The impact of luteal phase support on gene expression of extracellular matrix protein and adhesion molecules in the human endometrium during the window of implantation following controlled ovarian stimulation with a GnRH antagonist protocol

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Objective: To evaluate the impact of two different luteal phase support protocols on gene expression of extracellular matrix (ECM) proteins and adhesion molecules in the human endometrium.
Design: Eighty-four ECM protein and adhesion molecule genes were analyzed using array-based reverse-transcription polymerase chain reaction.
Setting: Academic hospital.
Patient(s): Nine oocyte donors.
Intervention(s): Endometrial biopsies were obtained on the day of oocyte retrieval (group I) and 3–5 days later (group II) after randomization into 3 groups. Group IIa had no luteal phase support, group IIb had luteal support with micronized progesterone, and group IIc had luteal support with progesterone plus 17β-estradiol.
Main Outcome Measure(s): Gene expression profiles in relation to different types of luteal phase support protocols.
Result(s): Compared with the day of retrieval (group I), 24 genes were significantly up-regulated (4 in group IIa, 14 in group IIb, 22 in group IIc) and 14 genes were down-regulated (5 in group IIa, 2 in group IIb, 10 in group IIc) on day 3–5 ($P<.05$). In the luteal support group, up- regulation occurred predominantly in genes encoding for matrix metallopeptidases (MMP10, MMP3, MMP9), integrins (ITGA3, ITGA5, ITGB3, ITGB4), and laminin (LAMB3). In contrast, the most significant suppression was documented in genes encoding for catenin-D2, collagen-11A1, and the laminins (LAMA1 and LAMA3). Significant changes between groups IIb and IIc were also observed in 9 genes.
Conclusion(s): Luteal phase support following controlled ovarian stimulation has a profound impact on the ECM pathway targeted genes. (Fertil Steril® 2010;94:2264–71. ©2010 by American Society for Reproductive Medicine.)

Key Words: Ovarian stimulation, luteal phase, microarray, extracellular matrix, adhesion molecules, implantation

The spatial and temporal expression of specific extracellular matrix (ECM) proteins and adhesion molecule genes creates a profile that is crucial for successful embryo implantation (1, 2).

Ovarian stimulation protocols for IVF have been invariably associated with luteal phase deficiency and poor implantation rates (3, 4). Although the exact reasons for this phenomenon are still unclear, luteal phase support, with medication aimed to improve endometrial characteristics and facilitate the implantation process, has been a standard practice. Progesterone (P) is universally accepted as a sine qua non agent for luteal phase support and can be administered orally, intramuscularly, or vaginally (5, 6). Estrogens in the form of 17β-E_2 or E_2 valerate also have been used for luteal phase support with the concept that there is also a lack of estrogens during the luteal phase after oocyte retrieval (7). So far, studies aimed to evaluate the concept of estrogen addition during the luteal phase have led to inconclusive results (3, 8). Reports on the effect of luteal phase support in GnRH antagonist protocols are limited, and there is no agreement regarding the optimal supplementation scheme during the luteal phase (9, 10).

In the present study, we investigated the effects of two commonly used luteal phase support protocols (P alone and P plus estrogen), on the gene expression profiles for ECM and adhesion molecules in the human endometrium following ovarian stimulation with a gonadotropin/GnRH antagonist protocol.

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MATERIALS AND METHODS

Oocyte Donors

All donors were 21–31 years of age and underwent a standard screening protocol for oocyte donation, in accordance with the recommendations of the American Society for Reproductive Medicine (11). A total of nine healthy oocyte donors completed this study. The study was approved by the Johns Hopkins Hospital Institutional Review Board.

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Study subjects underwent ovarian stimulation according to a gonadotropin/GnRH antagonist protocol as decribed previously (12).

Luteal Phase Support and Tissue Collection

On the day of oocyte retrieval, the study participants were randomized into three treatment categories: Category A did not receive any luteal phase support, category B received micronized P in the form of vaginal suppositories (200 mg every 6 h starting from the day after retrieval), and category C received a daily oral dose of 2 mg 17β-E_2 in addition to the micronized P. Up to the that point, all patients had been stimulated according to the same protocol and they had received approximately the same amount of medication. Therefore, performing an endometrial biopsy on the day of oocyte retrieval was thought be the most appropriate time to define a baseline status for the endometrium.

Endometrial biopsies were performed using a Pipelle catheter (Unimar, Wilton, CT) on the day of retrieval (day 14 of the ideal cycle) and then 3–5 days after retrieval, corresponding to ideal cycle day 17–19. For the convenience of comparisons, biopsy specimens were divided into four groups: Group I included biopsies obtained on the day of retrieval, which served as baseline; group IIa included biopsies obtained 3–5 days after retrieval in donors that received no luteal phase support; group IIb included biopsies obtained 3–5 days after retrieval from the donors that had luteal phase support with micronized P only; and group IIc included biopsies obtained from the group that received both E_2 and P (P+E). Immediately after the biopsy all specimens were stored in liquid nitrogen tanks at $-196^{\circ}C$.

