Prostaglandins and the initiation of blastocyst implantation and decidualization

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Abstract

The process of blastocyst implantation in mammals is remarkably variable, especially in the extent of trophoblast invasion of the endometrium. In most species studied, the earliest macroscopically identifiable sign of blastocyst implantation is an increase in endometrial vascular permeability in areas adjacent to the blastocysts. This is followed in species with invasive implantation by decidualization, again localized to areas adjacent to the blastocysts. In some species, the application of a stimulus to the endometrium can result in increased endometrial vascular permeability and decidualization. Based initially on studies utilizing inhibitors of prostaglandin (PG) synthesis and more recently on studies using the techniques of transgenics, considerable evidence has accumulated indicating that PGs have an important role in the early events of implantation and artificially induced decidualization. However, which PGs are involved remains controversial. There may be differences between species, and different PGs may be involved at different times.

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Introduction

While many different molecules have been implicated in the signaling that occurs between the embryo and the endometrium at the initiation of implantation, this review will concentrate on the role of prostaglandins (PGs). More is known about the role of PGs in implantation in rodents than in other species, and consequently this is the focus. In addition, the involvement of PGs in artificially induced decidualization is reviewed because this reaction has been widely and profitably used as a model of implantation.

Initiation of implantation and decidualization

Implantation of the embryo involves a complex series of sequential interactions between the embryo and the uterus, beginning with attachment of the blastocyst to the luminal epithelium of the endometrium and ending with the formation of the definitive placenta (see Fig. 1 for the timing of events in the rat). Embryo–endometrial interactions can only be initiated when the embryo and the endometrium have reached a precise stage of maturity; the embryo must be at the blastocyst stage, and hormone-dependent changes leading to the development of a ‘receptive’ endometrium must have occurred (Psychoyos 1973).

The process of blastocyst implantation in mammals is remarkably variable, especially in the extent of

trophoblast invasion of the endometrium. In some species there is little or no invasion of trophoblast cells into the endometrium, whereas in others the invasion is extensive (Psychoyos 1973). In those species with no or limited invasion, the endometrium undergoes little change in response to implantation. By contrast, in those species with extensive invasion, the endometrium undergoes substantial morphological and functional changes referred to as the decidual transformation of the endometrium. In most species, this transformation primarily involves the endometrial stroma and is localized to implantation sites. The fibroblastic stromal cells proliferate and differentiate into decidual cells, which ultimately form the maternal component of the placenta.

In all species investigated – with the exception of humans – there is an increase in the endometrial vascular permeability localized to areas adjacent to the blastocysts (Psychoyos 1973). In mice and rats, this occurs ~4–8 h after the initial blastocyst attachment, is the first macroscopically identifiable event in implantation, and is commonly taken as defining the beginning of implantation. The increase in endometrial vascular permeability is thought to be an essential prerequisite for decidualization. The localized nature of the endometrial vascular permeability response and subsequent decidualization suggests that they occur in response to interactions between the embryo and the endometrium.

Figure 1 Timing of events occurring early in pregnancy in the rat. For mice, uterine closure and the increased endometrial vascular permeability occur ~18 h earlier.

Signaling between the embryo and the endometrium

What is the nature of the signals interchanged between the embryo and the endometrium? Many different molecules have been implicated (for reviews, see [Paria et al. 2002](#), [Dey et al. 2004](#)). However, it is possible that the primary signal given by the blastocyst may be physical rather than chemical in nature. The fact that a variety of non-specific stimuli applied to the endometrium of rodents and some other species at an appropriate time will increase endometrial vascular permeability and result in decidualization suggests that the endometrium is able to produce many, if not all, of the signaling molecules involved. In addition, in the human a conceptus does not have to be present to initiate decidualization; it begins 'spontaneously' in the late luteal phase of the menstrual cycle with the differentiation of 'predecidual cells' ([Kennedy 2003](#)). An exposure of the endometrium to progesterone beyond the normal nonpregnant luteal phase results in a decidual transformation of the endometrium. Presumably because a signal from the embryo is not required for the initiation of decidualization, the transformation in the human uterus is generalized rather than localized to the site of implantation.

