Molecular and Cellular Endocrinology 433 (2016) 47–55

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

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

Local administration of platelet-derived growth factor B (PDGFB) improves follicular development and ovarian angiogenesis in a rat model of Polycystic Ovary Syndrome

Mariana Di Pietro [a], Leopoldina Scotti [a], Griselda Irusta [a], Marta Tesone [a, b], Fernanda Parborell [a], Dalhia Abramovich [a, *]

[a] Instituto de Biología y Medicina Experimental (IByME-CONICET), Vuelta de Obligado 2490, Buenos Aires, Argentina
[b] Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 4 February 2016
Received in revised form
4 May 2016
Accepted 27 May 2016
Available online 30 May 2016

Keywords:
Polycystic ovary syndrome
Platelet derived growth factor
Angiogenesis
Ovary

ABSTRACT

Alterations in ovarian angiogenesis are common features in Polycystic Ovary Syndrome (PCOS) patients; the most studied of these alterations is the increase in vascular endothelial growth factor (VEGF) production by ovarian cells. Platelet-derived growth factor B (PDGFB) and D (PDGFD) are decreased in follicular fluid of PCOS patients and in the ovaries of a rat model of PCOS. In the present study, we aimed to analyze the effects of local administration of PDGFB on ovarian angiogenesis, follicular development and ovulation in a DHEA-induced PCOS rat model. Ovarian PDGFB administration to PCOS rats partially restored follicular development, decreased the percentage of cysts, increased the percentage of corpora lutea, and decreased the production of anti-Müllerian hormone. In addition, PDGFB administration improved ovarian angiogenesis by reversing the increase in periendothelial cell area and restoring VEGF levels. Our results shed light into the mechanisms that lead to altered ovarian function in PCOS and provide new data for potential therapeutic strategies.

1. Introduction

Polycystic ovary syndrome (PCOS) is a common disorder that affects between 5% and 8% of women in reproductive age (Norman et al., 2007); its features comprise both metabolic and reproductive alterations. Due to the heterogeneity of the symptoms, PCOS diagnosis requires continuous revision (Franks, 1995). The ovary of these women presents an aberrant angiogenic process, with increased concentration of Vascular Endothelial Growth Factor (VEGF) as the most studied alteration (Agrawal et al., 1998; Artini et al., 2006; Kamat et al., 1995). These alterations lead to a higher risk of developing Ovarian Hyperstimulation Syndrome (OHSS) when these patients are treated with high gonadotropin doses during assisted reproductive techniques (Agrawal et al., 1998). Moreover, defects in the formation of blood vessels could be in part responsible for the alteration in follicular development in these women.

While the proliferation and migration of endothelial cells during angiogenesis involves mainly VEGF, the formation of a mature and functional vasculature is a tightly regulated process that involves several angiogenic factors (Ribatti et al., 2011). The main factors that regulate maturation and quiescence of new vessels are angiopoietins (ANGPTs) (Fagiani and Christofori, 2013) and platelet-derived growth factors (PDGFs) (Armulik et al., 2005; Hellberg et al., 2010). These angiogenic factors regulate vessel stability and permeability throughout the angiogenic process (Ribatti et al., 2011). PDGFs are members of a family that comprises homo- and heterodimers assembled by four different polypeptide chains, the classical A and B chains (Claesson-Welsh, 1996; Heldin and Westermark, 1999) and the C and D chains (LaRochelle et al., 2001; Li et al., 2000). These chains form four homodimeric

Abbreviations: PDGF, Platelet Derived Growth Factor; PCOS, Polycystic Ovary Syndrome; VEGF, Vascular Endothelial Growth Factor; OHSS, Ovarian Hyperstimulation Syndrome; ANGPT, Angiopoietin; TGFβ, Transforming Growth Factor beta; AMH, Anti-Müllerian Hormone; DHEA, Dehydroepiandrosetrone; FLK1, Fetal Liver Kinase 1.

* Corresponding author. Instituto de Biología y Medicina Experimental (IByME-CONICET), Vuelta de Obligado 2490, C1428ADN Buenos Aires, Argentina.
E-mail addresses: dipietromari@gmail.com (M. Di Pietro), leopoldinascotti@gmail.com (L. Scotti), irustag@gmail.com (G. Irusta), martatesone.uba@gmail.com (M. Tesone), fparborell@gmail.com (F. Parborell), dnabramovich@gmail.com (D. Abramovich).

http://dx.doi.org/10.1016/j.mce.2016.05.022
0303-7207/

isoforms (PDGF-AA, PDGF-BB, PDGF-CC and PDGF-DD) and one heterodimeric isoform (PDGF-AB). PDGFs bind and activate two different receptors: PDGFR-α and PDGFR-β. PDGFB and PDGFD signaling on their receptor PDGFR-β is critical for pericyte and perivascular cell proliferation as well as for migration along the new vessel (Armulik et al., 2005). Knockout mice of *pdgfb* or *pdgfr-β* die perinatally due to defects in vascular development (Leveen et al., 1994; Soriano, 1994). The main defect observed in these mice is the lack of pericytes covering the vasculature (Lindahl et al., 1997). In addition, lack of PDGFR-β action leads to a compensatory increase in VEGF concentration in these animals, which causes further defects in the vasculature (Hellstrom et al., 2001).

Platelet-derived growth factor B, PDGFD and the receptor PDGFR-β are expressed in different types of ovarian cells. Pinkas et al. (2008) described expression of PDGFA, PDGFB and their receptors in oocytes, theca and stromal cells of ovaries obtained from human fetuses and women (Pinkas et al., 2008). In addition, expression of PDGFA, PDGFB and PDGFR-β has been detected in granulosa cells from women (Pinkas et al., 2008). PDGFB and PDGFR-β have also been found in oocytes and granulosa cells from mouse ovaries (Yoon et al., 2006) and in oocytes from rat ovaries (Sleer and Taylor, 2007a). It has been demonstrated that PDGF signaling is involved in theca cell development and in steroid production in the ovary (Schmahl et al., 2008). We have also demonstrated that PDGFB is necessary for correct follicular development (Pascuali et al., 2015). Furthermore, PDGFB/PDGFR-β signaling has been recognized as one of the pathways involved in early folliculogenesis, promoting primordial follicle activation (Nilsson et al., 2006; Pinkas et al., 2008; Sleer and Taylor, 2007a). In this sense, the effects of PDGFB are opposite to those of the anti-Müllerian hormone (AMH). AMH is a member of the transforming growth factor-beta (TGF-β) superfamily and, in the ovary, it is produced by ovarian granulosa cells of primary, preantral and small antral follicles (Durlinger et al., 2002b). AMH inhibits primordial follicle growth, acting as an inhibitory factor during early stages of folliculogenesis (Durlinger et al., 2002a). Luteinizing hormone (LH) has been described as one of the regulators of PDGF synthesis in human luteinized granulosa cells since it is able to increase mRNA levels of *pdgfd* and to decrease mRNA levels of *pdgfb* (Hwu et al., 2009). In this sense, we have demonstrated that ovarian concentration of PDGFB and PDGFD are decreased in a rat OHSS model, suggesting a role of these proteins in the pathogenesis of OHSS (Scotti et al., 2013).

Different gynecological conditions present alterations in ovarian angiogenesis (Carmeliet, 2003). In particular, we and others have previously demonstrated that women with PCOS have higher follicular fluid concentrations of VEGF and ANGPT1 (Kamat et al., 1995; Scotti et al., 2014; Stanek et al., 2007) and an increase in ovarian stromal blood flow (Aleem and Predanic, 1996; Battaglia et al., 1995; Zaidi et al., 1995). We have recently described that PDGFB and PDGFD concentration in follicular fluid from PCOS patients are decreased compared to those of control patients (Scotti et al., 2014). We have also described similar reductions of these proteins in the ovary of a dehydroepiandrosterone (DHEA)-induced PCOS rat model (Di Pietro et al., 2015). Several rodent models have been used to mimic the main features of PCOS. However, no animal model has yet to mimic the full range of signs and symptoms of PCOS (Walters et al., 2012). Nevertheless, we have demonstrated that the DHEA-induced PCOS rat model presents, not only the main ovarian features of human PCOS such as anovulation, absence of cyclicity, and alterations in steroidogenesis, but also increased serum insulin concentration (Di Pietro et al., 2015). Interestingly, the ovaries of these rats have the same alterations in angiogenic factor concentration as PCOS women, such as increased VEGF and ANGPT1, and decreased PDGFB and PDGFD (Abramovich et al., 2012; Di Pietro et al., 2015; Scotti et al., 2014). Therefore, in the present study, we aimed to analyze the effects of local administration of PDGFB on ovarian angiogenesis, follicular development and ovulation in a DHEA-induced PCOS rat model.