Purification and Preparation of RNA

Total RNA was isolated from individual endometrial samples using Trizol reagent (Invitrogen, Carlsbad, CA) and chloroform following the manufacturer's protocol. The integrity of all total mRNA samples was examined by visual inspection of intact 28S and 18S ribosomal RNA bands on agarose gels before further processing. The quality of total RNA samples was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA).

Superarray reverse-transcription polymerase chain reaction (QRT-PCR) Analysis

Reverse transcription was performed on total RNA isolated from human endometrial biopsies using SABiosciences's RT^2 First Strand Kit (cat. no. C-03; Gaithersburg, MD) to generate single-stranded complementary DNA (cDNA) according to the manufacturer's protocol. QRT-PCR was performed using the RT^2 Profiler PCR Array from SABioscience. RT^2 Profiler PCR Arrays are designed for relative quantitative QRT-PCR based on Sybr Green detection and are performed on a one sample/one 96-well plate format using primers for a preset list of genes (84 ECM and adhesion molecule genes and 12 control genes) corresponding to a particular biologic pathway. Five of the control genes are beta-2-microglobulin (B2M), hypoxanthine phosphoribosyltransferase (HGPRT/HPRT), ribosomal protein L13a (RPL13A), glyceraldehyde-3-phosphate dehydrogenase (G3PD/GAPD), and beta-actin (PS1TP5BP1). Two of these genes (GAPDH and RPL13A) were chosen for use as normalizing genes, based on having the least variance among all samples (SD 0.71–0.81 Ct). The specific array was the human ECM PCR Array (PAHS-013). In brief, cDNA volumes were adjusted to ~2.5 mL with Superarray RT^2 Real-Time Sybr Green/ROX PCR 2× Master Mix (PA-012). A total of 25 μL cDNA mix was added to all wells. The PCR plate was sealed and spun at 1500 rpm for 4 minutes. Real-time PCR was performed on an Applied Biosystems (Foster City, CA) 7300 Real-Time PCR System. Instrument settings included setting reporter dye as "SYBR," passive reference "ROX," delete UNG Activation, and add Dissociation Stage.

Confirmation of Microarray Data by Real-Time PCR

To validate data derived from microarray analysis, real-time PCR studies were carried out using total RNA isolated from tissue and processed with Applied Biosystems High-Capacity cDNA Archive kit first-strand synthesis system for real-time PCR according to the manufacturer's protocol. QRT-PCR was performed using the TaqMan assay system from Applied Biosystems. All PCR amplifications were carried out in duplicate on an Applied Biosystems ABI Prism 7300 Sequence Detection System, using a fluorogenic 5′-nuclease assay (TaqMan probes).

Statistical Analysis

Relative gene expressions were calculated by using the $2^{-\Delta\Delta Ct}$ method, in which Ct indicates cycle threshold, the fractional cycle number where the fluorescent signal reaches detection threshold (13). The normalized ΔCt value of each sample was calculated using three endogenous control genes (RPL13A, GAPDH, and ACTB). Fold change values are presented as average fold change $= 2^{-(\text{average } \Delta\Delta Ct)}$ for genes in treated relative to control samples. For comparison between groups, a paired t test was used. A P value of $<.05$ (two sided) was considered to be significant.

RESULTS

Gene Expression Profiles of Endometrial ECM Protein and Adhesion Molecules

Total RNAs from 12 endometrial samples were extracted and subjected to Superarray focused on the ECM protein and adhesion molecule pathway. Triplicates of each group's samples were used. The expression profiles for 84 genes were analyzed. The overall fold changes for individual genes between the comparison groups are shown in Table 1. Initially, transcript expression at baseline (day of retrieval) was compared with transcript expression on day 3–5 in the three treatment groups (see Table 1, day 3–5 vs. day 0). Next, biopsies obtained on day 3–5 in the three treatment groups were compared to each other (see Table 1, day 3–5 vs. day 3–5). Transcript expression changes ranged from a 42-fold down-regulation (for the CTNND2 in the no luteal support group) to 152-fold up-regulation (for the MMP10 in the P+E support group) compared with baseline.

Expression of ECM Protein and Adhesion Molecules According to Different Luteal Phase Support Groups

Gene expressions in the endometrial samples obtained on the day of retrieval were compared with those obtained 3–5 days later. A twofold change in gene expression was arbitrarily selected as a cut-off level. In the group with no luteal phase support, 18 genes were up-regulated and 17 down-regulated; in the P-alone group, 25 genes were up-regulated and 7 down-regulated; and in the P+E support group, 33 genes were up-regulated and 14 down-regulated (Fig. 1A).

