Review

Lysophosphatidic acid (LPA) signaling in vertebrate reproduction

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Lysophosphatidic acid (LPA) is a cell membrane phospholipid metabolite that can act as an extracellular signal. Its effects are mediated through at least five G protein-coupled receptors, LPA_{1-5}, and probably others as well. Studies in multiple species including LPAR-deficient mice and humans have identified or implicated important roles for receptor-mediated LPA signaling in multiple aspects of vertebrate reproduction. These include ovarian function, spermatogenesis, fertilization, early embryo development, embryo implantation, embryo spacing, decidualization, pregnancy maintenance and parturition. LPA signaling can also have pathological consequences, influencing aspects of endometriosis and ovarian cancer. Here we review recent progress in LPA signaling research relevant to female and male reproduction.

Overview

Lysophosphatidic acid (LPA) is an extracellular lipid signaling molecule that has a broad range of cellular influences on processes including survival, differentiation, proliferation, morphological changes, migration and others. In vertebrates, LPA signaling has been implicated in numerous physiological and pathological processes affecting most, if not all, organ systems [1–4]. A key area that LPA influences is reproduction, both male and female. This review briefly introduces LPA signaling and reviews its effects on reproduction. Additional details on LPA signaling can be found in recent reviews that also note a related lysophospholipid, sphingosine 1-phosphate (S1P) that has other influences on reproduction, but is not discussed here [5–12].

LPA production and metabolism

LPA is present in many biological fluids such as serum (up to micromolar concentrations), plasma, saliva, blister fluids, tears, chicken egg white, follicular fluid, seminal plasma and ascites fluid. LPA can be produced by many different cell types that include postmitotic neurons, adipocytes, mast cells, other lymphoid cells, endometrial cells, erythrocytes and cancer cells, as well as activated platelets [2,13,14]. There are different species of LPA because of various acyl chain lengths and degrees of saturation and positions on the glycerophosphate backbone. A form commonly used in the laboratory is 18:1 or oleoyl-LPA [5]. Although precise and accurate mechanisms accounting for LPA metabolism within most cell types are still unclear, two general pathways of LPA production have been demonstrated. One pathway involves the generation of precursor lysophospholipids (LPLs) from various membrane phospholipids (PLs) by phospholipase A 1 (PLA1) and PLA2, followed by the action of autotaxin (ATX), which is a lysophospholipase D. A second pathway involves the formation of phosphatidic acids (PAs) from PL cleavage by phospholipase D (PLD) or from diacylglycerol by diacylglycerol kinase (DGK) and deacylation of PA by PLA-type enzymes. LPA can be metabolized to monoacylglycerol (MAG) by lipid phosphate phosphatases 1–3 (LPP1–3) and lysophospholipases. This process can be reversed by MAG kinase, whereas the actions of a variety of acyl transferases can also remove LPA from signaling or structural pools [12,14] (Figure 1). It has been reported that lysophospholipids are the main precursors for LPA production in serum, plasma and adipocytes, whereas in platelets and some cancer cells, LPA is mainly derived from PAs [14]. These differing routes of LPA biosynthesis probably reflect multiple levels of regulation – or dysregulation in cancers – available to cells having different lineages or functions. These distinctions are reflected not only in the penultimate precursor molecule, but also in the enzymes used to produce LPA. For example, ATX, which can also be produced locally, plays a key role in determining circulating LPA levels in adult animals [15,16] by acting on lysophosphatidyl choline (LPC) to produce LPA, whereas cytoplasmic PLA2 (cPLA2) acts on PAs. These respective enzymes are in turn regulated in different ways such as transcriptional or post-translational processes that are themselves influenced by both cell autonomous and non-cell autonomous signals. In addition to their biological significance, these different signaling pathways have clear therapeutic repercussions because compounds targeting ATX [3,17] would differ from those targeting other LPA biosynthetic pathways. Extracellular LPA is normally bound to proteins such as albumin, fatty acid binding protein, other lipoproteins and gelsolin, all of which increase the stability of LPA and aid in its transport [18–21], representing yet another level of control.