2. Materials and methods

2.1. Animal treatment

Immature (21 days old) female Sprague Dawley rats were injected sc with DHEA (6 mg per 100 g body weight per 0.2 ml sesame oil) daily for 15 d to induce the hyperandrogenic PCOS condition. Control animals were injected with 0.2 ml of sesame oil (control group; n = 6). For PDGFB administration, on day 15 of DHEA treatment, PCOS animals were anesthetized with ketamine HCl (80 mg/kg; Holliday-Scott S.A., Buenos Aires, Argentina) and xylazine (4 mg/kg; König Laboratories, Buenos Aires, Argentina), and the ovaries were exteriorized through an incision made in the dorsal lumbar region. Then, DHEA-treated rats were divided into two groups. One of them was injected under the bursa of both ovaries with 25 ng of recombinant PDGFB (kindly donated by Beta Laboratories S.A. Argentina, provided by Denver Farma S.A. Argentina) in 5 μl of saline (PDGFB-PCOS group; n = 6), whereas the other received vehicle under the bursa (basal-PCOS group; n = 6). Six rats per group were used in each experiment and each experiment was performed three times. After injection, the ovaries were returned to the peritoneal cavity and the incisions closed with skin adhesive. Rats were killed by CO_2 asphyxiation on day 16, as previously (Abramovich et al., 2006). The ovaries were removed and cleaned of adhering tissue in culture medium. One ovary was then frozen, and the other one was fixed in Bouin's solution (Biopur S.R.L, Rosario, Argentina) for subsequent assays. All protocols and experiments were approved by the Animal Experimentation Committee of the Instituto de Biología y Medicina Experimental-Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina- Protocol number: CE-022) and conducted according to the guide for the care and use of laboratory animals of the National Institutes of Health (USA).

2.2. Ovarian morphology

To evaluate changes in general structure, the ovaries were removed and immediately fixed in Bouin's solution for 12 h and then embedded in paraffin. Step sections (5 μm) were mounted at 50-μm intervals onto microscope slides to prevent counting the same structure twice, according to the method described by Woodruff et al. (Woodruff et al., 1990). One set of slides was stained with hematoxylin-eosin (H&E) to count the number of different structures per ovary section, and another set was used for immunohistochemistry, histochemistry and immunofluorescence assays. Follicles were classified as primordial (presence of one squamous granulosa cell layer), primary (presence of one cubic granulosa cell layer), preantral or early antral, according to the presence or absence of an antrum and corpora lutea. Morphological characteristics of atretic follicles include the degeneration and detachment of the granulosa cell layer from the basement membrane, the presence of pyknotic nuclei in this cell type, and oocyte degeneration (Andreu et al., 1998; Sadrkhanloo et al., 1987). A cystic follicle was considered as a follicle bigger than or equal in size to a preovulatory follicle, containing four or five plicated layers of granulosa cells surrounding a very large antrum (Convery et al., 1990; Lara et al., 2000) or a large fluid-filled structure with an attenuated granulosa cell layer and thickened theca interna cell layer (Lara et al., 2000). The number of different structures was determined in four ovarian sections from each ovary (six ovaries per group).

2.3. Histochemistry and immunohistochemistry

Tissue sections were deparaffinized in xylene and rehydrated by graduated ethanol washes. Endogenous peroxidase activity was blocked with hydrogen peroxide in PBS solution, and nonspecific binding was blocked with 2% BSA overnight at 4 °C. Sections were incubated with mouse polyclonal anti-smooth muscle cell alpha-actin antibody (Abcam 18147, 1:100; Abcam Inc., Cambridge, MA, USA) or biotinylated lectin *Bandeiraea simplicifolia-1* (BS-1 L3759 20 μg/ml, 1:100; Sigma Aldrich, MO, USA) overnight at 4 °C. After washing, the slides were incubated with biotinylated anti-mouse IgG (except for lectin BS-1) and after 30 min with avidin-biotinylated horseradish peroxidase complex (Vectastain ABC system; Vector Laboratories, Burlingame, CA, USA). Protein expression was visualized with diaminobenzidine staining (catalog number 11718096001; Roche, Mannheim, Germany). The reaction was stopped with distilled water, stained with hematoxylin, and dehydrated before mounting with mounting medium (Canada Balsam Synthetic; Biopack, Buenos Aires, Argentina). Negative controls were obtained in the absence of primary antibody or in the absence of lectin BS-1. Positive controls were obtained by staining HUVECs (for lectin BS-1) and vascular smooth muscle cells (VSMCs) obtained from adult rat aortas (for smooth muscle cell alpha-actin).

The images were digitized using a camera (Nikon, Melville, NY, USA) mounted on a conventional light microscope (Nikon). Finally, the images were converted to TIFF format (bilevel scale) for their analysis. The percentages of the endothelial and periendothelial areas were processed using Image Pro Plus 3.0 (Media Cybernetics). Areas were determined in four ovarian sections from each ovary and six ovaries per group. Only follicles and stroma were counted in the ovarian sections to avoid errors due to the differences in the percentage of CL between groups. Stained zonae pellucidae in the lectin BS-1 histochemistry were not considered in the analysis. The microphotographs were analyzed by an observer blinded to the treatment type.

2.4. Immunofluorescence

Tissue sections were deparaffinized in xylene and rehydrated by graduated ethanol washes. Nonspecific binding was blocked with blocking buffer for 60 min (1× PBS/5% normal serum/0.3% Triton™ X-100). Sections were then incubated with mouse polyclonal anti-smooth muscle cell alpha-actin antibody (Ab 18147, 1:200; Abcam Inc., Cambridge, MA, USA) in antibody dilution buffer (1× PBS/1% normal serum/0.3% Triton™ X-100) overnight at 4 °C. After washing, sections were incubated with rabbit anti-von Willebrand (A 0082, 1:100; Dako, Denmark) in antibody dilution buffer overnight at 4 °C. Slides were washed and incubated with goat anti-rabbit IgG FITC conjugate (Zymax™ 81-6111, 1/1000; Invitrogen, CA, USA) for 1–2 h at room temperature, washed and incubated with Normal mouse IgG, Fluor ^{R} 488 conjugate (Millipore 16-240, 1/1000; Temecula, CA, USA). After washing, the slides were mounted with Vectashield mounting medium for fluorescence (H-100, Vector Laboratories, CA, USA). Negative controls were obtained in the absence of primary antibodies. Positive controls were obtained by staining HUVECs (for von Willebrand factor) and vascular smooth muscle cells (VSMCs) obtained from adult rat aortas (for smooth muscle cell alpha-actin). The images were digitized using a confocal laser-scanning system attached to a microscope. For specificity control, a western blot was performed with the anti smooth muscle cell alpha-actin antibody using the ovarian protein extracts and a negative (protein extract from HUVEC cell line culture) and positive (protein extract from vascular smooth muscle cell (VSMC) primary culture). Immunocytochemistry was also performed with the anti von Willebrand and the anti smooth muscle cell alpha-actin antibodies on HUVEC and VSMCs (supplemental figure).

