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Steroid hormones induce *in vitro* human first trimester trophoblast tubulogenesis by the lysophosphatidic acid pathway

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ABSTRACT

Successful implantation and placentation requires that extravillous cytotrophoblast acquires an endovascular phenotype and remodels uterine spiral arteries. Progesterone (P4) and estradiol (E2) control many of the placental functions, but their role in vascular remodeling remains controversial. Here, we investigated whether P4 and E2 regulate the acquisition of the human first trimester trophoblast endovascular phenotype, and the participation of the lysophosphatidic acid pathway. For this purpose, human first trimester HTR-8/SVneo cells were seeded on Geltrex and assayed for capillary-like tube formation. P4 and E2 increased HTR-8/SVneo tube formation in a concentration-dependent manner and this effect is mediated by the LPA3 receptor. Moreover, sex steroids increased the mRNA levels of the main enzyme that produce lysophosphatidic acid (lysophospholipase-D) but did not regulate LPA3 mRNA levels. Overall, we demonstrate that steroid hormones regulate HTR-8/SVneo trophoblast capillary-like structures formation and we propose that this process could be modulated directly or indirectly by mechanisms associated to the LPA/LPA3 pathway.

1. Introduction

Spiral artery remodeling at the maternal-fetal interface is crucial and involves extravillous trophoblast differentiation into an endovascular trophoblast. In this mechanism, the endovascular trophoblast invades maternal spiral arteries, disrupts the endothelium-myometrium interactions and replaces endothelial and myometrial cells. These adaptations of the maternal vessels ensure an adequate blood flow in response to the increasing metabolic demands of the embryo (Demir et al., 2010). Failures in this process are correlated with severe obstetric complications such as implantation failure and preeclampsia (Zhou et al., 1997; Plaisier et al., 2009).

Sex steroids, P4 and E2, are the master hormones that orchestrate most of the reproductive events during implantation and placentation in mammals. P4 and E2 intervene to achieve appropriate maternal vascular adaptations and placental vasculature during the early stages of pregnancy (Clark et al., 2017). Although steroid hormones modulate uterine blood flow following remodeling of uterine arteries, placental angiogenesis and vasculogenesis (Chen et al., 2012; Maliqueo et al., 2016), their specific role in vascular remodeling at the maternal-fetal interface remains controversial.

Lysophosphatidic acid (LPA) is a phosphorylated lipid mediator that regulates several female reproductive functions through G protein-coupled receptors (Ye et al., 2005; Hama et al., 2007). LPA modulates blood vessel development and is involved in vascular pathologies in different biological systems (Mueller et al., 2015). In this sense, we have previously shown that LPA augments the production of vascular mediators in the rat uterus during implantation (Sordelli et al., 2012; Beltrame et al., 2013). Recently, we reported that LPA binding to LPA3 receptor participates in angiogenesis at the implantation sites in the rat (Sordelli et al., 2017) and promotes the acquisition of the endovascular phenotype by the human first trimester trophoblast (Beltrame et al., 2018). Interestingly, patients displaying recurrent implantation failure and endometriosis show reduced levels of LPA3 in the endometrium (Achache et al., 2010; Wei et al., 2009). Furthermore, it has been shown that P4 and E2 modulate uterine receptivity by LPA3-mediated signaling in mice during early pregnancy (Diao et al., 2015).

Based on these antecedents, we decided to investigate whether P4 and E2 regulate human first trimester trophoblast tube formation, and if LPA pathway participates in this process.

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2. Methods

2.1. Cell culture

The immortalized human first trimester trophoblast cell line HTR-8/SVneo (H8, a kind gift from Dr. Udo Markert, Plancenta Lab, Department of Obstetrics, Jena University Hospital, Jena, Germany) was maintained as previously described (Beltrame et al., 2018). H8 trophoblast cells were obtained from explant cultures of human first trimester placenta (8–10 weeks of gestation) and immortalized by transfection with a cDNA construct that encodes the SV40 large T antigen (Graham et al., 1993). These cells are non-tumourigenic, non-metastatic and highly invasive *in vitro*. However, they are not tumorigenic when injected into nude mice. H8 cells exhibit various properties of extravillous cytotrophoblast including the expression of cytokeratins 7, 8, and 18, placental alkaline phosphatase, uPAR, human leukocyte antigen framework antigen W6/32, IGF-II mRNA and protein, as well as an integrin profile characteristic of invasive cytotrophoblast (Graham et al., 1993; Irving et al., 1995). These cells have been shown to secrete variable levels of hCG (Armant et al., 2006) and to express cytoplasmic and membrane-associated HLAG (Kilburn et al., 2000; Kalkunte et al., 2008).

2.2. Tube formation assay

H8 cell line and tube formation assay were used to model the acquisition of the trophoblast endovascular phenotype at the maternal-fetal interface (Beltrame et al., 2018). Briefly, 96 well plates were coated with 50 μL/well of Geltrex (Gibco, Invitrogen, Argentina) and incubated at 37 °C for 30 min to promote solidification. H8 were seeded on the top of the gel (15000/well) and incubated at 37 °C with 5% CO_2 in DMEM/F12 medium without fetal bovine serum. Cells were treated with P4 (medroxyprogesterone 17-acetate, Sigma Aldrich Co., Argentina), E2 (17β-estradiol, Sigma Aldrich Co., Argentina), BMT (BMT-183172-01-002, LPA1 antagonist, Bristol Myers Squibb, Pennington, USA), DGPP (diacylglycerol pyrophosphate 8:0, LPA3 antagonist, Sigma Aldrich Co., Argentina) or BrP-LPA (1-bromo-3(S)-hydroxy-4-(palmitoyloxy)-butyl-phosphonate, LPA1 to LPA4 antagonist, Echelon Biosciences, Inc., Pennsylvania, USA). After 6 h, tubules were observed in an inverted light microscope (10x, IMT2 Olympus) and photographed with a digital camera (Olympus C-5060). Five different fields per well were analyzed and extreme edges were excluded due to gel meniscus formation. Image J (open source) software package was used to quantify tubule length of the capillary network formation. The length of each tubule was determined by drawing a line over each tubule and the mean length of the lines (pixels) drawn in each image was calculated. The number of capillary interconnections or branch points between cells was counted manually.