Receptor-mediated LPA signaling

The study of receptor-mediated LPA signaling mechanisms began with the cloning of LPA receptors and the establishment of receptor-mediated functions. So far, five

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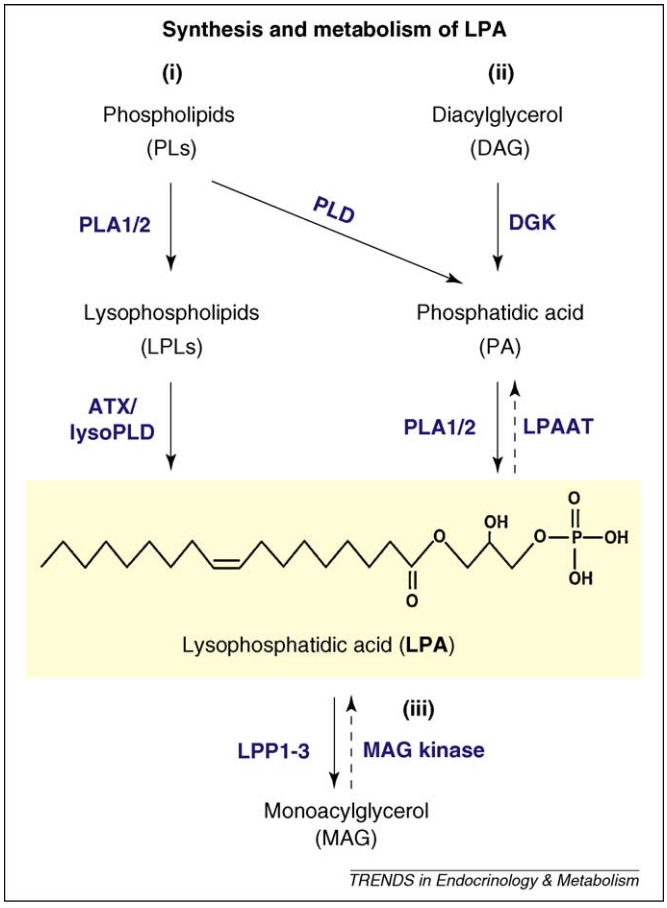

Figure 1. Synthesis and metabolism of LPA. LPA can be generated via two general pathways. **(i)** One involves the generation of LPLs from PLs by PLA1/2 and transformation of LPLs to LPA by ATX/lysoPLD. **(ii)** The other involves the formation of PAs from PLs by PLD or from DAG by DGK and transformation of PA to LPA by PLA1/2. **(iii)** LPA can be metabolized to MAG by LPP1–3 and lysophospholipases, a process that can be reversed by MAG kinase. LPA can also be removed by a variety of LPAATs. ATX/lysoPLD, autotaxin/lysophospholipase D; DGK, diacylglycerol kinase; LPA, lysophosphatidic acid (shown for the 18:1 chemical species of LPA); LPAAT, LPA acyltransferase; LPPs, lipid phosphate phosphatases; LPLs, lysophospholipids; MAG, monoacylglycerol; PA, phosphatidic acid; PLD, phospholipase D; PLA1/2, phospholipase A1 and A2; PLs, phospholipids.

bona fide LPA receptors, $LPA_{1–5}$, have been identified [1,5,9,12,22]. Their human genes are designated *LPARx* with x=1–5 (human genome organization, HUGO), whereas mouse names are *Lparx*, with x=1–5 (Genome Informatix, MGI) [5]. P2Y5 has been reported as a new LPA receptor, LPA_6 [23,24]. Two additional possible LPARs, GPR87 and P2Y10, have also been reported [25,26], but await validation. LPARs are all cell-surface seven transmembrane spanning G protein-coupled receptors (GPCRs). They can differentially couple to $G_{\alpha12/13}$, $G_{\alpha q}$, $G_{\alpha i/o}$ and, in one instance, $G_{\alpha s}$ to activate downstream signaling pathways leading to gene regulation and LPA-induced cellular functions (Table 1) [1,12,24,27]. In addition, LPARs have preferences for certain chemical ligand structures. For example, LPA_3 has a relatively high affinity for 2-acyl-LPA-containing unsaturated fatty acids (with the ester-linked fatty acid in the 2 position), whereas other receptors such as LPA_1 and LPA_2 do not discriminate 1-acyl- and 2-acyl-LPA [28–30]. LPARs also have overlapping and differential gene expression patterns [2]. These LPAR characteristics contribute to receptor activities and functions [10,31]. A critical strategic approach in determining the biological roles for LPA signaling has been via the creation and study of mice null for one or more LPARs [5–12]. In this way, roles for receptor-mediated signaling in reproduction were identified: LPA_3 affects embryo spacing and embryo implantation [32,33] and three receptors, LPA_1, LPA_2 and LPA_3, combine to affect spermatogenesis [34] (Table 2).

