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Restoration of On-Time Embryo Implantation Corrects the Timing of Parturition in Cytosolic Phospholipase A2 Group IVA Deficient Mice[1]

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ABSTRACT

Cytosolic phospholipase A2 (cPLA2, PLA2G4A) catalyzes the release of arachidonic acid for prostaglandin synthesis by cyclooxygenase 1 (PTGS1) and cyclooxygenase 2 (PTGS2). Mice with *Pla2g4a* deficiency have parturition delay and other reproductive deficits, including deferred onset of implantation, crowding of implantation sites, and small litters. In this study, we examined the contribution of PLA2G4A to parturition in mice. *Pla2g4a* mRNA and protein expression were discretely localized in the term and preterm uterine luminal epithelium and colocalized with *Ptgs1*, but not *Ptgs2*, expression. The levels of PGE2, PGF2alpha, 6-keto-PGF1alpha, and TxB2 were significantly decreased in *Pla2g4a*-null uterine tissues, similar to *Ptgs1*-null uteri, consistent with predominance of PLA2G4A-PTGS1-mediated prostaglandin synthesis in preparation for murine parturition. Litter size was strongly associated with the timing of parturition in *Pla2g4a*-null mice but could not fully account for the parturition delay. *Pla2g4a*-null females that received PGE2 + carbaprostacyclin at the time of implantation delivered earlier (20.5 ± 0.2 days vs. 21.6 ± 0.2 days, $P < 0.01$), although litter size was not improved (4.6 vs. 4.4 pups per litter, $P = 0.6$). After correction for small litter size, multivariate analysis indicated that *Pla2g4a*-null mice given prostaglandin treatment to improve implantation timing had gestational length that was similar to wild-type and *Pla2g4a* heterozygous mice. These results indicate that, despite specific *Pla2g4a* expression and function in term gestation uteri, the delayed parturition phenotype in *Pla2g4a*-null mice is primarily due to deferral of implantation. The role of PLA2G4A in timely parturition appears to be critically related to its actions in early pregnancy.

cPLA2, mouse, parturition, phospholipase, PLA2G4A, pregnancy, prostaglandin, uterus

INTRODUCTION

Prostaglandins are essential mediators of the parturition process. The generation of prostaglandins from lipid precursors involves multiple enzymes, including phospholipases, cyclooxygenase 1 (PTGS1), cyclooxygenase 2 (PTGS2), and specific downstream prostaglandin synthases. Among the phospholipases (PL), PLA2 is the predominant enzyme for hydrolysis of membrane fatty acids to produce lysophospholipids and the release of arachidonic acid from glycerophospholipids. This step forms the first critical event in the production of prostaglandins, thromboxanes, leukotrienes, and other bioactive lipid signaling molecules. The PLA2 superfamily is composed of distinct enzymes with different sizes, substrate specificities, cofactor utilizations, and subcellular localizations [1], predisposing their placement into one of five principal groups, including secretory PLA2s (sPLA2, PLA2G1–3, 5, 9–14), cytosolic PLA2s (cPLA2, hereafter PLA2G4), calcium-independent PLA2s (iPLA2, PLA2G6), PAF acetylhydrolase, and lysosomal PLA2s [2].

The PLA2G4 family itself consists of A–F isoforms and differs from other PLA2 members through their preference for esterified arachidonic acid in the *sn-2* position. After a physiological or pathological stimulus, changes in intracellular calcium stimulate PLA2G4A translocation from the cytoplasm to the nuclear envelope or endoplasmic reticulum and phosphorylation by MAP kinase [3–5]. In contrast, sPLA2 isoforms localize to the cell surface or perinuclear space, depending on enzyme isotype [6]. sPLA2 enzymes have diverse functions that include arachidonic acid release, but there is uncertainty regarding the contribution of sPLA2s to eicosanoid production [7, 8]. PLA2G4A-induced liberation of arachidonic acid within the lipid bilayer of cell membranes occurs in response to both internal and external cellular cues, providing a direct source of arachidonic acid as substrate for the inwardly facing catalytic cleft of the PTGS enzymes in intracellular and plasma membranes [9]. The close relationship of PLA2G4A with PTGS1 and PTGS2 and their downstream synthases has been considered to represent a functional coupling between these enzymes for the production of prostaglandins in various cells and tissues [10–15]. However, the role of PLA2G4A and its interaction with PTGS enzymes in the uterus for control of parturition is less clear.

In the mouse uterus, PTGS1 plays a critical role in parturition, as evidenced by high levels of *Ptgs1* expression in the uterine luminal epithelium and parturition failure in *Ptgs1*-deficient mice [16–19]. Peak *Ptgs1* expression during midgestation suggests that prostaglandins from this isoform are involved in uterine activation and preparation for labor [16, 18, 20–22]. On the other hand, *Ptgs2* is not upregulated until late in the parturition process [20, 21, 23] and is primarily expressed in the placental labyrinth and decidua [18]. Females with *Ptgs2* deficiency have multiple reproductive deficits [24–26] that preclude a complete examination of its role in parturition, although there is evidence that mice with *Ptgs2* deletion can deliver full-term offspring [27] but may have some delay in the onset of labor due to deferral in the timing of implantation [28].

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Pharmacological studies also reveal a role for PTGS2 at a later time point in parturition, after the decline in serum progesterone [29], or in response to infection [30], suggesting an ordered sequence of PTGS1- and PTGS2-mediated events that are required to complete successful parturition in the mouse.

Given the hierarchical relationship between PLA2G4A, PTGS1, and PTGS2, a critical role for PLA2G4A in parturition would be anticipated. However, previous studies showed that PLA2G4A expression and functional activity in the mouse uterus is unchanged over the course of pregnancy [22, 31]. Mice with targeted deletion of the *Pla2g4a* gene have a significant delay in parturition and marked perinatal lethality [32, 33], similar to *Ptgs1*-null mice, suggesting that PLA2G4A makes an important contribution to parturition. However, *Pla2g4a*-deficient females also have early reproductive abnormalities, including delay in the onset of implantation, abnormal embryo spacing [34], and small litter size [32–34], that can lead to parturition delay or failure. Thus, the contribution of PLA2G4A to parturition remains obscured by coexisting reproductive deficits. We hypothesized that PLA2G4A plays an essential role in parturition in addition to its other effects on female reproduction. To determine whether the prolonged gestational period of *Pla2g4a*-null mice is due to impaired parturition per se or occurs as a result of delayed implantation, reduced litter size, or other factors, we examined *Pla2g4a* expression and function in wild-type and knockout females. The effect of genetic background on the *Pla2g4a*-null phenotype was evaluated. Prostaglandin supplementation was also performed in an attempt to rescue the parturition phenotype of *Pla2g4a*-deficient mice by improving the timing of embryo implantation.

