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Ovarian angiogenesis in polycystic ovary syndrome

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Abstract

Polycystic ovary syndrome (PCOS) is the most prevalent endocrine pathology among women in reproductive age. Its main symptoms are oligo or amenorrhea, hyperandrogenism and the presence of ovarian cysts. It is also associated with infertility, obesity and insulin resistance. Mainly due to its heterogeneity, PCOS treatments are directed to manage its symptoms and to prevent associated diseases. The correct formation and regression of blood vessels during each ovarian cycle is indispensable for proper follicular development, ovulation and corpus luteum formation. The importance of these processes opened a new and promising field: ovarian angiogenesis. Vascular alterations characterize numerous pathologies, either with increased, decreased or abnormal angiogenesis. In the last years, several anomalies of ovarian angiogenesis have been described in women with PCOS. Therefore, it has been suggested that these alterations may be associated with the decreased – or lack of – ovulation rates and for the formation of cysts in the PCOS ovaries. Restoration of a proper vessel formation in the ovaries may lead to improved follicular development and ovulation in these patients. In the present review, we attempt to summarize the alterations in ovarian angiogenesis that have been described in women with PCOS. We also discuss the therapeutic approaches aimed to correct these alterations and their beneficial effects on the treatment of infertility in PCOS.

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Introduction

Polycystic ovary syndrome (PCOS) is the most prevalent endocrine pathology, affecting 5–10% of women in reproductive age (Norman *et al.* 2007). PCOS was first described in 1935 by Stein and Leventhal as a condition consisting of enlarged polycystic ovaries and amenorrhea (Stein & Leventhal 1935). Since then, numerous studies have been performed to unravel the molecular mechanisms of PCOS and achieve better management of these patients. Besides, PCOS is a complex interplay between genetic and environmental factors (Rosenfield & Ehrmann 2016). PCOS heterogeneity has opened an in-depth discussion that is still ongoing concerning its diagnosis and treatment. In 2003, a consensus was reached in Rotterdam to diagnose PCOS in women who present at least two of the following features: clinical or biochemical hyperandrogenemia, oligomenorrhea or amenorrhea and the presence of ovarian cysts in ultrasound (Rotterdam 2004). However, these features are usually accompanied by other comorbidities, such as increased risk of developing infertility, cardiovascular disease (CVD), metabolic syndrome, type-2 diabetes and endometrial cancer (Norman *et al.* 2007). Moreover, pregnant women with PCOS have increased risk of pregnancy complications, such as preeclampsia, miscarriage, gestational diabetes and preterm delivery (Norman *et al.* 2007). For all these reasons, PCOS is still a medical challenge. The current insufficiency of

knowledge, eighty years after its first description, makes continuous research crucial to unravel the mechanisms involved in PCOS pathophysiology. Thus, there are still many controversies and discussion on PCOS treatments, which therefore target to manage its symptoms and to prevent associated diseases.

The female reproductive system presents periodic growth and regression of blood vessels together with changes in blood flow, with the ovary as one of the few adult tissues in which angiogenesis is an active process (Hazzard & Stouffer 2000). Ovarian angiogenesis has proved to be an interesting field, since the correct formation and regression of vascular vessels during each cycle is indispensable for a proper follicular development, ovulation and corpus luteum formation. Small follicles are avascular and depend on stromal vessels for nutrition and hormone supply. When the thecal compartment begins to grow, blood vessels develop within this layer and each follicle relies on its thecal vascular network to survive and maturate (Hazzard & Stouffer 2000). Formation and regression of the vasculature is tightly regulated by angiogenic factors synthesized mainly by ovarian cells. Vascular endothelial growth factor (VEGF) is the main angiogenic factor that promotes endothelial cell proliferation and migration and vascular permeability. It binds to VEGF receptor 2 (VEGFR2 or kinase insert domain receptor, KDR), which is expressed not only in endothelial cells, but also in granulosa and theca cells (Reynolds & Redmer 1998,

Abramovich *et al*. 2009). In addition, we have shown that VEGF acts directly on follicular cells independently of its angiogenic actions, regulating follicular cell function and fate (Irusta *et al*. 2010). VEGF can also bind to VEGFR1 (also known as fms-related tyrosine kinase 1, FLT1), which has a kinase activity one order of magnitude weaker than that of VEGFR2 (Waltenberger *et al*. 1994). Besides, numerous proteins promote endothelial cell proliferation and migration and act in coordination with VEGF, such as transforming growth factor B (TGFB1), basic fibroblast growth factor (bFGF) and placental growth factor (PlGF) (Pandya *et al*. 2006).

