[Frontiers in Bioscience 12, 1329-1343, January 1, 2007]

Role of the prostaglandins in labour and prostaglandin receptor inhibitors in the prevention of preterm labour

David M. Olson, and Christina Ammann

Perinatal Research Centre, Departments of Obstetrics and Gynecology, Pediatrics, and Physiology, University of Alberta, Edmonton, Alberta, Canada

TABLE OF CONTENTS

1. ABSTRACT

Parturition is composed of five separate but integrated physiological events: fetal membrane rupture, cervical dilatation, myometrial contractility, placental separation, and uterine involution. Prostaglandins (PGs) have central roles in each of these events, but the most studied is myometrial contraction. Elevated uterine PGs or the enhanced sensitivity of the myometrium to PGs leads to contractions and labour. The primary regulator of PG synthesis is the mRNA expression of PG H Synthase (PGHS-2 or COX-2). Given the central role of PGs in labour, this enzyme becomes an obvious therapeutic target for the prevention of preterm labour, the major cause of perinatal mortality and morbidity. Unfortunately, even though the non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit PGHS, are usually successful in suppressing preterm labour or prolonging pregnancy in animal and human studies, the NSAIDS have had adverse effects on fetal physiology and development. Therefore, other means to suppress PG synthesis or action to arrest preterm labour need to be investigated. The PGF_{2alpha} receptor, FP, may prove to be a reasonable target for tocolysis. FP mRNA increases in the mouse uterus at preterm birth, whereas PGF_{2alpha} concentrations do not increase, suggesting elevated uterine sensitivity to contractile agonists is one mechanism for preterm labour initiation. New data shows that administration of a specific FP antagonist, Theratechnologies (THG) 113.31, delays preterm birth in mice and sheep with no observable maternal or fetal side effects. Hence antagonizing PG action offers new hope for delaying preterm birth.

2. THE PRETERM BIRTH PARADIGM AND THE ROLES OF PROSTAGLANDINS IN LABOUR

Preterm birth is the deadliest and most damaging of all the complications of pregnancy, and it is imperative that societal resources be allocated to study and ultimately lower the preterm birth rate. The rate is on the rise in most western countries and is now greater than 9% in parts of Canada(1) and is 12.3% in the United States.(2) It is the leading cause of perinatal mortality and morbidity. Based on data from the Canadian Perinatal Surveillance Report, 81.6% of all preterm births are low birth weight (LBW, <2500 g at birth), and about 60.0% of all infant deaths occur among LBW/preterm infants.(3) Roughly 20% of preterm infants are thought to be small for gestational age, and these intrauterine growth restricted infants are at greater risk for being delivered before 37 weeks. LBW and preterm delivery are key risk factors for neonatal and infant morbidity, including neurodevelopmental problems, chronic respiratory conditions, infections, and visual and hearing deficits. Furthermore, the lower the gestational age at delivery, the greater the risk for long-term impairment.(4-7) The purpose of this review article will be to tie together the role of the prostaglandins (PGs) and their receptors in the process of term and preterm birth and to discuss some of the current strategies to inhibit PG synthesis or antagonize their actions in attempts to delay preterm labour and prolong pregnancy.

Parturition is comprised of five separate but integrated physiological events: membrane rupture, cervical dilatation, myometrial contractility, placental separation

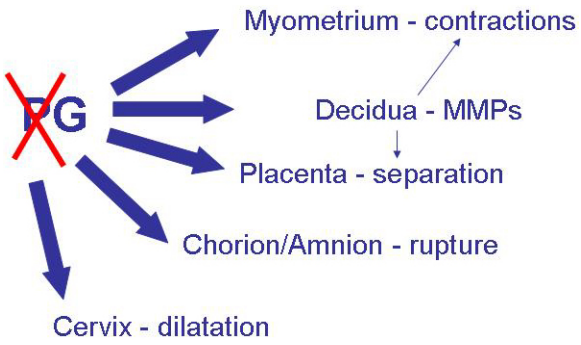

Figure 1. Prostaglandin synthesis inhibitors decrease concentrations of all PGs in intrauterine tissues thereby attenuating their physiological effects related to parturition and delaying delivery.

and uterine involution.(8) Increasingly, evidence supports a role for PGs in each of these events (Figure 1). Prostaglandins lead to fetal membrane rupture through the stimulation of matrix metalloproteinase (MMP) activity(9) that leads to extracellular matrix protein remodeling, and they also induce cell apoptosis.(10) A large literature illustrates the roles of PGs in cervical dilatation and ripening.(11-13) The data are also consistent for a PG role in placental separation. Noort *et al.*(14) report an association between elevated human PGF_{2alpha} plasma levels and placental separation, whereas an association exists between PGE_2 administered for induction of labour and placental abruption.(15) In cows with retained fetal membranes due to the non-separation of the fetal and maternal cotyledons, placenta tissue concentrations of PGF_{2alpha} were significantly lower than in normally separated cotyledons at 6h after parturition.(16) Increasingly the human decidua is being recognized as a site of PGF_{2alpha} action. Prostaglandin F_{2alpha} stimulates the production of MMP-2 and -9 and decreases the production of the progesterone receptor isoforms, A, B and C(17,18) and these actions indirectly lead to the termination of pregnancy. Uterine involution is very closely associated with plasma levels of PGF_{2alpha} in cows and sheep,(19,20) and the administration of PGF_{2alpha} promotes uterine involution in cows.(21) However, the largest amount of information about PGs in parturition addresses their role in promoting myometrial contractility.

3. UTERINE ACTIVATION GENES AND THEIR PROTEINS

It is well accepted that myometrial contractile activity at birth is the product of enhanced uterine activation and increased levels of contractile stimulators.(22) Several uterine activation genes and their proteins (UAPs) enhance the ability of the myometrium to respond to contractile agonists and to develop and propagate the depolarization signal efficiently between smooth muscle cells. These UAPs include the oxytocin receptor (OTR), PGF_{2alpha} and PGE_2 receptors (FP, $EP_{1\text{-}4}$), and the primary gap junction protein, Connexin-43 (Cx-43). We now add prostaglandin endoperoxide H synthase (PGHS-2) to this group due to the similarities in expression with the other UAPs,(23) and recent evidence from our laboratory demonstrates that both protein mass and enzymatic activity of the matrix metalloproteinases, MMP-2 and -9, increase in human decidua. The contractile stimulators include oxytocin and PGs.(22) The PGs are now recognized as the "triggers" of labour(24) because the myometrium contracts in response to exogenous PGs *in vivo* and *in vitro*,(25-27) PG synthetic enzymes and levels in tissues and fluids increase before or at the time of labour,(28-34) and inhibitors of PG synthesis delay birth and prolong pregnancy.(24,35)

4. PROSTAGLANDIN SYNTHESIS, METABOLISM AND TRANSPORT

Prostaglandins are 20-carbon chain fatty acids that function as local hormones and are produced by all cells of the body. Biologically active eicosanoids (PGs, leukotrienes, lipoxins, and other 20-carbon fatty acids) are formed from the polyunsaturated fatty acid, arachidonic acid (5,8,11,14-cis eicosatetraenoic acid). Arachidonic acid is a common constituent of phospholipids in all cell membranes, cholesteryl esters and triglycerides. The liberation of arachidonic acid from phospholipids is the initial step in the synthesis of PGs. This is accomplished directly by the catalytic action of members of the phospholipase A_2 (PLA_2) family of enzymes, or indirectly by the action of phospholipase C (PLC). PLA_2 is present in extracellular types I-IV as well as cytosolic PLA_2 forms, but only Type II PLA_2 (PLA_2 II), Type IV PLA_2 and cytosolic PLA_2 ($cPLA_2$) have been identified in the human gestational tissues at present. The majority of the data to date is on PLA_2 II. PLA_2 II mRNA and immunoreactive protein is present in the placenta, choriodecidua and amnion. PLA_2 II increased with labour in the amnion and placenta, and PLA_2 II enzyme activity increased with labour in the amnion, choriodecidua and placenta(36,37) Rice proposed a model to explain the initial step in the synthesis of PGs.(38) Extracellular (or secreretory) Type II PLA_2 is released by the gestational tissues and acts extracellularly to metabolize phospholipid substrates in the outer leaflet of the plasma membrane. Fatty acid metabolites, especially arachidonic acid, become available for intracellular processing following sn-2 hydrolysis. Arachidonic acid is then available for oxygenation by either of PGHS-1 or -2. Exogenous PLA_2 or arachidonic acid stimulates prostaglandin synthesis, supporting this proposed model.(38) Also, $cPLA_2$ shuttles between the cytoplasm (inactive form) and the cell membrane (active form) and may therefore also contribute to the hydrolysis of arachidonic acid.(38)

The second step in PG synthesis is the oxygenation and reduction of arachidonic acid to form an unstable intermediate endoperoxide. This step is catalyzed by PG H synthase (PGHS or cyclooxygenase, COX). Two isoforms of PGHS have been identified and well studied, PGHS-1 and –2 (COX-1 and COX-2). They are both homodimeric, haeme-containing, glycosylated proteins which catalyze two enzyme reactions, a cyclooxygenase reaction and a peroxidase reaction, forming, first, PGG_2, which then undergoes a two electron reduction to PGH_2.

Inhibitors of PGHS enzymes decrease synthesis of all PGs and are known as the non-steroidal anti-inflammatory drugs (NSAIDs).

The third enzymatic step of PG synthesis is the conversion of PGH_2 to one of the biologically active PGs (D_2, E_2, F_{2alpha}, I_2, or TXA_2) by specific PG synthases. An increase in PGF synthase mRNA relative to PGE synthase mRNA was noted in the sheep placenta at term or dexamethasone-induced delivery,(39) although researchers have little evidence to suggest that this step is either rate limiting or regulated in the human fetal membranes.(40) We believe there may be some regulated isomerase or reductase activity in the human decidua, but further research is necessary to demonstrate a role in parturition.

