REPRODUCTION
REVIEW

Novel role for lysophosphatidic acid in vascular remodeling at the maternal–fetal interface

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Abstract

Lysophosphatidic acid (LPA) belongs to the group of phosphorylated lipids reported as crucial mediators in the physiology of reproduction. LPA binds to G-protein-coupled receptors and regulates a wide range of female reproductive functions. This bioactive lipid has also been implicated in vascular functions during physiological and pathological conditions. In this regard, the establishment of a successful pregnancy requires proper coordination of vascular processes and remodeling of maternal blood vessels during early gestation. During this process, first trimester cytotrophoblast changes from an invasive to an endovascular phenotype and transforms uterine spiral arteries which are the nutrient supply for placenta and fetus. Here we present an overview of LPA participation in vascular remodeling and highlight the importance of LPA–LPA3 signaling during early gestation at the maternal–fetal interface.

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Implantation, decidualization and placentation

Embryo implantation into the maternal uterus comprises highly coordinated events and is a crucial step for the establishment of a healthy pregnancy in mammals (Wang & Dey 2006, Cha *et al.* 2012).

In humans, implantation begins with the apposition of the blastocyst until the extravillous cytotrophoblast invades the maternal endometrium. The blastocyst could implant only during the window of implantation which lasts for a limited period of time after ovulation. At this transient and unique moment, the active embryo interacts with the receptive endometrium, which supports embryo growth, attachment and the following stages of implantation (Zhang *et al.* 2013). In mice, the uterus is fully receptive approximately 72 h after ovulation. However, in humans, uterine receptivity occurs between 7 and 10 days after ovulation.

The process of implantation is extremely complex and could be classified into three stages: apposition, adhesion, and invasion (Fig. 1) (Hertig *et al.* 1956, Lindenberg 1991). Once the blastocyst apposes on the uterine epithelium, the outer layer of the blastocyst, the trophectoderm, increases the physical contact and establishes a strong and stable adhesion to the endometrium. During the invasion, the trophectoderm penetrates between the luminal epithelial cells to the basal lamina and extends to the uterine stroma, therefore establishing a stable maternal–fetal interface (Pijnenborg *et al.* 1980).

Embryo implantation promotes extensive modifications of the uterine stroma. The endometrial fibroblasts rapidly grow and differentiate into decidual cells. Decidualization is a crucial event in early pregnancy and is fundamental for the success of gestation in humans and mice (Dey & Lim 2006). In mice, the presence of an active blastocyst in the uterus is the stimulus for the decidual reaction. After attachment on day 4 of pregnancy, stromal cells surrounding the implanting blastocyst begin to proliferate extensively and differentiate (Tan *et al.* 2002). In women, pre-decidualization around the terminal spiral arteries of the superficial endometrial layer is under maternal control and occurs in the mid-luteal phase of the cycle in response to increasing levels of progesterone and cAMP (Wilcox *et al.* 1999). In the case of pregnancy, there is an extensive and profound burst in the decidual reaction. The decidua controls trophoblast invasion, regulates the activation of the immune system and protects and nourishes the growing embryo until the placenta is completely formed and functional.

Besides decidualization, a successful pregnancy includes the development of neovasculature, spiral arteries remodeling, and subsequent placentation. The placenta, a unique organ in mammals, reassures that the blood flow and nutrients exchange are sufficient during the whole gestation. During human placental development, the trophoblast differentiates to comprise pivotal mechanisms that allow fetal and maternal adaptations to pregnancy (Liu *et al.* 2018, Vento-Tormo *et al.* 2018). The villi are the functional units of the placenta and consist of an outer epithelial trophoblast layer and a stromal cell core. The mature human

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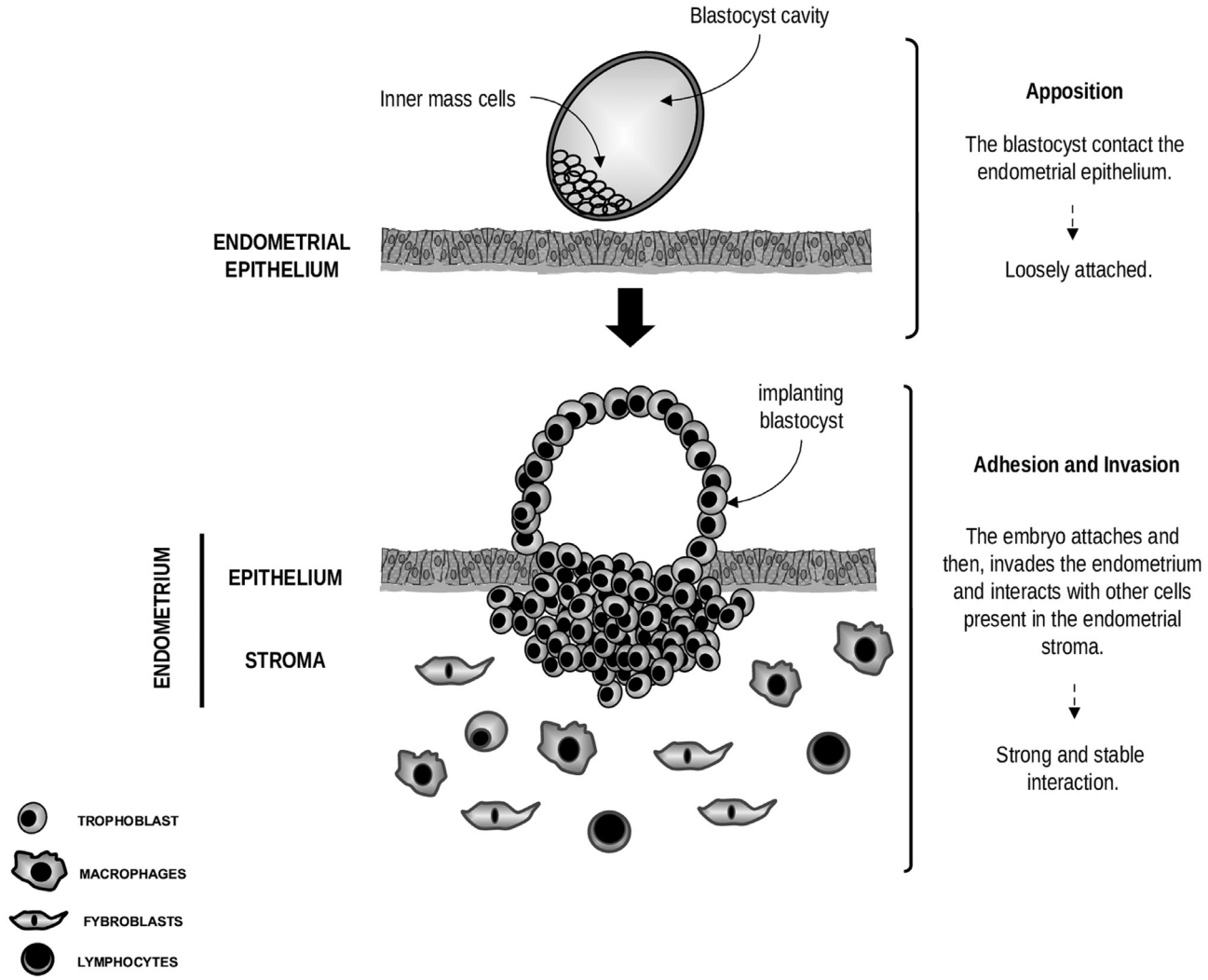