2.3. RNA isolation and polymerase chain reaction analysis (PCR)

H8 cells were plated in a 6 well plate (400000/well) and were treated with P4 10^{-7} M + E2 10^{-5} M. Total RNA isolation, cDNA synthesis and real time PCR were performed as previously described (Sordelli et al., 2011; Beltrame et al., 2013). First strand cDNA was synthesized from total RNA (3 μg). The PCR conditions in all cases started with a denaturation step at 95 °C for 5 min and followed by up to 40 cycles of denaturation, annealing and primer extension (lysophospholipase-D, LPA3 and GADPH: 94 °C 5 min, 59 °C 30 seg, 72 °C 1 min). PCR primers are detailed for lysophospholipase-D (NM_006209.4, Forward 5′-GGCACACACTCTCCCTACAT-3′, Reverse 5′-GTTCCAGCTTTCACCCCTTG-3′, product: 233 bp), LPA3 (NM_012152.2, Forward 5′-CTTAGGGGCGTTTGTGGTAT-3′, Reverse 5′-GTGCCATACATGTCCTCGTC-3′, product: 177 bp) and GAPDH (NM_000572.2, Forward 5′-CACATCGCTGAGACACCATG-3′, Reverse 5′-GATGACAAGCTTCCCGTTCTC-3′, product: 224 bp). A melting curve analysis was performed to confirm the amplification specificity. Lysophospholipase-D and LPA3 mRNA levels were normalized against levels of human GAPDH using the $2^{-\Delta\Delta Ct}$ method. GAPDH was chosen as the housekeeping gene because its expression did not change under the present experimental conditions.

2.4. Statistical analyses

All values represent mean ± S.E.M. Data was normally distributed according to the Shapiro-Wilk normality test. Comparisons between values of different groups were performed using analysis of variance (ANOVA) and significance was determined using Bonferroni or Tukey *post hoc* tests. A number of three replicates were used per treatment and each experiment was repeated 4–6 times. Differences between means were considered significant when $p < 0.05$. Statistical analysis was performed using the InfoStat Program (Córdoba, Argentina).

3. Results

3.1. A fine balance of steroid hormones is necessary to induce H8 tubulogenesis

First, we investigated the effect of P4 and E2 on trophoblast tubulogenesis. H8 cells were incubated with increasing concentrations of P4 (10^{-8} - 10^{-4} M) or E2 (10^{-10} - 10^{-4} M) for 6 h. We observed that P4 and E2 stimulated the formation of tubules in a concentration-dependent manner. The incubation with P4 10^{-7} M or 10^{-6} M increased the length of the tubules and the number of branches compared to the control (Fig. 1A). In contrast, the incubation with P4 10^{-8} M or 10^{-5} M did not show differences when compared to the basal tube formation. In the case of E2, we observed that the incubation with E2 10^{-8} M stimulated the formation of the tubular network (Fig. 1B). In contrast, E2 10^{-10}, 10^{-9}, 10^{-7}, 10^{-6} or 10^{-5} M showed no effect. Finally, the treatment with P4 10^{-4} M or E2 10^{-4} M inhibited tubulogenesis below the control.

In the uterine microenvironment, P4 and E2 are present simultaneously regulating the processes triggered before and after blastocyst implantation. Therefore, we evaluated the effect of the co-incubation of P4 + E2 on trophoblast tubulogenesis. For this purpose, H8 cells were incubated with P4 10^{-7} M and increasing concentrations of E2 (10^{-10} - 10^{-4} M) or with E2 10^{-8} M and increasing concentrations of P4 (10^{-8} - 10^{-4} M). Although P4 10^{-7} M and E2 10^{-8} M increased H8 tubule network when incubated separately, the combination of P4 10^{-7} M + E2 10^{-8} M did not change H8 tubulogenesis compared to the control (Fig. 2). We found that only the incubation of H8 cells with P4 10^{-7} M + E2 10^{-5} M stimulated trophoblast tube formation. The incubation with P4 10^{-4} M + E2 10^{-8} M suppressed the formation of tubules below control levels (Fig. 2). However, the treatment with these concentrations of P4 and E2 separately did not modify the tubule network (Fig. 1A and B). The effect of P4 10^{-4} M + E2 10^{-8} M was due to an inhibition in the process of tubulogenesis and not to deleterious actions of steroid hormones on cell viability or survival (data not shown). However, P4 10^{-3} M + E2 10^{-8} M showed a negative action on the survival of H8 cells (Fig. 2), as this combination of steroid hormones exert a toxic effect in H8 cell line (data not shown).

