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Potential Sites of Prostaglandin Actions in the Periimplantation Mouse Uterus: Differential Expression and Regulation of Prostaglandin Receptor Genes[1]

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ABSTRACT

Prostaglandins (PGs), especially PGE_2 and $PGF_{2\alpha}$, are considered important for blastocyst spacing, implantation, and decidualization in the rodent uterus. However, information regarding uterine sites of PG actions in these processes is lacking. PGE_2 or $PGF_{2\alpha}$ interacts with specific G protein-coupled membrane receptors. PGE_2 receptors are classified into four subtypes, EP_1, EP_2, EP_3, and EP_4. While EP_1 is coupled to Ca^{2+} mobilization, activation of EP_2 and EP_4 triggers stimulation of adenylyl cyclase. In contrast, activation of EP_3 inhibits adenylyl cyclase. $PGF_{2\alpha}$ receptor (FP) is coupled to stimulation of phospholipase C-inositol trisphosphate (IP_3) pathway and Ca^{2+} mobilization. This investigation demonstrates that PGE_2 and $PGF_{2\alpha}$ receptor genes are expressed in a temporal and cell-specific manner in the periimplantation mouse uterus. In the mouse, the attachment reaction occurs in the evening (2200–2300 h) of Day 4 of pregnancy and is preceded by embryo spacing, uterine edema, and luminal closure resulting in an intimate apposition of the blastocyst with the uterine luminal epithelium. Expression of EP_3 and FP primarily in the circular muscle of the myometrium on Days 3–5 of pregnancy suggests that the circular muscle, not the longitudinal muscle, is the primary target for PG-mediated uterine contractions required for embryo transport, spacing, and/or accommodation in the uterus. In contrast, expression of EP_3 in a subpopulation of cells in the stromal bed at the mesometrial side, and of EP_4 in the epithelium and stroma on these days, suggests that PGE_2 effects on uterine preparation for implantation (such as epithelial cell differentiation, stromal cell proliferation, uterine edema, luminal closure, and increased localized endometrial vascular permeability at the sites of blastocyst attachment) are mediated by these receptor subtypes. Similar expression patterns of EP_3 and EP_4 in the Day 4 pseudopregnant mouse uterus or in the ovariectomized uterus under combined treatment with estrogen and progesterone suggest that these genes are regulated by ovarian steroids rather than by the embryo during the preimplantation period (Days 1–4). In contrast, the expression of these genes during the postimplantation period (Days 5–8) is associated with the onset of decidualization.

INTRODUCTION

Cyclooxygenase (COX) is a rate-limiting enzyme that produces prostaglandins (PGs) from arachidonic acid. COX exists in two isoforms: COX-1, a constitutive enzyme, and COX-2, an enzyme induced by a variety of stimuli [1]. The most extensively studied members of this family are PGE_2, $PGF_{2\alpha}$, and PGI_2. However, the physiological significance of PGD_2 and PGJ_2 has recently been addressed [2–4]. In general, PGs are implicated in the regulation of growth, differentiation, and/or homeostasis [1]. In the rodent, major preimplantation events leading to successful implantation are fertilization of eggs in the oviduct, development of embryos into blastocysts, their spacing in the uterus, escape of blastocysts from their zona pellucidae, and a "two-way" interaction between the blastocyst and the receptive uterus. One of the earliest prerequisite events in the process of implantation is an increased endometrial vascular permeability at the sites of blastocyst apposition. This event coincides with the attachment reaction [5, 6]. Thus, PGs could be involved in uterine contractions to facilitate sperm transport after mating or blastocyst spacing prior to implantation. These mediators are also considered to be involved in embryo development and blastocyst zona dissolution, implantation, and decidualization. The involvement of PGs in implantation is indicated by their higher levels at the implantation sites, interference with implantation by PG synthesis inhibitors, and reversal of this inhibition by coadministration of PGs [7–11]. Further, increases in uterine PG and COX-1 levels prior to implantation in the rodent suggest that these mediators could be involved in uterine edema and luminal closure important for the attachment reaction [8, 12, 13]. PGs are also implicated in decidual cell reaction [14, 15]. Recognition of the importance of PGs in blastocyst development and hatching is derived from the observation that PG synthesis inhibitors or antagonists interfere with these processes in vitro [16, 17].

In spite of considerable evidence regarding the possible functions of PGs in the process of implantation, little is known about the sites of PG actions in the uterus and/or embryo during the periimplantation period. PGE_2 or $PGF_{2\alpha}$ interacts with specific G protein-coupled receptors to modulate the levels of second messengers such as cAMP, Ca^{2+}, and inositol trisphosphate (IP_3). PGE_2 receptors are classified into four subtypes, EP_1, EP_2, EP_3, and EP_4 [18–23]. EP_1 is coupled to Ca^{2+} mobilization. On the basis of pharmacological analysis, the receptor subtype that was cloned as EP_2 is now considered EP_4 [19, 21]. However, an EP_2 receptor subtype has also been cloned [22]. Both EP_2 and EP_4 receptor subtypes are coupled to stimulation of adenylyl cyclase, while EP_3 is coupled to inhibition of adenylyl cyclase. $PGF_{2\alpha}$ receptor (FP) is functionally coupled to the stimulation of phospholipase C that produces IP_3 and Ca^{2+} mobilization [24]. Receptors for PGD_2, PGI_2, or PGJ_2 have also been identified [3, 4, 25]. Since most of the known effects of PGs in uterine biology and embryo-uterine interactions center around PGE_2 and $PGF_{2\alpha}$, the present study examined the temporal and cell type-specific expression of PGE_2 and $PGF_{2\alpha}$ receptors in the periimplantation mouse uterus. Uterine events during the periimplantation period are regulated primarily by the coordinated and cell-specific

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effects of estrogen and progesterone (P_4). Therefore, we also examined the regulation of the PG receptor genes in the ovariectomized adult mouse uterus under the stimulation of these ovarian steroids.

MATERIALS AND METHODS

Animals and Tissue Preparation

Under the approval of NIH and institutional guidelines, CD-1 mice (Charles River Laboratory, Raleigh, NC) were housed in the animal care facility at the University of Kansas Medical Center. They were maintained on a 14L:10D schedule with lights-on at 0600 h. Adult female mice (20–25 g, 48–60 days old) were mated with fertile males of the same strain. The morning a vaginal plug was found was designated Day 1 of pregnancy. Mice were killed between 0830 and 0900 h on Days 1–8 of pregnancy. To collect uteri at the onset of the attachment reaction, mice were killed between 2200 and 2300 h on Day 4 of pregnancy. Whole uteri were collected on Days 1–7 of pregnancy, while the deciduum from Day 8 uteri was separated by surgical procedure. No attempt was made to separate the embryos from the decidua. Early implantation sites on Day 4 (2200–2300 h) and Day 5 (0830–0900 h) were visualized by i.v. injections (0.1 ml/mouse) of a Chicago Blue B dye solution (1% in saline). Mice were killed 5 min later [5,13]. Uteri were processed for Northern blotting and in situ hybridization. Mice in which pseudopregnancy was produced by mating with vasectomized males were also killed at these times to examine whether embryonic influences are important for expression of PG receptors in the uterus during early pregnancy. Uteri from 10–15 mice were pooled for each day of pregnancy and used for RNA isolation. For in situ hybridization, uteri were cut into small pieces and stored at −80°C until used.

