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Comprehensive expression analysis of prostanoid enzymes and receptors in the human endometrium across the menstrual cycle

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ABSTRACT: Prostanoids are well-described primary mediators of inflammatory processes and are essential for the normal physiological function of the female reproductive system. The aim of this study was to determine the temporal expression of the prostanoid biosynthetic enzymes (PTGS1, PTGS2, PTGES, PTGES2, PTGES3, AKR1B1, AKR1C3, CBR1, HPGDS, PTGDS, PTGIS, TBXAS1 and HPGD) and the prostanoid receptors (PTGER1, PTGER2, PTGER3, PTGER4, PTGFR, PTGDR, GPR44, PTGIR and TBXA2R) in the human endometrium throughout the menstrual cycle. The analysis identified PTGFR to have a distinct expression profile compared with other components of the prostanoid system, as expression is maximal during the proliferative phase. Immunohistochemical analysis for PTGER1 suggests a dual function for this receptor depending on its temporal (proliferative versus secretory) and spatial (nuclear versus cell membrane) expression. The expression profiles of the $PGF_{2\alpha}$ synthases identified AKR1B1 and CBR1 as the likely regulators of $PGF_{2\alpha}$ production during the menstrual phase. Immunohistochemical analysis for AKR1B1, CBR1 and AKR1C3 suggest expression to be in the glandular epithelium and vasculature. This study represents the first comprehensive analysis of the components of prostanoid biosynthetic and signalling pathway in the human endometrium. The expression profiles described have the potential to identify specific prostanoid components that may be dysregulated in inflammatory-associated disorders of the endometrium.

Key words: endometrium / menstruation / prostanoid / prostaglandin / inflammation

Introduction

The prostanoids are part of a family of biologically active lipids derived from arachidonic acid which include the prostaglandins, prostacyclins and thromboxanes. Prostanoids are well-described primary mediators in pathological conditions such as inflammation, hypertension and cancer but are also essential for normal physiological function such as in the female reproductive system (Loftin *et al.*, 2002). The importance of prostaglandins in the female reproductive tract is well documented in the mouse where targeted disruption of prostaglandin endoperoxidase synthase 1 or 2 (PTGS1 and PTGS2, previously known as COX1 and COX2) genes result in reduced reproductive efficiency (Lim *et al.*, 1997; Gross *et al.*, 1998). In humans, dysregulated endometrial prostanoid production has been described as a contributing factor in menstrual cycle disorders, infertility and uterine malignancies (Jabbour *et al.*, 2006; 2009; Achache *et al.*, 2010). Non-steroidal anti-inflammatory drugs (NSAIDS) are well documented to

inhibit prostanoid synthesis and are prominent analgesic/anti-inflammatory/antipyretic medication. However, a major drawback is that they target the early steps of biosynthesis thus blocking all prostanoids. Therefore, targeting of selective prostanoid synthases or receptors seem a logical progression in the development of new therapeutic strategies.

Prostanoids are not stored within cells, but are synthesized as required in response to stimuli. The first step in their synthesis is the release of arachidonic acid from the cellular phospholipids, by the action of the enzyme phospholipase A_2. Arachidonic acid is converted by one of two related enzymes, PTGS1 and PTGS2, to form firstly prostaglandin PGG_2 and subsequently reduced to prostaglandin PGH_2. PGH_2 is an unstable intermediate from which all other prostanoids are derived by a variety of different prostanoid synthases and isomerases (reviewed in Watanabe, 2002; Fortier *et al.*, 2008; summarized in Fig. 1). Thus PGH_2 is converted to PGD_2, PGI_2, TXA_2 PGE_2 and $PGF_{2\alpha}$ by prostaglandin D synthases (PTGDS and
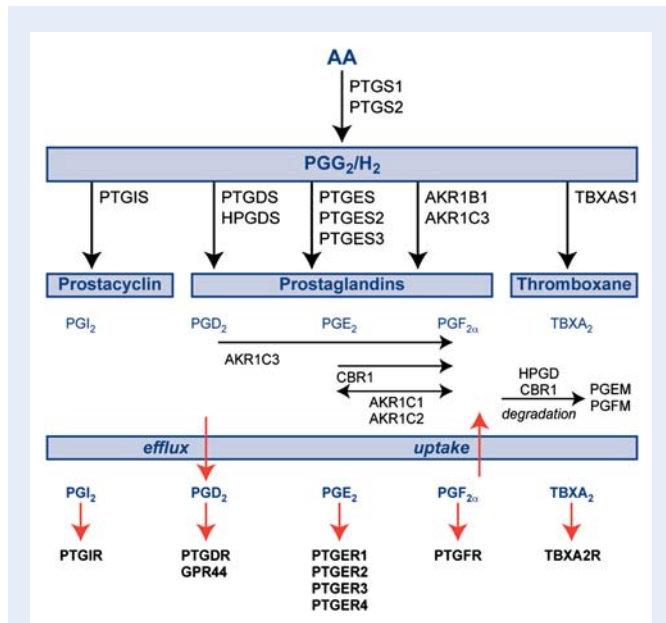
Figure 1 Prostanoid biosynthesis and signalling pathways. Arachidonic acid (AA) is converted by PTGS1 and PTGS2 to PGG_2 and PGH_2. PGH_2 is then converted into one of the active prostanoids by specific terminal synthases such as PGE synthases (PTGES, PTGES2 and PTGES3), PGF synthases (AKR1B1 and AKR1C3), PGD synthases (PTGDS, HPGDS), PGI synthase (PTGIS) and thromboxane synthase (TBXAS1). PGE_2 and $PGF_{2\alpha}$ may also be synthesized by interconversion by $PGF_{2\alpha}$ isomerases AKR1C1 and AKR1C2. $PGF_{2\alpha}$ may also be synthesized by conversion of PGD_2 by AKR1C3 or PGE_2 by CBR1. Synthesized prostanoids cross the plasma membrane via a carrier mediated process and bind to their respective cell surface receptors. Prostanoids may also be actively transported into the cytosol for degradation to inactive metabolites (PGEM and PGFM) by HPGD and CBR1. For abbreviations see Table I.

