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Research Article

Progesterone regulation of implantation-related genes: new insights into the role of oestrogen

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Abstract. Genomic profiling was performed on explants of late proliferative phase human endometrium after 24-h treatment with progesterone (P) or oestradiol and progesterone (17β-E_2+P) and on explants of menstrual phase endometrium treated with 17β-E_2+P. Gene expression was validated with real-time PCR in the samples used for the arrays, in endometrium collected from early and mid-secretory phase endometrium, and in additional experiments performed on new samples collected in the menstrual and late proliferative phase. The results show that late proliferative phase human endometrium is more responsive to progestins than menstrual phase endometrium, that the expression of several genes associated with embryo implantation (*i.e.* thrombomodulin, monoamine oxidase A, SPARC-like 1) can be induced by P *in vitro*, and that genes that are fully dependent on the continuous presence of 17β-E_2 during P exposure can be distinguished from those that are P-dependent to a lesser extent. Therefore, 17β-E_2 selectively primes implantation-related genes for the effects of P.

Keywords. human endometrium, explant cultures, global gene expression, oestradiol, progesterone.

Introduction

Optimal development of the endometrium is an essential prerequisite for successful blastocyst implantation. Progesterone (P) is essential for secretory differentiation of endometrium, and the need for oestrogen in cooperation with P in regulating the implantation process is species-specific [1]. Our current knowledge of the cellular and molecular events orchestrating endometrial growth and differentiation prior to implantation is limited.

In the natural cycle, the human endometrium is receptive during a short period, approximately 19 to 24 days after the onset of menstruation [2–6]. Prior to and during this period, the endometrium undergoes extensive morphological and physiological changes to facilitate implantation of the embryo [2, 6, 7]. These changes are tightly controlled by oestrogen and P [6, 8, 9]. The responsiveness of the endometrium to P is partly dependent upon the pre-ovulatory changes that have occurred under the influence of oestrogen. This is illustrated by the fact that high oestrogen levels and/

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or prolonged oestrogen exposure accelerates endometrial maturation, thus disturbing the synchrony of embryo and endometrial development and subsequent implantation [10, 11]. Currently, there is no clear definition and understanding of human endometrial maturation and only limited knowledge about the cellular mechanisms involved. We define mature endometrial tissue as the physiological state of the human endometrium that allows a correct response to the luteal P, resulting in implantation of the embryo and maintenance of pregnancy.

Our limited understanding of the processes underlying endometrial maturation and P-controlled differentiation prior to and during implantation is largely due to the lack of relevant model systems to evaluate endometrial responses under physiologically relevant conditions. Previous work has demonstrated that explant culture of human endometrial tissue is a suitable model to study the response to oestrogen and P, most likely due to the preservation of the tissue context [12–14]. Using this model we showed previously that the responsiveness of the endometrium to oestrogen changes throughout the proliferative phase with regard to the regulation of gene expression and proliferation [12].

The present study was designed to gain more insight into the responses of human endometrium to P with regard to gene expression and into the influence of 17β-oestradiol (17β-E_2) on this process. To this end, global gene expression analysis was performed on human endometrial tissue fragments collected from the menstrual and late proliferative phases after short-term culture in the presence of P and 17β-E_2.

Materials and methods

Human endometrial tissue. Endometrial tissue was collected from 26 women (20–45 years of age) with regular menstrual cycles who underwent surgery for benign indications. The tissue was collected from hysterectomy specimens for benign indications or by pipelle biopsies during laparoscopy for sterilisation (Pipelle catheter, Unimar Inc., Prodimed, Neuilly-Enthelle, France). It was documented that the women were not on any kind of steroid medication. All women who agreed to participate in the study signed an informed consent form according to a protocol approved by the Medical Ethical Committee of the Academic Hospital Maastricht. Tissue was transported to the laboratory in DMEM/Ham's F12 medium on ice. A portion of each sample was fixed in 10% buffered formalin for evaluation by histology. The endometrium was dated according to clinical information with respect to the start of the last menstrual period, which was reconfirmed by histological examination of the tissue [15]. Of the 26 biopsy specimens, 11 were collected in the proliferative phase [menstrual phase, cycle day (CD)1–5, n=6; late proliferative phase, CD11–14, n=5], and 15 were collected in the secretory phase [early secretory (ES), CD15–18, n=7; mid-secretory (MS), CD19–24, n=8]. Of the 11 biopsy specimens collected from the proliferative phase, 4 were used for microarray studies, and 7 were used for validation purposes with real-time PCR analysis. The biopsy specimens collected from the secretory phase were used for validation only.

Explant cultures. Human endometrium explant cultures were prepared from menstrual phase and late proliferative phase endometrium as described by Punyadeera et al. [16]. In brief, human endometrial tissue was cut into 2–3 mm^3 pieces. A total of 24 explants were placed in Millicell-CM culture inserts (0.4 μm pore size, 30 mm diameter; Millipore, France) in 6-well plates containing 1.2 ml phenol red-free DMEM/Ham's F12 medium (Life Technologies, Grand Island, NY), supplemented with L-glutamine (1%), penicillin and streptomycin (1%, P/S). Cultures were performed for 24 h. Previous experiments have shown that collagenase activity remains very low in proliferative endometrial tissue during the first 24 h of culture [17] and that the tissue viability is not affected after 24 h of culture [13].

The explants prepared from late proliferative phase endometrium were cultured in the presence of vehicle (0.1% ethanol), 17β-E_2 and P (1 nM each), or P alone (1 nM). The 17β-E_2 was included to maintain the *in vivo* oestrogen support. In order to make inferences with regard to the responsiveness of the endometrium before and after prolonged *in vivo* oestrogen exposure, we also treated explant cultures prepared from menstrual phase endometrium (CD3 and CD4) with 17β-E_2 and P. To test the importance of 17β-E_2 in the response of late proliferative phase endometrium to P, 17β-E_2 was also omitted from some cultures. The steroid hormones were provided by Organon N.V. (Oss, The Netherlands).

Total RNA extraction and cDNA synthesis. Total cellular RNA from explants was extracted using the SV total RNA isolation kit (Promega, USA) according to the manufacturer's protocol, with slight modifications: The concentration of DNase-1 during DNase treatment of the RNA samples was doubled, and the incubation time was extended by 15 min in order to completely remove genomic DNA. Total RNA was eluted from the column in 50 μl RNase-free water and stored at −70°C until further analysis. The quality of the RNA samples was determined with the Agilent bioanalyzer 2100 lab-on-a-chip (Agilent, USA). All the samples analysed gave 28S to 18S ratios higher than 1.5. PCR for a housekeeping gene, GAPDH, was performed to confirm that the RNA samples were free of genomic DNA.

Total RNA (1 μg) was incubated with random hexamers (1 μg/μl, Promega) at 70°C for 10 min. The samples were chilled on ice for 5 min. To this mixture, a reverse transcriptase (RT) mix consisting of 5× RT buffer (4 μl), 10 mM dNTP mix (1 μl; Pharmacia, Uppsala, Sweden), 0.1 M DTT (2 μl; Invitrogen, CA, USA), and superscript II reverse transcriptase (200 U/μl; Invitrogen) was added, and the samples were incubated at 42°C for 1 h, after which the reverse transcriptase was inactivated by heating the samples at 95°C for 5 min. The cDNA was stored at −20°C until further use. In each real-time PCR reaction, 50 ng cDNA template was used.

Affymetrix gene chip microarrays. Pooling of the RNA samples was performed according to the phase of the menstrual cycle and treatment conditions, *i.e.* two RNA samples from the menstrual phase (CD3 and CD4) and two RNA samples from the late proliferative phase (CD12 and CD13) were pooled. cRNA was generated from the pooled RNA and was labelled with biotin according to the Affymetrix protocol (Santa Clara, USA). cDNA was hybridised to the Affymetrix HU-133A chips, which contains approximately 22 000 human oligonucleotide probe sets, including 68 controls. The chip hybridisations were carried out in triplicate. After washing, the chips were scanned and analysed using the MicroArray suite MAS5. A detail description of the Affymetrix chip content is available at the NetAffy analysis web page (http://www.affymetrix.com/analysis/index.affx).

Microarray data analysis. Following gene chip data quality control, data files (.EXP, .DAT, .CEL) generated by MAS5 were transferred by FTP to the server housing the Rosetta Resolver Gene Expression Data Analysis System. Rosetta Resolver uses Affymetrix gene chip error models to transform the raw data into a processed form that can be used in various expression analyses and allows normalization of sample data of triplicate hybridizations using one-way analysis of variance (ANOVA) [18]. Changes in expression levels between the control and the treated samples were calculated using two criteria: (1) the absolute fold change (>2-fold)

(*e.g.* the ratio between treated and control samples) and (2) a corresponding *p*-value less than 0.01.

The use of microarrays results in a massive amount of data, which requires special tools to filter and extract relevant information. By combining the fold changes or log ratios and the *p*-value, we generated a so-called significance code, which simplifies the selection and extraction of genes of interest, especially when analyzing various conditions. The significance code assigned to the genes was based on ANOVA-retrieved *p*-values and up- or down-regulation compared to the untreated samples. A significance code of 1 (increased or up-regulated) was used for genes with $p<0.01$ and a log ratio >0; a significance code of -1 (decreased or down-regulated) was used for genes with $p<0.01$ and log ratio <0. For genes that didn't show significant regulation, the significance code was 0 (log ratio $=0$ and $p>0.01$ independent of log ratio).

Data were then exported from Rosetta Resolver to the Spotfire decision site 7.1 (Spotfire, Göteborg, Sweden), in which gene sets of interest were visualized and subsequently selected. Data were analyzed through the use of Ingenuity Pathways Analysis (IPA, Ingenuity® Systems, www.ingenuity.com). The data set containing the significantly up- and down-regulated genes and the corresponding expression values were uploaded into the application. Each gene identifier was mapped to its corresponding gene object in the Ingenuity Pathways Knowledge Base. These genes, called focus genes, were overlaid onto a global molecular network developed from information contained in the Ingenuity Pathways Knowledge Base. Networks of these focus genes were then algorithmically generated based on their connectivity.

A network is a graphical representation of the molecular relationships between gene products. The gene products are represented as nodes, and the biological relationship between two nodes is represented as a line. All lines are supported by at least one reference in literature, textbook, or canonical information stored in the Ingenuity Pathways Knowledge Base. The intensity of the node colour indicates the degree of up- (red) or down- (green) regulation. Nodes are displayed using various shapes that represent the functional class of the gene product.

Validation of array data using real-time PCR analysis. A selection of genes was validated with q-PCR to confirm expression in the samples used for microarray analysis. In addition, the expression of these genes was evaluated in an independent series of experiments. To confirm that the genes induced by P *in vitro* are indeed up-regulated during the implantation window, we also assessed their expression levels in endometrial tissue collected in the ES and MS phases of the cycle.

Primers and probes were purchased from Perkin-Elmer Applied Biosystems as pre-developed assays. Human cyclophilin A was selected as an endogenous RNA control in order to normalize for differences in the amount of total RNA added to each reaction. Uncultured human endometrial tissue was included as a positive control. All PCR reactions were performed using an ABI Prism 7700 sequence detection system (Perkin-Elmer Applied Biosystems). The thermal cycling conditions comprised an initial decontamination step at 50°C for 2 min, a denaturation step at 95°C for 10 min, and 40 cycles of 15 s at 95°C, followed by 1 min at 60°C. Experiments were performed for each sample in duplicate. Quantitative values were obtained from the threshold cycle number (Ct), at which the increase in the signal associated with exponential growth of PCR products was first detected with the ABI Prism 7700 sequence detector software (Perkin-Elmer, Foster city, CA) The fold-change in expression was calculated using the $\Delta\Delta$ Ct method, with cyclophilin A mRNA as an internal control [19]. For a detailed description of the procedure, please refer to the ABI user manual (http://www.uk1.unifreiburg.de/core/facility/tagman/user_bulletin_2.pdf).