MATERIALS AND METHODS

Animals and Tissue Preparation

All studies were conducted in accordance with the standards of humane animal care described in the NIH Guide for the Care and Use of Laboratory Animals using protocols approved by the Vanderbilt University institutional animal care and use committee. C57BL/6J and CD1 (Charles River Laboratory, Raleigh, NC) mice were housed in an AAALAC-approved animal care facility. Adult female mice (20–25 g, 48–60 days old) were mated with fertile males of the same strain and genotype to produce timed pregnancies. The morning of finding a vaginal plug was designated as Day 1 of pregnancy. Delivery at term gestation typically occurs on the evening of Day 19 in this colony. Mice were anesthetized with isoflurane and killed by cervical dislocation between 0830 and 0930 h on Day 15 or 19 of pregnancy. Uterine wall or whole utero-fetal segments were excised, snap-frozen, and stored at −80°C for later analysis.

Mice with targeted deletion of the *Pla2g4a* gene were generated on a C57BL/6J genetic background and genotyped as previously described [32]. *Pla2g4a*-null females were mated with homozygous null males to produce litters of nullizygous offspring. Due to the existence of naturally occurring sPLA2 mutations in C57BL/6J and other inbred mouse strains [35, 36], *Pla2g4a* deficiency was reestablished in albino CD1 outbred mice through 10-generation outcross matings to minimize concerns for potential sPLA2 effects and to create *Pla2g4a* deletion in a strain with vigorous reproductive characteristics. *Pla2g4a*-null mice on the CD1 background have early reproductive deficits similar to C57BL/6J mice [34], but their parturition outcome after outcross breeding is unknown. Parturition timing was determined by continuous video recording of pregnant females under infrared-filtered light in rooms with 12L:12D (light 0700–1900 h). The time of parturition was designated as the complete delivery of the first pup.

A subset of *Pla2g4a*-null females was treated with an established regimen for prostaglandin supplementation to circumvent prostaglandin deficiency at the time of implantation [34]. Briefly, PGE2 and carbaprostacyclin (cPGI), a stable PGI2 analog, were stored in 100% ethanol at −20°C (Cayman Chemical, Ann Arbor, MI). At 1000 h on Day 4 of pregnancy, separate i.p. injections of 5 μg of PGE2 and 5 μg of cPGI were given (in 10% ethanol/90% saline [vol/vol]). A second set of PGE2 and cPGI injections was similarly administered at 1800 h on Day 4. Parturition outcomes were monitored by video recording.

Prostaglandin Content

Levels of PGE2, PGF2α, 6-keto-PGF1α (the stable hydrolysis product of PGI2), and thromboxane B2 were quantitatively determined by gas chromatographic/negative ion chemical ionization mass spectrometric assays. These species were chosen in order to compare the prostaglandin content to previous measurements obtained in wild-type and *Pla2g4a*-null uteri during the peri-implantation period [34]. Briefly, uterine tissues stored at −80°C were pulverized in liquid nitrogen. The nitrogen was evaporated and the lipids extracted with 5 ml of ice-cold methanol containing 10^{-6} M indomethacin. To this solution, 1 ng of [$^2H_4$]-15-F_{2t}-IsoP ([$^2H_4$]-8-iso-PGF_{2a}), [$^2H_4$]-PGD_2, [$^2H_4$]-PGE_2, [$^2H_3$]-11-dehydro-thromboxane B_2 (11-dehydro-TxB_2), and [$^2H_4$]-6-keto-PGF_{1a} (Cayman Chemicals) was added as internal standard, and the prostanoids were purified, derivatized, and analyzed as described [37]. Levels of endogenous eicosanoids in the biological samples are calculated from the ratio of intensities of the [$^2H_0$] and [$^2H_4$] ions. The interday variability for each assay is <10%. The precision for each assay is ±5%, while the accuracy for each assay is 95%.

In Situ Hybridization

Templates for cRNA probes were generated from RT-PCR-derived cDNAs for *Pla2g4a*, *Ptgs1*, and *Ptgs2* [18, 34]. Partial cDNAs were subcloned (TOPO TA; Invitrogen, Carlsbad, CA) and sequenced to verify gene identity and orientation within the vector. Sense and antisense ^{35}S-labeled cRNA probes were generated using the appropriate polymerases. Probes had specific activity of approximately 2×10^9 dpm/μg.

In situ hybridization was performed as previously described [18]. Briefly, small segments of uterine tissues were snap-frozen in liquid Super Friendly Freeze-It (Fisher Scientific, St. Louis, MO). Frozen sections (11 μm) were mounted onto poly-L-lysine-coated slides, fixed in cold 4% paraformaldehyde solution in PBS, acetylated, and hybridized at 45°C for 4 h in hybridization buffer containing the ^{35}S-labeled probes. After hybridization, sections were incubated with ribonuclease A (RNase A; 20 μg/ml) at 37°C for 20 min. RNase A-resistant hybrids were detected by autoradiography using Kodak NTB-2 liquid emulsion (Eastman Kodak, Rochester, NY). Parallel sections were hybridized with sense cRNA probes to serve as negative controls. Slides were developed after 3- to 5-wk exposure periods. Sections were briefly poststained with hematoxylin and eosin.

Immunohistochemistry

A rabbit anti-human polyclonal antibody (ab53105; Abcam Inc., Cambridge, MA) that detects PLA2G4A when phosphorylated at the serine 505 residue was used to examine the cellular localization of activated PLA2G4A. Rabbit polyclonal antibodies raised against the N-terminal region of mouse PTGS1 and the C-terminal region of mouse PTGS2 [38] were a generous gift from Dr. S.K. Dey (Cincinnati Children's Research Foundation, Cincinnati, OH). Frozen sections of mouse utero-fetal tissues on Days 16 and 19 of pregnancy were thaw-mounted onto poly-L-lysine-coated slides and fixed in 4% paraformaldehyde or Bouin solution for 10 min at 4°C. Immunolocalization was performed by serial washing steps, blocking nonspecific staining with 10% nonimmune serum for 10 min and overnight incubation with a 1:500 dilution of each primary antibody at 4°C according to the manufacturer's recommendations (Zymed Laboratories Inc., San Francisco, CA). Slides were then washed and incubated with secondary antibody for 10 min, briefly exposed to 0.23% periodic acid to block endogenous peroxidase activity, washed, and exposed to peroxidase substrate. Slides were observed under direct visualization to determine maturity of the reaction. Immunoreactive protein was detected as red-brown deposits. Sections were lightly counterstained with hematoxylin. Negative controls were similarly treated except were incubated overnight with 10% nonimmune serum without addition of the primary antibody. Replicate experiments were performed at least three times with tissues from two to three mice at each time point.

