



Sex Steroids Modulate Uterine-Placental Vasculature: Implications for Obstetrics and Neonatal Outcomes

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Adequate blood supply to the uterine-placental region is crucial to ensure the transport of oxygen and nutrients to the growing fetus. Multiple factors intervene to achieve appropriate uterine blood flow and the structuring of the placental vasculature during the early stages of pregnancy. Among these factors, oxygen concentrations, growth factors, cytokines, and steroid hormones are the most important. Sex steroids are present in extremely high concentrations in the maternal circulation and are important paracrine and autocrine regulators of a wide range of maternal and placental functions. In this regard, progesterone and estrogens act as modulators of uterine vessels and decrease the resistance of the spiral uterine arteries. On the other hand, androgens have the opposite effect, increasing the vascular resistance of the uterus. Moreover, progesterone and estrogens modulate the synthesis and release of angiogenic factors by placental cells, which regulates trophoblastic invasion and uterine artery remodeling. In this scenario, it is not surprising that women with pregnancy-related pathologies, such as early miscarriages, preterm delivery, preeclampsia, and fetal growth restriction, exhibit altered sex steroid concentrations.

Keywords: placental angiogenesis, uterine blood flow, progesterone, androgen, estrogen

INTRODUCTION

During pregnancy, the placenta has important nutritional, metabolic, and endocrine functions that constitute the link between the mother and the fetus. The transfer of oxygen and essential nutrients from maternal blood to the fetal bloodstream requires an adequate uterine perfusion and a placental vascular network. Abnormalities in these processes are associated with an increased risk for miscarriage, preterm delivery, preeclampsia, and fetal growth restriction (FGR) (Regnault et al., 2002).

The formation of blood vessels involves two consecutive processes: (1) vasculogenesis, which involves the structuring of primitive vessels from mesenchymal cells; and (2) angiogenesis, which is the generation of new blood vessels from preexisting vessels to form the vascular placental network (Charnock-Jones et al., 2004). Both processes are driven and regulated by multiple factors, including oxygen concentration, growth factors, cytokines, and steroid hormones. Sex steroids are essential to maintain a normal pregnancy, and they participate in the control of a wide range of maternal and placental functions as well as in the normal development of fetal organs such as the

lungs and adrenal glands (Seaborn et al., 2010; Ishimoto and Jaffe, 2011). Moreover, variations in maternal serum concentrations of sex steroids have been described in conditions associated with abnormal placentation that impact placental perfusion, thus leading to pregnancy-related pathologies. Therefore, the aim of the present review is to summarize the current knowledge regarding the role of progesterone, androgens, and estrogens in the uterine-placental vasculature.

REGULATION OF UTERINE VASCULAR TONE

During pregnancy, uterine blood flow increases dramatically mainly through a decrease in the uterine vascular resistance as a result of uterine arteries dilation and remodeling. Many of these effects are produced by changes in the muscular tone of uterine arteries that are mediated by the action of nitric oxide (NO) and prostanoids (prostaglandins, prostacyclins, and thromboxane). NO increases uterine blood flow through the relaxation of uterine arteries by a mechanism that involves a decrease in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) in vascular smooth muscle cells (VSMC). NO originates from the metabolism of L-arginine by the action of endothelial NO synthase (eNOS) in endothelial cells. Prostacyclin (PGI₂) also induces vasodilation. However, has been observed that PGI₂ exerts a compensatory action when NO production is reduced (Beverelli et al., 1997). Prostaglandin F_{2α} (PGF_{2α}) and thromboxane A₂ (TXA₂) induce vasoconstriction. Prostanoids are produced by the action of the cyclooxygenase (COX) enzymes, COX-1 and COX-2, on arachidonic acid. Of note, during pregnancy, serum concentrations of PGI₂ increase dramatically, whereas PGF_{2α} and TXA₂ remain constant, thus favoring vasodilation (Mills et al., 1999).

Other regulators of the uterine vascular tone during pregnancy include adrenomedullin (Ross et al., 2010) and the components of the renin-angiotensin system, mainly angiotensin-(1–7) (Merrill et al., 2002). In rat uterine arteries, adrenomedullin induces relaxation mediated by the NO–cGMP-pathway (Ross et al., 2010). Angiotensin (1–7) is released from syncytiotrophoblasts, which act as a potent vasodilator in contrast to angiotensin II, which induces vasoconstriction (Valdes et al., 2006).

