Reproduction

Reproduction (2025) 169 e240372
https ://doi.org/10.1 530/REP-2 4-0372

Received 3 October 2024
Accepted 6 March 2025
Available online 6 March 2025
Version of Record published 19 March 2025

RESEARCH

Progesterone supplementation after postovulatory mifepristone reduce changes in human endometrial gene expression

AlejandroTapia-Pizarro [1] ,NicolásSantander [2] , Abril Salinas [1], Andrea Torres , [1]Denise Vega[1], Miguel del Rio[3] and Pilar Vigil[3]

[1]Institute of Mother and Child Research, Faculty of Medicine, Universidad de Chile, Santiago, Chile
[2]Instituto de Ciencias de la Salud, Universidad de O'Higgins, Rancagua, Chile
[3]Reproductive Health Research Institute, Santiago, Chile

Correspondence should be addressed to P Vigil: pilarvigil@rhri.cl

Abstract

In brief: Progesterone supplementation reverses 83% of transcript changes in the secretory endometrium induced by postovulatory mifepristone, potentially mitigating its antiprogestogenic effects.

Abstract: Mifepristone (RU486) antagonizes progesterone signaling in human endometrium interfering in the secretory phenotype after estradiol priming. The objective of the present study was to determine effect in the endometrial transcript profile of progesterone supplementation after the administration of 200 mg of the antiprogestin mifepristone 48 h after the LH peak (LH+2, LH+0 = LH peak). Endometrial samples were obtained on LH+7 after vaginal administration of micronized progesterone 200 mg/day for 3 days in nine women of proven fertility, each one contributing with one cycle treated with progesterone and another with a placebo. In addition, endometrial samples were obtained in LH+7 from a subgroup of four women with no administration of mifepristone, with each one contributing with one cycle treated with vaginal progesterone supplementation or placebo as a reference. RNA-seq was used to identify transcripts significantly regulated under the administration of progesterone vs placebo with or without postovulatory mifepristone. We observed that 713 transcripts changed significantly in the endometrium under mifepristone after progesterone supplementation in group A. Of these, progesterone reversed approximately 83% of the transcripts affected by mifepristone in the secretory endometrium. Bioinformatic analyses revealed that these transcripts were enriched in genes associated with mitochondrial function, particularly oxidative phosphorylation. In addition, NR2C2 and DLX1 were identified as potential transcription factors that may mediate the effects of progesterone in the endometrium. We conclude that progesterone supplementation after postovulatory mifepristone administration can reverse the antiprogestogenic effects for most of the affected endometrial transcripts.
Keywords: mifepristone; RU486; progesterone; secretory endometrium; transcript profile

Introduction

The human endometrium is regulated by the ovarian steroid hormones estradiol and progesterone (P4). One crucial role of progesterone is to transform an endometrium primed by estrogen to establish an adequate milieu for embryo implantation and pregnancy. In a non-conceptional cycle, there is a regression of the corpus luteum, leading to a reduction in P4 levels and the subsequent induction of endometrial

Published by Bioscientifica Ltd.
https://rep.bioscientifica.com

Reproduction (2025) 169 e240372
https ://doi.org/10.1 530/REP-2 4-0372

shedding with the menses. P4 primarily exerts its effects on target cells through the nuclear P4 receptor (PR), which belongs to the ligand-activated transcription factors superfamily (Misrahi et al. 1987, Tsai & O'Malley 1994). Upon binding with P4, PR activates its gene regulatory functions (Graham & Clarke 1997, Chabbert-Buffet et al. 2005) by interacting with the regulatory sequences of specific target genes, which influences their expression. There are two known PR isoforms, PR-A and PR-B, transcribed from the same gene that has two distinct promoters (O'Malley & Tsai 1992). In addition, P4 can trigger rapid signaling events independently of the transcriptional regulation or even without expression of nuclear receptors (Gellersen et al. 2009). These swift non-genomic signaling events, coupled with the comparatively slower genomic actions, determine the functional response to P4 in a cell type- and environment-specific manner. Although genomic responses are well understood, the mechanisms behind non-genomic P4 actions are yet to be fully elucidated (Gellersen et al. 2009).

Mifepristone (RU486) is a synthetic 19-nor-steroid and a progesterone receptor (PR) antagonist (Spitz 2003) that inhibits P4-mediated gene transcription. Although its molecular mechanism of action is not fully understood, studies suggest that mifepristone binding to PR does not disrupt receptor activation, dissociation of heat shock proteins, dimerization or binding to P4 response elements in DNA. This suggests that its antiprogestogenic activity is primarily driven by the interaction and recruitment of coregulators (Liu et al. 2002, Chauchereau et al. 2003, Dasgupta & O'Malley 2014, Szwarc et al. 2015). In addition, mifepristone may act as a P4 antagonist for certain non-genomic responses induced by P4 (Chien et al. 2009). Its effects on the endometrium depend on the dose and the timing of administration within the menstrual cycle (Gemzell-Danielsson et al. 1993). When a single 200 mg dose of mifepristone is administered 2 days after ovulation (i.e. 2 days after the luteinizing hormone peak, LH+2), it does not alter menstrual cycle length or serum estrogen and P4 levels. However, it profoundly affects endometrial morphology by delaying endometrial development and inhibiting glandular secretory activity (Swahn et al. 1990). In addition, our group found that the administration of mifepristone 200 mg on LH+2 has a profound effect on the endometrial transcript profile during the mid-luteal phase (LH+7) (Cuevas et al. 2016). This result, along with the data from clinical studies and the effects on endometrial morphology, provides robust evidence that the changes induced in the endometrium by postovulatory mifepristone are sufficient to prevent the secretory phenotype development. The aim of this study was to explore the effects of P4 supplementation following the postovulatory administration of mifepristone (200 mg) on the mid-luteal endometrial transcript profile. Specifically, our goal was to determine whether P4 could antagonize the effects of mifepristone and to identify the biological processes whose dysregulation by mifepristone could potentially be reversed by exogenous P4 administration. This approach aims to enhance our understanding of the plasticity of the endometrial response to transient P4 withdrawal.

Materials and methods

Subjects

Healthy women aged 18–43 were eligible to participate in the study if they had proven fertility, regular menstrual cycles (21–35 days) and had undergone surgical sterilization at least 1 year before enrollment for reasons unrelated to this study. Exclusion criteria included chronic medical conditions, abnormal screening blood test results, ovarian masses, symptomatic endometriosis, uterine leiomyomas, chronic medication use or the use of hormones or drugs that could alter steroid hormone metabolism within the previous 3 months. This study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the review board of the Servicio de Salud Metropolitano Central, Government of Chile (Acta No. 16/01 No. 95/2022). Written informed consent was obtained from each subject before participating. A total of nine women of Hispanic ethnicity were recruited for the study, with the nine participating in group A and four of them participating in group B. Functional and anthropometric and parameters of participating women are presented in Table 1.