Statistical analysis of real-time PCR results. Statistical tests were carried out using the SPSS 11 (SPSS Inc., Chicago, IL) statistical analysis package. The effects of 17β-E_2+P and P alone on cultured explants were analysed using the nonparametric paired Wilcoxon signed rank test at a confidence level of 95%. The nonparametric unpaired Mann-Whitney U test at a confidence level of 95% was employed to analyse the real-time PCR data generated from uncultured ES phase endometrial tissue and uncultured MS phase endometrial tissue.

Results

Validation of array data with quantitative real-time PCR. Eight genes were selected from the initial dataset on the basis of fold-change (≥2-fold) and on literature-documented expression during the implantation window: (1) four genes previously described in literature to be up-regulated during the implantation window and selectively stimulated by 17β-E_2+P in late proliferative phase but not menstrual phase endometrium (Dickkopf homolog 1, DDK1; thrombomodulin, THBD; monoamine oxidase A, MAOA; gastrin, GAS) [2, 20, 21]; (2) two genes not yet reported that were selectively stimulated by 17β-E_2+P in late proliferative phase explants but not in menstrual phase explants (cytidine deaminase, CDA; SPARC-like 1, SPARCL1); and (3) two genes that were selectively stimulated by 17β-E_2+P in menstrual phase explants but not in late proliferative phase explants (trefoil factor 1, TFF1; mammaglobin 1).

The real-time PCR results corroborated well with the array data (Table 1). We performed additional independent experiments to validate the observed effects of treatment with 17β-E_2+P and P alone (Fig. 1). From the validated genes, DKK1, MAOA and SPARCL1 were significantly stimulated by P in late proliferative and menstrual phase explants both in the presence and absence of 17β-E_2. The induction of SPARCL1 expression by P was significantly decreased in the presence of 17β-E_2 in both menstrual and late proliferative phase explants.

The response of DKK1 to P was higher in the late proliferative phase explants than in the menstrual phase explants, whereas the induction of mammaglobin expression by 17β-E_2+P and P alone was more pronounced in menstrual phase than in late proliferative phase endometrium. Thrombomodulin expression was induced only by P in late proliferative phase explants.

The expression of DKK1, THBD, MAOA, GAS, CDA and SPARCL1 was also assessed in an independent series of ES and MS endometrial samples to confirm selective up-regulation in the implantation window. The expression levels are presented in Fig. 2. The expression of DKK1, MAOA, CDA and SPARCL1 was significantly higher in MS endometrium compared to ES endometrium.

Gene expression in menstrual and late proliferative phase endometrial tissue explants after 17β-E_2+P or P treatment. Treatment of late proliferative phase endometrial tissue with 17β-E_2+P up-regulated (≥2-

Table 1. Validation results of the microarray findings for selected genes.

Gene	Real-time PCR individual samples								Pooled array samples		
	E_2+P M phase		E_2+P LP phase		P alone M phase		P alone LP phase		E_2+P	E_2+P	P alone
	CD3	CD4	CD12	CD13	CD3	CD4	CD12	CD13	M phase	LP phase	LP phase
DKK1	1.80	1.93	2.67	12.68	3.20	2.96	4.27	13.69	1.58	6.03	5.01
THBD	1.11	1.14	2.72	4.08	2.24	3.59	2.67	3.48	1.30	2.95	2.43
MAOA	0.97	1.19	5.64	1.85	1.82	1.80	6.36	1.19	1.10	2.59	2.00
GAS	0.69	0.26	1.07	3.07	1.51	0.57	1.95	2.49	1.00	2.19	1.58
CDA	1.27	0.62	1.97	4.01	1.09	1.34	1.02	3.61	0.32	2.82	1.86
SPARCL1	1.47	2.43	6.96	3.02	1.11	1.87	4.07	2.36	1.29	2.00	2.04

Gene transcript levels of DKK1, THBD, MAOA, GAS, CDA and SPARCL1 were assessed with quantitative real-time PCR in the individual samples used for microarray hybridization. Data are presented as fold change [P, progesterone; E_2, 17β-oestradiol; CD, cycle day; M, menstrual (n=2); LP, late proliferative (n=2)].

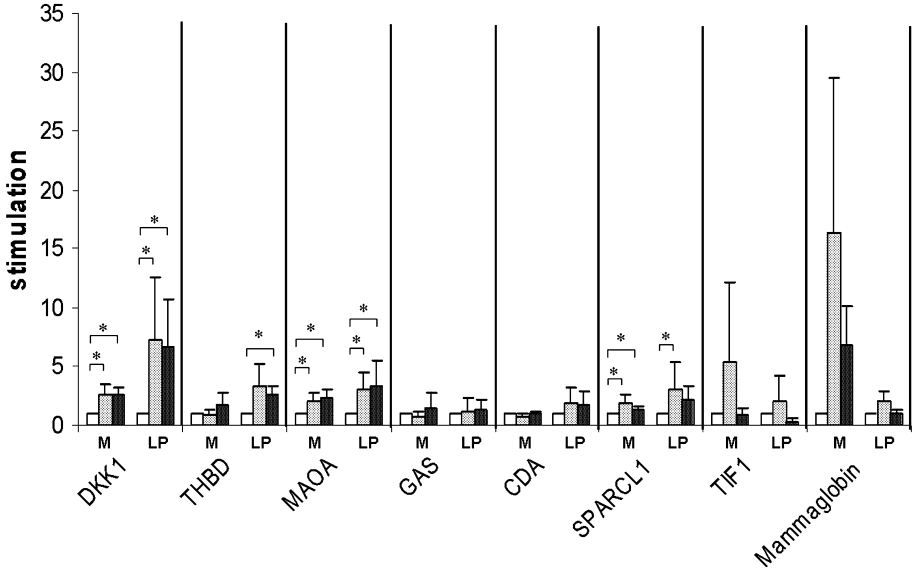

Figure 1. Mean fold changes found for DKK1, THBD, MAOA, GAS, CDA and SPARCL1 in menstrual phase (M, n=4) and late proliferative phase (LP, n=3) explants treated with 17β-oestradiol and progesterone (17β-E_2+P, dark grey bars) or P alone (light grey bars). Controls (open bars) were cultured with vehicle alone. Data are presented as fold changes (*p<0.05).

fold) the expression of 110 gene transcripts and down-regulated (≥2-fold) the expression of 109 gene transcripts when compared to the control (vehicle) (Table 2). Treating late proliferative phase explants with P alone up-regulated (≥2-fold) the expression of 107 gene transcripts and down-regulated (≥2-fold) the expression of 54 gene transcripts when compared to the control (vehicle) (Table 3). A total of 77/107 up-regulated and 42/54 down-regulated genes were also modulated by 17β-E_2+P treatment in late proliferative phase explants (Table 3).

The response of menstrual phase endometrium to 17β-E_2+P was less pronounced than that of late proliferative phase endometrium. Treatment of menstrual phase endometrial tissue with 17β-E_2+P up-regulated (≥2-fold) the expression of only 38 gene transcripts and down-regulated (≥2-fold) the expression of 79 gene transcripts when compared to the control sample (vehicle) (Table 4).

Almost all genes modulated by 17β-E_2+P in late proliferative phase endometrium were specific for that phase of the cycle. Of the 110 up-regulated (≥2-fold) gene transcripts, 100 were expressed in late proliferative phase explants and not menstrual phase explants; of these, 10 gene transcripts were documented to be up-regulated during the window of implantation (Table 5). Of the 107 down-regulated (≥2-fold) gene transcripts, 102 were selective for late proliferative phase explants; of these, 7 genes were documented to be down-regulated during the implantation window (Table 5). The genes regulated by 17β-E_2+P in both menstrual and late proliferative phase explants are presented in Table 6.

Ingenuity Pathways Analysis. Ingenuity Pathways Analysis revealed various significant networks of interconnected focus genes after treatment with 17β-E_2+P. In late proliferative phase endometrium, five highly significant networks were identified. Network 1

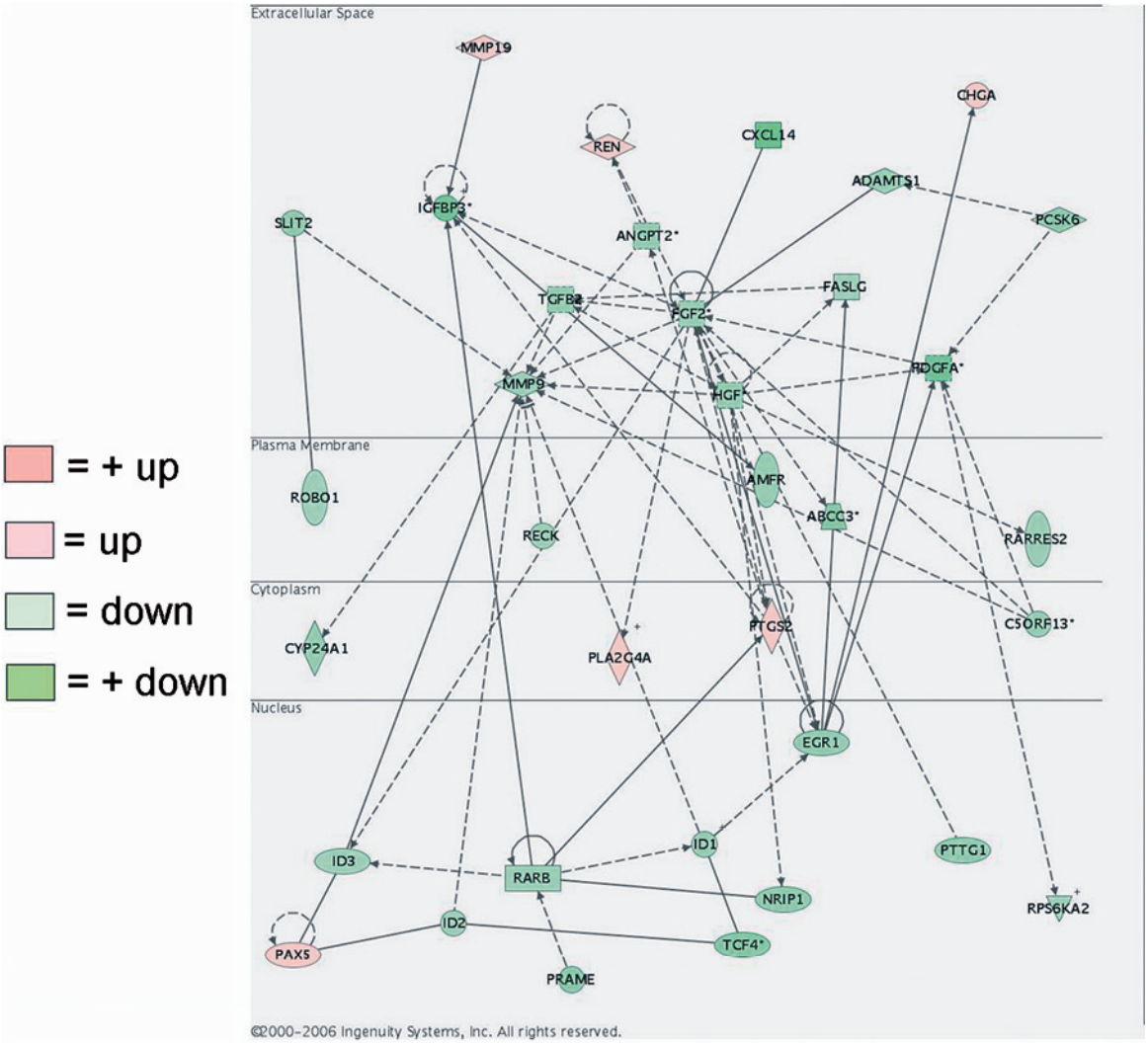

Figure 2. Example of a highly significant network identified in the gene expression profile of menstrual phase endometrium treated with 17β-oestradiol and progesterone (17β-E_2+P) as determined by the Ingenuity Pathways Analysis program. Green indicates down-regulated genes, and pink indicates up-regulated genes.

connected nodes IL1B, PLAU, MMP1, MMP3, MMP7, MMP9, SERPINE1 and EDN1; network 2 connected IL8, MMP14, FGF2, PDGFB, ITGB3, PDGFRA, PDGFRB, PTGS2 and EGR1; network 3 related TGFβ2, TGFβ3, INHBA, PTHLH, JUN, SMAD3 and SMAD7; network 4 linked IGF1, TNFSF11 and HOXA9; and network 5 coupled ICAM1, CXCL10, IL15, SOCS1, RARα and ARNT2. Network 1 is illustrated in Figure 3.

