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11-deoxy prostaglandin $F_{2\alpha}$, a thromboxane A2 receptor (TP) agonist, partially alleviates embryo crowding in *Lpar3*$^{(-/-)}$ females

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Abstract

Objective—To determine cyclooxygenase (COX)-derived prostanoid signaling in alleviating embryo crowding in the *Lpar3*$^{(-/-)}$ females.

Design—Experimental mouse model.

Setting—Research laboratories.

Animal(s)—Wild type, *Lpar3*$^{(+/-)}$, and *Lpar3*$^{(-/-)}$ mice.

Intervention(s)—Intraperitoneal (i.p.) administration of prostaglandin E_2 (PGE_2), cPGI (a stable analogue of PGI_2), and 11-deoxy prostaglandin $F_{2\alpha}$ (11-deoxy $PGF_{2\alpha}$, a thromboxane A_2 (TxA_2) receptor (TP) agonist) to preimplantation gestation day 3.5 *Lpar3*$^{(-/-)}$ females.

Main Outcome Measure(s)—Implantation sites were detected by blue dye reaction and embryo spacing was determined by the distribution of the implantation sites along the uterine horns on gestation day 4.5; pregnancy outcome was measured by litter size at birth.

Result(s)—Administration of PGE_2 + cPGI on gestation day 3.5 *Lpar3*$^{(-/-)}$ females restored on-time implantation but did not affect embryo spacing or the number of implantation sites detected on gestation day 4.5; PGE_2 + cPGI treatment increased litter size at birth. Administration of PGE_2 + cPGI + 11-deoxy $PGF_{2\alpha}$ on gestation day 3.5 *Lpar3*$^{(-/-)}$ females rescued on-time implantation, partially dispersed the clustered implantation sites normally observed in the *Lpar3*$^{(-/-)}$ females, increased the number of implantation sites detected on gestation day 4.5, and increased litter size at birth.

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Conclusion(s)—The TP agonist 11-deoxy $PGF_{2\alpha}$ can partially alleviate embryo crowding in the *Lpar3*$^{(-/-)}$ females and embryo crowding likely contributes to reduced litter size in the *Lpar3*$^{(-/-)}$ females.

Keywords

lysophosphatidic acid (LPA) receptor 3; COX-derived prostanoids; prostanoid receptors; prostanoid receptor agonists; 11-deoxy $PGF_{2\alpha}$; embryo implantation; embryo spacing

Introduction

Embryo spacing in polyovulating animals tends to be evenly spaced along each uterine horn, which may minimize early embryo mortality resulting from local overcrowding. Even embryo spacing is thought to be achieved through uterine myometrial contractions (1–3).

Lysophosphatidic acid (LPA) is an extracellular lipid mediator with myriad actions that include cell proliferation, survival, and migration. LPA signals through six known G protein-coupled receptors (GPCRs), LPA_{1-6} (4, 5). LPA signaling has been implicated in many aspects of female reproduction, such as fertilization, embryo development, ovum transportation in the oviduct, uterine contraction, embryo implantation, and pregnancy maintenance (reviewed in (6, 7)). LPA_3-mediated signaling is specifically involved in two separate events: implantation timing and embryo spacing (8, 9). Deletion of *Lpar3* in mice results in delayed embryo implantation and aberrant embryo spacing, which is reflected by the crowding of implantation sites on the cervical half of a uterine horn (proximal uterine segment) and multiple embryos sharing a single placenta (8). *Lpar3*$^{(-/-)}$ uterine horns lose LPA_3 agonist-induced uterine contraction (9), supporting the theory that dysregulated myometrial activities may contribute to embryo crowding in the *Lpar3*$^{(-/-)}$ females. LPA_3 has also been implicated in the aberrant embryo spacing induced by transient β_2-adrenoceptor activation (10).

Delayed embryo implantation and aberrant embryo spacing phenotypes in *Lpar3*$^{(-/-)}$ females have also been observed in rats and mice treated with indomethacin, a cyclooxygenase (COX) inhibitor (11–14), and *Pla2g4a*$^{(-/-)}$ mice deficient for cytosolic phospholipase $A_{2\alpha}$ ($cPLA_{2\alpha}$) (15). Both *Lpar3*$^{(-/-)}$ females and *Pla2g4a*$^{(-/-)}$ females have reduced uterine expression of COX-2, the rate limiting enzyme for prostagnoid synthesis (8, 15). These observations reveal the importance of COX-derived prostanoid signaling in on-time embryo implantation and even embryo spacing (8, 11–15).

