rat preadipocyte terminal differentiation and suggested that the mechanism underlying these responses involved the regulation of genes encoding adipogenic transcriptional factors. Among these factors, PPAR-γ was considered as one of the master regulator genes of the adipoconversion process. The authors showed that PPAR-γ expression was increased after E2 exposure in epididymal adipocytes from male rats and in parametrial adipocytes from ovariectomized rats, whereas T levels slightly decreased the expression of PPAR-γ in epididymal adipocytes. One mechanism that could explain difference between the actions of E2 and T on PPARs expression is a availability and number of receptors for steroid hormones; in the ovary prevail estrogen receptor. However, future study indicating molecular mechanism of observed difference in steroid action on PPARs expression are needed. Based on the obtained results, we suggest that ovarian expression of PPARs are regulated by gonadotropin and steroid hormones. This hypothesis was partly confirmed by Bogacka and Bogacki (40), that proposed a correlation between PPARs gene expression and steroids hormones in porcine endometrium. These authors observed lower expression of PPAR-α/-β at the early stages of the luteal phase and later during the follicular phase of the estrous cycle, when the P4 level is diminished, but when estrogens play a crucial role (40). Moreover, *in vitro* effect of PPAR-γ receptor ligand on prostaglandin E2 synthesis and secretion by porcine endometrium during estrous cycle suggested additional mechanistic mechanisms of PPAR-γ-induced regulation of ovary (41).

There is evidence of possible interaction between PPAR and estrogen signaling in regulation of ovarian function. PPARs can effect on ovarian function by modifying the ability of E2 to elicit cellular responses. PPARs are able to bind to estrogen response elements - EREs (42, 43), and can act as competitive inhibitors (42). Moreover, PPAR-γ can also stimulate ubiquitination of estrogen receptor α, leading to its degradation (44) and regulates the activity and expression of aromatase, an enzyme involved in the biosynthesis of estrogen. In human breast adipose tissue and granulosa-lutein cells, activation of PPAR-γ decreased aromatase activity (21, 45). The activation of PPAR-γ has also been shown to inhibit P4 production by cultured porcine (46) and human (47) granulosa cells by inhibition of the activity of 3β-hydroxysteroid dehydrogenase. PPARs regulated also other factors in the ovary, such as endothelin-1 (48), nitric oxide synthase (49-51), and cyclooxygenase-2 (52), indicating that there are a number of ways PPARs could regulate the ovarian function.

In summary, our results provide novel insights into regulation of PPAR expression in ovarian follicles. We observed that FSH increased mRNA and protein expression of all PPARs isoforms, while LH only increased PPAR-α and -γ. Steroids like progesterone and estradiol increased expression of PPAR-α and -γ without affecting the -β isoform, while testosterone had no effect on all PPARs expression. We have clearly demonstrated that both gonadotropin and steroid hormones are regulators of PPARs isoforms expression in porcine ovarian follicles. Numerous studies have revealed that PPARs are functionally expressed in ovary indicating their important role in female reproduction physiology and pathology. Cui *et al*. (5) have shown that tissue specific deletion of PPAR-γ in ovaries of mice has led to impairment in fertility. Moreover, role of PPARs has been evoked in ovarian dysfunction related to obesity, polycystic ovarian syndrome, dyslipidemia, hyperandrogenemia and insulin resistance (14). Results of our data describing regulators of PPARs expression in porcine ovary are useful for studying the role of PPARs in metabolic and reproduction, and hold promise for future therapeutic use in human ovarian disease pathologies involving PPARs. Hence, results of presented data clearly suggesting that hormonal regulation of PPARs might be useful as novel tools for studying PPARs functions in the ovary and as agents for controlling fertility.

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Conflict of interests: None declared.

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