Study design

This study consisted of two parts, both placebo-controlled trials. The first and main part included nine women (group A), four of whom (group B) also participated in the second part of our protocol, which was a complementary trial. Volunteers in group A received a postovulatory dose of mifepristone 200 mg. Each subject participated in one placebo-treated cycle (Mife+Pla) and one P4-treated cycle (Mife+P4) in a randomized order. A single endometrial sample was taken on day LH+7

Characteristicsofwomenparticipatinginthestudyand

Table1

parameters evaluated during the hormonal replacement cycle.

Data are presented as the mean ± SD.

	Group A* (n =9)	Group B (n =4)
Age (years)	34.7 ± 4.4	35 ± 4.7
Body mass index	26.9 ± 2.6	26.1 ± 1.5
Hemoglobin (g/dL)	13.5 ± 1.4	14.1 ± 0.45
Live births, n	2.8 ± 0.98	2.8 ± 0.96

*Subjects received mifepristone + progesterone or placebo. †Subjects received progesterone or placebo.

(LH+0 = day of LH peak) of each cycle, in which mifepristone was administered on LH+2. After the first cycle, women had a resting cycle with no intervention before the second cycle, swapping the intervention.

To control the timing of the interventions and assure normality of the cycle under study, each woman was instructed to use an in-home urinary LH kit to detect LH peak. The urinary LH surge was detected in first morning urine using a self-test (ClearBlue, Swiss Precision Diagnostics GmbH – SPD, Switzerland) at home, assessed daily starting from the mid-follicular phase (days 7–10). A single pill containing mifepristone 200 mg (APROFA, Chile) was administered 48 h after the LH peak (LH+2) during the experimental and control cycles. Treatment was given at the hospital under supervision of a midwife in all cases and the ovary and endometrial thickness were assessed by ultrasound with Doppler ultrasonography using a 7.5 MHz transvaginal ultrasound probe (Medison SA 6000, Korea) to confirm follicular rupture and the adequacy of endometrial proliferative response. Then, micronized progesterone (200 mg/day) or a placebo of similar appearance to the progesterone pill was self-administered vaginally for the following 3 days (LH+3 to LH+5). On day LH+7, the ovary and endometrial thickness were assessed by ultrasound with Doppler ultrasonography and then an endometrial sample was collected on LH+7 along with a blood sample to determine circulating P4 levels. The endometrial samples were collected under sterile conditions from the uterine fundus using Pipelle catheters (Laboratoire C.C.D., France). The second part of the study involved a subgroup of four volunteers (group B) who had previously participated in group A. Women in group B underwent the same follow-up for LH peak detection in urine as described above, with each volunteer contributing two non-consecutive cycles: one placebo-treated cycle (control) and one progesterone-treated cycle experimental). In a randomized order, No mifepristone was administered in these cycles; instead, a placebo pill resembling mifepristone was given 48 h after the LH peak (LH+2). Over the following three days (LH+3 to LH+5), each subject received either vaginal micronized progesterone (Pla+P4) at the same dose as in group A or a vaginal placebo of similar appearance to the progesterone pill (Pla+Pla). On day LH+7, the ovary and endometrial thickness were assessed by ultrasound with Doppler ultrasonography and then an endometrial sample was collected, as mentioned above, along with a blood sample to determine circulating P4 levels. After the first cycle, women had a resting cycle, with no which intervention before the second cycle, in intervention was swapped.

For both parts of the study (group A and group B), each endometrial sample was divided into two parts. One piece was incubated in RNAlater (Thermo Fisher Scientific, USA) overnight and then stored at −80°C for subsequent RNA isolation. The remaining piece was placed into 10% buffered formalin and processed for histological assessment by an independent pathologist under blind conditions, using the criteria described by Noyes et al. (1975). It was considered a delay in endometrial dating when the histological date of an endometrial biopsy was ≥3 days behind the actual day the sample was collected (LH+7).

The present study has been registered at the ClinicalTrials.gov database under the identifier NCT06616077.

Isolation of RNA

Total RNA was extracted from each tissue sample using the RNeasy kit (Qiagen, USA), following the manufacturer's instructions. RNA concentration was measured by absorbance at 260 nm (A260), while purity was assessed by calculating the A260/A280 ratio, which ranged between 1.8 and 2.1, using a Nanodrop spectrophotometer. RNA quality was further evaluated using Agilent's Lab-on-a-Chip Total RNA Nano Biosizing Assay (Agilent Technologies, Inc., USA).

RNA sequencing transcriptome analysis

Sequencing libraries were synthesized using the NEBNext Ultra II RNA Library Prep Kit (New England Biolabs, USA). Unstranded libraries were sequenced using a HiSeq4000 system (Illumina, USA) with 150 bp paired-end settings. After demultiplexing, fastq files were aligned to the human genome (GRCh38) with the Rsubread 2.10 and quantified using FeatureCounts. Differential expression was tested with DESeq2 1.36. DESeq2 fits a generalized linear model for each gene, assuming a negative binomial distribution, and performs a Wald test to evaluate statistical significance. Since we obtained two or more samples from each person, we built the model for group A gene patient + treatment1 and for group B patient + treatment1 + treatment2 + treatment1:treatment2, where 'gene' is the expression level as log2FoldChange of the gene, 'patient' is the person id, 'treatment1' refers to P4 or placebo and treatment2 refers to mifepristone or placebo. Next, we obtained each contrast of interest with the as contrast() command, considering an FDR <0.1 1.14 statistically significant. The EnhancedVolcano while package was used to draw volcano plots, Heatmaps were made with the pheatmap 1.0. package. using Overrepresentation PANTHER 17.0, and GSEA 4.2 was performed gene set enrichment analysis. RNASeq raw data of endometrial samples has been made publicly available at the Gene Expression Omnibus (GEO) database under accession GSE263868.

Pairedwise comparisons were performed for the RNA differentially expressed genes between progesterone and placebo groups under postovulatory mifepristone (group A) or placebo (group B). Given that not all women from group A sequencing analyses to identify the

Reproduction (2025) 169 e240372
https ://doi.org/10.1 530/REP-2 4-0372

proceeded to group B, two separate analyses were performed: the first one focused on the evaluation of DEGs between progesterone- and placebo-treated women after mifepristone. The second analysis focused on the comparison of DEGs between progesterone- and placebo-treated women without mifepristone.

Overlapping genes among differentially expressed transcripts in various comparisons were identified and visually represented using Venn diagrams.

Statistical analysis

A paired t-test was used to analyze endometrial thickness and circulating progesterone levels, while a chi-square test was applied to histological dating to assess statistically significant differences. Analyses were conducted separately for groups A and B, with a P-value <0.05 considered as statistically significant.