Transforming growth factor B (TGFB) belongs to the TGFB superfamily of proteins, which comprises activins, inhibins and the anti-Müllerian hormone (AMH). There are three TGFB isoforms (TGFB1–3) that participate in the regulation of different cellular processes such as angiogenesis, proliferation and apoptosis in humans (Laiho *et al*. 1990, Yang & Moses 1990, Satterwhite & Moses 1994). Basic fibroblast growth factor (bFGF) is a potent angiogenic factor that promotes endothelial cell proliferation both *in vivo* and *in vitro* (Gospodarowicz *et al*. 1987). Due to its high affinity to heparan sulfate proteoglycans, it is sequestered to the extracellular matrix after secretion and needs to be released by proteases/heparinases in order to signal. Thus, bFGF acts mainly in a paracrine way near the secretion site (Turner & Grose 2010). Placental growth factor (PlGF) is a member of the VEGF family that binds exclusively to the FLT1 receptor (Park *et al*. 1994). It is able to dimerize with VEGF to promote vessel growth (Cao *et al*. 1996).

The angiopoietin (ANGPT) system participates in the regulation of vascular stability and permeability by binding to the TIE2 receptor. ANGPT1 is the key member of this family regarding stability, since it promotes the maturation of newly formed blood vessels, contributing to quiescence and integrity of adult vasculature (Davis *et al*. 1996). ANGPT2 is a context-dependent agonist or antagonist of TIE2, which constrains vasculature stabilization (Maisonpierre *et al*. 1997, Shim *et al*. 2007). In the presence of VEGF, ANGPT2 triggers angiogenic sprouting by promoting detachment of mural cells, an essential step for endothelial cell proliferation, tubule formation and vessel growth. In the absence of VEGF, ANGPT2 promotes vascular destabilization and regression (Gale *et al*. 2002). Furthermore, we have observed that protein expression of ANGPT1, ANGPT2 and their receptor TIE2 increase during follicular development in the rat ovary (Abramovich *et al*. 2009).

Platelet-derived growth factors (PDGFs) belong to a protein family that encompasses five ligands (PDGFAA, PDGFBB, PDGFAB, PDGFCC and PDGFDD) and two tyrosine kinase receptors (PDGFRA and PDGFRB). The receptor involved in angiogenesis regulation is the PDGFRB , which only binds to the PDGFBB and PDGFDD ligands. Therefore, these two ligands are crucial factors involved in the regulation of vessel formation by inducing pericyte and smooth muscle cell recruitment to the newly formed vessels, promoting their maturation and stability (Hoch & Soriano 2003, Betsholtz 2004). PDGFBB and PDGFDD are widely expressed in platelets, smooth muscle cells and endothelial cells (Heldin & Westermark 1999), while PDGFRB is mainly expressed in fibroblasts, pericytes and vascular smooth muscle cells (VSMCs) (Nissen *et al*. 2007). In physiological conditions, protein expression of PDGF family members has been previously detected in the rat, mouse and human ovaries (Sleer & Taylor 2007, Pinkas *et al*. 2008). In this regard, we have shown that PDGF system is necessary for follicular development induced by gonadotropins (Pascuali *et al*. 2015).

The entire angiogenic process is also regulated by antiangiogenic factors that inhibit endothelial cell proliferation and migration and control the end of the process, avoiding an excessive growth of blood vessels and the formation of tortuous vasculature. The main antiangiogenic factors are thrombospondins (TSP), a family that comprises five members (TSP 1–5), endostatin and angiostatin (Pandya *et al*. 2006). Soluble receptors, as soluble FLT1 (sFLT1) also act as antiangiogenic factors by trapping pro-angiogenic proteins and decreasing their bioavailability (Pandya *et al*. 2006).

In adults, most of the vasculature is quiescent and physiological angiogenesis occurs only in certain tissues, such as reproductive tissues and in wound healing. A myriad of pathologies display either increased angiogenesis (psoriasis, ocular neovascularization, rheumatoid arthritis and others), decreased angiogenesis (impaired wound or ulcer healing, myocardial ischemia, atherosclerosis, cerebral ischemia and others) or abnormal angiogenesis (telangiectasias, tumor vessels, preeclampsia, PCOS and others) (Ollauri-Ibanez *et al*. 2017). Thus, targeting angiogenesis has become a growing therapeutic strategy in many of those disorders.

Recent studies have proven that there exist several angiogenesis anomalies in the ovaries of women with PCOS (Fig. 1). Moreover, it has been observed that these anomalies modify ovarian blood flow and possibly irrigation of follicles within the ovaries, leading to abnormal oxygen, nutrient and hormone supply. Hence, these vascular alterations may be partly responsible for deficient ovulation rates and for the formation of cysts in PCOS ovaries. Thus, it is not surprising that improvement in blood vessel formation has emerged as a new approach in PCOS fertility treatment.