Table 1. LPA receptors and their coupling to G proteins

Receptor (synonyms)	G proteins	References
LPA_1 (EDG-2, VZG-1, LP_{A1})	$G_{\alpha12/13}$, $G_{\alpha i/o}$, $G_{\alpha q}$	[70]
LPA_2 (EDG-4, LP_{A2})	$G_{\alpha12/13}$, $G_{\alpha i/o}$, $G_{\alpha q}$	[103]
LPA_3 (EDG-7, LP_{A3})	$G_{\alpha i/o}$, $G_{\alpha q}$	[28,93]
LPA_4 (p2y$_9$, GPR23)	$G_{\alpha12/13}$, $G_{\alpha i/o}$, $G_{\alpha q}$, $G_{\alpha s}$	[36]
LPA_5 (GPR92)	$G_{\alpha12/13}$, $G_{\alpha q}$	[104,105]
LPA_6 (P2Y5)*	$G_{\alpha12/13}$	[23,24]

*Putative 6th LPA receptor.

LPA signaling in ovary and breast

LPA signaling has been extensively studied in ovarian cells and the ovary. In one study, mRNA expression of three receptors, *LPAR1*, *LPAR2* and *LPAR3*, was detected in granulosa lutein cells from women undergoing *in vitro* fertilization (IVF) [35]. LPA_4 is highly expressed in human and mouse ovary [36,37]. LPA itself is present at significant levels in follicular fluid of human preovulatory follicles [38]. Serum ATX activity in patients subject to ovarian stimulation was higher than in women with natural cycles [35] and LPA was induced in incubated human follicular fluid by ATX [38], suggesting that ovarian stimulation in women might increase LPA levels. High amounts of LPA are also known to be present in chicken eggs [39,40].

Table 2. LPA receptor-mediated signaling in reproduction

Site	Effects	Receptors identified[a]	References
Testis	Germ cell survival	LPA_1, LPA_2, LPA_3	[34]
Oocyte	Maturation	LPA_1?, LPA_2?	[106]
Xenopus oocyte	Early embryo shape maintenance	$XLPA_{1–2}$	[101]
Ovary	IL-6, IL-8, and growth factor induction	LPA_1, LPA_2	[45]
	Cancer cell growth, survival, migration and invasion	LPA_2, LPA_3	[107–110]
Blastocyst	Differentiation	LPA_1?, LPA_2?	[99]
Uterus	Uterine receptivity	LPA_3	[34]
	Embryo spacing	LPA_3	[32,33]
	Uterine contraction	LPA_1?, LPA_2?, LPA_3	[33,82,87]
	Endometrial angiogenesis	LPA_1?	[72]
Placenta	Placental angiogenesis	LPA_1?	[72]
	Pregnancy hypertension	LPA_2?, LPA_3?	[73]

[a]?, proposed but not fully confirmed receptors.

LPA signaling plays multiple roles in ovarian function [2]. LPA induces Ca^{2+}-activated Cl^- currents in naked *Xenopus laevis* oocytes via $G_{\alpha i}$. LPAR, $G_{\alpha i}$ and extracellular signal-regulated kinase (ERK)/p38 signaling pathways were implicated in mouse oocyte maturation *in vitro*. LPA induced expression of angiogenic cytokines interleukin-6 (IL-6) and IL-8 in granulosa lutein cells in women undergoing IVF. LPA_1, $G_{\alpha i}$, mitogen-activated protein kinase (MAPK)–p38, phosphoinositol 3-kinase (PI3K)–Akt and NF-κB signaling pathways were shown to be involved in LPA-induced IL-8 expression and LPA_2, $G_{\alpha i}$, MAPK–p38 and NF-κB signaling pathways in LPA-induced IL-6 expression. Excessive induction of these angiogenic cytokines by LPA from multiple corpora lutea of stimulated ovaries might pathophysiologically contribute to ovarian hyperstimulation syndrome, a complication of some fertility medications, with symptoms of abdominal bloating and nausea among others [35]. LPA also induced Chinese hamster ovary (CHO) cell growth through $G_{\alpha i}$ [41], suggesting its physiological and pathological roles in CHO cells. LPA signaling also affects bovine ovarian theca cells and luteal cells, inducing ERK phosphorylation through the LPA_1–$G_{\alpha 12/13}$ signaling pathway, redistribution of protein kinase Cδ (PKCδ) from the cytosol to the perinuclear area, morphological changes and steroid synthesis [42–44].