Then we investigated gene expressions between different treatment groups during the putative window of receptivity (3–5 days after retrieval). Increased levels of expression (more than twofold) was observed in the P-alone group for 15 genes and in the P+E support group for 34 genes compared with the no supplementation group (Fig. 1B). In the P+E group, 18 genes were up-regulated and 9 down-regulated more than twofold compared with the P-alone group.

Genes with more than twofold changes in common for all three groups are shown in Figure 2. Panel A shows changes in gene expression between day 3–5 and day of retrieval. Among the three comparison groups, 19 genes were shared by all three groups and 21–27 genes were shared between two groups. Panel B compares groups at day 3–5. Four genes were shared among three groups and 6–20 genes were shared between two groups.

Significant changes in gene expression ($P<.05$) and their fold changes are indicated in Table 1. MMP10 was the gene up-regulated the most during the implantation window (3–5 days after retrieval) with 38-, 61-, and 152-fold increase in the no luteal support, P support, and P+E support groups, respectively. Other ECM protein

TABLE 1

Endometrial extracellular matrix and adhesion molecule gene profiles and fold changes between six comparison groups.

		(A) Day 3–5 vs. day 0			(B) Day 3–5 vs. day 3–5		
Gene symbol	Description	IIa vs. I (no vs. no)	IIb vs. I (P vs. no)	IIc vs. I (P+E vs. no)	IIb vs. IIa (P vs. no)	IIc vs. IIa (P+E vs. no)	IIc vs. IIb (P+E vs. P)
ADAMTS1	ADAM metallopeptidase with thrombospondin type 1 motif, 1	+2.80	+3.40[a]	+4.55[a]	+1.21	+1.63	+1.34
ADAMTS13	ADAM metallopeptidase with thrombospondin type 1 motif, 13	−1.72	+1.18	−1.60	+2.03	+1.08	−1.88
ADAMTS8	ADAM metallopeptidase with thrombospondin type 1 motif, 8	+1.07	+1.99	−2.11	+1.86	−2.26	−4.21[b]
CD44	CD44 molecule (Indian blood group)	−1.46	−1.09	+1.39[a]	+1.33	+2.02	+1.52
CDH1	Cadherin 1, type 1, E-cadherin (epithelial)	+3.48[a]	+3.08	+4.28[a]	−1.13	+1.23	+1.39
CLEC3B	C-type lectin domain family 3, member B	+3.99	+5.84[a]	+4.59[a]	+1.46	+1.15	−1.27
CNTN1	Contactin 1	−2.18	−1.47	−2.04	+1.49	+1.07	−1.39
COL11A1	Collagen, type XI, alpha 1	−3.15	−4.70	−5.24[a]	−1.49	−1.66	−1.11
COL12A1	Collagen, type XII, alpha 1	−5.90[a]	−2.26	−1.06	+2.61	+5.57[a]	+2.13
COL14A1	Collagen, type XIV, alpha 1	−2.27	−1.57	−2.17[a]	+1.44	+1.05	−1.38[a]
COL15A1	Collagen, type XV, alpha 1	+1.27	+2.26[a]	+2.03[a]	+1.78	+1.60	−1.11
COL16A1	Collagen, type XVI, alpha 1	−1.75	−1.52	−3.28[a]	+1.15	−1.87	−2.16
COL1A1	Collagen, type I, alpha 1	−1.84	+1.06	−1.48	+1.96	+1.24	−1.58
COL4A2	Collagen, type IV, alpha 2	−2.02	−1.14	−1.01	+1.77	+1.99	+1.13
COL5A1	Collagen, type V, alpha 1	−1.57	+1.11	+1.17	+1.75	+1.84	+1.05
COL6A1	Collagen, type VI, alpha 1	+1.02	+1.57	+1.50	+1.54	+1.47	−1.05
COL6A2	Collagen, type VI, alpha 2	−1.12	+1.84	+1.73	+2.06	+1.94	−1.06
COL7A1	Collagen, type VII, alpha 1	−2.23	−1.46	−1.33	+1.53	+1.68	+1.10
COL8A1	Collagen, type VIII, alpha 1	+2.40	+3.98	+6.29	+1.66	+2.62	+1.58
CTGF	Connective tissue growth factor	−1.89	−1.03	+1.19	+1.84	+2.26	+1.23
CTNNA1	Catenin (cadherin-associated protein), alpha 1, 102 kDa	+1.43	+1.43	+1.35	−1.00	−1.06	−1.06
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88 kDa	+1.04	+1.20	−1.21	+1.16	−1.25	−1.46
CTNND1	Catenin (cadherin-associated protein), delta 1	−1.12	+1.16	−1.09	+1.29	+1.03	−1.26
CTNND2	Catenin (cadherin-associated protein), delta 2	−42.71[a]	−10.20	−29.33[b]	+4.19	+1.46	−2.88
ECM1	Extracellular matrix protein 1	−2.32	−1.58	−3.30[a]	+1.47	−1.42	−2.09[b]
FN1	Fibronectin 1	−2.71	−1.71	−2.21[a]	+1.59	+1.23	−1.30
HAS1	Hyaluronan synthase 1	+2.90	+1.15	−1.53	−2.52	−4.44[b]	−1.76
ICAM1	Intercellular adhesion molecule 1 (CD54)	−1.01	+1.60	+3.59[a]	+1.62	+3.62[a]	+2.24
ITGA1	Integrin, alpha 1	−2.32	−1.34	−1.94	+1.74	+1.20	−1.45
ITGA2	Integrin, alpha 2	+1.31	+1.14	+2.72	−1.15	+2.07	+2.37
ITGA3	Integrin, alpha 3	+2.50	+2.75[a]	+7.12[a]	+1.10	+2.84	+2.59
ITGA4	Integrin, alpha 4	+1.31	+2.15	−1.21	+1.64	−1.59	−2.60
ITGA5	Integrin, alpha 5	+1.16	+1.67[a]	+2.25[a]	+1.43	+1.93	+1.35
ITGA6	Integrin, alpha 6	+1.13	+1.89	+1.04	+1.67	−1.09	−1.82[a]
ITGA7	Integrin, alpha 7	−1.94	+1.32	+1.09	+2.56	+2.11	−1.21
ITGA8	Integrin, alpha 8	−3.55	−1.71	−2.32[b]	+2.07	+1.53	−1.35
ITGAL	Integrin, alpha L	−1.04	+1.58	+2.05	+1.65	+2.14	+1.30
ITGAM	Integrin, alpha M	+1.29	+1.33	+3.13	+1.03	+2.42	+2.34
ITGAV	Integrin, alpha V	−1.60	−1.30	−1.63	+1.23	−1.02	−1.25
ITGB1	Integrin, beta 1	−1.41	+1.15	−1.10	+1.63	+1.28	−1.27