In contrast, in menstrual phase endometrium only two highly significant networks were extracted from the data. One network connected CCL5, TNFS11, INTGB3, MAPK8 and ESR1. The second network linked IFGBP3, TGFβ2, FGF2, HGF, PDGFA, MMP9, PTGS2, RARβ and EGR1. The latter network is presented in Figure 2.

Discussion

Previous work in our laboratory has shown that explant cultures of human endometrial tissue are biologically relevant *in vitro* models to investigate oestrogen regulation of gene expression and proliferation [12, 16]. With regard to progestins, it has been shown that tissue cultures of human endometrium are also responsive, as evidenced by the suppressive effects on the production and activation of MMPs [12–14]. The present study was designed to gain more insight into the responses of human endometrium to P with regard to gene expression and the influence of 17β-E_2. The results show that in explant cultures of human endometrium, the expression of genes that have been implicated in the process of embryo implantation can be modulated by 17β-E_2 and P.

Table 2. Genes affected by 17β-oestradiol and progesterone (17β-E2+P) in explants of late proliferative phase endometrium.

Gene	Gene Symbol	fold change	Accession #	Function
cytochrome P450, family 26, subfamily A, polypeptide 1	CYP26A1	33.11	NM_000783.1	metabolism
hemoglobin, alpha 2	–-	28.18	V00489	–-
calpain 6	CAPN6	19.05	NM_014289.2	metabolism
heart and neural crest derivatives expressed 2	HAND2	10.47	NM_021973.1	transcription factor
secretoglobin, family 1D, member 2	SCGB1D2	10.00	NM_006551.2	extracellular matrix
hemoglobin, alpha 1	HBA1	9.55	AF105974.1	transport
FK506 binding protein 5	FKBP5	7.76	NM_004117.1	metabolism
chemokine (C-X-C motif) ligand 11	CXCL11	7.59	AF030514.1	signal transduction
carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 7	CHST7	7.08	NM_019886.1	metabolism
glycine-N-acyltransferase	GLYAT	6.17	AW024233	metabolism
hemoglobin, beta	HBB	6.17	M25079.1	transport
dickkopf homolog 1 (Xenopus laevis)	DKK1	6.03	NM_012242.1	growth factor
Homo sapiens mutant beta-globin (HBB) gene, complete cds.	–-	5.50	AF059180	–-
neuronal pentraxin II	NPTX2	5.50	U26662.1	cell adhesion
PDZ domain containing 3	PDZK3	5.50	AF338650.1	signal transduction
apolipoprotein D	APOD	4.90	NM_001647.1	transport
alkaline phosphatase, placental (Regan isozyme)	ALPP	4.79	NM_001632.2	metabolism
keratin 6A	KRT6A	4.68	J00269.1	structural protein
G protein-coupled receptor 105	GPR105	4.47	NM_014879.1	signal transduction
solute carrier family 7, member 8	SLC7A8	4.37	NM_012244.1	transport
hypothetical protein FLJ11539	FLJ11539	4.17	NM_024748.1	–-
a disintegrin-like and metalloprotease with thrombospondin type 1 motif, 8	ADAMTS8	3.98	NM_007037.1	cell growth
integrin, beta-like 1	ITGBL1	3.80	NM_004791.1	cell adhesion
potassium inwardly-rectifying channel, subfamily J, member 8	KCNJ8	3.80	NM_004982.1	transport
RGC32 protein	RGC32	3.80	NM_014059.1	cell growth
prostaglandin-endoperoxide synthase 1	PTGS1	3.72	NM_000962.1	metabolism
regulator of G-protein signalling 2, 24kDa	RGS2	3.72	NM_002923.1	signal transduction
cannabinoid receptor 1 (brain)	CNR1	3.55	U73304	signal transduction
hemoglobin, delta	HBD	3.39	NM_000519.2	transport
keratin 6B	KRT6B	3.39	L42612.1	structural protein
sushi-repeat-containing protein, X-linked	SRPX	3.24	NM_006307.1	cell adhesion
thrombomodulin	THBD	3.24	NM_000361.1	membrane protein
delta sleep inducing peptide, immunoreactor	DSIPI	3.16	AL110191.1	transcription factor
cytochrome P450, family 4, subfamily B, polypeptide 1	CYP4B1	3.09	J02871.1	metabolism
hemoglobin, gamma A	HBG1	3.09	NM_000559.1	transport
paired basic amino acid cleaving system 4	PACE4	3.09	NM_002570.1	signal transduction
insulin receptor substrate 2	IRS2	3.02	BF700086	signal transduction

Table 2 *(Continued)*

Gene	Gene Symbol	fold change	Accession #	Function
metallothionein 1K	MT1K	2.95	R06655	transport
amylase, alpha 1A; salivary	AMY1A	2.88	NM_004038.1	metabolism
fibrinogen-like 2	FGL2	2.88	NM_006682.1	extracellular matrix
latent transforming growth factor beta binding protein 1	LTBP1	2.88	NM_000627.1	protein binding
monoamine oxidase A	MAOA	2.88	NM_000240.1	transport
retinol binding protein 4, plasma	RBP4	2.88	NM_006744.2	transport
cytidine deaminase	CDA	2.82	NM_001785.1	metabolism
potassium voltage-gated channel, subfamily G, member 1	KCNG1	2.82	AI332979	transport
mitogen-activated protein kinase kinase 6	MAP2K6	2.82	NM_002758.1	signal transduction
solute carrier family 15 (H+/peptide transporter), member 2	SLC15A2	2.82	BF223679	transport
hemoglobin, gamma G	HBG2	2.75	AI133353	transport
protein kinase, X-linked	PRKX	2.75	NM_005044.1	metabolism
suppressor of cytokine signaling 1	SOCS1	2.75	AB005043.1	signal transduction
KIAA0924 protein	KIAA0924	2.69	NM_014897.1	nuclear
secretoglobin, family 1D, member 1	SCGB1D1	2.69	NM_006552.1	extracellular matrix
serine (or cysteine) proteinase inhibitor, clade E, member 1	SERPINE1	2.69	NM_000602.1	metabolism
chloride channel 4	CLCN4	2.63	AA071195	transport
fatty-acid-Coenzyme A ligase, long-chain 2	FACL2	2.63	NM_001995.1	metabolism
monoamine oxidase B	MAOB	2.63	NM_000898.1	transport
secretoglobin, family 2A, member 1	SCGB2A1	2.63	NM_002407.1	hormone binding
ATPase, H+ transporting, lysosomal 70kDa, V1 subunit A	ATP6V1A	2.57	NM_001690.1	transport
dipeptidylpeptidase 4	DPP4	2.57	M80536.1	metabolism
nuclear factor I/B	NFIB	2.57	AI186739	transcription factor
creatine kinase, brain	CKB	2.51	NM_001823.1	metabolism
cytokine receptor-like factor 1	CRLF1	2.51	NM_004750.1	signal transduction
iroquois homeobox protein 4	IRX4	2.51	NM_016358.1	transcription factor
Homo sapiens mRNA; cDNA DKFZp586B0220	–-	2.45	AL049435.1	–-
chemokine (C-X-C motif) ligand 10	CXCL10	2.45	NM_001565.1	signal transduction
hypothetical protein FLJ20701	FLJ20701	2.45	NM_017933.1	–-
insulin-like growth factor binding protein 1	IGFBP1	2.45	NM_000596.1	signal transduction
Norrie disease (pseudoglioma)	NDP	2.45	NM_000266.1	signal transduction
zinc finger protein 145	ZNF145	2.45	NM_006006.1	protein binding
hypothetical protein FLJ20366	FLJ20366	2.40	NM_017786.1	–-
peroxisome proliferative activated receptor, gamma, coactivator 1	PPARGC1	2.40	NM_013261.1	DNA binding
S100 calcium binding protein A2	S100A2	2.40	NM_005978.2	transport
Arg/Abl-interacting protein ArgBP2	ARGBP2	2.34	NM_021069.1	structural protein
interleukin 1 receptor-like 1	IL1RL1	2.34	NM_003856.1	signal transduction

Table 2 *(Continued)*

Gene	Gene Symbol	fold change	Accession #	Function
NPD009 protein	NPD009	2.34	AF237813.1	–-
Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	CITED2	2.29	NM_006079.1	transcription factor
colony stimulating factor 2 receptor, alpha, low-affinity	CSF2RA	2.29	BC002635.1	immune response
secreted and transmembrane 1	SECTM1	2.29	BF939675	membrane protein
H.sapiens mRNA for interleukin-15	–-	2.24	Y09908.1	–-
GREB1 protein	GREB1	2.24	NM_014668.1	–-
adiponectin receptor 2	ADIPOR2	2.19	NM_024551.1	membrane protein
hypothetical protein DKFZp434B044	DKFZP434B044	2.19	AL136861.1	extracellular matrix
gastrin	GAS	2.19	NM_000805.2	signal transduction
alkaline phosphatase, liver/bone/kidney	ALPL	2.14	X14174.1	metabolism
chromosome 1 open reading frame 29	C1orf29	2.14	NM_006820.1	–-
Fas apoptotic inhibitory molecule	FAIM	2.14	NM_018147.1	–-
KIAA0089 protein	KIAA0089	2.14	AA135522	metabolism
POU domain, class 5, transcription factor 1	POU5F1	2.14	NM_002701.1	transcription factor
serum amyloid A2	SAA2	2.14	M23699.1	immune response
SEC14-like 1 (S. cerevisiae)	SEC14L1	2.14	AV748469	transport
solute carrier family 26 (sulfate transporter), member 2	SLC26A2	2.14	AI025519	transport
CDC14 cell division cycle 14 homolog B (S. cerevisiae)	CDC14B	2.09	AU145941	metabolism
hypothetical protein FLJ11795	FLJ11795	2.09	NM_024669.1	–-
likely ortholog of mouse tumor necrosis-alpha-induced adipose-related protein	FLJ23153	2.09	NM_024636.1	transport
KIAA0960 protein	KIAA0960	2.09	BF447246	–-
oxysterol binding protein-like 11	OSBPL11	2.09	NM_022776.1	transport
protein tyrosine phosphatase, receptor type, R	PTPRR	2.09	NM_002849.1	signal transduction
fibrinogen, A alpha polypeptide	FGA	2.04	NM_021871.1	cell adhesion
interleukin 6 signal transducer (gp130, oncostatin M receptor)	IL6ST	2.04	AB015706.1	signal transduction
KIAA0367 protein	KIAA0367	2.04	AL138349	–-
KIAA0711 gene product	KIAA0711	2.04	NM_014867.1	protein binding
ADP-ribosyltransferase 3	ART3	2.00	U47054.1	metabolism
cut-like 2 (Drosophila)	CUTL2	2.00	AB006631.1	transcription factor
dual specificity phosphatase 1	DUSP1	2.00	AA530892	metabolism
eukaryotic translation initiation factor 2, subunit 3 gamma, 52kDa	EIF2S3	2.00	NM_001415.1	translation
interleukin 20 receptor, alpha	IL20RA	2.00	NM_014432.1	signal transduction
PRO2000 protein	PRO2000	2.00	NM_014109.1	DNA binding
solute carrier family 7, member 2	SLC7A2	2.00	NM_003046.1	transport
SPARC-like 1 (mast9, hevin)	SPARCL1	2.00	NM_004684.1	–-
toll-like receptor 2	TLR2	2.00	NM_003264.1	immune response