Statistical Analysis

Data are summarized as mean ± SD or mean changes with 95% confidence intervals (CI) and *P*-values. Student *t*-test was used to compare prostaglandin content in wild-type and knockout tissues. Linear regression was used to examine the association between length of gestation, genotype, and prostaglandin treatment in unadjusted models and multivariate models that controlled for litter size. Controlling for litter size using a nonlinear function (2 *df* spline) was considered, but no evidence was found that this model provided a better fit to the dataset. Nonparametric methods as well as interaction of litter

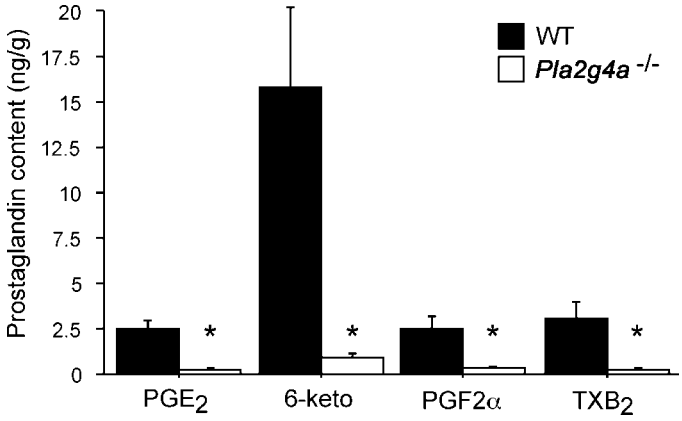

FIG. 1. Prostaglandin levels in wild-type and *Pla2g4a*-null uteri. The prostaglandin content of uterine tissues from pregnant wild-type (WT; n = 5) and *Pla2g4a*-null (n = 6) dams on Day 19 of gestation was determined by gas chromatography-mass spectrometry. Data are mean ± SD. *$P < 0.01$, compared to WT.

size, genotype, and treatment were also considered, but these more complex models did not alter the conclusions of the analysis.

RESULTS

Reduced Prostaglandin Content in Pla2g4a-*Null Uteri*

Uterine tissues, excluding the placenta and implantation site, were obtained on the morning of anticipated delivery (Day 19), when prostaglandins play critical roles in myometrial activation and contractility, cervical ripening, and luteolysis. Wild-type uterine tissues had the expected pattern of prostaglandin levels at term gestation [18], including high levels of the stable prostacyclin derivative 6-keto-PGF1α. In contrast, *Pla2g4a*-null uteri had significantly reduced levels of PGE2, 6-keto-PGF1α, PGF2α, and thromboxane compared to wild-type uteri (Fig. 1), with over 10-fold reduction in each prostanoid species.

Localization of Pla2g4a *in Utero-Placental Tissues*

To better understand the reduced prostaglandin levels in *Pla2g4a*-null uteri and the abnormal reproductive phenotypes of *Pla2g4a*-deficient mice, the cell-specific localization pattern of *Pla2g4a* was examined by in situ hybridization. Antisense cRNA probes to *Pla2g4a* demonstrated specific gene expression in the uterus and placenta (Fig. 2). Autoradiographic signals were primarily localized in the uterine luminal epithelium and were present along the interface of the placenta and uterus up to the point where the epithelium stopped at the site of placental attachment (Fig. 2, a and b). *Pla2g4a* expression in the luminal epithelium was observed around the full circumference of the uterus as it encircled the fetus (not shown). There were no differences in the levels of *Pla2g4a* gene expression or its localization pattern between Day 16 and Day 19 uterine tissues. In Day 16 placental tissues, *Pla2g4a* expression was occasionally noted in undefined cells between the labyrinth and spongiotrophoblast layers (Fig. 2a). Antisense *Pla2g4a* probes hybridized to Day 19 tissues from *Pla2g4a*-null mice that were mounted on the same glass slides as Day 16

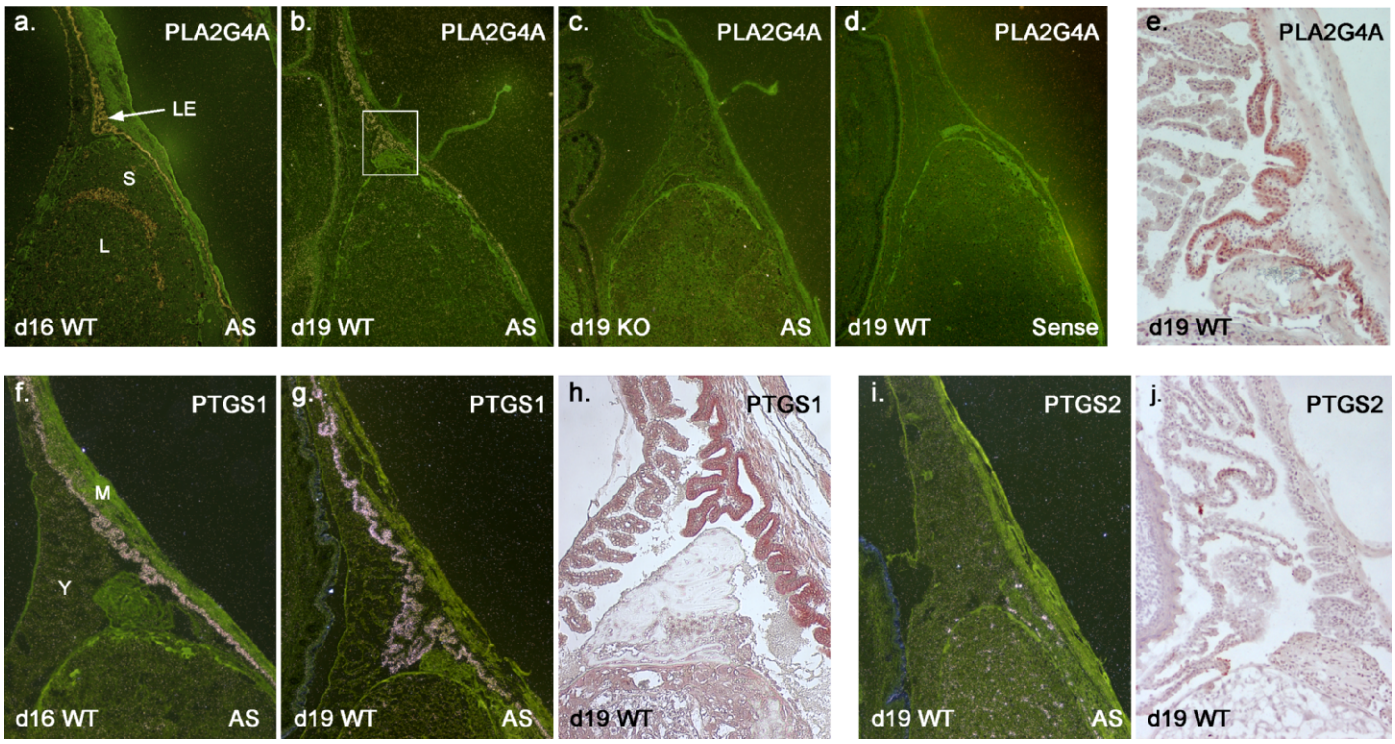

FIG. 2. Localization of PLA2G4A and PTGS enzymes in utero-placental tissues. Cell-specific localization patterns for *Pla2g4a*, *Ptgs1,* and *Ptgs2* mRNA and protein were determined by in situ hybridization and immunohistochemistry. Dark-field images show autoradiographic signal accumulation of antisense (AS) probes in the Day 16 (d16) wild-type (WT) placenta (**a**) and uterine luminal epithelium (LE) (**a**, **b**). Antisense probes failed to detect *Pla2g4a* expression in *Pla2g4a* knockout (KO) uteri (**c**); sense *Pla2g4a* probes were negative at sites of specific hybridization (**d**). Antibodies specific for the activated form of PLA2G4A localized to the LE in parallel tissue sections (**e**, inset from **b**; original magnification ×100). ^{35}S-labeled *Ptgs1* hybridized to the uterine LE of Day 16 (d16) and Day 19 (d19) uteri, similar to *Pla2g4a* (**f**, **g**). PTGS1 immunostaining was also similar to PLA2G4A (**h**). *Ptgs2* mRNA and protein were not detected in the uterine LE (**i**, **j**), but were present in the placenta and deciduas (not shown). Images are representative of replicates from three-five animals. M, myometrium; L, labyrinth; S, spongiotrophoblast; Y, yolk sac and fimbria. Original magnification ×40 (**a–d** and **f–j**).

