

—Mini Review—

Roles of Prostaglandins during Oocyte Maturation: Lessons from Knockout Mice

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Abstract: Prostaglandins (PGs) are implicated in various physiological and pathological functions because of their vasoactive, mitogenic, and differentiating properties. PGs have long been known to participate in various female reproductive functions. The cyclooxygenase (COX) isozymes, COX-1 and COX-2, are the rate-limiting enzymes of PGs. Gene targeting studies have revealed that COX-2, but not COX-1, derived PGs are essential for ovulation, fertilization, implantation, and decidualization. However, the roles of PGs in oocyte maturation remain controversial. We have clarified that COX-2-deficient mice have defective oocyte meiotic and cytoplasmic maturation associated with defective cumulus expansion *in vivo* and *in vitro*. Lack of COX-2-derived PGE2 leads to impaired cumulus cell-oocyte interactions, which are critical for the production of fertilization-competent oocytes. This review summarizes the published data regarding the roles of PGs in oocyte maturation especially in gene targeting studies.

Key words: Prostaglandin, COX-2, PGE2, Oocyte maturation, Cumulus expansion

Introduction

Prostanoids are a group of lipid mediators that consist of prostaglandins (PGs) and thromboxanes (TXs). PGs are implicated in various physiological and pathological functions because of their vasoactive, mitogenic, and differentiating properties [1]. PGs are involved in various female reproductive functions, such as ovulation, fertilization, embryo development, implantation, and decidualization [2, 3], and are

synthesized from arachidonic acid by cyclooxygenase (COX) and various PG-specific synthetases. There are two types of COX enzymes, COX-1 and COX-2. Although COX-1 is considered to be constitutive, COX-2 is induced by inflammatory stimuli, such as cytokines and growth factors. Gene targeting experiments in mice have revealed the distinct functions of these isozymes. COX-1-deficient females are mostly fertile but show parturition defects [4]. In contrast, COX-2-deficient (COX-2^{-/-}) females are infertile because of severely impaired ovulation, fertilization, and implantation [5]. Thus, COX-2-derived PGs are essential for female reproduction.

The diverse actions of PGs are normally mediated by the G protein-coupled receptors on plasma membranes. Cell-surface receptors for PGE2, PGF2 α , PGD2, and PGI2 have been cloned as EP, FP, DP, and IP, respectively [1]. The luteinizing hormone (LH) surge or hCG stimulation leads to production of large amount of PGE2, PGF2 α , and 6-keto-PGF1 α (a metabolite of PGI2), within the ovaries of various species [6–11]. Whereas the PGs induced by LH or hCG reverse ovulatory defects treated with indomethacin, a non-specific COX inhibitor [10, 12, 13], PGE2, but not PGF2 α , restores anovulation in COX-2^{-/-} mice [6]. Thus, PGE2 is the dominant PG in the ovary and has important roles in the peri-ovulation period. The actions of PGE2 are mediated by four receptor subtypes, EP1, EP2, EP3, and EP4. Gene targeting studies have shown that EP2-deficient (EP2^{-/-}) females have similar phenotypes, such as impaired ovulation and fertilization, in COX-2^{-/-} mice [14–16]. EP1- and EP3-deficient mice are apparently fertile [17]. However most EP4-deficient mice die shortly after birth because of patent ductus arteriosus [18, 19], and studies on reproductive phenotypes have not been possible.

The mechanisms underlying the coordination of the

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maturation of follicles and their enclosed oocytes are not fully understood. Intracellular communication between oocytes and surrounding somatic cells, granulosa and cumulus cells, is of pivotal importance for oocyte maturation. Oocytes are arrested at the germinal vesicle stage before the LH surge. The LH surge induces meiotic resumption of oocytes through its receptors on theca and granulosa cells. Recent findings show that epidermal growth factor (EGF)-like growth factors are induced by human chorionic gonadotropin (hCG), and that EGF-like growth factors promote meiotic resumption and cumulus expansion by depositing cell matrix in a process involving activating COX-2-derived PGE2 [20]. As the meiotic resumption of oocytes is closely associated with cumulus expansion, cumulus expansion is a prerequisite for ovulation, fertilization, and subsequent embryo development [21]. Moreover, PGE2 stimulates cumulus expansion of cumulus oocyte complexes (COCs) in various species [22–25]. Thus, PGE2 possibly plays important roles in oocyte maturation. This review focuses on the roles of COX-2-derived PGE2 during oocyte maturation.