The metabolism of PGs can be as important a regulator of absolute PG concentrations in a tissue as the synthesis of PGs.(41) The primary enzyme metabolizing biologically active to inactive PGs in the human uterus is 15-hydroxy prostaglandin dehydrogenase (PGDH).(42) It is found principally in the chorion cytotrophoblast cells, but also in placenta, decidua, myometrium and cervix.(43-45) Its mRNA and protein levels are high during pregnancy and decrease with the onset of labour at term and preterm. This metabolism can be increased (leading to lowered concentrations of biologically active PGs) by progesterone, progesterone mimetics, or IL-10, or decreased by cortisol and synthetic glucocorticoids, and the pro-inflammatory cytokines, IL-1β and TNFα.(46-50) In spite of these changes in expression of PGDH at delivery or induced by various factors in cell cultures, there is controversy whether the actual enzyme activity changes at parturition.(50,51)

A PG transport protein (PGT) has been discovered in bovine uterus and fetal membranes. Its expression is higher in caruncles (placentae) than elsewhere, and in this tissue only its expression increases in late gestation. It was first proposed and cloned in 1996 when it was determined that PGT avidly facilitates the uptake of PGD_2, PGE_2 and PGF_{2alpha} into cells.(52) The transporter has also been identified in human endometrium throughout the menstrual cycle and in decidualized endometrial stromal cells,(53,54) and although it is found in human placenta,(52) it has not yet been well characterized in the pregnant or parturient human uterus. The precise role of this transporter in parturition has not been determined, although it is speculated that it may facilitate PG transfer across the uterus and the metabolism of PGs.(55)

4.1. Prostaglandin Endoperoxide H Synthase

Considerable attention has been addressed towards inducible PGHS in recent years and its association with term and preterm birth. As mentioned, it catalyses the second step in PG synthesis: the oxygenation and reduction of arachidonic acid to an unstable intermediate endoperoxide. PGHS-1 and –2 catalyze, first, a cyclooxygenase reaction to form PGG_2, and then a peroxidase reaction to reduce PGG_2 to PGH_2. The mature, processed forms of PGHS-1 and –2 have a great deal of homology in their 576 and 587 amino acids, respectively,(56) between 60-65% identity between isoforms within a species and 85-90% sequence identity for similar isoforms between species. The main exceptions are six residues in from the C-terminal region of PGHS-2, an extra 18 amino acids are inserted. The last four may facilitate binding to the nuclear and endoplasmic reticulum membranes or the entire insertion may mark the isoform for rapid proteolysis**.**(57) PGHS-2 also lacks 17 amino acids that are present in the N-terminal region of PGHS-1. However, the amino acid residues thought to be important for catalysis are conserved, (58) and the two isoforms have about the same affinity (K_m) and capacity (V_{max}) to convert arachidonic acid to PGH_2.(59)

The single best characterized distinction between PGHS-1 and PGHS-2 is their differential regulation of expression. PGHS-1 can be detected in most tissues although not within all cells of a tissue and is therefore considered to be 'constitutive,' or constantly expressed.(60) On the other hand, PGHS-2 is found at variable levels in tissues and is expressed only in response to cytokines, growth factors, or tumor promoters,(61) hence it is known as the 'inducible' enzyme. PGHS-2 is more highly concentrated on the nuclear envelope than PGHS-1,(62) and oxygenates lower concentrations of arachidonic acid (<1mM) more efficiently than PGHS-1,(63) suggesting that arachidonate can be streamed within a cell for preferential oxygenation by PGHS-2. Cortisol and synthetic glucocorticoids can stimulate cultured human amnion cell PGHS-2 mRNA and protein expression as we showed.(64)

PGHS-2 mRNA is present in human fetal membranes, decidua and myometrium and increases before labour onset.(28-34) The X-ray crystallographic forms of PGHS-1 and –2 are nearly superimposable. But it is the substitution of valine in PGHS-2 for isoleucine in PGHS-1 at positions 434 and 523 (the residues in PGHS-2 are given the equivalent number as their counterparts in PGHS-1) that permits the design of inhibitors that are specific for PGHS-2 or –1. In PGHS-2, the smaller size of the Valine at amino acid position 523 exposes a side-pocket off the main substrate channel, which increases the volume of the PGHS-2 active site, a fact that is exploited to produce specific inhibitors of PGHS-2. The longer side chain of isoleucine in PGHS-1, on the other hand, prevents access to this side pocket and thereby considerably lowers specificity of PGHS-2 inhibitors or nonselective non-steroidal anti-inflammatory drugs (NSAIDs) to PGHS-1.(65) Hence, several specific inhibitors of PGHS-1 and –2 have been developed.

A third distinct isoform of PGHS has been discovered, PGHS-3 or COX-3.(66) Along with a partial COX-1 protein, it is derived from PGHS-1 but retains intron 1 in its mRNA. It is found in the cerebral cortex of dogs followed by heart and in lesser amounts in other tissues including placenta and fetal tissues. COX-3 is inhibited more readily by acetaminophen than is PGHS-1, but aspirin and indomethacin are among its most potent inhibitors. Indeed, its presence was predicted by the properties of acetaminophen, an NSAID with potent antipyretic and analgesic actions, but relatively little anti-

inflammatory function, which was not characteristic of other NSAIDs that are better inhibitors of PGHS-1 or –2**.** (67,68) At this time there is no evidence that COX-3 synthesizes PGs for the physiological processes of birth.

4.2. Sites of prostaglandin synthesis

Prostaglandins are produced in many vertebrate tissues, including the intrauterine tissues. In humans, the sites of prostaglandin synthesis have been studied at term caesarian-section (CS) and at term spontaneous labour (SL). PGE_2, $PGF_{2\alpha}$, and 6-keto $F_{1\alpha}$ (the stable metabolite of PGI_2) are produced in the amnion, chorion, decidua, and placenta at term CS(69,70). A significant increase in PGE_2, $PGF_{2\alpha}$, and 6-keto $F_{1\alpha}$ occurs at SL compared to at term CS in the amnion and chorion.(71) However, we found that there is no significant increase in PGE_2, $PGF_{2\alpha}$, and 6-keto $F_{1\alpha}$ at SL compared to CS in the decidua or placenta.(71) Skinner and Challis, on the other hand, found that PGE_2 and $PGF_{2\alpha}$ output increases at SL in the decidua.(70) During cervical ripening PGE_2 production rose in the cervix.(72)Mitchell *et al*. measured PGD_2 production in the amnion, chorion, decidua, and placenta and found that it was present before the onset of labour (CS) in all four tissues, but production did not significantly increase at SL. Interestingly, production of PGD_2 was three-fold greater in the placenta than in other tissues studied.(72)

4.3 Prostaglandin synthesis regulation and labour

Prostaglandin synthesis regulation is a changing dynamic that is dependent upon the time of gestation and the nature of its stimulatory factors. This is clearly evident when preterm and term parturition are examined. One example is the abundance or activity of PG synthetic or metabolic enzymes and concentrations of maternal plasma and fetal amnion tissue and fluid PGs. For instance, the concentrations of tissue PGE_2 and PGF_{2alpha} in amnion and placenta are significantly lower at preterm birth (<37 weeks gestation) than at term birth.(73) This is due to the specific activity of PGHS-1 and -2, which we showed is considerably lower at preterm labour than at term labour in human fetal membranes. The specific activity in the human amnion rises from 6±2 to 28±7 pg PGE_2/microgram protein/min in women delivered by cesarean section preterm but not in labour to those following spontaneous preterm labour.(74) This contrasts to the term birth levels, which are much higher, rising from 18±4 to 39±6 pg PGE_2/microgram protein/min in women delivered by elective cesarean section at term to those delivering spontaneously at term. The situation in the chorion is similar except that the preterm levels rise proportionally less, from approximately 9±2 to 22±3 pg PGE_2/microgram protein/min compared to from 17±3 to 32±2 pg PGE_2/microgram protein/min (all increases are $p<0.05$) at term labour.(29,32,75) These data are confirmed by Sadovsky *et al.*(76) whereby they showed that although human amnion PGHS-2 protein mass and PGE_2 concentrations rise with labour at preterm or term, the levels at preterm labour are equal to or lower than in non-labouring term tissues. Hence the PG synthetic capacity of membranes is considerably lower at preterm birth than at term birth when there are no signs of infection.

These fetal membrane PGs may interact with receptors in the fetal membranes(77) or diffuse or be transported to decidua or myometrium. However, a high level of chorionic prostaglandin 15-hydroxy dehydrogenase (PGDH), the primary enzyme that catalyzes PGs into inactive metabolites, prevents intact PGs from the fetal or maternal compartments from crossing over to the other side.(51,78) In light of information (below) regarding high decidual PG synthetic capacity throughout gestation, this metabolic barrier may be acting opposite to conventional thought, that is it may prevent maternal PGs from interacting with fetal membrane PG receptors which might lead to activation of mediators, such as MMPs,(9) that promote membrane rupture. In pathological (infected) preterm birth or in about 15% of idiopathic preterm births, however, the specific activity of PGDH in chorion decreases, potentially allowing greater diffusion or transport of PGs across the chorion to facilitate myometrial contraction.(79) This capacity for PGs to traverse the chorion intact under these conditions remains to be shown directly.

The PGHS synthetic capacity in decidua throughout gestation is different than in fetal membranes. Neither PGHS-1 nor -2 mRNA abundance, enzyme activity or protein concentrations change in decidua during gestation or with labor onset in women.(29,76) The specific activity of (total) PGHS is very high (111±3 pg PGE_2/microgram protein/min) in decidua, about 3- to 4-fold greater than in fetal membranes. Some controversy exists whether PG concentrations or output from the decidua increase with term labour onset as some studies showed no changes(69,76) while others suggest that there is an increase in PG output from decidual cells with labour at term,(80,81) which may reflect changes in the specific PGE and F synthases. One report indicates there is no increase in PG output in decidua with preterm labour.(76) The human myometrium does demonstrate an increase in PGHS-2 mRNA with term labour onset,(34) but no increase in PGE2 output occurs with preterm or term labour.(76) The myometrium produces mostly PGI_2, which was not examined, and which more likely has an effect upon vascular tone than uterine contractility.(24)

Prostaglandin synthesis changes in tissues obviously lead to changes in PG concentrations in fluids, which provide more valuable information about the changing nature of PG synthesis. Evidence exists to suggest that amniotic fluid PG concentrations may be relatively low at some preterm births, an observation that reflects the low synthetic capacity of amnion at preterm birth without infection. Romero and Mitchell and others(82) showed that amniotic fluid levels of PGE_2 did not rise at preterm labour compared to not-in-labour matched controls (the mean values were actually lower), but PGF_{2alpha} concentrations did increase from 252±53 pg/ml to 731±363 pg/ml, although this rise was not statistically significant. Only with infection-associated preterm labour (which occurs in 30-40% of all preterm births) was there a significant increase in amniotic fluid PG levels. During normal birth maternal plasma levels of the PGF_{2alpha} metabolite, PGFM,

rise with cervical dilatation, increasing considerably from late pregnancy not in labour (59±8 pg/ml) to 143±32 pg/ml in early labour and 283±55 pg/ml in late labour. In contrast, the plasma levels were 63±17 pg/ml in preterm labour without infection, which were not different from term not-in-labour values.(83,84)

Another key association with PG synthesis that changes as gestation advances is the infection rate. For instance, 70% of spontaneous preterm birth ≤ 30 weeks gestational age is associated with intrauterine infection, whereas after 30 weeks a decreasing rate from 40 to 30% (at term) of spontaneous birth is associated with infection.(85) Pregnancies and births associated with infection are characterized by increased levels of cytokines (interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-α) in the fetal membranes,(86) amniotic fluid,(87-90) lower genital tract,(91) and in the lower segment of the uterus(92) Interestingly, normal pregnancies in the third trimester and term deliveries without infection are also associated with increased levels of IL-8 in myometrium(93) and increased levels of IL-1beta and IL-8 in the amnion and chorio-decidua(94) This is particularly relevant as the synthesis of PGs is stimulated by inflammatory agents(95,96), which leads to increases in intrauterine fluid and tissue concentrations of PGs and a decrease in PG metabolism.(79) It is clear therefore, that the conditions associated with and responsible for PG synthesis change as gestation advances. When infection is not present, preterm labour is characterized by very low PG synthetic capacities and concentrations and very high metabolic capacities. Conversely, when infection is present, PG synthesis and levels are very high, and metabolism is decreased.