Table 2 *(Continued)*

Gene	Gene Symbol	fold change	Accession #	Function
coagulation factor XIII, A1 polypeptide	F13A1	−2.00	NM_000129.2	metabolism
protein kinase (cAMP-dependent, catalytic) inhibitor alpha	PKIA	−2.00	NM_006823.1	metabolism
glutaminyl-peptide cyclotransferase (glutaminyl cyclase)	QPCT	−2.00	NM_012413.2	metabolism
TGF2_HUMAN Transforming growth factor beta 2 precursor (TGF-beta 2)	–-	−2.04	BF061658	–-
cadherin 5, type 2, VE-cadherin (vascular epithelium)	CDH5	−2.04	NM_001795.1	cell adhesion
cellular retinoic acid binding protein 2	CRABP2	−2.04	NM_001878.2	signal transduction
drebrin 1	DBN1	−2.04	NM_004395.1	structural protein
dimethylarginine dimethylaminohydrolase 2	DDAH2	−2.04	AJ012008	metabolism
early growth response 1	EGR1	−2.04	NM_001964.1	transcription factor
hypothetical protein FLJ11082	FLJ11082	−2.04	NM_018317.1	–-
matrix metalloproteinase 1 (interstitial collagenase)	MMP1	−2.04	NM_002421.2	metabolism
matrix metalloproteinase 3 (stromelysin 1, progelatinase)	MMP3	−2.04	NM_002422.2	metabolism
matrix metalloproteinase 9	MMP9	−2.04	NM_004994.1	metabolism
reticulon 3	RTN3	−2.04	NM_023941.1	membrane protein
stanniocalcin 1	STC1	−2.04	U46768.1	signal transduction
Thy-1 cell surface antigen	THY1	−2.04	AL558479	membrane protein
tumor necrosis factor receptor superfamily, member 21	TNFRSF21	−2.04	NM_016629.1	signal transduction
basic helix-loop-helix domain containing, class B, 3	BHLHB3	−2.09	BE857425	transcription factor
chromosome 21 open reading frame 7	C21orf7	−2.09	NM_020152.1	–-
glycoprotein A repetitions predominant	GARP	−2.09	NM_005512.1	–-
regulator of G-protein signalling 3	RGS3	−2.09	NM_021106.1	signal transduction
trefoil factor 1	TFF1	−2.09	NM_003225.1	growth factor
ATP-binding cassette, sub-family A (ABC1), member 8	ABCA8	−2.14	NM_007168.1	transport
hypothetical gene BC008967	BC008967	−2.14	BE299456	–-
solute carrier family 14 (urea transporter), member 1	SLC14A1	−2.14	NM_015865.1	transport
a disintegrin and metalloproteinase domain 12 (meltrin alpha)	ADAM12	−2.19	NM_003474.2	metabolism
aquaporin 3	AQP3	−2.19	AB001325	transport
carcinoembryonic antigen-related cell adhesion molecule 6	CEACAM6	−2.19	BC005008.1	signal transduction
chloride channel, calcium activated, family member 4	CLCA4	−2.19	NM_012128.2	transport
chloride intracellular channel 2	CLIC2	−2.19	AI768628	transport
DVS27-related protein	DVS27	−2.19	AB024518.1	–-
hypothetical protein FLJ31737	FLJ31737	−2.19	N91149	–-
fascin homolog 1, actin-bundling protein	FSCN1	−2.19	NM_003088.1	structural protein
synuclein, alpha interacting protein (synphilin)	SNCAIP	−2.19	NM_005460.1	protein binding
embryonal Fyn-associated substrate	EFS	−2.24	NM_005864.1	cell adhesion
integral membrane protein 2C	ITM2C	−2.24	NM_030926.1	–-
keratin 23 (histone deacetylase inducible)	KRT23	−2.24	NM_015515.1	–-
matrix metalloproteinase 27	MMP27	−2.24	NM_022122.1	metabolism

Table 2 *(Continued)*

Gene	Gene Symbol	fold change	Accession #	Function
pre-B-cell leukemia transcription factor 1	PBX1	−2.24	BF967998	transcription factor
suppression of tumorigenicity	ST7	−2.24	NM_013437.1	–-
Homo sapiens mRNA, chromosome 1 specific transcript KIAA0509.	–-	−2.29	AB007978.1	–-
apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B	APOBEC3B	−2.29	NM_004900.1	metabolism
ARF-GAP, RHO-GAP, ankyrin repeat and plekstrin homology domains-containing protein 3	ARAP3	−2.29	NM_022481.1	signal transduction
carboxypeptidase Z	CPZ	−2.29	BC006393.1	metabolism
melanoma antigen, family D, 4	MAGED4	−2.29	NM_030801.1	–-
matrilin 4	MATN4	−2.29	NM_003833.2	extracellular matrix
regulator of G-protein signalling 4	RGS4	−2.29	AL514445	signal transduction
chromosome 6 open reading frame 59	C6orf59	−2.34	NM_020133.1	–-
aldo-keto reductase family 1, member B10 (aldose reductase)	AKR1B10	−2.40	NM_020299.1	metabolism
angiopoietin 2	ANGPT2	−2.40	AF187858.1	signal transduction
dapper homolog 1, antagonist of beta-catenin (xenopus)	DACT1	−2.40	NM_016651.2	nuclear
G protein-coupled receptor 17	GPR17	−2.40	NM_005291.1	signal transduction
glutathione peroxidase 2 (gastrointestinal)	GPX2	−2.40	NM_002083.1	metabolism
microfibrillar-associated protein 2	MFAP2	−2.40	NM_017459.1	extracellular matrix
transforming growth factor, beta 3	TGFB3	−2.40	J03241.1	growth factor
WNT1 inducible signaling pathway protein 2	WISP2	−2.40	NM_003881.1	signal transduction
hepatocyte growth factor (hepapoietin A; scatter factor)	HGF	−2.45	M77227.1	growth factor
KIAA1277 protein	KIAA1277	−2.45	AA127623	–-
matrix metalloproteinase 14 (membrane-inserted)	MMP14	−2.45	AU149305	metabolism
Ras family member Ris	RIS	−2.45	NM_016563.1	signal transduction
thymosin, beta, identified in neuroblastoma cells	TMSNB	−2.45	NM_021992.1	structural protein
latexin protein	LXN	−2.51	NM_020169.1	–-
serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 2	SERPINB2	−2.51	NM_002575.1	cell growth
carbonic anhydrase II	CA2	−2.57	M36532.1	metabolism
deleted in malignant brain tumors 1	DMBT1	−2.57	NM_004406.1	cell growth
fibronectin leucine rich transmembrane protein 2	FLRT2	−2.57	NM_013231.1	cell adhesion
orosomucoid 1	ORM1	−2.57	NM_000608.1	transport
stathmin-like 2	STMN2	−2.57	BF967657	signal transduction
synaptojanin 2	SYNJ2	−2.57	AF318616.1	metabolism
carcinoembryonic antigen-related cell adhesion molecule 5	CEACAM5	−2.63	NM_004363.1	membrane protein
ectodermal-neural cortex (with BTB-like domain)	ENC1	−2.63	NM_003633.1	protein binding
mucin 4, tracheobronchial	MUC4	−2.63	AJ242547.1	signal transduction
protocadherin 16 dachsous-like (Drosophila)	PCDH16	−2.63	BF222893	cell adhesion
interleukin 24	IL24	−2.69	NM_006850.1	signal transduction

Table 2 *(Continued)*

Gene	Gene Symbol	fold change	Accession #	Function
transforming growth factor, beta 2	TGFB2	−2.69	NM_003238.1	growth factor
integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	ITGB3	−2.75	M35999.1	cell adhesion
plasminogen activator, urokinase	PLAU	−2.75	NM_002658.1	metabolism
chromosome 20 open reading frame 42	C20orf42	−2.82	NM_017671.1	–-
four jointed box 1 (Drosophila)	FJX1	−2.82	NM_014344.1	–-
ephrin-B2	EFNB2	−2.88	BF001670	cell growth
parathyroid hormone-like hormone	PTHLH	−2.88	BC005961.1	signal transduction
tribbles homolog 2	TRB2	−2.88	NM_021643.1	metabolism
twist homolog 1	TWIST1	−2.88	X99268.1	DNA binding
gap junction protein, alpha 4, 37kDa (connexin 37)	GJA4	−2.95	NM_002060.1	transport
integrin, beta 6	ITGB6	−2.95	NM_000888.3	cell adhesion
bradykinin receptor B1	BDKRB1	−3.02	NM_000710.1	signal transduction
solute carrier family 21 (organic anion transporter), member 11	SLC21A11	−3.02	NM_013272.2	transport
myristoylated alanine-rich protein kinase C substrate	MARCKS	−3.09	M68956.1	membrane protein
angiopoietin-like 2	ANGPTL2	−3.16	NM_012098.1	signal transduction
tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	TNFRSF11B	−3.16	NM_002546.1	signal transduction
acid phosphatase, prostate	ACPP	−3.24	NM_001099.2	metabolism
homeo box A11	HOXA11	−3.24	NM_005523.3	transcription factor
hypothetical protein FLJ38993	FLJ38993	−3.31	AF070524.1	signal transduction
matrix metalloproteinase 11 (stromelysin 3)	MMP11	−3.31	AI761713	metabolism
pleckstrin 2	PLEK2	−3.31	NM_016445.1	signal transduction
SRY (sex determining region Y)-box 9	SOX9	−3.31	NM_000346.1	DNA binding
lymphocyte-specific protein tyrosine kinase	LCK	−3.39	NM_005356.1	signal transduction
BDG-29 proten	BDG29	−3.47	AL117532.1	DNA binding
deiodinase, iodothyronine, type II	DIO2	−3.47	U53506.1	metabolism
SRY (sex determining region Y)-box 4	SOX4	−3.47	AI989477	transcription factor
cysteine knot superfamily 1, BMP antagonist 1	CKTSF1B1	−3.63	AF154054.1	–-
chromogranin A (parathyroid secretory protein 1)	CHGA	−3.72	NM_001275.2	transport
cystic fibrosis transmembrane conductance regulator, ATP-binding cassette	CFTR	−4.07	NM_000492.2	transport
Ras-induced senescence 1	RIS1	−4.07	BF062629	–-
hypothetical protein FLJ10640	FLJ10640	−4.17	NM_024703.1	metabolism
ribosomal protein S20	RPS20	−4.27	AF113008.1	protein biosynthesis
SRY (sex determining region Y)-box 11	SOX11	−4.68	AB028641.1	transcription factor
platelet-derived growth factor beta polypeptide	PDGFB	−5.75	NM_002608.1	growth factor
ribosomal protein L27a	RPL27A	−5.75	BE737027	protein biosynthesis

Gene transcripts regulated (≥2-fold) by 17β-E_2+P in late proliferative phase explants when compared to the vehicle-treated controls. Data are presented as fold changes.

Table 3. Genes affected by progesterone alone in explants of late proliferative phase endometrium.