3.2. LPA mediates the induction of P4 + E2 on trophoblast tube formation

We have previously reported that LPA promotes the acquisition of the human first trimester trophoblast endovascular phenotype (Beltrame et al., 2018). Therefore, we decided to evaluate the participation of LPA in the effect of P4 + E2 on trophoblast tubulogenesis. H8 cells were incubated with P4 10^{-7} + E2 10^{-5} M alone or in the presence of DGPP 100 μM (a selective LPA3 antagonist), BrP-LPA 5 μM (a broad LPA1 to LPA4 antagonist) or BMT 10 μM (a selective LPA1 antagonist) for 6 h. The concentrations of the antagonists were selected

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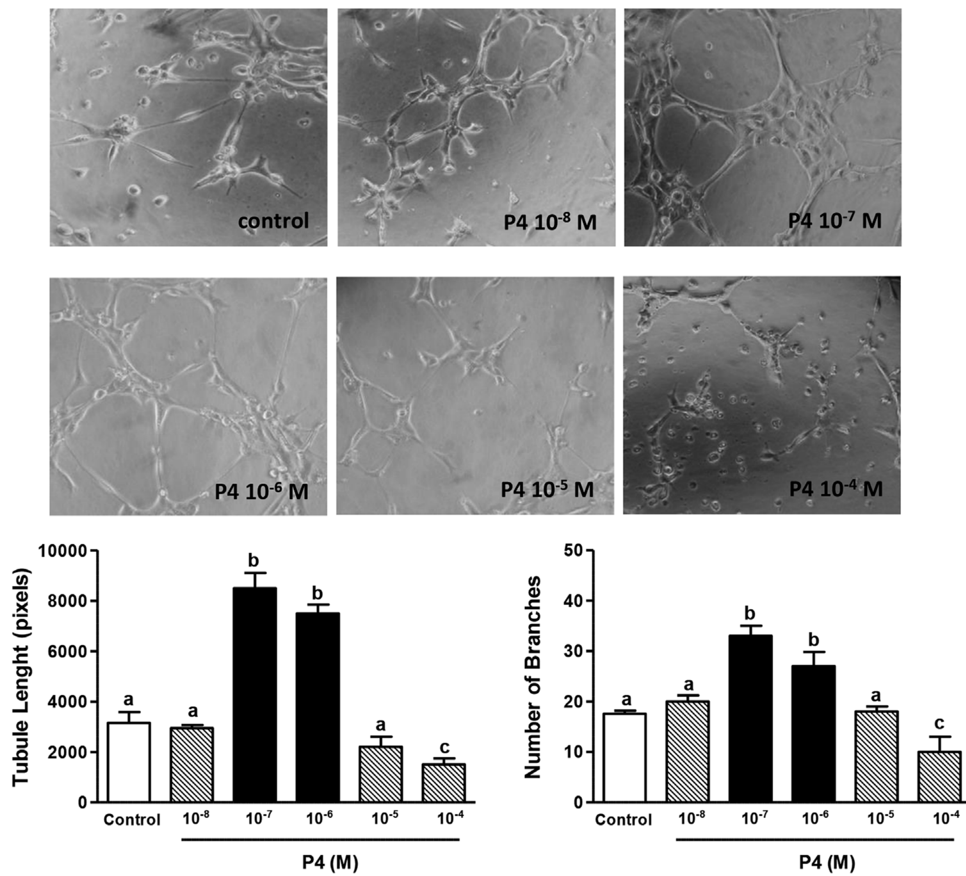