In the situation of preterm labour without infection, when PG levels are very low and at the levels of intact non-labouring term pregnancies, the question derives of how do PGs lead to the initiation and maintenance of labour? We have data in the mouse at preterm labour induced by ovariectomy,(97) ethanol,(23) or lipopolysaccharide(98) that uterine PGF_{2alpha} concentrations do not increase above levels in age-matched pregnancy-intact non-labouring controls – a situation similar to humans without infection. However, the uterine mRNA expression for FP, the PGF_{2alpha} receptor, increases significantly, suggesting that uterine sensitivity to PGs increases rather than the absolute level of PGs. But these deliveries are dependent upon PG synthesis as administration of the specific PGHS-2 inhibitor, NS-398, delays preterm labour.(99) Thus the threshold for uterine responsiveness to PGs is lowered. By extension, it is possible that enhanced human uterine responsiveness to low concentrations of PGs at preterm in the absence of uterine infection may be responsible for the initiation and maintenance of labour.

4.4. Inhibiting PGHS-2 and other strategies to delay preterm birth

Because investigators and caregivers are realizing just how multifactorial and complex preterm birth is, the strategies for dealing with it are evolving into early and late interventions. Early intervention strategies are approaching the problem through dealing with maximizing the environment for pregnant women such as improved antenatal care and lifestyles. Late interventions are largely pharmacological with the intention of decreasing the preterm contractions of the uterine myometrium. One of the goals of late interventions has been to achieve a delivery delay of 48 hours for transport to a tertiary center and administration of glucocorticoids for lung maturation. But clearly, the potential for the late intervention strategy can be much more. Prostaglandins, the "triggers of parturition,"(24) have been at the center of late intervention attempts to delay preterm labor. Administration of non-specific PGHS inhibitors or inhibitors specific to PGHS-2 decrease uterine contractility, *in vitro*,(76) and delay birth and prolong pregnancy, *in vivo,*(24,25) confirming the central role of PGs in labour initiation at term and preterm (Figure 1).

Although early trials showed that NSAIDs delayed preterm birth by 48 hours,(14,100) other trials revealed an association between NSAIDs and adverse fetal effects including oligohydramnios, patent ductus arteriosus, necrotizing enterocolitis, intraventricular hemorrhage, persistent pulmonary hypertension of the newborn,(19) and renal failure.(20,21) Revived hope emerged when studies in the mid 1990's demonstrated that much of the PGs synthesized by intrauterine tissues at preterm labor were derived from the inducible isoenzyme PGHS-2. This discovery provided new hope for the delay in labour since several very specific inhibitors of PGHS-2 that had little effect on PGHS-1 activity existed. If these inhibitors could selectively inhibit uterine PGHS-2 activity, then perhaps preterm birth might be delayed with minimal side effects upon the fetus. The first observational trial using a specific PGHS-2 inhibitor, Nimesulide, appeared to be beneficial in women at high risk of preterm delivery. There were no long-term adverse effects; Nimesulide did not constrict the ductus arteriosus, but oligohydramnios was present in 54% of the patients.(101) Unfortunately in another study from this group, administration of the specific PGHS-2 inhibitor, Rofecoxib (Vioxx), to women at risk of preterm delivery in a randomized, double-blind, placebo-controlled study led to reversible renal effects and did not delay preterm birth.(102) Hence reversible oligohydramnios is a consistent consequence of PGHS-2 inhibition, but delay of preterm birth is inconsistent. It is doubtful prolonged administration of PGHS-2 inhibitors will ever be recommended.

However, lower doses of selective PGHS-2 inhibitors or intermittent administration of these compounds might prove effective along with combining treatments with other drugs. For instance, 200 mg/day Celecoxib, half the normal test dose, in combination with an epidermal growth factor receptor tyrosine kinase inhibitor, Tarceva (Erlotinib), has proven effective in treating mouse models of colon cancer**.**(103) This information might apply also to managing preterm labor, as we demonstrated a few years ago that tyrosine kinase inhibitors (tyrphostins) were as effective as the PGHS-2 inhibitor, NS-398, in preventing LPS-induced preterm birth in mice.(104) One effect of tyrphostins is to decrease

inflammatory reactions and reduce cytokine levels.(105,106) Perhaps a combination of a low dose PGHS-2 inhibitor to arrest formed cyclooxygenase activity plus a tyrphostin to decrease PGHS-2 gene expression(107-109) might arrest labor without fetal toxicity.

Unfortunately, a 48-hour delay to improve immediate fetal outcome is a long way from decreasing the overall preterm birth rate in western society, and thereby improving many long term infant outcomes. It is uncertain whether the synthesis of all PGs can be inhibited. In fact, this might be the wrong strategy. PGHS inhibitors have a limited ability to differentiate between those PGs important in maternal and fetal physiological and developmental processes and those that are responsible for labor itself. Another, and perhaps better, strategy to block PG synthesis is to antagonize the activity of a single PG, rather than many PGs. This antagonism is possible through the inhibition of specific PG receptors.

5. PROSTAGLANDIN RECEPTORS

Prostaglandins were originally thought to interact directly with the cell membrane, thereby altering its characteristics and modulating cell physiology. However, their different activity profiles and generation of second messengers, principally cyclic AMP (cAMP), phosphatidylinositol turnover and Ca^{2+} shifts, suggested that they interact with discreet receptors and that different receptors exist for each PG.(110) In the 1980s, studies by Coleman and colleagues using specific antagonists further characterized these receptors pharmacologically.(111) They classified the receptors as one each for PGs D_2, F_{2alpha}, I_2, and TXA_2, as DP, FP, IP, and TP, and four for PGE_2, as $EP_{1\text{-}4}$. The first of these receptors that was isolated and cloned was the TP receptor.(112) The protein is a G-protein linked member of the rhodopsin family of receptors with seven transmembrane domains. Subsequently, homology screening using mouse cDNA libraries led to the identification of the structures of all eight receptors,(113) each encoded by a different gene. More recently, several splice variants have been identified for human EP_3 (8 isoforms), sheep and bovine FP (2 isoforms),(114) rat EP_1 (2 isoforms), and human TP (2 isoforms)(115) They vary largely in their cytoplasmic C-terminal regions where coupling with G proteins occurs. The amino acid sequences have been fully described for human and mouse receptors, and partially described for rat and sheep. There is only 20-30% homology between amino acid sequences of various receptors within a species, but 79-89% homology exists between like receptors of different species.

Prostaglandin $F_{2\alpha}$ action is mediated by the FP receptor, which is coupled to G-protein mediated signal transduction pathways leading to the mobilization of intracellular Ca^{2+}. The human FP receptor consists of 359 amino acid residues with a predicted molecular mass of 40,060 Da.(116) In human myometrium, PGE_2 interacts with EP_1 receptors, which elevate $[Ca^{2+}]_i$ independently from PLC, but involving a G_i protein and plasma membrane calcium channels; EP_2 receptors stimulate adenylate cyclase; EP_{3A} receptors inhibit adenylate cyclase activity through G_i activation; and EP_{3D} receptors activate PLC through a pertusis toxin-insensitive pathway and also elevate $[Ca^{2+}]_i$.(117) Overall, the receptors can be grouped into two categories; the EP_1, EP_3 FP, and TP stimulate contraction, and the DP, EP_2, EP_4, and IP are relaxatory receptors.

5.1. Prostaglandin receptors in pregnancy and birth

PG receptor expression varies considerably from the non-pregnant state to pregnancy to birth, and the relative level or type of receptors may dictate the degree of uterine quiescence or contractility. The expression of the contractile EP_3 gene in pregnant human myometrium was 40% less than that in non-pregnant myometrium, and expression of the FP gene in human myometrium also decreased during pregnancy by 45% compared to levels in non-pregnant myometrium.(118) Similarly, uterine FP mRNA expression was much lower in pregnant than non-pregnant myometrium(118) and declined significantly with gestational age in patients not in labor and then at term increased significantly with labor.(119) These data correlate very well with the *in vitro* human myometrial contractile data. The contractile ED_{25} for $PGF_{2\alpha}$ administered to non-pregnant human myometrium, *in vitro*, was 0.04 nmol versus the much higher 0.5 nmol in non-labouring term tissue. The values were similar for contractile PGE_2 responses suggesting the number of contractile receptors is reduced during pregnancy to maintain uterine quiescence.(25-27) Conversely, labour in the pregnant baboon myometrium was associated with a lower abundance of the relaxatory EP_2 receptor mRNA,(120) and similarly in the rat uterus, EP_2 expression increased during pregnancy then decreased in labour.(121) In the myometrium of pregnant rats, expression of FP mRNA increased significantly from late gestation until delivery, returning to prepartum levels by one day postpartum while the levels of the relaxatory EP_2 receptor were the inverse to those of FP.(122) In one exception, rat myometrial FP mRNA levels were higher on GD18 than in nonpregnant tissues, but were statistically similar to high term levels and may have already begun the labour-associated rise.(121) In one study the myometrium of the sheep displayed a significant increase in the mRNA expression of FP and EP_3 with the onset of labour at term, but interestingly, there was also an increase in the relaxatory EP_2, which may have some role for passage of the fetus (see below).(123) Another ovine study did not observe any changes in FP or the four EP receptor subtypes in myometrium at term, suggesting that changes in the expression of these receptors may not be large and subject to both stimulatory and inhibitory influences.(124)

The maintenance of pregnancy is likely related to an increase in relaxatory EP receptor responses (primarily EP_2)(27) as well as increased coupling of $G_{\alpha s}$ to adenylate cyclase,(125) because there is much less of this coupling at parturition. These events correlate with an increase in EP_2 mRNA expression in the myometrium of women late in gestation, and not in labor, when compared to women at term not in labor.(119) Data from pregnant baboons(120) (as mentioned above) and mice(126) support these findings. Thus, myometrial active quiescence may change to an

active contractile state in concert with an up-regulation of contractile receptors and loss of relaxatory receptors and decreased cAMP production.

The location of receptors within the tissue may also be important for uterine function. Contraction-promoting EP_1 and EP_3 receptors are found in greater abundance in the fundus whereas relaxation-promoting EP_2 and EP_4 receptors are found in the lower uterine segment,(127) where they may be involved in uterine relaxation to allow passage of the fetal head and shoulders. However, the lower segment responds by contraction to $PGF_{2\alpha}$ challenge and lower doses of PGE_2 in *in vitro* studies,(27) so it may have the dual function of contraction and relaxation.

Only one study has examined the relative levels of myometrial PG receptors at human preterm birth.(122) FP mRNA levels from lower uterine segment myometrium were higher at preterm birth with and without labour than at term without labour. The EP_2 mRNA levels were highest at preterm not in labour and decreased to term. None of the patients had clinical signs of infection, but a direct examination of tissues or amniotic fluid for signs of infection(128,129) or stratification of patients to gestational age is warranted to identify more subtle changes in the gestational situation. Such information may provide clues as to potential regulation of the genes for the PG receptors.

