SOFIA BOUCHER-KOVALIK

CARACTÉRISATION DE L'EXPRESSION DES ENZYMES DE BIOSYNTHÈSE DES PROSTAGLANDINES DANS DES LIGNÉES CELLULAIRES ENDOMÉTRIALES IMMORTALISÉES

Mémoire présenté
à la Faculté des études supérieures de l'Université Laval
dans le cadre du programme de maîtrise en biologie cellulaire et moléculaire
pour l'obtention du grade de Maître ès sciences (M.Sc.)

FACULTÉ DE MÉDECINE
UNIVERSITÉ LAVAL
QUÉBEC

2010

Résumé

Les prostaglandines (PGs) sont des régulateurs importants de la physiologie humaine et médient, entre autres, l'inflammation, diverses fonctions vasculaires ainsi que la reproduction. Elles sont également impliquées dans de multiples désordres gynécologiques. L'objectif principal de notre laboratoire est d'étudier l'implication des PGs en reproduction humaine. Pour ce faire, des lignées cellulaires immortalisées de l'endomètre humain ont été créées. Dans mon projet de recherche, j'ai caractérisé ces lignées et analysé les processus de biosynthèse des PGs dans les cellules d'endomètre humain. L'expression des enzymes de biosynthèse et des protéines a également été étudiée dans le tissu provenant de biopsies de femmes souffrant de ménorragies. Cela nous a permis d'observer l'expression des PGs synthases dans l'endomètre de femmes souffrant d'hyperménorrhées et de quantifier l'expression d'une nouvelle PGF synthase dans l'endomètre humain. Les résultats de ces travaux devraient faciliter la mise en place de nouvelles études en reproduction et permettre de mieux comprendre l'impact des PGs chez la femme.

Publications

- Chapdelaine, P., Kang, J., **Boucher-Kovalik, S.**, Caron, N., Tremblay, J.P., Fortier, M.A. *Decidualization and maintenance of a functional prostaglandin system in human endometrial cell lines following transformation with SV40 large T antigen.* Mol hum Reprod, 2006.12(5) : p.309-19

- Bresson, E., **Boucher-Kovalik, S.**, Chapdelaine, P., Madore, E., Harvey, N., Laberge, P. Y., Leboeuf, M., and Fortier M. A. *The Human Aldose Reductase AKR1B1 Qualifies as the Primary Prostaglandin F Synthase in the Endometrium.* J Clin Endocrinol Metab published October 13, 2010 as doi:10.1210/jc.2010-1589

- Fortier MA, Krishnaswamy K, Danyod G, **Boucher- Kovalik S**, Chapdelaine P and Arosh JA. *A Postgenomic Integrated View of Prostaglandins in Reproduction:Implications for Other Body Systems*. Journal of Physiology and Pharmacology, 2008

Avant-propos

Je souhaite remercier chaleureusement tous les membres du laboratoire qui m'ont aidé pendant ma maîtrise: Pierre Chapdelaine, Nathalie Harvey, Ewa Bresson, Ghislain, Nani, et bien sûr le directeur de ma maîtrise Michel A. Fortier. Ils m'ont soutenu dans l'apprentissage des techniques de recherche et m'ont démontré l'importance d'être méticuleux et persévérant. Je vous souhaite à tous une carrière riche et stimulante où que vous soyez et pour ceux qui prendront leur retraite prochainement, profitez en bien.

Table des matières

Liste des tableaux

Chapitre 1

Chapitre 2

Chapitre 3

Liste des figures

Chapitre 1

Chapitre 2

Chapitre 3

Abréviations

AA : acide arachidonique

AINS : anti-inflammatoires non-stéroïdiens

AMPc : Adénosine 3'-5' monophosphate cyclique

ARN : acide ribonucléique

AUB : Abnormal uterine bleeding

COX : cyclooxygénase

cPLA$_2$: Phospholipase cytosolique A$_2$

cPGES: Prostaglandine E synthétase cytoplasmique

DP: Récepteur de la PGD$_2$

EP : Récepteur de PGE$_2$

ER : Récepteur de l'oestrogène

FP : Récepteur de PGF$_{2\alpha}$

FSH : Hormone folliculo-stimulante

GnRH : Hormone de libération des gonadotrophines

GST-μ : Glutathione S-transférase

HPETE: acide hydroperoxy-eicosatetraenoïque

HSD : activité hydroxystéroïde réductase

IL-1: interleukines 1

IP : Récepteur de la prostacycline (PGI$_2$)

LH : Hormone lutéinisante

LT: Leucotriènes

mPGES (1 ou 2): Prostaglandine E synthétase microsomale de type 1 ou 2

OATP : 12-transmembrane Organic Anion Transporting Polypeptide

PD : population doubling

PG (s) : prostaglandine (s)

PGD$_2$: Prostaglandine D$_2$

PGE$_2$: Prostaglandine E$_2$

PGES : PGE synthases

$PGF_{2\alpha}$: Prostaglandine $F_{2\alpha}$

PGFS : PGF synthases

PGT: Transporteur des prostaglandines

PKA: protéine kinase A

PLC: Phospholipase C

PPAR : peroxisome proliferator-activated receptor

PR : Récepteur de la progestérone

SV40: virus simien 40

Tx : Thromboxanes

TxA_2: Thromboxane A_2

ω-3 : Oméga-3

ω-6 : Oméga-6

CHAPITRE 1

INTRODUCTION GÉNÉRALE ET REVUE DE LITTÉRATURE

Introduction générale

Problématique

Les prostaglandines (PGs) se révèlent essentielles en physiologie humaine. Ces molécules ubiquitaires sont parmi les principaux régulateurs locaux des fonctions cellulaires. Elles sont impliquées dans diverses étapes de la physiologie reproductive chez la femme (ovulation, lutéinisation, contractions utérines). Les PGs de l'endomètre dictent la durée du cycle et la fonction du corps jaune chez l'animal, alors que chez l'humain ces médiateurs semblent plutôt contrôler les menstruations (Milne, Perchick et al. 2001; Jabbour, Kelly et al. 2006). À cet égard, les deux PGs les plus abondantes dans l'endomètre, $PGF_{2\alpha}$ et PGE_2, jouent un rôle primordial et souvent opposé chez toutes les espèces étudiées, à l'instar du duo PGI_2 et TxA pour le système vasculaire. L'action complexe des PGs peut être régulée à divers niveaux dont : la biosynthèse, le transport transmembranaire, les récepteurs et le catabolisme.

En plus de leurs rôles physiologiques, les PGs sont impliquées dans diverses pathologies d'ordre gynécologiques ou autres. Entre autres, les désordres menstruels sont couramment rencontrés chez la femme. Parmi les troubles les plus fréquents, on retrouve l'hyperménorrhée (ménorragies ou abnormal uterine bleeding (AUB) en anglais) et la dysménorrhée (crampes menstruelles). Il semblerait que 10 à 30 % des femmes en âge de se reproduire souffrent de ménorragies (Fraser, Critchley et al. 2007) et que 40 à 90 % souffrent de dysménorrhées.(Albers, Hull et al. 2004) Les PGs semblent être des facteurs importants dans l'élaboration de ces dysfonctions. C'est pourquoi une des pierres angulaires du traitement des troubles menstruels est l'inhibition complète de la synthèse des prostaglandines à l'aide d'anti-inflammatoires non stéroïdiens (AINS) tels que l'ibuprofène et le naproxen.

Les travaux de Fortier *et al.* ont permis d'identifier des rôles spécifiques et déterminants pour des joueurs au niveau de la cascade de biosynthèse des PGs comme les

phospholipases, les synthases terminales ou les transporteurs, certains étant identifiés récemment dans son laboratoire.(Kang, Chapdelaine et al. 2005; Parent, Chapdelaine et al. 2005) Pour la réalisation de ces études, des cultures cellulaires provenant d'endomètre humain ont été isolées selon leur type (épithélial ou stromal), puis immortalisées. Une partie de mon travail a consisté caractériser ces lignées cellulaires. Par la suite, nous avons évalué le patron d'expression des enzymes de biosynthèse des PGs dans les tissus endométriaux humains au cours du cycle menstruel grâce à diverses techniques d'analyse des protéines et de l'ARN. Nous avons également évalué l'effet de diverses substances stimulatrices et inhibitrices des PGs sur l'expression des enzymes de biosynthèse des PGs.

L'axe hypothalamo-hypophyso-gonadique

Le cycle menstruel chez l'humain et les primates de l'Ancien Monde se distingue de celui des autres animaux par le fait que la couche superficielle de l'endomètre est renouvelée à chaque cycle. Le cycle débute le premier jour des règles. Il dure en moyenne 28 jours, mais peut varier de 24 à 35 jours sans être pathologique. La durée normale des règles est de 3 à 6 jours et les pertes sanguines se situent autour de 25 à 35 ml.(Hacker, Moore et al. 2004)

La régulation du cycle menstruel se fait via l'axe hypothalamo-hypophyso-gonadique. Les neurones de l'hypothalamus sécrètent l'hormone GnHR, une neurohormone, qui est responsable de la stimulation de la synthèse et de la libération de LH et de FSH par l'adénohypophyse. Ces processus sont contrôlés par l'amplitude et la fréquence de libération du GnRH. La sécrétion de GnRH en petite quantité stimule la libération de FSH tandis que les impulsions de grande amplitude stimulent la sécrétion de LH. Le GnRH cause habituellement une plus grande libération de LH que de FSH, c'est pourquoi cette hormone se nomme également LH-releasing hormone (LHRH) ou LH-releasing factor (LRF). Le GnRH est sécrété de façon pulsatile tout au long du cycle menstruel. L'hypothalamus contrôle également les fonctions reproductrices en produisant

de la dopamine qui exerce une inhibition chronique sur la libération de prolactine par les cellules lactotrophes de l'hypophyse.

L'hypophyse, l'organe régi par l'hypothalamus, sécrète différentes hormones régulant les fonctions reproductives. La neurohypophyse (lobe postérieur) sécrète l'ocytocine dont le rôle principal est la stimulation des contactions utérines lors de l'accouchement et l'initiation de la lactation. L'adénohypophyse (lobe antérieur) sécrète, grâce à différents types cellulaires, six hormones dont trois sont impliquées dans la physiologie reproductive femelle soit : la FSH, la LH et la prolactine. La FSH et la LH sont des glycoprotéines constituées de deux sous-unités (α et β). La sous-unité α est identique pour ces deux hormones ainsi que pour le hCG (human chorionic gonadotropin) sécrété par le placenta lors de la grossesse. Elles régulent la production hormonale ovarienne et par le fait même le cycle menstruel. (Figure 1) La prolactine, quant à elle, est sécrétée par les cellules lactotrophes de l'hypophyse. Contrairement aux autres hormones produites par l'adénohypophyse, la sécrétion de prolactine est contrôlée par la dopamine, un neurotransmetteur provenant de l'hypothalamus, qui inhibe l'activité hypophysaire. Les taux de prolactine s'élèvent lors de la grossesse afin de stimuler la production lactée des glandes mammaires. Les hormones FSH et LH sont les principaux régulateurs du cycle reproducteur féminin et dictent la production hormonale des ovaires.

Le cycle ovarien et endométrial

Le cycle menstruel est subdivisé en deux phases. Il débute par la phase folliculaire au cours de laquelle certains follicules ovariens se développent grâce à la stimulation par le FSH. La durée de cette phase peut varier, mais la moyenne se situe autour de 13 jours. L'ovaire produit à ce moment une concentration croissante d'oestradiol jusqu'au moment de l'ovulation. Cette hormone stimule principalement la croissance de l'endomètre. Vers le 13^{e} jour, l'oestradiol atteint une concentration suffisante pour stimuler de façon ponctuelle la libération de LH de l'hypophyse. L'ovulation survient environ 24 heures après le pic de LH.

La seconde phase, nommée phase lutéale, survient après l'ovulation. C'est à ce moment que les cellules thécales et la granulosa résiduelle de l'ovaire se transforment en corps jaune (*corpus luteum*), dont la principale fonction est la production d'hormones stéroïdiennes (progestérone et oestrogènes). Ces hormones permettent à l'endomètre de maturer et devenir propice à l'implantation. La diminution brutale de la concentration de ces hormones à la fin de la phase lutéale cause la desquamation de la couche superficielle de l'endomètre. La phase lutéale a une durée constante de 14 jours en l'absence d'une grossesse.

Les hormones stéroïdiennes produites par l'ovaire stimulent les cellules de l'endomètre. Les modifications de concentrations hormonales induisent des changements structuraux et fonctionnels au niveau de l'endomètre tout au long du cycle menstruel. C'est pourquoi le cycle endométrial contient deux phases en relation avec les phases folliculaires et lutéales de l'ovaire. La portion superficielle (couche fonctionnelle) de l'endomètre passe, à chaque cycle, à travers une série d'étapes de prolifération, de différenciation, puis de destruction afin d'être régénérée au cycle suivant.(Jabbour, Kelly et al. 2006)

La phase proliférative de l'endomètre coïncide avec la phase folliculaire ovarienne. Les niveaux graduellement progressifs d'œstrogènes au cours de la première phase du cycle menstruel mènent à une prolifération de l'endomètre et à l'épaississement de la couche tissulaire qui va accueillir l'embryon suite à la fécondation. Il y a alors prolifération et différenciation équivalente des cellules glandulaires (épithéliales) et stromales de la couche superficielle de l'endomètre.

La seconde phase endométriale, nommée sécrétoire, correspond à la phase lutéale de l'ovaire. C'est une période de prolifération principalement glandulaire et de production de glycogène par les cellules stromales. La maturation de l'endomètre est stimulée par l'œstradiol et la progestérone du corps jaune ce qui lui permet de devenir propice à l'implantation du blastocyste. Si la fécondation n'a pas lieu, le cycle se poursuit et la concentration hormonale chute radicalement en fin de phase sécrétoire. Ceci mène à la destruction et à l'évacuation de la couche superficielle de l'endomètre lors des règles.

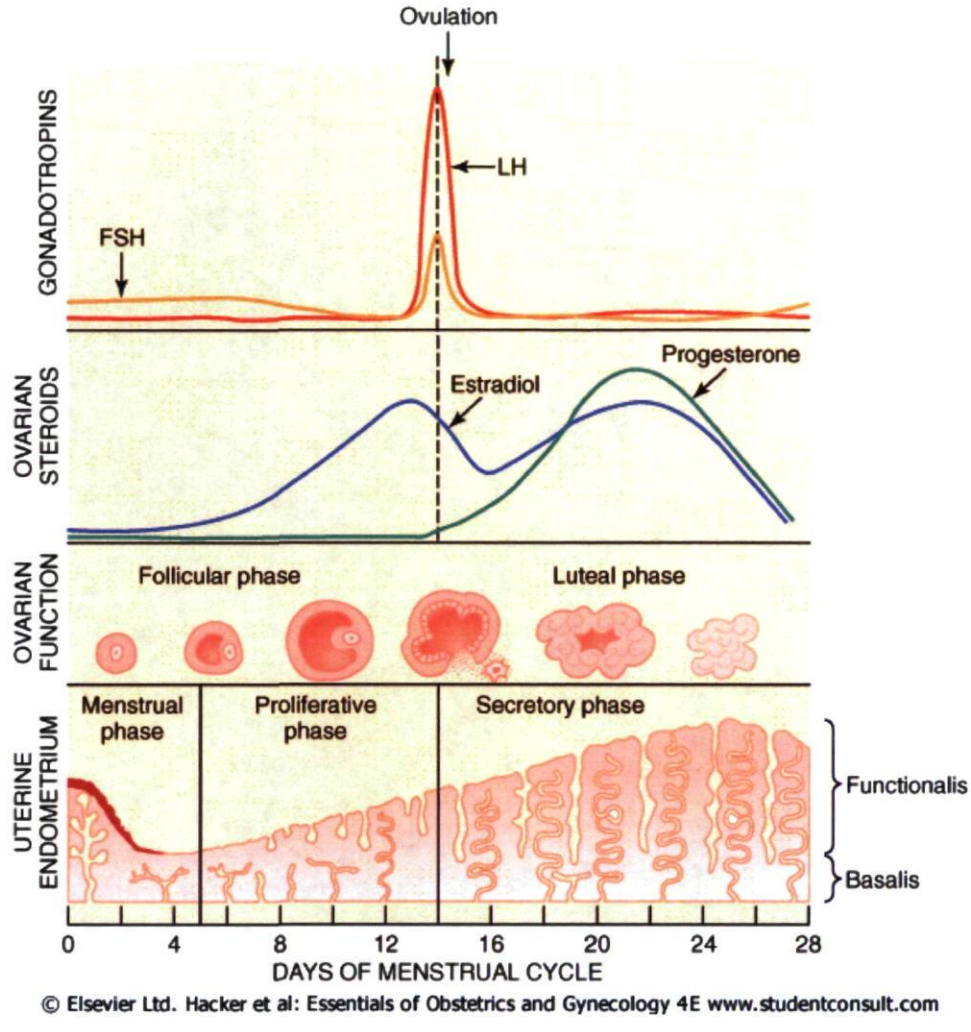


Figure 1. Le cycle menstruel : concentrations hormonales hypophysaires et gonadiques au cours du cycle menstruel, développement folliculaire et prolifération endométriale. Tiré du manuel : Hacker, N.F., J.G. Moore, and J.C. Gambone, *Essentials of obstetrics and gynecology*. 4th ed. 2004, Philadelphia, Pa.: Saunders. xvii, 520 p.

Les prostaglandines

Les PGs sont une famille de lipides ubiquitaires dans l'organisme humain. Elles participent à une grande variété de processus physiologiques et pathologiques. Les PGs sont exprimées, entre autres, dans les réactions inflammatoires, la prolifération cellulaire et le cancer. (Bos, Richel et al. 2004) Outre les hormones stéroïdiennes, les PGs sont parmi les principaux régulateurs de la fonction reproductrice chez la femme. Les PGs sont synthétisées localement et médient leurs actions via des récepteurs couplés à des protéines G. Ces récepteurs sont présents sur la membrane plasmique des cellules environnantes. C'est pourquoi les PGs ne peuvent être considérées comme des hormones, mais plutôt comment des régulateurs locaux paracrines et autocrines des fonctions cellulaires.

Elles font partie intégrante d'une plus grande classe de molécules, les eicosanoïdes, qui comprennent les PGs, les thromboxanes (Tx), les leucotrinènes (LT), les lipoxines et les hépoxylines.(Smith and Song 2002) Les eicosanoïdes proviennent principalement, chez l'humain, de la libération et de la transformation de l'acide arachidonique (AA), un acide gras oméga-6 (ω-6). Les prostanoïdes sont des acides gras oxygénés contenant 20 carbones. Les PGs sont subdivisées en 9 types (A à I). Les variétés A, B et C ne semblent pas être présentes dans les tissus vivants, mais peuvent être synthétisées en laboratoire. Les PGD, E, F et I sont présentes dans les tissus humains sains. Les PGs de type I sont également nommées prostacyclines. Les Tx, quant à elles, sont subdivisées en deux types (A et B). En plus du type de PG, celles-ci sont caractérisées par le nombre de doubles liaisons présentes dans la molécule. C'est ainsi qu'il existe trois séries de PGs (ex. PGE_1, PGE_2, PGE_3). (Kudo and Murakami 2002; Smith and Song 2002; Bos, Richel et al. 2004) Les précurseurs moléculaires des PGs déterminent leur structure. Il en existe donc 3 types qui sont : l'acide γ-homolinolénique (ω-6, série 1), l'acide arachidonique (ω-6, série 2) et l'acide 5,8,11,14,17-eicosapentaenoïque (ω-3, série 3). La série 2 est prédominante chez les mammifères, puisque l'acide arachidonique est le précurseur le plus abondant.

La synthèse des PGs s'effectue en trois étapes : (1) la libération par la phospholipase A_2 d'acides gras, tels que l'acide arachidonique (AA), à partir des phospholipides membranaires suite à un stimulus, (2) la transformation de l'AA en PGH_2 par les cyclooxygénases (COX 1 et 2), (3) puis la conversion de la PGH_2 en PGs particuliers par des prostaglandines synthases spécifiques à chaque sous-type de PG. (Figure 2) Tel que mentionné précédemment, les principales PGs synthétisées par l'endomètre sont PGE_2 et $PGF_{2\alpha}$ qui sont produites respectivement par des PGE et PGF synthases à partir du PGH_2.

Les phospholipases

Les prostaglandines sont des lipides provenant de l'acide arachidonique (AA), un acide gras essentiel retrouvé dans la membrane plasmique des cellules. Pour être métabolisé, l'AA doit être détaché des phospholipides membranaires. La libération de l'AA se fait principalement par la phospholipase A_2 (PLA_2) ou la C (PLC) et ceci constitue l'étape limitante de la production des PGs. (Sales and Jabbour 2003; Bos, Richel et al. 2004) L'AA est par la suite transformé en eicosanoïdes. Tel que mentionné précédemment, les eicosanoïdes incluent les PGs, les Txs et les LTs. (Kudo and Murakami 2002) Il existe différentes variétés de phospholipases : sPLA2, $cPLA_2$, $iPLA_2$, PAF-AH. Cependant, celle qui semble être la plus importante pour la production de PGs est la phospholipase cytosolique A_2 ($cPLA_2$) puisqu'elle semble utiliser de préférence l'AA comme substrat.(Kudo and Murakami 2002) Il existe trois isotypes de $cPLA_2$ (α, β et γ). $cPLA_2\alpha$ est exprimée dans de nombreux tissus humains et est également présente dans l'endomètre.(Leslie 1997) Ce serait la phospholipase impliquée dans la production des PGs au niveau de l'utérus humain.

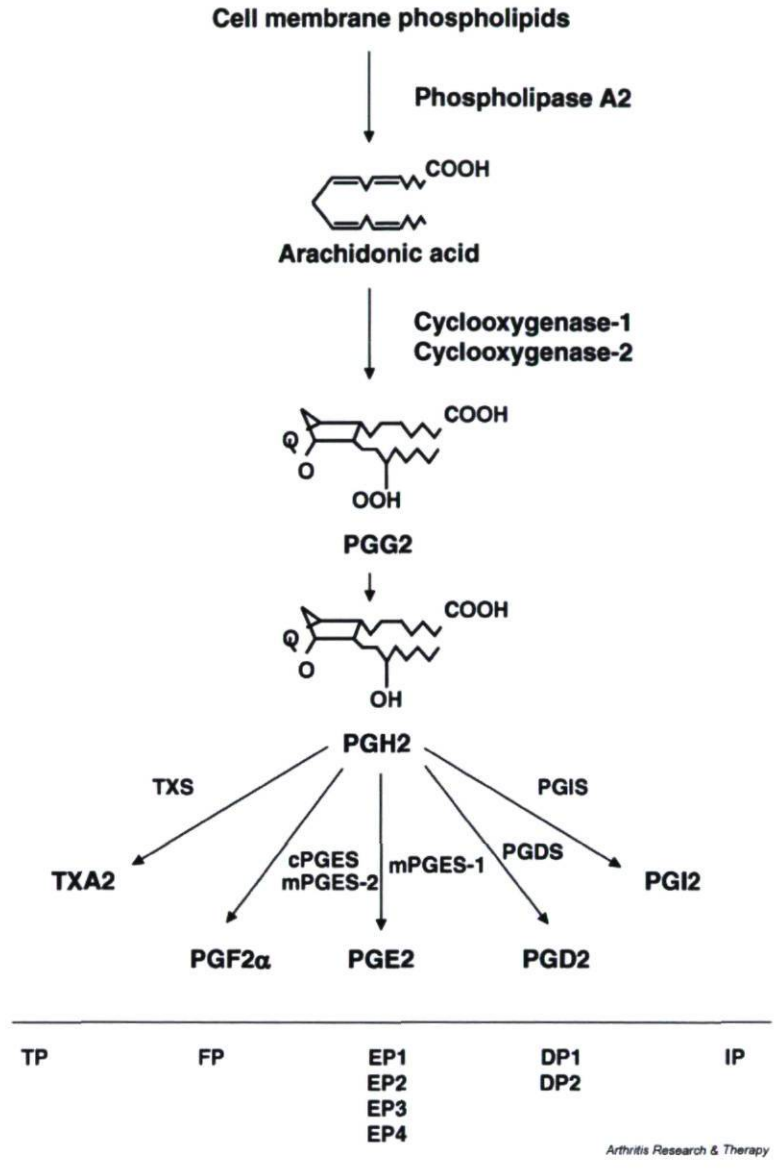


Figure 2. Voie de biosynthèse des prostaglandines de la série 2 chez l'humain. Transformation de l'acide arachidonique à partir de la membrane cellulaire en prostaglandines terminales via diverses enzymes (phospholipase A2, cyclooxygénases et les PG synthases spécifiques) Tiré de : Sampey *et al. Arthritis Research & Therapy* 2005 **7**:114

Les cyclooxygénases

Par la suite, l'AA est transformé en PGH_2 par les enzymes cyclooxygénases (COX). Il existe 2 formes principales de cyclooxygénases : COX-1, une enzyme constitutive, et COX-2, une enzyme inductible. (Jabbour and Sales 2004) Récemment, une 3e enzyme de la famille des COX a été clonée (COX-3 ou COX-1b). Il s'agit d'une variante de COX-1 dont les fonctions sont encore mal connues et qui ne semble pas avoir le même profil d'inhibition par les anti-inflammatoires non stéroïdiens (AINS) que les autres COX.(Chandrasekharan, Dai et al. 2002; Hersh, Lally et al. 2005)

Il est habituellement considéré que COX-1 est associée aux fonctions physiologiques normales des cellules, tandis que COX-2 s'exprime principalement lors des pathologies.(Sales and Jabbour 2003) Cependant, il a été récemment démontré que COX-1 joue, tel que COX-2, un rôle important dans l'établissement de nombreux cancers. (Hwang, Scollard et al. 1998; Sales, Katz et al. 2002; Daikoku, Tranguch et al. 2006; Sugimoto, Koizumi et al. 2007) On retrouve une expression basale de COX-1 dans la majorité des tissus chez l'humain. Cependant, le gène semble être inductible dans certaines conditions et dans certains tissus humains et animaux. L'enzyme COX-2, quant à elle est peu exprimée de façon constitutive, mais est stimulée par divers oncogènes, facteurs de croissance et d'inflammation.(Morita 2002)

Le gène de COX-2 peut être inhibé par les glucocorticoïdes (dexaméthasone) et par des cytokines anti-inflammatoires.(Niiro, Otsuka et al. 1997) De plus, COX-1 et COX-2 sont les cibles principales de l'inhibition par les anti-inflammatoires non stéroïdiens (AINS). (Smith and Song 2002; Sales and Jabbour 2003) Les AINS sont des inhibiteurs compétitifs des cyclooxygénases et empêchent la liaison des acides gras tels que l'AA à ces enzymes.

Les prostaglandines synthases

La PGH_2, synthétisée par les cyclooxygénases, est la molécule précurseure de toutes les PGs et est convertie en diverses formes de prostaglandines (PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2 et TxA_2) par des synthases terminales spécifiques nommées en fonction de la PG qu'ils produisent.(Helliwell, Adams et al. 2004) Chaque PG ayant des fonctions particulières, leur production varie en fonction du stimulus impliqué. Le taux de production des différentes PGs suite à la libération d'AA, dépend en grande partie du niveau d'expression des PG synthases.

Les synthases terminales de la série E (PGES)

Le rôle principal des PGES est d'effectuer la transformation du PGH_2 obtenu grâce aux cyclooxygénases (COX-1 ou COX-2) en PGE_2. Il existe quatre sous-types de cette enzyme : une qui se retrouve au niveau du cytoplasme (cPGES), deux sous-types se retrouvant dans les membranes (mPGES-1 et 2) et une glutathione S-transférase (GST-μ). (Murakami and Kudo 2004) Le taux d'expression de chaque sous-type de PGES varie d'un tissu à l'autre. Certains organes possèdent une expression basale de PGES, dont principalement de cPGES. Cependant, l'expression génique des mPGES peut être induite par divers stimuli cellulaires, dont l'inflammation. Il semble exister une corrélation, lors des processus pro-inflammatoires, entre l'induction de COX-2, mais non de COX-1, et celle de mPGES. Cette stimulation peut être prévenue par un traitement à la dexamethasone, un corticostéroïde, ayant une action anti-inflammatoire.(Stichtenoth, Thoren et al. 2001)

Pour ce qui est du système reproducteur féminin. La PGE synthase (mPGES-1) semble être la principale PGES exprimée dans les cellules stromales, épithéliales et endothéliales de l'endomètre humain. (Milne and Jabbour 2003) mPGES-1 semble être également exprimé au niveau du placenta et des membranes fœtales lors de la grossesse chez l'humain.(Alfaidy, Sun et al. 2003) Présentement, il existe peu de données sur le rôle des divers PGES au niveau de la fonction reproductive féminine. Puisque PGE_2 est

d'une importance capitale en reproduction, autant au cours de la gestation que du cycle menstruel; les connaissances de l'expression, de la stimulation et le rôle des divers PGES au niveau des tissus gynécologiques devraient être approfondies.

Les synthases de la série F (PGFS)

La synthèse de $PGF_{2\alpha}$ se fait via l'action de la 9,11- endoperoxide réductase. Cette enzyme transforme PGH_2 en $PGF_{2\alpha}$. Divers sous-types de PGFS ont été identifiés chez l'animal, dont deux isotypes de la PGD 11-ketoreductase qui ont été nommés PGFS I (sous-type pulmonaire) et PGFS II (sous-type rénal). PGFS I est exprimé dans les tissus pulmonaires, les neurones ainsi qu'au niveau de l'endothélium vasculaire chez le rat.(Helliwell, Adams et al. 2004) Cependant, AKR1C3 était le seul isoforme identifié à ce jour dans les tissus humains. Cette enzyme de la famille des aldoketoreductase, a une activité hydroxystéroïde réductase (HSD) et PGFS. Vous verrez dans le troisième chapitre de ce document que notre laboratoire a réussi à identifier une nouvelle PGFS, AKR1B1. Elle est exprimée au niveau de l'endomètre humain et pourrait être l'enzyme induite dans diverses situations physiologiques nécessitant une production de $PGF_{2\alpha}$. Cependant, pour l'instant, de la même façon que PGES, il existe très peu d'informations sur l'expression et la régulation des divers PGFS au niveau du système reproducteur féminin humain.

Le transport des PGs

Les PGs diffusent difficilement à travers la paroi cellulaire et doivent être transportées en dehors de la cellule afin d'exercer leurs actions. Le transport des PGs est encore matière à discussion dans la communauté scientifique. Il peut se faire soit par diffusion ou à l'aide d'un transporteur. Il a été estimé que le taux de diffusion des PGs à travers les parois cellulaires serait trop lent pour expliquer les élévations rapides de concentrations extra-cellulaires. C'est pourquoi il semble qu'un transporteur soit effectivement nécessaire. PGT, a été la première molécule transporteuse de PGs à être clonée.(Schuster 2002) Elle appartient à la famille des 12-transmembrane Organic Anion

Transporting Polypeptide (OATP). Elle possède une affinité pour PGE_2, $PGF_{2\alpha}$ et PGD_2 et est exprimée dans de nombreux tissus humains dont l'utérus et les ovaires. Il a été suggéré que PGT participerait aussi bien à l'efflux qu'à l'influx des PGs dans les cellules, cependant, il n'y a pas encore de consensus à ce sujet. MRP4, un autre récepteur dont l'affinité pour les PGs a été récemment démontrée dans notre laboratoire pourrait contribuer au transport des PGs hors de la cellule.

Les récepteurs des PGs

Les prostaglandines médient leurs effets autocrines et paracrines via des récepteurs de la membrane cellulaire couplés à des protéines G. Ces récepteurs sont désignés par le type de prostaglandine qu'ils reconnaissent en priorité (EP, FP, DP, IP et TP). Les principales PGs du système reproducteur féminin, PGE_2 et $PGF_{2\alpha}$, exercent leur activité par l'intermédiaire des récepteurs EP et FP respectivement. (Figure 3) Des études récentes ont démontré que les récepteurs EP2 et FP sont essentiels aux fonctions reproductives chez la femme.(Kennedy, Zhang et al. 1999; Narumiya and FitzGerald 2001)

Il existe 4 sous-types de récepteurs EP (EP1, EP2, EP3, EP4), tous spécifiques à PGE_2. Ces récepteurs activent des cascades de signalisation cellulaires distinctes.(Narumiya, Sugimoto et al. 1999) Les récepteurs EP2 et EP4 sont associés à l'adénylate cyclase et mènent à la production d'AMPc qui active la voie de signalisation des PKA. Ils médient des effets relaxants telle la vasodilatation. Le récepteur EP1 est lié à la phospholipase C (PLC) qui produit l'inositol triphosphate et le diacyl glycérol comme seconds messagers. L'activation de ce récepteur amène une libération de calcium intracellulaire et active la protéine kinase C. Ce récepteur médie plutôt des effets tonifiants, telle la vasoconstriction.(Coleman, Smith et al. 1994) Le récepteur EP3, quant à lui, produit divers ARNm par épissage alternatif. La voie de signalisation diffère selon l'ARNm produit et est spécifique au tissu stimulé.(Sugimoto, Namba et al. 1992; Narumiya, Sugimoto et al. 1999; Sales and Jabbour 2003)

Pour ce qui est du récepteur FP, il n'en existe qu'une seule variante connue à ce jour. Le récepteur FP médie des effets semblables au récepteur EP1. Il est couplé à la protéine G Gq, qui active la PLC et libère de l'inositol triphosphate et du diacylglycérol. Dans l'endomètre humain, le récepteur FP semble être exprimé dans les cellules épithéliales et péri-vasculaires et de façon majoritaire pendant la phase proliférative.(Milne and Jabbour 2003)

La reproduction humaine et les prostaglandines

Les PGs sont des médiateurs importants de la fonction reproductive de la femme. Ils concourent autant à l'établissement du cycle menstruel (menstruations, ovulation, lutéolyse) qu'à celle de la gestation (implantation, contractilité du myomètre, dilatation du col…).(Sales and Jabbour 2003) Les menstruations et l'implantation, entre autres, sont des phénomènes gynécologiques qui impliquent une réponse de type inflammatoire de l'endomètre, ce qui peut expliquer en partie l'importance des PGs. Tel que mentionné précédemment, les PGs sont générées par les cyclooxygénases (COX-1 et COX-2). Des études de suppression génique ont démontré l'importance des COX, de FP et du récepteur EP2 en reproduction, puisque l'abolition de l'expression de ces gènes mène à de nombreux problèmes reproducteurs.(Lim, Paria et al. 1997; Sugimoto, Yamasaki et al. 1997; Kennedy, Zhang et al. 1999) Les souris déficientes en COX-2 possèdent de nombreux problèmes reproducteurs tandis que celles déficientes en COX-1 demeurent fertiles. Le gène de COX-2 semble être impliqué dans l'ovulation, la fécondation ainsi que dans l'implantation.(Lim, Paria et al. 1997) COX-1, quant à elle, est exprimée de façon constitutive au niveau de l'endomètre humain. Pour ce qui est de COX-2, elle ne se retrouve pas de façon constitutive dans les cellules de l'endomètre, toutefois, son expression peut être induite par un stimulus, de type inflammatoire par exemple.

Pour ce qui est de l'expression des PGs, on en retrouve principalement deux types au niveau de l'endomètre humain; PGE_2 et $PGF_{2\alpha}$.(Smith and Kelly 1988) Elles sont sécrétées par les cellules stromales et épithéliales de ce tissu. Leurs rôles semblent être

antagonistes. On retrouve également de la prostacycline et de la TxA2 au niveau des vaisseaux sanguins irrigants le tissu endométrial.

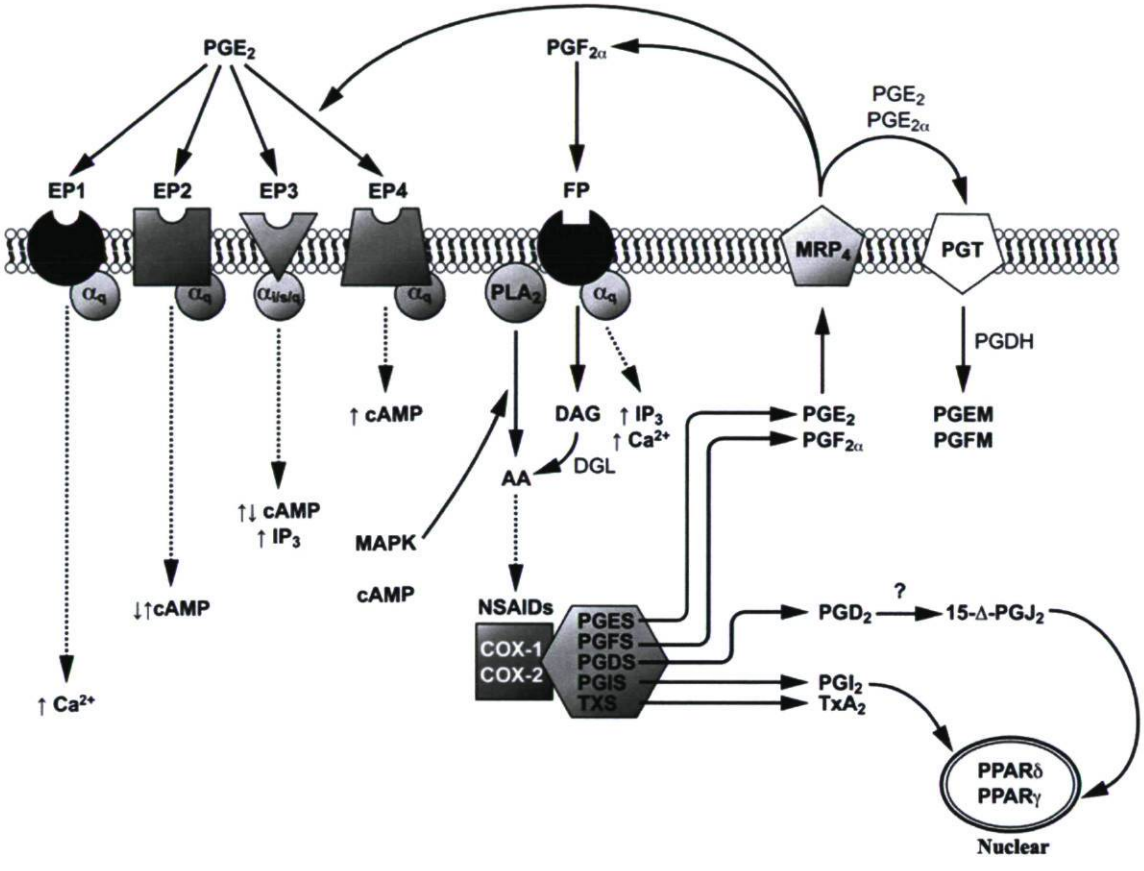


Figure 3. Voie de signalisation des prostaglandines. Après leur production, les PGs sortent des cellules par diffusion ou à l'aide de transporteurs (MRP4). Les PG médient leurs actions via des récepteurs transmembranaires couplés à des protéines G. Les récepteurs transmembranaires de PGE_2 et $PGF_{2\alpha}$ sont les EP (1 à 4) et FP respectivement. Ils activent divers signaux de transduction dans les cellules.Tirée de Fortier MA, et al. 2008 (Voir appendice 1)

Le cycle menstruel régulé par les prostaglandines.

L'endomètre est subdivisé en deux portions, la couche basale et la couche fonctionnelle, celle qui est éliminée à chaque menstruation. Lors de la phase proliférative du cycle menstruel, l'endomètre se développe en réponse à la concentration croissante d'oestradiol. Un endomètre mature est essentiel au processus d'implantation du blastocyste. Lors des menstruations, seule la portion superficielle de l'endomètre est éliminée. Il demeure une couche de cellules basales qui permettent la régénération de la couche fonctionnelle.(Jabbour, Kelly et al. 2006) Les PGs sont impliquées dans de multiples étapes du cycle menstruel. Elles sont essentielles lors du processus de croissance de l'endomètre en début de cycle ; principalement $PGF_{2\alpha}$, qui agit sur la prolifération des cellules épithéliales au niveau de l'endomètre. (Milne and Jabbour 2003; Sales and Jabbour 2003)

Les PGs sont également d'une grande importance dans le système vasculaire, tant au niveau du système reproducteur féminin que dans tout l'organisme. Le PGs médient, entre autres, la vasoconstriction et la vasodilatation des vaisseaux, ce qui régule en grande partie le débit sanguin. $PGF_{2\alpha}$ et la TxA_2, de par leur action sur les récepteurs FP et TP respectivement, provoquent de la vasoconstriction au niveau de l'endomètre.(Milne and Jabbour 2003) PGE_2 et PGI_2 entrainent, quant à eux, de la vasodilatation. De plus, PGE_2 et $PGF_{2\alpha}$ semblent contrôler l'angiogenèse au niveau de l'endomètre.(Jabbour, Sales et al. 2006) Le réseau vasculaire de l'utérus s'avère d'une importance cruciale pour tous les processus de prolifération, de fécondation et d'embryogenèse puisqu'ils apportent les nutriments nécessaires à la croissance utérine et fœtale. Une partie non négligeable de leur contrôle se fait via les PGs, ce qui leur confère un rôle essentiel à ce niveau pour la reproduction féminine.

En fin de phase lutéale, $PGF_{2\alpha}$ est la principale molécule induisant la lutéolyse tant chez l'humain que chez certaines espèces animales.(Olofsson and Leung 1994; Sales and Jabbour 2003; Arosh, Banu et al. 2004) Ceci se caractérise par l'involution du corps

jeune en l'absence de grossesse. Il y a production et libération, au niveau de l'endomètre, de $PGF_{2\alpha}$ via l'action de l'ocytocine. Ce processus s'accompagne de la diminution drastique des concentrations de progestérone à la fin de la phase sécrétoire ce qui induit l'expression de médiateurs de l'inflammation au niveau de l'endomètre humain. Certaines cytokines (IL-8, MCP-1), ainsi que la COX-2, semblent induites au niveau des cellules endométriales. (Critchley, Kelly et al. 1996; Jones, Kelly et al. 1997; Jabbour, Kelly et al. 2006) L'expression plus élevée de COX-2 précédent les menstruations coïncide avec la migration de leucocytes dans l'endomètre. La stimulation de COX-2 augmente à un niveau encore plus élevé la production de PGE_2 et de $PGF_{2\alpha}$ au niveau de l'endomètre. Lors des règles, les contractions utérines et la vasoconstriction observées au niveau de l'utérus sont en grande partie attribuées à l'action de $PGF_{2\alpha}$ au niveau des vaisseaux et du myomètre de l'utérus. Ces mécanismes sont essentiels à l'élimination de la couche superficielle de l'endomètre afin de débuter un nouveau cycle.