HPGDS), prostacyclin synthase (PTGIS) and thromboxane A synthase (TBXAS1), prostaglandin E synthases and prostaglandin F synthases, respectively. A number of distinct forms of prostaglandin E synthase exist, a membrane-bound type 1 (PTGES), a type 2 (PTGES2) which has various isoforms and a cytosolic (PTGES3) synthase. $PGF_{2\alpha}$ is synthesized via various routes, although not all routes of synthesis have been demonstrated in human endometrial cells. $PGF_{2\alpha}$ can be produced directly from PGH_2 by aldo-keto reductase family 1 members B1 and C3 (AKR1B1 and AKR1C3). Alternatively, it can be synthesized via PGE_2 by the action of the enzymes carbonyl reductase 1 (CBR1), AKR1C1 and AKR1C2 (Terada et al., 2001; Dozier et al., 2008) or by reduction of PGD_2 by AKR1C3. Prostanoids such as PGI_2 and TXA_2 are deactivated spontaneously before they are exported into the circulation as inactive metabolites. Other prostanoids, such as PGE_2 and $PGF_{2\alpha}$, can be actively metabolized by hydroxyprostaglandin dehydrogenase (HPGD) or CBR1 into keto-prostanoids (Casey et al., 1980; Terada et al., 2001).

Following biosynthesis, prostanoids exert their function through G protein coupled receptors. The receptors have preferential binding to specific ligands; PTGER1–4 for PGE_2, PTGFR for $PGF_{2\alpha}$, PTGDR1 and GPR44 for PGD_2, PTGIR for PGI_2 and TBXA2R for TXA_2 (Abramovitz et al., 2000). Prostanoids may also exert their function through nuclear receptors such as binding of PGI_2 to peroxisome proliferator-activated receptor (PPARδ) (Dussault and Forman, 2000).

The aim of this study was to identify the temporal expression profile of the prostanoid system in the human endometrium across the menstrual cycle. To our knowledge this is the first quantitative and fully comprehensive analysis of the prostanoid system across the menstrual cycle in the human endometrium. The data are discussed in relation to key inflammatory events during the menstrual cycle.

Materials and Methods

Patients and tissue collection

Endometrial biopsies were obtained from women (age range 21–39 years) with regular menstrual cycles who had not received hormonal preparation in the 3 months preceding biopsy collection, and dated according to Noyes criteria (Noyes et al., 1975) by a pathologist. Circulating estradiol and progesterone concentrations were measured and were consistent with the histological assessment. Ethical approval was obtained from Lothian research ethics committee and written informed patient consent obtained before tissue collection.

Quantitative RT–PCR

RNA was extracted with TRI-reagent (Thermo Scientific, Cramlington, UK) following manufacturers guidelines using phase lock tubes (Eppendorf, Cambridge, UK). RNA samples were reverse transcribed using MultiScribe (Applied Biosystems, Foster City, CA, USA) primed with random hexamers according to the manufacturer's instructions. PCR reactions were carried out using an ABI Prism 7500 (Applied Biosystems). Primer and FAM (6-carboxyfluorescein)-labelled probe sequences are shown in Table I. Multiple transcript variants have been reported for human PTGFR (Liang et al., 2008). However, only the full length receptor was detected in the human endometrium in this study. Multiple transcript variants have been described for PTGER3 (Kotani et al., 1997), TBXA2R (Miggin and Kinsella, 1998) and TBXAS1 (Swanson et al., 1992). The primers utilized in this study did not distinguish between these isoforms. Pre-validated primers and probes were purchased for AKR1B1, PTGES3, HPGDS and GPR44 (Assay-on-Demand, Applied Biosystems). Primer-only amplification products were detected using SYBR Green (Applied Biosystems) incorporation during the PCR reaction. Gene expression was normalized to 18S ribosomal RNA (Applied Biosystems) as an internal standard. Results are expressed as relative to a standard (pooled endometrial tissue cDNA) included in all reactions.

Immunohistochemistry

Five micrometres of paraffin-embedded sections were de-waxed and rehydrated through graded alcohols before antigen retrieval was carried out using 0.01 M citrate phosphate buffer in a pressure cooker for 5 min at maximum pressure. After release of pressure, the sections were incubated for a further 21 min before endogenous peroxidase activity was blocked by 5 min incubation in 3% solution of hydrogen peroxide: methanol. Immunohistochemistry was performed using an automated Visions Biosytems 'Bond' Immunohistochemistry system (Bond, CA, USA). Sections were incubated for 120 min with primary antibodies such as mouse anti-AKR1C3 (1:5000; A6229; Sigma, Dorset, UK); rabbit anti-AKR1B1 (1:50; Ab71405; Abcam, Cambridge, UK); goat anti-CBR1 (1:500; Ab4148; Abcam); rabbit anti-PTGDR (1:100; 101640; Cayman Chemicals, Ann Arbor, MI, USA); rabbit anti-PTGER1 (1:500; 101740; Cayman Chemicals) and a corresponding post-primary protocol. Sections were

Table I QPCR primer and probe sequences.