5.2. Mechanisms regulating prostaglandin receptor expression

Surprisingly, there is little work published on the regulation of the PG receptors in uterine myometrium. Interestingly, in rat, FP rose in pregnancy compared to the nonpregnant state, but rose even more during labour.(121) Administration of the progesterone receptor antagonist, RU486, increased FP and decreased EP_2 mRNA. Further, antagonism of estrogen action decreased FP mRNA whereas administration of estradiol enhanced FP expression. Progesterone administration alone promoted EP_2 expression, and when this was supplemented with estradiol, the increase was greater. Hence steroids regulate PG receptors in the rat.(121)

Similarly, little is known of the cellular regulation of PG receptors. Some information may be available from studies examining the other UAPs that have implicated pro-inflammatory cytokines in their regulation. For instance, PGHS-2 mRNA increases in human myometrium prior to the onset of term labour,(34) and transcription of PGHS-2 is stimulated by IL-1beta through activation of the transcription factor, NFκB.(130) In cultured human ULTR myometrial cells, IL-1beta (10 ng/ml for 24h) stimulated an 8-fold increase in FP mRNA abundance compared to untreated control levels (6.87±1.69 units normalized to GAPDH vs. 0.85±0.12 units, n=4, $p<0.05$).(131)

In addition, evidence of FP regulation was found through characterization of the FP promoter. We isolated, cloned and sequenced a ~4.1 kb fragment containing intron 1, exon 1 and ~2.5 kb upstream of the human FP gene.(132) The transcription start site at the beginning of exon 1, several AP-1 sites, a STAT-1 site, a potential ½ estrogen response element, and a ½ progesterone response element were identified. In addition, a NF kappa B site was identified. Hence cytokines and steroids are possible regulators of FP expression at term and preterm.

A potential negative regulator of FP expression is its own ligand, PGF_{2alpha}. Both exogenous and endogenous PGs decreased the number of binding sites in rat myometrium, suggesting ligand-induced receptor down regulation.(133) Srinivasan demonstrated that PGF_{2alpha} induced the internalization of FP_A, an isoform of the ovine FP gene, in HEK-293 cells.(134) Furthermore, our own data demonstrate that PGF_{2alpha} added to human myometrial-derived cells in culture (ULTR cells) decrease the mRNA expression of FP.(135)

5.3. FP receptor

The FP gene has been sequenced from human,(116) bovine,(136) mouse,(137) and sheep,(138) and there is general agreement in the literature on its structure. The gene is approximately 10 kb in length and is composed of 3 exons separated by 2 introns. The translation initiation start site is located within exon 2, while exon 1 is non-coding and exon 3 also contains large regions of non-coding sequence. Two mRNA species are reported to arise from alternative splicing within exon 3 of the ovine FP gene, and have been called the FP_A and FP_B isoforms.(114)

5.3.1. Effect of FP knockout in mice

Mice in which the FP gene is deleted become pregnant, but hold their pups past normal term because luteolysis is prevented and the fall in progesterone does not occur.(97,139) However, ovariectomy on GD19 in FP knockout mice leads to a precipitous drop in circulating progesterone concentrations and normal birth at term on GD20(140) that is associated with mRNA increases in both OTR and PGHS-2. These data appear to be contradictory to the notion that FP is a key component to labour because it is dispensable to the birth process. However, when the other uterine activation proteins are examined, none can be proven to be essential to the birth process. The oxytocin knockout mouse delivers at term birth;(141,142) the OTR knockout is lethal to the embryonic mouse (Lou Muglia – personal communication); the PGHS-1 knockout mouse does not deliver on time, but that is due to the loss of $PGF_{2\alpha}$ for luteolysis;(143,144) the PGHS-2 knockout mouse rarely survives to adulthood, but when it does, it cannot reproduce because PGHS-2 is required for ovulation;(145,146) and connexin-43 (CX-43) knockout fetuses die at birth as a result of a failure in pulmonary gas exchange caused by a swelling and blockage of the right ventricular outflow from the heart.(147)

We used other means, therefore, to address the need for the four uterine activation proteins in mice by studying their mRNA levels and uterine PGF_{2alpha} concentrations at term birth, preterm birth induced by ovariectomy, and under conditions in which birth was delayed at term by an implanted pellet that released

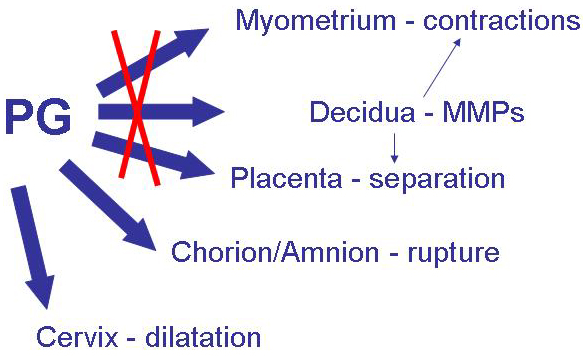

Figure 2. A prostaglandin F_{2alpha} receptor antagonist administered to the mother is effective because it arrests several parturition-related PGF_{2alpha} actions in maternal tissues. The PGE_2 actions (membrane rupture and cervical dilatation) would not be arrested.

progesterone at a low rate.(148) These treatments created conditions of variable expression of the UAPs. These data demonstrated that the FP receptor mRNA increases at each birth - preterm, term and postterm, that its expression is decreased at expected term birth when elevated P_4 levels blocked birth, and that it is the only UAP that exhibits these characteristics. Further, birth occurs if expression of three of the four UAPs are increased, suggesting that redundant mechanisms affect birth. But when only two of the UAPs increase their expression, birth does not occur. Thus it may be possible to delay preterm birth by blocking the expression or action of two of the UAPs. By extension, antagonizing the activity or action of either PGHS-2 or FP effectively blocks two of the UAPs, and this may be the reason selective PGHS-2 inhibitors are so effective in delaying preterm labour(35). This concept also predicts that antagonizing the FP receptor will delay preterm labour. The following studies tested this concept using a specific antagonist of FP action.

5.3.2. A specific FP receptor antagonist

THG113 (Theratechnologies, Montreal, PQ) is a specific, noncompetitive, reversible peptide inhibitor of the FP receptor that blocks the interaction of the receptor with $G_{alpha\ q}$, ultimately preventing the increase in intracellular $[Ca^{2+}]$. It binds specifically to FP-expressing but not to native (non FP-expressing) HEK293 cells.(149) In FP-expressing HEK293 cells, THG113 significantly reduces PGF_{2alpha} -induced IP_3 generation with an IC_{50} of 30 nM. Similarly, PGF_{2alpha} -stimulated retinal arteriole contraction is non-competitively blocked (ca. 90%, IC_{50} 340 nM) by THG113 whereas neither agonists requiring homologous receptors (PGE_2 (EP_1 receptor) and U46619 (TP receptor)) nor numerous other contractile agents (*e.g.* PAF, endothelin, or angiotensin II) are affected by THG113. Only 1% or less crosses the mouse placenta (Dr. K. Peri, Theratechnologies, personal communication), a labyrinthine, hemotrichorial model (two layers of syncytiotrophoblast and a single mononuclear cell layer).(150) The rate of transfer across other placenta types, especially the hemochorial human placenta, is unknown, but hopefully is small, thereby minimizing the effect of THG113 upon the fetus and qualifying THG113 as a candidate tocolytic.

THG113 was effective in delaying preterm birth induced by administration of LPS (50 microgram, ip, twice at 3 h interval) on GD 16 to pregnant mice.(149) In control dams, preterm birth occurred within 18h of LPS administration in 100% of cases. THG113 administered (10 micromol/kg bolus followed by 0.8 micromol/kg/h) 4-6h after administration of LPS, when the PG effect of luteolysis was past, delayed preterm birth >40h in LPS-treated dams by antagonizing PGF_{2alpha} responses in the uterus. Fetuses from THG113 treated dams were born alive with higher birth weights than control-treated dams and appeared healthy.

Increases in the abundance of contractile FP and EP_3 mRNA and in the relaxatory EP_4 mRNA were evident in sheep myometrium at term in one study(123) but not in another study(124). No studies have explored the changes in receptors at preterm birth in sheep to our knowledge. We therefore determined that a variant of the FP receptor antagonist, THG113.31, is effective in delaying RU486-induced preterm labour and delivery in a sheep infused for 9h (the length of the infusion) beyond the time an untreated ewe delivered, indicating that FP is involved in generating uterine contractile activity at preterm labour.(151) The effectiveness of this FP antagonist is likely due to the multiple roles of PGF_{2alpha} in mediating several of the physiological effects of labour. (Figure 2)

6. NUCLEAR FACTOR-KAPPA B – INVOLVEMENT WITH PG SYNTHESIS-RECEPTOR CASCADE

Considerable evidence accumulated since 1999 has shown that the nuclear transcription factor, nuclear factor-kappa B (NF kappa B), is involved with many aspects of PG synthesis and action in the intrauterine tissues. The NF kappa B family of transcription factors is associated with inflammation and can be activated by the pro-inflammatory cytokines associated with labour to affect PG synthesis in uterine-derived tissues and cells. This mechanism includes TNF-alpha,(152,153) IL-1beta,(130) and LPS,(154) although this latter effect is not always consistent.(155) It appears that NF kappa B mediates IL-1beta action at several levels of the PG synthesis-receptor cascade, including secretory type II PLA_2,(154) PGHS-2,(130,153,156) and the FP receptor.(131) The possibility exists that NFκB may also regulate specific PG synthases and PG metabolism (PGDH), but there is no evidence at present to support or refute this notion. An inhibitor of NF kappa B, SN-50, was able to delay preterm birth when administered into the amniotic fluid of mice,(157) and infusion of sulfasalazine, an anti-inflammatory and NF kappa B inhibitor, decreased uterine electromyographic activity in pregnant ewes induced to enter preterm labour with RU486, the progesterone receptor blocker (I.R. Young, personal communication). These studies demonstrate the participation of NF kappa B in preterm labour.