Data available for *Lpar* null mice indicate that LPA signaling might not be critical for ovulation in mice. LPA_1, LPA_2 and LPA_4 are expressed in mouse ovary [37,45], whereas expression data for LPA_3 in mouse ovary are inconsistent [32,45]. However, deletion of any of these receptors or of $LPA_{1–3}$ does not cause any obvious defect in ovulation [32,37,46]. LPA signaling in human ovulation has yet to be determined.

LPA signaling might play a significant role in ovarian pathology especially relevant to ovarian cancer through both LPA levels and LPAR upregulation [2,20]. LPA levels are elevated in the plasma and ascites of ovarian cancer patients. LPA_2 and LPA_3, but not LPA_1, were upregulated in ovarian cancer tissues. LPA promoted ovarian cancer cell proliferation and migration and was suggested as a potential biomarker of ovarian cancers [5,47], although this relationship remains controversial [48]. A complex interplay of many other molecular factors modulate LPA signaling in ovarian cancer, examples of which include glycodelin, thyroid receptor-interacting protein 6 (TRIP6), telomerase, granulin epithelin precursor, Fas (which internalizes from the cell membrane to the cytosol), IL-6, IL-8, cyclooxygenase-2 (COX-2), growth-regulated oncogene α (GROα) and urokinase plasminogen activator (uPA) [49]. Several LPA-induced downstream signaling pathways have been identified in ovarian cancer cells. For example, uPA upregulation by LPA was mainly mediated through the $G_{\alpha i}$–Ras–PKCα–CARMA3–NF-κB signaling pathway [50,51]. LPA-induced IL-6 expression via the $G_{\alpha i}$–PI3K–Akt–NF-κB pathway. Both $G_{\alpha i}$–Ras–MAPK kinase kinase 1 (MEKK1) and $G_{\alpha 12/13}$–RhoA–Rho-associated kinase (ROCK) signaling pathways contributed to LPA-stimulated ovarian cancer cell migration.

In addition, LPA signaling induces breast cancer cell proliferation and chemotaxis. LPA_2 is upregulated in mammary gland carcinoma tissue and it has been suggested that LPA signaling might also play a role in breast cancer progression [2]. The involvement of LPA signaling in female reproductive organ function and dysfunction indicates common mechanistic threads that should provide insights into normal function, disease and future therapeutic strategies. LPA signaling in both ovarian and breast cancers might facilitate the development of novel treatments, particularly in cases in which these cancers are co-morbid.

LPA signaling in the oviduct

It was suggested that the receptor-mediated $G_{\alpha i}$–Ca^{2+} signaling pathway is involved in LPA-induced mouse ovum transport [52]. However, in mice, deletion of LPA_1, LPA_2 or LPA_3, the three $G_{\alpha i}$-coupled LPARs expressed in the oviduct, did not seem to affect embryo transport to the uterus when the blastocysts were examined on embryonic day 3.5 (E3.5) mouse uterus (E0 is defined as the day of mating) [32,46]. This suggests that (i) receptor-mediated LPA signaling is involved in embryo transport in the oviduct and that the effect was not detectable on E3.5 but at an earlier time point not determined by the authors; (ii) LPA signaling is not critical for embryo transport in the oviduct under physiological conditions; and/or (iii) if LPA signaling is indeed important for ovum transport, LPA_4 and/or other as yet unidentified $G_{\alpha i}$-coupled LPAR(s) in mouse oviduct can compensate for null mutations of normally expressed LPA_1, LPA_2 or LPA_3, a possibility that remains to be examined.