To determine the effects of estrogen and P_4, mice were ovariectomized without regard to the stage of the estrous cycle and rested for 2 wk. They were then treated with an injection of estradiol-17β (E_2, 100 ng/mouse; Sigma Chemical Co., St. Louis, MO), P_4 (1 mg/mouse; Sigma), or a combination of the same doses of P_4 and E_2. All steroids were dissolved in sesame oil and injected s.c. Controls received the vehicle only (0.1 ml/mouse). Mice were killed at various times after hormone injections, and their uteri were collected for RNA extraction and in situ hybridization.

Hybridization Probes

Mouse-specific cDNAs to EP_1, EP_3, EP_4, and FP were used for the present investigation. These cDNAs were subcloned in appropriate vectors for synthesis of both sense and antisense cRNA probes [18, 19, 21, 23, 24]. For Northern hybridization, antisense ^{32}P-labeled cRNA probes were generated, while for in situ hybridization, sense and antisense ^{35}S-labeled cRNA probes were generated using the appropriate polymerases. A cDNA clone of human fibroblast cytoplasmic β-actin [26] was subcloned into a pGEM vector containing a promotor for SP6 polymerase and used as a template for synthesis of a ^{32}P-labeled antisense cRNA probe [27]. Probes had specific activities of about 2×10^9 dpm/μg.

Northern Blot Hybridization

Total RNAs were extracted from whole uteri by a modified guanidine thiocyanate procedure [28]. Poly(A)$^+$ RNAs were isolated from total RNAs by oligo(dT)-cellulose column chromatography [29]. Total (6 μg) or poly(A)$^+$ (2 μg) RNAs were denatured, separated by formaldehyde-agarose gel electrophoresis, and transferred to nylon membranes. RNAs were cross-linked to the membranes by UV irradiation (Spectrolinker, XL-1500; Spectronics Corp., Westbury, NY). The blots were prehybridized, hybridized, and washed as described previously [30]. After hybridization, the blots were washed under stringent conditions, and the hybrids were detected by autoradiography. The blots were stripped and rehybridized with a β-actin probe as described previously [31]. Total RNA blots were sequentially hybridized with the EP_3 and EP_4 probes, while poly(A)$^+$ RNA blots were similarly hybridized with the EP_1 and FP probes. For total RNA analysis, duplicate gels were run and stained with acridine orange to confirm the integrity of RNA samples. Autoradiograms were subjected to densitometric scanning (Personal Densitometer SI; Molecular Dynamics, Sunnyvale, CA) for quantitation of PG receptor mRNA levels relative to β-actin mRNA levels.

In Situ Hybridization

In situ hybridization was performed as described previously [32]. Uteri were cut into 4- to 6-mm pieces and flash frozen in freon. Frozen sections (10 μm) from Days 1–4 or Days 5–8 of pregnancy were mounted onto poly-L-lysine-coated slides and fixed in cold 4% paraformaldehyde solution in PBS. Sections were then acetylated, prehybridized, and hybridized at 45°C for 4 h in hybridization buffer containing ^{35}S-labeled antisense or sense cRNA probes. After hybridization and washing, the slides were incubated with RNase A (20 μg/ml) at 37°C for 15 min. RNase A-resistant hybrids were detected by autoradiography using Kodak NTB-2 (Eastman Kodak, Rochester, NY) liquid emulsion. Sections from the same uterus were hybridized with the antisense or corresponding sense probes. Sections were poststained with hematoxylin and eosin.

RESULTS

Northern Blot Analysis of PG Receptor mRNAs in the Periimplantation Uterus

As reported previously [18, 24], a 2.4-kilobase (kb) transcript for EP_1 and two transcripts (6.0 kb and 2.3 kb) for FP were detected only in poly(A)$^+$ uterine RNAs. The use of poly(A)$^+$ for detecting these transcripts suggests their low abundance in the periimplantation uterus. The steady-state uterine levels of EP_1 mRNA showed only modest increases on Days 4–7 of pregnancy (Fig. 1). In contrast, the levels of FP transcripts were relatively higher on Days 1–3 of pregnancy, but declined thereafter (Fig. 1). As reported previously [19, 22, 23], 2.3-kb and 3.9-kb transcripts for EP_3 and EP_4 mRNAs, respectively, were detected in total RNA samples, suggesting their relatively higher abundance in the periimplantation uterus. The levels of these mRNAs were higher on Days 3 and 4 of pregnancy (Fig. 2). Because of the high sequence similarity between β-actin and α-actin, the β-actin probe hybridizes with both the uterine β-actin and α-actin mRNAs [33]. The presence of the smaller transcript in whole uterine RNA samples reflects the abundance of α-actin mRNA in the myometrium [33]. This transcript was not detected in decidual RNA. The results are shown as the average of two separate experiments with about 10% variation between the duplicate experiments.

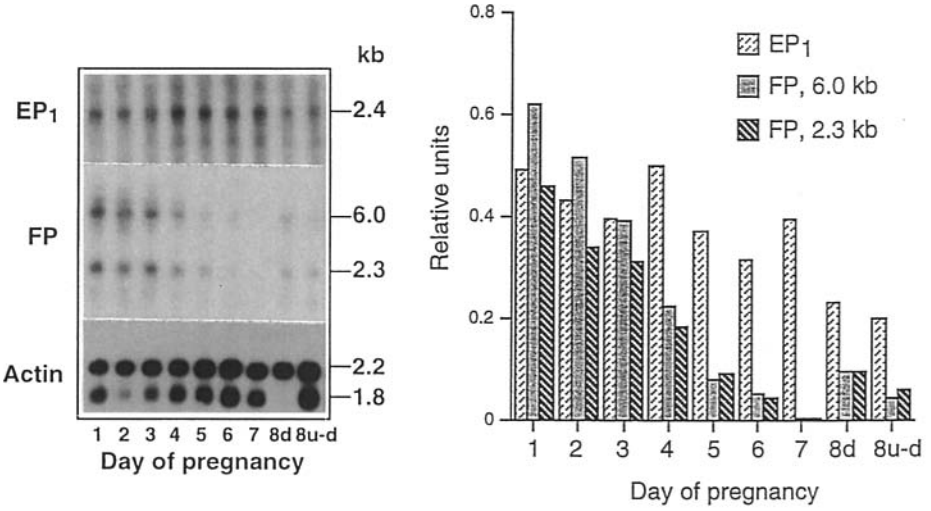

FIG. 1. Northern blot analysis of EP_1 and FP mRNAs in the mouse uterus during the periimplantation period. **Left**) Steady-state levels of uterine EP_1 and FP mRNAs were detected in whole uterine poly(A)$^+$ RNA samples obtained on Days 1–7 or in the separated deciduum (d) and uterus minus the deciduum (u-d) on Day 8 of pregnancy. Poly(A)$^+$ RNAs (2 μg) were separated by formaldehyde-agarose gel electrophoresis, transferred to nylon membranes, UV cross-linked, and hybridized to ^{32}P-labeled cRNA probes specific for mouse EP_1 and FP mRNAs. The same blot was stripped and rehybridized to a β-actin probe. The autoradiographic exposures were 15 h, 48 h, and 0.5 h for EP_1, FP, and β-actin, respectively. **Right**) Quantitation of receptor mRNA levels relative to those of the β-actin mRNA. The presence of the smaller transcript in whole uterine RNA samples reflects the abundance of α-actin mRNA in the myometrium [33]. This transcript was not detected in decidual RNA. The results are an average of two separate experiments.