COX-derived prostanoids include prostaglandins PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and thromboxane A_2 (TxA_2), which activate their respective GPCRs, DP_{1-2}, EP_{1-4}, FP, IP, and TP (16, 17). These GPCRs are expressed in the uterus and mediate the effects of COX-derived prostanoids in myometrial activity (18, 19). EP_1, EP_3, FP, and TP have a contractile effect on the myometrium, while DP_{1-2}, EP_2, EP_4, and IP have a relaxant effect (16, 18, 20, 21). It has been demonstrated that PGE_2 and cPGI (a stable analogue of PGI_2) can restore normal implantation timing but fail to rescue embryo spacing defect in *Lpar3*$^{(-/-)}$ females and *Pla2g4a*$^{(-/-)}$ females (8, 15). It has not been determined if any specific COX-derived prostanoid signaling affects embryo spacing. PGE_2 can induce contractile (via EP_1 and EP_3) and relaxant (via EP_2 and EP_4) effects whereas cPGI only induces relaxant (via IP) effects. Since they do not have an obvious effect on embryo spacing yet can rescue on-time implantation in *Lpar3*$^{(-/-)}$ females (8), it was hypothesized that COX-derived prostanoid(s) that can induce a contractile effect rather than a relaxant effect on the myometrium may alleviate embryo crowding in *Lpar3*$^{(-/-)}$ females.

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Materials and Methods

Animals

Wild type, *Lpar3*$^{(+/-)}$, and *Lpar3*$^{(-/-)}$ mice (129/SvJ and C57BL/6 mixed background) were generated and genotyped as described (8). The animals were housed on a 12-hour light/dark cycle (6:00 AM to 6:00 PM) at 23±1°C with 30–50 relative humidity. All methods used in this study were approved by the Animal Subjects Programs of The Scripps Research Institute and the University of Georgia and conform to National Institutes of Health guidelines and public law.

Mating, administration of prostanoid receptor agonists, detection of implantation sites, gestation period, and litter size

Females were mated with fertile males and checked for a vaginal plug next morning (midnight of mating night was designated as day 0 00:00). Implantation sites were detected by intravenous injection of blue dye on gestation day 4.5 for the control mice (including wild type and *Lpar3*$^{(+/-)}$, both of which have normal embryo implantation (8)), which was designated as Group 1 in this study, and on gestation day 5.5 for the *Lpar3*$^{(-/-)}$ females (undetectable implantation sites on gestation day 4.5 due to delayed implantation (8)), which was designated as Group 2. The previously reported treatment regimen was followed (8) to determine COX-derived prostanoid signaling in embryo spacing in *Lpar3*$^{(-/-)}$ females. Briefly, gestation day 3.5 *Lpar3*$^{(-/-)}$ females were intraperitoneally injected (at 10:00 and 18:00, respectively) with 100 μl of PGE_2 (5 μg) + cPGI (5 μg) in vehicle (10% ETOH with saline), which was designated as Group 3, or 100 μl of PGE_2 (5 μg) + cPGI (5 μg) + 11-deoxy $PGF_{2\alpha}$ (5 μg) in vehicle, which was designated as Group 4. Implantation sites were detected on gestation day 4.5 as previously described (8). The uterine horns without visible implantation site(s) were flushed with 1×PBS to examine the presence of healthy-looking blastocyst(s) thus the pregnancy status. The non-pregnant mice without implantation sites and healthy-looking blastocysts were excluded in this study. The numbers of mice included in the above four study groups were: N=16 (Group 1), 12 (Group 2), 8 (Group 3), and 13 (Group 4), respectively. Another set of animals from each of these four groups was used to determine the gestation period and litter size at birth. Only the animals delivered pups were included. The numbers of mice were: N=29 (Group 1), 17 (Group 2), 5 (Group 3), and 12 (Group 4), respectively. In addition, there were also three other groups of *Lpar3*$^{(-/-)}$ females for the following treatments: a vehicle-injected *Lpar3*$^{(-/-)}$ group (N=12), a (+)-Fluprostenol (5 μg)-injected *Lpar3*$^{(-/-)}$ group (N=5), and an 11-deoxy $PGF_{2\alpha}$ (5 μg)-injected *Lpar3*$^{(-/-)}$ group (N=16) following the same protocol as the designated Groups 3 and 4 above. Fluprostenol is a metabolically stable analog of $PGF_{2\alpha}$ and an FP agonist. Since these three different treatments only partially rescued or failed to rescue on-time implantation detected on gestation day 4.5 (Fig. 1), these three groups were not included in the analysis of embryo spacing at gestation day 4.5. All the mice were dissected between 11:00 and 12:00 hours on gestation day 4.5 except Group 2 (untreated *Lpar3*$^{(-/-)}$ *Lpar3*$^{(-/-)}$ females) on gestation day 5.5. All the prostanoid receptor agonists used in this study were purchased from Cayman Chemical Company (Ann Arbor, MI, USA).