Ovarian vascularization in PCOS

The first reports that assessed ovarian blood flow in PCOS are from Battaglia and coworkers (1995) and Zaidi and coworkers (1995). In those studies, the authors compared ovarian blood flow in control and PCOS patients by color Doppler analysis. Women were diagnosed with PCOS when they had oligo or
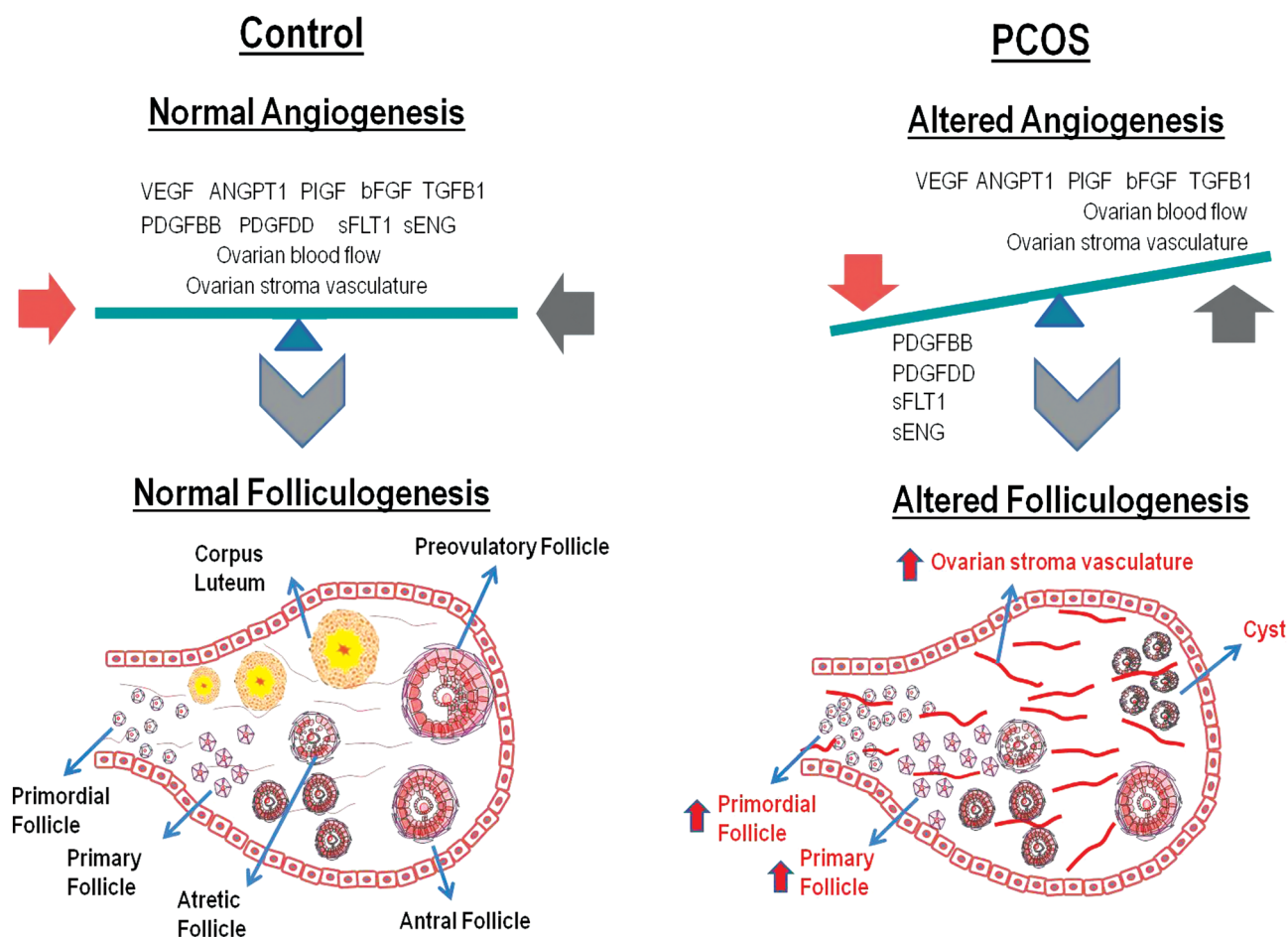
Figure 1 Deregulation of ovarian angiogenesis contributes to abnormal follicular development in women with PCOS. Ovarian angiogenesis is a tightly regulated process that needs a delicate balance of angiogenic factors, which is disrupted in women with PCOS, who present anomalous ovarian blood flow and angiogenesis. This altered angiogenesis may contribute to the ovarian features of PCOS such as abnormal follicular development, increase in the quantity of small follicles and failure in the selection of the dominant follicle, with anovulation and cyst formation. The figure summarizes the main angiogenic and ovarian alterations observed in women with PCOS.

amenorrhea, acne or seborrhea, hirsutism, increased LH/FSH ratio and enlarged ovaries with a high number of subcapsular follicles. Interestingly, they found an increase in ovarian stromal vascularization, an increase in the pulsatility index (PI) and a decrease in the resistance index (RI) in the ovaries from patients with PCOS compared to control women. The authors hypothesized that ovarian neoangiogenesis may occur to a higher extent in these patients than in control patients (Battaglia et al. 1995, Zaidi et al. 1995). One of the studies also suggested that the increase in stromal vascularization may be involved in the increased risk of developing ovarian hyperstimulation syndrome (OHSS) and could serve as a diagnostic criterion for PCOS (Zaidi et al. 1995). One of those research groups then compared obese PCOS patients with lean PCOS patients and oligo vs amenorrheic PCOS patients. These authors have found that vascularization and Doppler parameter abnormalities increase with BMI and amenorrhea, in comparison with women with a milder PCOS phenotype