LPA signaling in uterus and pregnancy maintenance

LPA can be locally produced and released in bovine endometrium during estrous and early pregnancy [53]. It is also detected in ovine uterus in early pregnancy stages [54]. LPA containing varied fatty acid acyl chains was also detected in porcine uterine lumen on day 12 of both the estrous cycle and pregnancy [55]. LPA production in mouse uterus has not been specifically determined; however, LPA-producing enzymes, PLD1 and PLD2, are expressed in young adult mouse uterus (unknown stage of estrous cycle) [56].

Gene expression patterns of LPA_3 in porcine uterus suggest that this isoform plays a role during early pregnancy as the predominant LPAR subtype of four examined receptors ($LPA_{1–4}$) and is regulated by pregnancy and estrous cycle [55]. *Lpar3* gene expression peaked on day 12 of pregnancy, when the embryo undergoes a dramatic elongation process prior to implantation [57], localized in the luminal and glandular epithelium and induced by estrogen in the endometrium [55]. By comparison, studies in mouse uterus revealed that LPA_3 is mainly localized in the luminal epithelium, upregulated by progesterone and, unlike in the porcine uterus, downregulated by estrogens [32,58]. In addition, embryo presence induces porcine uterine LPA_3 expression [59], in contrast to the situation in mice, in which *Lpar3* gene expression patterns in uteri from early pregnant and pseudopregnant (mating with a vasectomized male) females were similar [32,58].

A dramatic effect of LPA signaling in uterus was identified in *Lpar3* deficient mice, in which loss of LPA_3

influenced embryo implantation and spacing [32,33]. Embryo implantation involves a competent embryo, a receptive uterus and their reciprocal interaction [60]. Embryo spacing refers to the regular and approximately equidistant implantation of embryos along the uterus of polytocous (multiple offspring in a single birth) species. Deletion of *Lpar3* in mice led to uneven embryo spacing and delayed embryo implantation [32]. This was associated with delayed embryonic development, prolonged pregnancy duration and ~50% embryonic lethality. Decidualization was not adversely affected in the *Lpar3* deficient uterus. Embryo crowding and delayed implantation seem to be two segregated events because restoration of on-time implantation by exogenous prostaglandins PGE_2 and PGI_2 failed to correct embryo crowding in *Lpar3* deficient females and a single embryo transferred to a pseudopregnant *Lpar3* deficient uterus did not exhibit delayed implantation [32,33].

Prostaglandins (PGs) were identified to be at least partially responsible for the phenotypes of *Lpar3* deficient females. Expression of COX-2, important in PG synthesis, as well as levels of the PG forms PGE_2 and PGI_2, was suppressed in pre-implantation E3.5 *Lpar3* deficient uteri. Exogenous PGE_2 and PGI_2 rescued delayed implantation in *Lpar3* deficient females, highlighting the importance of PGs in embryo implantation [61–63]. However, PGE_2 and PGI_2 failed to correct embryo spacing, suggesting that other PGs that control uterine contraction or possibly non-PG mechanisms are responsible for embryo spacing [33].

LPA signaling, COX-2 and PGs have also been examined in porcine and bovine uterus. LPA can induce COX-2 expression in porcine uterine endometrium, suggesting that LPA produced in the uterine endometrium plays a role in porcine uterine endometrial function [55]. In bovine uterus, LPA is locally produced and released from the endometrium. LPA can induce progesterone and PGE_2 secretion and increase the $PGE_2/PGF_{2\alpha}$ ratio. These effects can be inhibited by a dual LPA_1 and LPA_3 antagonist (Ki16425) [53,64]. A further study indicated that LPA induces PGE_2 production in endometrial stromal cells but not in epithelial cells via upregulation of PGE_2 synthesis enzymes, COX-2 and PGE_2 synthase. It was suggested that LPA_1 is the main receptor that mediates this process [65]. Thus, LPA might play autocrine and/or paracrine roles in the maintenance of early bovine pregnancy [53,64,65].