Spatio-temporal Expression of COX during Oocyte Maturation

The gonadotropin-induced expression of COX of periovulatory follicles is regulated in a spatio-temporal manner [26–29]. There are quite different expression profiles of COX-1 and COX-2 during ovulation. Both COX-1 and COX-2 mRNA are not significantly expressed in the follicle without LH or hCG stimulation. While COX-1 mRNA is expressed in granulosa cells from 8 to 16 hrs after hCG stimulation in C57BL mice [27, 29], it is first observed at 4 hrs after hCG stimulation and is followed by persistent expression through to 16 hrs post-hCG stimulation in CD-1 mice [29]. In contrast to COX-1, COX-2 expression induced by hCG has a multiphasic profile. In mouse follicles, COX-2 mRNA is first detected in granulosa cells as early as 2 hrs after hCG stimulation, and is followed by peak expression in both granulosa and cumulus cells at 4 and 12 hrs, respectively, after hCG stimulation [26]. The COX-2 protein is expressed as early as 2 hrs after hCG stimulation in mouse cumulus cells with sustained high levels until ovulation (see Fig. 1A). The COX-2, but not the COX-1 protein, is also expressed in oocytes as well as cumulus cells in ovulated mouse COCs [3, 5] (see Fig. 1B). Similar to the mouse, COX-2 mRNA is expressed as early as 4 hrs post-hCG stimulation in the

rat follicle [28]. However, COX-2 mRNA in bovine and equine follicles is expressed at 18 and 30 hrs post-hCG stimulation, respectively [30, 31]. Although there are large differences in the time-course of COX-2 induction among different species, the interval time from the first COX-2 induction to ovulation, nearly 10 hrs, is remarkably conserved [10]. These results support the proposal that COX-2 may serve as a molecular determinant that sets the alarm of the mammalian ovulatory clock [32]. On the other hand, there are few studies available on the expression of COX-2 in human preovulatory follicles. Tokuyama *et al.* reported that COX-2 is expressed in the granulosa cells of secondary and developing follicles, but is not detected in primary and Graafian follicles [33]. COX-2 is also present in human luteinized granulosa cells obtained from patients undergoing *in vitro* fertilization [34]. In monkey follicles, as a primate model with a similar ovulatory cycle, the expression of COX-2 mRNA is first detected in granulosa cells at 12 hrs post hCG stimulation, and is maintained for 24 hrs [9].

Spatio-temporal Expression of PGE2 Receptors during Oocyte Maturation

There are few available reports of gonadotropin-induced expression patterns of PGE2 receptors, EP1, EP2, EP3, and EP4. Similar to COX expression, the expressions of EP1–4 in the ovary are species and strain specific. Segi *et al.* reported the gonadotropin-induced expression of EP2 and EP4 in the mouse ovary [27]. While EP2 mRNA is expressed in theca and interstitial cells, without hCG stimulation, EP4 mRNA is mainly expressed in the oocytes of small preantral follicles in immature mouse ovaries. Both EP2 and EP4 mRNA are expressed in granulosa and cumulus cells at 3 hrs after hCG stimulation. Although the expression of EP4 mRNA is decreased in both granulosa and cumulus cells, the expression of EP2 mRNA is maintained at a high level in cumulus cells, but not in granulosa cells, at 9 hrs after hCG stimulation. It is also reported that there is no expression of EP1 in murine and bovine ovaries, whereas EP3 mRNA is expressed in the murine and bovine COCs [23, 35]. In murine COCs, EP3 is localized in the cumulus cells, and is not found in oocytes [35]. EP3 is also expressed in cumulus cells in bovine COCs during *in vitro* culture [23, 36]. In monkey follicles, EP1–3, but not EP4, is expressed in granulosa cells after hCG stimulation [37]. There are no available human data on the gonadotropin-induced expression of PGE2 receptors in the ovary.