Gene symbol	Forward primer	Reverse primer	Probe
PTGS1 (COX1), Prostaglandin-endoperoxidase synthase 1	tgt tcg gtg tcc agt tcc aat a	acc ttg aag gag tca ggc atg ag	cgc aac cgc att gcc atg gag t
PTGS2 (COX2), Prostaglandin-endoperoxidase synthase 2	cct tcc tcc tgt gcc tga tg	aca atc tca ttt gaa tca gga agc t	tgc ccg act ccc ttg ggt gtc a
PTGES (mPGES1), Prostaglandin E synthase	cgg agg ccc cca gta ttg	ggg tag atg gtc tcc atg tcg tt	cga ccc gac gtg gaa cg
PTGES2 (mPGES2) Prostaglandin E synthase 2	cct cat cag caa gcg act	cca ctt gtc agc agc ctc a	agc agg cac cgc ctc cag
PTGES3 (mPGES3), Prostaglandin E synthase 3	Assay-on-Demand Hs01566250_gH		
AKR1B1, Aldo-keto reductase 1, member B1	Assay-on-Demand Hs00739326_ml		
AKR1C3, Aldo-keto reductase 1, member C1	ggg atc tca acg aga caa acg	aaa gga ctg ggt cct cca aga	tgg acc cga act ccc cgg tg
CBR1, Carbonyl reductase 1	ctg atc cca cac cct ttc at	gct cct cct cag tga tgg tc	n/a
PTGDS, Prostaglandin D2 synthase	gct gca gga gaa tgg cta ct	agg aag gtg gag gtc agg tt	n/a
HPGD (PDGH), Hydroxyprostaglandin dehydrogenase	gtg aag gcg gca tca tta tc	tga atc aaa cta tgc cat gc	n/a
PTGIS, Prostaglandin I2 (prostacyclin) synthase	acg cag atg tgg aga tcc ct	gtc gtg ttc cgg ctg ca	cct cag cag gta cgg ctt cgg tct g
TBXAS1, Thromboxane A synthase 1	cag gtg ttg gtt gag aac ttc agt a	tgt cgg cta ccg act tga act	caa acc cga cgc cat tct gtt ggt aaa g
HPGDS, Haematopoietic prostaglandin D synthase	Assay-on-Demand IDHs00183950_ml		
PTGER1, Prostaglandin E receptor 1	aga tgg tgg gcc agc ttg t	gcc acc aac acc agc att g	cag cag atg cac gac acc acc atg
PTGER2, Prostaglandin E receptor 2	gac cgc tta cct gca gct gta c	tga agt tgc agg cga gca	cca ccc tgc tgc tgc ttc tca ttg tct
PTGER3, Prostaglandin E receptor 3	gac ggc cat tca gct tat gg	ttg aag atc att ttc aac atc att atc a	ctg tcg gtc tgc tgg tct ccg ctc
PTGER4, Prostaglandin E receptor 4	acg ccg cct act cct aca tg	aga gga cgg tgg cga gaa t	acg cgg gct tca gct cct tcc t
PTGFR, Prostaglandin F receptor	gca gct gcg ctt ctt tca a	cac tgt cat gaa gat tac tga aaa aaa tac	cac aac ctg cca gac gga aaa ccg
PTGDR, Prostaglandin D2 receptor	tga tga ccg tgc tct tca ct	cac cgg ctc ctg tac cta ag	n/a
GPR44 (CRTH2), G protein-coupled receptor 44	Assay-on-Demand, IDHs00173717_ml		
PTGIR, Prostaglandin I2 receptor	gcc ctc ccc ctc tac caa	ttt tcc aat aac tgt ggt ttt gt g	cca aga gcc agc ccc ctt tct gc
TBXA2R, Thromboxane A2 receptor	tgg tgg tgg cca gcg t	cgg gtt tcg cag cac tgt	tgc ccc ttc tgg tct tca tcg ccc

then counterstained in haematoxylin and mounted in pertex. Sections were imaged using a Provis AX70 microscope (Olympus, Southend-on-Sea, UK) and photographed using an AxioCam HRc with AxioVision Release 4.8 (Carl Zeiss Ltd, Berlin, Germany). Immunohistochemistry was performed in duplicate on three to five different endometrial samples for each phase of the menstrual cycle and the most representative section was imaged.

Statistics

Data were subjected to statistical analysis using a one-way analysis of variance with Tukey's multiple comparison post test (GraphPad Prism, GraphPad Software, Inc., La Jolla, CA, USA).

Results

RNA transcript expression of prostanoid enzymes across the menstrual cycle

Quantitative real-time PCR (Q-RT–PCR) was performed to determine the RNA transcriptional profiles of prostanoid enzymes in endometrial tissue collected across the menstrual cycle. The temporal pattern of expression for all synthase enzymes is illustrated in Fig. 2. The analyses demonstrate that expression of prostanoid synthases are predominantly elevated in the secretory and menstrual phases

of the cycle with a decrease in expression in the proliferative phase (Fig. 2 and Table II).

Significant mean fold increases in expression across the menstrual cycle for each prostanoid enzyme are shown in Table II. The table illustrates that expression for all prostanoid synthases, except PTGES2, during the menstrual phase is higher by 2-fold or more compared with the proliferative phase. A 10-fold or more increase in expression was observed for PTGS1 (15.5-fold, MS versus LS), PTGS2 (16.5-fold, Mn versus P), PTGES3 (17.1-fold, Mn versus LS), CBR1 (10.7-fold, Mn versus P), HPGDS (12.6-fold, LS versus P) and PTGDS (36.6-fold, MS versus Mn). The expression of HPGD, a regulator of prostanoid catabolism, was significantly decreased in the proliferative and late secretory phases.