In addition to data from non-human species, a recent report indicates that LPA_3 may also have a function in human reproduction. The study demonstrated that LPA_3 levels decrease in middle and later secretory endometrium (implantation occurs in the secretory phase) of patients with endometriosis [66], a medical condition in which endometrial tissue grows outside of the uterus associated with a higher rate of subfertility, affecting 5–10% of women of reproductive age in the USA [67]. LPA_3 and other putative uterine receptivity biomarkers (glycodelin A, osteopontin, and HOXA10) examined in the study are all regulated by progesterone. Reduced expression of these genes might explain the progesterone resistance associated with endometriosis [66]. However, increased levels of COX-2-derived PGE_2 are detected in the endometrium, especially in ectopic endometrial tissue from women with endometriosis [67]. In addition to evidence that LPA signaling stimulates COX-2 expression in endometrial and endothelial cells [32,65,68], it is possible that LPA signaling might play a role in endometriosis and associated subfertility.

In human decidual cells, LPA can increase embryo outgrowth and induce actin stress fiber formation [69], a result consistent with the earliest known effects of receptor-mediated LPA signaling [70,71]. RhoA signaling mediates these LPA effects in decidual cells, which might contribute to embryo development and differentiation after attachment [69]. LPA can induce IL-8-enhanced migration, permeability, capillary tube formation and proliferation of human endometrial microvascular endothelial cells. Of the three LPA receptors examined, LPA_1, but not LPA_2 or LPA_3, is highly expressed in human endometrial stromal cells. LPA_1 mediates LPA-induced IL-8 expression via an NF-κB-dependent pathway. A role of LPA in angiogenesis of endometrium and placenta through induction of IL-8 in endometrial stromal cells during pregnancy has thus been suggested [72].

It has also been suggested that LPA signaling has pathological roles during pregnancy. Although LPA_2 and LPA_3 are not detectable in human endometrial stromal cells, high levels of *LPAR2* and *LPAR3* gene expression were detected in placentas of patients with gestational hypertension and pre-eclampsia (pregnancy-induced hypertension in association with significant amounts of protein in the urine) [73]. In addition, LPA can potentiate endothelin-1-induced vasoconstriction [74]. These data suggest that LPA signaling might contribute to gestational hypertension and pre-eclampsia. These hypertensive disorders can worsen with pregnancy progression, and LPA levels are known to increase during pregnancy [75]. It is unknown whether even higher levels of LPA are present in pregnant patients with complicated hypertensive disorders. LPA levels increase during blood clotting, which can promote wound healing processes. However, aberrant accumulation of LPA in blood can lead to adverse effects such as endothelial barrier dysfunction [76] and platelet aggregation [77], which can contribute to thrombosis during pregnancy. LPA signaling might also be associated with preterm labor and pre-eclampsia [78] and influence the growth of uterine tumors such as leiomyomas and fibroids (tumors of uterine smooth muscle) [79]. LPA signaling might also play a role in endometrial cancer. A recent study indicated that LPA can promote endometrial cancer invasion. LPA_2 and matrix metalloproteinase-7 are implicated in this process [80]. The functions of LPA signaling in these processes await further exploration.

It has also been suggested that LPA signaling plays a role in maintenance of human pregnancy. Serum ATX activity, a key enzyme for LPA production, and LPA levels increase during pregnancy [75]. High lysophospholipase activity was detected in human placental tissues and was highest in the amnion [81]. Although the amnion has been implicated in the initiation of labor, presumably through the release of arachidonic acid, high lysophospholipase activity in the amnion suggests that lysophospholipid

substrates, including LPA, might also be involved in the regulation of labor. These issues deserve further examination.

LPA signaling could potentially regulate uterine contractility and load bearing during pregnancy and labor. An early study indicated that LPA had similar effects to those of $PGF_{2\alpha}$ on rat smooth muscle contraction and intrauterine pressure [82]. Although the effect of LPA signaling in parturition *per se* has not been established *in vivo*, deletion of FP, the GPCR for $PGF_{2\alpha}$, led to parturition failure [83]. The potential roles of LPA signaling in uterine smooth muscle can be further demonstrated by the following studies. LPA stimulated myosin light chain phosphorylation through RhoA signaling in myometrial tissue from pregnant women [84], specifically through $G_{\alpha 12/13}$–Rho kinase signaling [85]. In addition, the $G_{\alpha i/o}$ signaling pathway involving $[Ca^{2+}]_i$ regulation controls LPA-induced cell proliferation of human myometrial smooth muscle cells [86]. A follow-up study identified the critical role of Ca^{2+}/calmodulin-dependent protein kinase in this effect and detected LPA_1, LPA_2 and LPA_3 in human myometrial smooth muscle cells [87]. The involvement of LPA_3 in uterine smooth muscle contraction was demonstrated in mice using the LPA_3-specific agonist T13 and *Lpar3* deficient uterus [33]. These data indicate that LPA signaling can regulate uterine contractility, which might underlie its roles not only in embryo spacing in mice, but also in load bearing during pregnancy and eventually labor in humans.