La grossesse

Les PGs jouent un rôle essentiel dans le maintien et la poursuite de la gestation chez l'humain, principalement de par leurs actions sur le myomètre. La régulation de la grossesse est un phénomène d'une grande complexité et nécessite une multitude de modulateurs chimiques. Certains de ces modulateurs nécessaires durant la grossesse ont un impact sur la production de PGs. L'IL-1β, une cytokine impliquée dans l'interaction entre l'endomètre et l'embryon lors de l'implantation, stimule la production de PGE_2 et de $PGF_{2\alpha}$.(Simon, Mercader et al. 1997; Huang, Liu et al. 1998; Tamura, Sebastian et al. 2002) L'expression de COX-2 et des PGs augmente à ce moment au niveau de la déciduale suite à la stimulation de l'IL-1β.(Tamura, Sebastian et al. 2002) À l'aide d'études de délétion de gènes chez la souris, il a été démontré que les PGs sont en effet essentielles à l'implantation du blastocyste. Les souris n'exprimant pas les gènes de COX-2 et de $cPLA_2$ ont des déficits d'implantation et de décidualisation, ce qui indique l'importance de la production de PGs pour l'établissement de la gestation.(Lim, Paria et al. 1997; Song, Lim et al. 2002) L'administration exogène de PGs à ces souris permet de rétablir le processus d'implantation et leur fertilité.(Song, Lim et al. 2002) COX-2 est

exprimé durant la période d'implantation au niveau de l'utérus de nombreuses espèces, incluant l'humain.(Marions and Danielsson 1999) Des études utilisant les souris COX-2 (-/-) ont indiqué que PGI_2 serait également impliquée dans les processus d'implantation et de décidualisation. Son effet serait médié par l'activation de PPARδ plutôt que par son récepteur traditionnel IP. PGE_2 semblerait également jouer un rôle, en second plan, avec PGI_2.(Lim, Gupta et al. 1999) Les connaissances à ce jour sur l'importance physiologique de PPARδ sont toutefois plutôt limitées.

L'importance des PGs durant la grossesse ne se limite pas à l'implantation. Elles sont également nécessaires au maintien de la grossesse et à l'accouchement. $PGF_{2\alpha}$ est essentielle pour l'instauration des contractions lors de la parturition tandis que PGI_2, à l'inverse, participe à la sauvegarde de la relaxation utérine au cours de la grossesse.(Challis, Matthews et al. 2000) Les PGs sont même utilisées sous forme de médication en obstétrique afin de provoquer le travail et la maturation du col chez les femmes à terme. (Ueland and Conrad 1983) En effet, PGE_2, $PGF_{2\alpha}$ et PGI_2 jouent un rôle essentiel pour les modifications du col utérin en fin de grossesse. Les PGs participent à la dilatation et à l'effacement du col en stimulant la libération d'enzymes pouvant dégrader le collagène soutenant le tissu cervical.(Yoshida, Sagawa et al. 2002)

Les prostaglandines et les désordres menstruels

Les désordres associés aux règles représentent un des motifs de consultation les plus importants en gynécologie. Leur fréquence dans la population de femmes en âge de se reproduire varie entre 10% et 90% selon les pathologies. Cette catégorie regroupe des maladies d'étiologies bien différentes (dysménorrhées, ménorragies, aménorrhée, oligoménorrhée…).(Hacker, Moore et al. 2004) Certaines sont causées par un dérèglement de l'axe hypothalamo-hypophyso-gonadique ou par une anomalie structurelle de l'appareil génital. Toutefois, certaines de ces maladies semblent également être causées par des anomalies de production des PGs.(Sales and Jabbour 2003; Fortier, Krishnaswamy et al. 2008)

La dysménorrhée

La dysménorrhée (crampes menstruelles) se définit comme étant des douleurs pelviennes accompagnant les menstruations. Elles surviennent habituellement au début des saignements ou pendant les heures précédentes. La classification de cette pathologie se fait selon l'étiologie de la douleur et selon l'âge de la patiente au moment du diagnostic. La dysménorrhée peut être subdivisée en quatre catégories : essentielle ou organique et primaire ou secondaire.(Hacker, Moore et al. 2004)

La dysménorrhée essentielle, celle qui est la plus fréquente chez les jeunes femmes en âge de se reproduire, se traduit par des douleurs apparaissant à chaque règle. À la suite d'investigations cliniques, aucune anomalie anatomique ou hormonale ne semble être reliée au problème. La dysménorrhée essentielle apparaît habituellement près de la ménarche et son intensité varie peu chaque mois.(Harel 2006) La dysménorrhée organique est associée à une autre pathologie du système gynécologique, habituellement des léiomyomes, des adénomyomes ou de l'endométriose. Elle est plus fréquente chez les femmes plus âgées, avant la ménopause. La douleur survient habituellement à l'âge adulte et peut également être surajoutée à une dysménorrhée essentielle présente de longue date. Ce qui caractérise cette dysménorrhée est la présence de douleurs progressivement croissantes au fil des mois. L'apparition d'autres symptômes accompagnant la dysménorrhée tels que la dyspareunie profonde ou des ménorragies peut orienter le diagnostic plus vers une dysménorrhée d'étiologie organique.

La dysménorrhée est de type primaire lorsqu'elle débute à l'adolescence peu après le commencement des règles. Tandis que la dysménorrhée secondaire commence plusieurs années après l'apparition des premières règles. On peut considérer une dysménorrhée comme secondaire lorsqu'une dysménorrhée primaire s'aggrave au fil des mois, plusieurs années après la puberté.

La douleur de la dysménorrhée essentielle provient d'une hypercontractilité du myomètre engendrant une vasoconstriction des artérioles et de l'ischémie locale. $PGF_{2\alpha}$

semble être présente en plus grande quantité chez les femmes souffrant de cette forme de dysménorrhées et pourrait causer, du moins en partie, l'état d'hypercontractilité. (Sales and Jabbour 2003) Il a été démontré que PGE_2 et $PGF_{2\alpha}$ se retrouvent en plus grande quantité dans le liquide menstruel des femmes souffrant de dysménorrhée que chez les femmes saines. (Lundstrom, Green et al. 1976) L'élévation de la concentration de $PGF_{2\alpha}$ est beaucoup plus forte que celle de PGE_2, ce qui expliquerait l'activité plus contractile. De plus, une infusion de $PGF_{2\alpha}$ au niveau de l'utérus cause une hypercontractilité du myomètre et provoque des douleurs similaires à la dysménorrhée.(Lundstrom 1977)

Étant donné l'implication des PGs dans la pathophysiologie de la dysménorrhée essentielle, les anti-inflammatoires non stéroïdiens (AINS), tels l'ibuprofène et le naproxen, se révèlent être un traitement de choix. (Hacker, Moore et al. 2004; Proctor and Farquhar 2006) Cette classe de médication est très efficace dans le traitement de la dysménorrhée. Parmi les AINS les plus couramment employées nommons l'ibuprofène, le naproxen, et l'acide méfénamique. Les AINS agissent en inhibant l'activité des COX et par le fait même, la production de PGs. Les inhibiteurs spécifiques de COX-2 (VIOXX, Celebrex), une nouvelle forme de médication plus ciblée récemment mise sur le marché, semblent également avoir des effets bénéfiques chez les femmes souffrant de dysménorrhées. (Connolly 2003)

Les ménorragies

La fréquence des ménorragies est grande dans la population féminine, 10-30 % chez les femmes en âge de se reproduire et jusqu'à 50% chez celles en pré-ménopause.(Sales and Jabbour 2003) Ce n'est pas une pathologie qui menace la vie des gens, mais il n'en demeure pas moins qu'elle cause de l'inconfort pour les patientes et peut engendrer une anémie ferriprive suffisamment sévère pour nécessiter une hystérectomie. Le traitement de ces patientes améliore grandement leur qualité de vie et diminue leur absentéisme au travail.

La majorité des cas de ménorragies surviennent près de la ménarche et en pré-ménopause.(Hacker, Moore et al. 2004) Le flot menstruel normal est en moyenne de 25-35 ml, mais peut varier de 5 à 60 ml. La durée moyenne des menstruations est de 4 à 6 jours. Les ménorragies sont des saignements menstruels excédant la durée normale des règles (> 7 jours) et d'abondance supérieure à 80 ml. Elles peuvent également se présenter uniquement par des règles de durées normales, mais trop abondantes (> 80 ml / cycle).(Jabbour, Kelly et al. 2006)

De façon analogue à la dysménorrhée, diverses étiologies sont à la base des ménorragies. Elles peuvent être causées, entre autres, par des processus néoplasiques bénins ou malins au niveau de l'utérus (myomes, polypes, adénocarcinome), ainsi qu'à des anomalies de l'hémostase ou de la coagulation.(Apgar, Kaufman et al. 2007) Cependant, la majorité des patientes ne souffrent d'aucune autre pathologie. Dans ces situations, de nombreuses études ont identifié l'implication des PGs dans l'apparition de ménorragies.(Sales and Jabbour 2003; Smith, Jabbour et al. 2007) PGE_2 et PGI_2, des PGs à action vasodilatatrice, sont plus abondantes au niveau de l'endomètre des femmes souffrant de ménorragies.(Hagenfeldt 1987; Jabbour, Sales et al. 2006) Chez ces femmes, le ratio de $PGE_2/PGF_{2\alpha}$ s'accroît comparativement aux femmes saines.(Smith, Abel et al. 1981) Il semblerait que ces vasodilatateurs à action locale auraient un impact sur la physiopathologie des ménorragies. De plus, puisque PGE_2 semble être impliqué dans l'angiogenèse au niveau de la couche fonctionnelle de l'endomètre, il a été également suggéré qu'une anomalie de la maturation des vaisseaux sanguins au niveau de l'endomètre pourrait également contribuer au processus.(Jabbour, Sales et al. 2006) En effet, une modification de l'expression de l'angiopoïétine, avec déstabilisation des vaisseaux sanguins, a été observée dans l'endomètre de femmes ménorragiques.(Blumenthal, Taylor et al. 2002) Il semblerait que cette modification soit régulée par PGE_2.(Sales, Katz et al. 2002)

Traitements des ménorragies

Une étude a démontré que chez environ 80% des femmes ayant subi une hystérectomie pour contrôler des ménorragies, l'utérus s'avérait entièrement normal en pathologie.(Clarke, Black et al. 1995) Sous cette optique, un traitement médicamenteux optimal pourrait réduire le nombre de chirurgies de résection utérines ou endométriales. Utilisée en première intention, la médication permet de diminuer les symptômes des ménorragies chez les femmes en âge de se reproduire et qui veulent préserver leurs fonctions reproductrices.

Le stérilet au lévonorgestrel (Mirena) est la médication la plus efficace pour arrêter les saignements excessifs causés par les ménorragies. Le lévonorgestrel, un progestatif synthétique, cause une atrophie de l'endomètre et, par le fait même, diminue le flux menstruel.(Lete, Obispo et al. 2008; Goni, Lacruz et al. 2009) Toutefois, les femmes ne peuvent l'utiliser si elles désirent une grossesse de par son effet contraceptif. Les progestatifs oraux sont, quant à eux, de loin la médication la plus prescrite pour traiter les ménorragies.(Apgar, Kaufman et al. 2007) Leur mode d'action est le même que le stérilet, cependant, leur efficacité demeure moindre pour contrôler les saignements. Il n'en demeure pas moins que les femmes désirant une grossesse ne peuvent utiliser ce type de médication.

Les anti-inflammatoires non stéroïdiens (AINS) sont une médication importante pour les femmes souffrant de ménorragies puisqu'elles inhibent la synthèse des PGs. (Lethaby, Augood et al. 2007) Il a été démontré que l'utilisation d'AINS diminue le flot menstruel.(Cameron, Haining et al. 1990) Les AINS sont utiles chez les femmes désirant une grossesse et ne souffrant pas d'autres pathologies gynécologiques. Ils agiraient principalement sur les cas de ménorragies fonctionnelles en diminuant la production de PGE_2 et de PGI_2 qui agissent comme vasodilatateurs et inhibent l'agrégation plaquettaire. Malgré leur efficacité, les effets secondaires d'une utilisation à long terme des AINS doit être prise en compte, puisque leur inhibition n'est pas spécifique aux PGs impliquées dans les ménorragies, mais comprend la production globale de PGs. Tel qu'il a été

mentionné précédemment, les PGs possèdent diverses fonctions physiologiques essentielles au bon fonctionnement des organes.

Sommaire

La prévalence des désordres menstruels dans la population féminine en santé et en âge de procréer est très élevée. C'est un problème majeur autant pour la qualité de vie de ces femmes que pour l'impact sur la société de par le nombre d'heures d'absentéisme et la baisse d'activité de ces femmes. Les PGs sont impliqués dans la physiopathologie des problèmes menstruels les plus fréquents. Elles s'avèrent donc une cible thérapeutique de premier choix, d'autant plus que leur inhibition ponctuelle n'affecte pas la capacité de reproduction chez les femmes désirant une grossesse.

Les anti-inflammatoires non stéroïdiens

Les AINS sont parmi les médicaments les plus utilisés au monde. Ils sont connus depuis des siècles, tel l'acide salicylique, pour le traitement de la fièvre et de l'inflammation.(Rao and Knaus 2008) Les AINS agissent en inhibant les COX et en empêchant par le fait même la production de PGs.

Les AINS classiques

Les AINS traditionnels (aspirine, naproxen, ibuprofen etc.) sont non-spécifiques et inhibent conjointement les deux formes de COX. Ce sont parmi les anti-inflammatoires les plus utilisés en santé. Cette médication sert au traitement d'une grande variété de pathologies inflammatoires (polyarthrite rhumatoïde, arthrose, tendinites, bursites, etc.) ainsi que comme prophylaxie des complications cardio-vasculaires (aspirine). Les processus inflammatoires induisent principalement l'expression de COX-2. La production de PGs au niveau des tissus amplifie le processus inflammatoire et augmente la perméabilité vasculaire et l'œdème.(Seibert, Zhang et al. 1994) Ce phénomène est présent dans de nombreuses affections courantes et concoure à leur pathophysiologie.

Le revers de la médaille des AINS classiques demeure leurs multiples effets indésirables. Chaque médicament a son profil d'effets secondaires et certains AINS, principalement à faible dose, peuvent être très bien tolérés. Cependant, certaines personnes plus vulnérables, telles les personnes âgées, demeurent plus à risque. Les effets secondaires les plus fréquents des AINS traditionnels sont gastro-intestinaux. Il semblerait que ce soit dû à l'inhibition de la production des PGs protectrices au niveau de la paroi gastrique. Ces PGs sont produites par l'enzyme COX-1.(Allison, Howatson et al. 1992) L'inhibition des PGs de l'estomac favorise la formation de gastrites ainsi que d'ulcères gastriques et duodénaux pouvant engendrer des perforations et des hémorragies. De nombreux autres effets secondaires sont possibles avec l'utilisation des AINS, ce qui limite leur utilisation surtout chez certains patients. Ils peuvent engendrer des bronchospasmes, une insuffisance rénale aiguë, une élévation de la tension artérielle, une aggravation de l'insuffisance cardiaque, des céphalées et des vertiges ainsi qu'une diminution de la fertilité chez la femme. Ainsi, c'est dans l'optique de diminuer le taux d'effets secondaires qu'une nouvelle classe d'AINS a été créée.

Les AINS spécifiques à COX-2

Tel que mentionné, une nouvelle classe d'AINS a récemment été développée. Elle exerce une inhibition spécifique sur l'enzyme COX-2. À l'origine, cette classe de médicaments a été produite afin d'inhiber les PGs produites dans un contexte inflammatoire tout en conservant les PGs produites par COX-1, une enzyme constitutive. L'idée était d'augmenter l'effet anti-inflammatoire de cette médication tout en diminuant les effets secondaires, principalement gastro-intestinaux.(Smith and Song 2002) Celecoxib (Celebrex®), produite par Pfizer, a été la première molécule de cette catégorie. Par la suite, Merck a produit la molécule rofecoxib (Vioxx®). Cependant, peu de temps après leur mise en marché, il s'est avéré que ces médicaments augmentaient le risque d'événements cardio-vasculaires.(Mukherjee, Nissen et al. 2001) Il semblerait que COX-2, malgré son implication dans diverses pathologies, contribuerait également à la physiologie normale de certains systèmes organiques.

De nombreuses études in vitro ont été effectuées afin de déterminer la sélectivité envers COX-2 des AINS présents sur le marché. (Riendeau, Charleson et al. 1997; Pairet and van Ryn 1998) Ils ont été subdivisés selon leur sélectivité envers COX-2 (ratio IC_{50} COX-1 / IC_{50} COX-2). Ce ratio a été obtenu en mesurant la concentration requise des divers AINS pour inhiber 50 % de l'activité enzymatique (IC_{50}) de COX-1 et COX-2.(Rao and Knaus 2008) (Tableau 1)

Tableau 1 : Les principaux AINS présentés en ordre selon leur spécificité pour l'inhibition de COX-2. Les IC_{50} sont également indiqués.

	Cox-1	Cox-2	Ratio Cox-2/Cox-1
AINS	IC_{50} (μM)		
Aspirine	1.2	15.8	13.1
Flurbiprofen	0.1	0.4	4
Ibuprofen	3.3	37	11.4
Indométhacine	0.1	0.9	9
Acide méfénamique	25	2.9	0.12
Naproxen	1.1	36	32.7
Celecoxib (Celebrex*)	1.2	0.83	0.7
Nimesulide	12.5	0.4	0.03
NS-398	28.9	0.04	0.001
Rofecoxib (Vioxx*)	15	0.018	0.0012
Valdecoxib (Bextra*)	150	0.005	0.00003
Ketorolac	0.0014	0.14	100
SC-560	0.009	6.3	700

L'aspirine, un AINS ayant plus une spécificité pour COX-1, mais pouvant inhiber les deux cyclooxygénases dépendamment de la concentration, semble avoir un effet anti-coagulant et cardioprotecteur. C'est pourquoi il est prescrit en prophylaxie des maladies cardio-vasculaires. Tandis que le Celebrex, un AINS de nouvelle génération est plus spécifique à COX-2, et sert principalement à traiter les pathologies inflammatoires. Chaque catégorie d'AINS semble avoir son utilité et sa gamme d'effets secondaires. Pour l'instant, il n'existe pas sur le marché une médication pouvant inhiber spécifiquement les

PG synthases. Cette médication pourrait permettre de cibler plus spécifiquement les PGs mis en cause dans chaque pathologie, tout en conservant l'expression des autres PGs à effet physiologique.

Contribution à la recherche

Les PGs sont cruciales dans la fonction reproductive de la femme et sont reconnues comme étant impliquées dans de nombreuses pathologies de la sphère reproductive : carcinomes, endométriose, dysménorrhées, ménorragies, infertilité, accouchements prématurés.(Sales and Jabbour 2003) Cependant, les mécanismes cellulaires spécifiques impliqués dans la physiopathologie de la majorité de ces maladies ne sont pas entièrement connus. L'objectif principal de nos études est de tenter de déterminer les différentes cascades enzymatiques impliquées dans la biosynthèse des PGs au niveau de l'endomètre humain et leurs implications dans les pathologies du système reproducteur. Ce projet de maîtrise visait la caractérisation de l'expression des différentes enzymes de biosynthèse des prostaglandines, dont certains nouveaux joueurs (AKR1B1), dans des lignées cellulaires immortalisées de l'endomètre humain ainsi que dans des tissus endométriaux au cours du cycle menstruel.

Notre laboratoire a développé des lignées cellulaires endométriales stromales et épithéliales immortalisées à partir de biopsies (Chapitre 2). Ces lignées sont nécessaires afin de faciliter les études in vitro. Au cours de ma maîtrise, la similitude de ces cellules avec les cultures primaires a été déterminée en étudiant le spectre d'expression des différentes enzymes de la cascade des PGs. De nombreuses expériences de stimulation et d'inhibition de l'expression des PGs ont été effectuées sur ces lignées cellulaires et analysées tant au niveau de l'ADN, de l'ARN et des protéines. La réceptivité de nos lignées aux hormones stéroïdiennes a également été analysée in vitro. Cela nous a permis d'établir que nos cultures immortalisées semblaient adéquates pour de futures études ainsi que d'observer l'expression différentielle des principales enzymes de biosynthèse des PGs dans les cellules stromales et épithéliales de l'endomètre.

Ces lignées cellulaires ont par la suite été utilisées pour l'étude de la fonction de l'enzyme AKR1B1, une présumée PGF synthase humaine découverte par notre laboratoire. (Chapitre 3) Cette étude a été principalement menée par une étudiante au

postdoctorat Ewa Zarzewska. Mes travaux ont permis d'analyser l'expression des enzymes AKR1B1, AKR1C3, COX-1 et 2 par RT-PCR quantitatif et par immunohistochimie dans des tissus endométriaux de femmes consultant le gynécologue pour ménorragies. Nous avons pu déterminer la variation d'expression ainsi que la localisation de ces enzymes dans l'endomètre humain au cours du cycle menstruel. COX-1 et COX-2 semblent plus exprimés au cours du cycle sécrétoire et se retrouvent tant au niveau des cellules stromales que épithéliales. AKR1B1 et AKR1C3 sont, quant à eux, présent tout au long du cycle, sans variation significative. Cette étude démontre pour la première fois que l'enzyme AKR1B1 est une PGF synthase fonctionnelle dans l'endomètre humain et a permis de certifier l'utilité de nos lignées cellulaires récemment immortalisées dans des études in vitro sur l'expression des principaux joueurs de la cascade de biosynthèse des PGs.

CHAPITRE 2

IMMORTALISATION DE LIGNÉES CELLULAIRES

Decidualization and maintenance of a functional prostaglandin system in human endometrial cell lines following transformation with SV40 large T antigen

Pierre Chapdelaine[1], Jihong Kang[1], **Sofia Boucher- Kovalik[1]**, Nicolas Caron[2], Jacques P.Tremblay[2] and Michel A. Fortier[1]

[1]Unité de Recherche en Ontogénie et Reproduction, Centre de Recherche du CHUL, CHUQ,
Faculté de Médecine, Université Laval, Sainte-Foy, Québec, Canada

[2]Unité de Recherche en Génétique Humaine, Centre de Recherche du CHUL, CHUQ,
Faculté de Médecine, Université Laval, Sainte-Foy, Québec, Canada

Correspondence should be addressed to:
Michel A. Fortier, Ph.D.,
Unité de recherche en Ontogénie et Reproduction
Centre de Recherche du CHUL,
2705, boul Laurier,
Sainte-Foy, Québec,
G1V 4G2, Canada
E-mail : Michel.A.Fortier@crchul.ulaval.ca

Résumé

Les prostaglandines (PGs) sont des régulateurs clés de la fonction reproductive et sont également associées à plusieurs désordres gynécologiques. Les cultures cellulaires de l'endomètre apparaissent comme la meilleure approche pour étudier les mécanismes moléculaires associés à l'action des PGs chez l'humain. Notre objectif était d'établir des lignées cellulaires stables par infection avec un rétrovirus recombinant défectif contenant la protéine SV40 Large T Antigen (TAG). Deux clones de chaque type cellulaire ont été sélectionnés de par leurs rapidités de prolifération, leurs taux de production de PGs et leurs réponses à l'interleukine-1β (IL-1β). Les cellules stromales (HIESC) et épithéliales (HIEEC) résultantes retiennent les caractéristiques morphologiques et phénotypiques de nature épithéliale (cytokératine) et stromale (vimentine) pour minimalement 40 passages. Les lignées cellulaires sélectionnées expriment les récepteurs de l'oestradiol (ERα) et de la progestérone (PR) tant au niveau de l'ARNm que de la protéine. De plus, par contraste avec les lignées cellulaires préexistantes ISHIKAWA et KLE, la production de $PGF_{2\alpha}$ et de PGE_2 par les HIESC et les HIEEC est induite suivant un traitement avec l'IL-1β. Ces lignées expriment également les principales enzymes de biosynthèse nécessaires à la production de PGs : la phospholipase cytosolique A_2 ($cPLA_2$), les cyclooxygenases 1 (COX-1) et 2 (COX-2), la PGF synthase (AKR1B1), la prostaglandine E synthase microsomiale ($mPGES_1$) et les récepteurs correspondants EP2, EP3, EP4 et FP. L'inhibiteur sélectif de COX-2, NS398, inhibe complètement l'élévation de la production des PGs induite par l'IL-1β chez les deux lignées cellulaires, tandis que la dexamethasone exerce une inhibition plus importante sur les cellules HIESC que sur les HIEEC. Cette caractéristique peut s'expliquer par l'expression constitutionnelle plus grande de COX-1 dans les HIEEC. Les lignées épithéliales et stromales de l'endomètre que nous avons produites apparaissent idéales pour l'étude de la contribution des PGs dans la régulation de la fonction de l'endomètre humain ainsi que dans ses pathologies associées.

Summary

Prostaglandins (PGs) are key regulators of reproductive function and associated pathologies. We have established stable endometrial stromal and epithelial cell lines with SV40 Large T Antigen as a model to study prostaglandin action in the human endometrium. Two clones for each cell type were selected for rapid growth, PG production and response to Interleukin-1β (IL-1β). The resulting stromal (HIESC) and epithelial (HIEEC) cells retain their characteristics for at least 40 population doublings. The selected clones express progesterone (PR) and oestrogen receptor-ϙ (ER-α) at both mRNA and protein levels. By contrast with the existing known human endometrial cell lines ISHIKAWA and KLE, HIESC and HIEEC increase their production of $PGF_{2\alpha}$ and PGE_2 and COX-2 protein expression in response to IL-1β. The latter cells also express the main biosynthetic enzymes involved in PG production, cytosolic phospholipase A_2, cyclooxygenases 1 (COX-1) and 2 (COX-2), PGF synthase and PGE synthase and the corresponding EP2, EP3, EP4 and FP receptors. The selective COX-2 inhibitor NS-398 inhibits completely the increased production of PGs induced by IL-1β in both cell types, whereas dexamethasone exerts a stronger inhibition in HIESC than in HIEEC. The latter observation may be related to the higher expression of COX-1 measured in HIEEC. Based on the present characterisation and previous determination of corresponding primary cell cultures, HIESC and HIEEC appear appropriate to study the contribution of prostaglandins in the regulation human endometrium function and associated pathologies.

Introduction

The human endometrium undergoes cyclical changes necessary to develop uterine receptivity in response to the ovarian steroid hormones, oestradiol and progesterone. In addition to sex steroids, prostaglandins (PGs) appear as primary regulators of female reproductive function. PGs are involved in the regulation of ovulation, implantation, menstruation and several other aspects of endometrial function (for a review, see Sales *et al.,* 2003). PGs are also involved in pathologies of the endometrium including carcinomas, menorrhagia, dysmenorrhoea and endometriosis (for a review, see Sales *et al.,* 2003). The biosynthetic pathway leading to PGs production begins by the activation of phospholipase A_2 (PLA_2) or C (PLC) liberating arachidonic acid (AA) from membrane phospholipids (for a review, see Kudo *et al.,* 2002). AA is then converted to PGG_2 and PGH_2 by PG synthases, suicide enzymes also known as cyclooxygenases for which there are two isotypes, COX-1 and COX-2 (for a review, see Simmons *et al.,* 2004). PGH_2 is the common precursor for all PGs produced through terminal enzymes like PGE synthase for PGE_2 and PGF synthase for $PGF_{2\alpha}$ (for a review, see Helliwell *et al.,* 2004). The importance of PGs in reproduction was evidenced by exhibition of multiple reproductive failures in mice where the prostaglandin synthase 2 (*COX-2)* (Lim *et al,* 1997), as well as *FP* (Sugimoto *et al,* 1997) *and EP2* (Kennedy *et al.*, 1999) genes were disrupted. We have described the expression and regulation of PG biosynthetic enzymes and receptors in the bovine (Parent *et al.*, 2005; Arosh *et al.*, 2004; Madore *et al.*, 2003; Parent *et al.*, 2003) and human endometrium (Kang *et al.*, 2004).

In the endometrium, PGE_2 and $PGF_{2\alpha}$ are the main prostanoids produced (Sales *et al.*, 2003; Smith *et al.*, 1988) and their autocrine and paracrine actions are mediated by G protein coupled EP and FP receptors. Altered production of PGE_2 and $PGF_{2\alpha}$ has been found in uterine fluids in association with gynaecological disorders like menorrhagia, dysmenorrhoea and endometriosis (Sales *et al.*, 2003). Cytokines such as IL-1β are involved in endometrial-embryonic crosstalk at the time of implantation (Simon *et al.*, 1997) and stimulate the production of prostaglandins E_2 (Tamura *et al.*, 2002) and

$F_{2\alpha}$ (Huang *et al.*, 1998). The increased production of PG in human decidual stromal cells is associated with an increased number of cells positive for COX-2 in vivo (Ishara *et al.*, 1995) and increased COX-2 protein expression in vitro (Tamura *et al.*, 2002). We have studied the effect of IL-1β on MCP1 a chemokine involved in uterine receptivity and endometriosis, and PGs production using human endometrial stromal and epithelial cells in primary culture (Kang *et al.*, 2004). That study highlighted the difficulties and limitations of biopsies and primary cultures for extensive molecular and cellular studies in the human endometrium. The endometrium is composed mainly of epithelial, stromal and infiltrating haematopoietic cells. Isolated stromal cells in primary culture can go through 10 to 20 population doublings (PD) (Rinehart *et al.*, 1991) with minimal morphological changes whereas epithelial cells do not grow as well in primary culture, and exhibit limited ability for subculture (Matthews *et al.*, 1992). Clearly, the difficulty to obtain endometrial biopsies of sufficient size to prepare large populations of cells in primary culture and the inherent variability between such cultures make it difficult to develop a working model for the study of cellular and molecular regulation of prostaglandin or related systems.

It has been reported that stable endometrial stromal (Krikun *et al.*, 2004) and glandular epithelial (Kyo *et al.*, 2003) cells could be generated using telomerase to overcome senescence observed particularly in epithelial cells in primary culture (Matthews *et al.*, 1992). Other reports have described the use of SV 40 large T antigen (TAG) to extend the lifespan of human endometrial cells in culture (Rinehart *et al.*, 1991; Merviel *et al.*, 1995) but there is only limited availability of normal epithelial cell lines to compare side by side with stromal cells. It has been reported that immortalization of epithelial or other cells with telomerase (hTERT) alone, could yield cell lines with limited lifespan (Barbier *et al.*, 2005; Kyo *et al.*, 2003). Therefore, we have elected to use TAG as proposed by Merviel et al (1995).

In the present study, we have developed a strategy based on the use of a defective retrovirus SSR69 (Westerman *et al.*, 1996) coding for the TAG protein to extend the lifespan of human endometrial stromal and epithelial cells. We have then examined if the

resulting HIESC and HIEEC cell lines exhibited the phenotypic characteristics of the primary cultures. Finally, we have characterized PG biosynthesis and its modulation by IL-1β.

Materials and methods

PG production and IL-1β response in established endometrial cell lines

Ishikawa cells, an oestrogen responsive endometrial cancer line, and KLE cells (ATCC# CRL1622) were grown to confluence and treated or not with IL-1β (1ng/ml) for 24 h. The culture medium was recovered for measurement of PG by ELISA as described below. The cells were processed as described for cells lines below and expression of PG biosynthetic enzymes, COX-1 and COX-2 was evaluated by Western Blot. Response to IL-1β treatment was evaluated by measurement of alteration of the above parameters.

Isolation and culture of primary endometrial cells

Endometrial biopsies were taken from women with regular menstrual cycles undergoing gynaecological investigation for benign conditions. Informed consent for donation of anonymous endometrial samples was obtained before tissue collection. Biopsies were classified first according to the stated last menstrual period (LMP) and confirmed by histological examination by the hospital pathologist using standard criteria (Noyes *et al.*, 1950). The pathologist also confirmed the absence of neoplasia and endometritis. The research protocol was approved by Centre Hospitalier Universitaire de Québec (CHUQ) ethics committee on human research. The tissue samples were placed in sterile Hank's balanced salt solution containing 100 IU/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B at 4°C and transported to the laboratory immediately. Primary human endometrial stromal and epithelial cells were prepared as we described recently (Kang *et al.*, 2004). Cells were cultured in Dulbecco's modified Eagle's (DMEM/F12) (Sigma, ON, Canada) supplemented with 10% foetal bovine serum (Wisent Inc, QUE, Canada), 10 μg/ml insulin (Invitrogen, ON, Canada), 5 μg/ml transferrin, 50 IU penicillin-streptomycin (Wisent Inc, QUE, Canada).

The primary cultures used to generate purified stromal and epithelial cells in the present study were prepared from biopsies taken respectively from a 40 years old woman on day 17 of her menstrual cycle and a 37 years old woman on day 12. The purity of the cultures was assessed morphologically under a phase contrast microscope and immunohistochemically according to the expression of vimentin and cytokeratin as described previously (Kang *et al.*, 2004).

Retrovirus infection and establishment of SV40 T-Antigen Cell Lines

The retroviral vector SSR69 containing SV40 Large T antigen (TAG) and a gene resistant to hygromycin (Figure 1) was transfected with effectene (Qiagen, ON, Canada) in the mouse amphotropic packaging cell line PA 317 (Westerman *et al.*, 1996). The resulting colonies resistant to hygromycin (800 μg/ml, Roche, ON, Canada) were cultured and the supernatants containing amphotropic viruses were collected and used to infect separately, purified stromal and epithelial cells in primary culture. Endometrial cells grown in 6-well plates were infected in presence of polybrene (8 μg/ml, Sigma, ON, Canada) for 6 hours and the procedure was repeated 24 hours later. The day following last infection, the cells were trypsinised and seeded in 10 mm dishes in presence of hygromycin (400 μg/ml). The cultures were grown for 7 to 8 days until the TAG infected cells formed colonies while control non infected cells died in presence of the antibiotic. A total of 17 clones (17 colonies) for stromal cells and 50 for epithelial cells were picked by clonal selection (cloning o-ring) and grown in 24-well plates until confluency and then seeded in T-25 Flasks. The population doubling (PD) for TAG clones was calculated as follows: n(PD) = log (final cells count) – log (inoculation cell count) / 0.301. Since colonies are produced from a single cell, we calculated that at confluency, the initial PD in T-25 flask was 19.2. The TAG clones were maintained in complete culture medium unless specified differently. The clones were then selected according to their growth rate, production of prostaglandins and response to IL1-β. Four cell lines, two of stromal origin (HIESC, clones 2 and 16) and two of epithelial origin (HIEEC clones 18 and 22) were selected and characterized thoroughly in the present study.

Characterization of HIESC and HIEEC

Stromal and epithelial TAG clones were grown on Lab Tek 4-chamber slides (Nalge Nunc International, Rochester, NY, USA), fixed and processed as previously described (Kang *et al.*, 2004). Monoclonal antibodies against vimentin (Sigma, ON, Canada) or cytokeratin (recognizing 4, 5, 6, 8, 13 and 18 isoforms, Sigma, ON, Canada) were used as first antibodies and a fluorescent IgG antibody (Alexa Fluor® 488 goat anti-mouse Molecular Probes inc, Eugene, OR, USA) as a second antibody to characterize endometrial cells by immunofluorescence. The fluorescence was visualized using a Zeiss Axiovert 100-Inverted microscope (Zeiss, Germany) and images were captured and integrated using the Northern Exposure program (Empix Imaging Inc., ON, Canada)

Integration of viral SV40 large T antigen DNA in the genome of TAG clones was analysed by polymerase chain reaction (PCR) directly from genomic DNA prepared as described elsewhere (Chapdelaine *et al.*, 2001). The primer pairs were 5'-CAGGTTAAGATCAAGGTCTT-3'(forward) and 5'-GTGTCGTCCATCACAGTT-3'(reverse) corresponding respectively to the psi sequence and hygromycin sequence carried by the retrovirus SSR69. Briefly, PCR was performed with 250 ng of genomic DNA and cycle program was the same as described for RT-PCR in the section below. Dystrophin exon 50 amplified with the specific primers 5'-AGGAAGTTAGAAGATCT GAGCTCT-3'(forward) and 5'-AGGCTCCAATAGTGGTCAGTCCA-3' (reverse) were used as internal standard. Protein TAG detection was analysed by immunofluorescence directly on the cells with antibody specific to the protein (SV40 T Ag (ab-2) (Oncogen Research Products, San Diego, CA) and the molecular weight (90 kDa) was determined by Western blot. The karyotype was evaluated for two representative cell lines HIESC-2 (PD 47) and HIEEC-22 (PD 42) using the G-banding procedure to identify the chromosomes (Mitelman, 1995). Sex steroid responses were evaluated either by treatment (24 hours) with 17 β-Estradiol 100 nM or up to 10 days with medroxyprogesterone acetate (MPA) and assessment of ER and PR protein and ^{3}H-Thymidine incorporation.

Detection of steroid and prostaglandin receptors by RT-PCR

The expression of estrogen (ERα and ERβ) and progesterone (PR) receptor mRNAs was analyzed by RT-PCR. The primer pairs used were 5'-GCAGACAGGGAGCTGGTTCA-3'(forward) and 5'-AGAGATGCTCCATGCCTTTG-3'(reverse) for ERα (524bp) (accession no X03635), 5'-TCACATCTGTATGCGGAACC-3' (forward) and 5'-CGTAACACTTCCGAAGTCGG-3' (reverse) for ERβ (345 bp) (acc. no BC024181), 5'-AACATGTCAGTGGGCAGATG-3' (forward) and 5'-GCAGCAATAACTTCAGACATC-3' (reverse) for PR (438 bp) (acc. no NM_000926). Total RNA was extracted from cells grown in T-75 Flasks and 1 ml of TRIZOL reagent (Invitrogen, ON, Canada) was added to isolate RNA according to the manufacturer's instructions. The first strand of cDNA was synthesized at 42°C (50 minutes) from 2.5 μg of RNA using superscript II reverse transcriptase RNAse H-(Invitrogen, ON, Canada) as described by the manufacturer with random hexamer primers (250ng) (Amersham Biosciences, Piscataway, NJ, USA). Usually, 2 μl aliquots of the first-strand cDNA were amplified with the primers (20 pmol) previously described and polymerase chain reaction (PCR) was performed using Taq DNA polymerase (Amersham Biosciences, Piscataway, NJ, USA) for 35 cycles: 95°C (1 min) as denaturing temperature, 55°C (1min) as annealing temperature, 72°C (1 min 30 sec) as elongating temperature and a final step at 72°C 10 minutes. The PCR products were analysed on 1.5 % agarose gels stained with ethidium bromide (Sigma, ON, Canada). For the PG receptors, the primer pairs used were 5'-TTGTCGGTATCATGGTGGTG-3' (forward) and 5'-ATGTACACCCAAGGGTCCAG-3' (reverse) for EP1 (159 bp) (acc. no NM_000955), 5'-GTCTGCTCCTTGCCTTTCAC-3' (forward) and 5'-CGACAACAGAGGACTGAACG-3' (reverse) for EP2 (175 bp) (acc. no NM_000956), 5'-AGCTTATGGGGATCATGTGC-3'(forward) and 5'ACAGCAGGTAAACCCAAGG A-3' (reverse) for EP3 (197 bp)(acc.no NM_000957), 5'-CTGGTGGTGCTCATCTGCT -3' (sense) and 5'-TATCCAGGGGTCTAGGATGG-3' (reverse) for EP4 (149 bp) (acc. no NM_000958), 5'-TCTGGTTACAATGGCCAAC A-3' (forward) and 5'-ATGCACTC CACAGCATTGAC-3'(reverse) for FP (183 bp) (acc.no NM_000959). Human β-actin was used as the positive control and the primers used were 5'-ACGGCTGCTTCCAGCT

CCTCC-3'(forward) and 5'-AGCCATGCCAATCTCATCTTGT-3'(reverse) for a PCR product of 524 bp (acc. no BC013835). RNA preparation and cDNA transcription procedures were the same as described above for steroid receptors.

In vitro decidualisation of HIESC and prolactin assay

Induction of decidualisation was performed as described for primary stromal cell cultures by Brosens *et al, (1999).* Briefly, confluent HIESC were grown in six-well plates in RPMI without phenol-red (Gibco-BRL (Invitrogen), Mississauga, Ontario, Canada) containing 2% dextran-coated charcoal-treated FBS (DCC-FBS), and 1% penicillin-streptomycin in presence or absence of 0.5mM 8-bromo-cAMP (Sigma, St-Louis, USA) and 10^{-6}M medroxyprogesterone acetate (MPA, Pharmacia Canada, Mississauga, Ontario, Canada). As a control, the epithelial cell line HIEEC was submitted to the same treatment. The culture medium was changed every other day and supernatants were collected at days 1, 6 and 10 of treatment for PRL assay. PRL measurement was done by ELISA using the ADVIA Centaur® immunoassay system (Bayer HealthCare LLC, USA). The lower detection limit was 0.8 µg/l. The intra- and inter-assay coefficients of variation (N=144) were 1.9-4.4% and 2-5.3% respectively.

Propagation and treatment of TAG clones

The culture and propagation of HIESC 2 and 16 and HIEEC 18 and 22 was done in RPMI 1640 (Invitrogen, ON, Canada) without phenol red, containing 10% foetal bovine serum (FBS) (Wisent inc., QUE, Canada) and 50 UI penicillin-streptomycin (Wisent Inc., QUE, Canada). Briefly, cells were grown at 37°C under a humidified atmosphere of 5% CO_2 and 95% air. The cells at PD 40 were first cultured in T-Flasks (75 cm^2) until early confluency and then plated at a 1/2 split ratio in 24-well plates. After 24 hours, the culture medium was replaced with fresh medium supplemented with 10 % FBS depleted of steroids by dextran-charcoal (DC) extraction and cells grown for 4 days with a medium change every other day. After this period the culture medium was replaced for RPMI without serum and cells were stimulated or not with IL1-β (1ng/ml) for 24 hours. Cells were stimulated in presence or absence of a specific COX-2 inhibitor

NS-398 (1 μM) or an other known inhibitor of PG biosynthesis, dexamethasone (from 0 to 1 μM) At the end of the treatment period, the culture medium was recovered and stored at -20°C until protein (Chapdelaine et al., 2001) and PGs analysis.

Enzyme immunoassays (EIA) of prostaglandins

PGE_2 and $PGF_{2\alpha}$ were assayed by EIA using acethylcholinesterase-linked PG tracers (Cayman, Ann Arbor, Michigan, USA) as described previously (Asselin *et al.*, 1996). Rabbit anti-PGE_2 (kindly provided by Dr TG Kennedy) (Evans *et al.*, 1982) and sheep anti-$PGF_{2\alpha}$ (Bio-Quant, Ann Arbor, MI) were used as selective antibodies. The inter and intra assay coefficient of variation (n=12) were 16% and 10% respectively.

Measurement of protein expression by Western blot analysis

HIESC and HIEEC were washed twice in phosphate buffered saline (PBS 1X), and proteins were extracted and measured directly in SDS-PAGE loading buffer as previously described (Chapdelaine *et al.*, 2001). Aliquots of 10 μg protein were loaded in each well and separated on 10 % or 12.5% SDS-PAGE, and then transferred onto nitrocellulose membranes (Bio-Rad, ON, Canada). The membranes were blocked overnight at 4°C in 5% (w/v) non fat dried milk (BLOTTO) in PBS containing 0.05% Tween-20 (PBS-T) and then incubated with first antibodies for one hour at room temperature. The first antibody dilutions were as follows: anti ERα (sc-538, Santa-Cruz biotechnology CA, USA) dilution 1/1000, anti PR (sc-543, Santa-Cruz biotechnology CA, USA)) dilution 1/500, anti Phospho-$cPLA_2$ (Ser505, Santa-Cruz biotechnology CA, USA)) dilution 1/1000 , anti-mPGES-1 (Cayman) dilution 1/500, anti-COX-2 (Merck 243) and anti-COX-1 (Merck 241) dilution 1/3000 were kindly provided by Dr S Kargman (Merck, QUE, Canada), anti-AKR1B1 a polyclonal antiserum raised from recombinant protein in our laboratory (Madore *et al.,* 2002) was used at a 1/2000 dilution, anti-β-Actin (Sigma ON, Canada) was used as an internal standard at a 1/5000 dilution. After incubation of the membranes with first antibodies in PBS-T containing 5% BLOTTO, three washings were performed at room temperature in PBS-T (10 minutes each). Then, the membranes were incubated for 1 hour at room temperature with the

second antibodies, goat anti-rabbit or anti-mouse conjugated with horseradish peroxidase (Jackson Immunoresearch Laboratories, Cedarlane, ON, Canada) diluted 1/10000 in PBS-T with 5% BLOTTO followed by three washes (10 minutes) in PBS-T. The chemiluminescence signal was analysed with an autoradiography film (2 minutes exposure) after treatment of the membrane with Renaissance reagent (NEN, Perkins Elmer, Boston, MA, USA).