Ultrastructural studies of the early stages of implantation have demonstrated physical interactions between trophoblastic cells of the blastocyst and endometrial luminal epithelial cells. The endometrial vascular permeability response is preceded by interdigitation of microvilli on trophoblastic and epithelial cells, followed by broad areas of apposition of cell membranes ([Parkening 1976](#), [Enders & Schlafke 1979](#), [Segalen & Chambon 1983](#)). These interactions between the blastocyst and the endometrium are facilitated by the

phenomenon of uterine 'closure' which results from the removal of fluid from the uterine lumen in the peri-implantation period, thereby bringing the embryo into close contact with the luminal epithelium ([Martin et al. 1970](#), [Hedlund et al. 1972](#), [Ljungkvist 1972](#)). The intimate contact between trophoblastic and epithelial cells that results may be sufficient to signal the presence of the blastocyst. [Finn & Porter \(1975\)](#) have summarized the evidence against the initial signal being physical, but did not take into account the possibility that the blastocyst may have an active role in physically perturbing the luminal epithelium. When cultured *in vitro*, rat and mouse blastocysts undergo repetitive contractions and dilations ([Cole 1967](#), [Bitton-Casimiri et al. 1970](#), [Hurst & MacFarlane 1981](#)). If these contractions and dilations also occur *in vivo*, they will presumably amplify the physical signal resulting from the close contact between the blastocyst and the luminal epithelium.

If the initial signal is physical in nature, it will then be necessary for the luminal epithelial cells to convey the signal to the endometrial stroma since it is the stroma that is vascularized and undergoes decidualization. This would require the conversion of the physical signal to a chemical signal by the epithelial cells. *In vivo*, the luminal epithelium has an essential role in decidualization as indicated by the work of [Ferrando & Nalbandov \(1968\)](#) and [Lejeune et al. \(1981\)](#), which demonstrated that if the luminal epithelium is destroyed or removed, decidualization cannot be obtained in response to stimuli that would otherwise be decidualogenic. These observations are consistent with the concept that the luminal epithelium responds to natural and artificial decidualogenic stimuli with the production of compounds

which then result in decidualization. Whether an increase in endometrial vascular permeability can be obtained in the absence of the luminal epithelium is not known.

PG synthesis and action

PGs are widely produced in mammalian cells from free arachidonic acid (reviewed by Simmons *et al.* 2004). Arachidonic acid is present mainly in membrane phospholipids and is released from these by the action of phospholipases, principally phospholipase A₂. Arachidonic acid can be converted to PGH₂ by PG-endoperoxide synthases (PTGSs); there are at least two PTGS isozymes in mammals: PTGS1 and PTGS2. In general, PTGS1 is constitutively expressed, whereas PTGS2 is inducible. PGH₂ is converted to the biologically active PGs by specific synthases. Phospholipase A₂ and PTGS activities are rate-limiting steps in PG production. The action of PGs is mediated through G-protein-coupled cell-surface receptors, including four subtypes of the PGE receptor (PTGER1, PTGER2, PTGER3, and PTGER4), the PGF receptor (PTGFR), and the PGI₂ receptor (PTGIR). In addition, PGI₂ may act through the nuclear peroxisome proliferator-activated receptor- δ (PPAR δ ; *vide infra*).

PGs and the initiation of implantation/decidualization

In general, investigation of the role of PGs in implantation/decidualization can be separated into two phases. In the first, a pharmacological approach was taken, whereas in the second, more recent phase the techniques of transgenics have been used. Both approaches have their limitations. A concern with the pharmacological approach is the specificity of the drugs being utilized. The use of transgenics is largely limited to mice, and there are concerns as to what extent findings in mice are generally applicable.

In retrospect, it is possible to attribute the first indications of the involvement of PGs in implantation to Horan (1971), who reported the effects of a non-steroidal anti-inflammatory drug on implantation before it had been established that these drugs inhibited PG synthesis. Since then, there have been numerous reports that non-steroidal anti-inflammatory drugs such as indomethacin delay or inhibit the localized increase in endometrial vascular permeability and implantation in a variety of species (reviewed by Kennedy 1990, 1994). Further evidence for the involvement of PGs in implantation was provided by observations demonstrating that the intrauterine administration of PG antagonists at the expected time of implantation reduced the number of implantation sites (Biggers *et al.* 1981).