Results

Anthropometric and functional parameters of subjects

A total of 26 endometrial biopsies were obtained from nine women, recruited as explained in Fig. 1 and the flowchart in Fig. 2. Eighteen samples were obtained from mifepristone-treated cycles with P4 or placebo (group A, n = 9). Another eight samples were obtained from cycles without mifepristone with progesterone or placebo (group B, n = 4). Women in both groups (A and B) had at least one 'wash-out' cycle in between intervened

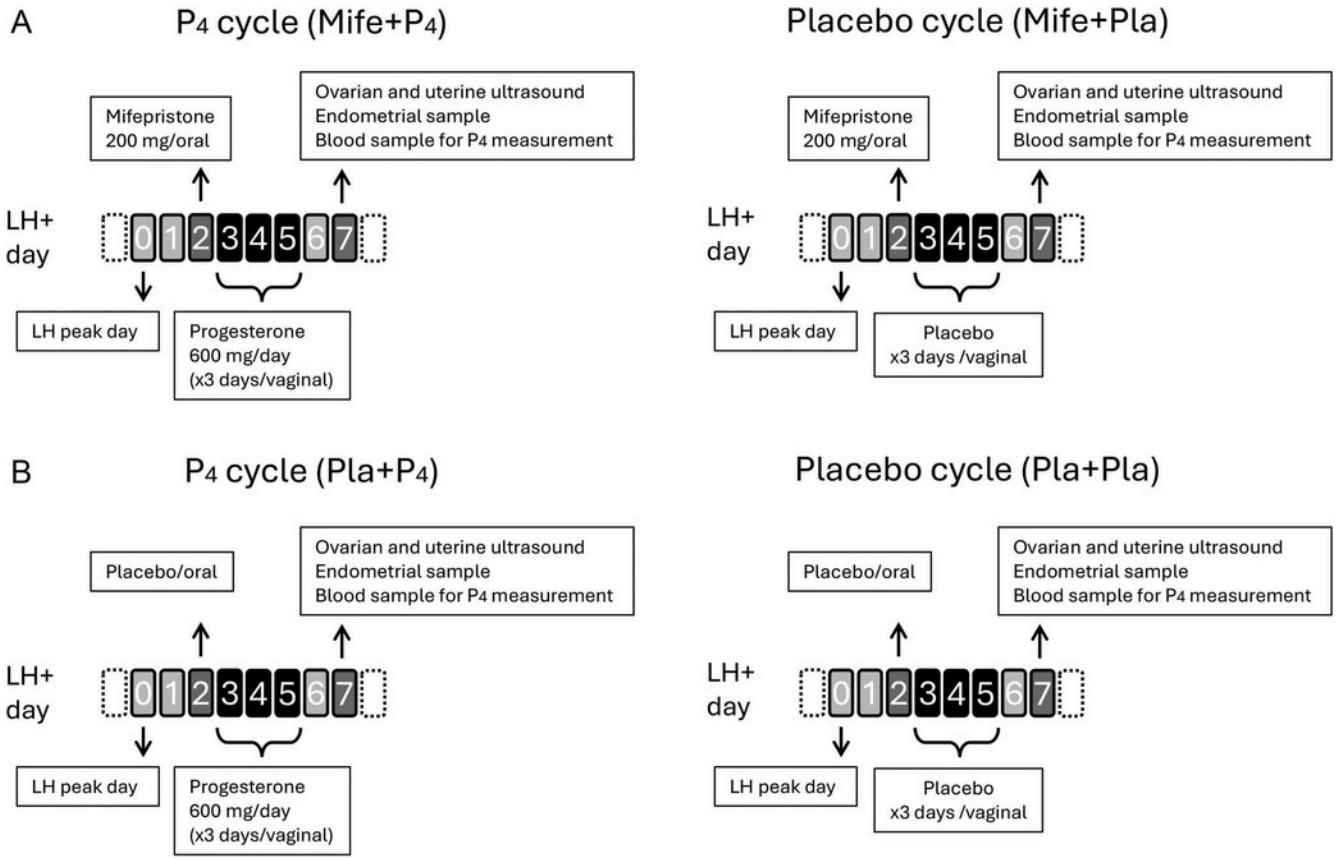

Figure 1

Schemes for the study protocols used for groups A and B. (A) Group A participated in two non-consecutive study cycles. In each cycle, participants received a single dose of mifepristone (200 mg) on LH+2, followed by either 600 mg/day of vaginal progesterone (MIFE+P4) or placebo (MIFE+Pla) for the next 3 days. (B) Group B was a subgroup of group A and underwent an additional two non-consecutive study cycles. In these cycles, participants received a placebo pill on LH+2 and then received either 600 mg/day of vaginal progesterone (Pla+P4) or placebo (Pla+Pla) between LH+3 and LH+5 (B). In all study cycles, LH peak was determined through urine analysis (LH peak = LH+0) and participants underwent vaginal ultrasonography on LH+7 to assess the ovaries and endometrium. On the same day (LH+7), a blood sample was collected to measure circulating progesterone levels and an endometrial sample was obtained for histological and transcriptomic analyses.

Reproduction (2025) 169 e240372
https ://doi.org/10.1 530/REP-2 4-0372

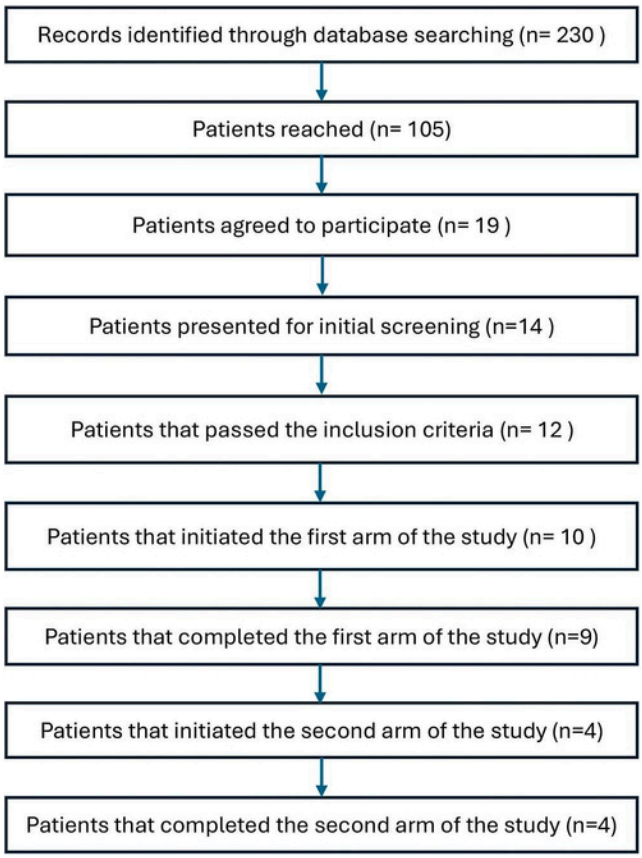

Figure 2
Participant flowchart of women who received postovulatory mifepristone 200 mg or placebo followed by progesterone or placebo. Ten women initially started the study cycles but one volunteer dropped out because of personal reasons unrelated to the study, rendering an n = 9 for group A.

cycles. The relevant anthropometric and functional parameters of subjects are presented in Table 1.