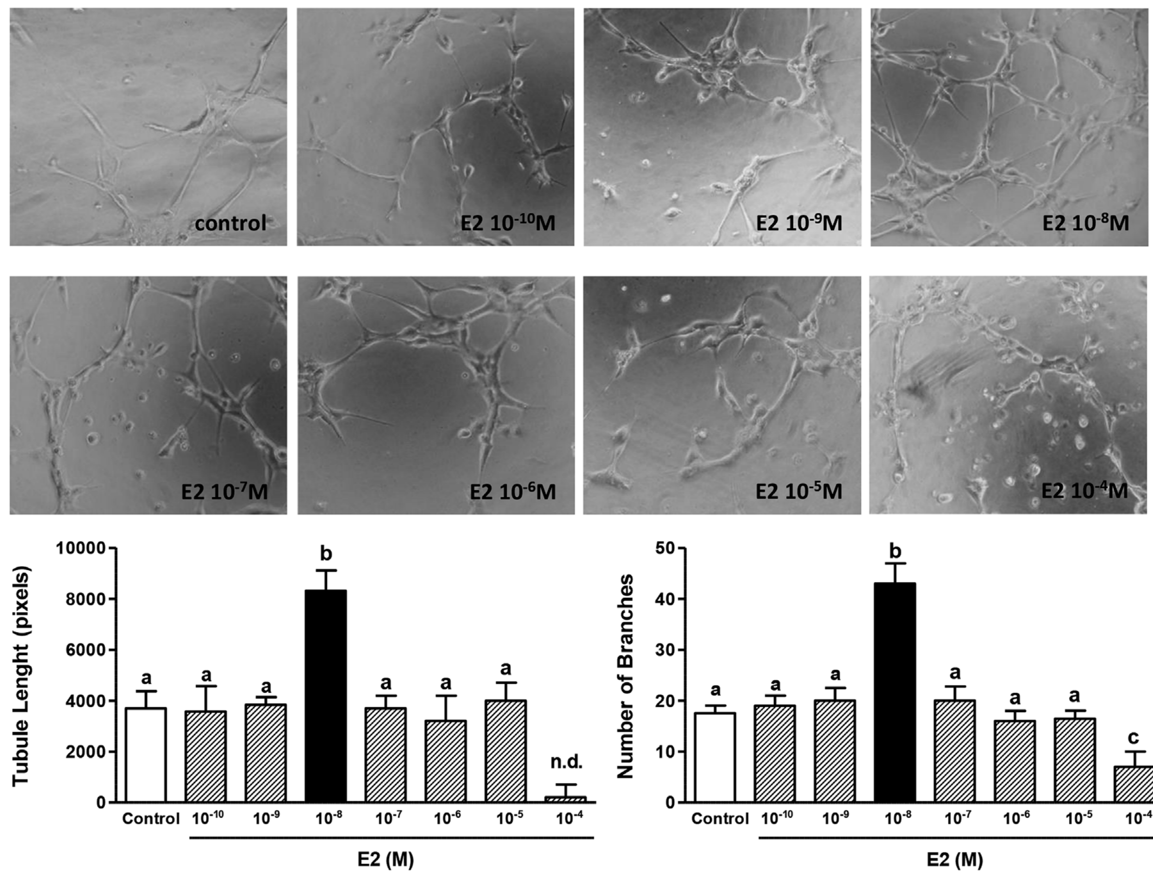

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Fig. 1. P4 or E2 stimulate H8 tubulogenesis in a concentration-response manner. H8 cells were seeded onto Geltrex, incubated with medium (control), (A) P4 (10^{-8} to 10^{-4} M) or (B) E2 (10^{-10} to 10^{-4} M) and assayed for tube formation for 6 h. Representative photographs are shown (10x). Data represent mean ± S.E.M. Different letters indicate statistical differences ($p < 0.05$). Three replicates per treatment and each experiment was repeated 4–6 times.

based on previous results from our own laboratory (Beltrame et al., 2018). The incubation of P4 + E2 with DGPP or with BrP-LPA decreased the tubule length and the number of branching points (Fig. 3). BMT did not modify the stimulatory action of P4 + E2 on trophoblast tube formation (Fig. 3).

3.3. P4 + E2 increase lysophospholipase-D mRNA levels in H8 cells

We show that the incubation with P4 10^{-7} M + E2 10^{-5} M increased H8 tubulogenesis and DGPP partially reversed the stimulatory action. These results suggest that endogenous LPA participates in steroid hormones stimulation and this effect is mediated at least in part by LPA3. Thus, we investigated if P4 + E2 modulated the expression of lysophospholipase-D (the main enzyme involved in LPA synthesis) and LPA3. H8 cells were incubated with P4 10^{-7} M + E2 10^{-5} M for 24 h and lysophospholipase-D and LPA3 mRNA levels were determined by qRT-PCR. We observed that P4 10^{-7} M + E2 10^{-5} M stimulated lysophospholipase-D mRNA level (Fig. 4A) and did not modify LPA3 (Fig. 4B).