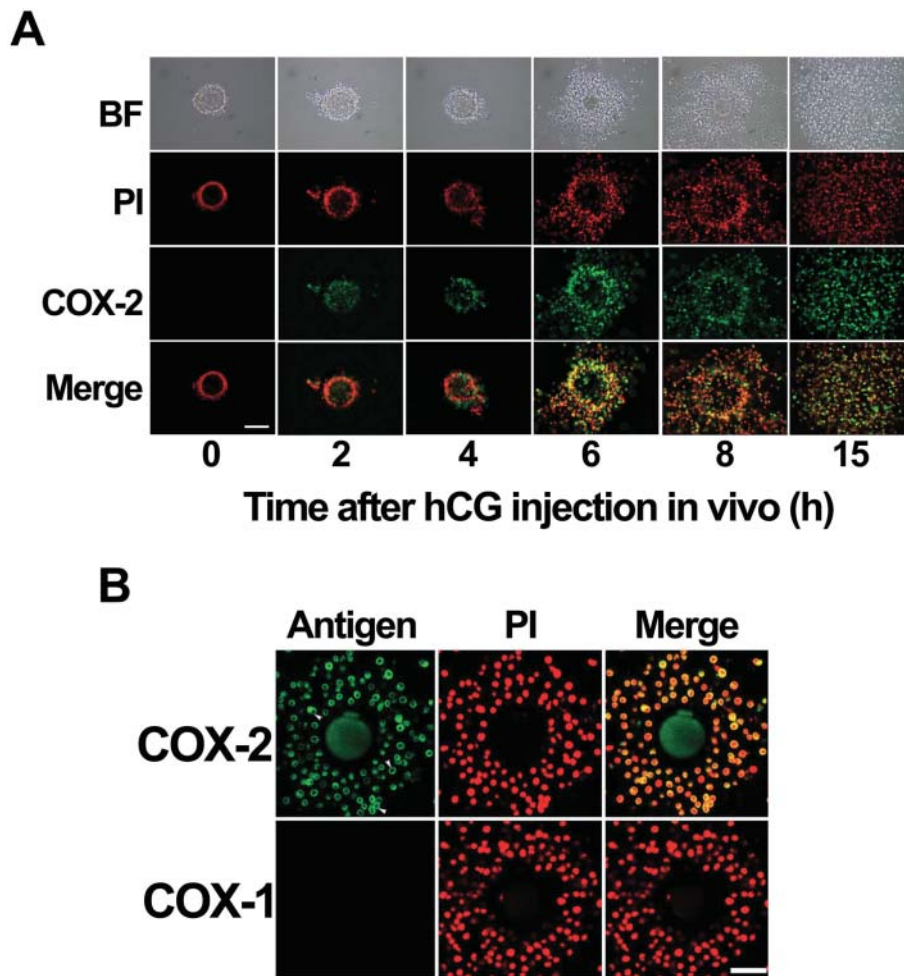


Fig. 1. Gonadotropin-induced COX-2 expression in mouse cumulus-oocyte complexes. (A) At indicated times after hCG injection *in vivo*, cumulus-oocyte complexes (COCs) were collected from wild-type mice and subjected to immunofluorescence analysis of COX-2. Propidium iodide (PI) was used as nuclear staining. BF, bright field. Bar, 100 μm . (B) Immunofluorescence analyses of COX-1 and COX-2 in ovulated COCs after 15 hrs hCG injection. Bar, 50 μm . This photograph is provided by Wang H. with partial modification. Reprinted with permission from Ref. [3].

Evidence of the Importance of Prostaglandins in Oocyte Maturation

There are two different kinds of oocyte maturation, meiotic and cytoplasmic maturation. While meiotic maturation is characterized by nuclear progression, from germinal vesicle breakdown (GVBD) to metaphase II, cytoplasmic maturation is considered to be essential for the acquisition of fertilization and embryo development capabilities. The coordination of nuclear and cytoplasmic oocyte maturation is indispensable in normal fertilization and embryo development in mammals [38].

Prostaglandins and oocyte meiotic maturation in early studies

Early studies showed that PGE₂ induces oocyte meiotic maturation in rodents *in vivo* [39] and *in vitro* [40–42], and indomethacin, a nonspecific COX inhibitor, attenuates gonadotropin-induced cumulus expansion and GVBD in mouse and sheep oocytes [43–46]. The inhibitory effects of indomethacin on oocyte meiotic maturation in sheep ovaries are reversed by the intrafollicular injection of PGE₂ [46]. On the other hand, although indomethacin prevents ovulation induced by gonadotropin-releasing hormone and angiotensin II by