In Situ Hybridization of PG Receptor mRNAs in the Periimplantation Uterus

The results of Northern blot hybridization of whole uterine RNA samples suggested that PG receptor genes are expressed in the uterus during the preimplantation period. However, if PG receptors are important for periimplantation events, it should be through cell-specific expression in the uterus in a temporal manner. Thus, the distribution of PG receptor mRNAs in the periimplantation uterus was examined by in situ hybridization. On Days 1–5 of pregnancy, only low levels of signals for EP_1 mRNA were present in all major uterine cell types without any distinct cell-specific accumulation. However, from Day 6 onward, localized EP_1 mRNA accumulation was evident in a subpopulation of cells in the decidual bed at the mesometrial pole. Autoradiographic signals in representative Day 1 and 8 pregnant uterine sections are shown (Fig. 3). With respect to EP_3 mRNA (Fig. 4), no specific localized signals were observed on Days 1 and 2 of pregnancy. However, this mRNA was clearly localized in the circular muscle of the myometrium and in a subpopulation of cells in the stromal bed at the mesometrial pole on Days 3 and 4 of pregnancy. This pattern persisted with the onset of the attachment reaction at 2300 h on Day 4 and the progression of implantation on Day 5. A similar expression pattern was also observed in the Day 4 pseudopregnant uterus. While low levels of signals persisted in the circular muscles on Day 6 of pregnancy, EP_3 mRNA was primarily detected in the secondary decidual zone on Day 7. On Day 8 of pregnancy, no specific signals were present in any uterine cell types. Auto-

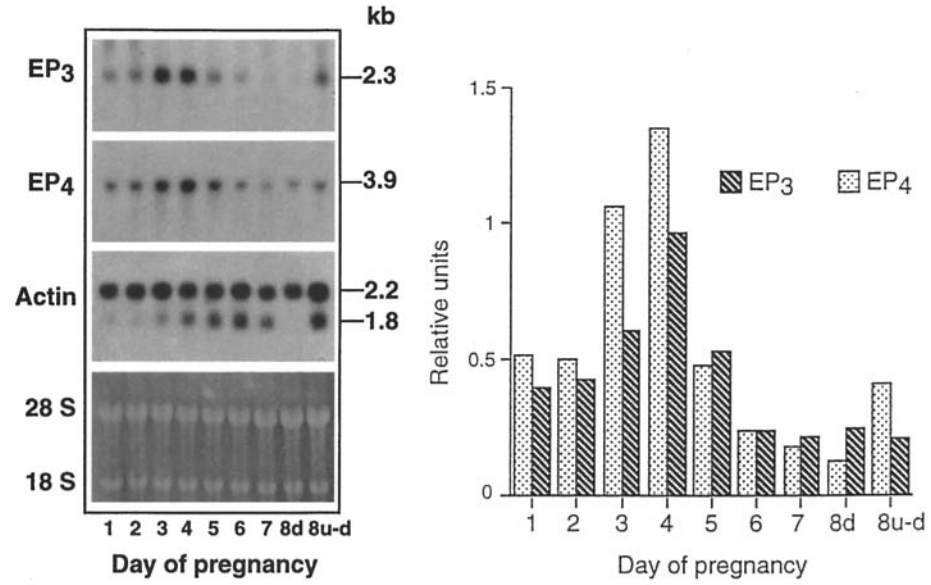

FIG. 2. Northern blot analysis of EP_3 and EP_4 mRNAs in the mouse uterus during the periimplantation period. **Left**) Steady-state levels of uterine EP_3 and EP_4 mRNAs were detected in whole uterine total RNA samples obtained on Days 1–7 or in the separated deciduum (d) and uterus minus the deciduum (u-d) on Day 8 of pregnancy. Total RNAs (6 μg) were separated by formaldehyde-agarose gel electrophoresis, transferred to nylon membranes, UV cross-linked, and hybridized to ^{32}P-labeled cRNA probes specific for mouse EP_3 and EP_4 mRNAs. The same blot was stripped and rehybridized to a β-actin probe. RNA samples in duplicate gels were stained with acridine orange to ensure integrity of RNA. The mobilities of 28S and 18S rRNAs are indicated. The autoradiographic exposures were 5 h, 3 h, and 0.5 h for EP_3, EP_4, and β-actin, respectively. **Right**) Quantitation of receptor mRNAs relative to β-actin mRNA. The presence of the smaller transcript in whole uterine RNA samples reflects the abundance of α-actin mRNA in the myometrium [33]. This transcript was not detected in decidual RNA. The results are an average of two experiments.

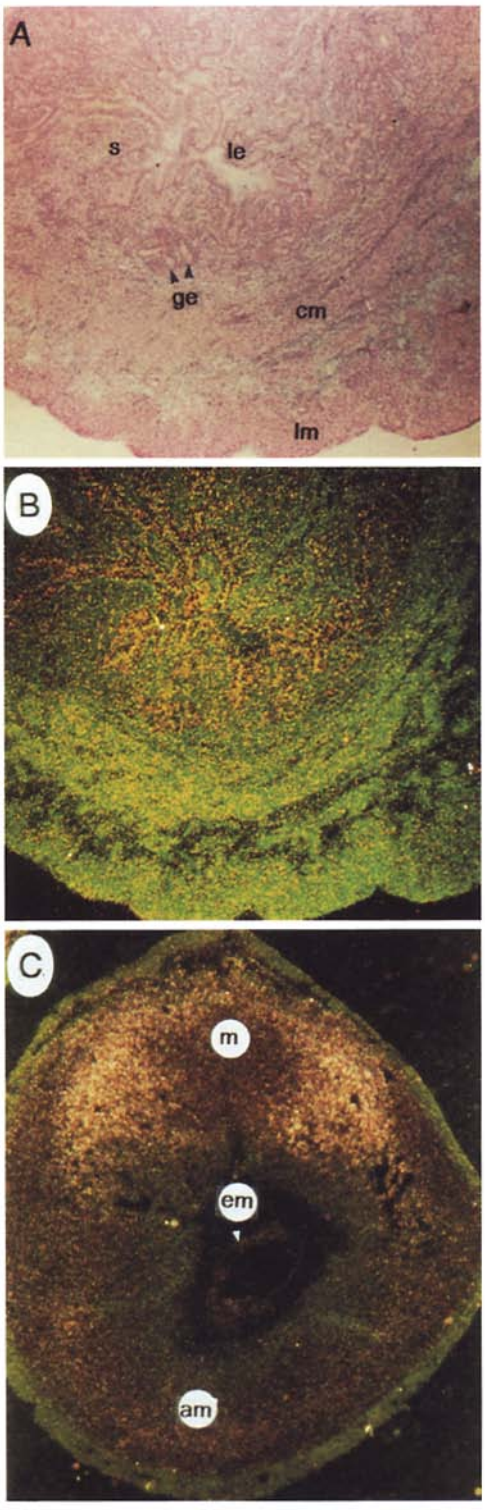

FIG. 3. In situ hybridization of EP_1 mRNA in the periimplantation mouse uterus using sections hybridized with a ^{35}S-labeled EP_1 antisense cRNA probe; sections from Days 1–4 or Days 5–8 of pregnancy were mounted onto the same slide. RNase A-resistant hybrids were detected after 10–15 days of autoradiography using Kodak NTB-2 liquid emulsion. EP_1 mRNA distribution in representative Day 1 (**A**, brightfield and **B**, darkfield; ×100) and Day 8 (**C**, darkfield; ×40) is shown. le, luminal epithelium; ge, glandular epithelium; s, stroma; cm, circular muscle; lm, longitudinal muscle; em, embryo; m, mesometrial pole; am, antimesometrial pole.