Zhao. Luteal support and endometrial ECM gene profile. Fertil Steril 2010.

TABLE 1

Continued.

Gene symbol	Description	(A) Day 3–5 vs. day 0			(B) Day 3–5 vs. day 3–5		
		IIa vs. I (no vs. no)	IIb vs. I (P vs. no)	IIc vs. I (P+E vs. no)	IIb vs. IIa (P vs. no)	IIc vs. IIa (P+E vs. no)	IIc vs. IIb (P+E vs. P)
ITGB2	Integrin, beta 2	+1.30	+1.93	+3.06[a]	+1.48	+2.35[a]	+1.59
ITGB3	Integrin, beta 3	+2.50	+1.84	+5.48[a]	−1.35	+2.19	+2.97
ITGB4	Integrin, beta 4	+1.58	+6.24[b]	+2.88[a]	+3.96	+1.82	−2.17[b]
ITGB5	Integrin, beta 5	−1.35	−1.29	+1.33	+1.04	+1.80	+1.72[a]
KAL1	Kallmann syndrome 1 sequence	−1.08	+2.02	+1.04	+2.18	+1.12	−1.95
LAMA1	Laminin, alpha 1	−19.14[b]	−5.70[b]	−6.06[a]	+3.36	+3.16	−1.06
LAMA2	Laminin, alpha 2	+1.34	+1.34[a]	+1.01	+1.00	−1.32	−1.33
LAMA3	Laminin, alpha 3	−3.85[a]	−3.06[b]	−3.38	+1.26	+1.14	−1.10
LAMB1	Laminin, beta 1	+1.17	+1.59	+1.30	+1.36	+1.11	−1.22
LAMB3	Laminin, beta 3	+6.03[a]	+2.94[b]	+25.30[a]	−2.05	+4.19	+8.61[a]
LAMC1	Laminin, gamma 1	+1.33	+1.29	+1.96[a]	−1.03	+1.48	+1.52
MMP1	Matrix metallopeptidase 1	+9.73	+7.44	+30.38	−1.31	+3.12	+4.08
MMP10	Matrix metallopeptidase 10	38.44[a]	+61.48[b]	+152.17[b]	+1.60	+3.96	+2.47
MMP11	Matrix metallopeptidase 11	−8.94[a]	−5.22	−4.31	+1.71	+2.07[a]	+1.21
MMP12	Matrix metallopeptidase 12	+2.44	+3.49[a]	+3.74	+1.43	+1.53	+1.07
MMP13	Matrix metallopeptidase 13	+2.62	+4.55	+2.09	+1.74	−1.25	−2.18
MMP14	Matrix metallopeptidase 14	−1.86	+1.03	+1.06	+1.93	+1.98	+1.03
MMP15	Matrix metallopeptidase 15	−2.68	+1.13	−1.39	+3.02	+1.93	−1.56
MMP16	Matrix metallopeptidase 16	−3.61	−2.24[a]	−3.58[b]	+1.61	+1.01	−1.60[a]
MMP2	Matrix metallopeptidase 2	−1.20	+1.32	+1.53	+1.57	+1.83	+1.16
MMP3	Matrix metallopeptidase 3	+17.24[a]	+9.80	72.35[a]	−1.76	+4.20	+7.38
MMP7	Matrix metallopeptidase 7	−1.23	+1.57	+3.40	+1.92	+4.16	+2.17
MMP8	Matrix metallopeptidase 8	+3.95	+5.52[a]	+1.01	+1.40	−3.90	−5.46
MMP9	Matrix metallopeptidase 9	+3.76	+8.11[a]	+25.34[a]	+2.16	+6.75[a]	+3.12