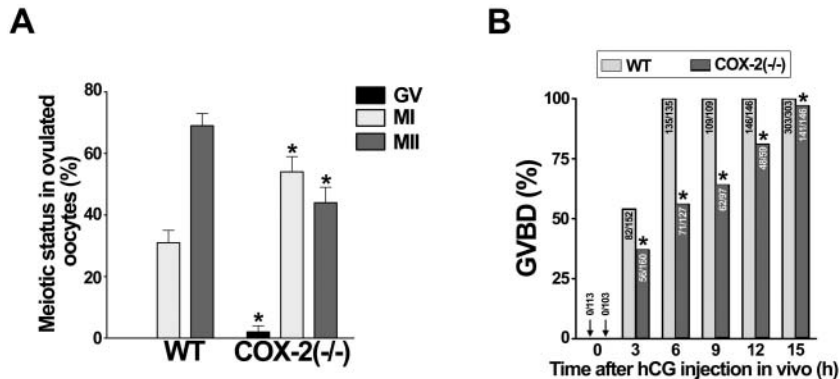


Fig. 2. COX-2 deficiency compromises gonadotropin-induced oocyte meiotic maturation *in vivo*. (A) Ovulated oocytes were collected from wild-type (WT) and COX-2^{-/-} mice 15 hrs after hCG injection. Oocytes were examined and classified as germinal vesicle (GV), metaphase I (MI), or metaphase II (MII) by nuclear staining. The percentage of oocytes classified for each stage of meiosis for wild-type and COX-2^{-/-} mice was calculated by dividing the number of oocytes at each stage of meiosis by the total number of ovulated oocytes. Data are presented as means \pm S.E. (B) Germinal vesicle breakdown (GVBD) was scored for cumulus oocyte complexes isolated at various times after hCG injection. Numbers inside bars indicate the number of oocytes with GVBD/total numbers of oocytes examined. Asterisks indicate significantly different from WT mice ($P < 0.01$) [35].

inhibiting PGs production, it fails to inhibit GVBD in *in vitro* perfused rabbit ovaries [47, 48]. These results are not consistent with the roles of PGs in oocyte meiotic maturation.

Oocyte meiotic maturation in COX-2^{-/-} mice

Gene targeting studies have shown that ovarian follicular development, with or without stimulation by gonadotropin, is apparently normal in COX-2^{-/-} mice [6, 49], but it remains unclear whether loss of COX-2 affects gonadotropin-induced oocyte meiotic maturation. Lim *et al.* initially reported that ovulated oocytes induced by gonadotropin from COX-2^{-/-} mice have very few oocytes showing first polar body extrusion [5]. In addition, unfertilized oocytes with signs of fragmentation are frequently observed in COX-2^{-/-} mice on day 2 of pregnancy, suggesting defective oocyte maturation [29]. In contrast, Davis *et al.* reported that the oocytes with GVBD are observed in COX-2^{-/-} mice at 6–8 hrs after hCG stimulation [6]. Moreover, COX-2^{-/-} oocytes show spontaneous meiotic maturation *in vitro* [16]. These results are not consistent with PGs roles in oocyte meiotic maturation. Therefore, we examined the roles of PGs in gonadotropin-induced oocyte meiotic maturation *in vivo* and *in vitro* using COX-2^{-/-} mice [35]. We reported that the ratio of

metaphase II to metaphase I-stage oocytes was decreased in superovulated COX-2^{-/-} mice compared with wild-type mice (see Fig. 2A). We also reported that hCG-induced resumption of meiosis, such as GVBD, was delayed in COX-2^{-/-} oocytes compared with wild-type oocytes (see Fig. 2B). These results suggest that loss of COX-2-derived PGs impairs gonadotropin-induced oocyte meiotic maturation *in vivo*. Moreover, although there were no differences with respect to spontaneous meiotic maturation *in vitro*, follicle-stimulating hormone (FSH)-induced meiotic maturation *in vivo* was significantly compromised in COX-2^{-/-} mice compared with wild-type mice. Collectively, these findings are consistent with the idea that the loss of COX-2-derived PGs impairs gonadotropin-induced oocyte meiotic maturation *in vivo* and *in vitro*.

Oocyte meiotic maturation in EP2^{-/-} mice

Although there are four types of PGE₂ receptors, EP1–4, only EP2^{-/-} mice have similar reproductive phenotypes to COX-2^{-/-} mice, such as ovulation, cumulus expansion, and fertilization failure [14–16, 50]. In the only report available regarding oocyte meiotic maturation in EP2^{-/-} mice, Matsumoto *et al.* reported that spontaneous meiotic maturation *in vitro* is not compromised in EP2^{-/-} mice compared with wild-type

mice [16]; no reports are available regarding gonadotropin-induced oocyte meiotic maturation *in vivo*.