Analysis of embryo spacing

Each uterus was placed on a weighing paper and stretched evenly to a comparable length for photographing. Embryo spacing was determined by analyzing the distribution of implantation sites along the uterine horns: 1) each uterine horn was divided into 100 even parts, from 0 at the ovary side to 100 at the cervix side, 0~50 and 51~100 were also assigned as distal and proximal uterine segments, respectively (Fig. 2A); 2) each blue band (implantation site) was located by a number, indicating its position on a uterine horn (Fig. 2); 3) the percentage of total implantation sites within the distal and the proximal uterine

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segments from the same group was then determined (Fig. 3A); 4) the mean localization (Mean), the standard deviation (STD), and the coefficient of variation (CV= STD/Mean) for each female with implantation sites were calculated (both STD and CV indicating the relative level of dispersion of implantation sites on the uterine horns); and 5) since each animal had one set of data from step 4, the average Mean, STD, and CV in each group were then calculated (Fig. 3B–3D). This procedure was used to calculate the relative distribution of implantation sites for all four studied groups: Group 1. untreated gestation day 4.5 control; Group 2. untreated gestation day 5.5 *Lpar3*$^{(-/-)}$ females; Group 3. PGE_2 + cPGI-treated gestation day 4.5 *Lpar3*$^{(-/-)}$ females; and Group 4. PGE_2 + cPGI + 11-deoxy $PGF_{2\alpha}$-treated gestation day 4.5 *Lpar3*$^{(-/-)}$ females.

Statistical analysis

Data were expressed as mean ± SEM. χ^2 test was used to analyze the percentage of implantation sites on the distal and proximal uterine segments. Unequal variance student t-test was used for the rest statistical analysis. The significance level was set at $p<0.05$.

Results and Discussion

Vehicle treatment on gestation day 3.5 partially rescued on-time implantation in *Lpar3*$^{(-/-)}$ females examined on gestation day 4.5. Among the 12 vehicle-treated pregnant *Lpar3*$^{(-/-)}$ (pregnancy was defined as the presence of visible implantation site(s) and/or hatched healthy-looking blastocyst(s)), three of them showed 1, 3, and 5 implantation sites, respectively. Some implantation sites appeared to be faint and less defined, suggesting delayed implantation (22), and the two females with multiple implantation sites had uneven embryo spacing (Fig. 1A and data not shown). Nine of the 12 vehicle-treated pregnant *Lpar3*$^{(-/-)}$ females had no detectable implantation sites (Fig. 1B) but hatched healthy-looking blastocysts were flushed from their uterine horns (data not shown). These observations ruled out significant contribution of vehicle injection to the effects from treatments with prostanoid receptor agonists.

Loss-of-function studies have implicated COX-derived prostanoids in embryo spacing (8, 11–15) but the specific COX-derived prostanoid(s) in embryo spacing has not been determined. Myometrial contractions are thought to play an important role in embryo spacing (1–3). Since PGE_2, which induces both contractile (via EP_1 and EP_3) and relaxant (via EP_2 and EP_4) effects, and cPGI, which only induces relaxant (via IP) effect, failed to show any obvious effect on embryo spacing yet could rescue on-time implantation in *Lpar3*(−/−) females (8), we began to test the hypothesis that COX-derived prostanoid signaling with contractile effects on the myometrium may alleviate embryo crowding in *Lpar3*$^{(-/-)}$ females. Among the GPCRs that mediate the signaling of COX-derived prostanoids, FP and TP exclusively mediate uterine contractility (18). We examined an FP agonist, Fluprostenol (a metabolically stable analog of $PGF_{2\alpha}$), and a TP agonist, 11-deoxy $PGF_{2\alpha}$ (a synthetic analog of $PGF_{2\alpha}$) (23).