PLACENTAL VASCULATURE

The placenta originates from the differentiation of trophoblastic cells from the pre-implantation embryo into cytotrophoblasts and syncytiotrophoblasts (Gerbaud and Pidoux, 2015). Two weeks after conception, the blastocyst cells acquire the ability to invade and migrate through the endometrial wall. The decidualization reaction of stromal endometrial cells subsequently results in an important increment in tissue permeability and vascular density. This reaction favors the migration of extravillous cytotrophoblasts (EVT) across the decidua to reach the endothelial cells of the terminal segments of the uterine arteries occluding their lumen (**Figure 1**), which restricts blood flow into the intervillous space and leads to a

drop in oxygen concentration (**Figure 1**). Between weeks 11–12 until weeks 18–20 of gestation, EVT remodel the uterine spiral arteries. The remodeling allows the uterine spiral arteries to acquire a large capacitance and low resistance, thus gradually increasing maternal blood flow and oxygen levels (Rodesch et al., 1992).

The growth and development of the placental vascular network occurs through branching angiogenesis, which involves the formation of new vessels by the sprouting of preexisting vessels and a subsequent increase in the number of capillaries; it also occurs through non-branching angiogenesis, which involves the elongation of vessels and leads to the formation of capillary loops (Charnock-Jones et al., 2004).

The members of the vascular endothelial growth factor (VEGF) family are central in the regulation of placental vasculogenesis and angiogenesis (Demir et al., 2004). VEGF family members are produced by trophoblastic cells, Hofbauer cells, and maternal decidual cells (**Figure 1**) (Clark et al., 1996). The VEGF family has five members encoded by individual genes, including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PlGF (placenta growth factor). VEGF-A increases vascular permeability in endothelial cells, inducing placental vasculogenesis, and angiogenesis. Moreover, VEGF-A stimulates the expression of placental eNOS and NO production, thus inducing vasodilatation and promoting endothelial cell proliferation (Papapetropoulos et al., 1997) (**Figure 1**).

In general, VEGF-A, VEGF-B, and PlGF bind to VEGFR-1 (or Flt-1), whereas VEGF-A also binds to VEGFR-2 (or KDR). VEGF-A exhibits an increased affinity for Flt-1. However, KDR is more active in angiogenic stimulation (Stuttfield and Ballmer-Hofer, 2009). In the human placenta, Flt-1 is located in syncytiotrophoblasts and endothelial cells of the placental villi (Helske et al., 2001). On the other hand, KDR is almost exclusively expressed in endothelial cells, which mostly occurs during the first trimester of gestation in parallel to the high angiogenic activity at that time (Yamazaki and Morita, 2006). The action of VEGF on angiogenesis is regulated by an impressive paracrine negative feedback system in which the soluble form of Flt-1 (sFlt-1) acts as a potent inhibitor of angiogenesis that is regulated by VEGF.

The hypoxic environment induces the expression of factors regulating the angiogenesis process, and hypoxia-inducible factor (HIF)-1α is one of the main factors (Kingdom and Kaufmann, 1999). Of note, villous trophoblasts cultured under hypoxic conditions (1% O₂) express high levels of VEGF-A, Flt-1, and sFlt-1 mRNA (Munaut et al., 2008). Interestingly, recent evidence suggests that HIF-1α is also activated by non-hypoxic stimuli, such as growth factors, immunogenic cytokines, and sex steroids (Patel et al., 2010).