Circulating progesterone and ultrasound assessments

All women had a normal ovulatory cycle pattern, as assessed by a urinary LH surge and corpus luteum visualization by ultrasound on LH+2. In addition, plasma progesterone levels on LH+7 were >6 ng/mL except for one woman in the Mife+Pla group (3.26 ng/mL). Circulating P4 levels at the biopsy day (LH+7) along with the endometrial thickness documented by ultrasonography for all groups are presented in Table 2. No significant differences were obtained for circulating P4 levels and endometrial thickness for the comparisons in group A and group B.

Table 3 Endometrial dating.

Condition	Samples delayed*, n	Samples in phase, n
Mifepristone + placebo	7	2
Mifepristone + progesterone	5	4
Placebo + placebo	2	2
Placebo + progesterone	1	3

*Samples delayed 3 or more days.

Histological analysis

All endometrial biopsies performed on LH+7 had sufficient tissue for histologic dating. Women who received mifepristone had an endometrial maturation delay when compared with women that did not receive it. Twelve biopsy specimens had a maturation delay of >3 days in group A (with mifepristone, 66.6%) and three in group B (no mifepristone, 37.5%). The results of the endometrial dating evaluation for each group are summarized in Table 3. No significant differences were obtained for the histological dating in the comparisons within group A and group B.

Transcriptome analyses

This study primarily aimed to determine whether exogenous P4 could reverse the effects of mifepristone at the gene expression level and to identify biological processes disrupted by mifepristone that might be restored by P4 supplementation. Specifically, we sought to identify genes and gene sets whose mifepristone-induced alterations can be reversed by exogenous P4. We determined the transcriptomic profiles of endometrial tissue in biopsies from women in each group included in the study design. In group A, we sought to identify gene sets of interest, and then evaluated progesterone-mediated reversal effects in the endometrial tissue by comparing all treatments to the Pla+Pla group.

By comparing gene expression in Mife+P4 vs Mife+Pla, we observed a differential expression of 713 genes resulting from the administration of exogenous P4, with most of them showing over 1.5-fold change (Fig. 3A). Then, we analyzed the gene expression profile of these 713 genes in the endometrial samples obtained from women that participated in both groups A and B. The gene

Table 2 Circulating progesterone levels and endometrial thickness assessed by ultrasound. Data are presented as the mean ± SD.

	Group A		Group B	
	Mife + P_4	Mife + PLA	PLA + P_4	PLA + PLA
Plasma progesterone (ng/mL), LH+7	20.7 ± 12.4	16.1 ± 11.8	16.2 ± 8.7	15.9 ± 6.9
Endometrial thickness (mm) on LH+2	9.2 ± 2.2	8.2 ± 1.9	8.9 ± 1.9	7.5 ± 1.4
Endometrial thickness (mm) on LH+7	6.6 ± 1.8	7.2 ± 2.4	9.3 ± 2.4	8.0 ± 0.8

Mife, mifepristone; P4, exogenous progesterone; PLA, placebo.

Reproduction (2025) 169 e240372
https ://doi.org/10.1 530/REP-2 4-0372

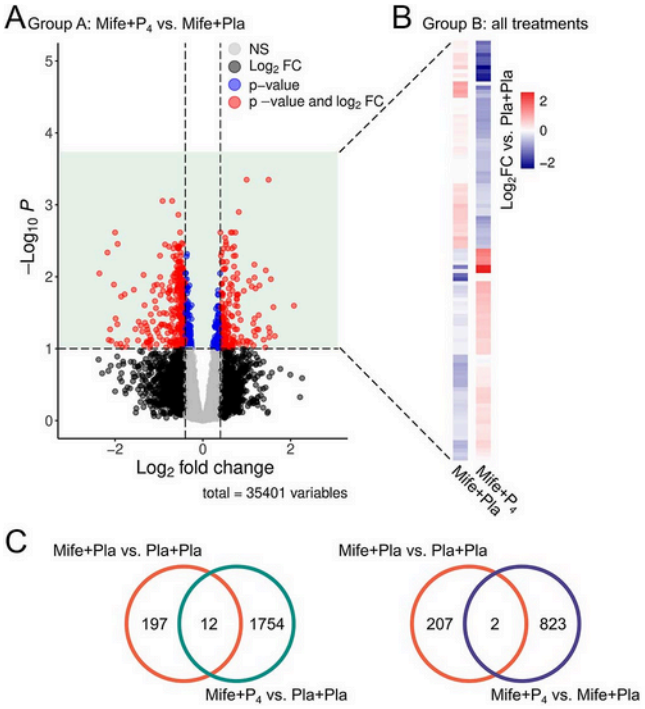

Figure 3

Endometrial transcriptomic changes after mifepristone are partially counteracted by progesterone. (A) Volcano plot showing changes in the expression levels for all transcripts detected in Mife+P4 versus Mife+Pla, in all samples from group A. Differentially expressed genes with >1.5-fold change are indicated in red. (B) Differentially expressed genes in (A) were selected and expression levels in samples of group B were analyzed, relative to the control condition (Pla+Pla). Shown are genes with inverse direction in Mife+Pla and Mife+P4, independently of statistical significance. (C) Number of differentially expressed genes before or after progesterone in the presence of mifepristone. There is only a small number of genes in the intersections. Statistical treatment of sequencing data is detailed in the Methods section. Mife, mifepristone; P4, progesterone; PLA, placebo.