(Battaglia et al. 1996, 1997). Several reports assessed ovarian and uterine blood flow in PCOS patients and, despite their differences regarding the Doppler indices analyzed, all studies confirmed the increase in ovarian stroma vascularization regardless of the methodology used (Agrawal et al. 1998a,b, Resende et al. 2001, Abd El Aal et al. 2005, Carmina et al. 2005, Alcazar & Kudla 2012). The study authored by Agrawal and coworkers drew especial attention to the relationship between the increase in stroma vascularization in PCOS with an increase in the levels of VEGF and the risk of developing OHSS in these patients (Agrawal et al. 1998a). That report was the first to recognize the influence of altered ovarian vascularization on systemic alterations seen in the PCOS patients. In the last years, another report evaluated these parameters by using a 4D spatiotemporal image correlation-high definition flow transvaginal ultrasound approach. In this study, PCOS was defined according to the Rotterdam criteria. Once again, the authors found evidence of higher vascularization and

lower impedance to flow in ovarian stromal vessels from women with PCOS (Alcazar & Kudla 2012).

There are considerable differences among these reports concerning the methodology used to assess ovarian blood flow and also the PCOS diagnosis criteria, which has suffered many changes through the years and consensus meetings. Nonetheless, they have all demonstrated the same alterations, which strengthen the relevance of the angiogenic component in women with PCOS.

Angiogenic factors in PCOS

Vascular endothelial growth factor (VEGF)

Modifications in the VEGF family were the first and best-described angiogenic alterations in the ovaries from women with PCOS. Early in 1995, Kamat and coworkers found an increase in VEGF expression in granulosa, theca and luteal cells in ovarian tissue from PCOS patients analyzed by immunohistochemistry (Kamat *et al*. 1995). Originally, the aim of their study was to analyze the presence of VEGF in other ovarian compartments besides the corpora lutea. However, as they included three patients with PCOS and patients that had indication for surgery for other pathologies as a control group, they were able to describe that PCOS patients had an increased protein expression of VEGF. Afterwards, that preliminary result was confirmed by various groups. Agrawal and coworkers found a positive correlation between the levels of serum VEGF and ovarian blood flow, providing the first explanation for the increase in stromal vascularization in PCOS ovaries (Agrawal *et al*. 1998*b*). Increased levels of VEGF were associated with the increase in ovarian blood flow, vascularization and OHSS by many authors (Ferrara *et al*. 2003, Abd El Aal *et al*. 2005, Gomez *et al*. 2010, Scotti *et al*. 2014). Interestingly, we and other authors have demonstrated that the protein concentration of VEGF in follicular fluids (FF) was higher than the serum concentration, suggesting that the source of increased serum VEGF is the ovary (Artini *et al*. 2006, 2009, Scotti *et al*. 2014).

Soluble Fms-like tyrosine kinase 1 (sFLT1) is a soluble receptor that binds to VEGF and blocks its interaction with the membrane receptor, regulating VEGF bioavailability. Patients with PCOS present lower serum and FF sFLT1 concentration than control patients, making VEGF bioavailability in PCOS patients even higher (Artini *et al*. 2009, Tal *et al*. 2014).

Different *VEGF* gene polymorphisms have been described in the promoter, intronic and untranslated regions, some of them associated with differences in the levels of VEGF protein expression (Vural *et al*. 2009). Therefore, several studies have evaluated *VEGF* polymorphisms in women with PCOS in different

populations. In the Korean population, single-nucleotide polymorphisms (SNP) at +9812 and +13553 sites are likely to be associated with PCOS (Lee *et al*. 2008). In South Indian women, the *VEGF* +405 G/C polymorphism is associated with an increased risk of PCOS (Guruvaiah *et al*. 2014). Almawi and coworkers showed a significant relationship between the −583 T>C and the −634 G/C variants and PCOS (Almawi *et al*. 2016) in Bahraini population. Although these results are interesting and promising, further investigations with larger study groups and with unrelated populations are needed to fully associate those polymorphisms to the PCOS phenotype.

Most of these alterations have also been observed by us and other authors in animal models of PCOS, such as postnatal DHEA injection or postnatal estradiol valerate injection in rats (Abramovich *et al*. 2012, Karimzadeh *et al*. 2013, Di Pietro *et al*. 2015, 2016). Despite their differences with the human pathology, these animal models are useful preclinical ways to study ovarian angiogenesis in PCOS and the potential interventions to manage it. So far, angiogenic factor levels in other PCOS animal models have not been assessed. In a DHEA rat model of PCOS, we showed increased ovarian VEGF protein levels and decreased FLK1 protein levels (Abramovich *et al*. 2012). Expression levels of the main VEGF membrane receptor in the ovaries of women with PCOS have not been studied yet.

