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Effect of single post-ovulatory administration of mifepristone (RU486) on transcript profile during the receptive period in human endometrium

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Abstract

Progesterone regulates uterine function during the luteal phase and is essential for the acquisition of endometrial receptivity. The objective of the present study was to identify endometrial transcripts whose expression is altered during the window of implantation after the administration of 200 mg of the antiprogestin mifepristone, 48 h after the LH peak (LH + 2, LH + 0 = LH peak), and to determine the relationship of these transcripts with those regulated during the acquisition of receptivity. Endometrial samples were obtained in LH + 7 from seven women of proven fertility, each one contributing with one cycle treated with placebo and another with mifepristone. Additionally, endometrial samples were obtained in LH + 2 and LH + 7 during a single untreated spontaneous cycle from seven normal fertile women as a reference. DNA microarrays were used to identify transcripts significantly regulated (defined as ≥2.0-fold change with false discovery rate below 1% using *t*-test) with the administration of mifepristone vs placebo, or during the transition from pre-receptive to receptive (LH + 2 vs LH + 7). Approximately 2000 transcripts were significantly regulated in both comparisons (mifepristone vs placebo and LH + 2 vs LH + 7), but only 777 of them were coincident and displayed opposite regulation except for 25. The mRNA level for eight selected genes regulated by mifepristone was confirmed by real-time RT-PCR. We conclude that not all changes in endometrial transcript levels occurring in the transition from LH + 2 to LH + 7 seem to be regulated by the progesterone receptor and ~37% of the genes whose transcript levels changed by effect of mifepristone could be associated with the acquisition of receptivity.
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Introduction

Human endometrium displays characteristic morphological and molecular changes during each stage of development during menstrual cycle under the control of the ovarian hormones estradiol and progesterone (P_4). Both epithelial and stromal cells undergo mitosis in response to rising estradiol serum levels during the proliferative phase of the endometrial cycle whereas the secretory phase is driven by P_4 (Bagchi *et al*. 2003), which triggers a highly coordinated and sequential responses beginning with the suppression of the estrogen-dependent epithelial cell proliferation and inducing endometrial differentiation and maturation characterized by the secretory transformation of the glands, infiltration of immune cells and stromal edema (Strowitzki *et al*. 2006, Gellersen *et al*. 2007). At functional level, P_4 renders the uterus in an adequate morphological and functional state for embryo implantation during a self-limited time (Paria *et al*. 2002). This period, so-called window of implantation, lasts for ~5 days starting on day 20 (day LH +7–11) of a 28-days menstrual cycle (Navot *et al*. 1991, Wilcox *et al*. 1999). On target cells, P_4 exerts its effects primarily through the nuclear P_4 receptor (PGR) (Tsai & O'Malley 1994), a member of the superfamily of ligand-activated transcription factors (Misrahi *et al*. 1987). PGR is a ligand activated transcription factor (Chabbert-Buffet *et al*. 2005) which upon P_4 binding activates its gene regulatory functions (Graham & Clarke 1997). The hormone–receptor complex interacts with specific target genes, modulating their expression. Two PGR isoforms have been described, PGR-A and PGR-B, which are transcribed from a single gene with two distinct promoters (O'Malley & Tsai 1992). In addition, P_4 may elicit several rapid signaling events, independent of

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transcriptional regulation or even in the absence of its nuclear receptors (Gellersen *et al*. 2009). It is accepted that these rapid non-genomic signaling events along with the relatively slower genomic actions, determine the functional response to P_4 in cell type- and milieu-specific manner. While the genomic responses are better understood, the mechanisms underlying non-genomic P_4 actions are mostly yet to be determined (Gellersen *et al*. 2009).

In the endometrium, P_4 regulates the expression and repression of genes during the period of receptivity that eventually induce a physiological state required to initiate and support pregnancy (Tabibzadeh 1998, Wang & Dey 2006). In women, most of P_4-regulated genes in the endometrium that might be relevant in embryo implantation have been identified using DNA microarrays by comparing the endometrial gene expression profile of women during mid-secretory phase (peak circulating P_4) with proliferative or early secretory phase (low circulating P_4) in spontaneous cycles. Unfortunately, few genes that were significantly and similarly regulated during the window of implantation have been reported considering the total number of regulated transcripts comprising each list of six different reports (Tapia *et al*. 2011). Since P_4 drives endometrial receptivity, chemical compounds that block P_4 action interfere with its transition to the secretory phenotype and avoid or interrupt pregnancy. One of this compounds is mifepristone (RU486), a synthetic 19 nor-steroid antagonist of the PGR (Spitz 2003) that inhibits P_4-mediated gene transcription and, if administered post-implantation, ultimately leads to *conceptus* abortion (Baulieu 1989). The molecular mechanism of mifepristone action is not completely understood although it has been described that receptor activation, heat shock proteins dissociation, dimerization and binding to P_4 response elements in the DNA do not appear to be affected upon mifepristone binding to PGR. This suggests that the interaction and recruitment of corregulators (Liu *et al*. 2002) are the main factors driving its antiprogestin activity (Chauchereau *et al*. 2003, Dasgupta & O'Malley 2014, Szwarc *et al*. 2015). In addition, mifepristone may act as P_4 antagonist for some non-genomic responses elicited by P_4 (Chien *et al*. 2009).

The effects of mifepristone in the endometrium depend on the dose and day of the menstrual cycle it is administered (Gemzell-Danielsson *et al*. 1993). Its administration during the proliferative phase inhibits follicular development and delays the LH peak retarding ovulation. Consequently, the menstrual cycle length is extended without effects on endometrial morphology (Liu *et al*. 1987, Swahn *et al*. 1988). When mifepristone is administered during the mid or late luteal phase induces endometrial bleeding a few days after its administration (Schaison *et al*. 1985, Shoupe *et al*. 1987, Swahn *et al*. 1988). When a single dose of mifepristone (200 mg) is administered 2 days after ovulation (i.e. 2 days after the LH surge, LH+2) affects neither the menstrual cycle length nor serum estrogen and P_4 levels, however it profoundly affects endometrial morphology, retarding endometrial development and inhibiting the glandular secretory activity (Swahn *et al*. 1990) which ultimately prevents pregnancy (Gemzell-Danielsson *et al*. 1993, Hapangama *et al*. 2001). Gemzell-Danielsson *et al*. (1993) reported the contraceptive effects of a single dose of mifepristone 200 mg in LH+2 to a group of women as their only contraceptive method. In 124 out of 157 cycles, sexual intercourse occurred during the fertile period and only one pregnancy was documented. Similar results were obtained in other study (Hapangama *et al*. 2001) using the same administration protocol and dose in which 136 out of 178 studied cycles were ovulatory and exposed, reporting only one pregnancy. Although these studies do not allow a pregnancy rate calculation (Croxatto 2003), they reveal a clear contraceptive effect upon the administration of a single mifepristone dose during the early luteal phase (day LH+2). The evidence from the clinical studies and the reported effects on the endometrial morphology provide robust evidence that the changes produced in the endometrium during the receptive phase are enough for preventing embryo implantation. In addition, studies using co-culture of human embryos with *in vitro* endometrial constructions showed that mifepristone inhibits embryo adhesion to the endometrial layer (Lalitkumar *et al*. 2007). Mifepristone has been used previously to evaluate endometrial gene expression *in vitro* using tissue explants obtained during the mid-secretory phase (Catalano *et al*. 2003). However, this model does not reflex the steroids dynamics occurring *in vivo*. Tissue explants are appropriate for evaluating immediate responses in human endometrium to ovarian steroids *ex vivo* but do not fully address the entire set of genes associated to embryo implantation (Catalano *et al*. 2007, Dassen *et al*. 2007*a*). The endometrial response to 200 mg of mifepristone in day LH+8 was evaluated in women after 6 or 24 h of treatment (Catalano *et al*. 2007). Although this model was designed to identify P_4-regulated genes involved in the endometrial receptivity, also pinpoints transcripts related to the induction of menstruation, introducing a confounding factor in the determination of uterine receptivity genes. The effect of mifepristone administration during the early luteal phase on human endometrial gene expression during the receptive period *in vivo* has not yet been studied. We hypothesize that the administration of mifepristone on LH+2 will inhibit P_4-induced gene expression changes required for endometrial receptivity. Such design does not induces menses prematurely nor alters circulating estrogen and P_4 levels, allowing the evaluation of gene expression changes induced by mifepristone in receptive endometrium, under conditions that inhibit implantation without inducing menses. The objective of the present study is

to identify endometrial genes whose transcript levels on LH+7 is altered upon administration of mifepristone on LH+2 and to determine their relation with the gene expression profile of the endometrium during the transition from pre-receptive (LH+2) to receptive (LH+7).

Materials and methods

Participants

The present study was conducted using a protocol in accordance with the guidelines in the Declaration of Helsinki and approved by the Ethical and Scientific Review Committee from Instituto Chileno de Medicina Reproductiva (ICMER). Each volunteer signed an informed consent form before participating. Two groups of seven women were recruited for the study, all of them having good health as determined by medical history, physical and gynecological examination. All volunteers were of Hispanic ethnicity, surgically sterilized at least 1 year before participating in the study for reasons unrelated to this study, regular menstrual cycles within the range of 26–35 days. Age, BMI and hemoglobin levels of all participating women are presented in Table 1. No one was under chronic medication or taking hormones or drugs able to modify the metabolism of steroid hormones in the 3 preceding months. Functional and anthropometric and parameters of participating women are presented in Table 1.

Study design

This study was comprised of two parts. The first one was a placebo controlled, cross-over, double-blinded and randomized trial that included seven volunteers. Each subject contributed with one placebo-treated cycle (control) and one mifepristone-treated cycle (experimental) in a randomized order and one endometrial biopsy was taken on day LH+7 (LH+0=day of LH peak) of each cycle. Three women started with the control cycle and the other four with the experimental cycle. The following two cycles, no treatment was given (resting cycles) and no biopsy was taken. At the fourth consecutive cycle, a corresponding swap with experimental and control cycles was done.

In order to control the timing of the interventions and assure normality of the cycle under study, serum LH was measured daily starting from the mid follicular phase (days 7–10) and follicular growth was monitored by transvaginal ultrasound (TVU). Blood sampling and TVU were discontinued the following day after ovulation was detected. When the echo-image of the leading follicle was found to have disappeared or reduced at least 50%, was taken as evidence that ovulation had occurred. A single pill containing mifepristone 200 mg (HRA Pharma, Paris, France) or placebo was administered 48 h after the LH peak (LH+2) during the experimental and control cycles respectively and both types of pills were identical in appearance. Treatment was given at the clinic under supervision of a nurse in all cases. Endometrial samples were collected on LH+7 under sterile conditions from the uterine fundus, using pipelle catheters (Laboratoire C.C.D., Paris, France). An additional blood sample was taken on the biopsy day to determine P_4 serum level. The second part of the study involved another group of seven volunteers that met the same inclusion criteria as the group from the first part of our protocol. A straightforward follow up for a single menstrual cycle was done for these women that included daily serum LH measurements and TVU as described above. Two endometrial biopsies were obtained within that menstrual cycle on which no treatment was given. One biopsy was taken during the early secretory (LH+2) and another during the mid-secretory (LH+7) phase.