NCAM1	Neural cell adhesion molecule 1	+1.98	+1.54	+1.88	−1.29	−1.05	+1.22
PECAM1	Platelet/endothelial cell adhesion molecule	+2.83	+4.11	+2.73	+1.45	−1.04	−1.50
SELE	Selectin E	−1.23	+3.98	+12.17[a]	+4.91	+15.00[a]	+3.06
SELL	Selectin L	−1.13	+1.43	+3.51	+1.62	+3.98	+2.45
SELP	Selectin P	+1.70	+2.69	+4.80	+1.59	+2.83	+1.79
SGCE	Sarcoglycan, epsilon	−1.20	+1.21	−1.35	+1.46	−1.12	−1.63
SPARC	Secreted protein, acidic, cysteine-rich	−2.18	−1.51	−1.29	+1.44	+1.69	+1.17
SPG7	Spastic paraplegia 7	−1.13	+1.20	−1.03	+1.35	+1.10	−1.23
SPP1	Secreted phosphoprotein 1	+4.89	+8.21	+19.59[a]	+1.68	+4.00[a]	+2.39
TGFBI	Transforming growth factor, beta-induced	−1.68	−1.29	+1.26	+1.30	+2.11	+1.62
THBS1	Thrombospondin 1	−1.58	−1.24	+1.38	+1.27	+2.18	+1.72
THBS2	Thrombospondin 2	+3.92	+3.45	+7.81[a]	−1.13	+1.99[a]	+2.26
THBS3	Thrombospondin 3	−1.26	+1.48	+1.02	+1.87	+1.28	−1.46
TIMP1	TIMP metallopeptidase inhibitor 1	+1.83	+2.04[a]	+4.44[b]	+1.11	+2.43[a]	+2.18[a]

Zhao. Luteal support and endometrial ECM gene profile. Fertil Steril 2010.

TABLE 1

Continued.

Gene symbol	Description	(A) Day 3–5 vs. day 0			(B) Day 3–5 vs. day 3–5		
		IIa vs. I (no vs. no)	IIb vs. I (P vs. no)	IIc vs. I (P+E vs. no)	IIb vs. IIa (P vs. no)	IIc vs. IIa (P+E vs. no)	IIc vs. IIb (P+E vs. P)
TIMP2	TIMP metallopeptidase inhibitor 2	−1.65	−1.08	+1.18	+1.52	+1.95	+1.28
TIMP3	TIMP metallopeptidase inhibitor 3	−1.24	−1.64	−3.67[b]	−1.32	−2.96[a]	−2.24
TNC	Tenascin C (hexabrachion)	−1.28	+1.73	+3.19[b]	+2.22	+4.09[a]	+1.84
VCAM1	Vascular cell adhesion molecule 1	+1.76	+2.10	+4.20	+1.20	+2.39	+2.00
VCAN	Versican	+1.05	+1.69	+2.45	+1.61	+2.33	+1.45
VTN	Vitronectin	+1.99	+2.38	+3.11[a]	+1.20	+1.56	+1.31

Note: ADAM = A disintegrin and metalloproteinase, no = no luteal support; P = progesterone support; P+E = progesterone + estrogen support; TIMP = tissue inhibitor of metalloproteinases. (A) between day 3–5 and baseline (day 0) in the three groups; (B) between the three groups on day 3–5.

[a] $P<.05$.

[b] $P<.01$.