Other regulators of placental angiogenesis include angiopoietin (Ang)-1, Ang-2, and their receptor Tie-1. These proteins are complementary to the VEGF system but participate in the later stages of angiogenesis. In early pregnancy, Ang-2 is more highly expressed than Ang-1. However, Ang-2 decrease during the course of pregnancy (Geva et al., 2002). Finally, endoglin (Eng), a homodimeric transmembrane glycoprotein that belongs to the TGF-β (transforming growth factor beta)

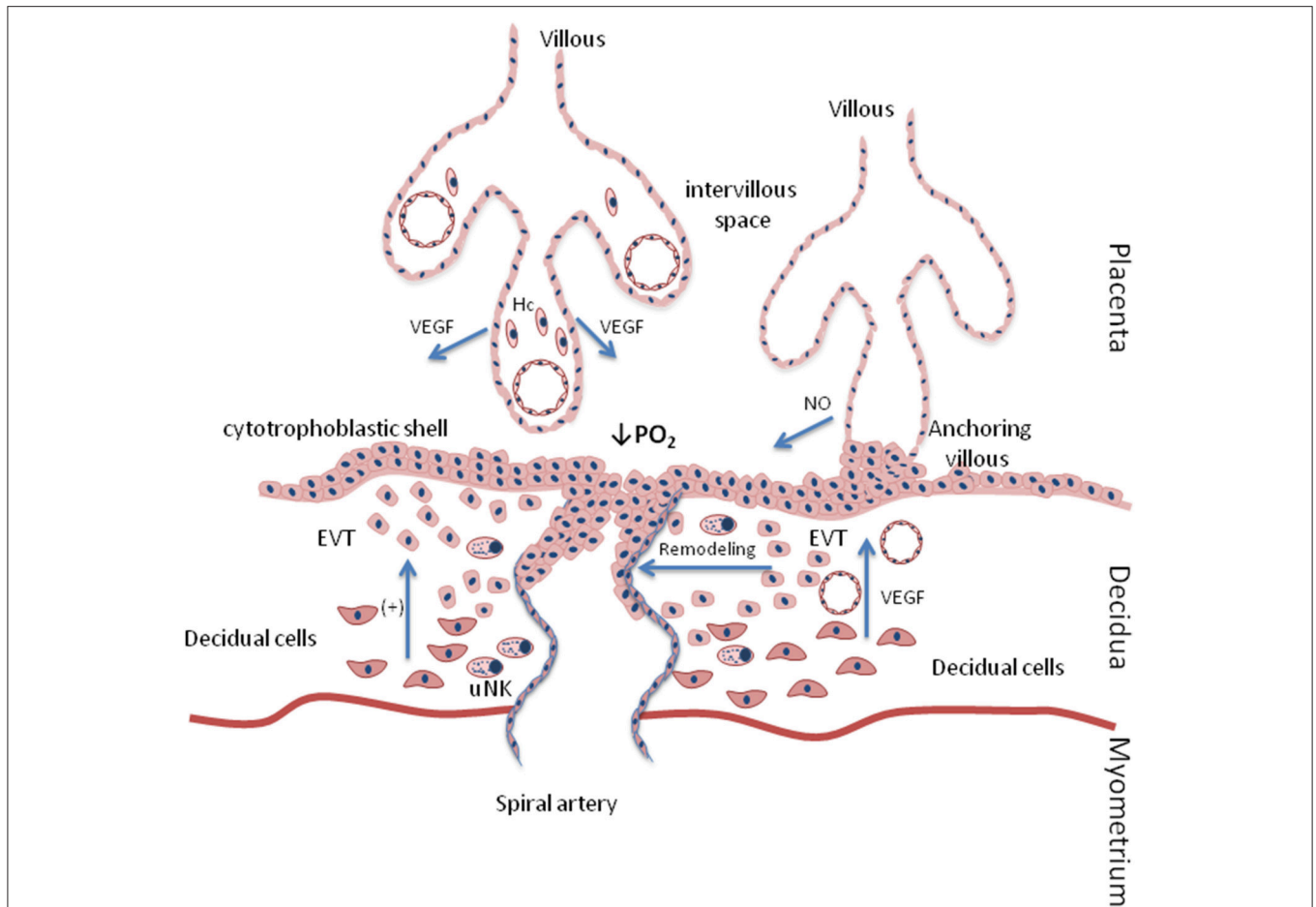


FIGURE 1 | Placental angiogenesis during early pregnancy. The reaction of decidualization of stromal endometrial cells promotes the migration of extravillous cytotrophoblasts (EVT) across the decidua to reach the endothelial cells of the terminal segments of the uterine arteries occluding their lumen, which restricts the blood flow into the intervillous space and leads to reduced oxygen concentrations. Moreover, EVT remodel uterine spiral arterioles to increase maternal blood flow. On the other hand, trophoblastic cells, Hofbauer cells (Hc), and maternal decidual cells secrete VEGF, thus promoting angiogenesis. In addition, trophoblasts increase NOS activity, thus stimulating nitric oxide (NO) production and vasodilatation.

complex, contributes to placental angiogenesis; however, a placenta-derived soluble endoglin isoform (sEng) acts as an anti-angiogenic protein that inhibits TGF- β 1 signaling in endothelial cells.