radiographic signals for Days 4, 5, and 7 of pregnancy as well as for Day 4 of pseudopregnancy are shown in Figure 4. With respect to EP_4 mRNA (Fig. 5), low levels of accumulation were evident primarily in the luminal epithelium on Days 1 and 2 of pregnancy, while distinct signals were detected in both the luminal epithelial and stromal cells on Days 3 and 4. Some signals were also noted in the glandular epithelium. A similar pattern of expression was also noted in the Day 4 pseudopregnant uterus. With the initiation of the attachment reaction on Day 4 (2300 h), EP_4 mRNA accumulation was still maintained in these cells. On Day 5 of pregnancy, this mRNA was localized in the luminal epithelium and decidualizing stroma around the implanting blastocyst. In the Day 5 pseudopregnant uterus, EP_4 mRNA was detected only in stromal cells. On Days 6–8 of pregnancy, signals were present in both the primary and secondary decidual cells and in undifferentiated stroma below the myometrium. Embryonic cells also exhibited accumulation of this mRNA on Day 8. Autoradiographic signals for Days 1, 4, and 5 of pregnancy and Day 4 of pseudopregnancy are shown in Figure 5.

In situ localization of FP mRNA also exhibited an interesting expression pattern. Low levels of signals were detected in some stromal cells and in the circular muscle layer on Day 1 of pregnancy. The stromal cell signals were not detected on Day 2, while myometrial signals showed a gradual increase reaching peak levels on Days 3 and 4. On Day 5, the accumulation in the circular muscle declined, and the decline was followed by undetected levels on Days 6–8. However, on Day 6, FP mRNA accumulation became highly localized to a subpopulation of cells in the decidual bed at the mesometrial pole and in undifferentiated stromal cells underneath the myometrium. On Days 7–8, cells accumulating this mRNA at the mesometrial pole became more polarized toward the lumen, and accumulation in the undifferentiated stromal cells markedly declined. Autoradiographic signals for Days 1, 4, and 6–8 of pregnancy are shown in Figure 6.

Sections hybridized with the corresponding sense probes exhibited only background nonspecific signals on any day of pregnancy examined. Background levels of autoradiographic signals of EP_1 (Day 8), EP_3 (Day 4), EP_4 (Day 4), and FP (Day 6) are shown in Figure 7. Further, different probes exhibiting signals at different cellular locations on sections from the same uterus served as internal controls. For example, serial sections of a Day 4 pregnant uterus hybridized with the EP_3 (Fig. 4, A and B) or EP_4 (Fig. 5, C and D) showed different localization of the respective mRNAs. The results shown are representative of data generated from at least 4 different mice for each day of pregnancy examined.

Regulation of the PGE_2 Receptor Genes in the Uterus by Ovarian Steroids

Since ovarian steroids regulate uterine functions in a cell-specific manner, in situ hybridization was performed to localize EP_3 and EP_4 mRNAs in the adult ovariectomized mice after E_2 and/or P_4 treatments. With respect to EP_3, no specific accumulation of this mRNA could be detected in the ovariectomized uterus treated with the vehicle alone (Fig. 8, a and b). While an injection of P_4 clearly induced the EP_3 mRNA accumulation in the circular muscle (Fig. 8, e and f), an injection of E_2 produced a similar response in both the circular and longitudinal muscle layers (Fig. 8, c and d). Treatment with the com-

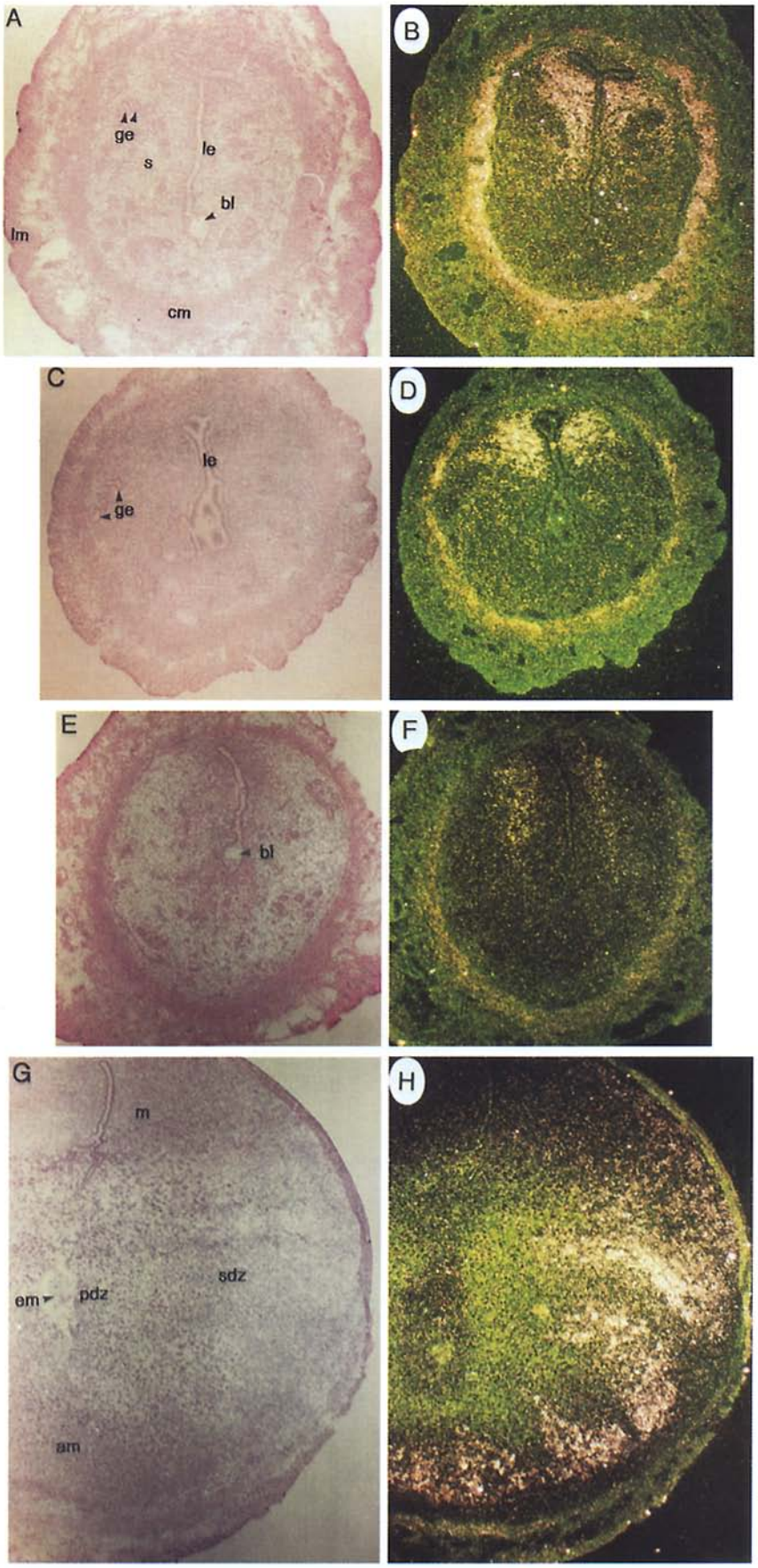

FIG. 4. In situ hybridization of EP_3 mRNA in the periimplantation mouse uterus. Uterine sections from Days 1–4 or Days 5–8 of pregnancy were mounted onto the same slide. Sections were hybridized with a ^{35}S-labeled EP_3 antisense cRNA probe. RNase A-resistant hybrids were detected by autoradiography after 5–7 days of exposure. Uterine EP_3 mRNA distribution on Days 4 (**A, B**), 5 (**E, F**), and 7 (**G, H**) of pregnancy or on Day 4 (**C, D**) of pseudopregnancy is shown in bright-field (left column) and darkfield (right column) photomicrographs at ×40. bl, blastocyst; pdz, primary decidual zone; sdz, secondary decidual zone; other abbreviations as for Figure 3.