RNA transcript expression of prostanoid receptors across the menstrual cycle

Q-RT–PCR was performed to determine the RNA transcriptional profiles of prostanoid receptors in endometrial tissue collected across the menstrual cycle. The temporal pattern of expression for prostanoid receptors is illustrated in Fig. 3. The analysis demonstrated that expression of the prostanoid receptors are predominantly elevated in the secretory phase of the cycle except for the expression
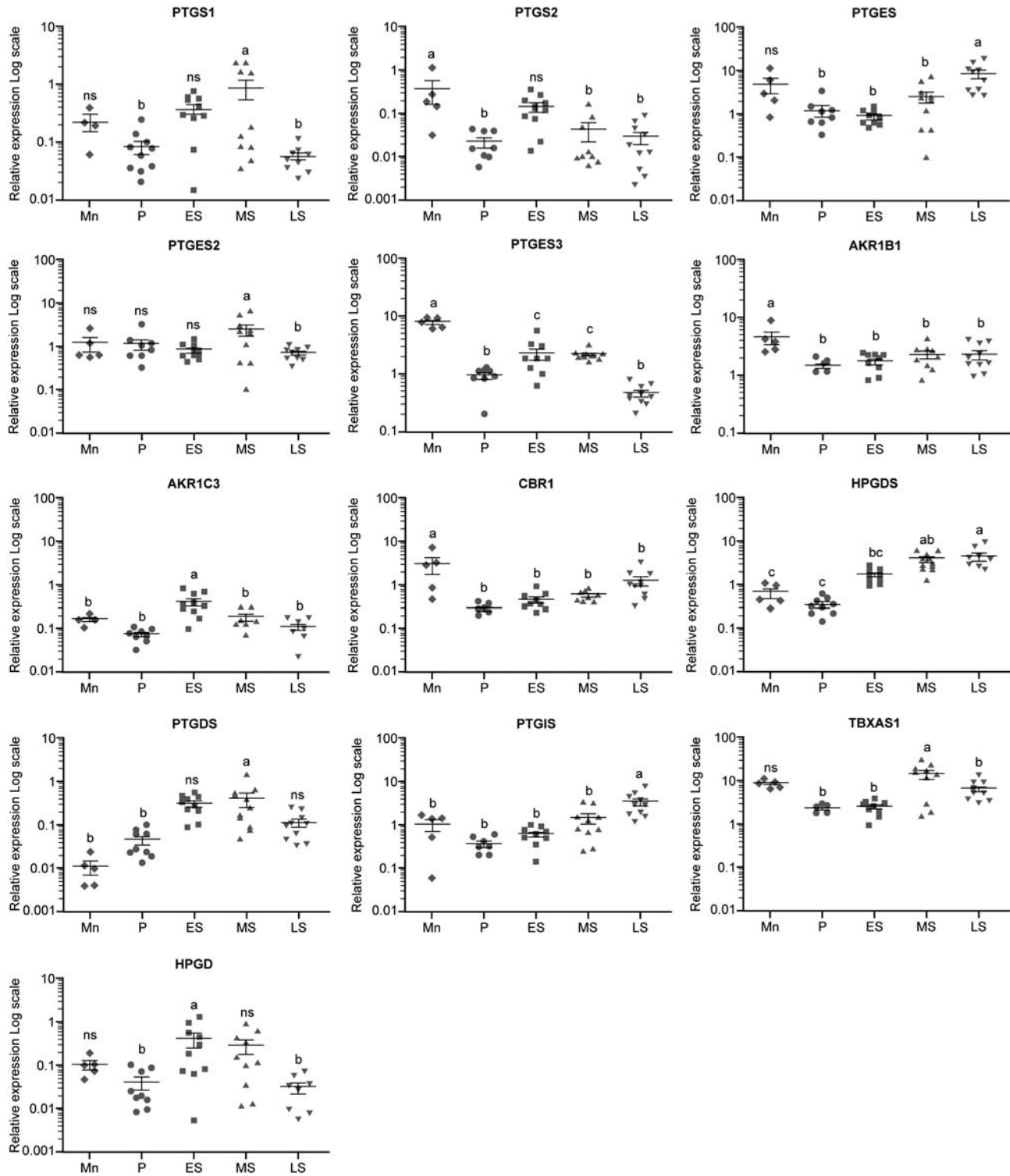
Figure 2 QPCR expression analysis of prostanoid enzymes across the menstrual cycle. Relative levels of RNA transcripts were quantified by RT−PCR from endometrial samples taken from the menstrual (Mn), proliferative (P), early secretory (ES), mid-secretory (MS) and late secretory (LS) phase of the menstrual cycle. Expression levels were measured compared with a calibrator RNA sample. The graphs show individual values for each sample, mean expression levels in arbitrary units normalized to 18S ribosomal RNA and error bars represent SEM. Mn ($n = 5$), P ($n = 10$), ES ($n = 10$), MS ($n = 10$) and LS ($n = 10$); Different letters (a−c) represent statistically significant differences between groups (see Table II for P-values); ns, not significant.

Table II Summary of significant increases in expression of prostanoid enzymes across the menstrual cycle; menstrual (Mn), proliferative (P), early secretory (ES), mid-secretory (MS) and late secretory phase (LS).

Comparison	*P*-value	Mean fold increase
PTGS1		
MS versus P	$P < 0.05$	10.5
MS versus LS	$P < 0.01$	15.5
PTGS2		
Mn versus P	$P < 0.01$	16.5
Mn versus MS	$P < 0.01$	8.9
Mn versus LS	$P < 0.01$	12.9
PTGES		
LS versus P	$P < 0.001$	6.9
LS versus ES	$P < 0.001$	9.6
LS versus MS	$P < 0.01$	3.3
PTGES2		
MS versus LS	$P < 0.05$	3.5
PTGES3		
ES versus P	$P < 0.05$	2.4
ES versus LS	$P < 0.001$	4.9
MS versus P	$P < 0.05$	2.4
MS versus LS	$P < 0.01$	4.8
Mn versus P	$P < 0.001$	8.5
Mn versus ES	$P < 0.001$	3.5
Mn versus MS	$P < 0.001$	3.6
Mn versus LS	$P < 0.001$	17.1
AKR1B1		
Mn versus P	$P < 0.001$	3.2
Mn versus ES	$P < 0.01$	2.6
Mn versus MS	$P < 0.05$	2
Mn versus LS	$P < 0.05$	2
AKR1C3		
ES versus P	$P < 0.001$	5.5
ES versus MS	$P < 0.01$	2.2
ES versus LS	$P < 0.001$	3.7
ES versus Mn	$P < 0.05$	2.4
CBR1		
Mn versus P	$P < 0.001$	10.7
Mn versus ES	$P < 0.001$	6.6
Mn versus MS	$P < 0.01$	5.2
Mn versus LS	$P < 0.05$	2.4
HPGDS		
MS versus Mn	$P < 0.01$	5.9
MS versus P	$P < 0.001$	11.1
LS versus Mn	$P < 0.01$	6.7
LS versus P	$P < 0.001$	12.6
LS versus ES	$P < 0.05$	2.5

Continued

Table II *Continued*

Comparison	*P*-value	Mean fold increase
PTGDS		
MS versus P	$P < 0.05$	8.9
MS versus Mn	$P < 0.05$	36.6
PTGIS		
LS versus P	$P < 0.001$	9.4
LS versus ES	$P < 0.001$	5.6
LS versus MS	$P < 0.01$	2.4
LS versus Mn	$P < 0.01$	3.3
TBXAS1		
MS versus P	$P < 0.001$	6.1
MS versus ES	$P < 0.001$	5.7
MS versus LS	$P < 0.05$	2.2
HPGD		
ES versus P	$P < 0.05$	9.9
ES versus LS	$P < 0.05$	12.6

of PTGFR which is significantly elevated in the proliferative phase (Fig. 3 and Table III).

The significant mean fold increase in expression across the menstrual cycle for each prostanoid receptor is shown in Table III. A dramatic increase in expression is observed in the proliferative phase for PTGFR (10.3-fold, P versus Mn), in the early secretory phase for PTGER1 (12.2-fold, ES versus Mn), in the mid-secretory phase for PTGER2 (15.1-fold, MS versus P) and TBXR (13.0-fold, MS versus Mn) and in the late secretory phase for PTGIR (22.3-fold, LS versus P) and PTGDR (9.0-fold LS versus ES).