Statistical analysis

Data are presented as the mean ± SEM. Statistical analysis was performed using ANOVA followed by Fischer's Protected LSD, Duncan New Multiple Range and Student-Newman-Keuls multiple comparison tests (Super ANOVA; Abacus Concepts, Berkeley, CA, USA).

Results

PG production and IL-1β response in established endometrial cell lines

The production of PGE_2 and $PGF_{2\alpha}$ by Ishikawa and KLE cells is presented in Table 1, and compared with that of primary stromal and epithelial cell cultures. The production of the two PGs is much higher in primary endometrial cells than in the two cell lines both in absence and presence of IL-1β (1ng/ml). Western analysis shows that low production of PGs in Ishikawa and KLE occurs in spite of high expression of COX-2 and significant expression of COX-1 (Figure 2). However, in agreement with the measured production of PGs, IL-1β does not stimulate COX-1 or COX-2 protein expression. Low production of PGs by comparison with primary endometrial cell cultures (Table 1), and absence of effect on COX-1 and COX-2 expression (Figure 2), show that these cell lines do not represent a working model to study the PG system of endometrial cells.

Phenotypic characteristics of endometrial stromal and epithelial TAG clones.

Endometrial stromal and epithelial clones were expanded and maintained in culture for at least 40 population doublings (PD) with minimal morphological changes. While HIESC and HIEEC could be maintained in continuous culture for over 40 PD without apparent senescence, non transformed stromal cells exhibited a change in shape at passage 15 whereas epithelial cells could not be passaged. Figure 3 illustrates the morphological characteristics of primary stromal (A) and epithelial (B) cells compared with the corresponding stromal (C, E) and epithelial (D, F) TAG clones. Among the selected clones, HIESC-2 and HIEEC-22 were fast growing lines reaching confluency after 3-4 days whereas HIESC-16 and HIEEC-18 were slightly slower, taking 5 days to reach confluency following a standard 1:3 split ratio.

Immunofluorescence was used to confirm intermediate filament protein expression in HIESC and HIEEC at PD 40. In figure 3, panels C and D illustrate that vimentin is expressed in both stromal HIESC-2 and epithelial HIEEC-22 clones whereas panels E and F illustrate that cytokeratin is present only in HIEEC-22. These observations on TAG transformed cells correlate well with other (Matthews *et al.*, 1992; Nisolle *et al.*, 1995) and our own characterization of primary endometrial cells (Kang *et al.*, 2004). The same patterns of expression were found for the other stromal and epithelial clones tested (results not shown).

Karyotype analysis of the representative HIESC-2 and HIEEC-22 cell lines is illustrated in table 2. The stromal HIESC-2 cell line has an intact 46 XX karyotype whereas the epithelial HIEEC-22 has two deletions, 1 X and 1 #5 chromosome.

Presence of SV40 Large T Antigen in infected endometrial cell lines

The expression of SV 40 Large T antigen was evaluated by immunofluorescence with an antibody specific to TAG protein. Figure 4A shows that both HIESC-2 and HIEEC-22 at PD 40 express a strong nuclear fluorescence in all cells. The same was observed in all HIESC and HIEEC clones tested (results not shown). Moreover, viral

integration was confirmed by amplification of a specific DNA fragment (expected size of 750 bp) from genomic DNA extracted from TAG clones and analysed by RT-PCR (figure 4B). Similarly, a positive signal was found at the expected molecular weight (90 kDa) following Western analysis of the same cell lines with a specific antibody against SV40 Large T antigen protein (figure 4C).

Expression of steroid receptors in endometrial cell lines

The expression of steroid receptors was evaluated by RT-PCR (figure 5). Oestrogen receptor (ERα) and progesterone receptor (PR) mRNAs were present in every cell line tested (figure 5A) whereas ERβ was expressed minimally and not detected in every cell line (result not shown). At the protein level, both isoforms of PR, A (~80-90 kDa) and B (~110 kDa) are recognised by the sc-543 antibody and could be detected in both epithelial and stromal cell lines by Western blot analysis (figure 5B and C). ERα is also present in the two cell types. In HIESC, following treatment with MPA and cAMP to induce decidualisation, there is a time dependent decrease in the expression of PR and to a lesser extent ERα. Treatment of the cells with 17β-Estradiol did not influence expression of ER or PR in HIEEC (figure 5C) or HIESC (not shown) and did not stimulate cell proliferation as assessed by ^{3}H-Thymidine incorporation (result not shown).

Expression of prostanoid receptors in endometrial cell lines

The expression of prostanoid receptor mRNAs was evaluated by RT-PCR. Every cell lines including stromal cells in primary culture used as a reference, expressed EP2, EP3, EP4 and FP whereas EP1 (not shown) was not found (Figure 6). These observations could be reproduced for the transformed cell lines at PD 40 and PD 50 without any change in the pattern of expression.

Decidualisation of HIESC and prolactin production

Treatment of confluent HIESC with 8-bromo-cAMP (0.5mM) in combination with MPA (1μM) induced a transformation of morphology from spindle-shaped to ovoid

cells with abundant cytoplasm characteristic of decidual cells (left, figure 7A). By comparison, the same treatment did not alter significantly the morphology of HIEEC-22 (right figure 7A). Prolactin (PRL) levels remained at or below the lower detection limit in epithelial (HIEEC-22) and non treated stromal (HIESC-2) cell lines. Treatment stimulated PRL to its maximum on day 6 (4.3±1 µg/l) and PRL decreased slowly to reach 2.6±0.6 µg/l on day 10(Figure 7B) in HIESC-2 but had no effect in HIEEC-22.

Correlation between Prostaglandin E_2 and $F_{2\alpha}$ production and expression of biosynthetic enzymes in HIESC and HIEEC .

We have examined the effect of IL-1β (1 ng/ml) on PGE_2 and $PGF_{2\alpha}$ production in HIESC and HIEEC cells. Figure 8 illustrates that PGE_2 (A,C) and $PGF_{2\alpha}$ (B,D) levels were highly stimulated by IL-1β in HIESC-2 (A, B) and HIEEC-22 (C, D) and that inhibition of COX-2 by the selective inhibitor NS-398 blocked the stimulation. The levels of PGE_2 produced in presence of IL-1β were always higher (5 to 10 times) than those of $PGF_{2\alpha}$. Similar data were obtained for HIESC-16 and HIEEC-18. (results not shown)

The expression of the 110 kDa, phosphorylated cytosolic PLA_2 ($cPLA_2$) (Kudo *et al.*, 2002), 72 kDa COX-1, 70-72 kDa COX-2, 36 kDa endometrial PGF synthase (AKR1B1) (Madore *et al.*, 2002), 16 kDa microsomal PGE synthase (mPGES1) and 45 kDa β-Actin used as internal standard was evaluated by Western analysis. Analysis was performed on whole cell proteins extracted from confluent cultures of HIESC and HIEEC treated or not with IL-1β. First, as illustrated in figure 8 E and F, treatment of the cells with IL-1β for 24 hours stimulated the expression of all the enzymes studied. The most striking increase was observed in HIESC-2 (E) for all but COX-1 enzymes. Interestingly, COX-1 protein was barely detected in HIESC-2 while a good basal expression level, stimulated by IL-1β was observed in HIEEC-22 (Figure 8F). As illustrated in G and H, all the PG biosynthetic enzymes (phospholipase A_2, COX-2, AKR1B1 and mPGES-1) were significantly increased by IL-1β treatment in both HIESC and HIEEC. COX-2 expression was not affected by treatment with NS-398 (results not shown).

Effect of Dexamethasone on PG production and COX2 expression in HIESC and HIEEC treated with IL-1β

We have evaluated the effect of dexamethasone (DEX), a synthetic anti-inflammatory steroid analogous to cortisol, on the accumulation of PGE_2 and $PGF_{2\alpha}$ in presence of IL-1β (figure 9). We have observed a dose dependent decrease of PGE_2 and $PGF_{2\alpha}$ levels in both HIESC-2 (A) and HIEEC-22 (B) in response to DEX. However, the inhibition at 10^{-6}M was more complete in HIESC-2 (>90%) than in HIEEC-22 (<50%). We have also studied the effect at the level of COX-2, the rate limiting enzyme in PG production. As shown in Figure 9 C and E and 9 D and F dexamethasone used at 10^{-7} and 10^{-6}M decreased significantly COX-2 protein levels in HIESC-2 but not HIEEC-22. We observed an almost complete inhibition of COX-2 in HIESC-2 whereas there was a minimal effect in HIEEC-22. These observations correlate well with the level of inhibition of PG production shown in panels A and B and suggest that DEX may act at both protein expression and activity levels. Moreover, it suggests that epithelial and stromal cells have distinct sites of regulation of PG biosynthesis.

Discussion

Prostaglandins are primary regulators of female reproductive function. This has been highlighted by disruption of COX-1 and COX-2 genes in the mouse where it induced parturition and multiple reproductive failures respectively (Lim *et al.*, 1997; Morham *et al.*, 1997); Langenbach *et al.*, 1997). In animals, endometrial PGs regulate uterine contractility, fertilization, implantation and luteal function (Arosh *et al.*, 2004); Doualla-Bell *et al.*, 1998). In humans, PGs appear to regulate menstruation and abnormal production of PGE_2 and $PGF_{2\alpha}$ is associated with menstrual disorders like menorrhagia and dysmenorrhoea (Sales *et al.*, 2003) Regulation of PG production with nonsteroidal anti-inflammatory drugs (NSAIDs), functional inhibitors of COXs, is commonly used to help relieve the symptoms of menstrual disorders.

The cellular and molecular mechanisms associated with human disorders, especially their dynamic regulation, are best studied in vitro using cell cultures. Primary

cultures appear as the ideal in vitro model because of the relative proximity with the corresponding cells in situ. Some studies have used endometrial cell cultures where stromal and epithelial cells were grown together (Arnold *et al.*, 2001) but most studies were done with endometrial stromal cells alone (Tamura *et al.*, 2002; Hang *et al.*, 1998). Indeed, fibroblastic cells are typically more viable and easier to grow in culture whereas epithelial cells such as those found in endometrial glands exhibit limited viability and barely survive two weeks in culture (Matthews *et al.*, 1992; Kyo *et al.*, 2003). Finally, the biological material is limited and difficult to obtain and primary cultures are plagued by limited viability and inherent variability. Therefore, the aim of the present work was to develop stable cell lines derived from normal human endometrium in order to establish a model to study prostaglandin biosynthesis and receptor systems in this tissue.

Some groups have succeeded in expanding human endometrial cells in culture using SV40 Large T antigen (SV40 TAG) (Rinehart *et al.*, 1991; Merviel *et al.*, 1995) or human telomerase reverse transcriptase (hTERT) (Krikun *et al.*, 2004; Kyo *et al.*, 2003) and the resulting cell lines expressed some characteristics of the original phenotype. One group has developed immortalized human endometrial epithelial and stromal cells by liposome transfection with SV40 TAG but only a few clones were analysed and prostaglandin analysis was not reported (Merviel *et al.*, 1995). Unfortunately none of the previously described cell lines were characterized in relation with the PG system, neither available commercially. We present here the characteristics of endometrial stromal and epithelial cell lines derived following infection with a defective retrovirus containing the protein SV40 Large T antigen. The introduction of the viral oncoprotein within the genome of the endometrial cells extends their life span until at least PD60. The SV 40 Large T antigen is known to interact with tumour suppressor protein p53 preventing apoptosis and Rb proteins leading to a proliferative state (Klawitz *et al.*, 2001). SV40 Large T antigen (TAG) introduced in the cells may modify normal structure and functional characteristics of the original cells (Ray *et al.*, 1995).

The karyotype of HIESC-2 is normal at 46XX and overall morphological and functional properties appear identical to primary stromal cells. The HIEEC-22 epithelial

line has a slightly altered karyotype where 1 X and 1 #5 chromosome were deleted. Nevertheless, a copy of each of the 23 chromosomes is represented in the karyotype. Although less than ideal, chromosome alteration is common in cell lines and in the present case it did not appear to affect the PG system. In the case of chromosome X, deletion of one copy is expected to exert minor impact because it is reported that 1 of these is normally inactivated in somatic cells (Chow *et al.*, 2005). This chromosome deletion likely results from the transformation of primary cells with TAG. However, HIEEC-22 cells have maintained a PG system comparable with that of primary cultures especially with respect to their response to IL-1β.

In the present study, the stromal and epithelial clones were used between PD 40 and 50 and cells were still expressing the nuclear SV40 TAG (detected by immunofluorescence). Under the phase contrast microscope, stromal cells exhibit a characteristic elongated fibroblast shape whereas epithelial cells are polygonal. The original phenotypic characteristics of epithelial cells expressing cytokeratin and vimentin and stromal cells expressing vimentin only are also conserved. Interestingly, the pattern of cytokeratin expression with variable immunofluorescence intensity among the HIEEC cells was also observed in endometrial glandular cells immortalized with telomerase (Kyo *et al.*, 2003). We have found that the mRNAs of main prostanoid receptors EP_2, EP_3, EP_4 and FP are present in both TAG clones and stromal cells in primary culture. HIESC and HIEEC cells express steroid receptors ERα and PR at both the mRNA and protein levels, but we failed to detect a measurable response in terms of proliferation, steroid receptor expression or PG production following treatment with 17β-estradiol. We cannot tell at the present time if ERα is non functional, partially functional or constitutively activated because we did not conduct a thorough study on every oestrogen responsive genes. Alteration in ER function in the presence of SV40 Large T Antigen was reported to be consequent to interaction with the CREB binding protein (CBP) an essential component of the oestrogen-receptor complex (Barbier *et al.*,2005; Ali *et al.*,2001). Functional PR are present in stromal HIESC-2 because treatment with cAMP and MPA, a synthetic progesterone, induced decidualisation, evidenced by altered morphology and increased PRL secretion. As it was reported elsewhere (Brosens *et al.*, 1999; Tessier *et al.*, 2000),

we have observed a PR and ERα protein decrease during decidualisation of HIESC. The same treatment was ineffective in the epithelial HIEEC-22 line. In vitro decidualisation was previously reported following immortalization of endometrial stromal cells with SV40 Large T antigen but the prostaglandin system was not studied (Brosens *et al.*, 1996).

Our results on the regulation of PG production are particularly interesting. First it is noticeable that both HIESC and HIEEC are highly responsive to IL-1β as was found by us and others in human endometrial cells in primary culture (Kang *et al.*, 2004; Tamura *et al.*, 2002; Huang *et al.*, 1998). Similarly, the inhibition of PG production with NS-398 and Dexamethasone is comparable to what was observed for endometrial stromal cells in primary culture (Huang *et al.*, 1998). Together, these observations suggest that the original phenotype of stromal cells with regard to PG production was maintained in HIESC. Interestingly, the HIEEC clones appear to produce higher levels of both PGE_2 and $PGF_{2\alpha}$ especially in presence of IL-1β. This increased production of PG in epithelial clones is correlated with higher basal expression of all biosynthetic enzymes. Also, although NS-398 achieves a similar level of inhibition in HIESC and HIEEC clones, DEX is much more efficient in stromal HIESC (>90%) than in epithelial HIEEC (40-50%). As mentioned earlier, the human endometrial epithelial cells have not been studied extensively, but it has been reported that glucocorticoid receptors are primarily found in the stromal compartment of the human endometrium (Bamberger *et al.*, 2001). This can explain the difference between HIEEC and HIESC following treatment with DEX. The high levels of PGE_2 and $PGF_{2\alpha}$ produced by HIESC and HIEEC and its stimulation by IL-1β is comparable to what was observed in primary endometrial cells (Kang et al 2004). By contrast, established endometrial cell lines such as KLE and Ishikawa, in spite of being oestrogen responsive (Holika *et al.*, 1986) and expressing high basal levels of COX-2 protein do not possess a functional PG system comparable to primary endometrial cells. These observations confirm that any cell line should be validated prior to being used for the study of specific, physiologically relevant systems.

Analysis of the biosynthetic enzymes involved in $PGF_{2\alpha}$ and PGE_2 production in HIESC and HIEEC reveals that $cPLA_2$, COX-2 and the two terminal synthases mPGES-1 and AKR1B1 are expressed and increased in response to IL-1β. Also, COX-1 is barely detectable in HIESC cells by comparison with HIEEC cells thus highlighting an additional distinction between the two types of cells. The last observation correlates well with the pattern of expression of COX-1 and COX-2 in epithelial and stromal cells studied by immunohistochemistry in the human endometrium (Stavreus-Evers *et al.*, 2005). In the present study, COX-2 protein level was not affected by NS-398, demonstrating that the inhibition of PGs production induced by this inhibitor was on enzyme activity rather than transcription or translation of the gene product. By contrast, dexamethasone induced a dose dependent reduction in COX-2 protein, but only in HIESC stimulated with IL-1β .

In summary, we have successfully established human endometrial stromal (HIEEC) and epithelial (HIEEC) cells by infection with the SV40 retrovirus Large T antigen directly on purified stromal or epithelial cells in primary culture. The cell lines produced maintain many morphological and functional characteristics of endometrial cells and tissue. A major advantage of these cell lines is first to document the properties of endometrial epithelial cells which have only been marginally studied due to limited viability in primary culture (Matthews *et al.*, 1992) and difficulty in establishing an immortalized phenotype (Kyo *et al.*, 2003). In addition, the availability of the two endometrial cell types allowed highlighting functional differences in the pattern of expression of PG biosynthetic enzymes and their inhibition by glucocorticoids. HIESC and HIEEC globally appear as very good models for in vitro study of the PG synthesis and receptor systems, but as with any other cell culture model, expression of the endometrial cell properties to be tested must be checked and data generated utilized with caution until validation in vivo. HIESC and HIEEC cells appear ready for the study of the major transcriptional factors responsible for the expression of PGs biosynthetic and catabolic enzymes, the PG receptors and the PGT transporter that we have identified in the human endometrium (Kang *et al.*, 2005). Ultimately, this will contribute to the

understanding of the contribution and the management of the PG system in menstrual disorders.

Figure legends

Figure 1. Schematic representation of the SSR69 retrovirus containing SV 40 Large T Antigen.

Recombinant viruses containing the SV40 TAG and hygromycin resistance genes were packaged in the amphotropic cell line PA-317. The virus released by the cells was used to infect separated human endometrial stromal and epithelial cells in primary culture.

Figure 2. Western Blot analysis of COX-1 and COX–2 in Ishikawa and KLE. Ishikawa and KLE cells were grown to confluency and treated or not with increasing IL-1β concentrations (0.01, 0.1 and 1 ng/ml). COX-1 (lower panel) and COX–2 (upper panel) expression was evaluated by Western analysis of cell protein extracted as described in material and methods.

Figure 3. Morphological and phenotypic characteristics of endometrial cells in culture.

Endometrial stromal (A) and epithelial B) were isolated and grown to confluency before infection with the SV 40 Large T Antigen described in figure 1. The resulting stromal HIESC-2 (C, E) and epithelial HIEEC-22 (D, F) shown at PD 40, expressed morphological characteristics comparable with the original primary cultures (A, B). The expression of the cytoskeleton filaments vimentin and cytokeratin was studied by immunofluorescence as described in materials and methods. Vimentin was expressed by both HIESC (C) and HIEEC (D) but cytokeratin (E, F) was expressed in HIEEC (F) only. Clones 2 and 22 shown, other clones tested showed same properties Magnification 100X.

Figure 4. Integration of the recombinant retroviral TAG within the genome of HIESC and HIEEC.

TAG expression was analysed by immunofluorescence (A) in HIESC and HIEEC, clones 2 and 22 shown respectively, magnification 100X. Panel B represents the PCR analysis where viral integration results in an amplified product at 750 bp. HIESC clones 2 and 16 and HIEEC clones 18 and 22 are represented. Genomic DNA extracted from stromal cells (SC) in primary culture or from retroviral plasmid (P) were used as negative and positive controls respectively. Amplification of dystrophin (exon 50) giving a PCR product of 100 bp was performed as an internal standard. Panel C represents the analysis of TAG protein expression by Western analysis. A band at the expected MW of 90 kDa can be seen in HIESC (clones 2 and 16) and HIEEC (clones 18 and 22) but not in non infected SC.

Figure 5. Expression of steroid receptors in HIESC and HIEEC.

The expression of steroid receptors ERα and PR mRNAs was analysed by RT-PCR (**A**). The corresponding bands were found at the expected MW for all clones and primary stromal cells (SC). ERα and PR (isoforms a and b) proteins were evaluated by Western analysis. In HIESC-2, all three forms are expressed and down-regulated during the decidualisation process induced by MPA and cAMP (**B**). In HIEEC cells, all steroid receptors tested are expressed but not influenced by treatment with 17-β Oestradiol (**C**). In panels **B** and **C** arrows on the left of gel show the molecular weight (MW) markers and on the right, the apparent MW of PR isoforms a and b. Data represent analysis of protein extracts from two independent experiments.

Figure 6. Expression of prostaglandin receptors in HIESC and HIEEC.

Expression of prostanoid receptors EP2, EP3, EP4 and FP mRNAs were analysed by RT-PCR. The corresponding bands were found at the expected MW for all clones and primary stromal cells (SC).

Figure 7. Induction of decidualisation following treatment with c-AMP and MPA.

Treatment with c-AMP (0.5mM) and MPA (10^{-6} M) for 10 days induced a morphological change in HIESC–2 from a spindle to an ovoid shape (A, left) but had no effect in HIEEC-22 (A, right), magnification 100X. Induction of PRL-secretion by the same treatment in HIESC-2 and HIEEC-22 is shown in B, at different days of culture (1, 6 and 10). A significant increase was found at days 6 and 10 with maximal levels observed at day 6 ($p< 0.05$). The data represent the means ± SEM from three independent experiments run in duplicate.

Figure 8. Correlation between PG production and expression of biosynthetic enzymes.

HIESC-2 (A, C, E) and HIEEC-22 (B, D, F) were grown to confluency and treated with IL-1β (1ng/ml) in presence or absence of the COX-2 inhibitor NS-398 (1μM). PGE_2 (A, C) and $PGF_{2\alpha}$ (B, D) production shown for HIESC (A, B) and HIEEC (C, D) at basal (C) and IL-1β stimulated levels in absence (IL) and presence (IL+NS-398) of NS-398. A to D, results are the mean ± SEM of 3 experiments run in quadruplicate. Western analysis of the expression of the different biosynthetic enzymes shown for HIESC-2 (E) and HIEEC-22 (F) after treatment in absence or presence of IL-1β (results are representative of one out of 2 experiments where lanes 1, 2 and 3, 4 are duplicates). Panels G (HIESC-2) and H (HIEEC-22) represent the quantitation of the autoradiography signal (Integrated density value (IDV) for the Western blot analysis shown in panels E and F and are the mean ± SEM of 2 experiments run in duplicate.

Figure 9. Effects of Dexamethasone on PGs production and COX-2 expression.

PGE_2 and $PGF_{2\alpha}$ production (ng/ml) was measured in the culture medium of HIESC-2 (A) and HIEEC-22 (B) treated with IL-1β (1ng/ml) in absence or presence of dexamethasone at the indicated concentrations. The results represent the mean ± SEM

of 2 experiments run in duplicate. Western analysis of COX-2 expression in HIESC-2 (C) and HIEEC-22 (D) treated with IL-1β (1ng/ml) in absence or presence of DEX at the indicated concentrations (1 representative out of 2 experiments). E,G represent respectively the relative integrated density value (IDV) for Cox-2 and β-actin from panel C and a replicate experiment (HIESC-2) whereas F, H represent the same for panel F and a replicate experiment. Values are the mean ± SEM of 2 experiments run in duplicate.

TABLE 1

COMPARISON OF PROSTAGLANDIN PRODUCTION BETWEEN PRIMARY ENDOMETRIAL AND ENDOMETRIAL ADENOCARCINOMA CELLS

	PRIMARY ENDOMETRIAL CELLS				**CELL LINES**			
	Stromal		**Epithelial**		**Ishikawa**		**KLE**	
	PGE_2	$PGF_{2\alpha}$	PGE_2	$PGF_{2\alpha}$	PGE_2	$PGF_{2\alpha}$	PGE_2	$PGF_{2\alpha}$
Control	550±150	330±80	560±80	250±50	<35	<35	<35	<35
IL-1β (1ng/ml)	3100±70	3600±110	1100±250	1090±30	<35	100	<35	<35

PGs were measured in triplicate wells from 3 separate experiments (n=3). PGE_2 and $PGF_{2\alpha}$ levels are expressed as mean ±SE pg/ml of culture medium from confluent cultures following treatment or not with IL-1β for 24 hours.

TABLE 2

KARYOTYPE ANALYSIS OF TAG CELLS

Cells		Karyotype
HIESC-2	46, XX	
HIEEC-22	45, X	-X, -5, + mar

References

Ali SH, Decaprio JA (2001) Cellular transformation by SV40 large T antigen: interaction with host proteins. Semin Cancer Biol 11: 15-22

Arosh JA, Banu SK, Chapdelaine P, Madore E, Sirois J, Fortier MA (2004) Prostaglandin biosynthesis, transport, and signaling in corpus luteum: a basis for autoregulation of luteal function. Endocrinology 145(5):2551-60

Arosh JA, Banu SK, Chapdelaine P, Fortier MA (2004) Temporal and tissue-specific expression of prostaglandin receptors EP2, EP3, EP4, FP, and cyclooxygenases 1 and 2 in uterus and foetal membranes during bovine pregnancy. Endocrinology 145(1):407-17

Arnold JT, Kaufman DG, Seppala M, Lessey BA (2001) Endometrial stromal cells regulate epithelial cell growth in vitro: a new co-culture model. Hum Reprod 16(5):836-45

Asselin E, Goff AK, Bergeron H and Fortier MA (1996) Influence of sex steroids on the production of prostaglandins F2 alpha and E2 and response to oxytocin in cultured epithelial and stromal cells of the bovine endometrium. Biol Reprod 54: 371-379

Bamberger AM, Milde-Langosch K, Loning T, Bamberger CM (2001) The glucocorticoid receptor is specifically expressed in the stromal compartment of the human endometrium. J Clin Endocrinol Metab. 86(10):5071-4

Barbier CS, Becker KA, Troester MA and Kaufman DG (2005) Expression of exogenous human telomerase in cultures of endometrial cells does not alter their hormone responsiveness. Biol Reprod 73:106-114

Brosens JJ, Hayashi N and White JO (1999) Progesterone receptor regulates decidual prolactin expression in differentiating human endometrial stromal cells. Endocrinology 140: 4809-4820

Brosens JJ, Takeda S, Acevedo CH, Lewis MP, Kirby PL, Symes EK, Krausz T, Purohit A, Gellersen B and White JO (1996) Human endometrials fibroblasts immortalized by Simian Virus 40 Large T Antigen differentiate in response to a decidualisation stimulus. Endocrinology 137: 2225-2231

Chapdelaine P, Delahaye S, Gauthier E, Tremblay RR, Dube JY (1993) A one-hour procedure for the preparation of genomic DNA from frozen tissues. Biotechniques 14(2):163-4

Chapdelaine P, Vignola K and Fortier MA (2001) Protein estimation directly from SDS-PAGE loading buffer of lysed cells or tissue homogenates for standardization of samples

from cell lysates or tissue homogenates before western blot analysis. Biotechniques 31(3) 478-482

Chow JC, Yen Z, Ziesche SM, Brown CJ (2005) Silencing of the mammalian X chromosome. Annu Rev Genomics Hum Genet. 6:69-92

Doualla-Bell F, Guay JM, Bourgoin S, Fortier MA (1998) Prostaglandin G/H synthase (PGHS)-2 expression in bovine myometrium: influence of steroid hormones and PGHS inhibitors. Biol Reprod. 59(6):1433-8

Evans CA, Kennedy TG and Challis JRG: Gestational changes in prostanoid concentration in intrauterine tissues and foetal fluids from pregnant sheep and the relation to prostanoid output. Biol. Reprod 1982, 27: 1-11

Helliwell RJ, Adams LF, Mitchell MD (2004) Prostaglandin synthases: recent developments and a novel hypothesis. Prostaglandins Leukot Essent Fatty Acids 70:101-13

Holinka CF, Hata H, Kuramoto H, Gurpide E. (1986) Responses to estradiol in human endometrial adenocarcinoma cell line (Ishikawa). J Steroid Biochem. 24 (1):85-9.

Huang JC, Liu DY, Yadollahi S, Wu KK, Dawood MY (1998) Interleukin-1 beta induces cyclooxygenase-2 gene expression in cultured endometrial stromal cells. J Clin Endocrinol Metab 83(2):538-41

Ishihara O, Matsuoka K, Kinoshita K, Sullivan MH, Elder MG (1995) Interleukin-1 beta-stimulated PGE2 production from early first trimester human decidual cells is inhibited by dexamethasone and progesterone. Prostaglandins 49(1):15-26

Kang J, Akoum A, Chapdelaine P, Laberge P, Poubelle PE, Fortier MA (2004) Independent regulation of prostaglandins and monocyte chemoattractant protein-1 by interleukin-1beta and hCG in human endometrial cells. Hum Reprod 19(11):2465-2473

Kang J, Chapdelaine P, Parent J, Madore E, Laberge PY, Fortier MA (2005) Expression of Human Prostaglandin Transporter in the Human Endometrium across the Menstrual Cycle. J Clin Endocrinol Metab 90 (4): 2308-13

Kennedy CR, Zhang Y, Brandon S, Guan Y, Coffee K, Funk CD, Magnuson MA JA, Breyer MD, Breyer RM (1999) Salt-sensitive hypertension and reduced fertility in mice lacking the prostaglandin EP2 receptor. Nat Med 5(2):217-20

Klawitz I, Preuss U, Scheidtmann KH (2001) Interaction of SV40 large T antigen with components of the nucleo/cytoskeleton. Int J Oncol 19(6):1325-32

Krikun G, Mor G, Alvero A, Guller S, Schatz F, Sapi E, Rahman M, Caze R, Qumsiyeh M, Lockwood CJ (2004) A novel immortalized human endometrial stromal cell line with normal progestational response. Endocrinology 145(5):2291-6

Kudo I, Murakami M (2002) Phospholipase A2 enzymes. Prostaglandins Other Lipid Mediat Aug 68-69:3-58

Kyo S, Nakamura M, Kiyono T, Maida Y, Kanaya T, Tanaka M, Yatabe N, Inoue M (2003) Successful immortalization of endometrial glandular cells with normal structural and functional characteristics. Am J Pathol 163(6):2259-69

Langenbach R, Morham SG, Tiano HF, Loftin CD, Ghanayem BI, Chulada PC, Mahler, JF, Davis BJ, Lee CA (1997) Disruption of the mouse cyclooxygenase 1 gene. Characteristics of the mutant and areas of future study. Adv Exp Med Biol. 407:87-92

Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, Trzaskos JM, Dey SK (1997) Multiple female reproductive failures in cyclooxygenase 2-deficient mice. Cell 91(2):197-208

Madore E, Harvey N, Parent J, Chapdelaine P, Arosh JA, Fortier MA (2003) An aldose reductase with 20 alpha-hydroxysteroid dehydrogenase activity is most likely the enzyme responsible for the production of prostaglandin f2 alpha in the bovine endometrium. J Biol Chem 278(13):11205-12

Madore E, Parent J, Laberge P and Fortier MA: Modulation of enzymes involved in the production of endometrial prostaglandins in vivo and in vitro. Annual Meeting of the endocrine Society, San Francisco, CA, USA, 2002, June 18-22, Endocrinology, #P2-403

Matthews CJ, Redfern CP, Hirst BH, Thomas EJ (1992) Characterization of human purified epithelial and stromal cells from endometrium and endometriosis in tissue culture. Fertil Steril 57(5):990-7

Mitelman F (1995) An international system for human cytogenetic nomenclature. Karger ed. Memphis, Tennessee, USA

Merviel P, Degeorges A, Salat-Baroux J, Calvo F (1995) Normal human endometrial cells in culture: characterization and immortalization of epithelial and stromal cells by SV 40 large T antigen. Biol Cell 84(3):187-93

Morham SG, Langenbach R, Mahler J, Smithies (1997) Characterization of prostaglandin H synthase 2 deficient mice and implications for mechanisms of NSAID action. Adv Exp Med Biol 407:131-8

Nisolle M, Casanas-Roux F, Donnez J (1995) Coexpression of cytokeratin and vimentin in eutopic endometrium and endometriosis throughout the menstrual cycle: evaluation by a computerized method. Fertil Steril 64(1):69-75

Noyes RW, Hertig AT, Rock J (1950) Dating the endometrial biopsy. Fertil Steril 1:3-25

Parent J, Fortier MA (2005) Expression and Contribution of Three Different Isoforms of Prostaglandin E Synthase in the Bovine Endometrium. Biol Reprod. 73(1): 36-44

Parent J, Villeneuve C, Fortier MA (2003) Evaluation of the contribution of cyclooxygenase 1 and cyclooxygenase 2 to the production of PGE2 and PGF2 alpha in epithelial cells from bovine endometrium. Reproduction 126(4):539-47

Ray AF (1995) Simian virus large T antigen induces chromosome damage that precedes and coincides with complete neoplastic transformation. DNA Tumor Virus. Edited by G Barbanti-Bridano, M Bendinelli, H Frieman, New York, Plenum, pp 15-26

Rinehart CA, Haskill JS, Morris JS, Butler TD, Kaufman DG (1991) Extended life span of human endometrial stromal cells transfected with cloned origin-defective, temperature-sensitive simian virus 40. J Virol 65(3):1458-65

Sales KJ, Jabbour HN (2003) Cyclooxygenase enzymes and prostaglandins in reproductive tract physiology and pathology. Prostaglandins & other Lipid Mediators 71: 97-117

Sales KJ, Jabbour HN (2003) Cyclooxygenase enzymes and prostaglandins in pathology of the endometrium. Reproduction 126(5):559-67

Simmons DL, Botting RM, Hla T (2004) Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. Pharmacol Rev 56(3):387-437

Simon C, Mercader A, Gimeno MJ, Pellicer A (1997) The interleukin-1 system and human implantation. Am J Reprod Immunol 37(1):64-72

Smith SK, Kelly RW (1988) The release of PGF2 alpha and PGE2 from separated cells of human endometrium and decidua. Prostaglandins Leukot Essent Fatty Acids 33(2):91-6

Stavreus-Evers A, Koraen L, Scott JE, Zhang P, Westlund P (2005) Distribution of cyclooxygenase-1, cyclooxygenase-2, and cytosolic phospholipase in the luteal phase human endometrium and ovary. Fertil Steril 83(1):156-62

Sugimoto Y, Yamasaki A, Segi E, Tsuboi K, Aze Y, Nishimura T, Oida H, Yoshida N, Tanaka T, Katsuyama M, Hasumoto K, Murata T, Hirata M, Ushikubi F, Negishi M (1997) Failure of parturition in mice lacking the prostaglandin F receptor. Science 277(5326):681-3

Tamura M, Sebastian S, Yang S, Gurates B, Fang Z, Bulun SE (2002) Interleukin-1beta elevates cyclooxygenase-2 protein level and enzyme activity via increasing its mRNA

stability in human endometrial stromal cells: an effect mediated by extracellularly regulated kinases 1 and 2. J Clin Endocrinol Metab 87(7):3263-73

Tessier C, Deb S, Prigent-Tessier A, Ferguson-Gottschall S, Gibori GB, Shiu RPC, Gibori G (2000) Estrogen Receptors alpha and beta in rat decidua cells : Cell-specific Expression and Differential Regulation by Steroid hormones and Prolactin. Endocrinology 141: 3842-3851

Westerman KA, Leboulch P (1996) Reversible immortalization of mammalian cells mediated by retroviral transfer and site-specific recombination. Proc Natl Acad Sci USA 20;93(17):8971-6

Figure 1

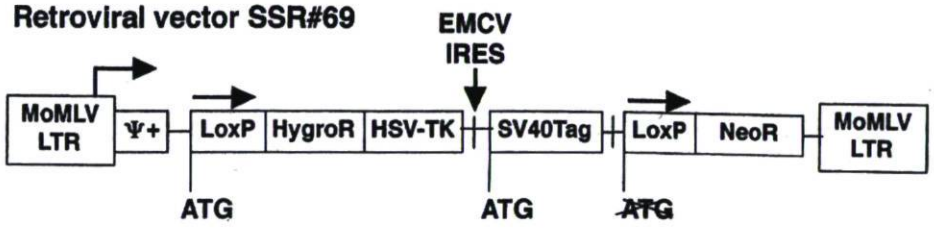

Retroviral vector SSR#69
EMCV
IRES
MoMLV
LTR
Ψ+
LoxP
HygroR
HSV-TK
SV40Tag
LoxP
NeoR
MoMLV
LTR
ATG
ATG
ATG

Figure 2

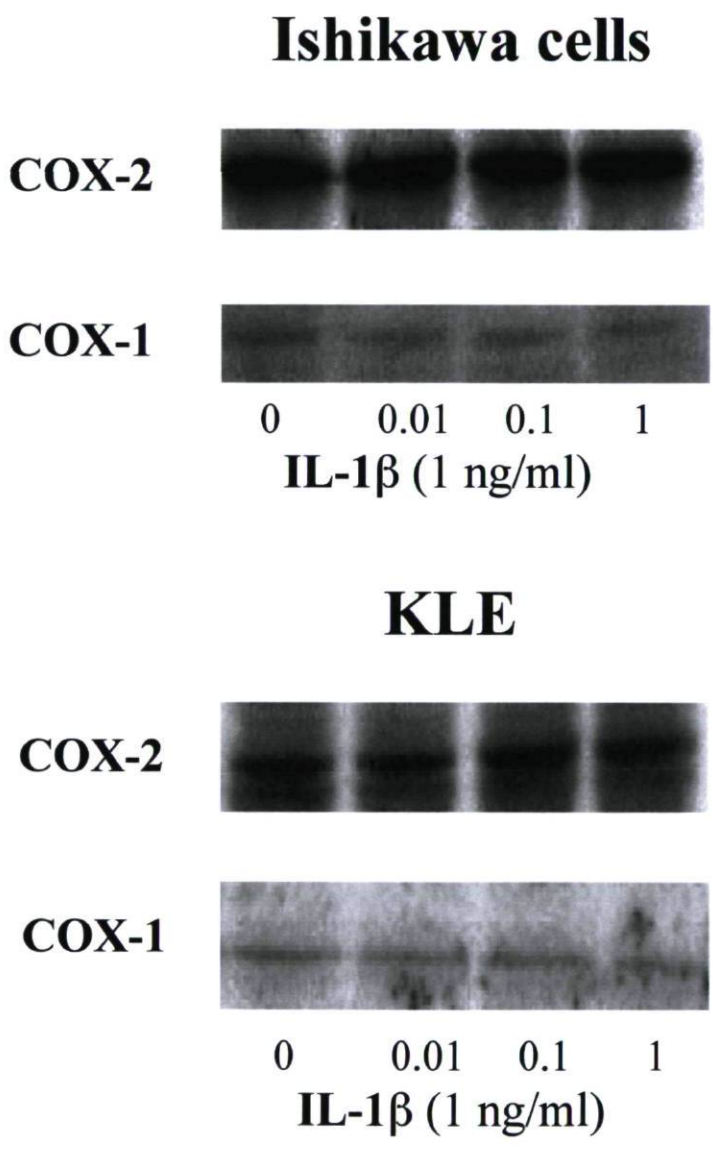

Ishikawa cells
COX-2
COX-1
0
0.01
0.1
1
IL-1β (1 ng/ml)
KLE
COX-2
COX-1
0
0.01
0.1
1
IL-1β (1 ng/ml)

Figure 3

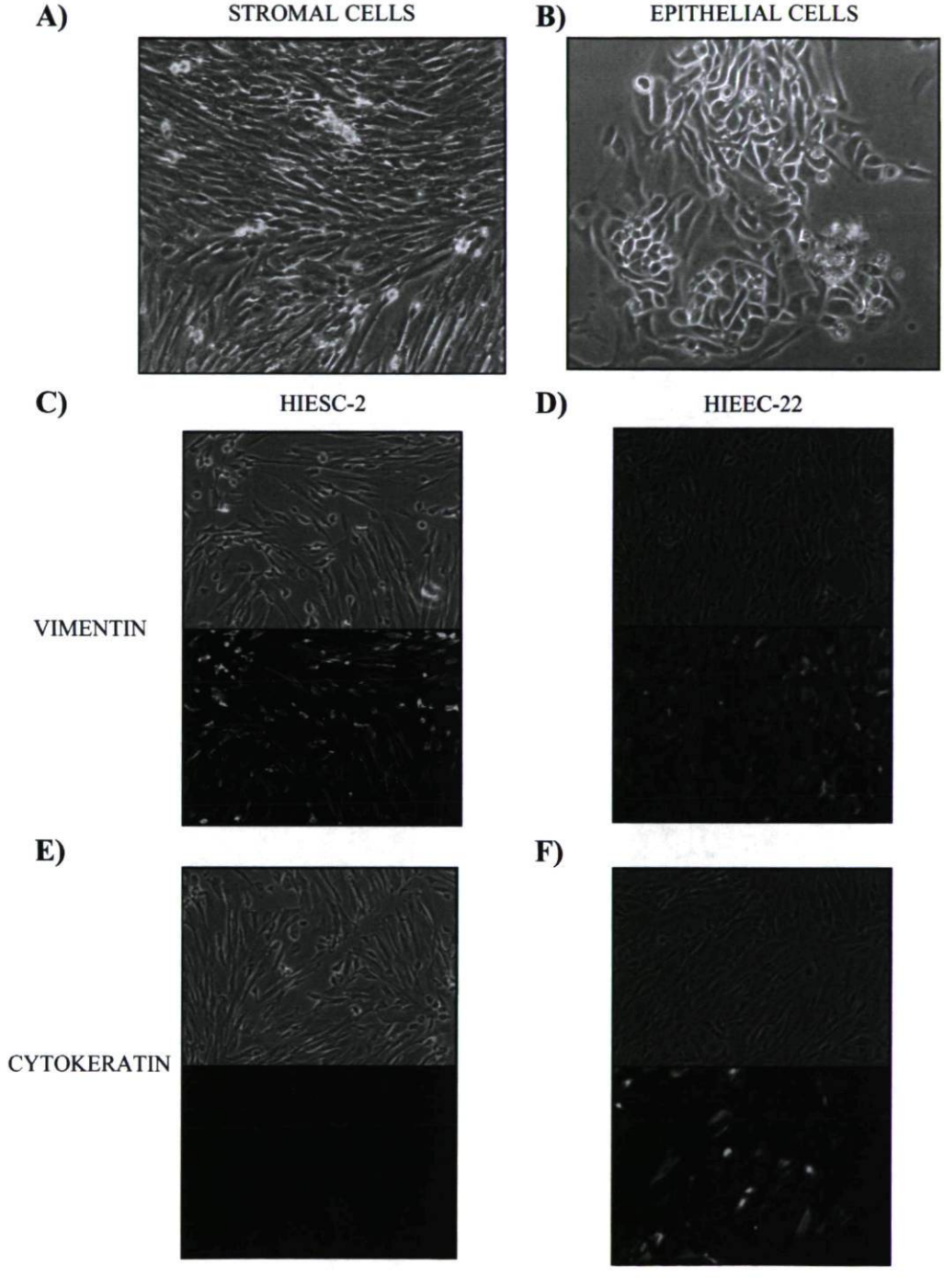
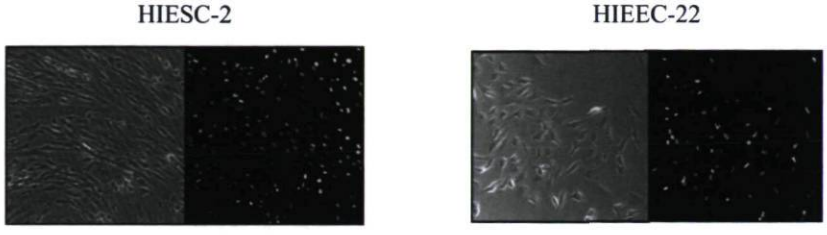
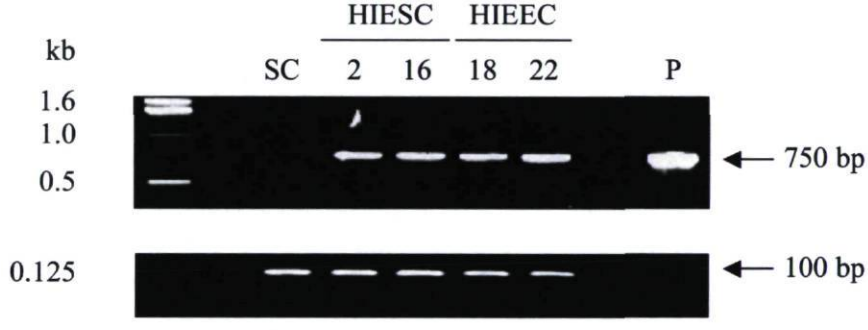