LH and P_4 serum levels were determined as described before (Croxatto *et al.* 2004). For both parts of the study, each endometrial sample was divided into two and one piece was flash frozen in liquid nitrogen and stored at −80 °C for subsequent RNA isolation. The remainder piece was processed for histological assessment by an independent pathologist, under blind conditions, using the criteria described by Noyes *et al.* (1975).

Isolation of RNA

Total RNA was isolated from each tissue sample using Trizol reagent (Invitrogen) according to the instructions from the manufacturer. RNA concentration was determined by absorbance at 260 nm (A_{260}) and purity by calculating the A_{260}/A_{280} ratio which varied between 1.8 and 2.1. The quality of the RNA was checked using the Agilent's Lab-on-a-Chip total RNA nano biosizing assay (Agilent Technologies, Inc., Palo Alto, CA, USA). The RNA integrity number was >9.0 for all samples analyzed.

GeneChip hybridization

Human Genome U133 plus 2.0 GeneChip oligonucleotide microarrays (Affymetrix, Sunnyvale, CA, USA) corresponding to 47 000 transcripts and variants, including 38 500 well-characterized human genes were used for gene expression analysis. Complementary RNA (cRNA) synthesis and array hybridization were performed according to the Affymetrix Expression Analysis Technical Manual. Briefly, 2 μg of purified

Table 1 Characteristics of women participating in the study and parameters evaluated during the hormonal replacement cycle. All participating women had a BMI ≤30 and hemoglobin levels ≥12 g/dl. Mean and range for each parameter is indicated in parenthesis.

	Group MIF/placebo (*n*=7)	**Group untreated LH+2/LH+7** (*n*=7)
Age (years)	36.1 (32–39)	35.4 (30–40)
BMI	25.2 (23.4–30)	25.6 (20.8–29.7)
Hemoglobin (g/dl)	14.6 (13.7–15.9)	13.5 (12.4–14.7)
Number of live births	3.3 (2–4)	3.0 (2–4)
Plasma progesterone[a] (nmol/l)	49.1 (28.4 –104.9)/42.7 (24.1–58.7)	10.5 (6.7–14.7)/35.4 (17.7–51.1)

[a]On the day of biopsy.

endometrial RNA was reverse transcribed using Whole Transcript cDNA Synthesis Kit (Affymetrix). After second strand synthesis biotin labeled cRNA from all endometrial samples was generated from the double strand template using T7 RNA polymerase (Affymetrix). *In vitro* transcription was performed to produce cRNA that was verified by Agilent's Lab-on-a-Chip total RNA nano biosizing assay. Twenty micrograms of biotin-labeled cRNA were hybridized to the microarrays chip for 16 h at 45 °C in premixed hybridization solution containing labeled hybridization control prokaryotic genes (bioB, bioC, bioD and cre). Replicate spots for each control gene are present on the chip. Washing and fluorescent staining with streptavidin–phycoerithrin were performed in the GeneChip Fluidics Station 450 and using the Affymetrix Staining Kit (Affymetrix). Microarrays were immediately scanned at a resolution of 6 µm using a GeneChip Scanner 3000 (Affymetrix). Replicate hybridizations were performed for each RNA sample.

Microarray data analysis

Intensity values for the probes generated with the Affymetrix GeneChip Miroarray suite v 1.4 were exported to the Partek Genomics suite v 6.3 beta software (Partek Incorporated, St Louis, MO, USA) to determine gene expression differences and statistical analyses.

The sample size for this study was 14 subjects divided in two groups of seven. Since each women provided two endometrial samples (placebo/mifepristone or LH+2/LH+7), a total of 28 samples were analyzed in duplicate. Data from all 56 arrays were normalized using Robust Multichip Average method (Irizarry *et al.* 2003) and used for further statistical analysis. To identify the differentially expressed genes between mifepristone and placebo groups; and LH+2 and LH+7 samples, a pairwise *t*-test was applied. To assess the results, the *P* value <0.001 was used as a cutoff and a false discovery rate (FDR) of 1% was applied to the lists of genes. Finally, in addition to the appropriate correction for multiple testing, it was combined with a fold change of gene expression ≥ 2.0, calculated as the average log-ratio between two groups to determine up- and down-regulated genes between groups.

Overlapping genes from differentially expressed transcripts among comparisons were determined and graphically represented using Venn Diagrams.

Principal component analysis

Principal component analysis (PCA) is a statistical technique for simplifying large amounts of data derived from microarray analysis (Joliffe & Morgan 1992). The method reduces the dimensionality of multivariate data while preserving as much of the relevant information as possible. As a form of unsupervised learning, relies entirely on the input data itself without reference to the corresponding target data. Mathematically is the transformation of the data to a new coordinate system with three dimensions, such that the variables from the new set (the principal components) are linear functions of the original variables. This simplification determines the key variables that explain the differences between samples based on the expression profiles, allowing the summarization and further analysis of the microarray data. We applied the unbiased PCA algorithm using the Partek software to all samples using 54 675 genes and ESTs represented on the microarray chip to look for expression patterns and underlying cluster structures.

Hierarchical clustering

Hierarchical clustering of endometrial samples was performed as another method of data structure visualization, using differentially expressed transcripts based on uncentered correlations with average linkage clustering. The resulting dendogram allows data structure visualization of endometrial samples according to total gene expression, revealing similar patterns of gene expression and relationships between the specimens and groups.

Over represented transcription factor binding sites detection in promoter regions of endometrial genes regulated by mifepristone

For a systematic search for potential over represented transcription factor binding sites (TFBS), the promoter region of our genes of interest defined as the region proximal to the transcription-start site of genes transcribed by RNA polymerase II, was analyzed. For that we used three bioinformatic approaches and platforms to increase the likelihood of our results: MotifScanner on software TOUCAN (Aerts *et al.* 2003), The Transcription Element Listening System (Cole *et al.* 2005) and Gene Annotation Tool to Help Explain Relationships (GATHER) (Chang & Nevins 2006).

Functional clustering

The list of significantly regulated endometrial transcripts with mifepristone compared with placebo, obtained as described in the microarrays data analysis section, was used for functional clustering analyses in order to gain more insights about the biological process related to the regulated transcripts. The web-based tools Database for Annotation, Visualization and Integrated Discovery (DAVID) (Dennis *et al.* 2003) and GATHER (Chang & Nevins 2006) were used as described before (Tapia *et al.* 2011). Both web-tool services obtain the biological meaning of submitted genes by retrieving their functional annotations from the Kyoto Encyclopedia of Genes and Genomes (Kanehisa *et al.* 2006), Biocarta pathways (http://www.biocarta.com) and Gene Ontology (GO) (Ashburner *et al.* 2000) databases. No directionality is associated with the obtained relationships (i.e. the function should not be interpreted as being increased or decreased).

Validation of microarray data by real-time RT-PCR

Verification of microarrays data was performed by real-time RT–PCR analysis of selected genes using an ABI PRISM 7000 sequence detection system according to the manufacturer's instructions (Applied Biosystems). Briefly, first-strand cDNA was synthesized from total RNA of each endometrial sample in duplicate by reverse transcription using the SuperScript III reverse transcriptase (Invitrogen), according to the manufacturer's protocol. Pre-validated primers and TaqMan probes (Assays-on-demand, PE Applied Biosystems) were used for all

transcripts in Table 2 to determine their respective transcript levels and GAPDH was used as a reference housekeeping gene. Quantitative analysis was based on the relative quantification of each gene of interest in the endometrial samples from of each group by using the ΔΔ*C*T method (Livak & Schmittgen 2001). Statistical significance was determined with the Wilcoxon two-sample paired signed rank test.

Results

Anthropometric and functional parameters of subjects in placebo and mifepristone treated cycles

A total of 28 endometrial biopsies were obtained. Sixteen samples were obtained from mifepristone- and placebo-treated cycles ($n=7$ for each group) with two 'wash-out' cycles in between. Another 14 samples were obtained from spontaneous cycles on LH+2 and LH+7 days ($n=7$ for each of the 2 collection days).

All P_4 serum levels obtained on LH+7 were within expected values for a spontaneous normal menstrual cycle (>30 nmol/l) with no statistically significant differences between the mifepristone- and placebo-treated groups (Table 1) in agreement with previous reports (Swahn *et al*. 1990). No unscheduled bleeding or spotting was reported.

PCA and hierarchical clustering

PCA showed a clear segregation of samples according to their respective groups (Fig. 1A). As expected, endometrial gene expression profiles from the LH+7 group clustered together with those from placebo group. Interestingly, biopsies taken on LH+2 grouped along with the mifepristone-treated samples. Unsupervised hierarchical clustering analysis was applied for gene expression profiles from endometrial samples obtained from microarrays data. A dendogram was generated with the data from the endometrial samples from all groups in a tree-structured graph. The dendogram obtained displayed a striking segregation of samples into two major clustering branches, one corresponding to the LH+2 and mifepristone groups and the other to the LH+7 and placebo groups (Fig. 1B).

In the PCA and hierarchical clustering we found that the endometrial transcript profile during the window of implantation upon mifepristone administration is more similar to those obtained for early secretory endometrium during natural cycle. On the other hand, the endometrial transcript profiles from endometrial samples obtained in mid secretory phase upon placebo where indistinguishable from those obtained during the window of implantation during a non-treated spontaneous cycles and clustered separately from the other two groups.

Table 2 Transcripts submitted to real-time RT-PCR confirmation.

UniGene ID	Gene name	Description
Hs.404466	*CRISP3*	Cysteine-rich secretory protein 3
Hs.204096	*SCGB1D2*	Secretoglobin, family 1D, member 2
Hs.491232	*SLC39A14*	Solute carrier family 39 (zinc transporter), member 14
Hs.70327	*CRIP1*	Cysteine-rich protein 1 (intestinal)
Hs.40499	*DKK1*	Dickkopf homolog 1 (Xenopus laevis)
Hs.183109	*MAOA*	Monoamine oxidase A
Hs.278959	*GAL*	Galanin prepropeptide
Hs.116651	*MPZL2*	Myelin protein zero-like 2

Transcripts with differential expression in the endometrium after post ovulatory administration of mifepristone

We identified a total of 2119 transcripts corresponding to known genes whose expression altered significantly in the uterus at the time of implantation in response to mifepristone which represents a ∼4.7% of the total number of transcripts represented in the microarrays chip. In total, 766 and 1353 transcripts were up- and down-regulated respectively in receptive endometrium upon administration of mifepristone 200 mg on day LH+2 of the menstrual cycle compared with the time matched placebo group. In Tables 3 and 4 are shown the top 100 endometrial genes whose transcript levels increased and decreased respectively, upon mifepristone administration.