Immunohistochemical localization of prostanoid enzymes and receptors in the human endometrium

Immunohistochemical analysis was performed for selected genes which have not been studied previously in the human endometrium across the menstrual cycle. These included AKR1C3, CBR1, AKR1B1, PTGDR and PTGER1. The analysis aimed to determine whether the RNA transcripts detected by Q-RT–PCR were translated into protein products and to determine their spatial and temporal expression across the menstrual cycle. All transcripts detected by RT–PCR were shown to be translated into protein products (Fig. 4). The staining intensity of the immunohistochemistry suggested protein expression was comparable to RNA expression obtained by Q-RT–PCR except for staining of AKR1C3 and AKR1B1 in the mid-secretory phase and PTGER1 in the proliferative phase. These differences suggested a modest decrease in protein expression compared with the RNA expression profile, although caution must be exercised when interpreting immunohistochemistry. Quantitatively interpreting immunostaining between a section with intense localized staining and a section with moderate staining intensity throughout may give a misleading impression of differences in global expression levels.
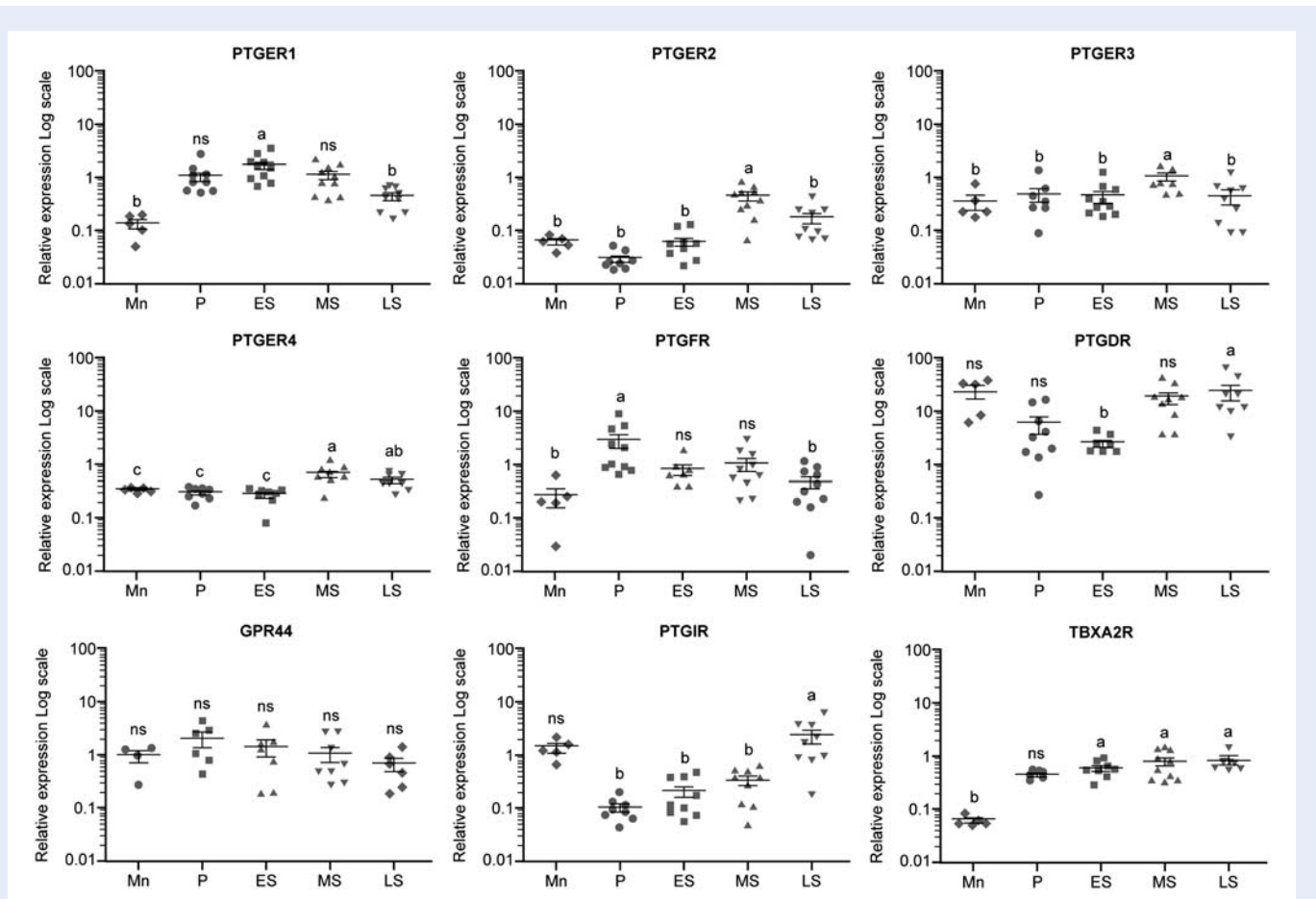
Figure 3 QPCR expression analysis of prostanoid receptors across the menstrual cycle. Relative levels of RNA transcripts were quantified by RT−PCR from endometrial samples taken from the menstrual (Mn), proliferative (P), early secretory (ES), mid-secretory (MS) and late secretory (LS) phase of the menstrual cycle. Expression levels were measured compared with a calibrator RNA sample. The graphs show individual values for each sample, mean expression levels in arbitrary units normalized to 18S ribosomal RNA and error bars represent SEM. Mn ($n = 5$), P ($n = 10$), ES ($n = 10$), MS ($n = 10$) and LS ($n = 10$); Different letters (a−c) represent statistically significant differences between groups (see Table III for P-values); ns, not significant.