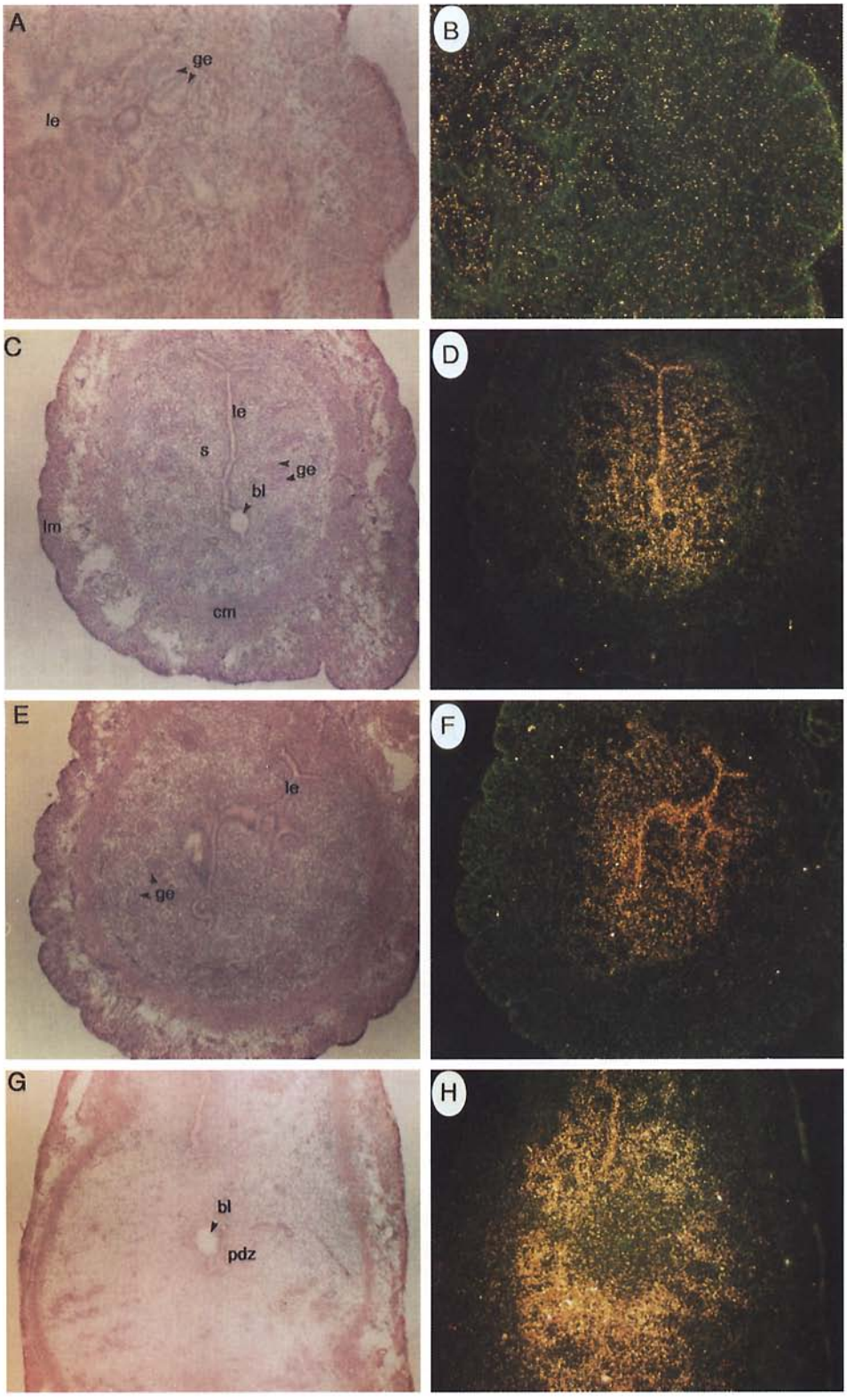

FIG. 5. In situ hybridization of EP_4 mRNA in the periimplantation mouse uterus. Uterine sections from Days 1–4 or Days 5–8 of pregnancy were mounted onto the same slide. Sections were hybridized with a ^{35}S-labeled EP_4 antisense cRNA probe. RNase A-resistant hybrids were detected by autoradiography after 5–7 days of exposure. Uterine EP_4 mRNA distribution on Days 1 (**A, B**), 4 (**C, D**), and 5 (**G, H**) of pregnancy or on Day 4 (**E, F**) of pseudopregnancy is shown in bright-field (left column) and darkfield (right column) photomicrographs at ×40. bl, blastocyst; pdz, primary decidual zone; other abbreviations as for Figure 3.

bination of these steroids induced this gene in the circular muscle as well as in a subpopulation of stromal cells at the mesometrial pole (Fig. 8, g and h), similar to what was observed in the Day 4 pregnant or pseudopregnant uterus. With respect to EP_4, low levels of this mRNA were detected in the luminal epithelium, stroma, and circular muscle of ovariectomized mice treated with the vehicle (oil) alone (Fig. 8, i and j). An injection of E_2 considerably up-regulated the accumulation of EP_4 mRNA in the luminal epithelium (Fig. 8, k and l), whereas an injection of P_4 alone did so in the stroma and luminal epithelium (Fig. 8, m and n). A co-injection of E_2 with P_4 did not change the expression pattern as observed after P_4 treatment alone (data not shown). The results shown are representative of data derived from 3–4 mice for each treatment group.