Figure 1 Stages of implantation. During implantation, the embryo apposes and adheres to the luminal endometrium before invasion begins.

placenta has three major types of epithelial trophoblast: the syncytiotrophoblast, the villous cytotrophoblast and the extravillous cytotrophoblast (Maltepe & Fisher 2015). The villous cytotrophoblast form a single layer that lines the stromal cell core and are the source of the replenishment of the syncytiotrophoblast and the extravillous trophoblast. Villous cytotrophoblast cells fuse and form the multinucleated syncytiotrophoblast which has mainly secretory functions and exerts an active role in the nutrition and gas exchange with the developing embryo. In the extravillous pathway, villous cytotrophoblast cells differentiate into interstitial extravillous trophoblast or endovascular extravillous trophoblast. In humans, the initial wave of invasion occurs via the formation of a primitive syncytium through which the cytotrophoblast escapes from the villi and acquire an invasive phenotype (Da Silva-Arnold *et al.* 2015). Invasive extravillous trophoblast is derived from column cytotrophoblast progenitors located at the tips of anchoring villi, migrates through the uterine parenchyma, and invades the decidua until the third portion of the myometrium. On the other hand, the endovascular extravillous trophoblast remodels the maternal vasculature (Zhou *et al.* 1997*a*, Kam *et al.* 1999). The main goal of cytotrophoblast invasion is to reach the uterine spiral arteries and remodel the pre-existing vessels, to increase blood flow and subsequent supply of nutrients and oxygen to the growing embryo (Zhou *et al.* 1997*a*,*b*).

When apposition, adhesion or invasion do not take place or are incomplete, the embryo fails to implant. Each step depends on the success of the preceding event. Early pregnancy loss is a worldwide concern and often occurs due to defects before, during or immediately after implantation (Wilcox *et al.* 1988, Zinaman *et al.* 1996). Despite advances in human reproductive technology, pregnancy rate remains low related to implantation failure (Cha *et al.* 2012, Miller *et al.* 2012, Patrizio &

Silber 2017). Therefore, understanding the implantation mechanism is a challenge to alleviate early pregnancy loss and infertility problems.

Vascular remodeling at the maternal–fetal interface

Vascular remodeling is a key mechanism triggered after invasion and lacunae formation. Uterine blood supply consists of multiple branches that decreases in diameter while invading the endometrium and myometrium. During pregnancy, the vascular bed of the uterus changes dramatically, where new vessels are formed and the existing vessels dilate. Particularly, blood flow increases and vascular resistance decreases as a result of a highly coordinated process known as spiral artery remodeling. In this sense, coiled appearance vessels named spiral arteries are transformed from strained and rigid muscular walls arteries into flaccid sinusoidal sacs that allow a significant increase in blood flow to sustain normal growth (Fig. 2) (Harris *et al.* 2006, Leach *et al.* 2006, Demir *et al.* 2010).

Coordination of vascular processes at the maternal–fetal interface is essential for maintenance of gestation and requires a deep reorganization of uterine and fetal tissues. Several mechanisms are proposed for spiral artery remodeling including cellular migration, apoptosis, inhibition of proliferation and endovascular differentiation (Cartwright *et al.* 2010, Cartwright & Whitley 2017). Although the precise pathways are not fully elucidated, it is accepted that spiral artery remodeling involves an active role of the extravillous cytotrophoblast.

Throughout placentation, human extravillous cytotrophoblast begins an active differentiation while migrates, invades decidual stroma and surrounds the spiral arteries. Afterward, the extravillous cytotrophoblast penetrates into the vascular lumen and replaces the endothelial cells of these vessels. This 'remodeling of spiral arteries' or 'trophoblast angiogenesis' involves a switch in cytotrophoblast phenotype and function from an invading to an endovascular one (Kam *et al.* 1999, Benirschke & Kaufmann 2000, Espinoza *et al.* 2006, Pijnenborg *et al.* 2006). Endovascular trophoblast mimics endothelial cells profile of blood vessels changing the expression pattern of adhesion molecules allowing interaction with different cell types (Burrows *et al.* 1994). Initially, cytotrophoblast expresses epithelial type adhesion molecules such as integrin α6/β4 and α6/β1 and E-cadherin. During the differentiation from cytotrophoblast to endovascular cytotrophoblast it begins to express integrins α1/β1 and α5/β3, as well as VE-cadherin, PECAM-1 and VCAM-1, classically expressed by the endothelium that it will replace. In particular, Zhou *et al.* (1997*a*) observed that E-cadherin expression by trophoblast inhibits invasion affecting vascular remodeling. It is worth mentioning that in preeclampsia, cytotrophoblast does not express endothelial markers, suggesting a failure in changing the trophoblast profile necessary for vascular remodeling (Zhou *et al.* 1997*b*, McMaster *et al.* 2004).