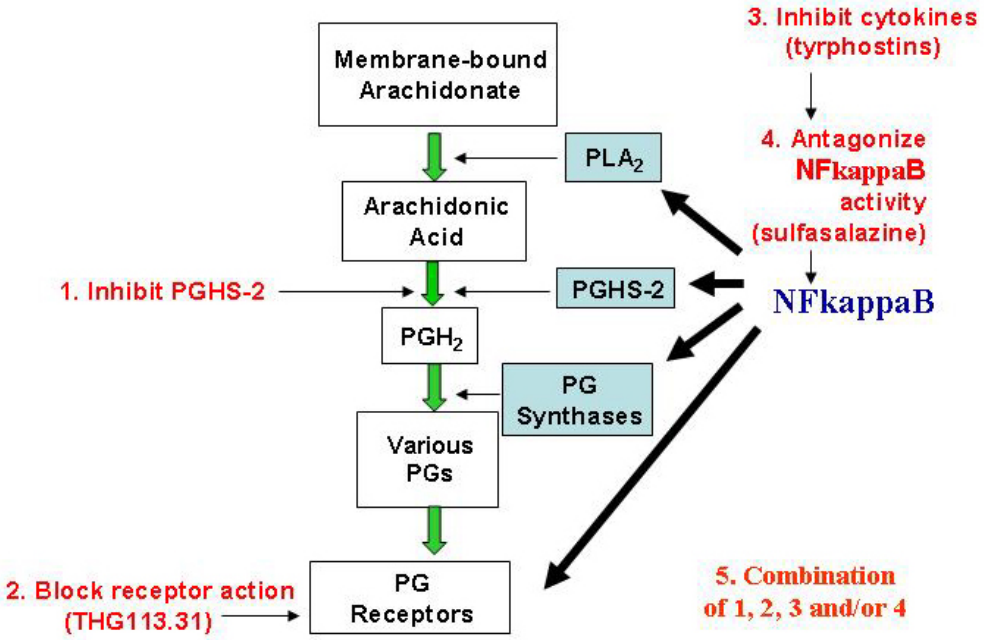

Figure 3. Combined prostaglandin-targeted strategies for delaying preterm labour. 1. Specific PGHS-1 or PGHS-2 inhibitors or mixed PGHS inhibitors decrease synthesis of all prostaglandins. This approach has side effects that affect fetal physiology, particularly in the kidney. 2. Antagonizing the action of a specific prostaglandin receptor. Fewer physiological actions of prostaglandins are affected, but greater specificity of targeting specific prostaglandin actions can be achieved and, with certain antagonists, they may not cross the placenta to the fetus. 3. The tyrphostins presumably inhibit cytokine action, but this remains to be demonstrated in intrauterine tissues in addition to assessing their side effects on mother and/or fetus. 4. The transcription factor, NF kappa B is involved in the IL-1beta-induced expression of at 3-4 steps of the PG synthesis-receptor pathway. Inhibiting NF kappa B should decrease overall PG synthesis and action, but this needs to be demonstrated in the whole animal. Also, the side effects of inhibiting NF kappa B must be determined. 5. It is likely that effective tocolysis may occur using combinations of these approaches which could either utilize lower, less harmful doses of certain inhibitors, or permit arrest of preterm labour at critical windows of development.

7. THE FUTURE FOR PG-RELATED TOCOLYSIS

Increasingly, strategies for inhibiting PG synthesis or action for the delay of preterm labour are evolving. (Figure 3) The administration of PGHS inhibitors has met with variable success and in most cases, results in fetal side effects, principally decreasing renal urine production. Another target of the PG synthesis-receptor cascade that does have potential as a tocolytic target is antagonizing the action of the PGF_{2alpha} receptor, FP, as it combines tocolytic effectiveness with minimal side effects. This derives from the fact that PGF_{2alpha} has relatively few effects in the fetus (or in the mother outside of uterine effects). Arresting cytokine production or activation is clearly an important area for exploration. The tyrphostins (inhibitors of tyrosine kinases) presumably arrest cytokine activation, although this needs to be confirmed in relation to preterm birth. If so, then levels of key cytokines such as IL-1beta, IL-6, IL-8, and TNF-alpha, may be reduced and not able to stimulate PLA_2, PGHS-2, PG synthases, or PG receptors when tyrphostins are administered. Accumulating evidence demonstrates that an important mechanism of IL-1beta, TNF-alpha and perhaps other cytokines, and lipopolysaccharide (LPS), is to stimulate NF kappa B and its upregulation of several components of the PG synthesis-receptor cascade. Targeting NF kappa B would potentially decrease the expression of the PG synthesis-receptor cascade. Lastly, while some of these approaches have concerns regarding fetal or maternal safety, and others have not been studied for their potential side effects, it is likely that investigators will continue to explore their possibilities for prolonging pregnancy because of the central importance of PGs in mediating the final common pathways for preterm birth. We anticipate that investigators will continue to explore the effectiveness of PGHS inhibitors, perhaps testing lower doses, seeking developmental windows where inhibiting fetal PGHS activity and PG synthesis has minimal side effects, administrating drugs intermittently, or possibly combining PGHS-1 and -2-specific inhibitors together or with other inhibitors. These approaches may be the hope for the future of successfully manipulating the PG synthesis-receptor cascade for successful maintenance of pregnancy and delay of preterm birth.

8. ACKNOWLEDGEMENTS

Work in the authors' laboratory was supported by the Canadian Institutes of Health Research (Institute for Human Development, Child and Youth Health) and the Alberta Heritage Foundation for Medical Research. The authors thank Ms. Sheila McManus for her editorial assistance.

9. REFERENCES

1. Vital Statistics. In: Statistics Canada; (2002)
2. March of Dimes Birth Defects Foundation. http://www.modimes.org/files/pm_launch_factsheet.doc. *March of Dimes.* (2006)
3. Health Canada. Preterm Birth (Canadian Perinatal Surveillance fact sheet). In. Ottawa, ON: Bureau of Reproductive and Child Health; (1999)
4. Kramer M. S. Determinants of low birth weight: methodological assessment and meta-analysis. *Bull World Health Organ* 65, 663-737 (1987)
5. Berkowitz G. S. & E. Papiernik. Epidemiology of preterm birth. *Epidemiol Rev* 15, 414-443 (1993)
6. Moutquin J. M. & E. Papiernik. Can we lower the rate of preterm birth". *Bull SOGC* 19-20 (1990)
7. Hack M., N. Klein & H. Taylor. Long-term development outcomes of low birth weight infants. *Future Child* 5, 176-196 (1995)
8. Olson D. M., J. E. Mijovic & D. W. Sadowsky. Control of human parturition. *Semin Perinatol* 19, 52-63. (1995)
9. McLaren J., D. J. Taylor & S. C. Bell. Prostaglandin E(2)-dependent production of latent matrix metalloproteinase-9 in cultures of human fetal membranes. *Mol Hum Reprod* 6, 1033-1040. (2000)
10. Keelan J., R. Helliwell, B. Nijmeijer, E. Berry, T. Sato, K. Marvin, M. Mitchell & R. Gilmour. 15-deoxy-delta12,14-prostaglandin J2-induced apoptosis in amnion-like WISH cells. *Prostaglandins Other Lipid Mediat* 66, 265-282 (2001)
11. Fletcher H. M., S. Mitchell, D. Simeon, J. Frederick & D. Brown. Intravaginal misoprostol as a cervical ripening agent. *Br J Obstet Gynaecol* 100, 641-644. (1993)
12. Keirse M. J., M. Thiery, W. Parewijck & M. D. Mitchell. Chronic stimulation of uterine prostaglandin synthesis during cervical ripening before the onset of labor. *Prostaglandins* 25, 671-682. (1983)
13. Keirse M. J. Prostaglandins in preinduction cervical ripening. Meta-analysis of worldwide clinical experience. *J Reprod Med* 38, 89-100. (1993)
14. Noort W. A., B. van Bulck, A. Vereecken, F. A. de Zwart & M. J. Keirse. Changes in plasma levels of PGF2 alpha and PGI2 metabolites at and after delivery at term. *Prostaglandins* 37, 3-12. (1989)
15. Leung A., P. Kwok & A. Chang. Association between prostaglandin E2 and placental abruption. *Br J Obstet Gynaecol* 94, 1001-1002 (1987)
16. Takagi M., S. Fujimoto, M. Ohtani, A. Miyamoto, M. P. Wijagunawardane, T. J. Acosta, K. Miyazawa & K. Sato. Bovine retained placenta: hormonal concentrations in fetal and maternal placenta. *Placenta* 23, 429-437 (2002)
17. Ulug U., S. Goldman, I. Ben-Shlomo & E. Shalev. Matrix metalloproteinase (MMP)-2 and MMP-9 and their inhibitor, TIMP-1, in human term decidua and fetal membranes: the effect of prostaglandin F(2alpha) and indomethacin. *Mol Hum Reprod* 7, 1187-1193. (2001)
18. Goldman S., A. Weiss, I. Almalah & E. Shalev. Progesterone receptor expression in human decidua and fetal membranes before and after contractions: possible mechanism for functional progesterone withdrawal. *Mol Hum Reprod* 11, 269-277 (2005)
19. Lindell J. O., H. Kindahl, L. Jansson & L. Edqvist. Postpartum release of prostaglandin F2alpha and uterine involution in the cow. *Theriogenology* 17, 237-245 (1982)
20. Thompson F. N., R. D. Page, C. B. Cook & A. B. Caudle. Prostaglandin F2 alpha metabolite levels in normal and uterine-infected postpartum cows. *Vet Res Commun* 11, 503-507 (1987)
21. Lindell J. O. & H. Kindahl. Exogenous prostaglandin F2 alpha promotes uterine involution in the cow. *Acta Vet Scand* 24, 269-274 (1983)
22. Lye S. J. & J. R. Challis. Paracrine and endocrine control of myometrial activity at term. In: Gluckman P. D., B. M. Johnston & P. W. Nathanielsz, eds. *Advances in Fetal Physiology*. Ithaca: Perinatology Press; 1989:361-375.
23. Cook J. L., D. B. Zaragoza, D. H. Sung & D. M. Olson. Expression of myometrial activation and stimulation genes in a mouse model of preterm labor: myometrial activation, stimulation, and preterm labor. *Endocrinology* 141, 1718-1728 (2000)
24. Challis J. R. & D. M. Olson. Parturition. In: Knobil E. & J. Neill, eds. *The Physiology of Reproduction*. New York: Raven Press; 1988:2177-2216.
25. Senior J., K. Marshall, R. Sangha, G. S. Baxter & J. K. Clayton. In vitro characterization of prostanoid EP-receptors in the non- pregnant human myometrium. *Br J Pharmacol* 102, 747-753 (1991)
26. Senior J., R. Sangha, G. S. Baxter, K. Marshall & J. K. Clayton. In vitro characterization of prostanoid FP-, DP-, IP- and TP-receptors on the non-pregnant human myometrium. *Br J Pharmacol* 107, 215-221 (1992)
27. Senior J., K. Marshall, R. Sangha & J. K. Clayton. In vitro characterization of prostanoid receptors on human myometrium at term pregnancy. *Br J Pharmacol* 108, 501-506 (1993)
28. Hirst J. J., T. Teixeira, T. Zakar & D. M. Olson. Prostaglandin endoperoxide-H-synthase-1 and -2 messenger reibonucleic acid levels in human amnion with spontaneous labor onset. *Journal of Clinical Endocrinology & Metabolism* 80, 517-523 (1995)
29. Hirst J. J., J. E. Mijovic, T. Zakar & D. M. Olson. Prostaglandin endoperoxide H synthase-1 and -2 mRNA levels and enzyme activity in human decidua at term labor. *J Soc Gynecol Investig* 5, 13-20. (1998)
30. Mijovic J. E., T. Zakar, J. Angelova & D. M. Olson. Prostaglandin endoperoxide H synthase mRNA expression in the human amnion and decidua during pregnancy and in the amnion at preterm labour. *Mol Hum Reprod* 5, 182-187. (1999)
31. Mijovic J. E., T. Zakar, T. K. Nairn & D. M. Olson. Prostaglandin endoperoxide H synthase (PGHS) activity and PGHS-1 and -2 messenger ribonucleic acid abundance in human chorion throughout gestation and with preterm labor. *J Clin Endocrinol Metab* 83, 1358-1367. (1998)
32. Mijovic J. E., T. Zakar, T. K. Nairn & D. M. Olson. Prostaglandin-endoperoxide H synthase-2 expression and activity increases with term labor in human chorion. *Am J Physiol* 272, E832-840. (1997)
33. Slater D. M., L. C. Berger, R. Newton, G. E. Moore & P. R. Bennett. Expression of cyclooxygenase types 1 and 2 in human fetal membranes at term. *Am J Obstet Gynecol* 172, 77-82 (1995)