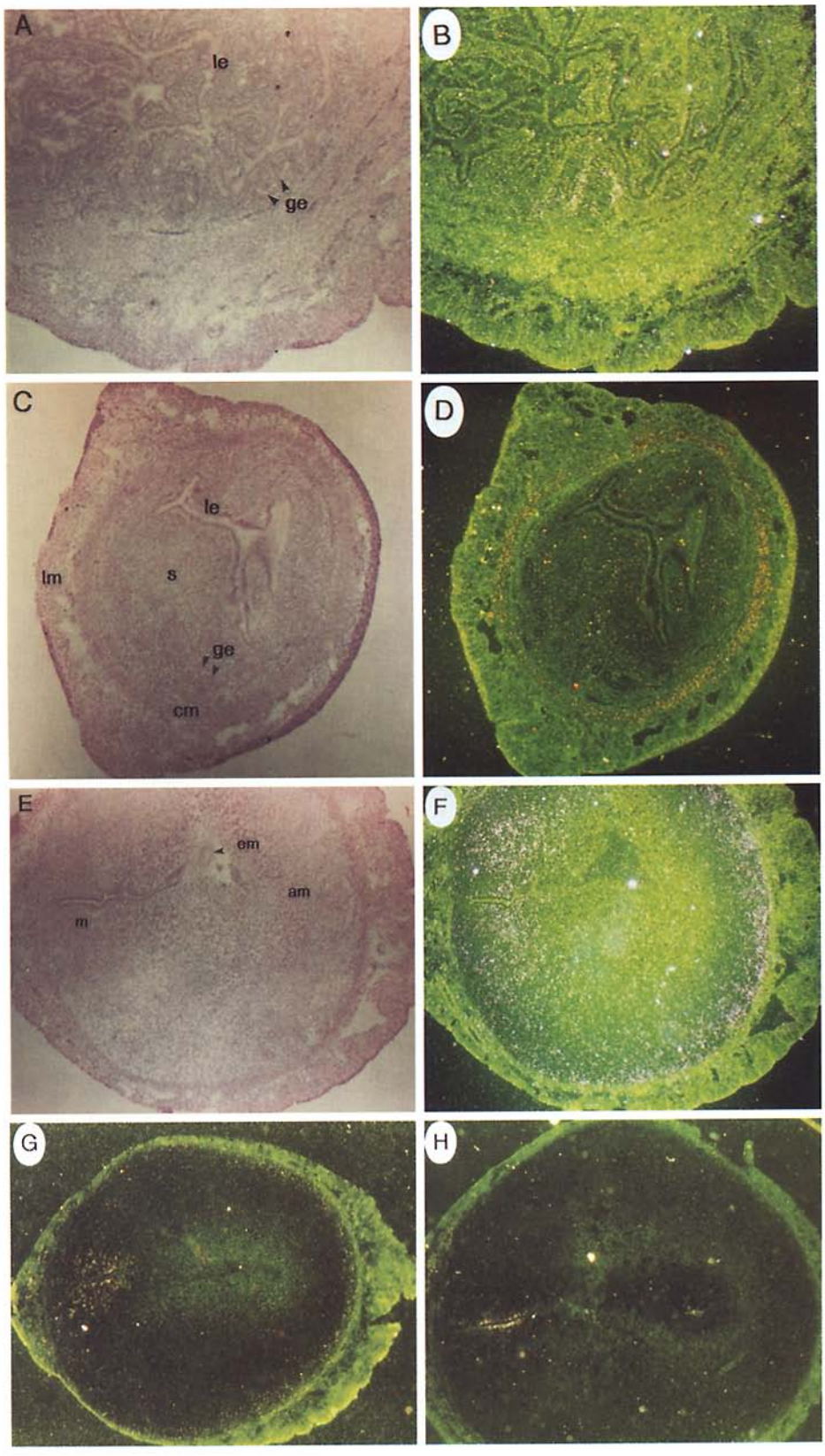

FIG. 6. In situ hybridization of FP mRNA in the periimplantation mouse uterus. Uterine sections from Days 1–4 or Days 5–8 of pregnancy were mounted onto the same slide. Sections were hybridized with a ^{35}S-labeled FP antisense cRNA probe. RNase A-resistant hybrids were detected by autoradiography after 10 days of exposure. Uterine FP mRNA distribution on Days 1 (**A, B**), 4 (**C, D**), and 6 (**E, F**) of pregnancy is shown in brightfield (left column) and darkfield (right column) photomicrographs, while the distribution on Day 7 (G) and Day 8 (H) is shown in darkfield photomicrographs at ×40. Abbreviations are the same as for Figure 3.

DISCUSSION

Although PGs influence a wide range of events in the uterus during pregnancy, the mechanisms by which PGs influence specific effects and the sites of these effects in the uterus are not known. Thus, we examined the spatiotemporal expression and regulation of PGE_2 and PGF_{α} receptors in the periimplantation mouse uterus in order to better understand the roles of these mediators during early pregnancy. This study constitutes the first evidence for the potential sites of actions of PGE_2 and $PGF_{2\alpha}$ in the mouse

