Roles of prostaglandin receptors in female reproduction

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Prostaglandins (PGs) have long been known to play roles in various processes of female reproduction; however, the molecular mechanisms therein remained unsolved until recently. This review summarizes the recent progress towards understanding the molecular mechanisms underlying PG actions in fertilization and parturition. A series of studies using EP2-deficient mice demonstrated that after ovulation chemokine signalling in the cumulus cells stimulates integrin activation and cumulus extracellular matrix (ECM) assembly through the RhoA/ROCK/actomyosin pathway, although excessive chemokine signalling disturbs sperm penetration. PGE2-EP2 signalling suppresses such a chemokine signalling and stimulates cumulus ECM disassembly, which contributes to successful fertilization. A series of studies using EP-FP-deficient mice revealed that PGF2α-FP signalling induces parturition at least by terminating progesterone production; however, some other EP signals are likely to be involved in parturition by inducing myometrial contraction. Therefore, it should be clarified as to which EP and/or FP receptor signals are physiologically essential for myometrial contraction and successful parturition.

Keywords: cumulus cell/fertilization/luteolysis/parturition/prostanoid receptor.

Abbreviations: COC, cumulus-oocyte complex; COX, cyclooxygenase; ECM, extracellular matrix; FSH, follicle-stimulating hormone; LH, luteinizing hormone; NSAID, non-steroidal anti-inflammatory drug; PG, prostaglandin; PL, phospholipase; TX, thromboxane; WT, wild type.

Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and indomethacin are known to exert adverse effects on female reproduction in many species, including humans (1). NSAIDs have been shown to affect gonadotropin-releasing hormone release (2), ovulation, fertilization, luteolysis and parturition (3). Because most NSAID actions are exerted by inhibition of the enzymatic activity of cyclooxygenases (COXs), endogenously synthesized COX products such as prostaglandins (PGs) were believed to play important roles in such processes in female reproduction. Indeed, gene disruption of each PG synthase and PG receptor in mice clearly revealed that type-E and type-F PGs play critical roles in these processes (Fig. 1); however, the molecular mechanisms therein remained unsolved until recently. This review summarizes the recent progress regarding the molecular mechanisms underlying PG-induced fertilization and parturition.

Molecular Basis of PG Synthesis and Receptors

PG synthesis
PGs are a group of C20 lipid mediators (eicosanoids) synthesized from arachidonic acid (AA) by COX as a key enzyme. The COX pathway produces five bioactive metabolites consisting of four kinds of PGs and a thromboxane (TX): PGE2, PGD2, PGF2, PGI2 and TXA2, which are often referred to as ‘prostanoids’. The biosynthesis of PGs is triggered by cleavage of the ‘sn-2’ site of membrane phospholipids by phospholipase A2 (PLA2) enzymes and the release of AA. The COX isozyme, either constitutive COX-1 or inducible COX-2, converts AA into PGH2, and this unstable PG precursor is immediately converted into each PG by the respective synthases (4) (Fig. 2). Synthesized PGs are released from the cells and act as autacoids in the vicinity of their production sites to maintain local homeostasis. PGs exert a wide variety of actions in the body, which are mediated by G protein-coupled receptors (GPCRs) expressed on neighbouring target cells (5).

PG receptors
In the 1980s, PG receptors were pharmacologically characterized using several bioassay systems, including contraction-relaxation assays on various smooth muscles and the platelet aggregation assay (6, 7). These receptors are classified into five basic types, termed EP (type E Prostanoid receptor), FP, DP, IP and TP, on the basis of their sensitivity to the five primary prostanoids, PGE2, PGF2α, PGD2, PGI2, and TXA2, respectively. Moreover, pharmacological analyses predicted the existence of multiple EP subtypes: EP1, EP2, EP3 and EP4. Molecular identification of these receptors was accomplished by their cDNA cloning, which revealed that the PG receptors are GPCRs and that there is indeed a family of eight GPCRs corresponding to the pharmacologically defined receptors (5, 8). In 2001, Hirai et al. (9) revealed the presence of a second receptor for PGD2, which was originally called CRTH2 (chemoattractant receptor-homologous...
Although CRTH2 belongs to the chemoattractant receptor family, it is currently known as DP2, in reference to its endogenous ligand and the initially identified DP receptor, which is designated as DP1. PGE2 is the most widely and abundantly found in animal species and exhibits the most versatile actions. Because each EP subtype has very different signal transduction properties, PGE2 is able to exert diverse actions; EP1 is coupled to intracellular Ca\textsuperscript{2+} mobilization via G\textsubscript{i/o}, EP2 and EP4 are coupled to the stimulation of adenylyl cyclase via G\textsubscript{s}. 