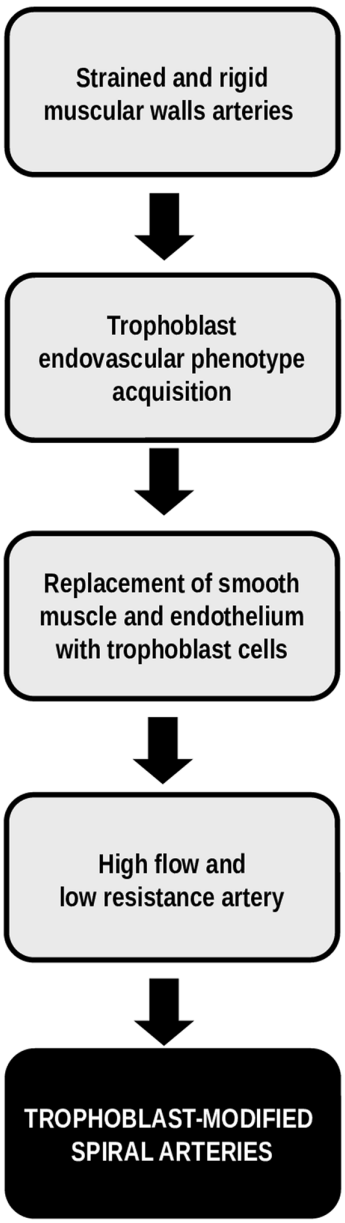

Figure 2 Spiral artery remodeling. Proposed mechanisms involved in the transformation of the uterine spiral arteries from strained and rigid vessels into flaccid sinusoidal sacs.

In addition to endovascular cytotrophoblast phenotype, spiral arteries transformation implicates loss or re-arrangement of smooth muscle layers and endothelial cells of maternal vessels, comprising the activation and vacuolization of endothelial cells as well as disorganization of smooth muscle cells (Cartwright *et al.* 2010). One question to be addressed is what directs trophoblast cells toward maternal spiral arteries. It is proposed that endovascular trophoblast

interacts directly with endothelial cells, while interstitial trophoblast interacts with smooth muscle cells (Cartwright & Whitley 2017). Particularly, the presence of endovascular cytotrophoblast was detected in spiral arteries after 8 weeks of gestation and is more prevalent at 10 weeks (Pijnenborg *et al.* 1980). This reorganization is accomplished when the endovascular trophoblast is surrounded by fibrin deposits giving structure to the remodeled vessel. As result of this mechanism, approximately 150 human spiral arteries acquire greater caliber and blood flow with low resistance, shifting the average luminal size from 200 µm to 2 mm (Boyd & Hamilton 1970, Benirschke & Kaufmann 2000, Lyall 2005). The importance of these events occurring in a regulated manner is evidenced by obstetric pathologies associated with insufficient spiral arteries remodeling during early pregnancy (implantation failures and recurrent miscarriage) and late pregnancy (preeclampsia and intrauterine growth retardation) (Khong *et al.* 1986). Understanding the potential causes of the disorders related to inadequate vascular remodeling would help to design new approaches to alleviate these pathologies.

General characteristics of LPA

LPA is the simplest natural lysophospholipid yet discovered with one fatty acid chain, a glycerol backbone with a hydroxyl group and a phosphate group as a polar head (Fig. 3) (Tokumura 1995, Moolenaar 1999, Tigyi & Parrill 2003). For many years, scientists thought that LPA was only an intermediate in *de novo* lipid synthesis and a component of plasma membranes. However, LPA is a family of structurally related compounds composed by saturated fatty acids (16:0, 18:0) and unsaturated fatty acids (16:1, 18:1, 18:2, 18:3, 20:4) which show differential biological activities by activating different receptors in human and rat (Tokumura *et al.* 1986, Gerrard & Robinson 1989, Eichholtz *et al.* 1993, Baker *et al.* 2000, Sano *et al.* 2002, Sugiura *et al.* 2002). In biological fluids, LPA is generally bound to chaperones

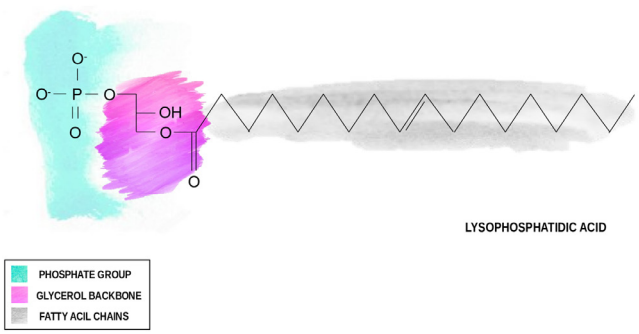

Figure 3 Molecular structure of LPA. LPA is formed by one fatty acid chain, a glycerol backbone with a hydroxyl group and a phosphate group.

(Mills & Moolenaar 2003, Blaho & Chun 2018). In plasma, 16:0 is the predominant species, while 18:0, 18:1, 18:2, 18:3 and 20:4 forms are present at lower concentrations (Akira *et al.* 1986, Choi *et al.* 2010).

So far, it has been reported that activated platelets produce high concentrations of LPA in the organism (Eichholtz *et al.* 1993, Leblanc *et al.* 2018). However, these are not the exclusive source of this bioactive lysophospholipid. Although LPA is produced after platelet activation, the amount of LPA released is insufficient to explain its levels in serum. The concentration of LPA in serum increases more than ten-fold compared to plasma (1 vs 0.1 µM) (Eichholtz *et al.* 1993). As mentioned later, these levels are tightly regulated by dynamic synthesis and degradation mechanisms.