FP agonist Fluprostenol failed to rescue on-time embryo implantation in *Lpar3*$^{(-/-)}$ females. All seven *Lpar3*$^{(-/-)}$ females (5 pregnant + 2 non-pregnant) treated with FP agonist Fluprostenol on gestation day 3.5 did not show any implantation sites on gestation day 4.5. Compared to vehicle-treated (Fig. 1A, 1B), 11-deoxy $PGF_{2\alpha}$-treated (Fig. 1D), PGE_2 + cPGI-treated (Fig. 2B), or PGE_2 + cPGI + 11-deoxy $PGF_{2\alpha}$-treated (Fig. 2C) *Lpar3*$^{(-/-)}$ females, all seven uteri from Fluprostenol injection had a distended / swollen appearance regardless of the presence (in 5 females, Fig. 1C) or absence (in 2 females, data not shown) of healthy-looking hatched blastocysts (data not shown). Similar result was also observed in all 5 Fluprostenol-injected *Lpar3*$^{(+/-)}$ females (data not shown), which would have normal implantation without the treatment (8). Although Fluprostenol has a contractile effect on

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isolated uterine muscles (24), these data demonstrate that Fluprostenol is a luteolytic factor when administered prior to embryo implantation *in vivo* (Fig. 1C), similar to FP agonist $PGF_{2\alpha}$ as a luteolytic factor (25). Therefore, we focused on the TP agonist 11-deoxy $PGF_{2\alpha}$(23).

TP agonist 11-deoxy $PGF_{2\alpha}$ alone did not fully rescue on-time implantation but seemed to partially alleviate embryo crowding in *Lpar3*$^{(-/-)}$ females. Among the 16 pregnant *Lpar3*$^{(-/-)}$ females treated with 11-deoxy $PGF_{2\alpha}$ alone on gestation day 3.5, 12 of them had detectable implantation sites on gestation day 4.5 with some implantation sites showing delayed implantation (Fig. 1D), the rest four only had hatched healthy-looking blastocysts (data not shown). The percentage of 11-deoxy $PGF_{2\alpha}$-treated pregnant *Lpar3*$^{(-/-)}$ mice with implantation sites (12/16=75%) was significantly higher than that in the vehicle-treated group (3/12=25%). These data demonstrate that 11-deoxy $PGF_{2\alpha}$ alone can partially rescue on-time implantation in *Lpar3*$^{(-/-)}$ females. The average number of implantation sites was 5.08±0.26, which was lower than Group 4 (PGE_2 + cPGI + 11-deoxy $PGF_{2\alpha}$-treated, with on-time implantation and partial alleviation of embryo crowding) (Fig. 4A). The smaller number of implantation sites may reflect incomplete restoration of implantation timing by 11-deoxy $PGF_{2\alpha}$ alone. Regardless, the percentage of the detectable implantation sites on the distal segment (26/61=42.6%) upon 11-deoxy $PGF_{2\alpha}$ treatment was significantly higher than that in the untreated *Lpar3*$^{(-/-)}$ females (Group 2, 13/57=22.8%) (Fig. 3A), and so was the coefficient of variation (0.27±0.02 vs. 0.19±0.02) (Fig. 3D), demonstrating the effect of TP agonist 11-deoxy $PGF_{2\alpha}$ alone on dispersing the implantation sites in *Lpar3*$^{(-/-)}$ uterine horns. To fully evaluate the effect of 11-deoxy $PGF_{2\alpha}$ on embryo spacing without the concern for implantation timing, 11-deoxy $PGF_{2\alpha}$ was injected along with PGE_2 + cPGI, which can rescue on-time implantation in 100% of pregnant *Lpar3*$^{(-/-)}$ females (8), into gestation day 3.5 *Lpar3*$^{(-/-)}$ females. The effects from PGE_2 cPGI + 11-deoxy $PGF_{2\alpha}$ treatment were compared to that from PGE_2 + cPGI treatment to assess the contributions of 11-deoxy $PGF_{2\alpha}$ on embryo spacing at gestation day 4.5 and pregnancy outcome at birth.