A)
STROMAL CELLS
B)
EPITHELIAL CELLS
C)
HIESC-2
D)
HIEEC-22
VIMENTIN
E)
F)
CYTOKERATIN

Figure 4

A SV 40 Large T antigen (immunofluorescence)



B VIRAL INTEGRATION (PCR)



C SV40 Large T Antigen (WESTERN BLOT)

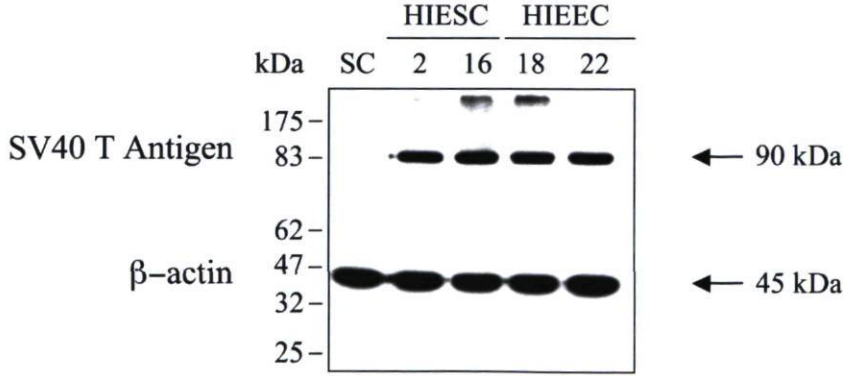
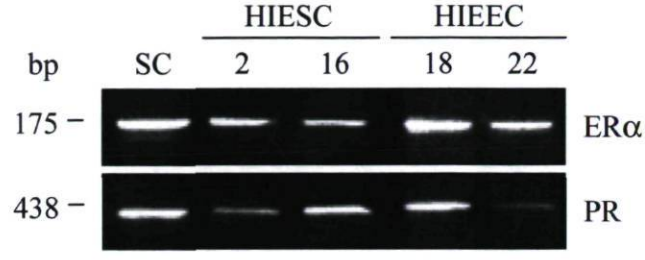
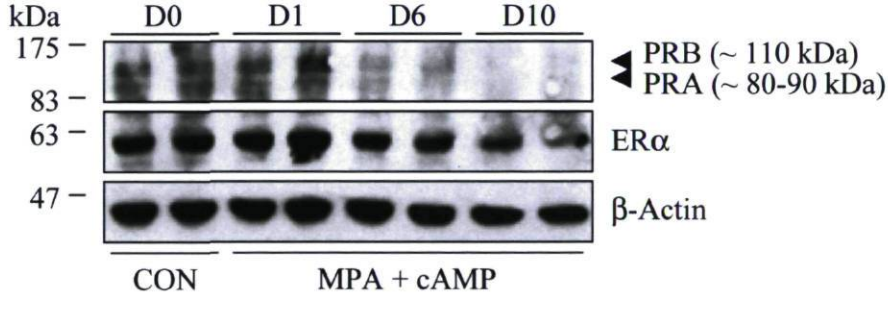


Figure 5

A **STEROID RECEPTORS (RT-PCR)**



B **HIESC-2 DECIDUALISATION (WESTERN BLOT)**



C **HIEEC-22 (WESTERN BLOT)**

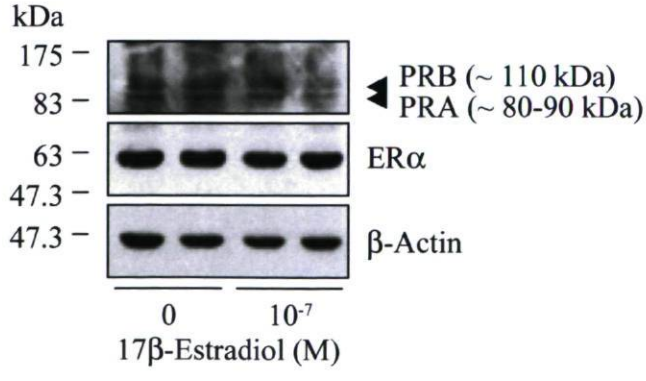


Figure 6

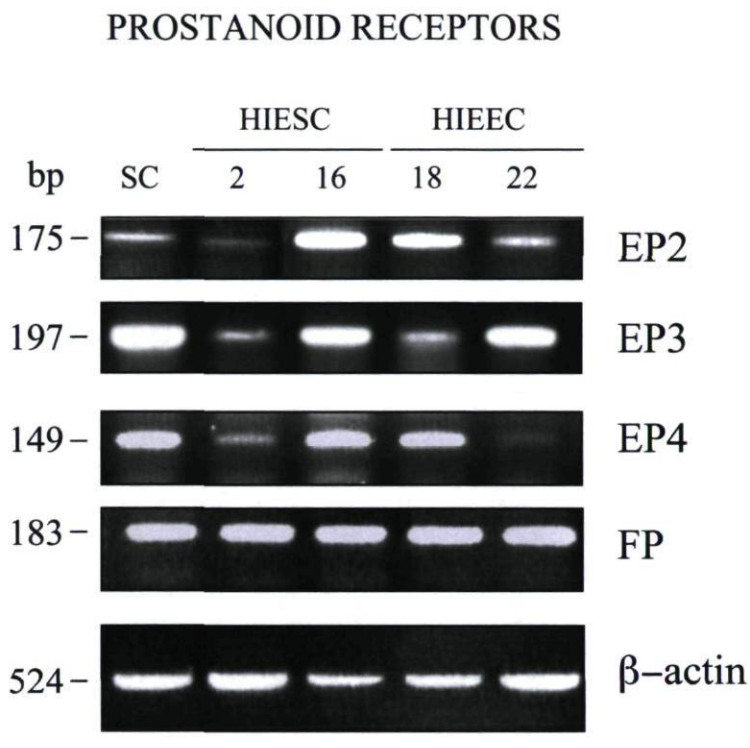

PROSTANOID RECEPTORS
HIESC
HIEEC
bp
SC
2
16
18
22
175 –
197 –
149 –
183 –
524 –
EP2
EP3
EP4
FP
β–actin

Figure 7

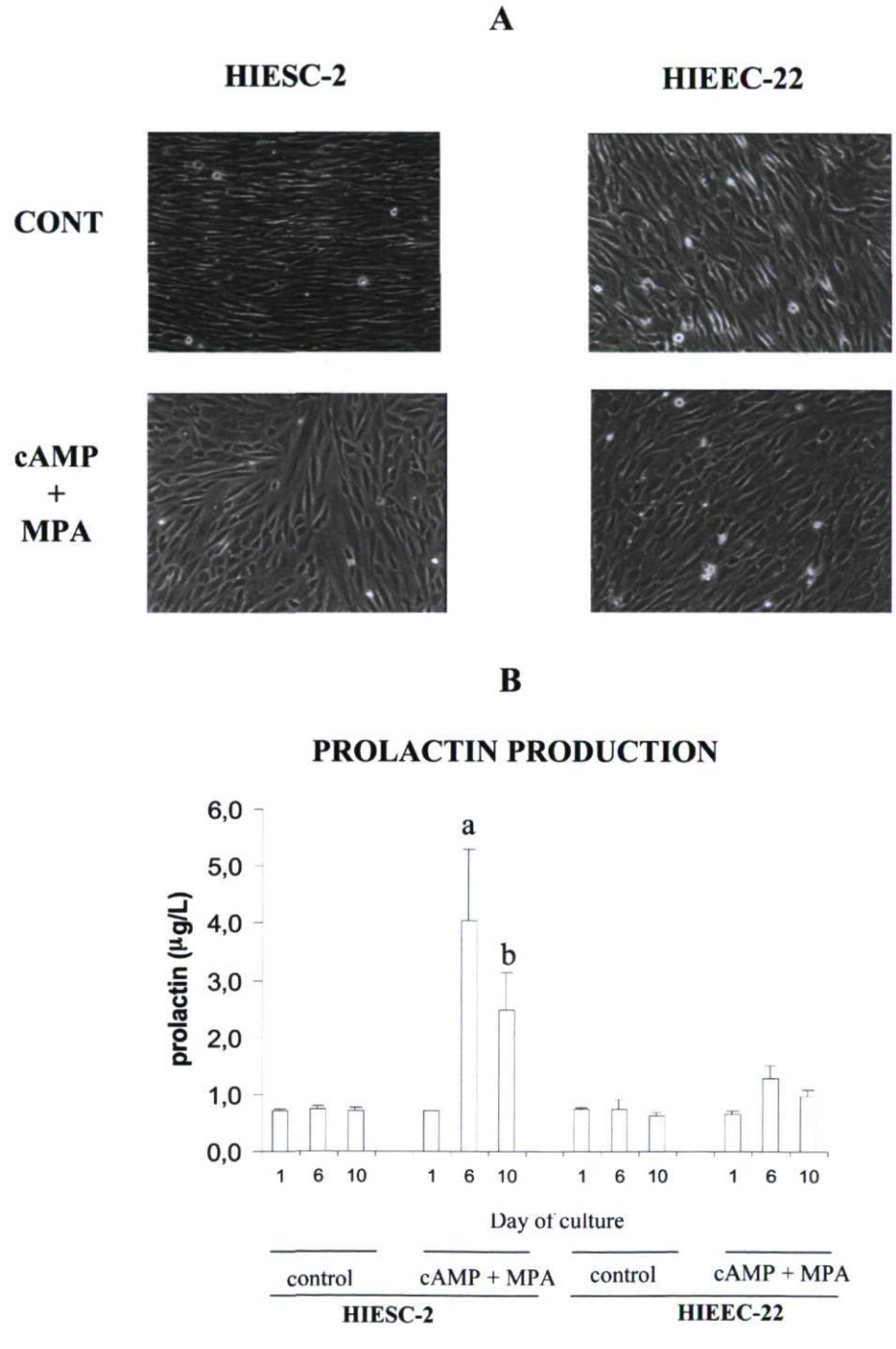

A
HIESC-2
HIEEC-22
CONT
cAMP
+
MPA
B
PROLACTIN PRODUCTION
prolactin (µg/L)
6,0
5,0
4,0
3,0
2,0
1,0
0,0
a
b
1 6 10 1 6 10 1 6 10 1 6 10
Day of culture
control
cAMP + MPA
control
cAMP + MPA
HIESC-2
HIEEC-22

Figure 8

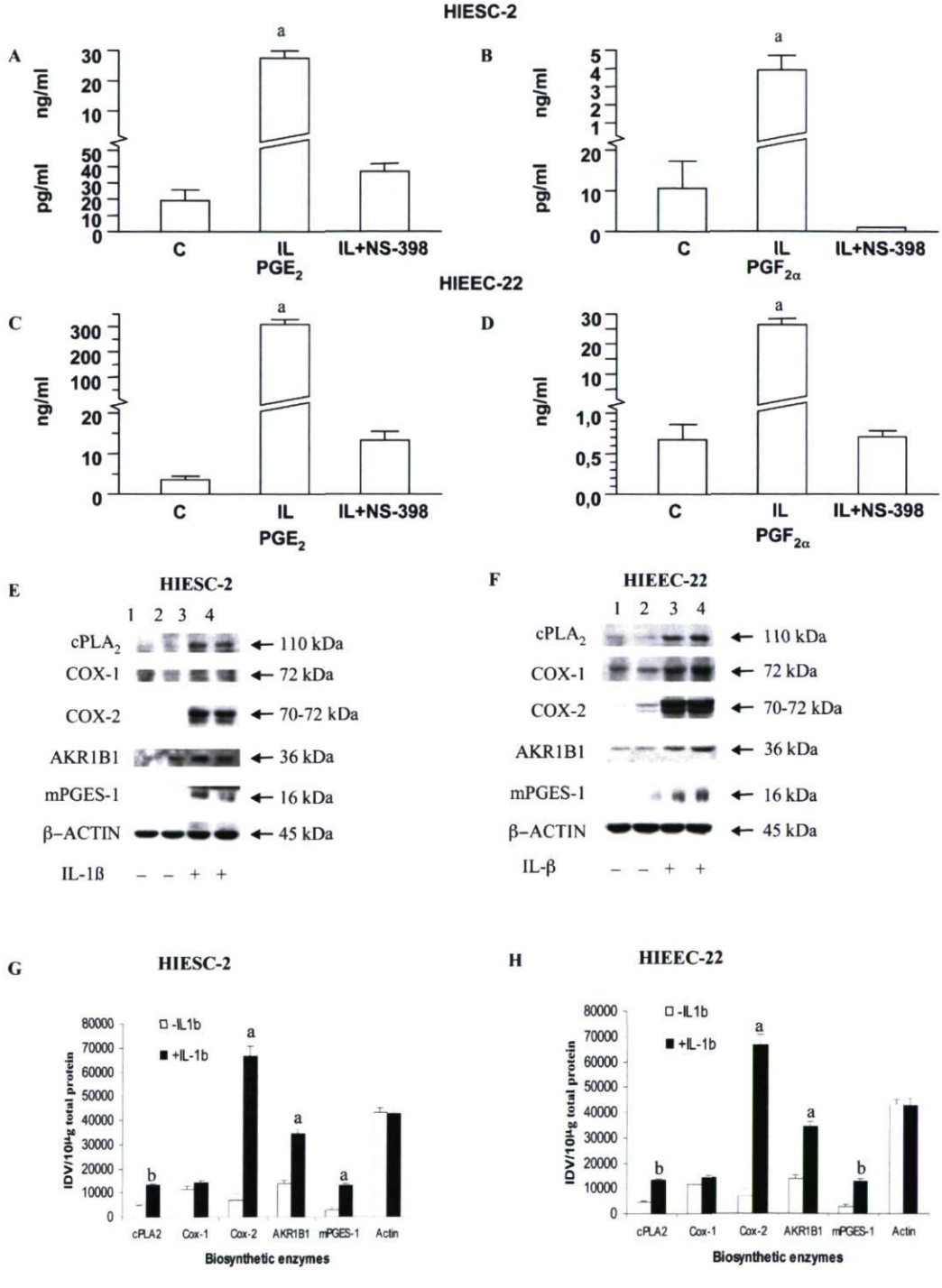
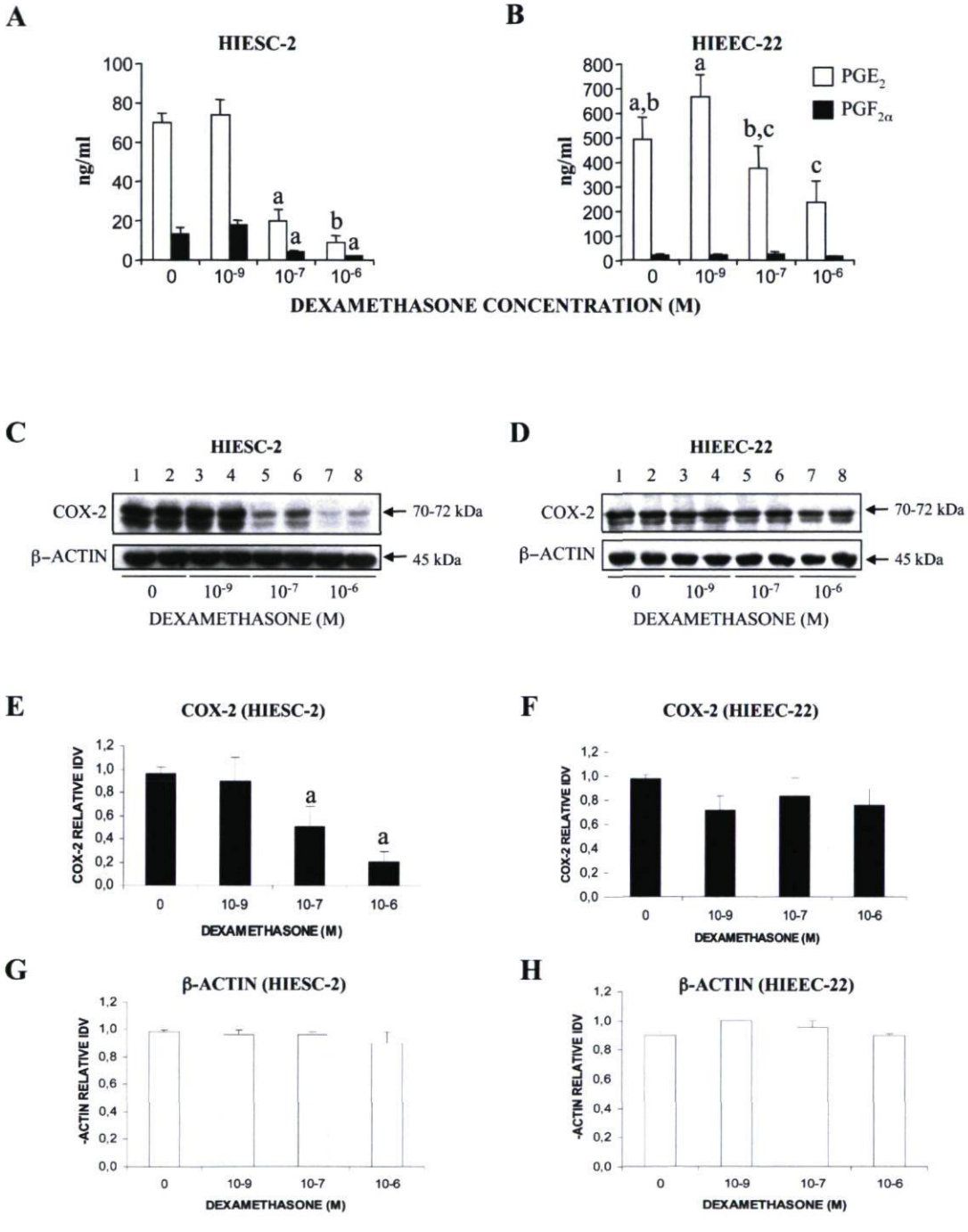

HIESC-2
A
ng/ml
pg/ml
C
IL
IL+NS-398
PGE2
B
PGF2α
HIEEC-22
C
D
E
F
cPLA2
COX-1
COX-2
AKR1B1
mPGES-1
β–ACTIN
IL-1ß
IL-β
110 kDa
72 kDa
70-72 kDa
36 kDa
16 kDa
45 kDa
G
H
-IL1b
+IL-1b
IDV/10Hg total protein
Biosynthetic enzymes
Actin

Figure 9


A
HIESC-2
ng/ml
100
80
60
40
20
0
a
a
b
a
0
10-9
10-7
10-6
B
HIEEC-22
ng/ml
800
700
600
500
400
300
200
100
0
a
a,b
b,c
c
PGE2
PGF2α
0
10-9
10-7
10-6
DEXAMETHASONE CONCENTRATION (M)
C
HIESC-2
1 2 3 4 5 6 7 8
COX-2
β-ACTIN
70-72 kDa
45 kDa
0
10-9
10-7
10-6
DEXAMETHASONE (M)
D
HIEEC-22
1 2 3 4 5 6 7 8
COX-2
β-ACTIN
70-72 kDa
45 kDa
0
10-9
10-7
10-6
DEXAMETHASONE (M)
E
COX-2 (HIESC-2)
COX-2 RELATIVE IDV
1,2
1,0
0,8
0,6
0,4
0,2
0,0
a
a
0
10-9
10-7
10-6
DEXAMETHASONE (M)
F
COX-2 (HIEEC-22)
COX-2 RELATIVE IDV
1,2
1,0
0,8
0,6
0,4
0,2
0,0
0
10-9
10-7
10-6
DEXAMETHASONE (M)
G
β-ACTIN (HIESC-2)
-ACTIN RELATIVE IDV
1,2
1,0
0,8
0,6
0,4
0,2
0,0
0
10-9
10-7
10-6
DEXAMETHASONE (M)
H
β-ACTIN (HIEEC-22)
-ACTIN RELATIVE IDV
1,2
1,0
0,8
0,6
0,4
0,2
0,0
0
10-9
10-7
10-6
DEXAMETHASONE (M)

CHAPITRE 3

THE HUMAN ALDOSE REDUCTASE AKR1B1 QUALIFIES AS THE PRIMARY PROSTAGLANDIN F SYNTHASE IN THE ENDOMETRIUM.

THE HUMAN ALDOSE REDUCTASE AKR1B1 QUALIFIES AS THE PRIMARY PROSTAGLANDIN F SYNTHASE IN THE ENDOMETRIUM.*

Bresson, E., **Boucher-Kovalik, S.**, Chapdelaine, P., Madore, E., Harvey, N., Laberge, P. Y., Leboeuf, M., and Fortier M. A.

Axe de Recherche en Reproduction, Pédiatrie et Santé Périnatale, Centre Hospitalier Universitaire de Québec (CHUL), Centre de Recherche en Biologie de la Reproduction (CRBR), and Département d'Obstétrique et Gynécologie, Université Laval, Ste-Foy, QC, Canada G1V4G2

Keywords: prostaglandins, biosynthesis, regulation, endometrium, menstrual cycle

Supported by grants from CIHR and a Wyeth-Ayerst/CIHR postdoctoral fellowship[1].

Correspondence and reprints request : Dr Michel A. Fortier, Reproduction Pédiatrie et Santé Périnatale, Centre Hospitalier Universitaire de Québec (CHUL),
Université Laval, 2705 boulevard Laurier, Ste-Foy, QC, Canada G1V 4G2.
Fax : (418) 654-2765, mafortier@crchul.ulaval.ca

*Publié dans J Clin Endocrinol Metab published October 13, 2010 as doi:10.1210/jc.2010-1589

Résumé

Les prostaglandines (PGs) sont principalement connues comme étant des médiateurs de la douleur et de l'inflammation, mais elles sont également nécessaires pour la fonction reproductrice normale de la femme. PGE_2 et $PGF_{2\alpha}$ sont les principales prostaglandines produites par l'endomètre humain et un taux approprié de leur expression est important pour obtenir un cycle menstruel normal et un taux de fertilité adéquat. Les désordres menstruels sont liés à une production excessive de PGs. En effet, les dysménorrhées et les ménorragies sont associées à une production anormale de $PGF_{2\alpha}$ ou de PGE_2 respectivement. PGE_2 et sa biosynthèse par PGES ont été extensivement étudiées dans diverses conditions pathologiques, cependant, peu est connu de la biosynthèse de $PGF_{2\alpha}$ par PGFS. AKR1C3 est la seule PGFS identifiée chez l'humain, mais son profil et ses sites d'expression ne peuvent expliquer à eux seuls la production entière de $PGF_{2\alpha}$ dans les cellules endométriales *in vivo* et *in vitro*. Nous avons démontré récemment qu'une autre aldose réductase, AKR1B1, est exprimée et modulée, en association avec la production de $PGF_{2\alpha}$, en réponse à l'IL-1β dans les cellules endométriales humaines en culture. Dans cette étude, nous avons déterminé si AKR1B1 (gene ID : 231) est une $PGF_{2\alpha}$ synthase fonctionnelle dans l'endomètre humain. Nous avons observé qu'AKR1B1 est exprimé à de hauts niveaux pendant le cycle menstruel et à des taux maximaux durant la phase sécrétoire. Au niveau protéique, AKR1C3 a été retrouvé dans les cellules épithéliales uniquement, tandis qu'AKR1B1 est exprimé à de hauts niveaux autant dans les cellules épithéliales que stromales. La protéine recombinante AKR1B1 est capable de convertir PGH_2 en $PGF_{2\alpha}$ in vitro. En présence d'acide arachidonique (AA), les transfections de cellules endométriales avec l'ADNc de AKR1B1 augmentent la production de $PGF_{2\alpha}$. Au contraire, la diminution de l'expression d'AKR1B1 à l'aide de siRNA ou par inhibition à l'aide de l'aldose reductase inhibiteur Statil réduit la production de $PGF_{2\alpha}$ en réponse à l'IL-1β. Dans des explants d'endomètre, l'IL-1β stimule la production d'AKR1B1 ainsi que de $PGF_{2\alpha}$, tandis que le Statil diminue l'expression de $PGF_{2\alpha}$. Ces résultats démontrent pour la première fois que l'enzyme AKR1B1 est une PGF synthase fonctionnelle dans l'endomètre humain.

Summary

Prostaglandins (PGs) are primarily known as mediators of pain and inflammation, but they are also necessary for normal reproductive function in the female. PGE2 and PGF2α are the main prostanoids produced in the human endometrium and proper balance in their relative production is important for normal menstruation and optimal fertility. Menstrual disorders are associated with increased production of PGs. Dysmenorrhea or menstrual pain is associated with increased production of PGF2α relative to PGE2 whereas menorrhagia or abnormal uterine bleeding is associated with increased PGE2 in menstrual fluid. PGE2 and its biosynthesis by PGES have been studied extensively in association with pathological conditions in different systems, but little is known on the pathways leading to the production of PGF2α by PGFS. AKR1C3 is the only PGFS currently identified in the human, but its site and pattern of expression cannot explain entirely the production of PGF2α in endometrial cells *in vivo* and *in vitro*. We have demonstrated recently that another aldose reductase, AKR1B1, is expressed and modulated in association with PGF2α production in response to IL-1β in human endometrial cells in culture. In the present study, we address whether AKR1B1 (gene ID: 231) is a functional PGF2α synthase in the human endometrium. We have found that AKR1B1 is expressed at a high level during the menstrual cycle with maximal expression during the secretory phase. At the protein level, AKR1B1 is expressed at high levels in both epithelial and stromal cells whereas AKR1C3 was only found in epithelial cells. The recombinant AKR1B1 protein is able to convert PGH2 into PGF2α *in vitro* and transfection of endometrial cells with AKR1B1 cDNA increases PGF2α production in presence of arachidonic acid (AA). By contrast, down regulation of AKR1B1 protein using small interfering RNA (siRNA) or inhibition using the aldose reductase inhibitor Statil reduced PGF2α production in response to IL-1β. In endometrial explants, IL-1β is able to increase both AKR1B1 expression and PGF2α production whereas Statil reduced PGF2α production. Globally, our data demonstrate for the first time that AKR1B1 is a functional PGF synthase in the human endometrium and can be a target for treatment of menstrual disorders.

Introduction

Prostaglandins (PGs) are recognized as primary regulators of female reproductive function (ovulation, uterine receptivity, implantation and parturition) and associated pathologies including endometrial carcinomas, menorrhagia, dysmenorrhea, endometriosis and premature labor. Among the different PGs, PGE_2 and $PGF_{2\alpha}$ are the main prostanoids produced in the human endometrium (1, 2). PGs are synthesized from arachidonic acid (AA) and converted to PGG_2 and PGH_2 by PGH synthases (PGHS), also known as cyclooxygenases (COX). There are two isoforms of PGHS, the constitutive COX-1 and the inducible COX-2 encoded by two distinct genes (3). PGH_2 produced by COXs is the common precursor of all PGs generated by specific terminal synthases such as PGF synthase for $PGF_{2\alpha}$ and PGE synthase for PGE_2. There are three known PGE synthases and expression and localization of the inducible microsomal PGE synthase (mPGES-1) was studied in the human endometrium and found present in stromal, epithelial and endothelial cells throughout the menstrual cycle (4). In spite of its demonstrated role as the PG responsible for dysmenorrhea no data is available on PGF synthase activity and its localisation in relation with $PGF_{2\alpha}$ production within the human endometrium.

$PGF_{2\alpha}$ is a biologically active prostanoid belonging to the eicosanoid family of bioactive lipids (5). Its biosynthesis occurs via different pathways involving reduction of PGH_2 by a 9,11-endoperoxyde reductase (6). Several PGFS have been identified in animals (7), but AKR1C3 is the only isoform currently identified in human (8). AKR1C3 (Gene ID: 8644), an aldoketoreductase of the 1C family generally associated with hydroxysteroid dehydrogenase (HSD) activity, has been studied primarily for its type V 17β -hydroxysteroid dehydrogenase (17β-HSD) activity and in this respect, was found to be expressed in the human endometrium (9).

In the bovine endometrium we have shown a strong association between AKR1B5 recently renamed as *bos taurus* AKR1B1 (Gene ID: 317748), and $PGF_{2\alpha}$ production a new function for this enzyme previously known for its 20α -HSD and glucose

metabolism activities (7). The human and bovine AKR1B1 both belong to the aldoketoreductase 1B family and share 86% identity or homology. The human AKR1B1 (Gene ID: 231) also known as the aldose reductase is highly expressed in the placenta for glucose metabolism and in the eye and kidney for osmotic regulation (10).

After identifying the bovine AKR1B5 as a potential PGFS (7), we have accumulated several lines of evidence suggesting that it could play the same role in the human endometrium. We have found that AKR1B1 expression was associated with $PGF_{2\alpha}$ production in human endometrial cell lines (11) and in decidualized stromal cells (12). However, expression of AKR1B1 within the human endometrium and its ability to act as a PGF synthase to produce $PGF_{2\alpha}$ remain to be demonstrated. In a recent study, Talbi et al (13) used high throughput DNA array to investigate the expression of more than 54,600 genes in the human endometrium throughout the menstrual cycle. Among the full list of genes available online at GeoProfile, COX-2 and AKR1B1 were shown to be modulated during the menstrual cycle and increased in association with IL-1β supporting the work of Rossi (14) who showed up regulation of AKR1B1 mRNA by IL-1β in human endometrial stromal cells using cDNA microarray analysis.

Therefore, we have studied the expression of both AKR1B1 and AKR1C3 at the mRNA and protein levels in non pregnant human endometrium across the menstrual cycle. We have also investigated their association with the production of $PGF_{2\alpha}$ and response to inflammatory IL-1β using human endometrial biopsies and cell lines.

Materials and methods

Reagents

RPMI 1640 culture medium without phenol, Superscript II reverse transcriptase, TRIzol, lipofectamine 2000, pCR3.1 and pEF6/V5 TA cloning vectors were purchased from Invitrogen (Burlington, Canada). TAQ DNA polymerase and buffer used for polymerase chain reaction (PCR) were from Pharmacia (General Electric, Montreal, QC,

Canada) and QuantiTectTM Syber® Green PCR kit (Qiagen, Mississauga, ON, Canada). RiboMax polymerase kit was purchased from Promega (Madison, Wi, USA) and Qiaquick gel extraction kit was bought from Qiagen (Mississauga, ON, Canada). All oligonucleotide primers were chemically synthesized using ABT 394 synthase (Perkin-Elmer, Foster City, CA). [α-^{32}P]dCTP radioactivity was bought from Perkin-Elmer Life Sciences (Markham, Ontario, Canada). Bright Star-Plus nylon membrane and UltraHyb solution were purchased from Ambion Inc. (Austin,TX, USA). Goat polyclonal antibodies to AKR1C3 were purchased from Abcam inc. (Cambridge, MA, USA) and rabbit COXs antibodies were kindly provided by Dr S. Kargman (Merck, Que, Canada). AKR1B1 was produced in our laboratory using whole recombinant protein as described previously (11). Biotinylated secondary antibodies (goat anti-rabbit IgG or rabbit anti-goat IgG) were bought from Dako Diagnostic of Canada, Inc. (Mississauga, Ontario, Canada). Vectastain Elite ABC kit was purchased from Vector Laboratories Inc. (Burlingame, CA, USA). Goat anti-rabbit horseradish peroxidase–conjugated IgG was bought from Jackson Immunoresearch Laboratories (West Grove, PA,USA). Enhanced chemiluminescence system (Renaissance) was purchased from NEN life Science Products (Boston, MA, USA). Arachidonic acid (AA) and mPGES-1 polyclonal antibodies were from Cayman Chemicals (Ann Arbor, MI, USA) and recombinant human IL-1β was purchased from Research and Diagnostic Systems (Minneapolis, MN, USA). Statil an inhibitor of aldose reductase was purchased from Tocris Bioscience (Ellisville, Missouri,USA)

Endometrial tissue collection and cell lines.

Endometrial tissue was obtained either from biopsy or hysterectomy. Biopsies representing the functionalis layer were collected with an endometrial curette (Pipelle) and obtained from women aged between 25 to 50 years undergoing gynecological investigation for infertility or menorrhagia. Uterine tissues obtained by hysterectomy originated from cycling women aged between 29 to 53 years presenting with chronic pelvic pain, leiomyomas and menorrhagia. In these samples, the functionalis layer of the endometrium was isolated by gentle scraping of the internal lining of intact portions of the uterus selected by the hospital pathologist. Inclusion criteria for all samples included

regular menstrual cycles (21-35 days) and absence of hormonal treatment in the 3 months prior to biopsy collection. Informed consent for donation of anonymous endometrial samples was obtained before tissue collection. Endometrial samples were dated according to the stated last menstrual period. The stage of the cycle (proliferative or secretory) was then confirmed by histological examination using the criterion of Noyes (15) and samples with conflicting dating were discarded. The research protocol was approved by the Ethics Committee on Human Research of Centre Hospitalier Universitaire de Québec. The samples were washed, divided and portions processed differentially for explants culture, RNA and protein analysis. The human endometrial stromal and epithelial cell lines HIESC-2 and HIEEC were generated in our laboratory and their characterization was published previously (11).

Assessment of COX-2, AKR1B1 and AKR1C3 mRNAs by quantitative RT- PCR (qRT-PCR).

For qRT-PCR, endometrial biopsies (N=48) were processed immediately upon reception. RNA was prepared in TRIzol Reagent according to the manufacturer' instructions and samples stored at -80°C until used for qRT-PCR analysis. Quantitative RT-PCR (qRT-PCR) reactions were performed using a Roche Light Cycler (Roche) and QuantiTectTM Syber® Green PCR kit (Qiagen). Samples from different endometrial tissues were run in duplicate. Primers for COX-1, COX-2, AKR1B1 and AKR1C3 mRNAs were designed in such a way that the amplified region spanned over intron–exon boundaries to avoid coamplification of genomic DNA (Table 1). Total RNA (5µg) from biopsies was analyzed on 1.2% agarose gel formadehyde to verify the quality and integrity of the 28S and 18S rRNA and was reverse-transcribed by SuperScriptTM II RT (Invitrogen) in a 20 µl reaction mixture according to the manufacturer's instructions. A RT-minus control (no enzyme) was included as a negative control. 2 µl cDNA were used as the template in a final PCR reaction volume of 20 µl; a two µl aliquot of diluted (1 in10) cDNA 18S rRNA was used to normalize the level of each sample analyzed. Thermocycling was initiated by a 15 min incubation at 95°C, followed by 38 cycles for COX-1, 40 cycles for 18S rRNA, 45 cycles for COX-2, 50 cycles for AKR1C3 and AKR1B1 followed for all by PCR denaturing step at 95°C for 15 sec; annealing step,

55°C for 30 sec; and extension time at 72°C for 20 sec, and a single fluorescence reading was taken at the end of each cycle at 78°C (AKR1C3), 80°C (AKR1B1,18S rRNA, COX-1 and COX-2). Each run was monitored with a melting curve analysis to confirm the specificity of amplification and lack of primer dimers. Comparative threshold cycle (Ct) values were determined by software using a fluorescence threshold automatically and further analysis was done. The amplified products were fractionated by agarose gel electrophoresis which showed single bands of predicted sizes for each sample and no product for the negative controls (water instead of cDNA). 18S RNA gene served as an internal control to normalize the expression of each gene.

Immunohistochemistry and evaluation of immunostaining

Thin tissue sections (3 μm) from endometrial biopsies taken at different periods of the menstrual cycle (N=18; 6 periods and 3 samples per period) were fixed in 4% paraformaldehyde and prepared as paraffin-embedded sections. Slides were deparaffinized in xylene and rehydrated using decreasing grades of ethanol. Endogenous peroxidase activity was blocked by dipping in 3% H_2O_2 in methanol. Antigen retrieval was done by heating sections in 1M urea solution for 15 minutes in a microwave oven at medium power. Tissue sections were then blocked with 10% goat serum for 1 hour in a humidified chamber at room temperature followed by overnight incubation at 4°C (cold room) with primary antibodies at optimal dilutions (AKR1B1 1/250, AKR1C3 1/200, COX-1 1/500 and COX-2 1/750). Non-immune rabbit serum was used as the negative control. The next day, sections were washed in PBS and incubated 30 minutes at room temperature with 1/200 dilutions of biotinylated Goat anti-rabbit IgG (AKR1B1, COX-1 and COX–2) or Rabbit anti-goat IgG (AKR1C3) as secondary antibodies. After washing with PBS, sections were treated with avidin-biotin-peroxidase complex (Vectastain Elite ABC kit) followed by staining with 3-amino-9-ethyl carbazole (AEC). Finally, sections were washed with water and counterstained with Harris hematoxylin reagent (Sigma, Mississauga, Canada). The staining was evaluated subjectively by three blinded observers not involved with the present study using a scoring system of immunostaining intensity

interpreted as absent (1), weak (2), moderate (3), or intense (4). Individual scores for each slide were averaged and expressed as relative expression level.

Endometrial explants culture.

Explants cultures were prepared according to the procedure of Li et al., (2005). Briefly, endometrial biopsy samples were washed several times in Phosphate Buffer saline solution (DPBS, Wisent, Montreal, Canada), cut uniformly into 1–2 mm^3 pieces with a sterile scalpel blade, washed and placed in 5 ml RPMI (w/o Phenol Red) containing 2% steroid-stripped FBS, antimycotic and penicillin-streptomycin and incubated overnight at 37°C in a humidified atmosphere of 5% CO2 and 95% air. The next day, explants were washed at least three times with RPMI and plated on 24 well-plates. Each well contained 1 ml of RPMI containing 10ng/ml of IL-1β in the presence or absence of 100 μM Statil and cultured at 37°C for 6 hours. At the end of the incubation, the medium was harvested by centrifugation for measurement of PGF2α whereas explants tissue (pellet) were suspended in 200 μl lysis solution (20 mM Tris-HCl pH 7.5, 1mM Dithiothreitol, 1% SDS, 1mM PMSF) and homogenized 3 times (5-10 sec) using a handheld homogenizer (BF20, 7x95mm saw tooth (Troemner, NJ, USA). Subsequently, 600 μl of methanol was added to the homogenate to precipitate protein as described previously (16) for analysis by Western Blotting. Proteins were quantified by image analysis using the AlphaImager 2000 software (Alpha Innotech Corporation, San Leandro, CA).

AKR1B1 specific siRNA

Specific siRNAs for AKR1B1 were designed using a program (17) facilitating the identification of oligonucleotides optimal for production of short interfering RNA with T7 RNA polymerase forward: 5'-aaattgttgagcaggagacggctatagtgag-tcgtattacc-3'and the reverse: 5'-aagccgtctcctgctcaacaactatagtgagt cgtattacc-3'. Using these oligonucleotides, we followed exactly the procedure of Donze (18) using RiboMax kit. The resulting siRNA products were purified by ethanol precipitation and 100 ng/ml were used for transfection of cells grown in 6 or 24-well plates using lipofectamine 2000.

Western blot

Western blot analysis was performed as we described (11). Briefly, around 20 μg total protein extracted from cultured cells was loaded in each lane and electrophoresed on 10 % SDS-PAGE followed by electrotransfer onto nitrocellulose membrane. The primary antibodies used for the present study were rabbit anti human AKR1B1 (dilution 1/1000) and anti COX-2 (dilution 1/6000) anti-serums and goat anti AKR1C3 (dilution 1/500) anti-serum. As an internal control, a β-actin monoclonal antibody (1/5000, Sigma, Mississauga, ON, Canada) was used. The goat anti-rabbit IgG conjugated with horse radish peroxidase (HRP), rabbit anti-goat IgG HRP or goat anti-mouse IgG HRP were used as secondary antibodies. The chemiluminescence was analyzed with autoradiography films at optimal times of exposure following treatment of the membranes with Renaissance reagent (NEN, Perkins Elmer, Boston, MA, USA). Chemiluminescence on the film was quantified by image analysis using the AlphaImager 2000 software (Alpha Innotech Corporation, San Leandro, CA).

Northern blot

Northern blot analysis was performed as follows: total RNA (~20 μg) was extracted from endometrial cells in culture, loaded on a 1.2% formaldehyde-agarose gel and electrophoresed at 100 V in 1X MOPS buffer. After electrophoresis, RNA was transferred overnight onto a nylon membrane in 10X saline-sodium citrate (SSC). The AKR1B1 cDNA probe was generated by labeling the ~500 bp fragment with [α-^{32}P]dCTP (3000 Ci/mmol) using the Ready-To-Go DNA labeling Kit (Amersham/Pharmacia). Prehybridization (2-4 hours) and hybridization (overnigth) were done at 45°C using UltraHyb solution (Ambion Inc.). The blots were then washed at 65°C twice for 15 minutes in 0.5 X SSC and exposed for 24 to 48 hours at -80°C on BioMAx films to quantitate the hybridization signal intensity. 18S ribosomal RNA stained with ethidium bromide was used to confirm uniform loading of RNA samples.