The production of LPA is controlled by different phospholipase pathways (Aoki *et al.* 2002). However, studies in mice and human revealed that majority of extracellular LPA is produced by the secreted glycosylated enzyme lysophospholipase-D (LYSO-PLD) (van Meeteren *et al.* 2006, Benesch *et al.* 2015). LYSO-PLD is identical to a soluble form of cell motility-stimulating factor autotaxin and synthesizes LPA from lysophosphatidylcholine (the most abundant lysophospholipid precursor in plasma, ~200 µM) or from lysophosphatidylserine and lysophosphatidylethanolamine (Aoki *et al.* 2002).

The mechanism of LPA signaling was not elucidated until 1996 when the first LPA receptor was cloned (Hecht *et al.* 1996). The bioactive fraction of LPA is bound to albumin and gelsolin in circulation (Meerschaert *et al.* 1998) and this may have an important impact on its bioavailability and binding to specific receptors. It is known that LPA exerts its action through different G-protein-coupled receptors (Yung *et al.* 2014). Up to now, there are six *bona fide* LPA receptors. From these receptors, LPA1, 2 and 3 belong to the Edg family, while LPA4, 5 and 6 are classified into non-Edg receptors from the purinergic family (P2Y). Other orphan G-protein-coupled receptors are proposed to facilitate LPA signaling (GPR35, GPR87, P2Y10 and RAGE) (Benesch *et al.* 2018).

Most cells express different combinations of LPA receptors that share intracellular signaling pathways dependent on heterotrimeric G-proteins including Gαi, Gα12/13, Gαq and Gαs (Noguchi *et al.* 2009). LPA receptors are expressed in different cell types amplifying the possibility of its action. Moreover, activation of LPA receptors modulates downstream effectors such as adenylyl cyclase, cAMP, intracellular Ca^{2+}, mitogen-activated protein kinases, phospholipase C, PI3K/AKT and small GTPases (Ras, Rho, Rac) (Ishii *et al.* 2004). Due to these signaling characteristics, LPA regulates multiple cellular processes as cell survival, proliferation, cytoskeleton re-arrangement, motility, cytokine secretion and cell differentiation. Therefore, LPA redundancy has to be considered carefully according to each physiological and pathological situations.

Extracellular LPA signaling is rapidly terminated through degradation by a family of three transmembrane exophosphatases known as lipid phosphate phosphatases which convert LPA into inorganic phosphate and monoacylglycerol by dephosphorylation (Benesch *et al.* 2015).

LPA as a vascular mediator

Lipids are not encoded in the genome. Therefore, genetic studies performed to dissect the importance of lipid signaling in physiology and pathology have been directed toward their receptors, transporters and metabolizing enzymes. Analysis of transgenic mice revealed that lipid mediators are involved in a wide range of physiological events including inflammation, immunity and angiogenesis. Moreover, vascular network formation is precisely controlled by pro- and anti-angiogenic molecules and lipid factors.

LPA could be considered a 'vascular lipid' since it is mainly produced by platelets and its metabolic pathways depend on extracellular enzymes present in circulation. Early in 1960, it was reported that a new vasopressor factor induced the contraction of isolated rabbit duodenum preparations (Khairahllah & Page 1960). The authors observed that this factor was different from adrenergic amines and pressor polypeptides and was probably a lysophosphatide produced by an enzymatic reaction in plasma. They also described that similar vasopressor effects could be produced by the action of rattlesnake venom on lecithin. After 18 years, the vasopressor factor in soybean lecithin was identified as LPA (Tokumura *et al.* 1978*a*). Since then, numerous works were published describing vascular actions of LPA in different *in vitro* and *in vivo* biological systems.

Studies performed in mice and human revealed that smooth muscle cells (Panchatcharam *et al.* 2008, Subramanian *et al.* 2010, Dancs *et al.* 2017), endothelial cells (Zhou *et al.* 2011) and platelets (Haserück *et al.* 2004, Pamuklar *et al.* 2008) present in the vasculature respond to LPA (Cheng *et al.* 2009, Morris *et al.* 2009*a*,*b*). These interactions are involved in the regulation of blood vessel development and contribute to vascular biology and pathology (Mueller *et al.* 2015). Moreover, the effect of LPA on the vasculature has been also demonstrated in cat, rabbit, guinea pig (Tokumura *et al.* 1978*b*) and non-mammal species such as zebrafish (Yukiura *et al.* 2011).

As mentioned earlier, a wide range of actions of LPA in vascular events was discerned from studies involving transgenic mice. Both downregulation and overexpression of LYSO-PLD produce defects in angiogenesis triggering embryonic lethality due to severe vascular phenotype in the yolk sac and embryos as well as neural tube defects at embryonic day 9.5–10 (Tanaka *et al.* 2006, van Meeteren *et al.* 2006, Koike *et al.* 2011, Yukiura *et al.* 2015). Single and multiple deletions of LPA receptors produce differing vascular phenotypes. While LPA1 and LPA4 double knockdown embryos exhibit vascular defects similar to *Lyso-PLD* knockdown, LPA4-knockout mice are embryonic lethal due to hemorrhages and edema. Nevertheless, their phenotypes are not as severe as *Lyso-PLD*-knockout mice (Sumida *et al.* 2010). Endothelial-specific lipid phosphate phosphatases-3-knockout mice also exhibit lethal vascular leakage and hemorrhage as observed in *Lyso-PLD* knockout and overexpressing mice (Panchatcharam *et al.* 2014, Chatterjee *et al.* 2016).

Altogether, these data strongly suggest that LPA turnover and action via its receptors are complex mechanisms by which vascular adaptations could be regulated.