Treatment with exogenous PGE_2 + cPGI rescued on-time implantation, but not embryo spacing in *Lpar3*$^{(-/-)}$ females. Compared to the untreated gestation day 5.5 *Lpar3*$^{(-/-)}$ females (Group 2) (8), this treatment regimen did not significantly affect the mean localization of the implantation sites (Fig. 2B, 3B). It did not have an obvious effect on the dispersion of implantation sites along the uterine horns, as evaluated by the percentage of implantation sites on the distal and proximal segments (Fig. 3A), the standard deviation (Fig. 3C) or the coefficient of variation (Fig. 3D) of the localizations of the implantation sites. This treatment regimen also did not have an effect on the number of implantation sites detected on gestation day 4.5 (Fig. 4A).

Treatment with exogenous PGE_2 + cPGI + 11-deoxy $PGF_{2\alpha}$ rescued on-time implantation and partially alleviated embryo crowding in *Lpar3*$^{(-/-)}$ females. All the treated pregnant *Lpar3*$^{(-/-)}$ females had defined implantation sites on gestation day 4.5 indicating on-time implantation. There was a comparable percentage of implantation sites on the distal uterine segment as the untreated gestation day 4.5 control (Group 1), both of which were significantly higher than that observed in the untreated gestation day 5.5 *Lpar3*$^{(-/-)}$ females (Group 2) or PGE_2 + cPGI-treated gestation day 4.5 *Lpar3*$^{(-/-)}$ females (Group 3). Conversely, the percentage of implantation sites on the proximal uterine segment was significantly higher in the Group 2 and Group 3 than that in the Group 1 and Group 4 (Fig. 3A). The distribution of implantation sites on the distal and proximal uterine segments in Group 2 (untreated *Lpar3*$^{(-/-)}$ females at 11:00~12:00 hours on gestation day 5.5) and Group 3 (PGE_2 + cPGI-treated *Lpar3*$^{(-/-)}$ females at 11:00~12:00 hours on gestation day 4.5) was comparable to the localizations of preimplantation embryos observed in the untreated *Lpar3*$^{(-/-)}$ females at 19:00 hours on gestation day 3.5, which showed 12 implantation sites (75%) on the proximal uterine segment and 4 implantation site (25%) on the distal uterine segment (9). These observations indicate that embryo spacing has been roughly determined by the time embryo implantation initiates ~gestation day 4.0 (22). The mean localization of the implantation sites in the PGE + cPGI + 11-deoxy $PGF_{2\alpha}$-treated group (Group 4) was also comparable to the control (Group 1), both of which were significantly lower than that of the untreated gestation day 5.5 (Group 2) or PGE_2 + cPGI-treated gestation day 4.5 *Lpar3*$^{(-/-)}$ females (Group 3) (Fig. 3B). These data reflected more implantation sites on the proximal uterine segment in Group 2 and Group 3 (Fig. 3A). Standard deviation (Fig. 3C) and coefficient of variation (Fig. 3D) of the distribution of implantation sites, which correlated with the dispersion of implantation sites along the uterine horns, were significantly higher in the PGE_2 + cPGI + 11-deoxy $PGF_{2\alpha}$-treated group (Group 4) than the untreated gestation day 5.5 (Group 2) or PGE_2 + cPGI-treated gestation day 4.5 *Lpar3*$^{(-/-)}$ females (Group 3). However, these parameters in Group 4 were still significantly lower than those in the gestation day 4.5 control (Group 1). Similar pattern was also observed with the numbers of implantation sites in these four groups (Fig. 4A). These data (Fig. 3 & 4A) demonstrate that TP agonist 11-deoxy $PGF_{2\alpha}$ can partially disperse the implantation sites along the *Lpar3*$^{(-/-)}$ uterine horns resulting in more implantation sites within the distal uterine segment. However, the partial alleviation of embryo crowding suggests that the treatment regimen in this study doesn't fully mimic the optimal physiological conditions required for even embryo spacing in mice. Consistent with rescued on-time implantation, the gestation periods for both PGE_2 + cPGI-treated (Group 3) and PGE_2 + cPGI + 11-deoxy $PGF_{2\alpha}$-treated (Group 4) *Lpar3*$^{(-/-)}$ females were significantly shorter than the untreated *Lpar3*$^{(-/-)}$ females (Group 2) but comparable to the control females (Group 1, data not shown). At birth, the litter size from the PGE_2 + cPGI + 11-deoxy $PGF_{2\alpha}$-treated *Lpar3*$^{(-/-)}$ females (Group 4) was significantly larger than that from *Lpar3*$^{(-/-)}$ females only receiving PGE_2 + cPGI (Group 3), which was itself significantly larger than that from the untreated *Lpar3*$^{(-/-)}$ females (Group 2). However, the litter sizes from the above three groups were all significantly smaller than the control (Group 1) (Fig. 4B). These data demonstrate that the two segregated events, delayed implantation and embryo crowding, can both contribute to the reduced litter size in *Lpar3*$^{(-/-)}$ females (8, 9).