When the endometrial samples obtained on day LH+2 were compared with those obtained on day LH+7 during the same spontaneous cycle, 1915 transcripts were differentially expressed (∼3.5% of transcripts assayed in the microarrays assay) with 893 and 1022 transcripts up- and down-regulated respectively. Seven hundred and seventy seven transcripts out of the total of differentially expressed genes from pre-receptive (LH+2) to receptive endometrium (LH+7) were common to those obtained with the administration of mifepristone compared with placebo (Fig. 2). Interestingly, 752 of them displayed opposite regulation directionalities whereas 25 changed in the same direction as mifepristone. It should be pointed out that such concordance of differentially expressed transcripts with opposed regulation is likely to be the main driver for the cluster structures found with PCA and hierarchical clustering.

Integration and cross-validation of human endometrial transcriptome during the window of implantation reported by different groups could increase the confidence in the detection of regulated transcripts for many more genes than is tractable with classical validation (Kemmeren *et al*. 2002, Rhodes *et al*. 2002). The available data sets comparing endometrial gene expression profiles during spontaneous cycles from the proliferative vs mid secretory phase (Kao *et al*. 2002, Borthwick *et al*. 2003), from early secretory vs mid secretory phase (Carson *et al*. 2002, Riesewijk *et al*. 2003, Mirkin *et al*. 2005, Talbi *et al*. 2006) and with a single dose of mifepristone 200 mg on LH+8 after 6 and

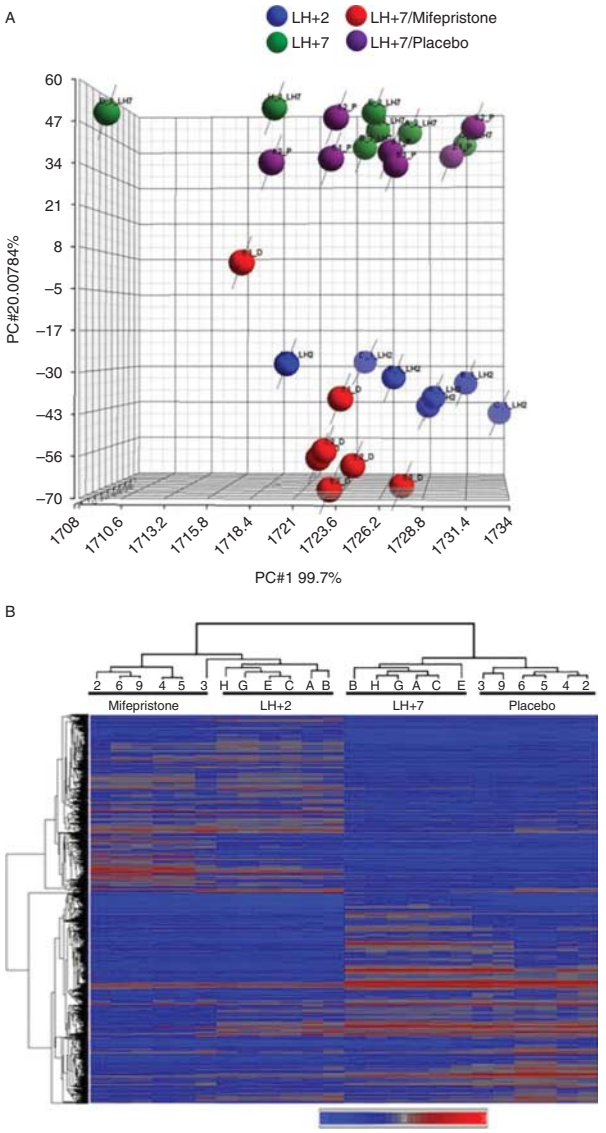

Figure 1 Unsupervised analyses of microarrays data. Principal component analysis (PCA) plot (A) and Hierarchical clustering (B) of gene expression profiles from endometrial samples. PCA included 28 endometrial samples whose gene expression profiles were obtained by microarray analysis. Blue dots = samples obtained on LH + 2, green dots = samples obtained on LH + 7, purple dots = samples obtained in LH + 7 with administration of placebo and red dots = samples obtained on LH + 7 with administration of mifepristone. Endometrial samples from the reference group on LH + 2 and LH + 7 (blue and green dots) were obtained during the same spontaneous cycle of each women. The profiles from the mifepristone group (red dots) cluster together with those for the LH + 2 reference group whereas the samples from the placebo group cluster along with the LH + 7 reference group. Hierarchical clustering analysis represented in a tree-like dendogram revealing the similarities on gene expression profiles of 28 endometrial samples. Each row represents a transcript and each column the gene expression profile of a particular endometrial sample. A clear segregation of samples into two major clustering branches, one with samples from mifepristone and LH + 2 groups and the other with samples from placebo and LH + 7 groups, is shown. Most gene expression profiles self-cluster together with others from their respective group.

24 h of administration vs placebo (Catalano *et al.* 2007) were contrasted with our data sets from microarrays analysis. We found 14 transcripts regulated by mifepristone (13 down- and 1 up-regulated) that also have been reported in the opposite direction during the window of implantation in at least four different studies and in our reference group (Table 5). The transcript CLDN4 was coincident with five reports but not significantly regulated in our reference group. Interestingly, we found only two coincident transcripts (MMP7 and CXCL12) in the study of Catalano *et al.* (2007) displaying the same regulatory direction with our study group and that have also been reported for the acquisition of receptivity; however the described regulatory behavior during spontaneous cycles is not the opposite in all of them.

Identification of overrepresented consensus sequences for TFBS sites of transcripts regulated by mifepristone

To identify potential common regulatory pathways in endometrial genes regulated by mifepristone, we performed an analysis for detection of over-represented promoter sequences using three bioinformatics tools. First, the potential TFBS were detected and then those statistically over-represented in our set of regulated endometrial genes were determined. The results are listed in Table 6 for up- and down-regulated transcripts respectively. Interestingly, DNA binding sites for seven transcription factors were identified as overrepresented in both increased and decreased transcripts whereas other 16 and 24 overrepresented TFBS where unique for up- and down-regulated genes respectively.

Functional clustering of endometrial transcripts regulated by mifepristone

In order to gain further understanding of the potential functional roles of dysregulated endometrial transcripts from group A, we obtained the functional annotations from each gene and determined the enriched processes associated with them from two different web-based tools. Within the up-regulated transcripts, the functional classifications related to cell adhesion and proliferation, were found to be statistically over-represented in both web-based tools used (Tables 7 and 8). The down-regulated transcript list was not enriched with transcripts related to coincident functions in the two analysis performed for functional annotation clusters.

Real-time RT-PCR confirmation

In order to confirm differences in transcript levels found with the microarrays, five genes (*CRISP3*, *GAL*, *MAOA*, *SLC39A14* and *DKK1*) whose mRNA steady-state level increased in the comparison LH + 2 vs LH + 7 and showed an opposite regulation upon mifepristone

Table 3 Top 100 endometrial transcripts most down-regulated on LH+7 after oral administration of mifepristone 200 mg on day LH+2.