34. Slater D. M., W. J. Dennes, J. S. Campa, L. Poston & P. R. Bennett. Expression of cyclo-oxygenase types-1 and -2 in human myometrium throughout pregnancy. *Mol Hum Reprod* 5, 880-884. (1999)

35. Poore K. R., I. R. Young & J. J. Hirst. Efficacy of the selective prostaglandin synthase type 2 inhibitor nimesulide in blocking basal prostaglandin production and delaying glucocorticoid-induced premature labor in sheep. *Am J Obstet Gynecol* 180, 1244-1253. (1999)

36. Rice G. E. Glycerophospholipid metabolism and human labour. *Reprod Fertil Dev* 7, 613-622 (1995)

37. Slater D. M., S. Astle, P. R. Bennett & S. Thornton. Labour is associated with increased expression of type-IIA secretory phospholipase A2 but not type-IV cytosolic phospholipase A2 in human myometrium. *Mol Hum Reprod* 10, 799-805 (2004)

38. Rice G. E. Secretory type II phospholipase A2 and the generation of intrauterine signals. *Reprod Fertil Dev* 7, 1471-1479 (1995)

39. Palliser H. K., G. T. Ooi, J. J. Hirst, G. Rice, N. L. Dellios, R. M. Escalona & I. R. Young. Changes in the expression of prostaglandin E and F synthases at induced and spontaneous labour onset in the sheep. *J Endocrinol* 180, 469-477 (2004)

40. Meadows J. W., A. L. Eis, D. E. Brockman & L. Myatt. Expression and localization of prostaglandin E synthase isoforms in human fetal membranes in term and preterm labor. *J Clin Endocrinol Metab* 88, 433-439 (2003)

41. Flower R. J. & J. R. Vane. Inhibition of prostaglandin biosynthesis. *Biochem Pharmacol* 23, 1439-1450 (1974)

42. Keirse M. J. Biosynthesis and metabolism of prostaglandins in the pregnant human uterus. *Adv Prostaglandin Thromboxane Res* 4, 87-102 (1978)

43. Sangha R. K., J. C. Walton, C. M. Ensor, H. H. Tai & J. R. Challis. Immunohistochemical localization, messenger ribonucleic acid abundance, and activity of 15-hydroxyprostaglandin dehydrogenase in placenta and fetal membranes during term and preterm labor. *J Clin Endocrinol Metab* 78, 982-989. (1994)

44. Ramirez M. M., L. J. Fraher, D. Goltzman, G. N. Hendy, S. G. Matthews, R. Sangha & J. R. Challis. Immunoreactive parathyroid hormone-related protein: its association with preterm labor. *Eur J Obstet Gynecol Reprod Biol* 63, 21-26 (1995)

45. Giannoulias D., F. A. Patel, A. C. Holloway, S. J. Lye, H. H. Tai & J. R. Challis. Differential changes in 15-hydroxyprostaglandin dehydrogenase and prostaglandin H synthase (types I and II) in human pregnant myometrium. *J Clin Endocrinol Metab* 87, 1345-1352 (2002)

46. Brown N. L., S. A. Alvi, M. G. Elder, P. R. Bennett & M. H. F. Sullivan. Regulation of prostaglandin production in intact fetal membranes by interleukin-1 and its receptor antagonist. *J Endocrinol* 159, 519-526 (1998)

47. Mitchell M. D., V. Goodwin, S. Mesnage & J. A. Keelan. Cytokine-induced coordinate expression of enzymes of prostaglandin biosynthesis and metabolism: 15-hydroxyprostaglandin dehydrogenase. *Prostaglandins Leukot Essent Fatty Acids* 62, 1-5 (2000)

48. Patel F. A., V. L. Clifton, K. Chwalisz & J. R. Challis. Steroid regulation of prostaglandin dehydrogenase activity and expression in human term placenta and chorio-decidua in relation to labor. *J Clin Endocrinol Metab* 84, 291-299 (1999)

49. Patel F. A., J. W. Funder & J. R. Challis. Mechanism of cortisol/progesterone antagonism in the regulation of 15-hydroxyprostaglandin dehydrogenase activity and messenger ribonucleic acid levels in human chorion and placental trophoblast cells at term. *J Clin Endocrinol Metab* 88, 2922-2933 (2003)

50. Pomini F., F. A. Patel, S. Mancuso & J. R. Challis. Activity and expression of 15-hydroxyprostaglandin dehydrogenase in cultured chorionic trophoblast and villous trophoblast cells and in chorionic explants at term with and without spontaneous labor. *Am J Obstet Gynecol* 182, 221-226 (2000)

51. Mitchell B. F., K. Rogers & S. Wong. The dynamics of prostaglandin metabolism in human fetal membranes and decidua around the time of parturition. *J Clin Endocrinol Metab* 77, 759-764. (1993)

52. Lu R., N. Kanai, Y. Bao & V. L. Schuster. Cloning, in vitro expression, and tissue distribution of a human prostaglandin transporter cDNA(hPGT). *J Clin Invest* 98, 1142-1149 (1996)

53. Kang J., P. Chapdelaine, J. Parent, E. Madore, P. Y. Laberge & M. A. Fortier. Expression of human prostaglandin transporter in the human endometrium across the menstrual cycle. *J Clin Endocrinol Metab* 90, 2308-2313 (2005)

54. Kang J., P. Chapdelaine, P. Y. Laberge & M. A. Fortier. Functional characterization of prostaglandin transporter and terminal prostaglandin synthases during decidualization of human endometrial stromal cells. *Hum Reprod* 21, 592-599 (2006)

55. Banu S. K., J. A. Arosh, P. Chapdelaine & M. A. Fortier. Expression of prostaglandin transporter in the bovine uterus and fetal membranes during pregnancy. *Biol Reprod* 73, 230-236 (2005)

56. Xie W. L., J. G. Chipman, D. L. Robertson, R. L. Erikson & D. L. Simmons. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proc Natl Acad Sci U S A* 88, 2692-2696. (1991)

57. Otto J. C. & W. L. Smith. Photolabeling of prostaglandin endoperoxide H synthase-1 with 3-trifluoro-3-(m-[125I]iodophenyl)diazirine as a probe of membrane association and the cyclooxygenase active site. *J Biol Chem* 271, 9906-9910 (1996)

58. DeWitt D. L., E. A. Meade & W. L. Smith. PGH synthase isoenzyme selectivity: the potential for safer nonsteroidal antiinflammatory drugs. *Am J Med* 95, 40S-44S. (1993)

59. Percival M. D., M. Ouellet, C. J. Vincent, J. A. Yergey, B. P. Kennedy & G. P. O'Neill. Purification and characterization of recombinant human cyclooxygenase-2. *Arch Biochem Biophys* 315, 111-118 (1994)

60. Smith W. L., R. M. Garavito & D. L. DeWitt. Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. *J Biol Chem* 271, 33157-33160 (1996)

61. Herschman H. R. Prostaglandin synthase 2. *Biochim Biophys Acta* 1299, 125-140 (1996)

62. Morita I., M. Schindler, M. K. Regier, J. C. Otto, T. Hori, D. L. DeWitt & W. L. Smith. Different intracellular

locations for prostaglandin endoperoxide H synthase-1 and -2. *J Biol Chem* 270, 10902-10908 (1995)

63. Swinney D. C., A. Y. Mak, J. Barnett & C. S. Ramesha. Differential allosteric regulation of prostaglandin H synthase 1 and 2 by arachidonic acid. *J Biol Chem* 272, 12393-12398 (1997)

64. Zakar T., J. J. Hirst, J. E. Mijovic & D. M. Olson. Glucocorticoids stimulate the expression of prostaglandin endoperoxide H synthase-2 in amnion cells. *Endocrinology* 136, 1610-1619 (1995)

65. Luong C., A. Miller, J. Barnett, J. Chow, C. Ramesha & M. F. Browner. Flexibility of the NSAID binding site in the structure of human cyclooxygenase-2. *Nat Struct Biol* 3, 927-933 (1996)

66. Chandrasekharan N. V., H. Dai, K. L. Roos, N. K. Evanson, J. Tomsik, T. S. Elton & D. L. Simmons. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. *Proc Natl Acad Sci U S A* 99, 13926-13931 (2002)

67. Botting R. M. Mechanism of action of acetaminophen: is there a cyclooxygenase 3? *Clin Infect Dis* 31 Suppl 5, S202-210 (2000)

68. Warner T. D. & J. A. Mitchell. Cyclooxygenase-3 (COX-3): filling in the gaps toward a COX continuum? *Proc Natl Acad Sci U S A* 99, 13371-13373 (2002)

69. Olson D. M., K. Skinner & J. R. Challis. Prostaglandin output in relation to parturition by cells dispersed from human intrauterine tissues. *J Clin Endocrinol Metab* 57, 694-699. (1983)

70. Skinner K. A. & J. R. Challis. Changes in the synthesis and metabolism of prostaglandins by human fetal membranes and decidua at labor. *Am J Obstet Gynecol* 151, 519-523. (1985)

71. Olson D. M., M. A. Opavsky & J. R. Challis. Prostaglandin synthesis by human amnion is dependent upon extracellular calcium. *Can J Physiol Pharmacol* 61, 1089-1092. (1983)

72. Sellers S. M., H. T. Hodgson, M. D. Mitchell, A. B. Anderson & A. C. Turnbull. Raised prostaglandin levels in the third stage of labor. *Am J Obstet Gynecol* 144, 209-212. (1982)

73. Reece M. S., J. A. McGregor, K. G. Allen, M. M. Mathias & M. A. Harris. Prostaglandins in selected reproductive tissues in preterm and full-term gestations. *Prostaglandins Leukot Essent Fatty Acids* 55, 303-307 (1996)

74. Teixeira F. J., T. Zakar, J. J. Hirst, F. Guo, D. W. Sadowsky, G. Machin, N. Demianczuk, B. Resch & D. M. Olson. Prostaglandin endoperoxide-H synthase (PGHS) activity and immunoreactive PGHS-1 and PGHS-2 levels in human amnion throughout gestation, at term, and during labor. *J Clin Endocrinol Metab* 78, 1396-1402. (1994)

75. Mijovic J. E., T. Zakar & D. M. Olson. Prostaglandin endoperoxidase H synthase (PGHS)-1 and -2 expression and activity in human chorion and decidua. *Trophoblast Research* 11, 209-228 (1998)

76. Sadovsky Y., D. M. Nelson, L. J. Muglia, G. A. Gross, K. C. Harris, A. Koki, J. L. Masferrer & L. M. Olson. Effective diminution of amniotic prostaglandin production by selective inhibitors of cyclooxygenase type 2. *Am J Obstet Gynecol* 182, 370-376 (2000)