In addition, the concentrations of PGs are elevated in the areas of increased endometrial vascular permeability associated with the initiation of implantation (Kennedy 1977, Evans & Kennedy 1978, Kennedy & Zamecnik 1978, Sharma 1979, Lim *et al.* 1999, Wang *et al.* 2004b) and exogenous PGs can reverse, at least partially, the effects of indomethacin on implantation (Oettel *et al.* 1979, Holmes & Gordashko 1980, Garg & Chaudhury 1983).

In species in which the application of a decidualogenic stimulus to the sensitized uterus induces decidualization, the extent of the decidual response and the increase in endometrial vascular permeability which precedes it are reduced by indomethacin (reviewed by Kennedy 1990, 1994). Uterine concentrations of PGs are elevated by decidualogenic stimuli before there is a detectable increase in permeability, a time course which is consistent with the elevated levels of PGs being a cause rather than a consequence of the permeability increase. PGs administered into the uterine lumen of animals in which endogenous PG production has been inhibited restore the endometrial responses.

PGs are probably involved not only in the initial vascular changes but also throughout the process of decidualization, as indicated by observations that indomethacin administration 12 h (Tobert 1976) or up to 48 h (Kennedy & Lukash 1982) after the application of a decidualogenic stimulus decreased the extent of decidualization. Because the vascular permeability response to a decidualogenic stimulus occurs by 12 h (Kennedy 1979, Milligan & Mirembé 1984), these findings suggest that PGs are also involved in the later stages of the decidual cell reaction. Support for this proposal comes from observations that, while the injection of PGE₂ into the uterine lumen of indomethacin-treated rats restored the vascular permeability response to that seen in the absence of inhibition of PG synthesis (Kennedy 1979), decidualization was not restored (Kennedy & Armstrong 1981). However, the intrauterine infusion of PGE₂ restored both the permeability response and the decidualization. The differences in responses to injected and infused PGs suggest that, following injection, the PGs do not remain within the uterus at sufficiently high concentrations long enough to bring about decidualization. Additional indirect evidence that PGs are involved throughout the process of decidualization came from the observation that combined administration of indomethacin, both subcutaneously before a decidualogenic stimulus and intraluminally into the uterine lumen during the decidual cell reaction, resulted in substantially lesser decidualization than either treatment alone (Kennedy 1985). The combined treatment with indomethacin was presumably more effective in inhibiting endometrial PG production in the 5 days during which the decidual reaction occurs than either treatment alone.

PGs as signaling molecules – the second phase

The interest in PGs as mediators of the early events in implantation/decidualization waned from around 1985 when investigators concentrated on the roles of locally produced growth factors/cytokines in the processes. However, some investigators explored the possibility that some of the effects of growth factors/cytokines within the endometrium might be a consequence of the modulation of PG production (see, for example, Bany & Kennedy 1995a, 1995b, 1999). The interest was rekindled when it was reported that female mice with a targeted deletion of *Ptgs2* have multiple reproductive deficits, including failure of blastocyst implantation and decidualization (Lim *et al.* 1997). However, the failure of blastocyst implantation is apparently background specific. Using mice purportedly with the same genetic background, Cheng & Stewart (2003) found that implantation occurred in these *Ptgs2* knockout mice, but that the initial rate of decidual growth after implantation was retarded by ~24 h. One of the possible reasons advanced by Cheng & Stewart (2003) to explain why their results differed from those of Lim *et al.* (1997, 1999) was the possibility that different breeding protocols may have resulted in the segregation of a critical modifier gene affecting the requirement for PTGS2 during the process of implantation. Subsequently, Wang *et al.* (2004a) reported that *Ptgs2* null mice on a CD-1 background have an improved implantation relative to null mice on a C57BL/6J/129 background and this was a consequence of the upregulation of *Ptgs1*. Despite the differences, all these results are consistent with the notion that PGs are involved in implantation.