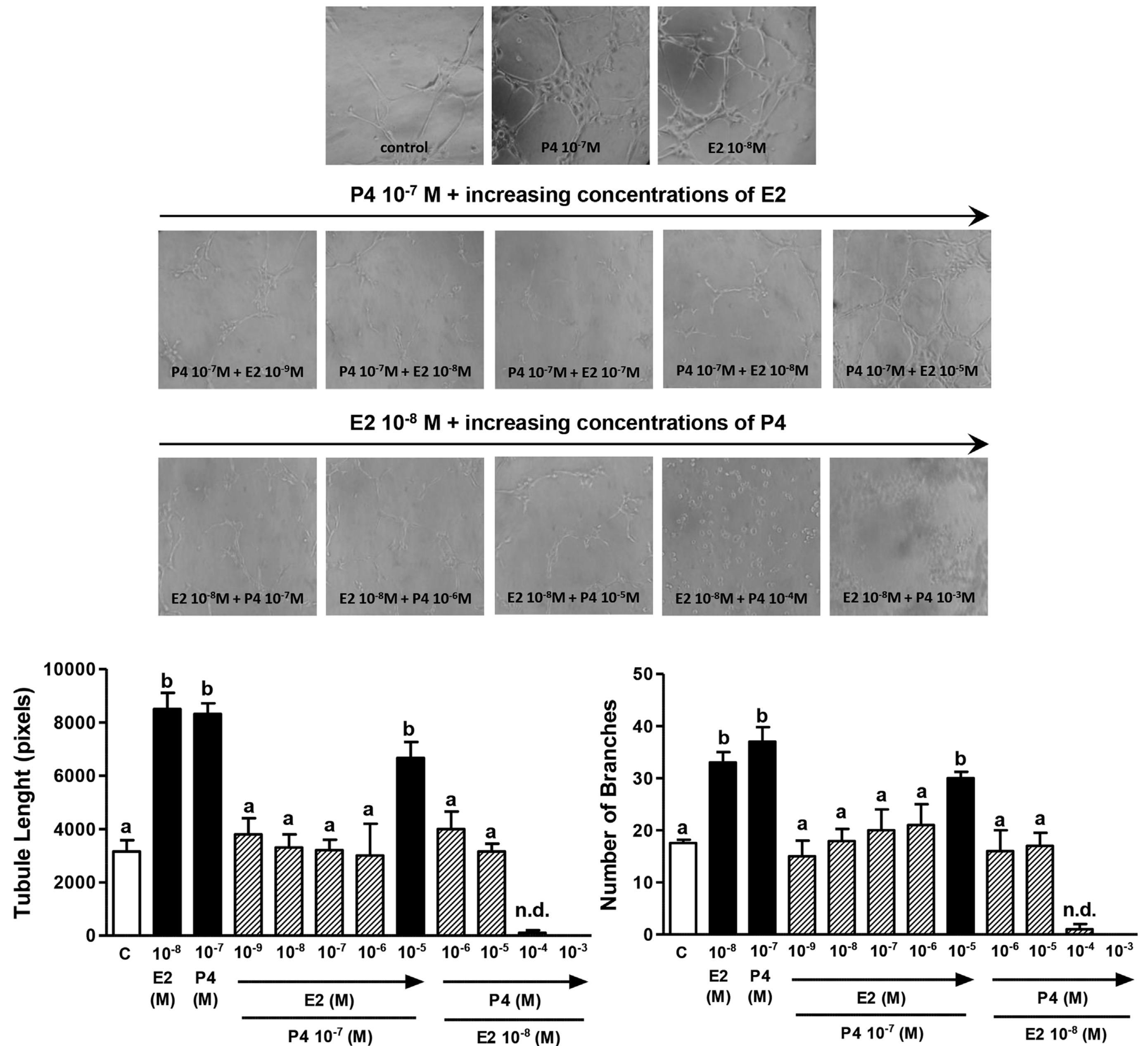

Fig. 2. A fine balance of P4 + E2 is necessary to induce H8 tubulogenesis. H8 cells were seeded onto Geltrex, incubated with medium (control), P4 10^{-7} M + E2 (10^{-10} - 10^{-4} M) or E2 10^{-8} M + P4 (10^{-8} - 10^{-4} M) and assayed for tube formation for 6 h. Representative photographs are shown (10x). Data represent mean ± S.E.M. Different letters indicate statistical differences ($p < 0.05$). Three replicates per treatment and each experiment was repeated 4–6 times.

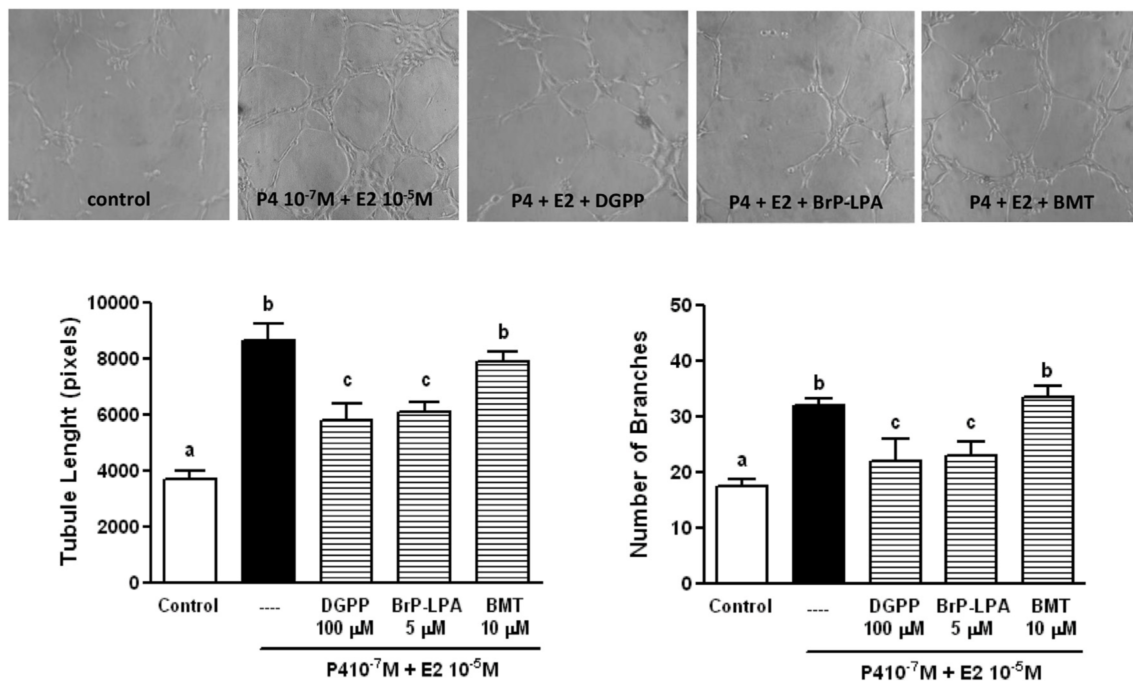

Fig. 3. LPA mediates the induction of P4 + E2 on trophoblast tube formation. H8 cells were seeded onto Geltrex, incubated with medium (control), P4 10^{-7} + E2 10^{-5} M alone or in the presence of DGPP 100 µM (a selective LPA3 antagonist), BrP-LPA 5 µM (a broad LPA1 to LPA4 antagonist) or BMT 10 µM (a selective LPA1 antagonist) and assayed for tube formation for 6 h. Representative photographs are shown (10x). Data represent mean ± S.E.M. Different letters indicate statistical differences ($p < 0.05$). Three replicates per treatment and each experiment was repeated 4–6 times.

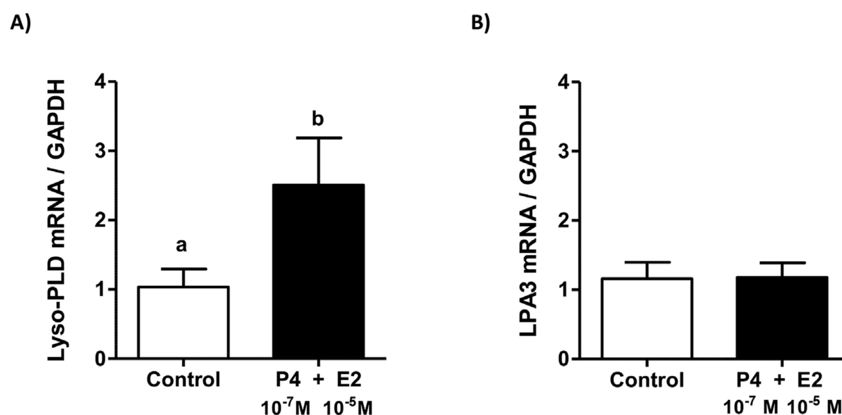

Fig. 4. P4 + E2 increase lysophospholipase-D mRNA levels in H8 cells. H8 cells were incubated in the presence of medium (control) or P4 10^{-7} + E2 10^{-5} M. Detection of mRNA levels of A) Lyso-PLD and B) LPA3 was assessed by qRT-PCR. Target genes were normalized against human GAPDH used as internal control. Data represent mean ± S.E.M. Different letters indicate statistical differences ($p < 0.05$). Three replicates per treatment and each experiment was repeated 4–6 times. Lyso-PLD: lysophospholipase-D.