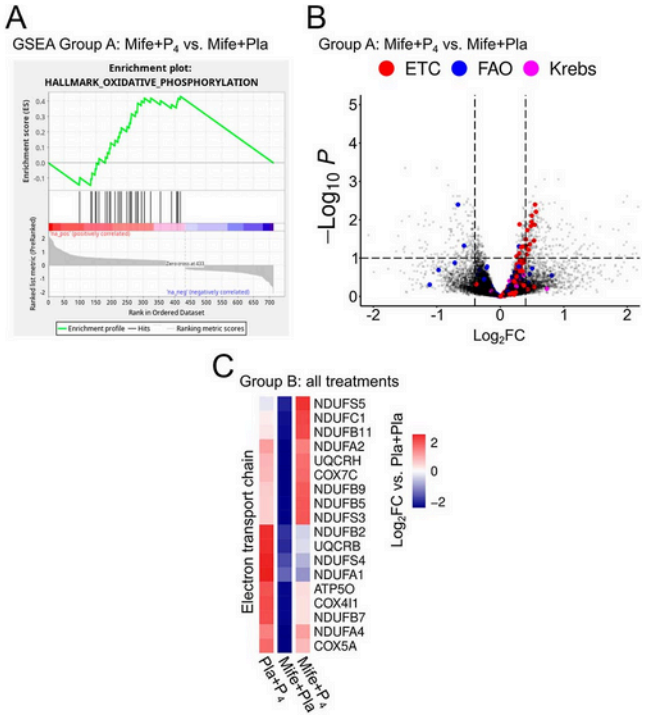

Figure 4

Progesterone stimulates the expression of genes involved in mitochondrial function. (A) Gene set enrichment analysis of data from Arm 1 using the MsigDb database. (B) Volcano plot showing all genes in gray, ETC genes in red, FAO genes in blue and Krebs cycle genes in magenta. (C) Heatmap of expression changes relative to Pla+Pla of genes involved in the ETC from group B.

expression profile of the endometrial samples obtained from women treated with Mife+Pla vs Pla+Pla and Mife+P4 vs Pla+Pla for the 713 transcripts is shown as a heatmap in Fig. 3B. The heatmap shows changes in the transcript profile of women receiving Mife+P4 and Mife+Pla, displaying genes with opposite changes in those cycles. Of the entire gene set (713 genes), 266 genes showed this opposite trend, whereas 123 seemed to maintain the same trend in both cycles. Compared with placebo, we observed reversing effects of P4 administration to the anti-progestogenic action of mifepristone in the endometrium in most of the analyzed genes, suggesting that the progesterone dose used in this study may overcome most of the changes in endometrial gene expression induced by mifepristone (Fig. 3B). Interestingly, the antagonization seems to be stronger in those genes downregulated by mifepristone. The overlapping genes from differentially expressed transcripts among the comparisons Mife+Pla vs Pla+Pla and Mife+P4 vs Pla+Pla along with Mife+Pla vs Pla+Pla and Mife+P4 vs Mife+Pla are graphically represented in Venn diagrams from Fig. 3C.

Next, we used the list of differentially expressed genes found in group A to identify enriched biological processes. We observed significant enrichment of gene ontology (GO) terms associated with mitochondrial respiration (e.g., electron transport chain (ETC)) and protein translation. As an orthogonal approach to determine changes in gene sets, we used GSEA comparing Mife+P4 vs Mife+Pla with the MSigDB (Fig. 4A). Consistently with the first approach, we detected enrichment of genes associated with 'oxidative phosphorylation' with the administration of P4 after mifepristone (Fig. 4A). Notably, almost every gene associated with the ETC and the Krebs cycle was upregulated in Mife+P4 compared to Mife+Pla supporting the concept that exogenous progesterone may overcome the effects of mifepristone in gene expression (Fig. 4B). By contrast, fatty acid oxidation (FAO), which is another mitochondrial oxidative process, did not show this expression pattern and genes were upregulated or downregulated with about equal probability in this category (Fig. 4B).

Reproduction (2025) 169 e240372
https ://doi.org/10.1 530/REP-2 4-0372

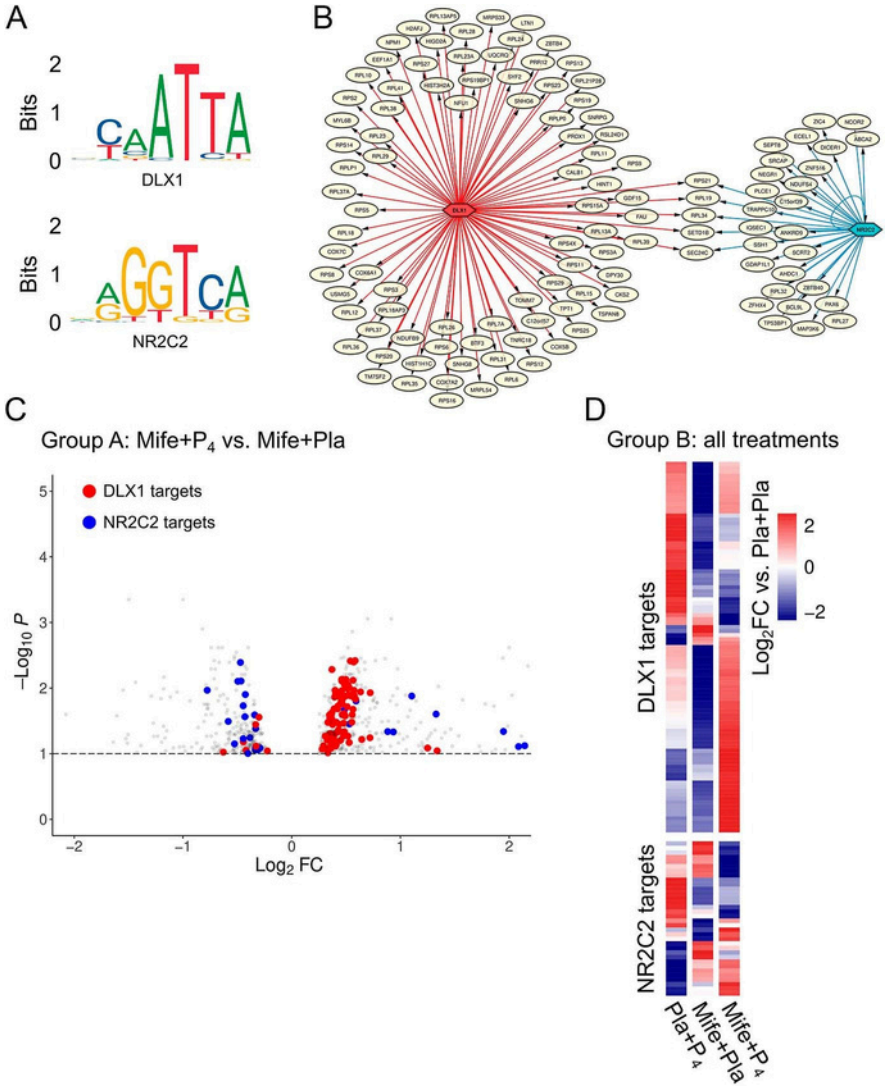

Figure 5

Putative upstream regulators of the endometrial response to progesterone. (A) Putative upstream regulators driving transcriptomic changes in samples from group A were determined with iRegulon. Logos for the hits DLX1 and NR2C2 were extracted from JASPAR 2024. (B) Genes downstream of DLX1 or NR2C2 and differentially expressed in group A are arranged in a regulatory network. (C) Volcano plot showing expression of DLX1 and NR2C2 targets in group A, showing that almost all DLX1 targets are upregulated after progesterone administration. (D) Expression changes in samples from group B are shown in the heatmap. Most changes show opposite directions when mifepristone is administered with or without progesterone.