SEX STEROIDS AND UTERINE VASCULAR TONE

The role of progesterone and estrogen in the regulation of the uterine vascular tone has been recognized for a long time. However, the effects of testosterone have only been recently addressed. In the placenta, androgens are metabolized to estrogens by the P450 aromatase. Dihydrotestosterone (DHT), which cannot be metabolized to estrogen, is subsequently reduced by aldo-keto reductase family 1 C into androstenediol (5 α -androstane-3 β , 17 β -diol [3 β -diol]), which has estrogen-like activity through ER β (Lund et al., 2004). Therefore, androgenic and estrogenic effects cannot be easily separated in this tissue.

Progesterone

Progesterone plays an important role in uterine vessel vasodilation before the 10th week of gestation (Dickey and Hower, 1996). This feature, along with the decreased resistance of the placental bed, contributes to a reduction of systemic blood pressure until 28 weeks of gestation.

Progesterone binds to its own receptors located in the nucleus and on the plasma membrane, mediating genomic, and non-genomic actions. In general, nuclear progesterone receptor (PR) comprises five isoforms, of which PR-A (81 kDa) and PR-B (116 kDa) are the most widely expressed in different tissues (Li and O'malley, 2003).

Progesterone has been implicated in the rapid increase of eNOS activity and the production of NO in human endothelial cells (Simoncini et al., 2007). In a similar manner, progesterone stimulates PGI₂ production because it enhances the expression and activity of COX-1 and COX-2 (Hermenegildo et al., 2005). In addition, membrane progesterone receptors (mPRs) are present in VSMC, and they promote the decrease of the i[Ca²⁺] and lead

to vasodilation (Minshall et al., 2002; Moussatche and Lyons, 2012). In this regard, in human umbilical vein endothelial cells (HUVECs), progesterone induces NO production through mPR α (Pang et al., 2015).

Androgens

Testosterone promotes the proliferation of human myometrial microvascular endothelial cells through the activation of the MAPK/ERK-kinase pathway and VEGF-A production (Dietrich et al., 2011). In rats, the administration of androgen during pregnancy reduced uterine blood flow and elevated the maternal blood pressure due to an increased resistance of uterine vessels, which was due to the suppression of eNOS activity (Chinnathambi et al., 2013). Moreover, testosterone contracted the uterine arteries and reduced vascular relaxation due to the decline of endothelial NO production and the expression of prostacyclin and small conductance calcium-activated channel-3 (SK3). In contrast, hypoxia-responsive genes were increased, indicating poor uterine oxygenation induced by testosterone (Chinnathambi et al., 2014).

Estrogens

Estrogens have an important function in the regulation of blood flow and microvascular volume because they control specific genes involved in vascular tone (Pastore et al., 2012). In this regard, NO is stimulated by both estrogen receptor isoforms (ER α and ER β). Of interest, NO inhibition blunts the action of estradiol, suggesting that estrogen relaxation of myometrial arteries is mediated by both NO-dependent and -independent mechanisms (Rosenfeld et al., 1996). In addition, estrogens act on a 7-transmembrane G protein-coupled receptor named GPER. However, its activation reduces vascular tone in the rat uterus during pregnancy (Tropea et al., 2015).

Estradiol relaxes precontracted human myometrial and placental arteries by binding to ER α and ER β . However, this effect is lower than that in the myometrial than placental vessels (Corcoran et al., 2014). Moreover, estradiol can stimulate PGI₂ synthesis due to upregulation of COX-1 expression, as observed in HUVEC and ovine fetal pulmonary artery cells. This effect is Ca²⁺ dependent and mediated by the activation of ER β (Jun et al., 1998; Calkin et al., 2002; Sherman et al., 2002).