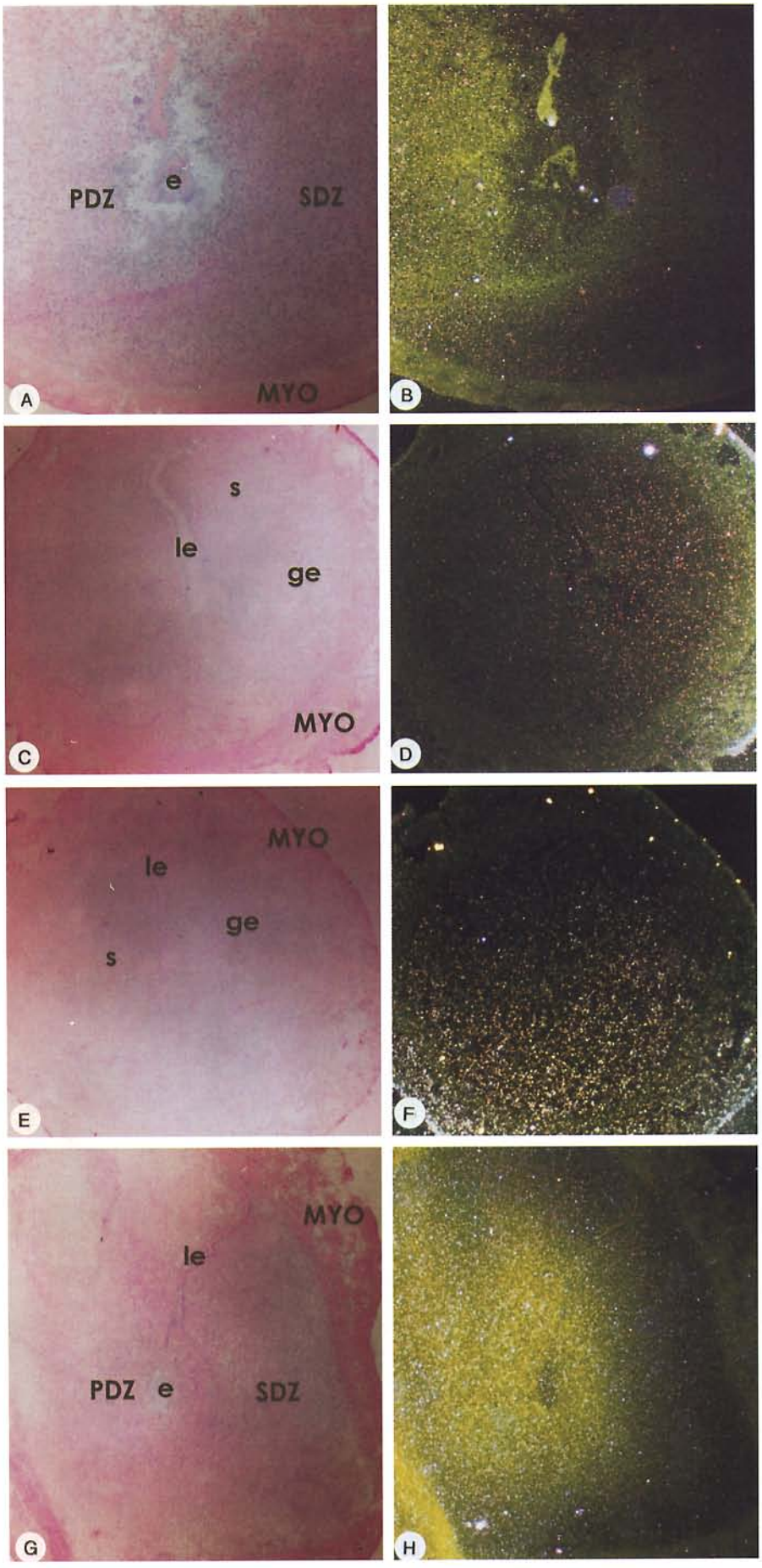

FIG. 7. Negative controls (sense probes) of EP_1, EP_3, EP_4, and FP mRNAs in the uterus. Uterine sections from Days 1–4 or Days 5–8 of pregnancy were mounted onto the same slide. Sections were hybridized with a ^{35}S-labeled sense cRNA probes. RNase A-resistant hybrids were detected by autoradiography after similar days of exposure corresponding to the antisense probes. Uterine mRNA distributions for EP_1 on Day 8 (**A, B**), EP_3 on Day 4 (**C, D**), EP_4 on Day 4 (**E, F**), and FP on Day 6 (**G, H**) of pregnancy are shown in bright-field (left column) and darkfield (right column) photomicrographs at ×40. MYO, myometrium; PDZ, primary decidual zone; SDZ, secondary decidual zone; other abbreviations as for Figure 3.

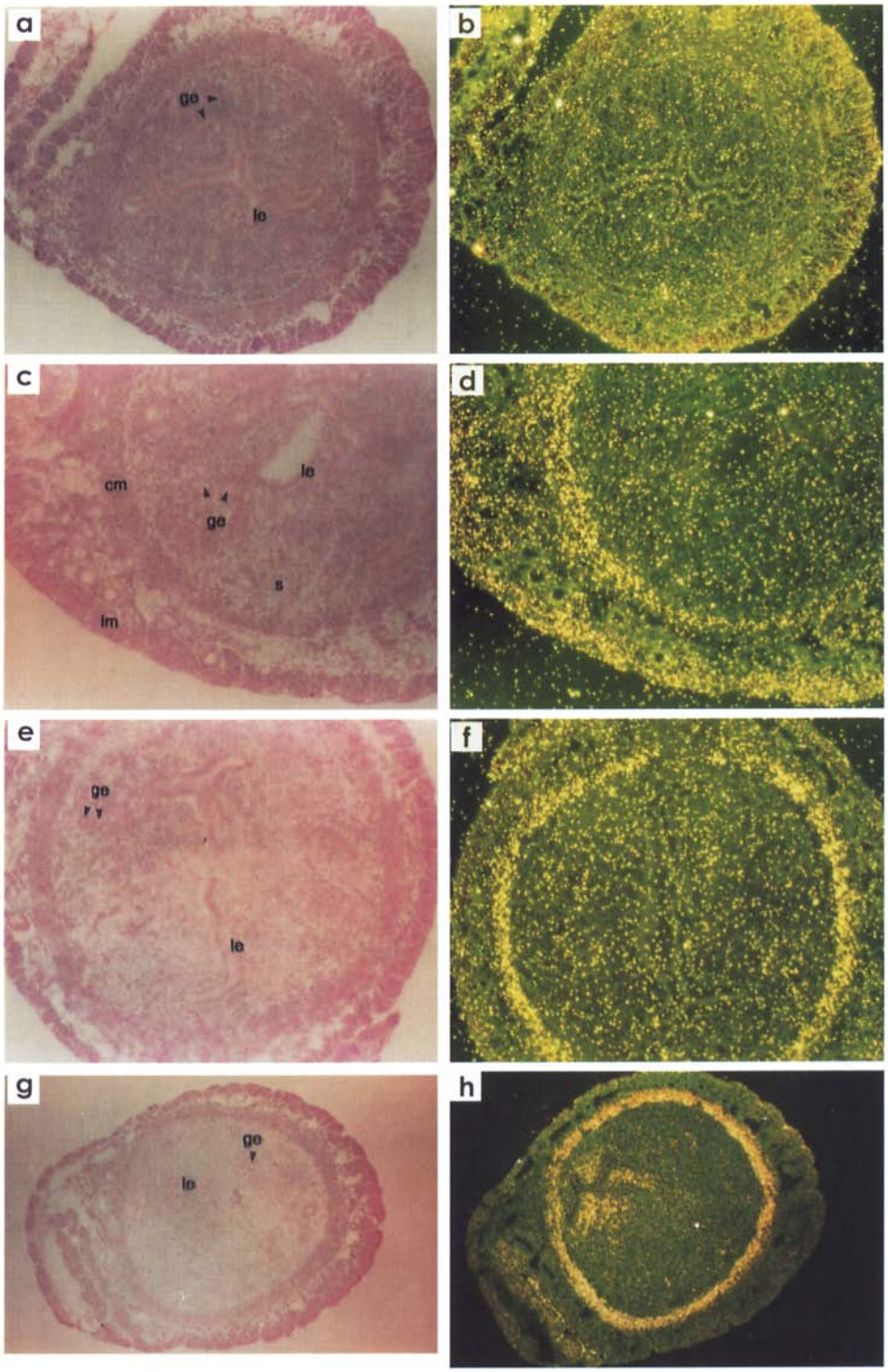

FIG. 8. In situ hybridization of EP_3 and EP_4 mRNAs in steroid-treated adult ovariectomized mice. Mice were given an injection of oil (0.1 ml/mouse), E_2 (100 ng/mouse), P_4 (1 mg/mouse), or a combination of the same doses of E_2+P_4 and killed 24 h later. EP_3 and EP_4 mRNA distributions are shown in brightfield (left column) and darkfield (right column) photomicrographs. EP_3 mRNA: oil (**a, b**), E_2 (**c, d**), P_4 (**e, f**), and E_2+P_4 (**g, h**). EP_4 mRNA: oil (**i, j**), E_2 (**k, l**), and P_4 (**m, n**). All magnifications are shown at ×100, except **g, h**, which are shown at ×40. Abbreviations are the same as for Figure 3.

uterus during the periimplantation period and under specific steroid hormonal stimulations. Furthermore, the results provide evidence for the diversification of PG actions with respect to specific developmental stages and uterine cell types during early pregnancy.

PGE_2 is likely to exert multiple effects during the periimplantation period depending upon the expression of PGE receptor subtypes. Since EP_1 is coupled to Ca^{2+} channels [34], the expression of EP_1 mRNA primarily in the secondary decidual zone (SDZ) at the mesometrial pole on Days 6–8 could be involved in angiogenesis required for the establishment of the placenta. The role of Ca^{2+} in angiogenesis is well documented [35]. In contrast, increased levels of cAMP resulting from the activation of EP_2 or EP_4 may participate in the process of implantation and decidualization. Indeed, previous reports [36–41], as well as the present observation of luminal epithelial and stromal cell expression of EP_4 on Days 4 and 5, suggest the involvement of PGE_2 and cAMP in these processes. Since cAMP can exert opposite effects on cell proliferation depending on the cell types involved [42], differential expression of EP_3 and EP_4 in the decidualizing stroma on Days 5–8 sug-