Cell transfection and treatments

Immortalized human endometrial stromal (HIESC-2) and epithelial (HIEEC-22) cells were produced and used as we described recently (11). Briefly, HIESC-2 (passages 15-22) and HIEEC-22 (passages 15-22) were cultured in RPMI 1640 without phenol red, containing 50 IU penicillin-streptomycin supplemented with 10% FBS. Once cells have reached confluency medium was replaced with fresh RPMI containing 10% steroid stripped FBS for 24 hours. Knockdown with AKR1B1 specific siRNAs and knock-in transfection of cells with AKR1B1 or AKR1C3 cDNAs in pCR3.1 expression vectors was performed with lipofectamine 2000 for 4 hours in culture medium without antibiotic. After 36 hours, cells were treated for 24 hours with IL-1β (1ng/ml) or arachidonic acid (AA) 10 μM in RPMI 1640 medium without serum. For Western blot analysis (described above), the cells were grown in 24-well plates whereas for Northern blots analysis (described above), cells were plated on 6-well plates. At the end of the treatment period, the culture medium was recovered and stored at -20°C until evaluation of $PGF_{2\alpha}$ production.

AKR1B1 enzyme activity

AKR1B1 recombinant protein was overexpressed in Escherichia Coli (BL21), purified and enzymatic activity was determined as we described for bovine AKR1B5 (7). Briefly, AKR1B1 was inserted in the Nde1 restriction site of pET16B and the HIS-TAG proteins were produced and purified on Nickel-sepharose column (Novagen). Enzymatic activity was measured by monitoring NADPH degradation at 340 nm. The assays were performed in 1 ml of 50 mM Tris-HCl pH7.5, 100 μM NADPH with 10 to 100 μg of enzyme and variable concentrations of PGH_2. The production of PGF2α was confirmed by TLC using silica plates. Migration was performed in ethyl acetate [110:50:20] water saturated solvent and detection was achieved by spraying phosphomolybdic acid 10% (v/v) in methanol followed by heating on a plate at 120°C for 10 minutes (19).

$PGF_{2\alpha}$ EIA

$PGF_{2\alpha}$ was measured by enzyme immunoassay and acetylcholinesterase-linked $PGF_{2\alpha}$ tracer (Cayman) was used as described previously (20). Sheep anti- $PGF_{2\alpha}$ Bio-Quant, Ann Arbor, MI, USA) was used as the selective antibody. Inter- and intra- assay coefficients of variations (N = 12) were 16 and 10% respectively.

Statistical analysis

Data are presented as the mean ± SEM. Statistical significance was assessed by one ANOVA using GraphPad Prism 5. If the null hypothesis was rejected, Tukey's multiple comparison was used as a *post-hoc* test to find the critical difference between pairs of treatment means. In all the experiments, confidence level was set at 95% to determine the significance of difference ($P<0.05$).

Results

Gene expression of COX-1,-2, AKR1B1 and AKR1C3

Analysis of mRNA expression for COX-1, COX-2, AKR1B1 and AKR1C3 was performed by quantitative RT-PCR (Fig. 1) in endometrial biopsies collected at different periods of the menstrual cycle. The results were variable among samples, a result coherent with their involvement with inflammation responses, but showed that mRNA was expressed for all four genes tested. COX-1 mRNA expression was globally higher in the secretory than in the proliferative phase. COX-2 mRNA levels were highest at the early proliferative phase. AKR1C3 and AKR1B1 mRNA were present throughout the cycle without significant variation.

Immunohistochemical analysis of AKR1B1 and AKR1C3 protein

Immunohistochemical staining for COX-1, COX-2, AKR1B1 and AKR1C3 was performed in endometrial samples collected at different phases of the menstrual cycle and

evaluated by subjective analysis (Fig. 2 and 3). At the protein level COX-1 was present at all periods except late proliferative phase (Fig. 3). COX-2 protein followed a similar pattern in epithelial and stromal compartments and was highest in the mid and late secretory phase. AKR1C3 protein staining is intense and fairly constant throughout the cycle in epithelial cells but completely absent in the stromal compartment. AKR1B1 protein is present in luminal and glandular epithelial as well as in stromal cells of the endometrium. Higher expression was found in early proliferative and mid-late secretory compared with other phases of menstrual cycle when both epithelial and stromal cell compartments are considered simultaneously (Fig. 2 and 3).

Downregulation of AKR1B1 expression by siRNA

Gene and protein expression of AKR1B1 was tested in absence or presence of IL-1β, a known regulator of PG production, and correlated with the production of PGF2α in cultured endometrial stromal cells. Northern and Western blot analysis of stromal cell line HIESC-2 treated with IL-1β (1ng/ml) shows that AKR1B1 mRNA and protein are significantly reduced following transfection of the cells with AKR1B1 siRNA (Fig. 4A). The same observation was found for the epithelial cell line HIEEC-22 (results not shown). AKR1B1 siRNA does not affect COX-2 protein level following treatment with IL-1β (Fig. 4B). The decrease in AKR1B1 protein level by specific siRNA knock-down was associated with a significant reduction of PGF2α production ($P < 0.05$) (Fig. 4C).

PGF synthase activity of AKR1B1

AKR1B1 recombinant protein was produced in *Escherichia coli* and purified on a nickel-nitrilotriacetic column. The recombinant protein was found to functionally reduce phenanthrenequinone and NADPH at a rate of 10 nmole/min/mg in presence of 40 μM PGH_2 as monitored by absorbance at 340 nm (Fig. 5A). The conversion of PGH_2 into PGF2α was confirmed by TLC where a spot corresponding to the PGF2α marker is detected (Fig. 5A). AKR1B1 PGF synthase activity within cells *in vitro* was confirmed by gain of function and compared with AKR1C3. The expression vector pCR3.1 containing the CMV promoter able to drive full length AKR1B1 or AKR1C3 cDNA was

transfected in HIESC-2 cells to overexpress the proteins (Fig.5B). Treatment of the transfected cells with 10 μM AA results in much higher production of PGF2α in the culture medium following transfection with AKR1B1 than AKR1C3 (Fig.5C). Together, these observations confirm AKR1B1 protein as a functional PGF synthase in endometrial cells.

Association between AKR1B1 and PGF2α production in endometrial explants.

To confirm the physiological relevance of our observations in vitro, fresh endometrial biopsies were used to study the effect of IL-1β on AKR1B1 stimulation and PGF2α production. In endometrial explants stimulated with IL-1β (10 ng/ml), we observed elevated COX-2 and AKR1B1 protein levels (Fig. 6A) associated with increased PGF2α production (Fig. 6B).

Inhibition of PGFS activity of AKR1B1 with the aldose reductase inhibitor Statil in human endometrial cell lines and explants.

Endometrial HIEEC-22 and HIESC-2 cells were stimulated with IL-1β in presence of increasing concentrations of Statil, a functional aldose reductase inhibitor developed to inhibit the conversion of glucose to sorbitol by AKR1B1 (21). Interestingly, Statil induced a dose dependent inhibition of PGF2α production in both stromal and epithelial cells (Fig. 7A) cells. The inhibition of PGF2α production by Statil appears more complete in the stromal (HIESC-2) than in the epithelial cell line (HIEEC-22). When endometrial explants were treated with IL-1β at 10 ng/ml, Statil was also able to block PGF2α production even below unstimulated levels.

Discussion

Prostaglandins are important regulators of female reproductive function and contribute to gynecological disorders. Normal menstruation depend on an equilibrium between vasoconstrictors such as PGF2α (1, 22) and vasodilators such as PGE2 or nitric oxide (NO) (23). Excessive production of contracting prostaglandins create an ischemia-

reperfusion response causing painful menstruation or dysmenorrhea (24) whereas increased vasodilatation leads to abundant menstrual bleeding (25). NSAIDs represent the most important and widely used drugs on the market and they are all efficient to treat menstrual disorders at some level. However these drugs act at an early step of biosynthesis common for all PGs and not only the one responsible for the pathological response. While the short duration of NSAID needed to treat menstrual pain should not lead to serious side effects, long term treatment with the most efficient treatment of dysmenorrhea such as naproxen leads to serious gastrointestinal side effects (26) whereas chronic use of any NSAID can be associated with impaired timing of ovulation and reduced fertility (27). Because of its notorious role on inflammation and pain, the biosynthetic pathway leading to PGE2 has been studied extensively, but that of PGF2α is poorly documented. The data presented in this manuscript describe for the first time the expression in the human endometrium of two gene candidates, AKR1B1 and AKR1C3 and the corresponding proteins and their functional association with PGF2α production. In the human endometrium, it has been reported that production of PGF2α is higher in late secretory and menstrual periods of the menstrual cycle (28). Accordingly, both AKR1B1 and AKR1C3 enzymes are present in the endometrium throughout the menstrual cycle. By contrast with AKR1B1 expressed in both stromal and glandular epithelial cells and modulated in accordance with endometrial PGF2α production, AKR1C3 protein is absent in stromal cells as was reported previously (9). The absence of the only currently accepted human PGFS AKR1C3 in stromal cells (Fig. 2, 3) was surprising because we and others have shown that human endometrial stromal cells produce high levels of PGF2α (11, 12, 29). Because of a similar finding in the bovine endometrium and our identification of AKR1B5 as a potential PGFS in that system (7), we hypothesized that the corresponding human enzyme AKR1B1, could also possess PGFS activity in the human endometrium. We have shown in previous studies that indeed AKR1B1 was expressed in human endometrial cells and modulated in parallel with PGF2α production. However, because of the major impact of this finding on our understanding of the contribution of AKR1B1 to normal body function, its PGFS activity remained to be demonstrated unequivocally.

In a global study of endometrial transcriptome from endometrium of women with mild pathologies, Talbi et al have documented the expression of 54600 genes using high throughput DNA array where they have shown that expression of COX and AKR genes were significantly altered (13). In the present study we confirm the expression of AKR1B1, AKR1C3, COX-1 and COX-2 mRNA with significant variation among samples and some regulation during the cycle. The variation among the different samples is not surprising given that biopsies were obtained from women with benign endometrial pathologies. Moreover, PGs are recognized as mediators of inflammation easily and rapidly triggered by microbial infections which may have been present in the endometrium or introduced during the sampling procedure. Finally, the potential modulation of mRNA during the cycle may represent a variation in the relative proportion of epithelial and stromal cells for those genes with heterogeneous expression between the cell types. At the protein level COX-2 (Fig. 3) expression is increased in both endometrial cell types at the end of the proliferative period together with high expression of AKR1B1 (Fig. 2 and 3). This is consistent with the proposed role of PGF2α in the initiation of menstruation and its association with menstrual pain at the beginning of the cycle.

We illustrate the ability of the purified recombinant human AKR1B1 to release PGF2α and metabolize PGH2 *in vitro* in presence of NADPH (Fig. 5A). As was found for the bovine isoform, the human AKR1B1 is able to metabolize PGH2 and form PGF2α with a high efficiency. In fact, AKR1B1 uses PGH2 at concentrations well within the physiological range whereas it processes glucose only at supra-physiological concentrations found primarily under pathological conditions (10). Recently, using different recombinant AKR proteins Kabututu et al have shown that AKR1B1 is 20 times more potent than AKR1C3 to produce PGF2α from its precursor PGH2 (30). We also show here that transfection of endometrial cells to overexpress AKR1B1 increased their ability to generate PGF2α. By contrast, downregulation of AKR1B1 using siRNA (Fig 4) significantly reduced the production of PGF2α in IL-1β stimulated cells. Similarly, Statil, a known aldose reductase inhibitor, dose-dependently reduced PGF2α production in the same cells (Fig 6).

The physiological relevance of PGF2α production by AKR1B1 in the human endometrial cells was verified using fresh endometrial explants treated with IL-1β (Fig.6) and Statil (Fig.7). It was interesting to note that the pattern of response was similar to that of endometrial cell lines. Endometrial explants were developed initially to allow metabolic labeling of secretory proteins synthesized *de novo* in the porcine endometrium (31) and more recently for the study of apoptosis associated with menstruation in human endometrium (32). This appears to be the experimental model closest to *in vivo* that we can use with living human tissues. There are several successful examples of use of endometrial explants to study the mechanisms underlying PGs biosynthesis (33, 34). More recently, human endometrial explants were used to show increased PGE2 response in women with AUB (35).

We have found that transfection of epithelial cells with AKR1B1 also induced increased production of PGF2α whereas knocking down its expression with specific siRNA reduced PGF2α production (results not shown). We have also confirmed the PGFS activity of AKR1C3 following transfection of endometrial stromal cells and found slightly increased PGF2α production compared with non-transfected cells in presence of exogenous AA (results not shown). Because AKR1C3 is expressed only in epithelial cells that represent only a small fraction of endometrial functionalis and since this enzyme is neither modulated during the cycle nor stimulated by IL-1β, its contribution to the release of endometrial PGF2α is probably marginal. This is supported by our observation that AKR1C3 is much less efficient than AKR1B1 to trigger increased PGF2α production following overexpression of the 2 enzymes (Figure 5).

IL-1β is an important regulator of endometrial PG production that also induces apoptosis in epithelial cells of the endometrium (36) thus contributing to the initiation of menstruation (37). Interestingly, a cDNA microarray study of 15164 sequence-verified clones has identified AKR1B1 as an important gene upregulated by IL-1β in human endometrial cells (14) supporting our observation that it is a key inducible endometrial protein (11). This finding was confirmed in the endometrial gene profiling experiment

reported by Talbi et al (13) and a cluster analysis of the genes considered in the present study using data available on Geo Profile revealed that AKR1B1 and IL-1β exhibited a similar expression profile.

Together, these results suggest that AKR1B1 is the primary candidate to be considered as the functional PGFS responsible for PGF2α production in the human endometrium. AKR1B1 is a well known and widely studied enzyme, but its contribution to prostaglandin production had never been suspected until we proposed a PGFS activity for AKR1B5 (7). AKR1B1 has been traditionally associated with reduction of glucose and diabetes-induced oxidative stress (38). Accordingly, AKR1B1 knockout mice have been used to study the pathogenesis of various diseases associated with diabetes mellitus such as cataract, retinopathy, neuropathy and nephropathy (39). Interestingly, transgenic mice overexpressing human AKR1B1 were more prone to myocardial ischemic injury (40) whereas knockout mice appeared protected against cerebral ischemic injury (41). These observations suggest that AKR1B1 is involved in the regulation of vascular tone by mechanisms distinct from those of glucose metabolism. Interestingly, ischemia is a documented function for PGF2α and its FP receptor (42), and menstrual pain which can be defined as uterine ischemia can be induced with exogenous PGF2α (43).

PGs induce a wide variety of responses mediated by distinct receptors for each isoform and using several second messenger systems (5, 44). In the vascular system, TXA2 and PGI2 exert opposing action on coagulation and vascular tone to regulate hemostasis (42). In the reproductive system, a similar opposing action is often observed for PGE2 and PGF2α (45). There have been reports showing that some terminal synthases are preferentially associated with a specific COX such as mPGES-1 with COX-2 or mPGES-2 with COX-1 (46). Intriguingly, in spite of significant and stimulus sensitive production of PGF2α, no co-localization or association was found between COXs and PGF synthases (47). Such associations would imply that inhibition of a specific COX could exert some selectivity on the release of a specific PG. In this respect, ASA, the first marketed NSAID (ASPIRIN) exhibits a slight preference for COX-1 and platelets thus yielding preferential inhibition of TXA2 over PGI2 in the vascular system

(48). Similarly, the recently developed coxibs such as BEXTRA and VIOXX are COX-2 selective and have proven extremely efficient to reduce pain and inflammation induced by PGE2 (49). Unfortunately, the chronic use of these drugs was associated with increased risk of heart failure whereas other common NSAIDs such as ibuprofen, acting on both COX with no distinction between COX-1 and COX-2 (50) appeared safer. Therefore, acting at the level of terminal synthases responsible for the release of specific PG isotypes appears as a promising avenue to control the release of bad PGs while allowing the action of the good ones (51).

AKR1B1 was first identified as a key enzyme of the polyol pathway and more recently as a detoxification enzyme involved in the reduction of a wide range of carbonyl compound including benzaldehyde derivatives, quinones, sugars and many lipid peroxidation end products such as 4-hydroxy trans-2-nonenal (HNE) and acrolein (10). The present finding that in the human endometrium AKR1B1 is a functional PGFS liberating the bioactive PGF2α metabolite acting through a specific and selective receptor, was unexpected and is highly challenging. This result opens entirely new avenues to study the mechanisms underlying the contribution of this key enzyme in menstrual disorders (Fig. 8). ARIs such as Statil were developed to inhibit reduction of glucose by AKR1B1 and most if not all proved inefficient to prevent diabetic complications but were generally non toxic. Since the conversion of glucose into sorbitol by AKR1B1 may exhibit enzyme dynamics different from biosynthesis of PGF2α there may be existing inhibitors which have gone through toxicity tests and different phases for human use that could represent a new class of medications to treat menstrual disorders.

References

1. **Sales KJ, Jabbour HN** 2003 Cyclooxygenase enzymes and prostaglandins in pathology of the endometrium. Reproduction 126:559-567
2. **Smith SK, Kelly RW** 1988 The release of PGF2 alpha and PGE2 from separated cells of human endometrium and decidua. Prostaglandins Leukot Essent Fatty Acids 33:91-96
3. **Smith WL, Garavito RM, DeWitt DL** 1996 Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. J Biol Chem 271:33157-33160
4. **Milne SA, Jabbour HN** 2003 Prostaglandin (PG) F(2alpha) receptor expression and signaling in human endometrium: role of PGF(2alpha) in epithelial cell proliferation. J Clin Endocrinol Metab 88:1825-1832
5. **Narumiya S, FitzGerald GA** 2001 Genetic and pharmacological analysis of prostanoid receptor function. J Clin Invest 108:25-30
6. **Watanabe K** 2002 Prostaglandin F synthase. Prostaglandins Other Lipid Mediat 68-69:401-407
7. **Madore E, Harvey N, Parent J, Chapdelaine P, Arosh JA, Fortier MA** 2003 An aldose reductase with 20 alpha-hydroxysteroid dehydrogenase activity is most likely the enzyme responsible for the production of prostaglandin f2 alpha in the bovine endometrium. J Biol Chem 278:11205-11212
8. **Komoto J, Yamada T, Watanabe K, Woodward DF, Takusagawa F** 2006 Prostaglandin F2alpha formation from prostaglandin H2 by prostaglandin F synthase (PGFS): crystal structure of PGFS containing bimatoprost. Biochemistry 45:1987-1996
9. **Pelletier G, Luu-The V, Tetu B, Labrie F** 1999 Immunocytochemical localization of type 5 17beta-hydroxysteroid dehydrogenase in human reproductive tissues. JOURNAL OF HISTOCHEMISTRY & CYTOCHEMISTRY 47:731-738
10. **Srivastava SK, Ramana KV, Bhatnagar A** 2005 Role of aldose reductase and oxidative damage in diabetes and the consequent potential for therapeutic options. Endocr Rev 26:380-392

11. **Chapdelaine P, Kang J, Boucher-Kovalik S, Caron N, Tremblay JP, Fortier MA** 2006 Decidualization and maintenance of a functional prostaglandin system in human endometrial cell lines following transformation with SV40 large T antigen. Mol Hum Reprod 12:309-319

12. **Kang J, Chapdelaine P, Laberge PY, Fortier MA** 2006 Functional characterization of prostaglandin transporter and terminal prostaglandin synthases during decidualization of human endometrial stromal cells. Hum Reprod 21:592-599

13. **Talbi S, Hamilton AE, Vo KC, Tulac S, Overgaard MT, Dosiou C, Le Shay N, Nezhat CN, Kempson R, Lessey BA, Nayak NR, Giudice LC** 2006 Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women. Endocrinology 147:1097-1121

14. **Rossi M, Sharkey AM, Vigano P, Fiore G, Furlong R, Florio P, Ambrosini G, Smith SK, Petraglia F** 2005 Identification of genes regulated by interleukin-1beta in human endometrial stromal cells. Reproduction 130:721-729

15. **Noyes R, Hertig A, Rock J** 1950 Dating the endometrial biopsy. Fertil Steril 1:3-25

16. **Chapdelaine P, Vignola K, Fortier MA** 2001 Protein estimation directly from SDS-PAGE loading buffer for standardization of samples from cell lysates or tissue homogenates before Western blot analysis. Biotechniques 31:478, 480, 482

17. **Dudek P, Picard D** 2004 TROD: T7 RNAi Oligo Designer. Nucleic Acids Res 32:W121-123

18. **Donze O, Picard D** 2002 RNA interference in mammalian cells using siRNAs synthesized with T7 RNA polymerase. Nucleic Acids Res 30:e46

19. **Green K, Hamberg M, Samuelsson B, Frolich JC** 1978 Extraction and chromatographic procedures for purification of prostaglandins, thromboxanes, prostacyclin, and their metabolites. Adv Prostaglandin Thromboxane Res 5:15-38

20. **Asselin E, Goff AK, Bergeron H, Fortier MA** 1996 Influence of sex steroids on the production of prostaglandins F2 alpha and E2 and response to oxytocin in

cultured epithelial and stromal cells of the bovine endometrium. Biol Reprod 54:371-379

21. **Sochor M, Kunjara S, McLean P** 1988 The effect of aldose reductase inhibitor Statil (ICI 128436) on the glucose over-utilization in kidney of diabetic rats. Biochem Pharmacol 37:3349-3356
22. **Lockwood CJ, Schatz F** 1996 A biological model for the regulation of peri-implantational hemostasis and menstruation. J Soc Gynecol Investig 3:159-165
23. **Tschugguel W, Schneeberger C, Unfried G, Brautigam G, Stonek F, Wieser F, Vytiska-Binstorfer E, Czerwenka K, Weninger W, Kaider A, Bursch W, Breitschopf H, Huber JC** 1999 Elevation of inducible nitric oxide synthase activity in human endometrium during menstruation. Biol Reprod 60:297-304
24. **Okazaki M, Matsuyama T, Kohno T, Shindo H, Koji T, Morimoto Y, Ishimaru T** 2005 Induction of epithelial cell apoptosis in the uterus by a mouse uterine ischemia-reperfusion model: possible involvement of tumor necrosis factor-alpha. Biol Reprod 72:1282-1288
25. **Sales KJ, Jabbour HN** 2003 Cyclooxygenase enzymes and prostaglandins in reproductive tract physiology and pathology. Prostaglandins Other Lipid Mediat 71:97-117
26. **Garcia Rodriguez LA, Barreales Tolosa L** 2007 Risk of upper gastrointestinal complications among users of traditional NSAIDs and COXIBs in the general population. Gastroenterology 132:498-506
27. **Norman RJ, Wu R** 2004 The potential danger of COX-2 inhibitors. Fertil Steril 81:493-494
28. **Downie J, Poyser NL, Wunderlich M** 1974 Levels of prostaglandins in human endometrium during the normal menstrual cycle. J Physiol 236:465-472
29. **Huang JC, Liu DY, Yadollahi S, Wu KK, Dawood MY** 1998 Interleukin-1 beta induces cyclooxygenase-2 gene expression in cultured endometrial stromal cells. J Clin Endocrinol Metab 83:538-541
30. **Kabututu Z, Manin M, Pointud JC, Maruyama T, Nagata N, Lambert S, Lefrancois-Martinez AM, Martinez A, Urade Y** 2009 Prostaglandin F2alpha

synthase activities of aldo-keto reductase 1B1, 1B3 and 1B7. J Biochem 145:161-168

31. **Basha SM, Bazer FW, Roberts RM** 1980 Effect of the conceptus on quantitative and qualitative aspects of uterine secretion in pigs. J Reprod Fertil 60:41-48
32. **Li A, Felix JC, Hao J, Minoo P, Jain JK** 2005 Menstrual-like breakdown and apoptosis in human endometrial explants. Hum Reprod 20:1709-1719
33. **Charpigny G, Reinaud P, Tamby JP, Creminon C, Martal J, Maclouf J, Guillomot M** 1997 Expression of cyclooxygenase-1 and -2 in ovine endometrium during the estrous cycle and early pregnancy. Endocrinology 138:2163-2171
34. **Burns PD, Mendes JO, Jr., Yemm RS, Clay CM, Nelson SE, Hayes SH, Silvia WJ** 2001 Cellular mechanisms by which oxytocin mediates ovine endometrial prostaglandin F2alpha synthesis: role of G(i) proteins and mitogen-activated protein kinases. Biol Reprod 65:1150-1155
35. **Smith OP, Jabbour HN, Critchley HO** 2007 Cyclooxygenase enzyme expression and E series prostaglandin receptor signalling are enhanced in heavy menstruation. Hum Reprod 22:1450-1456
36. **Tanaka T, Umesaki N, Mizuno K, Chang L, Ohtaki S, Ogita S** 1998 Enhancement of apoptotic susceptibility by interleukin-1 beta in human endometrial epithelial cells. Gynecol Endocrinol 12:315-319
37. **Kokawa K, Shikone T, Nakano R** 1996 Apoptosis in the human uterine endometrium during the menstrual cycle. J Clin Endocrinol Metab 81:4144-4147
38. **Chung SS, Ho EC, Lam KS, Chung SK** 2003 Contribution of polyol pathway to diabetes-induced oxidative stress. J Am Soc Nephrol 14:S233-236
39. **Ho HT, Chung SK, Law JW, Ko BC, Tam SC, Brooks HL, Knepper MA, Chung SS** 2000 Aldose reductase-deficient mice develop nephrogenic diabetes insipidus. Mol Cell Biol 20:5840-5846
40. **Hwang YC, Kaneko M, Bakr S, Liao H, Lu Y, Lewis ER, Yan S, Ii S, Itakura M, Rui L, Skopicki H, Homma S, Schmidt AM, Oates PJ, Szabolcs M, Ramasamy R** 2004 Central role for aldose reductase pathway in myocardial ischemic injury. Faseb J 18:1192-1199

41. **Lo AC, Cheung AK, Hung VK, Yeung CM, He QY, Chiu JF, Chung SS, Chung SK** 2007 Deletion of aldose reductase leads to protection against cerebral ischemic injury. J Cereb Blood Flow Metab 27:1496-1509

42. **Norel X** 2007 Prostanoid receptors in the human vascular wall. ScientificWorldJournal 7:1359-1374

43. **Coco AS** 1999 Primary dysmenorrhea. Am Fam Physician 60:489-496

44. **Narumiya S, Sugimoto Y, Ushikubi F** 1999 Prostanoid receptors: structures, properties, and functions. Physiol Rev 79:1193-1226

45. **Arosh JA, Banu SK, Chapdelaine P, Madore E, Sirois J, Fortier MA** 2004 Prostaglandin biosynthesis, transport, and signaling in corpus luteum: a basis for autoregulation of luteal function. Endocrinology 145:2551-2560

46. **Ueno N, Takegoshi Y, Kamei D, Kudo I, Murakami M** 2005 Coupling between cyclooxygenases and terminal prostanoid synthases. Biochem Biophys Res Commun 338:70-76

47. **Nakashima K, Ueno N, Kamei D, Tanioka T, Nakatani Y, Murakami M, Kudo I** 2003 Coupling between cyclooxygenases and prostaglandin F(2alpha) synthase. Detection of an inducible, glutathione-activated, membrane-bound prostaglandin F(2alpha)-synthetic activity. Biochim Biophys Acta 1633:96-105

48. **FitzGerald GA** 2002 Cardiovascular pharmacology of nonselective nonsteroidal anti-inflammatory drugs and coxibs: clinical considerations. Am J Cardiol 89:26D-32D

49. **Zeilhofer HU, Brune K** 2006 Analgesic strategies beyond the inhibition of cyclooxygenases. Trends Pharmacol Sci 27:467-474

50. **Rainsford KD** 2005 Introduction - The coxib controversies. Inflammopharmacology 13:331-341

51. **Zeilhofer HU** 2007 Prostanoids in nociception and pain. Biochem Pharmacol 73:165-174

Fig 1: Expression of COX-1, COX-2, AKR1B1 and AKR1C3 mRNA during the menstrual cycle. Endometrial biopsies (30) were obtained at different days of the menstrual cycle and total RNA was extracted. Target mRNAs were evaluated by quantitative RT-PCR as described in material and methods. Data are presented at six periods of the menstrual cycle. Results are expressed as mRNA quantity relative to 18S rRNA for each enzyme. Bars represent the mean + SEM for each group n=3 to 5 for each bar. The same extracts were used for all enzymes tested.

Fig 2: Immunohistochemical analysis of AKR1B1 and AKR1C3 protein expression in human endometrium during the menstrual cycle. Representative examples of endometrial samples labelled with AKR1B1 (top) or AKR1C3 (bottom) antibodies. On the left we can see background coloration in presence of pre-immune serum. For each enzyme results are shown at the mid-proliferative (MP) or mid-secretory (MS) period. Note abundant expression of AKR1B1 in luminal and glandular epithelium and stroma during the secretory phase and absence of AKR1C3 in the stromal compartment.

Figure 3: Subjective quantitation and localisation of PG biosynthetic enzymes in the human endometrium. Endometrial biopsies were incubated with antibodies against AKR1B1, AKR1C3, COX-1 and COX-2, and IHC staining intensity levels were quantitated subjectively by three different observers. Staining in epithelial and stromal cells was considered separately and intensity scored between 1 (no staining) and 4 (clear heavy staining). Results represent the mean = SEM of the scores of the three observers.

Figure 4: AKR1B1 silencing. Northern and Western blot analyses of COX-2 and AKR1B1 mRNA and protein were done in HIESC2 cells transfected with a selective AKR1B1 siRNA to down regulate gene expression and compared with non transfected cells. (A) AKR1B1 expression was evaluated at the mRNA (Northern) and protein (Western) levels and selectivity verified on COX-2 (B) protein expression. PGF2α production was measured following treatment in absence or presence of IL-1β for 24 hours (C).

Figure 5: PGFS activity of AKR1B1. A) AKR1B1 activity was measured *in vitro* using 100 µg of purified recombinant enzyme in presence of 40 µM PGH2. Prostaglandins were extracted and analyzed by TLC as described in the material and methods section. B) Western analysis of AKR1B1 and AKR1C3 proteins following transfection of HIESC cells to overexpress these enzymes. C) Effect of increased AKR1B1 and AKR1C3 protein level on PGF2α production in presence and absence of exogenous AA.

Figure 6: Regulation of PGF2α production by AKR1B1 in endometrial explants cultures. Three different endometrial explants were prepared from fresh biopsies and treated with IL-1β as described in materials and methods. A) Effect of IL-1β (10ng/ml) treatment for 6 hrs on COX-2 and AKR1B1 protein levels analysed by Western blot. B) PGF2α production following stimulation with IL-1β for 6 hrs for the same endometrial explants.

Figure 7: Effect of the aldose reductase inhibitor Statil on PGF2α production in endometrial cells and endometrial explants. A) Endometrial epithelial (HIEEC) and stromal (HIESC) cells were grown to confluency and treated with increasing doses of Statil in presence of IL-1β at 10 ng/ml. After 6 hrs the culture medium was recovered for analysis of PGF2α by EIA. Results are expressed as % of production in presence of IL-1β alone and represent the mean + SEM of 3 experiments. B) Three different endometrial explants (different from those in Figure 6) have been stimulated by IL-1β for 6 hrs in presence or absence of Statil. Culture medium was harvested for the measurement of PGF2α expressed in terms of pg/µg of proteins.

Figure 8: The PGF2α production pathway in the human endometrium. The present study demonstrates that in endometrium, PGF2α is preferentially released by AKR1B1 from PGH_2 produced by COX-1 and COX-2. IL-1β (inflammation) increased PGF2α release by stimulating two enzymatic steps, COX-2 and AKR1B1. PGF2α production can be inhibited at the level of COXs by dexamethasone as well as selective (coxibs) and non selective (NSAIDs) COX inhibitors. This will also block the release of all other PGs sharing the same PGH2 substrate. A key finding of the present study is that PGF2α

production can be inhibited specifically by Statil an inhibitor of aldose reductase opening the way for selective inhibition of specific PGs. In the present case, ARIs such as Statil can be potentially efficient to treat menstrual pain.

Table1. Primers used for qRT-PCR

GENE SYMBOL	Name	Sequence ID	Forward primer	Reverse primer
AKR1B1	AKR1B1 aldo-keto reductase family 1, member B1 (aldose reductase)	NM_001628	5'-CTACCTTATTCACTGGCCGACT-3' (pos 395-416)	5'-GTTGGAGATGCCAATAGCTTTC-3' (pos 557-536)
AKR1C3	AKR1C3 aldo-keto reductase family 1, member C3 (3-alpha hydroxysteroid dehydrogenase, type II)	NM_003739	5'-TCTCTAAAGCCAGGTGAGGAAC-3' (pos 430-451)	5'-TACTTGAGTCCTGGCTTGTTGA-3' (pos 620-599)
PTGS1 (Cox-1)	PTGS1 prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	NM_000962	5'-CCCACCAGTTCTTCAAAACTTC-3' (pos 749-770)	5'-CCTGGTACTTGAGTTTCCCATC-3' (pos 898-877)
PTGS2 (Cox-2)	PTGS2 prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	NM_000963	5'-ATGAGTACCGCAAACGCTTTAT-3' (pos 1486-1507)	5'-GAATGGTGCTCCAACTTCTACC-3' (pos 1682-1661)
18S RNA	Human 18S rRNA gene	M10098	5'-GTAACCCGTTGAACCCCATT-3' (pos 1678-1697)	5'-CCATCCAATCGGTAGTAGCG-3' (pos 1828-1809)

Figure 1

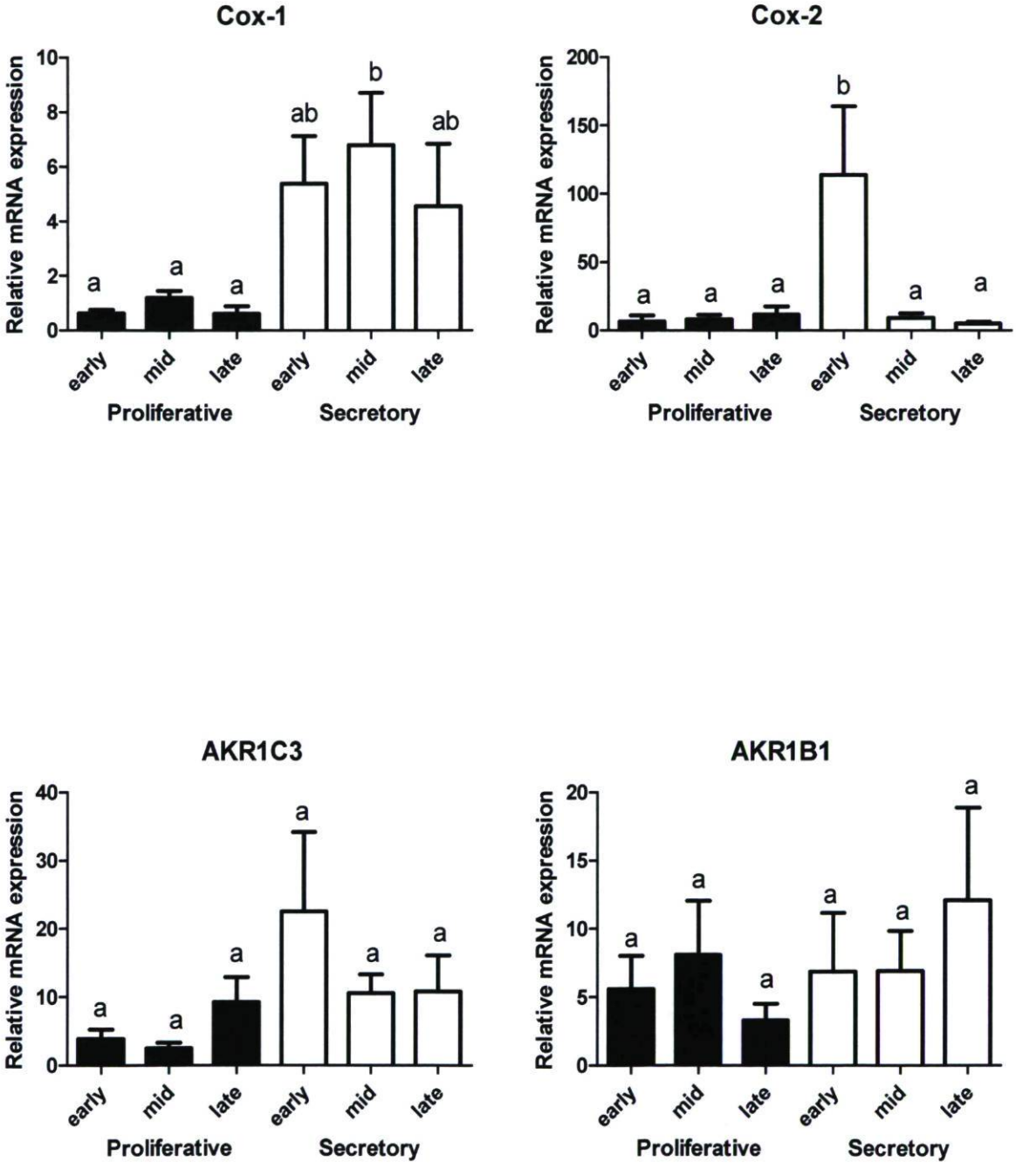

Cox-1
Relative mRNA expression
10
8
6
4
2
0
a
a
a
ab
b
ab
early
mid
late
early
mid
late
Proliferative
Secretory
Cox-2
Relative mRNA expression
200
150
100
50
0
a
a
a
b
a
a
early
mid
late
early
mid
late
Proliferative
Secretory
AKR1C3
Relative mRNA expression
40
30
20
10
0
a
a
a
a
a
a
early
mid
late
early
mid
late
Proliferative
Secretory
AKR1B1
Relative mRNA expression
20
15
10
5
0
a
a
a
a
a
a
early
mid
late
early
mid
late
Proliferative
Secretory

Figure 2

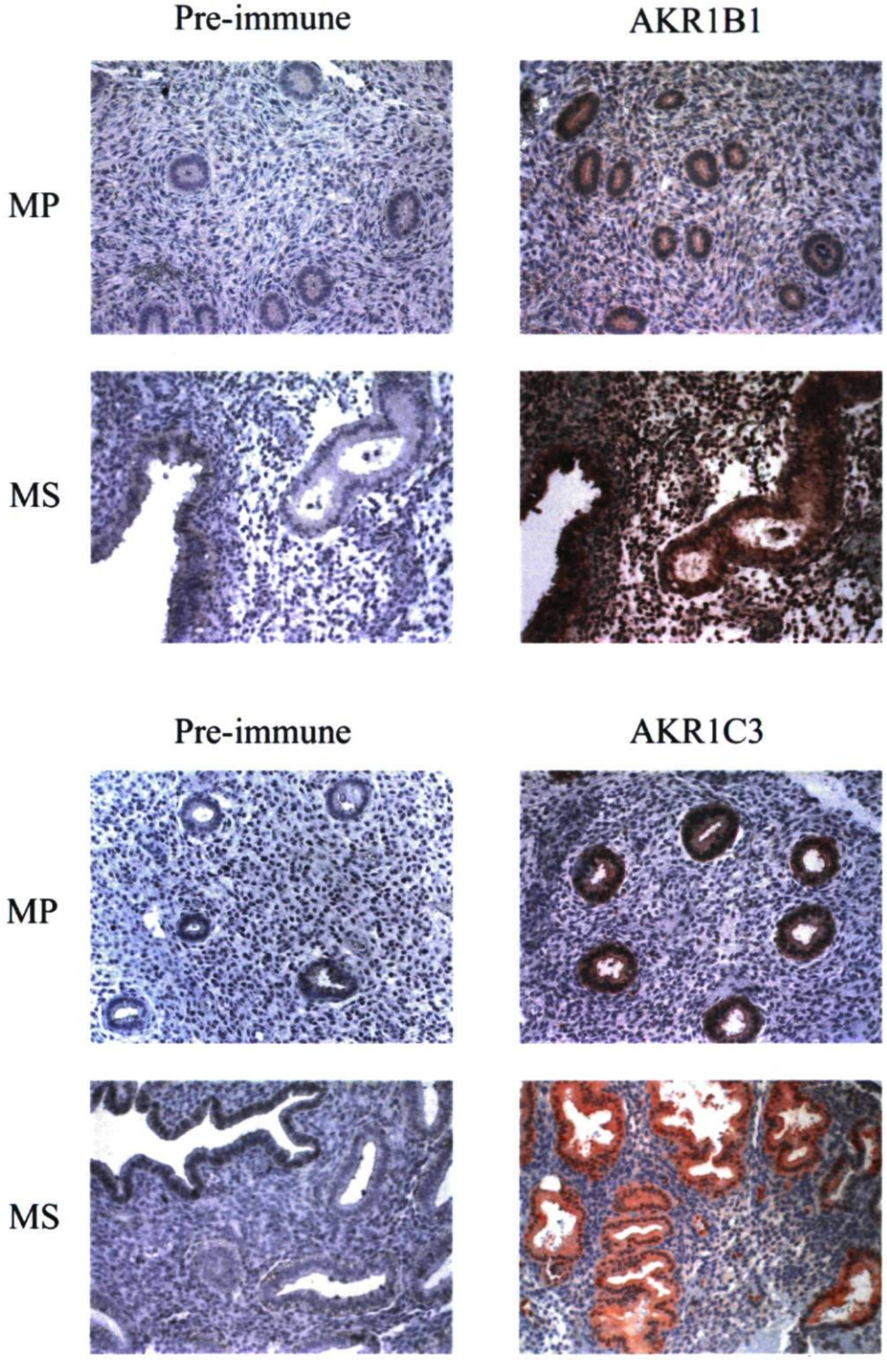
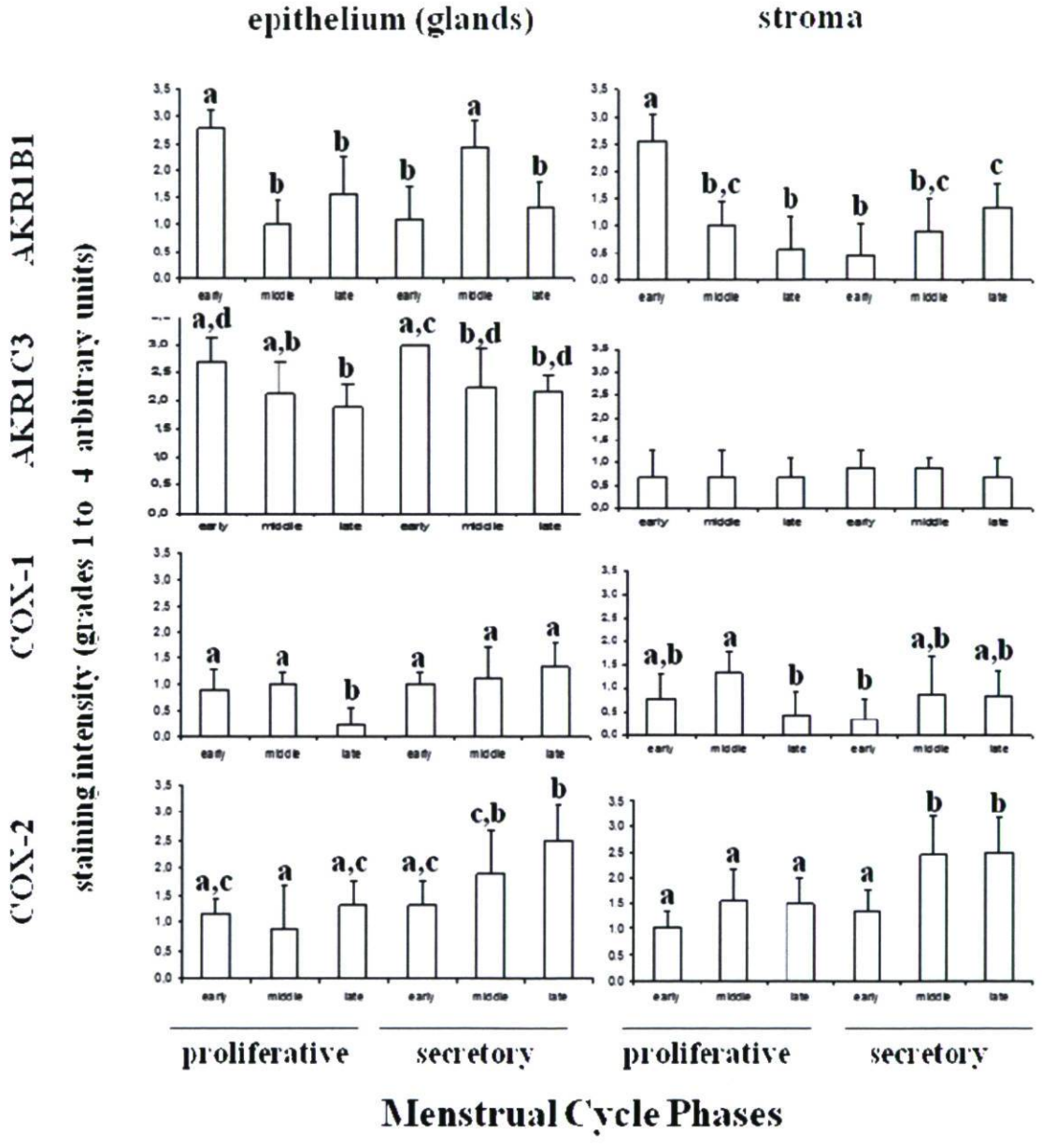