Based on the implantation/decidualization phenotype observed in *Ptgs2* null mice, it follows that PTGSs should be expressed within the endometrium in the peri-implantation period and following artificially induced decidualization. Their expression has been investigated in a number of species, and there are differences between species. In pregnant mice, *Ptgs1* expression in the endometrial luminal epithelium is downregulated at the time of blastocyst attachment, while *Ptgs2* expression was observed in the luminal epithelium and subepithelial stromal cells at the antimesometrial pole surrounding the blastocyst at this time (Chakraborty *et al.* 1996). The expression of *Ptgs2* was not detected in the inter-implantation areas, suggesting that the induction of its expression at the implantation site was induced by the blastocyst. However, it should be noted that the localized increase in endometrial vascular permeability had already occurred at the time these observations were made, and consequently, it cannot be concluded that PTGS2 was responsible for the PGs that resulted in the permeability response. If it could be shown that *Ptgs2* expression is increased at implantation sites prior to the increase in permeability, this would provide suggestive evidence that this enzyme might be responsible.

However, for technical reasons related to the difficulty of identifying implantation sites prior to the increase in permeability, no such data exist, and consequently the question of which isoforms of PTGS are involved is unresolved. In pseudopregnant mice, the application of a decidualogenic stimulus to sensitized endometrium induced the expression of *Ptgs2* in the luminal epithelial cells at 2 h (Lim *et al.* 1999), followed by a decrease at 8 h (Lim *et al.* 1997) and subsequently an expression in the endometrial stroma at 24 h (Lim *et al.* 1999). Under these conditions, no changes in *Ptgs1* mRNA were observed (Lim *et al.* 1997). The time course for the changes in *Ptgs2* gene expression is consistent with the view that PGs produced by PTGS2 are responsible for the increase in the endometrial vascular permeability that follows the application of a decidualogenic stimulus.

In the rat, PTGS1 immunostaining was reported to be strong in the luminal epithelium at implantation sites on day 6 of pregnancy, with less staining in the inter-implantation areas (Cong *et al.* 2006). PTGS2 was localized to the subepithelial stromal cells on the morning of day 5 before the initiation of implantation, and continued to be expressed in the stroma at both implantation sites and inter-implantation areas through to day 6. In pseudopregnant rats not given a decidualogenic stimulus, these investigators reported a high level of PTGS1 immunostaining in the luminal epithelium on days 5 and 6. On day 5 of pseudopregnancy, PTGS2 immunostaining was localized to the antimesometrial subluminal stroma, but this signal was not found on day 6 (Cong *et al.* 2006). It is not known why the expression of *Ptgs2* in the pseudopregnant uterus declined on day 6, whereas it was maintained in the inter-implantation areas in pregnant rats at the equivalent time. Overall, these results suggest that, in contrast to the mouse, a stimulus provided by the blastocyst or a decidualogenic stimulus is not required in rats for endometrial *Ptgs2* expression. Potentially, this might explain why the endometrium of the rat is more sensitive to decidualogenic stimuli than that of the mouse (Finn & Porter 1975). Furthermore, it explains why uterine PG levels in the rat increase rapidly following the application of a decidualogenic stimulus to sensitized endometrium (Kennedy 1979, 1980a, 1980b, Kennedy *et al.* 1980).

On day 3 of pregnancy in the hamster, prior to the initiation of implantation, both *Ptgs1* and *Ptgs2* mRNA are expressed in the endometrial luminal epithelium (Wang *et al.* 2004a). During the initiation of implantation on the afternoon of day 4 of pregnancy, *Ptgs1* mRNA expression was decreased in the luminal epithelium at the implantation sites, but not in the inter-implantation areas. By contrast, *Ptgs2* mRNA levels were high in the luminal epithelium and in the subepithelial stroma at implantation sites when compared with the inter-implantation areas. Thus, as in rats, a signal from the blastocyst is not required in hamsters to induce endometrial *Ptgs2* expression,

but the presence of a blastocyst subsequently upregulates *Ptgs2* expression.