77. Spaziani E. P., J. C. Tsibris, L. T. Hunt, R. R. Benoit & W. F. O'Brien. The effect of interleukin-1 beta and interleukin-4 on the expression of prostaglandin receptors EP1 and EP3 in amnion WISH cells. *Am J Reprod Immunol* 38, 279-285 (1997)

78. McCoshen J. A., D. R. Hoffman, J. V. Kredentser, C. Araneda & J. M. Johnston. The role of fetal membranes in regulating production, transport, and metabolism of prostaglandin E2 during labor. *Am J Obstet Gynecol* 163, 1632-1640 (1990)

79. Van Meir C. A., R. K. Sangha, J. C. Walton, S. G. Matthews, M. J. Keirse & J. R. Challis. Immunoreactive 15-hydroxyprostaglandin dehydrogenase (PGDH) is reduced in fetal membranes from patients at preterm delivery in the presence of infection. *Placenta* 17, 291-297. (1996)

80. Willman E. A. & W. P. Collins. Distribution of prostaglandins E2 and F2 alpha within the foetoplacental unit throughout human pregnancy. *J Endocrinol* 69, 413-419. (1976)

81. Norwitz E. R., P. M. Starkey, A. Lopez Bernal & A. C. Turnbull. Identification by flow cytometry of the prostaglandin-producing cell populations of term human decidua. *J Endocrinol* 131, 327-334. (1991)

82. Romero R., M. Emamian, M. Wan, R. Quintero, J. C. Hobbins & M. D. Mitchell. Prostaglandin concentrations in amniotic fluid of women with intra- amniotic infection and preterm labor. *Am J Obstet Gynecol* 157, 1461-1467. (1987)

83. Mitchell M. D., A. P. Flint, J. Bibby, J. Brunt, J. M. Arnold, A. B. Anderson & A. C. Turnbull. Plasma concentrations of prostaglandins during late human pregnancy: influence of normal and preterm labor. *J Clin Endocrinol Metab* 46, 947-951. (1978)

84. Sellers S. M., M. D. Mitchell, J. G. Bibby, A. B. Anderson & A. C. Turnbull. A comparison of plasma prostaglandin levels in term and preterm labour. *Br J Obstet Gynaecol* 88, 362-366. (1981)

85. Goldenberg R. L., J. C. Hauth & W. W. Andrews. Intrauterine infection and preterm delivery. *N Engl J Med* 342, 1500-1507 (2000)

86. McLaren J., D. J. Taylor & S. C. Bell. Increased concentration of pro-matrix metalloproteinase 9 in term fetal membranes overlying the cervix before labor: implications for membrane remodeling and rupture. *Am J Obstet Gynecol* 182, 409-416. (2000)

87. Cherouny P. H., G. A. Pankuch, R. Romero, J. J. Botti, D. C. Kuhn, L. M. Demers & P. C. Appelbaum. Neutrophil attractant/activating peptide-1/interleukin-8: association with histologic chorioamnionitis, preterm delivery, and bioactive amniotic fluid leukoattractants. *Am J Obstet Gynecol* 169, 1299-1303. (1993)

88. Rizzo G., A. Capponi, D. Rinaldo, D. Tedeschi, D. Arduini & C. Romanini. Interleukin-6 concentrations in cervical secretions identify microbial invasion of the amniotic cavity in patients with preterm labor and intact membranes. *Am J Obstet Gynecol* 175, 812-817. (1996)

89. Romero R., D. T. Brody, E. Oyarzun, M. Mazor, Y. K. Wu, J. C. Hobbins & S. K. Durum. Infection and labor. III. Interleukin-1: a signal for the onset of parturition. *Am J Obstet Gynecol* 160, 1117-1123 (1989)

90. Romero R. & M. Mazor. Infection and preterm labor. *Clin Obstet Gynecol* 31, 553-584. (1988)

91. Inglis S. R., J. Jeremias, K. Kuno, K. Lescale, Q. Peeper, F. A. Chervenak & S. S. Witkin. Detection of tumor necrosis factor-alpha, interleukin-6, and fetal fibronectin in the lower genital tract during pregnancy: relation to outcome. *Am J Obstet Gynecol* 171, 5-10. (1994)
92. Willi M. J., M. Winkler, D.-C. Fischer, R. T., H. Maul & W. Rath. Chorioamnionitis: elevated interleukin-6 and interleukin-8 concentrations in the lower uterine segment. *J Perinat Med* 30, 292-296 (2002)
93. Elliott C. L., D. M. Slater, W. Dennes, L. Poston & P. R. Bennett. Interleukin 8 expression in human myometrium: changes in relation to labor onset and with gestational age. *Am J Reprod Immunol* 43, 272-277. (2000)
94. Elliott C. L., J. A. Loudon, N. Brown, D. M. Slater, P. R. Bennett & M. H. Sullivan. IL-1beta and IL-8 in human fetal membranes: changes with gestational age, labor, and culture conditions. *Am J Reprod Immunol* 46, 260-267. (2001)
95. Mitchell M. D., D. J. Dudley, S. S. Edwin & S. L. Schiller. Interleukin-6 stimulates prostaglandin production by human amnion and decidual cells. *Eur J Pharmacol* 192, 189-191. (1991)
96. Mitchell M. D., S. S. Edwin, S. Lundin-Schiller, R. M. Silver, D. Smotkin & M. S. Trautman. Mechanism of interleukin-1 beta stimulation of human amnion prostaglandin biosynthesis: mediation via a novel inducible cyclooxygenase. *Placenta* 14, 615-625. (1993)
97. Cook J. L., M. C. Shallow, D. B. Zaragoza, K. I. Anderson & D. M. Olson. Mouse placental prostaglandins are associated with uterine activation and the timing of birth. *Biol Reprod* 68, 579-587 (2003)
98. Olson D. M., D. B. Zaragoza, M. C. Shallow, J. L. Cook, B. F. Mitchell, P. Grigsby & J. Hirst. Myometrial activation and preterm labour: evidence supporting a role for the prostaglandin F receptor--a review. *Placenta* 24 Suppl A, S47-54 (2003)
99. Cook J. L., D. B. Zaragoza, N. M. White, C. L. Randall & D. M. Olson. Progesterone and prostaglandin H synthase-2 involvement in alcohol- induced preterm birth in mice. *Alcohol Clin Exp Res* 23, 1793-1800. (1999)
100. Zuckerman H., E. Shalev, G. Gilad & E. Katzuni. Further study of the inhibition of premature labor by indomethacin. Part I. *J Perinat Med* 12, 19-23 (1984)
101. Sawdy R. J., K. M. Groom & P. R. Bennett. Experience of the use of nimesulide, a cyclo-oxygenase-2 selective prostaglandin synthesis inhibitor, in the prevention of preterm labour in 44 high-risk cases. *J Obstet Gynaecol* 24, 226-229 (2004)
102. Groom K. M., A. H. Shennan, B. A. Jones, P. Seed & P. R. Bennett. TOCOX--a randomised, double-blind, placebo-controlled trial of rofecoxib (a COX-2-specific prostaglandin inhibitor) for the prevention of preterm delivery in women at high risk. *Bjog* 112, 725-730 (2005)
103. Vanchieri C. Researchers plan to continue to study COX-2 inhibitors in cancer treatment and prevention. *J Natl Cancer Inst* 97, 552-553 (2005)
104. Mijovic J. E., T. Zakar, D. B. Zaragoza & D. M. Olson. Tyrphostins inhibit lipopolysaccharide induced preterm labor in mice. *J Perinatal Medicine* 30, 297-300 (2002)
105. Balachandra S., T. Genovese, E. Mazzon, R. Di Paola, C. Thiemerman, A. K. Siriwardena & S. Cuzzocrea. Inhibition of tyrosine-kinase-mediated cellular signaling by tyrphostins AG 126 and AG556 modulates murine experimental acute pancreatitis. *Surgery* 138, 913-923 (2005)
106. Shouda T., K. Hiraoka, S. Komiya, T. Hamada, M. Zenmyo, H. Iwasaki, T. Isayama, N. Fukushima, K. Nagata & A. Yoshimura. Suppression of IL-6 production and proliferation by blocking STAT3 activation in malignant soft tissue tumor cells. *Cancer Lett* 231, 176-184 (2006)
107. Zakar T., J. E. Mijovic & D. M. Olson. Prostaglandin endoperoxide H synthase-2 expression in human amnion cells: involvement of tyrosine kinases in the regulation. *Adv Exp Med Biol* 433, 415-418 (1997)
108. Zakar T., J. E. Mijovic, K. M. Eyster, D. Bhardwaj & D. M. Olson. Regulation of prostaglandin H2 synthase-2 expression in primary human amnion cells by tyrosine kinase dependent mechanisms. *Biochim Biophys Acta* 1391, 37-51. (1998)
109. Zakar T., J. E. Mijovic, D. Bhardwaj & D. M. Olson. Tyrosine kinase inhibitors block the glucocorticoid stimulation of prostaglandin endoperoxide H synthase expression in amnion cells. *Can J Physiol Pharmacol* 77, 138-142. (1999)
110. Kennedy I., R. A. Coleman, P. P. Humphrey, G. P. Levy & P. Lumley. Studies on the characterisation of prostanoid receptors: a proposed classification. *Prostaglandins* 24, 667-689. (1982)
111. Coleman R. A., W. L. Smith & S. Narumiya. International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. *Pharmacol Rev* 46, 205-229 (1994)
112. Hirata M., Y. Hayashi, F. Ushikubi, Y. Yokota, R. Kageyama, S. Nakanishi & S. Narumiya. Cloning and expression of cDNA for a human thromboxane A2 receptor. *Nature* 349, 617-620. (1991)
113. Narumiya S., Y. Sugimoto & F. Ushikubi. Prostanoid receptors: structures, properties, and functions. *Physiol Rev* 79, 1193-1226 (1999)
114. Pierce K. L., T. J. Bailey, P. B. Hoyer, D. W. Gil, D. F. Woodward & J. W. Regan. Cloning of a carboxyl-terminal isoform of the prostanoid FP receptor. *J Biol Chem* 272, 883-887 (1997)
115. Pierce K. L. & J. W. Regan. Prostanoid receptor heterogeneity through alternative mRNA splicing. *Life Sci* 62, 1479-1483 (1998)
116. Abramovitz M., Y. Boie, T. Nguyen, T. H. Rushmore, M. A. Bayne, K. M. Metters, D. M. Slipetz & R. Grygorczyk. Cloning and expression of a cDNA for the human prostanoid FP receptor. *J Biol Chem* 269, 2632-2636 (1994)
117. Asboth G., S. Phaneuf & A. L. Lopez Bernal. Prostaglandin E receptors in myometrial cells. *Acta Physiol Hung* 85, 39-50 (1997)
118. Matsumoto T., N. Sagawa, M. Yoshida, T. Mori, I. Tanaka, M. Mukoyama, M. Kotani & K. Nakao. The prostaglandin E2 and F2 alpha receptor genes are expressed in human myometrium and are down-regulated during pregnancy. *Biochem Biophys Res Commun* 238, 838-841 (1997)
119. Brodt-Eppley J. & L. Myatt. Prostaglandin receptors in lower segment myometrium during gestation and labor. *Obstet Gynecol* 93, 89-93 (1999)