Gene	Gene Symbol	fold change	Accession #	Function
cytochrome P450, family 26, subfamily A, polypeptide 1	CYP26A1	28.84	NM_000783.1	metabolism
calpain 6	CAPN6	19.50	NM_014289.2	metabolism
hemoglobin, alpha 2	**--**	**12.88**	**V00489**	**--**
heart and neural crest derivatives expressed 2	HAND2	10.96	NM_021973.1	transcription factor
secretoglobin, family 1D, member 2	SCGB1D2	9.55	NM_006551.2	extracellular matrix
carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 7	CHST7	7.59	NM_019886.1	metabolism
glycine-N-acyltransferase	GLYAT	7.08	AW024233	metabolism
FK506 binding protein 5	FKBP5	6.31	NM_004117.1	metabolism
PDZ domain containing 3	PDZK3	6.17	AF338650.1	signal transduction
neuronal pentraxin II	NPTX2	6.03	U26662.1	cell adhesion
chemokine (C-X-C motif) ligand 11	CXCL11	5.50	AF030514.1	signal transduction
solute carrier family 7, member 8	SLC7A8	5.25	NM_012244.1	transport
a disintegrin-like and metalloprotease with thrombospondin type 1 motif, 8	ADAMTS8	5.01	NM_007037.1	metabolism
dickkopf homolog 1 (Xenopus laevis)	DKK1	5.01	NM_012242.1	growth factor
keratin 6A	KRT6A	4.90	J00269.1	structural protein
alkaline phosphatase, placental (Regan isozyme)	ALPP	4.79	NM_001632.2	metabolism
apolipoprotein D	APOD	4.68	NM_001647.1	transport
G protein-coupled receptor 105	GPR105	4.68	NM_014879.1	signal transduction
prostaglandin-endoperoxide synthase 1	PTGS1	4.68	NM_000962.1	metabolism
TU3A protein	**TU3A**	**4.68**	**AL050264.1**	**--**
hemoglobin, alpha 1	HBA1	4.57	AF105974.1	transport
mitogen-activated protein kinase kinase 6	MAP2K6	4.27	NM_002758.1	signal transduction
keratin 6B	KRT6B	4.07	L42612.1	structural protein
claudin 5	**CLDN5**	**3.98**	**NM_003277.1**	**structural protein**
regulator of G-protein signalling 2, 24kDa	RGS2	3.98	NM_002923.1	signal transduction
RGC32 protein	RGC32	3.89	NM_014059.1	cell growth
integrin, beta-like 1	ITGBL1	3.80	NM_004791.1	cell adhesion
solute carrier family 15, member 2	SLC15A2	3.72	BF223679	transport
delta sleep inducing peptide, immunoreactor	DSIPI	3.55	AL110191.1	transcription factor
myosin heavy chain Myr 8	**MYR8**	**3.55**	**AI522028**	**metabolism**
potassium inwardly-rectifying channel, subfamily J, member 8	KCNJ8	3.31	NM_004982.1	transport
paired basic amino acid cleaving system 4	PACE4	3.31	NM_002570.1	signal transduction
cannabinoid receptor 1 (brain)	CNR1	3.24	U73304	signal transduction
hypothetical protein FLJ11539	FLJ11539	3.24	NM_024748.1	--
protein kinase, X-linked	PRKX	3.24	NM_005044.1	metabolism
latent transforming growth factor beta binding protein 1	LTBP1	3.16	NM_000627.1	protein binding
KIAA0960 protein	KIAA0960	3.09	BF447246	--
nuclear factor I/B	NFIB	3.02	AI186739	transcription factor

Table 3 *(Continued)*

Gene	Gene Symbol	fold change	Accession #	Function
sushi-repeat-containing protein, X-linked	SRPX	3.02	NM_006307.1	cell adhesion
cytochrome P450, family 4, subfamily B, polypeptide 1	CYP4B1	2.95	J02871.1	metabolism
insulin receptor substrate 2	IRS2	2.95	BF700086	signal transduction
potassium voltage-gated channel, subfamily G, member 1	KCNG1	2.95	AI332979	transport
Arg/Abl-interacting protein ArgBP2	ARGBP2	2.88	NM_021069.1	structural protein
hemoglobin, beta	HBB	2.88	M25079.1	transport
Homo sapiens mutant beta-globin (HBB) gene, complete cds.	**--**	**2.82**	**AF059180**	**--**
RAR-related orphan receptor B	**RORB**	**2.82**	**NM_006914.1**	**transcription factor**
S100 calcium binding protein A2	S100A2	2.75	NM_005978.2	transport
serum amyloid A2	SAA2	2.75	NM_030754.1	immune response
absent in melanoma 1-like	**AIM1L**	**2.69**	**NM_017977.1**	**--**
RIM binding protein 2	**KIAA0318**	**2.69**	**AB002316.1**	**transport**
thrombomodulin	THBD	2.69	NM_000361.1	signal transduction
cytokine receptor-like factor 1	CRLF1	2.63	NM_004750.1	signal transduction
v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)	**MAF**	**2.51**	**NM_005360.2**	**transcription factor**
monoamine oxidase B	MAOB	2.51	NM_000898.1	transport
secretoglobin, family 1D, member 1	SCGB1D1	2.51	NM_006552.1	extracellular matrix
interleukin 15	**IL15**	**2.45**	**NM_000585.1**	**signal transduction**
hypothetical protein FLJ20701	FLJ20701	2.45	NM_017933.1	--
secretoglobin, family 2A, member 1	SCGB2A1	2.45	NM_002407.1	hormone binding
dipeptidylpeptidase 4	DPP4	2.40	M80536.1	metabolism
immunoglobulin kappa constant	**IGKC**	**2.40**	**BC005332.1**	**immune response**
immunoglobulin heavy constant gamma 3 (G3 m marker)	**IGHG3**	**2.34**	**M87789.1**	**immune response**
iroquois homeobox protein 4	IRX4	2.34	NM_016358.1	transcription factor
killer cell immunoglobulin-like receptor, two domains, short cytoplasmic tail, 1	**KIR2DS1**	**2.34**	**NM_014512.1**	**immune response**
metallothionein 1K	MT1K	2.34	R06655	transport
amylase, alpha 1A; salivary	AMY1A	2.29	NM_004038.1	metabolism
creatine kinase, brain	CKB	2.29	NM_001823.1	metabolism
cut-like 2 (Drosophila)	CUTL2	2.29	AB006631.1	transcription factor
fatty-acid-Coenzyme A ligase, long-chain 2	FACL2	2.29	NM_001995.1	metabolism
PRO2000 protein	PRO2000	2.29	NM_014109.1	DNA binding
serine (or cysteine) proteinase inhibitor, clade E , member 1	SERPINE1	2.29	NM_000602.1	metabolism
adiponectin receptor 2	ADIPOR2	2.24	NM_024551.1	membrane protein
alkaline phosphatase, liver/bone/kidney	ALPL	2.24	X14174.1	metabolism
N-acylsphingosine amidohydrolase (acid ceramidase)-like	**ASAHL**	**2.24**	**AK024677.1**	**metabolism**
hypothetical protein FLJ20366	FLJ20366	2.24	NM_017786.1	--
Norrie disease (pseudoglioma)	NDP	2.24	NM_000266.1	signal transduction
zinc finger, BED domain containing 2	**ZBED2**	**2.24**	**NM_024508.1**	**DNA binding**

Table 3 *(Continued)*

Gene	Gene Symbol	fold change	Accession #	Function
zinc finger protein 145	ZNF145	2.24	NM_006006.1	protein binding
Fas apoptotic inhibitory molecule	FAIM	2.19	NM_018147.1	---
insulin-like growth factor binding protein 1	IGFBP1	2.19	NM_000596.1	signal transduction
interleukin 20 receptor, alpha	IL20RA	2.19	NM_014432.1	signal transduction
mesothelin	**MSLN**	**2.19**	**NM_005823.2**	**---**
secreted and transmembrane 1	SECTM1	2.19	BF939675	membrane protein
CDC14 cell division cycle 14 homolog B	CDC14B	2.14	AU145941	metabolism
cathepsin E	**CTSE**	**2.14**	**NM_001910.1**	**metabolism**
LIM and cysteine-rich domains 1	**LMCD1**	**2.14**	**NM_014583.1**	**---**
monoamine oxidase A	MAOA	2.14	NM_000240.1	transport
toll-like receptor 2	TLR2	2.14	NM_003264.1	signal transduction
Homo sapiens mRNA; cDNA DKFZp586B0220	**---**	**2.09**	**AL049435.1**	**---**
hypothetical protein DKFZp434B044	DKFZP434B044	2.09	AL136861.1	extracellular matrix
glutamyl aminopeptidase	**ENPEP**	**2.09**	**L12468.1**	**metabolism**
epithelial V-like antigen 1	**EVA1**	**2.09**	**NM_005797.1**	**cell adhesion**
fibulin 2	**FBLN2**	**2.09**	**NM_001998.1**	**extracellular matrix**
KIAA0924 protein	KIAA0924	2.09	NM_014897.1	nuclear
KIAA1609 protein	**KIAA1609**	**2.09**	**AA195017**	**---**
neuroligin 4	**NLGN4**	**2.09**	**AI338338**	**cell adhesion**
peroxisome proliferative activated receptor, gamma, coactivator 1	PPARGC1	2.09	NM_013261.1	DNA binding
solute carrier family 26, member 2	SLC26A2	2.09	AI025519	transport
CDC42 effector protein 3	**CDC42EP3**	**2.04**	**AI754416**	**---**
GREB1 protein	GREB1	2.04	NM_014668.1	---
interleukin 1 receptor-like 1	IL1RL1	2.04	NM_003856.1	signal transduction
leucine-rich repeat-containing 1	**LRRC1**	**2.04**	**NM_018214.1**	**---**
protein kinase, AMP-activated, gamma 2 non-catalytic subunit	**PRKAG2**	**2.04**	**NM_016203.1**	**metabolism**
SPARC-like 1 (mast9, hevin)	SPARCL1	2.04	NM_004684.1	---
chromosome 18 open reading frame 1	**C18orf1**	**2.00**	**NM_004338.1**	**membrane protein**
choline phosphotransferase 1	**CHPT1**	**2.00**	**AF195624.1**	**metabolism**
endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 7	**EDG7**	**2.00**	**NM_012152.1**	**signal transduction**
retinol binding protein 4, plasma	RBP4	2.00	NM_006744.2	transport
carcinoembryonic antigen-related cell adhesion molecule 5	CEACAM5	−2.00	NM_004363.1	membrane protein
chromosome condensation 1	**CHC1**	**−2.00**	**NM_001269.1**	**cell growth**
cytochrome P450, family 27, subfamily B, polypeptide 1	**CYP27B1**	**−2.00**	**NM_000785.1**	**metabolism**
DVS27-related protein	**DVS27**	**−2.00**	**AB024518.1**	**---**
four jointed box 1 (Drosophila)	FJX1	−2.00	NM_014344.1	---
interleukin 24	IL24	−2.00	NM_006850.1	cell growth
matrix metalloproteinase 11 (stromelysin 3)	MMP11	−2.00	AI761713	metabolism
synaptojanin 2	SYNJ2	−2.00	AF318616.1	metabolism
aldo-keto reductase family 1, member B10 (aldose reductase)	AKR1B10	−2.04	NM_020299.1	metabolism