TP is a GPCR that can activate the phospholipase C pathway to increase intracellular calcium levels. The binding of calcium to calmodulin activates myosin light chain (MLC) kinase, which phosphorylates the MLC on the myosin heads. MLC phosphorylation facilitates the interaction between the myosin heads and the actin filaments, leading to myometrial contraction (26). Although the molecular mechanism for embryo spacing has not been fully understood, myometrial contractions seem to play critical roles on embryo spacing (1–3). Since TP mediates a contractile effect on the myometrium (18, 26), the partial alleviation of embryo crowding in the *Lpar3*$^{(-/-)}$ females by TP agonist 11-deoxy $PGF_{2\alpha}$ (Fig. 2~4) supports myometrial contraction in embryo spacing.

Studies have demonstrated that myometrial contractions are related to embryo spacing. For example, inhibition of α_1-adrenoceptor (whose activation leads to smooth muscle contraction via increased intracellular calcium levels) or activation of β_2-adrenoceptor (whose activation leads to smooth muscle relaxation via increased cAMP levels) can both induce embryo crowding (10, 27). Nicotine and relaxin, both of which could decrease uterine contractions, can also cause embryo crowding (3, 28). Although embryo crowding could happen along the uterine horns, it seems that more embryo crowding occurs in the distal uterine segment (close to the ovary side) after the above pharmacological treatments that either inhibit myometrial contraction (3, 27, 28) or promote myometrial relaxation (10). However, more embryo crowding is seen in the proximal uterine segment (close to the cervix side) in *Lpar3*(−/−) and *Pla2g4a*$^{(-/-)}$ females (8, 15). It is understandable to state

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that embryo crowding indicates disrupted myometrial activities required for even embryo spacing.

However, the mechanism for the differential localization of embryo crowding is unknown. It has been demonstrated that *Lpar3* expression is decreased in the β_2-adrenoceptor agonist-treated uteri, which have more embryo crowding in the distal uterine segment (10), and that the localization of more embryo crowding in the *Lpar3*$^{(-/-)}$ females is shifted from the distal uterine segment at 12:00 hours on gestation day 3.5 to the proximal uterine segment at 19:00 hours on gestation day 3.5 and later (9). Based on these observations, we speculate that the localization of embryo crowding may reflect the timing and degree of the disrupted coordination between myometrial contraction and relaxation. For example, the effects of pharmacological treatments are associated with the treatment timing and the pharmacokinetics of the drugs, whereas the effects from gene deletion (e.g., *Lpar3*$^{(-/-)}$ and *Pla2g4a*$^{(-/-)}$ females) are constant. These differences may have influences on the localizations of embryo crowding along the uterine horns.

Although studies have shown that LPA can induce uterine contraction (29), LPA_3 can mediate LPA_3 agonist-induced uterine contraction (9), and the contractile TP agonist 11-deoxy $PGF_{2\alpha}$ can partially alleviate embryo crowding in the *Lpar3*$^{(-/-)}$ females (Fig. 2~4), it remains to be determined how LPA_3, whose mRNA is specifically detected in the uterine epithelium (8), influences embryo spacing (8, 9), a process involving myometrial contraction (1–3, 10, 27, 28).