Taken together, all these studies highlight the relevant role of ovarian VEGF alterations in the pathogenesis of PCOS. Moreover, VEGF emerges as a possible therapeutic target for follicular development and ovulation improvement in these patients.

Basic fibroblast growth factor (bFGF)

As previously stated, bFGF promotes endothelial cell proliferation, migration and survival (Turner & Grose 2010). Regarding its relationship with PCOS, only one work analyzed the levels of bFGF in serum and FF from women with PCOS (Artini *et al*. 2006). The authors reported increased serum bFGF protein in PCOS patients undergoing ovarian stimulation with gonadotropins compared to control patients, only at day of hCG administration, as well as increased bFGF in FF. It is interesting that the authors have not found differences in bFGF levels between PCOS and control patients before FSH stimulation. The increment in serum bFGF in PCOS patients was found only after FSH stimulation, suggesting that FSH may be inducing bFGF expression differentially among PCOS and control women (Artini *et al*. 2006). More studies are needed to further explain these findings.

These findings indicate that bFGF deregulation plays an important role in PCOS ovaries after FSH stimulation. Increased bFGF levels could be involved in the higher risk of patients with PCOS to develop OHSS.

Transforming growth factor B (TGFB)

An increasing number of studies have addressed TGFB deregulation in patients with PCOS, since they regulate diverse biological processes such as angiogenesis, cell proliferation and tissue fibrosis (Laiho *et al*. 1990, Yang & Moses 1990, Satterwhite & Moses 1994), all of them related to PCOS. It has been established that TGFB1 is increased in serum from patients with PCOS compared to control patients (Raja-Khan *et al*. 2010, Tal *et al*. 2013*b*, Liu *et al*. 2015). Moreover, soluble endoglin (sENG), a soluble receptor that binds to TGFB1 and 3, is decreased in serum of patients with PCOS, leading to higher TGFB1 bioavailability (Tal *et al*. 2013*b*).

These results suggest that increased serum TGFB1 levels and bioavailability may contribute to augmented ovarian angiogenesis and a greater risk of ovarian hyperstimulation after gonadotropin administration in these patients.

Placental growth factor (PlGF)

Tal and coworkers (2014) analyzed PlGF protein levels in serum and FF from women with PCOS and concluded that, while no differences were found in serum levels between control and PCOS patients, this factor is increased in FF from patients with PCOS. Moreover, due to a decrease in sFLT1 in FF, PlGF bioavailability was also increased in FF (Tal *et al*. 2014). These findings suggest a pertinent role for PlGF in ovarian angiogenesis deregulation in PCOS. However, more studies should be conducted to confirm the involvement of PlGF in this pathology.

Angiopoietins (ANGPTs)

The levels of ANGPT1 and 2 have been found altered in patients with PCOS, as well as in animal models of PCOS. Sova and coworkers evaluated serum levels of ANGPT2 protein in control and PCOS patients and found no differences between these two groups (Sova *et al*. 2010). This result was confirmed by Tal and coworkers when assessing the protein levels of ANGPT1 and 2 in both serum and FF from control and PCOS patients (Tal *et al*. 2013*a*). Nevertheless, they did report an increase in ANGPT1 with no changes in ANGPT2 in serum from patients with PCOS. In FF from patients with PCOS, ANGPT2 was increased with no changes in ANGPT1, leading to a higher ANGPT2/ANGPT1 ratio, which correlates with an increase in vascularization. In our laboratory, we investigated FF ANGPTs levels and observed an increase in ANGPT1 with no changes either in ANGPT2 or in soluble TIE2 (Scotti *et al*. 2014). In those patients, VEGF was also higher in FF compared to control women. We proposed that elevated ANGPT1 may be compensating for the increase in permeabilization elicited by the high VEGF

concentration (Scotti *et al*. 2014). The differences among these studies may respond to variations in stimulation protocols or to diversity in the population included in the study. Further studies are needed to determine whether the dynamic pattern of expression of ANGPTs in the ovary is altered in PCOS patients.

A recently published study examined the differences between women with PCOS that ovulated after clomiphene citrate (CC) and PCOS women resistant to CC (Wang *et al*. 2017). By using a cytokine antibody arrange confirmed by ELISA, one of the differences found was the circulating ANGPT2 levels, since PCOS women resistant to CC presented lower levels of ANGPT2 than sensitive ones. The authors conclude that CC-resistant women had an excessive ovarian angiogenesis that compromise their responsiveness to ovulation induction (Wang *et al*. 2017).

Furthermore, in a PCOS rat model developed by DHEA injection, we found an increase in ANGPT1, a decrease in ANGPT2 and an increase in ovarian TIE2 membrane receptor (Abramovich *et al*. 2012). The levels of ovarian TIE2 have not been studied in PCOS women yet.

Taken together, all these studies demonstrate that the ANGPT system is altered in PCOS, which may be related to the deregulation of ovarian angiogenesis in this pathology.