2.5. Western blots

Ovaries were immediately frozen at −70 °C until protein extraction. Ovaries were resuspended in 400 μl of lysis buffer (20 mM Tris–HCl, pH 8; 137 mM NaCl; 1% Nonidet P-40; and 10% glycerol) supplemented with protease inhibitors (0.5 mM phenylmethylsulfonyl fluoride, 0.025 mM *N*-CBZ-L-phenylalanine chloromethyl ketone, 0.025 mM *N-p*-tosyl-lysine chloromethyl ketone, and 0.025 mM L-1-tosylamide-2-phenyl-ethylchloromethyl ketone) and homogenized with an Ultra-Turrax (IKA-Werke GmbH & Co., Staufen, Germany) homogenizer. Samples were centrifuged at 4 °C for 10 min at 10,000 *g*, and the resulting pellets were discarded. Protein concentration in the supernatant was measured by the Bradford assay. After boiling for 5 min, 40 μg protein was applied to 8%–12% sodium dodecyl sulfate-polyacrylamide gel, and electrophoresis was performed at 150 V for 1.5 h. The resolved proteins were transferred for 2 h onto nitrocellulose membranes. The blot was preincubated in blocking buffer [5% nonfat milk, 0.05% Tween 20 in 20 mM TBS (4 mM Tris-HCl; 100 mM NaCl, pH8)] for 1 h at room temperature and incubated with appropriate primary antibodies (AMH, Santa Cruz, sc 6886, 1:200; ANGPT1, Abcam 8451, 1:500; ANGPT2, Abcam 65835, 1:500; FLK1, Cell Signaling, 2479 S, 1:500; PDGFRβ, Santa Cruz, sc-432, 1:3000 and β-actin, Cell Signaling, 4967 S, 1:10,000) in blocking buffer overnight at 4 °C. Then, the blot was incubated with anti-rabbit (A-4914, 1:1000; Sigma Aldrich, MO, USA) or anti-mouse secondary antibodies conjugated with horseradish peroxidase (HAF007, 1:1000; R&D Systems, MN, USA) and finally detected by chemiluminescence and autoradiography using X-ray film. The density in each band was normalized to the density of the β-actin band that was used as an internal control.

2.6. Semi-quantification for the western blot assay

For quantification, a screening was performed on blots with X-ray film using different times of exposure to optimize the signal. The levels of protein were compared and analyzed by densitometric studies using Scion Image for Windows (Scion, Frederick, MD, USA). Data are expressed as arbitrary units ± SEM (n = 6).

2.7. Enzyme-linked immunosorbent assay (ELISA)

Ovarian protein extracts were used to measure ovarian VEGF concentration. For this purpose, we used a commercially available ELISA kit (Quantikine rat VEGF kit; RRV00; R&D Systems, MN, USA) according to the manufacturer's instructions. This kit is designed to measure rat VEGF 164. The intra- and interassay coefficients of variation for VEGF were 3.7 and 7.9%, respectively.

2.8. Data analysis

Statistical analysis was performed using the statistical program Prism v4.0. Firstly, normal distribution (Kolmogroc-Smirnov test) of data was verified. All the data followed a normal distribution with the exception of the % of CL. Therefore, statistical analysis was performed using one-way ANOVA following Tukey post-test and the % of CL analysis was performed using Kruskal-Wallis test followed by the Dunn's post test. To analyze the contingency table, a $\chi 2$ test was performed. Values of $P < 0.05$ were considered significant. Data are expressed as the mean ± SEM. Representative gels and tissue sections are shown in the figures.

3. Results

3.1. Follicular development

To evaluate the effect of PDGFB on ovarian morphology in the PCOS rat model, ovarian histological slides were stained with H&E to analyze the percentage of different follicular stages. The percentage of primordial follicles from PDGFB-treated PCOS rats was significantly lower when compared to non-treated PCOS rats (basal-PCOS: 21.41 ± 3.80%; PDGFB-PCOS: 9.73 ± 1.92%; $P < 0.05$ $n = 6$). On the contrary, PDGFB did not reverse the increased percentage of primary (Control: 2.74 ± 1.48%; basal-PCOS: 18.59 ± 1.11%; PDGFB-PCOS: 13.87 ± 2.62%; Control vs basal-PCOS $P < 0.001$; Control vs PDGFB-PCOS $P < 0.01$ $n = 6$) and atretic follicles (Control: 21.11 ± 3.69%; basal-PCOS: 31.00 ± 2.14%; PDGFB-PCOS: 33.00 ± 1.44%; Control vs basal-PCOS $P < 0.05$; Control vs PDGFB-PCOS $P < 0.01$; $n = 6$). No changes were found in the percentages of preantral and antral follicles between groups (Fig. 1).

3.2. AMH levels

To analyze the effect of PDGFB administration on the levels of AMH, we evaluated the levels of this hormone in the ovaries of the three groups by western blot. Basal-PCOS ovaries presented increased AMH protein levels (Control: 0.63 ± 0.21; basal-PCOS: 1.47 ± 0.06 arbitrary units; $P < 0.01$ $n = 6$). Interestingly, PDGFB administration reversed this increase in AMH levels (PDGFB-PCOS: 0.56 ± 0.08 arbitrary units; $P < 0.001$ vs basal-PCOS, $n = 6$) (Fig. 2).

3.3. CL and cyst formation

PDGFB administration significantly increased the percentage of CL in the ovaries of PCOS rats compared to untreated PCOS rats (basal-PCOS: 0.18 ± 0.18%; PDGFB-PCOS: 3.20 ± 1.17%; $P < 0.05$ $n = 6$) (Fig. 3A). Based on this result, we compared the number of rats that presented CL in histological slides versus the number of rats that did not present CL in the three experimental groups. The number of basal-PCOS rats that presented CL was significantly lower than that of control rats (control: 76.9%, ten of thirteen; PCOS: 6.7%, one of fifteen; $P < 0.001$). PDGFB administration increased the number of rats that presented CL (PDGFB-PCOS: 54.5%, six of eleven, $P < 0.05$ compared to basal-PCOS group) (Fig. 3B).

When we analyzed raw CL number, basal-PCOS rats presented a lower number of CL in histological slides compared to the control

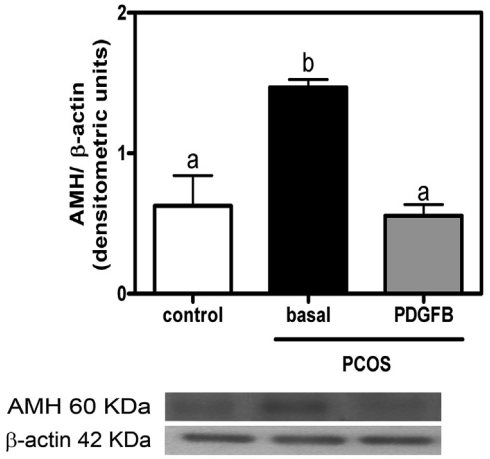


Fig. 2. Ovarian AMH levels in Control, basal-PCOS and PDGFB-PCOS rats measured by western blot. The density in each band was normalized to the density of the β-actin band. Differences between groups were tested for significances using One-way ANOVA, followed by Tukey's test. Different letters indicate significant differences (Control vs basal-PCOS, $P < 0.05$; basal-PCOS vs PDGFB-PCOS, $P < 0.01$; $n = 6$).