AKR1C3 immunostaining was localized to the glandular and luminal epithelium and the blood vessels (Fig. 4A−E). Immunostaining was very prominent throughout the cycle, and in the menstrual phase (Fig. 4E) staining in the blood vessels became more prominent. CBR1 immunostaining was localized predominantly to the glandular and luminal epithelium (Fig. 4F−J). Staining was observed in the stroma throughout the menstrual cycle but was faint and inconsistent. AKR1B1 immunostaining was localized predominantly to the glandular and luminal epithelium and the blood vessels with less intense and scattered stromal perinuclear staining (Fig. 4K−O). In the late secretory phase and menstrual phase (Fig. 4N and O) immunostaining was observed in a subset of stromal cells. PTGDR immunostaining was localized to the glandular epithelium, blood vessels and throughout the stroma but not to all cells (Fig. 4P−T). Intense immunostaining was observed in aggregating cells in the stroma in the secretory phase and menstrual phase (Fig. 4S and T). PTGER1 was shown to be localized to the glandular epithelium. Immunostaining of proliferative phase and early secretory phase (Fig. 4U and V) endometrium was weak and confined to the nuclear region. In the mid- and late secretory phases (Fig. 4W−X) immunostaining was intense and localized predominantly

to the apical membrane of the glandular epithelium. Menstrual phase immunostaining was faint and localized to the nuclear region of the glandular epithelium (Fig. 4Y).

Discussion

Inflammatory events have been described in the human endometrium during the period of endometrial receptivity and menstruation. These events are characterized by an increase in endometrial capillary dilation, permeability, stromal edema, blood flow, leucocyte number and secretion of pro-inflammatory factors (Fraser et al., 1987; Johannisson et al., 1987; Peek et al., 1992). Pro-inflammatory enzymes PTGS1 and PTGS2 are key mediators of these inflammatory processes. However, the downstream prostanoids and receptors that contribute to these inflammatory events remain unclear. The aim of this study was to provide expression profiles across the menstrual cycle of the genes which constitute the prostanoid biosynthetic and signalling pathways. The data generated in this study are summarized in Fig. 5 and indicate the stage of the menstrual cycle where maximum transcript expression occurs for each gene. The data suggest that distinct components of the

Table III Summary of significant increases in expression of prostanoid receptors across the menstrual cycle; menstrual (Mn), proliferative (P), early secretory (ES), mid-secretory (MS) and late secretory phase (LS).

Comparison	P-value	Mean fold increase
PTGER1		
ES versus LS	$P < 0.001$	3.8
ES versus Mn	$P < 0.001$	12.2
PTGER2		
MS versus P	$P < 0.001$	15.1
MS versus ES	$P < 0.001$	7.3
MS versus LS	$P < 0.001$	2.6
MS versus Mn	$P < 0.001$	7
PTGER3		
MS versus P	$P < 0.05$	2.2
MS versus ES	$P < 0.01$	2.4
MS versus LS	$P < 0.05$	2.3
MS versus Mn	$P < 0.05$	2.9
PTGER4		
MS versus P	$P < 0.001$	2.2
MS versus ES	$P < 0.001$	2.4
MS versus Mn	$P < 0.01$	1.9
LS versus ES	$P < 0.05$	1.9
PTGFR		
P versus LS	$P < 0.01$	5.8
P versus Mn	$P < 0.05$	10.3
PTGDR		
LS versus ES	$P < 0.05$	9
PTGIR		
LS versus P	$P < 0.001$	22.3
LS versus ES	$P < 0.001$	11.1
LS versus MS	$P < 0.01$	6.8
TBXA2R		
ES versus Mn	$P < 0.05$	9.4
MS versus Mn	$P < 0.001$	13
LS versus Mn	$P < 0.001$	12.7

prostanoid system are associated with specific stages of the menstrual cycle and expression predominantly increases from the mid-secretory phase through to, and including, menstruation.

The proliferative phase of the menstrual cycle is characterized by endometrial re-growth consisting of endometrial cell proliferation and angiogenesis. This study confirms that the expression of PTGFR is maximal during the proliferative phase (Milne and Jabbour, 2003) and therefore distinct to other components of the prostanoid biosynthetic and signalling pathway. PTGFR is predominantly expressed in the glandular epithelium and also the stromal and perivascular cells (Milne and Jabbour, 2003). $PGF_{2\alpha}$ signalling via PTGFR in endometrial epithelial cells has been shown to induce changes in epithelial cell morphology and migration (Sales et al., 2008), proliferation (Sales et al.,

2007) and angiogenesis (Keightley et al., 2010) suggesting an important role for this receptor in tissue remodelling during endometrial re-growth. $PGF_{2\alpha}$ production in the endometrium has been shown to rise significantly in the mid-proliferative phase (Downie et al., 1974). This increase may be due to the reduction of HPGD expression at this time which would reduce $PGF_{2\alpha}$ degradation.

The secretory phase is dependent on the rise in progesterone secretion following ovulation and induces endometrial differentiation. During the secretory phase prostanoid production continues to increase (Downie et al., 1974), which can be accounted for by the elevated expression of PTGS1 increasing the availability of PGH_2 for enzymatic conversion. During the early secretory phase PTGER1 expression is maximal. PTGER1 has been reported to be expressed strongly in the luminal and glandular epithelium (Carson et al., 2002). We demonstrate that PTGER1 immunostaining in the proliferative, early secretory and menstrual phases is localized to the nuclear region of the glands similar to reports for PGE_2 receptors 3 and 4 (Bhattacharya et al., 1998) and during the mid- and late secretory phases localizes to the apical plasma membrane of the glands. This suggests that PTGER1 may have two distinct functions in the endometrium depending on its localization. The expression of the prostanoid receptors PTGER2, 3 and 4 peaked during the mid-secretory phase. The increased expression of these receptors coincides with increased stromal oedema, blood vessel permeability and endometrial blood flow in the human endometrium (Fraser et al., 1987; Johannisson et al., 1987; Peek et al., 1992). Evidence supporting a role for PGE_2 receptors in these events is demonstrated by infusion of PGE_2 into rodent uteri which has been shown to increase uterine vascular permeability, uterine extracellular fluid volume (Hamilton and Kennedy, 1994) and act as an immunomodulator inhibiting lytic activation of maternal leucocytes (Parhar et al., 1989).