Probeset ID	UniGene ID	Gene title	Gene symbol	Fold change	*P* value
206799_at	Hs.204096	Secretoglobin, family 1D, member 2	SCGB1D2	−139.44	4.8×10^{-14}
207802_at	Hs.404466	Cysteine-rich secretory protein 3	*CRISP3*	−107.26	3.3×10^{-17}
217546_at	Hs.647370	Metallothionein 1M	*MT1M*	−62.48	2.8×10^{-19}
206424_at	Hs.150595	Cytochrome P450, family 26, subfamily A, polypeptide 1	*CYP26A1*	−60.94	1.1×10^{-12}
241031_at	Hs.202656	C2 calcium-dependent domain containing 4A	*C2CD4A*	−58.65	2.1×10^{-15}
219597_s_at	Hs.272813	Dual oxidase 1	*DUOX1*	−49.25	1.2×10^{-12}
203946_s_at	Hs.226007	Arginase 2	*ARG2*	−47.87	4.2×10^{-16}
232737_s_at	Hs.486489	Ectonucleotide pyrophosphatase/phosphodiesterase 3	*ENPP3*	−46.28	2.0×10^{-12}
224840_at	Hs.407190	FK506 binding protein 5	*FKBP5*	−46.04	1.2×10^{-17}
218002_s_at	Hs.483444	Chemokine (C–X–C motif) ligand 14	*CXCL14*	−39.86	1.0×10^{-08}
224412_s_at	Hs.272225	Transient receptor potential cation channel, subfamily M, member 6	*TRPM6*	−38.60	1.6×10^{-08}
229254_at	Hs.567714	Major facilitator superfamily domain containing 4	*MFSD4*	−37.73	9.2×10^{-17}
205242_at	Hs.100431	Chemokine (C–X–C motif) ligand 13	*CXCL13*	−36.90	1.4×10^{-11}
204745_x_at	Hs.433391	Metallothionein 1G	*MT1G*	−36.05	5.1×10^{-20}
215813_s_at	Hs.201978	Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	*PTGS1*	−35.61	9.9×10^{-12}
210029_at	Hs.738619	Indoleamine 2,3-dioxygenase 1	*IDO1*	−33.02	3.1×10^{-14}
227253_at	Hs.558314	Ceruloplasmin (ferroxidase)	*CP*	−29.11	3.1×10^{-13}
220724_at	Hs.479703	Cell wall biogenesis 43 C-terminal homolog (*Saccharomyces cerevisiae*)	*CWH43*	−28.82	5.9×10^{-10}
213629_x_at	Hs.513626	Metallothionein 1F	*MT1F*	−28.55	1.0×10^{-18}
241994_at	Hs.250	Xanthine dehydrogenase	*XDH*	−26.67	8.8×10^{-08}
1555434_a_at	Hs.491232	Solute carrier family 39 (zinc transporter), member 14	*SLC39A14*	−26.41	2.2×10^{-11}
1552715_a_at	Hs.196119	Relaxin/insulin-like family peptide receptor 1	*RXFP1*	−26.22	4.9×10^{-15}
225987_at	Hs.521008	STEAP family member 4	*STEAP4*	−26.12	5.2×10^{-11}
204602_at	Hs.40499	Dickkopf WNT signaling pathway inhibitor 1	*DKK1*	−25.75	1.4×10^{-13}
203180_at	Hs.459538	Aldehyde dehydrogenase 1 family, member A3	*ALDH1A3*	−25.53	4.8×10^{-11}
244780_at	Hs.591604	Sphingosine-1-phosphate phosphatase 2	*SGPP2*	−25.01	2.9×10^{-08}
230673_at	Hs.170128	Polycystic kidney and hepatic disease 1 (autosomal recessive)-like 1	*PKHD1L1*	−23.73	2.2×10^{-07}
229839_at	Hs.591833	Scavenger receptor class A, member 5 (putative)	*SCARA5*	−22.88	1.4×10^{-11}
206378_at	Hs.46452	Secretoglobin, family 2A, member 2	*SCGB2A2*	−22.00	2.5×10^{-11}
202965_s_at	Hs.496593	Calpain 6	*CAPN6*	−21.95	3.8×10^{-08}
231372_at	Hs.720329	Solute carrier family 25, member 48	*SLC25A48*	−21.71	8.2×10^{-09}
209555_s_at	Hs.120949	CD36 molecule (thrombospondin receptor)	*CD36*	−21.61	6.8×10^{-11}
206461_x_at	Hs.438462	Metallothionein 1H	*MT1H*	−21.27	9.6×10^{-18}
201739_at	Hs.510078	Serum/glucocorticoid regulated kinase 1	*SGK1*	−20.91	8.2×10^{-14}
214240_at	Hs.278959	Galanin/GMAP prepropeptide	*GAL*	−20.36	7.9×10^{-11}
204326_x_at	Hs.374950	Metallothionein 1X	*MT1X*	−20.10	4.8×10^{-15}
212859_x_at	Hs.744893	Metallothionein 1E	*MT1E*	−19.86	6.5×10^{-21}
212741_at	Hs.183109	Monoamine oxidase A	*MAOA*	−18.16	4.0×10^{-17}
236161_at	–	Uncharacterized LOC102659288	*LOC102659288*	−18.02	1.0×10^{-12}
204818_at	Hs.162795	Hydroxysteroid (17-(β) dehydrogenase 2	*HSD17B2*	−17.85	7.0×10^{-06}
204595_s_at	Hs.25590	Stanniocalcin 1	*STC1*	−17.77	5.9×10^{-12}
205934_at	Hs.153322	Phospholipase C-like 1	*PLCL1*	−17.57	2.1×10^{-13}
203780_at	Hs.116651	Myelin protein zero-like 2	*MPZL2*	−16.13	1.5×10^{-14}
205382_s_at	Hs.155597	Complement factor D (adipsin)	*CFD*	−15.57	2.9×10^{-14}
206859_s_at	Hs.532325	Progestagen-associated endometrial protein	*PAEP*	−15.50	1.1×10^{-4}
209723_at	Hs.104879	Serpin peptidase inhibitor, clade B (ovalbumin), member 9	*SERPINB9*	−15.48	1.8×10^{-08}
226863_at	Hs.8379	Family with sequence similarity 110, member C	*FAM110C*	−14.63	6.8×10^{-16}
204942_s_at	Hs.87539	Aldehyde dehydrogenase 3 family, member B2	*ALDH3B2*	−14.09	4.9×10^{-11}
212805_at	Hs.262857	Prune homolog 2 (*Drosophila*)	*PRUNE2*	−13.96	6.5×10^{-15}
203434_s_at	Hs.307734	Membrane metallo-endopeptidase	*MME*	−13.56	2.0×10^{-13}

(continued)

Table 3 *Continued.*

Probeset ID	UniGene ID	Gene title	Gene symbol	Fold change	*P* value
205081_at	Hs.70327	Cysteine-rich protein 1 (intestinal)	*CRIP1*	−13.18	5.9×10^{-14}
210064_s_at	Hs.271580	Uroplakin 1B	*UPK1B*	−13.17	5.0×10^{-08}
1554648_a_at	Hs.356664	Dual oxidase maturation factor 1	*DUOXA1*	−13.16	2.9×10^{-12}
227241_at	Hs.407152	Mucin 15, cell surface associated	*MUC15*	−13.15	6.8×10^{-06}
209758_s_at	Hs.512842	Microfibrillar associated protein 5	*MFAP5*	−13.00	1.8×10^{-10}
224209_s_at	Hs.494163	Guanine deaminase	*GDA*	−13.00	3.8×10^{-13}
236761_at	Hs.659164	Lipoma HMGIC fusion partner-like 3	*LHFPL3*	−13.00	2.5×10^{-12}
203574_at	Hs.599756	Nuclear factor, interleukin 3 regulated	*NFIL3*	−12.69	4.3×10^{-15}
203961_at	Hs.5025	Nebulette	*NEBL*	−12.45	8.0×10^{-12}
204061_at	Hs.390788	Protein kinase, X-linked	*PRKX*	−12.21	5.7×10^{-11}
206002_at	Hs.146978	G protein-coupled receptor 64	*GPR64*	−12.14	3.1×10^{-05}
204351_at	Hs.2962	S100 calcium binding protein P	*S100P*	−12.14	5.1×10^{-07}
228195_at	Hs.389311	Chromosome 2 open reading frame 88	*C2orf88*	−12.07	8.9×10^{-15}
209183_s_at	Hs.93675	Chromosome 10 open reading frame 10	*C10orf10*	−12.07	2.3×10^{-09}
223484_at	Hs.112242	Chromosome 15 open reading frame 48	*C15orf48*	−12.04	1.1×10^{-07}
235649_at	Hs.271605	ADAM metallopeptidase with thrombospondin type 1 motif, 8	*ADAMTS8*	−12.01	2.7×10^{-08}
230084_at	Hs.143545	Solute carrier family 30 (zinc transporter), member 2	*SLC30A2*	−11.87	6.2×10^{-12}
206268_at	Hs.656214	Left-right determination factor 1	*LEFTY1*	−11.75	1.8×10^{-07}
226164_x_at	Hs.504670	Ribosomal modification protein rimK-like family member B	*RIMKLB*	−11.58	2.0×10^{-08}
219867_at	Hs.283725	Chondrolectin	*CHODL*	−11.56	6.6×10^{-07}
202856_s_at	Hs.500761	MicroRNA 6787/solute carrier family 16 (monocarboxylate transporter), member 3	*MIR6787*	−11.17	1.1×10^{-10}
217080_s_at	Hs.459142	Homer homolog 2 (*Drosophila*)	*HOMER2*	−10.98	2.1×10^{-10}
238063_at	Hs.122456	Transmembrane protein 154	*TMEM154*	−10.82	1.3×10^{-13}
209278_s_at	Hs.438231	Tissue factor pathway inhibitor 2	*TFPI2*	−10.82	1.1×10^{-06}
219959_at	Hs.354068	Molybdenum cofactor sulfurase	*MOCOS*	−10.65	5.0×10^{-10}
201926_s_at	Hs.126517	CD55 molecule, decay accelerating factor for complement (Cromer blood group)	*CD55*	−10.56	3.8×10^{-11}
209283_at	Hs.53454	Crystallin, alpha B	*CRYAB*	−10.19	2.3×10^{-13}
218963_s_at	Hs.9029	Keratin 23 (histone deacetylase inducible)	*KRT23*	−10.17	1.1×10^{-08}
213880_at	Hs.604364	Leucine-rich repeat containing G protein-coupled receptor 5	*LGR5*	−10.09	2.4×10^{-07}
230964_at	Hs.253994	FRAS1 related extracellular matrix protein 2	*FREM2*	−10.03	1.9×10^{-07}
205857_at	Hs.596992	Solute carrier family 18 (vesicular monoamine transporter), member 2	*SLC18A2*	−9.88	9.2×10^{-09}
232523_at	Hs.438709	Multiple EGF-like-domains 10	*MEGF10*	−9.88	1.8×10^{-08}
201525_at	Hs.522555	Apolipoprotein D	*APOD*	−9.66	7.2×10^{-12}
228055_at	Hs.636624	Napsin B aspartic peptidase, pseudogene	*NAPSB*	−9.54	5.2×10^{-10}
1555600_s_at	Hs.115099	Apolipoprotein L, 4	*APOL4*	−9.52	1.4×10^{-09}
230577_at	Hs.170953	Long intergenic non-protein coding RNA 844	*LINC00844*	−9.43	1.1×10^{-07}
205654_at	Hs.1012	Complement component 4 binding protein, α	*C4BPA*	−9.40	8.0×10^{-07}
209875_s_at	Hs.313	Secreted phosphoprotein 1	*SPP1*	−9.37	4.7×10^{-10}
202723_s_at	Hs.370666	Forkhead box O1	*FOXO1*	−9.31	3.0×10^{-14}
211737_x_at	Hs.371249	Pleiotrophin	*PTN*	−9.28	1.2×10^{-10}
209546_s_at	Hs.114309	Apolipoprotein L, 1	*APOL1*	−9.21	1.3×10^{-13}
212670_at	Hs.647061	Elastin	*ELN*	−9.20	5.0×10^{-10}
229160_at	Hs.10653	Melanoma associated antigen (mutated) 1-like 1	*MUM1L1*	−9.17	2.0×10^{-12}
205671_s_at	Hs.1802	Major histocompatibility complex, class II, DO beta	*HLA-DOB*	−9.13	1.2×10^{-05}
210096_at	Hs.436317	Cytochrome P450, family 4, subfamily B, polypeptide 1	*CYP4B1*	−9.05	1.0×10^{-09}
205373_at	Hs.167368	Catenin (cadherin-associated protein), α 2	*CTNNA2*	−9.03	8.2×10^{-07}
219403_s_at	Hs.44227	Heparanase	*HPSE*	−8.81	1.2×10^{-06}
209443_at	Hs.159628	Serpin peptidase inhibitor, clade A (α-1 antiproteinase, antitrypsin), member 5	*SERPINA5*	−8.79	3.2×10^{-10}
209016_s_at	Hs.411501	Keratin 7	*KRT7*	−8.78	5.8×10^{-10}
226517_at	Hs.438993	Branched chain amino-acid transaminase 1, cytosolic	*BCAT1*	−8.71	2.3×10^{-11}

Table 4 Top 100 endometrial transcripts most up-regulated on LH+7 after oral administration of mifepristone 200 mg on day LH+2.