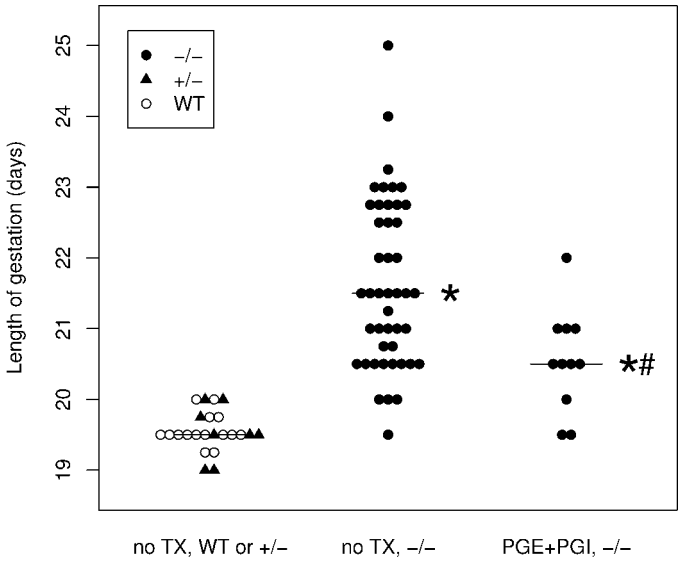

FIG. 3. Length of gestation by genotype and treatment group. Parturition timing was monitored by infrared video recording and clustered in quartile blocks. Mean length of gestation is represented by the horizontal line. Outcomes for individual wild-type (WT; white circles; n = 15), heterozygous (+/–; black triangles; n = 8), or homozygous pregnant females (–/–; black circles) are presented. *Pla2g4a*-null animals received no treatment (no TX; n = 45) or two serial injections of PGE2 and carbaprostacyclin (PGI; n = 11) on the morning and evening of Day 4 of pregnancy to improve the timing of embryo implantation. *$P < 0.01$, compared to WT; #$P < 0.01$, compared to untreated *Pla2g4a*-null mice.

and Day 19 wild-type tissues revealed the absence of *Pla2g4a* expression in the placenta and uterine luminal epithelium (Fig. 2c). In addition, parallel tissue sections hybridized with sense probes were negative at sites of specific hybridization

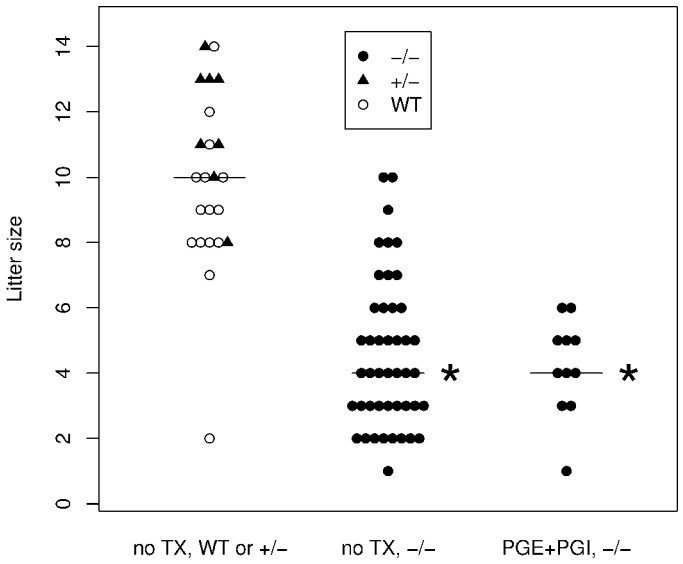

FIG. 4. Litter size by genotype and treatment group. Litter size was determined at completion of parturition by video recording and direct inspection. Mean litter size is represented by the horizontal line. Outcomes for individual wild-type (WT; white circles; n = 15), heterozygous (+/–; black triangles; n = 8), or homozygous pregnant females (–/–; black circles) are presented. *Pla2g4a*-null animals received no treatment (no TX; n = 45) or two serial injections of PGE2 and carbaprostacyclin (PGI; n = 11) on the morning and evening of Day 4 of pregnancy to improve the timing of embryo implantation. *$P < 0.01$, compared to WT.

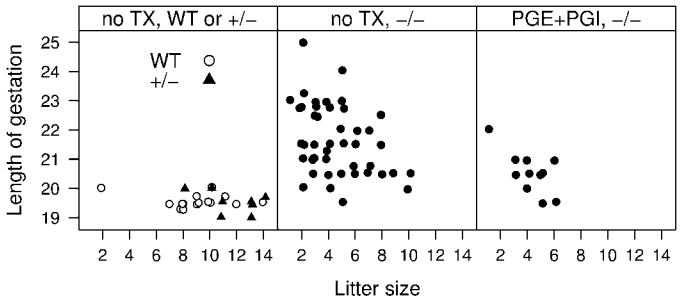

FIG. 5. Relationship between length of gestation and litter size by genotype and treatment group. Length of gestation was plotted as a function of litter size. Outcomes for individual wild-type (WT; white circles; n = 15), heterozygous (+/–; black triangles; n = 8), or homozygous pregnant (–/–; black circles) females are presented. *Pla2g4a*-null animals received no treatment (no TX; n = 45) or two serial injections of PGE2 and carbaprostacyclin (PGI; n = 11) on the morning and evening of Day 4 of pregnancy to improve the timing of embryo implantation. Litters with similar outcomes are shown as off-centered overlapping points.

(Fig. 2d). Serial tissue sections were also used to determine the localization of PLA2G4A protein. Similar to its gene expression pattern, immunoreactive PLA2G4A was observed in the uterine luminal epithelium and was absent in the adjacent myometrial, placental, and yolk sac tissues on Day 19 of gestation (Fig. 2e). Cytoplasmic localization of the phosphorylated, active form of PLA2G4A was consistent with its expression in positive controls and its established intracellular distribution pattern.

The cell-specific expression pattern of *Ptgs1* mRNA in Day 16 and Day 19 utero-placental tissues was very similar to *Pla2g4a* gene expression (Fig. 2, f and g). PTGS1 protein also localized to the cytoplasm of uterine luminal epithelial cells, similar to PLA2G4A (Fig. 2h). By comparison, *Ptgs2* gene expression is highly localized to the decidual layer of the placenta in Day 19 tissues [18] (not shown) and sparingly expressed in giant cells and other cells scattered throughout the placental labyrinth (Fig. 2i). In contrast to the distribution of *Pla2g4a* and *Ptgs1*, *Ptgs2* gene and protein expression was absent in the uterine luminal epithelium (Fig. 2, i and j).