LPA relevance in vascular remodeling at the maternal–fetal interface

Studies in animal models - in vivo

The relevance of LPA–LPA3 in embryo implantation was first reported by Ye *et al.* (2005). These authors performed elegant studies identifying strong phenotypic changes in *Lpa3*-deficient mice such as reduced litter size, altered positioning or crowding of embryos and embryonic death. On the other hand, *Lpa1* and *Lpa2* deletion shows no effects on embryo implantation, revealing other roles for these receptors (Contos *et al.* 2000, 2002). These results suggest that LPA3 is the major LPA receptor involved in embryo implantation (Ye *et al.* 2005, 2011, Wei *et al.* 2009, Achache *et al.* 2010). To explore this possibility, we examined the role of LPA3 at the sites of implantation in an *in vivo* rat model. The intrauterine administration of an LPA3 antagonist to pregnant rats in day 5 of gestation causes profound alterations in the vasculature and decidual damage that affects placental and embryo development (Sordelli *et al.* 2017). Interestingly, we observed a dissimilar vascularization pattern between the resorbed units compared with controls. The LPA3 antagonist decreased the cross-sectional length of uterine and arcuate arteries, suggesting a decline in the supply of nutrients and oxygen to the developing embryos being finally resorbed. In addition, the microvasculature is also altered. Resorbed implantation sites and placentas present fewer vessels, and these vessels show larger perimeter. We speculate that this could provoke increased oxygen levels with subsequent excessive oxidative stress that induces inflammation and resorption. In this sense, Plaisier *et al.* (2009) reported that decidual vascularization differs between fertile women and those who miscarry (i.e. fewer vessels with a larger circumference in miscarriages). In line with this, Sharkey *et al.* (2000) demonstrated that oxygen concentration modulates the architecture of the vasculature as well as the expression of certain angiogenic molecules. Furthermore, LPA3 blockade decreased *Il-10*, *Vegf-a* and *Vegf-r1* mRNA levels in resorbed implantation sites, indicating that macro

and microvascular changes after the administration of the LPA3 antagonist are accompanied by molecular alterations (Sordelli *et al.* 2017).

Defects in the angiogenic process due to LPA3 antagonism are paralleled by histological anomalies in decidua and placenta (Sordelli *et al.* 2017). Decidual and placental damage is characterized by cellular disorganization, hemorrhage, fibrin deposition and the infiltration of neutrophils, which are typical events associated with embryo resorption. Since the decidua supports embryo growth until the placenta is entirely formed and secretes molecules that participate in neovascularization, failures in vascular and decidual formation seriously compromise the success of early pregnancy. Therefore, we hypothesize that the defects described in uterine and arcuate arteries affect decidualization and placentation.

Our findings together with those obtained from other authors suggest that endogenous LPA regulates decidualization and angiogenesis at the maternal–fetal interface. Moreover, LPA3 blockade produces significant irreversible post-implantation defects (embryo crowding and fetal resorption), indicating that this receptor might be involved in mechanisms associated with the development of the embryo and its supporting tissues. Interestingly, *Lpa3*-knockout mice exhibit similar phenotypic changes (Ye *et al.* 2005). Ye *et al.* (2005) also demonstrated that the expression of cyclooxygenase-2 (COX-2), as well as the production of prostaglandin E2, are reduced in *Lpa3*-deficient uteri. Two years later, Hama *et al.* (2007) showed that LPA–LPA3 signaling participates in implantation timing and embryo spacing independently. Prostaglandins are crucial regulators of uterine contractions which are necessary for embryo spacing. Therefore, altogether these observations support the notion that, besides angiogenesis and decidualization, LPA3 receptor might be also involved in the contractions that allow accurate location of the embryos along the uterine horn.

The relevance of LPA during gestation has been demonstrated in other mammalian species. LPA is locally produced in the endometrium of cows (Woclawek-Potocka *et al.* 2009). Also, LPA regulates the ratio of prostaglandin E2/prostaglandin F2 alpha in the bovine uterus. This mechanism seems to be involved in the luteotropic effect of LPA as it stimulates the secretion of progesterone from the bovine corpus luteum (Woclawek-Potocka *et al.* 2010). In addition, it has been described that the porcine uterus expresses the LYSO-PLD enzyme which regulates the synthesis of LPA locally (Seo *et al.* 2012). This result suggests that LPA participates in the interaction between the mother and the conceptus in pigs.

In conclusion, disruption of endogenous LPA signaling modifies uterine vasculature development with detrimental consequences in decidualization, placenta development and embryo growth.

Studies in animal models – in vitro

As mentioned, several evidences suggest that LPA is a key factor in embryo implantation (Ye *et al.* 2005, 2011, Wei *et al.* 2009, Achache *et al.* 2010). Taking this knowledge into account, during the last years our group described that LPA promotes blastocyst implantation regulating pivotal events as decidualization and vascular remodeling during early gestation. We demonstrated that LPA modulates the level of important lipid mediators, namely prostaglandins, that prepare uterine milieu for embryo invasion during the window of implantation in the rat (Sordelli *et al.* 2012). The incubation of uterine strips obtained from pregnant rats on day 5 of gestation with LPA increases the production of COX-2 derived prostaglandin E2. Prostaglandin E2 and prostacyclin synthetized by COX-2 increase vascular permeability, being pro-angiogenic factors during early gestation in mice (Matsumoto *et al.* 2002, Sookvanichsilp & Pulbutr 2002). In addition, we described that the LPA effect is mediated by LPA3 in the rat uterus. LPA3 is confined to the glandular and luminal epithelium of the endometrium, being the latter expressed in maternal tissues in close contact with the invasive trophoblast (Sordelli *et al.* 2012). The fact that LPA3 is differentially regulated during the peri-implantation period (day 4 vs day 5 and day 6 of gestation) indicates that the expression of this receptor might depend on the presence of the blastocyst and its state of activation. Interestingly, the endometrium from women with recurrent implantation failure showed reduced LPA3 and COX-2 levels (Achache *et al.* 2010). Hence, LPA3 seems to be the subtype of LPA receptor with major participation during early gestation.