Probeset ID	UniGene ID	Gene title	Gene symbol	Fold change	*P* value
219525_at	Hs.232054	Solute carrier family 47 (multidrug and toxin extrusion), member 1	*SLC47A1*	33.94	7.4×10^{-13}
206622_at	Hs.182231	Thyrotropin-releasing hormone	*TRH*	33.89	1.8×10^{-13}
204052_s_at	Hs.608988	Secreted frizzled-related protein 4	*SFRP4*	33.87	4.0×10^{-14}
226777_at	Hs.594351	ADAM metallopeptidase domain 12	*ADAM12*	32.54	8.1×10^{-15}
203878_s_at	Hs.143751	Matrix metallopeptidase 11 (stromelysin 3)	*MMP11*	21.38	2.6×10^{-12}
202833_s_at	Hs.525557	Serpin peptidase inhibitor, clade A (α-1 antiproteinase, antitrypsin), member 1	*SERPINA1*	21.29	2.5×10^{-09}
206100_at	Hs.654387	Carboxypeptidase M	*CPM*	15.24	7.3×10^{-09}
228010_at	Hs.479069	Protein phosphatase 2, regulatory subunit B, gamma	*PPP2R2C*	13.58	1.8×10^{-10}
205432_at	Hs.1154	Oviductal glycoprotein 1, 120 kDa	*OVGP1*	12.32	8.0×10^{-07}
242064_at	Hs.435719	Sidekick cell adhesion molecule 2	*SDK2*	11.23	7.5×10^{-14}
201645_at	Hs.143250	Tenascin C	*TNC*	10.91	1.64×10^{-08}
220192_x_at	Hs.485158	SAM pointed domain containing ETS transcription factor	*SPDEF*	10.57	1.1×10^{-11}
202037_s_at	Hs.213424	Secreted frizzled-related protein 1	*SFRP1*	10.33	4.3×10^{-09}
202920_at	Hs.599220	Ankyrin 2, neuronal	*ANK2*	10.24	1.1×10^{-10}
209687_at	Hs.522891	Chemokine (C-X-C motif) ligand 12	*CXCL12*	9.93	2.8×10^{-07}
205347_s_at	Hs.56145	Thymosin beta 15a	*TMSB15A*	9.79	8.0×10^{-13}
213661_at	Hs.55044	Peptidase domain containing associated with muscle regeneration 1	*PAMR1*	9.65	7.1×10^{-11}
213652_at	Hs.368542	Proprotein convertase subtilisin/kexin type 5	*PCSK5*	9.39	6.6×10^{-09}
213131_at	Hs.522484	Olfactomedin 1	*OLFM1*	9.04	3.0×10^{-11}
204319_s_at	Hs.501200	Regulator of G-protein signaling 10	*RGS10*	8.93	3.7×10^{-12}
214247_s_at	Hs.292156	Dickkopf WNT signaling pathway inhibitor 3	*DKK3*	8.86	2.2×10^{-11}
208305_at	Hs.32405	Progesterone receptor	*PGR*	8.52	2.8×10^{-09}
229358_at	Hs.654504	Indian hedgehog	*IHH*	8.30	4.1×10^{-12}
230424_at	Hs.745061	Neuronal regeneration related protein	*NREP*	8.26	6.2×10^{-08}
229802_at	Hs.492974	WNT1 inducible signaling pathway protein 1	*WISP1*	8.15	1.7×10^{-08}
218885_s_at	Hs.47099	Polypeptide N-acetylgalactosaminyltransferase 12	*GALNT12*	7.81	4.2×10^{-10}
219478_at	Hs.36688	WAP four-disulfide core domain 1	*WFDC1*	7.75	5.3×10^{-12}
203305_at	Hs.335513	Coagulation factor XIII, A1 polypeptide	*F13A1*	7.75	4.5×10^{-06}
222450_at	Hs.517155	Prostate transmembrane protein, androgen induced 1	*PMEPA1*	7.70	9.3×10^{-10}
202729_s_at	Hs.619315	Latent transforming growth factor beta binding protein 1	*LTBP1*	7.60	2.6×10^{-11}
203184_at	Hs.519294	Fibrillin 2	*FBN2*	7.57	2.0×10^{-11}
225288_at	Hs.494892	Collagen, type XXVII, α 1	*COL27A1*	7.51	1.4×10^{-09}
210809_s_at	Hs.136348	Periostin, osteoblast specific factor	*POSTN*	7.22	1.2×10^{-04}
213791_at	Hs.104920	Proenkephalin	*PENK*	7.04	5.1×10^{-08}
208399_s_at	Hs.1408	Endothelin 3	*EDN3*	7.03	1.3×10^{-07}
210026_s_at	Hs.57973	Caspase recruitment domain family, member 10	*CARD10*	6.94	3.3×10^{-10}
202291_s_at	Hs.365706	Matrix Gla protein	*MGP*	6.93	3.2×10^{-07}
227742_at	Hs.473695	Chloride intracellular channel 6	*CLIC6*	6.84	5.2×10^{-08}
228233_at	Hs.50850	FRAS1 related extracellular matrix 1	*FREM1*	6.80	2.6×10^{-15}
226576_at	Hs.610471	Rho GTPase activating protein 26	*ARHGAP26*	6.71	8.6×10^{-08}
1555520_at	Hs.494538	Patched 1	*PTCH1*	6.45	1.2×10^{-10}
202935_s_at	Hs.647409	SRY (sex determining region Y)-box 9	*SOX9*	6.33	1.1×10^{-07}
219197_s_at	Hs.523468	Signal peptide, CUB domain, EGF-like 2	*SCUBE2*	6.17	1.0×10^{-11}
205306_x_at	Hs.731056	Kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)	*KMO*	6.17	8.3×10^{-07}
238332_at	Hs.355689	Ankyrin repeat domain 29	*ANKRD29*	6.03	1.5×10^{-07}
209757_s_at	Hs.25960	v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog	*MYCN*	6.02	3.4×10^{-10}
226455_at	Hs.372924	cAMP responsive element binding protein 3-like 4	*CREB3L4*	6.01	8.0×10^{-11}
203726_s_at	Hs.436367	Laminin, α 3	*LAMA3*	5.95	4.4×10^{-12}
229641_at	Hs.34333	Collagen and calcium binding EGF domains 1	*CCBE1*	5.89	7.7×10^{-09}
201341_at	Hs.104925	Ectodermal-neural cortex 1 (with BTB domain)	*ENC1*	5.86	5.3×10^{-10}
201506_at	Hs.369397	Transforming growth factor, (β-induced, 68 kDa	*TGFBI*	5.81	4.3×10^{-09}

(continued)

Table 4 *Continued.*

Probeset ID	UniGene ID	Gene title	Gene symbol	Fold change	*P* value
202202_s_at	Hs.654572	Laminin, α 4	*LAMA4*	5.77	7.6×10^{-07}
1553179_at	Hs.23751	ADAM metallopeptidase with thrombospondin type 1 motif, 19	*ADAMTS19*	5.76	1.3×10^{-07}
219225_at	Hs.520463	PiggyBac transposable element derived 5	*PGBD5*	5.75	1.5×10^{-06}
218638_s_at	Hs.302963	Uncharacterized LOC100130872	*LOC100130872*	5.68	7.4×10^{-11}
204304_s_at	Hs.614734	Prominin 1	*PROM1*	5.62	1.3×10^{-06}
204607_at	Hs.592695	3-Hydroxy-3-methylglutaryl-CoA synthase 2 (mitochondrial)	*HMGCS2*	5.62	6.0×10^{-06}
212944_at	Hs.302742	Solute carrier family 5 (sodium/myo-inositol cotransporter), member 3	*SLC5A3*	5.51	1.3×10^{-09}
228731_at	Hs.24321	Guanylate cyclase 1, soluble, α 2	*GUCY1A2*	5.42	1.8×10^{-07}
203417_at	Hs.389137	Microfibrillar-associated protein 2	*MFAP2*	5.39	1.2×10^{-06}
204712_at	Hs.284122	WNT inhibitory factor 1	*WIF1*	5.37	2.6×10^{-04}
228821_at	Hs.609912	ST6 β-galactosamide α-2,6-sialyltranferase 2	*ST6GAL2*	5.33	2.3×10^{-06}
225817_at	Hs.148989	Cingulin-like 1	*CGNL1*	5.28	3.4×10^{-10}
228547_at	Hs.597143	Neurexin 1	*NRXN1*	5.27	3.0×10^{-07}
227070_at	Hs.631650	Glycosyltransferase 8 domain containing 2	*GLT8D2*	5.26	8.3×10^{-09}
239457_at	Hs.306212	ATPase, aminophospholipid transporter, class I, type 8B, member 3	*ATP8B3*	5.24	3.1×10^{-07}
202478_at	Hs.467751	Tribbles pseudokinase 2	*TRIB2*	5.19	1.7×10^{-07}
201291_s_at	Hs.156346	Topoisomerase (DNA) II α 170 kDa	*TOP2A*	5.11	5.8×10^{-08}
215363_x_at	Hs.654487	Folate hydrolase (prostate-specific membrane antigen) 1	*FOLH1*	5.07	9.3×10^{-10}
204487_s_at	Hs.613018	Potassium voltage-gated channel, KQT-like subfamily, member 1	*KCNQ1*	5.01	5.1×10^{-11}
228570_at	Hs.271272	BTB (POZ) domain containing 11	*BTBD11*	4.98	3.5×10^{-08}
204310_s_at	Hs.78518	Natriuretic peptide receptor 2	*NPR2*	4.92	1.7×10^{-09}
229381_at	Hs.29190	Chromosome 1 open reading frame 64	*C1orf64*	4.90	1.1×10^{-08}
236420_s_at	Hs.58785	Anoctamin 4	*ANO4*	4.86	2.5×10^{-07}
232111_at	Hs.455955	TCL1 upstream neural differentiation-associated RNA	*TUNAR*	4.84	1.0×10^{-04}
37892_at	Hs.523446	Collagen, type XI, α 1	*COL11A1*	4.84	5.8×10^{-06}
227048_at	Hs.270364	Laminin, α 1	*LAMA1*	4.74	2.0×10^{-07}
221019_s_at	Hs.464422	Collectin sub-family member 12	*COLEC12*	4.67	2.2×10^{-06}
227705_at	Hs.21861	Transcription elongation factor A (SII)-like 7	*TCEAL7*	4.67	5.9×10^{-08}
238066_at	Hs.422688	Retinol binding protein 7, cellular	*RBP7*	4.65	1.5×10^{-06}
223475_at	Hs.436542	Cysteine-rich secretory protein LCCL domain containing 1	*CRISPLD1*	4.58	4.0×10^{-08}
202016_at	Hs.270978	Mesoderm specific transcript	*MEST*	4.57	6.1×10^{-07}
210839_s_at	Hs.190977	Ectonucleotide pyrophosphatase/phosphodiesterase 2	*ENPP2*	4.54	1.7×10^{-06}
225626_at	Hs.266175	Phosphoprotein membrane anchor with glycosphingolipid microdomains 1	*PAG1*	4.54	2.0×10^{-05}
219142_at	Hs.596555	RAS-like, family 11, member B	*RASL11B*	4.51	5.4×10^{-07}
225242_s_at	Hs.477128	Coiled-coil domain containing 80	*CCDC80*	4.49	1.5×10^{-05}
209596_at	Hs.369422	Matrix-remodelling associated 5	*MXRA5*	4.44	1.3×10^{-06}
205381_at	Hs.567412	Leucine rich repeat containing 17	*LRRC17*	4.42	1.0×10^{-08}
229281_at	Hs.603919	Neuronal PAS domain protein 3	*NPAS3*	4.41	5.0×10^{-07}
238125_at	Hs.619000	ADAM metallopeptidase with thrombospondin type 1 motif, 16	*ADAMTS16*	4.41	5.5×10^{-09}
228235_at	Hs.416379	Uncharacterized protein MGC16121	*MGC16121*	4.41	2.1×10^{-07}
203440_at	Hs.464829	Cadherin 2, type 1, N-cadherin (neuronal)	*CDH2*	4.34	1.5×10^{-09}
205489_at	Hs.924	Crystallin, μ	*CRYM*	4.33	9.0×10^{-07}
236064_at	Hs.118918	Solute carrier family 25, member 35	*SLC25A35*	4.31	3.1×10^{-09}
219277_s_at	Hs.17860	Oxoglutarate dehydrogenase-like	*OGDHL*	4.31	1.3×10^{-06}
226047_at	Hs.501898	Murine retrovirus integration site 1 homolog	*MRVI1*	4.30	1.1×10^{-06}
204259_at	Hs.2256	Matrix metallopeptidase 7 (matrilysin, uterine)	*MMP7*	4.25	3.5×10^{-04}
240145_at	Hs.326475	Diacylglycerol kinase, η	*DGKH*	4.24	1.1×10^{-08}
225275_at	Hs.482730	EGF-like repeats and discoidin I-like domains 3	*EDIL3*	4.23	1.8×10^{-06}
219213_at	Hs.517227	Junctional adhesion molecule 2	*JAM2*	4.21	1.2×10^{-10}