Figure 3



grade intensity: 1: 0
2: +
3: ++
4: +++

Figure 4

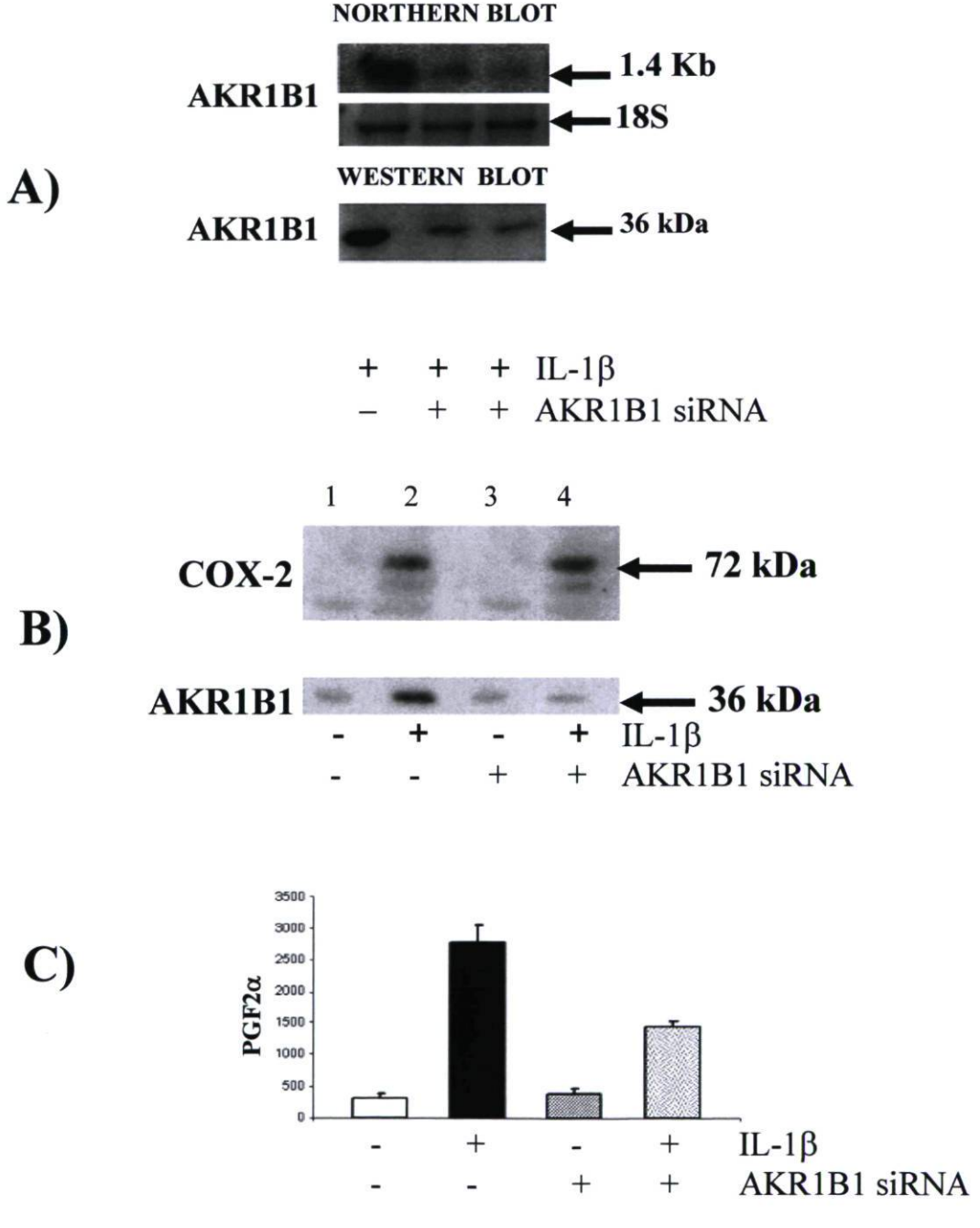

A)
NORTHERN BLOT
AKR1B1
1.4 Kb
18S
WESTERN BLOT
AKR1B1
36 kDa
+ + + IL-1β
– + + AKR1B1 siRNA
B)
1 2 3 4
COX-2
72 kDa
AKR1B1
36 kDa
- + - + IL-1β
- - + + AKR1B1 siRNA
C)
PGF2α
3500
3000
2500
2000
1500
1000
500
0
- + - + IL-1β
- - + + AKR1B1 siRNA

Figure 5

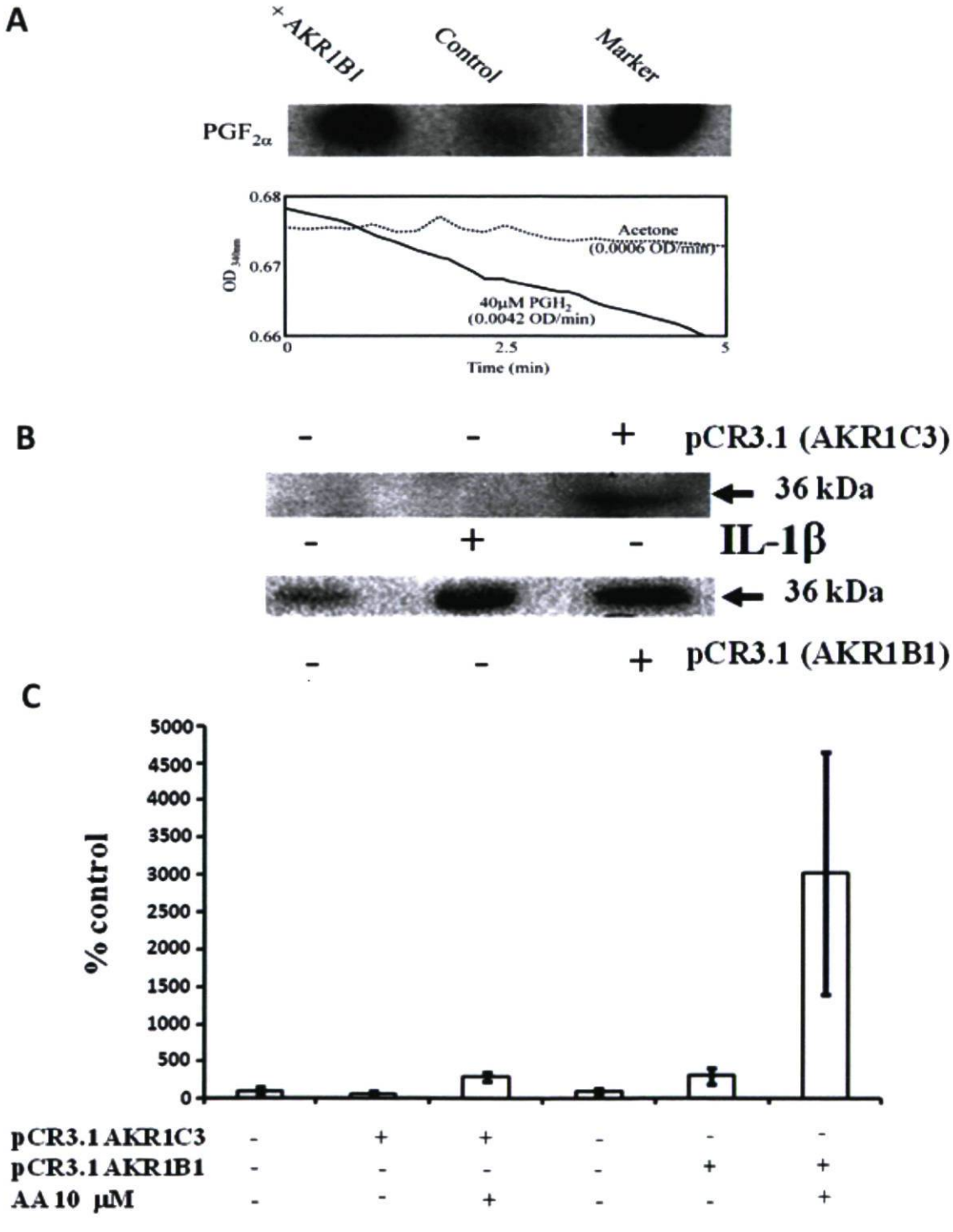

A
+AKR1B1
Control
Marker
PGF2α
0.68
0.67
0.66
OD 340nm
Acetone
(0.0006 OD/min)
40μM PGH2
(0.0042 OD/min)
0
2.5
5
Time (min)
B
- - + pCR3.1 (AKR1C3)
36 kDa
- + - IL-1β
36 kDa
- - + pCR3.1 (AKR1B1)
C
% control
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
pCR3.1 AKR1C3 - + + - - -
pCR3.1 AKR1B1 - - - - + +
AA 10 μM - - + - - +

Figure 6

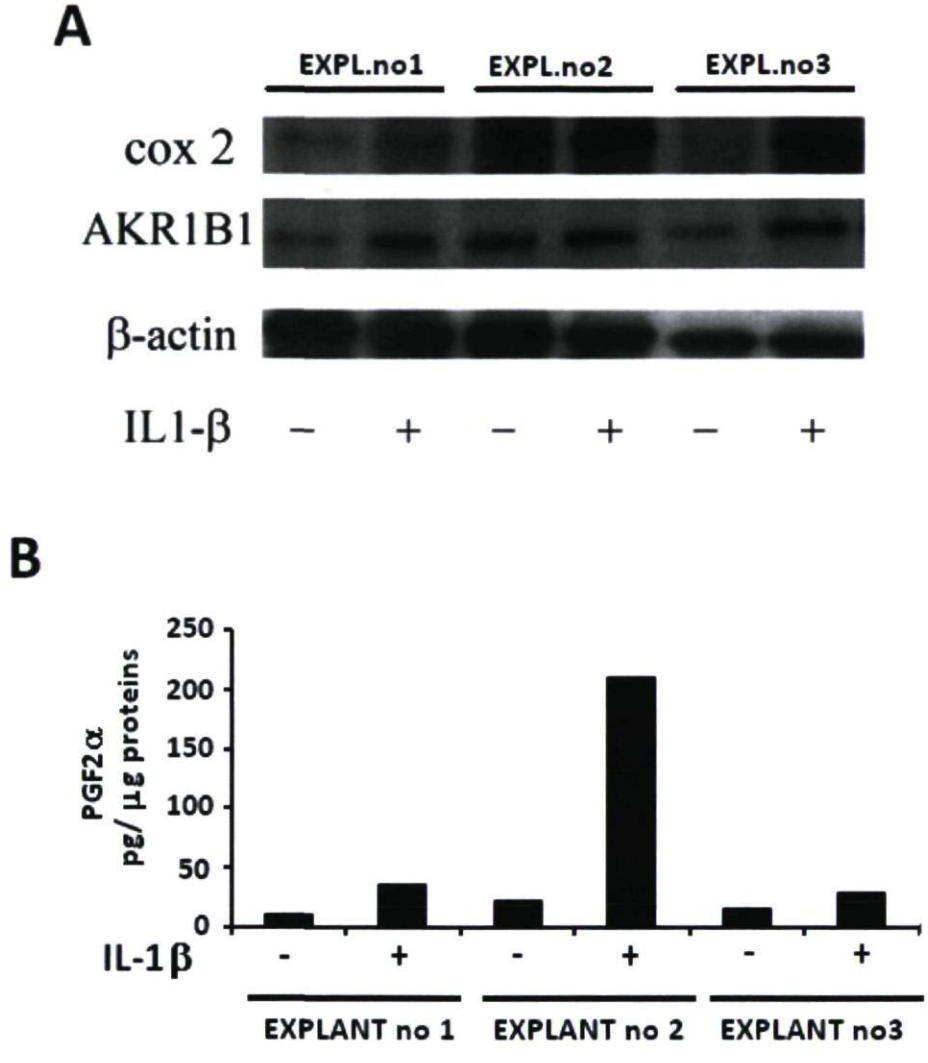
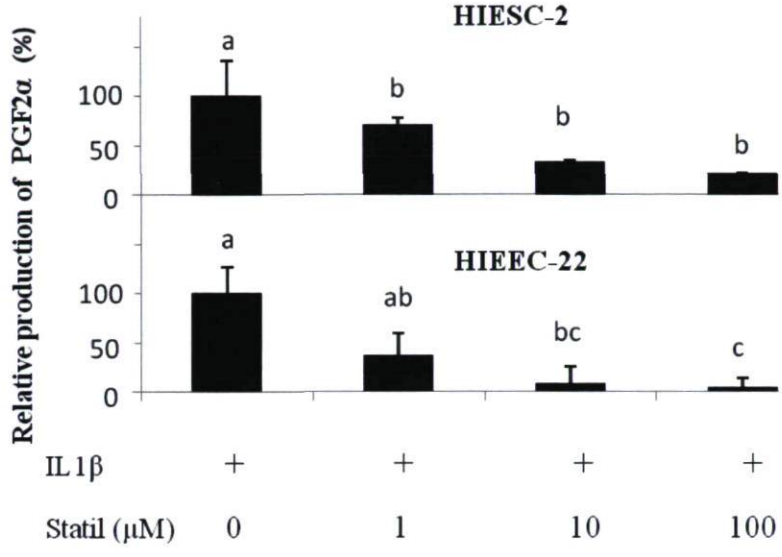

A
EXPL.no1
EXPL.no2
EXPL.no3
cox 2
AKR1B1
β-actin
IL1-β – + – + – +
B
PGF2α pg/ µg proteins
250
200
150
100
50
0
IL-1β - + - + - +
EXPLANT no 1
EXPLANT no 2
EXPLANT no3

Figure 7

A


HIESC-2
HIEEC-22
Relative production of PGF2α (%)
100
50
0
a
b
b
b
a
ab
bc
c
IL1β + + + +
Statil (μM) 0 1 10 100

B

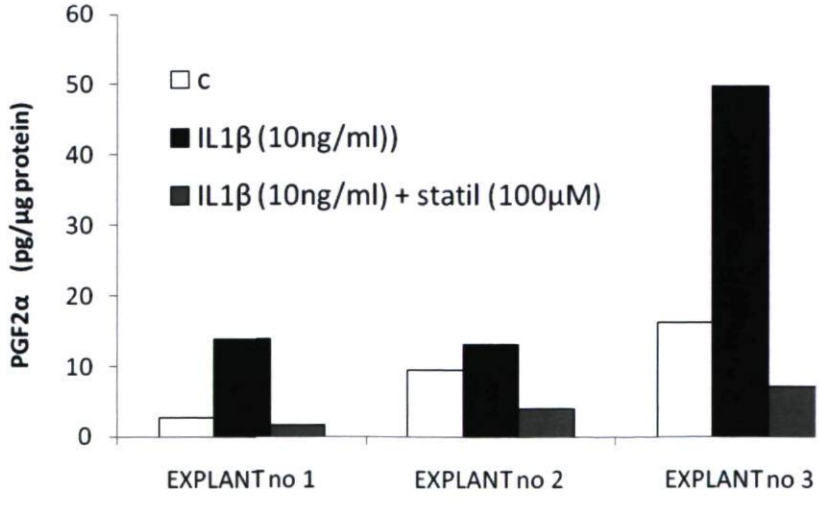
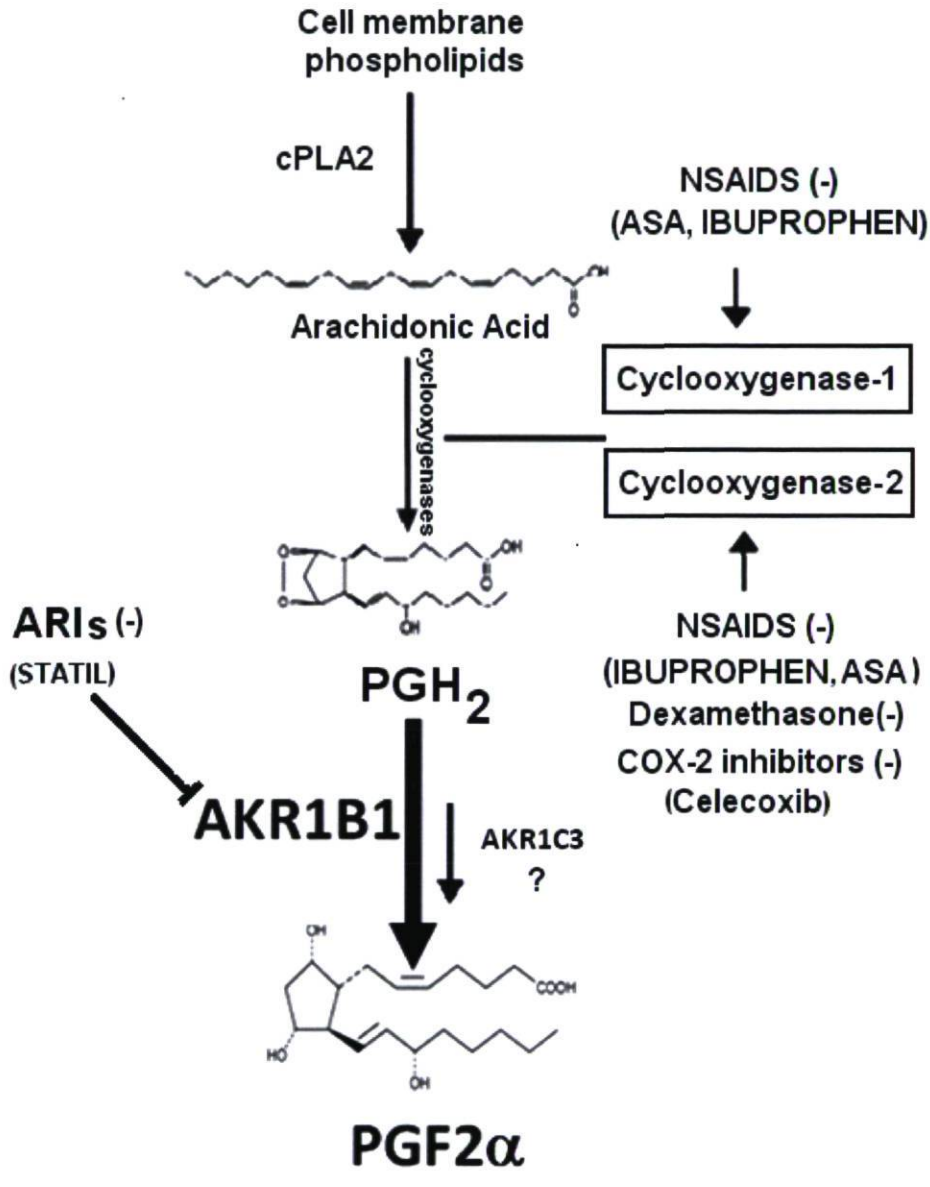

PGF2α (pg/μg protein)
60
50
40
30
20
10
0
c
IL1β (10ng/ml))
IL1β (10ng/ml) + statil (100μM)
EXPLANT no 1
EXPLANT no 2
EXPLANT no 3

Figure 8


Cell membrane phospholipids
cPLA2
Arachidonic Acid
NSAIDS (-)
(ASA, IBUPROPHEN)
Cyclooxygenase-1
Cyclooxygenase-2
cyclooxygenases
NSAIDS (-)
(IBUPROPHEN, ASA)
Dexamethasone(-)
COX-2 inhibitors (-)
(Celecoxib)
ARIs (-)
(STATIL)
PGH2
AKR1B1
AKR1C3
?
PGF2α

CHAPITRE 4

DISCUSSION GÉNÉRALE

Discussion générale

Les prostaglandines sont des régulateurs essentiels de la fonction reproductive chez la femme. L'inhibition des gènes COX-1 et COX-2 chez la souris l'a démontré en engendrant de multiples désordres du système reproducteur. (Lim, Paria et al. 1997) Les anomalies d'expression des PGs sont impliquées dans diverses pathologies du système reproducteur, dont les désordres menstruels (dysménorrhée, ménorragies). Des études in vitro s'avèrent essentielles pour étudier les mécanismes cellulaires et moléculaires associés à ces pathologies. Le premier choix de tissus lors de ces études sont les cultures primaires puisqu'elles reproduisent plus fidèlement le modèle in vivo. Elles ont d'ailleurs été utilisées, principalement les cultures mixtes et stromales, dans de nombreuses études sur la biosynthèse des PGs. Toutefois, étant donné la viabilité limitée des cellules épithéliales in vitro ainsi que la variabilité engendrée par l'utilisation de tissus provenant de diverses biopsies, notre équipe a décidé de produire des lignées cellulaires endométriales stromales et épithéliales.

Des lignées cellulaires stables et qui retiennent les caractéristiques morphologiques et phénotypiques des cellules in vivo ont donc été immortalisées. (Chapitre 2) Les cultures que nous avons produites apparaissent idéales pour étudier la contribution des PGs dans la régulation de la fonction de l'endomètre humain ainsi que dans les désordres menstruels. L'immortalisation de cellules épithéliales et stromales séparément permettera l'étude des différences entre ces deux types cellulaires, principalement au niveau de la voie de biosynthèse des PGs. Ces lignées ont servi par la suite à la caractérisation d'une nouvelle PGF synthase, AKR1B1, et à déterminer son patron d'expression dans les cellules endométriales in vitro (Chapitre 3). Ceci démontre l'utilité de nos cultures immortalisées dans les études in vitro. AKR1B1 est exprimé dans les cellules stromales et épithéliales de l'endomètre. Notre étude a également démontré que cette enzyme synthétise $PGF_{2\alpha}$ et cette enzyme semble donc être une vrai PGF synthase. AKR1B1 semble être la principale PGF synthase impliquée dans la production de $PGF_{2\alpha}$ dans l'endomètre humain, une prostaglandine essentielle à de nombreuses étapes du système reproducteur. De plus, nous avons étudié l'expression des

cyclooxygénases ainsi que des PGF synthases (AKR1B1, COX-1, COX-2, AKR1C3) chez les femmes lors du cycle menstruel (Chapitre 3).

De nombreuses études indiquent une corrélation entre les saignements menstruels abondants, les périodes douloureuses et la production de PGs. (Sales and Jabbour 2003; Jabbour and Sales 2004) Les anti-inflammatoires non stéroïdiens (AINS) sont couramment utilisés pour soulager les symptômes des désordres menstruels, sans pour autant connaître leur spécificité d'inhibition des PGs. Chaque type d'AINS a un profil particulier d'inhibition des enzymes de biosynthèse des prostaglandines, les plus connus sont les COX-1 et COX-2. Les lignées cellulaires pourront être utilisées pour l'étude de l'impact des différentes classes d'AINS sur l'endomètre humain, des médicaments clés dans le traitement de pathologies du système reproducteur (dysménorrhées, ménorragies, déclenchements d'accouchements…) ainsi que de nombreuses maladies touchant tout le corps humain (maladies cardio-vasculaires, maladies inflammatoires, troubles oculaires, fièvre, douleur et j'en passe…). Les AINS sont les médicaments les plus consommés en Amérique du Nord et la majorité se retrouvent en vente libre. Ils causent pourtant de nombreux effets secondaires, dont la physiologie n'est pas comprise entièrement. Les lignées cellulaires nouvellement immortalisées nous ont ouvert la porte pour faire des études plus approfondies sur l'inhibition spécifique des différentes prostaglandines par les AINS et leurs mécanismes d'action intra-cellulaires. L'organe d'étude étant l'endomètre humain, un tissu déjà réceptif à l'action des prostaglandines de par sa fonction en reproduction. À l'aide des lignées cellulaires, nous avons obtenu les IC_{50} pour l'inhibition de $PGF_{2\alpha}$ et de PGE_2 des AINS les plus couramment utilisés (résultats à venir). Le modèle cellulaire habituellement utilisé pour déterminer les IC_{50} est la TxA_2 et la PGI_2 dans le système vasculaire. Notre nouveau modèle cellulaire endométrial pourra distinguer la spécificité des AINS pour inhiber chaque type de PG et pourra éclairer leur contribution au soulagement des désordres menstruels.

Très peu d'études ont été faites pour identifier des variations dans les voies de biosynthèse des prostaglandines liées aux pathologies de l'endomètre et spécifiquement

en ce qui concerne les synthases terminales. L'objectif à long terme du laboratoire est d'évaluer l'expression distincte des diverses enzymes de biosynthèse des PGs dans les tissus endométriaux provenant de femmes souffrant de troubles menstruels (dysménorrhée primaire, ménorragies) comparativement aux femmes saines. Nous croyons que les différents désordres menstruels se caractériseront par un patron d'expression distinct. Cela permettrait de mieux connaître la pathophysiologie de ces désordres et de développer des stratégies de traitement plus spécifiques pour les femmes souffrant de ces désordres menstruels. Ces nouveaux traitements pourraient permettre aux femmes de conserver leur fertilité et de minimiser les effets secondaires aux traitements.

Bibliographie

Albers, J. R., S. K. Hull, et al. (2004). "Abnormal uterine bleeding." Am Fam Physician **69**(8): 1915-26.

Alfaidy, N., M. Sun, et al. (2003). "Expression of membrane prostaglandin E synthase in human placenta and fetal membranes and effect of labor." Endocrine **20**(3): 219-25.

Allison, M. C., A. G. Howatson, et al. (1992). "Gastrointestinal damage associated with the use of nonsteroidal antiinflammatory drugs." N Engl J Med **327**(11): 749-54.

Apgar, B. S., A. H. Kaufman, et al. (2007). "Treatment of menorrhagia." Am Fam Physician **75**(12): 1813-9.

Arosh, J. A., S. K. Banu, et al. (2004). "Prostaglandin biosynthesis, transport, and signaling in corpus luteum: a basis for autoregulation of luteal function." Endocrinology **145**(5): 2551-60.

Asselin, E., A. K. Goff, et al. (1996). "Influence of sex steroids on the production of prostaglandins F2 alpha and E2 and response to oxytocin in cultured epithelial and stromal cells of the bovine endometrium." Biol Reprod **54**(2): 371-9.

Blumenthal, R. D., A. P. Taylor, et al. (2002). "Abnormal expression of the angiopoietins and Tie receptors in menorrhagic endometrium." Fertil Steril **78**(6): 1294-300.

Bos, C. L., D. J. Richel, et al. (2004). "Prostanoids and prostanoid receptors in signal transduction." Int J Biochem Cell Biol **36**(7): 1187-205.

Cameron, I. T., R. Haining, et al. (1990). "The effects of mefenamic acid and norethisterone on measured menstrual blood loss." Obstet Gynecol **76**(1): 85-8.

Challis, J. R. G., S. G. Matthews, et al. (2000). "Endocrine and paracrine regulation of birth at term and preterm." Endocr Rev **21**(5): 514-50.

Chandrasekharan, N. V., H. Dai, et al. (2002). "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**(21): 13926-31.

Chapdelaine, P., J. Kang, et al. (2006). "Decidualization and maintenance of a functional prostaglandin system in human endometrial cell lines following transformation with SV40 large T antigen." Mol Hum Reprod **12**(5): 309-19.

Chung, S. S., E. C. Ho, et al. (2003). "Contribution of polyol pathway to diabetes-induced oxidative stress." J Am Soc Nephrol **14**(8 Suppl 3): S233-6.

Clarke, A., N. Black, et al. (1995). "Indications for and outcome of total abdominal hysterectomy for benign disease: a prospective cohort study." Br J Obstet Gynaecol **102**(8): 611-20.

Coleman, R. A., W. L. Smith, et al. (1994). "International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes." Pharmacol Rev **46**(2): 205-29.

Connolly, T. P. (2003). "Cyclooxygenase-2 inhibitors in gynecologic practice." Clin Med Res **1**(2): 105-10.

Critchley, H. O., R. W. Kelly, et al. (1996). "Sex steroid regulation of leukocyte traffic in human decidua." Hum Reprod **11**(10): 2257-62.

Daikoku, T., S. Tranguch, et al. (2006). "Cyclooxygenase-1 is overexpressed in multiple genetically engineered mouse models of epithelial ovarian cancer." Cancer Res **66**(5): 2527-31.

Downie, J., N. L. Poyser, et al. (1974). "Levels of prostaglandins in human endometrium during the normal menstrual cycle." J Physiol **236**(2): 465-472.

Dudek, P. and D. Picard (2004). "TROD: T7 RNAi Oligo Designer." Nucleic Acids Res **32**(Web Server issue): W121-3.

FitzGerald, G. A. (2002). "Cardiovascular pharmacology of nonselective nonsteroidal anti-inflammatory drugs and coxibs: clinical considerations." Am J Cardiol **89**(6A): 26D-32D.

Fortier, M. A., K. Krishnaswamy, et al. (2008). "A postgenomic integrated view of prostaglandins in reproduction: implications for other body systems." J Physiol Pharmacol **59 Suppl 1**: 65-89.

Fraser, I. S., H. O. Critchley, et al. (2007). "Can we achieve international agreement on terminologies and definitions used to describe abnormalities of menstrual bleeding?" Hum Reprod **22**(3): 635-643.

Goni, A. Z., R. L. Lacruz, et al. (2009). "The levonorgestrel intrauterine system as an alternative to hysterectomy for the treatment of idiopathic menorrhagia." Gynecol Endocrinol: 1-6.

Green, K., M. Hamberg, et al. (1978). "Extraction and chromatographic procedures for purification of prostaglandins, thromboxanes, prostacyclin, and their metabolites." Adv Prostaglandin Thromboxane Res **5**: 15-38.

Hacker, N. F., J. G. Moore, et al. (2004). Essentials of obstetrics and gynecology. Philadelphia, Pa., Saunders.

Hagenfeldt, K. (1987). "The role of prostaglandins and allied substances in uterine haemostasis." Contraception **36**(1): 23-35.

Harel, Z. (2006). "Dysmenorrhea in adolescents and young adults: etiology and management." J Pediatr Adolesc Gynecol **19**(6): 363-71.

Helliwell, R. J., L. F. Adams, et al. (2004). "Prostaglandin synthases: recent developments and a novel hypothesis." Prostaglandins Leukot Essent Fatty Acids **70**(2): 101-13.

Hersh, E. V., E. T. Lally, et al. (2005). "Update on cyclooxygenase inhibitors: has a third COX isoform entered the fray?" Curr Med Res Opin **21**(8): 1217-26.

Ho, H. T., S. K. Chung, et al. (2000). "Aldose reductase-deficient mice develop nephrogenic diabetes insipidus." Mol Cell Biol **20**(16): 5840-6.

Huang, J. C., D. Y. Liu, et al. (1998). "Interleukin-1 beta induces cyclooxygenase-2 gene expression in cultured endometrial stromal cells." J Clin Endocrinol Metab **83**(2): 538-41.

Hwang, D., D. Scollard, et al. (1998). "Expression of cyclooxygenase-1 and cyclooxygenase-2 in human breast cancer." J Natl Cancer Inst **90**(6): 455-60.

Hwang, Y. C., M. Kaneko, et al. (2004). "Central role for aldose reductase pathway in myocardial ischemic injury." Faseb J **18**(11): 1192-9.

Jabbour, H. N., R. W. Kelly, et al. (2006). "Endocrine regulation of menstruation." Endocr Rev **27**(1): 17-46.

Jabbour, H. N. and K. J. Sales (2004). "Prostaglandin receptor signalling and function in human endometrial pathology." Trends Endocrinol Metab **15**(8): 398-404.

Jabbour, H. N., K. J. Sales, et al. (2006). "Prostaglandin receptors are mediators of vascular function in endometrial pathologies." Mol Cell Endocrinol **252**(1-2): 191-200.

Jones, R. L., R. W. Kelly, et al. (1997). "Chemokine and cyclooxygenase-2 expression in human endometrium coincides with leukocyte accumulation." Hum Reprod **12**(6): 1300-6.

Kang, J., P. Chapdelaine, et al. (2006). "Functional characterization of prostaglandin transporter and terminal prostaglandin synthases during decidualization of human endometrial stromal cells." Hum Reprod **21**(3): 592-599.

Kang, J., P. Chapdelaine, et al. (2005). "Expression of human prostaglandin transporter in the human endometrium across the menstrual cycle." J Clin Endocrinol Metab **90**(4): 2308-13.

Kennedy, C. R., Y. Zhang, et al. (1999). "Salt-sensitive hypertension and reduced fertility in mice lacking the prostaglandin EP2 receptor." Nat Med **5**(2): 217-20.

Kokawa, K., T. Shikone, et al. (1996). "Apoptosis in the human uterine endometrium during the menstrual cycle." J Clin Endocrinol Metab **81**(11): 4144-7.

Komoto, J., T. Yamada, et al. (2006). "Prostaglandin F2alpha formation from prostaglandin H2 by prostaglandin F synthase (PGFS): crystal structure of PGFS containing bimatoprost." Biochemistry **45**(7): 1987-96.

Kudo, I. and M. Murakami (2002). "Phospholipase A2 enzymes." Prostaglandins Other Lipid Mediat **68-69**: 3-58.

Leslie, C. C. (1997). "Properties and regulation of cytosolic phospholipase A2." J Biol Chem **272**(27): 16709-12.

Lete, I., C. Obispo, et al. (2008). "The levonorgestrel intrauterine system (Mirena) for treatment of idiopathic menorrhagia. Assessment of quality of life and satisfaction." Eur J Contracept Reprod Health Care **13**(3): 231-7.

Lethaby, A., C. Augood, et al. (2007). "Nonsteroidal anti-inflammatory drugs for heavy menstrual bleeding." Cochrane Database Syst Rev(4): CD000400.

Lim, H., R. A. Gupta, et al. (1999). "Cyclo-oxygenase-2-derived prostacyclin mediates embryo implantation in the mouse via PPARdelta." Genes Dev **13**(12): 1561-74.

Lim, H., B. C. Paria, et al. (1997). "Multiple female reproductive failures in cyclooxygenase 2-deficient mice." Cell **91**(2): 197-208.

Lo, A. C., A. K. Cheung, et al. (2007). "Deletion of aldose reductase leads to protection against cerebral ischemic injury." J Cereb Blood Flow Metab **27**(8): 1496-509.

Lockwood, C. J. and F. Schatz (1996). "A biological model for the regulation of peri-implantational hemostasis and menstruation." J Soc Gynecol Investig **3**(4): 159-65.

Lundstrom, V. (1977). "The myometrial response to intra-uterine administration of PGF2alpha and PGE2 in dysmenorrheic women." Acta Obstet Gynecol Scand **56**(3): 167-72.

Lundstrom, V., K. Green, et al. (1976). "Prostaglandins, indomethacin and dysmenorrhea." Prostaglandins **11**(5): 893-907.

Madore, E., N. Harvey, et al. (2003). "An aldose reductase with 20 alpha-hydroxysteroid dehydrogenase activity is most likely the enzyme responsible for the production of prostaglandin f2 alpha in the bovine endometrium." J Biol Chem **278**(13): 11205-12.

Marions, L. and K. G. Danielsson (1999). "Expression of cyclo-oxygenase in human endometrium during the implantation period." Mol Hum Reprod **5**(10): 961-5.

Milne, S. A. and H. N. Jabbour (2003). "Prostaglandin (PG) F(2alpha) receptor expression and signaling in human endometrium: role of PGF(2alpha) in epithelial cell proliferation." J Clin Endocrinol Metab **88**(4): 1825-32.

Milne, S. A., G. B. Perchick, et al. (2001). "Expression, localization, and signaling of PGE(2) and EP2/EP4 receptors in human nonpregnant endometrium across the menstrual cycle." J Clin Endocrinol Metab **86**(9): 4453-4459.

Morita, I. (2002). "Distinct functions of COX-1 and COX-2." Prostaglandins Other Lipid Mediat **68-69**: 165-75.

Mukherjee, D., S. E. Nissen, et al. (2001). "Risk of cardiovascular events associated with selective COX-2 inhibitors." JAMA **286**(8): 954-9.

Murakami, M. and I. Kudo (2004). "Recent advances in molecular biology and physiology of the prostaglandin E2-biosynthetic pathway." Prog Lipid Res **43**(1): 3-35.

Nakashima, K., N. Ueno, et al. (2003). "Coupling between cyclooxygenases and prostaglandin F(2alpha) synthase. Detection of an inducible, glutathione-activated, membrane-bound prostaglandin F(2alpha)-synthetic activity." Biochim Biophys Acta **1633**(2): 96-105.

Narumiya, S. and G. A. FitzGerald (2001). "Genetic and pharmacological analysis of prostanoid receptor function." J Clin Invest **108**(1): 25-30.

Narumiya, S., Y. Sugimoto, et al. (1999). "Prostanoid receptors: structures, properties, and functions." Physiol Rev **79**(4): 1193-226.

Niiro, H., T. Otsuka, et al. (1997). "Regulation by interleukin-10 and interleukin-4 of cyclooxygenase-2 expression in human neutrophils." Blood **89**(5): 1621-8.

Norel, X. (2007). "Prostanoid receptors in the human vascular wall." ScientificWorldJournal **7**: 1359-74.

Noyes, R., A. Hertig, et al. (1950). "Dating the endometrial biopsy." Fertil Steril **1**: 3-25.

Okazaki, M., T. Matsuyama, et al. (2005). "Induction of epithelial cell apoptosis in the uterus by a mouse uterine ischemia-reperfusion model: possible involvement of tumor necrosis factor-alpha." Biol Reprod **72**(5): 1282-8.

Olofsson, J. and P. C. Leung (1994). "Auto/paracrine role of prostaglandins in corpus luteum function." Mol Cell Endocrinol **100**(1-2): 87-91.

Pairet, M. and J. van Ryn (1998). "Experimental models used to investigate the differential inhibition of cyclooxygenase-1 and cyclooxygenase-2 by non-steroidal anti-inflammatory drugs." Inflamm Res **47 Suppl 2**: S93-101.

Parent, J., P. Chapdelaine, et al. (2005). "Molecular cloning and tissue distribution of microsomal-1 and cytosolic prostaglandin E synthases in macaque." Prostaglandins Other Lipid Mediat **78**(1-4): 27-37.

Pelletier, G., V. Luu-The, et al. (1999). "Immunocytochemical localization of type 5 17beta-hydroxysteroid dehydrogenase in human reproductive tissues." J Histochem Cytochem **47**(6): 731-8.

Proctor, M. and C. Farquhar (2006). "Diagnosis and management of dysmenorrhoea." Bmj **332**(7550): 1134-8.

Rainsford, K. D. (2005). "Introduction - The coxib controversies." Inflammopharmacology **13**(4): 331-41.

Rao, P. and E. E. Knaus (2008). "Evolution of nonsteroidal anti-inflammatory drugs (NSAIDs): cyclooxygenase (COX) inhibition and beyond." J Pharm Pharm Sci **11**(2): 81s-110s.

Riendeau, D., S. Charleson, et al. (1997). "Comparison of the cyclooxygenase-1 inhibitory properties of nonsteroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors, using sensitive microsomal and platelet assays." Can J Physiol Pharmacol **75**(9): 1088-95.

Rossi, M., A. M. Sharkey, et al. (2005). "Identification of genes regulated by interleukin-1beta in human endometrial stromal cells." Reproduction **130**(5): 721-9.

Sales, K. J. and H. N. Jabbour (2003). "Cyclooxygenase enzymes and prostaglandins in pathology of the endometrium." Reproduction **126**(5): 559-67.

Sales, K. J. and H. N. Jabbour (2003). "Cyclooxygenase enzymes and prostaglandins in reproductive tract physiology and pathology." Prostaglandins Other Lipid Mediat **71**(3-4): 97-117.

Sales, K. J., A. A. Katz, et al. (2002). "Cyclooxygenase-1 is up-regulated in cervical carcinomas: autocrine/paracrine regulation of cyclooxygenase-2, prostaglandin e receptors, and angiogenic factors by cyclooxygenase-1." Cancer Res **62**(2): 424-32.

Schuster, V. L. (2002). "Prostaglandin transport." Prostaglandins Other Lipid Mediat **68-69**: 633-47.

Seibert, K., Y. Zhang, et al. (1994). "Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain." Proc Natl Acad Sci U S A **91**(25): 12013-7.

Siebert, P. D. and J. W. Larrick (1992). "Competitive PCR." Nature **359**(6395): 557-8.

Simon, C., A. Mercader, et al. (1997). "The interleukin-1 system and human implantation." Am J Reprod Immunol **37**(1): 64-72.

Smith, O. P., H. N. Jabbour, et al. (2007). "Cyclooxygenase enzyme expression and E series prostaglandin receptor signalling are enhanced in heavy menstruation." Hum Reprod **22**(5): 1450-6.

Smith, S. K., M. H. Abel, et al. (1981). "Prostaglandin synthesis in the endometrium of women with ovular dysfunctional uterine bleeding." Br J Obstet Gynaecol **88**(4): 434-42.

Smith, S. K. and R. W. Kelly (1988). "The release of PGF2 alpha and PGE2 from separated cells of human endometrium and decidua." Prostaglandins Leukot Essent Fatty Acids **33**(2): 91-6.

Smith, W. L., R. M. Garavito, et al. (1996). "Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2." J Biol Chem **271**(52): 33157-33160.

Smith, W. L. and I. Song (2002). "The enzymology of prostaglandin endoperoxide H synthases-1 and -2." Prostaglandins Other Lipid Mediat **68-69**: 115-28.