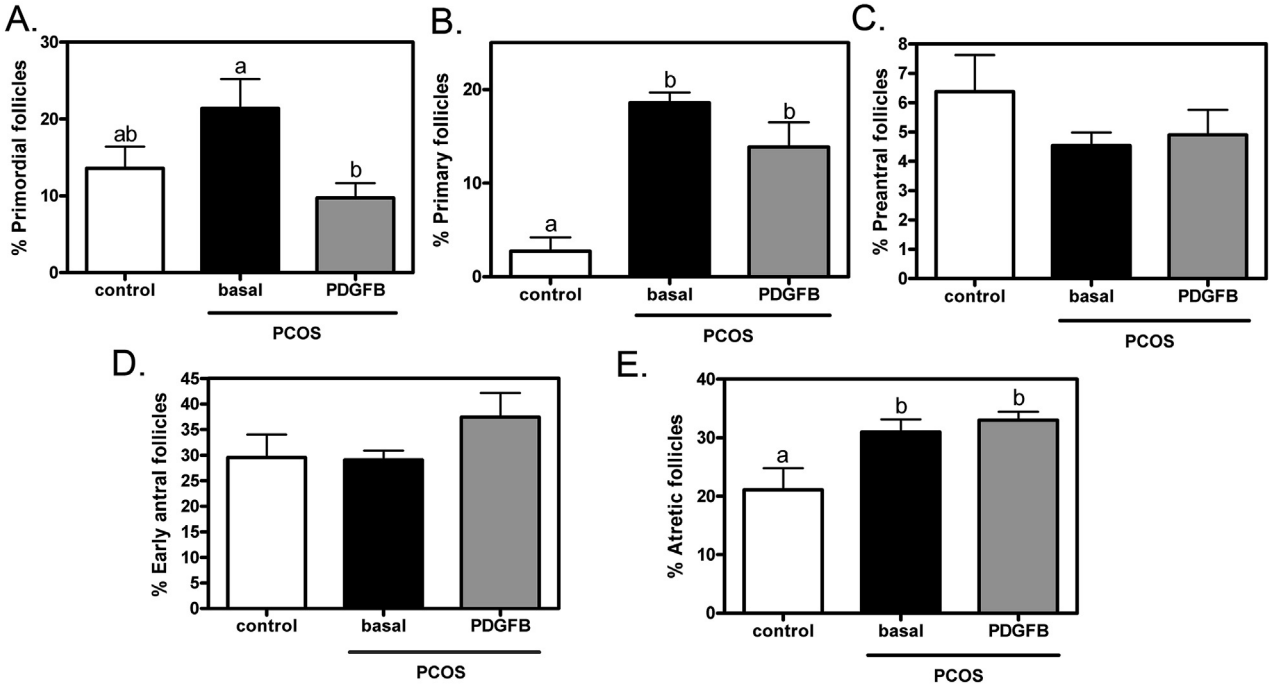


Fig. 1. Follicular development in Control, basal-PCOS and PDGFB-PCOS rats. The graphs show the percentage of each structure in H&E-stained ovarian sections. A. % of primordial follicles (a vs b: $P < 0.05$, $n = 6$); B. % of primary follicles (Control vs basal-PCOS, $P < 0.001$; Control vs PDGFB-PCOS, $P < 0.05$; $n = 6$); C. % of preantral follicles; D. % of early antral follicles E. % atretic follicles (Control vs basal-PCOS, $P < 0.05$; Control vs PDGFB-PCOS, $P < 0.01$; $n = 6$). Differences between groups were tested for significances using One-way ANOVA, followed by Tukey's test. Different letters represent significant differences.

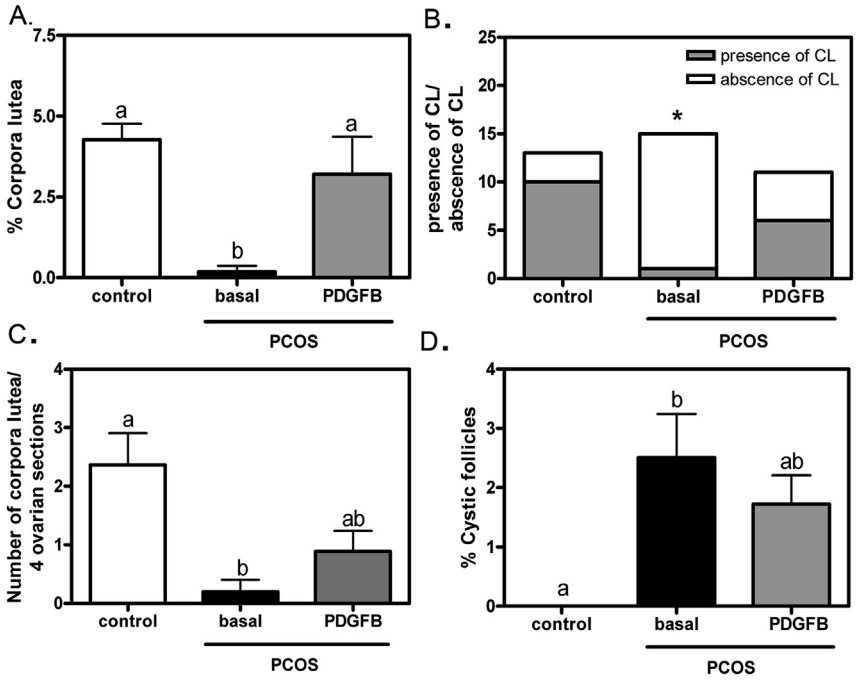


Fig. 3. Corpora lutea and cyst formation in Control, basal-PCOS and PDGFB-PCOS rats. A. % of corpora lutea in H&E-stained ovarian sections. Differences between groups were tested for significances using Kruskal-Wallis test followed by Dunn's Multiple Comparison Test (a vs b: $P < 0.05$; $n = 6$). B. Number of rats that presented CL in histological slides versus the number of rats that did not present CL in the three experimental groups. Differences between groups were tested for significances using a $\chi 2$ test. C. Number of CL in H&E-stained ovarian sections. Differences between groups were tested for significances using Kruskal-Wallis test followed (a vs b: $P < 0.05$; $n = 6$). D. % of cysts in H&E-stained ovarian sections. Differences between groups were tested for significances using One-way ANOVA, followed by Tukey's test (a vs b: $P < 0.05$; $n = 6$).

group (control: 2.4 ± 0.5; basal-PCOS: 0.2 ± 0.2, $P < 0.01$, $n = 6$). PDGFB administration increased the number of CL to values similar to those of the control rats (PDGFB-PCOS: 0.9 ± 0.4, $P > 0.05$ compared to control group; $n = 6$) (Fig. 3C).

Regarding cyst formation, control rats showed no cysts, and PDGFB was able to reduce the percentage of cysts to values similar to those of the control rats (basal-PCOS: 2.51 ± 0.73%; PDGFB-PCOS: 1.72 ± 0.48%; Control vs basal-PCOS $P < 0.05$; Control vs PDGFB-PCOS $P > 0.05$; $n = 6$) (Fig. 3d).

3.4. Endothelial and periendothelial cell area

To evaluate whether PDGFB administration affects blood vessel formation and stabilization, we analyzed endothelial and peri-endothelial cell area by immunohistochemistry, using BS-1 lectin and alpha-actin antibody respectively.

No changes were observed in endothelial cell area between the groups analyzed (Fig. 4A). In contrast, the percentage of peri-endothelial cell area was increased in basal-PCOS ovaries (Control: 1.52 ± 0.08%; basal-PCOS: 4.26 ± 0.33%; $P < 0.001$; $n = 6$). Interestingly, PDGFB reduced the periendothelial cell area compared to basal-PCOS ovaries (PDGFB-PCOS: 2.35 ± 0.21%; $P < 0.001$; $n = 6$) (Fig. 4B). Representative pictures of the ovarian vasculature in the three groups, showing dual staining of endothelial and peri-endothelial cells are depicted in Fig. 4C.