120. Smith G. C. S., W. X. WU & P. W. Nathanielsz. Baboon labour is associated with a decreased uterine prostanoid EP2 receptor expression. *Journal of the Society for Gynecological Investigation* 6, 128A-129A (1999)

121. Dong Y. L. & C. Yallampalli. Pregnancy and exogenous steroid treatments modulate the expression of relaxant EP(2) and contractile FP receptors in the rat uterus. *Biol Reprod* 62, 533-539 (2000)

122. Brodt-Eppley J. & L. Myatt. Changes in expression of contractile FP and relaxatory EP2 receptors in pregnant rat myometrium during late gestation, at labor, and postpartum. *Biol Reprod* 59, 878-883 (1998)

123. Wu W. X., X. H. Ma, T. Yoshizato, N. Shinozuka & P. W. Nathanielsz. Differential expression of myometrial oxytocin receptor and prostaglandin H synthase 2, but not estrogen receptor alpha and heat shock protein 90 messenger ribonucleic acid in the gravid horn and nongravid horn in sheep during betamethasone-induced labor. *Endocrinology* 140, 5712-5718. (1999)

124. Gyomorey S., S. J. Lye, W. Gibb & J. R. Challis. Fetal-to-maternal progression of prostaglandin H(2) synthase-2 expression in ovine intrauterine tissues during the course of labor. *Biol Reprod* 62, 797-805 (2000)

125. Europe-Finner G. N., S. Phaneuf, A. M. Tolkovsky, S. P. Watson & A. Lopez Bernal. Down-regulation of G alpha s in human myometrium in term and preterm labor: a mechanism for parturition. *J Clin Endocrinol Metab* 79, 1835-1839. (1994)

126. Docherty C. C., A. Sathananthan, L. A. Fraine, B. Davis & P. W. Nathanielsz. Altered PG receptor function in murine myometrium around the time of parturition. *Society for Gynecological Investigation* 6, 160A (1999)

127. Smith G. C., W. X. Wu & P. W. Nathanielsz. Effects of gestational age and labor on expression of prostanoid receptor genes in baboon uterus. *Biol Reprod* 64, 1131-1137. (2001)

128. Armer T. L. & P. Duff. Intraamniotic infection in patients with intact membranes and preterm labor. *Obstet Gynecol Surv* 46, 589-593. (1991)

129. Romero R., B. H. Yoon, M. Mazor, R. Gomez, M. P. Diamond, J. S. Kenney, M. Ramirez, P. L. Fidel, Y. Sorokin, D. Cotton & et al. The diagnostic and prognostic value of amniotic fluid white blood cell count, glucose, interleukin-6, and gram stain in patients with preterm labor and intact membranes. *Am J Obstet Gynecol* 169, 805-816. (1993)

130. Belt A. R., J. J. Baldassare, M. Molnar, R. Romero & F. Hertelendy. The nuclear transcription factor NF-kappaB mediates interleukin-1beta- induced expression of cyclooxygenase-2 in human myometrial cells. *Am J Obstet Gynecol* 181, 359-366. (1999)

131. Zaragoza D. B., R. R. Wilson, B. F. Mitchell & D. M. Olson. The interleukin-1beta induced upregulation of human prostaglandin F2alpha receptor mRNA in human myometrial cells (ULTR) is mediated by the transcription factor, NF-kappa B. *J Soc Gynecol Investig* Supplement 1st International Summit Preterm Birth, 70 (2005)

132. Zaragoza D. B., R. Wilson, K. M. Eyster & D. M. Olson. Cloning and characterization of the promoter region of the human prostaglandin F2 receptor gene. *Biochim Biophys Acta* doi:10.1016/j.bbaexp.2003.11.004, (2003)

133. Molnar M. & F. Hertelendy. PGF2 alpha and PGE2 binding to rat myometrium during gestation, parturition, and postpartum. *Am J Physiol* 258, E740-747 (1990)

134. Srinivasan D., H. Fujino & J. W. Regan. Differential internalization of the prostaglandin F2 alpha receptor isoforms: role of protein kinase C and clathrin. *J Pharmacol Exp Ther* 302, 219-224 (2002)

135. Zaragoza D. B., K. George & D. M. Olson. Regulation of Prostaglandin $F_{2\alpha}$ receptor (FP) mRNA by $PGF_{2\alpha}$ in human myometrial ULTR cells. *J Soc Gynecol Investig* 12, 244A (2006)

136. Sakamoto K., T. Ezashi, K. Miwa, E. Okuda-Ashitaka, T. Houtani, T. Sugimoto, S. Ito & O. Hayaishi. Molecular cloning and expression of a cDNA of the bovine prostaglandin F2 alpha receptor. *J Biol Chem* 269, 3881-3886 (1994)

137. Sugimoto Y., K. Hasumoto, T. Namba, A. Irie, M. Katsuyama, M. Negishi, A. Kakizuka, S. Narumiya & A. Ichikawa. Cloning and expression of a cDNA for mouse prostaglandin F receptor. *J Biol Chem* 269, 1356-1360 (1994)

138. Graves P. E., K. L. Pierce, T. J. Bailey, B. R. Rueda, D. W. Gil, D. F. Woodward, A. J. Yool, P. B. Hoyer & J. W. Regan. Cloning of a receptor for prostaglandin F2 alpha from the ovine corpus luteum. *Endocrinology* 136, 3430-3436 (1995)

139. Sugimoto Y., A. Yamasaki, E. Segi, K. Tsuboi, Y. Aze, T. Nishimura, H. Oida, N. Yoshida, T. Tanaka, M. Katsuyama, K. Hasumoto, T. Murata, M. Hirata, F. Ushikubi, M. Negishi, A. Ichikawa & S. Narumiya. Failure of parturition in mice lacking the prostaglandin F receptor. *Science* 277, 681-683 (1997)

140. Tsuboi K., Y. Sugimoto, A. Iwane, K. Yamamoto, S. Yamamoto & A. Ichikawa. Uterine expression of prostaglandin H2 synthase in late pregnancy and during parturition in prostaglandin F receptor-deficient mice. *Endocrinology* 141, 315-324 (2000)

141. Young W. S., 3rd, E. Shepard, J. Amico, L. Hennighausen, K. U. Wagner, M. E. LaMarca, C. McKinney & E. I. Ginns. Deficiency in mouse oxytocin prevents milk ejection, but not fertility or parturition. *J Neuroendocrinol* 8, 847-853. (1996)

142. Nishimori K., L. J. Young, Q. Guo, Z. Wang, T. R. Insel & M. M. Matzuk. Oxytocin is required for nursing but is not essential for parturition or reproductive behavior. *Proc Natl Acad Sci U S A* 93, 11699-11704. (1996)

143. Langenbach R., S. G. Morham, H. F. Tiano, C. D. Loftin, B. I. Ghanayem, P. C. Chulada, J. F. Mahler, C. A. Lee, E. H. Goulding, K. D. Kluckman & et al. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell* 83, 483-492. (1995)

144. Gross G., T. Imamura & L. J. Muglia. Gene knockout mice in the study of parturition. *J Soc Gynecol Investig* 7, 88-95 (2000)

145. Dinchuk J. E., B. D. Car, R. J. Focht, J. J. Johnston, B. D. Jaffee, M. B. Covington, N. R. Contel, V. M. Eng, R. J. Collins, P. M. Czerniak & et al. Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. *Nature* 378, 406-409. (1995)

146. Morham S. G., R. Langenbach, C. D. Loftin, H. F. Tiano, N. Vouloumanos, J. C. Jennette, J. F. Mahler, K. D. Kluckman, A. Ledford, C. A. Lee & et al. Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. *Cell* 83, 473-482. (1995)

147. Reaume A. G., P. A. de Sousa, S. Kulkarni, B. L. Langille, D. Zhu, T. C. Davies, S. C. Juneja, G. M. Kidder & J. Rossant. Cardiac malformation in neonatal mice lacking connexin43. *Science* 267, 1831-1834. (1995)
148. Cook J. L., M. C. Shallow, D. B. Zaragoza, K. I. Anderson & D. M. Olson. Mouse fetal prostaglandins influence the timing of birth and uterine activation. *Biology of Reproduction* In Press (2002)
149. Quiniou C., K. Peri, X. Hou, D. Abran & S. Chemtob. PHG113 is a selective PGF2α receptor antagonist which delays preterm labor. *Journal of the Society for Gynecological Investigation* 8, 79A (2001)
150. Leiser R. & P. Kaufmann. Placental structure: in a comparative aspect. *Exp Clin Endocrinol* 102, 122-134 (1994)
151. Hirst J. J., H. C. Parkington, I. R. Young, H. K. Palliser, K. G. Peri & D. M. Olson. Delay of preterm birth in sheep by THG113.31, a prostaglandin F2alpha receptor antagonist. *Am J Obstet Gynecol* 193, 256-266 (2005)
152. Hertelendy F., M. Molnar & R. Romero. Interferon gamma antagonizes interleukin-1beta-induced cyclooxygenase-2 expression and prostaglandin E(2) production in human myometrial cells. *J Soc Gynecol Investig* 9, 215-219 (2002)
153. Kniss D. A., B. Rovin, R. H. Fertel & P. D. Zimmerman. Blockade NF-kappaB activation prohibits TNF-alpha-induced cyclooxygenase-2 gene expression in ED27 trophoblast-like cells. *Placenta* 22, 80-89 (2001)
154. Lappas M., K. Yee, M. Permezel & G. E. Rice. Lipopolysaccharide and TNF-alpha Activate the Nuclear Factor Kappa B Pathway in the Human Placental JEG-3 Cells. *Placenta* (2005)
155. Lappas M., M. Permezel, H. M. Georgiou & G. E. Rice. Regulation of phospholipase isozymes by nuclear factor-kappaB in human gestational tissues in vitro. *J Clin Endocrinol Metab* 89, 2365-2372 (2004)
156. Allport V. C., D. Pieber, D. M. Slater, R. Newton, J. O. White & P. R. Bennett. Human labour is associated with nuclear factor-kappaB activity which mediates cyclo-oxygenase-2 expression and is involved with the 'functional progesterone withdrawal'. *Mol Hum Reprod* 7, 581-586. (2001)
157. Condon J. C., P. Jeyasuria, J. M. Faust & C. R. Mendelson. Surfactant protein secreted by the maturing mouse fetal lung acts as a hormone that signals the initiation of parturition. *Proc Natl Acad Sci U S A* 101, 4978-4983 (2004)

Key Words: Prostaglandins, PGHS-2, COX-2, COX-2 Inhibitors, Prostaglandin Receptors, FP, Receptor Antagonist, Preterm Birth, Tocolytics, Review

Send correspondence to: David M. Olson, Ph.D., Perinatal Research Centre, 220 HMRC, University of Alberta, Edmonton, Alberta, Canada T6G 2S2, Phone: 780-492-2765, Fax: 780-492-1308, Email: david.olson@ualberta.ca

http://www.bioscience.org/current/vol12.htm