Role of prostaglandins on oocyte cytoplasmic maturation

Oocyte cytoplasmic maturation is essential for the acquisition of fertilization and embryo development capabilities. Since surrounding follicle cells, particularly cumulus and granulosa cells, have been demonstrated to be important for cytoplasmic maturation [51, 52], inhibition or loss of PGs, which are secreted by cumulus and granulosa cells, might affect the cytoplasmic maturation of oocytes. Although there are several reports available on the association between PGs and fertilization, very little is known about the association of PGs and embryo development after fertilization. Lim *et al.* first reported that *in vivo* fertilization is severely compromised in COX-2^{-/-} mice [5]. Wang *et al.* also reported *in vivo* fertilization failure in COX-2^{-/-} mice [29]. Although *in vivo* fertilization is severely impaired in EP2^{-/-} mice as well as that in COX-2^{-/-} mice [14, 15, 50], the *in vitro* fertilization rate of oocytes from EP2^{-/-} mice is not different from that of oocytes from wild-type mice [14, 50]. Moreover, Matsumoto *et al.* reported that *in vitro* fertilization and blastocyst formation rates of oocytes matured *in vivo* or *in vitro* collected from COX-2^{-/-} or EP2^{-/-} mice are almost similar to those of wild-type mice [16]. Because of the severe ovulation defects, only a small number of oocytes were available for *in vitro* fertilization experiments using oocyte from COX-2^{-/-} or EP2^{-/-} mice on the C57BL/6J/129 background [16]. As the numbers of ovulated oocytes are higher in CD1-background COX-2^{-/-} mice than in C57BL/6J/129 [29], we examined whether or not COX-2^{-/-} oocytes have normal fertilization and embryo development competency using CD1-background COX-2^{-/-} mice [35]. We reported that *in vitro* fertilization and blastocyst formation rates of oocytes from CD1-background COX-2^{-/-} mice were significantly decreased compared with of wild-type mice. These results are different from those reported by Matsumoto. Matsumoto *et al.* performed partial zona dissection to assist sperm penetration through the zona in *in vitro* fertilization experiments [16]. There is a reason for similar *in vitro* fertilization rates seen for C57BL/6J/129-background COX-2^{-/-} and wild-type mice. For embryo development, the differences in blastocyst formation rates between COX-2^{-/-} (77%) and wild-type (94%) mice are not so large. As we inseminated with spermatozoa from wild-type male mice, the embryos of fertilized COX-2^{-/-} oocytes were heterozygous, and produced COX-2-derived PGI₂ by

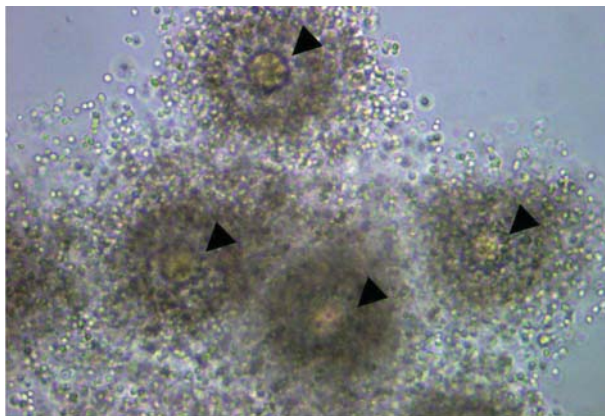
themselves, and PGI₂ analogue stimulates hatching of blastocyst [53, 54]. Further study will be needed to clarify the roles of PGs in embryo development using COX-2-null embryos. Collectively, loss of PGs mainly affects *in vivo* fertilization failure, which might be related to cumulus cell function.