3.5. Angiogenic factor levels

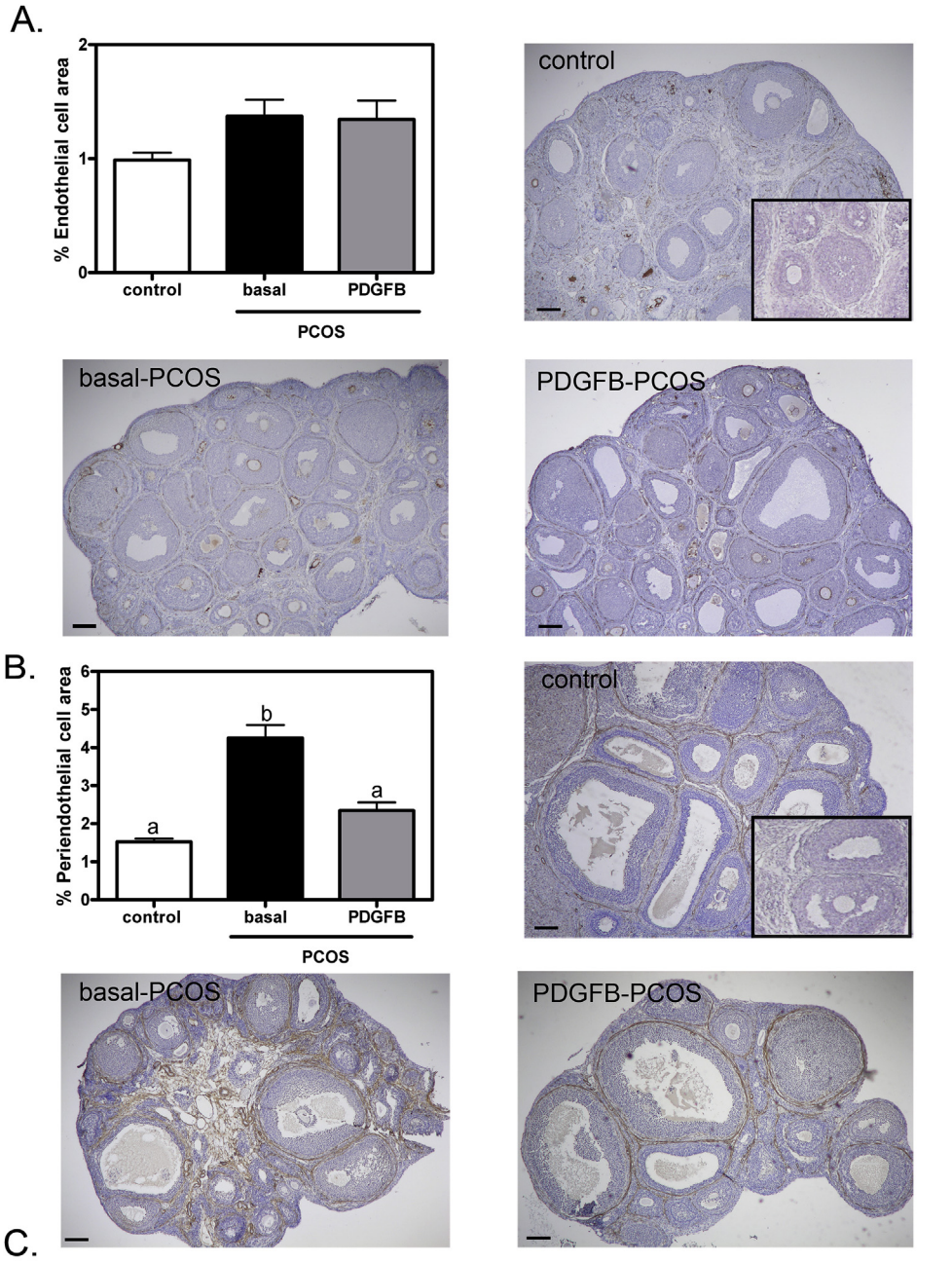
To corroborate differences in the levels of the PDGF family between control and PCOS rats, we analyzed the PDGFB, PDGFD and PDGFR-β protein levels in the ovaries from the three experimental groups. The levels of the three proteins analyzed were decreased in basal-PCOS rats (PDGFB: Control, 0.51 ± 0.09; basal-PCOS, 0.17 ± 0.02 arbitrary units; $P < 0.01$; PDGFD: Control, 3.62 ± 0.54; basal-PCOS 2.10 ± 0.24 arbitrary units; $P < 0.05$; PDGFR-β: Control, 0.69 ± 0.07; basal-PCOS 0.41 ± 0.03 arbitrary units; $P < 0.05$; $n = 6$). Interestingly, PDGFR-β levels increased after ligand administration (PDGFB-PCOS: 0.67 ± 0.08 arbitrary units; basal-PCOS vs PDGFB-PCOS, $P < 0.05$; $n = 6$).

Then, we aimed to analyze whether PDGFB administration could affect other angiogenic systems. Basal-PCOS rats showed increased ovarian VEGF concentration compared to control rats (Control: 50.09 ± 5.93 pg/mg protein; basal-PCOS: 83.36 ± 13.09 pg/mg protein; $P < 0.05$; $n = 6$). PDGFB administration decreased VEGF concentration to values similar to those of the control group (PDGFB-PCOS: 61.66 ± 12.53 pg/mg protein; $P > 0.05$ vs control group; $n = 6$) (Fig. 5A). Regarding its receptor, basal-PCOS rats showed lower levels of FLK1 than control rats (Control: 0.49 ± 0.06; basal-PCOS: 0.20 ± 0.02 arbitrary units; $P < 0.05$; $n = 6$). PDGFB administration increased the levels of FLK1 in PCOS rats to values similar to those of the control group (PDGFB-PCOS: 0.36 ± 0.1 arbitrary units; $P > 0.05$ vs control group; $n = 6$) (Fig. 5B).

ANGPT1 was significantly increased in the basal-PCOS group compared to the control group (Control: 0.49 ± 0.05; basal-PCOS: 0.74 ± 0.7 arbitrary units; $P < 0.05$; $n = 6$). PDGFB administration reduced these levels to values similar to those of the control group (PDGFB-PCOS: 0.66 ± 0.05 arbitrary units; $P > 0.05$ vs control group; $n = 6$) (Fig. 6A). Regarding ANGPT2, PDGFB had no effect on the levels of this protein (Fig. 6B). No changes were found in the ANGPT1/ANGPT2 ratio after PDGFB administration (Fig. 6C).

4. Discussion

Fertility management in women with PCOS is still a medical challenge. Thus, research in the mechanisms that lead to impaired follicular development and ovulation, together with exploration of


A.
% Endothelial cell area
2
1
0
control
basal
PDGFB
PCOS
control
basal-PCOS
PDGFB-PCOS
B.
% Periendothelial cell area
6
5
4
3
2
1
0
a
b
a
control
basal
PDGFB
PCOS
control
basal-PCOS
PDGFB-PCOS
C.
control
basal-PCOS
PDGFB-PCOS

new strategies to restore ovarian function are essential. In the present work, we describe, for the first time, that ovarian PDGFB administration in a rat model of PCOS partially restores follicular development and ovarian angiogenesis.

There is increasing evidence of the importance of angiogenic alterations in PCOS ovaries that are likely to be involved in the impaired fertility in these patients (Tal et al., 2015). The alterations first described in women with PCOS were the increased VEGF concentration and ovarian blood flow (Aleem and Predanic, 1996; Battaglia et al., 1995; Kamat et al., 1995; Zaidi et al., 1995). Besides other angiogenic-related alterations in women with PCOS ovaries (Scotti et al., 2014; Tal et al., 2013a, 2013b, 2014; Xu et al., 2011); (Artini et al., 2006; Hammadeh et al., 2003), we have previously shown that the concentration of ovarian PDGFB and PDGFD is decreased in the DHEA-induced PCOS rat model (Di Pietro et al., 2015) and in follicular fluid from PCOS patients (Scotti et al., 2014).