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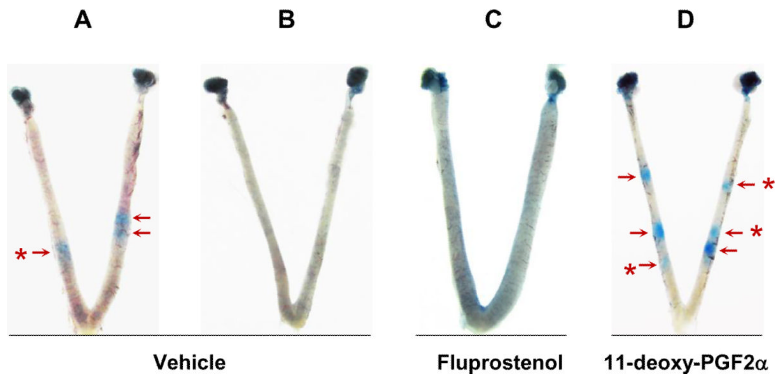

Figure 1.

Effects of vehicle, Fluprostenol (a metabolically stable analog of $PGF_{2\alpha}$ and an FP agonist), and 11-deoxy $PGF_{2\alpha}$ (a TP agonist) T **on embryo implantation in *Lpar3*$^{(-/-)}$ females detected on gestation day 4.5.** A. One of three vehicle-treated *Lpar3*$^{(-/-)}$ uteri with implantation sites. Red arrows indicate implantation sites. B. A representative of nine vehicle-treated pregnant *Lpar3*$^{(-/-)}$ uteri without detectable implantation sites but with hatched and healthy-looking blastocysts. C. A representative Fluprostenol-treated pregnant *Lpar3*$^{(-/-)}$ uterus showing distended appearance. D. A representative 11-deoxy $PGF_{2\alpha}$-treated *Lpar3*$^{(-/-)}$ uterus. Red arrows indicate implantation sites; red stars show fainter/less defined blue bands, indicating delayed implantation.

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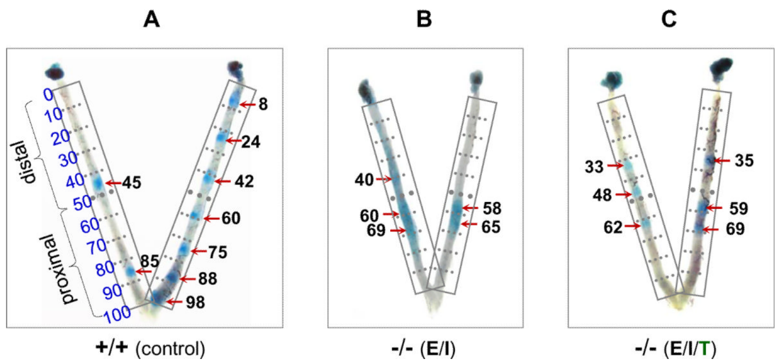

Figure 2. Relative localization of implantation sites

A. A representative gestation day 4.5 wild type (+/+) uterus showing how the uterine horns are divided to give each implantation site a number for its relative localization. **B.** A representative gestation day 4.5 *Lpar3*$^{(-/-)}$ uterus treated with PGE_2 (E) and cPGI (I). **C.** A representative gestation day 4.5 *Lpar3*$^{(-/-)}$ uterus treated with E, I and 11-deoxy $PGF_{2\alpha}$ (**T**), a TP agonist. Red arrows indicate implantation sites and the numbers indicate the relative localizations of the implantation sites on the uterine horns.

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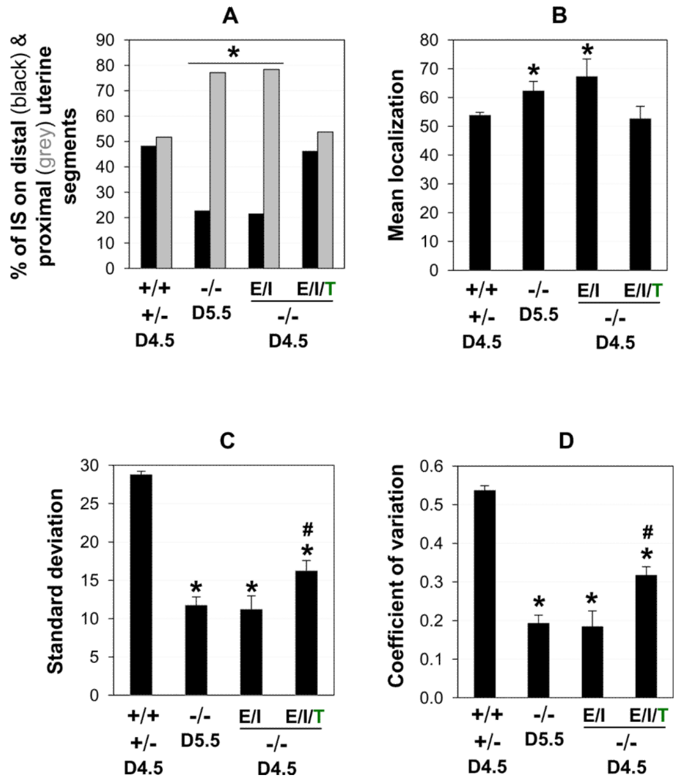