Prostaglandins and Cumulus Expansion

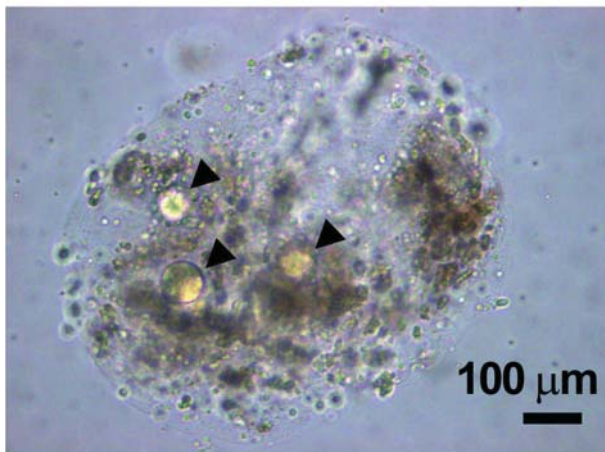
Cumulus expansion is characterized by the synthesis and assembly of highly mucoelastic extracellular matrix (ECM), mainly hyaluronan (HA) deposition, by cumulus cells [55]. This process facilitates detachment of the COC from the follicle wall, its extrusion at ovulation, and its capture by oviductal fimbria [56]. Many studies have shown that abnormal cumulus expansion is crucial for female fertility, as without it there is ovulation and fertilization failure [56–59]. There are many genes involved in regulating gonadotropin-induced cumulus expansion, and one of the key molecules is COX-2-derived PGE₂ [58].

Defective cumulus expansion in COX-2^{-/-} and EP2^{-/-} mice

Early studies showed that indomethacin inhibits hCG-induced cumulus expansion of mouse COCs *in vivo* [43, 45]. In contrast, although PGE₂ dose-dependently stimulates cumulus expansion, FSH-induced cumulus expansion *in vitro* is not inhibited by indomethacin in mouse COCs [22]. Gene targeting studies have shown that hCG-induced cumulus expansion *in vivo* is impaired in COX-2^{-/-} [5, 6, 29, 35] (see Fig. 3) and EP2^{-/-} mice [14]. The defective cumulus expansion of COX-2^{-/-} mice is rescued by PGE₂ treatment *in vivo* [6]. What mechanisms of defective cumulus expansion are involved in COX-2^{-/-} and EP2^{-/-} mice? There are important structural components of the cumulus ECM, such as HA, and HA-binding proteins. The expression of HA is regulated by hyaluronan synthase-2 (HAS-2), which is induced by hCG treatment [60]. There are several HA-binding proteins: tumor necrosis factor- α -induced protein 6 (TSG-6), long pentraxin 3 (PTX3), and inter- α -trypsin inhibitor (ITI). These HA-binding proteins bind to each other and stabilize HA within the cumulus ECM. We and other groups have reported that TSG-6 expression of cumulus cells is decreased in COX-2^{-/-} and EP2^{-/-} mice compared with wild-type mice [35, 61]. In addition, PGE₂ treatment induces TSG-6 expression in cumulus cells and cumulus ECM *in vitro* [35, 61]. Thus, the defective cumulus expansions in COX-2^{-/-} and EP2^{-/-} mice are related to the decreased



WT



COX-2(-/-)

Fig. 3. Defective cumulus expansion in COX-2^{-/-} mice *in vivo*. Cumulus-oocyte complexes with fewer layers of outer cumulus cells than in wild-type (WT) mice were observed in COX-2^{-/-} mice. Arrows indicate the central oocytes.

expression of TSG-6, which is a downstream protein of PGE2 and stabilizes the cumulus ECM. In fact, TSG-6-deficient female mice show impaired cumulus expansion with sterility due to fertilization failure [62]. On the other hand, loss of other HA-binding proteins, such as PTX3 and ITI, also lead to inability to assemble cumulus ECM, and these knockout mice have a phenotype of decreased fertility [63–66].

PGE2 Restores Defective Oocyte Maturation and Cumulus Expansion in COX-2^{-/-} Mice

Davis *et al.* reported that PGE2 restores defective ovulation and cumulus expansion in COX-2^{-/-} mice *in vivo* [6], but they did not examine the effects of PGE2 on oocyte maturation in COX-2^{-/-} mice. We examined whether PGE2 could rescue the defective oocyte maturation in COX-2^{-/-} mice *in vivo* and *in vitro* [35]. PGE2 treatment restored normal oocyte meiotic maturation *in vivo* in COX-2^{-/-} mice (see Fig. 4A). In addition, COX-2^{-/-} oocytes with PGE2 pretreatment *in vivo* during the preovulatory period exhibited normal fertilization and embryo development *in vitro* (see Figs. 4B and 4C). On the other hand, in an *in vitro* culture system using oocytes from COX-2^{-/-} mice, PGE2 treatment did not improve the percentages of GVBD and metaphase II-stage oocytes, whereas a treatment with both FSH and PGE2 significantly increased those percentages. These findings suggest that FSH and PGE2 have additive effects for oocyte meiotic maturation in COX-2^{-/-} oocytes *in vitro* culture. In the same context, we also examined whether PGE2 could rescue the defective cumulus expansion of oocytes from COX-2^{-/-} mice in *in vitro* culture. Interestingly, PGE2 treatment alone was sufficient to induce cumulus expansion in both wild-type and COX-2^{-/-} COCs. No additive effects were observed when COCs of either genotype were cotreated with FSH and PGE2, while FSH did exert some stimulatory effects on cumulus expansion in COX-2^{-/-} mice. Taken together, it indicates that there are other signaling molecules in addition to PGE2 that are involved in regulating gonadotropin-induced oocyte meiotic maturation as well as cumulus expansion.