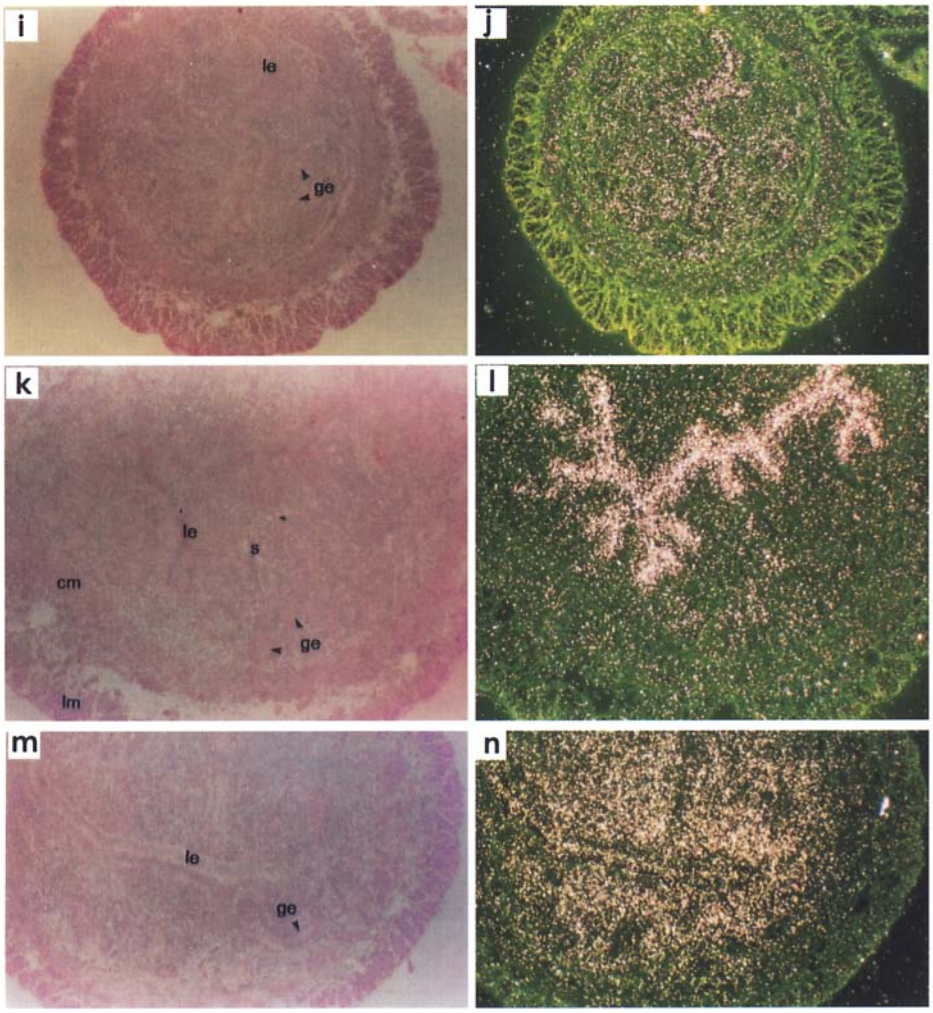

FIG. 8. Continued

gests that PGE_2 effects on decidualization (proliferation and differentiation) should involve these receptor subtypes. However, the significance of expression of EP_4 and FP in the undifferentiated stroma underneath the myometrium during this period is presently not clear.

Although PGs are considered important mediators for the localized uterine vascular permeability at the sites of implantation [8], the mechanism by which they mediate this response is unknown. PGs themselves are poor inducers of edema, but they can enhance this response induced by other factors [34]. Vascular endothelial growth factor (VEGF) is angiogenic and stimulates vascular permeability (reviewed in [43]). Since PGs are known inducers of VEGF [44, 45], it is possible that increased endometrial vascular permeability at the sites of implantation is mediated by VEGF induced by PGE_2 via interaction with EP_4. Indeed, the expression patterns of VEGF and its receptors in the periimplantation mouse uterus are consistent with this assumption [43].

The mechanism(s) that determines embryo spacing in the uterus of polytocous species is poorly understood. Myometrial contraction presumably contributes to uterine spacing of blastocysts [46, 47]. Thus, initial uterine spacing of embryos is considered to be mediated by myometrial contractility, while completion of this process after the attachment reaction is perhaps achieved by the elongation and differential growth of the uterus [48, 49]. Although PGs are implicated in uterine contraction, their mechanism(s) of action is not known. While increases in cytosolic Ca^{2+} levels stimulate smooth muscle contraction, increases in intracellular cAMP levels inhibit it. The present study suggests that PG-mediated uterine contractions could be regulated via activation of EP_3 and/or FP in the circular muscle. Since activation of EP_3 inhibits adenylyl cyclase [20] and that of FP stimulates IP_3 levels and Ca^{2+} mobilization [24], regulated myometrial activity could be achieved by an appropriate ratio between the inhibitory and stimulatory factors. The role of PG-mediated Ca^{2+} mobilization in uterine contractions has been reported [50–52]. Thus, the expression of EP_3 and FP in the circular muscle on Days 3–5 could function in a cooperative manner in myometrial contractility for embryo spacing. In fact, inhibition of uterine PG synthesis by indomethacin during early pregnancy in the rat interferes with uterine spacing of embryos [8]. After embryo spacing and initiation of implantation, the myometrium remains in a state of quiescence until the time of parturition. The down-regulation of the EP_3 and FP genes in the circular muscle on Days 6–8 is compatible with this event. EP_3 or FP was not expressed in the longitudinal muscle, suggesting that this muscle layer is perhaps not the target for PGE_2 or $PGF_{2\alpha}$ to elicit uterine contractions. However, the possibility that other mediators influence the

longitudinal muscle for uterine contractions cannot be excluded.

The significance of the unique expression of EP_3 in a subpopulation of cells in the stromal bed at the mesometrial site on Day 4 is not clear. Since PGE_2 appears to be involved in Na^+ and water reabsorption in the kidney [53], EP_3 could be involved in uterine edema and subsequent luminal closure required for the attachment reaction. Further, it is also interesting to note that the location of these subpopulations of cells at the mesometrial stromal bed is the presumptive site of angiogenesis and placentation, favoring increased blood flow because of the presence of afferent arterioles of the mesometrial triangle and the efferent endometrial circumferential venules [54].

In the mouse, uterine preparation to achieve the receptive state for blastocyst implantation on Day 4 is primarily regulated by rising ovarian P_4 plus a small amount of estrogen; it is not dependent upon the presence of the embryo [55]. This is consistent with the observation of a similar expression pattern of EP_3 or EP_4 on Day 4 of pregnancy or pseudopregnancy. Further, similar uterine expression of these receptor subtypes under E_2 and/or P_4 treatment suggests that these uterine genes are primarily regulated by ovarian steroids during the preimplantation period. In this respect, high-affinity binding of PGE_2 was detected in rat and human stromal cell membranes, and this binding in the rat uterus was P_4-dependent [56–58]. In contrast, uterine $PGF_{2\alpha}$ binding was either undetectable or very low in the rat and human uterus [58, 59]. However, defining diverse functions of PGs will require continuing investigation because of the addition of new members to the PG receptor gene family [22, 60, 61]. Nonetheless, our present studies on the temporal and cell-specific expression of certain PG receptor subtypes and of COX-1 and COX-2 [13] in the mouse uterus provide evidence for possible sites of synthesis and actions of PGs in the mouse uterus during the periimplantation period. Development of specific antagonists to or mutation of the PG receptor subtypes will be required to support or refute their proposed roles in uterine biology.

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