Platelet-derived growth factor (PDGF)

Among the PDGF family, PDGFRB, which binds to the PDGFBB and PDGFDD ligands, is the main receptor involved in angiogenesis, as described in the previous section. For this reason, we studied the levels of PDGFBB and PDGFDD in FF of PCOS women that undergo assisted reproductive techniques (ART) and found that both proteins were decreased compared to control women (Scotti *et al*. 2014). Moreover, in a DHEA rat model of PCOS, we also observed decreased levels of ovarian PDGFBB, PDGFDD and PDGFRB (Di Pietro *et al*. 2015). It is important to mention that PDGFRB signaling has been linked to the regulation of early folliculogenesis (Nilsson *et al*. 2006, Sleer & Taylor 2007, Pinkas *et al*. 2008), since granulosa cells from primordial follicles express this receptor and PDGF stimulation promotes primordial to primary follicle transition (Nilsson *et al*. 2006). Hence, decreased levels of ovarian PDGF proteins in PCOS may not only be associated to deregulation of ovarian angiogenesis but also to the abnormal small follicle accumulation observed in these patients (Scotti *et al*. 2014).

Because of its involvement in primordial follicle activation and in newly formed vessel maturation, PDGF deregulation in PCOS is worth being investigated since its normalization may be directly connected to these two key processes, which are altered in this pathology.

Therefore, PDGF is a good candidate to target in a future therapy for fertility improvement.

Antiangiogenic factors in PCOS

As angiogenesis is a complex process extremely regulated by factors that promote vessel growth and factors that inhibit this growth, it is not surprising that deregulation of blood vessel formation and proangiogenic factor levels is accompanied by alterations in antiangiogenic factors in PCOS. In this regard, some factors involved in physiologic inhibition of new vessel formation have been analyzed in patients and in animal models of PCOS.

As previously mentioned, sFLT1 is a soluble form of the VEGF receptor FLT1. It carries only six immunoglobulin domains of the full length form by alternative splicing (Kendall *et al.* 1996) and binds with high affinity to VEGF and PlGF, blocking their signaling through other receptors (Kendall *et al.* 1996). The levels of this soluble receptor have been found decreased in serum and FF of patients with PCOS (Artini *et al.* 2009, Tal *et al.* 2014), exacerbating the increase in its ligand levels.

It has been demonstrated that thrombospondin 1 (TSP1), a heparin-binding protein with potent antiangiogenic activity, is decreased in serum and subcutaneous and omental adipose tissue from patients with PCOS (Tan *et al.* 2009). This decrease is independent of either body mass index or PCOS phenotype, suggesting a role for TSP1 in the angiogenic alterations in this syndrome (Liu *et al.* 2015).

No other antiangiogenic molecules have been studied in PCOS, opening a big field in PCOS research since these compounds could serve as therapeutic tools to improve ovarian angiogenesis. Moreover, evidence of deregulation of antiangiogenic factors in combination with deregulation of proangiogenic factors strengthens the importance of the angiogenic process in the pathophysiology of PCOS. Table 1 summarizes the alterations in pro and antiangiogenic factors reported in PCOS.

Therapeutic strategies to restore a correct ovarian angiogenesis

The concept of restoring altered angiogenesis as a potential therapy has seen increasingly rapid advances in many pathologies, such as cancer (Albiges *et al.* 2011, Leite *et al.* 2011, Sharma *et al.* 2011, Socinski 2011), inflammatory diseases, retinopathies and age-related macular degeneration (Bauditz & Lochs 2007, Mitchell 2011, Ribeiro *et al.* 2011). As abnormal ovarian angiogenesis is a main feature of the PCOS, restoring this process has been proposed as a possible PCOS therapy to improve ovulation and fertility. To this end, many studies have analyzed the effect of different therapeutic approaches on ovarian angiogenesis (Table 2).

Laparoscopic ovarian drilling (LOD) is indicated in women with anovulatory PCOS that are resistant to clomiphene citrate (Thessaloniki 2008, Hueb *et al.* 2015). It consists of multiple ovarian punctures performed by laser or diathermy (2008). This treatment decreases LH and androgen levels and increases FSH in serum (Greenblatt & Casper 1987). Although the exact mechanisms remain unknown, LOD improves ovulation in these patients (Hueb *et al.* 2015). Regarding angiogenesis, LOD reduces serum VEGF (Amin *et al.* 2003, El Behery *et al.* 2011) and ovarian blood flow velocities (Amin *et al.* 2003, El Behery *et al.* 2011, Elmashad 2011, Giampaolino *et al.* 2017) in women with PCOS, leading to a decrease in the risk of developing OHSS if these patients need to be stimulated with gonadotropins after LOD (El Behery *et al.* 2011). Therefore, restoration of normal ovarian VEGF levels and blood flow could be one of the mechanisms that contribute to ovulation in these patients.

Another approach that aims to improve ovarian angiogenesis in PCOS is the use of the biguanide metformin (MET). MET is an oral hypoglycemic drug, widely spread for the treatment of type 2 diabetes. Despite the controversy of using MET to improve fertility and/or ART success in women with PCOS, MET has been reported to enhance fertility parameters, including live

Table 1 Alterations in angiogenic factors in women with PCOS.