LPA might also have relevance to infection-related preterm labor. Significantly higher levels of lysophosphatidylcholine (LPC), the substrate for LPA production by ATX, was detected in human uterine endometrial cells on exposure to extracts from common anaerobes involved in intrauterine infection, accompanied by elevated arachidonic acid, a key precursor for PG synthesis in regulating labor. LPA signaling is involved in lipid metabolism [88,89]. LPA and LPC are important components in the LPA metabolism pathway [19], so it is reasonable to expect that LPA signaling might also be involved in infection-related preterm labor.

In the bovine reproductive tract, LPA stimulates progesterone and PGE_2 secretion, indicating a possible supporting role for LPA in the corpus luteum. Hence, it was suggested that LPA is involved in the maintenance of early bovine pregnancy [53]. LPA signaling has also been implicated in the establishment and maintenance of porcine pregnancy [90].

LPA signaling in testis and prostate

Three lines of evidence suggest that LPA signaling has potential roles in male reproduction [2]. First, LPA biosynthetic enzymes, including PLA1, PLA2 and ATX, are present in testis. Second, *Lpar1–3* are highly expressed in mouse testis [34,91] and *LPAR1–4* are detected in human testis [5,36]. Third, transgenic mice overexpressing LPP1, which degrades LPA, show impaired spermatogenesis [92]. *In situ* hybridization demonstrated *Lpar1–3* expression in male germ cells. Deletion of these receptors in mice led to a testosterone-independent reduction in mating activity and sperm count, with an increased prevalence of azoospermia in aging animals. Increased germ cell apoptosis was responsible for the consequent reduction in germ cell proliferation and diminished sperm count, implicating LPA signaling as a germ cell survival factor in spermatogenesis [34].

LPA signaling might have functions in the pathology of the prostate. *LPAR1–3* were detected in prostate, with significantly higher expression levels in human prostate malignancies compared with benign tissues [93,94]. LPA signaling might have multiple roles in prostate cancer by facilitating early prostate cancer development, inducing prostate cancer cell proliferation, survival, morphological changes, migration and invasion. LPA_1 seems to be the key receptor in mediating LPA-induced prostate cancer cell proliferation and migration because expression of this receptor is correlated with these LPA-induced cellular events in cultured prostate cancer cells [94]. NF-κB is constitutively activated in prostate cancer but not in benign prostate tissues. LPA can activate the Akt–NF-κB pathway in cultured prostate cancer cells. It has been suggested that the LPAR–Akt–NF-κB signaling axis mediates LPA-induced prostate cell survival [95]. LPA might also play roles in prostate cells via secondary factors such as cytokines, CYR61 and RhoA [2].

LPA signaling in fertilization

The spermatic acrosome reaction is a main step in fertilization, involving the binding and fusion of sperm and egg. LPA can activate spermatic PKCα, implicated in the acrosome reaction, and could promote actin polymerization, a process necessary for spermatozoa penetration into the egg cytoplasm [2]. LPA and Rho GTPases are involved in the latter process, consistent with receptor-mediated phenomena [96,97]. However, the LPAR(s) mediating this process is (are) unknown. LPA signaling does not seem to alter sperm motility in either mice deficient for three LPARs ($LPA_{1–3}$) or in studies of bovine motility. No obvious deficiencies in fertilization were observed in *Lpar3* deficient mice [32]. To determine the potential function of LPA signaling in fertilization, especially the acrosome reaction, a systematic study needs to be carried out. Currently, expression levels of different LPARs in sperm remain unknown, although several are detected in the testis [5,34,36].