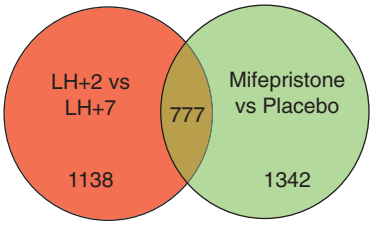

Figure 2 Venn diagram from differentially expressed transcripts in the experimental and reference groups. The green circle indicates the number of transcripts significantly regulated in the endometrium of women on day LH+7 with oral postovulatory administration of mifepristone (mifepristone vs placebo). The red circle indicates those significantly regulated during the acquisition of endometrial receptivity (LH+2 vs LH+7).

administration, were submitted to real-time RT-qPCR analysis. Additionally, transcript level for other three genes (*SGB1D2*, *EVA1* and *CRIP1*) that were shown to decrease in the mifepristone-treated group but did not show significant variation in the comparison LH+2 vs LH+7, was analyzed by RT-qPCR. The mRNA levels for the eight genes analyzed were reduced in the mifepristone treated group compared with the placebo group in agreement with the microarray data (Fig. 3). The transcript level of *CRISP3*, *GAL*, *MAOA*, *SLC39A14* and *DKK1* increased in the LH+7 group compared with the LH+2 group, however the level for *SCGB1D2*, *EVA1* y *CRIP1* was not statistically significant amongst these groups (Fig. 3); in line with the microarrays results.

Discussion

We determined the endometrial gene expression profile in human endometrium under four different conditions analyzing 54 675 transcripts that cover most of the known human genes reported. The endometrial samples were characterized at the molecular level organized in the unsupervised analyses PCA and hierarchical clustering. Each sample seems to have an expression profile that self-cluster with its own group. The gene expression profile of endometrial samples on LH+7 with mifepristone is similar to the one obtained with samples obtained in LH+2, and the profile of samples obtained in LH+7 with no treatment or with placebo are similar amongst them but different to the other two groups. For microarrays data validation, we confirmed by qPCR the transcript level of eight genes that were differentially expressed in the microarrays analysis during the window of implantation after mifepristone administration on LH+2. Although a relatively small number of transcripts were confirmed, considering that in other studies using the same microarrays platform we used have given a good correlation for gene expression confirmation, we consider such validation confers a reasonable validity to the groups of regulated transcripts.

The 2119 transcripts whose abundance was found altered 5 days after mifepristone administration on LH+2 in comparison with the placebo group is the most novel finding of this work. Since the most remarkable pharmacological property of mifepristone is to block P_4 action, we presume these genes are regulated either directly or indirectly by P_4. Such presumption is in line with the finding that almost all transcripts that changed its level in the transition from LH+2 to LH+7 changed in the opposite direction of the mifepristone group compared with placebo. However such fact did not occur for 25 transcripts whose expression level changed significantly but in the same direction. This transcript regulation could be explained by an agonistic effect on these P_4 regulated transcripts. The agonistic effect of mifepristone has been described before in the endometrium from postmenopausal women with the induction of secretory changes after inducing proliferation with exogenous estradiol (Gravanis *et al.* 1985). Such progestogenic effect of mifepristone has been documented *in vitro* in endometrial cell lines as well. In HeLa cells co-transfected with reporter genes and an expression vector with the PGR, an agonistic effect of mifepristone was described on gene expression (Meyer *et al.* 1990, Tung *et al.* 1993, Jackson *et al.* 1997) which depends on the isoform of the PGR mifepristone binds to and the cell context (Meyer *et al.* 1990). The gene expression profile analysis of Ishikawa cells in presence of P_4 or mifepristone showed that mifepristone induces a transcriptional behavior with both agonistic and antagonistic activity of P_4 (Tamm-Rosenstein *et al.* 2013). Regardless of the unexpected behavior of these 25 transcripts, it is unlikely they are involved in the anti-implantation effect driven by mifepristone.

The endometrium is composed of an heterogeneous population of cells including mesodermal-derived glandular and luminal epithelial cells that are supported by a basement membrane and uterine fibroblasts, vascular smooth cells, endothelial cells and lymphoid cells in the connective stroma. These cells have been described to respond differentially to the ovarian steroidal hormones. The endometrial samples analyzed in the present study, are processed combining all the cell types composing the tissue, hence it is difficult to put the regulated transcripts in context of a complex tissue. In this sense it is required further identification of the endometrial cell types expressing the genes of interest by techniques such as immunohistochemistry or *in situ* hybridization. An alternative approach has been to analyze separately the endometrial compartments during the window of implantation by laser capture microdissection (Torres *et al.* 2002, Evans *et al.* 2012, Evans *et al.* 2014). These studies may contribute to the understanding on the regulation exerted by progesterone on specific endometrial compartments.

In the present study, we used two independent clustering strategies to analyze the microarrays data

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Table 5 Genes previously described to be regulated during the window of implantation in spontaneous cycles and/or regulated in endometrium of women with mifepristone administration on LH+8 that are coincident with our datasets.

			Mifepristone treated			Pre-receptive to receptive endometrium					
UniGene ID	**Probeset ID**	**Gene title**	Gene symbol	Our study (mifepristone vs placebo)	Catalano *et al.* (2007) (6 h, 24 h)	Our study (LH+2 vs LH+7)	Kao *et al.* (2002)	Carson *et al.* (2002)	Borthwick *et al.* (2003)	Riesewijk *et al.* (2003)	Mirkin *et al.* (2005)	Talbi *et al.* (2006)
Down-regulated transcripts												
Hs.313	209875_s_at	Secreted phosphoprotein 1 (osteopontin)	SPP1	−9.37	–, –	13.77	↑	↑	↑	↑	↑	↑
Hs.105806	205495_s_at	Granulysin	GNLY	−5.20	–, –	3.71	↑	↑	↑	↑	–	↑
Hs.40499	204602_at	Dickkopf homolog 1 (Xenopus laevis)	DKK1	−25.75	–, –	9.93	↑	↑	↑	↑	–	↑
Hs.522555	201525_at	Apolipoprotein D	APOD	−9.66	–, –	3.60	↑	↑	↑	↑	–	↑
Hs.527653	201926_s_at	CD55 molecule, decay accelerating factor for complement	CD55	−10.56	–, –	17.41	↑	–	↑	↑	↑	↑
Hs.654378	205992_s_at	Interleukin 15	IL15	−6.86	–, –	4.10	↑	↑	↑	–	↑	↑
Hs.1012	205654_at	Complement component 4 binding protein, alpha	C4BPA	−9.40	–, –	44.42	↑	–	↑	↑	–	↑
Hs.155597	205382_s_at	Complement factor D (adipsin)	CFD	−15.57	–, –	19.85	↑	–	↑	↑	–	↑
Hs.183109	212741_at	Monoamine oxidase A	MAOA	−18.16	–, –	14.30	↑	–	↑	↑	↑	–
Hs.186486	203836_s_at	Mitogen-activated protein kinase kinase kinase 5	MAP3K5	−4.67	–, –	5.23	↑	–	↑	↑	↑	–
Hs.384598	200986_at	Serpin peptidase inhibitor, clade G (C1 inhibitor), member 1	SERPING1	−4.37	–, –	7.07	–	–	↑	↑	↑	↑
Hs.386567	202748_at	Guanylate binding protein 2, interferon-inducible	GBP2	−8.58	–, –	8.44	–	↑	↑	↑	–	↑
Hs.524224	212067_s_at	Complement component 1, r subcomponent	C1R	−3.08	–, –	2.33	↑	–	↑	–	↑	↑
Hs.647036	201428_at	Claudin 4	CLDN4	−5.38	–, –	–	↑	↑	↑	↑	–	↑
Up-regulated transcripts												
Hs.522484	213131_at	Olfactomedin 1	OLFM1	3.71	–, –	−13.54	↓	↓	↓	↓	–	↓
Hs.2256	204259_at	Matrix metallopeptidase 7 (matrilysin, uterine)	MMP7	4.25	–, ↑	–	↓	↓	↓	↑	–	–
Hs.522891	209687_at	Chemokine (C–X–C motif) ligand 12 (stromal cell-derived factor 1)	CXCL12	5.82	↑, ↑	–	–	↓	↓	↑	–	↑

Data are expressed as fold change for endometrial genes regulated ≥2-fold in groups mifepristone vs placebo and groups LH+2 vs LH+7. Upward and downward arrows mean transcript reported as up- and down-regulated respectively. –, not reported as significantly changed.

Table 6 Transcription factor binding sites (TFBS) over represented in up- and down- regulated genes in the endometrium from the mifepristone group.