Endometrial *PTGS* expression has also been determined during early pregnancy in the ewe (Chapigny *et al.* 1997, Kim *et al.* 2003), cow (Emond *et al.* 2004), sow (Blitek *et al.* 2006), and mare (Boerboom *et al.* 2004). With the exception of the mare, pregnancy is associated with the upregulation of *PTGS2* gene expression, primarily in the endometrial luminal epithelium. In those studies in which it was investigated, *PTGS1* gene expression was reported to be weak (Emond *et al.* 2004) or unchanged by pregnancy (Chapigny *et al.* 1997). By contrast, on day 15 of pregnancy in the mare, the presence of the conceptus blocked the induction of *PTGS2* expression that was observed on day 15 of the cycle (Boerboom *et al.* 2004). Presumably, the upregulation of *PTGS2* on day 15 of the cycle results in the production of $\text{PGF}_{2\alpha}$, which is luteolytic, and the inhibition of this upregulation during pregnancy is an anti-luteolytic mechanism.

In the baboon, endometrial *PTGS1* expression is decreased in early pregnancy, whereas *PTGS2* expression is high at the implantation site, especially in the stromal cells undergoing decidualization (Kim *et al.* 1999).

Which PG?

The identity of the PGs involved in the initiation of implantation and decidualization is uncertain. It is possible that different PGs are involved at different times. For example, the endometrial vascular permeability response may be mediated by one PG and another may mediate the subsequent proliferation and differentiation of endometrial stromal cells to decidual cells. In addition, it is possible that there may be differences between species. In attempting to identify the PGs involved, use has been made of uterine PG measurements, responses to exogenously administered PGs, and the assessment of expression of PG receptors within the endometrium.

No single PG has been unequivocally identified as the mediator of endometrial vascular changes as a consequence of PG measurements. Based on the measurements of PG concentrations in implantation sites, there appear to be species-dependent differences. In rats, the concentrations of PGs of the E, F, and I series (the latter measured as 6-keto- $\text{PGF}_{1\alpha}$) are elevated in implantation sites (Kennedy 1977, Kennedy & Zamecnik 1978). In the mouse, Pakrasi (1997) reported that PGE_2 concentrations were higher in implantation sites, whereas PGF levels were higher in inter-implantation areas. By contrast, Lim *et al.* (1999) reported that PGE_2 and PGI_2 concentrations were higher in implantation sites but found no difference in the $\text{PGF}_{2\alpha}$ levels. In hamsters, only PGE_2 is elevated in implantation sites (Evans & Kennedy 1978, Wang *et al.* 2004b). In rabbits, Sharma (1979)

reported that the PGE_2 , but not the $\text{PGF}_{2\alpha}$, concentrations were higher at implantation sites, whereas Pakrasi & Dey (1982) found both to be elevated. Following the application of an artificial deciduogenic stimulus, uterine concentration of PGs of the E, F, and I series are rapidly increased in the rat (Kennedy 1979, 1980a, 1980b, Kennedy *et al.* 1980). For mice, there are varying reports for the effects of deciduogenic stimuli. Jonsson *et al.* (1979), Rankin *et al.* (1979) and Milligan & Lytton (1983) all found the $\text{PGF}_{2\alpha}$ concentrations to be rapidly increased after stimulation. Jonsson *et al.* (1979) reported that PGE_2 was also increased, while Milligan & Lytton (1983) found PGE_2 as well as PGI_2 to be unchanged.

Uterine responses to exogenously administered PGs have been no more enlightening than their measurements. Indirect evidence that $\text{PGF}_{2\alpha}$ may be involved has come from reports that this PG can induce implantation in rats and mice when given systemically (Saksena *et al.* 1976, Oettel *et al.* 1979). However, when administered into the uterine lumen, $\text{PGF}_{2\alpha}$ was found to be less effective than PGE_2 at inducing implantation in mice (Holmes & Gordashko 1980). More recently, Lim *et al.* (1999) reported that carbaprostacyclin (cPGI_2), a stable analog of PGI_2 , which induces DNA binding and transcriptional activation by PPARD (Forman *et al.* 1997), is able to induce blastocyst implantation in *Ptgs2* null mice when given systemically. Cicaprost, another stable analog of PGI_2 that is not a ligand for PPARD, was ineffective. Based on these and other observations, Lim *et al.* (1999) concluded that PGI_2 derived from *PTGS2* mediates implantation in the mouse. It is somewhat surprising that the administration of cPGI_2 did not induce decidualization in the entire uterus in these studies since the same group had previously reported (Lim *et al.* 1997) that this compound induced decidualization when given into the uterine lumen (*vide infra*). Presumably elevated endometrial concentrations of PPARD ligands are by themselves insufficient; an additional stimulus provided by blastocysts or a deciduogenic stimulus is required for decidualization.