To determine whether the mitochondrial effect associated with progesterone administration after mifepristone is also observed in cycles with progesterone supplementation without mifepristone, we analyzed the data from group B for this purpose. P4 supplementation with no previous mifepristone was associated with an overexpression of nearly all the genes involved in the ETC that were differentially expressed in group A (Fig. 4C). By contrast, Mife+Pla was associated with a strong reduction in the expression of all ETC genes. This effect was completely reversed in Mife+P4 group (Fig. 4C).

Using the data from group A, we analyzed putative upstream regulators responsible for the observed differential expression using iRegulon. We identified several potential regulators, of which only two binding sites (representing DLX1 and NR2C2 sites) were also differentially expressed in the dataset, suggesting they could be regulated by progesterone and contribute to the observed changes in the endometrium (Fig. 5A). These two transcription factors regulate a large part of the observed differentially expressed genes in a simple network with limited overlap between the two transcription factors (Fig. 5B). Notably, expression changes of the target genes in group A closely followed those of these two transcription factors. DLX1 was upregulated over two-fold and NR2C2 was downregulated around 0.8-fold. Accordingly, almost all DLX1 target genes were upregulated in Mife+P4 vs Mife+Pla, localizing to the right side of the volcano plot (Fig. 5C). In contrast, NR2C2 targets were mostly downregulated, but there were some exceptions (Fig. 5C). These data suggest that DLX1 could be an important downstream effector of progesterone in the endometrium. To strengthen this idea, we next tested reversal of the effect of mifepristone using group B data. Consistent with the concept of an effector

downstream of P4 in the endometrium, mifepristone was associated with the downregulation of most of DLX1 target genes (Fig. 5D). We observed reversal of the effect of mifepristone in Mife+P4 group for most target genes (Fig. 5D), which further supports DLX1 as a downstream effector of P4.

Discussion

In the present study, we leveraged RNASeq technologies to obtain insights into the endometrial response to exogenous progesterone after postovulatory mifepristone administration in the transcript profile of human endometrium during the mid-luteal phase.

In our protocol, P4 was administered through the vaginal route, which has shown to be highly effective in inducing the secretory transformation of the endometrium with minimal systemic side effects (Tavaniotou et al. 2000). We observed that the histological features in the endometrium were altered in most of the women receiving a single postovulatory dose of mifepristone (200 mg), as described before (Maentausta et al. 1993, Gemzell-Danielsson & Hamberg 1994, Cameron et al. 1997). The administration of P4 after mifepristone showed that the number of endometrial samples with histological dating discordant by ≥3 days relative to the actual day of the menstrual cycle when the sample was taken was reduced, suggesting that exogenous P4 may modulate the endometrial response promoting the secretory phenotype development. Mifepristone acts as an antagonist at the PR, inhibiting P4-mediated gene transcription (Spitz 2003). It has been shown that upon administration of a single dose of mifepristone (200 mg) 2 days after ovulation (LH+2), the menstrual cycle length and serum estrogen and progesterone levels remain unaffected; however, it significantly impacts the endometrial morphology, delaying secretory development and suppressing glandular activity (Gemzell-Danielsson et al. 1993, Hapangama et al. 2001). In addition, the endometrial transcriptomic profile at mid-secretory phase is profoundly modified, becoming similar to that of the proliferative phase (Cuevas et al. 2016). When we compared the endometrial transcriptomic profile between Mife+Pla and Pla+Pla, we found 713 genes differentially expressed, suggesting that exogenous P4 after mifepristone induces an important regulatory effect on the endometrial transcript profile. Since mifepristone antagonizes the PR, we determined the gene expression response of those 713 transcripts by including in the comparison the groups Pla+Pla and Pla+P4 to determine their differential regulation under these conditions. Out of the 713 genes differentially expressed from the comparison Mife+Pla and Mife+P4, we found that 266 transcripts exhibited an opposite regulatory response in their expression levels when we compared Mife+Pla vs Pla+Pla and Mife+P4 vs Pla+Pla. The fact that the reversal was stronger in downregulated transcripts by mifepristone suggests that the exogenous P4 may overcome inhibition of the PR. Interestingly, only two transcripts with differential expression were common out of the 209 from Mife+Pla and Pla+Pla and the 825 from Mife+Pla and Mife+P4, suggesting that most of the genes whose expression level was modified by mifepristone were not significatively changed once P4 was administered after mifepristone.

We observed that the analysis of enrichment of GO terms for the gene list, with differential expression in the endometrium upon administration of progesterone after mifepristone, was associated with mitochondrial respiration (e.g., ETC). The fact that FAO, which is another mitochondrial oxidative process, did not show a coordinated regulatory behavior in the comparison Mife+Pla and Mife+P4 suggests that mitochondrial functions specifically involved in ATP synthesis could be important for the effects of mifepristone on the endometrium. Endometrial decidualization is initiated in the mid-secretory phase driven by progesterone. In particular, the endometrial stromal cells (ESCs) decidualization is characterized by the differentiation from an elongated fibroblast-like shaped cell into rounded epithelial-like decidual cells with secretory features (Gellersen & Brosens 2014). This process involves multiple changes in cellular metabolism and energy regulation (Prigione & Adjaye 2010), in which the mitochondria and its energy metabolism play an important role (Prigione & Adjaye 2010, Fernandez-Marcos & Auwerx 2011, Zhang et al. 2020). In addition, a proteomic analysis of the decidua from patients with recurrent pregnancy loss showed a dysfunction related with mitochondrial oxidative stress, suggesting a major role of mitochondrial oxidative stress in endometrial function. The role of mitochondria in decidualization highlights the importance of P4 in regulating mitochondrial energy metabolism, dynamics and proper related regulatory mechanisms in the functioning and differentiation of ESCs.

The in-silico analysis of putative transcription factors that could be mediating the effects of P4 identified nuclear receptor subfamily 2 group C member 2 (NR2C2) and distal-less homeobox-1 (DLX1) as potential upstream regulators. NR2C2, also known as testicular nuclear receptor 4 (TR4), belongs to the nuclear receptor superfamily (Chang et al. 1994). Polyunsaturated fatty acid (PUFA) metabolites 13-hydroxyoctadecadienoic acid (13-HODE) and 15-hydroxyeicosatetraenoic acid (15-HETE), along with the thiazolidinedione (TZD) family of antidiabetic drugs, have shown to transactivate NR2C2. Interestingly, in cultured MCF-7 cells, a direct interaction between NR2C2 and the estrogen receptors (ERs) has been described, which inhibits ER homodimerization and its binding the the estrogen response elements (Shyu et al. 2012). The suppression of ER target genes,

induction of NR2C2 by P4 could explain the antiestrogenic effects of P4 observed in the endometrium.