	Up-regulated genes			Down-regulated genes		
Tool for TFBS analysis	Matrix ID	Transcription factor	*P* value	Matrix ID	Transcription factor	*P* value
MotifScanner	**PAX4_04**	**Paired box 4**	0.0007	**E47_02**	**Transcription factor E47**	<0.0001
				SREBP1_01	Sterol regulatory binding protein 1	<0.0001
				PAX4_04	**Paired box 4**	<0.0001
				AREB6_03	ARE (Atp1a1 regulatory element) B6	0.0005
				TAL1BETAITF2_01	Tal-1β/ITF-2 heterodimer	0.0015
				TAL1ALPHAE47_01	Tal-1α/E47 heterodimer	0.0017
				MEF2_02 - 04	Myocyte-specific enhancer factor 2A	0.0024
Transcription Element Listening System	USF_02	Upstream stimulating factor	0.0004	**SP1_Q6**	**Stimulating protein 1**	<0.0001
	CAP_01	Cap signal for transcription initiation	0.0005	SP1_01	Stimulating protein 1	0.0001
	SP1_Q6	**Stimulating protein 1**	0.0028	**ELK1_02**	**Elk-1**	0.0006
	NRF2_01	**Nuclear respiratory factor 2**	0.0039	**NRF2_01**	**Nuclear respiratory factor 2**	0.0008
	E47_02	**Transcription factor E47**	0.0069	**MZF1_01**	**Myeloid zinc finger protein MZF1**	0.0014
	ELK1_02	**Elk-1**	0.0073	**GC_01**	**GC box elements**	0.0015
	MZF1_02	Myeloid zinc finger protein MZF1	0.0119	CETS1P54_02	c-Ets-1(p54)	0.0116
	YY1_01	Yin and Yang 1	0.0126	VMYB_01	v-Myb, viral myb	0.0216
	AP2_Q6	Activator protein 2	0.0151	CETS1P54_01	c-Ets-1(p54)	0.0224
	GC_01	**GC box elements**	0.0176	CDXA_01	Caudal-type homeodomain protein	0.0246
	MYCMAX_02	c-Myc/Max heterodimer	0.0204	VMYB_02	v-Myb, viral myb	0.0258
	PBX1_01	Homeo domain factor Pbx-1	0.0275	AP4_Q6	Activator protein 4	0.0260
	CAAT_C	Cellular and viral CCAAT box	0.0306	CHOP_01	Heterodimers of CHOP and C/EBPalpha	0.0295
	MZF1_01	**Myeloid zinc finger protein MZF1**	0.0325	EGR3_01	Early growth response gene 3 product	0.0358
	ER_Q6	Estrogen receptor-α	0.0402	OCT1_03	Octamer-binding factor 1	0.0365
	GATA2_01	GATA-binding factor 2	0.0484	OCT1_01	Octamer-binding factor 1	0.0396
				MEF2_01	Myogenic enhancer factor 2	0.0400
				HNF4_01	Hepatic nuclear factor 4, DR1 sites	0.0495
Gene Annotation Tool to Help Explain Relationships	**NRF2_01**	**Nuclear respiratory factor 2**	<0.0001	NFKAPPAB_01	NFκB	<0.0001
	GABP_B	GA repeat binding protein	<0.0001	NFKB_Q6	NFκB	0.0001
	KROX_Q6	Egr-1,2,3,4	0.0002	NRF2_01	nuclear respiratory factor 2	0.0001
	CETS168_Q6	c-Ets	0.0003	YY1_02	Yin and Yang 1	0.0002
	E2F1_Q3_01	E2F Transcription Factor 1	0.0008	MAZR_01	MAZ related factor	0.0003
	DEC_Q1	Dec transcription factor	0.001			
	NFY_Q6_01	Nuclear factor Y	0.001			

Transcription factors predicted by more than one analysis tool appear in bolded style.

from the endometrial tissues and to analyze how samples cluster together based on similarities in their transcript profiles. Both clustering methods using different algorithms generated equivalent patterns of segregation of samples suggesting that the endometrial samples from day LH+7 obtained from women with mifepristone have a gene expression profile that does not progress from LH+2, highlighting the P_4 signaling restrain.

When mifepristone is administered immediately after ovulation, it prevents the PGR and estrogen receptor down-regulation induced by P_4 which occurs during the luteal phase (Maentausta *et al*. 1993) suggesting a sustained endometrial stimulation by estrogen upon mifepristone administration. P_4 signaling inhibits the estrogen signaling pathways in the uterus (Hsueh *et al*. 1975) and this inhibitory relationship involving both hormone pathways orchestrate the regulatory mechanisms required for endometrial receptivity and embryo implantation. Amongst the regulated transcripts in the mid-secretory endometrium from women with mifepristone administration we found several genes involved in the P_4 signaling axis including modulators and effectors with down regulation of *NCOA2* (*SRC-2*, (Mukherjee *et al*. 2006, Jeong *et al*. 2007)), *IHH* (Matsumoto *et al*. 2002, Takamoto *et al*. 2002), *ERRFI1* (*MIG6*, (Kim *et al*. 2010)) *PTCH1* (Lee *et al*. 2006) and *DKK1* (Tulac *et al*. 2006); and up-regulation of *ESR1* (Curtis *et al*. 1999), *PRA* (Conneely & Lydon 2000), *FKBP5* (Tranguch *et al*. 2005), *FOXO1A* (Kim *et al*. 2005), *KLF9* (Simmen *et al*. 2004) and *PTGS1* (Wang *et al*. 2004).

With regard to the identification of key genes responsible for endometrial receptivity, Catalano *et al*. (2003) analyzed the endometrial gene expression profile

Table 7 Functional annotation clusters for up- and down-regulated transcripts in the endometrium from the mifepristone group, obtained through Gene Annotation Tool to Help Explain Relationships (GATHER) webtool.

Database	Functional annotation	Number of genes	*P* value
Up-regulated transcripts			
GO:0007067 [8]	**Mitosis**	**23**	**<0.0001**
GO:0000087 [7]	**M phase of mitotic cell cycle**	**23**	**<0.0001**
GO:0000278 [6]	**Mitotic cell cycle**	**27**	**<0.0001**
GO:0007155 [4]	**Cell adhesion**	**47**	**<0.0001**
GO:0000280 [7]	**Nuclear division**	**23**	**<0.0001**
GO:0000279 [6]	**M phase**	**23**	**<0.0001**
GO:0007275 [2]	Development	91	<0.0001
GO:0009653 [3]	Morphogenesis	62	<0.0001
GO:0007049 [5]	Cell cycle	43	<0.0001
GO:0008283 [4]	Cell proliferation	56	<0.0001
GO:0000910 [5]	Cytokinesis	14	<0.0001
GO:0016055 [6]	WNT receptor signaling pathway	12	<0.0001
Down-regulated transcripts			
GO:0006955 [4]	Immune response	66	<0.0001
GO:0009607 [4]	Response to biotic stimulus	76	0.0001
GO:0007186 [6]	G-protein coupled Receptor signaling pathway	22	0.0001
GO:0006952 [5]	Defense response	68	0.0002

Enriched functional annotations found in GATHER (Table 7) and Database for Annotation, Visualization and Integrated Discovery (Table 8) appear in bold.

under mifepristone stimulation using the endometrial tissue explant during the receptive phase. Explants were analyzed with a DNA microarray that investigated ~1000 genes involved in cell adhesion, signaling, apoptosis, cell cycle regulation, extracellular matrix remodeling and angiogenesis.

When mifepristone is administered during the mid-luteal phase of the menstrual cycle, it induces uterine bleeding due to endometrial breakdown within 72 h of administration in a similar way as P_4 withdrawal induces menstruation (Swahn *et al*. 1988). Catalano identified genes regulated by P_4 in human endometrium *in vivo* with the administration of 200 mg of mifepristone during mid-secretory phase. Under these circumstances, P_4 had already started to exert its effects in the endometrium and mifepristone induces menses within 48 h. Hence, the block of P_4 action during mid-secretory phase not only renders the endometrium unreceptive but also induces processes involved in menstruation generating a confounding effect that difficult the identification of transcripts involved in endometrial receptivity. When mifepristone is administered immediately after ovulation, the length of the menstrual cycle and plasmatic levels of estradiol and P_4 are not affected (Swahn *et al*. 1990), however induces a dramatic effect on endometrial development (Dockery *et al*. 1997, Danielsson *et al*. 2003). For these reasons we chose for the present study, administering mifepristone on the

Table 8 Functional annotation clusters for up- and down-regulated transcripts in the endometrium from the mifepristone group, obtained through Database for Annotation, Visualization and Integrated Discovery (DAVID) webtool.

Database	Functional annotation	Number of genes	*P* value
Up-regulated transcripts			
GOTERM_BP_FAT	**Cell adhesion**	**59**	**<0.0001**
GOTERM_BP_FAT	Biological adhesion	59	<0.0001
GOTERM_BP_FAT	**Mitosis**	**30**	**<0.0001**
GOTERM_BP_FAT	**Nuclear division**	**30**	**<0.0001**
GOTERM_BP_FAT	**M phase of mitotic cell cycle**	**30**	**<0.0001**
GOTERM_BP_FAT	Organelle fission	30	<0.0001
GOTERM_BP_FAT	**Mitotic cell cycle**	**38**	**<0.0001**
GOTERM_BP_FAT	Cell cycle phase	39	<0.0001
GOTERM_BP_FAT	**M phase**	**34**	**<0.0001**
GOTERM_BP_FAT	Cell cycle process	46	<0.0001
GOTERM_BP_FAT	**Cell cycle**	**55**	**<0.0001**
GOTERM_BP_FAT	Cell division	30	<0.0001
Down-regulated transcripts			
GOTERM_BP_FAT	Anti-apoptosis	28	<0.0001
GOTERM_BP_FAT	Negative regulation of programmed cell death	39	<0.0001
GOTERM_BP_FAT	Negative regulation of cell death	39	<0.0001
GOTERM_BP_FAT	Negative regulation of apoptosis	38	<0.0001
GOTERM_BP_FAT	Response to organic substance	61	<0.0001
GOTERM_BP_FAT	Regulation of cell proliferation	65	<0.0001
GOTERM_BP_FAT	Response to endogenous stimulus	39	<0.0001

Enriched functional annotations found in Gene Annotation Tool to Help Explain Relationships (Table 7) and DAVID (Table 8) appear in bold.