PDGFs have been described as factors involved in the activation of primordial follicles and, acting together with other growth factors, they are likely to be necessary for early follicular development (Nilsson et al., 2006; Pinkas et al., 2008). In contrast, AMH, a member of the TGF-β superfamily, inhibits primordial follicle activation, leading to accumulation of these follicles and to a decrease in the number of follicles of other stages (Nilsson et al., 2007; Weenen et al., 2004). AMH has been shown to stimulate VEGF synthesis in cultured secondary follicles from nonhuman primates (Xu et al., 2013). To our knowledge, no studies have evaluated a possible regulation of AMH on other angiogenic factors. In this study, we showed that PCOS rats presented an increase in AMH levels together with a decrease in PDGFB and PDGFD. PDGFB administration decreased the percentage of primordial follicles with no changes in other follicular structures, compared to PCOS without treatment. These results suggest that PDGFB is promoting primordial follicle growth without affecting other follicular stages. Furthermore, we found an increase in the percentage of CL while the percentage of cystic structures was reversed to values similar to the control group. This suggests that PDGFB administration not only improves follicular development but also decreases cyst formation leading to an increase in ovulation. Since in this work we analyzed the percentage of follicular structures and not the raw number of each follicular stage, another possibility is that PDGFB could promote the whole follicular development process, leading to an increase in CL formation together with a lesser formation of cysts. We further analyzed the number of rats that presented CL in histological slides (Abramovich et al., 2012). This number was smaller in the PCOS group than in the control group. Interestingly, PDGFB administration increased the number of rats with CL, reflecting an increase in the number of rats that ovulated. Moreover, when we counted the number of CL in the ovaries, we found that basal-PCOS rats presented a decrease in this number, while PDGFB-treated rats presented an increase in the number of CL that was similar to that of the control group. Our findings are in agreement with a study regarding the role of PDGFB/PDGFR-β in luteogenesis that describes an increase in mRNA expression of several PDGFs and their receptors after LH surge in rats and a decrease in CL formation when PDGF signaling is inhibited by using a receptor inhibitor (Sleer and Taylor, 2007b). Taken together, these results strengthen the fact that PDGFB can partially restore follicular development and increase ovulation rate in the DHEA-induced rat model of PCOS.

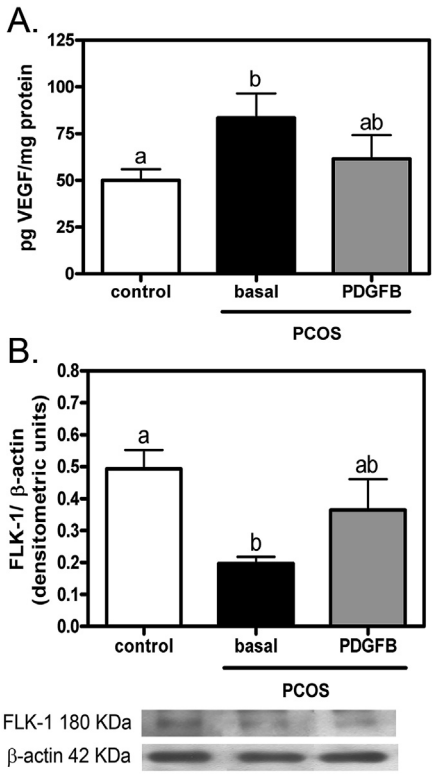


Fig. 5. Ovarian VEGF and FLK1 levels in Control, basal-PCOS and PDGFB-PCOS rats. A. VEGF concentration in ovarian protein extracts was measured by ELISA. B. FLK1 levels in ovarian protein extracts were measured by Western blotting. The density in each band was normalized to the density of the β-actin band. Differences between groups were tested for significances using One-way ANOVA, followed by Tukey's test. Different letters indicate significant differences (a vs b: $P < 0.05$, $n = 6$).

Follicular development relies on the correct formation of a vascular network that allows nutrients and hormones to reach each follicle (Stouffer et al., 2001). PDGFB is one of the main factors responsible for the proliferation and recruitment of pericytes and other perivascular cells (Andrae et al., 2008). In this work, we measured endothelial and periendothelial cell area in the three experimental groups. As we have previously demonstrated (Abramovich et al., 2012; Di Pietro et al., 2015), PCOS rats show an increase in the percentage of periendothelial cell area, with no changes in the endothelial cell area. Surprisingly, PDGFB administration reversed the increase in the percentage of periendothelial cell area in PCOS rats and had no effect on the endothelial cell area. As vessel formation and maturation are processes that require tight regulation and as the actions of angiogenic factors also depend on

Fig. 4. Effect of PDGFB administration on ovarian vessels. A. Endothelial cell staining in Control, basal-PCOS, and PDGFB-PCOS rat ovaries. Graph: Quantification of endothelial cell area in ovarian sections stained with lectin BS-1 in the three groups analyzed. The photographs show representative histological sections of Control, basal-PCOS and PDGFB-PCOS ovaries stained with lectin BS-1. Scale bars, 100 μm. Areas were determined in four ovarian sections from each ovary; six ovaries per group. B. Immunostaining of periendothelial cells in Control, basal-PCOS, and PDGFB-PCOS rat ovaries. Graph: Quantification of periendothelial cell area in ovarian sections stained with anti-smooth muscle cell β-actin antibody in the three groups analyzed. The photographs show representative histological sections of Control, basal-PCOS, and PDGFB-PCOS ovaries stained with anti-smooth muscle cell β-actin antibody. Scale bars, 100 μm. Insets: negative controls. Differences between groups were tested for significances using one-way ANOVA, followed by Tukey's test. Different letters indicate significant differences (a vs b: $P < 0.001$; $n = 6$). Areas were determined in four ovarian sections from each ovary; six ovaries per group. C. Representative photographs of ovarian vasculature in the three groups, showing dual staining of endothelial and periendothelial cells. Green color: von Willebrand Factor (endothelial cell marker). Red color: smooth muscle cell alpha-actin (periendothelial cell marker) (Scale bars, 100 μm). Lower panels show sections of each photograph at higher magnification. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

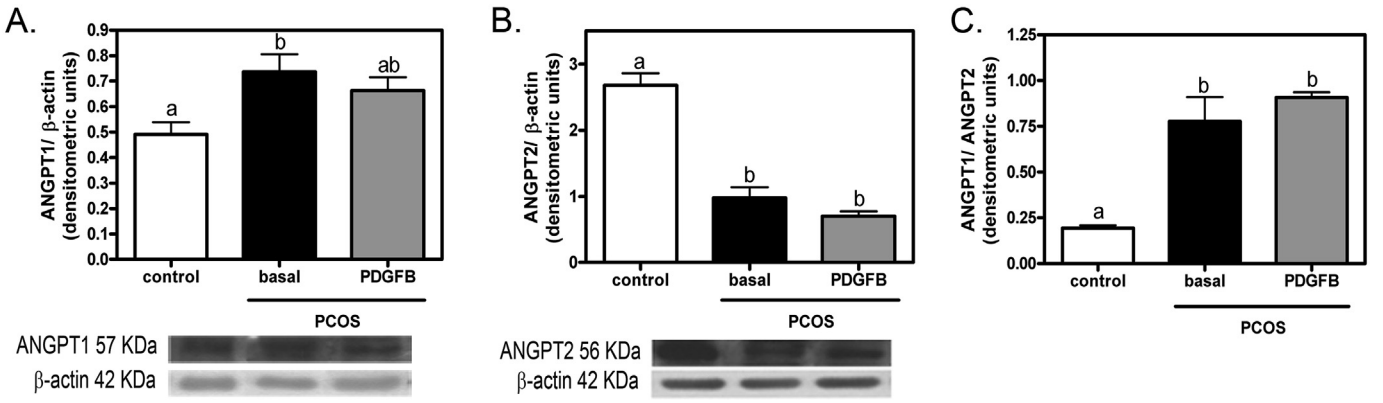


Fig. 6. Levels of ANGPT proteins in control, basal-PCOS, and PDGFB-PCOS rat ovaries. The graphs show the densitometric analysis for each protein. The density in each band was normalized to the density of the β-actin band. Lower panels show a representative blot for each protein analyzed. Differences between groups were tested for significances using One-way ANOVA, followed by Tukey's test. Different letters indicate significant differences. A. Densitometric quantification of ANGPT1 in the rat ovaries of the three groups analyzed (a vs b: $P < 0.05$; $n = 6$). B. Densitometric quantification of ANGPT2 in the rat ovaries of the three groups analyzed (a vs b: $P < 0.001$; $n = 6$). C, ANGPT1/ANGPT2 ratio in the rat ovaries (a vs b: $P < 0.001$; $n = 6$).

the levels of other factors, restoration of the PDGFB concentration could improve the whole angiogenic process by influencing the levels of other factors. To confirm this hypothesis, we measured the levels of other proteins involved in angiogenesis that are altered in PCOS ovaries. As we have previously shown, ovarian VEGF concentration is increased in the DHEA-induced model of PCOS (Abramovich et al., 2012). Interestingly, PDGFB administration decreased ovarian VEGF concentration to values similar to those of the control group. Hellstrom et al. demonstrated that in PDGFB and PDGFR-β-deficient mice, VEGF is upregulated during gestation, probably due to a compensation mechanism to maintain tissue homeostasis (Hellstrom et al., 2001). The increased VEGF concentration, together with the decreased PDGFB and PDGFR-β levels observed in the DHEA-induced PCOS rat model, are in agreement with the results of Hellstrom et al. Whether the increased VEGF concentration is a cause, a consequence, or not related to the decreased levels of the PDGFB/PDGFR-β system remains to be addressed. Furthermore, when PDGFB is administered locally in the ovary, VEGF concentration decreases suggesting that PDGFB is able to regulate VEGF concentration.