Role of PLA2G4A in Timely Parturition

To determine whether PLA2G4A makes a significant contribution to the parturition process independent of its effects on implantation timing [34] or litter size [32, 33], we examined the delayed parturition phenotype of *Pla2g4a*-null mice in more detail. Wild-type and *Pla2g4a* heterozygous mice had an average length of gestation of 19.5 ± 0.3 days. *Pla2g4a*-null mice had an average length of gestation of 21.6 ± 1.2 days, which was 2.1 days longer than heterozygotes/wild types ($P < 0.001$; 95% CI = [1.6, 2.6]; Fig. 3). There was no difference in the parturition delay phenotype of *Pla2g4a*-null mice between C57BL/6J (n = 16) and CD1 (n = 29) genetic backgrounds; thus, these animals were considered as a single group for the remaining analyses. In an effort to overcome the effects of *Pla2g4a* deficiency on the timing of embryo implantation, pregnant *Pla2g4a*-null females were treated with PGE2 and cPGI, a stable prostacyclin analogue, on Day 4 of pregnancy according to an established regimen [34]. Prostaglandin-treated *Pla2g4a*-null mice had an average length of gestation of 20.5 ± 0.7 days, which was 1.1 days shorter than untreated *Pla2g4a*-null mice ($P = 0.001$; 95% CI = [0.5, 1.7]). Although prostaglandin supplementation significantly reduced the parturition delay in *Pla2g4a*-null females, their

length of gestation was still 1.0 ± 0.4 days longer than heterozygous or wild-type females ($P < 0.01$; 95% CI = [0.3, 1.7]; Fig. 3).

Wild-type and heterozygous females had a mean litter size of 9.9 ± 2.7 pups. *Pla2g4a*-null females had significantly smaller litters (4.6 ± 2.3 pups, $P < 0.001$), consistent with prior reports [32, 33] (Fig. 4). Although treatment of *Pla2g4a*-null mice with PGE2 and cPGI caused a significant reduction in the length of gestation (Fig. 3), this improvement was not because of an increase in the number of pups that were carried to term gestation, since the litter size of treated and untreated *Pla2g4a*-null females was similar ($P = 0.63$); Fig. 4). When gestational length was considered as a simple function of litter size (Fig. 5), an inverse relationship between length of gestation and litter size became apparent in the *Pla2g4a*-null treated and untreated groups, prompting further analysis.

In multiple regression models controlling for litter size, *Pla2g4a*-null mice were found to have a length of gestation that was 1.3 days longer than heterozygotes/wild types ($P < 0.001$; 95% CI = [0.7, 2.0]). Treatment of *Pla2g4a*-null mice with PGE2 and cPGI reduced the parturition delay by 1.1 days compared to untreated *Pla2g4a*-null mice ($P < 0.001$; 95% CI = [0.5, 1.7]). The length of gestation of PGE2- and cPGI-treated *Pla2g4a*-null mice was only 0.2 days longer than heterozygotes/wild types, after controlling for litter size, and was not statistically different ($P = 0.63$; 95% CI = [−0.6, 1.0]), suggesting that rescue of implantation timing [34] may be partially responsible for the improvement in parturition timing seen in prostaglandin-treated *Pla2g4a*-null mice. Regression analysis confirmed the significant negative association of litter size and length of gestation in the treated and untreated *Pla2g4a*-null groups (Fig. 5), such that every one-pup increase in litter size was associated with a 0.14 day decrease in length of gestation (95% CI = [−0.23, −0.05]).

DISCUSSION

Phospholipases mediate the earliest step in prostaglandin synthesis and are expressed in gravid intrauterine tissues of numerous species, suggesting a regulatory role in parturition. Contrary to our hypothesis, the major finding of this study is that PLA2G4A is not essential for the parturition process in mice. Despite our observation that *Pla2g4a* is highly expressed and restricted to the uterine epithelium, and the finding of significantly reduced prostaglandin levels in *Pla2g4a*-null uterine tissues, the present studies demonstrate that parturition in the mouse can occur in a timely manner if early reproductive deficits and litter size are accounted for when *Pla2g4a* is genetically inactivated. These results further suggest that the timing of parturition is more strongly associated with the timing of embryo implantation than litter size, and extend earlier findings on the relationship between on-time embryo implantation and subsequent fetal development to include the timing of labor.

Phospholipase-catalyzed release of arachidonic acid from membrane phospholipids is a critical enzymatic checkpoint for the production of prostaglandins. The continual identification of new PLA2 family members has complicated efforts to assess the contribution of each isoform to parturition, although expression or function of PLA2 enzymes has been reported in gestational tissues of most species [6]. Less is known about PLA2G4A actions in parturition than other PLA2 isoforms. However, *Pla2g4a* expression has been demonstrated in human amnion, chorion, placenta, and myometrium [39–44]. Accumulation of PLA2G4A protein was noted in the amnion, chorion, and decidua vera of laboring compared to non-laboring women [44]. PLA2G4A activity also increased in human amnion with advancing gestational age [41], although it remained unchanged in the myometrium during pregnancy and parturition [42, 43]. *Pla2g4a* gene and protein expression is increased in association with spontaneous term labor or induced preterm labor in both the endometrium and myometrium of sheep [45]. PLA2G4A expression is also increased in the amnion of rabbits at term gestation [46]. In rodents, Kurusu et al. [47] showed that PLA2G4A activity is increased with advancing gestation in the uterus of rats, with peak activity at the time of parturition and decline soon after birth. Although peak PLA2G4A activity occurred after the decline in serum progesterone levels, treatment with a progesterone receptor agonist or antagonist did not change uterine PLA2G4A activity [48]. Farina et al. [49] found similar results in the rat uterus and additionally reported that PLA2G4A expression and activity decline after treatment with an oxytocin antagonist but not an antiestrogen, suggesting that PLA2G4A is insensitive to changes in ovarian steroid hormones at the time of parturition. Due to the prolonged length of gestation in *Pla2g4a*-null mice and a presumptive role for PLA2G4A in other species, a regulatory role for PLA2G4A in murine parturition was considered. Although we observed discrete localization of *Pla2g4a* in the uterine luminal epithelium (endometrial epithelium), qualitative assessment did not indicate any increase in expression between preterm and term tissues. These findings are in agreement with two prior reports in the mouse. Sato et al. [31] found a significant increase in *Pla2g4a* expression in placentae and decidual caps at the time of labor and an upward trend in fetal membranes by RT-PCR, but there was no change in uterine *Pla2g4a* expression throughout gestation. Winchester et al. [22] also reported steady levels of *Pla2g4a* mRNA and protein expression in whole uteri during murine pregnancy. Although a downward trend was noted in PLA2G4A protein levels and catalytic activity in late gestation, these findings were not statistically significant. Thus, though PLA2G4A expression and activity do not appear to be developmentally regulated during murine gestation, the delayed parturition phenotype of mice lacking *Pla2g4a* suggested that PLA2G4A interactions with PTGS1, PTGS2, or other downstream members of the prostaglandin-signaling axis depend on PLA2G4A functions for timely parturition.