Mechanisms Underlying Fertilization Failure in Loss of PGE2-EP2 Signaling

Recently, the mechanism of fertilization failure in EP2^{-/-} mice has been reported by Sugimoto *et al.* [67, 68]. They reported that the mRNA expression of chemokines, such as CCL2, CCL7, and CCL9, is increased in EP2^{-/-} cumulus cells compared with wild-type mice. CCL7 facilitates not only sperm migration to COCs but also integrin-fibronectin-mediated cumulus ECM assembly to protect oocytes. Since the expression of CCL7 in cumulus cells is downregulated by PGE2, chronic CCL7 signaling results in excessive accumulation in the cumulus ECM in EP2^{-/-} mice. The excessive accumulation of cumulus ECM results in

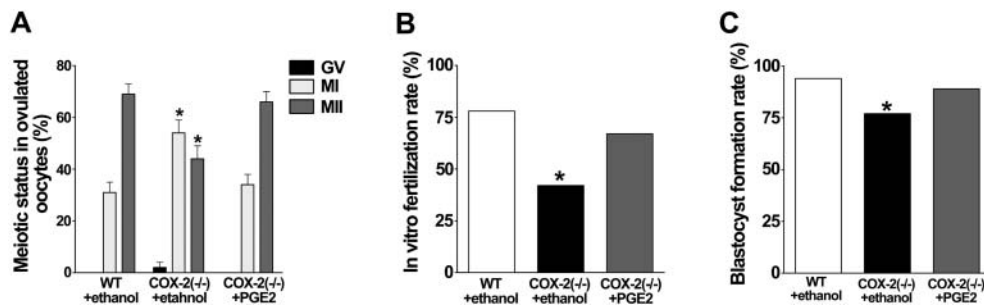


Fig. 4. PGE2 rescues oocyte meiotic maturation, *in vitro* fertilization, and embryo development in COX-2^{-/-} mice. (A) Superovulated oocytes were collected from wild-type (WT) and COX-2^{-/-} mice 15 hrs after hCG injection. Oocytes were examined and classified as germinal vesicle (GV), metaphase I (MI), or metaphase II (MII) by nuclear staining. The percentage of oocytes classified for each stage of meiosis for wild-type and COX-2^{-/-} mice was calculated by dividing the number of oocytes at each stage of meiosis by the total number of ovulated oocytes. Data are presented as means \pm S.E. Asterisks indicate significantly different from WT mice ($P < 0.01$). (B) Superovulated oocytes were subjected to *in vitro* fertilization. The oocytes with two pronuclei indicate successful fertilization. (C) The number of blastocysts formed from fertilized oocytes was recorded after 5 days of culture [35].

resistance to sperm hyaluronidase, and sperm penetration is prevented in EP2^{-/-} mice. Sugimoto *et al.* have also demonstrated that CCL7 facilitates RhoA activation followed by accumulation of integrin-fibronectin in the cumulus ECM. Therefore, PGE2-EP2 signaling negatively regulates the action of chemokines preventing excessive ECM assembly in cumulus cells, thereby promoting successful fertilization.

Conclusion

During the past decade, gene targeting studies have shown that COX-2-derived PGE2 plays major role in the oocyte maturation associated with cumulus expansion. Loss of COX-2-derived PGE2 leads to impairment of cumulus cell-oocyte interactions, which are critical for the production of fertilization-competent oocytes [69]. However, as well as PGE2 there are other molecules, which are involved in oocyte maturation and cumulus expansion. Therefore, identification of these key molecules and their signaling pathways within the oocyte-cumulus cell regulatory loop will help us to understand the mechanisms underlying oocyte maturation.

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