Regarding the ANGPT system, also involved in vessel stabilization, we analyzed the levels of ovarian ANGPT1 and ANGPT2 after PDGFB administration. PDGFB administration partially reversed the increase in ANGPT1 levels, but had no effects on ANGPT2 levels, leading to an ANGPT1/ANGPT2 ratio similar to that of the PCOS group. These results suggest that, at the dose and the time point analyzed, PDGFB is not regulating the ANGPT system in this model. It is known that regulation of angiogenic factor expression by other angiogenic factors is a context-dependent process and depends on multiple factors that lead to a correct angiogenic signal. Several factors are involved in this regulation, and the result depends on the interrelationship between all the angiogenic signals.

In summary, local PDGFB administration partially restores the primordial follicle accumulation observed in PCOS rats, reduces cyst formation and increases corpora lutea formation. The mechanism could be through an improvement in follicular vessel formation and a decrease in VEGF concentration, which lead to a better vascularization and nutrition to the developing follicles. PDGFB may also act on primordial and preovulatory follicles to improve early follicular development and corpus luteum formation, besides its role in angiogenesis.

5. Conclusions

This study demonstrates, for the first time, a beneficial effect of local PDGFB administration on luteal development and ovarian angiogenic factors in a rat model of PCOS. The results presented contribute to the knowledge of the ovarian mechanisms that lead to altered ovarian function in PCOS and provide new data for potential therapeutic strategies. Studies in women are needed to fully translate these findings to PCOS patients.

Disclosure summary

The authors have nothing to disclose.

Acknowledgments

This study was supported by ANPCyT (PICT 2012/897), CONICET (PIP 1223), Roemmers Foundation and René Barón Foundation, Argentina.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.mce.2016.05.022.

References

Abramovich, D., Irusta, G., Bas, D., Cataldi, N.I., Parborell, F., Tesone, M., 2012. Angiopoietins/TIE2 system and VEGF are involved in ovarian function in a DHEA rat model of polycystic ovary syndrome. Endocrinology 153, 3446–3456.
Abramovich, D., Parborell, F., Tesone, M., 2006. Effect of a vascular endothelial growth factor (VEGF) inhibitory treatment on the folliculogenesis and ovarian apoptosis in gonadotropin-treated prepubertal rats. Biol. Reprod. 75, 434–441.
Agrawal, R., Sladkevicius, P., Engmann, L., Conway, G.S., Payne, N.N., Bekis, J., Tan, S.L., Campbell, S., Jacobs, H.S., 1998. Serum vascular endothelial growth factor concentrations and ovarian stromal blood flow are increased in women with polycystic ovaries. Hum. Reprod. 13, 651–655.
Aleem, F.A., Predanic, M., 1996. Transvaginal color Doppler determination of the ovarian and uterine blood flow characteristics in polycystic ovary disease. Fertil. Steril. 65, 510–516.
Andrae, J., Gallini, R., Betsholtz, C., 2008. Role of platelet-derived growth factors in physiology and medicine. Genes Dev. 22, 1276–1312.
Andreu, C., Parborell, F., Vanzulli, S., Chemes, H., Tesone, M., 1998. Regulation of follicular luteinization by a gonadotropin-releasing hormone agonist: relationship between steroidogenesis and apoptosis. Mol. Reprod. Dev. 51, 287–294.
Armulik, A., Abramsson, A., Betsholtz, C., 2005. Endothelial/pericyte interactions. Circ. Res. 97, 512–523.