4. Discussion

In this manuscript we show for the first time that steroid hormones regulate H8 capillary-like response through LPA pathway. The H8 cell line was used as a tool to model the acquisition of the endovascular phenotype by the invading trophoblast (Beltrame et al., 2018). There are multiple factors that intervene during the early stages of gestation to achieve an adequate blood flow into the uterus. During the first trimester, the extravillous trophoblast acquires an endovascular phenotype and remodels maternal vessels. Deregulation of the spiral artery adaptations impairs the supply of nutrient and oxygen to the embryo, and has a major clinical impact resulting in several pregnancy complications such as implantation failure and preeclampsia. Steroid hormones are present in extremely high concentrations in the maternal circulation and orchestrate most of the reproductive events. However, their role in vascular remodeling at the maternal-fetal interface remains controversial (Chen et al., 2012; Maliqueo et al., 2016).

The concentrations of P4 and E2 used in our experiments were selected according to the levels reported in plasma and in placental tissue of women in the first trimester of pregnancy (Chen et al., 2012). It has been described that H8 cells constitutively express both P4 (PR-A and PR-B) and E2 (ER-α and ER-β) receptors (Lee et al., 2014; Patel et al., 2015). P4 and E2 increases H8 tube formation in a concentration dependent manner and only the combination of P4 10^{-7}M + E2 10^{-5}M stimulates tubulogenesis. These results suggest that there must be a fine regulation of the steroid hormones tone at the maternal-fetal interface during endovascular differentiation, which strengthen the concept that the balance of female sex hormones is critical for the establishment of pregnancy (Ramathal et al., 2010; Lim and Wang, 2010; Cha et al., 2012). In humans, P4 regulates the invasion and migration of the extravillous cytotrophoblast *in vitro* (Chen et al., 2011; Halasz and Szekeres-Bartho, 2013) and is postulated that stimulates the differentiation of a subtype of decidual cells (subfraction $CD31^-CD146^-$) into endothelial and smooth muscle cells. These evidences suggest that P4 could participate in the regulation of vascular remodeling (Wang et al., 2013). In addition, E2 increases the percentage of the vascular area and the density of vessels in the placental tissue (Albrecht et al., 2004; Robb et al., 2004; Albrecht and Pepe, 2010). It is worth mentioning that we

observed that the highest concentrations of P4 alone or in combination with E2 suppressed the basal tubular formation. In this sense, it has been reported that preeclampsia, a pathology associated with placental vascular deficiencies, is correlated with higher P4 levels that seem to affect placenta development (Walsh and Coulter, 1989). Our results support the hypothesis that steroid hormones present at the maternal-fetal interface participate in the acquisition of the human first trimester trophoblast endovascular phenotype.

Some of the most widely studied mediators of physiological responses in reproduction are the phosphorylated lipids such as LPA. Previously we demonstrated that LPA and its LPA3 receptor play major roles in vascular remodeling at the implantation sites (Beltrame et al., 2013, 2018; Sordelli et al., 2017). Moreover, Diao et al. (2015) reported that P4 and E2 contribute to define uterine receptivity by modulating LPA3-mediated signaling in mice. Therefore, LPA/LPA3 appears as a key system that could be activated downstream steroid hormones. In the present work we demonstrate that P4 and E2 increase the formation of capillary-like structures in H8 cells which implies the participation of LPA and at least its LPA3 receptor. Furthermore, P4 + E2 increase the mRNA levels of the main enzyme that produces LPA, lysophospholipase-D. Altogether, these results suggest that the induction of the first trimester endovascular phenotype by P4 + E2 is mediated by an increase in LPA production that activates LPA3 present in the first trimester trophoblast cells. This reinforces the relevance of LPA in the utero-placental vasculature at the maternal-fetal interface. In this sense, the importance of LPA signaling during gestation has been also supported by other authors. LPA is produced locally at the maternal-fetal interface as lysophospholipase-D is expressed in the human placental trophoblast (Irving et al., 1995). In addition, lysophospholipase-D activity increases in serum with the length of gestation producing micromolar levels of LPA (Tokumura et al., 2002a). However, the activity of this enzyme decreases to non-pregnant levels soon after delivery (Tokumura et al., 2002b), suggesting that the main source of LPA during gestation is the placental trophoblast (Irving et al., 1995). The participation of LPA3 in gestation has been also demonstrated in humans and mice (Ye et al., 2005; Wei et al., 2009; Ye et al., 2011; Aikawa et al., 2017). The importance of LPA-LPA3 in utero-placental vasculature is highlighted by the fact that LPA3 levels are diminished in the endometrium of patients displaying recurrent implantation failure and endometriosis (Plaisier et al., 2009; Wei et al., 2009; Achache et al., 2010). In this sense, the fine balance of P4 and E2 signaling is disrupted in the uterus of LPA3 knockout mice leading to a delayed embryo implantation (Diao et al., 2015). Therefore, the actions of P4 and E2 in early pregnancy appear to be mediated by LPA3 signaling. However, we could not rule out the involvement of other LPA receptors, such as LPA5 or LPA6. Moreover, prostaglandins and nitric oxide pathways could also be involved since they are modulated by LPA and steroid hormones in different reproductive processes (Farina et al., 2004, 2007; Sordelli et al., 2012; Beltrame et al., 2013, 2018).