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and adhesion molecule genes up-regulated significantly by P and P+E were ADAMTS1, CLEC3B, COL15A1, ITGA3, ITGA5, ITGB4, MMP9, and TIMP1, whereas MMP16 was down-regulated significantly in both the P and the P+E support groups. The addition of E_2 up-regulated the expression of following genes: CD44, ICAM1, ITGB2, ITGB3, LAMC1, SELE, SPP1, THBS2, TNC, and VTN, whereas the genes COL11A1, Clol14A1, COL16A1, ECM1, FN1, ITGA8, and TIMP3 were significantly down-regulated compared with baseline. Looking at gene expression on day 3–5 after retrieval, among the three groups (IIa, IIb, IIc), luteal support with P alone significantly up-regulated ten genes and down-regulated two, whereas in the E+P support group three genes were up-regulated and six down-regulated, compared with the no support group.

Validation of Array Data with Quantitative Real-Time PCR

We arbitrarily selected four endometrial ECM protein and adhesion molecule genes (huITGB3, huLAMB3, huMMP10, and huTIMP1) with significant expression changes to undergo real-time PCR analysis accompanying with three housekeeping genes, (huACTB, huGAPDH, and huPGK1) for validation. The levels of expression for huACTB, huGAPDH, and huPGK1 genes were consistent in all groups. Expression profiles and effect of treatment with P and P+E for the four genes extracted from real-time PCR tests were compared with the microarray data as shown in Figure 3. Results for huITGB3, huLAMB3, huMMP10, and huTIMP1 expression corroborated the regulation profiles observed in the microarray study.

DISCUSSION

The present study has demonstrated that luteal phase support with exogenous steroids such as progesterone and estrogen has a profound impact on the expression of ECM pathway targeted and adhesion molecule genes.

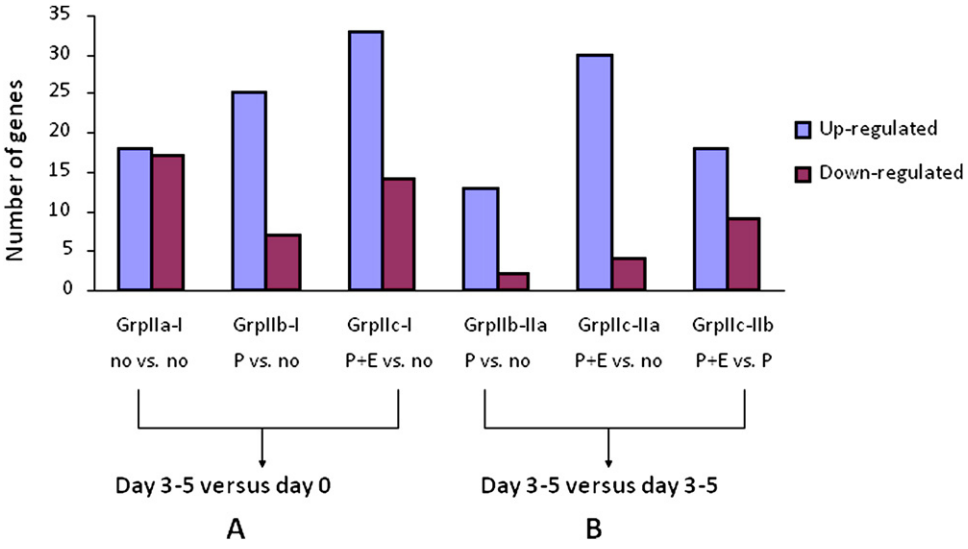
The implantation window opens approximately 4–5 days and closes 9–10 days after P exposure (14). Despite that, we purposely elected to obtain the endometrial biopsies at the time of the anticipated embryo transfer, which in most cases occurs around 3–5 days after retrieval. This is the period of the initial cross-talk between the embryo and the endometrium, which we believe has a pivotal role for subsequent attachment to the endometrium and successful implantation.

Recent studies using array technology revealed a large group of genes potentially related to endometrial receptivity (15, 16) in natural as well as in gonadotropin-stimulated cycles (17, 18). Haouzi et al. (17) reported, that although gene profiles during the endometrial transition from prereceptive to receptive phases are similar between patients, controlled ovarian stimulation regimens may alter endometrial receptivity compared with the natural cycle. Mirkin et al. (18) observed significant changes in endometrial gene expression between cycles using a GnRH agonist versus a GnRH antagonist (13 genes/expressed sequence tags and +1.42- to +2.10-fold changes). The effect however of different luteal phase supports on ECM protein and adhesion molecule gene profiles has not yet been addressed. Detection of P- and E_2-regulated genes and other gene pathways in the endometrium, after ovarian stimulation protocols, may assist in elucidating the cellular processes necessary for the development of an endometrial environment suitable for implantation.

Of the 86 genes represented on the array expressions, four genes were significantly up-regulated and five down-regulated at 3–5 days after oocyte retrieval in the group that had no luteal support, although 24 genes were significantly up-regulated and 14 down-regulated after luteal phase support with P or P+E treatment.