Endometrial PGD_2 release has been shown to peak during the mid-secretory phase (Rees and Kelly, 1986) and is produced by macrophages and glandular epithelial cells (Norwitz et al., 1992; Norwitz and Wilson, 2000). Two PGD_2 synthases have been characterized: lipocalin type (PTGDS) (Tanaka et al., 1997) and haematopoietic PGDS (HPGDS). The peak in PTGDS expression in the mid-secretory phase supports data demonstrating PTGDS to be regulated by progesterone, as expression decreases in the endometrium after administration of mifepristone (Catalano et al., 2007). The expression of HPGDS also peaked in the mid-secretory phase and was maintained throughout the late secretory phase. PGD_2 is involved in multiple aspects of inflammation through its dual receptor systems, PTGDR and GPR44 (previously known as CRTH2). GPR44 is a chemoattractant receptor expressed on T-helper (Th) and T-cytotoxic (Tc) 2 cells, eosinophils and basophils (Nagata et al., 1999; Tsuda et al., 2001). PTGDR expressed on dendritic cells has been shown to inhibit T cell migration (Gosset et al., 2005). In the endometrium PGD_2 through its two receptors regulates recruitment of Th2 and Tc2 cells into the implantation site (Michimata et al., 2002) and positively regulates trophoblast invasion (Chaouat et al., 1988). GPR44 expression levels were low and did not change throughout the menstrual cycle, whereas PTGDR expression increased from the mid-secretory to the menstrual phase. Immunostaining of PTGDR during the late secretory phase and menstrual phase was detected in cell aggregates in the stroma. This observation may reflect the influx of immune cells into the endometrium at these stages (Salamonsen
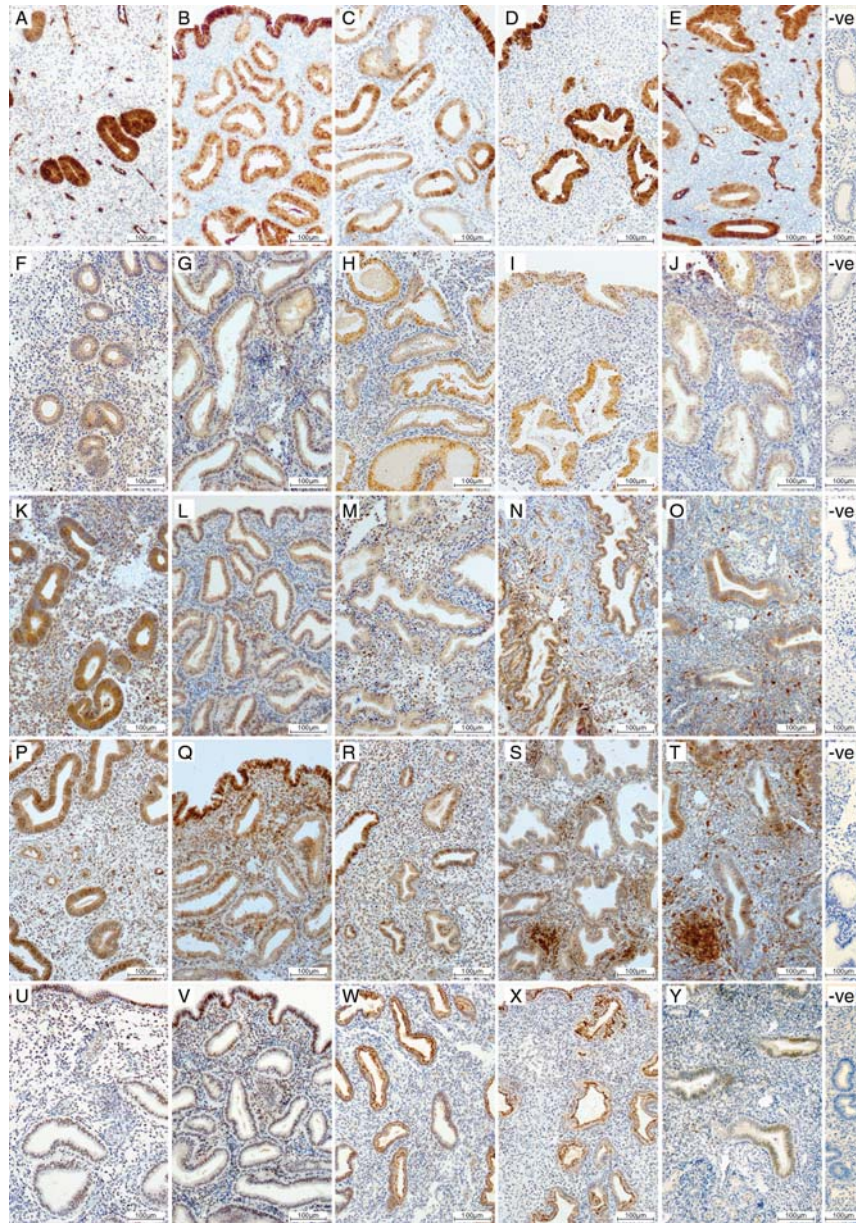
Figure 4 Immunohistochemical localization of AKR1C3 (**A**–**E**), CBR1 (**F**–**J**), AKR1B1 (**K**–**O**), PTGDR (**P**–**T**) and PTGER1 (**U**–**Y**) in the endometrium across the menstrual cycle; proliferative endometrium (A, F, K, P, U), early-secretory endometrium (B, G, L, Q, V), mid-secretory endometrium (C, H, M, R, W), late secretory endometrium (D, I, N, S, X) and menstrual endometrium (E, J, O, T, Y). Negative controls (−ve). For abbreviations see Table I.

and Lathbury, 2000) as dentritic cells, macrophages and natural killer cells have been shown to express PTGDR (Chen et al., 2007; Tajima et al., 2008). Immunostaining for PTGDR also localized to the blood vessels. This suggests PGD_2 may regulate menstrual blood loss during menstruation via known functions of PGD_2 on platelets and blood vessels, such as increasing blood flow and inhibiting platelet aggregation and smooth muscle contraction and relaxation (Braun and Schror, 1992; Foley et al., 2001).

Our data also demonstrate a role for thromboxane during the secretory phase. TBXAS1 expression peaked during the mid-secretory phase and TBXA2R expression was elevated throughout the secretory phase. TXA_2 is classically known to cause platelet aggregation and constriction of blood vessels and in the endometrium presumed to regulate blood flow (Swanson et al., 1992). TXA_2 has also been described to have other functions such as the regulation of angiogenesis, but this has not been investigated in the endometrium (Cathcart et al., 2010). TBXAS1 immunostaining has been observed in glandular epithelium, stroma and blood vessels and increased in the glandular epithelium during the secretory phase (Swanson et al., 1992).