DLX1 is part of the DLX gene family and is involved in embryo development and tumorigenesis (Lezot et al. 2010, Fujimoto et al. 2013). DLX1 interacts with the nucleosome remodeling and deacetylase (NuRD) complex, an epigenetic regulator that couple histone deacetylase and nucleosome remodeling activity, regulating gene expression (Price et al. 2022). In ovarian cancer, DLX1 was found to activate TGF-beta signaling promoting ovarian cancer aggressiveness (Chan et al. 2017).

Research on the interaction between NR2C2 or DLX1 and progesterone is limited, with no direct studies linking them specifically, so more research is required to confirm the relationship between P4 signaling with the transcription factors NR2C2 and DLX1 in the endometrium. The available literature on RNA-seq for measuring gene expression on a large scale suggests that validation of differentially expressed genes by qPCR and/or other approaches is not always required because this method is robust enough (Hughes 2009, Everaert et al. 2017). However, it is crucial to confirm that protein levels for these transcription factors are indeed regulated by exogenous P4 after mifepristone to understand the biological significance of the observed transcriptional changes.

Mifepristone also binds and antagonize the glucocorticoid receptor (Bertagna et al. 1984), which is strongly expressed in the stromal compartment of the endometrium (Bamberger et al. 2001, Sitruk-Ware & Spitz 2003). Hence, it is possible that some changes in the endometrial gene expression profile induced by mifepristone result from a regulation of the glucocorticoid receptor, which could not be fully reverted by P4 supplementation. In addition, the timing, dose and duration of P4 supplementation could also contribute to the effects of exogenous P4 on mifepristone in the endometrium. In the present study, a fixed dose of 600 mg/day was administered for 3 days, starting 24 h after mifepristone ingestion. Therefore, supplementation of P4 with a shorter delay after mifepristone, a higher dose or longer duration could potentially modify the extent of mifepristone antagonism.

P4 withdrawal from an estrogen–P4-primed endometrium leads to endometrial breakdown and shedding. In an ovariectomized macaque model, menses can be induced by discontinuing P4 administration at the end of an artificial menstrual cycle (late-luteal phase). However, if P4 is reintroduced within 36–40 h after withdrawal, menses is prevented (Brenner 2006). This suggests that the endometrium exhibits plasticity in response to transiently reduced P4 stimulation, lasting approximately 1–2 days. In our model, most genes regulated by mifepristone were reversed by exogenous P4 when administered 24 h later. This finding aligns with the concept of endometrial plasticity to a transient reduced P4 stimulation, as described in the macaque model. This information contributes to a better understanding of the hormonal regulation of the uterus in humans.

In conclusion we determined that the administration of exogenous P4 after postovulatory mifepristone could revert to a large extension, but not completely, the transcriptional effects of PR antagonization. These results should be taken with caution because of the limited sample size involved. Whether this reversion can restore the mid-secretory endometrial function remains to be elucidated.

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

Funding
This research was funded by ANID FONDECYT Iniciación 11240017 and the Reproductive Health Research Institute.

Author contribution statement
AT-P conceived the study, performed the experimental design, directed the investigation, analyzed the data and wrote the paper. NS collaborated with the experimental design, performed data curation and data analysis and assisted in reviewing and editing the paper. AS contributed with the experimental design, subject's assessments and analysis of ultrasonographic images. AT assisted in the methodology and subject's recruitment, management and follow-up. DV contributed with endometrial sample collections and ultrasonographic assessments. MdR assisted in funding acquisition and administrated the project. PV conceived the study, performed the experimental design, directed the investigation, analyzed the data, assisted with funding acquisition and collaborated in reviewing and editing the paper.

Acknowledge ments
We would like to thank Veronica Maturana for the blood collections and endometrial samples storage during non-working days, Dr Fernando Gabler for the histologic dating of endometrial samples and Dr Diego Fernández for his help in endometrial samples collection.

References

Bamberger AM, Milde-Langosch K, Loning T, et al. 2001 The glucocorticoid receptor is specifically expressed in the stromal compartment of the human endometrium. J Clin Endocrinol Metab 86 5071–5074. (https://doi.or g/10.1210/jcem.8 6.10.8101)

Bertagna X, Bertagna C, Luton JP, et al. 1984 The new steroid analog RU 486 inhibits glucocorticoid action in man. J Clin Endocrinol Metab 59 25–28. (https://doi.or g/10.1210/jcem- 59-1-25)

Brenner RM 2006 Basic and applied biology of the primate reproductive tract--a symposium in honor of the career of Dr Robert M Brenner: introduction and overview. Reprod Biol Endocrinol 4 (Supplement 1) S2. (https://doi.or g/10.1186/14 77-7827-4-s1- s2)

Cameron ST, Critchley HO, Buckley CH, et al. 1997 Effect of two antiprogestins (mifepristone and onapristone) on endometrial factors of potential importance for implantation. Fertil Steril 67 1046–1053. (https://doi.or g/10.1016/s001 5-0282(97)81 437-9)

Chabbert-Buffet N, Meduri G, Bouchard P, et al. 2005 Selective progesterone receptor modulators and progesterone antagonists: mechanisms of action and clinical applications. Hum Reprod Update 11 293–307. (https://doi.org/10.1093/humupd/dmi002)

Chan DW, Hui WW, Wang JJ, et al. 2017 DLX1 acts as a crucial target of FOXM1 to promote ovarian cancer aggressiveness by enhancing TGF-β/SMAD4 signaling. Oncogene 36 1404–1416. (https ://doi.org/10 .1038/onc.20 16.307)

Chang C, Da Silva SL, Ideta R, et al. 1994 Human and rat TR4 orphan receptors specify a subclass of the steroid receptor superfamily. Proc Natl Acad Sci U S A 91 6040–6044. (https://doi.org/10.1073/pnas.91.13.6040)

Chauchereau A, Amazit L, Quesne M, et al. 2003 Sumoylation of the progesterone receptor and of the steroid receptor coactivator SRC-1. J Biol Chem 278 12335–12343. (https://doi.org/10.1074/jbc.m207148200)

Chien CH, Lai JN, Liao CF, et al. 2009 Mifepristone acts as progesterone antagonist of non-genomic responses but inhibits phytohemagglutinin-induced proliferation in human T cells. Hum Reprod 24 1968–1975. (https ://doi.org/10 .1093/humrep/dep 099)

Cuevas CA, Tapia-Pizarro A, Salvatierra AM, et al. 2016 Effect of single post-ovulatory administration of mifepristone (RU486) on transcript profile during the receptive period in human endometrium. Reproduction 151 331–349. (https://doi.org/10.1530/rep-15-0458)