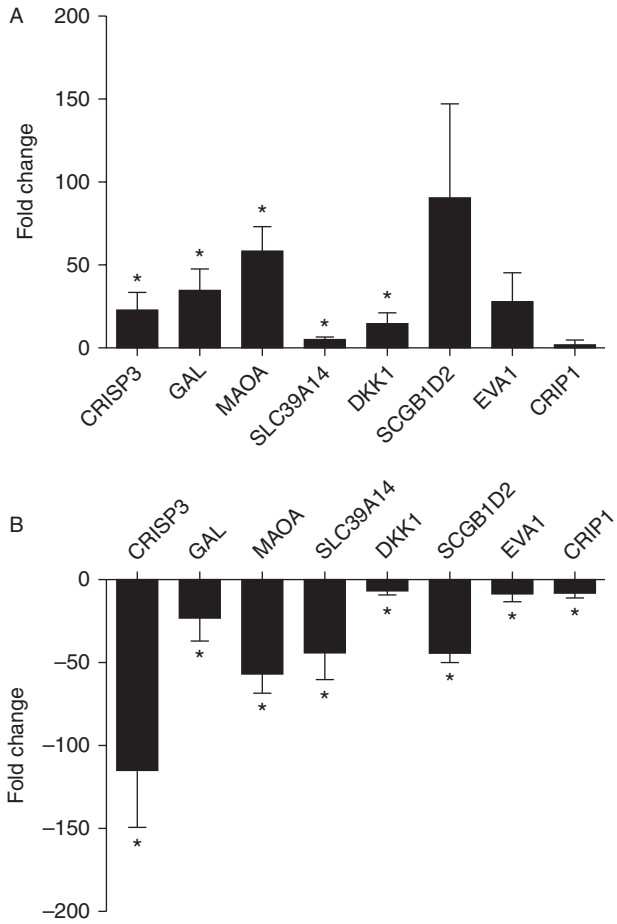

Figure 3 Validation of microarrays data by real-time RT-PCR. Real-time qPCR in endometrial samples was performed in pairwise comparisons of LH+2 vs LH+7 (A) and mifepristone vs placebo (B) after normalization to GAPDH. Each condition contains $n=7$ subject samples. Fold-change values were calculated from the $\Delta\Delta CT$ method for an endometrial gene expressed upon mifepristone vs placebo or LH+7 vs LH+2. Data are presented as mean ± S.E.M. above (A) or bellow (B) each gene and are plotted on the *y*-axis. *$P<0.05$, Wilcoxon two-sample paired signed rank test.

early secretory period (i.e. LH+2). Under these circumstances, P_4 action was antagonized from the beginning of the luteal phase and thus, avoiding the induction of early menses reflecting a lack of P_4 action in mid-secretory endometrium. After 5 days of mifepristone administration is difficult to speculate about the proportion of PGRs that remain blocked in the endometrium since to our knowledge there is no report regarding this matter so far. The studies performed with mifepristone administration during the early luteal phase showed an out-of-phase endometrial development in spite of high circulating P_4, suggesting a persistent block of the PGR (Swahn *et al.* 1990). The administration of mifepristone (200 mg) in LH+2 reaches a peak circulating level of ∼0.3–0.4 µmol/l within 2 h (Sarkar 2002) and is able to alter uterine gene expression in early pregnancy as early as 6 h after oral administration (Critchley *et al.* 1996). However, considering that plasmatic half-life of mifepristone is 30 h (Sarkar 2002) and its concentration (as well as its metabolites that retain anti-progestin properties) is maintained at the micromolar level for at least 72 h (Heikinheimo *et al.* 2003) with detectable levels in circulation for 6–7 days (Sitruk-Ware & Spitz 2003) we believe that most of the P_4 effects are still abrogated in the endometrium on LH+7. Thus, the endometrial phenotype observed 5 days after mifepristone administration results from the disruption of the events directly and indirectly driven by P_4 from the early secretory phase until the window of implantation.

Mifepristone also has affinity and antagonistic effects on the glucocorticoid receptor (Bertagna *et al.* 1984) present in human endometrial stroma (Bamberger *et al.* 2001, Sitruk-Ware & Spitz 2003). However for mifepristone to exert an anti-glucocorticoid effect, the dose that has to be administered is in the range of 5–20 mg/kg (Gaillard *et al.* 1984, Cadepond *et al.* 1997, Sitruk-Ware & Spitz 2003, Johanssen & Allolio 2007) which is a much higher dose than the used dose in the present study. Hence, we presume that the described changes on gene expression are mostly attributable to a blockade of P_4 action without ruling out that some of them are driven by mifepristone regulation of the glucocorticoid receptor.

Our microarrays analysis identified 1915 transcripts differentially expressed in the transition from the pre-receptive to receptive endometrium (i.e. from LH+2 to LH+7) in a spontaneous cycle. This number represents a 3.5% of the total number of analyzed transcripts. Other reports have using DNA microarrays technology have described a number of endometrial transcripts regulated during the receptive phase (highest P_4 levels) compared with earlier stages of the secretory phase (low P_4 levels) (Carson *et al.* 2002, Riesewijk *et al.* 2003, Mirkin *et al.* 2005, Talbi *et al.* 2006) or proliferative phase dominated by estrogen (Kao *et al.* 2002, Borthwick *et al.* 2003), suggesting a hormonal control of these genes. We contrasted our results with the reports afore mentioned. We found that the transcript for SPP1 was down-regulated upon mifepristone while it appears up-regulated in the six other studies we compared our results in our reference group (LH+2/LH+7). Other six transcripts are regulated with mifepristone and are also regulated in the opposite direction in other five reports as well as in our reference group. Given the rather small number of coincident endometrial transcripts across the different studies reported during the window of implantation (Tapia *et al.* 2011); such concordance of transcript regulation with mifepristone validates our results.

When P_4 action was blocked with mifepristone administration during the early luteal phase, the number of regulated transcripts during the receptive phase (2119, ∼3.9%) was similar to the one that change during the window of implantation. When we compared the identity of such transcripts, we found that only a sub group of 777 were regulated in both comparisons.

A rather small fraction of the differentially expressed genes upon mifepristone administration were shown to be associated with the acquisition of endometrial receptivity during a spontaneous cycle. To rule out the possibility that this proportion of overlapping transcripts was underestimated as a result of a significant amount of false–positive in each comparison, we performed the statistical analyses with a higher threshold (FDR 1%, FC $\geq$4) and found 594 and 470 regulated transcripts in the mifepristone and reference group (LH+2/LH+7) respectively. Only 130 transcripts (22%) were common in both the groups and all of them displayed opposite regulatory direction between groups, except for three down-regulated transcripts in the mifepristone group, supporting our main finding. Endometrial receptivity results from the expression and repression of genes controlled by ovarian steroidal hormones and other factors; however it is well established that P_4 is a critical determinant of endometrial function during the window of implantation (Rosario *et al*. 2003). Under this premise, it is surprising that only about a third of the genes that change its expression level at the moment of endometrial receptivity are also regulated under the action of mifepristone. A possible explanation to this is that not all the genes regulated during the window of implantation are necessarily involved in the acquisition of receptivity and it is possible that the gene regulation observed in a subgroup of transcripts is in response to other chemical messengers present during the secretory phase, independently of P_4. The endometrium is exposed to important levels of estradiol and relaxin of ovarian origin that could influence and control endometrial gene expression. The function of estradiol during the secretory phase in humans is not clear and its role on acquisition of receptivity is controversial. Using the oocyte donation model in women without ovarian function, Younis *et al*. (1994) showed that estrogen privation during the secretory phase does not affect the development of endometrial morphology. On the other hand, other study using estrogen receptor antagonism with clomiphene starting 2 days after the LH peak in spontaneous cycles and maintained until an endometrial biopsy was taken on day 13 showed a consistently delayed histological dating (Fritz *et al*. 1987). In addition, endometrial tissue explants incubated with P_4 or in combination with estradiol showed that the expression of a group of genes whose expression can be modulated only with P_4, is sensitive to its exposure to estradiol suggesting that endometrial expression of some genes regulated by P_4 are sensitive to the presence of estradiol (Dassen *et al*. 2007*b*).

Circulating relaxin (RLX) levels rise during the luteal phase (Bond *et al*. 2004, Hayes 2004) and its detection in the endometrium during natural cycles coincide with the period of endometrial receptivity (Yki-Jarvinen *et al*. 1985) suggesting a role in the early events of embryo implantation. Several studies show that RLX exerts a wide range of effects on the human endometrium mediating differentiation, vascularization and immunomodulation in this tissue (Goldsmith & Weiss 2009). Furthermore, RLX stimulates the secretion of several molecules in endometrial cells *in vitro* including prolactin (Telgmann & Gellersen 1998), Insulin-like binding protein-1 (IGFBP1, Bell *et al*. 1991), glycodelin (Tseng *et al*. 1999) and vascular endothelial growth factor (VEGF; Unemori *et al*. 1999, Palejwala *et al*. 2002). The results obtained in the present study cannot distinguish between genes that are regulated by P_4 and those whose transcript levels are regulated by the presence of estrogens, relaxin or other factors.

With regard to the functional clustering of regulated transcripts under mifepristone, the terms ‘cell adhesion’ and ‘proliferation’ were most consistent within the up-regulated transcripts. It has to be noted that the association of a particular function with a set of up- or down-regulated genes should not be interpreted as the function being regulated in the direction of the transcript level change. P_4 has been shown to completely inhibit estrogen-induced epithelial cell proliferation and DNA synthesis (Das & Martin 1973, Martin *et al*. 1973) which is in line with the functional clustering related with ‘proliferation.’ Moreover, a functional analysis of mifepristone responsive genes in Ishikawa cells identified a signaling pathway associated with adhesion and cell-to-cell interactions (Tamm-Rosenstein *et al*. 2013), which is in line with the term ‘cell adhesion’ we found. In relation to the over-represented TBFS analysis in the promoter regions of endometrial genes regulated by mifepristone, it is interesting that the cognate sequence for PGR was not over represented, suggesting that the transcriptional regulation exerted by the PGR is presumably mostly indirect.

In conclusion, in this study we determined changes in endometrial gene expression during the receptive period associated with a pharmacologic blockade of P_4 since LH+2. Approximately a 37% of the genes regulated with mifepristone appear to be also associated with endometrial receptivity considering those whose transcript level also changed during the window of implantation (i.e. from LH+2 to LH+7). Interestingly, out of the total of coincident transcripts with differential expression, almost all of them (96.8%) were found to be oppositely regulated, suggesting they are target of progesterone regulation and underlining their potential role in endowing the endometrium its receptive capacity. Additionally, more than 1000 transcripts whose endometrial levels change during period of receptivity see not to be regulated directly or indirectly by P_4 considering that mifepristone did not interfere with this change. Further analysis should establish the cellular phenotypes and temporal dynamics involved in transcripts regulation as well as the function in the uterus of each gene identified in this study to elucidate the mechanisms regulating endometrial receptivity.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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