5. Conclusions

Vascular remodeling at the maternal-fetal interface is essential for embryo growth and to achieve normal gestation. We demonstrate that steroid hormones regulate H8 trophoblast tubulogenesis and this process could be modulated directly or indirectly by mechanisms associated to the LPA/LPA3 pathway. The inadequate acquisition of the trophoblast endovascular phenotype due to abnormal LPA signaling may contribute to the development of pathologies related to an impaired vascular remodeling. Understanding the role of different regulators of the vascular dynamics will provide further insight into the origin of disorders associated with endovascular differentiation as implantation failure and preeclampsia.

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Declarations of interest

None.

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References

Achache, H., Tsafrir, A., Prus, D., Reich, R., Revel, A., 2010. Defective endometrial prostaglandin synthesis identified in patients with repeated implantation failure undergoing in vitro fertilization. Fertil. Steril. 94, 1271–1278.

Aikawa, S., Kano, K., Inoue, A., Wang, J., Saigusa, D., Nagamatsu, T., Hirota, Y., Fujii, T., Tsuchiya, S., Taketomi, Y., Sugimoto, Y., Murakami, M., Arita, M., Kurano, M., Ikeda, H., Yatomi, Y., Chun, J., Aoki, J., 2017. Autotaxin-lysophosphatidic acid-LPA3 signaling at the embryo-epithelial boundary controls decidualization pathways. EMBO J. 36 (14), 2146–2160.

Albrecht, E.D., Robb, V.A., Pepe, G.J., 2004. Regulation of placental vascular endothelial growth/permeability factor expression and angiogenesis by estrogen during early baboon pregnancy. J. Clin. Endocrinol. Metab. 89, 5803–5809.

Albrecht, E.D., Pepe, G.J., 2010. Estrogen regulation of placental angiogenesis and fetal ovarian development during primate pregnancy. Int. J. Dev. Biol. 54, 397.

Armant, D.R., Kilburn, B.A., Petkova, A., Edwin, S.S., Duniec-Dmuchowski, Z.M., Edwards, H.J., Romero, R., Leach, R.E., 2006. Human trophoblast survival at low oxygen concentrations requires metalloproteinase-mediated shedding of heparin-binding EGF-like growth factor. Development 133, 751–759.

Beltrame, J.S., Sordelli, M.S., Cella, M., Perez Martinez, S., Franchi, A.M., Ribeiro, M.L., 2013. Lysophosphatidic acid increases the production of pivotal mediators of decidualization and vascularization in the rat uterus. Placenta 34, 751–756.

Beltrame, J.S., Sordelli, M.S., Cañumil, V., Franchi, A., Ribeiro, M.L., 2018. Lysophosphatidic acid-triggered pathways promote the acquisition of trophoblast endovascular phenotype in vitro. J. Cell. Biochem. 119, 758–772.

Cha, J., Sun, X., Dey, S.K., 2012. Mechanisms of implantation: strategies for successful pregnancy. Nat. Med. 18, 1754–1767.

Chen, J.Z., Wong, M.H., Brennecke, S.P., Keogh, R.J., 2011. The effects of human chorionic gonadotrophin, progesterone and oestradiol on trophoblast function. Mol. Cell. Endocrinol. 342, 73–80.

Chen, J.Z., Sheehan, P.M., Brennecke, S.P., Keogh, R.J., 2012. Vessel remodelling, pregnancy hormones and extravillous trophoblast function. Mol. Cell. Endocrinol. 349, 138–144.

Clark, N.C., Pru, C.A., Pru, J.K., 2017. Novel regulators of hemodynamics in the pregnant uterus. Prog Mol Biol Transl Sci 145, 181–216.

Demir, R., Yaba, A., Huppertz, B., 2010. Vasculogenesis and angiogenesis in the endometrium during menstrual cycle and implantation. Acta Histochem. 112 (3), 203–214.

Diao, H., Li, R., El Zowalaty, A.E., Xiao, S., Zhao, F., Dudley, E.A., Ye, X., 2015. Deletion of lysophosphatidic acid receptor 3 (Lpar3) disrupts fine local balance of progesterone and estrogen signaling in mouse uterus during implantation. Biol. Reprod. 93, 123.

Farina, M., Ribeiro, M.L., Weissmann, C., Estevez, A., Billi, S., Vercelli, C., Franchi, A.M., 2004. Biosynthesis and catabolism of prostaglandin F2alpha (PGF2alpha) are controlled by progesterone in the rat uterus during pregnancy. J. Steroid Biochem. Mol. Biol. 91 (4–5), 211–218.

Farina, M., Billi, S., Leguizamón, G., Weissmann, C., Guadagnoli, T., Ribeiro, M.L., Franchi, A.M., 2007. Secretory and cytosolic phospholipase A2 activities and expression are regulated by oxytocin and estradiol during labor. Reproduction 134 (2), 355–364.

Graham, C.H., Hawley, T.S., Hawley, R.G., MacDougall, J.R., Kerbel, R.S., Khoo, N., Lala, P.K., 1993. Establishment and characterization of first trimester human trophoblast cells with extended lifespan. Exp. Cell Res. 206, 204–211.

Halasz, M., Szekeres-Bartho, J., 2013. The role of progesterone in implantation and trophoblast invasion. J. Reprod. Immunol. 97, 43–50.

Hama, K., Aoki, J., Inoue, A., Endo, T., Amano, T., Motoki, R., Kanai, M., Ye, X., Chun, J., Matsuki, N., Suzuki, H., Shibasaki, M., Arai, H., 2007. Embryo spacing and implantation timing are differentially regulated by LPA3-mediated lysophosphatidic acid signaling in mice. Biol. Reprod. 77 (6), 954–959.