Table 3 *(Continued)*

Gene	Gene Symbol	fold change	Accession #	Function
neuromedin B	**NMB**	**−2.04**	**NM_021077.1**	**signal transduction**
pleckstrin 2	PLEK2	−2.04	NM_016445.1	structural protein
transmembrane protease, serine 3	**TMPRSS3**	**−2.04**	**NM_024022.1**	**metabolism**
twist homolog 1	TWIST1	−2.04	X99268.1	DNA binding
hypothetical protein FLJ38993	FLJ38993	−2.09	AF070524.1	signal transduction
somatostatin	**SST**	**−2.09**	**NM_001048.1**	**signal transduction**
chromosome 21 open reading frame 7	C21orf7	−2.14	NM_020152.1	--
carboxypeptidase M	**CPM**	**−2.14**	**NM_001874.1**	**metabolism**
glutathione peroxidase 2 (gastrointestinal)	GPX2	−2.14	NM_002083.1	metabolism
orosomucoid 1	ORM1	−2.14	NM_000607.1	transport
serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 2	SERPINB2	−2.14	NM_002575.1	cell growth
solute carrier family 14 (urea transporter), member 1	SLC14A1	−2.14	NM_015865.1	transport
SRY (sex determining region Y)-box 4	SOX4	−2.14	AI989477	transcription factor
tribbles homolog 2	TRB2	−2.14	NM_021643.1	metabolism
chromosome 20 open reading frame 42	C20orf42	−2.19	NM_017671.1	--
dapper homolog 1, antagonist of beta-catenin (xenopus)	DACT1	−2.19	NM_016651.2	nuclear
ectodermal-neural cortex	**ENC1**	**−2.24**	**AF010314.1**	**protein binding**
keratin 23	KRT23	−2.24	NM_015515.1	--
deiodinase, iodothyronine, type II	DIO2	−2.29	U53506.1	metabolism
plasminogen activator, urokinase	PLAU	−2.29	NM_002658.1	metabolism
NY-REN-7 antigen	**NY-REN-7**	**−2.34**	**AL117630.1**	**--**
stanniocalcin 1	STC1	−2.40	U46768.1	signal transduction
carbonic anhydrase II	CA2	−2.45	M36532.1	metabolism
G protein-coupled receptor 17	GPR17	−2.45	NM_005291.1	signal transduction
high mobility group AT-hook 1	**HMGA1**	**−2.45**	**AF176039.1**	**transcription factor**
Ras-induced senescence 1	RIS1	−2.45	BF062629	--
trefoil factor 1	TFF1	−2.45	NM_003225.1	growth factor
WNT1 inducible signaling pathway protein 2	WISP2	−2.45	NM_003881.1	signal transduction
aquaporin 3	AQP3	−2.51	AB001325	transport
SRY (sex determining region Y)-box 9	SOX9	−2.51	NM_000346.1	transcription factor
bradykinin receptor B1	BDKRB1	−2.57	NM_000710.1	signal transduction
ephrin-B2	EFNB2	−2.57	U16797.1	signal transduction
gap junction protein, alpha 4, 37kDa (connexin 37)	GJA4	−2.57	NM_002060.1	transport
myristoylated alanine-rich protein kinase C substrate	MARCKS	−2.88	AW163148	structural protein
tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	TNFRSF11B	−2.88	NM_002546.1	signal transduction
small proline-rich protein 2B	**SPRR2B**	**−2.95**	**NM_006945.1**	**structural protein**
chromogranin A (parathyroid secretory protein 1)	CHGA	−3.02	NM_001275.2	transport

Table 3 *(Continued)*

Gene	Gene Symbol	fold change	Accession #	Function
Homo sapiens non-functional folate binding protein (HSAF000381), mRNA	**--**	**– 3.16**	**NM_013307.1**	**--**
acid phosphatase, prostate	ACPP	– 3.31	NM_001099.2	metabolism
integrin, beta 3	ITGB3	– 3.47	M35999.1	cell adhesion
platelet-derived growth factor beta polypeptide	PDGFB	– 3.47	NM_002608.1	growth factor
SRY (sex determining region Y)-box 11	SOX11	– 3.72	AB028641.1	transcription factor
cysteine knot superfamily 1, BMP antagonist 1	CKTSF1B1	– 3.89	AF154054.1	--
ribosomal protein S20	RPS20	– 6.17	AF113008.1	protein biosynthesis
ribosomal protein L27a	RPL27A	– 7.59	BE737027	protein biosynthesis

Gene transcripts regulated (≥2-fold) by progesterone alone in late proliferative phase explants when compared to the vehicle-treated controls. Data are presented as fold changes. The genes in bold were not found to be modulated by 17β-oestradiol and progesterone (17β-E_2+P).

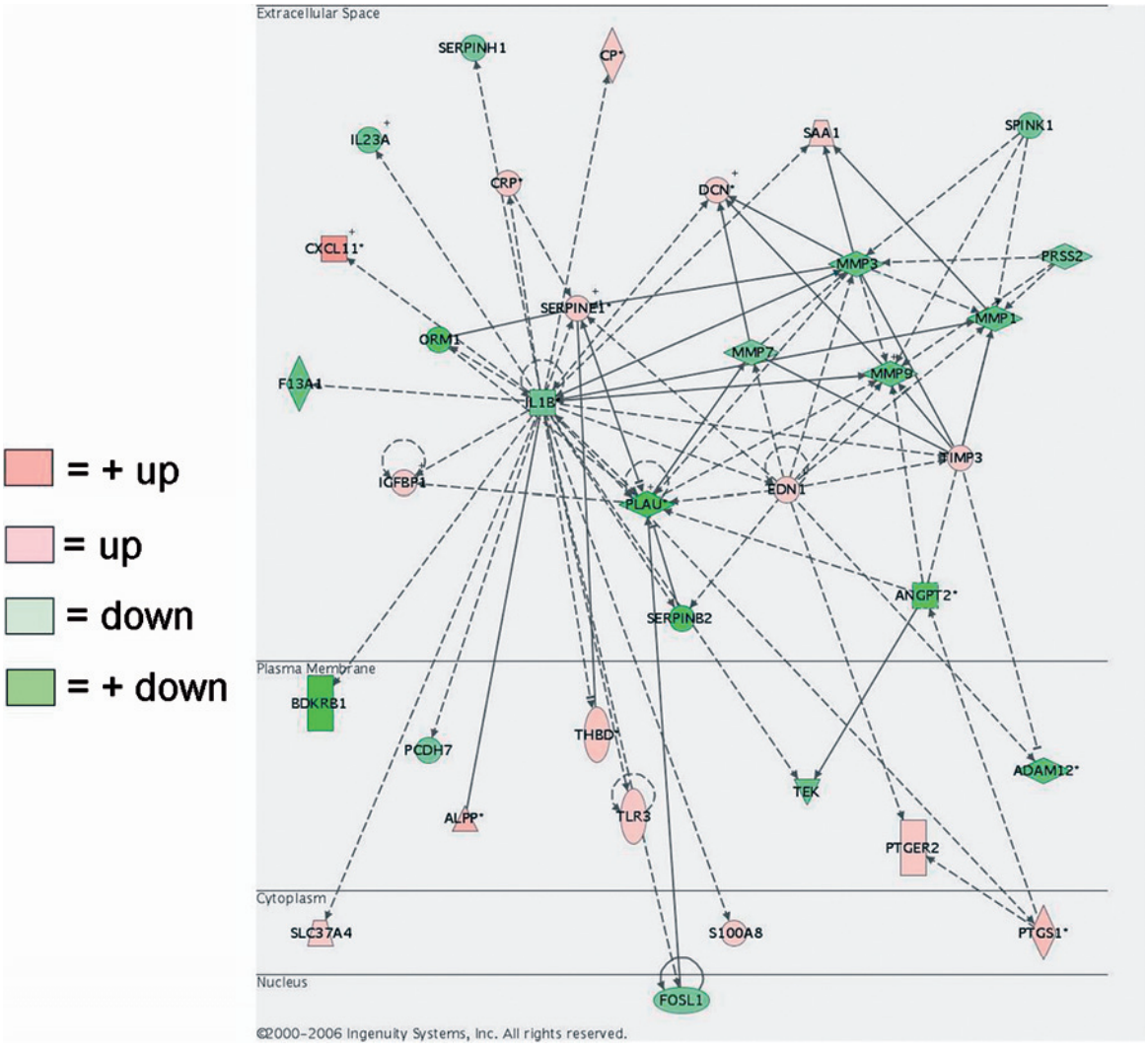

Figure 3. Example of a highly significant network identified in the gene expression profile of late proliferative phase endometrium treated with 17β-oestradiol and progesterone (17β-E_2+P) as determined by the Ingenuity Pathways Analysis program. Green indicates down-regulated genes, and pink indicates up-regulated genes.

Table 4. Genes affected by 17β-oestradiol and progesterone (17β-E_2+P) in explants of menstrual phase endometrium.

Gene	Gene Symbol	fold change	Accession #	function
secretoglobin, family 1D, member 2	SCGB1D2	60.26	NM_006551.2	extracellular matrix
alkaline phosphatase, placental (Regan isozyme)	ALPP	10.00	NM_001632.2	metabolism
hypothetical protein FLJ10847	FLJ10847	7.08	NM_018242.1	transport
secretoglobin, family 2A, member 1	SCGB2A1	6.92	NM_002407.1	hormone binding
secretoglobin, family 2A, member 2	SCGB2A2	6.03	NM_002411.1	hormone binding
trefoil factor 1	TFF1	5.13	NM_003225.1	growth factor
cytochrome P450, family 26, subfamily A, polypeptide 1	CYP26A1	4.68	NM_000783.1	transport
carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 7	CHST7	4.57	NM_019886.1	metabolism
hypothetical protein FLJ10640	FLJ10640	3.47	NM_024703.1	metabolism
hydroxysteroid (17-beta) dehydrogenase 2	HSD17B2	3.39	NM_002153.1	metabolism
paired box gene 5 (B-cell lineage specific activator protein)	PAX5	3.31	NM_016734.1	transcription factor
apolipoprotein D	APOD	3.09	NM_001647.1	transport
solute carrier family 7 (cationic amino acid transporter, y+ system), member 8	SLC7A8	2.75	NM_012244.1	transport
DNA segment on chromosome 4 (unique) 234 expressed sequence	D4S234E	2.69	NM_014392.1	nuclear
GREB1 protein	GREB1	2.69	NM_014668.1	--
anthrax toxin receptor 1	ANTXR1	2.63	NM_018153.1	membrane protein
histone 1, H2bd	HIST1H2BD	2.63	AL353759	DNA binding
prostaglandin-endoperoxide synthase 2	PTGS2	2.63	NM_000963.1	metabolism
heat shock 70kDa protein 6 (HSP70B')	HSPA6	2.57	NM_002155.1	metabolism
cyclin A1	CCNA1	2.45	NM_003914.1	cell growth
asparaginase like 1	ASRGL1	2.40	NM_025080.1	metabolism
apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B	APOBEC3B	2.34	NM_004900.1	metabolism
hypothetical protein FLJ20152	FLJ20152	2.34	AI816291	--
histone 1, H2bh	HIST1H2BH	2.34	NM_003524.1	DNA binding
Homo sapiens mRNA; cDNA DKFZp564G112	--	2.24	AA053967	--
crystallin, alpha B	CRYAB	2.19	AF007162.1	structural protein
colony stimulating factor 3 (granulocyte)	CSF3	2.19	NM_000759.1	signal transduction
histone 1, H1c	HIST1H1C	2.19	BC002649.1	DNA binding
insulin-like growth factor binding protein 1	IGFBP1	2.19	NM_000596.1	signal transduction
serine (or cysteine) proteinase inhibitor, clade A, member 3	SERPINA3	2.19	NM_001085.2	immune respons
apolipoprotein M	APOM	2.14	NM_019101.1	transport
piggyBac transposable element derived 5	PGBD5	2.14	NM_024554.1	--
trefoil factor 3 (intestinal)	TFF3	2.14	NM_003226.1	immune respons
histone 1, H2bi	HIST1H2BI	2.04	NM_003525.1	DNA binding
H2B histone family, member S	H2BFS	2.00	NM_017445.1	DNA binding
putative chemokine receptor	HM74	2.00	NM_006018.1	signal transduction
metallothionein 1X	MT1X	2.00	NM_002450.1	transport
TUWD12	TUWD12	2.00	NM_003774.2	--
hyaluronan binding protein 2	HABP2	−2.00	NM_004132.1	metabolism
interleukin 2 receptor, beta	IL2RB	−2.00	NM_000878.1	immune respons
myosin, light polypeptide kinase	MYLK	−2.00	NM_005965.1	signal transduction