SEX STEROIDS AND PLACENTAL ANGIOGENESIS

The role of sex steroids in placental angiogenesis has not been widely studied. Preliminary evidence suggests that sex steroids can regulate both endometrial and placental angiogenesis.

Progesterone

Progesterone has an important role in the activation of the decidual reaction in endometrial stromal cells, increasing the vascular permeability in the endometrial stroma through the activation of the nuclear receptor subfamily, group A, member 1 independently of VEGF action (Figure 2A; Goddard et al., 2014). Progesterone increases the number of uterine natural killer (uNK) cells (Bulmer and Lash, 2005), which most likely occurs

indirectly through the decidualization reaction (Figure 2A). In this regard, uNK cells secrete a wide variety of angiogenic factors during early pregnancy, including VEGF-C, PlGF, Ang-1, Ang-2, and TGF- β 1, promoting the vascular development of the decidua (Hanna et al., 2006).

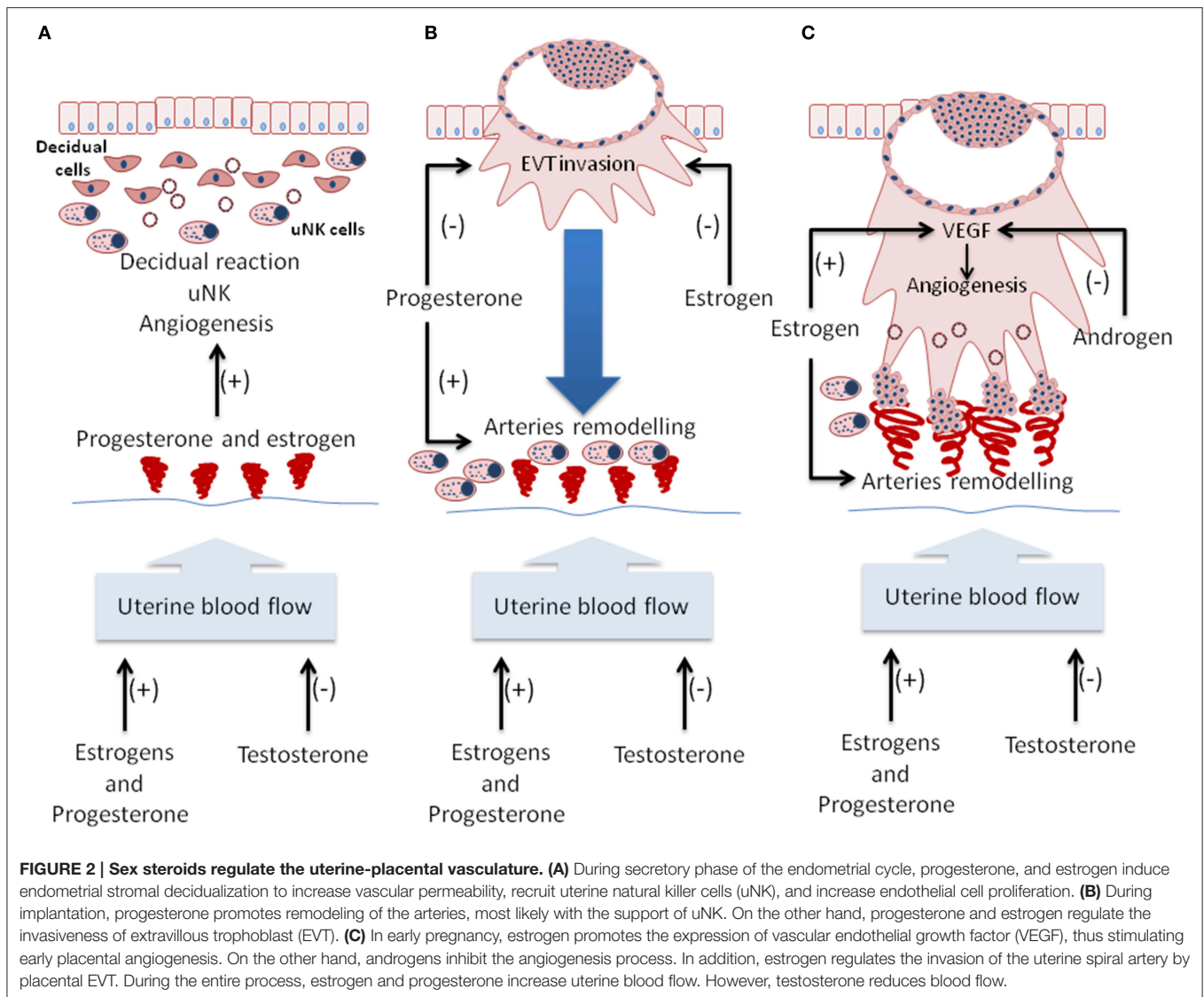
In early pregnancy, PR is expressed in the endothelial cells of decidual tissue, and the binding of progesterone stimulates endothelial cell proliferation. This process is partly mediated by VEGF with no necessary estrogen priming (Wang et al., 1992). Moreover, progesterone regulates early trophoblast invasion because it reduces the invasive properties of EVT *in vitro* and the secretion of matrix metalloproteinase (MMP)-2 and -9, which are primary mediators of vascular remodeling and angiogenesis in decidual tissue (Goldman and Shalev, 2006) (Figure 2B). However, progesterone promotes the migration of EVT by the upregulation of an insulin-like growth factor binding protein-1 and Dickkopf-related protein-1 (Halasz and Szekeres-Bartho, 2013). In addition, progesterone can promote the differentiation of a subfraction of decidual cells (named decidua-derived CD31⁻CD146⁻ subfraction of side population (SP) cells) into endothelial cells and smooth muscle cells, suggesting that progesterone may play a role in the formation of new blood vessels in the placenta (Wang et al., 2013).