Figure 3. Effects of PGE_2, cPGI, and 11-deoxy $PGF_{2\alpha}$ on embryo spacing

A. The percentages of implantation sites on the distal and proximal uterine segments among the following four groups: Group 1: Gestation day 4.5 (D4.5) untreated control females, which include both wild type (+/+) and *Lpar3* heterozygote (+/−); Group 2: Gestation day 5.5 (D5.5) untreated *Lpar3*$^{(-/-)}$ (−/−) females; Group 3: Gestation day 4.5 (D4.5) *Lpar3*$^{(-/-)}$ (−/−) females treated with PGE_2 (E) and cPGI (I); Group 4: Gestation day 4.5 (D4.5) *Lpar3*$^{(-/-)}$ (−/−) females treated with PGE_2 (E), cPGI (I) and 11-deoxy $PGF_{2\alpha}$ (**T**). The total number of implantation sites in each group was: 149, 57, 37, and 80, respectively. * P<0.05 compared to Group 1 or Group 4 for the percentage of implantation sites either on the distal (black bars) or on the proximal (grey bars) uterine segments. χ^2 test. **B.** The mean localization of the implantation sites. * P<0.05 compared to Group 1 or Group 4. **C.** The standard deviation of the implantation site distribution. **D.** The coefficient of variation of the implantation site distribution. B~D. N=16 (Group 1), 12 (Group 2), 8 (Group 3), and 13 (Group 4), respectively. C & D. * P<0.05 compared to Group 1; # P<0.05 compared to Group 2 or Group 3. B~D. Error bars represent standard errors. Unequal variance student t-test was used.

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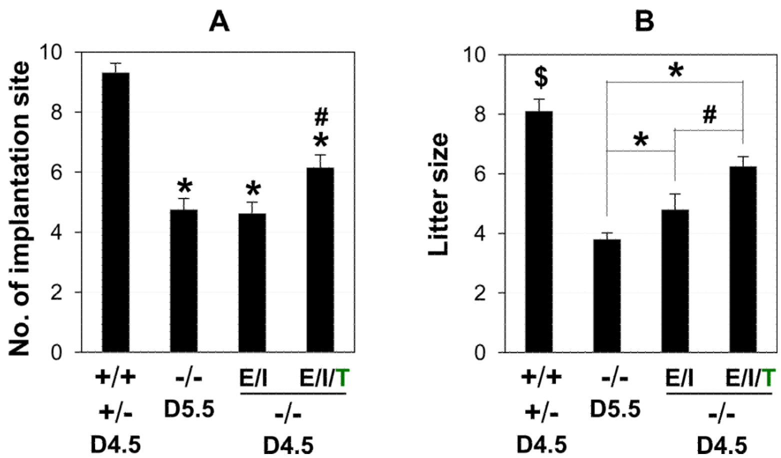

Figure 4. Effects of PGE_2, cPGI, and 11-deoxy $PGF_{2\alpha}$ on the number of implantation sites on gestation day 4.5 and litter size at birth

A. The number of implantation sites. N=16 (Group 1), 12 (Group 2), 8 (Group 3), and 13 (Group 4), respectively. * $P<0.05$ compared to Group 1; # $P<0.05$ compared to Group 2 or Group 3. **B.** Litter sizes for Groups 1–4. N=29 (Group 1), 17 (Group 2), 5 (Group 3), and 12 (Group 4), respectively. *, #, $ $P<0.05$; $, compared to Groups 2, 3, or 4. Error bars represent standard errors. Mice were treated on gestation day 3.5; implantation sites were detected on gestation day 4.5 for Groups 1, 3, and 4, and gestation day 5.5 for Group 2; litter sizes were recorded at birth. Unequal variance student t-test was used.