Afterward, we studied the role of nitric oxide that has been described as an important regulator of vascular biology in different physiological and pathological conditions. Nitric oxide synthase (NOS) catalyzes nitric oxide production and exists in three isoforms: endothelial NOS, neuronal NOS and inducible NOS (iNOS). In this sense, we observed that LPA increases the production of nitric oxide in the rat uterus as augments iNOS activity through the activation of LPA3 receptor (Beltrame *et al.* 2013). Also, NOS activity is increased at the sites of embryo implantation and depends on the presence of the blastocyst (Sordelli *et al.* 2011). Other authors reported that the regulation of nitric oxide tone and NOS expression is associated to vascularization defects leading to reduced implantation rates in the rat uterus (Ota *et al.* 1999, Purcell *et al.* 1999). Moreover, iNOS and COX-2 signaling pathways interact downstream the LPA effect, which is relevant for the physiology and progress of decidualization and angiogenesis (Beltrame *et al.* 2013). Interestingly, we found that LPA increases the expression of *Igfbp-1* and *Il-10* in the rat uterus (Sordelli *et al.* 2012) reinforcing the hypothesis that LPA is a local regulator of implantation (Fig. 4). This was the first time that LPA was related to the transformation of the

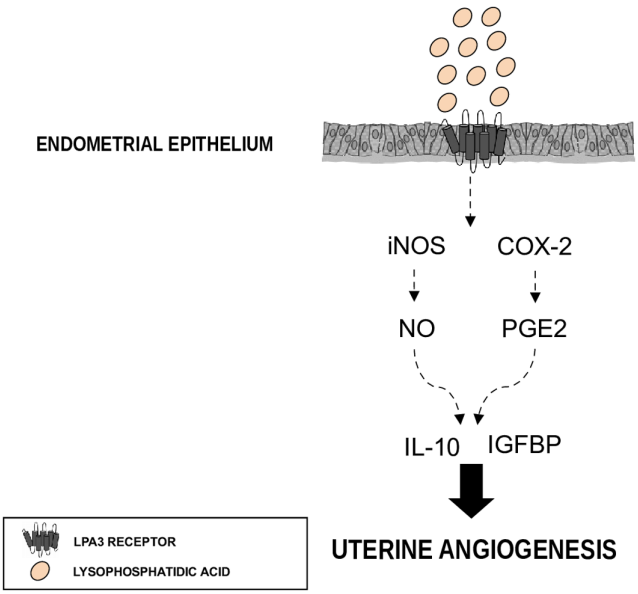

Figure 4 LPA regulates uterine angiogenesis in the rat uterus. LPA induces the activation of COX-2 and prostaglandin E2 synthesis and increases iNOS-derived nitric oxide. These pathways seem to regulate decidualization and angiogenesis through LPA3 receptors at implantation sites. COX-2, cyclooxygenase-2; iNOS, inducible nitric oxide synthase; NO, nitric oxide; PGE2, prostaglandin E2.

stroma into decidual cells. During decidualization, cells undergo highly active apoptosis and differentiation and LPA could promote these processes in other biological systems (Noguchi *et al.* 2009). The relevance of LPA in decidualization has been recently confirmed in mice by Aikawa and colleagues who demonstrated that LYSO-PLD/LPA/LPA3 signaling induces decidualization via the canonical HB-EGF and COX-2 pathways at the embryo epithelial boundary (Aikawa *et al.* 2017). LPA is produced near embryo implantation sites and activates LPA3 localized at the epithelial layer of the uterine endometrium in mice. The regulatory pathway activated by LPA finally induces the stromal layer and leads to the decidual reaction.

Overall, these results reinforce the hypothesis about LPA action at the maternal–fetal interface.

Studies performed in human cell lines

The results detailed before suggest that LPA may play a pivotal role in endometrium and placental angiogenesis in rodents. In fact, spatiotemporal and reciprocal interactions between the trophoblast and endothelium are required for angiogenesis during early gestation. Thus, abnormal LPA signaling at the beginning of implantation may result in aberrant regulation of endothelial adaptations, trophoblast functions and vascular remodeling leading to placental abruption, fetal demise and pregnancy loss.

Several authors reported that LPA is present at high concentration in different fluids during reproductive processes. Along gestation, serum LYSO-PLD activity increases in pregnant women and decreases to non-pregnant levels soon after delivery (Tokumura *et al.* 2002*a*). Therefore, the group of Iwasawa *et al.* (2009) proposed that the placental trophoblast is the main source of LPA during gestation. Additionally, LPA is present in human follicular fluid (Tokumura *et al.* 1999) and at micromolar concentrations in uterine flushings of domestic mammals (Seo *et al.* 2008, Liszewska *et al.* 2009).

As highlighted before, vessel remodeling requires interaction between diverse cell types and mediators from the maternal and fetal components. Much effort is being made to unravel the mechanisms by which invasive first trimester cytotrophoblast changes its phenotype to an endovascular one, and how the invasive and endovascular trophoblast transforms the spiral arteries. However, due to difficulties in studying early human pregnancy, the regulatory pathways that modulate cytotrophoblast behavior and contribute to spiral artery remodeling remains to be determined. The LPA role in endovascular profile acquisition by human first trimester trophoblast and in the interaction between endovascular trophoblast and endothelial cells were therefore examined. LPA via LPA3 stimulates HTR-8/SVneo cell line endovascular differentiation, migration and proliferation (Beltrame *et al.* 2018*a*). COX-2 participates in LPA-increased trophoblast tubulogenesis and LPA augments COX-2 nuclear accumulation without changes in its distribution (Beltrame *et al.* 2018*a*). Other studies reveal COX-2 nuclear localization, suggesting a role for this enzyme in regulating gene expression (Parfenova *et al.* 2001). Interestingly, prostaglandin E2 stimulates HTR-8/SVneo tubulogenesis, while prostaglandin F2alpha does not affect this process. It has been suggested that prostaglandin E2 contributes to maternal decidualization and angiogenesis, whereas prostaglandin F2alpha promotes uterine contractions with adverse consequences for embryo implantation (Hamilton & Kennedy 1994, Stocco & Deis 1998, Callegari *et al.* 2005). Moreover, COX-2 selective inhibition in LPA-induced trophoblast tubulogenesis is rescued by the incubation with prostaglandin E2, highlighting that this prostaglandin is the main COX-2-derived prostanoid involved in LPA effect on trophoblast tube formation (Beltrame *et al.* 2018*a*).