Artini, P.G., Monti, M., Matteucci, C., Valentino, V., Cristello, F., Genazzani, A.R., 2006. Vascular endothelial growth factor and basic fibroblast growth factor in polycystic ovary syndrome during controlled ovarian hyperstimulation. Gynecol. Endocrinol. 22, 465–470.
Battaglia, C., Artini, P.G., D'Ambrogio, G., Genazzani, A.D., Genazzani, A.R., 1995. The role of color Doppler imaging in the diagnosis of polycystic ovary syndrome. Am. J. Obstet. Gynecol. 172, 108–113.
Carmeliet, P., 2003. Angiogenesis in health and disease. Nat. Med. 9, 653–660.
Claesson-Welsh, L., 1996. Mechanism of action of platelet-derived growth factor. Int. J. Biochem. Cell Biol. 28, 373–385.
Convery, M., McCarthy, G.F., Brawer, J.R., 1990. Remission of the polycystic ovarian condition (PCO) in the rat following hemiovariectomy. Anat. Rec. 226, 328–336.
Di Pietro, M., Parborell, F., Irusta, G., Pascuali, N., Bas, D., Bianchi, M.S., Tesone, M., Abramovich, D., 2015. Metformin regulates ovarian angiogenesis and follicular development in a female polycystic ovary syndrome rat model. Endocrinology 156, 1453–1463.
Durlinger, A.L., Gruijters, M.J., Kramer, P., Karels, B., Ingraham, H.A., Nachtigal, M.W., Uilenbroek, J.T., Grootegoed, J.A., Themmen, A.P., 2002a. Anti-Mullerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. Endocrinology 143, 1076–1084.
Durlinger, A.L., Visser, J.A., Themmen, A.P., 2002b. Regulation of ovarian function: the role of anti-Mullerian hormone. Reproduction 124, 601–609.
Fagiani, E., Christofori, G., 2013. Angiopoietins in angiogenesis. Cancer Lett. 328, 18–26.
Franks, S., 1995. Polycystic ovary syndrome. N. Engl. J. Med. 333, 853–861.
Hammadeh, M.E., Fischer-Hammadeh, C., Hoffmeister, H., Huebner, U., Georg, T., Rosenbaum, P., Schmidt, W., 2003. Fibroblast growth factor (FGF), intracellular adhesion molecule (sICAM-1) level in serum and follicular fluid of infertile women with polycystic ovarian syndrome, endometriosis and tubal damage, and their effect on ICSI outcome. Am. J. Reprod. Immunol. 50, 124–130.
Heldin, C.H., Westermark, B., 1999. Mechanism of action and in vivo role of platelet-derived growth factor. Physiol. Rev. 79, 1283–1316.
Hellberg, C., Ostman, A., Heldin, C.H., 2010. PDGF and vessel maturation. Recent Results Cancer Res. 180, 103–114.
Hellstrom, M., Gerhardt, H., Kalen, M., Li, X., Eriksson, U., Wolburg, H., Betsholtz, C., 2001. Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. J. Cell Biol. 153, 543–553.
Hwu, Y.M., Li, S.H., Lee, R.K., Lin, M.H., Tsai, Y.H., Yeh, T.S., 2009. Luteinizing hormone increases platelet-derived growth factor-D gene expression in human granulosa-luteal cells. Fertil. Steril. 92, 2065–2068.
Kamat, B.R., Brown, L.F., Manseau, E.J., Senger, D.R., Dvorak, H.F., 1995. Expression of vascular permeability factor/vascular endothelial growth factor by human granulosa and theca lutein cells. Role in corpus luteum development. Am. J. Pathol. 146, 157–165.
Lara, H.E., Dissen, G.A., Leyton, V., Paredes, A., Fuenzalida, H., Fiedler, J.L., Ojeda, S.R., 2000. An increased intraovarian synthesis of nerve growth factor and its low affinity receptor is a principal component of steroid-induced polycystic ovary in the rat. Endocrinology 141, 1059–1072.
LaRochelle, W.J., Jeffers, M., McDonald, W.F., Chillakuru, R.A., Giese, N.A., Lokker, N.A., Sullivan, C., Boldog, F.L., Yang, M., Vernet, C., Burgess, C.E., Fernandes, E., Deegler, L.L., Rittman, B., Shimkets, J., Shimkets, R.A., Rothberg, J.M., Lichenstein, H.S., 2001. PDGF-D, a new protease-activated growth factor. Nat. Cell Biol. 3, 517–521.
Leveen, P., Pekny, M., Gebre-Medhin, S., Swolin, B., Larsson, E., Betsholtz, C., 1994. Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. Genes Dev. 8, 1875–1887.
Li, X., Ponten, A., Aase, K., Karlsson, L., Abramsson, A., Uutela, M., Backstrom, G., Hellstrom, M., Bostrom, H., Li, H., Soriano, P., Betsholtz, C., Heldin, C.H., Alitalo, K., Ostman, A., Eriksson, U., 2000. PDGF-C is a new protease-activated ligand for the PDGF alpha-receptor. Nat. Cell Biol. 2, 302–309.
Lindahl, P., Johansson, B.R., Leveen, P., Betsholtz, C., 1997. Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. Science 277, 242–245.
Nilsson, E., Rogers, N., Skinner, M.K., 2007. Actions of anti-Mullerian hormone on the ovarian transcriptome to inhibit primordial to primary follicle transition. Reproduction 134, 209–221.
Nilsson, E.E., Detzel, C., Skinner, M.K., 2006. Platelet-derived growth factor modulates the primordial to primary follicle transition. Reproduction 131, 1007–1015.
Norman, R.J., Dewailly, D., Legro, R.S., Hickey, T.E., 2007. Polycystic ovary syndrome. Lancet 370, 685–697.
Pascuali, N., Scotti, L., Abramovich, D., Irusta, G., Di Pietro, M., Bas, D., Tesone, M., Parborell, F., 2015. Inhibition of platelet-derived growth factor (PDGF) receptor affects follicular development and ovarian proliferation, apoptosis and angiogenesis in prepubertal eCG-treated rats. Mol. Cell. Endocrinol. 412, 148–158.
Pinkas, H., Fisch, B., Rozansky, G., Felz, C., Kessler-Icekson, G., Krissi, H., Nitke, S., Ao, A., Abir, R., 2008. Platelet-derived growth factors (PDGF-A and -B) and their receptors in human fetal and adult ovaries. Mol. Hum. Reprod. 14, 199–206.
Ribatti, D., Nico, B., Crivellato, E., 2011. The role of pericytes in angiogenesis. Int. J. Dev. Biol. 55, 261–268.
Sadrkhanloo, R., Hofeditz, C., Erickson, G.F., 1987. Evidence for widespread atresia in the hypophysectomized estrogen-treated rat. Endocrinology 120, 146–155.
Schmahl, J., Rizzolo, K., Soriano, P., 2008. The PDGF signaling pathway controls multiple steroid-producing lineages. Genes Dev. 22, 3255–3267.
Scotti, L., Abramovich, D., Pascuali, N., de Zúñiga, I., Oubina, A., Kopcow, L., Lange, S., Owen, G., Tesone, M., Parborell, F., 2013. Involvement of the ANGPTs/Tie-2 system in ovarian hyperstimulation syndrome (OHSS). Mol. Cell. Endocrinol. 365, 223–230.
Scotti, L., Parborell, F., Irusta, G., de Zúñiga, I., Bisioli, C., Pettorossi, H., Tesone, M., Abramovich, D., 2014. Platelet-derived growth factor BB and DD and angiopoietin1 are altered in follicular fluid from polycystic ovary syndrome patients. Mol. Reprod. Dev. 81, 748–756.
Sleer, L.S., Taylor, C.C., 2007a. Cell-type localization of platelet-derived growth factors and receptors in the postnatal rat ovary and follicle. Biol. Reprod. 76, 379–390.
Sleer, L.S., Taylor, C.C., 2007b. Platelet-derived growth factors and receptors in the rat corpus luteum: localization and identification of an effect on luteogenesis. Biol. Reprod. 76, 391–400.
Soriano, P., 1994. Abnormal kidney development and hematological disorders in PDGF beta-receptor mutant mice. Genes Dev. 8, 1888–1896.
Stanek, M.B., Borman, S.M., Molskness, T.A., Larson, J.M., Stouffer, R.L., Patton, P.E., 2007. Insulin and insulin-like growth factor stimulation of vascular endothelial growth factor production by luteinized granulosa cells: comparison between polycystic ovarian syndrome (PCOS) and non-PCOS women. J. Clin. Endocrinol. Metab. 92, 2726–2733.
Stouffer, R.L., Martinez-Chequer, J.C., Molskness, T.A., Xu, F., Hazzard, T.M., 2001. Regulation and action of angiogenic factors in the primate ovary. Arch. Med. Res. 32, 567–575.
Tal, R., Seifer, D.B., Arici, A., 2015. The emerging role of angiogenic factor dysregulation in the pathogenesis of polycystic ovarian syndrome. Semin. Reprod. Med. 33, 195–207.
Tal, R., Seifer, D.B., Grazi, R.V., Malter, H.E., 2013a. Angiopoietin-1 and angiopoietin-2 are altered in polycystic ovarian syndrome (PCOS) during controlled ovarian stimulation. Vasc. Cell 5, 18.
Tal, R., Seifer, D.B., Grazi, R.V., Malter, H.E., 2014. Follicular fluid placental growth factor is increased in polycystic ovarian syndrome: correlation with ovarian stimulation. Reprod. Biol. Endocrinol. 12, 82.
Tal, R., Seifer, D.B., Shohat-Tal, A., Grazi, R.V., Malter, H.E., 2013b. Transforming growth factor-beta1 and its receptor soluble endoglin are altered in polycystic ovary syndrome during controlled ovarian stimulation. Fertil. Steril. 100, 538–543.
Walters, K.A., Allan, C.M., Handelsman, D.J., 2012. Rodent models for human polycystic ovary syndrome. Biol. Reprod. 86, 149, 1-149,12.
Weenen, C., Laven, J.S., Von Bergh, A.R., Cranfield, M., Groome, N.P., Visser, J.A., Kramer, P., Fauser, B.C., Themmen, A.P., 2004. Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Mol. Hum. Reprod. 10, 77–83.
Woodruff, T.K., Lyon, R.J., Hansen, S.E., Rice, G.C., Mather, J.P., 1990. Inhibin and activin locally regulate rat ovarian folliculogenesis. Endocrinology 127, 3196–3205.
Xu, J., Xu, M., Bernuci, M.P., Fisher, T.E., Shea, L.D., Woodruff, T.K., Zelinski, M.B., Stouffer, R.L., 2013. Primate follicular development and oocyte maturation in vitro. Adv. Exp. Med. Biol. 761, 43–67.
Xu, N., Kwon, S., Abbott, D.H., Geller, D.H., Dumesic, D.A., Azziz, R., Guo, X., Goodarzi, M.O., 2011. Epigenetic mechanism underlying the development of polycystic ovary syndrome (PCOS)-like phenotypes in prenatally androgenized rhesus monkeys. PLoS One 6, e27286.
Yoon, S.J., Kim, K.H., Chung, H.M., Choi, D.H., Lee, W.S., Cha, K.Y., Lee, K.A., 2006. Gene expression profiling of early follicular development in primordial, primary, and secondary follicles. Fertil. Steril. 85, 193–203.
Zaidi, J., Campbell, S., Pittrof, R., Kyei-Mensah, A., Shaker, A., Jacobs, H.S., Tan, S.L., 1995. Ovarian stromal blood flow in women with polycystic ovaries—a possible new marker for diagnosis? Hum. Reprod. 10, 1992–1996.