Table 4 *(Continued)*

Gene	Gene Symbol	fold change	Accession #	function
SAM and SH3 domain containing 1	SASH1	−2.00	AK025495.1	cell growth
transglutaminase 2	TGM2	−2.00	BC003551.1	metabolism
adipose specific 2	APM2	−2.04	NM_006829.1	--
Microfibril-associated glycoprotein-2	MAGP2	−2.04	AW665892	extracellular matrix
3-phosphoinositide dependent protein kinase-1	PDPK1	−2.04	NM_002613.1	signal transduction
polymerase (RNA) II (DNA directed) polypeptide J, 13.3kDa	POLR2J	−2.04	AI738591	DNA binding
preferentially expressed antigen in melanoma	PRAME	−2.04	NM_006115.1	--
transmembrane protein 5	TMEM5	−2.04	BF224146	membrane protein
leucine-rich repeat-containing 5	LRRC5	−2.09	NM_018103.1	--
parathyroid hormone receptor 2	PTHR2	−2.09	NM_005048.1	signal transduction
retinoblastoma binding protein 6	RBBP6	−2.09	NM_006910.1	cell growth
cadherin 6, type 2, K-cadherin (fetal kidney)	CDH6	−2.14	AU151483	cell adhesion
v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)	MYCN	−2.14	BC002712.1	transcription factor
SRY (sex determining region Y)-box 4	SOX4	−2.14	AI989477	transcription factor
zinc finger, BED domain containing 2	ZBED2	−2.14	NM_024508.1	DNA binding
ATP-binding cassette, sub-family C (CFTR/MRP), member 3	ABCC3	−2.19	AF009670.1	transport
hypothetical protein LOC339290	LOC339290	−2.19	H49382	--
hypothetical protein MGC29643	MGC29643	−2.19	AL567376	--
transcription factor 4	TCF4	−2.19	AU118026	transcription factor
nudix (nucleoside diphosphate linked moiety X)-type motif 6	NUDT6	−2.24	NM_007083.1	growth factor
ribosomal protein S6 kinase, 90kDa, polypeptide 5	RPS6KA5	−2.24	AF074393.1	metabolism
heme oxygenase (decycling) 1	HMOX1	−2.29	NM_002133.1	metabolism
killer cell lectin-like receptor subfamily B, member 1	KLRB1	−2.29	NM_002258.1	signal transduction
PTPRF interacting protein, binding protein 2 (liprin beta 2)	PPFIBP2	−2.29	AI692180	DNA binding
ubiquitin D	UBD	−2.29	NM_006398.1	--
laminin, alpha 3	LAMA3	−2.34	NM_000227.1	structural protein
ribonucleotide reductase M2 polypeptide	RRM2	−2.34	BE966236	metabolism
Rho guanine nucleotide exchange factor (GEF) 17	ARHGEF17	−2.40	NM_014786.1	--
N-myristoyltransferase 1	NMT1	−2.40	AI570834	metabolism
Homo sapiens cDNA: FLJ22812 fis, clone KAIA2955	--	−2.45	AK026465.1	--
solute carrier family 16 (monocarboxylic acid transporters), member 6	SLC16A6	−2.45	NM_004694.1	transport
spondin 1, (f-spondin) extracellular matrix protein	SPON1	−2.45	AI885290	extracellular matrix
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit s (factor B)	ATP5S	−2.51	NM_015684.1	--
chemokine (C-C motif) ligand 5	CCL5	−2.51	M21121	signal transduction
CD96 antigen	CD96	−2.51	NM_005816.1	cell adhesion
growth associated protein 43	GAP43	−2.51	NM_002045.1	cell growth
histone H2A.F/Z variant	H2AV	−2.51	BF343852	DNA binding
tumor necrosis factor receptor superfamily, member 4	TNFRSF4	−2.51	AJ277151	immune respons

Table 4 *(Continued)*

Gene	Gene Symbol	fold change	Accession #	function
chemokine (C motif) ligand 1	XCL1	−2.57	U23772.1	signal transduction
Homo sapiens transcribed sequences	---	−2.63	BE045982	---
C-terminal binding protein 1	CTBP1	−2.63	AA053830	metabolism
fibroblast growth factor 9 (glia-activating factor)	FGF9	−2.63	NM_002010.1	growth factor
latexin protein	LXN	−2.63	NM_020169.1	---
protocadherin gamma subfamily C, 3	PCDHGC3	−2.63	AB002325.1	transport
cathepsin W (lymphopain)	CTSW	−2.75	NM_001335.1	metabolism
dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	DYRK2	−2.75	NM_006482.1	metabolism
integrin, beta 6	ITGB6	−2.75	NM_000888.3	signal transduction
hypothetical protein LOC284266	LOC284266	−2.75	AK025833.1	---
platelet-derived growth factor alpha polypeptide	PDGFA	−2.75	X03795.1	growth factor
chromosome 14 open reading frame 117	C14orf117	−2.82	NM_018678.1	---
chromosome 20 open reading frame 42	C20orf42	−2.82	NM_017671.1	---
insulin-like growth factor binding protein 3	IGFBP3	−2.82	BF340228	signal transduction
chloride intracellular channel 3	CLIC3	−2.88	NM_004669.1	signal transduction
hypothetical protein FLJ11082	FLJ11082	−2.88	NM_018317.1	---
glutathione S-transferase theta 1	GSTT1	−2.88	NM_000853.1	metabolism
B/K protein	LOC51760	−2.95	NM_016524.1	transport
retinoic acid receptor responder (tazarotene induced) 1	RARRES1	−2.95	AI669229	cell growth
Homo sapiens, clone IMAGE:4866926, mRNA	---	−3.09	AA631242	---
chemokine (C-X-C motif) ligand 14	CXCL14	−3.09	NM_004887.1	signal transduction
chemokine (C motif) ligand 2	XCL2	−3.09	NM_003175.1	signal transduction
cytidine deaminase	CDA	−3.16	NM_001785.1	metabolism
erythrocyte membrane protein band 4.1 like 4A	EPB41L4A	−3.39	NM_022140.1	structural protein
zinc finger protein 426	ZNF426	−3.39	NM_024106.1	transcription factor
regulator of G-protein signalling 5	RGS5	−3.63	AI183997	signal transduction
KIAA0924 protein	KIAA0924	−3.72	NM_014897.1	nuclear
serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5	SERPINB5	−4.07	NM_002639.1	cell adhesion
killer cell lectin-like receptor subfamily C, member 3	KLRC3	−4.17	NM_002260.2	immune response
calcium channel, voltage-dependent, alpha 1G subunit	CACNA1G	−4.27	NM_018896.1	transport
protein phosphatase 1, regulatory (inhibitor) subunit 16B	PPP1R16B	−4.47	AB020630	signal transduction
Homo sapiens mRNA; cDNA DKFZp564N1116	---	−4.68	BF344237	---
integral membrane protein 2A	ITM2A	−4.79	NM_004867.1	membrane protein
phosphoribosylformylglycinamidine synthase (FGAR amidotransferase)	PFAS	−4.90	AL044326	metabolism
major histocompatibility complex, class II, DR beta 3	HLA-DRB3	−5.13	BC005312.1	immune response
immunoglobulin lambda joining 3	IGLJ3	−5.62	X57812.1	---
fibroblast growth factor 18	FGF18	−6.92	BC006245.1	growth factor

Table 4 *(Continued)*

Gene	Gene Symbol	fold change	Accession #	function
cystic fibrosis transmembrane conductance regulator, ATP-binding cassette	CFTR	−8.32	NM_000492.2	transport

Gene transcripts regulated (≥2-fold) by 17β-E_2+P in menstrual phase explants when compared to vehicle-treated controls. Data are presented as fold changes.

Table 5. Genes affected by 17β-E_2+P and reported to be altered in the implantation window.

Gene	Symbol	fold change	Accession #	Kao et al.	Riesewijk et al.	Carson et al.
dickkopf homolog 1	DKK1	6.03	NM_012242.1	12.1	7	12.6
thrombomodulin	THBD	3.24	NM_000361.1		10	
fibrinogen-like 2	FGL2	2.88	NM_006682.1		5	
monoamine oxidase A	MAOA	2.88	NM_000240.1	7.5	15	
retinol binding protein 4, plasma	RBP4	2.88	NM_006744.2		6	
dipeptidylpeptidase 4	DPP4	2.57	M80536.1		15	
nuclear factor I/B	NFIB	2.57	AI186739		10	
H.sapiens mRNA for interleukin-15	–-	2.24	Y09908.1	3.7	3	2.2
gastrin	GAS	2.19	NM_000805.2		11	
KIAA0367 protein	KIAA0367	2.04	AL138349		4	
coagulation factor XIII, A1 polypeptide	F13A1	−2.00	NM_000129.2	−4.1		
microfibrillar-associated protein 2	MFAP2	−2.40	NM_017459.1	−3		
transforming growth factor, beta 3	TGFB3	−2.40	J03241.1			−2.44
gap junction protein, alpha 4, 37kDa (connexin 37)	GJA4	−2.95	NM_002060.1			−20
myristoylated alanine-rich protein kinase C substrate	MARCKS	−3.09	M68956.1	−2.2		
matrix metalloproteinase 11 (stromelysin 3)	MMP11	−3.31	AI761713			−10
deiodinase, iodothyronine, type II	DIO2	−3.47	U53506.1	−2.4		

Gene transcripts regulated (≥2-fold) by 17β-oestradiol and progesterone (17β-E_2+P) that have also been reported to be altered during the window of implantation (by Riesewijk et al. [2], Carson et al. [20] and Kao et al [21]).

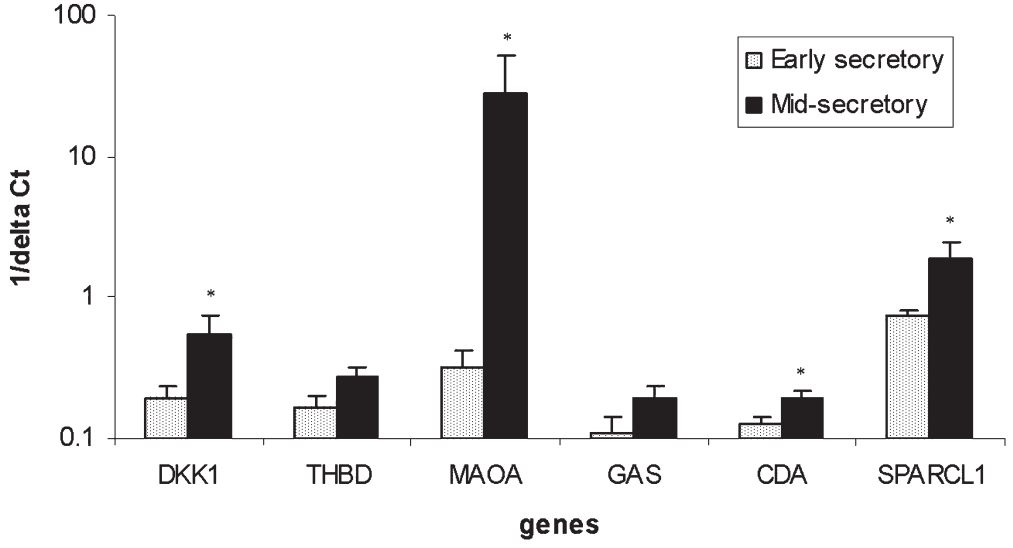

Figure 4. Relative expression levels of gene transcripts for DKK1, THBD, MAOA, GAS, CDA and SPARCL1 in early secretory (n=7) and mid-secretory (n=8) endometrium, which represent endometrial tissues exposed to low (pre-implantation window) and high (implantation window) progesterone concentrations, respectively (*p<0.05).