As COX-2, iNOS isoform participates in LPA-increased trophoblast tubulogenesis, is localized in HTR-8/SVneo nucleus and LPA increases its protein level (Beltrame *et al.* 2018*a*). This tied in with evidence from other authors demonstrating a specific role for nitric oxide in vascular adaptations for a healthy pregnancy (Dunk *et al.* 2000, Xu *et al.* 2014). In particular, Zhou *et al.* (1997*a*) show that nitric oxide promotes cytotrophoblast endovascular invasion. Notably, nitric oxide is produced by the trophoblast while invading the maternal spiral arteries in the wall of the uterus (Al-Hijji *et al.* 2003).

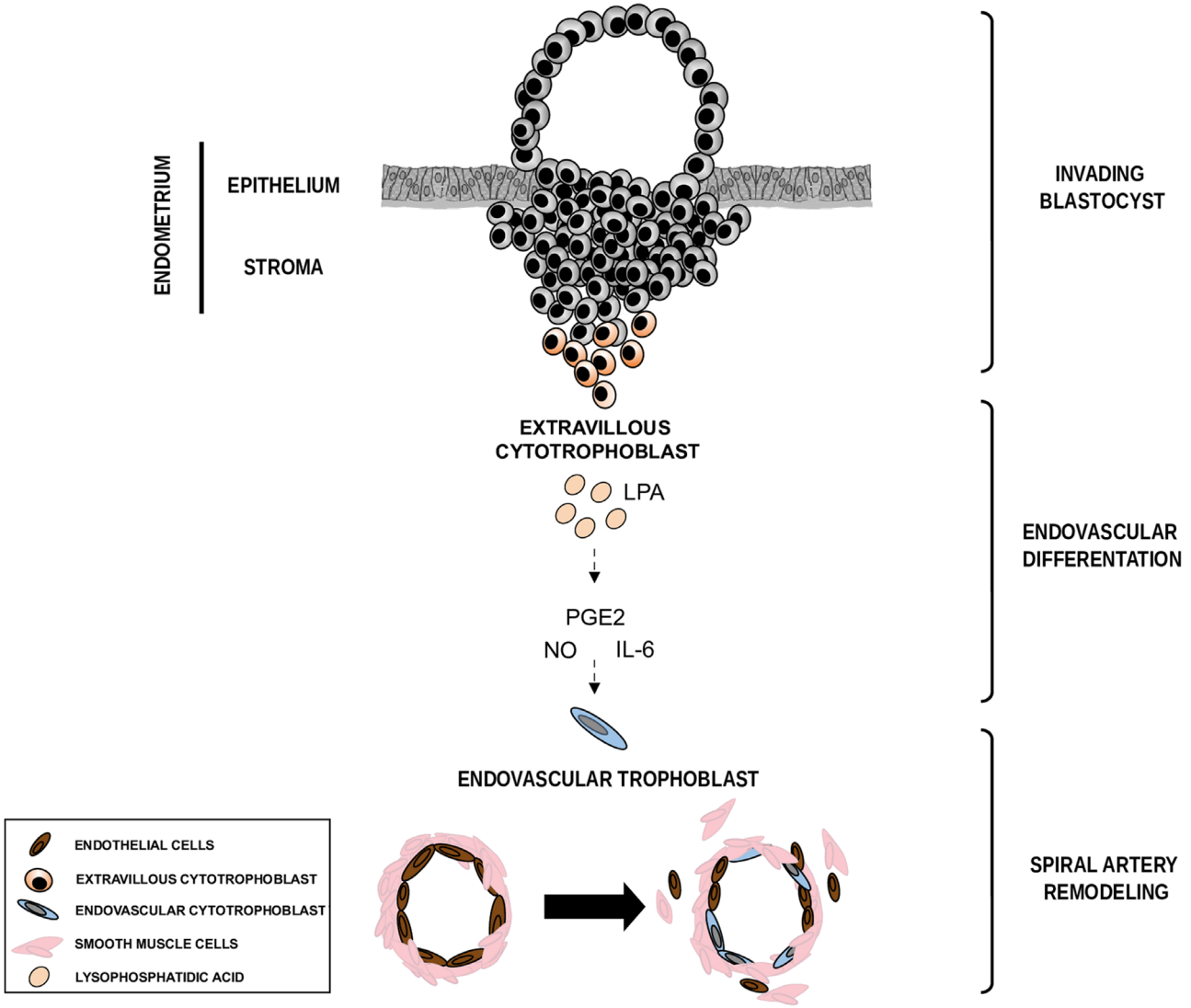

Figure 5 LPA and vascular remodeling. LPA promotes the acquisition of trophoblast endovascular phenotype by a mechanism involving PGE2, nitric oxide and IL-6. In addition, LPA-induced endovascular phenotype stimulates the release of soluble factors that induce endothelial cells migration away from spiral arteries. NO, nitric oxide; PGE2, prostaglandin-E2.

Based on these findings, we hypothesize that LPA produced at the maternal–fetal interface induces COX-2 derived prostaglandin E2 production and nitric oxide by iNOS promoting endovascular trophoblast differentiation. This mechanism might support cytotrophoblast participation in spiral artery remodeling, in order to sustain blood flow due to the increased metabolic demands of the embryo.