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1}
\caption{Schematic representation of the rodent female reproduction processes in which PGs are involved. The horizontal axis represents the time throughout pregnancy as day after post-conception. The processes in which PGs are involved are shown in white letters. The PG class, PG synthase gene and PG receptor gene involved in each process are shown at the bottom. GnRH: gonadotropin-releasing hormone; FSH: follicle-stimulating hormone; LH: luteinizing hormone; n.d.: not determined; P4: progesterone.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig2}
\caption{COX pathway and PG receptors. PLA\textsubscript{2} cleaves the 'sn-2' site of membrane phospholipids and releases AA, which is converted to PGH\textsubscript{2} by COX-1 or COX-2. Each prostanoid is produced by the action of their specific synthases. Immediately after synthesis, each prostanoid is released from the cells, and acts on GPCRs on various kinds of neighbouring cells. PGE\textsubscript{2} acts on four kinds of receptor subtypes (EP1–EP4), each of which has different signal transduction properties, and elicits diverse physiological functions. PGD\textsubscript{2} also acts on two different receptors, DP1 and DP2, each of which belongs to a phylogenetically different receptor family.}
\end{figure}
and EP3 is mainly coupled to the inhibition of adenyl cyclase via G, It should be noted that EP2 and EP4 receptors also activate phosphoinositide 3-kinase (PI3K) via the β-arrestin pathway (11, 12), which is a key property of these receptors to understand their roles in T cells (13) (Fig. 2).

Roles of PGE2 in Fertilization

Roles of cumulus cells and extracellular matrices in fertilization

Ovulation and fertilization, which are controlled by gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), are key processes in mammalian female reproduction. These hormones induce a series of processes including follicular development, oocyte maturation, cumulus expansion and rupture of the antral follicle (4). The ovulated egg moves into the oviduct, and timely interaction between an egg and a sperm then leads to successful fertilization (15, 16). Cumulus cells are a type of granulosa cell surrounding the oocyte in the antral follicle (17). Upon an LH stimulus, the cumulus cells start to produce extracellular matrices (ECMs), and the ECMs are deposited into the intercellular space and are stabilized by accessory proteins; these processes are called cumulus expansion. A major component of the ECM produced by the cumulus cells is hyaluronan, which provides the viscoelastic properties of the cumulus oophorus (cumulus cells and ECM). Cumulus cells also produce other ECM proteins, such as fibronectin and type IV collagen. The expanded cumulus oophorus forms a tight complex with an oocyte, and is ovulated together as the cumulus-oocyte complex (COC). During ovulation, the cumulus oophorus protects the oocyte from mechanical and proteolytic insults, and promotes oocyte movement by facilitating its capture by the ciliated epithelial cells of the oviduct (18). In the oviduct, the cumulus oophorus facilitates the access of the sperm to the oocyte by trapping and selecting sperm for successful fertilization (19). Thus, complex formation of the oocyte, cumulus cells and ECM is essential for successful fertilization. Indeed, studies using mice lacking ECM-stabilizing proteins show that the cumulus ECM is required for successful fertilization in vitro; female mice deficient in these molecules are sterile owing to a loss of the cumulus and ECM (20–23). On the other hand, once sperm reaches the COC, the cumulus ECM needs to be disassembled so that the sperm can find a passage through the ECM layer. Although it had been suggested that the cumulus ECM is disassembled by sperm motility and their hyaluronidase (24), it remained unknown whether cumulus ECM disassembly is also regulated by the cumulus-derived factors in an autocrine or paracrine manner.

PGE2-EP2 signalling stimulates cumulus ECM disassembly

PGE2, an arachidonate metabolite most abundantly synthesized within the follicle, is a key mediator in the ovulation-stimulating action of gonadotropin (3, 25). Upon gonadotropin stimulation, COX-2 (Ptgs2) is induced in all cells within the follicle (26), and a large amount of PGE2 is released into the follicular fluid (27). Indeed, ‘Cox2’ null mice show severely impaired ovulation (28). Similarly, mice lacking the PGE receptor EP2 (Ptger2), which is expressed in the cumulus cells, exhibit reduced ovulation (29). These results clearly show that PGE2-EP2 signalling in the cumulus plays roles in ovulatory processes. Notably, however, Ptger2−/− mice showed a higher rate of failure in fertilization than in ovulation; the ovulation number of Ptger2−/− mice is 80% that of wild-type (WT) mice, whereas the fertilization rate of Ptger2−/− mice is ~20% that of WT mice. Both Ptgs2 and Ptger2 genes are still highly expressed in cumulus cells even after ovulation. Intriguingly, COCs isolated from the oviduct of Ptger2−/− mice show a significantly reduced rate of in vitro fertilization compared with control WT COCs (29), suggesting that PGE2-EP2 signalling facilitates cumulus ECM disassembly or sperm penetration.