Intrauterine administration of $\text{PGF}_{2\alpha}$ results in decidualization in rats (Sananès *et al.* 1976) and rabbits (Hoffman *et al.* 1977) although in the latter study PGE_2 was more effective than $\text{PGF}_{2\alpha}$. Unfortunately, no inhibitors of endogenous PG production were used in these studies. Consequently, the responses may have been caused by the exogenous PG, endogenously produced PGs, or a combination of exogenous and endogenous PGs. Indeed, subsequent studies by Sananès *et al.* (1981) revealed that, while $\text{PGF}_{2\alpha}$ induced decidualization when given to rats in which endogenous PG synthesis was not inhibited, it was ineffective when given to indomethacin-treated animals. In an attempt to circumvent these problems of interpretation, Kennedy & Lukash (1982) infused PGs into the uterine lumen of rats treated with indomethacin. PGE_2 and $\text{PGF}_{2\alpha}$, alone or

combined, increased endometrial vascular permeability and produced decidualization. Subsequently, it was shown that when analogs of PGE₂ and PGF_{2 α} , which presumably could not be converted to the corresponding F- or E-type analog respectively, were infused, decidualization was only obtained with the E-analog (Kennedy & Doktorcik 1988). These data were interpreted as suggesting that PGE₂ mediates the decidual cell reaction in rats and that the response to PGF_{2 α} may have involved its conversion within the uterus to PGE₂. It should be noted that a complete restoration of decidualization was obtained in indomethacin-treated rats only if the PGs were infused into the uterine lumen. The intrauterine injection of PGs, although effective at restoring the vascular permeability response, did not bring about full decidualization (Kennedy 1979). These results have been interpreted as indicating that PG levels must be elevated within the uterus throughout decidualization, a notion consistent with observations that inhibition of PG synthesis up to 48 h after the application of a decidualogenic stimulus decreases the extent of decidualization (*vide supra*). More recently, Lim *et al.* (1997) reported that the intrauterine injection of cPGI₂ was more effective than PGE₂ at restoring decidualization in *Ptgs2* null mice. However, at best only a partial restoration of decidualization was obtained, possibly because elevated uterine concentrations of PGs were achieved only for a brief period following the injection. Additional evidence that PGI₂ may mediate the decidual cell reaction in mice comes from the work of Jonsson *et al.* (1979), who found that tranylcypromine, purportedly a selective inhibitor of PGI₂ synthesis, inhibits decidualization. However, the specificity of this inhibitory action of tranylcypromine has been questioned (Rajtar & de Gaetano 1979, Buxton & Murdoch 1982).

In an attempt to identify the PGs involved in the initiation of implantation, investigators have sought to identify the receptors for PGs that are expressed in the endometrium in the peri-implantation period. Binding sites, presumably representing receptors for PGE (Kennedy *et al.* 1983a), but not for PGF_{2 α} (Martel *et al.* 1985), are present in the rat endometrium. In these studies, PGE binding was detected in the endometrial stroma but not in the luminal epithelium of progesterone-treated ovariectomized rats (Kennedy *et al.* 1983b), whereas in the human binding was localized by autoradiography to stromal cells, glandular epithelium, arterioles, and erythrocytes (Chegini *et al.* 1986). More recently, Northern blot analyses and *in situ* hybridization have been used to investigate transcripts for PG receptors in the mouse and the rat uterus. In agreement with the binding studies in rats, *in situ* hybridization in mice did not detect *Ptgir* transcripts in the endometrium on day 4 of pregnancy (Yang *et al.* 1997). *Ptger1* mRNA levels were found to be very low or undetectable in the endometrium of mice (Katsuyama *et al.* 1997) and rats (Papay & Kennedy 2000) in the peri-implantation period.