LPA signaling in preimplantation embryo development

LPA signaling has been implicated in post-implantation embryo developmental processes such as vascular formation, vascular maturation and maintenance, heart development and brain formation [2]. In a study preceding LPAR identification, cultured embryos from the pronuclear stage in the presence of LPA significantly increased the success rate of the development of two- and four-cell embryos to blastocysts via a $G_{\alpha i}$ mechanism [98]. A more recent study reported *Lpar1* mRNA expression in differentiating mouse blastocysts, expression of *Lpar2* in late-stage blastocysts and no expression of *Lpar3* at any of the stages examined [99]. One potential mechanism could be that LPA elevates $[Ca^{2+}]_i$ levels to accelerate murine blastocyst differentiation. LPA induces the transient accumulation of heparin-binding EGF-like growth factor (HB-EGF) on the

Box 1. Outstanding questions

LPA ligand-related issues

- What chemical forms of LPA exist within the reproductive system?
- What are local LPA concentrations?
- Do LPA concentrations vary with reproductive stage or age?
- Are there LPA concentration gradients?
- Which cells produce LPA?
- What is most critical for LPA signaling with respect to the ligand: synthesis or degradation?
- Are there physiological or disease conditions that significantly alter LPA levels or gradients (e.g. obesity)?

LPA biosynthetic and degradative enzyme-related issues

- Which enzymes are most important in the reproductive system?
- How are their expression and activity controlled?
- Do LPA precursors and/or products create positive and negative feedback loops affecting activity?
- Are there unidentified enzymes that contribute to LPA enzymatic pathways?

LPA receptor-related issues

- Have all LPARs involved in reproduction been identified?
- What are the rate-limiting mechanisms in controlling LPAR activity?
- Which downstream signaling pathways are dominant for LPA-mediated reproductive effects?
- What is the relationship between LPA signaling and other lysophospholipid pathways?
- What is the relationship between LPA signaling and other signaling pathways involved in reproduction?

LPA-based therapeutic issues

- Which molecular targets are tractable for intervention?
- What physiological or disease indications could be therapeutically and safely accessed?

embryo surface, and interference with HB-EGF signaling through EGF receptors ErbB1 or ErbB4 could attenuate LPA-stimulated blastocyst differentiation [99]. However, there is no obvious defect in blastocyst development in *Lpar3* deficient or double *Lpar1/Lpar2* deficient mice [32]. Delayed post-implantation embryo development in *Lpar3* deficient uteri reflects delayed embryo implantation that is maternal in origin [32]. Nevertheless, LPA signaling can influence post-implantation embryo development. Deletion of ATX, a key enzyme in LPA production, leads to embryonic lethality via defects in blood vessel formation and brain development [15,16], suggesting roles for LPA signaling in embryo development. LPAR expression patterns in the embryo suggest potential functions of LPA signaling in organogenesis [31]. We have observed embryonic hematoma and embryonic lethality with incomplete but increased penetrance in *Lpar1*, *Lpar1/Lpar2* double knockout and *Lpar1/Lpar2/Lpar3* triple knockout mice [34,46,100]. However, these phenotypes do not mimic *Atx/Enpp2/NPP2* knockout mice. Considering that more LPARs are being identified, any single or a few LPARs might not be able to reproduce the ATX-null phenotype.

LPA signaling might play a role in reproduction in other species as well. $XLPA_1$ and $XLPA_2$ mediated LPA signaling is important for early embryo development in *Xenopus* via the maintenance of overall rigidity and shape of the embryo [101,102]. LPA_3 was detected in porcine concepti of day 12 and 15, and it was suggested that LPA produced in porcine uterine endometrium influences conceptus development during implantation and establishment of pregnancy [55]. A recent study showed multiple effects of LPA on ovine trophectoderm cells, such as activation of MAPK ERK1/2 phosphorylation, promotion of proliferation and cytoskeletal rearrangement and release of $PGF_{2\alpha}$ and PGE_2, suggesting a potential role of LPA signaling in the ovine conceptus at the time of implantation [54].

Summary

Progress over the past decade has revealed the importance of LPA signaling in female and male reproduction, as well as in fertilization and pre-implantation embryo development. All vertebrate species examined utilize LPA receptor-mediated mechanisms and this form of lysophospholipid signaling influences most elements of reproduction directly or indirectly. Numerous issues remain to be addressed in the future (Box 1), a partial list that underscores both the multitude of pathway elements to consider and open questions within each group. The therapeutic potential of LPA signaling represents an area of opportunity that awaits future investigations.

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