The present studies demonstrated highly localized *Pla2g4a* mRNA and protein expression in the mouse uterine epithelium, comparable to PLA2G4A immunolocalization in the rat [47]. In addition, we observed colocalization of *Pla2g4a* with *Ptgs1*, but not *Ptgs2*, in the uterine epithelium. Although the availability of human uterine tissues for similar studies is limited, PLA2G4A is expressed in the uterine luminal epithelium of women in the luteal phase, where it colocalizes with PTGS enzymes [50]. Combined with the marked reduction in uterine prostaglandin levels that we observed in *Pla2g4a*-null uteri, these findings suggest cooperative interaction of PLA2G4A and PTGS1 functions at the time of parturition in the mouse.

We previously reported a significant reduction in prostaglandin levels in *Ptgs1*-null uteri, similar in magnitude to those in the present studies [18]. Thus, the prolonged length of gestation in *Pla2g4a*-null mice might be anticipated to occur on the basis of cellular colocalization with *Ptgs1* and >10-fold reduction in prostaglandin levels, resulting in insufficient eicosanoid generation to complete parturition. However, while parturition failure is a prominent phenotype in *Ptgs1*-deficient mice [16–18], *Pla2g4a*-null mice also have abnormalities in litter size, embryo spacing, and delays in embryo implantation [34] that can also affect the timing of parturition. The

association between litter size in polytocous species and length of gestation may reflect the degree of myometrial stretch, since intrauterine volume or uterine distension acts as a stimulus for labor, and rats with small litters have a delay in parturition [51–54]. Litter size may also exert biochemical influences on the timing of parturition, since experimental reduction in the number of rat fetuses extends the period of prolactin surges [55], delays the antepartum decline in relaxin and progesterone [56], and alters the level of myometrial PGF2α receptors [57]. Litter size is also inversely related to the length of gestation in mice [58, 59]. However, very small rodent litters are only associated with a modest prolongation of gestation of about 24 h [56, 58]. Indeed, our data show that statistical correction for small litter size in *Pla2g4a*-null mice accounts for approximately one day of the >2-day delay in parturition. Although we only observed one wild-type animal with very small litter size, the delay in parturition was modest and did not approach the prolonged gestational periods of untreated *Pla2g4a*-null mice (Fig. 5), suggesting that other impairments might be contributory.

A recent in-depth analysis of the reproductive deficits of *Pla2g4a*-null female mice demonstrated abnormalities in the timing of embryo implantation, whereas ovulation, fertilization, and stromal cell decidualization were essentially intact [34]. Implantation is a highly regulated event that is dependent on synchronous development of the embryo to the blastocyst stage coupled with progression of normal uterine cyclicity to a state of cellular and hormonal receptiveness for implantation [60]. Pregnancy does not ensue if implantation-competent blastocysts and the brief window of uterine receptivity are not strictly coordinated [61]. However, implantation studies in *Pla2g4a*-null mice suggested another alternative—that implantation can be delayed but does not occur outside the window of receptivity, rather, the window appears to be shifted to a later time point in gestation [34]. Deferral of on-time embryo implantation did not result in infertility, but did result in reduction in the number of implanted embryos, abnormal crowding of implantation sites, and retarded feto-placental growth. The shift in the implantation window caused by *Pla2g4a* deficiency resulted in an approximately 1-day delay in the timing of implantation. In that study, exogenous PGE2 and cPGI partially compensated for reduced levels of prostaglandins at the time of implantation, resulting in significant improvement in implantation rates and restoration of the normal window of implantation [34]. The results of the present study show that this prostaglandin regimen significantly reduces the length of gestation in *Pla2g4a*-null females. Correction of the parturition delay was not due to improved litter size in *Pla2g4a*-null mice, since treated and untreated *Pla2g4a*-null females had similar litter sizes. Restoration of on-time embryo implantation did not completely resolve the delay in parturition timing (Fig. 5). However, multivariate analysis revealed that controlling for both the effects of litter size and improved implantation timing can account for most of the parturition delay in these mice. Interestingly, mice with deletion of the lysophosphatidic acid (LPA) receptor LPAR3 have a strikingly similar phenotype, with delayed and crowded embryo implantation, reduced litter size, impaired fetal growth, and delay in parturition [62]. Treatment of *Lpar3*-null females with exogenous prostaglandins restored on-time implantation without improvement in embryo spacing, similar to *Pla2g4a*-null mice, but the effects of prostaglandin rescue on parturition timing in *Lpar3*-null females have not been reported [62, 63]. LPA signaling via the LPAR3 receptor appears to act in concert with the prostaglandin biosynthetic system, since *Lpar3*-null uteri have reduced *Ptgs2* expression and prostaglandin levels. However, PLA2G4A expression and activity were unaffected [62]. Thus, prostaglandin synthesis in both *Pla2g4a*- and *Lpar3*-null mice is impaired but sufficient for eventual initiation of implantation. Though deferral of on-time embryo implantation may itself cause abnormalities in the regulation of implantation and subsequent reproductive events, a recent proteomic profile of *Pla2g4a*-null uteri suggests that disruption of specific signaling pathways may be more important than the effects of disrupted implantation timing alone [64].

In summary, these studies demonstrate the discrete localization and function of PLA2G4A for prostaglandin synthesis in the peri-partum mouse uterus. Despite an apparently critical role in parturition, the prolonged length of gestation in *Pla2g4a*-null mice is more strongly related to delay in the onset of implantation and reduction in litter size than prostaglandin insufficiency. It is somewhat surprising that parturition is governed by the timing of embryo implantation. On the other hand, these studies may highlight the importance of a fixed gestational period that is required for adequate fetal growth and development. As such, any perturbation in early pregnancy that does not end in fetal demise may result in a prolonged gestational period, which does not necessarily constitute an impaired mechanism for parturition, but rather a deferral of timely labor to accommodate fetal maturation. Whether fetal signals for the initiation of labor [65] are delayed in *Pla2g4a*, *Lpar3*, or similar knockout mice awaits further study.