By contrast, mRNA expression of *Ptger2*, *Ptger3*, and *Ptger4* was cell specific and correlated with endometrial preparation for implantation and decidualization in both mice (Yang *et al.* 1997) and rats (Papay & Kennedy 2000). For both mice and rats, *Ptger2* transcripts were localized to the luminal epithelium, whereas *Ptger4* transcripts were localized to both the luminal epithelium and the stroma in mice but were restricted to the subepithelial stroma in rats. *Ptger3* transcripts were localized to the glandular epithelium and subepithelial stroma of rats and to stromal cells at the mesometrial pole in mice. The failure to detect PGE-binding sites in membrane preparations from rat epithelial cells contrasts with the detection of *Ptger2* transcripts in these cells. This apparent discrepancy is almost certainly a consequence of the fact that the binding studies were performed on the luminal epithelium of progesterone-treated ovariectomized rats. *Ptger2* transcripts were detected in luminal epithelium only after the administration of estradiol to progesterone-treated rats (Papay & Kennedy 2000). It is intriguing that, in the rat, transcripts for *Ptger2* and *Ptger4* are concentrated in the antimesometrial pole of the uterus on the equivalent of day 5 of pseudopregnancy when the endometrium is sensitized for the decidual cell reaction (Papay & Kennedy 2000). The localization of these receptors could facilitate the initiation of decidualization, which begins in the antimesometrial region. However, it must be noted that mice deficient in each *Ptger* subtype have been generated and an implantation/decidualization phenotype has not been reported (see Sugimoto & Narumiya 2007), suggesting that these receptors are not essential for implantation/decidualization.

In the mouse, *Ptgir* mRNA levels are very low to undetectable in the uterus in the peri-implantation period (Lim *et al.* 1999), suggesting that this receptor is not critical for implantation and decidualization. In support of this conclusion is the fact that *Ptgir* null female mice are fertile (Murata *et al.* 1997). *Ppard* expression is undetectable in the mouse uterus prior to implantation but is upregulated in the stroma at implantation sites late on day 4 of pregnancy (Lim *et al.* 1999). This upregulation was detected after the increase in endometrial vascular permeability had occurred. If PPARD is to mediate this early event in implantation, then it should be expressed prior to the increase in endometrial vascular permeability. The study by Lim *et al.* (1999) does not provide information on this. However, Scherle *et al.* (2000) determined *Ppard* expression in the uterus of pseudopregnant mice 2, 8, and 24 h after a decidualogenic stimulus. *Ppard* expression was observed at 24 h but not at the earlier times. Since the endometrial vascular permeability increase is detectable within 4 h of a decidualogenic stimulus, it seems unlikely that PPARD mediates this early event in decidualization. It should be noted that PGI₂ itself has never been shown to be a ligand for PPARD, only that some stable analogs of PGI₂ are ligands. Furthermore, it is noteworthy that *Ppard* null

Figure 2 Schematic representation of changes in expression of genes involved in prostaglandin (PG) synthesis and action at implantation sites in rat endometrium on days 5–6 of pregnancy. BL, blastocyst; GE, glandular epithelium; LE, luminal epithelium; PTGS, prostaglandin-endoperoxide synthase; *Ptger*, prostaglandin E receptor; PDZ, primary decidual zone; St, stroma; SubE-St, sub-epithelia-stroma; ZP, zona pellucida.

mice have been independently generated by three laboratories (Peters *et al.* 2000, Barak *et al.* 2002, Nadra *et al.* 2006). All laboratories reported that breeding heterozygous mice resulted in fewer null mice than expected, but significantly all groups reported that the null females were fertile although Nadra *et al.* (2006) found that the litter size produced by null females was lower than control heterozygous mating. Overall, these results raise doubts about the involvement of PPARD in the early events of implantation/decidualization.

Concluding comments

Changes in the expression of genes involved in PG synthesis and action within the peri-implantation endometrium, with particular reference to the rat, are summarized in Fig. 2. While there are extensive data indicating that PGs have an important role in

implantation/decidualization in many species, there are still numerous gaps in our knowledge. In particular, the type(s) of PGs involved has not been definitively established. There may be species differences, and different PGs may be involved in different processes. Finally, given that implantation is such a crucial event, there may well be redundancy in the system.

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