Song, H., H. Lim, et al. (2002). "Cytosolic phospholipase A2alpha is crucial [correction of A2alpha deficiency is crucial] for 'on-time' embryo implantation that directs subsequent development." Development **129**(12): 2879-89.

Srivastava, S. K., K. V. Ramana, et al. (2005). "Role of aldose reductase and oxidative damage in diabetes and the consequent potential for therapeutic options." Endocr Rev **26**(3): 380-92.

Stichtenoth, D. O., S. Thoren, et al. (2001). "Microsomal prostaglandin E synthase is regulated by proinflammatory cytokines and glucocorticoids in primary rheumatoid synovial cells." J Immunol **167**(1): 469-74.

Sugimoto, T., T. Koizumi, et al. (2007). "Correlative expression of cyclooxygenase-1 (Cox-1) and human epidermal growth factor receptor type-2 (Her-2) in endometrial cancer." Kobe J Med Sci **53**(5): 177-87.

Sugimoto, Y., T. Namba, et al. (1992). "Cloning and expression of a cDNA for mouse prostaglandin E receptor EP3 subtype." J Biol Chem **267**(10): 6463-6.

Sugimoto, Y., A. Yamasaki, et al. (1997). "Failure of parturition in mice lacking the prostaglandin F receptor." Science **277**(5326): 681-3.

Tamura, M., S. Sebastian, et al. (2002). "Interleukin-1beta elevates cyclooxygenase-2 protein level and enzyme activity via increasing its mRNA stability in human endometrial stromal cells: an effect mediated by extracellularly regulated kinases 1 and 2." J Clin Endocrinol Metab **87**(7): 3263-73.

Tanaka, T., N. Umesaki, et al. (1998). "Enhancement of apoptotic susceptibility by interleukin-1 beta in human endometrial epithelial cells." Gynecol Endocrinol **12**(5): 315-9.

Tschugguel, W., C. Schneeberger, et al. (1999). "Elevation of inducible nitric oxide synthase activity in human endometrium during menstruation." Biol Reprod **60**(2): 297-304.

Ueland, K. and J. T. Conrad (1983). "Characteristics of oral prostaglandin E2-induced labor." Clin Obstet Gynecol **26**(1): 87-94.

Ueno, N., Y. Takegoshi, et al. (2005). "Coupling between cyclooxygenases and terminal prostanoid synthases." Biochem Biophys Res Commun **338**(1): 70-6.

Watanabe, K. (2002). "Prostaglandin F synthase." Prostaglandins Other Lipid Mediat **68-69**: 401-7.

Whitehouse, M. W. (2005). "Prostanoids as friends, not foes: further evidence from the interference by cycloxygenase-inhibitory drugs when inducing tolerance to experimental arthritigens in rats." Inflammopharmacology **12**(5): 481-92.

Yoshida, M., N. Sagawa, et al. (2002). "Prostaglandin F(2alpha), cytokines and cyclic mechanical stretch augment matrix metalloproteinase-1 secretion from cultured human uterine cervical fibroblast cells." Mol Hum Reprod **8**(7): 681-7.

Zeilhofer, H. U. (2007). "Prostanoids in nociception and pain." Biochem Pharmacol **73**(2): 165-74.

Zeilhofer, H. U. and K. Brune (2006). "Analgesic strategies beyond the inhibition of cyclooxygenases." Trends Pharmacol Sci **27**(9): 467-74.

Annexe A

A POSTGENOMIC INTEGRATRED VIEW OF PROSTAGLANDINS IN REPRODUCTION: IMPLICATIONS FOR OTHER BODY SYSTEMS.

JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2008, 59, Suppl 1, 65–89
www.jpp.krakow.pl

M.A. FORTIER, K. KRISHNASWAMY, G. DANYOD,
S.BOUCHER-KOVALIK, P. CHAPDELAINE J.A.

A POSTGENOMIC INTEGRATRED VIEW OF PROSTAGLANDINS IN REPRODUCTION: IMPLICATIONS FOR OTHER BODY SYSTEMS.

Unité de Recherche en Ontogénie et Reproduction, Centre de Recherche du CHUQ (CHUL), Québec, Québec GIV4G2, Canada and Centre de Recherche en Biologie de la Reproduction (CRBR) Département d'Obstétrique et Gynécologie, Université Laval, Ste-Foy, Québec GIK7P4, Canada.

Prostaglandins are primary mediators of pain and are involved in pathological conditions such as hypertension, cancer and inflammation but are also needed for normal function of the female reproductive system. This may hold true for other systems because long term use of selective COX-2 inhibitors such as VIOXX and BEXTRA was associated with heart failure, leading to their withdrawal. A thorough study of the contribution of prostaglandins in the regulation of normal body function is clearly needed. A major drawback of the current therapeutic strategies aiming at controlling PGs is that they aim at early steps of biosynthesis thus blocking all PGs, good and bad. However, PGs often work as opposing dyads such as PGI2-TXA2 in the vascular system and PGF2α-PGE2 in the female reproductive system. The paradigm thus appears as effecting selective synthesis, transport and action of individual PG isoforms. In this respect, the female reproductive system appears as an ideal study model. Data from human and animal genome projects allowed identifying the corresponding members of the biosynthetic and signal transduction components of the PG system in different animal species. Of particular interest was that PG terminal synthase shared similarities or identity with enzymes previously known for steroid or sugar metabolism and free radical detoxification. We present here an integrated view of PG action based on observations in the female reproductive system, but with potential strategic implications for cardiovascular and metabolic complications.

K e y w o r d s : Prostaglandis, female reproduction, fertility, aldose reductase, menstrual disorders, ischemia

Prostaglandin biosynthesis

Prostaglandins are notorious mediators of pain, fever, inflammation and hypertension, and their production has been a target for pharmacological therapy with non steroidal anti-inflammatory drugs (NSAIDs) for more than a century. PGs are produced by all nucleated cells of the body and act locally in a paracrine or autocrine fashion. The first limiting step in the generation of eicosanoids is the liberation of arachidonic acid from membrane phospholipids by phospholipases and the most relevant for the production of PGs from arachidonic acid is cPLA2α(1).

Arachidonate can then be sequentially transformed into leukotrienes, not covered in the present review, and different active prostanoids (Fig. 1). PGH2, the common precursor of all PGs is generated from arachidonic acid (AA) by prostaglandin synthase (PGHS or COX). There are two isoforms encoded by distinct genes (2) the constitutive isoform, COX-1, is widely expressed in a variety of tissues and cells, whereas the inducible form, COX-2, is regulated by factors such as: cytokines or tumour promoters (3). A splice variant of COX-1

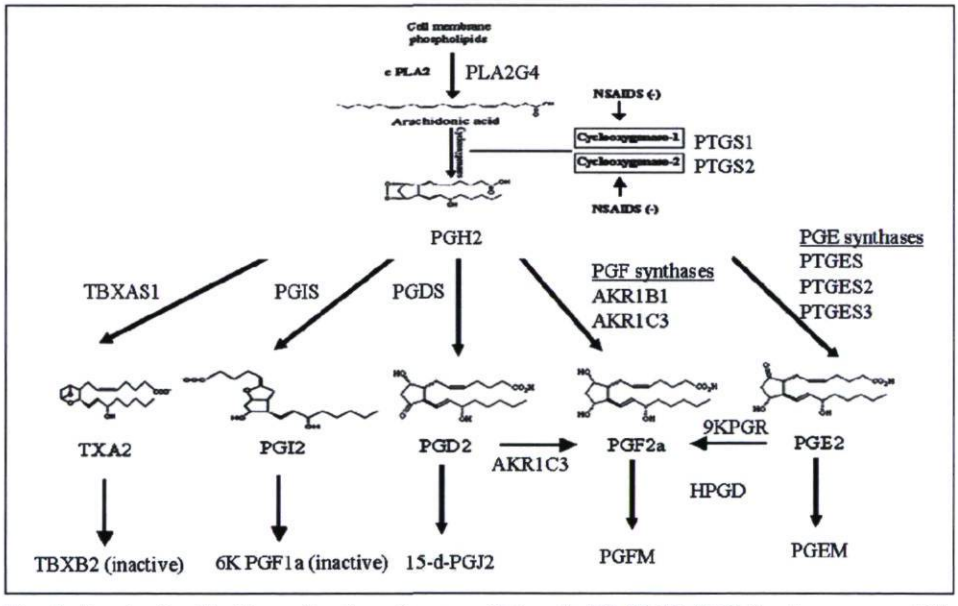


Fig. 1. Prostaglandin biosynthesis pathways. Cytosolic PLA2 (PLA2G4) releases arachidonic acid (AA) from membrane phospholipids and COX enzymes (PTGS1, PTGS2) convert it to PGG2 and PGH2, the common precursor for all PGs. PGH2 is then converted into one of the active PG by specific terminal synthases such as PGE synthases (PTGES, PTGES2, PTGES3), PGF synthases (AKR1B1, AKR1C3), PGD synthase (PGDS), PGI synthase (PGIS) and Thromboxane synthase (TXA1S). PGE2 and PGF2a are inactivated into PGEM and PGFM by HPGD (15-PGDH), PGD2 converts spontaneously into active PGJ2 whereas unstable PGI2 and TXA2 convert into inactive 6K-PGF1α and TXB2.

was referred to as COX-3, but its contribution to physiological or pathological conditions remains speculative. COX-1 is constitutively expressed in most tissues and responsible for housekeeping functions and immediate response to levels of AA above 10 μM. COX-2 is regulated by factors such as cytokines or tumour promoters and supports sustained production of PGs from relatively low levels of AA (below 2.5 μM) (4). PGH2 produced by COXs is the common precursor for generation of primary PGs including PGE2, PGF2α, PGD2, PGI2 and TxA2 by cell-specific isomerases and synthases such as PGES, PGFS, PGDS, PGIS and TXAS, respectively.

The physiological importance of prostaglandins has been confirmed in the mouse where targeted disruption of COX-1 (5) or COX-2 genes (6) resulted in severe nephropathy or reduced reproductive efficiency in homozygous null mice. In fact, female COX-2 null mice suffered from multiple failures in reproductive processes (7). Other studies have shown that COX-2 expressed in COX-1

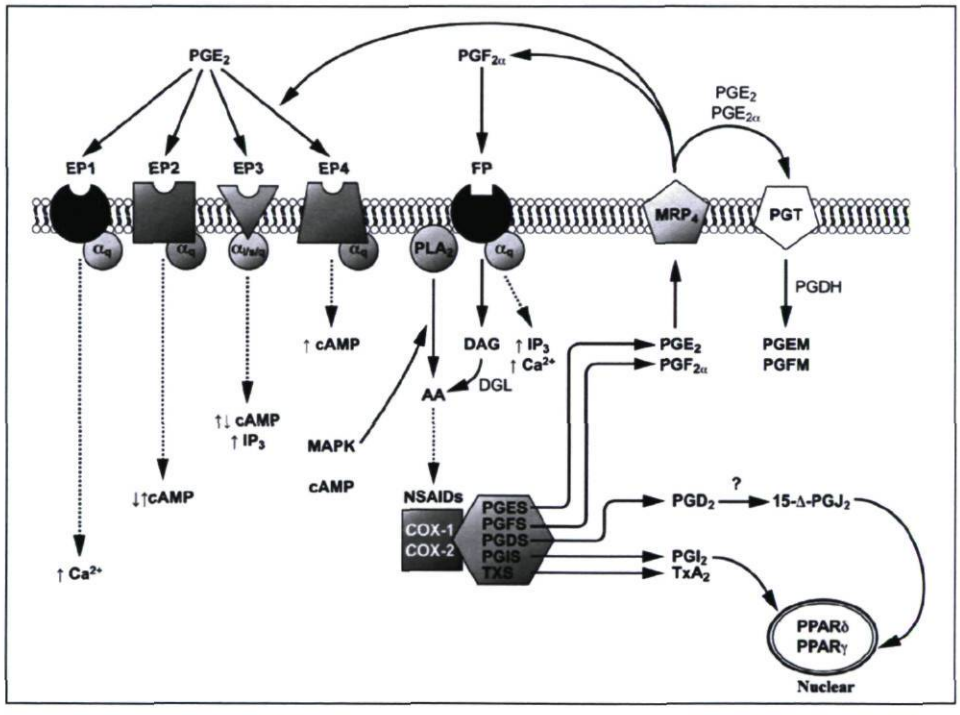


Fig. 2. Prostaglandin signalling pathways. Immediately upon biosynthesis, PGs exit the site of production passively or through constitutively expressed facilitated transport (MRP4) and either bind to specific membrane receptors in an autocrine or paracrine manner. PGE2 and PGF2α can travel across successive cell layers through PGT or enter target cells to act on nuclear receptors or be inactivated by 15-PGDH. The membrane DP, EP1-4, FP, IP and TP receptors are coupled to diverse G protein and second messengers as illustrated. PGJ2, the spontaneous active metabolite of PGD2 is the physiological ligand for the nuclear receptor PPARγ whereas PGI2 binds to PPARδ.

deficient mice was able to compensate at least partly (8). COX-1 -/- and COX-2-/- double knockouts induced death early after birth of the pups suggesting that PGs might be more important for survival than initially anticipated (9, 10).

Pharmacological control of PG biosynthesis is more than a century old. Indeed, Aspirin (ASA) was the first non steroidal anti-inflammatory drug (NSAID) commercialized. ASA shares with newer drugs like ibuprophen (ADVIL) the ability to inhibit non-selectively COX-1 and COX-2 activities (11). More recently, new inhibitors like NS-398 and SC-560 have been shown to specifically block either COX-2 (12) or COX-1 (13) opening the field for the development of more specific NSAIDs such as CELEBREX and VIOXX. However, severe side effects of COX-2 inhibition such as heart failure (14) and infertility (15) lead to the widely publicized withdrawal of VIOXX from the market. Total blockade of all PGs by NSAIDs provides a quick relieve of symptoms but unfortunately deprives from a physiological cure (16). In this respect, targeted action at the level of terminal synthases such as PGES and PGFS responsible for the selective production of PGE2 and PGF2α appears as promising and important to explore (17).

Prostaglandin signal transduction

The different prostaglandins exert a wide array of different or even opposite actions mediated by specific receptors sometimes taking multiple isoforms for a single prostaglandin (Fig. 2) (18, 19). PGs represent a class of local regulators with complementary or opposing actions depending on the type of PG or the receptor signalling their action. PGF2α acts through FP receptors coupled to Gq, PLC and Ca++ release whereas PGE2 acts through 4 classes of receptors, EP1 coupled to Gi and calcium channels, EP2 and EP4 coupled to Gs and cAMP generation, and EP3 for which there are 8 splice variants in the human coupled principally to the inhibitory Gi system (20). Considerable efforts were made to develop selective agonists and antagonists of PG receptors over the last 30 years, but most treatments aiming at controlling PG action are still based on systemic COX inhibition (21).

Peroxisome Proliferators-Activated Receptors (PPAR α,γ,δ) have been proposed as nuclear receptors for PGD_2 and PGI_2 (22). Recently EP2 and EP4 have been identified in the nuclear envelope suggesting the presence of functional nuclear receptors for PGE_2 (23). However, limited information is available on the putative actions of nuclear receptors.

Prostaglandins and reproduction

Apart from sex steroids, prostaglandins are probably the most important regulators of female reproductive function (ovulation, uterine receptivity, implantation and parturition) and associated pathologies (24). Reproductive tissues express different classes of prostaglandin receptors (25). Among the

different PGs, PGE2 and PGF2α are the main prostanoids produced in the human (26, 27) and bovine (28) endometrium. The physiological importance of PGs in reproduction has been confirmed in the mouse where targeted disruption of COX-1 or COX-2 genes reduced reproductive efficiency (5-7). Null mutation for cPLA2 a PG biosynthesis enzyme upstream of COX-2, also leads to an infertile phenotype (29). At the receptor level, deletion of the PGF2α receptor (FP) showed that it is necessary for parturition in the mouse (30) whereas EP2 receptors null mutants exhibit ovulation and peri-implantation problems (31, 32).

In the reproductive system, PGF2α and PGE_2 often exhibit opposite actions (Fig. 3) (33). The endometrial release of PGF2α in response to oxytocin is the initial signal triggering luteolysis in animals and ovarian PGF2α contributes to the luteolytic process in primates including humans (34). In presence of a viable embryo, the default luteolytic signal is counteracted by an antiluteolytic or a

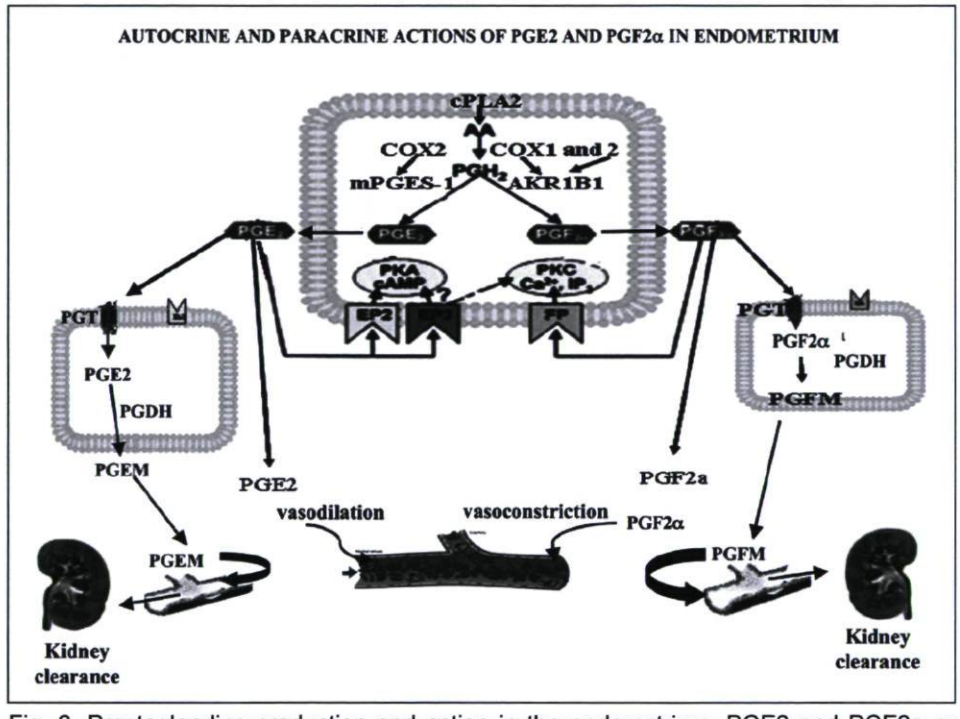


Fig. 3. Prostaglandins production and action in the endometrium. PGE2 and PGF2α are the primary PGs produced in the endometrium of all species studied so far. While endometrial cells produce both, epithelial cells preferentially release PGF2α and stromal cells PGE2. PGs can then act on the neighbouring cells to regulate endometrial function or travel across cells and tissues to reach the ovary and exert a luteolytic or luteotrophic effect to regulate progesterone production. When native PGs enter the vascular system, PGF2a exerts a TXA2 like contractile response whereas PGE2 induces a prostacyclin-like relaxation response. Native PGs are catabolised in the lung and metabolites cleared in the kidney.

luteotrophic signal or a combination of both to maintain the production of progesterone. PGF2α is also a potent constrictor of the myometrium and uterine blood vessels (21). By contrast, PGE2 is vasodilator able to exert a strong luteotrophic action in human (35). Prostaglandins, especially PGE2, are produced by early embryos and we have found that PGES (36) is increased at the time of maximal uterine receptivity. Similar observations in the mouse, suggest that PGE2 contribution to this process is well conserved among species (37, 38). At the time of implantation or recognition of pregnancy, PGE2 induces a local alteration in growth factors secretion and nutrients and increases vascular permeability (39, 40). PGE2 is a potent immunomodulator mediating the local maturation/differentiation processes (41) and inhibiting the lytic activities of both NK and lymphokine activated killer (LAK) cells (42) around the time of implantation in the endometrium. Consistent with the roles attributed to PGs, the treatment of pregnant females with NSAIDs inhibits implantation or at the very least reduces pregnancy rates (15). In humans, PGs interact with cytokines and PRL to regulate decidualization and with angiogenic and coagulation factors to regulate menstruation (43). During the menstrual cycle, the concentration of PGF2α is apparently higher than PGE2 during the secretory phase whereas levels of both PGs are low during the proliferative phase. The concentrations of PGE2 remain low whereas PGF2α goes higher during menstruation and lower during the implantation window (44). Our data and a recent reviews concur to state that across species, PGF2α and PGE2 are universally important in the regulation of endometrial function (43, 45).

SELECTIVE PGF2α AND PGE2 RELEASE

It is widely acknowledged that PGs play a critical role in reproductive processes. The expression of rate limiting enzymes such as phospholipase A2 (cPLA2α) and prostaglandin synthases 1 and 2 (PGHS-1/-2) also called cyclooxygenases (COX) regulates the rate of production of PGs as a group, but other mechanisms are needed for selective production of specific PGs. It is increasingly evident that the physiological action of PGs is regulated at multiple levels not only quantitatively, but also qualitatively by selective biosynthesis, expression of specific receptor subtypes, and specialized transport across cell membranes and compartments.

Very little has been done to identify the biosynthetic pathways leading to the formation of specific prostaglandins. Initially it was thought that a single type of PG was produced by distinct subsets of cells (46, 47). Our results with primary cultures now confirmed with clonal cell lines (48) demonstrated that endometrial cells can produce more than one PG. Therefore, conditions leading to the generation of a particular PG vary and must be set within individual cells.

Selective PGF2α production

PGF2α can be produced from three distinct pathways (Fig. 1) but most likely through reduction of PGH2 by 9, 11-endoperoxyde reduction referred to as PGFS activity. Several PGFS have been identified; three were isolated in the bovine: lung type prostaglandin F synthase (PGFS1) (49), lung type PGFS found in liver (PGFS2) (50) and liver type PGFS, also called dihydrodiol dehydrogenase 3 (DDBX) (51, 52). Others were identified respectively in human (AKR1C3) (53), sheep (54), Trypanosoma brucei (a protozoa) (55) and recently the porcine endometrium (56). All recognised mammal PGFSs belong to the aldoketoreductase 1C family, and are generally associated with hydroxysteroid dehydrogenase (HSD) activity. In the bovine endometrium we have shown that none of the presumed functional PGFS was expressed under any condition while we identified AKR1B5, an old enzyme with a new function, as a functional PGFS (28). We have studied the characteristics of various PGFS isoforms in relation with PGF2α production (Fig. 4). We found that aldoketoreductase 1B5 (AKR1B5) was the most likely PGFS involved in the production of PGF2a in bovine endometrium at the time of luteolysis (28). Interestingly, with its 20αHSD activity, this enzyme can also inactivate progesterone, another factor regulating endometrial function (Fig. 5). The human equivalent of the bovine AKR1B5 is AKR1B1 belonging to the AKR superfamily composed of 140 members divided into 15 families (57). AKR1B1 is one of 13 human AKRs catalyzing reactions on a broad and overlapping list of substrates making it difficult to find natural substrates and specific functions for any of these enzymes. AKR1B1 also known as the aldose reductase is highly expressed in the placenta for glucose metabolism and in the eye and kidney for osmotic regulation (58). We have accumulated several lines of evidence supporting the hypothesis that AKR1B1 is a functional PGFS in the human endometrium, but we are currently the only group exploring this avenue. We have studied AKR1B1 and demonstrated its association with PGF2α production in human endometrial cell lines (48) and in decidualized stromal cells (59). In a cell free system, purified AKR1B1 recombinant protein is able to produce PGF2α from PGH2. Endometrial cell lines transiently transfected with an expression vector coding for AKR1B1 exhibit increased ability to release PGF2α. In contrast, when AKR1B1 expression is knockdown with specific siRNAs, PGF2α production is decreased. We have found that the other potential PGF synthase (AKR1C3) is also expressed in endometrial cell cultures but its contribution to PGF2α production remains to be determined.

Selective PGE2 production

Three forms of PGE synthase (PGES) have been characterized so far (Fig. 4). Microsomal PGES-1 (mPGES-1) was the first identified and reported as inducible by agents such as cytokines and LPS (60). This enzyme is often coupled with COX-2 for delayed and sustained production of PGE2 (61). We have

described previously the regulation of mPGES-1 expression during the bovine oestrous cycle and its association with COX-2 (36). A cytosolic PGES (cPGES), identical to p23, a ubiquitous chaperone protein weakly bound to the steroid hormone receptor/hsp90 complex, was characterized and found coupled to COX-1 for immediate production of PGE2 (62). Enzymatic activity from a third PGES, microsomal PGES-2 (mPGES-2), was purified from bovine heart and cloning of homologous human and monkey sequences was done (63). This PGES is associated with both isoforms of COX with a slight preference for COX-2 (64) and we have documented its expression in the endometrium during the oestrous cycle (65) while we have cloned and sequenced the other two PGES from the macaque endometrium (66). In the bovine endometrium, all three PGES are expressed during the oestrous cycle with mPGES-1 dominating around ovulation. In cell cultures only mPGES-1 was found to increase in parallel with COX-2 when PGE2 production was stimulated with various factors (65).

In the human endometrium, the three known PGES, mPGES-1, mPGES-2 and cPGES are expressed during the menstrual cycle together with COX-1 and COX-2. At the mRNA level, mPGES-1 is expressed maximally during menses, mPGES-2 during the secretory phase and cPGES is expressed at a constant level. We have shown that mPGES-1 protein expression was stimulated following decidualization of stromal cells in vitro (59). In human endometrial cell lines, mPGES-1 mRNA and protein expression are highly stimulated by IL-1β and associated with PGE2 production (48). Accordingly, knockdown of mPGES-1 with a specific siRNAs decreased mRNA, protein and associated PGE2 production. It is worth noting that mPGES-1 appears to mediate most effects following stimulation of PGE2 production, but although mice with null mutation

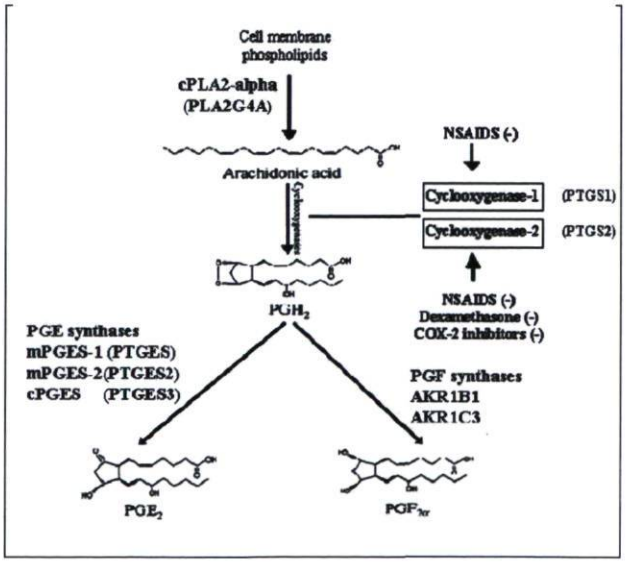


Fig. 4. Selective production of PGE2 and PGF2α. Following release of arachidonic acid (AA) from plasma membrane phospholipids primarily through cytosolic Phospholipase A2 (cPLA2), conversion into PGH2, the common precursor for all PGs, occurs through PGH synthase or COX, for which there are two isoforms COX-1 and COX-2 encoded by 2 genes, PTGS1 and PTGS2. PGH2 can then be converted into active PG by terminal synthases. We present two putative PGFS, AKR1B1 and AKR1C3 for PGF2α and three PGES, mPGES-1, mPGES-2 and cPGES for PGE2.

for this gene are insensitive to LPS and NFκB they do not exhibit the fertility problems found for COX-2, EP2 or cPLA2 knockout. Therefore, the mPGES-2 and cPGES are either able to compensate or are solicited as contributors for PGE2 production through an NFκB independent mechanism (67).

Physical association (team up) of PG biosynthetic enzymes

Biosynthesis of a specific PG requires simultaneous expression of the different members of the biosynthetic cascade (Fig. 1, Fig. 4). However, this does not rule out simultaneous expression of more than one terminal synthase. Therefore selective production may also involve functional association (compartmentalization) of complementary enzymes. This may include linking, association around scaffold proteins or grouping on a common structure. To date, no specific scaffolds involving PG biosynthesis have been identified. Terminal synthases are necessary to produce a specific PG but spatiotemporal association with upstream phospholipase and COXs is necessary to access the rate limiting precursors AA and PGH2. Functional associations between terminal synthases

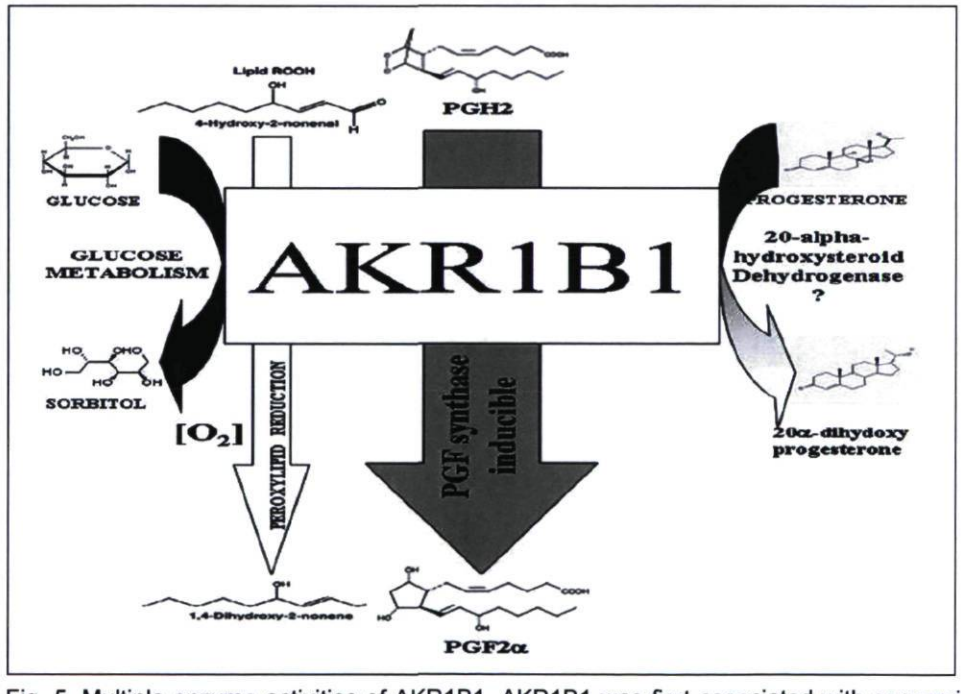


Fig. 5. Multiple enzyme activities of AKR1B1. AKR1B1 was first associated with conversion of glucose into sorbitol and accordingly named aldose reductase. The corresponding bovine AKR1B5 was first identified as a progesterone processing enzyme with 20α HSD activity. While both AKR1B5 and AKR1B1 express the latter activities, we have demonstrated that in both species, the primary activity of these enzymes is PGFS.

and upstream COXs have been described almost exclusively for the different PGES using transfected cell lines. Microsomal PGES-1 is often coupled with COX-2 for delayed and sustained production of PGE2 initiated by cytokines or LPS through an NFκB mediated mechanism (61). mPGES-2 is associated with both isoforms of COX with a slight preference for COX-2 (64) and cPGES is coupled to COX-1 for immediate release of PGE2 (62). There is no data available to link any PGFS with upstream enzymes of the PGF2α cascade at the functional or the transcription level (67), but our data suggest functional association of AKR1B1 with COX-2 (48) and potential association with COX-1. A study on AKR1C3 in transfected HEK-293 cells suggested preferential association with COX-1 (68). In bovine epithelial cells PGF2α production is stimulated preferentially by oxytocin through a PLC-PKC mediated pathway (69) and PGE2 with interferon (IFNt) (70) potentially through a NFκB mediated mechanism. In contrast, both PGs are increased simultaneously under all conditions tested so far in human endometrial cells (48, 71). There appear to be a trend for preferential stimulation of PGF2α and AKR1B1 in response to IL-1β in human endometrial cells, but significant increases in PGE2 and mPGES-1 are also observed.

Because the selective production of one PG such as PGE2 requires synchronous expression of at least 3 enzymes and because endometrial remodelling involves coordinate expression of multiple genes favouring cell proliferation and angiogenesis, key regulatory factors are likely to liberate transcription factors acting on a cassette of complementary genes. Some groups working on the involvement of PGs in cancer and inflammation have described important regulation by transcription factors such as TonE/NFAT5 (NFκB), NRF-2 and EGR-1, but very little has been done on reproductive tissues or non pathologic conditions such as pregnancy or menstruation. Our results showing a time and dose dependent increase in PGF2α associated with a parallel increase in AKR1B1 mRNA in response to IL-1β suggested transcriptional regulation of the AKR1B1 gene. Accordingly, we have cloned a 4.5 kb AKR1B1 promoter in the basic pGL3 vector coupled with the luciferase reporter gene. This construct is strongly activated by IL-1β. Progressive 5'deletions allowed to identify an IL-1β sensitive region located at -1177 to -1047. In the mouse AKR1B3 gene, homologous to the human AKR1B1, an Nrf2 binding motif is regulated by the antioxidant response element (ARE) present in the Multiple Stress Response Region (MSRR) (72). We have identified the corresponding putative trans-acting factors TonE/NFAT5, AP1, Nrf2 and NFκB in the AKR1B1 promoter. In the mouse, knocking out NFAT5 (TonE) leads to down-regulation of the AKR1B3 gene and poor embryo survival (73), disrupting the Nrf2 gene leads to a normal and fertile phenotype under controlled environment, but extreme susceptibility to oxidative stress, characteristic of PG biosynthesis (74). Interestingly, using constructs coupling different MSRR fragments of the AKR1B1 promoter with the SV40 pGL3-promoter, we have identified two AREs as important factors mediating the effect of IL-1β, potentially through Nrf2, in human endometrial

cells. In addition, mutations in the osmotic response element ORE (TONE) of the same MSSR fragment lead to decreased promoter activity following IL-1β stimulation. It was reported that NFκB was able to bind ORE of the AKR1B1 gene in human liver and lens cells treated with TNFα (75) whereas involvement of Nrf2 was shown in the regulation of TXA2 synthase an important vasoactive PG in platelets (76). Finally, numerous constructs and mouse mutation models used to characterize the NFκB system point to genes associated with AA metabolism as important targets (77). As observed for AKR1B1, PGE2 production and mPGES-1 mRNA exhibit a parallel increase in response to IL-1β suggesting transcriptional regulation of this gene. We have cloned a 4.2 kb promoter of the mPGES-1 gene and progressive deletion constructs showed that the -1059 +52 region conferred IL1B response whereas position -3096 to -2796 is associated with repressive activity. These are the first data describing promoter activity of a relatively long (4.2 kb) fragment for the human mPGES-1 gene. We hypothesize that Egr-1, an inducible zinc finger protein that recognizes the GC-rich consensus DNA sequence 5'-GCG(T/G)GGGCG-3'box present at the proximal promoter region -119/-112 and -108/-101 of the mPGES-1 gene is a functional transcription factor in endometrial cells. The same regions were found essential for the expression the mPGES-1 gene in osteoblasts and macrophage-like cells (78). IL-1β has also been reported to repress type II collagen gene in a chondrocyte cell line through Egr-1 (79) while it activates the Tissue Factor gene through Sp1 in Hela cells (80). Egr1 k/o mice have an infertile phenotype originating from lack of functional LH thus making it impossible to estimate its contribution on other aspects of endometrial function (81).

The proximal (1kb) promoters of cPLA2 and COX-2 genes contain several regions with putative cis-elements for NFκB (82). In human lens cells, NFκB proteins p50 and p65 interact with the ORE (osmotic response element) complex of the AKR1B1 promoter (75) corresponding precisely to the MSRR region mediating the effect of IL-1β in our endometrial cell lines. We believe that increased PGF2α production in response to IL-1β in endometrial cells is somewhat related to oxidative stress for which NFκB is considered a sensor (83). Interestingly, Egr-1 (also called Zif-268 or Krox-24) which is likely involved in the regulation of mPGES-1 is also identified as an oxidative stress-early inducible transcription factor when human lens epithelial cells are exposed to H2O2 (84). These data suggest that interactions between transcription factors and binding elements on the promoters of PG synthases, and especially ORE and ARE in the case of AKR1B1 and Egr1 for mPGES-1 provide a functional mean to achieve selective production of specific PGs.

Selective output of PGF2α and PGE2, local transport and catabolism

Prostaglandin transport: PGs predominate as charged anions and in spite of their lipid nature, diffuse poorly through plasma membranes. The mechanisms

responsible for the transport of newly synthesized PGs out of producing cells, either by simple diffusion (85), or a PG efflux transporter (86), are still in dispute. It has been shown that though anions cross the cell membrane by simple diffusion, the estimated flow rate would be too low for maintaining a biological function. Therefore, passive diffusion of PG into cells appears to be poor and is thought to be mediated by carriers (Fig. 2) such as prostaglandin transporter (PGT) (85). PGT was the first cloned PG transporter (87) and is a 12-transmembrane protein with a broad tissue expression. It is a functional uptake-carrier with high affinity for PGE2, PGF2α and PGD2 (85). PGT mRNA is expressed in reproductive tissues such as testis, ovary, and uterus (87, 88). PGT belongs to the super family of 12-transmembrane Organic Anion Transporting Polypeptide (OATP). It has been proposed that PGT mediates both the efflux of newly synthesised PGs to effect their biological actions through their cell surface receptors, and influx of PGs from the extra cellular milieu for their inactivation or action through specific nuclear receptors. PGT was found to be expressed preferentially in cell membranes of tissues capable of producing more PGs. Interestingly, PGT and cell surface PG receptors have comparable affinities for their substrates (85). Other members of the same transporter family such as CFTR are involved with efflux function and another member, MRP4 (86), has been proposed as a functional efflux carrier for PGs.

Our group has cloned bovine PGT (89) and characterized PGT as a key player in the action of PGs in the bovine reproductive system (89-92). Recently, we have shown the expression of PGT in the human endometrium (93). The co-expression of PGT and PGDH in a single cell type is believed to be associated with PG catabolism (94) whereas expression of PGT alone may favour transport of PGs across adjacent cells and tissues and mediate paracrine action of PGs (90-92). We have found that decidualization influenced the expression of hPGT and the distribution of PGF2a and PGE2 in the intra and extra-cellular compartments (59). We have also studied the expression of different members of the MRP and OATP transporters in the bovine endometrium and found that the former are preferentially expressed during the early part and the latter in the late part of the oestrous cycle. In vitro, the expression of both transporters was found to be modulated in parallel with PG biosynthesis in response to oxytocin and interferon.

PG catabolism: The first step for biological inactivation of PGs is effected by 15-PGDH (95) (96) and further catabolism by 15-13PGR generates the PGF2α and PGE2 metabolites PGFM and PGEM. There are two types of 15-PGDH, but only type I PGDH is associated with peripheral metabolism of PGs. We have found that endometrial 15-PGDH was modulated during the bovine oestrous cycle suggesting that local catabolism could exert a regulatory mechanism in the endometrium. In the mouse, it has been found that in preparation for parturition a peak of PGF2α is associated with an increase of COX-1 and PGF synthase and a decrease of 15-PGDH while cPLA2 and COX-2 are unaffected (97). In studies focusing on neoplasia pathways the COX-2-dependent production of PGE2 is associated with

tumorgenesis and this effect is exacerbated when 15-PGDH expression is reduced (98). In parallel, it has been shown that cytokines like IL-1β or TNF-α are able to reduce significantly 15-PGDH activity at the mRNA level and that the ratio of PGFM/ PGF2α is decreased significantly by steroid hormones (progesterone and dexamethasone) in trophoblast cells in culture (99). These results suggest that net PG production is regulated locally by a complex process involving both synthetic and catabolic enzymes. Preliminary results in the human endometrium indicate that the 15-PGDH protein is present in glandular epithelial cells during the early and mid secretory phases. Because treatment with PG biosynthesis blockers (NSAIDs) is efficient to treat many pathological conditions, we may assume that reduced peripheral catabolism may contribute to some of the disorders observed (100).

DIET AND PROSTAGLANDIN PRODUCTION

Free fatty acid composition and PGFnα and PGEn production

Prostaglandins are derived from eicosanoic (C20) fatty acids, and the trend to rely increasingly more on sn-6 PUFA yielding series 2 PGs potentially exacerbates adverse inflammatory and cardio-vascular conditions. In humans, the relative proportion of PUFA in body tissues depends on diet composition. In women, increased release of AA before menstruation is at the origin of increased PGF2α and PGE2 production considered responsible for dysmenorrhea (43, 101) and pre-menstrual syndrome (102). Recent circumstantial evidences suggest that altering even slightly (20%) the fat content of diet towards PUFA favouring series 1 or series 3 PGs can have a significant positive impact on health (Fig. 6). The biologically active series 2 prostaglandins are derived from arachidonic acid (AA) yielding the well known PGF2α and PGE2. Series 3 prostaglandins can be formed from eicosapentaenoic acid (EPA, 20:5n-3) the major fish oil omega 3, which gives rise to PGE3 and PGF3α. Series 1 prostaglandins are derived from dihomogamma linolenic acid (DGLA, 20:3n-6) which gives rise to PGH1, PGE1 and PGF1α. The biological activity of series 1 and 3 PGs vary among species and between tissues. Manipulation of the dietary intake of PUFAs in a variety of species and models was shown to impact on follicular development, ovulation, corpus luteum function, maternal recognition of pregnancy and parturition (100). It has been established that omega-3 can significantly reduce dysmenorrhea and PMS symptoms presumably through a competitive action of prostaglandins of the 3 series (102). The effects were accompanied by alteration of net output of urinary metabolites, but the exact mechanisms behind were not determined. It was reported that series 1 PGs are anti inflammatory, but long significant alteration of PUFA composition toward DGLA is difficult because of intrinsic conversion into AA the precursor of pro-inflammatory series 2 PGs. It must be stressed that even though many reports militate in favour of increased omega 3 consumption in the diet, recovery of full body function following complete deprivation of FFA is optimal with omega 6 FA such as AA (103).