Remarkably, LPA seems to modulate another crucial step in spiral artery remodeling. This lipid mediator promotes *in vitro* the crosstalk between human first trimester endovascular trophoblast and endothelial cells and induces the release of trophoblast-soluble factors derived from COX-2 and IL-6 pathways, which finally stimulate migration of endothelial cells without affecting apoptosis (Fig. 5). The change in trophoblast phenotype triggered by LPA and not LPA itself modulates trophoblast-endothelial dialog (Beltrame *et al.* 2019). COX-2 but not iNOS participates in the interaction between trophoblast and endothelium downstream LPA. Nitric oxide seems to modulate cytotrophoblast behavior without obvious effects on the interaction between endovascular trophoblast and the endothelium (Beltrame *et al.* 2018*a*, 2019). On the other hand, COX-2 is an active factor in vascular processes in different biological systems, and it is well known that COX-2-derived soluble factors induce endothelial cells migration (Daniel *et al.* 1999, Zhao *et al.* 2012). The relevance of LPA-COX-2 pathway is highlighted in LPA3$^{-/-}$ mice where COX-2 expression in

the uterus is decreased and administration of exogenous prostaglandin E2 and prostacyclin restores most of the normal phenotype (Ye *et al.* 2005). Altogether, these data point out the importance of prostaglandin E2 in the vascular remodeling processes during early gestation.

Within the wide range of mediators involved in vascular adaptations, different authors demonstrate the importance of cytokines in these processes. HTR-8/SVneo trophoblast cells as well as the syncytiotrophoblast and cytotrophoblast secrete IL-6 and express its receptor (Kameda *et al.* 1990, Jovanovic & Vicovac 2009, Champion *et al.* 2012). LPA increases *Il-6* mRNA which participates in HTR-8/SVneo capillary-like tube formation (Beltrame *et al.* 2019). Moreover, IL-6 mediates LPA-induced trophoblast-endothelial crosstalk and COX-2 mediates the increment in its expression. Interestingly, LPA increases *Il-6* mRNA, but does not modify other vascular cytokines (*Il-8, Vegf-c, Vegf-a*) in HTR-8/SVneo. Also, the group of Weiss *et al.* (2016) reported that IL-6 might be a chemoattractant factor guiding trophoblast cells toward endothelial cells. These observations together suggest a preponderant role for IL-6 in vascular remodeling at the maternal–fetal interface (Fig. 5).

Sex steroids orchestrate most of the reproductive events in mammals. In particular, progesterone and estradiol are in extremely high concentrations in maternal circulation and have an important role to accomplish proper maternal vascular adaptations during early pregnancy (Clark *et al.* 2017). Although it has been reported that progesterone and estradiol modulate uterine arteries remodeling and placental angiogenesis (Chen *et al.* 2012, Maliqueo *et al.* 2016), their specific role in vascular remodeling at the maternal–fetal interface remains controversial. In this sense, we observed that progesterone and estradiol regulate HTR-8/SVneo trophoblast capillary-like structures formation in a concentration-dependent manner and only a specific combination of progesterone + estradiol (10^{-7} M + 10^{-5} M) stimulates tube formation (Beltrame *et al.* 2018*b*). This sex steroid-triggered tubulogenesis could be modulated directly or indirectly by mechanisms associated with LPA/LPA3 pathway (Beltrame *et al.* 2018*b*). Particularly, progesterone + estradiol effect is partially mediated by an increase in LPA production that activates at least LPA3 receptor expressed in HTR-8/SVneo cells. Studies in mice reveal that the balance of progesterone and estrogen signaling is disrupted in *Lpa3* knock out uterus and leads to delayed embryo implantation (Diao *et al.* 2015). Therefore, there might be a fine regulation of steroid hormones tone during endovascular differentiation at the maternal–fetal interface. This reinforces the notion that the balance of female sex hormones is critical for pregnancy establishment and modifications in the circulating levels could lead to poor pregnancy outcomes (Lim & Wang 2010, Ramathal *et al.* 2010, Cha *et al.* 2012, Maliqueo *et al.* 2016). Interestingly, progesterone at highest concentrations alone or in combination with estradiol suppresses basal tubular formation (Beltrame *et al.* 2018*b*). In this regard, it has been reported that preeclampsia, a pathology associated with vascular insufficiencies, is correlated with higher progesterone levels (Walsh & Coulter 1989). Based on these data, LPA/LPA3 appears as a key system that could be triggered downstream by steroid hormones.

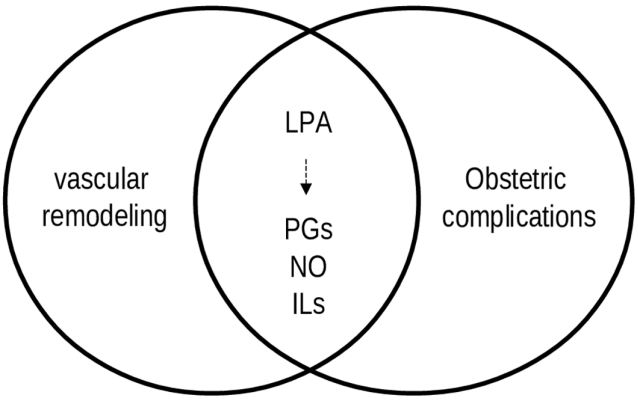

Figure 6 Importance of LPA signaling during early gestation. LPA and the molecules activated downstream modulate vascular events at the implantation site which seem to be relevant for a successful gestation. ILs, interleukins; NO, nitric oxide; PGs, prostaglandins.

Altogether, these data strongly suggest that inadequate endovascular trophoblast differentiation or abnormal interaction with spiral arteries endothelium due to aberrant LPA signaling may contribute to pathologies related to vascular deficiencies.

Conclusions

Observations in several experimental models show that LPA plays a crucial role in regulating vascular biology and pathology. Vascular remodeling at the maternal–fetal interface is essential to achieve normal gestation. This review presents evidence that supports the participation of LPA in the vascular adaptations that occur during early gestation, ensuring the adequate blood flow to sustain normal growth of the embryo. Elucidating the interaction between LPA and the molecules regulated downstream would provide new insights into the significance of LPA signaling in the vascular events that lead to a successful pregnancy. Therefore, we propose a new role for LPA as a pivotal lipid that could be related to severe obstetric complications associated with vascular diseases (Fig. 6).

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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Author contribution statement

J S B and M L R conceived the review. J S B, M S S and M L R wrote the manuscript. M S S and V A C corrected the text. J S B and M S S performed experiments. J S B and M L R analyzed the data. M S S and M L R contributed with their research grants.

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