Androgens

Androgen receptor is present in the cells of the syncytiotrophoblast and in the decidua during the first trimester of gestation (Horie et al., 1992). Rat models have shown that elevated androgen levels during pregnancy induce a reduction in placental weight and the activity of amino acid transporters (Sathishkumar et al., 2011; Sun et al., 2012). Moreover, androgens induce the downregulation of genes related to vascular development and angiogenesis (*Ccr3*, *Stra6*, *Dhcr7*, *Arid1a*, *Ptprj*, *Col1a2*, *Lef1*, *Col1a1*, and *Mmp2*) in the placenta (Figure 2C). Along with this antivascular gene expression profile, the authors reported a reduction in radial and spiral artery diameters and branching angiogenesis (Gopalakrishnan et al., 2016). Thus, androgens could negatively regulate placental oxygenation, which is reflected by an increase in pimonidazole binding and HIF-1 α levels (Gopalakrishnan et al., 2016).

Estrogens

P450 aromatase is expressed in stromal uterine cells, indicating a local production of estrogen. Here, estrogen appears to facilitate decidualization and uterine neovascularization (Figure 2A), inducing the expression of HIF2 α , Ang-2, Ang-4, and adrenomedullin (Das et al., 2009). Estrogen receptors (ER) α and β are expressed within villous trophoblasts of the human placentas (Bukovsky et al., 2003a,b). In this regard, the estrogen signaling has also been involved in the regulation of trophoblast differentiation and its invasive capacity in the hypoxic environment of the first trimester primate placenta. For example, similar to progesterone, estrogens act as regulators of the extent of remodeling during early pregnancy because they inhibit the invasive capacity of EVT (Figure 2B), reduce VEGF protein expression in the placenta anchoring villi and reduce the expression of integrins in cells from the anchoring



villi and the cytotrophoblastic shell (Bonagura et al., 2012). Moreover, estradiol can regulate placental angiogenesis by the degranulation of mast cells that secrete important amounts of VEGF, suggesting a role of inflammation in this process. In this regard, estradiol and progesterone attract mast cells to the uterus (Corcoran et al., 2014).

In many species, including humans, estradiol induces the expression of the VEGF protein in the cytotrophoblast and increases the percent of vascularized area and vessel density in placental tissue (Albrecht et al., 2004; Robb et al., 2004; Albrecht and Pepe, 2010) (Figure 2C). In baboon cytotrophoblasts, VEGF mRNA increases in parallel with the increase in serum estradiol levels during early pregnancy (Hildebrandt et al., 2001). However, during the last two-thirds of pregnancy, the inhibition of P450 aromatase does not affect VEGF action in blood vessel development, suggesting that the cytotrophoblast loses its control by estrogen action during pregnancy (Albrecht and Pepe, 2010).