Table 6. Genes affected by 17β-E2+P in explants of both menstrual and late proliferative phase endometrium.

Gene	Gene Symbol	fold change M	fold change LP	Accession #	Function
alkaline phosphatase, placental (Regan isozyme)	ALPP	10.00	4.79	NM_001632.2	metabolism
apolipoprotein D	APOD	3.09	4.90	NM_001647.1	transport
carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 7	CHST7	4.57	7.08	NM_019886.1	metabolism
cytochrome P450, family 26, subfamily A, polypeptide 1	CYP26A1	4.68	33.11	NM_000783.1	transport
GREB1 protein	GREB1	2.69	2.24	NM_014668.1	unknown
insulin-like growth factor binding protein 1	IGFBP1	2.19	2.45	NM_000596.1	signal transduction
secretoglobin, family 1D, member 2	SCGB1D2	60.26	10.00	NM_006551.2	unknown
secretoglobin, family 2A, member 1	SCGB2A1	6.92	2.63	NM_002407.1	signal transduction
solute carrier family 7 (cationic amino acid transporter, y+ system), member 8	SLC7A8	2.75	4.37	NM_012244.1	transport
chromosome 20 open reading frame 42	C20orf42	−2.82	−2.82	NM_017671.1	unknown
cystic fibrosis transmembrane conductance regulator	CFTR	−8.32	−4.07	NM_000492.2	transport
hypothetical protein FLJ11082	FLJ11082	−2.88	−2.04	NM_018317.1	unknown
integrin, beta 6	ITGB6	−2.75	−2.95	NM_000888.3	cell adhesion
latexin protein	LXN	−2.63	−2.51	NM_020169.1	unknown

Gene transcripts regulated (≥2-fold) by 17β-oestradiol and progesterone (17β-E_2+P) in both menstrual (M) and late proliferative (LP) phase explants when compared to their respective vehicle-treated controls. Data are presented as fold changes.

The number of gene transcripts regulated by P in late proliferative phase explants was almost twice the number regulated in menstrual phase explants, indicating that oestrogen priming sensitizes the endometrium for P regulation, most likely by induction of P receptor gene expression [16]. In addition, most of these genes were specifically modulated in the late proliferative phase endometrium. Of these genes (n=100), at least 17 were previously described to be regulated in the implantation window (Table 5) [2, 20, 21]. Three examples of such genes are DKK1, MAOA and SPARCL1. Regulation of expression by 17β-E_2+P and P alone was confirmed with real-time PCR in both explant cultures and endometrium biopsy specimens collected during the implantation window and ES phase. These findings demonstrate that the expression of genes associated with the implantation window can be modulated in explant cultures of human endometrium and that for most of these genes, prolonged *in vivo* exposure to 17β-E_2 is required for adequate P regulation. These findings also support the hypothesis that variations in the duration of 17β-E_2 priming can affect the response of the endometrium to P and therefore the subsequent implantation process [11, 22].

The number of implantation-associated gene transcripts, however, was rather low. This could be because the culturing of explants alters the physiology of the tissue and therefore its steroid responsiveness or because, as shown for prolactin and IGFBP1, in some cases prolonged exposure to P is required for genes to respond [23]; the latter finding is supported by a report from Kao and coworkers showing that many genes up-regulated in the implantation window are not yet regulated in ES endometrium, at which time the endometrium has been exposed to P for only a short time [21]. Explant cultures are therefore appropriate models to study immediate responses of human endometrium to oestrogens and progestins *ex vivo* but do not allow investigation of the entire spectrum of implantation-associated genes.

The low number of implantation-related genes identified may also be a result of the relatively low number of samples used for the initial microarray hybridizations, which increases the likelihood of missing relevant genes and the chance of generating false positives. At the time the microarray experiments were performed, we opted to carry out a limited number of array hybridizations so that we could apply rigorous statistical procedures and perform extensive validation of selected genes for both the array samples and samples from additional independent experiments. Rockett and Hellmann asked the questions: how many genes should we pick for validation, and which genes should we pick? The authors argue that genes can be selected to ensure successful confirmation, *i.e.* by selecting genes that have changed more than 4-fold [24] or by selecting genes that have been

reported to be changed in similar models or conditions [25]. We selected six genes primarily based on the fact that their expression is altered during the implantation window. In addition, we selected two genes that have not yet been reported in the endometrium. With the exception of DKK1 (more than 5-fold induction), the expression of the selected genes changed less than 3-fold. We could confirm steroid regulation for four of eight genes in independent experiments, which justifies our approach.

Rockett and Hellmann also questioned the additive value of corroborating the findings of microarray experiments with alternative means of quantitating the mRNA abundance of a limited number of genes of the array [25]. The vast majority of studies published state that the DNA array data can be corroborated, indicating that the array data are reliable as long as the experimental design and statistical analysis is sound. Even in high-impact journals, studies that have not been validated are being published; Goodman illustrated this by showing that our of 28 microarray papers in Science, Cell and Nature published in 2002, only 11 reported corroborative studies [26]. It is evident that clear standards, such as the guideline Minimal Information about a Microarray Experiment (MIAME), in the confirmatory studies area are necessary [25].

A clear distinction could be made between genes that are regulated by P irrespective of the presence of 17β-E_2 and genes for which the expression is clearly influenced by the continuous presence of 17β-E_2. Many genes modulated by P alone were similarly modulated in the 17β-E_2+P-treated explants (119/161 P-modulated genes), however, 42 of the P-modulated genes were not affected in the 17β-E_2+P-treated explants. Also, of the 219 17β-E_2+P-modulated genes, 117 were not modulated by treatment with P alone. This clearly indicates that the expression of a subset of genes is sensitive to the continuing presence of 17β-E_2. It also indicates that *in vivo* priming of CD12 and CD13 endometrium is remembered by the tissue *in vitro*, leading to similar expression patterns for certain genes induced both in the absence and presence of 17β-E_2.

A good example of genes for which expression is known to be suppressed by P, but which were only suppressed by P in the presence of 17β-E_2, are various members of the MMP family [12–14]. Only the expression of MMP11 was suppressed by P alone; the expression of MMP1, -3, -14 and -27 was only suppressed in the presence of 17β-E_2. Similarly, cystic fibrosis transmembrane conductance regulator (CFTR) was suppressed in 17β-E_2+P-treated explants but not in P-treated explants, suggesting that continued presence of 17β-E_2 is required for the down-regulation of CFTR. This corresponds with the finding that CFTR is highly expressed in the human endometrium around the ovulatory period [27] and is responsive to both 17β-E_2 and P.

Some genes were induced by 17β-E_2+P in both menstrual and late proliferative phase explants (*i.e.* alkaline phosphatase, ALPP; monoamine oxidase, MAOA; secretoglobin family 1, member D, SCGB1D2; CFTR; P450 cytochrome family 26 subfamily A, CYP26A), indicating that the expression of these genes does not depend on prolonged *in vivo* oestrogen priming of the endometrium. A particularly interesting observation in this regard is the up-regulation of expression of the CYP26A gene in both menstrual and late proliferative phase endometrium by 17β-E_2+P and, to a lesser extent, by P alone. This enzyme is responsible for the metabolism of the active retinoid metabolite *all*-trans retinoic acid. The importance of controlling retinoid levels in the uterus is illustrated by the fact that vitamin A deficiency in women, nonhuman primates and laboratory animals is associated with pregnancy failure and developmental defects [28–30], whereas excess vitamin A levels are detrimental to blastocyst development [31] and the decidualization process [32].

Uterine vitamin A levels in women increase in the presence of oestrogens [33, 34], most likely as the result of up-regulation of retinaldehyde dehydrogenase (RALDH2), a critical enzyme in retinoic acid (RA) biosynthesis [35]. Since retinoids are morphogens and essential for epithelial cell growth [36], they may be involved in the regeneration, growth and differentiation of the endometrial epithelium after menstruation. The induction of CYP26A expression by P in the secretory phase most likely serves to inactivate excessive amounts of retinoids.

Databases can be explored with several different bioinformatics tools. We have employed the Ingenuity Pathways Analysis (IPA) program, which has the added advantage that it is an evidence-based data mining tool. In contrast to most other bioinformatics tools, which annotate certain functions to gene products, the IPA program includes any reported interaction between two genes, whether it involves regulation of gene or protein expression, protein-protein interactions or enzymatic conversion (for example, phosphorylation). It is therefore a continuously growing database and, by the nature of its development, not complete. It is not unusual that the most affected genes are not presented in the networks. The networks present groups of genes that have a proven biological relationship. The nodes in these highly significant networks presumably represent genes that have important modulatory roles. When interpreting the data, one has to realize that the IPA

database is biased in that certain genes have received more attention than others and therefore have a higher likelihood to be included in a network. However, the continuously growing database will allow reanalysis of the data in the future, which may reveal novel unidentified relationships between genes or groups of genes.

The significant suppressive actions of P on nodes representing immunomodulators were immediately apparent; these included IL-1β, IL-8, COX2, the chemokine CCL5 and members of the TGF-β superfamily (TGF-β2 and -3, INHBA and their signalling molecules SMAD2 and -3). At the end of the secretory phase, a rapid influx of leukocytes, consisting mostly of NK cells and macrophages, into the endometrium can be observed; this is believed to be the result of the disappearance of P suppression on key inflammatory mediators [37, 38]. Apparently, these immunosuppressive actions of P can at least partly be mimicked in the explant model by short-term incubation with 17β-E_2 and P.

One of the few nodes present in highly significant networks identified by the IPA program in both 17β-E_2- and P-treated menstrual and late proliferative phase endometrium was FGF2 or basic fibroblast growth factor (bFGF). FGF2 expression is suppressed by P. The significance of this finding is illustrated by the fact that FGF2 inhibits the decidualization process in human endometrial stromal cells [39] and should therefore be controlled by P during the secretory phase. FGF2 is an important mitogenic and angiogenic factor that is expressed as different isoforms synthesized through the alternative use of translation initiation codons [40]. In human endometrium, only the smallest 18-kD isoform is present [41]. It is located predominantly in the cytoplasm and is stored in the extracellular matrix [42]. FGF2 is released mostly during menstruation and the early proliferative phase and is expressed in blood vessels throughout the menstrual cycle [41, 43]. The FGF receptors, however, are not expressed in blood vessels except during the MS (FGFR2) and late secretory phases (FGFR1 and FGFR2). Blood vessels may therefore not be the main target of FGF2. FGF2 receptors are predominantly found in the epithelial compartment [44], suggesting that FGF2 is involved in the control of regeneration and growth of epithelial cells in a paracrine fashion. FGF2 is known to regulate proliferation of various cell populations of the bone marrow [44], which were shown to be of eminent importance for regeneration of the human endometrium [45].

In conclusion, explant culture of human endometrium is a biologically relevant *in vitro* model system that allows the investigation of steroid regulation of gene expression in the tissue context. Regulation of the expression of several genes associated with embryo implantation can be mimicked *in vitro*. We showed that expression of thrombomodulin, monoamine oxidase A and SPARCL1 is regulated by progestins. Only a subset of implantation-associated genes was modulated in the short-term explant cultures; however, we clearly showed that we can distinguish genes that require continuous presence of 17β-E_2 from those that depend on P only. Therefore, 17β-E_2 selectively primes implantation-related genes for the effects of P.

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