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REFERENCES

1. Murakami M, Kudo I. Phospholipase A2. J Biochem 2002; 131:285–292.
2. Schaloske RH, Dennis EA. The phospholipase A2 superfamily and its group numbering system. Biochim Biophys Acta 2006; 1761:1246–1259.
3. Hirabayashi T, Shimizu T. Localization and regulation of cytosolic phospholipase A(2). Biochim Biophys Acta 2000; 1488:124–138.
4. Lin LL, Wartmann M, Lin AY, Knopf JL, Seth A, Davis RJ. cPLA2 is phosphorylated and activated by MAP kinase. Cell 1993; 72:269–278.
5. Schievella AR, Regier MK, Smith WL, Lin LL. Calcium-mediated translocation of cytosolic phospholipase A2 to the nuclear envelope and endoplasmic reticulum. J Biol Chem 1995; 270:30749–30754.
6. Lappas M, Rice GE. Phospholipase A2 isozymes in pregnancy and parturition. Prostaglandins Leukot Essent Fatty Acids 2004; 70:87–100.
7. Burke JE, Dennis EA. Phospholipase A2 biochemistry. Cardiovasc Drugs Ther 2009; 23:49–59.
8. Lambeau G, Gelb MH. Biochemistry and physiology of mammalian secreted phospholipases A2. Annu Rev Biochem 2008; 77:495–520.
9. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem 2000; 69:145–182.
10. Balsinde J, Balboa MA, Dennis EA. Functional coupling between secretory phospholipase A2 and cyclooxygenase-2 and its regulation by cytosolic group IV phospholipase A2. Proc Natl Acad Sci U S A 1998; 95: 7951–7956.
11. Murakami M, Kambe T, Shimbara S, Kudo I. Functional coupling between various phospholipase A2s and cyclooxygenases in immediate and delayed prostanoid biosynthetic pathways. J Biol Chem 1999; 274: 3103–3115.
12. Reddy ST, Herschman HR. Prostaglandin synthase-1 and prostaglandin synthase-2 are coupled to distinct phospholipases for the generation of prostaglandin D2 in activated mast cells. J Biol Chem 1997; 272:3231–3237.
13. Scott KF, Bryant KJ, Bidgood MJ. Functional coupling and differential regulation of the phospholipase A2-cyclooxygenase pathways in inflammation. J Leukoc Biol 1999; 66:535–541.
14. Takano T, Panesar M, Papillon J, Cybulsky AV. Cyclooxygenases-1 and 2 couple to cytosolic but not group IIA phospholipase A2 in COS-1 cells. Prostaglandins Other Lipid Mediat 2000; 60:15–26.
15. Ueno N, Murakami M, Tanioka T, Fujimori K, Tanabe T, Urade Y, Kudo

I. Coupling between cyclooxygenase, terminal prostanoid synthase, and phospholipase A2. J Biol Chem 2001; 276:34918–34927.
16. Gross GA, Imamura T, Luedke C, Vogt SK, Olson LM, Nelson DM, Sadovsky Y, Muglia LJ. Opposing actions of prostaglandins and oxytocin determine the onset of murine labor. Proc Natl Acad Sci U S A 1998; 95: 11875–11879.
17. Langenbach R, Morham SG, Tiano HF, Loftin CD, Ghanayem BI, Chulada PC, Mahler JF, Lee CA, Goulding EH, Kluckman KD, Kim HS, Smithies O. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. Cell 1995; 83:483–492.
18. Reese J, Paria BC, Brown N, Zhao X, Morrow JD, Dey SK. Coordinated regulation of fetal and maternal prostaglandins directs successful birth and postnatal adaptation in the mouse. Proc Natl Acad Sci U S A 2000; 97: 9759–9764.
19. Yu Y, Cheng Y, Fan J, Chen XS, Klein-Szanto A, Fitzgerald GA, Funk CD. Differential impact of prostaglandin H synthase 1 knockdown on platelets and parturition. J Clin Invest 2005; 115:986–995.
20. Gupta DK, Sato TA, Keelan JA, Marvin KW, Mitchell MD. Expression of prostaglandin H synthase-1 and −2 in murine intrauterine and gestational tissues from mid-pregnancy until term. Prostaglandins Other Lipid Mediat 2001; 66:17–25.
21. Tsuboi K, Sugimoto Y, Iwane A, Yamamoto K, Yamamoto S, Ichikawa A. Uterine expression of prostaglandin H2 synthase in late pregnancy and during parturition in prostaglandin F receptor-deficient mice. Endocrinology 2000; 141:315–324.
22. Winchester SK, Imamura T, Gross GA, Muglia LM, Vogt SK, Wright J, Watanabe K, Tai HH, Muglia LJ. Coordinate regulation of prostaglandin metabolism for induction of parturition in mice. Endocrinology 2002; 143: 2593–2598.
23. Cook JL, Zaragoza DB, Sung DH, Olson DM. Expression of myometrial activation and stimulation genes in a mouse model of preterm labor: myometrial activation, stimulation, and preterm labor. Endocrinology 2000; 141:1718–1728.
24. Dinchuk JE, Car BD, Focht RJ, Johnston JJ, Jaffee BD, Covington MB, Contel NR, Eng VM, Collins RJ, Czerniak PM, Gorry SA, Trzaskos JM. Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. Nature 1995; 378:406–409.
25. Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, Trzaskos JM, Dey SK. Multiple female reproductive failures in cyclooxygenase 2-deficient mice. Cell 1997; 91:197–208.
26. Morham SG, Langenbach R, Loftin CD, Tiano HF, Vouloumanos N, Jennette JC, Mahler JF, Kluckman KD, Ledford A, Lee CA, Smithies O. Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. Cell 1995; 83:473–482.
27. Wang H, Ma WG, Tejada L, Zhang H, Morrow JD, Das SK, Dey SK. Rescue of female infertility from the loss of cyclooxygenase-2 by compensatory up-regulation of cyclooxygenase-1 is a function of genetic makeup. J Biol Chem 2004; 279:10649–10658.
28. Cheng JG, Stewart CL. Loss of cyclooxygenase-2 retards decidual growth but does not inhibit embryo implantation or development to term. Biol Reprod 2003; 68:401–404.
29. Tsuboi K, Iwane A, Nakazawa S, Sugimoto Y, Ichikawa A. Role of prostaglandin H2 synthase 2 in murine parturition: study on ovariectomy-induced parturition in prostaglandin F receptor-deficient mice. Biol Reprod 2003; 69:195–201.
30. Gross G, Imamura T, Vogt SK, Wozniak DF, Nelson DM, Sadovsky Y, Muglia LJ. Inhibition of cyclooxygenase-2 prevents inflammation-mediated preterm labor in the mouse. Am J Physiol Regul Integr Comp Physiol 2000; 278:R1415–R1423.
31. Sato TA, Gupta DK, Keelan JA, Marvin KW, Mitchell MD. Cytosolic phospholipase A(2) and 15-hydroxyprostaglandin dehydrogenase mRNA expression in murine uterine and gestational tissues during late pregnancy. Prostaglandins Leukot Essent Fatty Acids 2001; 64:247–251.
32. Bonventre JV, Huang Z, Taheri MR, O'Leary E, Li E, Moskowitz MA, Sapirstein A. Reduced fertility and postischaemic brain injury in mice deficient in cytosolic phospholipase A2. Nature 1997; 390:622–625.
33. Uozumi N, Kume K, Nagase T, Nakatani N, Ishii S, Tashiro F, Komagata Y, Maki K, Ikuta K, Ouchi Y, Miyazaki J, Shimizu T. Role of cytosolic phospholipase A2 in allergic response and parturition. Nature 1997; 390: 618–622.
34. Song H, Lim H, Paria BC, Matsumoto H, Swift LL, Morrow J, Bonventre JV, Dey SK. Cytosolic phospholipase A2alpha deficiency is crucial for 'on-time' embryo implantation that directs subsequent development. Development 2002; 129:2879–2889.
35. Kennedy BP, Payette P, Mudgett J, Vadas P, Pruzanski W, Kwan M, Tang C, Rancourt DE, Cromlish WA. A natural disruption of the secretory group II phospholipase A2 gene in inbred mouse strains. J Biol Chem 1995; 270:22378–22385.
36. Markova M, Koratkar RA, Silverman KA, Sollars VE, MacPhee-Pellini M, Walters R, Palazzo JP, Buchberg AM, Siracusa LD, Farber SA. Diversity in secreted PLA2-IIA activity among inbred mouse strains that are resistant or susceptible to Apc Min/+ tumorigenesis. Oncogene 2005; 24:6450–6458.
37. DuBois RN, Awad J, Morrow J, Roberts LJ II, Bishop PR. Regulation of eicosanoid production and mitogenesis in rat intestinal epithelial cells by transforming growth factor-alpha and phorbol ester. J Clin Invest 1994; 93:493–498.
38. Chakraborty I, Das SK, Wang J, Dey SK. Developmental expression of the cyclo-oxygenase-1 and cyclo-oxygenase-2 genes in the peri-implantation mouse uterus and their differential regulation by the blastocyst and ovarian steroids. J Mol Endocrinol 1996; 16:107–122.
39. Freed KA, Moses EK, Brennecke SP, Rice GE. Differential expression of type II, IV and cytosolic PLA2 messenger RNA in human intrauterine tissues at term. Mol Hum Reprod 1997; 3:493–499.
40. Johansen B, Rakkestad K, Balboa MA, Dennis EA. Expression of cytosolic and secreted forms of phospholipase A(2) and cyclooxygenases in human placenta, fetal membranes, and chorionic cell lines. Prostaglandins Other Lipid Mediat 2000; 60:119–125.
41. Skannal DG, Brockman DE, Eis AL, Xue S, Siddiqi TA, Myatt L. Changes in activity of cytosolic phospholipase A2 in human amnion at parturition. Am J Obstet Gynecol 1997; 177:179–184.
42. Skannal DG, Eis AL, Brockman D, Siddiqi TA, Myatt L. Immunohistochemical localization of phospholipase A2 isoforms in human myometrium during pregnancy and parturition. Am J Obstet Gynecol 1997; 176:878–882.
43. Slater DM, Astle S, Bennett PR, Thornton S. Labour is associated with increased expression of type-IIA secretory phospholipase A2 but not type-IV cytosolic phospholipase A2 in human myometrium. Mol Hum Reprod 2004; 10:799–805.
44. Terakawa K, Itoh H, Sagawa N, Yura S, Yoshida M, Korita D, Kakui K, Fujii S. Site-specific augmentation of amnion cyclooxygenase-2 and decidua vera phospholipase-A2 expression in labor: possible contribution of mechanical stretch and interleukin-1 to amnion prostaglandin synthesis. J Soc Gynecol Investig 2002; 9:68–74.
45. Zhang Q, Wu WX, Brenna JT, Nathanielsz PW. The expression of cytosolic phospholipase A2 and prostaglandin endoperoxide synthase in ovine maternal uterine and fetal tissues during late gestation and labor. Endocrinology 1996; 137:4010–4017.
46. Jeng YJ, Liebenthal D, Strakova Z, Ives KL, Hellmich MR, Soloff MS. Complementary mechanisms of enhanced oxytocin-stimulated prostaglandin E2 synthesis in rabbit amnion at the end of gestation. Endocrinology 2000; 141:4136–4145.
47. Kurusu S, Ishii S, Kawaminami M, Hashimoto I. Enhanced activity of cytosolic phospholipase A2 in the rat uterus and cervix around parturition. J Reprod Dev 2002; 488:65–73.
48. Kurusu S, Ishii S, Kawaminami M. Changes in rat uterine and cervical phospholipase A2 activity following progesterone agonist or antagonist administration at term. J Reprod Dev 2007; 53:345–350.
49. Farina MG, Billi S, Leguizamon G, Weissmann C, Guadagnoli T, Ribeiro ML, Franchi AM. Secretory and cytosolic phospholipase A2 activities and expression are regulated by oxytocin and estradiol during labor. Reproduction 2007; 134:355–364.
50. Stavreus-Evers A, Koraen L, Scott JE, Zhang P, Westlund P. Distribution of cyclooxygenase-1, cyclooxygenase-2, and cytosolic phospholipase A2 in the luteal phase human endometrium and ovary. Fertil Steril 2005; 83: 156–162.
51. Bosc MJ, Nicolle A. Influence of photoperiod on the time of parturition in the rat, I: effect of the length of daily illumination on normal or adrenalectomized animals. Reprod Nutr Dev 1980; 20:735–745.
52. Ochiai K, Kato H, Kelly PA, Rothchild I. The importance of a luteolytic effect of the pituitary in understanding the placental control of the rat corpus luteum. Endocrinology 1983; 112:1687–1695.
53. Reynolds SRM. Duration of pregnancy and uterine growth. In: Editor(ed.), Physiology of the Uterus, 3rd ed. New York: Hafner Publishing; 1965: 253–255.
54. Yoshinaga K. Superimplantation rats as a tool for study of onset of implantation. In: Bhatnagar AJ (ed.), The Anterior Pituitary Gland. New York: Raven Press; 1983:367–375.
55. Yogev L, Terkel J. Timing of termination of nocturnal prolactin surges in pregnant rats as determined by the number of fetuses. J Endocrinol 1980; 84:421–424.
56. Sherwood OD, Downing SJ, Rieber AJ, Fraley SW, Bohrer RE, Richardson BC, Shanks RD. Influence of litter size on antepartum serum