Dasgupta S & O'Malley BW 2014 Transcriptional coregulators: emerging roles of SRC family of coactivators in disease pathology. J Mol Endocrinol 53 R47–R59. (https://doi.org/10.1530/jme-14-0080)

Everaert C, Luypaert M, Maag JLV, et al. 2017 Benchmarking of RNA-sequencing analysis workflows using whole-transcriptome RT-qPCR expression data. Sci Rep 7 1559. (https ://doi.org/10 .1038/s41598- 017-01617-3)

Fernandez-Marcos PJ & Auwerx J 2011 Regulation of PGC-1alpha, a nodal regulator of mitochondrial biogenesis. Am J Clin Nutr 93 884S–890S. (https ://doi.org/10 .3945/ajcn.110.0 01917)

Fujimoto S, Oisi Y, Kuraku S, et al. 2013 Non-parsimonious evolution of hagfish Dlx genes. BMC Evol Biol 13 15. (https ://doi.org/10 .1186/1471-2148 -13-15)

Gellersen B & Brosens J 2003 Cyclic AMP and progesterone receptor cross-talk in human endometrium: a decidualizing affair. J Endocrinol 178 357–372. (https://doi.org/10.1677/joe.0.1780357)

Gellersen B, Fernandes MS & Brosens JJ 2009 Non-genomic progesterone actions in female reproduction. Hum Reprod Update 15 119–138. (https ://doi.org/10 .1093/humupd /dmn044)

Gemzell-Danielsson K & Hamberg M 1994 The effect of antiprogestin (RU 486) and prostaglandin biosynthesis inhibitor (naproxen) on uterine fluid prostaglandin F2 alpha concentrations. Hum Reprod 9 1626–1630. (https ://doi.org/10 .1093/oxfordj ournals.humrep .a13876 3)

Gemzell-Danielsson K, Swahn ML, Svalander P, et al. 1993 Early luteal phase treatment with mifepristone (RU 486) for fertility regulation. Hum Reprod 8 870–873. (https://doi.org/10.1093/oxfordjournals.humrep.a138157)

Graham JD & Clarke CL 1997 Physiological action of progesterone in target tissues. Endocr Rev 18 502–519. (https://doi.org/10.1210/edrv.18.4.0308)

Hapangama DK, Brown A, Glasier AF, et al. 2001 Feasibility of administering mifepristone as a once a month contraceptive pill. Hum Reprod 16 1145–1150. (https://doi.org/10.1093/humrep/16.6.1145)

Hughes TR 2009 Validation' in genome-scale research. J Biol 8 3. (https ://doi.org/10 .1186/jbiol104)

Lezot F, Thomas BL, Blin-Wakkach C, et al. 2010 Dlx homeobox gene family expression in osteoclasts. J Cell Physiol 223 779–787. (https://doi.org/10.1002/jcp. 22095)

Liu Z, Auboeuf D, Wong J, et al. 2002 Coactivator/corepressor ratios modulate PR-mediated transcription by the selective receptor modulator RU486. Proc Natl Acad Sci U S A 99 7940–7944. (https://doi.or g/10.1073/pnas.1 22225699)

Maentausta O, Svalander P, Danielsson KG, et al. 1993 The effects of an antiprogestin, mifepristone, and an antiestrogen, tamoxifen, on endometrial 17 beta-hydroxysteroid dehydrogenase and progestin and estrogen receptors during the luteal phase of the menstrual cycle: an immunohistochemical study. J Clin Endocrinol Metab 77 913–918. (https://doi.or g/10.1210/jcem.7 7.4.8408465)

Misrahi M, Atger M, d'Auriol L, et al. 1987 Complete amino acid sequence of the human progesterone receptor deduced from cloned cDNA. Biochem Biophys Res Commun 143 740–748. (https://doi.or g/10.1016/00 06-291x(87)91416 -1)

Noyes RW, Hertig AT & Rock J 1975 Dating the endometrial biopsy. Am J Obstet Gynecol 122 262–263. (https://doi.or g/10.1016/s000 2-9378(16)33 500-1)

O'Malley BW & Tsai MJ 1992 Molecular pathways of steroid receptor action. Biol Reprod 46 163–167. (https://doi.org/10.1095/biolreprod46.2.163)

Price JD, Lindtner S, Ypsilanti A, et al. 2022 DLX1 and the NuRD complex cooperate in enhancer decommissioning and transcriptional repression. Development 149 dev199508. (https://doi.org/10.1242/dev.199508)

Prigione A & Adjaye J 2010 Modulation of mitochondrial biogenesis and bioenergetic metabolism upon in vitro and in vivo differentiation of human ES and iPS cells. Int J Dev Biol 54 1729–1741. (https://doi.or g/10.1387/ij db.103198ap)

Shyr CR, Hu YC, Kim E, et al. 2002 Modulation of estrogen receptor-mediated transactivation by orphan receptor TR4 in MCF-7 cells. J Biol Chem 277 14622–14628. (https://doi.org/10.1074/jbc.m110051200)

Sitruk-Ware R & Spitz IM 2003 Pharmacological properties of mifepristone: toxicology and safety in animal and human studies. Contraception 68 409–420. (https://doi.org/10.1016/s0010-7824(03)00171-9)

Spitz IM 2003 Progesterone antagonists and progesterone receptor modulators: an overview. Steroids 68 981–993. (https://doi.or g/10.1016/j.s teroids.2003 .08.007)

Swahn ML, Bygdeman M, Cekan S, et al. 1990 The effect of RU 486 administered during the early luteal phase on bleeding pattern, hormonal parameters and endometrium. Hum Reprod 5 402–408. (https://doi.or g/10.1093/ox fordjournals.hu mrep.a1 37111)

Szwarc MM, Lydon JP & O'Malley BW 2015 Steroid receptor coactivators as therapeutic targets in the female reproductive system. J Steroid Biochem Mol Biol 154 32–38. (https://doi.org/10.1016/j.jsbmb.2015.06.010)

Tavaniotou A, Smitz J, Bourgain C, et al. 2000 Comparison between different routes of progesterone administration as luteal phase support in infertility treatments. Hum Reprod Update 6 139–148. (https://doi.or g/10.1093/hum upd/6.2.139)

Tsai MJ & O'Malley BW 1994 Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 63 451–486. (https://doi.org/10.1146/annurev.biochem.63.1.451)

Zhang L, Zhang Y, Chang X, et al. 2020 Imbalance in mitochondrial dynamics induced by low PGC-1α expression contributes to hepatocyte EMT and liver fibrosis. Cell Death Dis 11 226. (https://doi.org/10.1038/s41419-020-2429-9)