Recently, Tamba et al. (30) reported a series of studies in which they explored the molecular basis of the impaired fertilization in Ptger2−/− mice by global gene expression analysis of cumulus cells. Cumulus cells start to express a set of genes such as complement components, cytokine receptors and CC chemokines, indicating that cumulus cells begin to exert immune cell-like functions upon ovulation. Moreover, gene expression of chemokinases such as Ccl7 is enhanced in the cumulus cells of Ptger2−/− mice when compared with WT cells (31). PGE2-EP2 signalling suppresses chemokine gene expression in cumulus cells through the cyclic AMP (cAMP) pathway and such chemokine signalling stimulates integrin engagement to the ECM proteins via the RhoA-ROCK-actomyosin pathway (32); CCL7-CCR signalling increases the viscosity of the cumulus ECM so that it is strong enough to endure mechanical stress, but this chemokine action leads to the suppression of fertilization (Fig. 3). Hence, once COCs reach the fertilization site (oviductal ampulla), such chemokine actions should be down-regulated and the cumulus ECM should be disassembled, so that sperm can penetrate the cumulus ECM layer; the PGE2-EP2 system contributes to this process, which is required for successful fertilization. If PGE2-EP2 signalling does not work properly, cumulus cells will capture a large number of sperm owing to the cross-linking of integrins (cumulus cell) and fertilin molecules (sperm). Thus, the PGE2-elicited disassembly of the cumulus ECM is a prerequisite for successful fertilization (Fig. 3). Do the cumulus-derived chemokines only work in an autocrine manner? Tamba et al. (31) further demonstrated that CCL7 facilitates fertilization by stimulating sperm migration towards COCs; cumulus-derived CCL7 functions as a chemoattractant for sperm. Indeed, Isobe et al. (33) previously reported that regulated on activation, normal T cell expressed and secreted (RANTES), an agonist for CCR1/3/5, stimulates chemotaxis of human sperm that express mRNAs for CCR1, CCR2 and CCR5. Thus, CC chemokines facilitate sperm migration towards the COCs and presumably stimulate the entrapment and selection of sperm by the COCs (Fig. 3).
Does the PGE2-EP2 signalling function similarly in primates? Recently, Peluffo et al. (34) reported quite interesting results in female macaques; the PTGS2 and PTGER2 genes are induced in their follicles upon gonadotropin stimulation, PGE2 can stimulate cumulus expansion in an EP2-dependent manner, and an EP2 antagonist exhibits pregnancy-preventive effects in female macaques without eliciting any undesirable effects. These results suggest that PGE2-EP2 signalling in cumulus cells works in a similar manner also in primates, and an EP2 receptor antagonist may be a suitable candidate as a contraceptive for women.

**Role of PGs in Parturition**

**Roles of PGs in luteolysis and parturition**

PGF2α has been accepted as an inducer of luteolysis in the estrous cycle, defined as the failure of the corpus luteum to secrete progesterone in domestic animals such as the sheep and cow. This is because luteolysis in these animals is evoked by a substance in the blood flow from the uterus, that contains PGF2α, is inhibited by treatment with NSAIDs, and this inhibition is reversed by exogenously added PGF2α (35, 36). However, species differences exist in the actions of

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**Fig. 3 Schematic model of the interactions of chemokine and PGE2 signalling in cumulus ECM disassembly and successful fertilization.** (A) Ovulation-associated signals induce gene expression and production of chemokines such as CCL7 in cumulus cells. CCL7 promotes cumulus ECM protein assembly to protect the oocyte, and facilitates sperm migration to COCs. (B) In cumulus cells, chemokine signalling activates the RhoA/ROCK/actomyosin pathway, and finally elicits the surface accumulation of integrins, which enhances fibronectin fibril formation (left). Once the COCs reach the fertilization site, PGE2/EP2/cAMP signalling downregulates such chemokine actions on cumuli and disassembles the cumulus ECM (right). However, in Ptger2−/− cumuli (left), chemokine signalling persists and interferes with sperm penetration owing to its hyaluronidase resistance and the direct binding between integrins (cumulus) and fertilin (sperm). The figure is reproduced from the original figure in (31) with some revisions.
possibly functions in a different physiological setting. In addition, pregnant *Ptgfr<sup>−/−</sup>* mice were shown to be unable to perform parturition, apparently owing to the lack of labour (41). *Ptgfr<sup>−/−</sup>* mice fail to undergo a propartum decline in progesterone, which is the hormone that acts in the maintenance of pregnancy. A reduction in progesterone levels due to ovariectomy 24 h before term caused an up-regulation of the uterine expression of the oxytocin receptor and normal parturition in *Ptgfr<sup>−/−</sup>* mice. These results indicate that a luteolytic action of PGF<sub>2α</sub> is required in mice to diminish progesterone levels and thus permit the initiation of labour. Similar parturition failure is also observed in COX-1-deficient (*Ptgs1<sup>−/−</sup>* (44)) or cytosolic-PLA<sub>2α</sub>-deficient (Pla<sub>2g4α</sub>−/−) mothers (45, 46). In contrast, both oxytocin-deficient and oxytocin receptor-deficient mothers show normal parturition, although they show defects in mothering behavior after parturition (47, 48). These results suggest that a luteolytic action of PGF<sub>2α</sub> is exerted in late pregnancy via the cPLA<sub>2α</sub>-COX-1-PGF<sub>2α</sub>-FP pathway.