Factor	Increased/decreased	Measurement	Studies
VEGF	Increased	Serum, follicular fluid, ovary	Kamat *et al.* (1995), Agrawal *et al.* (1998a,b), Artini *et al.* (2006, 2009), Scotti *et al.* (2014)
PlGF	Increased	Follicular fluid	Tal *et al.* (2014)
bFGF	Increased	Serum and follicular fluid post hCG	Artini *et al.* (2006)
TGFB1	Increased	Serum	Raja Khan *et al.* (2010), Tal *et al.* (2013b), Liu *et al.* (2015)
ANGPT1	Increased	Serum	Tal *et al.* (2013a)
PDGFBB	Decreased	Follicular fluid	Scotti *et al.* (2014)
PDGFDD	Decreased	Follicular fluid	Scotti *et al.* (2014)
sFLT1	Decreased	Serum, follicular fluid	Artini *et al.* (2006), Tal *et al.* (2014)
TSP1	Decreased	Serum, adipose tissue	Tan *et al.* (2009, 2010)
sENG	Decreased	Serum	Tal *et al.* (2013b)

ANGPT1, angiopoietin 1; bFGF, basic fibroblast growth factor; hCG, human chorionic gonadotropin; PDGFBB, platelet-derived growth factor BB; PDGFDD, platelet-derived growth factor DD; PlGF, placental growth factor; sFLT1, soluble FLT1 receptor; sENG, soluble endoglin; TGFB1, transforming Growth Factor; TSP1, Thrombospondin1; VEGF, Vascular Endothelial Growth Factor.

Table 2 Therapeutic strategies to restore a correct ovarian angiogenesis.

Treatment	Design	Parameters analyzed	Study
LOD	Woman with anovulatory PCOS, resistant to CC	Decreases VEGF	Amin et al. (2003), El Behery et al. (2011)
		Decreases ovarian blood flow velocities	Amin et al. (2003), El Behery et al. (2011), Elmashad (2011), Giampaolino et al. (2017)
MET	Woman with PCOS treated with gonadotropins after LOD	Decreases risk of developing OHSS	El Behery et al. (2011)
	Woman with PCOS undergoing gonadotropin stimulation	Decreases risk of developing OHSS	Khattab et al. (2006), Moll et al. (2007), Palomba et al. (2011, 2013)
	Overweight woman with PCOS. 6 months of MET treatment	Decreases Thrombospondin 1	Tan et al. (2009, 2010)
	Normal weight woman with PCOS. 3 months of MET treatment	Decreases ovarian blood flow parameters	Makled et al. (2014)
	DHEA rat model of PCOS. Metformin in the drinking water	Restores ovarian endothelial and periendothelial cell area	Di Pietro et al. (2015)
		Restores ovarian VEGF, ANGPT1 and PDGFs levels	
	Endothelial cell line treated with sera from woman with PCOS	Increase migration and tube formation	Tan et al. (2009, 2010)
		Decreases angiogenesis	
OCPs	Woman with PCOS treated with drospirenone and ethinylestradiol	Decreases ovarian vascularization	Okyay et al. (2014)
VEGF inhibition	DHEA rat model of PCOS. VEGF administration under the bursa	Restores ovarian periendothelial cell area	Abramovich et al. (2012)
		Increase ovulation and improves follicular development	
PDGFBB	DHEA rat model of PCOS. PDGFBB administration under the bursa	Decreases ovarian VEGF levels	Di Pietro et al. (2016)
		Improves follicular vascularization	
		Increases ovulation and improves follicular development	

ANGPT1, Angiopoietin 1; CC, Clomiphene Citrate; DHEA, dehydroepiandrosterone; LOD, Laparoscopic Ovarian Drilling; MET, Metformin; OCPs, Oral Contraceptive Pills; OHSS, Ovarian Hyperstimulation Syndrome; PCOS, Polycystic Ovary Syndrome; PDGFBB, Platelet-Derived Growth Factor BB; VEGF, Vascular Endothelial Growth Factor.

birth rate in some PCOS populations (Jungheim & Odibo 2010, Johnson 2011, Kjotrod et al. 2011, Tang et al. 2012, Palomba et al. 2013, 2014). Moreover, MET can regulate angiogenesis in different *in vivo* and *in vitro* models. For example, in ovarian cancer, MET was proposed as an antiangiogenic adjuvant to other therapies due to its capability of decreasing VEGF (Rattan et al. 2011, Liao et al. 2012, Shank et al. 2012, Wu et al. 2012). MET was also able to decrease angiogenesis in murine models of diabetes and obesity, thus preventing vascular damage (Abdelsaid et al. 2014, Dallaglio et al. 2014). Moreover, MET prevented OHSS in PCOS and non-PCOS women undergoing gonadotropin stimulation for ART (Khattab et al. 2006, Moll et al. 2007, Palomba et al. 2011, 2013). For these reasons, many researchers have started to evaluate the involvement of MET on regulation of ovarian angiogenesis in women with PCOS.