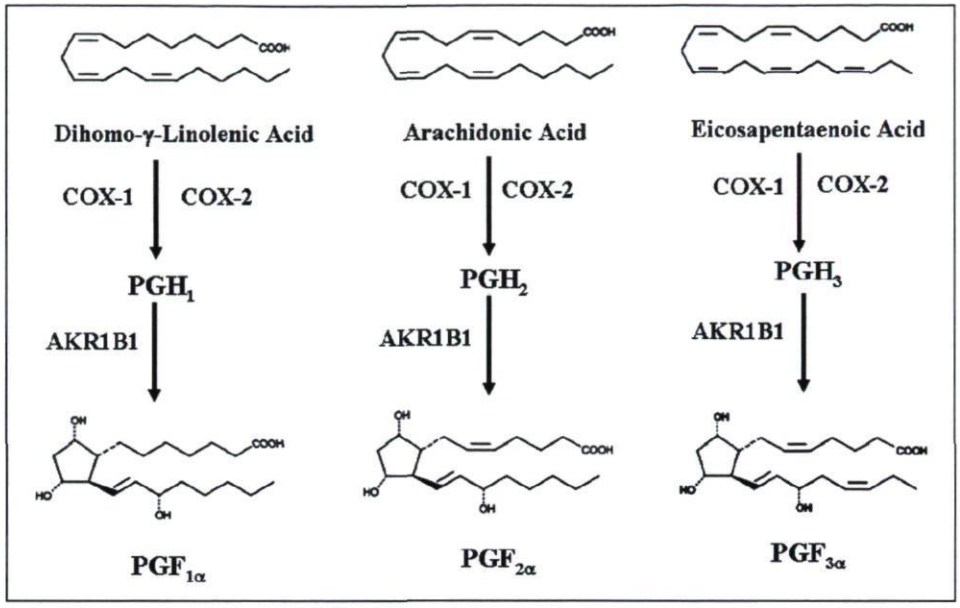


Fig. 6. Effect of dietary fatty acids on PG production. PGs can be generated from a variety of C-20 polyunsaturated fatty acids (PUFA) present in cell membranes in the form of phospholipids. These PUFA are poorly converted in mammals and must therefore be obtained from dietary intake. AA is the most abundant PUFA in the Western diet and is at the origin of the pro-inflammatory series 2 PGs. DGLA leads to the production of anti-inflammatory series 1 PGs whereas EPA, the omega 3 of fish origin leads to series 3 PGs. While there is no clear identification of the mechanisms responsible for the health benefits of omega 3 FFA, alteration in PG biosynthesis and signal transduction is a likely hypothesis.

Prostaglandin, ROS and antioxidants

There is a close association between the pathways generating reactive oxygen species (ROS) and PG biosynthesis. Reactive oxygen species (ROS) are generated by COXs during the process of PGs biosynthesis and can contribute directly to the regulation of reproduction (104) and initiation of menstruation (105). Interestingly, terminal PG synthases, especially aldose reductases can metabolize ROS. We have observed that AKR1B1 is increased by H2O2 whereas known antioxidants like curcumin vitamin E, N-acetyl cysteine and luteolin decreased in vitro PGs production in human endometrial cells treated with IL-1β.

PROSTAGLANDINS AND HUMAN PATHOLOGIES

Menstrual disorders

Menstrual disorders can affect women at any point in their childbearing years but are most prevalent during adolescence or the years just before menopause

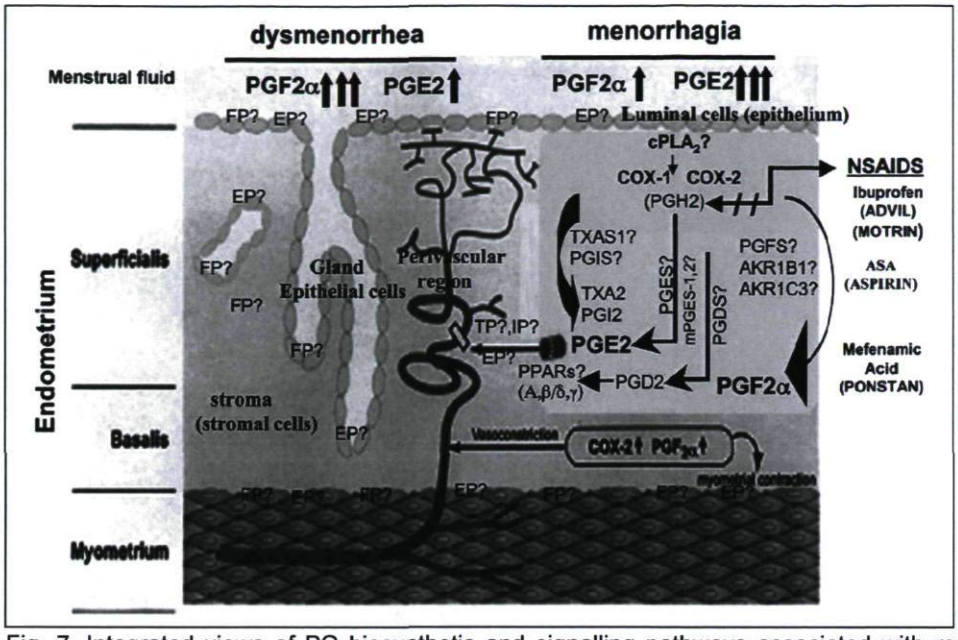


Fig. 7. Integrated views of PG biosynthetic and signalling pathways associated with menstrual disorders. The terminal synthases associated with the production of all PGs have been identified in the endometrium. However, PGE2 and PGF2α are produced in greater quantity and are the only members of the group with the chemical stability and penetration ability to generate the responses characteristic of menstrual disorders. Dysmenorrhea and menorrhagia are both associated with increased production of PGs, the former is preferentially associated with PGF2α inducing ischemia and pain and the latter with PGE2 exhibiting antithrombotic activity and hyperalgesia. The associations between specific biosynthetic enzymes, receptors and their precise sites of expression remain to be determined.

when sex hormones are shifting rapidly. The most common and debilitating menstrual disorders are dysmenorrhea or painful menstruation and menorrhagia or heavy menstrual bleeding (43). Locally released prostaglandins are considered as the primary mediators involved in the aberrant conditions and inhibition of their biosynthesis with non specific COX inhibitors (NSAIDs) is the primary therapeutic approach (Fig 7). Endometrial PGF2α is highest before the onset of menses. Vasoconstriction induced by PGF2α causes ischemia, accumulation of toxic catabolites, tissue necrosis, and desquamation. Myometrial contractility and abdominal discomfort (cramping) associated with menses are also caused by PGF2α. Increased concentrations of prostaglandins have been found in the endometrium and menstrual fluid of women who experience dysmenorrhea (106). The pain associated with uterine ischemia induced by PGF2α may be exacerbated by the hyper hyperalgesic effect of PGE2 on nerve terminals (107). The mechanisms behind dysfunctional uterine bleeding are not fully determined but PGs are again identified as important contributing factors. Increased PGE2 relative

to PGF2α levels in endometrium and menstrual fluid have been associated with menorrhagia (108), but altered PGI2 and TXA2 in the spiral arteries may also contribute significantly to this condition. In the case of menorrhagia, both NSAIDs and prothrombotic factors are used as therapeutic treatments. A recent review addresses the involvement of COX enzymes and prostaglandins in reproductive tract physiology and pathology (19). That review re-establishes the importance of COX expression in association with PGF2α and PGE2 in reproductive tract carcinoma, menorrhagia, dysmenorrhea and endometriosis through autocrine/paracrine mechanisms. Aberrations in uterine PG release or receptor expression were also demonstrated in association with premature labour (21).

Complications of metabolic disorders

Metabolic disorders result from complex interactions between genetic and environmental factors disturbing the normal immune and endocrine function. In turn, homeostasis is perturbed resulting in obesity, type 2 diabetes (T2D), increased cardiovascular morbidity and infertility. The contribution of prostaglandins (PGs) to the aetiology of metabolic disorders is poorly documented, but recent literature and our findings suggest that PGs may

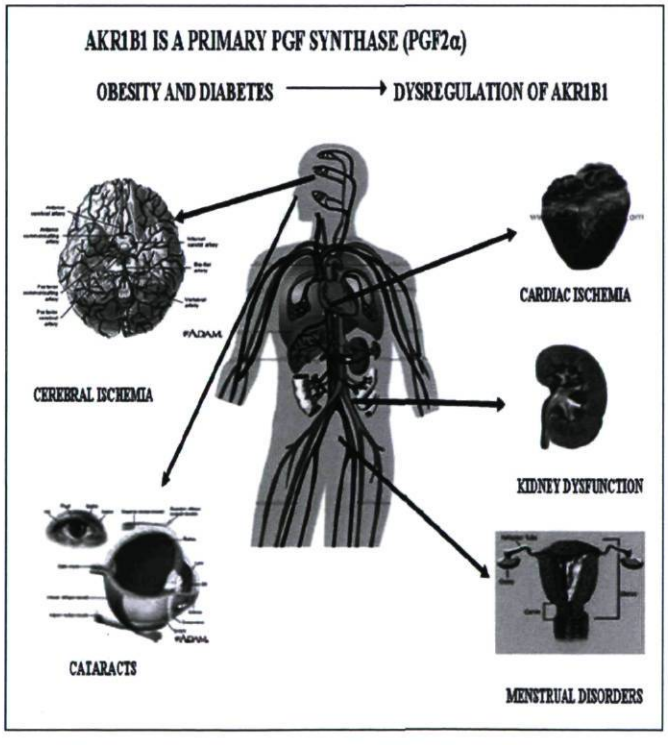


Fig. 8. PGFS activity and the aetiology of human pathologies. The generation of sorbitol from high levels of glucose observed in diabetes has been proposed as the primary cause of complications in many organs and systems. However, glucose at physiological concentrations is a poor substrate for AKR1B1. The demonstration of PGFS activity of AKR1B1 and increased expression in uterus, brain, heart and kidney in association with different pathologies, provide a strikingly coherent explanation of the effects observed in these organ and systems and warrant in depth investigation.

contribute to the development of associated complications. AKR1B1 and the polyol pathway responsible for conversion of glucose into sorbitol (Fig. 5) have been associated with several pathological conditions such as iron overload (109), alcoholic liver disease (110), heart failure (111), myocardial ischemia (112), vascular inflammation (113) and restenosis (114). Diabetes increases AKR1B1 expression and is associated with the impairment of NO-mediated vascular relaxation and decreased NO bioavailability, which may be a causative factor in other complications (115). However, recent studies have shown that AKR1B1 does not process glucose at physiological concentrations but is an excellent catalyst for the reduction of lipid peroxidation-derived aldehydes and their glutathione conjugates (116-121).

Interestingly, both AKR1B1 (122) and PGF2α (123) are increased in association with type 2 diabetes. This finding together with observation that this enzyme is expressed in adipose tissue, heart, skeletal muscle, eye and kidney (open an entire new field of investigation to study the potential contribution of PGF2α, its action relative to PGE2 and substrate interaction with steroids, glucose and their regulators in metabolic disorders such as obesity and diabetes. The newly described PGFS activity of AKR1B1 is also highly relevant to the documented association of this enzyme with cardiac (124-126) and cerebral ischemia (113, 127, 128) (Fig. 8).

Inhibitors of the aldose reductase activity of AKR1B1 were developed to correct aberrant responses associated with diabetes, but serious adverse side effects always occurred leading to their early withdrawal.

Table 1. Inhibitory effects and selectivity of some NSAIDs on Cox-1 and Cox-2 activity.

	Cox-1	Cox-2		
Drug	IC_{50} (ΠM)		Ratio Cox-2/Cox-1	Ref.
Aspirin	1.2	15.8	13.1	[130]
Flurbiprofen	0.1	0.4	4	[131]
Ibuprofen	3.3	37	11.4	[131]
Indomethacin	0.1	0.9	9	[131]
Mefenamic acid	25	2.9	0.12	[132]
Naproxen	1.1	36	32.7	[131]
Celecoxib (Celebrex*)	1.2	0.83	0.7	[132]
Nimesulide	12.5	0.4	0.03	[130]
NS-398	28.9	0.04	0.001	[130]
Rofecoxib (Vioxx*)	15	0.018	0.0012	[133]
Valdecoxib (Bextra*)	150	0.005	0.00003	[134]
Ketorolac	0.0014	0.14	100	[130]
SC-560	0.009	6.3	700	[135]

CONCLUSION

We have presented an integrated view of PGE2 and PGF2α biosynthesis, transport and signalling systems in the human and bovine endometrium. The net production of uterine PGs is governed by the anabolic enzymes COX-1, COX-2, PGES, PGFS and the catabolic enzyme PGDH (129) which are well conserved among species. Of particular interest is the identification of AKR1B1 as a functional PGFS. This activity appears as the missing link to understand the origin of diabetes complication affecting multiple tissues and systems and a promising pharmacological target to treat them (Fig. 8).

REFERENCES

1. Kudo I, Murakami M. Phospholipase A2 enzymes. Prostaglandins Other Lipid Mediat 2002; 68-69: 3-58.
2. Smith WL, Song I. The enzymology of prostaglandin endoperoxide H synthases-1 and -2. Prostaglandins Other Lipid Mediat 2002; 68-69: 115-128.
3. Sirois J. Richards JS. Purification and characterization of a novel, distinct isoform of prostaglandin endoperoxide synthase induced by human chorionic gonadotropin in granulosa cells of rat preovulatory follicles. J Biol Chem 1992; 267(9): 6382-6388.
4. Parent JC, Villeneuve S, Fortier MA. Evaluation of the contribution of cyclooxygenase 1 and cyclooxygenase 2 to the production of PGE2 and PGF2 alpha in epithelial cells from bovine endometrium. Reproduction 2003; 126(4): 539-547.
5. Langenbach R, Morham SG, Tiano HF, Loftin CD, Ghanayem BI, Chulada PC, Mahler JF et al. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. Cell 1995; 83(3): 483-492.
6. Morham SG, Langenbach R, Loftin CD, Tiano HF, Vouloumanos N, Jennette JC, Mahler JF et al. Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. Cell 1995; 83(3): 473-482.
7. Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, Trzaskos JM et al. Multiple female reproductive failures in cyclooxygenase 2-deficient mice. Cell 1997; 91(2): 197-208.
8. Reese J, Brown N, Paria BC, Morrow J, Dey SK. COX-2 compensation in the uterus of COX-1 deficient mice during the pre-implantation period. Mol Cell Endocrinol 1999; 150(1-2): 23-31.
9. Loftin CD, Trivedi DB, Tiano HF, Clark JA, Lee CA, Epstein JA, Morham SG et al. Failure of ductus arteriosus closure and remodeling in neonatal mice deficient in cyclooxygenase-1 and cyclooxygenase-2. Proc Natl Acad Sci U S A, 2001; 98(3): 1059-1064.
10. Reese J, Paria BC, Brown N, Zhao X, Morrow JD, Dey SK. Coordinated regulation of fetal and maternal prostaglandins directs successful birth and postnatal adaptation in the mouse. Proc Natl Acad Sci U S A 2000; 97(17): 9759-9764.
11. Priddy AR, Killick SR, Elstein M, Morris J, Sullivan M, Patel L, Elder M. The effect of prostaglandin synthetase inhibitors on human preovulatory follicular fluid prostaglandin, thromboxane, and leukotriene concentrations. J Clin Endocrinol Metab 1990; 71(1): 235-242.
12. Klein T, Nüsing RM, Pfeilschifter J, Ullrich V. Selective inhibition of cyclooxygenase 2. Biochem Pharmacol 1994; 48(8) p. 1605-1610.
13. Gretzer B, Maricic N, Respondek M, Schuligoi R, Peskar BM. Effects of specific inhibition of cyclo-oxygenase-1 and cyclo-oxygenase-2 in the rat stomach with normal mucosa and after acid challenge. Br J Pharmacol 2001; 132(7): 1565-1573.

14. Hudson M, Richard H, Pilote L. Differences in outcomes of patients with congestive heart failure prescribed celecoxib, rofecoxib, or non-steroidal anti-inflammatory drugs: population based study. BMJ 2005; 330(7504): 1370.
15. Norman RJ, Wu R. The potential danger of COX-2 inhibitors. Fertil Steril 2004; 81(3):493-494.
16. Whitehouse MW. Prostanoids as friends, not foes: further evidence from the interference by cycloxygenase-inhibitory drugs when inducing tolerance to experimental arthritigens in rats. Inflammopharmacology 2005; 12(5): 481-492.
17. Zeilhofer HU. Brune K. Analgesic strategies beyond the inhibition of cyclooxygenases. Trends Pharmacol Sci 2006; 27(9): 467-474.
18. Bos CL, Richel DJ, Ritsema T, Peppelenbosch MP, Versteeg HH. Prostanoids and prostanoid receptors in signal transduction. Int J Biochem Cell Biol 2004; 36(7):1187-1205.
19. Sales KJ, Jabbour HN. Cyclooxygenase enzymes and prostaglandins in reproductive tract physiology and pathology. Prostaglandins Other Lipid Mediat 2003; 71(3-4): 97-117.
20. Hata AN, Breyer RM. Pharmacology and signaling of prostaglandin receptors: multiple roles in inflammation and immune modulation. Pharmacol Ther 2004; 103(2): 147-166.
21. Olson DM. The promise of prostaglandins: have they fulfilled their potential as therapeutic targets for the delay of preterm birth? J Soc Gynecol Investig 2005; 12(7): 466-478.
22. Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev 1999; 20(5): 649-688.
23. Bhattacharya M, Peri K, Ribeiro-da-Silva A, Almazan G, Shichi H, Hou X et al. Localization of functional prostaglandin E2 receptors EP3 and EP4 in the nuclear envelope. J Biol Chem 1999; 274(22): 15719-15724.
24. LindstromT, Bennett P. Transcriptional regulation of genes for enzymes of the prostaglandin biosynthetic pathway. Prostaglandins Leukot Essent Fatty Acids 2004;70(2): 115-135.
25. Jabbour HN, Sales KJ. Prostaglandin receptor signalling and function in human endometrial pathology. Trends Endocrinol Metab 2004, 15(8): 398-404.
26. Sales KJ, Jabbour HN. Cyclooxygenase enzymes and prostaglandins in pathology of the endometrium. Reproduction 2003; 126(5): 559-567.
27. Smith SK, Kelly RW. The release of PGF2 alpha and PGE2 from separated cells of human endometrium and decidua. Prostaglandins Leukot Essent Fatty Acids 1988; 33(2): 91-96.
28. Madore E, Harvey N, Parent J, Chapdelaine P, Arosh JA, Fortier MA. An aldose reductase with 20 alpha-hydroxysteroid dehydrogenase activity is most likely the enzyme responsible for the production of prostaglandin f2 alpha in the bovine endometrium. J Biol Chem 2003; 278(13): 11205-11212.
29. Bonventre JV, Huang Z, Taheri MR, O'Leary E, Li E, Moskowitz MA et al. Reduced fertility and postischaemic brain injury in mice deficient in cytosolic phospholipase A2. Nature 1997; 390(6660): 622-625.
30. Sugimoto Y, Yamasaki A, Segi E, Tsuboi K, Aze Y, Nishimura T et al. Failure of parturition in mice lacking the prostaglandin F receptor. Science 1997; 277(5326): 681-683.
31. Kennedy CR, Zhang Y, Brandon S, Guan Y, Coffee K, Funk CD et al. Salt-sensitive hypertension and reduced fertility in mice lacking the prostaglandin EP2 receptor. Nat Med 1999; 5(2): 217-220.
32. Tilley SL, Audoly LP, Hicks EH, Kim HS, Flannery PJ, Coffman TM et al. Reproductive failure and reduced blood pressure in mice lacking the EP2 prostaglandin E2 receptor. J Clin Invest 1999; 103(11): 1539-1545.
33. Vincent DL, Inskeep EK. Role of progesterone in regulating uteroovarian venous concentrations of PGF2 alpha and PGE2 during the estrous cycle and early pregnancy in ewes. Prostaglandins 1986; 31(4): 715-733.

34. Nagle CA, Mendizábal AF, Lahoz MM, Porta MM, Torres MI. Transfer pathways between the ovaries and the uterus in the cebus monkeys (Cebus apella). Gen Comp Endocrinol 2005; 144(3): 248-256.
35. Hagstrom HG, Hahlin M, Bennegard-Edén B, Bourne T, Hamberger L. Regulation of corpus luteum function in early human pregnancy. Fertil Steril 1996; 65(1): p. 81-86.
36. Arosh JA, Parent J, Chapdelaine P, Sirois J, Fortier MA. Expression of cyclooxygenases 1 and 2 and prostaglandin E synthase in bovine endometrial tissue during the estrous cycle. Biol Reprod 2002; 67(1): 161-169.
37. Ni H, Sun T, Ding NZ, Ma XH, Yang ZM. Differential expression of microsomal prostaglandin e synthase at implantation sites and in decidual cells of mouse uterus. Biol Reprod 2002; 67(1): 351-358.
38. Ni H, Sun T, Ma XH, Yang ZM. Expression and regulation of cytosolic prostaglandin E synthase in mouse uterus during the peri-implantation period. Biol Reprod 2003; 68(3): 744-750.
39. Fortier MA, Boulet AP, Dugré FJ, Lambert RD. Local alteration in adenylate cyclase activity and stimulation response at implantation site in rabbit endometrium during early pregnancy. Biol Reprod 1990; 42(1): 106-113.
40. Psychoyos A, Nikas G, GravanisA. The role of prostaglandins in blastocyst implantation. Hum Reprod 1995; 10 (Suppl 2): 30-42.
41. Linnemeyer PA, Pollack SB. Prostaglandin E2-induced changes in the phenotype, morphology, and lytic activity of IL-2-activated natural killer cells. J Immunol 1993; 150(9): 3747-3754.
42. Bergeron D, Ouellette MJ, Lambert RD. PGE2, but not TGF beta 2, in rabbit blastocoelic fluid regulates the cytotoxic activities of NK and LAK cells. J Reprod Immunol 1997; 33(3): 203-219.
43. Jabbour HN, Kelly RW, Fraser HM, Critchley HO. Endocrine regulation of menstruation. Endocr Rev, 2006. 27(1): p. 17-46.
44. Fraser IS. Prostaglandins, prostaglandin inhibitors and their roles in gynaecological disorders. Baillieres Clin Obstet Gynaecol 1992; 6(4): 829-857.
45. Narumiya S. FitzGerald GA. Genetic and pharmacological analysis of prostanoid receptor function. J Clin Invest 2001; 108(1): 25-30.
46. Asselin E, Goff AK, Bergeron H, Fortier MA. Influence of sex steroids on the production of prostaglandins F2 alpha and E2 and response to oxytocin in cultured epithelial and stromal cells of the bovine endometrium. Biol Reprod 1996; 54(2): 371-379.
47. Kim JJ, Fortier MA. Cell type specificity and protein kinase C dependency on the stimulation of prostaglandin E2 and prostaglandin F2 alpha production by oxytocin and platelet-activating factor in bovine endometrial cells. J Reprod Fertil 1995; 103(2): 239-247.
48. Chapdelaine P, Kang J, Boucher-Kovalik S, Caron N, Tremblay JP, Fortier MA. Decidualization and maintenance of a functional prostaglandin system in human endometrial cell lines following transformation with SV40 large T antigen. Mol Hum Reprod 2006; 12(5): 309-319.
49. Watanabe K, Yoshida R, Shimizu T, Hayaishi O. Enzymatic formation of prostaglandin F2 alpha from prostaglandin H2 and D2. Purification and properties of prostaglandin F synthetase from bovine lung. J Biol Chem 1985; 260(11): 7035-7041.
50. Kuchinke W, Barski O, Watanabe K, Hayaishi O. A lung type prostaglandin F synthase is expressed in bovine liver: cDNA sequence and expression in E. coli. Biochem Biophys Res Commun 1992; 183(3): 1238-1246.
51. Chen LY, Watanabe K, Hayaishi O. Purification and characterization of prostaglandin F synthase from bovine liver. Arch Biochem Biophys 1992; 296(1): 17-26.
52. Suzuki T, Fujii Y, Miyano M, Chen LY, Takahashi T, Watanabe K. cDNA cloning, expression, and mutagenesis study of liver-type prostaglandin F synthase. J Biol Chem 1999; 274(1): 241-248.

53. Suzuki-Yamamoto T, Nishizawa M, Fukui M, Okuda-Ashitaka E, Nakajima T, Ito S et al. cDNA cloning, expression and characterization of human prostaglandin F synthase. FEBS Lett 1999; 462(3): 335-340.
54. Wu WX, Ma XH, Yoshizato T, Shinozuka N, Nathanielsz PW. Increase in prostaglandin H synthase 2, but not prostaglandin F2alpha synthase mRNA in intrauterine tissues during betamethasone-induced premature labor and spontaneous term labor in sheep. J Soc Gynecol Investig 2001; 8(2): 69-76.
55. Kubata BK, Duszenko M, Kabututu Z, Rawer M, Szallies A, Fujimori K et al. Identification of a novel prostaglandin f(2alpha) synthase in Trypanosoma brucei. J Exp Med 2000; 192(9): 1327-1338.
56. Waclawik A, Rivero-Muller A, Blitek A, Kaczmarek MM, Brokken LJ, Watanabe K et al. Molecular cloning and spatiotemporal expression of prostaglandin F synthase and microsomal prostaglandin E synthase-1 in porcine endometrium. Endocrinology 2006; 147(1): 210-221.
57. Jin Y, Penning TM. Aldo-keto reductases and bioactivation/detoxication. Annu Rev Pharmacol Toxicol 2007; 47: 263-292.
58. Srivastava SK, Ramana KV, Bhatnagar A. Role of aldose reductase and oxidative damage in diabetes and the consequent potential for therapeutic options. Endocr Rev 2005; 26(3): 380-392.
59. Kang J, Chapdelaine P, Laberge PY, Fortier MA. Functional characterization of prostaglandin transporter and terminal prostaglandin synthases during decidualization of human endometrial stromal cells. Hum Reprod 2006; 21(3): 592-599.
60. Jakobsson PJ, Mancini JA, Riendeau D, Ford-Hutchinson. Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. Proc Natl Acad Sci U S A 1999; 96(13): 7220-7225.
61. Murakami M, Naraba H, Tanioka T, Semmyo N, Nakatani Y, Kojima F et al. Regulation of prostaglandin E2 biosynthesis by inducible membrane-associated prostaglandin E2 synthase that acts in concert with cyclooxygenase-2. J Biol Chem 2000; 275(42): 32783-32792.
62. Tanioka T, Nakatani Y, Semmyo N, Murakami M, Kudo I. Molecular identification of cytosolic prostaglandin E2 synthase that is functionally coupled with cyclooxygenase-1 in immediate prostaglandin E2 biosynthesis. J Biol Chem 2000; 275(42): 32775-32782.
63. Tanikawa N, Ohmiya Y, Ohkubo H, Hashimoto K, Kangawa K, Kojima M et al. Identification and characterization of a novel type of membrane-associated prostaglandin E synthase. Biochem Biophys Res Commun 2002; 291(4): 884-889.
64. Murakami M, Nakashima K, Kamei D, Masuda S, Ishikawa Y, Ishii T et al. Cellular prostaglandin E2 production by membrane-bound prostaglandin E synthase-2 via both cyclooxygenases-1 and -2. J Biol Chem 2003; 278(39): 37937-37947.
65. Parent J, Fortier MA. Expression and contribution of three different isoforms of prostaglandin E synthase in the bovine endometrium. Biol Reprod 2005; 73(1): 36-44.
66. Parent J, Chapdelaine P, Fortier MA. Molecular cloning and tissue distribution of microsomal-1 and cytosolic prostaglandin E synthases in macaque. Prostaglandins Other Lipid Mediat 2005; 78(1-4): 27-37.
67. Helliwell RJ, Adams LF, Mitchell MD. Prostaglandin synthases: recent developments and a novel hypothesis. Prostaglandins Leukot Essent Fatty Acids 2004; 70(2): 101-113.
68. Nakashima K, Ueno N, Kamei D, Tanioka T, Nakatani Y, Murakami M et al. Coupling between cyclooxygenases and prostaglandin F(2alpha) synthase. Detection of an inducible, glutathione-activated, membrane-bound prostaglandin F(2alpha)-synthetic activity. Biochim Biophys Acta 2003; 1633(2): 96-105.
69. Asselin E, Drolet P, Fortier MA. Cellular mechanisms involved during oxytocin-induced prostaglandin F2alpha production in endometrial epithelial cells in vitro: role of cyclooxygenase-2. Endocrinology 1997; 138(11): 4798-4805.

70. Asselin E, Lacroix D, Fortier MA. IFN-tau increases PGE2 production and COX-2 gene expression in the bovine endometrium in vitro. Mol Cell Endocrinol 1997; 132(1-2): 117-126.
71. Kang J, Akoum A, Chapdelaine P, Laberge P, Poubelle PE, Fortier MA. Independent regulation of prostaglandins and monocyte chemoattractant protein-1 by interleukin-1beta and hCG in human endometrial cells. Hum Reprod 2004; 19(11): 2465-2473.
72. Nishinaka T, Yabe-Nishimura C. Transcription factor Nrf2 regulates promoter activity of mouse aldose reductase (AKR1B3) gene. J Pharmacol Sci 2005; 97(1): 43-51.
73. Lopez-Rodriguez C, Antos CL, Shelton JM, Richardson JA, Lin F, Novobrantseva TI et al. Loss of NFAT5 results in renal atrophy and lack of tonicity-responsive gene expression. Proc Natl Acad Sci U S A 2004; 101(8): 2392-2397.
74. Lee JM, Chan K, Kan YW, Johnson JA. Targeted disruption of Nrf2 causes regenerative immune-mediated hemolytic anemia. Proc Natl Acad Sci U S A 2004; 101(26): 9751-9756.
75. Iwata T, Sato S, Jimenez J, McGowan M, Moroni M, Dey A et al. Osmotic response element is required for the induction of aldose reductase by tumor necrosis factor-alpha. J Biol Chem 1999; 274(12): 7993-8001.
76. Yaekashiwa M, Wang LH. Nrf2 regulates thromboxane synthase gene expression in human lung cells. DNA Cell Biol 2003; 22(8): 479-487.
77. Lindstrom TM, Bennett PR. The role of nuclear factor kappa B in human labour. Reproduction 2005; 130(5): 569-581.
78. Naraba H, Yokoyama C, Tago N, Murakami M, Kudo I, Fueki M et al. Transcriptional regulation of the membrane-associated prostaglandin E2 synthase gene. Essential role of the transcription factor Egr-1. J Biol Chem 2002; 277(32): 28601-28608.
79. Tan L, Peng H, Osaki M, Choy BK, Auron PE, Sandell LJ et al. Egr-1 mediates transcriptional repression of COL2A1 promoter activity by interleukin-1beta. J Biol Chem 2003; 278(20): 17688-17700.
80. Cui MZ, Parry GC, Oeth P, Larson H, Smith M, Huang RP et al. Transcriptional regulation of the tissue factor gene in human epithelial cells is mediated by Sp1 and EGR-1. J Biol Chem 1996; 271(5): 2731-2739.
81. Lee SL, Sadovsky Y, Swirnoff AH, Polish JA, Goda P, Gavrilina G et al. Luteinizing hormone deficiency and female infertility in mice lacking the transcription factor NGFI-A (Egr-1). Science 1996; 273(5279): 1219-1221.
82. Lappas M, Permezel M, Rice GE. Release of proinflammatory cytokines and 8-isoprostane from placenta, adipose tissue, and skeletal muscle from normal pregnant women and women with gestational diabetes mellitus. J Clin Endocrinol Metab 2004; 89(11): 5627-5633.
83. Li N, Karin M. Is NF-kappaB the sensor of oxidative stress? FASEB J 1999; 13(10): 1137-1143.
84. Paron I, D'Elia A, D'Ambrosio C, Scaloni A, D'Aurizio F, Prescott A et al. A proteomic approach to identify early molecular targets of oxidative stress in human epithelial lens cells. Biochem J 2004; 378(Pt 3): 929-937.
85. Schuster VL. Prostaglandin transport. Prostaglandins Other Lipid Mediat 2002; 68-69: 633-647.
86. Reid G, Wielinga P, Zelcer N, van der Heijden I, Kuil A, de Haas M et al. The human multidrug resistance protein MRP4 functions as a prostaglandin efflux transporter and is inhibited by nonsteroidal antiinflammatory drugs. Proc Natl Acad Sci U S A 2003; 100(16): 9244-9249.
87. Kanai N, Lu R, Satriano JA, Bao Y, Wolkoff AW, Schuster VL. Identification and characterization of a prostaglandin transporter. Science 1995; 268(5212): 866-869.
88. Lu R, Kanai N, Bao Y, Schuster VL. Cloning, in vitro expression, and tissue distribution of a human prostaglandin transporter cDNA(hPGT). J Clin Invest 1996; 98(5): 1142-1149.
89. Banu SK, Arosh JA, Chapdelaine P, Fortier MA. Molecular cloning and spatio-temporal expression of the prostaglandin transporter: a basis for the action of prostaglandins in the bovine reproductive system. Proc Natl Acad Sci U S A 2003; 100(20): 11747-11752.

90. Arosh JA, Banu SK, Chapdelaine P, Madore E, Sirois J, Fortier MA. Prostaglandin biosynthesis, transport, and signaling in corpus luteum: a basis for autoregulation of luteal function. Endocrinology 2004; 145(5): 2551-2560.
91. Arosh JA, Banu SK, Kimmins S, Chapdelaine P, Maclaren LA, Fortier MA. Effect of interferon-tau on prostaglandin biosynthesis, transport, and signaling at the time of maternal recognition of pregnancy in cattle: evidence of polycrine actions of prostaglandin E2. Endocrinology 2004; 145(11): 5280-5893.
92. Banu SK Arosh JA, Chapdelaine P, Fortier MA. Expression of prostaglandin transporter in the bovine uterus and fetal membranes during pregnancy. Biol Reprod 2005; 73(2): 230-236.
93. Kang J, Chapdelaine P, Parent J, Madore E, Laberge PY, Fortier MA. Expression of human prostaglandin transporter in the human endometrium across the menstrual cycle. J Clin Endocrinol Metab 2005; 90(4): 2308-2313.
94. Nomura T, Chang HY, Lu R, Hankin J, Murphy RC, Schuster VL. Prostaglandin signaling in the renal collecting duct: release, reuptake, and oxidation in the same cell. J Biol Chem 2005; 280(31): 28424-28429.
95. Kankofer M. The enzymes responsible for the metabolism of prostaglandins in bovine placenta. Prostaglandins Leukot Essent Fatty Acids 1999; 61(6): 359-362.
96. Patel FA, Challis JR. Prostaglandins and uterine activity. Front Horm Res 2001; 27: 31-56.
97. Winchester SK, Imamura T, Gross GA, Muglia LM, Vogt SK, Wright J et al. Coordinate regulation of prostaglandin metabolism for induction of parturition in mice. Endocrinology 2002; 143(7): 2593-2598.
98. Backlund MG, Mann JR, Holla VR, Buchanan FG, Tai HH, Musiek ES et al. 15-Hydroxyprostaglandin dehydrogenase is down-regulated in colorectal cancer. J Biol Chem 2005; 280(5): 3217-3223.
99. Mitchell MD, Goodwin V, Mesnage S, Keelan JA. Cytokine-induced coordinate expression of enzymes of prostaglandin biosynthesis and metabolism: 15-hydroxyprostaglandin dehydrogenase. Prostaglandins Leukot Essent Fatty Acids 2000; 62(1): 1-5.
100. Brzozowski T, Konturek PC, Œliwowski Z et al. Interaction of nonsteroidal anti-inflammatory drugs (NSAID) with Helicobacter pylori in stomach of humans and experimental animals. J Physiol Pharmacol 2006; 57 (suppl. 3):67-79.
101. Alvin PE, Litt IF. Current status of the etiology and management of dysmenorrhea in adolescence. Pediatrics 1982; 70(4): 516-525.
102. Sampalis F, Bunea R, Pelland MF, Kowalski O, Duguet N, Dupuis S. Evaluation of the effects of Neptune Krill Oil on the management of premenstrual syndrome and dysmenorrhea. Altern Med Rev 2003; 8(2):171-179.
103. Lands WE. Biochemistry and physiology of n-3 fatty acids. FASEB J 1992; 6(8): 2530-536.
104. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil Steril 2003; 79(4): 829-843.
105. Sugino N, Karube-Harada A, Kashida S, Takiguchi S, Kato H. Reactive oxygen species stimulate prostaglandin F2 alpha production in human endometrial stromal cells in vitro. Hum Reprod 2001; 16(9): 1797-1801.
106. Poyser NL. The control of prostaglandin production by the endometrium in relation to luteolysis and menstruation. Prostaglandins Leukot Essent Fatty Acids 1995; 53(3): 147-195.
107. Nishihara I, Minami T, Uda R, Ito S, Hyodo M, Hayaishi. Effect of NMDA receptor antagonists on prostaglandin E2-induced hyperalgesia in conscious mice. Brain Res 1995; 677(1): 138-44.
108. Milne SA, Perchick GB, Boddy SC, Jabbour HN. Expression, localization, and signaling of PGE(2) and EP2/EP4 receptors in human nonpregnant endometrium across the menstrual cycle. J Clin Endocrinol Metab 2001; 86(9): 4453-4459.

109. Barisani D, Meneveri R, Ginelli E, Cassani C, Conte D. Iron overload and gene expression in HepG2 cells: analysis by differential display. FEBS Lett 2000; 469(2-3): 208-212.
110. O'Connor T, Ireland LS, Harrison DJ, Hayes JD. Major differences exist in the function and tissue-specific expression of human aflatoxin B1 aldehyde reductase and the principal human aldo-keto reductase AKR1 family members. Biochem J 1999; 343 (Pt 2): 487-504.
111. Yang J, Moravec CS, Sussman MA, DiPaola NR, Fu D, Hawthorn L et al. Decreased SLIM1 expression and increased gelsolin expression in failing human hearts measured by high-density oligonucleotide arrays. Circulation 2000; 102(25): 3046-3052.
112. Konturek SJ, Pawlik WW. Polish historical traces in cardiovascular pathophysiology and cardiac surgery. J Physiol Pharmacol 2006; 57 (suppl. 1): 3-4.
113. Rittner HL, Hafner V, Klimiuk PA, Szweda LI, Goronzy JJ, Weyand CM. Aldose reductase functions as a detoxification system for lipid peroxidation products in vasculitis. J Clin Invest 1999; 103(7): 1007-1013.
114. Ruef J, Liu SQ, Bode C, Tocchi M, Srivastava S, Runge MS et al. Involvement of aldose reductase in vascular smooth muscle cell growth and lesion formation after arterial injury. Arterioscler Thromb Vasc Biol 2000; 20(7): 1745-1752.
115. Kassab E, McFarlane SI, Sower JR. Vascular complications in diabetes and their prevention. Vasc Med 2001; 6(4): 249-525.
116. Srivastava S, Chandra A, Bhatnagar A, Srivastava SK, Ansari NH. Lipid peroxidation product, 4-hydroxynonenal and its conjugate with GSH are excellent substrates of bovine lens aldose reductase. Biochem Biophys Res Commun 1995; 217(3): 741-746.
117. Vander Jagt DL, Kolb NS, Vander Jagt TJ, Chino J, Martinez FJ, Hunsaker LA et al. Substrate specificity of human aldose reductase: identification of 4-hydroxynonenal as an endogenous substrate. Biochim Biophys Acta 1995; 1249(2): 117-126.
118. Srivastava S, Chandra A, Wang LF, Seifert WE Jr, DaGue BB, Ansari NH et al. Metabolism of the lipid peroxidation product, 4-hydroxy-trans-2-nonenal, in isolated perfused rat heart. J Biol Chem 1998; 273(18): 10893-10900.
119. Srivastava SK, Chandra A, Srivastava S, Petrash JM, Bhatnagar A. Regulation of aldose reductase by aldehydes and nitric oxide. Adv Exp Med Biol 1999; 463: 501-507.
120. Dixit BL, Balendiran GK, Watowich SJ, Srivastava S, Ramana KV, Petrash JM et al. Kinetic and structural characterization of the glutathione-binding site of aldose reductase. J Biol Chem 2000; 275(28): 21587-21595.
121. Ramana KV, Dixit BL, Srivastava S, Balendiran GK, Srivastava SK, Bhatnagar A. Selective recognition of glutathiolated aldehydes by aldose reductase. Biochemistry 2000; 39(40): 12172-12180.
122. Kasajima H, Yamagishi S, Sugai S, Yagihashi N, Yagihashi S. Enhanced in situ expression of aldose reductase in peripheral nerve and renal glomeruli in diabetic patients. Virchows Arch 2001; 439(1): 46-54.
123. Helmersson J, Vessby B, Larsson A, Basu S. Cyclooxygenase-mediated prostaglandin F2alpha is decreased in an elderly population treated with low-dose aspirin. Prostaglandins Leukot Essent Fatty Acids 2005; 72(4): 227-233.
124. Iwata K, Nishinaka T, Matsuno K, Kakehi T, Katsuyama M, Ibi M et al. The activity of aldose reductase is elevated in diabetic mouse heart. J Pharmacol Sci 2007; 103(4): 408-146.
125. Kaiserova K, Srivastava S, Hoetker JD, Awe SO, Tang XL, Cai J et al. Redox activation of aldose reductase in the ischemic heart. J Biol Chem 2006; 281(22): 15110-15120.
126. Kaneko M, Bucciarelli L, Hwang YC, Lee L, Yan SF, Schmidt AM et al. Aldose reductase and AGE-RAGE pathways: key players in myocardial ischemic injury. Ann N Y Acad Sci 2005; 1043: 702-709.

127. Lo AC, Cheung AK, Hung VK, Yeung CM, He QY, Chiu JF et al. Deletion of aldose reductase leads to protection against cerebral ischemic injury. J Cereb Blood Flow Metab 2007; 27(8): 1496-1509.
128. Ho EC, Lam KS, Chen YS, Yip JC, Arvindakshan M, Yamagishi S et al. Aldose reductase-deficient mice are protected from delayed motor nerve conduction velocity, increased c-Jun NH2-terminal kinase activation, depletion of reduced glutathione, increased superoxide accumulation, and DNA damage. Diabetes 2006; 55(7): 1946-1953.
129. Smith WL, Garavito RM, DeWitt DL. Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. J Biol Chem 1996; 271(52): p. 33157-33160.
130. Miralpeix M, Camacho M, López-Belmonte J, Canalías F, Beleta J, Palacios JM et al. Selective induction of cyclo-oxygenase-2 activity in the permanent human endothelial cell line HUV-EC-C: biochemical and pharmacological characterization. Br J Pharmacol 1997; 121(2): 171-180.
131. Gierse JK, Hauser SD, Creely DP, Koboldt C, Rangwala SH, Isakson PC, Seibert K. Expression and selective inhibition of the constitutive and inducible forms of human cyclo-oxygenase. Biochem J 1995; 305 (Pt 2): 479-484.
132. Warner TD, Giuliano F, Vojnovic I, Bukasa A, Mitchell JA, Vane JR. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis. Proc Natl Acad Sci U S A 1999; 96(13): 7563-7568.
133. Ehrich EW, Dallob A, De Lepeleire I, Van Hecken A, Riendeau D, Yuan W et al. Characterization of rofecoxib as a cyclooxygenase-2 isoform inhibitor and demonstration of analgesia in the dental pain model. Clin Pharmacol Ther 1999; 65(3): 336-347.
134. Gierse JK, Zhang Y, Hood WF, Walker MC, Trigg JS, Maziasz TJ et al. Valdecoxib: assessment of cyclooxygenase-2 potency and selectivity. J Pharmacol Exp Ther 2005; 312(3): 1206-1212.
135. Smith CJ, Zhang Y, Koboldt CM, Muhammad J, Zweifel BS, Shaffer A et al. Pharmacological analysis of cyclooxygenase-1 in inflammation. Proc Natl Acad Sci U S A 1998; 95(22): 13313-13318.

Received: July 10, 2008
Accepted: July 25, 2008

Author's address: Michel A. Fortier Ph.D., Professeur titulaire, Département de gynécologie-obstétrique Universite Laval, Ontogenié et reproduction-CRBR, CHUL Local T-1-49, Bureau 1-41. 2705 Boul. W. Laurier, Ste-Foy, Québec, G1V 4G2; Tél.: 418 656 4141 46141, FAX: 418 654 2765; http://www.crbr.ulaval.ca/