In the late secretory phase in a non-pregnant cycle the corpus luteum involutes and progesterone levels decline, triggering menstruation. The menstrual phase is characterized by ischaemia due to
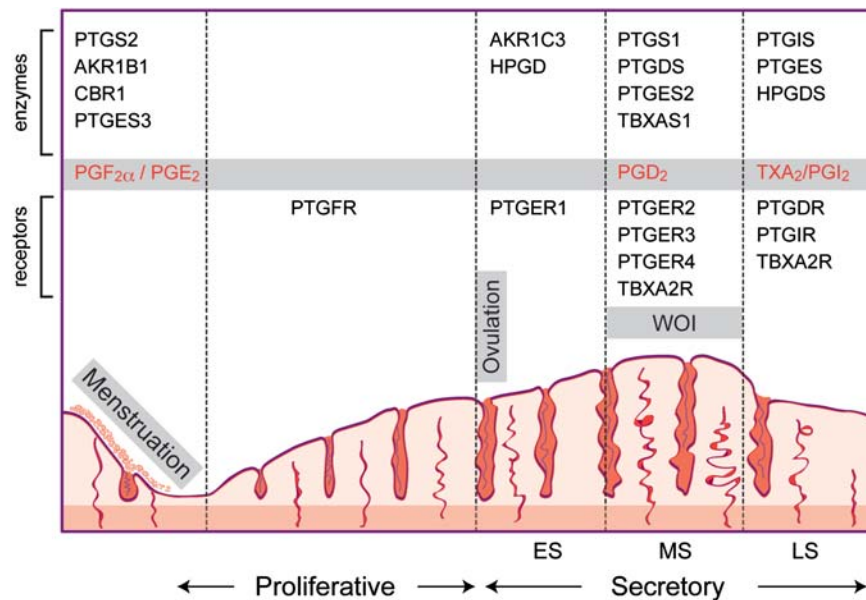
Figure 5 Expression of prostanoid biosynthetic enzyme and receptor genes in the menstrual cycle. Summary diagram illustrating the maximal expression during the menstrual cycle for each prostanoid biosynthesis and receptor gene analysed and reported maximum production during the menstrual cycle for each prostanoid (shown in red). WOI, window of implantation. For abbreviations see Table I.

vasoconstriction of endometrial vessels, rupture of the capillaries initiating bleeding and the consequent shedding of the functional layer of the endometrium. During the late secretory and menstrual phases $PGF_{2\alpha}$, PGE_2, TXA_2 and PGI_2 production rises significantly (Downie *et al.*, 1974; Liggins *et al.*, 1980; Goodfellow *et al.*, 1982). Our results from the expression analysis of the prostanoid enzymes provide data on how the production of $PGF_{2\alpha}$ and PGE_2 are regulated. The production of PGE_2 is regulated by different synthases predominating during the menstrual cycle with PTGES2 during the mid-secretory phase, PTGES during the late secretory phase and PTGES3 during the menstrual phase. This may reflect a mechanism by which PGE_2 production in the endometrium may be maintained under different regulatory conditions. Classically the role of $PGF_{2\alpha}$ via PTGFR during the menstrual stage is to mediate vasoconstriction of the endometrial vessels and contraction of the smooth muscles of the myometrium. Evidence supporting the functionality of the potential $PGF_{2\alpha}$ synthases (AKR1B1, AKR1C1, AKR1C2, AKR1C3 and CBR1) in the human endometrium has only been demonstrated for AKR1B1 (Chapdelaine *et al.*, 2006), AKR1C3 and CBR1 (Kruger and Schlegel, 1986; Pelletier *et al.*, 1999) therefore only these genes were analysed in this study. The rise in both $PGF_{2\alpha}$ and PGE_2 menstrually reflects the significant increase in PTGS2 and the terminal prostanoid synthases AKR1B1, CBR1 and PTGES3. The immuno-localization of CBR1, AKR1C3 and AKR1B1 are predominantly in the glandular epithelium and vasculature of the endometrium. This is in agreement with previous studies which report glandular epithelial cells to produce more $PGF_{2\alpha}$ than stromal cells (Lumsden *et al.*, 1984). AKR1C3 immunoreactivity has previously been shown in the luminal and glandular epithelium (Pelletier *et al.*, 1999). We also demonstrate AKR1B1 immunoreactivity in a subset of stromal cells in the late and menstrual phases. Immunostaining for AKR1B1 localized to the perinuclear region in stromal cells. Terminal prostanoid synthases show distinct functional coupling with upstream COX isozymes (Ueno *et al.*, 2005). The immunostaining supports previous data (Chapdelaine *et al.*, 2006) suggesting that AKR1B1 couples to PTGS2 which resides predominantly in the perinuclear membranes.

PGI_2 is the main prostanoid synthesized by the vascular endothelium and it causes blood vessel dilatation and inhibition of platelet aggregation (Smyth and FitzGerald, 2002): these effects have suggested a role for PGI_2 in menstruation. PTGIS and PTGIR immuno-reactivity in the endometrium is localized predominantly to the glandular and luminal epithelium with expression also detected in the vasculature and stroma (Battersby *et al.*, 2004). PGI_2 has been shown to be inhibited by progesterone (Levin *et al.*, 1992) which correlates with the expression of PGIS peaking in the late secretory phase as progesterone is withdrawn. PTGIR with a similar expression profile has also been demonstrated to be inhibited by progesterone as expression increases in the endometrium after administration of mifepristone (Catalano *et al.*, 2007).

This study represents the first comprehensive view of prostanoid action in the endometrium during the menstrual cycle. Identifying the expression profile of the prostanoid system and its association to key inflammatory events provides valuable insight into the role of specific signalling pathways in normal endometrial function. Menstrual cycle disorders such as primary dysmenorrhea and heavy menstrual blood loss in the absence of any pathology have been associated with an increase and an imbalance in prostanoid synthesis (Lundstrom and Green, 1978; Smith *et al.*, 1981; Makarainen and Ylikorkala, 1986). Hence the data presented herein provides the potential to identify which component(s) of the prostanoid system may contribute to

inflammatory-associated disorders of the endometrium such as heavy menstrual blood loss and dysmenorrhoea and may aid in the development of novel therapeutics.

Authors' roles

R.D.C.: major contribution to conception and design, analysis and interpretation of data, writing of manuscript. M.R.W.: design, acquisition of data, analysis and interpretation of data. S.C.B.: acquisition and analysis of data. H.N.J.: critically revising manuscript for important intellectual content and approval of the final version to be published.

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