**Other EP signals participate in parturition by exerting a uterotonic action**

Because ovariectomy at day 19 of pregnancy in *Ptgfr<sup>−/−</sup>* mothers is sufficient to restore parturition exactly 20 h later, even in the absence of the FP receptor in the myometrium, the uterotonic action of PGF<sub>2α</sub> is considered to be dispensable. Then, are the uterotonic actions of PGs really unnecessary for parturition? To assess this point, Tsuboi *et al.* (49) performed detailed uterine expression analysis of *Ptgs1* and *Ptgs2* in late pregnancy in WT and *Ptgfr<sup>−/−</sup>* mothers. As discussed earlier, COX-1 appears to play an indispensable role in uterine PGF<sub>2α</sub> synthesis and induction of luteolysis. Indeed, in WT mice, uterine gene expression of *Ptgs1* gradually increased from day 15 of pregnancy, reached maximal levels on day 17 and rapidly decreased after day 20, the day when parturition normally occurs. In contrast, the uterine *Ptgs1* expression is still at high levels on day 20 in *Ptgfr<sup>−/−</sup>* mice. This observation suggests that progesterone withdrawal could serve as a negative feedback system for uterine *Ptgs1* expression. On the other hand, *Ptgs2* expression is induced in the uteri at day 20 (when parturition occurs) in WT mice, but not in *Ptgfr<sup>−/−</sup>* mice. Interestingly, ovariectomy induces *Ptgs2* expression in the uteri of *Ptgfr<sup>−/−</sup>* mice 16 h later (4 h before delivery of the first pup). Tsuboi *et al.* (50) finally found that progesterone completely suppresses *Ptgs2* expression in myometrial cells, and once the ovaries (the source of progesterone) are removed, myometrial *Ptgs2* expression and delivery of the first pup occur simultaneously. Indeed, indomethacin as well as a COX-2 inhibitor postpones delivery of the first pup by >20 h in ovariectomized *Ptgfr<sup>−/−</sup>* mothers. Interestingly, administration of PGE<sub>2</sub> cancels the delaying effect of NSAIDs. Because mice deficient in a single EP receptor show normal parturition (8), the uterotonic actions of PGE<sub>2</sub> and PGF<sub>2α</sub> may be mediated by combination of multiple EP and FP receptors. Such a mechanism
underlying PG-induced parturition may be an interesting possibility to examine.

In rodents, the corpus luteum is the main tissue that produces progesterone throughout pregnancy, but in humans, the placenta begins to produce progesterone in late pregnancy. Moreover, a decline in progesterone levels does not always occur before parturition in humans. Therefore, mechanisms very different from PGF2α-induced luteolysis are presumably involved in the induction of human parturition. However, the uterotonic action of PGs is likely to be involved in the induction of parturition in many species including humans. Thus, PGs appear to work as a physiological uterotokin, which may be a reason why PGs have long been used as labour-inducing drugs.

**Concluding Remarks**

As described earlier, recent studies on PG receptors have uncovered the mechanisms underlying the physiological action of PGE2 on fertilization; PGF2α-EP2 signalling stimulates cumulus ECM disassembly, which is a prerequisite for the efficient penetration of sperm and successful fertilization. Of course, more extensive studies are necessary for the evaluation of whether such a mechanism really exists in humans, but an opposite interaction between PGs and chemokine signalling appear to be an attractive target to develop contraceptives for women. In contrast, although both PGE2 and PGF2α have long been used as a labour-inducing drug, their physiological roles in parturition have been unsolved. A series of studies using Ptgr−/− mothers demonstrated that COX-1-derived PGF2α-FP signalling induces parturition by terminating progesterone production, but some other EP signals derived from COX-2 are also involved in the induction of parturition by exerting myometrial contraction. It should be clarified as to which EP and/or FP receptor signals are physiologically essential for myometrial contraction and successful parturition.

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**Conflict of Interest**

None declared.

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