CLINICAL IMPLICATIONS

An abnormal blood supply to the uterine-placental region leads to early miscarriage, preterm delivery, preeclampsia, and FGR. In this regard, modifications to the circulating levels of sex steroids and/or uterine and placental sex steroids receptors are associated with poor obstetric and prenatal outcomes.

Women with unexplained recurrent pregnancy loss exhibit elevated uterine arterial impedance, which is negatively correlated with circulating progesterone levels. Of note, the administration of dydrogesterone, a synthetic progestin, reduced the resistance to blood flow in the uterine arteries, suggesting that insufficient progesterone action could be involved in a poor uterine blood supply and lead to miscarriage (Habara et al., 2002). Moreover, the elevated expression of Dickkopf-related protein-1 and low expression of PR-A have been observed in women with unexplained recurrent spontaneous miscarriage (Papamitsou et al., 2011; Bao et al., 2013). On the other

hand, in growth-restricted pregnancies, PR expression in the placental tissue is positively correlated with IGF-1 expression and infant anthropometry, and it is independent of the presence of pregnancy pathologies (Akram et al., 2011). A group of studies has demonstrated that preeclampsia is associated with increased levels of progesterone along with increased expression of CYP11A, which inhibits trophoblastic proliferation and potentially the production of prostacyclin; this association affects the development of placental vasculature (Walsh and Coulter, 1989; He et al., 2013). Another group of studies demonstrated low circulating levels of progesterone and aldosterone in women with preeclampsia affected the secretion of endothelin-1, which is a potent vasoconstrictor. These results indicate that progesterone could be involved in the maintenance of normal blood pressure (Kiprono et al., 2013; Uddin et al., 2014). Therefore, normal development of placental vasculature is potentially dependent on physiological ranges of progesterone concentrations.

Because estrogen is an important regulator of uterine blood flow and the production of angiogenic factors in placental tissue, it is possible to hypothesize that estrogens are involved in the pathophysiology of pregnancy-related pathologies. At the 27th gestational week, estradiol is positively associated with birth weight, birth length, and placental weight (Wuu et al., 2002). However, in rats, pharmacological doses of estradiol benzoate induce growth restriction, the reduction of placental weight, and trophoblastic degeneration (Matsuura et al., 2004). ER β appears to be an important inducer of vasoconstrictor prostanoids because it increases the resistance of the fetoplacental blood flow (Su et al., 2011).

Elevated androgen levels are a recurrent finding in preeclamptic women (Troisi et al., 2003; Salamalekis et al., 2006; Sharifzadeh et al., 2012), and this finding is likely related to a sex-related dysregulation in P450 aromatase (Steier et al., 2002; Sathishkumar et al., 2012). In women with polycystic ovary syndrome, which causes elevated androgen levels during pregnancy, the placenta presents an abnormal uterine blood flow

as well as placentation with reduced endovascular trophoblast invasion (Palomba et al., 2010, 2012, 2013). Interestingly, placental tissues from these patients exhibit increased ER α expression (Maliqueo et al., 2015). Therefore, abnormalities observed in PCOS women could be attributed to estrogen or androgen action. However, it is not clear whether these alterations are directly associated with elevated androgen levels, as PCOS mothers also exhibit elevated insulin levels and a pro-inflammatory pattern (Sir-Petermann et al., 2007; Palomba et al., 2014), which may also influence placental function.

CONCLUSIONS

Adequate uterine perfusion is necessary to achieve successful implantation. Moreover, placental vasculogenesis and angiogenesis ensure optimal transfer of oxygen and nutrients along with fetal detoxification. All these processes are essential for an adequate fetal development. The available data clearly note that sex steroids contribute to the modulation of uterine blood flow through the regulation of uterine vessels and placental vasculogenesis and angiogenesis, which involves controlling trophoblast invasion and the remodeling of uterine arteries.

AUTHOR CONTRIBUTIONS

MM conceived and wrote the manuscript; BE and NC contributed with the writing and revised critically the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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