relaxin and progesterone immunoactivity levels and on birth in the rat. Endocrinology 1985; 116:2554–2562.
57. Lintner F, Hertelendy F, Zsolnai B. The effect of fetectomy on prostaglandin receptors of the rat myometrium. Acta Chir Hung 1986; 27:177–184.
58. Biggers JD, Curnow RN, Finn CA, McLaren A. Regulation of the gestation period in mice. J Reprod Fertil 1963; 6:125–138.
59. McLaren A. Effect of foetal mass on gestation period in mice. J Reprod Fertil 1967; 13:349–351.
60. Paria BC, Reese J, Das SK, Dey SK. Deciphering the cross-talk of implantation: advances and challenges. Science 2002; 296:2185–2188.
61. Paria BC, Huet-Hudson YM, Dey SK. Blastocyst's state of activity determines the "window" of implantation in the receptive mouse uterus. Proc Natl Acad Sci U S A 1993; 90:10159–10162.
62. Ye X, Hama K, Contos JJ, Anliker B, Inoue A, Skinner MK, Suzuki H, Amano T, Kennedy G, Arai H, Aoki J, Chun J. LPA3-mediated lysophosphatidic acid signalling in embryo implantation and spacing. Nature 2005; 435:104–108.
63. 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. Embryo spacing and implantation timing are differentially regulated by LPA3-mediated lysophosphatidic acid signaling in mice. Biol Reprod 2007; 77:954–959.
64. Burnum KE, Tranguch S, Mi D, Daikoku T, Dey SK, Caprioli RM. Imaging mass spectrometry reveals unique protein profiles during embryo implantation. Endocrinology 2008; 149:3274–3278.
65. Condon JC, Jeyasuria P, Faust JM, Mendelson CR. Surfactant protein secreted by the maturing mouse fetal lung acts as a hormone that signals the initiation of parturition. Proc Natl Acad Sci U S A 2004; 101:4978–4983.