Tan and coworkers studied the effect of MET in an overweight PCOS population compared to overweight women without PCOS (Tan et al. 2009, 2010). These authors found that MET restores the decreased levels of serum and adipose tissue TSP1 in women with PCOS after 6 months of treatment. Additionally, while sera from women with PCOS increase migration and tube formation in an endothelial cell line, MET decreases these effects on *in vitro* angiogenesis (Tan et al. 2009, 2010). In normal weight women with PCOS, Makled et al. have shown that 3 months of MET treatment reduces ovarian blood flow parameters together with ovarian volume and corrects hormonal profiles (Makled et al. 2014). Whether the improvement in hormonal levels is a cause or a consequence of the improvement in ovarian stromal vascular bed still needs to be addressed.

In order to determine the mechanisms involved in MET regulation of ovarian angiogenesis, we evaluated the effect of MET in ovarian angiogenesis in a rat model of PCOS developed by prepubertal injection of DHEA (Di Pietro et al. 2015). We found that MET restores the ovarian endothelial and periendothelial cell area and the ovarian VEGF, ANGPT1 and PDGFs protein levels. MET also improves follicular development in the PCOS animals, decreasing ovarian cysts and increasing ovulation (Di Pietro et al. 2015). Since MET treatment has led to promising results regarding angiogenesis restoration, further studies are worth pursuing to deepen these findings.

Oral contraceptive pills (OCPs) are the first line of treatment after lifestyle modifications for women with PCOS who do not seek pregnancy (Vrbikova & Cibula 2005). OCPs decrease LH and androgen synthesis and increase sex hormone binding globulin (SHBG), leading to a decrease in the free androgen index (FAI) (Nader & Diamanti-Kandarakis 2007). Okyay et al. have observed that a 3-month treatment with drospirenone (3 mg) and ethinylestradiol (30 μg) reduces ovarian vascularization both in control and PCOS patients, with a stronger effect on PCOS population compared to control patients

(Okyay *et al.* 2014). The results presented in this study promote further research into the effect of OCPs on ovarian vascularization.

In our laboratory, we investigated the effect of local VEGF inhibition (Abramovich *et al.* 2012) and PDGFBB administration (Di Pietro *et al.* 2016) under the ovarian bursa of rats, previously injected with DHEA to mimic PCOS. We found that inhibiting ovarian VEGF by using a soluble receptor partially restores the accumulation of small follicles and reduces the formation of cysts in the PCOS rats. This strategy also increases ovulation and improves follicular development (Abramovich *et al.* 2012). Similarly, PDGFBB ovarian administration to PCOS rats partially restores primordial follicle accumulation, reduces cysts and increases corpora lutea formation. It also decreases ovarian VEGF levels and improves follicular vascularization (Di Pietro *et al.* 2016). Taken together, these findings support the hypothesis that normalization of ovarian angiogenesis can be considered a new strategy for improving follicular development, selection of the dominant follicle and ovulation in PCOS.

Vasculogenesis dysfunctions in PCOS

Endothelial progenitor cells (EPCs) are bone marrow-derived mononuclear cells that possess the ability to migrate to neovascularization sites and once there proliferate and differentiate into mature endothelial cells, participating in the formation of the new blood vessels. EPC alterations in number and/or function have been involved in the development of atherosclerosis and impaired glucose metabolism (Fadini *et al.* 2010). Given the high risk of developing endothelial dysfunction combined with the vascular deregulation present in women with PCOS, EPCs have become candidates possibly involved in these pathogenic processes (Dessapt-Baradez *et al.* 2011, Kao *et al.* 2013). In this regard, Dessapt-Baradez *et al.* have investigated the number and function of EPCs in non-obese women with PCOS compared to age and BMI-matched control women. Decreased circulating number and impaired function of EPCs were found in the PCOS group compared to the control group. These alterations are closely related to the increased risk of CVD and vascular aging in patients with PCOS (Dessapt-Baradez *et al.* 2011).

Based on these results, improving EPC function in PCOS ovaries may provide important insights to enhance ovarian vessel formation in these patients. More studies should be undertaken to confirm this hypothesis.

Conclusions and future remarks

In the last years, continuous research has contributed to better characterize PCOS and set new diagnostic and therapeutic strategies for its management. Ovarian angiogenesis dysfunction has thrived as a new field in the study of PCOS, not only as a marker of CVD but also as a marker of ovarian deregulation. An increasing body of evidence demonstrates that the impairment of blood vessel development is a central feature in the PCOS pathophysiology, contributing to the most important traits of the syndrome, such as infertility. Therefore, strategies that target angiogenesis in women with PCOS are being evaluated in order to manage different aspects of this syndrome. Moreover, ovarian angiogenic imbalance has been proposed as a new tool in PCOS diagnosis. Improving ovarian angiogenesis in women with PCOS appears to be a promising strategy to enhance ovulation and follicular development in these patients. Further studies are required to clarify the role of angiogenesis in PCOS and to develop new potential therapies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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