FIGURE 1

Number of ECM protein and adhesion molecule genes with more than twofold changes. (**A**) Between day 3–5 and day 0; (**B**) between the three groups on day 3–5. no = no luteal support; P = progesterone support; P+E = progesterone + estrogen support.

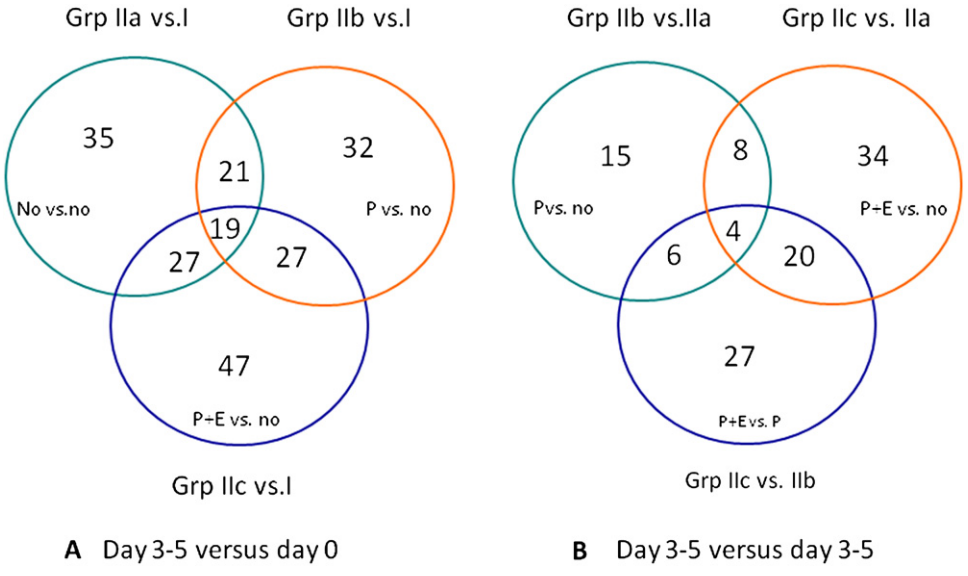


Zhao. Luteal support and endometrial ECM gene profile. Fertil Steril 2010.

Genes that were up-regulated included predominately those encoding for matrix metalloproteinases, such as MMP10, MMP3, and MMP9, and LAMB3, particularly after P or P+E treatment. MMPs not only degrade structural components of the ECM, thereby facilitating cell migration, but also affect cellular signaling and functions (19). MMP10 was increased 62-fold after P treatment and 152-fold after P+E, and MMP3 increased 72-fold after P+E support, suggesting an important role of these MMPs in the invasive process of implantation. Down-regulation of collagen-encoding genes, such as COL11A1, Col12A1, Col14A1, and CL16A1, mirrored the observations of elevated MMP gene expressions. Because the invading trophoblast produces MMP2, MMP9,

FIGURE 2

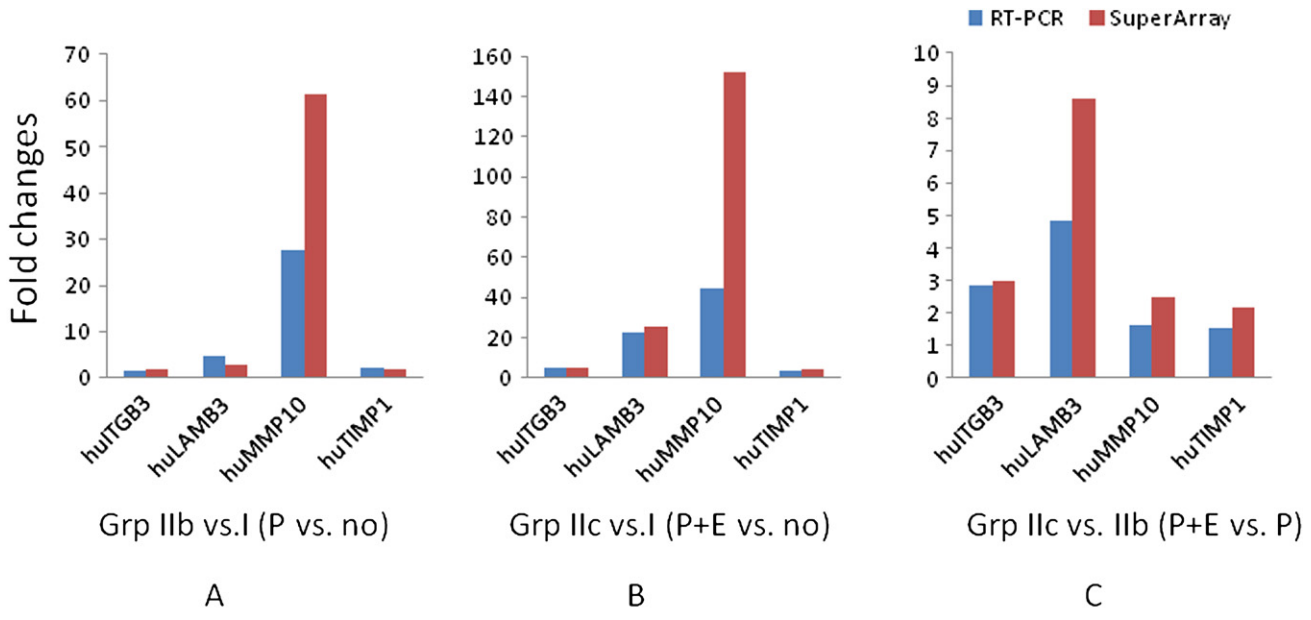
Venn diagram illustrations of the numbers of differentially expressed (at least twofold) ECM protein and adhesion molecule genes in the endometrium. (**A**) Changes within each group between day 3–5 and day 0; (**B**) changes between the three groups on day 3–5.