Irving, J.A., Lysiak, J.J., Graham, C.H., Hearn, S., Han, V.K., Lala, P.K., 1995. Characteristics of trophoblast cells migrating from first trimester chorionic villus explants and propagated in culture. Placenta 16, 413–433.

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Kalkunte, S., Lai, Z., Tewari, N., Chichester, C., Romero, R., Padbury, J., Sharma, S., 2008. In vitro and in vivo evidence for lack of endovascular remodeling by third trimester trophoblasts. Placenta 29, 871–878.

Kilburn, B.A., Wang, J., Duniec-Dmuchowski, Z.M., Leach, R.E., Romero, R., Armant, D.R., 2000. Extracellular matrix composition and hypoxia regulate the expression of HLAG and integrins in a human trophoblast cell line. Biol. Reprod. 62, 739–747.

Lee, B.H., Park, T.C., Lee, H.J., 2014. Influence of caffeine on the expression of human chorionic gonadotropin and progesterone receptors in human trophoblast cell lines. Acta Obstet. Gynecol. Scand. 93, 1334–1335.

Lim, H.J., Wang, H., 2010. Uterine disorders and pregnancy complications: insights from mouse models. J. Clin. Invest. 120, 1004–1015.

Maliqueo, M., Echiburú, B., Crisosto, N., 2016. Sex steroids modulate uterine-placental vasculature: implications for obstetrics and neonatal outcomes. Front. Physiol. 7, 152.

Mueller, P., Ye, S., Morris, A., Smyth, S.S., 2015. Lysophospholipid mediators in the vasculature. Exp. Cell Res. 333 (2), 190–194.

Patel, S., Kilburn, B., Imudia, A., Armant, D.R., Skafar, D.F., 2015. Estradiol elicits proapoptotic and antiproliferative effects in human trophoblast cells. Biol. Reprod. 93 (74), 1–10.

Plaisier, M., Dennert, I., Rost, E., Koolwijk, P., van Hinsbergh, V.W., Helmerhorst, F.M., 2009. Decidual vascularization and the expression of angiogenic growth factors and proteases in first trimester spontaneous abortions. Hum. Reprod. (Eynsham) 24, 185–197.

Ramathal, C.Y., Bagchi, I.C., Taylor, R.N., Bagchi, M.K., 2010. Endometrial decidualization: of mice and men. Semin. Reprod. Med. 28, 17–26.

Robb, V.A., Pepe, G.J., Albrecht, E.D., 2004. Acute temporal regulation of placental vascular endothelial growth/permeability factor expression in baboons by estrogen. Biol. Reprod. 71, 1694–1698.

Sordelli, M.S., Beltrame, J.S., Burdet, J., Zotta, E., Pardo, R., Cella, M., Franchi, A.M., Ribeiro, M.L., 2011. The effect of anandamide on uterine nitric oxide synthase activity depends on the presence of the blastocyst. PLoS One 6, e18368.

Sordelli, M.S., Beltrame, J.S., Cella, M., Gervasi, M.G., Perez Martinez, S., Burdet, J., Zotta, E., Franchi, A.M., Ribeiro, M.L., 2012. Interaction between lysophosphatidic acid, prostaglandins and the endocannabinoid system during the window of implantation in the rat uterus. PLoS One 7, e46059.

Sordelli, M.S., Beltrame, J.S., Zotta, E., Gomez, N., Dmytrenko, G., Sales, M.E., Blois, S.M., Davio, C., Martinez, S.P., Franchi, A.M., Ribeiro, M.L., 2017. Endogenous lysophosphatidic acid participates in vascularization and decidualization at the maternal-fetal interface in the rat. Reprod. Fertil. Dev. 29 (11), 2112–2126.

Tokumura, A., Kanaya, Y., Miyake, M., Yamano, S., Irahara, M., Fukuzawa, K., 2002a. Increased production of bioactive lysophosphatidic acid by serum lysophospholipase D in human pregnancy. Biol. Reprod. 67, 1386–1392.

Tokumura, A., Majima, E., Kariya, Y., Tominaga, K., Kogure, K., Yasuda, K., Fukuzawa, K., 2002b. Identification of human plasma lysophospholipase D, a lysophosphatidic acid-producing enzyme, as autotaxin, a multifunctional phosphodiesterase. J. Biol. Chem. 277, 39436–39442.

Walsh, S.W., Coulter, S., 1989. Increased placental progesterone may cause decreased placental prostacyclin production in preeclampsia. Am. J. Obstet. Gynecol. 161, 1586–1592.

Wang, Q., Shen, L., Huang, W., Song, Y., Xiao, L., Xu, W., Liu, Y., 2013. Vasculogenesis of decidua side population cells of first-trimester pregnancy. Stem Cell Res. Ther. 4 (3), 50.

Wei, Q., St Clair, J.B., Fu, T., Stratton, P., Nieman, L.K., 2009. Reduced expression of biomarkers associated with the implantation window in women with endometriosis. Fertil. Steril. 91 (5), 1686–1691.

Ye, X., Hama, K., Contos, J.J., Anliker, B., Inoue, A., Skinner, M.K., Suzuki, H., Amano, T., Kennedy, G., Arai, H., Aoki, J., Chun, J., 2005. LPA3-mediated lysophosphatidic acid signalling in embryo implantation and spacing. Nature 435 (7038), 104–108.

Ye, X., Herr, D.R., Diao, H., Rivera, R., Chun, J., 2011. Unique uterine localization and regulation may differentiate LPA3 from other lysophospholipid receptors for its role in embryo implantation. Fertil. Steril. 95 (6), 2107–2113.

Zhou, Y., Damsky, C.H., Fisher, S.J., 1997. Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome? J. Clin. Invest. 99 (9), 2152–2164.