Zhao. Luteal support and endometrial ECM gene profile. Fertil Steril 2010.

FIGURE 3

Validation of the microarray findings for four selected genes: huITGB3, huLAMB3, huMMP10, and huTIMP1. Comparison between real-time polymerase chain reaction (PCR) validation test and Superarray profile. (**A**) Progesterone (P) vs. no support; (**B**): progesterone plus estrogen (P+E) vs. no support; (**C**): P+E vs. P-only support. The real-time PCR results agreed with microarray data with similar trend and significance ($P < .05$, two sides *t* test) in the relative comparisons.



Zhao. Luteal support and endometrial ECM gene profile. Fertil Steril 2010.

and MMP10, with predominately greater amounts of MMP9 (20), the balance, interaction, and synchronization of these MMPs between trophoblast and endometrium may play a critical role in the implantation process.

Most MMPs are secreted as proenzymes and activated when cleaved by extracellular proteinases. Increased expression of ADAMTS1, an ECM proteinase, after P and P+E supplementation (Table 1) may act to enhance the activity of MMPs. The ratio of activated MMPs to their inhibitors plays a key role in regulating MMP activity. Tissue-specific inhibitors (TIMPs) inhibit MMPs by binding at a 1:1 ratio to the active sites of MMPs. Up-regulation of TIMP1 and down-regulation of TIMP3 (Table 1) after luteal support indicate the necessity of MMP and TIMP balance for creating a receptive endometrial environment. Likewise, down-regulation of ADAMTS8, with the addition of E_2 to the P support scheme, suggests a differential role for E_2 and P in modulating ECM pathways.

Integrins, a class of bioadhesion molecules, participate in cell-cell and cell-substratum interactions by anchoring extracellular proteins to the intracellular cytoskeletal components (21). The cycle-specific pattern of endometrial integrin expression suggests hormonal regulation (22). Increased expression of integrins in the midluteal phase has been proposed as a marker of the window of implantation (23). In the present study, P treatment significantly increased the expression of several integrin genes, including ITGA3, ITGA5, and ITGB4, and P+E treatment enhanced the expression of ITGA3, ITGA5, ITGB2, and ITGB3 and suppressed ITGA6, ITGA8, and ITGB4. These observations support the notion that P and E_2 have a coordinated effect on ITGA3 and ITGA5 gene expression in the endometrium during the peri-implantation period. Differences in the expression of integrin genes such as ITGB4, ITGB5, ITGA6, and ITGA8 between groups IIb and IIc indicate separate roles for P and E_2 on spatial and temporal distribution of integrin genes.

Different luteal support schemes with P and P+E have also altered the expression of many ECM protein and adhesion molecule genes. Compared with P support, only the addition of E_2 in the P+E group significantly suppressed the expression of genes such as ADAMTS8, COL14A1, ECM1, ITGA6, ITGB4, and MMP16 (Table 1) 3–5 days after oocyte retrieval. Whether this represents a desired effect remains unclear. The role of E_2 addition to luteal support schemes in IVF-ET cycles has been controversial. A recent meta-analysis suggested that following controlled ovarian hyperstimulation for IVF-ET, supplementary administration of E_2 to P for luteal phase support did not improve implantation and pregnancy rates in either GnRH agonist or antagonist cycles (24).

In conclusion, alterations in gene expression profiles according to the type of supplementation indicate the complexity of steroid impact on tissue remodeling during the window of receptivity. The mechanisms of ECM gene regulation, interaction of ECM proteins and adhesion molecules, and the significance of differential expression of ECM pathway genes under different luteal support schemes require further investigation.

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